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Effect of pomegranate (*Punica granutum*) and rosemary (*Rosmarinus officinalis* L.) extracts on shelf-life for chilled Greenland halibut (*Reinhardtius hippoglossoides)* fillets in modified atmosphere packaging at 2 ºC

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Abstract

The present study evaluated the effect of pomegranate extract (1% v/w) and rosemary extract (1% v/w) as natural preservatives as well as their combination (1% v/w) on shelf life extension of previously frozen and chilled Greenland halibut fillets in modified atmosphere packaging (MAP, 40%CO_2/60%N_2) at 2 °C. Parameters that were monitored were: microbiological (aerobic plate counts (APC), lactic acid bacteria (LAB), *Lactobacillus* spp., and *Photobacterium phosphoreum*), biochemical (pH, thio-***barbituric acid*** (TBA), trimethylamine (TMA) and total-volatile-nitrogen (TVN)), and sensory (color, flavor and texture) attributes. For microbiological results, irrespective of treatments, APC reached levels $\geq 10^7$ CFU/g during storage. The spoilage microflora of fillets in MAP was dominated by LAB, but the concentration of *Lactobacillus* was very low. During storage, *P.phosphoreum* was not detected in any sample. Among the chemical indices examined, TBA values of control samples exceeded the limit of 2 mg malondialdehyde (MDA)/kg (2.78 and 4.08 mg MDA/kg) on days 18 and 23 of storage, respectively, while TBA values for samples treated with extracts remained below the limit throughout the storage period. Final (TMA) and (TVN) values for all treatments ranged between 0.15 to 0.37 mg N/100 g and 16.90 to 24.10 mg N/100 g after 23 days of storage, respectively, not exceeding upper acceptability limit set by EU. Sensory analysis correlated well with TMA and TVN analysis, indicating a shelf life of longer than 23 days for all samples.
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Introduction

In recent years, there has been a growing interest in natural ingredients, especially of plant origin, due to their greater application in food industry for increasing consumer acceptability, palability, stability and shelf-life of food products and these ingredients has started to replace the synthetic preservatives. Compounds obtained from natural sources such as grains, oil seeds, spices, honey, fruits and vegetables have been investigated (1). Extracts from spices, rosemary, thyme, and sage are found to have antioxidant properties similar to or greater than BHA and BHT (2, 3). Pomegranate (*Punica granutum*) contains important bioactive compounds and has been widely consumed in many different cultures for thousands of years. Pomegranate is one of the important fruits grown in Turkey, Iran, USA, Middle East, Mediterranean and Arabic countries (4). Pomegranate fruit parts are rich in tannin and phenolic compounds and are known to possess considerable antioxidant effects (5) and antimicrobial activities (6, 7). (8) studied the effect of pomegranate sauce on the quality of marinated anchovy during refrigerated storage and found that the juice concentrate had a positive effect on the quality and shelf life of marinated fish. Another natural ingredient that could possess antioxidant activity is rosemary (*Rosmarinus officinalis* L.). In addition to inhibition of lipid oxidation, several authors have reported that some of the compounds present in rosemary extract/essential oil possess antibacterial properties (9, 10, 11). To the best of our knowledge, the antimicrobial and antioxidant effects of pomegranate and rosemary extracts on chilled Greenland halibut (*Reinhardtius hippoglossoides*) fillets have not previously been studied and reported. The objectives of this study were to evaluate the effect of pomegranate and rosemary extracts on changes in microbiological, physico-chemical and sensory quality attributes of MAP halibut stored at 2°C.

Materials and Methods

Materials Frozen

Greenland halibut fillets (*Reinhardtius hippoglossoides*) were obtained from Royal Greenland Seafood A/S, Aalborg, Denmark. 1% full extract of rosemary (*Rosmarinus officinalis*) was obtained from Food Quality Inc., Akureyri, Iceland.

Determination of total phenolic content

Freeze-dried ethanol pomegranate extracts were prepared by slight modification of the method given by (12). The total phenolic concentrations of extracts were determined using the Folin-Ciocalteu method described previously (13). Total phenolics of extracts were calculated from standard gallic acid solutions (0-0.5 mg/ml), and expressed as mg gallic acid equilavents (GAE) per g extract. The estimation of phenolic compounds in the extracts was carried out in triplicate and the results were averaged.

