



Microbiological quality of maatjes herring stored in air and under modified atmosphere at 4 and 10 °C

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Abstract

Microbiological and sensory changes of maatjes herring stored in air (experiment I) and under modified atmosphere (MAP) (experiments II and III) were evaluated during storage at 4 and 10 °C. Microbial (total and psychrotrophic viable bacteria, lactic acid bacteria and *Enterobacteriaceae*) counts and chemical analyses (chloride content, fat content, dry matter, ash and pH) were performed. A Quality Index Method (QIM) scheme developed for maatjes herring was used for sensory evaluation. The main reasons for sensory rejections at both storage temperatures were a strong rancid taste for herring stored in air (Experiment I) and a sour, bitter, rotten taste and an aftertaste like old flower water for MAP herring (Experiments II and III). A soft texture of freshly produced samples (Experiment II) was noticed. The sensory shelf-life of maatjes herring stored in air (Experiment I) was three days at both 4 and 10 °C. The MAP herring in Experiments II and III had a shelf-life of 5 and 6 days, respectively, at both storage temperatures. Rancidity due to oxidation of fat was the main spoilage indicator for air-stored maatjes herring. Autolytic enzymes may affect textural deterioration. The characteristic off-odour and off-taste in the MAP herring (Experiments II and III) were may well be attributable to microbial metabolism. On the day of sensory rejection, total viable counts for herring in all three experiments (Experiments I–III) stored at 4 °C did not reach 10⁶ cfu/g, which is considered the limit of acceptability for maatjes herring given by the Dutch fishery authorities. It appears that total viable counts have minor significance in the sensory assessment of maatjes herring.

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1. Introduction

Maatjes herring, a lightly salted ready-to-eat fish product, is very popular in the Netherlands. The term “maatjes” refers to herring caught just before its first spawning between May and July and is characterized by a distinct level of sub-cutaneous fat of 16–20%. After being caught, the herring is gibbed and lightly cured. The remaining appendices pyloricæ produce tryptic enzymes (Priebe, 1980; Luten, 1997), which stimulate a fermentation process resulting in typical maatjes product characteristics. After brining, the fish undergoes a ripening period up to 1 day before being vacuum-packaged and stored frozen until further use. For retail, the product is thawed, filleted and

sold loose or packaged under modified-atmosphere and stored at chilled temperatures.

Modified atmosphere packaging (MAP) in combination with chilled storage offers the fish industry and the consumer many advantages, such as extending the shelf-life of the products, longer transport distances of the product and reduced financial losses (Stammen et al., 1990; Farber, 1991; Rosnes et al., 1997; Bøknæs et al., 2000). MAP of fishery products including various atmospheres have been reviewed extensively and summarized in Sivertsvik et al. (2002). In the Netherlands, the consumption of MAP maatjes herring rose from 22% of total maatjes herring (unpacked and MAP) in 2001 to 27% in 2003 (Temminghof, 2004). However, to the authors' knowledge, there is no information available about the microbiological and sensory quality of maatjes herring stored under modified atmosphere.

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Maatjes herring undergoes many processing stages that can increase the risk of microbial contamination. Short shelf-life is exacerbated by fluctuating storage temperatures during thawing, transport and sale. Together with the low salt concentration of the fish product and the fact that it is eaten without further heating, these aspects highlight the need for research into microbiological development in maatjes herring. Very limited work has been carried out on investigating microbiological aspects during storage of these products. When studying packed herring (“Heringsfilet nach Matjesart”) at the retail level, Nieper and Stockemer (1995) determined 80% of total bacteria counts as LAB at the end of the shelf-life.

The aims of this study were (1) to determine the initial quality of maatjes herring produced in the Netherlands and (2) to characterize the microbiological and sensory changes of maatjes herring during storage in air and under modified atmosphere at 4 and 10 °C. The present paper can be considered as a related paper to Lyhs and Schelvis-Smit (2005) focusing on the development of a Quality Index Method (QIM) scheme for maatjes herring.

