# Keeping Quality of Desalted Cod Fillets in Consumer Packs

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ABSTRACT: To meet modern consumer demands, it is important to be able to offer "ready-to-use" desalted cod in consumer packs with sufficient keeping quality at chill temperatures. The aim of this work was to investigate the effect of potassium sorbate, citric acid, and modified atmosphere packaging (MAP) in varying combinations on the keeping quality of desalted cod fillets in consumer packs. After desalting, cod fillets were packed in trays and stored at 1.0  $\pm$  0.2 °C for up to 33 d. The fillets were packed in open bags, in modified atmosphere (MA) (CO<sub>2</sub>/N<sub>2</sub>/O<sub>2</sub>:75/20/5) only, or in MS following a potassium sorbate and/or a citric acid treatment. The rate of microbial growth was by far fastest in fillets in open bags. MAP alone decreased the growth rate considerably and still further decrease was obtained in MAP fillets treated with citric acid and/or sorbate solutions. The concurrent effect of these treatments was distinct. Quantitative descriptive analysis (QDA) was used to assess cooked samples. During the 1st d of storage, the samples were described by sweet and butter odor, salt taste, clammy and rubber-like texture, which became less evident with increasing storage time, but differed by groups and the least in samples treated with sorbate and citric acid/sorbate solutions. Sensory spoilage attributes and total volatile bases (TVB-N)/trimethylamine (TMA) measurements correlated well with microbial counts. Use of MAP increased the shelf life from 6 to 10 d to 18 to 24 d, MAP and citric acid to 24 to 28 d, while the addition of sorbate to MAP fillets extended the shelf life to at least 33 d.

Keywords: desalted cod, keeping quality, microorganisms, sensory evaluation, MAP

## Introduction

Salted fish has been exported from Iceland to some extent because the turn of the 20th century. It was in fact the main export product until World War II when frozen fish took over. In 2004, export of fishery products amounted to 60% of the total Icelandic export earnings. Salted fish, mainly cod (*Gadus morhua*), accounted for 6.6% of exported fishery products providing 17.4% of export value.

Salting of fish and fish products is a traditional preserving method. Many consumers, especially in southern Europe appreciate the special flavor and texture characteristics of desalted fish products (Skjerdal and others 2000). Therefore, salting is not only a method to prolong shelf life but is a method to produce fish products meeting the demands of selective consumers. During the salting process, chemical, flavor, and textural changes occur. These changes are responsible for the ripening of salted cod and remain during desalting and cooking. By far, the 2 main markets for salted cod are Spain and Portugal.

Fully salted cod contains about 20% salt and therefore has to be desalted before consumption. Desalting is a time-consuming and tedious process, and to meet modern consumer demands, it is important to be able to offer "ready to use" desalted cod in consumer packs with sufficient keeping quality at chill temperature.

The use of preservatives within the food industry is a common practice. One of the most used preservatives is sorbic acid, which has a wide spectrum of activity against yeasts, molds, and catalasepositive bacteria. It is, however, relatively ineffective against catalase-negative bacteria such as the lactic acid bacteria (Silliker 1980).

Few studies on the spoilage and preservation of desalted cod have been published. Pedro and others (2004) studied the effect of potassium sorbate and citric acid on spoilage isolates and desalted cod spiked with Shewanella putrefaciens and Pseudomonas fluorescens or putida. The combination of 0.1% potassium sorbate and 0.15% (w/v) citric acid fully inhibited the growth of Shewanella putrefaciens and partially that of Pseudomonas fluorescens or putida. The effect of different concentrations of salt, potassium sorbate, and citric acid on the microbial growth in desalted cod packed in air and vacuum was studied by Fernández-Segovia and others (2003). Microbial growth was not inhibited in desalted cod with 3.13% to 5.78% waterphase salt without the use of preservatives. By combining the lower salt content with potassium sorbate and citric acid, a synergistic effect was observed. Vacuum packaging did not significantly increase the keeping time of the product. Studies done in Norway on the spoilage microflora of rehydrated salt-cured and dried salt-cured cod (2% to 4% salt) found the dominating bacterium as belonging to the genus Psychrobacter. This bacterium did not produce trimethylamine (TMA) or H<sub>2</sub>S but led to spoilage characterized by a musty odor making the fish unacceptable within 7 to 10 d (Bjørkevoll and others 2003). However, P. fluorescens, P. putida, and S. putrefaciens were found as the most important microflora at chill storage of soaked cod (0.8% salt) made from salted and dry salted cod (Rodrigues and others 2003).

The use of modified atmosphere packaging (MAP) has been found to increase the keeping quality of fish products (see, for example, Reddy and others 1992; Sivertsvik and others 2002). Pellegrino and others (1990) studied cod fillets that were desalted for 24 and 36 h and packed in 97.8%  $CO_2$ . Shelf life of 21 d was reached for the former fillets, but the longer desalting process resulted in decreased shelf life.

