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[Sune Henning](#)<sup>\*</sup>, [Maretha Opperman](#), Sinazo Matika

Posted Date: 28 June 2024

doi: 10.20944/preprints202406.2032.v1

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## Article

# The Effects of Storage in Vegetable Oil on the Proximate Composition and Microbiological Quality of Hot Smoked South African Black Mussels (*Choromytilus meridionalis*) for Small-Scale Operations

Sune Henning <sup>1,\*</sup>, Maretha Opperman <sup>2</sup> and Sinazo Matika <sup>1</sup>

<sup>1</sup> Department of Food Science and Technology, Cape Peninsula University of Technology, Bellville, South Africa

<sup>2</sup> Functional Foods Research Unit, Cape Peninsula University of Technology, Bellville, South Africa

\* Correspondence: author: hennings@cput.ac.za

**Abstract:** Commercial production of *Choromytilus meridionalis* (black mussel) and *Mytilus galloprovincialis* (Mediterranean mussel) contributes to half of the South African marine aquaculture production per year. However, limited ready-to-eat black mussel products are available. The aim of this preliminary study was to investigate the effects of storage (15 days at room temperature) on the total viable counts (TVC), coliforms, proximate composition, and fat oxidation (TBARS) of a small-scale kitchen-based ready-to-eat hot smoked black mussel product preserved with vegetable oil. Three batches of black mussel meat were smoked at 80°C for 15 minutes. Half of each batch were packaged in sterile glass jars with heated (50°C) vegetable oil and half without oil. Microbiological analyses were done at days 1, 3, 9 and 15. Proximate composition was determined at day 1 and TBARS at days 1 and 15. Coliforms for all samples throughout the storage period were undetected. TVC plates for mussels stored without oil had no growth at days 1 to 5, however, reached TVC > 5 log<sub>10</sub>.g<sup>-1</sup> at day 15. TVC for mussels with oil increased from 0 at day 1 to > 5 log<sub>10</sub>.g<sup>-1</sup> at day 3 and remained at counts > 5 log<sub>10</sub>.g<sup>-1</sup>. There was no significant difference (P > 0.05) in moisture, protein, and ash for mussels stored with oil and those without oil, while significant differences (P < 0.001) in total fat and carbohydrates (10.27 ± 2.10% and 6.80 ± 2.22% with oil, and 5.05 ± 1.49% and 10.59 ± 3.00% without oil) were detected. These differences were due to the presence of additional fats from the vegetable oil. TBARS at day 15 showed no significant difference (P > 0.05) between mussels stored with oil and those without oil. There were no significant differences in TBARS from day 1 to 15 for both oil and without oil. Small-scale kitchen-based smoked black mussels preserved without vegetable oil could be stored for up to 9 days at room temperature.

**Keywords:** mussels; small-scale production; hot smoking; storage; vegetable oil; lipid oxidation

## 1. Introduction

Commercial mussel production contributes to half of the South African marine aquaculture production (DEFF 2021; Olivier et al., 2013). The South African West Coast is considered the major commercial production site of *Choromytilus meridionalis* (black mussel) and *Mytilus galloprovincialis* (Mediterranean mussel, also known as the blue mussel) (DEFF 2021; Marta et al., 2020; Probyn et al., 2015; Olivier et al., 2013). The black mussel is native to Southern Africa while the Mediterranean mussel was first detected on the West Coast in the 1970's (Griffiths et al., 1992). Commercial production of black and Mediterranean mussel comprised 51% (2,182.1 tonnes) of South African marine aquaculture production in 2018 and was the largest contributor to total marine aquaculture production in the same year (DEFF, 2021).

