Effect of Modified Atmosphere Packaging and Superchilled Storage on the Microbial and Sensory Quality of Atlantic Salmon (*Salmo salar*) Fillets

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ABSTRACT: Fresh Atlantic salmon fillets packaged under modified atmosphere (MA) (CO₂:N₂ 60:40) and air was stored at superchilled (–2 °C) and chilled (+4 °C) temperatures. Changes in sensory scores, microbial growth, headspace gas composition, water loss, and pH were monitored during 24 d of storage. The superchilled MA packaged salmon maintained a good quality, with negligible microbial growth (<1000 colony-forming units [CFU]/g) for more than 24 d based on both sensory and microbial analyses (aerobic plate count, H2S-producing, and psychrotrophic bacteria). Superchilled salmon in air had a 21-d sensory shelf life, whereas MA and air-stored fillets at chilled conditions was spoiled after 10 d and 7 d, respectively.

Keywords: modified atmosphere packaging, superchilling, partial freezing, Atlantic salmon, shelf life

Introduction

ODIFIED ATMOSPHERE PACKAGING (MAP) of fishery products has been thoroughly investigated since the initial works in the 1930s (Killeffer 1930; Coyne 1932, 1933; Stansby and Griffiths 1935) and in particular for the last 2 decades (Sivertsvik and others 2002). Shelf life extensions of fish packaged under MAP has been reported from 50% to 400% (Stammen and others 1990), but often it is only the period of moderate to low quality that has been extended and not the initial period of prime quality (Fletcher and Statham 1988). Usually the effect of MAP is conditioned by the concentrations and amount of CO₂ available in the gas atmosphere, availability of O2, the quality of the raw materials, and most importantly, the storage temperature (Sivertsvik and others 2002). CO2 inhibits growth of the normal spoilage flora in air (such as Pseudomonas and Shewanella putrefaciens) and during storage; CO2-tolerant microorganisms (such as Lactobacillus spp., Photobacterium phosphoreum, Brochothrix thermosphacta) will dominate the microflora. Usually a single species is responsible for the main sensory spoilage: the specific spoilage organisms (SSO). The numbers of SSO and the concentration of their metabolites can be used as objective indices of spoilage in shelf-life determinations. With knowledge on the microorganisms responsible for spoilage, a close relation between log-numbers of SSO and remaining shelf life can be expected

(Dalgaard 2000). At high CO_2 concentrations, *P. phosphoreum* has been found to be the main spoilage bacteria in both MA-packaged chilled cod (Dalgaard and others 1993; Dalgaard 1995) and salmon (Emborg and others 2002). Application of knowledge about specific spoilage organisms for determination, prediction, and extension of shelf life is of vital importance to extend the shelf life and to obtain a high raw material quality over a longer period.

Atlantic salmon (Salmo salar) is an important product both from an economical and nutritious perspective. The maximum shelf life for iced whole salmon is about at 20 d (Sveinsdottir and others 2002), and for salmon steaks/fillets in MAP at chilled temperatures (2 °C to 4 °C), shelf lives of 14 to 21 d have been observed (Pastoriza and others 1996; Randell and others 1999; de la Hoz and others 2000; Emborg and others 2002). This shelf life is sufficient to distribute both whole salmon and MAP salmon within markets closed to the salmon-farming industry, but for markets farther away from the main salmon producers in Norway, Scotland, and Chile, such as North America, Asia, and Oceania, the relative short shelf life either necessitates expensive air freight, frozen distribution, or inclusion of more preservative factors.