2. Material and methods

2.1. Product description

Herring (*Clupea harengus*) were caught in the North Sea in the Norwegian coastal area in May–June 2002 and 2003. The fish were stored in chilled seawater aboard the vessel. After delivery to shore, the ungutted but decapitated fish of ca. 70–80 g were cask brined by a processor in Norway resulting in a NaCl concentration of 2.0% (w/w) in the final product. After a ripening process lasting up to 1 day, the fish were vacuum-packaged (each package consisting of 17 kg fish and 3 kg brine) and in order to accelerate the freezing process frozen in a tunnel at an air temperature of –40 °C. After transport to the Netherlands, the packages were stored at –25 °C. For further processing, the fish were thawed for 24 h and machine filleted (Fieret, Barneveld, the Netherlands). For packaging the fish under modified atmosphere, individual filleted fish of approximately 75 g were placed at a time in a polypropylene tray using a Sealpac packaging machine (Ultrapak, Dronten, the Netherlands). The nominal values of the applied modified atmosphere were 30% CO₂ and 70% N₂. The producer declares a one day shelf-life for the unpacked herring. For MAP products at the beginning of the season (May–June) and after the season (July–April), the manufacturer declares a shelf-life of 3 days and 5 days, respectively, at a maximum storage temperature of 4 °C. The shorter shelf-life in the beginning of the season is due to the shorter period between freezing and processing. Longer freezing period after the season reduces the high initial enzymatic process. This inhibits the overripening of the product.

2.2. Storage characteristics and sampling

Immediately after processing, the unpacked and MAP samples were transported to the laboratory and stored at either 4 or 10 °C. Three series of storage experiments (I–III) were carried out in November 2002, February 2003, and June 2003 respectively. The fish of Experiments I and II were caught in May–June 2002 and those of Experiment III in May–June 2003. The maatjes herring in Experiment I were frozen for 6 months before further processing and then stored in air for 18 days. In Experiments II and III, the maatjes herring were frozen for 9 months and 6 days, respectively, and then stored under modified atmosphere for up to 28 days. In order to improve the accuracy of the growth curve models for different bacteria, the sampling was continued far beyond the shelf-life of the products until the bacteria counts were believed to have reached the maximum stationary levels. At appropriate intervals at each sampling time, two packages from each storage temperature were taken for microbiological and sensory analyses, respectively. It is noted that parallel analyses at the same time of the samples of all three experiments could have improved the reliability of the study; however, this was not possible due to limited financial and human resources.

2.3. Chemical analyses

Chemical analyses were carried out on three freshly produced maatjes herring fillets at the beginning of each storage Experiment (I–III), respectively. The chloride content was titrated according to Volhard’s method as described by Kolthoff and Sandell (1952). The fat content was determined gravimetrically after extraction following the Bligh and Dyer procedure (1959). Dry matter and ash were determined using method ISW-A034 (2001) and ISW-A105 (2002), respectively. The pH was determined from the first homogenate made for microbiological analysis by a WTW-537 Digital-pH-meter (Wissenschaftliche-Technische Werkstätten, Weilheim, Germany).

2.4. Headspace gas composition

Throughout the storage period, the headspace gas composition in the modified-atmosphere packages was determined at each sampling time in duplicate using an oxygen and carbon dioxide 1450 C Food Pack Gas Analyser (Servomex, Zoetermeer, the Netherlands). The gas was collected with a syringe after intrusion into the top foil. Calibrating was done with 100% carbon dioxide.

2.5. Microbiological analyses

A 10-g portion of fish was aseptically weighed into 90 ml of 0.9% NaCl (w/v) and 0.1% (w/v) peptone water in a sterile plastic bag, and then blended in a Stomacher 400

Lab Blender (Seward Medical, London, UK) for 60 s. Ten-fold serial dilutions were used for microbiological analyses.

Total viable counts (TVC) and counts of H₂S-producers (H₂S) were performed in pour plates with iron agar (IA) (Oxoid, Basingstoke, UK) according to the method of the Nordic Committee on Food Analysis (1994). Psychrotrophic viable counts (PVC) were done using the spread plate method on Long and Hammer's medium (LH) modified by Van Spreekens (1974) with an additional 1% w/v NaCl. Lactic acid bacteria count (LAB) was determined by the method of the Nordic Committee on Food Analysis (1991) using MRS agar (Tritium Mikrobiologie, Veldhoven, the Netherlands). The number of *Enterobacteriaceae* was determined by pour plating in Violet Red Bile Glucose (VRBG) agar (Tritium Mikrobiologie) according to the method of the Nordic Committee on Food Analysis (1992).

All iron agar plates were counted after 3 days' aerobic incubation at 21 °C. PVC and LAB plates were counted after 5 days' aerobic incubation at 15 °C and after 5–7 days' anaerobic incubation at 21 °C respectively. Typical *Enterobacteriaceae* colonies were counted after 2 days' aerobic incubation at 30 °C.

