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Original research article

Effect of storage temperature and osmotic pre-treatment with alternative solutes on the shelf-life of gilthead seabream (*Sparus aurata*) fillets

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ABSTRACT

The objective of the study was the kinetic modelling of the shelf-life of osmotically pre-treated fish during refrigerated and super-chilled storage. Fresh gilthead seabream (*Sparus aurata*) fillets were treated for 0–360 min at 15 °C in osmotic solutions 50:5 high dextrose equivalent maltodextrin:NaCl/100 g (HDM), 40:10:5 HDM:trehalose:NaCl/100 g (HDM + treh) and 40:10:5 HDM:glucosamine:NaCl/100 g (HDM + gluc). Water loss, solid gain, salt content and water activity were monitored throughout treatment. Slices untreated and osmotically pre-treated for 45 min were aerobically packed and stored isothermally at 15, 10, 5, 2.5, 0, −1 and −3 °C. Quality assessment was based on microbial growth (total viable count, *Pseudomonas* spp., *Brochothrix thermosphacta*, *Enterobacteriaceae* spp., H_2S-producing bacteria, lactic acid bacteria, yeasts and moulds), total volatile nitrogen (TVB-N), lipid oxidation (TBARs) and sensory scoring. Quality indices were kinetically modelled and temperature dependence of quality loss rates was modelled by Ratkowsky equation.

Osmotic pre-treatment led to significant shelf-life extension of fillets, in terms of microbial growth, chemical changes and organoleptic deterioration. The pre-treatment with the alternative solutes led to depression of the freezing point (−1.8, −2.6, −3.2 and −3.5 °C for the untreated samples and the osmotically pre-treated with HDM, HDM + treh and HDM + gluc, respectively). TVB-N values were higher in untreated samples, followed by osmotically treated fillets, mainly at higher storage temperatures (i.e. 10 and 15 °C). Based on the mathematical models for sensory evaluation scoring, the shelf-life was 12, 19, 22 and 22 days at 0 °C for untreated and osmotically pre-treated with HDM, HDM + treh and HDM + gluc fish slices, respectively, while the respective values at −3 °C were 21, 35, 38 and 38 days. The alternative solutes had no significant effect on the quality and shelf-life of pre-treated fish fillet during storage at refrigerated conditions.

1. Introduction

Gilthead seabream (*Sparus aurata*) is one of the most cultured species in the Mediterranean area. Greece is a significant world marine fish producer, specifically for gilthead seabream and European sea bass, with the combined production capacity of about 110,000 tonnes in 2015 (FAO, 2016). Although usually sold as whole fish, filleted products have high commercial potential but suffer from short shelf-life. Spoilage of refrigerated fresh and minimally processed fish is attributed mainly to bacterial activity and it manifests itself as changes in the sensory characteristics (Gram & Huss, 1996).

Lightly preserved fish products are uncooked or mildly cooked products, with low level of preservatives (NaCl<6%, pH>5). These products are usually produced from fresh seafood and further processing involves one or a few additional steps (e.g. drying, salting, cold smoking etc) (Leroi et al., 2008). Recent studies investigate the potential hurdles that might contribute to ensuring the quality of lightly preserved fish products.

Osmotic dehydration (OD) is a technique used to reduce water activity (a_w) in foods in order to improve nutritional, sensorial and functional properties of food. It consists of an immersion of the product into a concentrated solution (i.e. sugar, salt, sucralose etc.). A driving force for water removal is set up because of a difference in

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osmotic pressure between the food and its surrounding solution (Collignan, Bohuon, Deumier, & Poligné, 2001; Raoult-Wack, 1994; Rastogi, Raghavarao, Niranjan, & Knorr, 2002). The potential of OD to extend the shelf-life of fish fillets by reducing the initial load and delaying microorganisms' growth has been reported by Tsironi and Taoukis (2012, 2014).

Super-chilling is a technology that consists of storing food just above the initial freezing temperature and helps to inhibit most autolytic and microbial reactions (Huss, 1995). During super-chilling the temperature of the foodstuff is lowered often 1–2 °C below the initial freezing point of the product. Several studies evaluate the effect of super-chilling storage (−0.5 °C to −4 °C) on seafood products, including the use of flake or slurry ice (Bahuaud et al., 2008; Rodríguez, Carriles, Cruz, & Aubourg, 2008) and storage at subzero temperatures (Chang, Chang, Shiau, & Pan, 1998; Duun & Rustad, 2008; Olafsdottir, Lauzon, Martinsdøttir, Oehlenschläger, & Kristbergsson, 2006; Sivertsvik, Rosnes, & Kleiberg, 2003). At super-chilling temperatures, microbial activity is limited, however chemical and physical changes may progress (Magnussen, Haugland, Hemmingsen, Johansen, & Nordtvedt, 2008). Recent studies investigate the potential use of cryoprotective agents during frozen storage of fish products (Fukuma, Yamane, Itoh, Tsukamasa, & Ando, 2012; Kaale and Eikevik, 2014).

The objective of the study was to evaluate and model the combined effect of osmotic dehydration with alternative solutes and storage at refrigerated (0 to 15 °C) and super-chilled (−3 to −1 °C) conditions storage on the shelf-life of chilled fillets of marine cultured gilthead seabream (*Sparus aurata*) fillets.

2. Materials and methods

2.1. Raw material

Marine cultured gilthead seabream (*Sparus aurata*) fillets (weight: 90 ± 10 g, capture zone: Aegean Sea, Greece) came from the same batch and were provided by a leading Greek aquaculture company. Fillets were transported directly to the laboratory in polystyrene boxes with appropriate quantity of flaked ice (0 °C) within 2–4 h. A polyethylene film was placed between layers of fillets, to avoid contact between skin and meat sides of the fillets.

Fillets were cut into rectangular slices ($3 \times 3 \times 1 cm^3$, 10±1 g) in a laminar flow hood. Osmotic solution was prepared by dissolving HDM, high dextrose equivalent (DE) maltodextrin (GLUCIDEX® 47 Syrope de Glucose Dehydrate, Roquette, France) and distilled water at a concentration of 50%. NaCl (5% ww) was added in the osmotic solution to increase the driving force of the process and attenuate the low level sweetness that could result from the HDM uptake during osmotic pre-treatment (Lerici, Pinnavaia, Rosa, & Bartolucci, 1985). Osmotic solutions with 40% HDM, 5% NaCl and 10% trehalose (TREHA™ British sugar, Felixstowe P/No. 1-2, Japan) or 10% glucosamine (Bioibérica, Barcelona, Spain) were also used (coded as HDM + treh and HDM + gluc, respectively). The concentrations of trehalose and glucosamine in the osmotic solutions were based on their solubility in water.

