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High pressure effects on the quality and preservation of cold-smoked dolphinfish (*Coryphaena hippurus*) fillets

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Abstract

Different high pressure treatments (200–400 MPa) were tested to establish the best processing conditions for cold-smoked fish fillets of dolphinfish (*Coryphaena hippurus*). The water holding capacity (WHC) decreased at 400 MPa, while the shear strength by Kramer cell and Warner Bratzler and colour (L^*, a^*, and b^*) values rose at all the pressures applied; although in the case of the myotomes and myosepts the increase in shear strength was higher at 400 MPa, and the same was true for the a^* and b^* values. Lipid oxidation was prevented by the phenolic compounds from the smoking process. The best sensory attribute scores were achieved for cold-smoked dolphin fish pressurized at 300 MPa. During chilled storage the behaviour of WHC, shear strength, lipid oxidation, and colour in the sample pressurized at 300 MPa was quite stable, although sensory attributes declined over storage and fell off sharply after 65 d. Total volatile base levels rose over the storage period but did not exceed 35 mg TVBN/100 g. High pressure did not extend the shelf life, but it was able to diminish bacterial counts during early storage. Since pressurized products have achieved better acceptance than non pressurized ones, it could be utilised to obtain new products.

Keywords: High pressure; Smoking; Dolphinfish (*Coryphaena hippurus*); Preservation

1. Introduction

Cold-smoked fish is a high-value product that is very popular worldwide. Dolphinfish (*Coryphaena hippurus*) is much prized and abundant seafood, and it is usually prepared for consumption by cooking. Dolphinfish may offer an alternative to the species most commonly used for smoking, such as salmon, mackerel, trout, etc.

The quality of smoked fish is compromised mainly by the appearance of off-flavours; rancidity; changes in texture, colour or sensory attributes; the accumulation of spoilage products; and microbial growth. The shelf life of fish depends on these changes, which are in turn influenced by autolytic processes and microbial growth (Hansen, Gill, Røntved, & Huss, 1996). The latter is especially true for cold-smoked fish because the processing temperatures used do not produce microbial inactivation (Deng, Toledo, & Lillard, 1974). So, in these kind of products high pressure treatment is a potentially useful technology because it is known to delay the onset of spoilage of flesh by inhibiting microbial growth, but it may also bring about other negative or positive changes in the muscle tissue (Cheftel & Culioli, 1997).

Many studies have looked at the effects of high-pressure processing on muscle constituents, but few of these have reported on the changes taking place during storage of the pressurized muscle (Montero & Gómez-Guillén, 2005). Only a few studies have reported pressurization conditions that retain the appearance of raw muscle. For instance, turbot fillets were subjected to high pressures of between 100 and 200 MPa for 15 or 30 min and greater changes were observed with pressure and time (Chevalier, Le Bail, & Ghoul, 2001). In this study the fewest changes were recorded for treatment at 140 MPa/4 °C/15 min. Similar results have been published for hake (*Merluccius capen-*

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sis) muscle, which began to lose its raw appearance on pressurization of the muscle at 200 MPa (three 5-min pulses) at 7 °C (Hurtado, Montero, & Borderías, 2000). Other researchers (Amanatidou et al., 2000) studied the effect of high pressures on the sensory attributes of fresh salmon and on its chilled shelf life, noting that a product exhibiting the least sensory alteration and the longest shelf life was obtained by a treatment of 150 MPa/5 °C/10 min.

Attention has recently focused on using high pressure to improve the quality of cold-smoked salmon (Lakshmanan, Piggott, & Paterson, 2003), but it has not been studied on other species. Since low-temperature smoking produces less alteration in fish than hot smoking (Gómez-Guillén, Montero, Hurtado, & Borderías, 2000), cold-smoked fish could be affected by high pressure nearly in the same way as fresh fish.

Lakshmanan, Miskin, and Piggott (2005), studied the effect of high-pressure processing on the chilled storage of vacuum-packed cold-smoked salmon and observed that higher pressure levels (300 MPa) yielded products that were lighter in colour with higher levels of shear strength and lipid oxidation, resulting in perceptible alterations in the sensory attributes. Lower pressures of up to 200 MPa for 20 min produced a sensorially acceptable product, and the processing time was suitable for commercial production economies. Processing at 200 MPa at 9 °C for 20 min failed to prevent the growth of *Listeria monocytogenes* and spoilage microorganisms of chilled cold-smoked salmon (Lakshmanan & Dalgaard, 2004). Pressurization was also reported to affect proteins and proteolytic enzymes in cold-smoked salmon (Lakshmanan, Paterson, & Piggott, 2005). Thus, while the properties of pressurized cold-smoked salmon have been characterized in detail, the effects of pressurization of smoked fish will depend on the intrinsic properties of each species, such as lipid content, enzymatic activity levels, etc., as well as on the extent of salting and smoking. Having this in mind, no information is available on the effect of high-pressure processing on the quality and shelf life of other fish species with lower fat content and other muscle characteristics.

The object of this work was to study the effect of different levels of pressurization on the quality of vacuum-packed cold-smoked dolphinfish and on the subsequent chilled storage of the final product prepared using the most favourable treatment conditions.

2. Materials and methods

2.1. Sample preparation

Dolphinfish (*C. hippurus*) was caught off Mallorca island in the Mediterranean Sea and kept at 4 °C for 48 h. Fish were headed, gutted, and washed. Each fish was split in half lengthwise and the spine removed to produce two fillets. Mean individual weight was 1.29 ± 2.45 kg, length 0.51 ± 0.04 cm.