2.6. Sensory evaluation

For sensory evaluation of the samples, a QIM scheme developed for maatjes herring stored in air and under modified atmosphere (Lyhs and Schelvis-Smit, 2005) was used. Samples from all three storage experiments (I–III) were analysed by a sensory panel of five trained panellists. The following attributes and their changes during storage time were assessed: appearance of the skin side and bone side, colour of the blood, odour (rancidity and other), taste (rancidity and other), aftertaste and texture. In each session, the panel evaluated two samples per storage temperature and one control sample served in random order. The control sample was thawed before each session. For each sample, one fish with one fillet showing the skin side and the other the bone side was placed on a plate on ice 10 min before the evaluation and kept at constant temperature. All assessments were conducted individually under standardised conditions with as few interruptions as possible. The samples were coded with a randomized three-digit code unrelated to storage temperature and assessed under daylight conditions using lamps of >5000 K. A sample was deemed spoiled if at least three judges considered it unfit for human consumption.

2.7. Statistical data and growth curve analyses

TVC, PVC, counts of LAB and of *Enterobacteriaceae* at both storage temperatures were analysed as a function of storage time. The four parameter Gompertz function model and additional quantities as growth rate (log₁₀ count/day), lag-time (days) and time needed to reach a specific bacteria count level were analysed as described in

Lyhs et al. (2001a). A least-squares method was used to estimate the parameter values for the model considered. To reduce random error, mean values of the microbiological and sensory data obtained were used for samples having the same storage time and temperature.

3. Results

3.1. Chemical analyses

Table 1 shows the data of the chemical analyses and the determined pH values of the freshly produced maatjes herring at the beginning of each storage experiment (I–III). In Experiment I, final pH values of 6.50–6.69 were measured in samples stored at 4 °C and pH values of 6.90–7.04 in samples stored at 10 °C. In Experiments II and III, the pH values were relatively stable during the storage at both storage temperatures (data not shown).

3.2. Headspace gas composition

Initial CO₂ concentrations in Experiments II and III of 20.1–22.1% and 15.5–17.6%, respectively, were measured. Initially headspace O₂ concentrations were 0.6–1.7% and 1.2–5.1% in Experiments II and III, respectively. The CO₂ concentrations in both experiments (II and III) increased and the O₂ concentrations decreased throughout storage. In Experiment II, oxygen values up to 6.3% were measured in individual cases throughout storage time for samples stored at 4 °C. In Experiment III, oxygen values up to 3.0% were measured for samples at both storage temperatures.

3.3. Microbiological changes

Initial counts were about 10⁴ colony forming unit (cfu)/g in freshly produced unpackaged herring (Experiment I) and 10³–10⁵ cfu/g in freshly produced MAP samples (Experiments II and III) for TVC and PVC, respectively (Table 2). Growth of TVC, PVC, LAB and *Enterobacteriaceae* is presented as a function of storage time at 4 °C in Figs. 1a–d, respectively, and at 10 °C in Figs. 2a–d, respectively. The figures show that the bacterial counts

Table 1
Chemical analyses of freshly produced maatjes herring fillets (% on a wet weight basis)

Experiment	Ash (%)	Fat (%)	Dry matter (%)	Salt (% w/w)	pH
I	2.4	15.8	66.6	1.6	6.34
I	2.9	14.1	67.4	1.9	6.36
I	1.7	20.8	63.6	0.9	6.47
II	2.2	16.1	65.5	1.3	6.36
II	2.6	12.8	68.0	1.7	6.38
II	2.5	13.2	68.0	1.6	6.47
III	3.2	15.0	66.8	2.3	6.45
III	2.7	11.8	68.8	1.8	6.45
III	2.7	11.4	69.7	1.7	6.49

Table 2
Microbiological quality of freshly produced maatjes herring fillets

Bacterial groups (cfu/g) ^a	Experiment I	Experiment II	Experiment III
Total viable counts	1.0×10^4 – 1.4×10^4	4.0×10^3 – 3.9×10^5	1.6×10^4 – 2.2×10^4
Psychrotrophic viable counts	1.2×10^4 – 2.3×10^4	3.8×10^3 – 1.0×10^5	1.5×10^4 – 4.4×10^4
H ₂ S-producing bacteria counts	$< 10^2$	$< 10^2$	$< 10^2$
Lactic acid bacteria counts	2.7×10^2 – 1.5×10^3	$< 10^2$ – 3.6×10^2	2.0×10^3 – 4.7×10^3
Enterobacteriaceae counts	2.0×10^2 – 7.0×10^2	$< 10^2$ – 3.6×10^2	2.0×10^2 – 9.0×10^2

^aMinimum and maximum bacterial counts.