The Mediterranean and indigenous black mussel are used for exportation (66.17 tons in 2018). The Mediterranean mussels is also locally sold in the forms of fresh, frozen, or smoked and canned products (DEFF, 2021). However, the local utilisation of the black mussel is very low due to its soft texture and the dark colour of the female gonads (Firth, 2018; Heineken et al., 2017; Griffith & van Erkom Schurink, 1993). The most common use of the black mussel is its consumption among the coastal fishing communities, usually immediately after harvest. The black mussel is not available in any form of processed product. Further contributing to the underutilisation of the black mussel in the market are challenges in value addition at the level of small-scale producers, including local communities working out of their kitchens, consumer affordability, and safe processing of mussels for human consumption. With increasing populations both locally and internationally, mussels can contribute towards filling the void for the growing demand in food security (Olivier et al., 2013). To be able to meet this demand, value addition of mussel products is a major priority for small- and large-scale producers and public authorities (Avdelas et al., 2020). The need for value added mussel products are further driven by the decline in South African marine fish stocks due to overfishing which has led to a major negative impact on the livelihoods of the coastal fishing communities (Olivier et al., 2013).

The high water-activity ( $a_w > 0.95$ ) and pH (6.7–7.1) of mussels make them susceptible to microbial spoilage (Caglak et al., 2008; Sengör, 2004; Jay, 1992). Mussels are prone to contamination with *Vibrio parahaemolyticus*, *Pseudomonas*, *Enterobacter*, *Lactobacillus* and *Shewanella* spp. (Lamon et al., 2019; Goulas et al., 2005) which vary depending on the water quality where they are harvested, wash water and seasonal changes (Manousaridis et al., 2005). A fresh mussel has a shelf-life of less than 5 days (Caglak et al., 2008) at refrigerated conditions. Seafood cooking methods, such as smoking which include processing steps of brining, drying, and smoking with the use of wood chips, are not only to add value and create new products, but also to extend the shelf-life thereof (Doe & Olley, 1990). The primary purposes of smoking fish and fishery products include development of flavour, preservation action, creation of new products, protection from oxidation and rancidity, and development of colour (Horner, 1997). Smoke acts as a preservative since it inhibits microbial growth and retards fat oxidation (rancidity). Shelf-life extension of smoked fish is due to a combination of lowered water activity ( $a_w$ ) and the uptake by the product of bactericidal and antioxidant components of wood smoke. However, hygienic, and good manufacturing practices are of great importance as pathogens, such as *Listeria monocytogenes*, *Vibrio parahaemolyticus*, and *Vibrio cholera*, are associated with smoked mussels (Lamon et al., 2019; Brett et al., 1998). In most countries in Africa, smoking is the most widely practiced processing method to preserve fish and fishery products (Adeyeye & Oyewole, 2016).

In general, mussel meat is a good source of protein, essential omega-3 fatty acids, such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (Neri et al., 2021; Peycheva et al., 2021; Ackman, 2008) and micronutrients, such as zinc and iron (Peycheva et al., 2021; Kyriacou, 2017; Kyriacou et al., 2016; Kyriacou et al., 2014; Fuentes et al., 2009). However, nutritional content may exhibit substantial variation between habitat, region, season, gender, and diet (Peycheva et al., 2021; Neri et al., 2021; Zhou et al., 2014) as well as temporal and inter-species variations (Firth, 2018). Depending on species and season, raw mussels typically contain 77-85% moisture, 4-13% protein, 0-3% fat, 0-3% ash, and 1-7% carbohydrates (Neri et al., 2021; Kyriacou, 2017; Kyriacou et al., 2016; Kyriacou et al., 2014; Turan et al., 2008; Kyriazi-Papadopoulou et al., 2003).

To address the limited availability of safe and convenient South African black mussel products, value addition via product development for small-scale and community-based production is recommended. The implementation of food safety and quality assurance in small-scale fisheries and communities is always a challenge due to limited processing equipment, analytical capacity, and knowledge (GarridoGamarro et al., 2023). However, understanding of food safety risks for a specific fishery product within small-scale fisheries and communities, may result in education and potential investment in tools for food safety control. This may then aid in the improvement of food security within low-income fishing communities.

The aim of this study was to conduct a preliminary investigation on the effects of storage at ambient temperature of ready-to-eat hot smoked black mussels (*C. meridionalis*) in vegetable oil by analysing total viable counts, coliforms, proximate composition, and fat oxidation (TBARS), over a storage period of 15 days at ambient temperature. The smoking process and storage in oil was conducted as small-scale production trails at kitchen level for adoption by low-income communities where refrigeration remains a challenge.