Proximate analysis of the muscle was performed according to the AOAC (AOAC, 1984) for moisture (method 24003), ash (method 1821), and protein (method 24024). Crude fat was determined following the method of Bligh and Dyer (1959). The results were: crude protein $21.98 \pm 0.75\%$, moisture $75.08 \pm 1.09\%$, crude fat $1.53 \pm 0.19\%$, and ash $1.32 \pm 0.19\%$.

2.2. Salting and smoking

Samples were salted in brine (15-% NaCl) at 20 °C [ratio 4:1 (w/v)] for 30 min. The salt content in the muscle was $2.0 \pm 0.15\%$ as measured by a model SSX 56-NA salinometer (Ebro Electronic, Ingoldstadt, Germany), previously calibrated using different concentrations of sodium chloride.

After salting, fillets were quickly rinsed in water and immediately smoked in a smoking oven (model Micro 40, ELLER, Merino, Italy) at 26 ± 1 °C for 20 min using a traditional cold-smoking process with beech wood, followed by an air drying step during 90 min at the same temperature. The smoked fillets were then vacuum-packed in flexible bags (Cryovac BB4L, Barcelona, Spain).

2.3. High pressure treatment

In order to select the best high pressure conditions (first experiment), the vacuum-packed cold-smoked fillets were pressurized at 200, 300, or 400 MPa at 20 °C for 15 min in a pilot high pressure unit (ACB 665, GEC Alsthom, Nantes, France). The temperature of the immersion medium (distilled water) was regulated by a thermocouple connected to programmed thermostating equipment (model IA/2230 AC, INMASA, Barcelona, Spain). The pressure was increased by 2.5 MPa/s. A sample held at atmospheric pressure was used as the control.

Two batches were prepared to study the behaviour of both the pressurized and the unpressurized vacuum-packed cold-smoked fish during chilled storage (second experiment). One batch (HP) was pressurized at 300 MPa at 20 °C for 15 min and then stored at 5 °C, and the other batch (No HP) was stored directly at 5 °C without undergoing high pressure treatment.

2.4. Water holding capacity

The WHC was determined using the method described by Gómez-Guillén et al. (2000). Determinations were carried out at least in triplicate.

2.5. Thiobarbituric acid reactive substances

The thiobarbituric acid reactive substances (TBARS) were determined using a modified version of the method of Vyncke (1970), incubating at 20 °C for 15 h instead of heating. A standard curve was constructed with 1,1,3,3-tetraethoxypropane (Sigma Chemical Co., St. Louis, MO,

USA) as described by Botsoglou et al. (1994). Results have been expressed as mg of malonaldehyde per kg of muscle. Determinations were carried out at least in triplicate.

2.6. Rheological properties

The shear strength (N/g) of the fish muscle was determined using the Kramer shear test as described in Montero and Borderías (1990).

The shear strength (N) of myotomes and myosepta was determined using the Warner–Bratzler shear test, as described in Gómez-Guillén et al. (2000). Each point is the mean of at least five measurements.

2.7. Colour measurements

The colour parameters lightness (L^*), redness (a^*), and yellowness (b^*) were measured using a Hunter Lab colorimeter (Hunter Associates Laboratory Inc., Reston, VA, USA). Determinations were carried out on both whole fillets and slices made from them in the first experiment and in fillets in the second one. Each point is the mean of at least six measurements.

2.8. Sensory analysis

Descriptive sensory analysis was performed by nine laboratory-semitrained assessors to evaluate differences among the different pressure treatments. The attributes assessed were overall appearance, odour, taste, firmness, juiciness and general acceptability, rated on a scale of agreeable, neutral, and disagreeable. The results are expressed as the percentage of assessors that evaluated each attribute for each rate.

2.9. Total volatile basic nitrogen

The total volatile basic nitrogen (TVBN) determination was effected according to the method of Antonacopoulos and Vyncke (1989). The results have been expressed as mg of N per 100 g of muscle. Determinations were carried out at least in triplicate.

2.10. Microbiological analysis

An amount of 25 g of sample was homogenized in 225 ml of buffered peptone water (Scharlau 02-277) in a sterile Stomacher bag. This suspension was used to prepare decimal dilutions in peptone water.

Plate counts of aerobic bacteria at 15 °C, luminescent bacteria, and SH_2 producing bacteria were carried out using Iron agar (Scharlau 01-407) supplemented with 1% salt. Seeding was by surface looping of 0.1 ml, the total number of colonies being read following incubation at 15 ± 1 °C for 5 d. Luminescent bacteria were enumerated in a dark room after incubation in the same conditions as above for 3 d and 5 d. The SH_2 producing bacterial counts were effected after incubation for 5 d, likewise in the same conditions as above. Lactic acid bacterial counts were performed by inoculating on MRS agar supplemented with 3% salt (de Man, Rogosa, Sharpe) (Merck 1.10660) and covering with another layer of agar, followed by incubation at 30 ± 1 °C for 3 d. Determinations were carried out in duplicate.

2.11. Statistical analysis

One-way and two-way analyses of variance were carried out. The SPSS® computer program (SPSS Inc., Chicago, IL, USA) was used. Differences in pairs of mean values were evaluated by the Tukey-b test for a confidence interval of 95%.

