Refrigerated Storage (2 °C) of Sole (*Solea solea*) Fillets under CO_2-Enriched Atmospheres

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Microbial (total viable, *Brochothrix thermosphacta*, lactobacilli, and Enterobacteriaceae counts), biochemical [pH, total volatile nitrogen, nucleotide breakdown products, D(−) and L(+) lactic acid, and short-chain fatty acids], and sensory attributes (color and odor) of refrigerated (2 °C) sole (*Solea solea*) fillets under CO_2-enriched [CO_2/air (20/80) (v/v) and CO_2/air (40/60) (v/v)] atmospheres were determined. Sensory results showed shelf life extension of 4 and 8 days for 20 and 40% CO_2-enriched atmospheres, respectively. Microbial and biochemical results revealed that the 40%CO_2-enriched atmosphere was the most effective package type for refrigerated sole.

Keywords: *Modified atmospheres; shelf life; sole fillets*

INTRODUCTION

Sole (*Solea solea*) or Dover sole (OECD, 1990) is a fish species of the family Soleidae, which is highly appreciated throughout the world. It is mainly caught by trawlers (Anonymous, 1986; Collette, 1990) although it is also caught by other net types. Fresh sole is usually sold as fillets, although whole dressed fish are also available to the consumer. Some fillets are sold skin-on, particularly the white sides of the fish. Sole is very popular in Europe and can be defined as the classic European flatfish, considered to be the foundation of Continental seafood cuisine. Packaging fillets in a retail container at the end of the processing line provides an attractive seafood product. Forms of this type of packaging range from the standard packs with oxygen-permeable film to modified-atmosphere packaging and also fresh vacuum packs (Anonymous, 1986).

Bacterial spoilage in refrigerated fish under aerobic storage condition results from Gram-negative psychrotrophic organisms (Eklund, 1982; Van Spreekens, 1977) dominated by *Pseudomonas* and *Shewanella* (Hobbs, 1991; Lindsay et al., 1986). *Pseudomonas* species are effectively inhibited by atmospheres enriched with 20% or more carbon dioxide (Clark and Lentz, 1969; Christopher et al., 1979), although *Shewanella* is more resistant to CO_2 (Molin and Stenström, 1984) and growth of this microorganism is inhibited by higher carbon dioxide concentrations (∼40%) (López-Gálvez et al., 1995). Therefore, CO_2-enriched atmospheres have been increasingly used in the past few years for the distribution of red meats, poultry, and seafood. In Europe, modified atmosphere packaged (MAP) fish for end use represented 8% of the market in 1986 and 12% in 1990 (Brody, 1993) and increased even further in the following years.

The ability of modified atmospheres to extend fish shelf life has been studied by many authors, and some excellent reviews have been published (Stammen et al., 1990; Skura, 1991; Reddy et al., 1992; Davis, 1993). The objective of the present work was to study the effect of two CO_2-enriched atmospheres on both the bacterial flora and the changes occurring in certain selected chemical parameters during refrigerated storage of sole. However, the absence of sole from the wide list compiled by Davis (1993) reflects that very little research has been focused on this species.

MATERIALS AND METHODS

Preparation and Storage of Samples. Sole (*S. solea*) were caught during the spring (April−May) in the off-shore ground located at the VIIi and VIIh areas (north longitude 48−50°, west lattitude 5−12°) as defined by ICES (International Council for the Exploration of the Sea) Fisheries Statistical Areas. Captured fish, stored in flaked ice, usually arrive at the Spanish markets in <60 h. Sole (of ∼250 g each) were purchased in a local fish market and transported to the laboratory in crushed ice in <1 h. Fillets were obtained at the laboratory in a walk-in cold room (2 °C). For that, the head, viscera, and skin were removed, and four fillets were obtained from each animal. Samples (three to five fillets) were divided into three batches for packaging in three different atmospheres. Gas mixtures, supplied by Carburos Metálicos S.A., consisted of CO_2/air (20/80) (v/v), CO_2/air (40/60) (v/v), and air, which was used as control. Samples were individually packaged in laminated film bags (25 × 30 cm) of low gas permeability (Cryovac BB4L; diffusion coefficient of 35 $cm^3\ h^{-1}\ m^{-2}\ bar^{-1}$ to O_2 and 150 $cm^3\ h^{-1}\ m^{-2}\ bar^{-1}$ to CO_2). MAP was made in a Vapta model EUVAC 50 vacuum chamber; once the air was evacuated by vacuum pressure, plastic bags were flushed by injection with the selected gas mixture and heat sealed. The final gas/sample ratio in all bags was about 3:1 (v/w). All samples were stored in a walk-in cold room at 2 ± 1 °C until sampling. Two bags from each atmosphere were drawn for analysis. At each sampling time, the bag headspace gas odor was recorded.