2.2. Osmotic pre-treatment

Sliced samples were osmotically treated at 15 °C for 0, 20, 40, 60, 90, 120, 180, 240, 300 and 360 min as described by Tsironi and Taoukis (2010). The solution to sample ratio was 5:1 (w/w) to avoid significant dilution of the medium by water removal, which would lead to local reduction of the osmotic driving force during process (Medina-Vivanco, Sobral, & Hubinger, 2002). Three replicate samples were removed and measured each time and the average values were taken.

Moisture content was determined by drying at 110 °C (WTB BINDER 7200, Type E53, Tuttlingen, Germany) for 24 h. Salt content of fish fillets was determined titrimetrically using silver nitrate solution by the Mohr method (AOAC, 1990). Sample water activity was determined using an a_w-meter (Rotronic AG, AM3+Aw VD, Bassersdorf, Switzerland). Water loss (WL) and solid gain (SG) were calculated according to Tsironi, Salapa, and Taoukis (2009).

2.3. Determination of freezing point of fish

The cooling curve is one of the most simple, accurate and widely used methods to measure the freezing point of foods. The wide application of this method is due to its accuracy and simplicity. In order to determine the freezing point of fish slices, raw slices were placed in a freezer (Whirlpool AFG 610 M-B chest freezer, Italy), at temperature of −30 °C. A type T thermocouple was inserted into the centre of the parallelepiped, temperature was constantly monitored and the cooling curve of fish was developed. The initial freezing point was determined from the cooling curve as described by Rahman (1995).

2.4. Shelf-life kinetic study

Samples were stored at controlled isothermal conditions of 15, 10, 5, 2.5, 0, −1 and −3 °C in high-precision (±0.2 °C) low-temperature incubators (Sanyo MIR 153, Sanyo Electric, Ora-Gun, Gunma, Japan) and shelf-life study was carried out. Temperature in the incubators was constantly monitored with electronic, programmable miniature dataloggers (COX TRACER®, Belmont, NC). Samples were taken in appropriate time intervals to allow for efficient kinetic analysis of quality deterioration.

2.4.1. Microbiological analysis

For microbiological enumeration, a representative sample (10 g) was transferred to a sterile stomacher bag with 90 mL sterilized Ringer solution (Merck, Darmstadt, Germany) and was homogenized for 60 s with a Stomacher (BagMixer® interscience, France). Samples (0.1 mL) of 10-fold serial dilutions of fish homogenates were spread on the surface of the appropriate media in Petri dishes for enumeration of different spoilage bacteria. Total aerobic viable count was enumerated on Plate Count Agar (PCA, Merck, Darmstadt, Germany) after incubation at 25 °C for 72 h. *Pseudomonas* spp. were enumerated on Cetrimide Agar (CFC, Merck, Darmstadt, Germany) after incubation at 25 °C for 48 h. *Brochothrix thermosphacta* was enumerated on STAA Agar (CM 881, Oxoid, Cambridge, UK) supplemented with SR 151 (Oxoid, Cambridge, UK) which was incubated at 25 °C for 48 h. Yeasts and moulds were enumerated on Rose Bengal Chloramphenicol Agar (RBC, Merck, Darmstadt, Germany) incubated for 168 h at 25 °C. For *Lactobacilli*, *Enterobacteriaceae* and H_2S-producing bacteria enumeration the pour-plate method was used. Lactic acid bacteria (LAB) were enumerated on De Man-Rogosa-Sharpe Agar (MRS, Merck, Darmstadt, Germany) followed by incubation at 25 °C for 96 h. For *Enterobacteriaceae* sp. enumeration Violet Red Bile Dextrose Agar (VRBD, Merck, Darmstadt, Germany) was used, which was incubated at 25 °C for 48 h. For the H_2S-producing bacteria Iron Agar was composed as described by Gram, Trolle, and Huss (1987) and incubated at 25 °C for 96 h.

Two replicates of at least three appropriate dilutions were enumerated. The microbial growth was modelled using the Baranyi Growth Model (Baranyi & Roberts, 1995). For curve fitting the program DMFit was used (available at http://www.combase.cc/index.php/en/). The kinetic parameter, rate (k) of the microbial growth, was estimated at all tested temperature conditions.

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2.4.2. Measurements of chemical indices

2-Thiobarbituric acid reactive substances (TBARS) assay, to evaluate lipid oxidation, was performed according to the method of Loovas (Loovas, 1992). The absorbance was measured at 532 nm with a digital spectrophotometer (Unicam Helios, Spectronic Unicam EMEA, Cambridge, United Kingdom). The concentration of TBARS was calculated from a standard curve prepared by 1,1,3,3-tetraethoxypropane and expressed as mg malonaldehyde/kg muscle.

Total volatile basic nitrogen (TVB-N) analysis was conducted on a single TCA extraction by distillation in a Kjeldhal rapid distillation unit (Büchi 321 Distillation unit, Flawwil, Switzerland) and titration with sulphuric acid (Pivarnik, Ellis, Wang, & Reilly, 2001).

2.4.3. Sensory analysis

The sensory attributes of raw and cooked fish were evaluated by a trained sensory panel of 8, selected according to ISO 8586-1 (1993) standard and trained using discriminative tests with practice evaluation methods of determining spoilage characteristics in fish fillets (Botta, 1995). Gilthead seabream slices were cooked individually wrapped in aluminum foil, at 180 °C for 20 min, in preheated oven. Panellists developed a list of profiling attributes concerning appearance and odour of raw fillets and appearance, odour, texture and flavour of cooked samples.

The sensory parameters were evaluated using descriptive terms and recorded in appropriate forms, reflecting the organoleptic evolution of quality deterioration. A preference/acceptance test was also conducted. Rating was assigned separately for each parameter on a 1 to 9 scale (9 being the highest quality score and 1 the lowest). A sensory score of 5 was taken as the average score for minimum acceptability.