Very few studies have been published on sensory properties and shelf life of desalted cod. Barat and others (2006) studied the influence of raw material of different freshness on the sensory quality of desalted cod. They found that the main sensory differences were in texture where less fresh raw material, close to the limits of acceptable freshness quality, resulted in a flakier product. Raw mate-

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rial processed pre-rigor resulted in a harder product, but differences were also observed in odor or flavor.

A method such as quantitative descriptive analysis (QDA), which is used to describe all perceptible aspects of a product, can be used to provide useful information about sensory quality and shelf life of seafood (Sveinsdóttir and others 2002). In the case of determining the maximum shelf life, the limit of consumption may be determined at the point when the sensory panel detects spoilage attributes in the samples.

The purpose of this work was to investigate the effect of potassium sorbate, citric acid, and MA-packaging in varying combinations on the keeping quality of desalted cod fillets in consumer packs. Microbial counts, sensory evaluation, and chemical measurements were used to estimate the keeping quality.

## Materials and Methods

## **Experimental design**

Salted cod fillets with skin on were obtained from a large producer of salted fish located in southwest Iceland. Fresh line-caught cod was used for the experiments. Originally, the fillets were brine-injected, kept in brine for 3 d containing potassium and sodium di-, triphosphates (Carnal<sup>TM</sup>), and then dry salted for 2 wk. The salt content at this point was 21% to 21.5% (producer's information). The fillets were then packed in boxes and kept in a cold store (3 °C to 5 °C) for 5 wk before desalting. The desalting process was based on a method described by Thorarinsdottir and others (2004), and the aim was to obtain a final salt content of 1%. The ratio between fillets and water was 1:5, and desalting was carried out under chilled conditions (3 °C to 5 °C). Water change was performed after 7 and 24 h with stirring of the water 2 to 3 times a day. After completion of the 72 h desalting period, the fillets were drained and some treated with 0.2% citric acid solution (citric acid<sub>\*</sub>H<sub>2</sub>O and sodium citrate<sub>\*</sub>2H<sub>2</sub>O, BUFA B.V. Pharmaceutical Products, Holland) for 2 h before packaging. The rest of the fillets were further soaked in fresh water (ratio 1:5) for 2 additional hours. Just before packaging, some of the fillets were dipped in 3% (w/w) solution of potassium sorbate for 60 s. Fillet pieces (520 to 690 g) were packed in trays (expanded polystyrene, Linstar E 39-34) with a built-in absorption mat and vacuum bags (55PA/60LDPE,  $25 \times 40$ cm). After preliminary experiments with different gas mixtures and concentrations of citric acid and sorbate, the following experimental groups were chosen: Open bag (A1), MAP (A2), MAP with citric acid (A3), MAP with potassium sorbate (A4), and MAP with both citric acid and sorbate (A5). Sampling days of different groups are shown in Table 1. The gas mixture in experimental groups A2 to A5 was CO<sub>2</sub>/N<sub>2</sub>/  $O_2$ :75/20/5. All groups were kept at 1.0 ± 0.2 °C.

## Microbial counts

Total viable psychrotrophic counts (TVC) and counts of  $H_2S$ producing bacteria were evaluated on iron agar (IA) as described by Gram and others (1987) with the exception that 1% NaCl was used instead of 0.5%. Plates were surface-plated and incubated at 15 °C for 4 d. Bacteria forming black colonies on this medium produce  $H_2S$ from sodium thiosulphate and/or cysteine. Nitrite-Actidione-Polymyxin (NAP) agar was used for counts of lactic acid bacteria (LAB). The medium was prepared according to Davidson and Cronin (1977). Pour-plating was used and plates incubated at 22 °C for 4 d under microaerophilic conditions. Cephaloridine Fucidin Cetrimide (CFC) agar (Oxoid) was modified according to Stanbridge and Board (1994) and used for enumeration of presumptive pseudomonads. Pseudomonas Agar Base (Oxoid) with CFC Selective Agar Supplement was used (Oxoid). Plates were surface-plated and incubated at 22 °C for 4 d. *Pseudomonas* spp. form pink colonies

Table 1-Description of sample groups and sampling days

| Sample<br>groups | Sample<br>groups        | Sampling<br>days<br>(day after desalting)ª |  |  |  |  |
|------------------|-------------------------|--|--|--|--|--|
| Marking          | Description             |  |  |  |  |  |
| A1               | open bag                | 6, 10                                      |  |  |  |  |
| A2               | MAP                     | 6, 10, 18, 24                              |  |  |  |  |
| A3               | MAP/citric acid         | 6, 13, 24, 28, 33                          |  |  |  |  |
| A4               | MAP/sorbate             | 7, 13, 18, 25, 28, 33                      |  |  |  |  |
| A5               | MAP/citric acid/sorbate | 7, 18, 25, 28, 33                          |  |  |  |  |

<sup>a</sup>Storage at 1.0  $\pm$  0.2 °C.

on this medium. Dichloran Rose Bengal Chloramphenicol (DRBC) agar (Difco) was used for counts of yeasts and molds. Plates were surface-plated and incubated at 22 °C for 5 d.

