The effect of different cooking methods on fatty acid composition and antioxidant activity of n-3 fatty acids fortified tilapia meat with or without clove essential oil

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Abstract

Tilapia farmers are increasingly relying on dietary fish oil alternatives which substantially reduces health beneficial n-3 polyunsaturated fatty acids (PUFA) in tilapia products. This may be further exacerbated depending on the cooking method. This study aimed to evaluate the effects of different cooking methods on the fatty acid composition and oxidative stability of tilapia minced meat after prior fish oil fortifications with or without clove essential oil. Results showed that frying tilapia in either sunflower or palm oil significantly increased the saturated fatty acid and linoleic acid content, respectively, of tilapia. However, fish oil fortifications significantly increased the n-3 PUFA content, but tended to decrease oxidative stability, particularly when microwaving. This was mitigated by clove essential oil, which significantly improved oxidative stability after cooking. Results indicate that n-3 PUFA and clove essential oil fortifications is an effective method to deliver and protect these beneficial fatty acids for human consumers.

Key words

Clove essential oil, Fortification, Lipid oxidation, n-3 PUFAs, Tilapia

Introduction

Tilapia farming has quadrupled in production over the past few decades, and is currently the second most farmed fish in the world (FAO, 2011). This is due to many beneficial characteristics conducive to aquaculture, including their ability to accept and thrive on cheaper and more readily available terrestrially based ingredients such as fish oil alternatives (Ng et al., 2013). While more sustainable practice has greatly contributed to the exponential growth in the tilapia industry, this has also led to some public health concerns.

Tilapia have a relatively limited ability for the de novo synthesis of long chain polyunsaturated fatty acids (LC-PUFA) (Olsen et al., 1990; Tocher et al., 2002) and consequently, deficiencies in these beneficial fatty acids within their diets will also lead to low levels within their flesh (Ng et al., 2013; Szabó et al., 2011). Indeed, when various fish species were recently tested and compared for their fatty...
acid composition, tilapia was found to be least healthy due to relatively high n-6:n-3 fatty acid ratio along with high saturated fatty acid (SFA) content (Weaver et al., 2008; Young, 2009). Since these fatty acids are believed to increase the risk for heart disease and other degenerative diseases (Chow, 2007), tilapia’s public image as a healthy choice has suffered over the years.

In response, increasing attention has been made towards improving the nutritive benefits of tilapia. One method is developing “finishing” diets that typically contain high n-3 PUFA content, to enhance these healthy benefitting fatty acids in tilapia prior to harvesting (Ng et al., 2013; Shapira et al., 2009; Trushenski et al., 2009). For example, Ng et al., (2013) found that tilapia previously fed diets containing various fish oil alternatives had a low LC-PUFA content, but was significantly enhanced after one month of being fed a fish oil-based finishing diet. While the use of n-3 PUFA enriched finishing diets may be an effective practice, these diets are also often more expensive. As such, this provides little incentive for farmers to adopt such practices since tilapia are often sold as generic products (Ng and Romano, 2013).

However, in some cases when tilapia meat are minced and sold as branded sausages and burgers, these products can be directly fortified with n-3 PUFA by food companies and advertised as a healthier option. While this is likely to be a more cost-effective practice than finishing diets, important considerations need to be made since LC-PUFAs are more susceptible to peroxidation, which can deteriorate the quality of the product (Andrés et al., 2009; Panpipat and Yongsawatdigul, 2008) or off-flavors (Valencia O’Grady et al., 2008). Moreover, different cooking methods can also influence lipid peroxidation (Al-Saghir et al., 2004; Weber et al., 2008), as well as the proximate and/or fatty acid composition (Garcia-Arias Pontes et al., 2003; Gokoglu et al., 2004; Lee et al., 2006a). Subsequently, research on both terrestrial and aquatic animal meat products have been conducted to evaluate the efficacy of various anti-oxidants to improve oxidative stability during storage or after cooking (Lee et al., 2006a; Panpipat and Yongsawatdigul, 2008; Valencia et al., 2008). For example, while different cooking methods can increase lipid peroxidation of n-3 PUFA fortified meat, compared to non-fortified meat (Lee et al., 2006a,b), antioxidants can potentially mitigate this deterioration (Lee et al., 2006a,b; Pérez-Mateos et al., 2006; Valencia et al., 2008).