One of the few methods with the potential to extend the period of prime quality in fish is "superchilling" or "partial freezing" (Haard 1992). In this technique, originally introduced onboard trawlers in the 1960s (Pearson 1980), the temperature of the fish is reduced to 1 °C to 2 °C below the initial freezing point, and some ice is formed inside the product (Haard 1992; Sikorski and Sun 1994). How much of the water is frozen is highly temperature-dependent (Huss 1995). Freezing points for fishery products vary from about -1 °C to -2.5 °C, such as in salmon, shrimp, and mackerel at about -2.2 °C to carp at about -1.0 °C (Rahman and Driscoll 1994) and are usually dependent on the water content in the products (Chang and Tao 1981). Superchilling can either be used prior to traditional chilled distribution to store refrigerating capacity into the product (Magnussen and others 1998) or the superchilling temperature is maintained throughout the storage and distribution. Storage at superchilled conditions may enhance phospholipid hydrolysis and protein denaturation (Ashie and others 1996), but superchilling will inhibit most autolytic and microbial reactions, and thereby increase shelf life (Huss 1995; Chang and others 1998).

Few articles on the combined effect of superchilling under modified atmospheres have been published and, to our knowledge, none have been published on salmon fillets. Bulk-packaged, superchilled, whole, gutted salmon combined with a high-CO₂ atmosphere maintained a high microbiological and sensory quality for more than 3 wk (Sivertsvik and others 1999). A shelf life of

21 d was observed for mackerel at -2 °C in 100% CO₂ (Hong and others 1996), and for smoked blue cod, a doubling of shelf life was observed when lowering the storage temperature from 3 °C to -1.5 °C (Penney and others 1994).

The objective of this article was to compare the combined effect of MAP and superchilled conditions on the storage quality of salmon fillets.

Materials and Methods

Raw material, packaging, and storage

Three-d-old iced-stored, whole, gutted Atlantic salmon (Salmo salar) of size 3 to 4 kg was obtained from a commercial fish farm (Marine Harvest AS, Hielmeland, Norway). The salmon were filleted and cut into smaller fillets/steaks (131 ± 3 g each) without skin or bone. The filleting, deskinning, and cutting were carried out at the production area at the NORCONSERV Inst. of Fish Processing and Preservation Technology under high hygienic conditions to obtain raw material with low initial bacterial counts. The equal-sized fillets where individually packaged under modified atmosphere (MA) or in traditional overwrap packaging with exposure to air. The MA packages were high-density polyethylene semirigid trays (Dynopack, Polimoon, Kristiansand, Norway). The air was evacuated, and the gas mixture (60% CO₂ and 40% N₂) introduced into the package before heat sealing (lidding film: 15 my PE/75 my PA, Dynoseal ST 1575, Polimoon) on a semiautomatic packaging machine (Dyno VGA 462, Polimoon). Food grade CO₂ and N2 (AGA, Oslo, Norway) was mixed in a gas mixer (Witt KM 100-2m, Witt Gasetechnick, Witten, Germany) to obtain the gas mixture, and the gas volume-to-product (g/ p) ratio was approximately 1 (100 mL gas/ 100 g salmon).

The overwrap packages were expanded polystyrene trays with built in exudate absorber between layers in the bottom of tray (LinPac, Marietta, Ga., U.S.A.) and wrapped with cling film with low barrier properties (PVC-film, ITS Foil and Film Rewinding b.v., Apeldoorn, The Netherlands). Salmon packages of both types were stored in chill cabinets (Porkka CM710, Huurre Group, Hollola, Finland) set to -2 °C and +4 °C, respectively, and evaluated regularly from 0 (day of packaging) and up to 24 d according to the experimental design (Table 1).

Microbiological examinations

Samples of 25 g were as eptically taken from the salmon fillets and homogenized in 250 mL of 0.9% NaCl (w/v) and 0.1% pep-

Table 1-Experimental design and response variables				
Design variables	Levels			
Temperature (A): Packaging method (B): Storage time (C): Nr of experiments:	-2 or 4 °C Air exposed (overwrap) or anoxic CO_2 -exposed (MAP) 7, 10, 14, 17, 21, or 24 d 24 (full factorial design)			
Response variables	Analyses			
Microbiological:	Aerobic plate count (APC), H ₂ S-producing bacteria, psychrotrophic bacteria			
Sensory evaluation: Chemical/other:	Raw and cooked odor, cooked flavor, firmness, and juiciness Temperature, pH, water/drip loss, head-space gas composition			

