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5 Effect of oregano and thyme essential oils on the  
6 microbiological and chemical quality of refrigerated  
7 (4°C) ready-to-eat squid rings

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## SUMMARY

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3 The efficacy of oregano and thyme essential oils (OEO and TEO, respectively) in the  
4 quality retention of a refrigerated (4°C) squid (*Loligo vulgaris*) ring ready-to-eat (RTE)  
5 product was studied. Essential oils were added at different concentrations to the coating  
6 medium during processing. An inhibitory ( $p < 0.05$ ) effect of OEO on the microbial  
7 activity (aerobes, anaerobes, Enterobacteriaceae, psychrotrophs) of the squid rings was  
8 observed, with a more pronounced effect as OEO concentration increased. The addition  
9 of OEO also led to an inhibitory ( $p < 0.05$ ) effect on lipid oxidation, as determined by  
10 peroxide, thiobarbituric acid-reactive substance and interaction compound formation;  
11 however, no effect ( $p > 0.05$ ) of the OEO concentrations on lipid oxidation development  
12 was detected. The addition of TEO did not lead to an inhibitory effect ( $p > 0.05$ ) on the  
13 microbial activity of the refrigerated RTE squid, although a slight inhibitory ( $p < 0.05$ )  
14 effect on lipid oxidation was observed in the batches including the higher TEO  
15 concentrations.

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18 **Running Head:** Essential oils and refrigerated squid rings.

19 **Keywords:** Squid ring, oregano, thyme, ready-to-eat, microbial activity, lipid oxidation,  
20 quality.

## INTRODUCTION

1  
2 Marine species provide highly perishable products whose quality and freshness rapidly  
3 decline post-mortem due to different microbial and biochemical degradation  
4 mechanisms (Whittle *et al.*, 1990; Olafsdóttir *et al.*, 1997). A wide variety of  
5 preservation strategies have been satisfactorily tested to delay the spoilage of marine  
6 food while retaining its nutritional value and guaranteeing its safety. These strategies  
7 include chemical and/or physical treatments (Toledo-Flores & Zall, 1992; Ashie *et al.*,  
8 1996; Sanjuás-Rey *et al.*, 2011) and packaging employment (Ozen & Floros, 2001;  
9 Sivertsvik *et al.*, 2002; Corbo *et al.*, 2009).

10 With respect to the preservation techniques based on chemical methods, recent efforts  
11 have focused on the replacement of synthetic antioxidants by natural antioxidants. In  
12 this sense, essential oils have been reported to provide natural antimicrobial and  
13 antioxidant components with the potential to extend the shelf life of certain food  
14 products (Dziezak, 1989). Among the essential oils extracted from aromatic plants,  
15 oregano (*Origanum vulgare*) (Goulas & Kontominas, 2007; Giatrakou *et al.*, 2008;  
16 Mexis *et al.*, 2009) and thyme (*Thymus vulgaris*) (Del Nobile *et al.*, 2009; Kykkidou *et*  
17 *al.*, 2009; Erkan, 2010) oils have gained a great technological interest. The preservation  
18 effects found in both essential oils have been explained on the basis of the presence of  
19 different compounds such as carvacrol, thymol, p-cymene and  $\gamma$ -terpinene (Burt, 2004;  
20 Yanishlieva *et al.*, 2006).

21 A recent strategy to increase marine product distribution according to new trends in  
22 food consumption and lifestyle is represented by the increasing commercialisation of  
23 ready-to-eat (RTE) food products, which include a myriad of refrigerated, frozen, cured  
24 and canned seafood products (Manrique & Jensen, 2001). Thus, novel and attractive  
25 RTE products are increasingly available today in the market and restorer sectors, all of

1 which require strict safety controls and attempt to satisfy the consumer's expectations  
2 for taste, flavour and healthiness (Gilbert *et al.*, 2000; Gopinath *et al.*, 2007).  
3 Cephalopod catches have increased gradually in the last decade because of a growing  
4 market demand and the expansion of fisheries into new fishing grounds and deeper  
5 waters (FAO, 2007a). Among these cephalopods, squid species represent a major  
6 portion of the catches in many countries and are mainly exported as chilled or frozen  
7 products (FAO, 2007b).  
8 The present study focused on an RTE product consisting of pre-fried squid rings  
9 maintained under modified atmosphere packaging (MAP) conditions. The main  
10 objective of this work was to investigate the effects of the addition of oregano and  
11 thyme essential oils (OEO and TEO, respectively) on the quality and shelf life of such  
12 food products. For it, different concentrations of the essential oils were added to the  
13 coating mixture of the pre-fried squid rings. The microbial growth and lipid oxidation  
14 events in squid rings during the refrigerated storage (4°C) were observed and compared  
15 with those of control batches prepared in the absence of these essential oils.

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## **MATERIALS AND METHODS**

### **Raw material, processing and sampling**

19 Frozen individual peeled squid (*Loligo vulgaris*) mantle rings (weight: 19.0±1.0 g each  
20 squid ring) were acquired from a local seafood industry specialising in the  
21 commercialisation of RTE seafood products. Squid rings had been prepared from fresh  
22 squid and then kept frozen (-40°C) for 1 month. Once in our laboratory, two different  
23 experiments were conducted to assess the effect of the presence of OEO and TEO  
24 independently. In both experiments, the thawing step was performed by overnight  
25 storage at 4°C.

1 The first experiment concerned OEO (origanum oil, FCC grade, from *Thymus capitatus*;  
2 density: 0.939 g ml<sup>-1</sup> at 25°C; boiling point: 239°C; Sigma Aldrich, Madrid, Spain) and  
3 included squid rings (n = 306) that were randomly assigned into 4 batches (72 rings in  
4 each). Three of the batches were processed with a coating mixture supplemented with  
5 OEO at the following concentrations: 0.010% (O-1 batch), 0.025% (O-2 batch) or  
6 0.050% (O-3 batch) (w/w, OEO/squid ring). The fourth batch was prepared without  
7 oregano oil (control batch, C-O). The remaining rings (n = 18) were considered as the  
8 initial sample of the oregano experiment; they were distributed among 3 groups (6 rings  
9 in each) that were analysed independently.