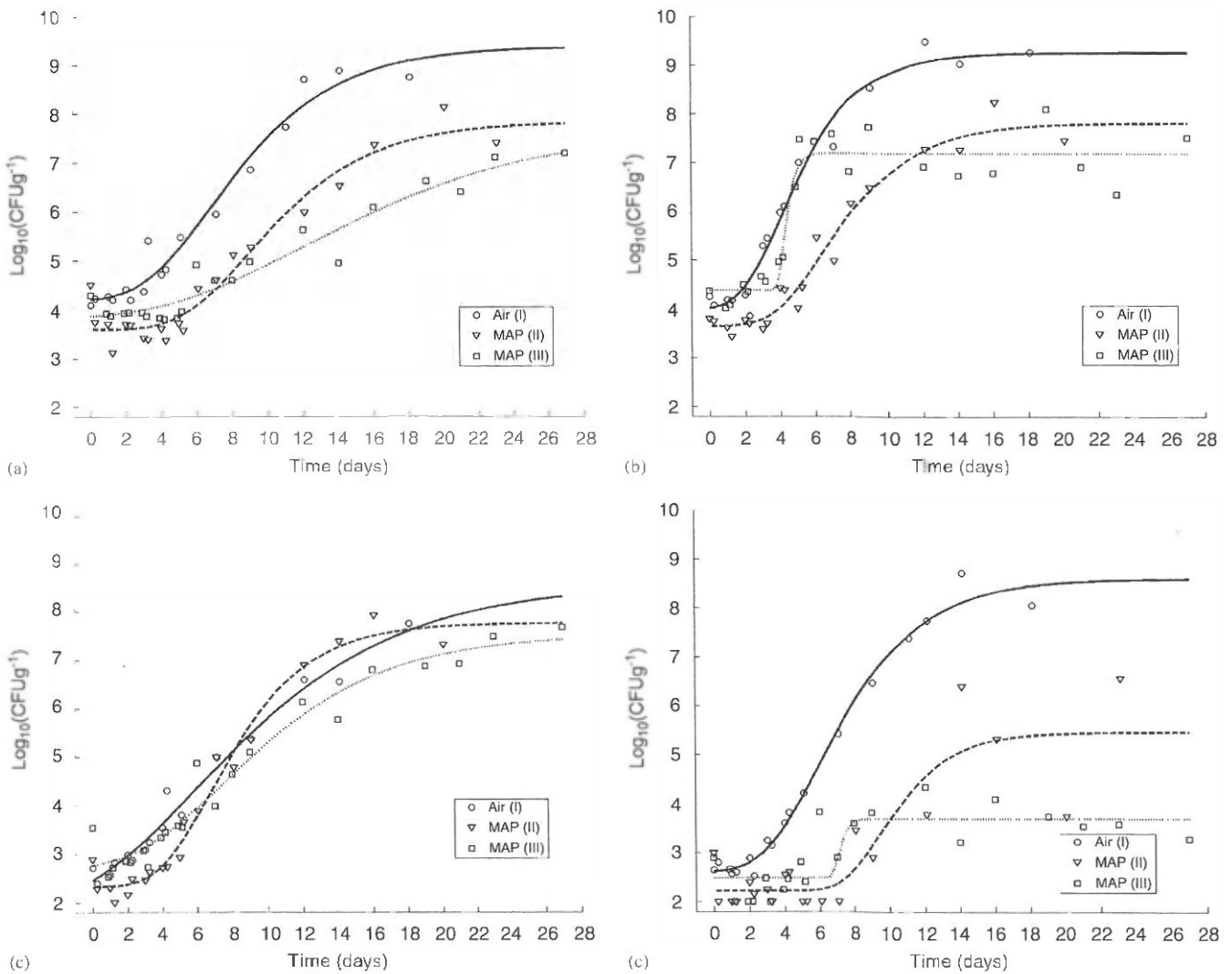


Fig. 1. (a) Total viable counts of maatjes herring stored in air. (b) Psychrotrophic bacteria counts of maatjes herring stored in air. (c) Lactic acid bacteria counts of maatjes herring stored in air. (d) Enterobacteriaceae counts of maatjes herring stored in air (fitted model is indicated by a continuous line ___ for Experiment I) and under modified atmosphere (dashed line---, Experiment II; dotted line...., Experiment III) at 4 °C.

for TVC, PVC and Enterobacteriaceae of samples stored in air (Experiment I) at both storage temperatures were higher than for MAP samples (Experiment II and III) at the end of sampling. Tables 3a–d show the lag phases,

growth rates and maximum growth for counts of TVC, PVC, Enterobacteriaceae and LAB at both temperatures.

