Journal of Food Engineering 115 (2013) 486–494

Contents lists available at SciVerse ScienceDirect

Journal of Food Engineering

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

Effects of chilled storage on quality of vacuum packed meagre fillets

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ARTICLE INFO

Article history:
Available online 18 September 2012

Keywords:
Meagre fillets
Quality
Vacuum packaging
Shelf life prediction

ABSTRACT

The aim of this study was to experimentally assess several quality indices of meagre *Argyrosomus regius* (Asso, 1801) fillets packed in air (AP) and vacuum (VP) stored chilled (+4 °C) for up to 13 days. Considering our experimental data on concentration of bacterial counts, shelf-life is estimated at ca. 6 days for AP fillets and an additional 3–5 days for VP meagre fillets. Total volatile basic nitrogen (TVB-N) and trimethylamine (TMA-N) did not reach the regulated limits (25–35 mg/100 g chilled fish). The models implemented in the software Seafood Spoilage and Safety Predictor predicted a relatively shorter shelf-life of 4.8–6.9 days for fish stored in air at +4 °C when compared to AP and VP fillets. Empirical data and the models implemented in the software were used to predict the shelf-life of fillets if packaged under different modified atmospheres (MAP). Chilled, MAP fillets are likely to have a longer shelf-life than AP or VP samples if equilibrium CO_2 concentration is substantially high.

1. Introduction

Meagre, *Argyrosomus regius* (Asso, 1801), is a highly valuable scianid fish that is widely distributed in the Mediterranean, e.g. Portugal, Spain, France, Italy, and Turkey. The demand for this species is increasing daily (Naylor et al., 2000) since its nutritional composition of about 20% proteins and 1.4% crude fat (Martins et al., 2006) favours its consumption as a "healthy food". Nonetheless, the commercialised forms of processed seafood consist of portion-sized products, e.g. frozen skinned and/or breaded fillets and cubes, whole gutted and/or ungutted, sliced, smoked (FAO, 2005-2011). Hence, the consumption of ready-to-eat minimally processed seafood products, e.g. those that can be derived from meagre, is preferable (Monfort, 2010).

Like other seafood (Frazier and Westhoff, 1988), fresh meagre is highly perishable (Genç, 2012) due to microbial activity and/or spoilage-specific chemical reactions (viz. oxidation and rancidity) (Dalgaard, 2000, 2006), and thus has limited shelf-life. "Light" preservation and/or proper packaging methods should be tested to help prolong the shelf-life (Huss et al., 2000; Poli et al., 2003).

Undoubtedly, vacuum packaging (VP) combined with chilled storage, prolongs the shelf-life of seafood products by limiting the availability of O_2 that is necessary for the growth of aerobic bacteria. Moreover, other advantages of VP have been reported, i.e. suitable moisture and gas permeability, correct assembly and protection from the contamination with undesirable substances from outer environment (Connel, 1995).

Modified atmosphere (MAP) and vacuum packaging (VP) of perishable (e.g. seafood) products maintains the hygienic characteristics (Özogul et al., 2004; Philips, 1996; Poli et al., 2006; Rotabakk et al., 2008; Torrieri et al., 2011). Moreover, the combination of MAP, VP and chilled storage has been found to extend the shelf life of fresh products (Pastoriza et al., 1996). There are several studies about VP fish, e.g. salmon (*Salmo salar*) (Dondero et al., 2004; Hansen et al., 2009), carp (*Cyrinus carpio*) (Krizek et al., 2004), rainbow trout (*Salmo gairdneri*) and Baltic herring (*Clupea harengus membras*) (Randell et al., 1997) and Atlantic herring (*Clupea harengus*) (Özoğul et al., 2000), but no available data for VP of meagre fillets.

The objective of this study was to experimentally assess several quality indices (microbiological and physical-chemical parameters) of meagre fillets packed in air and vacuum and stored chilled and by using empirical data and the models implemented in the software Seafood Spoilage and Safety Predictor to predict the shelf-life of fillets if packaged under different modified atmospheres.

2. Materials and methods

2.1. Materials

Whole meagre, *A. regius* (Asso, 1801), were obtained from the commercial circuit in Faro, Portugal. Specimens (mean

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http://dx.doi.org/10.1016/j.jfoodeng.2012.09.007

weight ± SE: 862.71 ± 18.17 g) were kept in polystyrene boxes covered with ice during transportation to the laboratory. The fish were washed with tap water and filleted under hygienic conditions. The average (±SE) weight of fillets was 124.5 g ± 4.51 g.

2.2. Packaging and storage conditions

Fillets were immediately packed in Combitherm® XX (Wolff Walsrode AG, Germany) bags (200 × 200 mm) under atmospheric air (AP, sealing only) and vacuum (VP, at ca. 380 mm Hg) using a multipack vacuum packaging system (Interdibipack S.p.a., Italy). The packaging film was coextruded laminate composed of an exterior cast polyamide (PA) layer, a co-extruded interior barrier layer containing ethylene vinylindene alcohol (EVOH) and a polyethylene (PE) sealing layer. The oxygen transmission rate (OTR) is 0.5 cm^3/m^2 d bar at 23 °C and 85% RH. Storage trials were carried out for 13 days at +4 °C ± 0.5 °C.