10 The coating formulation consisted of wheat flour and cold water in a 3/2 ratio, and the  
11 mixture was homogenised for 5 min in an Ultraturrax (Janke & Kunkel, Ultraturrax  
12 T25, Manasquan, NJ, USA) mixer. After mixing, OEO was added, and the mixture was  
13 homogenised for 1 min. Then, the squid rings were immersed into each coating solution  
14 for 2 min and deep-fried with refined sunflower oil at 180±2°C for 20 s in a Fritaurus  
15 Professional 4 domestic electric fryer (Taurus, Barcelona, Spain). After frying, the squid  
16 ring products were placed on polystyrene trays (20 cm x 12 cm), which were packed in  
17 retractile multiplayer co-extruded film bags. A mixture of gases consisting of 70% N<sub>2</sub>,  
18 25% CO<sub>2</sub> and 5% O<sub>2</sub> was injected into each sample at a ring/atmosphere ratio of 1/2  
19 (w/v). The bags were immediately sealed to obtain the final commercial RTE product.  
20 Each tray contained 3 squid rings, so 24 trays were prepared for each treatment. The  
21 packed samples were stored in a refrigerated room at 4°C. The samples were taken for  
22 analysis on days 3, 7, 10 and 14 of the refrigerated storage.

23 The second experiment concerned TEO (thyme oil white, FCC grade, from *Thymus*  
24 *vulgaris* and/or *Thymus zygis*; density: 0.917 g ml<sup>-1</sup> at 25°C; boiling point: 195°C;  
25 Sigma Aldrich) and included squid rings (n = 234) that were randomly assigned into 4

1 batches (54 rings in each). Three of the batches were processed with a coating mixture  
2 supplemented with TEO at the following concentrations: 0.010% (T-1 batch), 0.025%  
3 (T-2 batch) or 0.050% (T-3 batch) (w/w, TEO/squid ring). A control batch (C-T)  
4 without thyme oil was also prepared. The remaining rings (n = 18) were considered as  
5 the initial sample of the thyme experiment; they were distributed among 3 groups (6  
6 rings in each) that were analysed independently. The coating process, frying, packaging  
7 and storage temperature were the same as in the OEO experiment. Each tray contained 3  
8 squid rings, so 18 trays were prepared for each treatment. Samples were taken for  
9 analysis on days 5, 8 and 12 of refrigerated storage.

10 In both the oregano and the thyme experiments, 6 trays corresponding to each treatment  
11 were analysed at each refrigeration time. For this analysis, the trays were distributed  
12 into 3 groups (2 trays per group), and each group was analysed separately.

13 Previous preliminary trials were performed to establish the maximum concentration of  
14 each essential oil that could be employed without modifying the product odour.  
15 Analysis of squid ring odour was conducted by a sensory panel of five experienced  
16 judges. For it, three-digital-code samples were presented to the panellists and qualified  
17 as “modified”, “not modified” or “doubtful”. Under the present experimental  
18 conditions, it was concluded that if the essential oils were added at a 0.075% (w/w;  
19 essential oil/squid ring) concentration or higher, the presence of the essential oil odour  
20 was noticed in the squid ring RTE product. Accordingly, concentrations tested in the  
21 present research are below this value.

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### 23 **Microbiological analyses**

24 Squid rings were dissected aseptically from the coating medium in the refrigerated  
25 products; the initial squid rings were directly analysed. For this analysis, 10 g of squid

1 ring was mixed with 90 ml of 0.1% peptone water and homogenised in a stomacher  
2 (AES, Combourg, France) as previously described (Ben-Gigirey *et al.*, 1998, 1999). In  
3 all cases, serial dilutions of the microbial extracts were prepared in 0.1% peptone water.  
4 The total aerobe counts were determined in plate count agar (PCA, Oxoid Ltd., London,  
5 UK) after incubation at 30°C for 48 h as previously described (Ben-Gigirey *et al.*, 1998,  
6 1999). The anaerobe counts were also determined in PCA at 30°C, but the plates were  
7 introduced inside an anaerobiosis jar. The psychrotroph counts were also determined in  
8 PCA under aerobic conditions, but the incubation was carried out at 7-8°C for 7 days.  
9 The Enterobacteriaceae counts were determined by pour plating on Violet Red Bile  
10 Glucose Agar (VRBG) and subsequently incubated at 30°C for 24-48 h.  
11 In all cases, the bacterial counts were converted into log CFU g<sup>-1</sup> squid ring before  
12 undergoing statistical analysis. All analyses were performed in triplicate.

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#### 14 **Chemical analyses**

15 The squid rings were separated from the coating medium, minced, homogenised and  
16 employed for the different chemical analyses: pH, total lipids, peroxide value (PV),  
17 thiobarbituric acid index (TBA-i) and fluorescence ratio (FR). The initial squid rings  
18 were analysed after mincing and homogenising.

19 The evaluation of the pH values in the squid rings throughout the storage time was  
20 performed via a 6-mm diameter insertion electrode (Crison, Barcelona, Spain).

21 The lipids were extracted from the squid rings using the Bligh & Dyer (1959) method,  
22 which employs a single-phase solubilisation step of the lipids using a  
23 chloroform/methanol (1/1) mixture. The quantification of results was expressed as g  
24 lipid kg<sup>-1</sup> squid ring.

1 The PV was determined in the lipid extract by peroxide reduction with ferric  
2 thiocyanate according to the method of Chapman & McKay (1949). The results were  
3 expressed as meq active oxygen  $\text{kg}^{-1}$  lipids.

4 The TBA-i was determined according to the method of Vyncke (1970). This method is  
5 based on the reaction between a trichloroacetic acid extract of the squid ring and  
6 thiobarbituric acid. The concentration of thiobarbituric acid reactive substances  
7 (TBARS) was spectrophotometrically measured at 532 nm, and the results were  
8 expressed as mg malondialdehyde  $\text{kg}^{-1}$  squid ring.

9 The formation of fluorescent compounds was determined by measurements at 393/463  
10 nm and 327/415 nm as described by Aubourg & Gallardo (1997). The relative  
11 fluorescence (RF) was calculated as follows:  $\text{RF} = F/F_{\text{st}}$ , where F is the fluorescence  
12 measured at each excitation/emission maximum, and  $F_{\text{st}}$  is the fluorescence intensity of  
13 a quinine sulphate solution ( $1 \mu\text{g ml}^{-1}$  in  $0.05 \text{ M H}_2\text{SO}_4$ ) at the corresponding  
14 wavelength pair. The FR was calculated as the ratio between the two RF values:  $\text{FR} =$   
15  $\text{RF}_{393/463 \text{ nm}} / \text{RF}_{327/415 \text{ nm}}$ . Results on fluorescent compound formation are expressed as  
16 FR values and were determined in the lipid fraction resulting from the squid ring  
17 extraction (Bligh & Dyer, 1959).