To the best of our knowledge, no information is available regarding the effects of different combinations of antioxidant and n-3 PUFA fortifications on the oxidative stability of meat products. The aim of the current experiment was to examine the effects of different cooking methods on the lipid content, fatty acid composition and oxidative stability of tilapia minced meat after prior fish oil fortifications with or without clove essential oil.

**Materials and Methods**

**Fish samples** : Live red tilapia weighing 0.7-0.9 kg per fish were purchased from a local supermarket in Kuala Lumpur. Fish were immediately sacrificed by a blow in the head and transferred to the laboratory within 30 min. The fish head, tail, internal organs, skin and backbone were removed, and remaining samples were completely rinsed under tap water. The samples were minced using a commercial meat grinder through a 5 mm die and divided into two batches. One batch was fortified with menhaden fish oil as a source of omega-3 at 10% (w/w) ratio, while other batch was kept non-fortified. Both omega-3 fortified and non-fortified batches were then divided into two subgroups with one subgroup from each batch receiving clove essential oil (Sigma-Aldrich, 8000-34-8, Louis, USA) as a natural antioxidant at a concentration of 0.1% (w/w). Fifteen 100 g of homogenous samples were then prepared from each subgroup for immediate cooking, i.e. three 100 g samples were used for each heating treatment: Control (raw), grilling, baking in microwave, sunflower oil or frying in palm oil.

**Cooking methods** : Common ways of cooking were used. Microwave-baked samples were prepared in a microwave (Panasonic NN-ST557M) at potency 10, for 2 min. The mean core temperature immediately after cooking was 95±5 °C. Grilled samples were prepared in a griller (Panasonic AE 300N) with temperature set at 270 °C. The samples were grilled for 10 min (each side 5 min). The mean core temperature immediately after grilling was around 86±9 °C. The fish samples were deep-fried in sunflower oil and palm oil for 4 min. The frying temperature was around 210 and 225 °C in the sunflower oil and palm oil, respectively. Mean core temperatures immediately after frying in sunflower oil were 89±5 °C and after frying in palm oil were 92±6 °C. Samples of raw or cooked fish patties were immediately used for proximate and fatty acid composition, as well as the levels of lipid peroxidation, DPPH scavenging activity and ferric reducing antioxidant power (FRAP).

**Moisture and lipid content** : The samples were lyophilized for 48 hrs and the lost moisture was calculated. This was followed by crude lipid determination by ether-extraction.
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90°C for 60 min and cooled with water. After adding 3 ml of thiobarbituric acid solution. The mixture was then heated at µl of sodium dodecyl sulphate (SDS) and 2 ml of was mixed with 300 µl of distilled water, 35 µl of BHT, 165 1.15% KCl. After that, 200 µl of the homogenized sample modifications. One g of sample was homogenized in 4 ml of (TBARS) as described by Ohkawa was determined using thiobarbituric acid-reactive substances (Supelco, Bellfonte PA, USA) and menhaden oil (Sigma-

Determination of lipid peroxidation : Lipid peroxidation was determined using thiobarbituric acid-reactive substances (TBARS) as described by Ohkawa et al., (1979) with slight modifications. One g of sample was homogenized in 4 ml of 1.15% KCl. After that, 200 µl of the homogenized sample was mixed with 300 µl of distilled water, 35 µl of BHT, 165 µl of sodium dodecyl sulphate (SDS) and 2 ml of thiobarbituric acid solution. The mixture was then heated at 90 °C for 60 min and cooled with water. After adding 3 ml of n-butanol, the solution was centrifuged at 5,000 rpm for 10 min. The absorbance of the supernatant was measured at 532 nm by a spectrophotometer. The TBARS were calculated from a standard curve of 1, 1, 3, 3- tetraethoxypropane and expressed as mg malondialdehyde (MDA)/kg sample.

DPHH free radical scavenging activity : The DPPH free radical scavenging activity was determined according to method of Qwele et al. (2012) with slight modifications. A volume of 800 µl of DPPH methanolic solution (0.05 mM) was mixed with 200 µl of homogenized sample prepared, as described in section 2.3.3. After 20 min, the absorbance was recorded at 517 nm in a UV-visible microplate reader (Molecular Devices, Sunnyvale, CA). The inhibition percentage of the DPPH radicals was calculated using the following equation:

\[ \text{Inhibition(\%)} = \left( \frac{A_0 - A_1}{A_0} \right) \times 100\% \]

Where, \( A_0 \) is the absorbance of DPPH methanolic solution without sample and \( A_1 \) is the absorbance of DPPH methanolic solution having the sample after 20 min. Trolox was used as a positive control to convert the inhibition capability (%) to the trolox equivalent antioxidant capacity (TEAC) according to the method of Serpen et al. (2012).