In the “Code of Hygienic Practices” for retail trade fish products in the Netherlands (1997), the maximum limits

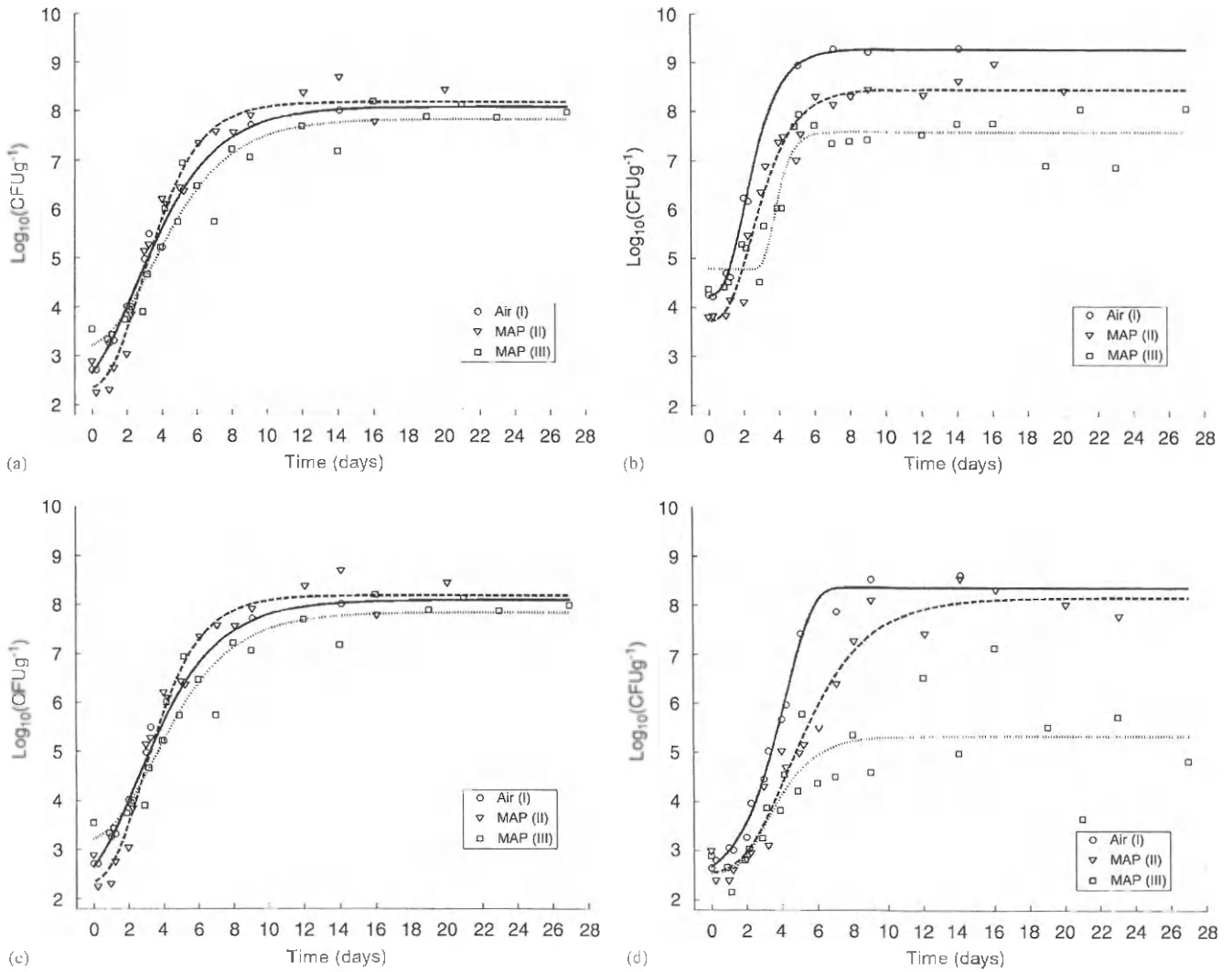


Fig. 2. (a) Total viable counts of maatjes herring stored in air. (b) Psychrotrophic viable counts of maatjes herring stored in air. (c) Lactic acid bacteria counts of maatjes herring stored in air. (d) *Enterobacteriaceae* counts of maatjes herring stored in air (fitted model is indicated by a continuous line — for Experiment I) and under modified atmosphere (dashed line-- --, Experiment II; dotted line...., Experiment III) at 10 °C.

for acceptability for unpacked and MAP maatjes herring are declared with TVC 10^6 cfu/g, PVC 5×10^5 cfu/g and *Enterobacteriaceae* counts 10^4 cfu/g. The times to reach the limit levels of bacteria counts according to the “Code of Hygienic Practices” for maatjes herring stored in air (Experiment I) and under modified-atmosphere (Experiments II and III) at 4 and 10 °C are summarized in Table 4.

