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Texture, color, lipid oxidation and sensory acceptability of gamma-irradiated marinated anchovy fillets

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HIGHLIGHTS

- Marinated anchovies were γ-irradiated at 2, 3 and 4 kGy and stored at 4 °C (10 months).
- Irradiation slightly hardened the texture and reduced its softening during storage.
- Irradiated marinades had good sensory acceptability without differences with controls.
- Irradiation improved the quality by reducing texture softening and color changes.

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ABSTRACT

The effect of gamma irradiation (0, 2, 3 and 4 kGy) on vacuum-packed marinated anchovy fillets was analyzed for their texture, color, lipid oxidation and sensory acceptability after 10 months under refrigeration. Marinated (3% acetic acid, 10% sodium chloride and 0.2% citric acid) *Engraulis anchoita* fillets were vacuum-packed and irradiated with a cobalt-60 source at a semi-industrial irradiation facility. The irradiation caused a slight increase in hardness values regardless of the applied dose but maintained a consistent texture over the 10 months, even though the control samples softened, most likely due to degradation. This hardness increase did not affect the textural sensory acceptability. Irradiation did not modify the color but still reduced color changes during storage, benefitting the product's quality. TBARS was increased in every sample throughout storage, but irradiation decreased these values. Sensory acceptability was not affected by gamma irradiation. Therefore, gamma irradiation could be successfully applied to this type of product for the purpose of shelf-life extension.

1. Introduction

The anchovy *Engraulis anchoita* is the most abundant pelagic species in the Southwestern Atlantic Ocean. It is found South of Brazil (24°S) to the Argentine Patagonia (48°S) and, due to its current underexploitation, has an intriguing economic potential (Massa et al., 2007; Pastous-Madureira et al., 2009). Argentina, which is a pioneer in the exploitation and processing of *E. anchoita*, is the main manufacturer of several products from this species, including marinated anchovies (Pastous-Madureira et al., 2009). Marination is performed by immersing the whole fish or portions of the fish in water solutions containing weak organic acids and sodium chloride as preservation agents in order to delay enzymatic and bacterial activities. The resulting product is a semipreserved, ready-to-eat fish with a characteristic flavor that is considered a high-value delicacy. This product has an extended but limited shelf-life (Capaccioni et al., 2011; Fuselli et al., 1994; Meyer, 1965), which is usually estimated at about six months based on food control regulatory organizations.

Fish and fish products are highly perishable foods because of their high protein and fat contents; much of the degradation is due to Gram negative bacteria (Huss, 1999). Gamma irradiation has been widely used to preserve a variety of foodstuffs, because it inactivates spoilage and pathogenic bacteria. After more than 60 years of intensive studies on its wholesomeness, gamma irradiation is considered a safe food preservation method by a number of global organizations, such as the WHO, FAO, FDA, USDA,

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among others (Josephson, 1983; Urbain, 1986; WHO, 1994, 1999). There have been many reports on the shelf-life extension of fish products by means of ionizing radiation (IAEA, 1969; ICGFI, 1991; Kilgen, 2001; Lescano et al., 1990; Narvaiz et al., 1989; Nickerson et al., 1983), including the inactivation of spoilage bacteria in anchovy (*Engraulis anchoita*) marinades (Tomac and Yeannes, in press). Despite its high potential efficacy as a preservation method, the effects of gamma irradiation on a product's sensory characteristics related to consumer acceptance should be assessed. Texture is one of the quality attributes that should be analyzed, as fish muscle can become softer due to autolytic activity (Huss, 1999). Regarding anchovy marinades, texture has been considered the main parameter determining the degree of marination for *Engraulis anchoita* (Yeannes and Casales, 1995; Cabrer et al., 2002). Firmness loss and color changes during storage are typical indicators of marinated fish spoilage (Günsen et al., 2011; Yeannes and Casales, 2008; Yeannes et al., 2007). Color is a very important qualitative characteristic of anchovy marinades due to the contributions of lipid oxidation to color changes. *Engraulis anchoita* is a fatty species with high amounts of unsaturated fatty acids, making it particularly sensitive to lipid oxidation (Massa et al., 2007; Yeannes and Casales, 1995).