Ferric reducing activity power (FRAP) : The FRAP was determined according to the method of Benzie and Strain (1999). This method is based on the reduction of 2,4,6-tripryridyl-s-triazine complex with a yellow color (Fe³⁺-TPTZ) to the ferrous form (Fe²⁺-TPTZ) with a blue color. A volume of 200µl of homogenized sample (prepared as described in section 2.3.3) was mixed with 800 µl of 10 mM ferric-TPTZ reagent and the changes in the absorbance after 20 min of incubation was measured at 593 nm using a UV-visible microplate reader (Molecular Devices, Sunnyvale, CA). Trolox was used as standard, and the FRAP of meat extract was reported as TEAC.

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Using a Soxtec System (AOAC, 1990).

**Fatty acid profile**: Lipid from each sample was extracted according to the method of Ebrahimi et al., (2012) with a chloroform:methanol (2:1 v:v) mixture as solvent. After a series of centrifugation with normal saline, liquid/liquid phase separation and saponification with KOH (R & M Chemicals, Essex, U.K.) in methanol, the lipid fraction was methylated using 14% methanolic boron trifluoride (Sigma-Aldrich, St. Louis, Missouri, USA) (Ramezani-Fard et al., 2012). Fatty acid methyl esters (FAMEs) were then analyzed using a gas chromatograph (Agilent 7890A; Agilent Technologies Inc., Santa Clara CA, USA) equipped with a split/splitless injector (ratio 1:10), a fused silica capillary column (Supelco, Bellefonte PA, USA; SP-2330: 30m × 0.25mm, 0.20 µm in film thickness ) and a flame ionization detector (FID). High purity hydrogen (99.99%) @ 40 ml min⁻¹ was used as a carrier gas and 1 µl of the sample was injected into the gas chromatograph by an automatic injector. Column temperature was set at 100 °C for 2 min, increased to 170 °C @ 10 °C min⁻¹, maintained for 2 min and increased again from 170 to 200 °C @ 7.5 °C min⁻¹ and maintained at 200 °C for 20 min. Injector and detector temperature were set at 250 and 300 °C, respectively. Fatty acids were identified by comparing their retention time with 37 component FAME mix standards (Supelco, Bellefonte PA, USA) and menhaden oil (Sigma-Aldrich, St. Louis, Missouri, USA). The results were expressed as the area percentage of total identified fatty acids.

**Table 1 :** Moisture and lipid content (g 100g⁻¹ meat) of non-fortified and n-3 pufa fortified tilapia meat after different cooking methods

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Non-fortified</th>
<th>n-3 PUFA fortified</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw</td>
<td>Grilled</td>
</tr>
<tr>
<td>Moisture</td>
<td>65.3±</td>
<td>49.6±</td>
</tr>
<tr>
<td>Lipid</td>
<td>9.2±</td>
<td>9.7±</td>
</tr>
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</table>
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778

E. Ramezani-Fard et al.

oil fortification on lipid peroxidation, DPPH free radical scavenging activity and ferric reducing antioxidant power within each cooking method were analysed using one-way ANOVA. Difference among the means were tested by Duncan's multiple range test at P < 0.05. All the analyses

Statistical analysis : All the experimental data was expressed as mean±SE. A two-way analysis of variance (ANOVA) was performed to compare possible differences in moisture, lipid and fatty acid values due to n-3 fortification and cooking method. Effect of n-3 PUFA and clove essential oil fortification on lipid peroxidation, DPPH free radical scavenging activity and ferric reducing antioxidant power within each cooking method were analysed using one-way ANOVA. Difference among the means were tested by Duncan's multiple range test at P < 0.05. All the analyses

Table 2 : Fatty acid composition (g fatty acid 100 g−1 total fatty acids) of non-fortified and n-3 pufa fortified tilapia meat after different cooking methods