2.5. Data analysis

Values of the different measured indices were plotted vs time for all temperatures studied and the apparent order of quality loss was determined based on the least square statistical fit. Temperature-dependence of the deterioration rate constants, k, was modelled by the Ratkowsky equation (1),

$$\sqrt{k} = b \cdot (T - T_0) \quad (1)$$

where b is the regression coefficient and T_0 is a hypothetical temperature which is an intrinsic property of the organism/quality index (Ratkowsky, Olley, McMeekin, & Ball, 1982).

2.6. Statistical analysis

Analysis of variance (ANOVA) at a significance level of 95% was used for the analysis of quality degradation rates of untreated and osmotically treated gilthead seabream fillets (STATISTICA® 7.0, StatSoft Inc., Tulsa, USA). Significant differences were calculated according to Duncan's multiple range test ($a = 0.05$).

3. Results

3.1. Osmotic pre-treatment

The osmotic pre-treatment caused a significant moisture loss from the fish flesh. The water loss, solid gain, %NaCl and a_w of gilthead seabream slices during osmotic pre-treatment are presented in Fig. 1. The water activity values decreased with time and averaged 0.879 after 360 min of osmotic pre-treatment. These results were in agreement with the respective values from previous studies for gilthead seabream slices treated with HDM + NaCl solutions (Tsironi & Taoukis, 2012; Tsironi et al., 2009). The use of the alternative solutes did not affect the mass transfer between the fish flesh and the osmotic solution. Processing time of 45 min was selected as the reference pre-treatment used in the shelf-life study, according to Tsironi and Taoukis (2010 and 2012). This processing time resulted in decrease of a_w at a level around 0.94–0.95, resulting in more stable products without significant quality and nutritional damage, observed by traditional methods. Neumeyer et al (1997) has reported a theoretical minimum a_w of 0.947 for *Pseudomonas* spp. growth, which can be the dominant spoilage microorganism in aerobic storage of fresh chilled fish. During this time, short enough for practical purposes, high rate of mass exchange is achieved, leading to a level of water loss-solid uptake that allows the product to retain optimum sensory characteristics. At the selected pre-treatment conditions (1:5 ratio of product to solution of 50% HDM+5% NaCl for 45 min at 15 °C) the fish flesh has 70.1 ± 0.3% moisture, 0.50 ± 0.04% NaCl and 0.94 ± 0.01 water activity.

3.2. Determination of freezing point of fish

The cooling curves for the untreated and the osmotically pre-treated gilthead seabream slices are shown on Fig. 2. The freezing point of untreated gilthead seabream was determined at −1.8 °C, being in agreement with the values reported in the literature for different fish species (Rahman, 1995). The pre-treatment with the alternative solutes led to depression of the freezing point (−2.6, −3.2 and −3.5 °C for HDM, HDM + treh and HDM + gluc, respectively). According to Jaczynski et al. (2012), cryoprotectants can depress the freezing point of the fish protein system, with the smaller molecular weight compounds leading in further depression compared to higher molecular weight compounds. Freezing point depression depends on the concentration of soluble particles, which lowers the effective number of solvent molecules that can produce phase transition from liquid to solid (Zaritzky, 2012). Similar findings of decrease in freezing point after osmotic dehydration have been reported in plant tissues (Cheng, Zhang, Adhikari, & Islam, 2014; Cornillon, 2000).

3.3. Microbial growth

Microbial counts for total flora, *Pseudomonas* spp., LAB, *B. thermosphacta*, *Enterobacteriaceae* spp., H_2S-producing bacteria and yeasts and moulds were plotted vs time, fitted to the Baranyi model and the growth kinetic parameters at each condition were determined. The growth rates of the measured microorganisms, in the various treatments and storage conditions are presented in Fig. 3.

Pseudomonas spp. dominated spoilage at 0–15 °C, being in agreement with the results reported for aerobically stored fresh chilled fish (Gram & Huss, 1996; Koutsoumanis & Nychas, 2000). As shown in Fig. 3, the osmotic pre-treatment led to significantly lower microbial growth rates ($P<0.05$) at all storage temperatures. Super-chilled storage further delayed microbial growth ($P<0.05$). Duun and Rustad (2008) and Sivertsvik et al. (2003) reported low growth in spoilage bacteria during super-chilled storage of Atlantic salmon. Super-chilled salmon fillets stored at −1.4 °C retained very good quality, with respect to growth of sulphide producing bacteria.

The temperature dependence of the rates of microbiological deterioration was adequately described by Ratkowsky model. The parameters and statistics of the Ratkowsky equation for the growth rate constants of all tested microorganisms are presented in Table 1. The calculated T_o for the tested microorganisms are in agreement with the respective T_o values reported by Ratkowsky et al. (1982)

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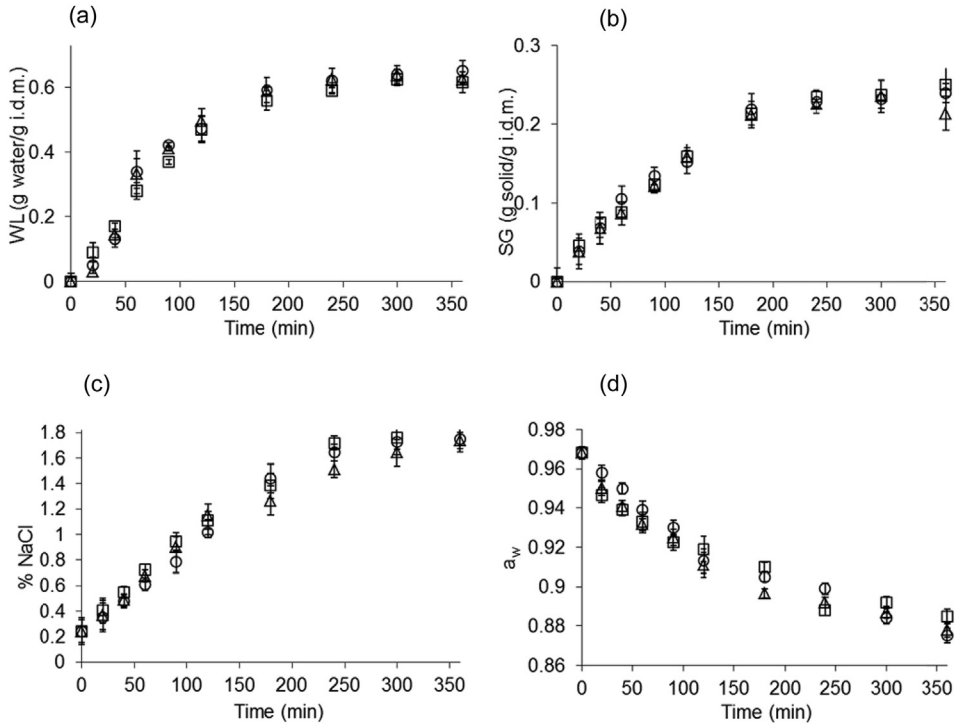