Microbiological Analyses. The methodology described by López-Gálvez et al. (1995) was followed to count, to characterize the dominant organisms, and to determine the incidence of *Brochothrix thermosphacta*. To do this, the bacteria were collected at each sampling time by swabbing a 4 cm^2 area of

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the fillet surface three times with sterile cotton swabs. Total viable counts (TVC) were made on 2% NaCl supplemented plate count agar (PCA, Oxoid) incubated at 24 °C for 36 h. Enterobacteriaceae were determined in double-layer violet-red-bile-glucose agar (VRBG, Oxoid) incubated at 32 °C for 48 h. Lactic acid bacteria (LAB) were counted in double-layer, pH 5.6, MRS agar (Oxoid) incubated at 30 °C for 3 days and *Br. thermosphacta* in STAA agar (Oxoid) with streptomycin sulfate (500 mg/L) (Oxoid), cycloheximide (50 mg/L) (Oxoid), and thallous acetate (50 mg/L) (Oxoid) incubated at 20 °C for 3 days.

To characterize the dominant organisms, 20% of the colonies from PCA plates were randomly chosen (Ordóñez, 1979) and subcultured into triptone soya broth (TSB). Gram-negative and Gram-positive microorganisms were characterized as described by Dainty et al. (1979). For the ascertainment of *Br. thermosphacta*, the cellular fatty acid content of isolates was determined. This was done according to the method of Dodd and Dainty (1992), which has been successfully applied for stored tuna in modified atmospheres (López-Gálvez et al., 1995). The fatty acid methyl esters of a culture of the isolates were analyzed by gas chromatography with a Konik KNK 3000-HRGC chromatograph equipped with a dual flame ionization detector. The capillary column (25 m, internal diameter 0.22 mm) was packed with BP5 (0.25 μm) on fused silica. The identification of different fatty acid methyl esters was made by comparison with authentic standards (Sigma). As a reference, one strain of *Br. thermosphacta* 847 (ATCC11589) and another of *Lactobacillus casei* 478 (ATCC393) provided by the Spanish Type Culture Collection were used.

Chemical Analyses. The pH measurements were made with a Crison-micro pH model 2001 pH meter on 10 g homogenates of fish muscle with 10 mL of distilled water.

Total volatile nitrogen and trimethylamine were determined according to the Conway microdiffusion method (Pearson, 1973).

For nucleotide breakdown product determination the HPLC method described by López-Gálvez et al. (1995) was used. The results were expressed as *K* values, by the following formula: *K* value (%) = 100 (inosine + hypoxanthine)/(ATP + ADP + AMP + IMP + inosine + hypoxanthine). The extraction of short-chain fatty acids from samples was carried out as previously reported (López-Gálvez et al., 1995) using a homogenate of fish (15 g), water (45 mL), and an internal standard (0.2 mL) of 0.6% hexanoic acid solution. The homogenate was centrifuged (10000*g* for 10 min) and the resulting pellet reextracted with 20 mL of distilled water and recentrifuged. The two supernatants were combined, and the volume was recorded. Proteins were precipitated by adding 5 N NaOH (final pH of 12.0) and 25% $ZnSO_4$ followed by heating in a boiling water bath. To remove protein precipitate, the mixture was filtered through a Whatman no. 54 paper. The filtrate was taken to dryness by freeze-drying. The quantification of the short-chain fatty acids was carried out as reported by Ordóñez et al. (1991) using a Konik KNK 3000-HRGC chromatograph equipped with a capillary column (30 m × 0.53 mm) packed with DB−FFAP (1 μm) on fused silica. The identification of different short-chain fatty acids and the response factor calculations for the quantifications were made by comparison with standards (BDH).