18 All analyses were performed in triplicate.

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## 20 **Statistical analyses**

21 The data from the different analyses were subjected to analysis of variance (ANOVA).  
22 PASW Statistics 18 (SPSS, Chicago, IL, USA) was used to explore the statistical  
23 significance of the differences due to the presence of the essential oil and the  
24 refrigeration time. Differences were considered significant for a confidence interval at  
25 the 95% level ( $p < 0.05$ ) in all cases.



## **RESULTS AND DISCUSSION**

### **Microbiological stability**

The comparative evolution of aerobes, anaerobes, Enterobacteriaceae and psychrotrophs during the refrigerated storage of RTE squid rings treated with OEO is shown in Table 1. Significantly ( $p < 0.05$ ) lower aerobic mesophile counts were measured in the squid rings treated with any of the three oregano concentrations relative to the control batch. Remarkably, aerobe counts below 3 log units were determined after 14 days of storage, whereas the control batch exhibited concentrations of approximately 6 log units on day 10. With respect to the development of anaerobic bacteria, lower counts at advanced storage times (10-14 days) were determined in the squid rings treated with any of the oregano concentrations tested than in the control batch. This inhibitory effect was found to be higher at day 10 for the two highest oregano concentrations (O-2 and O-3 conditions). It should be noted that the final anaerobe concentrations remained below 2 log units in the OEO batches, whereas these values were above 5 log units in the control batch on day 10.

With respect to Enterobacteriaceae, significant differences were found on day 7 between the three batches treated with oregano and the control batch. On day 14, significant differences between the control batch and the two most concentrated oregano batches (O-2 and O-3) were also observed. As in the case of aerobes and anaerobes, the Enterobacteriaceae assessment of oregano-treated squid samples exhibited a decreasing ( $p < 0.05$ ) value after processing and storing for 3 days. Since raw samples were analysed before the pre-frying step, the decrease in the microbial numbers determined for all batches on day 3 can be easily explained as a result of such frying step, which is required for the industrial elaboration of the RTE food product. However, when the pre-fried batches were compared (3-14-day period), the significant differences found among

1 OEO batches and controls for all microbial groups evidenced the inhibitory effect  
2 derived from the presence of such essential oil.

3 Differences in psychrotroph counts were also observed after 10 and 14 days of storage.  
4 Thus, on day 10, lower counts were obtained for the OEO batches than for the control  
5 batch. On day 10, differences between the control batch and the O-1, O-2 and O-3  
6 batches, respectively, were observed.

7 In conclusion, OEO in the coating medium of squid rings had a marked inhibitory effect  
8 on microbial development. Our study included a MAP strategy and refrigerated storage,  
9 which were combined with the OEO treatment. Our results are in agreement with those  
10 previous studies where OEO resulted in an enhancement of quality when combined with  
11 other preservation technologies. For example, an inhibitory effect of OEO on microbial  
12 activity development (total volatile base-nitrogen and trimethylamine-nitrogen  
13 assessment; TVB-N and TMA-N, respectively) was reported for the combination of  
14 OEO-MAP in salted sea bream (*Sparus aurata*) that were stored under refrigerated  
15 conditions ( $4\pm 0.5^{\circ}\text{C}$ ) (Goulas & Kontominas, 2007). Likewise, Giatrakou *et al.* (2008)  
16 reported positive results in microbial count and volatile amine assessment for the  
17 combination of OEO-MAP in swordfish (*Xiphias gladius*) fillets stored at  $4^{\circ}\text{C}$ . More  
18 recently, Pyrgotou *et al.* (2010) reported an inhibitory effect of the combination of OEO  
19 and MAP on the microbial activity (determined via the microbial counts and volatile  
20 amine formation) of salted rainbow trout (*Oncorhynchus mykiss*) fillets stored at  $4^{\circ}\text{C}$ . In  
21 a different type of combination (an OEO-package provided with an oxygen absorber),  
22 Mexis *et al.* (2009) found that microbial activity was partially inhibited (microbial count  
23 and volatile amine assessment) during the storage of rainbow trout (*O. mykiss*) fillets at  
24  $4^{\circ}\text{C}$ .

1 The assessment of microbial growth on the RTE squid rings from the TEO experiment  
2 is shown in Table 2. The comparative evolution of aerobic mesophiles did not  
3 significantly ( $p>0.05$ ) differ with the addition of TEO to the coating medium during  
4 processing. A marked decrease ( $p<0.05$ ) was observed in all cases after processing  
5 (namely, frying) and storage for 5 days. Then (5-12-day period), an increase in aerobe  
6 counts with storage time was observed for all types of squid batches; a marked effect of  
7 MAP conditions can be involved since aerobe numbers observed at the end of the  
8 experiment could be considered relatively low (counts under 3.50 log units) in all cases.  
9 Only slight differences in the number of anaerobes among batches were detected; thus,  
10 the presence of thyme in the coat did not have an effect ( $p>0.05$ ) on anaerobe growth.  
11 Contrary to the case of aerobes, a decreasing mean number of anaerobes with storage  
12 time was observed in most batches, and these decreases were significant ( $p<0.05$ ) in the  
13 case of the T-3 batch after processing and storage for 5 days and on day 8 for the  
14 remaining sample types (Table 2). As in the case of the aerobe numbers, the anaerobe  
15 numbers in all batches could be considered relatively low (scores under 3.20) and be the  
16 probable effect derived from the application of MAP conditions, especially of the  
17 presence of CO<sub>2</sub> in the packaging atmosphere.

18 The Enterobacteriaceae counts were markedly decreased ( $p<0.05$ ) after processing and  
19 storage for 5 days in all batches. Such a decrease can be mostly explained as a result of  
20 the pre-frying process, as stated above. Subsequently (5-8-day period), no significant  
21 ( $p>0.05$ ) differences due to storage time were observed, except for the control batch. In  
22 addition, no effect ( $p>0.05$ ) of the thyme addition at any concentration to the coating  
23 medium on the Enterobacteriaceae counts was observed (Table 2). As for  
24 Enterobacteriaceae, psychrotroph counts showed a marked decrease ( $p<0.05$ ) after  
25 processing and storage for 5 days; then, no significant differences ( $p>0.05$ ) were

1 observed for this microbial group, a result that may also be explained as an effect of  
2 MAP conditions on all kinds of samples.

3 Accordingly, an inhibitory effect of TEO in the coating medium on the microbiological  
4 quality of squid ring products subjected to MAP and refrigeration was not observed.