In all experiments cooled maximum recovery dilutent (MRD, Oxoid) was used for dilutions. Samples were analyzed in duplicate. All results are presented as an average. Analysis of variance (ANO-VA) was carried out on microbial and pH data in the statistical program NCSS 2000 (NCSS, Kaysville, Utah, U.S.A.) to compare the groups within different stages of storage (Table 2). The program calculates multiple comparisons using Tukey-Kramer multiple-comparison test. The significance level was set at 5%.

At the beginning of the experiment, samples of desalted cod fillets were tested for the presence of *Salmonella*, *Listeria*, *Staphylococcus aureus*, *Enterobacteriaceae*, and total/fecal coliforms. Methods used were based on the 4th edition of Compendium (Downes and Ito 2001) with the exception that Staphylococcus Medium 110 (Difco) with egg yolk was used for isolation of *S. aureus*. All these methods have gained accreditation according to the international standard ISO/IEC 17025.

## Chemical analysis

The method of Malle and Tao (1987) was used for total volatile bases (TVB-N) and trimethylamine (TMA) measurements. TVB-N was measured by steam distillation (Struer TVN distillatory, STRUERS, Copenhagen, Denmark) and titration after extracting the fish muscle with 7.5% aqueous trichloracetic acid solution. The distilled TVB-N was collected in boric acid solution and then titrated with sulfuric acid solution. TMA was measured in trichloroacetic acid (TCA) extract by adding 20 mL of 35% formaldehyde, an alkaline binding mono- and diamine, TMA being the only volatile and measurable amine. Trimethylamine oxide (TMAO) measurement was done by the picric acid method (AOAC 1990). The same extract was used as for TVB-N and TMA. TMA was 1st measured and then after TMAO reduction to TMA with titanium chloride. TMAO was calculated as the difference between the direct method and total TMA. Measurement of salt content was done with Titrino method (Volhard) according to AOAC (AOAC 1995) and water content according to ISO 6496 (ISO 1999). Measurement of sorbic acid was done with high-performance liquid chromatography (HPLC) at the Danish Veterinary and Food Administration in Copenhagen. The pH was measured in a mixture of 5 g mince and 5 mL deionized water using the Radiometer PHM 80.

Mince from 2 samples was combined for all chemical analysis except for pH where duplicate samples were measured.

## Sensory evaluation

Quantitative descriptive analysis, introduced by Stone and Sidel (1985), was used to assess cooked samples of desalted cod. Twelve panelists of the Icelandic Fisheries Laboratories' sensory panel participated in the QDA of the cooked samples. They were all trained according to international standards (ISO 1993), including the detection and recognition of tastes and odors, and were trained in the use