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Non-fortified</th>
<th>n-3 PUFA fortified</th>
<th>Statistical analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw</td>
<td>Grilled</td>
<td>Micro wave-baked</td>
</tr>
<tr>
<td>14:0</td>
<td>3.50±</td>
<td>3.42±</td>
<td>3.53±</td>
</tr>
<tr>
<td>14:1</td>
<td>0.01±</td>
<td>0.02±</td>
<td>0.03±</td>
</tr>
<tr>
<td>16:0</td>
<td>0.00±</td>
<td>0.23±</td>
<td>0.01±</td>
</tr>
<tr>
<td>16:1</td>
<td>6.70±</td>
<td>7.59±</td>
<td>6.71±</td>
</tr>
<tr>
<td>17:0</td>
<td>0.60±</td>
<td>0.92±</td>
<td>0.34±</td>
</tr>
<tr>
<td>17:1</td>
<td>0.00±</td>
<td>0.04±</td>
<td>0.00±</td>
</tr>
<tr>
<td>18:0</td>
<td>6.59±</td>
<td>7.06±</td>
<td>6.45±</td>
</tr>
<tr>
<td>18:1n-9</td>
<td>37.39±</td>
<td>35.84±</td>
<td>37.24±</td>
</tr>
<tr>
<td>18:2n-6</td>
<td>12.79±</td>
<td>12.41±</td>
<td>12.82±</td>
</tr>
<tr>
<td>18:3n-3</td>
<td>1.52±</td>
<td>1.66±</td>
<td>1.51±</td>
</tr>
<tr>
<td>18:4n-6</td>
<td>0.02±</td>
<td>0.53±</td>
<td>0.08±</td>
</tr>
<tr>
<td>18:5n-3</td>
<td>0.18±</td>
<td>0.07±</td>
<td>0.15±</td>
</tr>
<tr>
<td>20:4n-6</td>
<td>0.12±</td>
<td>0.27±</td>
<td>0.32±</td>
</tr>
<tr>
<td>20:5n-3</td>
<td>0.06±</td>
<td>0.02±</td>
<td>0.04±</td>
</tr>
<tr>
<td>22:5n-3</td>
<td>0.02±</td>
<td>0.63±</td>
<td>0.70±</td>
</tr>
<tr>
<td>22:6n-3</td>
<td>0.12±</td>
<td>0.03±</td>
<td>0.06±</td>
</tr>
<tr>
<td>∑SFA</td>
<td>37.26±</td>
<td>37.59±</td>
<td>37.51±</td>
</tr>
<tr>
<td>∑MUFA</td>
<td>44.58±</td>
<td>44.11±</td>
<td>44.45±</td>
</tr>
<tr>
<td>∑PUFA</td>
<td>15.48±</td>
<td>15.44±</td>
<td>14.89±</td>
</tr>
<tr>
<td>n-6</td>
<td>0.24±</td>
<td>0.47±</td>
<td>0.31±</td>
</tr>
<tr>
<td>n-3</td>
<td>2.69±</td>
<td>2.86±</td>
<td>3.14±</td>
</tr>
<tr>
<td>n-3:n-6</td>
<td>0.23±</td>
<td>0.08±</td>
<td>0.10±</td>
</tr>
<tr>
<td>ratio</td>
<td>0.17±</td>
<td>0.19±</td>
<td>0.21±</td>
</tr>
</tbody>
</table>

F: fortification; C: cooking method; F×C: interaction; ns: non-significant; *, P<0.05; **, P<0.01, ***P<0.001, *, sunflower
were performed by SPSS 21 for Windows (SPSS Inc., Chicago, IL, USA).

### Results and Discussion

**Cooking methods on the moisture and lipid content of unfortified and fish oil fortified tilapia meat**: The cooking method had a significant effect on the moisture content to both fortified and un-fortified tilapia meat (Table 1). The highest moisture content was raw tilapia meat, which was significantly higher than all the other treatments, followed by those that were grilled. Significantly lowest moisture content was observed in tilapia meat that was microwaved. Meanwhile, with the exception of microwaving, the addition of fish oil to tilapia meat led to significantly lower moisture content as compared to unfortified meat (Table 1).

The lipid content of both fortified and un-fortified tilapia meat was also significantly affected by the cooking method (Table 1). For un-fortified meat, microwaving or frying in either sunflower or palm oil led to a significantly higher lipid content as compared to those that were raw or grilled. However, no significant difference was detected between raw and grilled tilapia meat when fish oil was not added previously. After adding fish oil to meat, grilling led to a significantly higher lipid content as compared to raw tilapia meat, while microwaving or frying in either sunflower or palm oil led to significantly highest lipid content (Table 1).