Thus, the objective of this work was to analyze the effect of gamma irradiation on the texture, color, lipid oxidation and sensory acceptability of marinated anchovy fillets.

2. Materials and methods

2.1. Sample preparation

Whole specimens of *Engraulis anchoita* caught in May were frozen and stored at −18 °C for 7 months. The specimens were thawed, washed, beheaded, and gutted; their tails were cut off; and two fillets were obtained from each anchovy for marination in a water solution containing 3% acetic acid, 10% sodium chloride and 0.2% citric acid at a 1:1 w/w fish to solution ratio over two days at 16 °C and then 3 days at 5 °C. The fillets were removed from the marinating solution and centrifuged for 1 minute at 2800 rpm. The proximate composition of the marinated fillets was as follows: 65.3% water (AOAC, 1990; Sec. 984.25); 13.3% fat (Bligh and Dyer, 1959); 17.2% proteins (AOAC, 1990; Sec. 920.152); 2.6% ashes (AOAC, 1990; Sec. 945.46) and 1.1% acetic acid (Kirk et al., 1996). Approximately 150 samples consisting of 130 ± 5 g of fillets (approximately 26 fillets) were vacuum-packed using a Minimax 430 M machine (Servivac, Argentina) in heat-sealed bags of polyethylene/polyamide (Cryovac®, 125 μm thickness). Samples were stored at 4 ± 1 °C for 48 hours until they were transported 400 km to the semi-industrial cobalt-60 irradiation facility of the Ezeiza Atomic Center (National Atomic Energy Commission, Argentina. Activity: 2.22×10^{16} Bq). During transportation and irradiation, the samples were kept in polystyrene boxes with cooling gel ice packs. Samples were divided into four lots, each of which consisted of 37 bags. The lots were gamma-irradiated at 0, 2, 3 or 4 kGy at a dose rate of 10.91 kGy/h. The real minimum absorbed doses were 2.18; 3.12 and 3.85 kGy; the dose uniformity (maximum dose/minimum dose) was 1.13, 1.00 and 1.01, respectively. Doses were determined with silver dichromate dosimeters (ISO/ASTM 51401:2003(E), 2003).

Samples were kept at 4 ± 1 °C during irradiation, transportation and storage. Analyses were carried out in duplicate the day before irradiation and at 5, 125, 180, 243 and 330 days after irradiation.

2.2. Texture analysis

2.2.1. Sensory determination

The trained panel was composed of seven panelists between 30 and 55 years old with broad experience on the sensory evaluation of fish products, including marinated anchovies. They evaluated the marinades' hardness based on the sensory texture parameter *shear force*, which was defined as the *force required to cross cut the middle part of the fillet using a metal fork* (Yeannes and Casales, 1995). A continuous scale of increasing hardness intensity, ranging from 0 to 10, was used. The panelists defined the scale extremes and anchoring. The highest intensity of shear strength (10) corresponded to the hardness of a raw, non-marinated anchovy fillet, whereas a score of 5 corresponded to the typical hardness of the marinated anchovy fillet. The optimum degree of marinating was defined as a firm texture that could easily be cut with a fork (Yeannes and Casales, 1995). Four fillets (20 g approximately) from each sample were served at room temperature (20 ± 3 °C) on white plastic plates that were codified with random three-digit numbers. The fillet size was similar to that used in the instrumental determination in order to allow more precise comparisons among results. Evaluations were carried out in individual sensory cabins.

