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Combined effect of vacuum-packaging and oregano essential oil on the shelf-life of Mediterranean octopus (*Octopus vulgaris*) from the Aegean Sea stored at 4 °C

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ABSTRACT

The present study evaluated the use of vacuum packaging (alone) or with addition of oregano essential oil (EO), as an antimicrobial treatment for shelf-life extension of fresh Mediterranean octopus stored under refrigeration for a period of 23 days. Four different treatments were tested: A, control sample; under aerobic storage in the absence of oregano essential oil; VP, under vacuum packaging in the absence of oregano essential oil; and VO1, VO2, treated samples with oregano essential oil 0.2 and 0.4% (v/w), respectively, under VP. Of all the microorganisms enumerated, *Pseudomonas* spp., H_2S-producing bacteria and lactic acid bacteria (LAB) were the groups that prevailed in octopus samples, irrespective of antimicrobial treatment. With regard to the chemical freshness indices determined, thiobarbituric acid (TBA) values were low in all octopus samples, as could have been expected from the low fat content of the product. Both trimethylamine nitrogen (TMA-N) and total volatile basic nitrogen (TVB-N) values of oregano treated under VP octopus samples were significantly lower compared to control samples during the entire refrigerated storage period. Based primarily on sensory evaluation (odor), the use of VP, VO1 and VO2 extended the shelf-life of fresh Mediterranean octopus by ca. 3, 11 and 20 days, respectively.

1. Introduction

Cephalopods constitute an important part of the marine resources most suitable for human consumption. Cephalopods are currently recognized as the most promising seafood resource because of their abundance and rapid stock renewal, since their biological cycle lasts between 8 months and just under 2 years. Most of the cephalopods that are caught and subsequently stored are marketed in their frozen state. There are several reasons for this. Once caught, they deteriorate rapidly, since they contain a large amount of endogenous and bacterial enzymes that promote very rapid protein degradation. Such high proteolytic activity produces an increase in levels of muscle-derived nitrogen, hence favoring proliferation of degenerative flora and rapid decomposition (Hurtado et al., 1999a, b).

Given that the shelf-life of refrigerated cephalopods is relatively short and that there is a growing tendency of consumers for consumption of fresh rather than processed and frozen foods, research on the application of new preservation methods, which permit shelf-life extension of fresh cephalopods is required. Essential oils are regarded as "natural preservatives" to chemical preservatives and their use in foods meets the current demands of consumers for mildly processed or natural products (Nychas, 1995).

Essential oils (EO) are aromatic oily liquids obtained from plant material. Extracts from oregano, thyme, rosemary, clove, sage and mint are some of the EO that have been used to improve the sensory characteristics and extend the shelf-life of foods (Tsigarida et al., 2000; Burt, 2004).

To our knowledge, there are no studies in the literature on Mediterranean octopus (*Octopus vulgaris*) treated with oregano EO and stored under VP at 4 °C. Only very few data exist to date on the effect of essential oils, including oregano EO, on the shelf-life of fish and fish products (Mejlholm and Dalgaard, 2002; Harpaz et al., 2003; Mahmoud et al., 2004; Goulas and Kontominas, 2007).

With regard to cephalopods, limited work has been conducted; in one of these studies, the shelf-life of chilled, pressurized octopus was 43 days longer than the unpressurized product (Hurtado et al., 2001), whereas in another study on preservation of pota and octopus kept under chilled storage, the use of controlled atmospheres (60/15/25%; $CO_2/O_2/N_2$) increased their shelf-life by at least 54%. Finally, Vaz-Pires and Barbosa (2004) examined the sensory, microbiological and physical properties of iced whole common

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octopus (*Octopus vulgaris*). Recently, the spoilage potential of whole musky octopus (*Eledone moschata*) was determined by evaluating the changes in physicochemical, microbiological and sensory parameters (Lougovois et al., 2008). Whole musky octopus, stored in melting ice, had a storage life of 10 days. Both *Pseudomonas* spp. and *Shewanella putrefaciens* constituted a major part of the spoilage flora of ice-stored musky octopus.

Thus, the objective of the present work was to determine the effect of VP individually or in combination with the addition of oregano EO, as a natural preservative, on the shelf-life extension of fresh Mediterranean octopus (*Octopus vulgaris*) stored under refrigeration (4 ± 0.5 °C) by evaluating certain microbiological, chemical and sensory parameters.