**Fatty acid content of raw tilapia meat and after fish oil fortification**: Addition of fish oil fortifications to tilapia meat had a significant effect on the fatty acid composition. For un-fortified tilapia meat, the dominant saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) was palmitic acid (16:0) and oleic acid (18:1n-9), which significantly decreased when fish oil fortifications were made. Although the total MUFA content significantly decreased when tilapia meat was fish oil fortified, the total SFA did not significantly change. For example, myristic acid (14:0) significantly increased while there was a slight, but not significant, increase to margaric acid (17:0) when fish oil was added to tilapia meat (Table 2).

On the other hand, total n-3 PUFA fatty acids significantly increased from 2.69% to 18.84% when tilapia meat was fortified with fish oil. In particular, all the fatty acids with chain lengths longer than 20, including eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), significantly increased with fish oil additions. Meanwhile, total n-6 PUFA and n-3:n-6 fatty acid ratio significantly decreased and increased, respectively, for fish oil fortified tilapia meat (Table 2).

**Effects of different cooking methods on the fatty acid composition to unfortified and fish oil fortified tilapia meat**: For unfortified tilapia meat, grilling had no significant effect on the fatty acid composition, while only microwaving significantly reduced the arachidonic acid (ARA; 20:4n-6) content. However, frying unfortified tilapia meat in palm oil significantly increased 16:0, stearic acid (18:0), and 18:1n-9, while significantly decreasing 14:0, GLA and ARA. No significant effect was detected on n-3 PUFA. Frying unfortified tilapia meat in sunflower oil significantly decreased total SFA to include 14:0, 16:0, and 18:0, as well as total MUFA to include 18:1n-9 and 16:1. Meanwhile, there was a significant increase in LA and n-6 PUFA when unfortified tilapia meat was fried in sunflower oil, but no significant change was seen in n-3:n-6 PUFA ratio (Table 2).

For fish oil fortified tilapia meat, there was a similar trend with different cooking methods on the fatty acid composition. However, in contrast to the unfortified tilapia meat, microwaving led to significantly lower total n-3 PUFA content, which included 22:5n-3 and EPA, as compared to raw tilapia meat (Table 2).

**Different cooking methods on the oxidative stability of tilapia meat with or without n-3 PUFA or clove essential oil additions**

**Lipid peroxidation**: Generally, addition of fish oil and clove essential oil tended to increase and decrease lipid peroxidation in tilapia meat, respectively. Meanwhile, microwaving tended to increase lipid peroxidation among all the cooking methods. For example, combination that led to highest lipid peroxidation level was detected for microwaved tilapia meat that was fish oil fortified and without clove essential oil. Similar lipid peroxidation values were obtained from tilapia meat that were sunflower or palm oil fried or those that were grilled (Fig. 1).

**Scavenging activity on DPPH radicals**: Generally, the highest DPPH scavenging activity was obtained from tilapia meat with addition of clove essential oil. However, the highest DPPH value was obtained from unfortified tilapia meat that was microwaved and with clove essential oil, while microwaving fish oil fortified tilapia meat without clove essential oil drastically reduced the DPPH scavenging activity (Fig. 2).
Meat products can be cooked in different ways and heat is known to degrade and oxidize fatty acids (Lee et al., 2006a; Pérez-Mateos et al., 2006). Subsequently, the focus of several studies has been the effects of different cooking methods on product quality, which includes the proximate and/or fatty acid composition, as well as oxidative stability. In the current study, addition of fish oil to tilapia meat decreased and increased the moisture and lipid content, respectively. Meanwhile, cooking tended to decrease and increase the moisture and lipid content of tilapia meat, respectively, which is in agreement with the previous studies (Asghari et al., 2013; Gokoglu et al., 2004; Weber et al., 2008). One exception was un-fortified tilapia meat that was grilled, since the lipid content was unaffected as compared to raw tilapia meat.

Interestingly, among the various cooking methods in the current study, microwaving tilapia meat led to highest level of lipid peroxidation and oxidant production, as well as oxidative stability. In the current study, addition of fish oil to tilapia meat decreased and increased the moisture and lipid content, respectively. Considering the recommended EPA and DHA intake for human is approximately 1 g per day (Arts et al., 2001), it would be necessary to consume 31-52 g of n-3 PUFA fortified tilapia meat, as compared to 330-825 g of those that were unfortified. Moreover, there was also a proportional and significant decrease in monounsaturated fatty acids (MUFA) in all the treatments. Since, the health benefits of longer chain fatty acids are well known and can include reduce risk of various degenerative diseases (Chow, 2007), this practice can obviously have nutritive benefits for human consumers and thus, provide a strong incentive for feed companies to follow.