3. Results and discussion

3.1. Effect of high pressure conditions

The WHC of the cold-smoked dolphinfish pressurized at 200, 300, and 400 MPa and of the unpressurized cold-smoked fish (No HP batch) appears in Fig. 1. The WHC was similar in the unpressurized batch and in the samples pressurized at 200 and 300 MPa, but there was a significant decrease ($P \leqslant 0.05$) in the batch pressurized at 400 MPa. Although the moisture level was similar (70%) in all the samples, the lowest WHC in the 400 MPa treated batch could be the result of disintegration of the muscle tissue, thereby reducing water–protein interactions.

The dolphinfish muscle pressurized at 200, 300, and 400 MPa underwent an increase ($P \leqslant 0.05$) in shear strength (N/g) measured by the Kramer test compared with the control batch (Fig. 2), attributable to compaction produced by the high pressure. Working with the raw flesh of bluefish (*Pomatomus saltratix*) and cod (*Gadus morhua*), other researchers (Angsupanich & Ledward, 1998; Ashie, Simpson, & Ramaswamy, 1997) observed that under progressively applied pressure muscle hardness increased due to compaction, up to a maximum (at 100 MPa at ambient temperature in bluefish muscle and at 400–600 MPa in cod muscle) after which hardness decreased as a result of muscle disintegration.

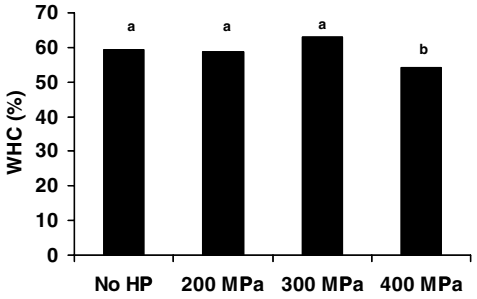

Fig. 1. Water holding capacity (WHC) of smoked dolphinfish processed at different conditions. The different letters (a, b) indicate significant differences ($P \leqslant 0.05$).

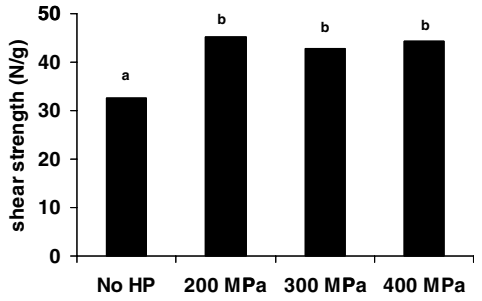

Fig. 2. Shear strength (N/g) measured by Kramer cell of smoked dolphinfish processed at different conditions. The different letters (a, b) indicate significant differences ($P \leqslant 0.05$).

The shear strength (N) of the myotomes and myosepts determined by the Warner–Bratzler cell also exhibited a slight increase at 200 and 300 MPa and a higher increase at 400 MPa both for the myotomes, which consist of myofibrillar protein, and for the myosepta, which are made up mainly of collagen (Fig. 3). The measurement of a peak associated with collagen rupture could be indicative of a mild smoking treatment, because at high temperature the collagen melts and the structure disappears (Gómez-Guillén et al., 2000). Amanatidou et al. (2000) found that pressure treatment at 100 MPa/5 °C/10 min did not increase the cutting strength of fresh Atlantic salmon muscle compared with an untreated batch, but higher values were obtained as the pressure increased. Similarly, Lakshmanan, Miskin et al. (2005) also reported an increase in the cutting strength of smoked salmon pressurized at 300 MPa, though not at 100 or 200 MPa.

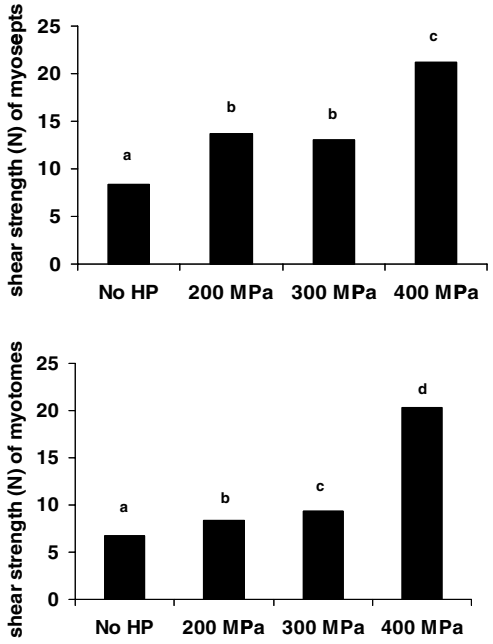

Fig. 3. Shear strength (N) measured by Warner Bratzler cell of smoked dolphinfish processed at different conditions. The different letters (a, b) indicate significant differences ($P \leqslant 0.05$).

Fig. 4 depicts the formation of thiobarbituric acid reactive substances (TBARS). The amount of TBARS in the pressurized fish was somewhat lower than in the unpressurized batch ($P \leqslant 0.05$), although high pressure treatment has been observed to cause lipid oxidation in fish muscle (Cheah & Ledward, 1996; Oshima, Ushio, & Koizumi, 1993; Pérez-Mateos, Gómez-Guillén, Hurtado, Solas, & Montero, 2002). One possible explanation is the presence of antioxidant phenolic compounds reported in smoked fish (Guillén, Errecalde, Salmerón, & Casas, 2006). Montero, Giménez, Pérez-Mateos, and Gómez-Guillén (2005) noted that phenol-based antioxidants such as rosemary and quercetin extracts protected both pressurized and unpressurized homogenized fish muscle from lipid oxidation. There were slight differences for the different high pressure conditions, with the lowest values ($P \leqslant 0.05$) being recorded for the sample treated at 300 MPa. A study carried out on pressurized cold-smoked salmon has shown that a pressure of 300 MPa induced greater lipid oxidation than 100 or 200 MPa (Lakshmanan, Miskin et al., 2005). Salmon is a fatty fish, whereas dolphinfish is a less fatty species, so the lower muscle fat content could result in lower levels of pressure-induced oxidation. Differing phenol contents from smoking could also account for the different lipid oxidation behaviours. Angsupanich and Ledward (1998) found that raw cod (a lean fish) muscle is very stable at pressures up to 400 MPa.