Preparation of fish samples

Frozen halibut fillet was stored at -40 °C and then thawed overnight at 8°C. Skinless and interleaved fillets prepared by a local fish processor were cut into pieces of appropriate size for packaging. 120 g fillet pieces for microbiological and chemical analysis and 100 g fillet pieces for sensory analysis were used. Fish samples were assigned to one of the following four treatments: Control (fish without any extract); P (pomegranate extract, 1% v/w); R (rosemary extract, 1% v/w); PR (pomegranate-rosemary extract combination (1% v/w)). After addition of extracts, samples placed in plastic bags were thoroughly tumbled. After treatment the fish fillets were kept in plastic bags for 1 h and then randomly placed in plastic trays and packed using a Multivac C500 packaging machine (Multivac Ltd., Vejle, Denmark). Fillets were packed in a modified atmosphere with 40% CO_2 and 60% N_2 (AGA, Copenhagen, Denmark). Packs of thawed halibut in MAP were stored at 2°C. The temperature of all sub-batches of halibut was followed during chilled storage by data loggers (Tinytag, Gemini Data Loggers Ltd., Chichester, UK).

Sampling and analyses

At each sampling time three packages were evaluated. The composition of the atmosphere in packs was determined by a gas analyser (Combi Check 9800-1, PBI Dansensor, Ringsted, Denmark), drip loss was measured as previously described (14) and sensory, microbiological and chemical analyses were carried out as indicated below.

Microbiological analyses

Each treatment of halibut fillets was analyzed for concentrations of aerobic plate counts (APC) and lactic acid bacteria (LAB) during chilled storage. Concentration of *P. phosphoreum* and *Lactobacillus* were determined on the first day and on the 18th day of chilled storage, and at the end of chilled storage, respectively. Twenty grams of skinless halibut flesh were diluted ten-fold in chilled physiological saline (PS) (0.85% NaCl and 0.1% Bacto-Peptone) and homogenized for 60 s in a Stomacher (Seward Laboratory Blender, London, UK). From this homogenate a series of ten-fold dilutions was prepared with chilled PS. APC were determined by spread plating on prechilled plates of Long and Hammer (LH) agar with 1% NaCl (15 °C, 7 days) (15). LAB were determined by pour plating in nitrate actidion polymyxin (NAP) agar with pH 6.2 (25 °C, 3 days) (16). *P. phosphoreum* was enumerated at 15°C by a conductance based incubation method using three vials for each halibut sample (17). *Lactobacillus spp*. was enumerated by spread plating on Rogosa Agar followed by incubation at 25°C for 2 days.

Physico-chemical analyses

To characterize halibut fillets, samples were homogenized using a blender and dry matter, lipid content, NaCl content, organic acids, pH, TMA, trimethylamine-N-oxide (TMAO) and TVN were determined in triplicate. These analyses were carried out prior to chilled storage and as previously described (14). The oxidative stability of halibut fish fillets were determined with the thiobarbituric acid-reactive substances (TBARS) assay as described by (18). ***TBA*** value was determined in triplicate. TBARS were calculated from a standard curve of MDA. TBA number was calculated as mg MDA per kg fish sample. During chilled storage of halibut pH, free fatty acids, concentration of TMAO, TVN, TMA and TBA value were determined.

Sensory analyses

At each sampling time two portions of cooked samples from each treatment of chilled halibut were evaluated by 3 trained people. Portions of 50 g were heated in coded porcelain bowls in a convection oven (100°C, 28 min) and then served to the panelists. In addition to halibut stored at 2 °C, freshly thawed halibut samples, previously kept at -40 °C, were included in each sensory evaluation as references. Color, flavour and texture of samples were evaluated by using a simple scale with three classes, where three corresponds to a spoiled sample (14). The shelf life of halibut at each treatment group was defined as the time when 50% or more of the panelists determined cooked samples to be in class three.