## 2. Materials and Methods

### 2.1. Sample Preparation

Twenty kilograms of frozen half-shell black mussel (*C. meridionalis*) was obtained from the Blue Ocean Mussels processing facility, Velddrif, Western Cape Province, South Africa. The mussels were commercially harvested from a mussel farm in Pepper Bay near Saldanha Bay. The frozen mussels were transported to the Cape Peninsula University of Technology on ice. Upon arrival the mussels were immediately stored at -18°C until processing commenced.

Three batches of smoked mussel meat were prepared by randomly pooling individual mussels into three groups. For the smoking process, the mussels were removed from the freezer (-18°C) and thawed overnight in a refrigerator at 5-7°C. The mussel meat was manually removed from the shell with the use of a knife and sorted by size, eliminating mussel meat smaller than 3 cm in length. Mussel meat between 3 and 4 cm in length were used for further process to ensure uniform cooking of the mussels during the smoking process. The mussel meat for each batch was steamed at 80°C for 5 minutes in a strainer over boiling water. The steamed mussel meat was then brined in a 4% (w/v) sodium chloride solution for 15 minutes at 45°C. The ratio of mussel meat to brine was 1:2. The brined mussel meat was laid out on a smoking tray and dried at 60°C for 13 minutes, followed by smoking at 80°C for 15 minutes in a smoker (Junior Butcherquip J536, South Africa) using generic French oak wine barrel sawdust (Lk's sawdust for smokers, South Africa). After smoking, half of each batch was packaged into sterile glass jars (Consol Glass, South Africa) and filled with heated (50°C) vegetable oil (1:1 blend of extra virgin olive and sunflower seed oil; Clover, South Africa) while the other half was packaged without oil and stored at ambient temperature (21°C) for 15 days. Sampling for microbiological analyses were conducted at days 1 (24 hours after processing), 3, 9 and 15, respectively. Sampling for proximate composition were done on day 1, and samples for TBARS were analysed on days 1 and 15, respectively.

### 2.2. Microbiological Analyses

Total viable counts (TVC) were determined by using plate count agar (PCA, Merck) and total coliforms by using Violet Red Bile Agar (VRBA, Merck). Aliquot samples of 10 g of mussel meat were aseptically removed from each glass jar with the use of sterilized tweezers. The 10 g mussel meat samples were mixed and homogenised with 90 ml sterile 0.1% peptone water (Merck) in sterile stomacher bags using a Stomacher (Stomacher 80 lab blender, CLC-570, England). Dilution series up to  $10^{-6}$  were prepared. Pour plates were prepared by adding 1 ml from each dilution into petri dishes, followed by tempered agar. Plates were prepared in triplicate for each dilution and incubated at 30°C for 72 hours for TVC and at 37°C for 24 hours for VRBA. Bacterial colonies on the agar were counted and expressed as  $\log_{10}$  CFU per gram mussel meat ( $\log_{10}$  cfu.g<sup>-1</sup>). Only agar plates with colony forming units (cfu) between 25 and 250 were considered for statistical analyses.

### 2.3. Proximate Composition Analyses

After aliquot samples of mussel meat for microbiological analyses were aseptically removed from each glass jar, the mussel meat from glass jars with vegetable oil was strained to remove excess oil. The mussel meat from each glass jar with oil and those without oil for day 1 (i.e. 24 hours after processing) were individually homogenised at 1500 Watts using a food processor (Kenwood, 220-240 V~ 50/60 Hz).



The AOAC Official methods were followed to determine crude protein (AOAC 2002a, method 992.15), moisture (AOAC 2002b, method 934.01) and crude ash (AOAC 2002c, method 942.05). The crude protein content was determined as a percentage (%) by multiplying the percentage of nitrogen by the protein conversion factor ( $N \times 6.25$ ) for meat, poultry, and fish. Crude fat content was determined using the chloroform:methanol (2:1) solvent extraction method described by Lee et al. (1996), containing 0.01% butylated hydroxytoluene as an antioxidant (Oyenihi et al., 2020). The carbohydrate content of each sample was determined by difference as % carbohydrates as follows:  $100 - (\% \text{ moisture} + \% \text{ ash} + \% \text{ protein} + \% \text{ lipid})$ .