Colour changes caused by pressure were observed in both the fillets and the slices (Fig. 5). The lightness value was in most cases significantly lower ($P \leqslant 0.05$) in the fillets than in the slices and increased with pressure, especially in the slices.

The redness value in the fillets increased ($P \leqslant 0.05$) with pressure. However, in the slices, for which the a^* values were negative, only limited changes ($P \leqslant 0.05$) were observed. Similarly, yellowness underwent a significant ($P \leqslant 0.05$) pressure-induced increase in the fillets but not in the slices. Pressure-related changes of this kind have been attributed to denaturation of the sarcoplasmic and myofibrillar proteins (Angsupanich & Ledward, 1998).

Changes in the appearance of fish muscle upon pressurization depend largely on the pressure level, the muscle becoming whiter and more opaque with increasing pressure

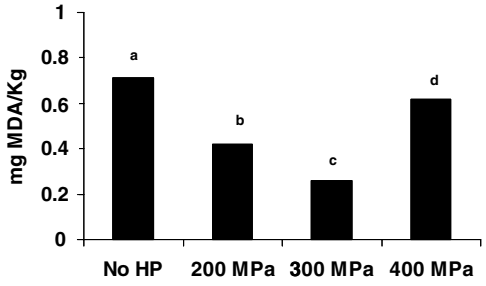

Fig. 4. Thiobarbituric acid reactive substances (TBARS) of smoked dolphinfish processed at different conditions. The different letters (a, b) indicate significant differences ($P \leqslant 0.05$).

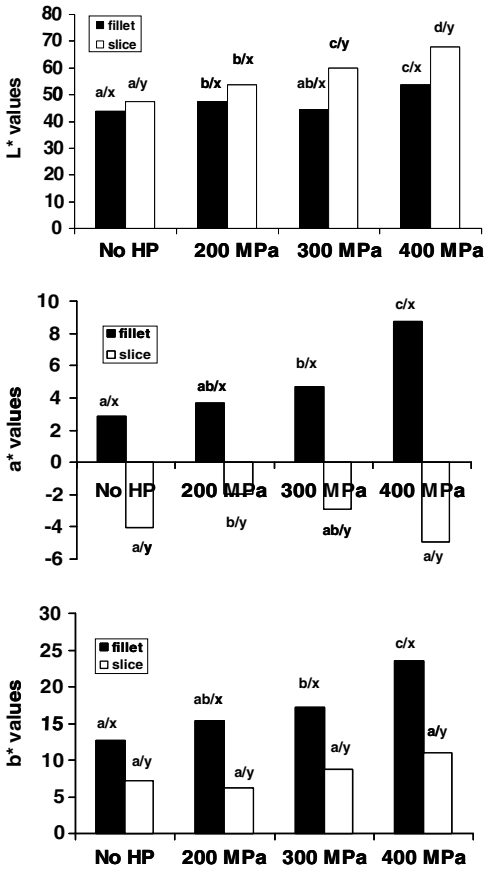

Fig. 5. Colour values (L^*, a^*, b^*) of smoked dolphinfish processed at different conditions. The different letters (a, b) indicate significant differences ($P \leqslant 0.05$) between processing conditions. The different letters (x, y) indicate differences ($P \leqslant 0.05$) between the fillets and the slices.

(Montero et al., 2005). Various studies have dealt with the changes induced by high pressure in raw fish (Amanatidou et al., 2000; Angsupanich & Ledward, 1998; Hurtado et al., 2000; Oshima et al., 1993), but there are very few dealing with smoked fish (Lakshmanan & Dalgaard, 2004; Lakshmanan, Miskin et al., 2005; Lakshmanan, Paterson et al., 2005). Working with pressurized vacuum-packed cold-smoked salmon, Lakshmanan, Miskin et al. (2005) also observed opaqueness and increased lightness values at 300 MPa during refrigerated storage, irrespective of storage time. They found that the redness values were not particularly affected by pressure levels ⩽200 MPa and that there were only minor changes in yellowness. Most studies agree that from 200 MPa upwards the changes in the appearance of fresh fish are so great that the muscle no longer looks fresh, although there can be slight differences depending on the species. These colour changes usually take the form of increases in the lightness (L^*) and yellowness (b^*) values and a decrease in the redness (a^*) value, especially in species with higher proportions of red muscle. Oshima et al. (1993) observed that in pressurized raw cod and mackerel muscle the L^* value increased with isostatic pressure. They also recorded a decrease in the a^* value in mackerel muscle, but no changes in the b^* value were observed. As indicators of the total colour difference, the ΔE ($\Delta E^2 = \Delta L^2 + \Delta a^2 + \Delta b^2$) and tristimulus colour values have shown that there were no differences in colour between sample treatments at up to 200 MPa at ambient temperature for 10 min in bluefish (*P. saltratix*) and sheepshead (Oshima et al., 1993). However, carp (*Cyprinus carpio*) muscle displayed appreciable differences at 100 MPa at 20–22 °C for 10 min and 300 MPa at 20–22 °C for 10 min (Yoshioka & Yamamoto, 1998). Differences arose in hake (*M. capensis*) muscle when the muscle was pressurized at 200 MPa in three 5-min pulses at 7 °C (Hurtado et al., 2000) and in turbot (*Scophthalmus maximus*) when the muscle was pressurized at 180 MPa at ambient temperature for 30 min (Chevalier et al., 2001). Processing at 100 MPa/5 °C/60 min and higher than 150 MPa at any time and temperature studied caused a significant rise in lightness in raw salmon, while redness values declined with increasing pressure and processing time (Amanatidou et al., 2000).