2.3. Microbiological analysis

Samples (10 g) of fillets were aseptically placed into sterile Stomacher® bags containing 90 ml of peptone water with NaCl (0.85% w/v) (Merck, Darmstadt, Germany) and homogenised for 1 min (Stomacher® 400, Seward Ltd., London, UK). Aliquots of 1 ml were poured in Petri dishes according to serial decimal dilutions before addition of appropriate media. For the enumeration of mesophilic aerobic and psychrophilic bacteria, PCA (Scharlau 01-161, Germany) was incubated at 30 °C for 2 days (ISO, 2003) and at 6.5 °C for 10 days (ISO, 2001), respectively. Enterobactericeae and lactic acid bacteria (LAB) were enumerated after inoculation of 1 ml aliquots into 10 ml of molten (at 45 °C) violet red bile glucose agar (VRBGA, Scharlau 01-295, Germany) and MRS agar (Scharlau 01-135, Germany), respectively. After settling, a 10 ml overlay of molten media was added and plates were incubated at 37 °C for 48 h for VRBGA plates and 30 °C for 5 days for MRS plates. For hydrogen sulfide (H_2S) producing bacteria, Iron Agar (IA) was prepared and used according to NMKL (2006). Specifically, a thin overlay of IA was poured on top of the IA to avoid fading of the black colonies due to oxidation of iron sulfide (FeS). Petri dishes were then incubated at 25 °C for 72 h and black colonies were counted as H_2S-producing bacteria. All plates were examined visually for typical colony types and morphological characteristics associated with each medium. Microbiological data, i.e. number of colony forming units per unit mass were log-transformed prior to analysis, log (cfu g^{-1}).

2.4. Chemical analysis

pH was determined directly from fish flesh using a digital meter (model Glp 21, Crison, Spain). Chemical spoilage was assessed by two indices: total volatile basic nitrogen (TVB-N) and trimethylamine (TMA-N). Both indexes were determined according to the Conway method (Conway and Byrne, 1933); specifically, TMA-N was determined after the addition of formaldehyde. The flesh content in TVB-N and TMA-N was expressed as mg TVB-N per 100 g of sample and mg TMA-N per 100 g of sample, respectively.

2.5. Physical analysis

Colour measurements were carried out directly on fresh and packed/chilled stored samples using a tristimilus colorimeter (model DR LANGE, Spectro-color, Spain) and examined according to the Hunter L,a,b colour scale (Hunter Associates Laboratory Inc., USA) where L refers to lightness (0 is black and 100 is white), a indicates greenness ($a < 0$) or redness ($a > 0$), and b measures blueness ($b < 0$) or yellowness ($b > 0$) of samples. As summary measure, total colour change (denoted ΔE) (Anon., 2008b) was calculated in accordance with:

$$\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{\frac{1}{2}}$$

where e.g. $\Delta L^2 = (L_t - L_0)^2$ and L refers to lightness at time t (L_t) and time 0 (L_0).

Hardness, i.e. the force required to attain a deformation of the products' surface (Szczesniak, 2002), was determined via a compression test. This test was carried out using a texturometer (LFRA Texture Analyzer, Brookfield Engineering Labs Inc., USA) equipped with a 12.7 mm-diameter stainless steel spherical probe which approached the sample at the speed of 1 mm^{-1} and compressed 5 mm into the fillets. Measurements (in kgf, where 1 kgf = 9.806 N) were analysed using TexturePro Lite v1.1 software (Brookfield Engineering Labs Inc., USA). Hardness was calculated as the peak force of the first compression cycle.

2.6. Experimental design and statistical analysis

Analyses described above were carried out on days 0, 1, 3, 8 for air packed samples and days 0, 1, 3, 8 and 13 for vacuum packed fillets. On each occasion, two fillets were sampled. Several measurements were made on each fillet and averaged: six for pH, three for colour, two for TVB-N and TMA-N and six for hardness. Results are reported as mean values ± standard errors. Two-way ANOVA with factors *storage time* and *package type* (AP and VP) was performed for each of the quality parameters analysed. A significant effect of the factors' interaction term was further studied using simple effects test to compare package type at each storage time (Esteves, 2011). The analyses were done using IBM® SPSS® 19 for Windows (IBM Company, NY, USA).

2.7. Modelling microbial growth of empirical data and prediction of shelf-life

The shelf-life of fillets packed under air and vacuum conditions was estimated based on the chemical (i.e. TVB-N content) and microbiological criteria (Bremner, 2002), specifically counts of mesophilic and psychrophilic aerobic and H_2S-producing bacteria. The guidelines of ICMSF (1986) and the PHLS working group (Anon., 2000) and the regulated methods for the control of fishery products (Anon., 2004, 2005, 2008a) were taken into consideration.

The microbial spoilage models implemented in the software Seafood Spoilage and Safety Predictor (SSSP v. 3.1, National Institute of Aquatic Resources, Technical University of Denmark (DTU Aqua), Denmark) were then used to model changes in the concentration of microorganisms over time, estimate kinetic parameters (e.g. maximum specific growth rate μ_{max}) and predict product shelf-life under specific storage conditions.

Specifically, the storage temperature (T, °C, in the range 3–5 °C) (Eq. 1) and the initial counts of mesophilic, psychrophilic and H_2S-producing bacteria (N_0, log cfu/g, of ca. 3.2) were firstly entered into the models of Dalgaard et al. (1993)

$$\sqrt{\mu_{max}} = 0.0299 \cdot (T + 7.08) \quad (1)$$

and

$$t_{SL} = \frac{[\log(10^7) - \log(N_0)] \times \ln(10)}{\mu_{max} \cdot 24} \quad (2)$$

to obtain estimates of μ_{max} (h^{-1}) and shelf-life (t_{SL}, in days). The primary growth model (a log-transformed three-parameter logistic model; Eq. (3)) supporting the above equations has been validated for several species stored aerobic conditions, e.g. cod, haddock, sea bream or snapper (Dalgaard, 1999):