5 Contrary to our results, the use of thyme extended the shelf-life of fresh fish, such as  
6 wild and farmed gilthead sea bream (*S. aurata*) fillets, by approximately 5 days as  
7 assessed by TVB-N, TMA-N, free amino acid formation and water-holding capacity  
8 assays (Attouchi & Sadok, 2010). The presence of TEO has also been reported to  
9 improve the microbial quality in terms of microbial counts, TMA-N and TVB-N  
10 assessment and the sensory quality of refrigerated ( $4.0\pm 0.5^{\circ}\text{C}$ ) sea bass (*Dicentrarchus*  
11 *labrax*) fillets when combined with MAP (Kostaki *et al.*, 2009). This preservation  
12 combination (TEO-MAP) led to an improvement in the microbial quality in blue fish  
13 burgers stored at  $4^{\circ}\text{C}$  for 28 days (Del Nobile *et al.*, 2009) and inhibited the microbial  
14 activity (microbial count, TMA-N and TVB-N assessment) and sensory quality loss of  
15 swordfish (*X. gladius*) during 18 days of storage at  $4^{\circ}\text{C}$  (Kykkidou *et al.*, 2009).

16 Additionally, the combination of TEO and vacuum packaging of hot smoked rainbow  
17 trout (*O. mykiss*) stored at  $2^{\circ}\text{C}$  was reported to inhibit the microbial activity (in terms of  
18 microbial count and TVB-N and TMA-N assessments) (Erkan, 2010).

19 In previous research, Mejlholm & Dalgaard (2002) found that the addition of OEO and  
20 TEO reduced microbial growth in cod (*Gadus morhua*) fillets and extended the shelf  
21 life of these fillets during refrigerated storage ( $2^{\circ}\text{C}$ ), and the antimicrobial activity of  
22 OEO was higher than that of TEO. However, Harpaz *et al.* (2003) showed that both  
23 OEO and TEO additions slowed the spoilage of Asian sea bass (*Lates calcarifer*) during  
24 storage at  $0-2^{\circ}\text{C}$  without showing a differential effect between both types of oils.

1 On contrast to these reports, the lack of effect of TEO on the microbial development in  
2 pre-fried squid rings observed in our study may be explained as follows: (i) unlike other  
3 previous studies, the initial frying step considered in our study might have exerted a  
4 negative effect on TEO stability; (ii) unlike other previous studies that considered  
5 aerobic storage or vacuum-packaging of fish, the squid rings considered in our study  
6 were subjected to MAP. The particular atmosphere considered in our study may imply  
7 the selection of a different type of spoilage microflora as compared with previous  
8 reports, and this might be related to the lack of effect of TEO in our study; and (iii)  
9 while most of the previous reports have been focused on fish fillets, the final TEO  
10 concentrations in elaborated products such as squid rings might need to be modified due  
11 to the potential different level of microbial contamination in this type of products. In all  
12 cases, and although these causes may explain the results obtained, the lack of effect of  
13 TEO in our study will need an additional research effort.

14

### 15 **Chemical stability**

16 In the oregano experiment (Table 3), lower pH values were obtained at all storage times  
17 for the two batches treated with the higher OEO concentrations. The differences in the  
18 pH values were significant ( $p < 0.05$ ) on day 7 for the squid rings in the O-3 batch  
19 compared with those of the control batch. However, in the thyme experiment (Table 4),  
20 a marked tendency could not be discerned when comparing the different batches. With  
21 respect to the effect of the storage time, both experiments (Tables 3-4) showed a marked  
22 pH increase ( $p < 0.05$ ) after processing and storage until day 3 and day 5 for the oregano  
23 and thyme experiments, respectively. After longer storage times, no differences  
24 ( $p > 0.05$ ) were observed for each essential oil because of the refrigerated storage  
25 conditions. In agreement with our results on the pH in the oregano experiment, other

1 authors have previously reported lower pH values as a result of the OEO treatment of  
2 sea bream (*S. aurata*) fillets refrigerated at  $4\pm 0.5^\circ\text{C}$  (Goulas & Kontominas, 2007) and  
3 rainbow trout (*O. mykiss*) fillets refrigerated at  $4^\circ\text{C}$  (Mexis *et al.*, 2009). Likewise,  
4 previous research has reported no effect of TEO treatment on the pH value when  
5 compared with the control; this observation accounts for the preservation of blue fish  
6 burgers during 28 days of storage (Del Nobile *et al.*, 2009) and of swordfish (*X.*  
7 *gladius*) fillets during storage at  $4^\circ\text{C}$  (Kykkidou *et al.*, 2009). However, other authors  
8 have reported lower pH values as a consequence of the application of TEO when  
9 compared with a control; such research refers to different fish species such as sea bass  
10 (*D. labrax*) during refrigerated storage at  $4\pm 0.5^\circ\text{C}$  (Kostaki *et al.*, 2009) and wild and  
11 farmed gilthead sea bream (*S. aurata*) fillets stored in ice (Attouchi & Sadok, 2010).

12 The lipid content of the initial non-fried squid ring samples was  $4.4\pm 0.5$  and  $4.5\pm 0.5$  g  
13  $\text{kg}^{-1}$  squid ring for the oregano and thyme experiments, respectively. The lipid content  
14 increased for all types of processed material such that the lipid contents were in the 8.0-  
15 11.0 and 8.5-11.5 g  $\text{kg}^{-1}$  ranges for the samples corresponding to the oregano and thyme  
16 experiments, respectively. The increase in lipid content is likely a result of the frying  
17 step (Huidobro *et al.*, 1995; Castrillón *et al.*, 1997). No differences ( $p>0.05$ ) in the lipid  
18 content of the squid rings could be found because of the addition of oregano or thyme or  
19 the storage time.

20 The occurrence of lipid oxidation in the squid rings was assessed via the formation of  
21 peroxide, TBARS and interaction compounds. The PV assessment indicated an  
22 inhibitory effect of OEO (Table 3), which was especially remarkable for the two  
23 batches with the higher OEO concentrations. This preservation effect could also be  
24 observed for the secondary (TBARS) and tertiary (fluorescence ratio) lipid oxidation  
25 product formation, although in these cases, no differences could be established among

1 the different OEO concentrations tested (Table 3). In all cases, the values determined  
2 for the PV and the TBA-i (below 6.0 and 0.80, respectively) can be considered  
3 relatively low.

4 Other authors have reported that the application of OEO has positive effects when  
5 combined with other preservation techniques. Thus, an inhibitory effect on lipid  
6 oxidation development (TBARS assessment) was observed when OEO was applied to  
7 salted sea bream (*S. aurata*) stored at  $4\pm 0.5^{\circ}\text{C}$  (Goulas & Kontominas, 2007) and in  
8 salted rainbow trout (*O. mykiss*) fillets stored at  $4^{\circ}\text{C}$  (Pyrgotou *et al.*, 2010). The  
9 combination of OEO and an oxygen absorber also reduced the rate of lipid oxidation  
10 (peroxide value and TBARS assessment) in rainbow trout (*O. mykiss*) fillets stored at  
11  $4^{\circ}\text{C}$  (Mexis *et al.*, 2009).