The sensory evaluation results (Fig. 6) indicated that only the 300 MPa treatment achieved 100% general acceptability. Nevertheless, other batches also attained high values for certain variables, and the large area shaded for the "agreeable" rating for the 200 MPa batch was indicative of the highly favourable scores awarded by the assessors. The unpressurized smoked fish batch received lower acceptability scores, but it was mainly rated as neutral, not as "disagreeable". The 400 MPa treatment was by far the worst for all the attributes assessed.

3.2. Chilled storage behaviour

The high pressure treatment chosen to study the behaviour during chilled storage was 300 MPa, which was the highest one with low changes in colour and high sensory acceptance. Water holding capacity values (Fig. 7) remained stable and were similar for both the pressurized (HP) and unpressurized (No HP) samples, but the values for the pressurized smoked dolphinfish were slightly lower ($P \leqslant 0.05$).

Shear strength was lower in the unpressurized smoked sample, although the differences recorded during storage were not always significant (Fig. 8). Only relatively small fluctuations were observed in both batches during chilled storage. Other workers have also reported constant shear strength values during storage in pressurized samples of such other species as octopus (*Octopus vulgaris*) (Hurtado, Montero, & Borderías, 2001) and prawns (*Penaeus japonicus*) (López-Caballero, Pérez-Mateos, Montero, & Borderías, 2000). Lakshmanan, Miskin et al. (2005) found that both pressurized (irrespective of treatment conditions) and unpressurized cold-smoked salmon became tougher as chilled storage advanced, with the pressurized batches being the firmest in all cases. Amanatidou et al. (2000) reported similar findings in fresh Atlantic salmon.

Fig. 9 depicts the formation of thiobarbituric acid reactive substances (TBARS) during chilled storage. As men-

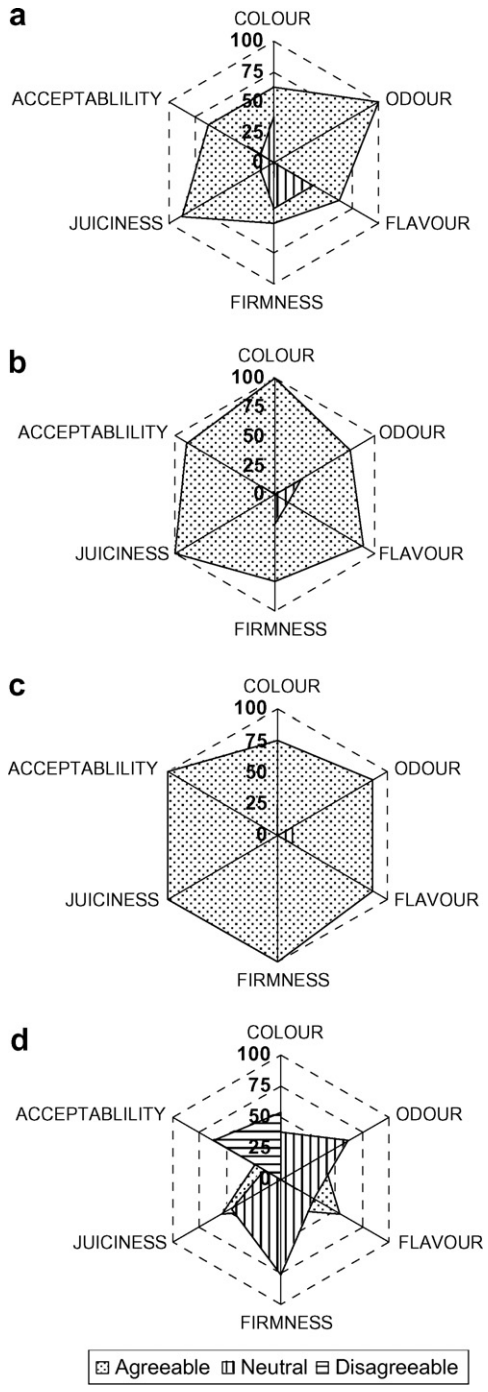

Fig. 6. Sensory analysis of smoked dolphinfish processed at different conditions. (a) No HP, (b) 200 MPa, (c) 300 MPa and (d) 400 MPa.

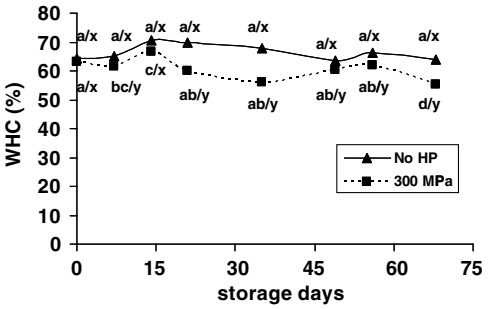

Fig. 7. Water holding capacity (WHC) of pressurized (300 MPa) and unpressurized smoked dolphinfish during chilled storage. The different letters (a, b) indicate significant differences ($P \leqslant 0.05$) within each treatment with storage time. The different letters (x, y) indicate significant differences ($P \leqslant 0.05$) between the batches on each sampling date.