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The results of the current study demonstrate that the cooking method, which included grilling, microwaving or sunflower or palm oil frying, can significantly affect both the oxidative stability and fatty acid composition of tilapia meat. Moreover, direct fish oil fortifications to tilapia meat significantly increased the n-3 PUFA content, which also increased the susceptibility to lipid peroxidation, although addition of clove essential oil enhanced oxidative stability.

**Ferric reducing antioxidant power**: Similarly to the DPPH results, the highest ferric reducing antioxidant power was obtained from tilapia meat with clove essential oil additions. Moreover, the lowest ferric reducing antioxidant power was, as obtained from microwaved tilapia meat. Significantly lowest values were obtained from fish oil fortified meat, regardless of the cooking method, followed by tilapia meat that was unfortified with either fish oil or clove essential oil (Fig. 3).
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781

2004; Gladyshev et al., 2006). This minimal transfer of vegetable oils to salmon meat is in contrast to the current study in which the use of palm or sunflower oil increased the SFA and 18:2n-6, respectively, as well as significantly decreased the n-3 PUFA content. This was likely due to the high SFA and 18:2n-6 content of palm and sunflower oil, respectively, being included in the fatty acid analysis and this finding is in agreement with Weber et al. (2008).

Nevertheless, there appeared to be some nutritional advantages of using palm oil as compared to sunflower oil. When tilapia meat was fried in palm oil, whether fortified with n-3 PUFA or not, this resulted in significantly higher n-3:n-6 ratio and oxidative stability, as well as significantly lower n-6 PUFA than those that were fried in sunflower oil. In terms of microwaving or grilling, Asghari et al. (2013) found that PUFA, including EPA and DHA, significantly increased in rainbow trout after being microwaved as compared to those that were raw or boiled. On the other hand, fatty acid composition of grass carp fillets were not significantly different when either microwaved or grilled (Zhang et al., 2013). Weber et al. (2008) and Zhang et al. (2013) both suggested this might have been due to loss of MDA products during frying or they were subsequently formed with proteins. While this may have occurred in the present study, it is worthy to note that many vegetable oils, including soybean, palm and sunflower oil are known to contain endogenous vitamin E, which is a powerful natural anti-oxidant (Duqan et al., 2011; Warner et al., 2008). As such, this might have contributed to this finding in the current study.

Although information on cooking methods, particularly microwaving to lipid peroxidation are relatively scarce, several studies have examined the subsequent effects of fatty acid composition of meat. In the current study, raw and grilled tilapia meat without any n-3 PUFA fortifications had a similar fatty acid composition. Similarly, heat had no significant effect on the fatty acid composition of salmon (Salmo salar) (Al-Saghir et al., 2004), humpback salmon (Oncorhynchus gorbuscha) (Gladyshev et al., 2006) or silver catfish (Weber et al., 2008). Moreover, when different vegetable oils were used to cook salmon, no significant effect on the fatty acid composition was noted. (Al-Saghir et al., 2004; Gladyshev et al., 2006). This minimal transfer of vegetable oils to salmon meat is in contrast to the current study in which the use of palm or sunflower oil increased the SFA and 18:2n-6, respectively, as well as significantly decreased the n-3 PUFA content. This was likely due to the high SFA and 18:2n-6 content of palm and sunflower oil, respectively, being included in the fatty acid analysis and this finding is in agreement with Weber et al. (2008).

Nevertheless, there appeared to be some nutritional advantages of using palm oil as compared to sunflower oil. When tilapia meat was fried in palm oil, whether fortified with n-3 PUFA or not, this resulted in significantly higher n-3:n-6 ratio and oxidative stability, as well as significantly lower n-6 PUFA than those that were fried in sunflower oil. In terms of microwaving or grilling, Asghari et al. (2013) found that PUFA, including EPA and DHA, significantly increased in rainbow trout after being microwaved as compared to those that were raw or boiled. On the other hand, fatty acid composition of grass carp fillets were not significantly different when either microwaved or grilled (Zhang et al., 2013). Similarly in the current study, microwaving unfortified tilapia meat led to no significant differences in the n-3 PUFA content as compared to those that were grilled. However, when fish oil fortifications were made to tilapia meat, the situation became different.