tone (w/v) for 120 s in a Stomacher 400 Laboratory Blender (Seward Medical, London, U.K.). Total viable counts, measured as aerobic plate counts (APC) and H₂S-producing bacteria were measured after a suitable dilution had been added to melted and temperate (44 °C) iron agar (Agar Lyngby, IA, Oxoid CM 867, Basingstoke, Hampshire, U.K.) supplemented with L-cysteine and stored at 20 ± 1 °C for 3 d. Black colonies were counted as H₂S-producing bacteria; APC were counted as the total of black and white colonies. The content of psychrotrophic bacteria was determined by a spread plate count method on solidified chilled plate count agar (PCA, Merck, Darnstadt, Germany) added 1% NaCl, and incubated at 8 °C for 5 to 7 d. Averaged results of triplicate measurements are presented as log colony-forming units (CFU) per gram salmon.

Sensory evaluation

Sensory evaluation was carried out on both raw- and cooked-salmon samples using a panel of 4 assessors trained according to the ISO-standard (ISO 1993). All sensory evaluations were carried out in randomized order of coded samples. The odor of salmon at package opening was evaluated in triplicate with a quality scale from 10 to 1 (10: sea fresh, typical of species; 1: faecal, indole and skatole, adopted from Shewan and others 1953). Test pieces (15- to 20-mm-wide cut of the stored samples) for cooked salmon evaluation were packaged in cook-plastic pouches (PA/PE 20/50) under slight vacuum (95%) and cooked in water vapor (80 °C in 10 min) without any salt or spice addition. The samples were evaluated in duplicate using a descriptive test (adopted from Shewan and others 1953) with a scale from 10 (fresh sea weedy odor, fresh sweet flavor and firm, succulent texture) to1 (putrid odor and flavor; sloppy, dry texture) for odor, flavor, and texture (firmness and juiciness). For both raw and cooked salmon, a score of 5 was chosen as the minimum acceptance level.

Temperature measurements

Sample's core temperatures were measured every 5 min during storage using electronic temperature loggers (Ebro Electronic, Ingolstadt, Germany) at both temperatures and in both packaging methods.

Gas analyses

The gas composition in the packages was determined in triplicate by injecting an aliquot (30 mL) of the headspace gas of the trays using an oxygen and carbon dioxide analyzer (M.A.P. Test 4000, Hitech Instruments, Luton; U.K.). The gas was collected with a syringe after intrusion of the top foil. The analyzer was calibrated against a certified gas mixture ($O_2:CO_2:N_2$ 1.1:44.1:54.8) and air before each sampling.

pH measurements were made in triplicate with a pH meter (Beckman 72, Dan Meszansky, Oslo, Norway) on 25 g of homogenate of salmon muscle with 25 mL of 0.1 *M* KCl in distilled water.

Formation of drip

The drip formed in the samples during storage was measured gravimetric. The mass of the drip (g) was divided by the initial mass of product (g) and reported as a percentage (%).

Statistical analysis

Partial least squares (PLS) regression with full cross-validation (leave-one-out) to find correlation (R^2) , random mean square error of prediction (RMSEP) and regression coefficients, and analyses of effects (multiple linear regression, MLR) to find the significant effects (Tukey) of the design variables on the responses were performed using Unscrambler/Guideline+7.6 (Camo, Oslo, Norway). Univariate analysis of variance were performed with Minitab 13.3 (Minitab, Coventry, U.K.) using Tukey's HSD test at level P < 0.05 (95%) to obtain confidence intervals for all pairwise differences between level means of temperature and packaging type on each sampling time (d of storage).