Statistical analysis

For the microbiological, chemical and physical analysis the effect of storage time and different treatments on chilled MAP halibut fillets were analyzed by one-way analysis of variance (ANOVA) method ($p<0.05$). Comparison of means was performed using Tukey's (HSD) test.

Results and Discussion

Total phenolic content

The freeze-dried ethanol pomegranate peel extract gave a yield of 21 mg g^{-1}. Pomegranate peel extract had high phenolic content of 481 mg GAE g^{-1} dry extract. 1% aqueous rosemary extract had 30.2 mg GAE g^{-1} dry extract (According the producer the solution was 1%). Fish fillets (100 g) contained 24 mg total phenolic content for pomegranate extract and 0.302 mg total phenolic content for rosemary extract as GA equivalent.

Microbiological changes

Fillets were stored at an average temperature of 2 °C with very little variation in the storage temperature. The initial concentration of APC of chilled halibut fillets in MAP was *ca* 10^3 CFU g^{-1} whereas counts on NAP corresponded to below or close to 10 CFU g^{-1}. During the storage period *P. phosphoreum* was not detected in any sample. Irrespective of treatments, APC reached levels $\geq 10^7$ CFU g^{-1} during storage. During chill storage no significant differences in APC ($P>0.05$) except storage of 10 day were observed in all four treatments. The spoilage microflora of fillets in MAP was dominated by LAB but the concentration of lactobacillus as determined on Rogosa agar was low (Table 1). The spoilage microflora was most likely dominated by *Carnobacterium* as these LAB are known to be resistant to frozen storage of fish and relatively resistant to the CO_2 concentrations in MAP. Absence of the the highly CO_2–resistant and Gram negative spoilage bacterium *P. phosphoreum* in the dominating microflora suggests it has been inactivated during the frozen storage of the studied fillets prior to thawing and chilled MAP storage (19, 20, 21).

Table 1. Microbiological changes of chilled MAP halibut fillets during chilled storage at 2 °C

Treatments	Storage Period (days)						
	0	3	7	10	14	18	23
Aerobic Plate Count							
A	3.10±0.07^{A}	3.34±0.34^{A}	4.76±0.70^{B}	5.14±0.18^{a,B}	6.64±1.24^{C}	7.55±0.03^{C}	9.37±0.04^{D}
B	3.51±0.60^{A}	3.34±0.37^{A}	5.04±0.24^{B}	6.12±0.17^{b,C}	7.07±0.21^{D}	7.75±0.34^{E}	9.46±0.01^{F}
C	3.31±0.38^{A}	3.57±0.08^{A}	5.25±0.30^{B}	6.40±0.06^{b,C}	7.74±0.07^{D}	7.76±0.11^{D}	9.38±0.14^{E}
D	2.97±0.28^{A}	3.51±0.44^{A}	5.26±0.17^{B}	5.98±0.46^{b,B}	7.18±0.14^{C}	7.93±0.28^{C}	9.50±0.05^{A}
Lactic Acid Bacteria							
A	1.00±0.00^{A}	1.36±1.23^{A}	3.98±1.43^{B}	4.17±0.18^{B}	5.18±1.49^{B}	6.48±0.06^{a,B}	9.09±0.23^{a,C}
B	0.68±1.18^{A}	1.10±0.95^{A}	3.26±0.03^{B}	4.85±0.40^{C}	5.64±0.31^{C}	7.06±0.19^{a,D}	9.26±0.08^{a,E}
C	1.85±0.47^{A}	1.97±0.03^{A}	3.39±0.58^{B}	5.59±0.26^{C}	6.14±0.67^{C}	5.53±0.37^{b,C}	8.88±0.24^{ab,D}
D	1.48±0.00^{A}	1.89±0.43^{A}	4.08±0.22 ^{B}	4.93±1.00^{B}	4.60±0.02^{B}	6.82±0.50^{a,C}	8.59± 0.13^{b,D}
Rogasa Agar, log (CFU g^{-1})							
A	-§	-	-	-	-	-	4.00±0.00
B	-	-	-	-	-	-	4.33±0.58
C	-	-	-	-	-	-	5.10±1.15
D	-	-	-	-	-	-	4.74±1.29

a-b : Different letters within each storage time denote significant difference at p<0.05.
A-F : Different letters within each treatment denote significant difference at p<0.05.
§ : Not measured