2.2.2. Instrumental determination

Shear force assays were carried out after six months of refrigerated storage with a TX.T2i Texture Analyser (Stable Micro System, Godalming, Surrey, UK) controlled by the Texture Expert® software. The machine was equipped with a standard knife blade of 3 mm thickness and 70 mm length and a 25 kg load cell. Marinated anchovy samples (n = 60) were 4.4 ± 0.4 mm thick; 17 ± 1 mm wide; and 80 ± 2 mm long. Thirteen anchovy fillets per radiation dose were cross cut in the middle. The maximum shear force (N), defined as the maximum force necessary to cut the sample, and the shear work (Ns), defined as the work needed to move the blade through the sample, were derived from the curves' shear force (N) against the time (s) obtained for each sample.

2.3. Color

The L^*, a^* and b^* parameters of the CIELAB color space system were measured with a portable colorimeter (Lovibond, SP60) using a D65 standard illuminant and a 10° standard observer (L^* = lightness, a^* = positive values for red color intensity and negative values for green color intensity, and b^* = positive values for yellow color intensity and negative values for blue color intensity) (C.I.E, 1978). Five marinated fillets per radiation dose were analyzed in triplicate.

2.4. Lipid oxidation. TBARS assay

Extraction was performed in duplicate according to Tironi et al. (2007). Incubation was carried out according to Botsoglou et al. (1994). Absorbance was measured with a Shimadzu® UV-1601 PC spectrophotometer (Kyoto, Japan) at a wavelength of 532 nm. TBARS was expressed as mg of MDA per kg of fish. Determinations were performed in duplicate.

2.5. Sensory acceptability

Sensory acceptability was determined using a pilot scale panel of 12 consumers, according to Witting de Penna (2001). The panel was composed of 60% women and 40% men, all of whom were between 25 and 60 years old. Three marinated fillets (15 g approximately) per radiation dose were served to the panelists at room temperature (20 ± 3 °C) in closed polypropylene containers coded with random three-digit numbers. Panelists evaluated the appearance, color, odor, taste, texture, and overall acceptability by means of a free translation into Spanish of the nine-point hedonic scale originally developed by Peryam and Pilgrim (1957).

The scale was categorized according to the following: *like extremely* (9)*; like very much* (8)*; like* (7)*; like slightly* (6); *neither like nor dislike* (5)*; dislike slightly* (4)*; dislike* (3)*; dislike very much* (2)*; and dislike extremely* (1). Panelists were asked to evaluate every attribute of each sample at a time before moving to the next one. The samples' order was randomized. Panelists were provided with water and crackers to rinse their mouths between samples.

2.6. Statistical analyses

Texture, color and lipid oxidation results were analyzed by a completely randomized design with two main factors: radiation dose (0, 2, 3 and 4 kGy) and storage days (0, 5, 125, 180, 240 and 330). The two-ways ANOVA test was used with a 5% significance level to analyze the effect of the main factors and their interaction. The Tukey test was used to compare means ($p < 0.05$). The sensory acceptability results were tested by Kruskal-Wallis ($p = 0.05$). The statistical analysis was carried out using R-Project software (R Development Core Team, 2008).

3. Results and discussion

3.1. Texture

3.1.1. Sensory results

The results of hardness sensory evaluation (shear force) indicated that five days after irradiation, the control samples' values corresponded to the optimum marination degree previously established for this product. Gamma irradiation caused a slight though significant ($p = 0.05$) increase in the shear force values, irrespective of the dose. The irradiated marinades' values remained constant during storage, whereas those of the control samples significantly ($p = 0.05$) decreased. In addition to its anti-bacterial effects, irradiation might also have decarboxylated part of the acetic acid that diffused into muscle during marinating (Josephson, 1983), lessening acidic proteolysis and thus protecting the texture. In this type of product, texture is modified during storage by enzymatic, bacterial, and acidic proteolysis due to marination, as reported by Yeannes and Casales (2008) and Yeannes et al. (2007) for anchovies and by Feeney (1977) and Meyer (1965) for other fish species. The present results agree with those of Lee et al. (2002) for irradiated semi-dried *Cololabis saira*, in which the shear force was not modified after 60 days under refrigeration, even though non-irradiated samples became softer.