Results and Discussion

THE GAS COMPOSITION IN THE MA \blacksquare packages was measured to 61.1% ± 0.3% CO_2 , 0.2% ± 0.1% O_2 immediately after packaging, and the remaining gas was N₂. After packaging, CO2 was dissolved into the product. Seven d after packaging, the CO₂ level in the headspace of the chilled MA packages was reduced to $34.0\% \pm 0.8\%$ and in the superchilled to 31.1% ± 0.5%, giving higher concentration of dissolved CO2 in the superchilled salmon compared with the chilled. Solubility of CO₂ in water increases with decreasing temperature (Carroll and others 1991); however, CO2 will not dissolve into ice (Monnin and others 2001). The fact that superchilled MA salmon dissolved more CO_2 (*P* < 0.01) compared with the chilled salmon indicates little ice formation in the superchilled product. The CO₂ level decreased linearly from day 7 (31.1%) to day



Figure 1-Growth of (a) aerobic plate count (APC), (b) H_2 S-producing bacteria, and (c) psychrotrophic bacteria in salmon fillet during storage. Legend \bigcirc : chilled, overwrap (air); \bigcirc : chilled, MAP; \bigcirc : superchilled, overwrap (air); and \blacksquare : superchilled, MAP.

Table 2–Regression coefficients (from PLS-2 model with 4 components) for the microbial analysis, water loss, and sensory scores against design variables and interactions. Significant effects (analysis of effect, MLR) of the design variables and interaction on the sensory score is marked with *** (P < 0.005), ** (P < 0.01), and * (P < 0.05).

Effect	Aerobic plate count	H₂S- producing bacteria	Psychro- trophic bacteria	Drip loss	
Temperature (A)	0.770***	0.618***	0.810***	0.517**	
MAP (B)	-0.526***	-0.573***	-0.442***	0.171	
Storage time (C)	0.268*	0.332*	0.297*	0.498	
A*B	0.087	-0.249**	0.199**	-0.406*	
A*C	-0.043	0.099	-0.032	-0.159	
B*C	-0.180	-0.245	-0.098	0.240	
RMSEP	0.683	0.517	0.679	0.937	
Correlation (R ²)	0.923 Odor, raw	0.893 Odor, cooked	0.937 Flavor	0.490 Firmness	Juiciness
Temperature (A)	-0.688***	-0.678***	-0.682***	-0.572***	-0.599***
MAP (B)	0.448***	0.293**	0.304*	0.400**	0.390**
Storage time (C)	-0.458*	-0.543**	-0.524*	-0.495*	-0.502*
A*B	0.095	0.139	0.012	0.210	0.202*
A*C	-0.260	-0.285*	-0.338	-0.375	-0.347*
B*C	0.092	-0.027	-0.035	0.071	0.061
RMSEP	0.430	0.784	0.717	0.572	0.478
Correlation (<i>R</i> ²)	0.935	0.864	0.873	0.826	0.856

24 (23.8%) of superchilled storage and even faster for the chilled-MA packages to 15% after 14 d, probably caused by permeation of CO₂ out of the HDPE trays. According to Henry's law, some of the dissolved CO₂ would be released from the product when the partial pressure of CO₂ in the packages atmosphere decreases due to the permeation of CO₂. Using a packaging material with better barrier properties would probably enhance the effect of MAP. since this would maintain a higher partial pressure of CO_2 inside the package, and the inhibiting effect on many spoilage microorganisms is dependent on the amount of dissolved CO₂ in the product (Devlieghere and Debevere 2000). Use of a larger g/p ratio would also increase the effect of MAP, since this would increase the dissolvement of CO₂. The O₂ in the MA packages never exceeded 1% during storage, and the O₂ permeating through the packaging materials would probably be consumed by aerobic bacteria inside the packages.

Atmosphere composition inside the overwrap packages was close to air composition throughout the storage time, 20.9% O_2 and 0.0% CO_2 during 21 d of superchilled air storage and during 10 d of chilled air storage, confirming good exposure of the salmon to air through the PVC film. For the chilled air salmon, a decrease in O_2 to about 17% and an increase in CO_2 level to about 2% of the packages atmospheres were observed, coinciding with high microbial activity (described subsequently).

The average temperature in the samples

stored at chilled conditions was 4.4 ± 0.2 °C (4.0/5.1 °C min/max), and for the superchilled samples the mean temperature was -1.9 ± 0.1 °C (-2.0/-1.9 °C min/max) during 24 d of storage. No differences in temperatures between the packaging methods were observed.