D(−)- and L(+)-lactic acid were determined enzymatically according to the lactic acid dehydrogenase method, with kits from Boehringer Mannheim, following the instructions provided by the manufacturer.

Odor and Color Assessment. At each sampling time, after 15 min of equilibration at room temperature, the odor and color of three samples were assessed by an untrained panel of 10 members composed by staff of the department, who possess moderate experience in sensory evaluation of seafood products. For assessing, the hedonic scale of Hansen (1980) was used. This scale ranged from ideal (10), very good (8), regular (6), borderline (4), poor (2) to very poor (0). Panelists were asked to score the color and odor of fillets. Additionally, when off-odors were detected, panelists were asked to describe them (pungent, sour, marinade, stale, cabbage, and putrid).

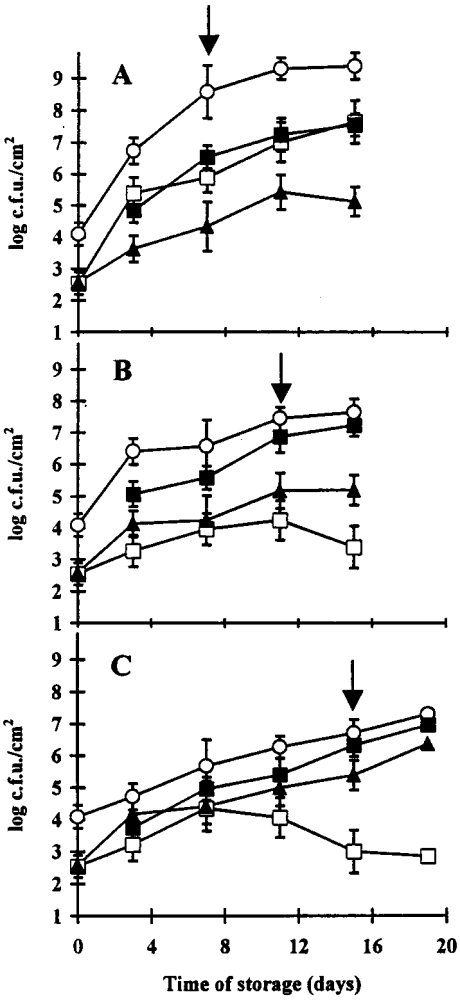

Figure 1. Changes in total viable (○), *Br. thermosphacta* (■), lactobacilli (▲), and Enterobacteriaceae (□) counts on refrigerated sole (*S. solea*) fillets stored in air (A), CO_2/air (20/80) (v/v) (B), and CO_2/air (40/60) (v/v) (C) atmospheres. The arrows indicate the end of the shelf life according to the panel assessment.

Likewise, panelists were required to record the slime presence. Final values were the mean and corresponding standard deviation calculated with the data obtained from each panelist for each of the three different sole fillets.

RESULTS AND DISCUSSION

Microbial Flora and Odor Assessment. The changes in the different microbial groups throughout storage of refrigerated sole fillets packaged in air, CO_2/air (20/80)(v/v), and CO_2/air (40/60)(v/v) are shown in Figure 1. No lag phase was clearly observed in the total viable count (TVC), at least in air storage. This may be because either the bacteria were adapted during aboard stowage in flaked ice or the lag phase occurred before the third day of sampling. After an initial TVC of $\sim 10^4$ cfu/cm^2, fillets packaged in air (Figure 1A) showed a microbial spoilage pattern similar to that previously described for refrigerated unpackaged fish (Hobbs, 1983). By the 3rd day of air packaging the TVC reached values close to 10^7 cfu/cm^2, on the 7th day values were $>10^8$ cfu/cm^2, and on the 11th day the counts were $>10^9$ cfu/cm^2. After 1 week of air packaging, fillets were coated with a bacterial slime and a noticeable off-odor (putrid, stale, pungent, and strong marinade) was detected (Figure 2). Enterobacteriaceae