3.4. Sensory changes

Freshly produced maatjes herring was described by the panellists as having firm texture with a good bite, a fresh and marine odour and a light salty, marine taste both odour and taste without any signs of rancidity. The colour of the blood was fresh red. The skin side of the fish was white-silver, light brown and shiny and the bone side creamy-white, clear and shiny.

The samples stored in air (Experiment I) were rejected on the basis of the development of a strong rancid taste. At the time of rejection, the taste of the MAP fish (II) was described as sour, bitter and compared with wet carton and rotten egg. The aftertaste was evaluated as bitter, sour, salty, like old flower water. Furthermore, a very soft texture even of the freshly produced samples was noticed. Important sensory properties of the MAP herring (III) for the judgment of unfitness for human consumption were taste and aftertaste, as described in Experiment II, but also rancidity. The sensory shelf-life of the maatjes herring stored in air (Experiment I) was three days at both 4 and 10 °C. The MAP herring in Experiments II and III had a shelf-life of 5 and 6 days, respectively, at both storage temperatures. Table 5 shows the bacterial levels of TVC, PVC, LAB and *Enterobacteriaceae* at the time of sensorial rejection for samples stored in air (Experiment I) and under

Table 3

Experiment	Storage temperature (°C)	Growth rate	Lag time	Maximum growth (days)
<i>(a) Growth rate, lag time and asymptotic maximum of total viable counts (TVC) using the Gompertz function for maatjes herring stored in air (Experiment I) and under modified-atmosphere (Experiments II and III) at 4 and 10 °C</i>				
I	4	0.48	2.89	13
I	10	0.98	1.42	6
II	4	0.40	5.10	15
II	10	0.70	1.66	9
III	4	0.18	4.10	25
III	10	0.58	1.72	8
<i>(b) Growth rate, lag time and asymptotic maximum of total psychrotrophic bacteria counts (PVC) using the Gompertz function for maatjes herring stored in air (Experiment I) and under modified-atmosphere (Experiments II and III) at 4 and 10 °C</i>				
I	4	0.80	1.71	8
I	10	1.68	0.97	4
II	4	0.53	3.61	11
II	10	1.31	1.23	4
III	4	2.71	3.84	5
III	10	1.76	3.13	5
<i>(c) Growth rate, lag time and asymptotic maximum of lactic acid bacteria counts (LAB) using the Gompertz function for maatjes herring stored in air (Experiment I) and under modified-atmosphere (Experiments II and III) at 4 and 10 °C</i>				
I	4	0.38	NE ^a	17
I	10	0.85	NE ^a	7
II	4	0.65	3.76	12
II	10	1.21	1.01	6
III	4	0.34	0.34	16
III	10	0.69	0.90	8
<i>(d) Growth rate, lag time and asymptotic maximum of Enterobacteriaceae counts using the Gompertz function for maatjes herring stored in air (Experiment I) and under modified-atmosphere (Experiments II and III) at 4 and 10 °C</i>				
I	4	0.67	2.76	11
I	10	1.43	5.80	1
II	4	0.54	7.33	13
II	10	0.84	1.85	8
III	4	1.22	1.22	7
III	10	0.67	1.58	5

^aNE, not estimated. Due to very short lag-phase, the Gompertz function failed to model lag time.

Table 4

Time to reach the limit levels of bacteria counts (Gesetz^a) for maatjes herring stored in air (Experiment I) and under modified-atmosphere (Experiments II and III) at 4 and 10 °C

Experiment	Storage temperature (°C)	Time to reach limit levels (days)		
		Total viable counts	Total psychrotrophic bacteria counts	Enterobacteriaceae counts
I	4	6.6	3.8	4.8
I	10	3.4	1.8	2.5
II	4	11.2	7.5	10.7
II	10	4.9	2.7	3.6
III	4	16.1	4.3	ND ^b
III	10	5.2	3.7	3.8

^aGuidelines, total viable counts 10⁶ cfu/g, total psychrotrophic bacteria counts 5 × 10⁵ CFU/g, Enterobacteriaceae counts 10⁴ cfu/g.

^bND, not determined because bacteria count 10⁴ cfu/g was not reached.

modified atmosphere (Experiments II and III) at both storage temperatures.