12 An inhibitory effect of thyme oil on the lipid oxidation of squid rings was observed  
13 (Table 4). Thus, the highest thyme concentration tested (T-3 condition) decreased the  
14 formation of peroxides (PV), TBARS and fluorescent compounds as compared with the  
15 control batch. Likewise, the T-1 and T-2 batches also exhibited some inhibitory effect;  
16 this effect was significant ( $p < 0.05$ ) only for TBARS formation on day 12 and on both  
17 days 8 and 12 for the T-1 and T-2 batches, respectively.

18 In agreement with our results, other authors have reported a partial inhibition of lipid  
19 oxidation due to the addition of TEO. For example, the use of TEO extended the lipid  
20 stability of wild and farmed gilthead sea bream (*S. aurata*) fillets by approximately 5  
21 days during chilled storage (Attouchi & Sadok, 2010). Likewise, the combination of  
22 TEO and MAP was also found profitable when compared with MAP alone; thus, lipid  
23 oxidation was inhibited in sword fish (*X. gladius*) fillets at  $4^{\circ}\text{C}$  during 18 days of  
24 refrigerated storage (Kykkidou *et al.*, 2009) and in hot smoked rainbow trout (*O.*  
25 *mykiss*) stored at  $2^{\circ}\text{C}$  (Erkan, 2010). Finally, thyme oil was also tested as a component

1 of a biodegradable film including hake (*Merluccius capensis*) proteins at different  
2 concentrations; the addition of TEO reduced the film thickness and water vapour  
3 permeability and exerted a significant antioxidant activity (Pires *et al.*, 2011).

## 4 5 **Conclusions**

6 The present study provides a first approach to the employment of two important  
7 essential oils (OEO and TEO) with the aim of extending the period of good quality of  
8 an RTE product, pre-fried squid ring, commercialised under MAP and refrigeration  
9 conditions. It can be concluded that there is a marked ( $p<0.05$ ) inhibitory effect on  
10 microbial activity (aerobes, anaerobes, Enterobacteriaceae and psychrotrophs) due to  
11 the addition of OEO to the coating at all OEO concentrations tested, and this effect was  
12 more relevant for the highest oregano concentration (0.050%). The addition of OEO  
13 also provoked the inhibition of lipid oxidation, as determined by the formation of  
14 peroxides, TBARS and interaction compounds. In contrast, the TEO experiment  
15 demonstrated that the addition of TEO to the coating did not result in an inhibitory  
16 effect ( $p>0.05$ ) of the microbial activity in RTE squid rings, although some inhibitory  
17 effect on lipid oxidation development could be noted, especially when using the highest  
18 (0.050%) TEO concentration.

## 19 20 21 **Acknowledgements**

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## REFERENCES

- 1
- 2
- 3 Ashie, I., Smith, J. & Simpson, B. (1996). Spoilage and shelf-life extension of fresh fish  
4 and shellfish. *Critical Reviews in Food Science and Nutrition*, **36**, 87-121.
- 5 Attouchi, M. & Sadok, S. (2010). The effect of powdered thyme sprinkling on quality  
6 changes of wild and farmed gilthead sea bream fillets stored in ice. *Food*  
7 *Chemistry*, **119**, 1527-1534.
- 8 Aubourg, S. & Gallardo, J. M. (1997). Fluorescence changes in amine model systems  
9 related to fish deterioration. *International Journal of Food Science and*  
10 *Technology*, **32**, 153-158.
- 11 Ben-Gigirey, B., Vieites Baptista de Sousa, J., Villa, T. & Barros-Velázquez, J. (1998).  
12 Changes in biogenic amines and microbiological analysis in albacore (*Thunnus*  
13 *alalunga*) muscle during frozen storage. *Journal of Food Protection*, **61**, 608–  
14 615.
- 15 Ben-Gigirey, B., Vieites Baptista de Sousa, J., Villa, T. & Barros-Velázquez, J. (1999).  
16 Histamine and cadaverine production by bacteria isolated from fresh and frozen  
17 albacore (*Thunnus alalunga*). *Journal of Food Protection*, **62**, 933–939.
- 18 Bligh, E. & Dyer, W. (1959). A rapid method of total extraction and purification.  
19 *Canadian Journal of Biochemistry and Physiology*, **37**, 911–917.
- 20 Burt, S. (2004). Essential oils: Their antibacterial properties and potential applications  
21 in foods- a review. *International Journal of Food Microbiology*, **94**, 223-253.
- 22 Castrillón, A., Navarro, P. & Álvarez-Pontes, E. (1997). Changes in chemical  
23 composition and nutritional quality of fried sardine (*Clupea pilchardus*)  
24 produced by frozen storage and microwave reheating. *Journal of the Science of*  
25 *Food and Agriculture*, **75**, 125-132.

1 Chapman, R. & McKay, J. (1949). The estimation of peroxides in fats and oils by the  
2 ferric thiocyanate method. *Journal of the American Oil Chemists' Society*, **26**,  
3 360-363.

4 Corbo, M<sup>a</sup> R., Di Giulio, S., Conte, A., Speranza, B., Sinigaglia, M. & Del Nobile, M.  
5 (2009). Thymol and modified atmosphere packaging to control microbiological  
6 spoilage in packed fresh cod hamburgers. *International Journal of Food Science*  
7 *and Technology*, **44**, 1553-1560.

8 Del Nobile, M. A., Corbo, M. R., Speranza, B., Sinigaglia, M., Conte, A. & Caroprese,  
9 M. (2009). Combined effect of MAP and active compounds on fresh blue fish  
10 burger. *International Journal of Food Microbiology*, **135**, 281-287.

11 Dziezak, J. (1989). Spice. *Food Technology*, **43**, 102-116.

12 Erkan, N. (2010). The effect of thyme and garlic oil on the preservation of vacuum-  
13 packaged hot smoked rainbow trout (*Oncorhynchus mykiss*). *Food and*  
14 *Bioprocess Technology*. DOI: 10.1007/s11947-010-0412-7.

15 FAO (2007a). Fishery statistics. Capture Production. In: *Food and Agriculture*  
16 *Organization of the United Nations*. Pp. 342-350. Vol. 100/1. Rome (Italy):  
17 Yearbook 2005.