2. Materials and methods

2.1. Preparation, processing ("tumbling") of samples, and packaging

Fresh octopus (*Octopus vulgaris*) was caught by professional home water fishers with fish traps (hooped nets) during the period November–December 2006 in the North Aegean Sea (Gulf of Pieria). Two experiments were conducted during this period and in each experiment, approximately 10–12 whole octopuses were used. The mean weight of each octopus sample was approximately 1.2 kg $\pm$ 150 g. After 8 h on ice at sea, octopus samples were landed in the fish port of Thessaloniki and were processed (tumbling) immediately, while still fresh (within 12 h from catch), because freezing itself can alter protein solubility and affect texture. Tumbling in the present study was performed using a custom-made tumbler, as previously described (Katsanidis, 2004). After tumbling the samples were repackaged in wooden boxes with ice, delivered to the laboratory in less than 3 h of landing. Immediately after delivery the octopus samples were gutted, rinsed in cold tap water and the tentacles removed (the tentacles being the focal point of this study). Octopus tentacles (120 $\pm$ 10 g) were separated into four lots: A, control sample; under aerobic storage in the absence of oregano essential oil; VP, under vacuum packaging in the absence of oregano essential oil; and VO1, VO2, treated samples with oregano essential oil 0.2 and 0.4% (v/w), respectively, under VP. Oregano oil (Kokkinakis, Athens, Greece) was added on the surface of octopus samples (tentacles) in appropriate volumes using a micropipette, followed by mild massage (directly with the fingers) of the oil for each sample. After addition of oregano oil, samples were packaged in food grade (EU 2002/72) polyethylene/polyamide (PE/PA, Flexo-Vacuum TH 100) barrier pouches (VER Pack, Thessaloniki, Greece), 100 μm in thickness, having an oxygen permeability of 55 cm^3/m^2 per 24 h/atm at 75% relative humidity (RH), 23 °C and a water vapor permeability of 4 g/m^2 per 24 h at 90% RH, 38 °C. Pouches, containing the octopus muscle tentacle, were heat-sealed using a vacuum sealer (Autovac, Kramer & Grebe, Wallau, Lahn, Germany) and stored under refrigeration (4 ± 0.5 °C) for a period of 9 (control) days and 23 (VP, VO1 and VO2) days. As previously mentioned, experiments were conducted twice and in each experiment triplicate samples from each lot were removed for subsequent analysis. Finally, fresh octopus was also stored at −30 °C to be used as reference sample in the sensory analyses. Samples were analysed at predetermined time intervals namely: 1, 3, 5, 7, 9, 13, 19 and 23 days of storage.

2.2. Microbiological analysis

A sample of 25 g was taken aseptically from each octopus arm, transferred to a stomacher bag and 225 ml of sterilized peptone water (Buffer Peptone Water, LAB M) were added, and the mixture was homogenized for 2 min with a stomacher (Stomacher 400, Lab. Blender, London, UK). Samples (0.1 ml) of serial dilutions of octopus homogenates were spread on the surface of the appropriate dry media in Petri dishes for determination of the total aerobic plate count (TPC) on Plate Count Agar (Oxoid, CM325), and incubated at 30 °C for 3 days. *Pseudomonas* spp. were determined on Cetrimide Fusidin Cephaloridine agar (Oxoid CM559 supplemented with selective supplement SR 103, Oxoid, Basingstoke, UK) after incubation at 20 °C for 2–3 days. For Enterobacteriaceae and H_2S-producing bacteria (including *Shewanella putrefaciens*), 1.0 ml was inoculated into 10 ml of molten (45 °C) violet red bile glucose agar (VRBGA, Oxoid CM485) and iron agar (IA, Oxoid CM867) respectively. After setting, a 10-ml overlay of molten medium was added. For the former, incubation was at 37 °C for 24 h. The large colonies with purple haloes were counted. IA plates were incubated at 25 °C and black colonies formed by the production of H_2S were enumerated after 3 days. Lactic acid bacteria (LAB) were enumerated on de Man Rogosa Sharpe agar (MRS, Oxoid, CM361) incubated at 30 °C for 5 days. Three replicates of at least three appropriate dilutions were enumerated. All plates were examined visually for typical colony types and morphological characteristics associated with each growth medium. Microbiological data were transformed into logarithms of the number of colony-forming units (CFU/g).

2.3. Biochemical analysis

TVB-N was determined using the European Union reference method (Malle and Poumeyrol, 1989). TMA-N was determined using the method of AOAC (AOAC, 1995). TVB-N and TMA-N contents were expressed as mg N/100 g octopus muscle. TBA was determined by a selective third-order derivative spectrophotometric method (Botsoglou et al., 1994). TBA content was expressed as μg of malondialdehyde (MDA)/kg octopus muscle. The pH value was recorded using a pH meter (Hanna Instruments, HI 9219, Woonsocket, RI, USA). Octopus muscle (10 g) was homogenized thoroughly with 90 ml of distilled water and the homogenate was used for pH determination.

2.4. Sensory evaluation

The sensory quality of raw octopus was evaluated on days 1, 3, 5, 7, 9 (control; untreated and treated with oil) and days 13, 19, 23 (oil treated) by a trained panel (among the staff from the laboratory). All panelists were trained for the period of 3 months in 1-h sessions three times a week (36 h total). Triangle tests were performed in order to select the five panelists who could detect off-flavors in raw product (octopus tentacle). Prior to sample evaluation, the five selected panelists participated in orientation sessions to familiarize themselves with the flavor (off-odor), appearance of raw (control and oil treated) octopus.