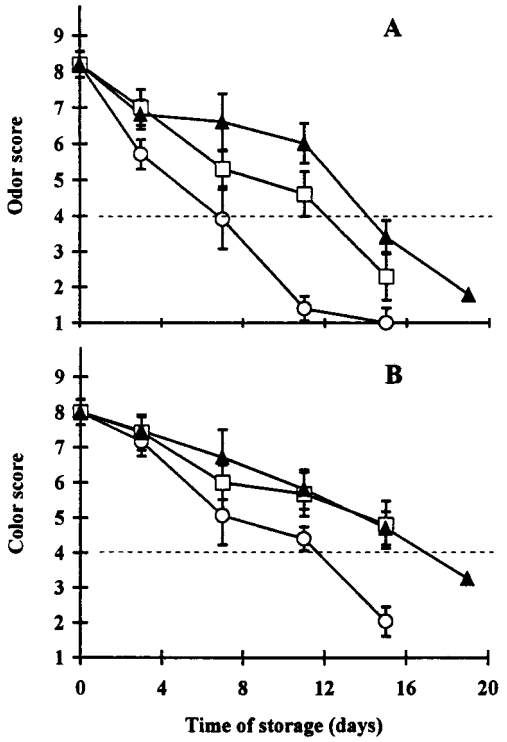

Figure 2. Changes in odor (A) and color (B) on refrigerated sole (*S. solea*) fillets stored in air (○), CO_2/air (20/80) (v/v) (□), and CO_2/air (40/60) (v/v) (▲) atmospheres. The horizontal dotted line indicates the sample-rejected level by the panel assessment.

counts were 2 log units lower than those of TVC (10^2 cfu/cm^2 at day 0, 10^5 cfu/cm^2 on the 3rd day, 10^6 cfu/cm^2 on the 7th day, and $>10^7$ cfu/cm^2 on the 15th day) and very similar to *Br. thermosphacta* counts obtained in STAA on the same sampling day.

On the seventh day of air storage (when samples were sensory rejected), the identification of the colonies on the PCA plates revealed that 95% of these were Gram-negative oxidase positive organisms, which could indicate that the spoilage flora was dominated by Gram-negative psychrotrophs, probably *Pseudomonas* and *Shewanella* types according to Stenström and Molin (1990) and Hobbs (1991).

Bacterial growth was delayed when sole fillets were packaged in atmospheres enriched with 20% CO_2 (Figure 1B). Values of TVC $>10^7$ cfu/cm^2 were reached after 11 days of storage. Afterward, only a slight increase in TVC content was recorded, and on day 15 the level of 10^8 cfu/cm^2 had not yet been reached. TVC levels in sole fillets packaged in refrigerated atmospheres enriched with 20% CO_2 were ~2 log units lower than those of TVC from sole fillets stored in air. Counts (~10^5 cfu/cm^2 after the 11th day) of MRS (lactic acid bacteria) in sole fillets were close to those found in samples packaged in air. However, the proportion of lactic bacteria in the TVC (0.1−1%) was higher in samples packaged in 20% CO_2 than in air. This indicates the low participation of this bacterial group in fish spoilage under 20% CO_2 atmosphere. Counts on VRBG (Enterobacteriaceae) reached a maximum value at 7−11 days of storage. Final counts after 15 days of storage were 4 log lower than the TVC. This bacterial group was efficiently inhibited by 20% CO_2. *Br. thermosphacta* (counts on STAA), not detected at day 0, showed an almost convergent trend with that of TVC from the third day of storage. Counts on STAA were ~1.5 log units lower than the TVC on the 3rd day of storage and about 0.3 log unit by the 11th day. These results indicated that *Br. thermosphacta* accounted for ~90% of the TVC in samples stored in 20% CO_2. The identification of colonies on the PCA on the 15th day revealed similar results with *Br. thermosphacta*, which accounted for ~95% of the colonies and the remaining 5% were *Lactobacillus* spp. This means that STAA agar is an appropriate selective medium for detecting *Br. thermosphacta* because no other bacteria may grow in it. However, it is not completely adequate for recovery of the total *Br. thermosphacta* present in the samples; that is, some cells of this bacterium are inhibited in this medium. If there was a total recovery of cells, an overlap of both graphs (that of *Br. thermosphacta* and that of the TVC) would be observed, but in fact the latter parameter is found to be ≈0.5 log unit higher in Figure 1B,C.

