Food Microbiology 26 (2009) 166–172

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

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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ARTICLE INFO

Article history:
Received 20 January 2008
Received in revised form
18 September 2008
Accepted 2 October 2008
Available online 1 November 2008

Keywords:
Cephalopods
Essential oils
Octopus
Oregano
Shelf-life
Vacuum packaging

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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doi:10.1016/j.fm.2008.10.005

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 ± 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