Since in our study octopus was used as tentacles (non-marinated and marinated in oregano oil) it was decided to base the rejection time on attribute of odor, which would be evaluated by the consumer after opening and just before cooking of the product. Similarly, the odor attribute was used as the decisive parameter in a related study of oregano essential oil on the shelf-life of sea bream (Goulas and Kontominas, 2007). It must be, however, stressed that extra care is needed by the consumers, with regard to seafood's quality and safety, especially in fish markets.

Octopus (tentacle) samples (ca. 100 g of muscle) after being defrosted (in a microwave oven at high power, 700 W, 3 min) were immediately presented to the panelists (each one evaluating approximately 20 g of tentacle sample) in Petri dishes covered with a lid in random order. Freshly thawed octopus samples (previously stored at −30 °C) was also presented to the panelists (reference sample). Sensory evaluation was conducted in individual booths under controlled conditions of light, temperature and humidity. Panelists were asked to score odor and appearance of tentacles using a 1–10 intensity scale with 10 corresponding to the most liked sample and 1 corresponding to the least-liked sample. Acceptability was

defined as having a score of >5, whereas the sample was classified as unacceptable after development of first off-odor (Botta, 1995).

2.5. Statistical analysis

Experiments were replicated twice on different occasions with different octopus samples. Triplicate samples were taken per replicate. Data from each replication were averaged and log transformed (CFU/g). These data were subjected to analysis of variance (ANOVA) using the statistical software Minitab 14.0 (Minitab, 2000; Petridis, 2000). Means and standard deviations were calculated, and, when F values were significant at the $P < 0.05$ level, mean differences were separated by the Student–Newman–Keuls (SNK) procedure (Steel and Torrie, 1980).

3. Results and discussion

3.1. Microbiological changes during storage

The changes in the microflora of octopus during storage under aerobic conditions and vacuum packaging with or without addition of oregano essential oil are shown in Fig. 1a–e. The initial (day 1) TPC (Fig.1a) was ca. 4.3 log CFU/g which is a relatively low bacterial load, taking into consideration the observed contamination of the tentacles, very common and difficult to remove in these species. Similarly low initial TPC (between 3 and 4 log CFU/g) has been reported for fresh common octopus (Hurtado et al., 2001; Vaz-Pires and Barbosa, 2004). TPC exceeded the value of 7 log CFU/g, which is considered as the upper acceptability limit for fresh water and marine species (ICMSF, 1986) on day 6 for samples A, on day 9 for VP and on day 13 for VO1, while VO2 samples did not reach this value throughout the 23-day storage period. Oregano treated under VP (VO2) octopus samples had significantly ($P < 0.05$) lower TPC count compared to all other samples. TPC in aerobically stored squid samples exceeded the microbiological limit of 7 log CFU/g after 8 days (Ohashi et al., 1991), whereas 2 log lower was observed in pressurized (400 MPa) octopus (*Octopus vulgaris*) muscle by Hurtado et al. (2001). With regard to the VO1 and VO2 samples, there are no reports in the literature on the use of VP in combination with oregano EO in seafood products. Limited work so far has been reported in related studies on meat. Fresh beef fillets, vacuum-packaged with addition of 0.8% (v/w) oregano EO, stored at 5 °C exceeded the limit of 7 log CFU/g on day 16 of storage (Tsigarida et al., 2000), while according to Skandamis and Nychas, 2002, the shelf-life of vacuum-packaged fresh beef increased from 20 to 27 days after the addition of oregano EO.

It is well documented that Gram-negative bacteria such as *Pseudomonas* and *Shewanella* spp. (of which *Shewanella putrefaciens* is the predominant species) grow during aerobic storage on chilled seafood products (Gram and Dalgaard, 2002). Initial (day 1) pseudomonads count was 3.3 log CFU/g and increased to reach final populations of ca. 8.05 log CFU/g (VP samples), 7.22 log CFU/g (A and VO1 samples) and 6.01 log CFU/g (VO2 samples) (Fig. 1b). As noted previously for TPC, *Pseudomonas* spp. population was significantly ($P < 0.05$) lower for VO2 samples compared to all other treatments. Interestingly in our study, pseudomonads being strictly aerobic bacteria reached high final counts not only in the air-packaged samples (A), as expected, but also in VP and VO1 samples. This is probably attributed to the permeability of the flexible pouches used for packaging of octopus (55 cm^3/m^2 per 24 h/atm at 75% RH and 23 °C). Borch et al. (1996) found that as the permeability of packaging bags of fresh beef was increased, the rate of growth and the maximum population of *Pseudomonas* spp. also increased. In addition according to Sutherland et al. (1975) the higher the initial population of *Pseudomonas* spp. the more intense is the effect of oxygen permeability of plastic films on their growth. In related studies it was found that addition of 0.8% (v/w) oregano EO in VP beef fillets reduced the population of *Pseudomonas* spp. (Tsigarida et al., 2000; Skandamis et al., 2002).