#### 2.4. Determination of the TBA Reactive Substances (TBARS)

Volumes of 100  $\mu\text{l}$  of the mussel-tissue fat extracts were pipetted into a 2 ml Eppendorf-tubes. The samples were mixed with a volume of 12.5  $\mu\text{l}$ , 4 mM of butylated hydroxytoluene and 100  $\mu\text{l}$  of 0.2 M ortho-phosphoric acid. The solutions were vortexed for ten seconds and 12.5  $\mu\text{l}$  thiobarbituric acid (TBA) was added to the mixtures. The mixtures were heated at 90°C for 45 minutes. After the heating was completed, the mixtures were placed into an ice-bath for 2 minutes to cool. The cooled mixtures were kept at ambient temperature for five minutes. A volume of 1 ml of n-butanol was added to the mixtures, followed by the addition of 100  $\mu\text{l}$  of saturated sodium chloride (NaCl) and vortexed for ten seconds. The mixture of solutions and extract were centrifuged at 6000 rpm for 5 minutes at 4°C. Aliquots of 300  $\mu\text{l}$  of the supernatants were placed into a 96 well plate in duplicate and absorbance was recorded at 532-572 nm using a microspectrophotometer (Fluostar omega microplate, Germany). The TBAR values were expressed as nmol malonaldehydes equivalents per gram of mussel meat ( $\text{MDA} \cdot \text{g}^{-1}$  mussel meat) and were calculated as follow:

$$\text{TBARS} = \frac{\text{Sample absorbance at 532} - \text{Sample absorbance at 572}}{\epsilon = 154000}$$

#### 2.5. Statistical Analysis

The treatment of storage with oil vs. without oil was replicated three times by preparing three different bathes of smoked mussels. Homogenates of mussel meat for different analyses were prepared per sampling day per storage method by homogenising 10 to 12 mussels. Microbiological and proximate analyses were done in triplicate, while TBARS were analysed in duplicate. The means and ( $\pm$ ) standard deviations were reported for each case. The effects of the independent variable storage in oil on the proximate composition was determined by subjecting the data to multivariate analysis of variance (MANOVA) using SPSS Statistics 28 (IBM, Chicago, USA, 2022). The effects of the independent variables, storage in oil, and storage time, on the dependent variables TVC and TBARS, was determined by subjecting the data to two-way analysis of variance (ANOVA) using SPSS, respectively. The least significant difference (LSD) procedure was used to test for differences between means of the dependable variables as confidence level 95%.

### 3. Results and Discussion

#### 3.1. Microbiological Analyses

The counts for total coliforms indicated no growth throughout the storage period of 15 days for both the mussels preserved with and without oil. Mohibbullah et al. (2018) reported similar results for coliforms during a storage period of 14 days at 10°C and 15°C, respectively, for superheated steam roasted and hot-smoked (75°C) pen shell mussel (*Atrina pectinate*).

The TVC significantly ( $P < 0.05$ ) increased from day 1 (no growth) to days 3 ( $7.34 \log_{10} \text{ cfu} \cdot \text{g}^{-1}$ ), 9 ( $5.55 \pm 1.93 \log_{10} \text{ cfu} \cdot \text{g}^{-1}$ ) and 15 ( $5.87 \pm 0.77 \log_{10} \text{ cfu} \cdot \text{g}^{-1}$ ) for the smoked mussels stored with oil (Table 1). However, there was no significant ( $P > 0.05$ ) difference in TVC between days 3, 9 and 15. The smoked mussels preserved without oil, showed no growth for days 1, 3 and 9. At day 15, the TVC for mussels without oil displayed a count of  $5.64 \pm 0.94 \log_{10} \text{ cfu} \cdot \text{g}^{-1}$ , which was similar ( $P > 0.05$ ) to that for mussels stored with oil ( $5.87 \pm 0.77 \log_{10} \text{ cfu} \cdot \text{g}^{-1}$ ). The increase in TVC at day 3 for the mussels with

oil, while there was no growth for mussels without oil, indicated potential contamination from the vegetable oil.