18 FAO (2007b). Fishery statistics. Commodities. In: *Food and Agriculture Organization*  
19 *of the United Nations*. Pp. 191-192. Vol. 101. Rome (Italy): Yearbook 2005.

20 Giatrakou, V., Kykkidou, S., Papavergou, A., Kontominas, M. G. & Savvaiddis, I. N.  
21 (2008). Potential of oregano essential oil and MAP to extend the shelf life of  
22 fresh swordfish: A comparative study with ice storage. *Journal of Food Science*,  
23 **73**, 167-173.

24 Gilbert, R., de Louvois, J., Donovan, T., Little, C., Nye, K., Ribeiro, C., Richards, J.,  
25 Roberts, D. & Bolton, F. J. (2000). Guidelines for the microbiological quality of

1           some ready-to-eat foods sampled at the point of sale. *Communicable Disease*  
2           *and Public Health*, **3**, 163-167.

3   Gopinath, S. P., Kochery Anthony, M. X., Nagarajarao, R. C., Jaganath, B. &  
4           Krishnaswamy, S. G. T. (2007). Standardisation of process parametres for ready-  
5           to-eat squid masala in indigenous polymer-coated tin-free steel cans.  
6           *International Journal of Food Science and Technology*, **42**, 148–1155.

7   Goulas, A. E. & Kontominas, M. G. (2007). Combined effect of light salting, modified  
8           atmosphere packaging and oregano essential oil on the shelf-life of sea bream  
9           (*Sparus aurata*): Biochemical and sensory attributes. *Food Chemistry*, **100**, 287-  
10          296.

11   Harpaz, S., Glatman, L., Drabkin, V. & Gelman, A. (2003). Effects of herbal essential  
12          oils used to extend the shelf life of freshwater-reared Asian sea bass fish (*Lates*  
13          *calcarifer*). *Journal of Food Protection*, **66**, 410–417.

14   Huidobro, A., Gómez-Guillén, C. & Borderías, J. (1995). Frozen storage of dressed and  
15          pre-fried portions of minced sardine muscle. *Zeitschrift für Lebensmittel-*  
16          *Untersuchung und -Forschung*, **200**, 178-181.

17   Kostaki, M., Giatrakou, V., Savvaidis, I. N. & Kontominas, M. G. (2009). Combined  
18          effect of MAP and thyme essential oil on the microbiological, chemical and  
19          sensory attributes of organically aquacultured sea bass (*Dicentrarchus labrax*)  
20          fillets. *Food Microbiology*, **26**, 475-482.

21   Kykkidou, S., Giatrakou, V., Papavergou, A., Kontominas, M. G. & Savvaidis, I. N.  
22          (2009). Effect of thyme essential oil and packaging treatments on fresh  
23          Mediterranean swordfish fillets during storage at 4°C. *Food Chemistry*, **115**,  
24          169-175.

- 1 Manrique, J. & Jensen, H. H. (2001). Spanish household demand for seafood. *Journal*  
2 *of Agricultural Economics*, **52**, 23-37.
- 3 Mejlholm, O. & Dalgaard, P. (2002). Antimicrobial effect of essential oils on the  
4 seafood spoilage micro-organism *Photobacterium phosphoreum* in liquid media  
5 and fish products. *Letters in Applied Microbiology*, **34**, 27-31.
- 6 Mexis, S. F., Chouliara, E. & Kontominas, M. G. (2009). Combined effect of an oxygen  
7 absorber and oregano essential oil on shelf life extension of rainbow trout fillets  
8 stored at 4° C. *Food Microbiology*, **26**, 598-605.
- 9 Olafsdóttir, G., Martinsdóttir, E., Oehlenschläger, J., Dalgaard, P., Jensen, B.,  
10 Undeland, I., Mackie, I., Henehan, G., Nielsen, J. & Nilsen, H. (1997). Methods  
11 to evaluate fish freshness in research and industry. *Trends in Food Science and*  
12 *Technology*, **8**, 258- 265.
- 13 Ozen, B. & Floros, J. (2001). Effects of emerging food processing techniques on the  
14 packaging materials. *Trends in Food Science and Technology*, **12**, 60-67.
- 15 Pires, C., Ramos, C., Teixeira, G., Batista, I., Mendes, R., Nunes, L. & Marques, A.  
16 (2011). Characterization of biodegradable films prepared with hake proteins and  
17 thyme oil. *Journal of Food Engineering*, **105**, 422-428.
- 18 Pyrgotou, N., Giatrakou, V., Ntzimani, A. & Savvaidis, I. (2010). Quality assessment of  
19 salted, modified atmosphere packaged rainbow trout under treatment with  
20 oregano essential oil. *Journal of Food Science*, **75**, M406-M410.
- 21 Sanjuás-Rey, M., García-Soto, B., Barros-Velázquez, J., Fuertes-Gamundi, J. &  
22 Aubourg, S. (2011). Effect of a two-step natural organic acid-treatment on  
23 microbial activity and lipid damage during blue whiting (*Micromesistius*  
24 *poutassou*) chilling. *International Journal of Food Science and Technology*, **46**,  
25 1021-1030.

- 1 Sivertsvik, M., Jeksrud, W. & Rosnes, T. (2002). A review of modified atmosphere  
2 packaging of fish and fishery products - significance of microbial growth,  
3 activities and safety. *International Journal of Food Science and Technology*, **37**,  
4 107-127.
- 5 Toledo-Flores, L. & Zall, R. (1992). Methods for extending the storage life of fresh  
6 tropical fish. In: *Advances in Seafood Biochemistry* (edited by G. Flick & R.  
7 Martin. Pp. 233-243. Lancaster, PA (USA): Technomic Publishing.
- 8 Vyncke, W. (1970). Direct determination of the thiobarbituric acid value in  
9 trichloroacetic acid extracts of fish as a measure of oxidative rancidity. *Fette*  
10 *Seifen Anstrichmittel*, **72**, 1084-1087.
- 11 Whittle, K., Hardy, R. & Hobbs, G. (1990). Chilled fish and fishery products. In:  
12 *Chilled foods. The state of the art* (edited by T. Gormley). Pp. 87-116. New  
13 York (USA): Elsevier Applied Science.
- 14 Yanishlieva, N., Marinova, E. & Pokorny, J. (2006). Natural antioxidants from herbs  
15 and spices. *European Journal of Lipid Science and Technology*, **108**, 776-793.