Fig. 1. (a) Water loss (WL: g of water/g initial dry matter), (b) solid gain (SG: g of total solids/g initial dry matter), (c) salt content (% NaCl) and (d) water activity (a_w) of gilthead seabream fillets during osmotic pre-treatment at 15 °C using different osmotic solutions: ○ HDM, △ HDM-treh and □ HDM-gluc (Mean of three measurements in three different samples ± standard deviation).

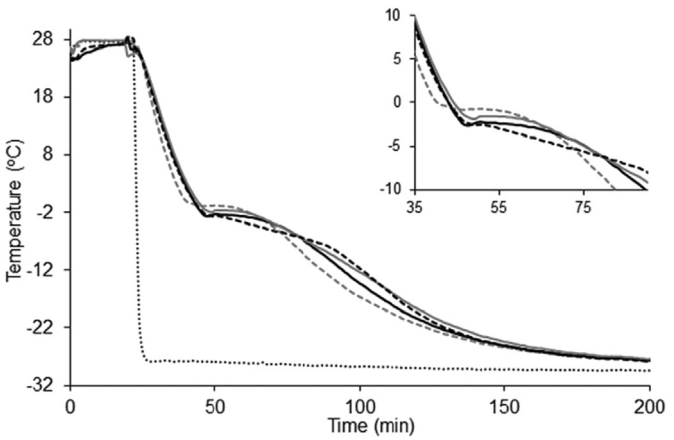

Fig. 2. Cooling curve of gilthead seabream slices: ----- untreated (control) and osmotically pre-treated using different osmotic solutions — HDM, — HDM + treh, ····· HDM + gluc (····· incubator temperature).

for *Pseudomonas* spp. and other psychrophilic bacteria. Processing conditions had no significant effect on the T_o values for microbial growth, chemical changes and sensory deterioration ($P>0.05$). Osmotic pre-treatment resulted in significantly lower *b* values for microbial growth (*Pseudomonas* spp., lactic acid bacteria, yeasts and moulds), chemical changes and sensory scoring decrease, compared to Control fillets ($P<0.05$). The addition of the alternative solutes in the osmotic solution further reduced the *b* values for TVB-N increase and overall impression scoring decrease ($P<0.05$).

3.4. Chemical changes

Initial TBAR levels were 0.23 ± 0.07 mg MDA/kg, being in agreement with the values reported for gilthead seabream and seabass (Duran-Montgé, Permanyer, & Belletti, 2015; Masniyom, Benjakul, & Visessanguan, 2005; Goulas & Kontominas, 2007). TBARs values increased with storage time and temperature, reaching 1.3–1.4 mg MDA/kg after 16–19 days at 2.5 °C. TBARs values were lower at subzero temperatures and reached 1.0 mg MDA/kg after more than 2 weeks at −1 °C and 3 weeks at −3 °C. Representative data for storage at −1 and 2.5 °C is shown in Fig. 4. The TBARs values in untreated and osmotically pre-treated samples were modelled by apparent zero order lines (Eq. (2), $R^2>0.87$),

$$C_{TBARs} = C_{TBARs,0} + k_{TBARs} \cdot t \quad (2)$$

where k_{TBARs} is the TBARs change rate constant, C_{TBARs} and $C_{TBARS,0}$ the TBARs values at storage times *t* and 0, respectively. The temperature dependence of the rates of TBARs changes were described by the Ratkowsky equation (Eq. (1)). The parameters of the Ratkowsky model for the rate constants of TBARs increase are shown in Table 1.

TVB-N was initially 12.5 ± 0.5 mg N/100 g and increased with storage time following apparent first order kinetics (Eq. (3), $R^2>0.92$) as shown in Fig. 5,

$$C_{TVB-N} = C_{TVB-N_0} \cdot e^{k_{TVB-N} \cdot t} \quad (3)$$

where $k_{TVB\text{-}N}$ is the TVB-N change rate constant estimated by a least square regression ($R^2>0.92$). TVB-N in super-chilled samples was significantly lower than the respective fillets stored at 2.5 °C. Osmotic pre-treatment further delayed TVB-N increase in fillets stored at 2.5 °C but had no significant additional effect on super-chilled samples. Trehalose and glucosamine did not affect significantly the TVB-N changes in super-chilled samples ($P>0.05$). The temperature dependence of the rate of TVB-N change was adequately described by Ratkowsky equation (Eq. (1)). The