At 15 days of storage at ambient temperature, TVC for the smoked mussels with and without oil reached log<sub>10</sub> values exceeding the total number of TVC of 5 log<sub>10</sub> cfu.g<sup>-1</sup> as governed by the South African Regulations for microbiological standards for cooked seafood and mussels (GNR, 1997).

**Table 1.** Mean (± standard deviations) log<sub>10</sub> cfu.g<sup>-1</sup> for TVC for smoked black mussels preserved in vegetable oil vs. smoked black mussels preserved without oil during a storage period of 15 days at ambient temperature.

Storage time (days)	Total viable counts (log <sub>10</sub> cfu.g <sup>-1</sup> )	
	Smoked mussels with oil	Smoked mussels without oil
Day 1	No growth	No growth
Day 3	7.34 ± 0.00 <sup>a</sup>	No growth
Day 9	5.55 ± 1.93 <sup>b</sup>	No growth
Day 15	5.87 ± 0.77 <sup>b</sup>	5.64 ± 0.94 <sup>b</sup>

<sup>ab</sup> Different superscripts differ significantly at P < 0.05.

Several studies (Bernárdez and Pastoriza, 2011; Caglak et al., 2008; Khan et al., 2005) reported initial total bacterial counts for raw mussels in the range of 3 to 5 log<sub>10</sub> CFU.g<sup>-1</sup>. During storage, these counts can increase up to more than 8 log<sub>10</sub> CFU.g<sup>-1</sup> after 8 to 14 days of storage at low temperatures. The smoked black mussels stored without oil had TVC values in the range of 5 log<sub>10</sub> CFU.g<sup>-1</sup> at day 15 (Table 1) clearly exhibiting the antimicrobial and inhibitory effects of smoking. For smoked black mussel meat, there was no growth for TVC at day 1 for both the mussels with oil and those without oil, indicating the antimicrobial preservation action of the hot smoking process (Kyriazi-Papadopoulou et al., 2003; Feiner, 2006; Miler & Sikorski, 1990). The antimicrobial effects of hot smoke processing are not only because of the salting, drying (i.e reduced water activity), and heating steps (Kyriazi-Papadopoulou, 2003), but also due to the antimicrobial compounds present in the smoke. The composition of wood smoke is complex and differs among the various types of sawdust or woodchips, but part of the inhibition action against bacteria is due to the presence of formaldehydes, aldehydes, phenols, and organic acids such as carboxylic acid (Feiner, 2006; Miler & Sikorski, 1990). The smoke components that exhibit the highest antimicrobial activity are the carboxylic acids and phenols. Depending on the concentration, smoke compounds either diminish the growth rate of micro-organisms, or reduce their count, with viable cells being more readily affected than spores.

3.2. Proximate Composition

Table 2 summarises the proximate composition at day 1 for smoked black mussels preserved in vegetable oil and those not preserved in oil. Since the same smoking method was used for all the batches, there was no significant difference (P > 0.05) in moisture, protein, and ash for smoked mussel meat between the two storage methods of oil vs. without oil. There was, however, a significant difference (P < 0.001) in total fat and carbohydrates between mussels with oil (10.27 ± 2.10% and 6.80 ± 2.22%) and those preserved without oil (5.05 ± 1.49% and 10.59 ± 3.00%). The higher fat content for the mussel meat preserved with oil was due to some vegetable oil remaining on the surface of the mussel meat after removing it from the oil (excess oil was drained from the mussels before analyses commenced). The lower carbohydrate value for the mussels stored with oil compared to those stored without oil is explained by the calculation of carbohydrates as a difference of the protein, moisture, ash, and fat constituents out of 100 (%). Due to the higher fat value for the mussels with oil, the calculation in difference for the carbohydrates equated to a lower value as appose to the mussels stored without oil. Similar results for carbohydrate content were documented by Mohibullah et al. (2018) for hot-smoked pen shell mussel (*Atrina pectinata*) from Asia. These authors reported a carbohydrate content of 10.53% for the abductor muscle of the pen shell mussel after treatments of superheated steam, followed by hot smoking. Neri et al. (2021) reported a carbohydrate content of between 3 and 5.2%, depending on the month of harvest, for fresh, raw *Mytilus edulis* mussels from

Korea. Higher values of carbohydrates for smoked mussels can be attributed to the concentration effect of the smoking process during the drying and heating steps.