#### Table 2-Statistical analysis of microbial counts (log numbers/g) and pH values<sup>a</sup>

|                            |              | A1         | A2    |       | A3      |       | A4    |        | A5    |       | р      |
|----------------------------|--------------|------------|-------|-------|---------|-------|-------|--------|-------|-------|--------|
| First storage period up to | o 10 d       |            |       |       |         |       |       |        |       |       |        |
| Storage time (d)           |              | 6          | 6     |       | 6       |       | 7     |        | 7     |       |        |
| Total counts               |              | 8.2a 6.2bc |       | oc    | 6.1cd   |       | 5.6d  |        | 5.9cd |       | 0.0001 |
| H2S-producing              | 6.5ab        |            | 4.4bc |       | 2.3cd   |       | 1.0d  |        | 0.0d  |       | 0.0013 |
| Pseudomonads               |              |            |       |       |         |       |       |        |       |       |        |
| Yeasts                     |              |            |       |       |         |       |       |        |       |       |        |
| Lactic acid bact.          |              |            |       |       |         |       |       |        |       |       |        |
| рН                         |              | 7.1ab      | 6.7bc |       | 6.6cd   |       | 6.5d  |        | 6.5d  |       | 0.0003 |
| Second storage period fro  | om 10 to 190 |            |       |       |         |       |       |        |       |       |        |
| Storage time (d)           | 10           | 13         | 10    | 18    | 1       | 3     | 13    | 18     |       | 18    |        |
| Total counts               | 8.7a         | 9.2a       | 6.2cd | 6.8bc | c 6.2cd |       | 5.8d  | 5.6d   | 5.7d  |       | 0.0000 |
| H <sub>2</sub> S-producing | 7.5ab        | 8.1ab      | 4.1c  | 6.0bc | 0.0d    |       | 1.0d  | 0.0d   | 0.0d  |       | 0.0000 |
| Pseudomonads               | 7.9b         | 8.9a       | 5.1ef | 6.2cd |         |       | 4.7fg | 5.5df  | 4.0g  |       | 0.0000 |
| Yeasts                     | 5.9ab        | 7.0a       | 4.6ce | 5.8ac |         |       | 4.5ce | 5.0bcd | 3.1fg |       | 0.0001 |
| Lactic acid bact.          | 5.9a         | 5.9a       | 4.3b  | 5.8a  |         |       | 3.9b  | 4.3b   | 4.4b  |       | 0.0001 |
| рН                         | 7.1bc        | 7.5ab      | 6.7cd | 6.3d  | d 6.7d  |       | 6.8d  | 6.4d   | 6.4d  |       | 0.0003 |
| Third storage period from  | 20 to 29 d   |            |       |       |         |       |       |        |       |       |        |
| Storage time (d)           |              |            | 24    |       | 24      | 28    | 25    | 28     | 25    | 28    |        |
| Total counts               |              |            | 7.9ab |       | 7.3bc   | 7.7ab | 6.3cd | 7.0    | 5.9d  | 5.7d  | 0.0033 |
| H2S-producing              |              |            | 7.4a  |       | 6.6a    | 7.5a  | 0.0b  | 0.0b   | 0.0b  | 0.0b  | 0.0000 |
| Pseudomonads               |              |            | 7.7ab |       | 6.2cd   | 7.0bc | 5.9d  | 6.5cd  | 3.6e  | 3.9e  | 0.0001 |
| Yeasts                     |              |            | 5.5   |       | 4.6     | 5.1   | 5.7   | 5.9    | 1.5   | 0.0   | 0.0367 |
| Lactic acid bact.          |              |            | 5.6b  |       | 6.2a    | 5.7b  | 5.0c  | 5.5b   | 5.1c  | 5.5b  | 0.0002 |
| рН                         |              |            | 6.6b  | 0     | 6.4cd   | 6.4cd | 6.5   | 6.7ab  | 6.4d  | 6.6ab | 0.0067 |
| Final sampling (33d)       |              |            |       |       |         |       |       |        |       |       |        |
| Storage time (d)           |              |            |       |       | 33      |       | 33    |        | 33    |       |        |
| Total counts               |              |            |       |       | 7.9     |       | 7.8   |        | 5.9   |       | 0.0497 |
| H2S-producing              |              |            |       |       | 7.4a    |       | 0.0b  |        | 0.0b  |       | 0.0006 |
| Pseudomonads               |              |            |       |       | 7.1     |       | 6.9   |        | 4.9   |       | 0.0654 |
| Yeasts                     |              |            |       |       | 5.7     |       |       |        | 4.9   |       | 0.1333 |
| Lactic acid bact.          |              |            |       |       | 6.2     |       | 5.8   |        | 6.1   |       | 0.2781 |
| рН                         |              |            |       |       | 6.8     |       | 6.7   |        | 6.6   |       | 0.3794 |

<sup>a</sup>Different letters indicate significant different values between samples within a line.

of scales and in the development and use of descriptors. The members of the panel were familiar with the QDA method and experienced in sensory analysis of cod. Four sessions were used for training of the panel using different samples of desalted cod (newly desalted, stored in open bags, MA-packed, treated with citric acid and/or sorbate) of different freshness categories. The panelists made a vocabulary of descriptors to describe the samples under the guidance of the panel leader. The panel was trained in describing the intensity of each attribute for a given sample using an unstructured scale (from 0% to 100%). The following 31 attributes evaluated were related to appearance (light/dark color, heterogeneity), odor (sweet, boiled milk, boiled potatoes, sea, butter, earth, tablecloth, characteristic, acid, sour, TMA, sulfur), flavor (salt, sweet, sour, sea-like, butter, earth, ripening, TMA, pungent, frozen storage), and texture (flakiness, juiciness, softness, tenderness, rubber, foamy, clammy). Samples were evaluated newly desalted (A0, referent sample) and samples from groups A1 to A5 were evaluated with a several-day interval (Table 1). Each panelist evaluated duplicates of each sample in a random order and with a maximum of 4 samples in each session.

All sample observations were conducted according to international standards (ISO 1988). Samples weighing 40 to 50 g were taken from the loin part of the fillets and placed in aluminum boxes coded with 3-digit random numbers. The samples were cooked at 95 °C to 100 °C for 7 min in a pre-warmed oven (Convotherm Elektrogeräte GmbH, Eglfing, Germany) with air circulation and steam and then served to the panel. A computerized system (FIZZ, Version 2.0, 1994-2000, Biosystèmes) was used for data recording.

Multivariate comparison of different attributes and samples

analyzed with QDA was conducted in the statistical program Unscrambler® (Version 8.0, CAMO, Trondheim, Norway), with partial least squares regression (PLS2) on mean level corrected attribute values (level effects caused by level differences between assessors and replicates removed). From a PLS2 model, the initial variance (signal) at zero PCs and the residuals variance (noise) after optimal PCs were plotted as a signal to noise (S/N) ratio for each sensory attribute (Thybo and Martens 2000) to select important sensory attributes. QDA data were also compared with microbial and chemical measurements with principal component analysis (PCA). The variables were scaled before the statistical analysis. Each element in the matrix was multiplied with the inverse of the standard deviation of the corresponding variable when variables had different ranges. A full cross-validation method was used.