Physico-chemical changes

Salt content, water activity, dry matter and lipid content of halibut fillets between first and last chilled storage days changed insignificantly (P>0.05) between 0.24-0.34%, 0.994-0.996, 24-30%, and 13-16%, respectively (Table 2). Just after packaging the modified atmosphere contained CO_2 concentrations from 33.90 to 35.70%. At the 3rd day of storage CO_2 concentrations significantly decreased (P<0.05) to 21.17-22.33%. After 3rd day of storage CO_2 concentrations remained almost constant for all four treatments (Table 2). Drip loss of fresh and thawed fish is strongly species dependent with less than 2% drip loss for MAP salmon (20) and above 10-15% during chilled storage of thawed MAP cod (19). Drip loss is crucial because every loss in weight cause economical loss and affect consumer preference negatively. These losses were not significant (P >0.05) between consecutive days; however, they were significant when the initial (*ca* 5%) and final sampling dates (*ca* 12%) were compared but the inclusion of drip pads helps to reduce the problem. Glycerides, glycolipids and phospholipids are hydrolyzed by lipases to free fatty acids, which then undergo further oxidation to produce low molecular compounds, such as aldehydes and ketones. These compounds are responsible for off-flavour and off-odour and taste of fish. The initial FFA content in the chilled MAP halibut sample was 3.3±0.25%. At the end of storage average FFA value of samples was 5.94±0.74 % with no significant difference (P>0.05) between the four treatments during chill storage (Table 2). The release of FFA significantly increased from the initial value to the final value (P<0.05) during the storage period. Since the release of FFA content increased with time as found in this study (Table 2),

Table 2 Physico-chemical changes of chilled MAP halibut fillets during chilled storage at 2 °C