Counts of H_2S-producing bacteria, like *Pseudomonas* spp., have been used as spoilage indicators of seafood products (Gram and Dalgaard, 2002). Especially the count of *Shewanella putrefaciens* is directly related to the remaining shelf-life of seafood products causing reduction of trimethylamine oxide (TMAO) to TMA-N in fish. H_2S-producing bacterial count was initially 2.78 log CFU/g, thereafter increasing to give final populations of ca. 6.92, 8.01, 6.82 and 5.55 log CFU/g for A, VP, VO1 and VO2 samples, respectively (Fig. 1c). H_2S-producing bacteria were significantly ($P < 0.05$) lower in treatment VO2, followed by treatment VO1. Our results for H_2S-producing bacteria in aerobically packaged octopus are lower than those reported for VP squid by Paarup et al. (2002).

As high populations of more than 10^7–10^8 CFU/g of the bacterium *Shewanella putrefaciens* are normally required to cause spoilage of fish and seafood (Gram and Dalgaard, 2002), it was assumed that this organism was also a major spoiler in the present study (6.92, 8.01 log CFU/g for A, VP samples, respectively, in contrast to the finding of Lougovois et al. (2007) for ice-stored musky octopus. Whilst the initial number of H_2S-producing bacteria, compared to the *Pseudomonas* spp., in octopus samples was lower (by ca. 0.6 log CFU/g) final populations of these bacterial species for A and VP samples were not significantly ($P < 0.05$) different. It has been previously postulated (Gram et al., 2002) that *Pseudomonas* spp., are able to produce a range of antibacterial compounds, i.e. siderophores that may cause a depression in the growth of H_2S-producing bacteria in an iron-limited environment. Such behavior for these bacterial species was not observed in the present study for A and VP octopus samples.

To our knowledge there is no information available in the literature on the effect of oregano EO on H_2S-producing bacteria in VP seafood. In the present study H_2S-producing bacteria were part of the natural flora of octopus but with *Pseudomonas* spp. being the dominant species, as already mentioned above.

LAB were found to be members of the microbial flora of octopus and the dominant species in samples VO2. Initial population of LAB was ca. 3 log CFU/g, while only for VP octopus samples a count of ca. 6.9 log CFU/g was reached on final day 23 of storage (Fig. 1d). LAB populations were highest in the VP octopus samples, as expected, compared to the control samples (Fig. 1d). The results of the present study are in good agreement with those reported for VP fillets of fresh beef (with addition of 0.8%, v/w, oregano EO). LAB, being a facultative anaerobic group, is tolerant to CO_2 and, therefore, may inhibit growth of other bacteria (because of the formation of lactic acid and bacteriocins) and this fact may contribute to their selective growth during spoilage of seafood products.

Enterobacteriaceae low initial count (2.1 log CFU/g, Fig. 1e) indicates both good hygiene of the marine environment from which the octopus was caught, as well as the adequate processing conditions during handling, transportation, cutting and packaging of octopus samples (Vaz-Pires and Barbosa, 2004). Enterobacteriaceae produced lower counts ($P < 0.05$) than the other species (examined in the present study) in control and oregano treated under VP octopus samples, and final counts were ca. 5.9 log CFU/g (control, day 9) and 5.1, 6.0, 6.7 log CFU/g (oregano treated, day 23, for VO2, VO1, VP samples, respectively).

Enterobacteriaceae, being psychrotolerant, are capable of growing at refrigeration temperatures; however, they cannot compete well with other Gram-negative spoilers (ICMSF, 1998). Our results for Enterobacteriaceae counts in oregano treated under VP (VO1) octopus samples (ca. 5.0 log CFU/g) are in agreement with those reported for iced-whole octopus (*Octopus vulgaris*) by Vaz-Pires and Barbosa (2004) and recently for ice-stored whole musky octopus (*Eledone moschata*) by Lougovois et al. (2007).