When tilapia meat was fortified with fish oil,
microwaving led to significantly lower n-3 PUFA as compared to those that were grilled. This finding, along with microwaving leading to the highest levels of lipid peroxidation, indicates that the added fish oil was likely a major contributor to oxide production. To the best of our knowledge, this is the first study on different cooking methods to the oxidative stability of n-3 PUFA fortified meat products. However, it is well known that LC-PUFA are more susceptible to peroxidation, and subsequently several studies have focused on different types of antioxidants to meat or fish products such rosemary extract (Pérez-Mateos et al., 2002), green tea (Pérez-Mateos et al., 2006), anti-oxidant cocktails (Lee et al., 2006a, b), potassium iodide (Panpipat and Yongsawatdigul, 2008), green coffee extract, green tea catechin (Valencia et al., 2008) or Vitamin E (Andrés et al., 2009). Among these tested antioxidants, their efficacy of protection to n-3 PUFA fortified products have been somewhat mixed. For example, potassium iodide was ineffective in protecting against lipid peroxidation to fish sausage fortified with refined tuna oil (Panpipat and Yongsawatdigul, 2008), while rosemary or green tea extract to fish oil fortified surimi was initially effective which was lost after nine months of storage (Pérez-Mateos et al., 2006). Meanwhile, when pork sausages were fortified with fish oil, green tea catechin significantly reduced lipid peroxidation as compared to use of green coffee extract (Valencia et al., 2008). Apparently better protection was obtained using an antioxidant cocktail, consisting of citrate, erythorbate and rosemary which, significantly increased the oxidative stability to various meat products fortified with algal oil (Lee et al., 2006a,b), while Vitamin E was suggested to contribute to limited lipid peroxidation in chicken sausage fortified with squid oil during storage (Andrés et al., 2009).

In the current study, addition of clove essential oil to tilapia meat generally had a beneficial effect to oxidative stability during cooking, which appeared to be particularly important when meat was fish oil fortified. For example, when tilapia meat was both fish oil and clove essential oil fortified, lipid peroxidation was not significantly different as compared to non-fortified meat when grilled or fried in either palm or sunflower oil. Moreover, even when tilapia was not fortified with fish oil, addition of clove essential oil also reduced lipid peroxidation as compared to without clove essential oil additions. The reason for the effectiveness of clove essential oil was likely due to their high antioxidant potential. In a recent comprehensive study, in which various food and food related ingredients were analysed for their antioxidant content, clove essential oil was among those with highest antioxidant content (Carlsen et al., 2010).

However, regardless of the treatment, none of the lipid peroxidation values exceeded 1 mg MDA/kg muscle, which would indicate a threshold for organoleptic rancidity detection.
Clove essential oil is categorized as a “generally regarded as safe” compound when not exceeding 1,500 ppm (Kildea et al., 2004), and the additions used in the current study are well below this level. However, when supplementing meat products with different fortifications, an important consideration is potential changes to physical or organoleptic qualities to the final product. Although clove essential oil was added in relatively small amounts, and no noticeable fragrance was evident during the cooking process, further investigations should be made regarding the organoleptic qualities. It is worthy to note that recently Salgado et al. (2013) reported that the addition of essential clove oil to wrapping films had no effect to color, texture, moisture or mechanical properties to sardine patties. Moreover, such additions also had a slight and initial benefit to reducing some bacterial counts during storage, but was lost over time (Salgado et al., 2013). Indeed, clove essential oil is known to have a broad spectrum of antimicrobial activities to bacteria, fungi, and viruses (Chatieb et al., 2007), which might provide additional benefits to such products.

In conclusion, fish oil fortifications to tilapia minced meat can, in turn, significantly enhance health beneficial n-3 PUFA, particularly the EPA and DHA content, even after different methods of cooking. Highest oxidative stability, as well as LC-PUFA content of fish oil fortified tilapia meat was after grilling. While the use of sunflower or palm oil significantly increased the linoleic acid and SFA content, respectively, the oxidative stability tended to be higher when fish oil fortified tilapia meat was microwaved. However, addition of clove essential oil significantly improved oxidative stability, and further experiments should be performed on the extent of lipid peroxidation, and even antimicrobial activities, during prolonged storage. In contrast to tilapia products that are sold whole and the price is based on weight rather than the fatty acid composition, fish oil fortifications to minced tilapia meat can provide food companies with an effective method to deliver beneficial fatty acids to human consumers.

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784
E. Ramezani-Fard et al.

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