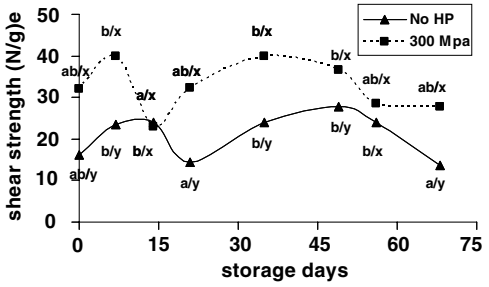

Fig. 8. Shear strength (N/g) measured by Kramer cell of pressurized (300 MPa) and unpressurized smoked dolphinfish during chilled storage. The different letters (a, b) indicate significant differences ($P \leqslant 0.05$) within each treatment with storage time. The different letters (x, y) indicate significant differences ($P \leqslant 0.05$) between the batches on each sampling date.

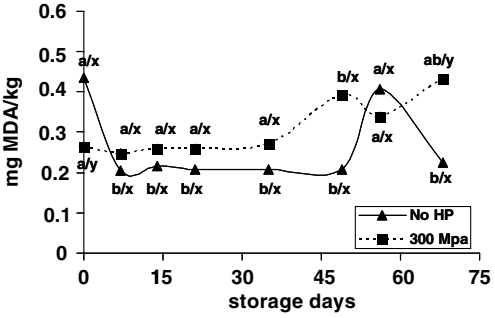

Fig. 9. Thiobarbituric acid reactive substances (TBARS) in pressurized (300 MPa) and unpressurized smoked dolphinfish during chilled storage. The different letters (a, b) indicate significant differences ($P \leqslant 0.05$) within each treatment with storage time. The different letters (x, y) indicate significant differences ($P \leqslant 0.05$) between the batches on each sampling date.

tioned previously, phenolic compounds are probably responsible for preventing high pressure-induced lipid oxidation. Furthermore, the TBARS level was stable in both batches during chilled storage although it was little higher but not always significant at the end of the storage for the HP batch. Conversely, Lakshmanan, Miskin et al. (2005) reported a very appreciable increase in TBARS levels during chilled storage in pressurized (300 MPa) cold-smoked salmon, the difference being attributable to the higher fat content of salmon and/or to different smoking compound residues. Amanatidou et al. (2000) found that high pressure treatment (150 MPa/5 °C/10 min) of raw salmon muscle did not raise TBARS values except when oxygen was added to the packaging atmosphere. Nevertheless, TBARS levels increased considerably after storage at 5 °C for 14 d due to the absence of phenolic compounds.

Fig. 10 plots changes in colour during chilled storage. Lightness was quite stable in the pressurized and unpressurized smoked dolphinfish slices, with values in the pressurized sample being higher than in the unpressurized batch, particularly during the first 14 d of storage ($P \leqslant 0.05$). As previously indicated, high-pressure processing barely affected redness, but during chilled storage the a^* value underwent a significant increase in both batches. Pressurization brought about a slight increase in yellowness, but values held relatively steady during chilled storage. In contrast, the yellowness value increased in batch No HP during storage. Lakshmanan, Miskin et al. (2005) found that there was a slight increase in the redness and yellowness values during storage of pressurized (100–200–300 MPa) and unpressurized cold-smoked salmon at 5 °C.

A study on hake (*M. capensis*) muscle pressurized (three 5-min pulses) at 200 MPa and 7 °C, which retained the appearance of raw fish, showed that the colour parameters stayed constant during storage at 2–3 °C (Hurtado et al., 2000). The same effect was observed in pressurized cod sausages (López-Caballero, Gómez-Guillén, Pérez-Mateos, & Montero, 2005) and raw salmon muscle (Amanatidou et al., 2000) during chilled storage.

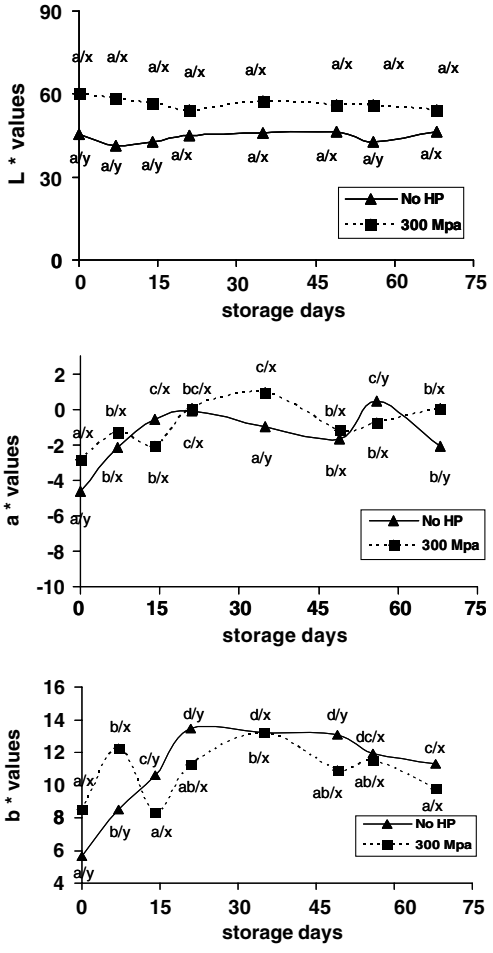

Fig. 10. Colour (L^*, a^*, b^*) of pressurized (300 MPa) and unpressurized smoked dolphinfish during chilled storage. The different letters (a, b) indicate significant differences ($p \leqslant 0.05$) within each treatment with storage time. The different letters (x, y) indicate significant differences ($p \leqslant 0.05$) between the batches on each sampling date.