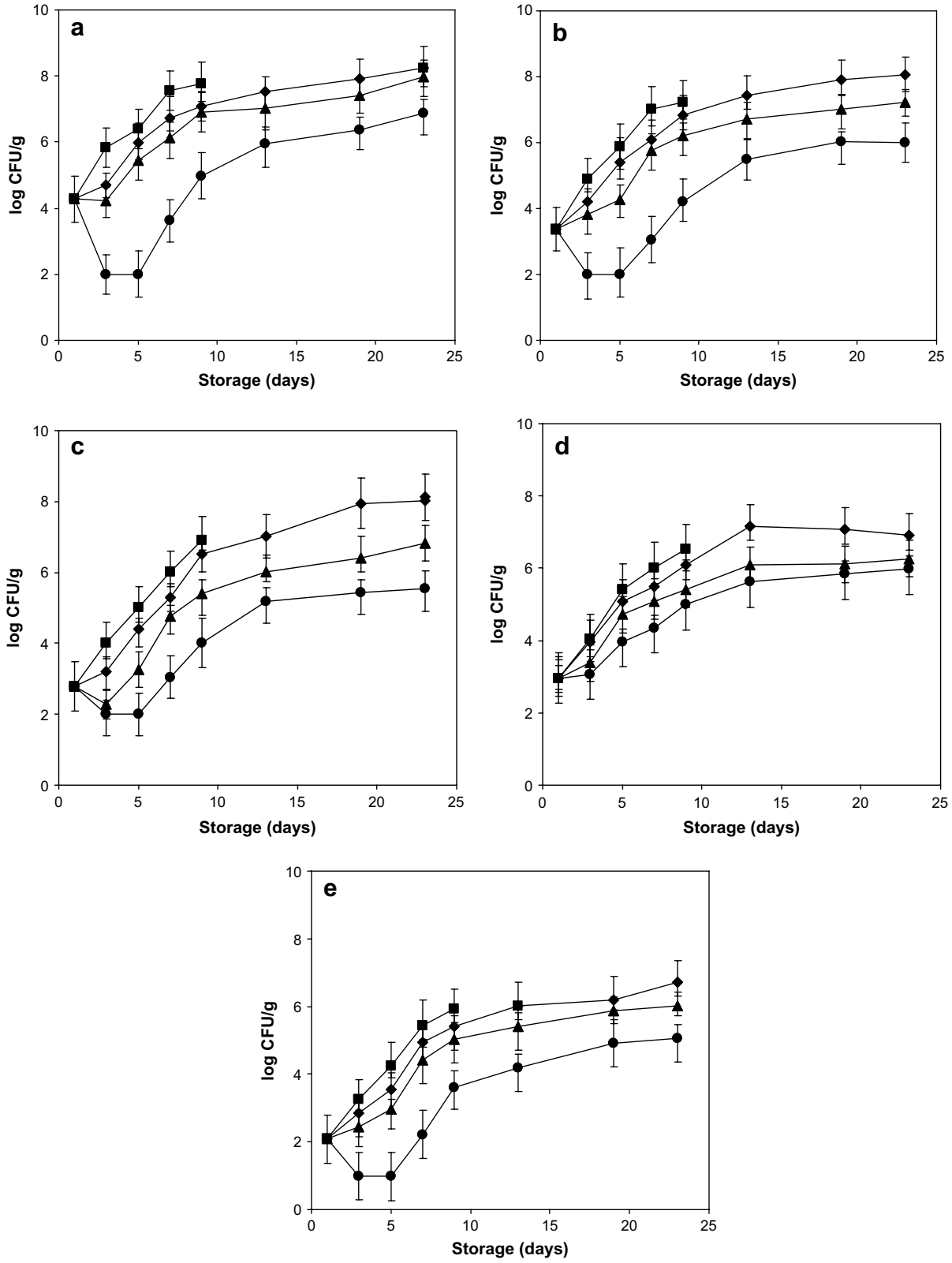

Fig. 1. Changes (log CFU/g) in: (a) total aerobic plate count (TPC); (b) *Pseudomonas* spp.; (c) H_2S-producing bacteria (including *Shewanella* putrefaciens); (d) LAB; and (e) Enterobacteriaceae of chilled fresh octopus without oregano oil stored in air (A, ■); without oregano oil stored under vacuum (VP, ◆); with oregano oil 0.2% (v/w) stored under vacuum (VO1, ▲) and with oregano oil 0.4% (v/w) stored under vacuum (VO2, ●). Each point is the mean of three samples taken from two replicate experiments ($n = 3 \times 2 = 6$). Error bars show SD.

3.2. Chemical changes during storage

The changes in pH, TVB-N, TMA-N and TBA (results not shown) of control (A), VP and oregano treated under VP (VO1, VO2) octopus during chilled storage are shown in Fig. 2a–c.

The initial (day 1) pH of octopus was ca. 6.1 (Fig. 2a). During the storage period at 4 °C the pH of control, VP and oregano treated under VP (VO1, VO2) octopus samples increased to reach final values of ca. 7.4, 7.2 and 6.6, 6.4, respectively. The significantly higher ($P < 0.05$) pH values recorded after days 5 and 13 of air and