Microbial analysis of the raw material after filleting and cutting showed that the salmon fillets were produced at very hygienic conditions and had a very high microbial quality (APC < 25 CFU/g salmon). APC, H₂S-producing bacteria, and psychrotrophic bacteria counts increased during storage (Figure 1a through 1c) for both temperatures and packaging types, but only negligible growth was observed for MA packaged salmon stored at superchilled temperature (CFU/g < 1000). Growth was always higher in salmon exposed to air compared with salmon in MA at same temperature and storage time.

The microbial counts had very good correlations (PLS-regression) with the design variable (R^2 [APC] = 0.92, R^2 [H₂S] = 0.89, and R^2 [psychrotrophic] = 0.94) and a prediction within the boundaries of the design levels, with less than 0.7 log CFU/g accuracy can be performed (RMSEP: 0.7, 0.5, and 0.7 for APC, H₂S-producing, and psychrotrophic counts, respectively).

PLS-regression coefficients and analysis of effects (Table 2) showed the highly significant effect of the storage temperature and packaging method (P < 0.005) on microbial growth for all 3 analyses. Not surprisingly, a significant effect of storage time Food Microbiology and Safety

(P < 0.05) also was observed; however, this effect is relatively smaller than the 2 other experimental variables. The only significant interaction effect was the temperaturepackaging method (AB) effect on H₂S-producing bacteria and psychrotrophic bacteria. The combination of CO₂ and superchilling had a synergistic effect on the inhibition of psychrotrophic growth, whereas the superchilled and overwrap packaging had a synergistic effect on H₂S-producing counts. The psychrotrophic counts for MAP salmon at 4 °C was about 20% higher than APC, while for the other samples the psychrotrophic counts was very similar to APC, indicating an enhanced growth of a psychrotrophic bacteria, in the chilled MAP salmon. The specific spoilage organism (SSO) of MAP salmon has recently been found to be P. phosphoreum (Emborg and others 2002), the same SSO as previously found for MAP cod (Dalgaard and others 1993; Dalgaard 1995). P. phosphoreum has been shown to grow on PCA-added salt in previous trials at our institute, but the colonies from this experiment were not characterized. Assuming the psychrotrophic count includes P. phosphoreum, then the low numbers indicated a very sparse growth under MA at -2 °C during 24 d of storage. Characterization of specific strains at superchilled temperatures should be included in further studies. The superchilled salmon exposed to air had relatively lower counts of H₂S-producing bacteria when compared with APC than chilled salmon exposed to air. S. putrefaciens may not contribute to all black colonies on iron agar, although this bacteria has often been shown to be the main H₂S producer on fresh fish stored in ice (Gram and others 1987; Dalgaard 1995); but if so, these results indicate an inhibitive effect on S. putrefaciens at superchilled conditions.

Water loss or drip formation from the samples was significantly increased by temperature (P < 0.01) and temperature–MAP interaction (P < 0.05) (Table 2). The drip was highest in the chilled overwrap samples (4% to 5%), and lowest in the superchilled overwrap (about 2%). The 2 MA packages had almost the same loss of water (about 3%), lower than in the chilled air but higher than in superchilled air storage. It has been suggested by several authors that dissolved CO₂ in the products could decrease the water-holding capacity (Sivertsvik and others 2002). However, for salmon this effect is not pronounced (Randell and others 1999): typical drip loss at about 2% under 60% CO2 atmosphere at 2 °C compared with about 1% for vacuum packaging and about 0.5% in air storage. Our results suggest that the storage temperature is more important, and super-

Table 3—Sensory evaluation of salmon fillets packaged in MA or air and store	d
at superchilled or chilled conditions. Mean results of 2 (3 for raw odor) repli	i -
cates times 4 judges.	