4. Discussion

The fat contents determined in the freshly produced maatjes herrings showed a relatively wide variation

(Table 1). In the Netherlands, at least 16% fat is prescribed in the whole fish. In the present study, in only two samples out of nine the required fat content was measured. Most of the fat is located in the belly of the fish, but in this study the fat content was determined in the fillets, thus resulting in an apparently lower level. The measured salt concentration varied from 0.9% to 2.3%. Over the last few years in the

Table 5
Shelf-life of maatjes herring in air (Experiment I) and under modified atmosphere (Experiments II and III) stored at 4 and 10 °C, estimated by sensory analyses in relation to the bacterial counts at the end of shelf-life

Experiment	Storage temperature (°C)	Shelf-life (days)	Bacterial counts (cfu/g) at the end of shelf-life				
			Total viable counts ^a	Total psychrotrophic bacteria counts	H ₂ S-producing bacteria counts	Lactic acid bacteria counts	Enterobacteriaceae counts
I	4	3	10 ⁴	10 ⁵ –10 ⁶	<10 ²	10 ³ –10 ⁴	<10 ² –10 ²
I	10	3	10 ⁶ –10 ⁷	10 ⁶ –10 ⁷	<10 ²	10 ⁴ –10 ⁶	10 ³ –10 ⁶
II	4	5	10 ³	10 ³ –10 ⁴	<10 ²	10 ² –10 ³	<10 ² –10 ³
II	10	5	10 ⁵ –10 ⁶	10 ⁷	<10 ²	10 ⁶	10 ⁴ –10 ⁵
III	4	6	10 ⁴ –10 ⁵	10 ⁷	<10 ²	10 ⁴	10 ³
III	10	6	10 ⁶	10 ⁷	<10 ²	10 ⁶	10 ⁵ –10 ⁶

^aMinimum and maximum bacterial counts.

Netherlands, the applied salt concentration has changed from heavily salted herring to current low salt concentrations of only 2%. Nevertheless, the present products can still be regarded as maatjes herring. It is worth noting that the guiding principles in Germany (Leitsätze für Fische, Krebs- und Weichtiere und Erzeugnisse daraus, 2003) dictate a fat content of at least 12%. Also, if the fish is sold to consumers as maatjes herring, salt concentration must be between a minimum of 6% and a maximum of 20% (so-called mildly salting process) in the fish tissue water. A similar herring-based product available on the German retail market is made from herring fillets, with a fat content of at least 10%, according to a special process involving edible acids and other ingredients. This product is called “Heringsfilet nach Matjesart” or “Heringsfilet, nach Matjesart gesalzen” (maatjes-like herring/herring à la maatjes).

To the authors' knowledge, no studies have been published on the initial bacterial counts of freshly produced maatjes herring fillets. However, the initial counts obtained in the present study are in agreement with previous studies reporting initial TVC between 10³ and 10⁵ cfu/g for different packaged (Özogul et al., 2000; Franzetti et al., 2001) and unpackaged (Taliadourou et al., 2003; Chytiri et al., 2004) not-heat-treated fish products other than maatjes herring. In contrast, Lyhs et al. (2001a) found TVC below 10²–10³ cfu/g for freshly produced vacuum-packaged ‘gravad’ rainbow trout. Higher initial bacterial counts as found in the present study are most probably associated with cross-contamination of the fish during the filleting process including different sources like house aerobic microflora, used utensils and the personnel (Taliadourou et al., 2003; Chytiri et al., 2004).

In the measurement II and III, the measured initial concentration of CO₂ was 20.1–22.1% and 15.5–17.6%, respectively. This is lower than the nominal value of the applied modified atmosphere in the package at processing time. A rapid decrease of the CO₂ concentration starts immediately after closing of the package and is due to CO₂ absorption in the herring flesh (Rosnes et al., 1997; Emborg

et al., 2002). In our case, there have been a few hours between packaging the fish and measuring the CO₂ concentration, which explains the lower values. Another potential explanation is the leakage of the packages; if present, that would contribute to the change of the gas composition.

Rancidity resulting from the oxidation of fat is the major spoilage indicator for air-stored maatjes herring leading to sensory rejection. Although the samples in Experiment III were packaged in MAP, rancidity was one of the main changes during storage. However, headspace gas composition analysis indicates that leakage might have occurred in the package during storage resulting in fat oxidation of the fish.