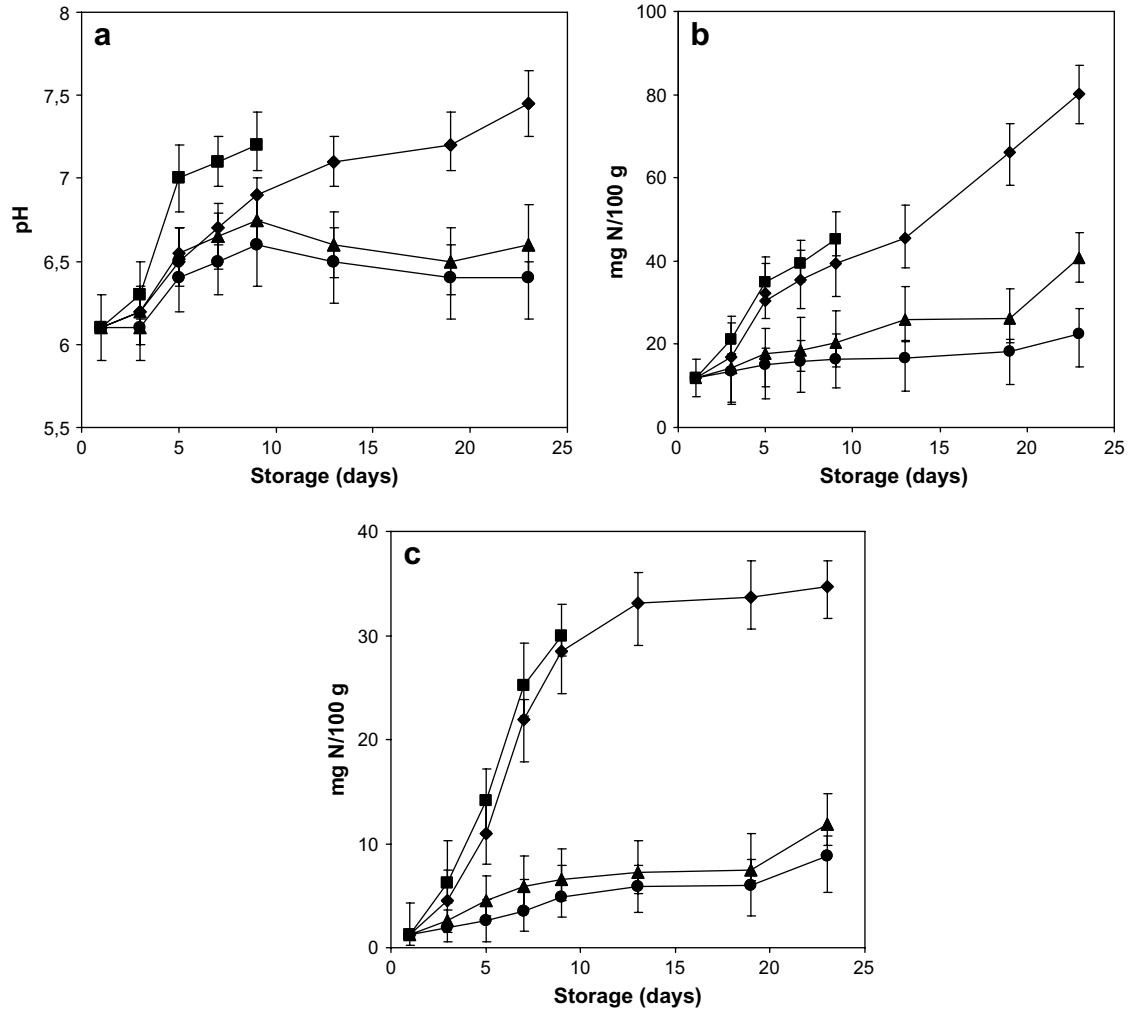

Fig. 2. Changes in (a) pH; (b) total volatile basic nitrogen (TVB-N) and (c) trimethylamine nitrogen (TMA-N) values of chilled fresh octopus without oregano oil stored in air (A, ■); without oregano oil stored under vacuum (VP, ◆); with oregano oil 0.2% (v/w) stored under vacuum (VO1, ▲); and with oregano oil 0.4% (v/w) stored under vacuum (VO2, ●). Each point is the mean of three samples taken from two replicate experiments ($n = 3 \times 2 = 6$). Error bars show SD.

vacuum-packaged octopus, respectively, may be attributed to the rapid spoilage of the product and the formation of alkaline compounds of autolysis and bacterial metabolites in the octopus muscle. Such behavior has also been noted for vacuum-packaged pressurized squid mantle (*Todaropsis eblanae*) by Paarup et al. (2002) and for ice-stored whole musky octopus (*Eledone moschata*) by Lougovois et al. (2007).