Fig. 11 depicts the results of the sensory evaluations carried out during storage. In the interest of clarity of the plots, some storage days have been omitted. The "agreeable" rating predominated in the scores for the unpressurized smoked dolphinfish in the early weeks of storage, achieving ratings of between 75% and 100% for all the sensory attributes evaluated. At day 65 scores were drastically reduced, and storage was considered to be at an end. The "neutral" rating was employed mainly at 65 d of storage, while "disagreeable" was assessed only for odour (<25%), flavour (30%), firmness (50%), juiciness (50%) and general acceptability (30%) at day 65. A similar scoring pattern is observable for the pressurized smoked dolphinfish. Scoring was mainly "agreeable", though percentages decreased over storage. The "neutral" rating increased, mainly around day 65. At this same time the "disagreeable" rating also increased in the pressurized smoked fish. On the whole, colour and odour were not rejected at any time during storage, the main causes of rejection being juiciness, firmness, and flavour. Lakshmanan, Miskin et al. (2005) found that pressurization of cold-smoked salmon at 200 MPa/20 °C/10 min changed sensory properties and in consequence, the product was not accepted. However, in the present study pressurized cold-smoked dolphinfish (300 MPa/20 °C/15 min) achieved better scores than non pressurized one. It could be due to the fact that panellists do not expect specific sensory properties as in smoked salmon occurs, since dolphinfish is not usually prepared by smoking. Moreover the different characteristics of this species could make it more suitable for high-pressure processing than smoked salmon.

Fig. 12 plots microbial growth during the chilled storage period. Aerobic and lactic acid bacteria were present at low levels in the No HP batch at the beginning of storage. High pressure treatment lowered counts of both aerobic and lactic acid bacteria to levels below the detection threshold for three weeks, as a result of the resulting stress on bacterial cells. After that time counts rose to 6 log cfus and were the same as in the unpressurized sample by day 65, which was therefore taken as marking the end of shelf life for both the pressurized and unpressurized batches. Neither luminescent nor SH_2-producing bacteria were observed in either of the two batches at any time during storage. High-pressure processing has been widely reported to extend the shelf life of foods, including fish products. Paarup, Sánchez, Peláez, and Moral (2002) observed that the higher the pressure, the longer the shelf life of squid muscle, the shelf life during chilled storage at 4 °C being lengthened from 7 d (without pressurization) to 28 d (400 MPa/20 °C/15 min). Similar findings were described for salmon, hake, octopus, and prawns (Amanatidou et al., 2000; Hurtado et al., 2000, 2001; López-Caballero et al., 2000). Less work has been carried out on the storage behaviour of high pressure-treated smoked fish. Lakshmanan and Dalgaard (2004) studied the effect of high-pressure processing