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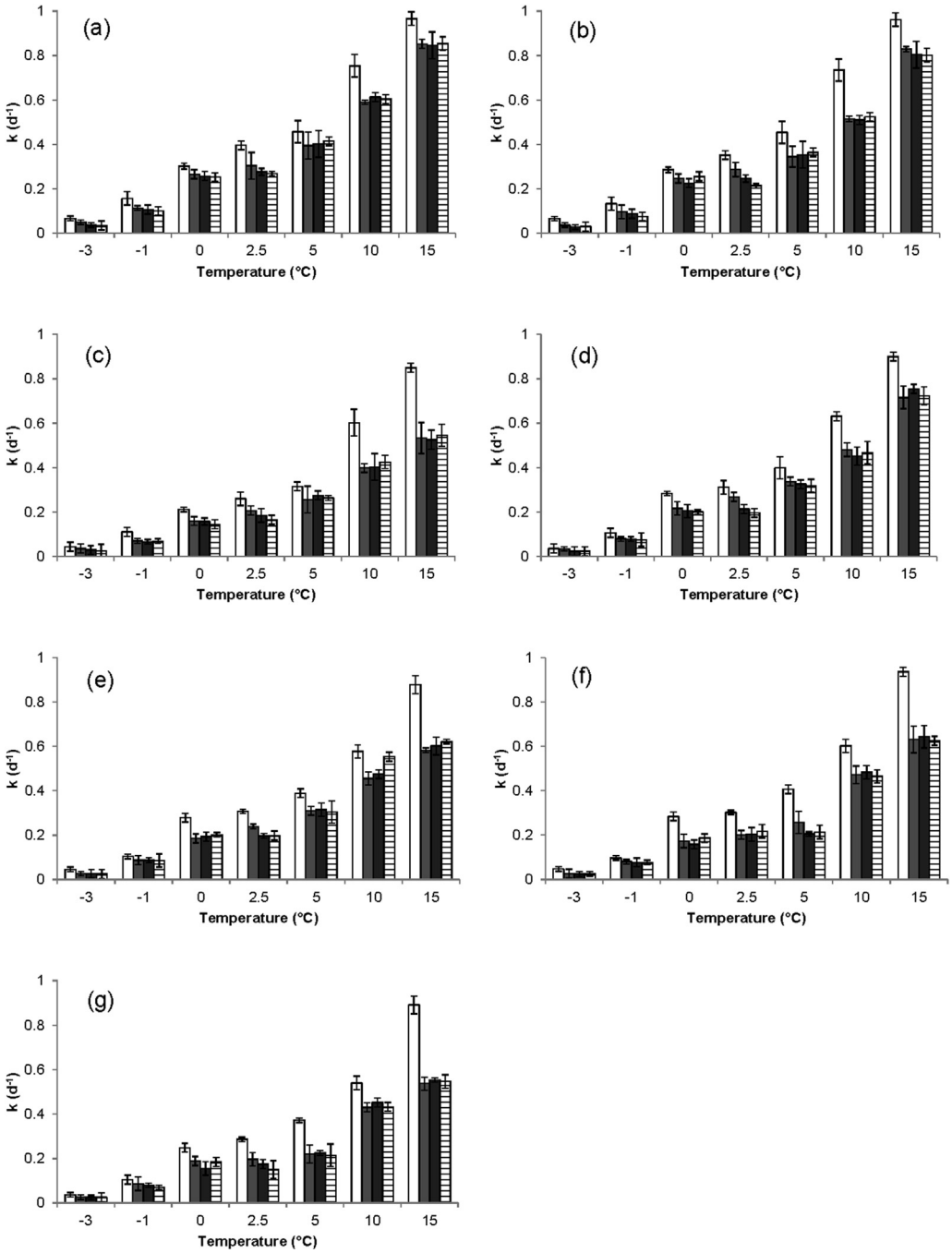

Fig. 3. Growth rates of (a) total viable count, (b) *Pseudomonas* spp., (c) lactic acid bacteria, (d) *Brochothrix thermosphacta*, (e) *Enterobacteriaceae* spp., (f) H_2S-producing bacteria and (g) yeasts and moulds in gilthead seabream slices (□ Control, ■ HDM, ■ HDM + treh, and ⊟ HDM + gluc) stored during isothermal storage at −3, −1, 0, 2.5, 5, 10 and 15 °C. (Mean values ± standard error based on the statistical variation of the kinetic parameters of the Baranyi growth model - regression analysis).

parameters of the Ratkowsky model for the rate constants of TVB-N increase are shown in Table 1.

3.5. Sensory evaluation and shelf-life determination

Appearance, flavour and overall acceptability of samples were evaluated by sensory analysis. The fillets were initially slightly translucent with a sweet seaweed-like odour. After osmotic treatment the fillets became slightly opaque, probably due to dissolution or denaturation of heme proteins (hemoglobin and myoglobin) (Haard, 1992). Additionally, osmotically pretreated fillets were more cohesive than the respective untreated samples. Osmotic treatment and super-chilled storage maintained fish freshness for longer times. Samples treated with the alternative solutes exhibited higher sensory scores at super-chilled conditions ($P<0.05$) with trehalose and glucosamine showing no significant differences.

A score of 5 for overall impression was judged as the lower limit of acceptability coinciding with slight off odour and off taste development. For super-chilled storage, quality degradation of fish fillets was attributed to odour and taste deterioration and texture changes, mainly increased hardness (Einen, Guerin, Fjæra, & Skjervold, 2002; Mackie, 1993). Textural hardness was

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Table 1
Parameters and statistics of the Ratkowsky model (Eq. (1)) for microbial growth, TBARs and TVB-N change and sensory scoring (odour, taste, overall impression) decrease rates in untreated (Control) and osmotically pre-treated gilthead seabream slices stored aerobically at the range −3 to 15 °C (Fitted parameter values ± standard error - Nonlinear regression analysis).