The results related with *Br. thermosphacta* dominance are in partial disagreement with previous research in tuna (López-Gálvez et al., 1995), which demonstrated how *Shewanella* growth was not completely inhibited by 20% CO_2. It is likely that in the present experiments the sole fillets were not initially contamined with *Shewanella*. However, because of the random nature of contamination, this is not necessarily the same as in other cases.

The spoilage pattern of sole fillets packaged in 20% CO_2 was very different from that observed in air. No slime was observed and a sour, light marinade odor indicative of spoilage was detected after the 11th day (Figure 2). Fillets stored in 20% CO_2 were rejected (score 4) by about the 11th day, whereas this occurred on the 7th day when samples were stored aerobically (Figure 2A). This means that it may be possible to extend the shelf life for 4 days.

Bacterial counts of fillets packaged in 40% CO_2-enriched atmospheres are shown in Figure 1C. A longer delay in bacterial growth than that of 20% CO_2 packaged fillets was found. Counts remained at a relatively low level ($<10^6$ cfu/cm^2) during 11−12 days, reaching values close to 10^7 cfu/cm^2 after 15 days of storage. Counts on MRS and STAA were about 1 and 0.5 log unit lower than those of TVC, respectively. Counts in VRBG were quite similar to those recorded in 20% CO_2-enriched atmospheres. Characterization of the PCA plate colonies on the 15th day showed a total flora comprised of *Br. thermosphacta* (66%) and *Lactobacillus* spp. (33%). As in the case of the 20% CO_2 atmosphere, no Gram-negative oxidase positive bacteria were detected, indicating that the *Pseudomonas*/*Shewanella* group was efficiently inhibited by 40% CO_2, which concurs with previous results (López-Gálvez et al., 1995). No slime was observed and the sour odor indicative of spoilage was detected after 14 and 18 days of storage, respectively. Samples were rejected by their odor after 14 days of storage, indicating that the shelf life of the fish may be extended from 7 to 15 days (Figure 2).

pH, Total Volatile Nitrogen, and Trimethylamine. The pH increased throughout the refrigerated storage of air-packaged sole (Figure 3). Only a slight increase was observed in samples stored in 20% CO_2, and no changes in the pH were observed when samples were stored in CO_2/air (40/60) (v/v). These findings are obviously related to the dominant bacteria in each atmosphere. The pH increased very quickly in the air

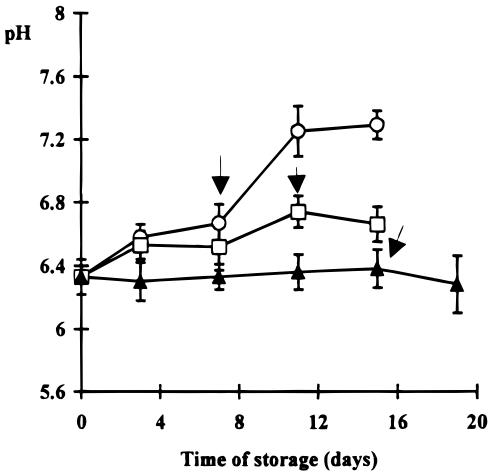

Figure 3. Changes in pH on refrigerated sole (*S. solea*) fillets stored in air (○), CO_2/air (20/80) (v/v) (□), and CO_2/air (40/60) (v/v) (▲) atmospheres. The arrows indicate the end of the shelf life according to the panel assessment.