3.1.2. Shear force instrumental results

The marinades' hardness was instrumentally analyzed after 6 months of refrigerated storage, which corresponds to the time estimated as the product′s shelf-life. The results of this test are shown in Table 1. Irradiated samples had significantly higher ($p < 0.05$) values than controls at the 6th storage month without significant differences among doses, in agreement with sensory results at that time. This radiation effect was also observed by Lescano et al. (1990) in vacuum-packed beheaded and eviscerated hake (*Merluccius hubbsi*), by Yoon (2003) in cooked chicken breast meat, and by Galán et al. (2010) in cow meat hamburgers. The increased hardness observed in irradiated muscular tissues could be explained by the contraction of the muscular myofibrils' diameters caused by irradiation. According to Yoon (2003), this contraction would be the main factor contributing to texture hardening. Irradiation would cause cross-links, affecting the structure of the meat muscle tissue when forming protein aggregates (Giroux and Lacroix, 1998). It has also been observed that soy protein irradiation induced aggregation, thereby increasing shear force (El-Moneim et al., 1988).

Table 1
Maximum shear force and shear work of marinated anchovy fillets at the 6th storage month*.

Dose (kGy)	Maximum shear force (N)		Shear work (Ns)	
0	26.7 ± 3.4	B	327.6 ± 40.4	B
2	34.0 ± 2.7	A	413.4 ± 56.3	A
3	31.1 ± 5.0	A	390.4 ± 65.3	A
4	32.0 ± 4.7	A	392.6 ± 62.7	A

* Means ± standard error. Different capital letters indicate significant differences due to the radiation dose ($p = 0.05$).

Lee et al. (2002) observed that irradiated semi-dried *Cololabis saira* maintained their initial shear-force values during storage, whereas control samples softened. Regarding the results of the present work, it is possible that tissue autolytic and chemical degradation during storage, with its consequent softening, would have been compensated by the effect of irradiation previously described on muscle tissues. Because texture softening closely relates to the quality loss of marinated anchovies (Cabrer et al., 2002; Yeannes et al., 2007), the hardening effects of irradiation can be considered beneficial.

3.1.3. Correlation between sensory and instrumental texture measurements

The results of the texture determinations by instrumental (maximum shear force) and sensory methods after the 6th month of storage were significantly correlated ($R^2 = 0.963$) indicating that both would be analogous, allowing the use of one or the other, depending on the need and circumstances.

3.2. Color

Fig. 1 shows the changes in the L*, a* and b* color parameters during storage.

Lightness (*L**) remained unchanged in irradiated marinades throughout the storage period, even as it was significantly decreased in control samples in relation to the darkening of the characteristic whitish/greyish color due to spoilage (Yeannes and Casales, 1995; Yeannes et al., 2007).

Regarding *a** (red color intensity), no irradiation effect was found; every value increased significantly though slightly during the storage time.

The values of *b** were observed to increase during storage, which can be attributed to lipid oxidation processes (Corzo and Bracho, 2006; Thanonkaew et al., 2006). This factor had previously been reported by Tomac and Yeannes (in press), although these authors found the increase was not so pronounced, most likely due to the lower lipid contents of their samples. Irradiation decreased the *b** values inversely from dose increases, resulting in this effect becoming significant from the 8th storage month onwards.

Thus, the irradiation treatment did not modify the initial characteristic color, but rather decreased the color changes associated with spoilage during refrigerated storage.