	Storage Period (days)						
	0	3	7	10	14	18	23
Dry matter, %							
A	28.72 ± 1.23	-§	24.60 ± 4.46	-	-	-	28.82 ± 3.02
B	28.72 ± 1.23	-	28.10 ± 2.32	-	-	-	29.87 ± 2.17
C	28.72 ± 1.23	-	29.65 ± 1.02	-	-	-	27.31 ± 2.68
D	28.72 ± 1.23	-	27.35 ± 1.37	-	-	-	28.83 ± 1.56
Water activity							
A	0.996 ± 0.002	-§	-	-	-	-	0.995 ± 0.002
B	0.996 ± 0.002	-	-	-	-	-	0.994 ± 0.004
C	0.996 ± 0.002	-	-	-	-	-	0.994 ± 0.002
D	0.996 ± 0.002	-	-	-	-	-	0.996 ± 0.002
Salt, *%*							
A	0.28 ± 0.02	-§	-	-	-	-	0.24 ± 0.06
B	0.28 ± 0.02	-	-	-	-	-	0.26 ± 0.04
C	0.28 ± 0.02	-	-	-	-	-	0.34 ± 0.01
D	0.28 ± 0.02	-	-	-	-	-	0.33 ± 0.04
Lipid, *%*							
A	16.88 ± 3.95^{A}	-§	14.23 ± 8.61^{A}	-	-	-	13.24 ± 0.52^{A}
B	16.88 ± 3.95^{A}	-	10.14 ± 2.42^{A}	-	-	-	12.64 ± 0.73^{A}
C	16.88 ± 3.95^{A}	-	13.74 ± 3.17^{A}	-	-	-	11.39 ± 2.06^{A}
D	16.88 ± 3.95^{A}	-	9.08 ± 0.63^{B}	-	-	-	12.60 ± 0.98^{AB}
Lactic acid, ppm							
A	312.0±173.49	-§	262.7±16.26	-	-	-	450.0±148.36
B	312.0±173.49	-	306.3±364.33	-	-	-	165.4±94.03
C	312.0±173.49	-	407.0±132.02	-	-	-	534.7±129.57
D	312.0±173.49	-	197.7±29.70	-	-	-	414.0±95.45
CO_2, (%)							
A	34.03 ± 0.76^{A}	21.70 ± 1.78^{B}	$21.40 \pm 1.41^{a,B}$	20.50 ± 0.26^{B}	20.97 ± 3.27^{B}	21.93 ± 1.23^{B}	$22.63 \pm 0.80^{a,B}$
B	33.90 ± 0.75^{A}	$21,17 \pm 3.47^{B}$	$20.23 \pm 0.81^{a,B}$	20.73 ± 1.24^{B}	22.13 ± 2.93^{B}	22.20 ± 5.69^{B}	$23.73 \pm 0.90^{a,B}$
C	35.70 ± 0.90^{A}	22.33 ± 0.35^{B}	$25.20 \pm 3.44^{b,B}$	23.10 ± 1.57^{B}	24.53 ± 4.62^{B}	21.20 ± 6.39^{B}	$19.20 \pm 1.18^{b,B}$
D	35.30 ± 0.53^{A}	21.67 ± 1.03^{B}	$20.50 \pm 0.46^{a,B}$	21.53 ± 0.95^{B}	21.37 ± 0.32^{B}	21.90 ± 0.92^{B}	$22.70 \pm 1.87^{a,A}$
Drip loss, % (w/w)							
A	4.71 ± 2.28^{A}	8.72 ± 1.50^{AB}	9.95 ± 2.72^{B}	12.38 ± 1.42^{B}	9.89 ± 2.10^{B}	9.58 ± 1.75^{B}	11.85 ± 2.56^{B}
B	3.39 ± 0.29^{A}	9.80 ± 3.17^{B}	10.52 ± 0.90^{B}	10.01 ± 1.84^{B}	10.57 ± 1.39^{B}	12.91 ± 2.71^{B}	11.90 ± 1.74^{B}
C	5.23 ± 1.14^{A}	7.98 ± 2.90^{AB}	10.26 ± 2.83^{AB}	11.85 ± 4.17^{AB}	14.05 ± 2.89^{B}	11.86 ± 2.9^{AB}	11.49 ± 1.41^{AB}
D	5.00 ± 1.63^{A}	7.75 ± 2.32^{B}	8.94 ± 1.36^{AB}	11.85 ± 4.17^{B}	12.30 ± 0.56^{B}	11.81 ± 0.91^{B}	11.52 ± 3.93^{B}
Trimethylamine-N-oxide (TMAO), mg-N 100 g^{-1}							
A	73.15 ± 8.45	-§	-	-	-	-	68.23 ± 16.18
B	73.15 ± 8.45	-	-	-	-	-	63.39 ± 14.35
C	73.15 ± 8.45	-	-	-	-	-	59.76 ± 7.66
D	73.15 ± 8.45	-	-	-	-	-	52.98 ± 7.12
Trimethylamine (TMA), mg-N 100 g^{-1}							
A	-3.37 ± 0.90	-§	-	-	-	-	0.15 ± 0.46
B	-3.37 ± 0.90	-	-	-	-	-	0.33 ± 1.34
C	-3.37 ± 0.90	-	-	-	-	-	0.37 ± 2.03
D	-3.37 ± 0.90	-	-	-	-	-	0.21 ± 0.33
Free Fatty Acid, *%*							
A	3.33 ± 0.25^{A}	-$^{\delta}$	5.03 ± 2.50^{B}	-	-	-	6.36 ± 1.59^{B}
B	3.33 ± 0.25^{A}	-	6.66 ± 0.65^{B}	-	-	-	4.89 ± 0.96^{AB}
C	3.33 ± 0.25^{A}	-	4.61 ± 1.82^{B}	-	-	-	5.95 ± 0.84^{B}
D	3.33 ± 0.25^{A}	-	6.79 ± 0.41^{B}	-	-	-	6.54 ± 1.75^{B}