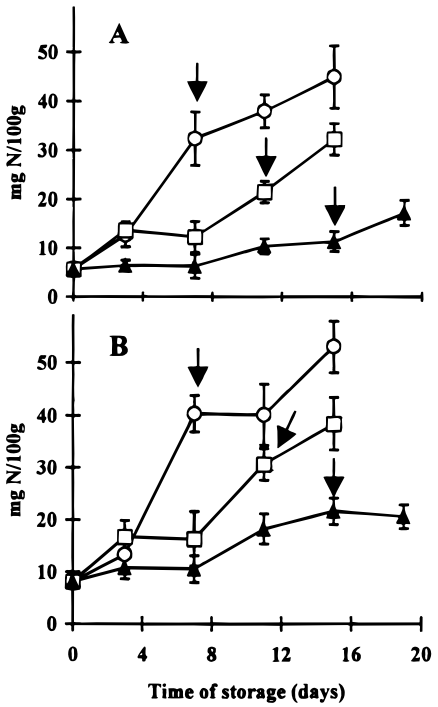

Figure 4. Changes in trimethylamine (A) and total volatile basic nitrogen (B) on refrigerated sole (*S. solea*) fillets stored in air (○), CO_2/air (20/80) (v/v) (□), and CO_2/air (40/60) (v/v) (▲) atmospheres. The arrows indicate the end of the shelf life according to the panel assessment.

atmosphere because of the growth and metabolism of the Gram-negative oxidase positive organisms, which cause the reduction of TMAO (Hebard et al., 1982) to TMA and other basic volatiles (Figure 4). The sole TMA and TBVN contents increased throughout the storage following a pattern similar to that observed for the pH. The formation of TMA and other basic volatiles was delayed by the use of CO_2/air atmospheres, and the effect was higher in the 40% CO_2 atmosphere than in the 20% CO_2 one, in total agreement with the higher inhibition of aerobic organisms by 40% CO_2 (Figure 1). Accordingly, the pH increases in the sole stored in both CO_2-enriched atmospheres were more gradual than corresponding increases in air. Similar findings have been described in fish and shellfish (Banks et al., 1980).

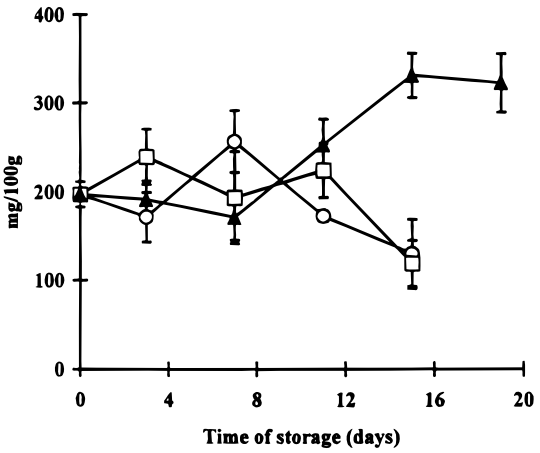

Figure 5. Changes in L(+)-lactic acid content on refrigerated sole (*S. solea*) fillets stored in air (○), CO_2/air (20/80) (v/v) (□), and CO_2/air (40/60) (v/v) (▲) atmospheres.

The almost constant pH in samples stored in CO_2/air (40/60) is probably due to the combined effect of microbial inhibition of aerobic flora with the concomitant low level of TMAO reduction (Banks et al., 1980; Wang and Brown, 1983) and the pH change resulting from the absorption of CO_2 on the sole surface and subsequent ionization of the carbonic acid formed, which acts as an additional microbial inhibitor (Genigeorgis, 1985).

Nucleotide Breakdown Products (*K* Value). The changes in the *K* value of the sole fillets were quite similar in samples stored in the three different atmospheres (data not shown). After an initial value of ~20%, a constant increase was observed throughout the storage, reaching values close to 80% by the 16th day. These results are similar to those previously reported for other fish stored aerobically in ice or CO_2-enriched atmospheres (Malle and Le Pezennec, 1992; López-Gálvez et al., 1995). No effect of the atmosphere composition on the *K* value was observed, which means that this phenomenon is completely independent of microbial growth.

D(−)- and L(+)-Lactic and Short-Chain Fatty Acids. D(−)-Lactic acid was not detected in samples stored in either the air or the CO_2/air (20/80) atmosphere throughout the storage period. However, this acid was detected in the CO_2/air (40/60) atmosphere after 1 day of storage, reaching at the end of the experiment (18 days) a value of 5 mg/100 g of wet fish (data not shown). As *Br. thermosphacta* is not a D(−)-lactic acid producer (Davidson et al., 1968), the low level determined must be formed by lactic acid bacteria, which reached a level $>10^6$ cfu/cm^2. These results are consistent with the microbiological changes (Figure 1) because in the other two atmospheres [air and CO_2/air (20/80)] the counts of lactic acid bacteria were 1−2 log units lower than that of CO_2/air (40/60).