Initial TVB-N value (11.9 mg N/100 g) indicates that the fresh octopus was of good quality, in agreement with the relatively low initial TPC count (4.3 log CFU/g) (Fig. 2b). Similar TVB-N values have been found for chilled pressurized octopus (*Octopus vulgaris*) (Hurtado et al., 2001) and for chilled squid and octopus stored under controlled atmospheres (Ruiz-Capillas et al., 2002). It is noteworthy that TVB-N values of oregano treated under VP (VO1, VO2) octopus samples were significantly lower ($P < 0.05$) than the control and VP samples during the entire chilled storage period. This may be attributed to the antibacterial properties of the phenolic constituents, carvacrol and thymol of the oregano essential oil (Burt, 2004). TVB-N levels in cephalopods are much higher than those in fish probably because of the higher autolytic activity in muscle of cephalopods. Especially in octopus, autolytic activity is 3 times greater than in squid and 25 times than in gadoids (Hurtado et al., 1999a, b). There is currently no legal TVB-N limit value for cephalopods; thus, a TVB-N value of 35 mg N/100 g suggested for fish freshness (Conell, 1990) was taken as the limit of acceptability of fresh octopus. Considering this TVB-N value, fresh chilled octopus stored under aerobic and vacuum packaging would be expected to have a shelf-life of ca. 5–6 days, extended to ca. 16 days for VO1 samples. Interestingly for VO2 samples this limit value was never attained during the entire refrigerated storage period. Higher TVB-N values (45 and 60 mg N/100 g) were suggested as the limit of acceptability for vacuum-packaged pressurized squid mantle (*Todaropsis eblanae*) by Paarup et al. (2002) and squid and octopus by Ruiz-Capillas et al. (2002). Interestingly, high TVB-N values correlated rather well when respective TPC values had reached 10^7 CFU/g). To our knowledge very little information is available in the literature on the effect of EO on the TVB-N values of vacuum-packaged fish, including seafood.

TMA-N is produced by the decomposition of trimethylamine oxide (TMAO) due to bacterial action and action of intrinsic enzymes (Connell, 1990). Currently as in the case of TVB-N, there are no legal limits for TMA-N in cephalopods in the European Union and, thus, the recommended limit for chilled fish of 12 mg N/100 g fish muscle (Connell, 1990) is used. Similarly, TMA-N production in the control, VP and oregano treated octopus samples under VP, followed the same pattern as the TVB-N production (Fig. 2c). The initial TMA-N content of octopus samples was low (ca. 1.3 mg N/100 g) indicating the freshness of the product. This value is in agreement with those reported by Hurtado et al. (2001) and Ruiz-Capillas et al. (2002) for octopus. On final day (9) of storage, a

TMA-N value for the control (A) sample was ca. 30 mg N/100 g, whereas the respective H_2S-producing bacterial (including *Shewanella putrefaciens*) count reached a level of 6.9 log CFU/g. As high cell concentrations of more than 7–8 log CFU/g of these bacteria are normally required for the reduction of TMAO to TMA-N, it is likely that other bacteria, such as *Photobacterium* spp. may also be involved in the formation of TMA-N in control samples. The presence of these bacteria in the microflora association of octopus samples was not investigated in the present study.

Assuming the TMA-N value of 12 mg N/100 g as limit of acceptability, octopus samples under aerobic storage and VP exceeded this value after 5 and 6 days, respectively, while oregano treated under VP (VO1, VO2) octopus samples never reached this limit value throughout the entire storage period. As before, TMA-N values of oregano treated under VP (VO1, VO2) octopus samples were significantly ($P < 0.05$) lower than the control and VP samples during the entire chilled storage period. The slow rate of TMA-N production in oregano treated under VP (VO1 and VO2) octopus samples can be attributed to the conditions of low oxygen tension that greatly influence the spoilage of fresh seafood (Gram et al., 2002). Recently, Goulas and Kontominas (2007) also reported low TMA-N content for fresh sea bream (*Sparus aurata*) stored under MAP with addition of oregano essential oil.

Present results on TVB-N and TMA-N production in oregano treated under VP (VO1 and VO2) octopus samples, show that the use of oregano EO may affect the degenerative flora and thus slow down rapid decomposition in octopus muscle. The latter hypothesis, however, is only speculative and further research is needed to support our findings.

According to Connell (1990), TBA values of 1000–1500 μg MDA/kg of fish muscle are usually regarded as the limit beyond which fish will normally develop an objectionable odor/taste. The initial TBA content of fresh octopus was low (29.4 μg MDA/kg) near the detection limit of the method (20 μg MDA/kg), as a result of the low fat content (0.2–1%) of octopus, in contrast to other mollusks and fish (Karakoltsidis et al., 1995; Vaz-Pires and Barbosa, 2004). In addition to the low fat content, use of VP (alone) or in combination with the antioxidant activity of oregano essential oil played a key role in maintaining TBA values at low levels in control, VP, VO1 and VO2 octopus samples throughout the entire storage period. Of the treatments examined in the present study, TBA content of oregano treated under VP octopus samples, especially VO2, was extremely low ($P < 0.05$) (results not shown). It has been stated that use of oregano essential oil and especially its phenolic constituents (mainly thymol and carvacrol) possessing strong antioxidant activity can control lipid oxidation in fish (Tsimidou et al., 1995).