	Packaging/		Days of storage*					
Parameter	storage	0	7	10	14	17	21	24
Odor, raw	Raw material	8.8						
,	Air, superchilled		7.5ab	6.8b	6.8a	6.4b	6.2b	6.1b
	Air, chilled		6.6b	5.7b	3.9c	ne	ne	ne
	MA, superchilled		8.4a	8.2a	7.7a	7.6a	7.4a	7.8a
	MA, chilled		7.0b	6.3b	5.8b	5.8b	4.6c	ne
Odor, cooked	Raw material	9.0						
	Air, superchilled		7.5	6.9a	5.7ab	6.6a	6.4a	5.0b
	Air, chilled		5.9	3.8b	2.6c	ne	ne	ne
	MA, superchilled		8.0	7.4a	7.0a	7.3a	6.3a	7.0a
	MA, chilled		7.6	6.8a	3.7bc	3.3b	3.1b	Ne
Flavor, cooked	Raw material	9.0						
	Air, superchilled		7.4ab	7.0a	5.5	6.1b	6.0a	5.3b
	Air, chilled		6.3b	4.0b	3.5	ne	ne	ne
	MA, superchilled		8.3a	7.8a	7.7	7.9a	7.0a	7.3a
	MA, chilled		8.0a	6.8a	5.3	3.3c	2.4b	ne
Firmness	Raw material	9.0						
	Air, superchilled		7.3	7.0ab	7.2	7.3ab	6.8	7.1
	Air, chilled		7.6	5.2b	5.0	ne	ne	ne
	MA, superchilled		8.0	7.3a	7.8	8.0a	6.9	7.9
	MA, chilled		8.1	7.1a	6.5	5.8b	5.5	ne
Juiciness	Raw material	9.0						
	Air, superchilled		6.8	7.1	7.3a	7.0ab	6.5	6.3
	Air, chilled		6.7	5.8	5.0b	ne	ne	ne
	MA, superchilled		7.8	7.5	7.2ab	7.8a	6.8	6.4
	MA, chilled		7.6	7.3	5.5ab	6.1b	5.4	ne

*Equal or no lowercase letter within column and parameter = no significant difference (P > 0.05). ne = not evaluated due to spoilage.

chilled storage at -2 °C does not lead to excessive drip caused by cell destruction due to freezing. However, the results from the drip measurements had large variance, and PLS regression against design variables showed low correlation ($R^2 = 0.49$) and a high RMSEP (0.93). No differences among design variables in salmon pH were observed; the pH was 6.2 to 6.3 for all measurements, except for a pH increase to 6.7 after spoilage in the overwrapped salmon stored at 4 °C for 21 d.

The sensory scores also showed good correlation with the design variables ($R^2 = 0.93$ [raw odor], 0.86 [cooked odor], 0.87 [flavor], 0.82 [firmness], and 0.86 [juiciness]). The PLS model is able to predict sensory score within half-a-score accuracy for raw odor (RMSEP = 0.4), and a little lower accuracy for cooked odor and flavor, RMSEP of 0.8 and 0.7, respectively. Sensory evaluation results (Table 2 and 3) confirmed the effects observed for the microbial counts. The temperature effect was highly significant (P < 0.005) for all sensory scores, and the use of MAP significantly increased sensory scores (P < 0.005 for raw odor, P < 0.05 for cooked flavor, and P < 0.01 for the rest). Sensory scores decreased as a function of storage time; however; the effect of using short storage time was less important than using superchilling and MAP on the sensory quality of salmon. Significant interaction effects were observed for the juiciness of salmon in the temperature * MAP combination and for juiciness and cooked odor in the temperature * storage combination, showing synergistic effects of these interactions.

Significant differences observed in the individual sensory parameters within each sampling days are shown in Table 3. The salmon exposed to air and stored at 4 °C had a shelf life of 7 d based on the sensory scores, and odor (cooked and raw) in particular. MA-stored salmon at the chilled temperature had a shelf life of 10 d, a 3-d increase as compared with overwrap package. The superchilled salmon had a considerable longer shelf life; both air and MA exposed was acceptable after 21 d of storage, but the MA superchilled salmon got higher scores throughout the storage period and at all sampling days. The superchilled MA salmon was evaluated of good quality after 24 d of storage, whereas the superchilled overwrap was not acceptable based on cooked odor scores.