The L(+)-lactic acid started at high levels (200 mg/100 g) as shown in Figure 5. Obviously, this was due to the anaerobic post-mortem glycolysis. During storage, a noticeable increase was observed only in the last third of the experiment in samples stored in the CO_2/air (40/60) atmosphere. This increase has been attributed to the growth of *Br. thermosphacta*, which was the dominant organism (Figure 1) and produces only this lactic acid isomer (Davidson et al., 1968) when it grows anaerobically (Hitchener et al., 1979).

Table 1 shows the levels of short-chain fatty acids at various times throughout the storage period. In samples

Table 1. Changes in Short-Chain Fatty Acids (Micrograms per Gram of Wet Muscle) in Refrigerated Sole Fillets Stored in Air, CO_2/Air (20/80) (v/v), and CO_2/Air (40/60) (v/v) Atmospheress

atmosphere	days	acetic	propionic	isobutyric	*n*-butyric	isovaleric
air	0	1.45 ± 0.36	ND[a]	ND	ND	ND
	3	2.54 ± 0.40	0.33 ± 0.07	0.27 ± 0.12	0.51 ± 0.09	0.49 ± 0.21
	7	19.70 ± 3.16	0.41 ± 0.10	2.75 ± 1.40	0.61 ± 0.20	2.80 ± 0.78
	11	12.75 ± 2.89	0.57 ± 0.04	5.85 ± 1.13	0.59 ± 0.16	6.65 ± 1.04
	15	17.29 ± 3.57	0.73 ± 0.23	8.49 ± 1.18	0.68 ± 0.16	7.27 ± 2.00
CO_2/air (20/80)	0	1.46 ± 0.36	ND	ND	ND	ND
	3	5.03 ± 1.12	0.34 ± 0.09	0.25 ± 0.04	0.73 ± 0.22	0.23 ± 0.12
	7	22.48 ± 4.61	0.45 ± 0.05	0.61 ± 0.09	0.93 ± 0.24	0.72 ± 0.19
	11	51.48 ± 6.42	0.71 ± 0.22	1.88 ± 0.26	1.40 ± 0.38	1.74 ± 0.31
	15	82.79 ± 12.28	1.51 ± 0.81	2.53 ± 0.91	1.01 ± 0.16	2.72 ± 0.80
CO_2/air (40/60)	0	1.46 ± 0.36	ND	ND	ND	ND
	3	4.03 ± 2.03	ND	0.22 ± 0.07	0.35 ± 0.08	0.41 ± 0.04
	7	2.52 ± 2.15	0.62 ± 0.15	0.29 ± 0.06	0.70 ± 0.15	0.36 ± 0.04
	11	16.68 ± 3.85	0.54 ± 0.08	0.35 ± 0.16	0.53 ± 0.15	0.59 ± 0.09
	15	26.75 ± 2.57	0.48 ± 0.43	0.72 ± 0.18	1.34 ± 0.48	0.88 ± 0.22
	19	29.32 ± 4.64	1.87 ± 0.70	0.64 ± 0.22	0.56 ± 0.16	0.59 ± 0.17

[a] ND, not detected.