Parameter	Control			HDM			HDM + treh			HDM + gluc		
	b	T_0 (°C)	R^2	b	T_0 (°C)	R^2	b	T_0 (°C)	R^2	b	T_0 (°C)	R^2
Total viable count	0.037 ± 0.004	−12.7 ± 2.1	0.927	0.035 ± 0.004	−12.0 ± 2.1	0.922	0.037 ± 0.005	−11.2 ± 2.0	0.919	0.037 ± 0.005	−10.9 ± 2.0	0.917
Pseudomonas spp.	0.041 ± 0.003	−11.0 ± 1.9	0.970	0.034 ± 0.005	−10.5 ± 2.1	0.910	0.032 ± 0.004	−10.6 ± 1.9	0.920	0.030 ± 0.005	−11.0 ± 2.3	0.885
Lactic acid bacteria	0.035 ± 0.002	−11.5 ± 1.2	0.986	0.028 ± 0.003	−12.1 ± 1.9	0.928	0.029 ± 0.004	−11.5 ± 2.0	0.919	0.031 ± 0.003	−10.5 ± 1.6	0.941
B. thermosphacta	0.034 ± 0.003	−13.1 ± 2.0	0.964	0.033 ± 0.004	−11.6 ± 2.2	0.904	0.035 ± 0.004	−10.3 ± 1.9	0.918	0.035 ± 0.004	−10.2 ± 1.8	0.921
Enterobacteriaceae spp.	0.033 ± 0.003	−13.2 ± 2.1	0.964	0.030 ± 0.004	−12.1 ± 2.4	0.900	0.031 ± 0.004	−11.5 ± 2.2	0.906	0.032 ± 0.004	−10.8 ± 2.1	0.908
H_2S-producing bacteria	0.035 ± 0.003	−12.5 ± 1.9	0.967	0.032 ± 0.003	−10.6 ± 1.6	0.939	0.033 ± 0.003	−10.8 ± 1.6	0.939	0.032 ± 0.005	−10.8 ± 2.1	0.918
Yeasts and moulds	0.035 ± 0.003	−12.1 ± 1.8	0.971	0.028 ± 0.004	−12.2 ± 2.5	0.894	0.031 ± 0.003	−10.7 ± 1.7	0.936	0.029 ± 0.004	−10.9 ± 2.3	0.892
TBARs	0.024 ± 0.001	−10.5 ± 1.1	0.970	0.019 ± 0.001	−10.3 ± 0.8	0.979	0.018 ± 0.002	−10.6 ± 1.3	0.944	0.017 ± 0.002	−10.6 ± 2.1	0.916
TVB-N	0.022 ± 0.002	−8.4 ± 1.5	0.932	0.018 ± 0.002	−9.0 ± 1.4	0.939	0.019 ± 0.002	−8.2 ± 1.3	0.939	0.015 ± 0.002	−9.6 ± 2.2	0.886
Sensory (odour)	0.051 ± 0.005	−9.7 ± 1.3	0.959	0.036 ± 0.005	−12.1 ± 2.5	0.900	0.037 ± 0.003	−10.0 ± 1.2	0.964	0.034 ± 0.005	−12.4 ± 2.5	0.898
Sensory (taste)	0.054 ± 0.006	−9.6 ± 1.6	0.936	0.038 ± 0.004	−11.8 ± 1.9	0.927	0.040 ± 0.005	−10.7 ± 1.9	0.917	0.036 ± 0.004	−12.5 ± 2.2	0.918
Sensory (ov.impression)	0.049 ± 0.004	−11.9 ± 1.6	0.952	0.039 ± 0.002	−11.6 ± 1.8	0.938	0.034 ± 0.002	−12.6 ± 2.1	0.922	0.033 ± 0.002	−12.8 ± 2.2	0.894

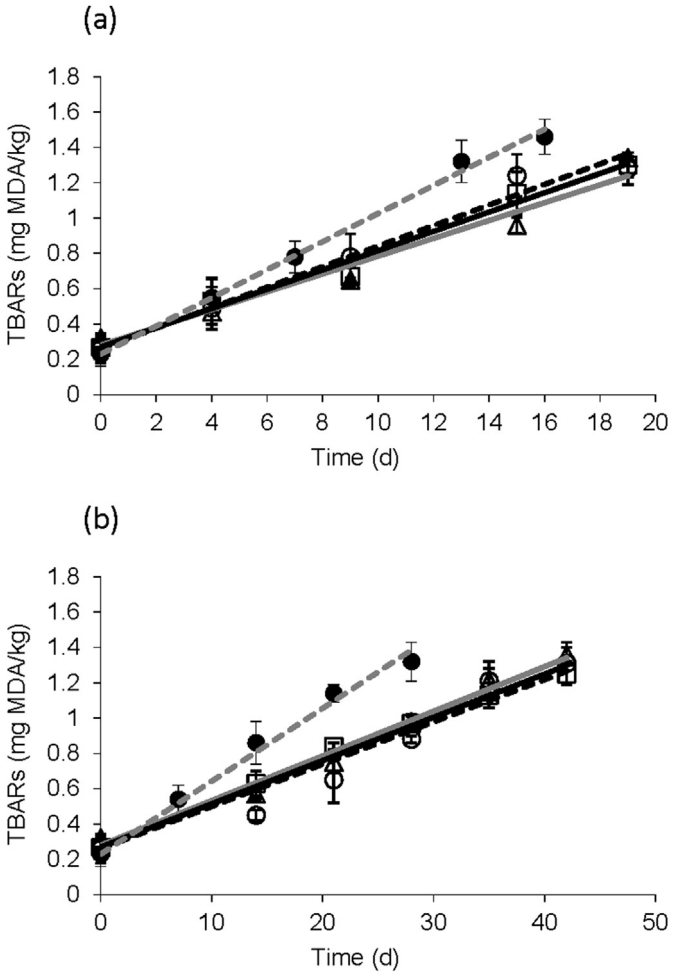

Fig. 4. TBARs change (mg MDA/kg) in gilthead seabream slices: ----- untreated (control) and osmotically pre-treated using different osmotic solutions — HDM, — HDM + treh, --- HDM + gluc during isothermal storage at (a) 2.5 °C and (b) −1 °C.