**TABLE 1: Microbiological count (log CFU g<sup>-1</sup> muscle) assessment\* in refrigerated squid rings treated with oregano essential oil (OEO)\*\***

Microbiological parameter	Storage time (days)	OEO treatment			
		C-O	O-1	O-2	O-3
Aerobes	Raw squid rings	a 3.39 (0.12)	c 3.39 (0.12)	b 3.39 (0.12)	b 3.39 (0.12)
	3	a 2.85 y (1.21)	a 1.99 z (0.00)	a 1.99 z (0.00)	a 1.99 z (0.00)
	7	ab 4.16 y (1.66)	a 1.99 z (0.00)	a 1.99 z (0.00)	a 1.99 z (0.00)
	10	b 5.94 y (0.41)	abc 2.63 z (0.91)	a 1.80 z (0.28)	b 2.77 z (0.66)
	14	ab 5.35 y (1.70)	b 2.57 z (0.38)	b 2.95 zy (0.83)	ab 2.85 zy (1.21)
Anaerobes	Raw squid rings	b 3.76 (0.63)	b 3.76 (0.63)	c 3.76 (0.63)	b 3.76 (0.63)
	3	ab 2.15 (1.20)	a 1.74 (0.37)	a 1.39 (0.12)	a 1.74 (0.37)
	7	a 1.99 (0.00)	a 1.99 (0.00)	b 1.99 (0.00)	a 1.99 (0.00)
	10	c 5.45 x (0.52)	b 3.92 y (0.45)	b 1.80 z (0.28)	a 1.65 z (0.49)
	14	c 5.42 y (1.03)	a 1.99 z (0.00)	abc 1.99 z (1.81)	a 1.99 z (0.00)
Enterobacteriaceae	Raw squid rings	b 1.89 (0.27)	b 1.89 (0.27)	b 1.89 (0.27)	b 1.89 (0.27)
	3	a 0.99 (0.00)	a 0.99 (0.00)	a 0.99 (0.00)	a 0.99 (0.00)
	7	b 2.56 y (0.78)	a 0.99 z (0.00)	a 0.99 z (0.00)	a 0.99 z (0.00)
	10	a 0.99 (0.00)	a 0.99 (0.00)	a 0.99 (0.00)	a 0.99 (0.00)
	14	b 2.36 y (0.52)	b 1.65 y (0.49)	a 0.99 z (0.00)	a 0.99 z (0.00)
Psychrotrophes	Raw squid rings	a 1.30 (0.43)	b 1.30 (0.43)	b 1.30 (0.43)	a 1.30 (0.43)
	3	ab 1.95 (1.34)	a 0.99 (0.00)	a 0.99 (0.00)	a 1.49 (0.43)
	7	b 1.99 (0.00)	c 1.99 (0.00)	c 1.99 (0.00)	b 1.99 (0.00)
	10	c 5.56 y (0.79)	d 3.62 z (0.74)	abc 2.15 z (1.63)	ab 2.33 z (1.89)
	14	abc 4.10 y (2.99)	c 1.99 z (0.00)	abc 1.99 y (1.08)	b 1.99 z (0.00)

\* Mean values of three independent replicates (n = 3); standard deviations are indicated in brackets. For each storage time, mean values in the same row followed by different letters (z, y, x) indicate significant (p<0.05) differences as a result of the OEO treatment. For each treatment, mean values in the same column followed by different letters (a-d) denote significant differences (p<0.05) as a result of the refrigeration time. No letters are indicated when significant differences are not found (p>0.05).

\*\* OEO concentrations (TEO/squid ring, w/w) employed in the coating medium: 0.000% (C-O; control), 0.010% (O-1), 0.025% (O-2) and 0.050% (O-3).

**TABLE 2: Microbiological count (log CFU g<sup>-1</sup> muscle) assessment\* in refrigerated squid rings treated with thyme essential oil (TEO)\*\***

Microbiological parameter	Storage time (days)	TEO treatment			
		C-T	T-1	T-2	T-3
Aerobes	Raw squid rings	b 3.72 (0.02)	b 3.72 (0.02)	c 3.72 (0.02)	b 3.72 (0.02)
	5	a 2.00 (0.00)	a 1.99 (0.00)	a 1.99 (0.00)	a 2.15 (0.21)
	8	ab 2.84 (1.21)	a 1.99 (0.00)	ab 2.65 (0.93)	a 1.99 (0.00)
	12	b 3.09 (0.87)	ab 2.74 (1.06)	b 3.34 (0.18)	b 3.27 (0.70)
Anaerobes	Raw squid rings	c 3.16 (0.45)	b 3.16 (0.45)	b 3.16 (0.45)	b 3.16 (0.45)
	5	bc 2.87 zy (0.80)	ab 2.42 zy (0.60)	b 3.00 y (0.00)	a 2.15 z (0.21)
	8	a 1.99 (0.00)	a 1.99 (0.00)	a 1.99 (0.00)	a 1.99 (0.00)
	12	ab 2.15 (0.21)	a 1.99 (0.00)	a 2.00 (0.01)	a 2.00 (0.01)
Enterobacteriaceae	Raw squid rings	c 3.39 (0.54)	b 3.39 (0.54)	b 3.39 (0.54)	b 3.39 (0.54)
	5	b 1.50 (0.71)	a 0.99 (0.00)	a 0.99 (0.00)	a 0.99 (0.00)
	8	a 0.99 (0.00)	a 0.99 (0.00)	a 0.99 (0.00)	a 0.99 (0.00)
	12	a 0.99 (0.00)	a 0.99 (0.00)	a 0.99 (0.00)	a 0.99 (0.00)
Psychrotrophes	Raw squid rings	b 3.14 (0.56)	b 3.14 (0.56)	b 3.14 (0.56)	b 3.14 (0.56)
	5	a 2.00 (0.01)	a 2.15 (0.22)	a 1.99 (0.00)	a 1.99 (0.00)
	8	a 1.99 (0.00)	a 1.99 (0.00)	a 1.99 (0.00)	a 1.99 (0.00)
	12	a 2.00 (0.01)	a 1.99 (0.00)	a 2.00 (0.01)	a 2.00 (0.01)

\* Mean values of three independent replicates (n = 3); standard deviations are indicated in brackets. For each storage time, mean values in the same row followed by different letters (z, y) indicate significant (p<0.05) differences as a result of the TEO treatment. For each treatment, mean values in the same column followed by different letters (a, b, c) denote significant differences (p<0.05) as a result of the refrigeration time. No letters are indicated when significant differences are not found (p>0.05).

\*\* TEO concentrations (TEO/squid ring, w/w) employed in the coating medium: 0.000% (C-T; control), 0.010% (T-1), 0.025% (T-2) and 0.050% (T-3).