stored in air, a clear rising trend in all acids was observed from the beginning, with the exception of *n*-butyric acid, in which, as in the case of both CO_2-enriched atmospheres, no consistent changes were observed. Increases of the other acids have been attributed to the dominant organisms, i.e., the aerobic flora. The *n*-butyric acid is probably not produced by the microorganisms present, according to phenomena occurring in both meat (Dainty et al., 1979; Ordóñez et al., 1991) and fish (tuna) stored under similar conditions (López-Gálvez et al., 1995). In both CO_2-enriched atmospheres an increase of the levels of acetic acid was also found. However, the increases in the CO_2/air (20/80) were clearly higher than those observed in the CO_2/air (40/60), although both counts at the end of the storage [11 days for the CO_2/air (20/80) and 15 days for the CO_2/air (40/60)] were practically the same and the dominant organisms were also the same (*Br. thermosphacta*). The levels of isobutyric and isovaleric acids increased throughout the experiment in samples stored in CO_2/air (20/80) atmospheres, but amounts fairly constant were recorded in the CO_2/air (40/60) atmosphere. The metabolism of *Br. thermosphacta* may be either aerobic and anaerobic. Under aerobic conditions, this bacterium generates acetoin and acetic, isobutyric, and isovaleric acids (Dainty and Hibbard, 1980, 1983; Dainty and Hofman, 1983) and 3-methylbutanal (Dainty and Hofman, 1983). On the other hand, under anaerobic conditions glucose is metabolized by *Br. thermosphacta* yielding, as the main metabolites, L(+)-lactic acid and ethanol in a ratio of almost 3:1 (Hitchener et al., 1979) but no acetoin (Hitchener et al., 1979; Dainty et al., 1979), and no (Hitchener et al., 1979), or small amounts of, short-chain fatty acids are produced (Dainty et al., 1979). According to these observations together with the results on short-chain fatty acids obtained in the present work, one can deduce that the metabolism of *Br. thermosphacta* is aerobic in samples stored in CO_2/air (20/80) atmosphere, as has been observed in tuna (López-Gálvez et al., 1995) and in meat (Ordóñez et al., 1991) stored in several modified atmospheres [CO_2/air (20/80), CO_2/O_2 (20/80), and (40/60)]. However, it seems that the metabolism of this bacterium becomes anaerobic in the CO_2/air (40/60) atmosphere, in which the percentage of oxygen is reduced from ~16% to 12%. This conclusion is also supported by the increase in L-(+)-lactic acid observed in samples during the last third of the storage period in the latter atmosphere (Figure 5). In this case, as *Br. thermosphacta* does not produce acetic acid under anaerobic conditions (Hitchener et al., 1979; Dainty et al., 1979), the acetic increases observed in the CO_2/air (40/60) atmospheres, clearly lower than those found in the CO_2/air (20/80) atmosphere, must be attributed to lactic acid bacteria, which reached levels clearly higher than those achieved in the CO_2/air (20/80). Similarly, the limitation of both glucose (as usually occurs in meat and fish after glycolysis post-mortem) and oxygen (as occurs in CO_2-enriched atmospheres) may cause the fermentation of glucose to switch the conversion of pyruvate to acetate instead of to lactic acid (Sedewitz et al., 1984; Murphy et al., 1985; Borch et al., 1991). A similar explanation has been given for the same phenomenon observed in poultry stored under modified atmospheres or vacuum (Kakouri and Nychas, 1994). The lower accumulation of short-chain fatty acids (including acetic, isobutyric, and isovaleric acids) and the higher content of lactic acid in the 40% CO_2-enriched atmosphere may have a beneficial sensory contribution for samples given that lactic acid has a less offensive flavor than that of the short-chain fatty acids.

An important inconvenience attributed to the use of modified atmospheres is the potential public health hazards related with *Clostridium botulinum*. It has been observed that *Cl. botulinum* type E and the nonproteolytic strains of types B and F may grow and produce toxin at 3.3 °C (Varnam and Evans, 1991), and botulinal toxin has been detected in some fishes packaged in modified atmospheres at temperatures below 5 °C, but the fish always showed evident spoilage signs (García et al., 1987; Post et al., 1985). However, at temperatures above 5 °C, toxin may be detected before spoilage (Post et al., 1985). For these reasons, no problem related with the botulinal toxin will occur if the temperature is mantained below 5 °C. Moreover, the inclusion of oxygen in the atmospheres reduces the risk of botulinal toxin production (Hotchkiss, 1988), which recommends the use of O_2 even in fish with low levels of myoglobin. Finally, it has been reported (Smith et al., 1990) that there is little conclusive evidence that

gas packaging represents a significantly greater hazard than packaging in air.

In conclusion, we recommend that an atmosphere of air enriched with 40% of carbon dioxide could be the most appropriate to extend the shelf life of sole fillets, and probably others from related lean fish (e.g. hake, cod, plaice, etc.) with a similar fat content. The enhanced shelf life achieved may constitute a very useful period for distribution of the fillets among consumers.

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