At the day of sensory rejection, TVC for herring in all three experiments (I–III) stored at 4 °C did not reach 10⁶ cfu/g, which is considered as the limit of acceptability for maatjes herring given by the Dutch fishery authorities (1997). Discrepancies between sensory and microbiological data has been reported by Magnússon and Traustadóttir (1982); Leroi et al. (1998) and Truelstrup Hansen et al. (1995, 1998) in vacuum-packaged cold-smoked fish products. In these studies fish with high microbiological numbers were not always spoiled. In contrast, Scott et al. (1984) observed with increasing TVC a decrease of sensory scores for modified atmosphere- and vacuum-packaged untreated snapper fillets; Lyhs et al. (2001a) observed the same for vacuum-packaged ‘gravad’ rainbow trout. In Experiments II and III of the present study, the herring was rejected owing to characteristic off-taste. In addition a very soft texture was noticed in Experiment II. It is known that autolytic enzymes from the fish tissue have a major impact on textural deterioration and therefore on spoilage (Truelstrup Hansen et al., 1996, 1998). Endogenous intestinal proteases, released from parts of viscera left in the herring, cause the hydrolysis of muscle proteins, thus creating a softer texture (Nielsen and Børresen, 1997). In chilled herring, enzymatic changes may precede and predominate bacterial spoilage (Huss, 1995). However, the development of off-odours and off-flavours is mainly a

result of microbiological activity (Paludan-Müller et al., 1998; Dondero et al., 2004). In this study, the herring were decapitated but gutted and strong intestinal enzymes probably caused the soft texture since they are not inactivated by heat during processing or before consumption. The sour, bitter and rotten off-taste described by the sensory panel probably results from microbial metabolism. In this study, there was no difference in the time of sensory rejection between both storage temperatures. Temperature controls the rate of both enzymatic and bacterial spoilage. Thus, for samples stored at 10 °C, the occurrence of both enzymatic and bacterial spoilage can be suggested.

Lactic acid bacteria (LAB) were found to be part of the microbial population at the time of sensory rejection in samples of all experiments (I–III) at both storage temperatures. The occurrence of LAB in high numbers in MAP fish products after a few weeks' storage at chilled temperatures has also been reported previously (Stenström, 1985; Nieper and Stockemer, 1995; Hong et al., 1996; Emborg et al., 2002; Franzetti et al., 2001). The counts in Experiment I are higher than in other studies dealing with untreated fish products stored in air. Molin and Stenström (1984) and Molin et al. (1983) reported findings between $<10^2$ and 10^3 cfu/g in herring fillets. However, Koutsoumanis et al. (1999) determined LAB counts in Mediterranean Gilt-head Sea Bream stored at 8 and 15 °C of 10^4 and 10^6 cfu/g, respectively. LAB usually do not dominate in the microflora of the raw material, and only certain species have been found in fish from temperate and cold marine waters and their surrounding environment (González et al., 2000; Ringø et al., 2000). It could be speculated that lactic acid bacterial strains thriving in the processing environment contaminate the maatjes herring after thawing during further processing. The exposure of fish products to in-house flora during several processing steps has been suggested earlier (Krüger, 1973; Lyhs et al., 2001b; Bagge-Ravn et al., 2003).

In all experiments (I–III) in herring stored at 4 °C, *Enterobacteriaceae* did not exceed the limit given by the Dutch fishery authorities ($<10^4$ cfu/g) at the time of sensory rejection. In the present study, CO₂ concentrations of 40–60% were measured at the end of the storage headspace in the modified-atmosphere packages (Experiments II and III). Previous studies including several MAP fish products showed that CO₂ concentrations of 20%, 40% and under 100% inhibited *Enterobacteriaceae* (Stenström 1985; Lopez-Galvez et al., 1998; De La Hoz et al., 2000; Arashisar et al., 2004). This is a possible reason for the low numbers in maatjes herring found in the present study.

The total absence of H₂S-producing bacteria in all three experiments (I–III) was unexpected. H₂S-producing bacteria are known as spoilage bacteria in aerobically stored and in MAP fish at chilled temperatures (Dalgaard et al., 1993; Rosnes et al., 1997; Koutsoumanis et al., 1999; Sveinsdóttir et al., 2002). H₂S-producing bacteria are favoured by a low O₂ level, but inhibited in environments

with high or increasing CO₂ levels as in MAP or vacuum-packaging (Jørgensen et al., 1988; Lyhs et al., 2001a; Rosnes et al., 1997). In vacuum-packaged 'gravad' rainbow trout, H₂S-producing bacteria constituted a large proportion of the PVC at the time of spoilage. However, studying the effect of smoke and salt on the microflora of vacuum-packaged cold-smoked salmon, Leroi et al. (2000) reported that salt produced a strong linear inhibition on H₂S-producing bacteria. The salt used in the processing of maatjes herring might have influenced these bacteria by suppressing their growth.

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