A-B : Different letters within each treatment denote significant difference at $p<0.05$.
§ : Not measured

it is reported that there is a relationship between FFA release and loss of freshness (22). The average pH value was 6.9±0.1 with a small reduction in pH recorded throughout the 23 days of storage (data not shown). Other chemical changes included the formation of lactic, acetic and citric acid. No significant difference ($P>0.05$) between all four treatments in the concentration of lactic acid was found during storage (Table 2). Acetic acid formation was initially below to detection limit and at the end of storage the

values reached 128 ppm but citric acid formation was below to detection limit during storage period (data not shown). The most common instrumental indices for assessing fish spoilage are TVN and TMA (23). 5-10 mg TMA 100 g^{-1} sample has been reported as the limit for sensory acceptability of fish (24). Although TMA values for the four treatments significantly increased ($P<0.05$) during the storage these value in all treatments were below limit level at the end of storage. Levels of 25 to 35 mg TVN 100 g^{-1} fish are regarded by the European Union as limits for sensory acceptability of fish. Initial concentration of TVN was 7.1±1.36 mg-N TVN 100 g^{-1}. No significant effect ($P>0.05$) of treatments in TVN formation was observed during the storage at 2 °C. Significantly higher TVN formation was only observed for all samples on day 23 at 2 °C. During storage TVN concentrations increased to 16.9-24.1 mg-N TVN 100 g^{-1} not exceeding upper acceptability limit set by EU (Figure 1). TBARS is one of the most widely used methods for measuring oxidative rancidity in food. The effects of addition of pomegranate and/or rosemary extracts on the oxidative stability of chilled MAP halibut fillets were shown in Figure 2. The TBA value significantly increased ($P<0.05$) in control samples throughout storage, however in treated samples the values increased insignificantly ($P>0.05$). Moreover, no difference was observed ($P>0.05$) between pomegranate and/or rosemary treated samples during the storage period and values in treated samples remained below or close to limit level (0.2 mg MDA kg^{-1} sample).

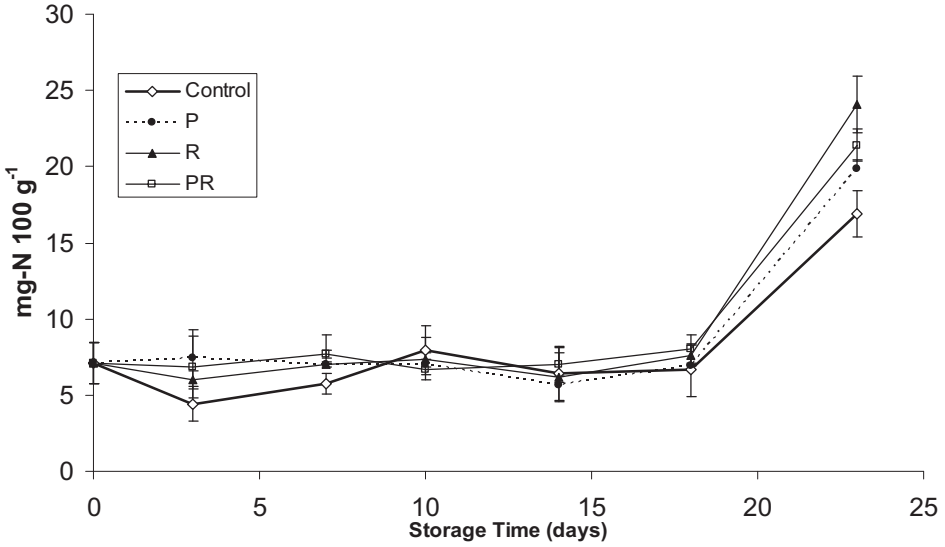

Figure 1. Changes in concentration of TVN of MAP halibut during chilled storage at 2 °C