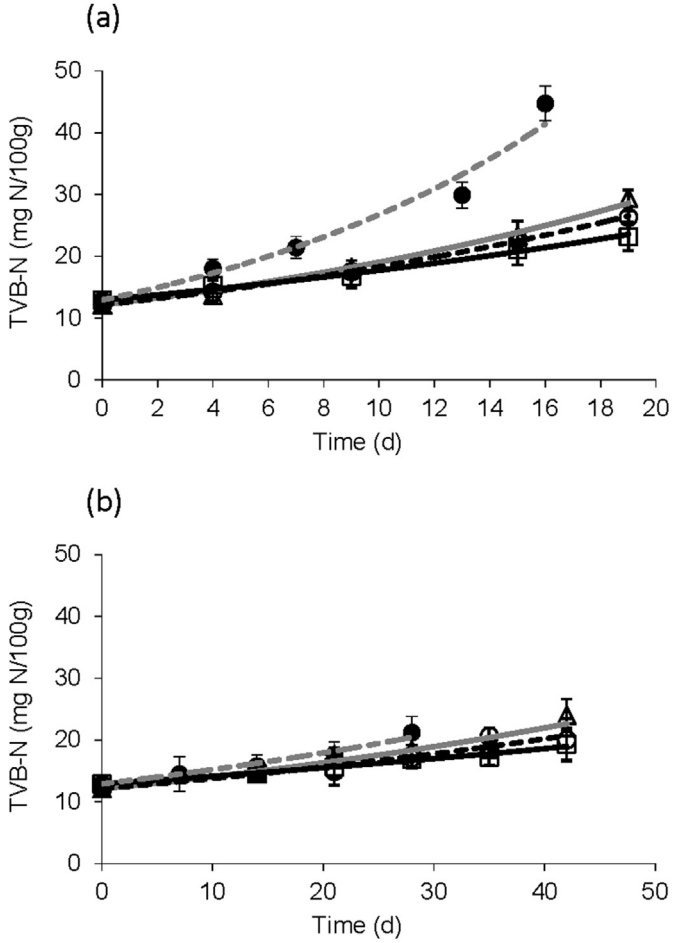

Fig. 5. TBV-N change (mg N/100 g) in gilthead seabream slices: ----- untreated (control) and osmotically pre-treated using different osmotic solutions — HDM, — HDM + treh, --- HDM + gluc during isothermal storage at (a) 2.5 °C and (b) −1 °C.

significantly higher in super-chilled salmon fillets stored at −3.6 °C compared to ice chilled samples (Duun & Rustad, 2008). Trehalose and glucosamine showed cryoprotective effect maintaining freshness characteristics and leading to higher sensory scores at subzero temperatures.

The sensory scores of untreated and osmotically pre-treated samples were modelled by apparent zero order lines (Eq. (4), R^2>0.90), as presented in Fig. 6,

$$s = -k_s t + s_0 \quad (4)$$

where k_s is the sensory scores change rate constant, s and s_0 the sensory scores at storage times t and 0, respectively. The temperature dependence of the rates of sensory deterioration were

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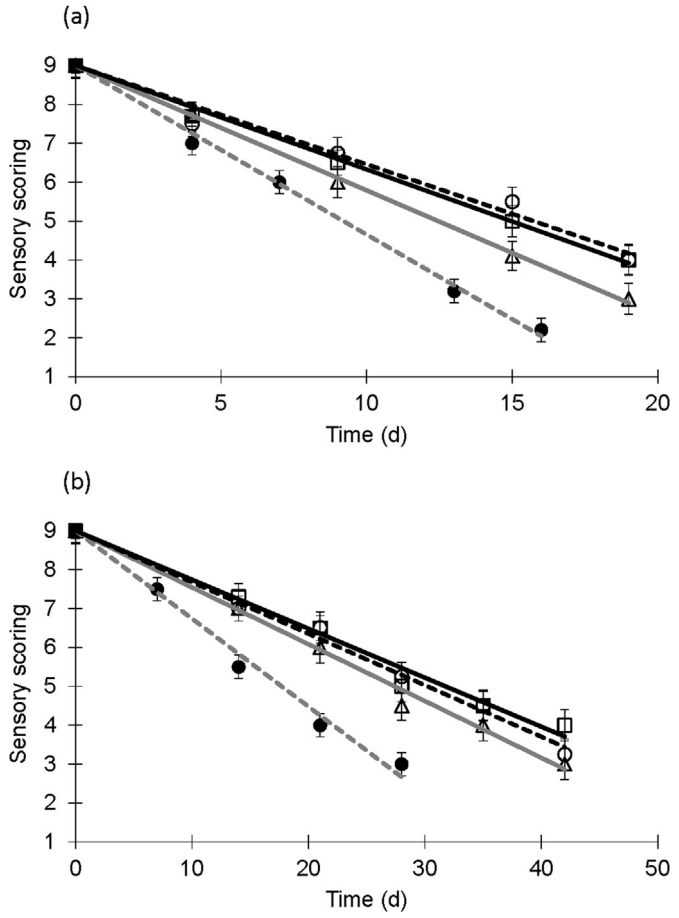

Fig. 6. Sensory scoring (overall impression) of gilthead seabream slices: ----- untreated (control) and osmotically pre-treated using different osmotic solutions — HDM, — HDM + treh, - - - HDM + gluc during isothermal storage at (a) 2.5 °C and (b) −1 °C.

described by the Ratkowsky equation (Eq (1)). The parameters of the Ratkowsky model for the rate constants of odour, taste and overall sensory impression scores are shown in Table 1.

At all temperatures studied, the time of sensory rejection correlated to a TBA value around 1.0 mg MDA/kg. According to Connell (1990) TBA values of 1–2 mg MDA/kg of fish flesh are usually regarded as the limit of acceptability, regarding the formation of unacceptable odour and flavour. The time of sensory rejection of refrigerated samples (0–15 °C) coincided also with a TVB-N concentration around 22 mg N/100 g, being in agreement with previous studies (Koutsoumanis & Nychas, 2000) and with *Pseudomonas* spp. load of 10^6 cfu/g.

Based on the limits indicating the end of the shelf-life for the different quality indices and the temperature dependence of their rate constants expressed by the Ratkowsky model (Table 1), equations to allow shelf-life determination at any storage temperature were developed. Shelf-life of gilthead seabream slices can be calculated by the following equations:

$$t_{SL} = \frac{\log N_l - \log N_0}{\left[b_{Ps} \cdot (T - T_{0,Ps})\right]^2}, \text{ based on } \textit{Pseudomonas} \text{ spp. growth (storage at } 0 - 15^{\circ}\text{C}) \quad (5)$$

$$t_{SL} = \frac{C_{TBARs,l} - C_{TBARs,0}}{\left[b_{TBARs} \cdot (T - T_{0,TBARs})\right]^2}, \text{ based on TBARs value} \quad (6)$$

$$t_{SL} = \frac{\ln C_{TVB-N,0} - \ln C_{TVB-N,l}}{\left[b_{TVB-N} \cdot (T - T_{0,TVB-N})\right]^2}, \text{ based on TVB} - \text{N value (storage at } 0 - 15^{\circ}\text{C}) \quad (7)$$