3.3. Lipid oxidation. TBARS assay

Fig. 2 shows TBARS results. The initial control sample's value was somewhat higher than is usually observed in these types of product (Günsen et al., 2011; Tomac and Yeannes, in press), most likely due to the high lipid content of these samples (13.3 ± 0.7%) and the previous 7 months of frozen storage to which the whole

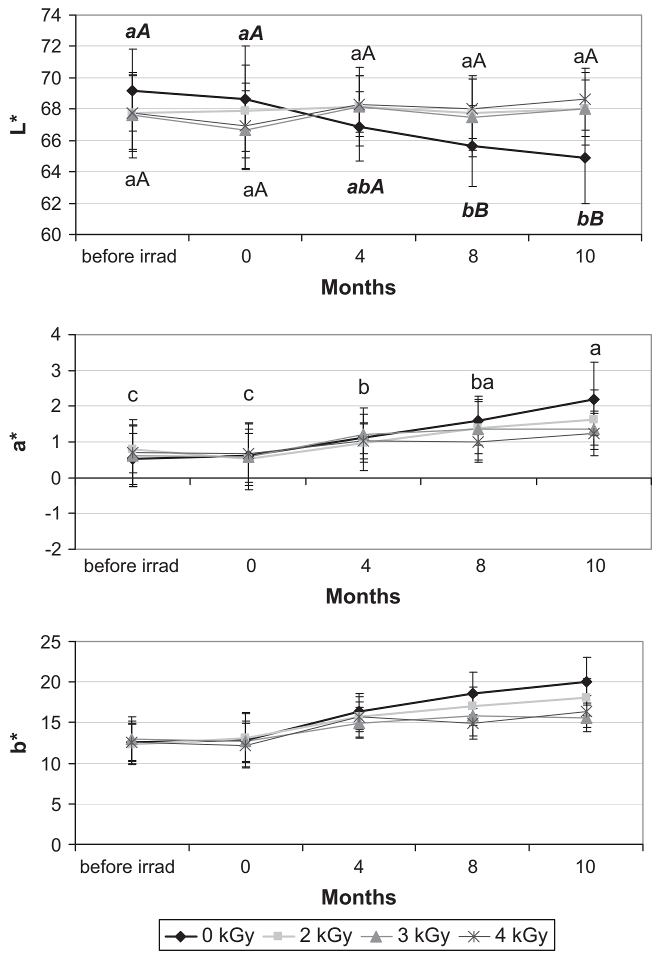

Fig. 1. L^*, a^* and b^* color parameters of marinated anchovies during refrigerated storage. Different capital letters indicate significant differences due to the radiation dose. Different lower case letters indicate significant differences due to storage time (p=0.05) (mean ± standard error, n=2).

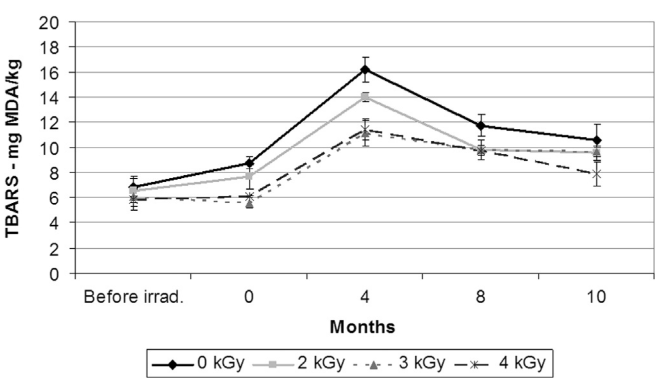

Fig. 2. TBARS evolution in control and irradiated marinated anchovy fillets during refrigerated storage (n=2).

anchovies were subjected before their use in marinades (Aubourg, 1999; Sahoo and Verma, 1999).

TBARS significantly increased in every sample throughout storage, as had already been reported in marinated fatty fish (Günsen et al., 2011; Kilinc and Cakli, 2005). A maximum was found at the 4th storage month; thereafter, the values decreased, possibly due to the further reactions of these lipid oxidation products reported by Chouliara et al. (2004) in sea bream, Özden et al. (2007) in sea bass and Lakshmanan et al. (1999) in anchovies.