## Gas and drip loss measurements

The gas composition in the headspace of 2 to 4 packs was measured in each group (A2-A5) at packaging and sampling. Septums were put on the MA-packs and the gas measured with a PBI Dansensor (CheckMate 9900). Gas sample was collected twice consecutively and the latter measurement recorded. Drip loss (%) was calculated from the water lost by the fillet (absorbed by tray) divided by initial fillet weight.

## **Results and Discussion**

## **Microbial counts**

After desalting, fillets were tested for the presence of Salmonella,

*Listeria, S. aureus, Enterobacteriaceae*, and total/fecal coliforms. These bacteria were not found.

Results of all microbial counts are shown in Figure 1 to 5. Statistical analysis of the microbial counts is shown in Table 2. In all cases, microbial numbers increased most rapidly in fillets stored aerobically (open bag). The effect of MA packaging alone (A2) was very effective in slowing down microbial growth. Total viable psychrotrophic counts and counts of  $H_2S$ -producing bacteria on iron agar are shown in Figure 1 and 2. The use of citric acid and especially sorbate led to an additional inhibitory effect toward the overall microflora, retarding its development considerably. Concurrent effect of these preservatives was evident in later stages of storage. In the presence of MS,  $H_2S$ -producing bacteria were very sensitive toward sorbate (A4) and their levels had decreased below detection level on day 18. A lesser effect was seen with citric acid treatment (A3) where growth of  $H_2S$ -producing bacteria was delayed only at early storage when compared with untreated MAP samples. Concurrent effects of citric acid and sorbate were observed, affecting  $H_2S$ -producing bacteria to such an extent that no cells could be detected over the whole storage period in that group (A5). It should be recalled that Pedro and others (2004) found that by combining 0.1% potassium sorbate and 0.15% citric acid, total inhibition was obtained for *S. putrefaciens* and partial for *P. fluorescens* or *putida*. The species *Shewanella* (formerly *Alteromonas*) *putrefaciens*, which

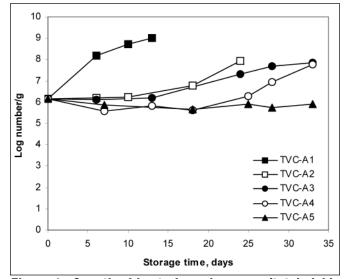


Figure 1-Growth of bacteria on iron agar (total viable count at 15 °C) during storage of desalted cod fillets at 1 °C (A1-Open bag, A2-modified atmosphere packaging [MAP], A3-MAP/citric acid, A4-MAP/sorbate, A5-MAP/citric acid/ sorbate). Means of 2 samples.

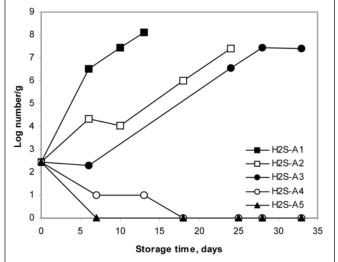


Figure 2–Growth of  $H_2$ S-producing bacteria on iron agar at 15 °C during storage of desalted cod fillets at 1 °C (A1-Open bag, A2-modified atmosphere packaging [MAP], A3-MAP/citric acid, A4-MAP/sorbate, A5-MAP/citric acid/sorbate). Means of 2 samples.



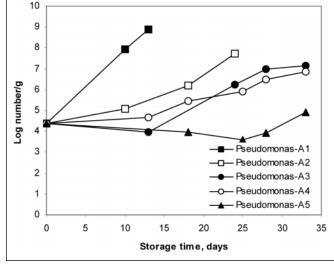


Figure 3-Growth of presumptive pseudomonads on Cephaloridine Fucidin Cetrimide (CFC) agar at 22 °C during storage of desalted cod fillets at 1 °C (A1-Open bag, A2-modified atmosphere packaging [MAP], A3-MAP/citric acid, A4-MAP/ sorbate, A5-MAP/citric acid/sorbate). Means of 2 samples.

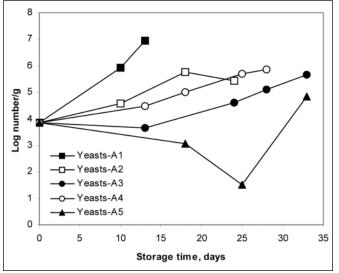


Figure 4–Growth of yeasts on Dichloran Rose Bengal Chloramphenicol (DRBC) agar at 22 °C during storage of desalted cod fillets at 1 °C (A1-Open bag, A2-modified atmosphere packaging [MAP], A3-MAP/citric acid, A4-MAP/ sorbate, A5-MAP/citric acid/sorbate). Means of 2 samples.