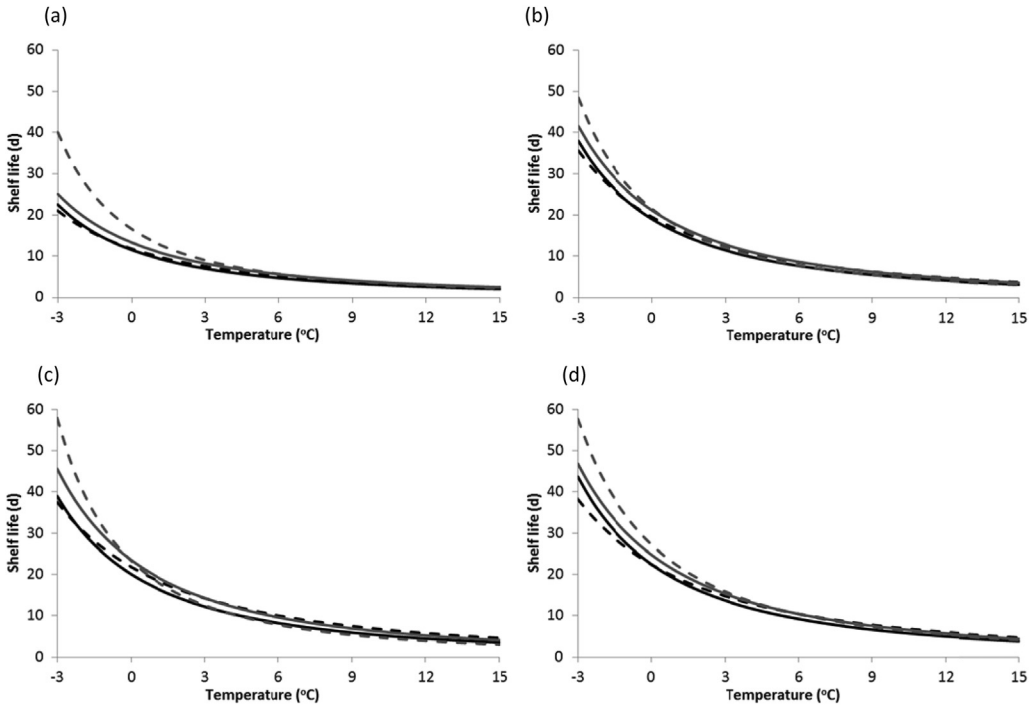

Fig. 7. Shelf-life (t_{SL} in days) of (a) untreated gilthead seabream slices (Control) and osmotically pretreated using osmotic solutions (b) 50% HDM (plus 5% NaCl) (HDM), (c) 40% HDM (plus 5% NaCl) and 10% trehalose (HDM + treh), (d) 40% HDM (plus 5% NaCl) and 10% glucosamine (HDM + gluc) stored aerobically at −3 to 15 °C, determined by - - - sensory evaluation (limit = 5 score for overall acceptability), — TBARs concentration (limit = 1 mg MDA/kg), — *Pseudomonas* spp. load (limit = 6 log cfu/g) and ----- TVB-N value (limit = 22 mg N/100 g).

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$$t_{SL} = \frac{S_0 - S_l}{\left[b_s \cdot (T - T_{0,s})\right]^2}, \text{ based on sensory scoring} \quad (8)$$

where t_{SL} is the shelf-life (d) of gilthead seabream slices, $\log N_l$ and $\log N_0$ are the limit ($\log N_l = 6$ log cfu/g) and initial *Pseudomonas* spp. counts, $C_{TBARs,l}$ is the limit TBARs concentration (1 mg MDA/kg), $C_{TBARs,0}$ is the initial TBARs concentration, $C_{TVB\text{-}N,l}$ is the limit TVB-N value (22 mg N/100 g), $C_{TVB\text{-}N,0}$ is the initial TVB-N value, S_l and S_0 are the limit ($S_l = 5$) and the initial sensory scores for overall acceptability, respectively, b and T_0 are the respective constants of the Ratkowsky equation (Table 1).

The shelf-life of untreated and osmotically pre-treated gilthead seabream with the different osmotic solutions calculated based on microbial growth, TBARs and TVB-N values and sensory scoring at the temperature range −3 to 15 °C using Eqs. (5)–(8) is presented in Fig. 7. Equations 6 and 8 result in the lowest shelf-life values, especially at sub-zero temperatures, leading in the assumption that shelf-life is limited by sensory scoring and TBARs value at the sub-zero temperature range. For refrigerated temperatures, i.e. 0–15 °C, the calculations by Eq. 1–4 do not differ significantly. Osmotic treatment maintained fish freshness for longer times compared to the Control samples. Super-chilled storage led to a significant shelf-life extension of untreated and osmotically pre-treated samples, as compared to refrigerated samples ($P<0.05$). Based on the mathematical models for overall sensory impression, the shelf-life was 21, 35, 38 and 38 days at −3 °C for untreated and osmotically pre-treated fish with HDM, HDM + treh and HDM + gluc, respectively, while the respective values at −1 °C were 14, 23, 26 and 26 days. Storage at −3 °C resulted in almost doubling of the shelf-life compared to 0 °C for all processing conditions. Similar results were reported for other super-chilled fish products. The storage time of vacuum packed salmon fillets was doubled by super-chilled storage at −1.4 °C and −3.6 °C compared to ice chilled storage (Duun & Rustad, 2008). In general, osmotic pre-treatment resulted in 60–70% shelf-life extension at all storage temperatures. The addition of the alternative solutes further extended the shelf-life of fish for 2–3 days at the range −3 to 0 °C.

4. Conclusions

The objective of the study was to evaluate the combined effect of osmotic dehydration and super-chilled storage on the quality characteristics of gilthead seabream. The results of the study show the potential of using osmotic pre-treatment with trehalose or glycosamine to delay microorganisms' growth and quality deterioration of fish products. Under this context, osmotic treatment combined with super-chilled storage may extend the shelf-life of gilthead seabream fillets. extend the shelf-life of fish products. Pre-treated samples were found to have improved quality stability during subsequent refrigerated or super-chilled storage, in terms of microbial growth, chemical and sensory degradation, resulting in a significant shelf life extension at all storage temperatures. The osmotic solutions with the alternative solutes resulted in higher shelf-life of the fish slices, especially at the lower temperatures (i.e. below 2.5 °C), with the effects of trehalose and glucosamine showing no significant differences. Super-chilled storage gave additional shelf-life increase of pre-treated fillets. The proposed models can be a reliable tool for predicting the shelf-life of minimally pre-treated gilthead seabream during super-chilled storage. Extending shelf life may reduce waste not only at consumer level, but also at retail, eliminating the financial burden of disposed products and environmental damage.

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