Irradiation decreased the TBARS values as a function of dose. This effect has also been observed in previous studies (Tomac and Yeannes, in press) and in other reports on sardine (Kasimoglu et al., 2003) and chub mackerel (Mbarki et al., 2009). According to Nawar (1983, 2000), in certain cases, ionizing radiation can produce protective substances, such as amino-hexose-reductones, that might improve lipid stability towards oxidation; further, such treatment could increase lipid oxidation in such a way that TBARS and peroxide value analysis would not be adequate, compared to the preferred utility of sensory analysis or fatty acids profiling. Other studies have suggested that irradiation can partially destroy malondialdehyde and other TBA-reactive substances; therefore, the lipid oxidation products caused by this treatment would react first with other compounds, such as proteins, before TBA (Diehl, 1983; Nawar, 1983; Tükenmez et al., 1997).

The present TBARS results help to explain the behavior of the $b*$ color parameter during storage because the lipid oxidation of fish species is strongly related to $b*$ increases (Lauritzsen et al., 1999; Thanonkaew et al., 2006). It has been proposed that hydroperoxide radicals and carbonyl compounds resulting from the oxidation of polyunsaturated fatty acids in cell membranes may react with free amino groups, resulting in the condensation of brown-colored polymeric pigments (Pokorny, 1981).

3.4. Sensory acceptability

These results are shown in Fig. 3. Scores were greater than 5 throughout storage indicated good acceptability without significant differences between control and irradiated samples. These findings concur with those of Tomac et al. (2012) for irradiated squid rings and of Badr (2012) for smoked salmon fillets. Moreover, Mbarki et al. (2009) reported that vacuum-packed irradiated *Scomber japonicus* was more accepted that non-irradiated samples.

In this work, the scores of the sensory attributes remained fairly stable throughout storage except on the 4th month, when they significantly diminished; coinciding with the higher TBARS values observed on that date. At the 10th storage month, irradiated samples were more accepted than controls as a function of dose, even though scores were not significantly different. This could partly be attributed to the less intense acid taste of irradiated samples found by the panelists and still partly due to the enhanced acetic acid decarboxylation in irradiated samples during storage (Josephson, 1983).

According to Günsen et al. (2011), the vacuum-packed refrigerated marinades of *Engraulis encrasicolus* were sensorily rejected at the 7th storage month, mainly due to their brownish color, texture softening and off-flavors. Sallam et al. (2007) also observed sensory acceptability decreases in vacuum-packed marinated *Cololabis saira* after 3 months of refrigerated storage. Gökoglu et al. (2004) reported that the sensory scores of sardine marinades decreased during refrigeration, culminating in spoiling at month 4, whereas Kilinc and Cakli (2005) observed a decrease in the sensory scores of marinated sardine (*Sardina pilchardus*) during refrigeration, with non-edible quality being reached at the 6th month.

In this work, anchovy marinades irradiated at 2, 3 and 4 kGy kept their sensory acceptability after 10 months under refrigeration.

4. Conclusions

Gamma irradiation at 2, 3 and 4 kGy slightly increased the refrigerated anchovy marinades' hardness, irrespective of the dose. Hardening was both sensorily and instrumentally measured, with a high correlation between these methods that would allow either to be used in the future. Irradiation improved the marinades' quality since the texture remained unchanged for 10 months,