both reduces TMAO to TMA and produces  $H_2S$ , has been reported as the main spoilage organism of fish stored at 0 °C. Gram and others (1987) studying whole fish and vacuum-packed fillets found that the majority of isolated black colonies ( $H_2S$ -producing) on iron agar was identified as the above species. However, in studies on thawed ocean perch fillets (Magnússon and Martinsdóttir 1995), 13 of 25 isolated colonies on iron agar produced  $H_2S$ . Of these, 8 were identified as *Alteromonas/Pseudomonas/Alcaligenes*, 4 as *Vibrio/Aeromonas* and 1 as *Enterobacteriaceae*. Only 6 of these 13 strains were able to reduce TMAO to TMA, all belonging to the 1st group. Therefore, it can not be stated that the  $H_2S$ -producing bacteria isolated in our experiments were *S. putrefaciens*. This species is very sensi-

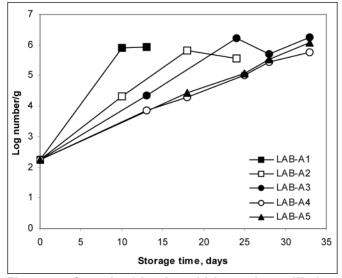


Figure 5-Growth of lactic acid bacteria on Nitrite-Actidione-Polymyxin (NAP) agar at 22 °C during storage of desalted cod fillets at 1 °C (A1-Open bag, A2-modified atmosphere packaging [MAP], A3-MAP/citric acid, A4-MAP/ sorbate, A5-MAP/citric acid/sorbate). Means of 2 samples.

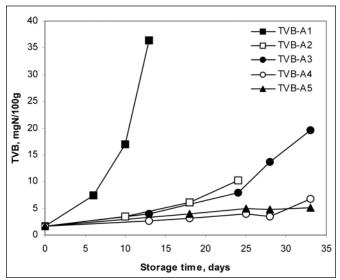


Figure 6—Changes in total volatile bases (total volatile bases [TVB-N]) during storage of desalted cod fillets at 1 °C (A1-Open bag, A2-modified atmosphere packaging [MAP], A3-MAP/citric acid, A4-MAP/sorbate, A5-MAP/citric acid/ sorbate).

tive toward  $CO_2$  (Dalgaard 1995). Hence, the high levels reached by the  $H_2S$ -producing bacteria under MS in groups A2 and A3 as storage progressed could indicate the presence of other species.

Counts of presumptive pseudomonads on CFC agar are shown in Figure 3. As expected, MAP retarded their proliferation to some extent. Additional growth delay was obtained in groups containing citric acid or sorbate. Growth curves for these 2 groups (A3 and A4) were similar. Concurrent effects in group A5 (MAP/citric acid/sorbate) were distinct, maintaining their initial level for most of the storage.

Counts of yeasts on DRBC agar are shown in Figure 4. Molds were not detected in any experimental groups during storage. Unlike the results from total counts, citric acid had a greater inhibitory effect on growth of yeasts than sorbate. Some concurrent effects were observed.

Counts of lactic acid bacteria on NAP agar are shown in Figure 5. Combined use of MAP and sorbate treatment was the most inhibitory toward LAB. No concurrent effects of citric acid and sorbate were observed.

## Chemical analysis

Directly after desalting, the salt content was 1.3% (1.5% water phase), water 84.0%, TVB-N 1.6 mg N/100 g, TMA not detected and TMAO 6.1 mg N/100 g. Initial pH was 6.7, which agrees with results obtained by Thorarinsdottir and others (2001). After 7 d from packaging the sorbic acid in groups A4 (MAP/sorbate) and A5 (MAP/ citric acid/sorbate) was 1100 and 730 mg/kg, respectively.

Results from TVB-N and TMA measurements over the storage period are shown in Figure 6 and 7. Both TVB-N and TMA increased rapidly in fillets stored aerobically (open bags, A1). When fillets in open bags were last examined on day 13, TMA was 6.0 mg N/100 g, which means that almost all TMAO initially present (6.1 mg N/100 g) had been reduced to TMA. The effect of MA-packaging alone was very effective in slowing down formation of TVB-N and TMA. In groups A4 and A5 where sorbate was used, hardly any increase was noticed in TVB-N and none in TMA over the whole storage period. As discussed earlier,  $H_2$ S-producing bacteria were very sensitive toward sorbate. These results strongly indicate that isolated  $H_2$ S-producing bacteria on iron agar (Figure 2) have been TMAO-reduc-

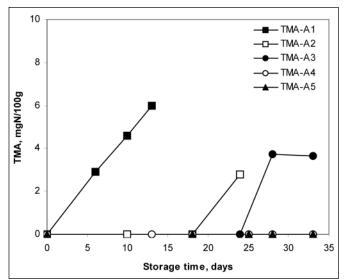


Figure 7—Changes in trimethylamine (TMA) during storage of desalted cod fillets at 1 °C (A1-Open bag, A2-modified atmosphere packaging [MAP], A3-MAP/citric acid, A4-MAP/sorbate, A5-MAP/citric acid/sorbate)

## Keeping quality of desalted cod . . .

ing. Results from pH measurements are shown in Figure 8. It is clear that pH increased rapidly in fillets packed in open bags. That is in line with the results from TVB-N and TMA measurements. No major changes in pH were noticed in the other groups, except for a decreasing trend upon addition of hurdles (MAP and sorbate) in groups A4 and A5. Slight increase in pH observed at late storage for groups A2 (MAP-d24) and A3 (MAP/citric acid-d33) could be linked to the detected increase in TMA/TVB-N content on these days.