3.3. Sensory analysis during storage

The limit of acceptability for odor was reached after 6 days in control samples (A), 9 days in VP samples, 17 and 23 days VO1 and VO2, respectively (Fig. 3). In other related studies, based on sensory evaluation, a shelf-life of ca. 7–8 days was recorded for fresh squid (Yamanaka et al., 1987). Hurtado et al. (1999a) stated that the high autolytic activity of cephalopods results in short shelf-life. Especially for fresh octopus, a shelf-life of ca. 6–7 days is estimated after capture even at low storage temperatures (2.5 °C). Similarly to our results obtained for VP octopus, a shelf-life of ca. 8 and 10 days was reported for ice-stored whole octopus (*Octopus vulgaris*) (Vaz-Pires and Barbosa, 2004) and ice-stored whole musky octopus (*Eledone moschata*) (Lougovois et al., 2007), respectively. A shelf-life of ca. 6 days at 4 °C for aerobically stored fresh octopus, obtained in the present study is typical for seafood, and agrees with other findings reported above. It must be noted that a longer shelf-life for fresh octopus would be expected at a lower temperature (i.e. 0 °C; melting ice), as also noted by Vaz-Pires and Barbosa (2004) for

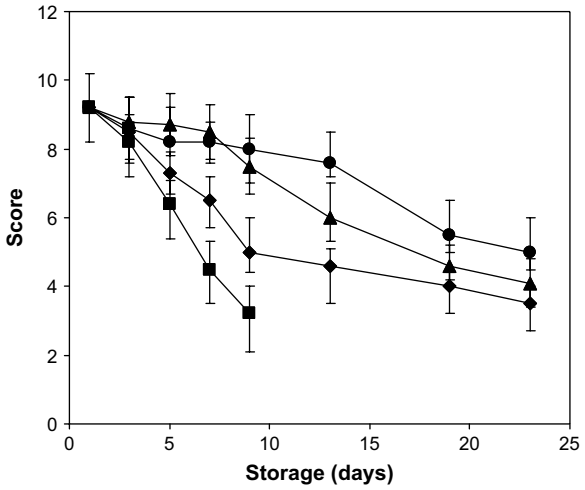

Fig. 3. Changes in odour scores of chilled fresh octopus without oregano oil stored in air (A, ■); without oregano oil stored under vacuum (VP, ◆); with oregano oil 0.2% (v/w) stored under vacuum (VO1, ▲); and with oregano oil 0.4% (v/w) stored under vacuum (VO2, ●). Each point is the mean of three samples taken from two replicate experiments ($n = 3 \times 2 = 6$). Error bars show SD.

iced-stored whole octopus (*Octopus vulgaris*). The temperature is a critical factor for storage of fresh seafood, including cephalopods, and current European regulations require a temperature of melting ice (EC 853/2004); however, this was not the objective in the present study.

Changes in appearance scores of control, VP and oregano treated under VP (VO1, VO2) octopus samples were not statistically significant ($P > 0.05$) (results not shown) and decreased at a slower rate than odor. Interestingly, all samples irrespective of treatment, by the end of storage period had acceptable scores (>5) (results not shown).

In our study, odor data of octopus samples correlated rather well with microbiological data (based on a TPC limit value of 7 log CFU/g, ICMSF, 1986), except for VO1 samples, in which the sensory acceptability limit was exceeded on day 17, whereas the respective microbiological (TPC) limit on day 13, meaning that the presence of oregano EO masked any unpleasant odor due to the bacterial action and spoilage of the product.

Concerning the sensorial effect of oregano essential oil concentration (0.2 and 0.4%, v/w) on the octopus tentacles in the present study, the use of the aforementioned concentrations gave a characteristic, desirable and pleasant (organoleptically acceptable) odor to the treated samples until days 17 and 23 days of storage, as judged by the sensory panel. It needs to be mentioned that VP (without oregano oil), VO1 and VO2 (treated with oregano oil 0.2 and 0.4%, v/w, respectively, under VP) octopus tentacles were cooked using a microwave (grill) and only samples taken from day −3 of storage were evaluated (results not shown). All cooked octopus samples (VP, VO1 and VO2) evaluated for both odor and taste by the sensory panel received acceptable scores, with octopus samples containing 0.2% oregano essential oil giving the highest acceptability score.

It is noteworthy that potential use of essential oils such oregano, thyme, etc., as preservative in foods needs to be carefully evaluated in terms of its sensorial acceptability. This study, in combination with current, limited knowledge on the potential use of oregano essential oil as a "natural" preservative in foods, leads to the general conclusion that oregano essential oil can expand its application to extend the shelf-life of cephalopods by (1) delaying of growth of specific spoilage organisms and (2) imparting a pleasant odor to the product, given that seafood treated with oregano (herb) is favorable to the consumers in the Mediterranean area.

To the best of our knowledge, this is the first study reporting on the combined use of VP and oregano EO for shelf-life extension of the Mediterranean octopus from the North Aegean Sea.

4. Conclusions

In conclusion, based primarily on sensory data the shelf-life of fresh Mediterranean octopus was 6 days (under aerobic storage), 9 days (under vacuum), 17 and 23 days treated with oregano oil (0.2 and 0.4%, v/w) under VP, respectively.

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