**TABLE 3: Assessment of chemical quality parameters\* in refrigerated squid rings treated with oregano essential oil (OEO)\*\***

Chemical parameter	Storage time (days)	OEO treatment			
		C-O	O-1	O-2	O-3
pH	Raw squid rings	a 6.42 (0.00)	a 6.42 (0.00)	a 6.42 (0.00)	a 6.42 (0.00)
	3	b 7.09 (0.30)	b 7.09 (0.30)	b 6.90 (0.21)	b 6.89 (0.04)
	7	b 7.06 y (0.08)	b 7.06 y (0.08)	b 6.98 zy (0.03)	b 6.87 z (0.10)
	10	b 7.15 (0.19)	b 7.15 (0.19)	b 7.07 (0.07)	b 6.99 (0.04)
	14	b 7.09 (0.06)	b 7.09 (0.06)	b 7.02 (0.19)	b 6.90 (0.13)
Peroxide value (meq oxygen kg <sup>-1</sup> lipids)	Raw squid rings	ab 2.36 (0.62)	c 2.36 (0.62)	b 2.36 (0.62)	a 2.36 (0.62)
	3	a 2.18 y (0.33)	a 0.82 z (0.24)	a 1.43 z (0.11)	a 1.43 z (0.41)
	7	bc 3.79 zy (1.18)	d 3.52 y (0.37)	b 2.16 z (0.50)	a 2.79 zy (1.08)
	10	c 3.90 y (0.21)	b 1.86 z (0.06)	b 2.16 z (0.55)	a 2.03 z (0.39)
	14	d 5.70 y (0.05)	e 5.70 y (0.33)	c 4.80 z (0.25)	b 5.21 z (0.11)
Thiobarbituric acid index (mg malondialdehyde kg <sup>-1</sup> muscle)	Raw squid rings	a 0.53 (0.15)	ab 0.53 (0.15)	a 0.53 (0.15)	a 0.53 (0.15)
	3	ab 0.74 (0.06)	bc 0.74 (0.06)	b 0.79 (0.05)	c 0.79 (0.03)
	7	b 0.78 y (0.04)	b 0.69 z (0.02)	ab 0.73 zy (0.12)	bc 0.75 zy (0.04)
	10	b 0.75 (0.03)	c 0.79 (0.04)	ab 0.74 (0.10)	bc 0.74 (0.04)
	14	b 0.79 y (0.01)	a 0.54 z (0.06)	a 0.65 z (0.05)	ab 0.66 z (0.07)
Fluorescence ratio	Raw squid rings	a 2.10 (0.10)	a 2.10 (0.10)	a 2.10 (0.10)	a 2.10 (0.10)
	3	a 2.15 (0.13)	a 1.98 (0.07)	a 1.98 (0.04)	ab 2.27 (0.29)
	7	b 2.54 (0.11)	b 2.43 (0.05)	b 2.36 (0.08)	b 2.47 (0.27)
	10	c 2.86 y (0.07)	ab 2.24 z (0.21)	ab 2.24 z (0.23)	b 2.44 z (0.11)
	14	d 3.38 y (0.12)	b 2.69 z (0.35)	b 2.43 z (0.12)	b 2.42 z (0.15)

\* Mean values of three independent replicates (n = 3); standard deviations are indicated in brackets. For each storage time, mean values in the same row followed by different letters (z, y) indicate significant (p<0.05) differences as a result of the OEO treatment. For each treatment, mean values in the same column followed by different letters (a-e) denote significant differences (p<0.05) as a result of the refrigeration time. No letters are indicated when significant differences are not found (p>0.05).

\*\* OEO concentrations employed as expressed in Table 1.



**TABLE 4: Assessment of chemical quality parameters\* in refrigerated squid rings treated with oregano essential oil (TEO)\*\***

Chemical parameter	Storage time (days)	TEO treatment			
		C-O	O-1	O-2	O-3
pH	Raw squid rings	a 6.32 (0.01)	a 6.32 (0.01)	a 6.32 (0.01)	a 6.32 (0.01)
	5	b 7.47 (0.14)	b 7.57 (0.29)	b 7.38 (0.04)	b 7.45 (0.05)
	8	b 7.47 (0.14)	b 7.37 (0.05)	b 7.45 (0.24)	b 7.47 (0.14)
	12	b 7.48 (0.21)	b 7.46 (0.33)	b 7.59 (0.26)	b 7.48 (0.17)
Peroxide value (meq oxygen kg <sup>-1</sup> lipids)	Raw squid rings	2.42 (0.45)	2.42 (0.45)	2.42 (0.45)	b 2.42 (0.45)
	5	2.38 (1.32)	1.89 (1.00)	1.67 (0.85)	a 1.59 (0.24)
	8	1.95 y (0.11)	2.07 y (0.14)	1.89 y (0.10)	a 1.57 z (0.16)
	12	2.66 y (0.70)	1.97 zy (0.74)	1.89 zy (0.27)	a 1.57 z (0.21)
Thiobarbituric acid index (mg malondialdehyde kg <sup>-1</sup> muscle)	Raw squid rings	a 0.28 (0.04)	a 0.28 (0.04)	a 0.28 (0.04)	0.28 (0.04)
	5	b 0.51 (0.14)	b 0.41 (0.04)	b 0.48 (0.03)	0.45 (0.14)
	8	b 0.53 y (0.10)	b 0.39 zy (0.07)	a 0.33 z (0.03)	0.31 z (0.02)
	12	c 1.64 x (0.33)	c 0.96 y (0.25)	ab 0.41 z (0.16)	0.52 z (0.14)
Fluorescence ratio	Raw squid rings	a 2.26 (0.08)	a 2.26 (0.08)	a 2.26 (0.08)	a 2.26 (0.08)
	5	a 2.32 (0.11)	a 2.41 (0.17)	b 2.42 (0.03)	a 2.33 (0.05)
	8	b 2.61 (0.12)	b 2.84 (0.13)	c 2.75 (0.25)	b 2.70 (0.12)
	12	b 2.82 y (0.15)	b 2.76 zy (0.11)	c 2.68 zy (0.07)	b 2.60 z (0.05)

\* Mean values of three independent replicates (n = 3); standard deviations are indicated in brackets. For each storage time, mean values in the same row followed by different letters (z, y, x) indicate significant (p<0.05) differences as a result of the TEO treatment. For each treatment, mean values in the same column followed by different letters (a, b, c) denote significant differences (p<0.05) as a result of the refrigeration time. No letters are indicated when significant differences are not found (p>0.05).

\*\* TEO concentrations as expressed in Table 2.