## Sensory evaluation

Signal to noise analysis showed that 20 of the evaluated sensory attributes had a S/N ratio greater than 1 and therefore a good descriptive power. These were: the appearance attribute heterogene-

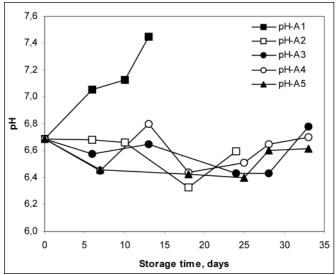
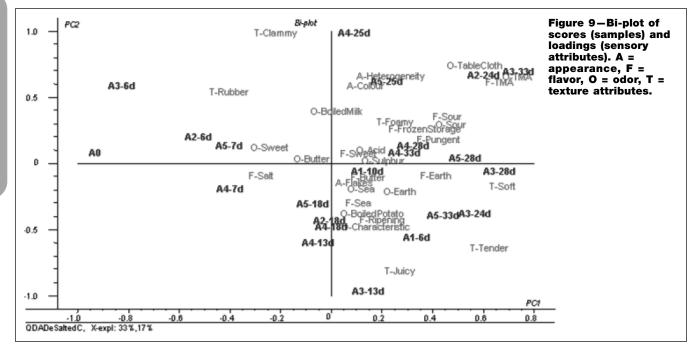


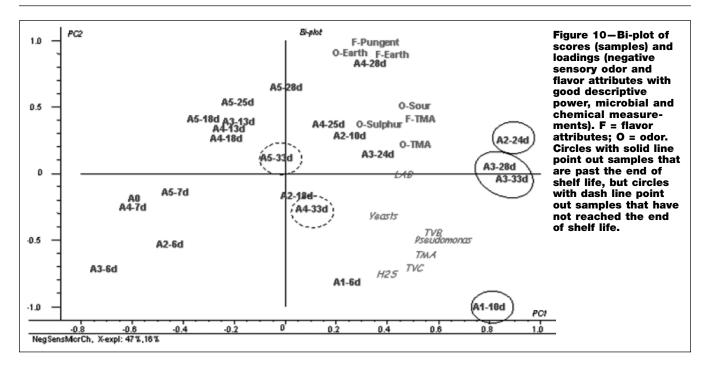
Figure 8-Changes in pH during storage of desalted cod fillets at 1 °C (A1-Open bag, A2-modified atmosphere packaging [MAP], A3-MAP/citric acid, A4-MAP/sorbate, A5-MAP/citric acid/sorbate). Means of 2 samples.

ity; the odor attributes sweet, boiled milk, boiled potatoes, butter, earth, characteristic, sour, TMA, and sulfur; the flavor attributes salt, sour, sea, earth, TMA, and pungent; and the texture attributes juicy, soft, tender, rubber, and clammy. Figure 9 shows how the different samples of desalted cod were described by the sensory attributes. The samples varied mainly with regard to differences in texture attributes, odor, and flavor along the 1st principal component (PC1), explaining 33% of the variation between the samples. The main difference appeared to occur with the storage time, as the sample groups are located to the left side at the beginning of storage but on the right side after longer storage (Figure 9). At the beginning of storage, sensory attributes such as sweet and butter odor, salt taste, and clammy and rubber texture were prominent. Those characteristics became less evident as storage time progressed, but differently by groups, with least changes in groups A4 (MAP/sorbate) and A5 (MAP/citric acid/sorbate). With increasing storage time, samples were described by characteristic and boiled potato odor and juicy texture, and then by earth, sour, and TMA flavor, sour, and TMA odor, heterogeneous color, and soft and tender texture. Those changes occurred over different time periods for each of the groups evaluated. Sample group A1 (open bag) was evaluated for 10 d, A2 (MAP) for 24 d, but groups A3 to A5 (MAP/ citric acid; MAP/sorbate; MAP/citric acid/sorbate) for 33 d. After this time, least changes had occurred in groups A4 and A5. Sample group A3 showed a trend toward juiciness, tenderness, and softness (Figure 9), indicating that citric acid might influence the texture. However, this trend was less obvious with the other sample group containing citric acid in addition to sorbate (A5). Beside this trend, no grouping of samples was observed. This indicated that MA packaging and the addition of citric acid and sorbate did not influence the sensory quality of the samples in other ways than to prolong their shelf life. Thus, the different treatments did not result in differences in appearance, odor, or flavor.