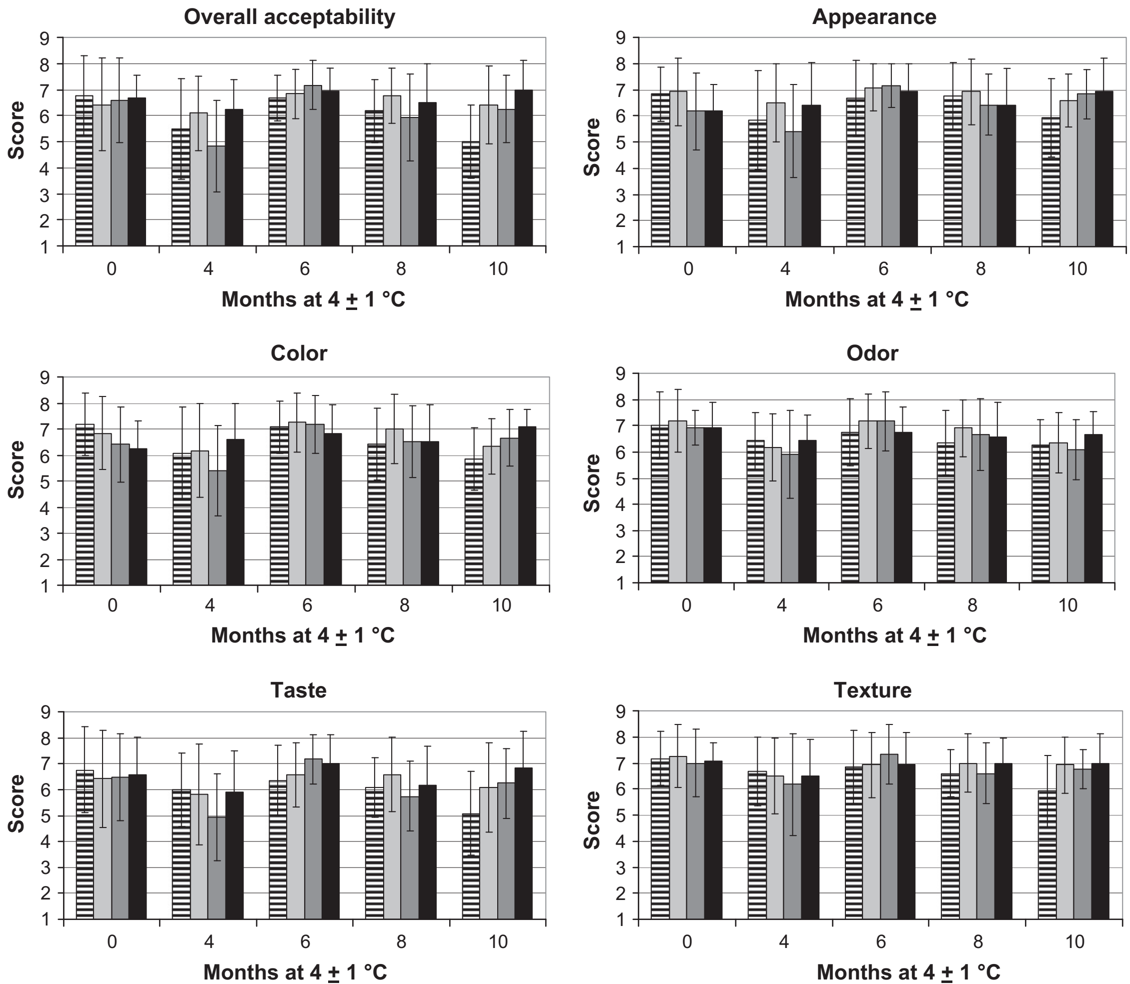

Fig. 3. Sensory acceptability, appearance, color, odor, taste and texture of marinated anchovies during refrigeration. 0 kGy (▬); 2 kGy (■); 3 kGy (■) and 4 kGy (■).

preventing the softening observed in non-irradiated samples. This treatment did not modify the characteristic colors of the marinades and reduced their changes during storage. Lipid oxidation did not increase with irradiation but did slightly with storage time. Irradiated marinades had good sensory acceptability during the 10 storage months and were not negatively affected by any irradiation-induced hardening. Therefore, gamma irradiation could be applied to this type of product for shelf-life extension and may allow for reductions in the intensity of other hurdles to food preservation.

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