These shelf lives for the salmon exposed to air is in good agreement with shelf lives estimated using the square root model (Storey 1985) and relative rate of spoilage compared with spoilage at 0 °C: Relative rate of spoilage = (0.1 * storage temperature [°C] + 1)²

Using 14 d of shelf life for salmon at 0 °C, this model gives a 6.8-d shelf life of airstored salmon at 4.4 °C, and a 21.3-d shelf life at -1.9 °C, which is the same as our results. Since the superchilled MA salmon was not stored for longer than 24 d, the end of shelf life was not determined; but taking into account the low microbial levels and high sensory scores, at least on some days longer shelf life should be possible, but at a more mediocre quality. Again, using the square-root model and shelf life of MA salmon of 10 d at 4.4 °C, as observed in this study, the shelf life at 0 °C would be about 21 d, and 32 d at -1.9 °C. For MA-packaged salmon, shelf lives up to 34 d (frozenthawed product at 2 °C) (Emborg and others 2002) and even 55 d to 62 d have been reported (Clostridium botulinum challenge test at 4 °C) (Reddy and others 1997). For the air-exposed samples, an APC of log 6 CFU/g corresponds well with the sensory spoilage time in this study (Figure 1a). Since spoilage is affected both by the numbers of specific spoilage organisms and their activity, the APC numbers cannot be directly related to time of spoilage without knowing the species involved. When the concentration of oxygen decreases and that of CO2 increases during vacuum and MAP storage, this gradually selects toward CO2-tolerant bacteria (Borch and others 1996). Dalgaard and others (1993) reported P. phosphoreum as the major spoilage bacterium of vacuumpackaged and gas-packaged (in mixtures of CO2 and N2) cod fillets stored at 0 °C. Fish stored under anaerobic conditions or in CO₂-containing atmosphere often contain lower number of psychrotrophic bacteria, such as S. putrefaciens and Pseudomonas, than aerobically stored fish. At these conditions, the numbers of psychrotrophic bacteria such as P. phosphoreum reach a level of 107 to 108 CFU/g at spoilage (Dalgaard and others 1993). In contrast to Dalgaard (1995), we did not observe high numbers of psychrotrophic bacteria in superchilled MApackaged salmon. The increased shelf life of superchilled salmon seems to be a suppression of SSOs in the product. Storage experiments of more than 32 d should therefore be carried out to confirm the estimates from the square root model.

The growth of psychrotrophic non-proteolytic *Clostridium botulinum* Type E has been a major concern for fish products packaged in oxygen-free environments, mainly because of their ability to grow and produce toxin under anaerobic conditions at temperature as low as 3.0 °C (Graham and others 1997). At superchilled conditions, the temperature in the products is several degrees lower than the minimum growth limit of C. botulinum Type E, excluding and health risks. Storage at chilled conditions (4 °C) makes growth possible, but time to toxin formation in inoculated MA-packaged salmon at this temperature is at least 50 d (Stier and others 1981; Garcia and Genigeorgis 1987; Reddy and others 1997). Because of the 10-d shelf life observed for chilled MA salmon in this study, there should be no risk for toxin formation prior to spoilage at this temperature. However, toxin formation has been found to precede spoilage in some cases at abuse temperatures (Sivertsvik and others 2002), so strict temperature control is vital both to ensure safety and shelf life. It is also imperative to keep the superchilled temperature as stable as possible since only 1 to 2 degrees decrease could lead to 30% to 50% freezing of the water in the product (Huss 1995) and lead to severe tissue damage and excessive drip.

Conclusions

OTH MODIFIED ATMOSPHERE AND SUPER-Dchilled conditions extended the shelf life of salmon fillets, and when combined, a synergistic effect was observed giving an additional effect, maybe caused by increased dissolvement of CO2 at the superchilled temperature. The combination of MA and superchilling gave a product of high quality in 24 d of storage with almost total bacterial growth inhibition. The shelf-life extension was at least 2.5 times that of traditional chilled MA salmon and at least 3.5 times that of chilled storage exposed to air. No negative texture differences were observed in the superchilled salmon, and the increased drip loss was insignificant.

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