## Comparison of microbial counts, chemical analysis, and sensory evaluation

Microbial counts (TVC, H<sub>2</sub>S, Pseudomonas, yeasts, and LAB) and





chemical measurements (TVB-N and TMA) were compared with selected negative sensory odor and flavor attributes increasing with storage time (earth, sour, sulfur, and TMA odor and pungent, earth, and TMA flavor) with PCA (Figure 10). The 1st dimension (PC1) explained 47% of the variation between the samples, which appeared to be storage time and increased values of negative sensory attributes, microbial counts, and chemical measurements located to the right in Figure 10. The sensory attributes appeared to covariate well with other measurements along PC1. Sour odor, TMA odor, and flavor covariated particularly well with the TVB-N and

TMA measurements as well as H<sub>2</sub>S, TVC, LAB, and Pseudomonas counts. The 2nd dimension (PC2) explained 16% of the variation due to difference between values of sensory attributes and the other measurements of samples. Some of this difference could be due to more formation of TMA and TVB-N in open bags (A1), as modified atmosphere packaging delayed and reduced the formation of those compounds. After 10 d of storage, sample group A1 was described by negative sensory attributes but even more by high values of microbial counts, TMA, and TVB-N. A similar trend was seen for sample group A2 after 24 d and sample group A3 after 28 to 33 d of storage. However, those groups did not have as high TMA and TVB-N values as A1 but slightly lower microbial counts. Samples within group A4 are all located close to the center of Figure 10 and were slightly described by negative sensory attributes, low microbial counts, TMA, and TVB-N content. Samples within group A5 are all located in the left side of Figure 10 and had therefore even lower values of the negative sensory attributes, microbial counts, and TMA and TVB.

End of shelf life is usually determined when sensory attributes related to spoilage, such as sour, pungent, and TMA odor and/or flavor become evident. When the average QDA score for those attributes is above 20 (on the scale 0 to 100), most panelists detect those negative attributes, which indicates that the sample is approaching the end of shelf life. The end of shelf life of sample groups A1-5 was determined when those sensory attributes were above 20 and the counts of  $H_2S$ -producing bacteria were about  $10^6$  to  $10^7$  CFU/g. Based on this, the expected shelf life should be 6 to 10 d for A1 (open bag), 18 to 24 for A2 (MAP), 24 to 28 for A3 (MAP/ citric acid), but A4 (MAP/sorbate) and A5 (MAP/citric acid/sorbate) did not reach the end of shelf life within the storage time of the experiment (33 d).

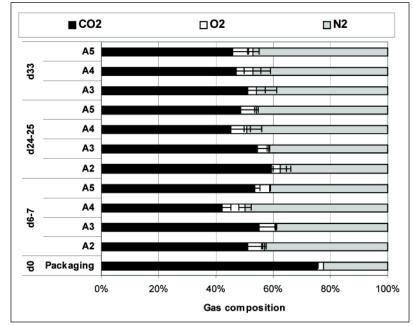


Figure 11—Changes in gas composition during storage of desalted cod fillets at 1 °C (A2-modified atmosphere packaging [MAP], A3-MAP/citric acid, A4-MAP/sorbate, A5-MAP/citric acid/sorbate)

## Gas and drip loss measurements

Results from gas measurements are shown in Figure 11. Initial gas composition directly after packaging was  $CO_2/N_2/O_2$ :75.5/22.4/ 2.1. As expected,  $CO_2$  concentration had decreased in the head-space of MA-packs after 6 to 7 d of cold storage while  $N_2$  and  $O_2$  had increased. At low storage temperatures,  $CO_2$  partly dissolves in the water phase of the fish flesh leading to changes in the gas composition. During prolonged storage, increasing numbers of microorganisms use more  $O_2$  and produce more  $CO_2$ . After 24 to 25 d of storage, most  $CO_2$  was formed in group A2 (MAP), less in A3 (MAP/citric acid) and least in A4 (MAP/sorbate) and A5 (MAP/citric acid/sorbate). As may be recalled, microbial growth was more rapid in groups A2 and A3 than in A4 and A5 (Figure 1).

The least drip loss was found in fillets packed in open bags (less than 1%). MA-packaging led to increased drip loss, most likely due to dissolved  $CO_2$  in the muscle, which lowers the pH and thus alters the food-water-holding capacity (Sivertsvik and others 2002). In groups A2 and A3, the drip loss was 2% to 4%, while in groups A4 and A5 it was 4% to 6%. The reason for increased drip loss in fillets where sorbate was used is not clear, but it could be linked to the lowest pH measured (about 6.4) in these groups possibly causing increased protein denaturation and decreased water-holding capacity.

#### Conclusions

It is concluded that the use of MAP along with citric acid and sorbate is a very effective way to increase the shelf life of desalted cod fillets in consumer packs. MAP alone increased the shelf life from 6 to 10 d to 18 to 24 d, but to 24 to 28 d when combined with citric acid, while the addition of sorbate extended the shelf life to at least 33 d. Determination of shelf life was based on results from sensory evaluation and microbial counts, and these correlated well with chemical analysis. The knowledge gained by this study might make it possible to export from Iceland "ready to use" desalted cod products to our main markets by sea.

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