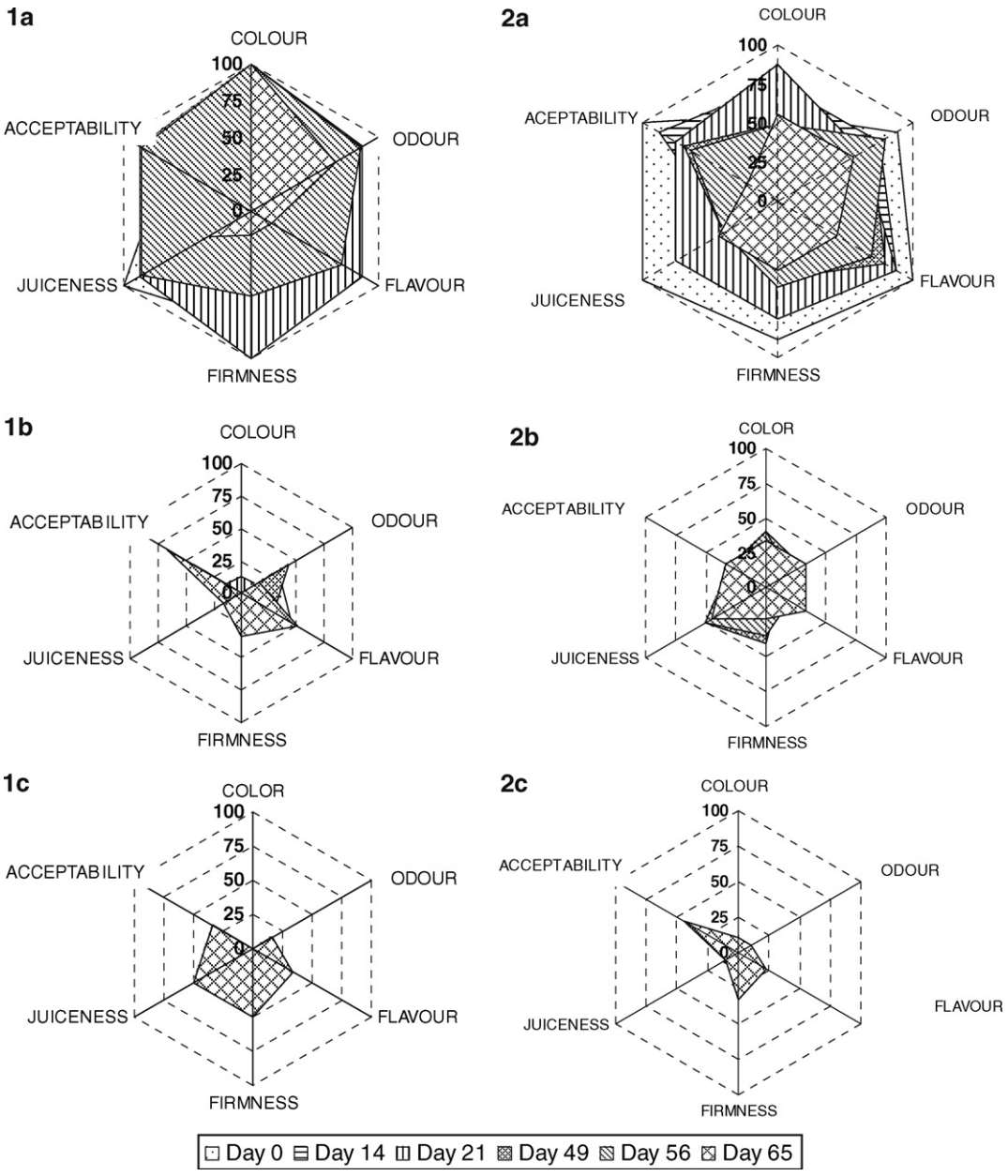

Fig. 11. Sensory analysis of smoked dolphinfish during chilled storage. (1) No HP batch; (2) HP batch; (a) agreeable, (b) neutral, (c) disagreeable.

(200 MPa/9 °C/20 min) on cold-smoked salmon during storage at 5.8 ± 0.4 °C. They found that both the pressurized and the unpressurized samples took between 60 and 67 d to reach 7 log units for both total aerobes and total lactic acid bacteria, 7 log units being the level deemed unacceptable and marking the end of shelf life for refrigerated fish. On the other hand, on chilled storage day 30 they observed that counts in the pressurized sample were 1–3 log units lower than the levels in the unpressurized control batch. These findings are similar to the observations made in this study, with a storage life of about 65 d in both HP and No HP batches and lower microbial counts during storage in HP batch.

The total volatile basic nitrogen (TVBN) present in fish is an index of spoilage and is related to microorganism growth (Fig. 13). Similar initial values of 15.41 ± 1.28 mg of TVBN/100 g in the unpressurized (No HP) smoked fish muscle and 15.13 ± 0.84 mg of TVBN/100 g in the pressurized (HP) smoked fish muscle were recorded on the first day of storage. The TVBN value increased over the storage period to 30 mg TVBN/100 g in batch No HP on day 49, where it held steady until the end of storage. Similar behaviour was observed in batch HP, although bacterial counts were under the detection threshold during the first three weeks of storage. The European Union establishes an upper limit between 25 and 35 mg TVBN per 100 g of fish muscle depending on the species (Commission decision 95/149/EEC). Nevertheless, it has not established limits for either all the species or processed products such as smoked fish. According to our results, an upper limit of 30 mg

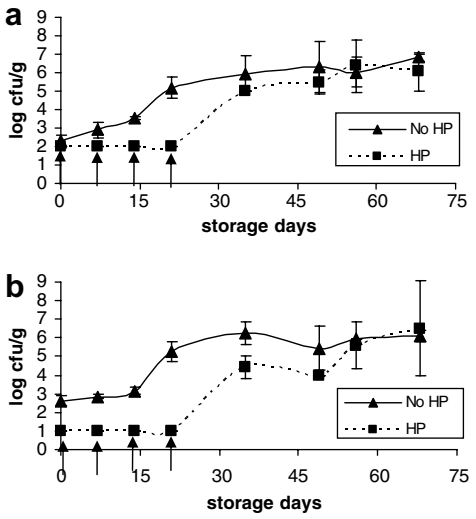

Fig. 12. Aerobic plate counts (a) at 15 °C and lactic acid bacterial counts (b) for pressurized (300 MPa) and unpressurized smoked dolphinfish during chilled storage. Arrows indicate values under the detection threshold.

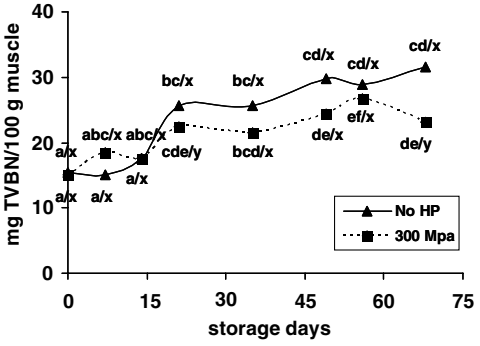

Fig. 13. Total volatile basic nitrogen in pressurized (300 MPa) and unpressurized smoked dolphinfish during chilled storage. The different letters (a, b) indicate significant differences ($P \leqslant 0.05$) within each treatment with storage time. The different letters (x, y) indicate significant differences ($P \leqslant 0.05$) between the batches on each sampling date.

TVBN per 100 g of fish muscle could be adequate for both pressurized and unpressurized cold-smoked dolphinfish.

In microbiological terms, high pressure did not prolong the shelf life though it did achieve better microbiological quality during chilled storage. So in this study both salting and smoking were sufficient to delay microbial growth. Although high pressure induces sensorial changes, in cold-smoked dolphinfish these are well accepted (300 MPa/20 °C/15 min) and so this technology may be useful in order to obtain new products.

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