**Table 2.** Mean ( $\pm$  standard deviation) protein, moisture, ash, and fat content (%) for smoked mussels stored with oil and smoked mussels stored without oil at day 1 (i.e. 24 hours after processing).

Storage method	Protein (%)	Moisture (%)	Ash (%)	Fat (%)	Carbohydrates (%)
Mussels in oil	16.55 $\pm$ 1.76 <sup>a</sup>	63.50 $\pm$ 2.51 <sup>a</sup>	2.88 $\pm$ 0.36 <sup>a</sup>	10.27 $\pm$ 2.10 <sup>a</sup>	6.80 $\pm$ 2.22 <sup>a</sup>
Mussels without oil	18.29 $\pm$ 2.10 <sup>a</sup>	63.54 $\pm$ 3.03 <sup>a</sup>	2.54 $\pm$ 0.51 <sup>a</sup>	5.05 $\pm$ 1.49 <sup>b</sup>	10.59 $\pm$ 3.00 <sup>b</sup>

<sup>ab</sup> Different superscripts within a column differs significantly at  $P < 0.05$ .

Previous studies (Kyriacou, 2017; Kyriacou et al., 2016; Kyriacou et al., 2014) investigating the protein content of black mussels along the Western Cape and KwaZulu-Natal coasts of South Africa reported a protein content ranging between 4.7 and 9.1  $\pm$  2.7% for raw tissue. The high protein content for smoked black mussels preserved with and without oil (16.55  $\pm$  1.76% and 18.29  $\pm$  2.10%, respectively) can be contributed to the concentration effect of the drying and cooking steps during the hot smoking process due to the loss of moisture (Turan et al., 2008; Kyriazi-Papadopoulou et al., 2003). In a study by Kyriazi-Papadopoulou et al. (2003) hot smoking of Mediterranean mussel (*Mytilus galloprovincialis*) reduced the moisture content to 62.60  $\pm$  3.19%, while increasing the protein (15.59  $\pm$  1.63%) and the fat (3.29  $\pm$  1.31%) content to values corresponding to this study (Table 1). In contrast, Turan et al. (2008) reported a much higher protein content (22.22%) for hot smoked Mediterranean mussel.

The fat content for the smoked black mussels preserved without oil (5.05  $\pm$  1.49%) was higher compared to that reported by Kyriacou (2017) and Kyriacou et al. (2016; 2014) for raw black mussel meat (ranging between 0.80 and 1.1  $\pm$  0.40%). This is contributed to the concentration effect of the hot smoking process due to loss of moisture. The fat content for smoked black mussels without oil was higher compared to hot smoked Mediterranean mussel (*Mytilus galloprovincialis*) reported by Kyriazi-Papadopoulou et al. (2003), while lower than reported by Turan et al. (2008) (10.04%). These differences can be attributed to species, regional, and seasonal affects (Neri et al., 2021; Fuentes et al., 2009; Turan et al., 2008). The high ash content for the smoked black mussels both preserved with (2.88  $\pm$  0.36%) and without oil (2.54  $\pm$  1.49%) may be contributed, in addition to the concentration effect of the smoking process, to the salt (NaCl) added as preservative in the brining step. Turan et al. (2008) also reported that hot smoking of Mediterranean mussel increased the ash (6.02%) content compared to that of the raw mussels (0.95%).