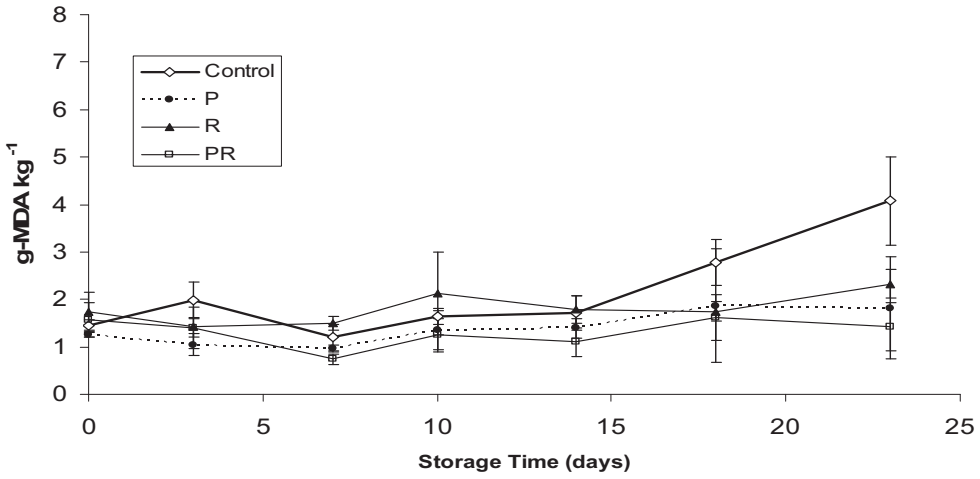

Figure 2. Changes in TBA values of MAP halibut during chilled storage at 2 °C

Sensory changes and shelf-life

The sensory spoilage characteristics of chilled MAP halibut were similar during storage and results for all treatments are shown Table 3. Dry texture and sour taste was the main sensory spoilage characteristics determined at all treatments. Particularly, the yellow colour of pomegranate extract-treated halibut samples (group P and PR) differed from other samples. In fact, at the first sensory analyses halibut fillets in group P and PR became light yellow, differed markedly from reference sample and samples in group Control and R. Difference between smell of halibut samples treated with or without extracts was not differentiated by panelists during the storage. For all treatments the sensory shelf life of chilled MAP Greenland halibut fillets was observed as longer than 23 days at 2 °C.

Table 3 Sensorial changes of chilled MAP halibut fillets during chilled storage at 2 °C

	Type of Treatments				
	Reference	A	B	C	D
Shelf-life (d)	>23	>23	>23	>23	>23
Sensory spoilage characteristics	White surface colour; good flavour; soft texture	White surface colour; sour flavour; chewy and dry texture	Yellow surface colour; sour flavour; dry texture	White surface colour; sour flavour; dry texture	Yellow surface and edges colour; sour flavour; chewy and dry texture

Conclusion

Sensory analysis correlated well with TMA and TVB-N analysis, indicating a shelf life of longer than 23 days for all samples. Despite no significant antimicrobial pomegranate and rosemary extracts, results provides first insight into their antioxidant effect on chilled halibut fillets in MAP and opens new frames for further investigations. From a microbiological point of view, the combined use of the tested natural preservatives and a packaging system with a 40% CO2, had no beneficial effect on microbial spoilage of the studied halibut fillets.

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PREFACE

It is our pleasure to introduce you **The International Food Congress** entitled **"Novel Approaches in Food Industry"** which will be held in Çeşme, Izmir, TURKEY. The congress will take place on 26-29 May, 2011 and include a variety of hot topics such as novel food products and technologies, thermal and non-thermal food processing technologies, applications of nanotechnology in food processing, innovations in food science and technology. This congress will highlight the most important areas of recent Research & Development in Food Science and Technology as well as explore relevant and interesting topics for the future. The congress will also provide accurate and updated scientific information and trends for the discipline of food science and technology. 400 leading scientists from all over 40 countries will contribute to the congress as oral or poster presentations.

This congress will provide a forum for the exchange of ideas and authoritative views by leading scientists, as well as business leaders and investors in the food industry. **More than 32 leading food industry companies became sponsor or supporting organization to our congress**. Outstanding keynote speakers and well-known leading scientists and experts from around the world will be sharing their knowledge with us. Company executives, as well as speakers from universities, research centers and governmental institutions will discuss scientific and technical developments in detail.

We would like to thank all contributors including authors of oral and poster presentations and our sponsors for contributing to the success of this congress.

On Behalf of the Executive Committee

Prof. Dr. Sebnem TAVMAN