3.3. TBA Reactive Substances (TBARS)

Thiobarbituric acid (TBA) reactive substances (TBARS) are indicative of changes in lipid oxidation, specifically the formation of secondary oxidation products (Rustad, 2010), and is related to off-flavour development in mussel meat (Cecchi et al., 2018). At day 15 of storage at ambient temperature, there was no significant difference ( $P > 0.05$ ) in TBARS (Table 3) between smoked mussels with oil and those without oil (Table 3). Although there was a slight reduction in TBARS between day 1 and 15 for smoked mussels preserved in oil, it was not significant ( $P > 0.05$ ). Similarly, the increase in TBARS from day 1 to day 15 for mussels preserved without oil, was not significant ( $P > 0.05$ ). TBARS values above 1 000 to 2 000 nmol MDA.g<sup>-1</sup> (1-2  $\mu$ mol MDA.g<sup>-1</sup> fat) is typically indicative of rancidity (Campo et al., 2006; Connell, 1975). The low TBARS values (< 2 nmol MDA.g<sup>-1</sup>) of the mussel meat in this study could be attribute to the antioxidant effect of smoke (Miler & Sikorski, 1990) from the hot smoking process. However, it is well documented that TBA can also react with proteins, amino acids, trace metals, sugars, and nucleic acids, resulting in TBARS values for different foods with the same level of oxidation, based on flavour scores, to vary significantly (Frankel, 2005; Nawar, 1996).

**Table 3.** Mean ( $\pm$  standard deviation) for TBARS (nmol  $^{\circ}$ MDA.g $^{-1}$ ) levels in mussel meat for smoked black mussels stored with and without oil at ambient temperature ( $\sim$ 21 $^{\circ}$ C) at days 1 and 15, respectively.

Storage time (Days)	Storage method	
	Mussels in oil	Mussels without oil
Day 1	1.099 $\pm$ 0.676 <sup>a</sup>	0.782 $\pm$ 0.238 <sup>a</sup>
Day 15	0.723 $\pm$ 0.274 <sup>a</sup>	0.871 $\pm$ 0.328 <sup>a</sup>

<sup>ab</sup> Different superscripts within columns and rows differs significantly at P < 0.05.  $^{\circ}$ MDA = malonaldehydes equivalents per gram of mussel meat.

Mohibbullah et al. (2018) demonstrated the inhibitory effect of hot smoking on the development of TBARS of processed pen shell (*Atrina pectinate*) abductor muscle during a shelf-life study of 13 days, showing a reduction from day 0 to day 13 at a storage temperature of 15 $^{\circ}$ C. In contrast to smoked mussels, Khan et al. (2005) documented a gradual increase in TBARS for raw blue mussels (*Mytilus edulis*) during 10 days of ice-storage, after which the values declined, possibly due to chemical interactions of the secondary oxidation products with proteins, amino acids, and other food components (Rustad, 2010).

4. Conclusions

In terms of macro-nutritional content, this study demonstrated that the use of vegetable oil to preserve a ready-to-eat, hot smoked mussel product, increased the total fat content of the final mussel meat due to residual oil. Storage at ambient temperature for up to 15 days had no significant effect on secondary lipid oxidation products of the smoked black mussel meat as the TBARS were less than 2 nmol MDA.g $^{-1}$  at the beginning and the end of the shelf-life period. From this study, preserving of hot smoked South African black mussels in vegetable oil had a significant effect on the total bacterial count (TVC) over a storage period of 15 days at ambient temperature. In contrast to the TVC, the coliform counts indicated a hygienic product. It is, however, unclear from this study whether the high TVC is due to only spoilage organisms and/or pathogens such as *Vibrio* species and *Salmonella*. Already at 3 days of ambient storage, the mussels in oil showed a high bacterial count (TVC), indicating potential contamination from the vegetable oil, limiting the shelf-life thereof in terms of microbial growth. The quality of the vegetable oil used in the preservation of smoked mussels at small-scale kitchen level is of great importance for food safety and quality. The hot smoked black mussels preserved in sterile glass jars without oil, had a shelf-life of up to 9 days when stored at ambient temperature, indicating the preservation action of the hot smoking process. In communities (low-income settlements) where the cold chain is not guaranteed to be maintained, or is completely absent, a product that does not require refrigeration would be ideal. In this study, the use of vegetable oil to preserve smoked mussels in glass jars limited the shelf-life thereof in terms of microbiological growth and thus cannot be recommend to the communities as a safe product. It is therefore recommended to investigate the potential shelf-life of a mussel pickle product, where heating of the oil together with spices and/or the mussel meat is to be a processing step, keeping in mind that the process is to be small-scale kitchen-based processing to address the adoption by low-income communities, who typically have limited access to large-scale processing equipment.

**Acknowledgements:** This study was funded by the Cape Peninsula University of Technology Pre-SEED Funds.

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