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Quality Changes in Gamma Irradiated Marinades of Anchovy (*Engraulis anchoita*) During Refrigerated Storage

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The effect of gamma irradiation (0, 1.8, and 3.3 kGy) on the microbiological, chemical, and color characteristics of marinated (7% acetic acid and 10% NaCl) and vacuum-packed anchovy fillets was analyzed during 20 months of refrigerated storage ($4 \pm 1°C$). Acidity, pH, water activity (a_w), total volatile basic nitrogen (TVBN), lipid oxidation, and color parameters were determined. Mesophilic and psychrotrophic bacteria, sulphite-reducing clostridia, total and fecal coliforms, *Staphylococcus* spp., yeasts, and molds were investigated. Gamma irradiation reduced the initial mesophilic bacterial counts and inhibited mesophils growth during 20 months. As a result, the production of TVBN during storage was lower in irradiated samples than in control. Also, lipid oxidation was lower in irradiated samples than in nonirradiated. The color of anchovy fillets was not affected by the irradiation treatment. Even if nonirradiated anchovy fillets presented a high stability in comparison with the traditional product (in flasks with vegetable oil and spices), gamma irradiation improved the microbiological and chemical quality of anchovy fillet marinades without inducing changes on its characteristic color for 20 months.

Keywords: ionizing radiation, fish, marinating, refrigeration, quality, preservation

INTRODUCTION

Engraulis anchoita is the most abundant pelagic species of the southwestern Atlantic Ocean, found in the waters from the South of Brazil (24° S) to the Argentine Patagonia (48° S). This species has an interesting economic potential because it is underexploited (Massa et al., 2012; Pastous-Madureira et al., 2009). Argentina is a pioneer in the exploitation and processing of *Engraulis anchoita*, and the main manufacturer of several products for human consumption. More than 80% of the elaborated products correspond to salted-ripened anchovy, which is mainly exported to European countries. It has also started to be exported as marinated anchovies (Cabrer et al., 2002; Yeannes and Casales, 2008; Pastous-Madureira et al., 2009). Marination is a preservation technique that consists of the immersion of fish or fish portions in marinating solutions that usually contain organic

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acids and salt as preservation agents (Meyer, 1965; Fuselli et al., 1994). Marinades can be packaged in glass or plastic containers with oils, sauces, or brines or vacuum-packed in plastic bags afterwards. Marinated fish are semi-preserved ready-to-eat fish products and are a high-value delicacy (Capaccioni et al., 2011). Weak organic acids (acetic or lactic) and salt are added to fish to retard the action of bacteria and enzymes; this process results in a product with characteristic flavor and extended but limited shelf life (Meyer, 1965; Fuselli et al., 1994, 1998). The aim of fish marination is not only to prevent spoilage from microorganisms and enzymes, but also to increase the value of the fish (Capaccioni et al., 2011). Research has been done on the marination of different fish species such as *Engraulis anchoita* (Fuselli et al., 1994; Cabrer et al., 2002), *Engraulis encrasicholus* (Gökoglu et al., 2009; Günsen et al., 2011), sardine (*Sardina pilchardus*; Gökoglu et al., 2004; Kilinc and Cakli, 2004), and in Pacific saury (*Cololabis saira*; Sallam et al., 2007).

In Argentina, marinated fillets of *E. anchoita* are usually commercialized in the domestic market; preparation includes covering with vegetable oil and spicing with black pepper grains, laurel dried leaves, red pepper powder, and others. Marinated fillets are exported in barrels covered with a weak marination solution. This product is similar to the "boquerón" (*Engraulis encrasicholus*) typically consumed in European countries, especially Spain.

Marinades are consumed with no further heat treatment. Since the microbial stability of the product might not be fully ensured by the hurdles of the marinating process, the application of another hurdle could be studied in order to extend shelf life (estimated as about 6 months for the traditional product).

Food irradiation is a nonthermal preservation method that has been intensively studied. More than 50 years of scientific studies support the benefits of irradiation processing on the preservation and microbial quality improvement of food (World Health Organization [WHO], 1994; Barbosa-Cánovas et al., 1999; Diehl, 2002). When food is exposed to ionizing radiation, the microorganism's cell is affected by two main actions: direct and indirect. In direct action, an ionizing particle or ray damages the DNA of cells in living organisms. In indirect action, the products of radiolysis, usually of water in most foods, affect the cell (Moseley, 1989). Processing seafood by ionizing radiation aims at shelf life extension as well as safety by eliminating pathogenic microorganisms of public health significance (International Consultative Group on Food Irradiation [ICGFI], 1999). The benefits of preservation and microbial quality improvement of fish and seafood through irradiation have been reviewed by Arvanitoyannis and Stratakos (2010). Shelf life of different fish products was improved by gamma irradiation—including whole anchovies (Lakshmanan et al., 1999), sea bream (Chouliara et al., 2004), and squid *Illex argentinus* (Tomac and Yeannes, 2012), among others. Taking into account that marinades have an extended but limited shelf life (Meyer, 1965; Capaccioni et al., 2011), low doses of gamma irradiation could be a suitable and effective method to extend marinade storage time.

Vacuum-packaging is a kind of modified atmosphere packaging technique that consists of the exclusion of air (and the oxygen in it) from the atmosphere that surrounds food. The reduction of oxygen slows the growth of aerobic bacteria and the oxidation of lipids, helping to increase the product shelf life (Phillips, 1996). Vacuum-packaging has proven to increase the shelf life of different fish products, such as sardines (*Sardina pilchardus*; Özogul et al., 2004) and *Scomber colias japonicus* (Stamatis and Arkoudelos, 2007).

Furthermore, free radicals induced by irradiation can easily oxidize double bonds of polyunsaturated fatty acids of fish, anchovy (*E. anchoita*) in this particular case. However, the absence of oxygen in the package has been reported to reduce the degree of rancidity in comparison with irradiation in air atmospheres (Brewer, 2009).

Thus, the aim of this work was to analyze the effect of gamma irradiation on microbial counts and chemical and color characteristics of vacuum-packed marinated fillets of *Engraulis anchoita* during storage at 4 ± 1°C.

MATERIALS AND METHODS

Raw Material Source, Treatment, and Storage

Whole specimens of *Engraulis anchoita* of 14.0 ± 1.1 cm long and 18.4 ± 2.3 g were caught in July and frozen for 6 months at −18°C. They were thawed, washed, beheaded, gutted, tails were cut off; two fillets were obtained from each specimen. At a company in the city of Mar del Plata (Argentina), fillets were subjected to marination with 7% acetic acid, 10% NaCl, and 0.2% citric acid solution, in a 1:1 fish to solution ratio for 2 days at 16°C, followed by 3 days at 5°C. Afterwards, the fillets were removed from the marinating solution and subjected to centrifugation (1 min at 2,800 rpm). The proximate composition of marinated fillets was determined prior to irradiation treatment. Fillets had 69.83% water; 6.32% fat; 16.71% proteins; and 3.28% ashes. More than 100 samples consisting of 140 ± 2 g of fillets (approximately 28 fillets) were vacuum-packed in a Minimax 430M machine (SERVIVAC, Buenos Aires, Argentina) in heat-sealed bags of LDPE/polyamide (125 μm) in our laboratory. Samples were transported under refrigeration to the semi-industrial Ezeiza Atomic Centre facility, National Atomic Energy Commission of Argentina (activity: 2.22×10^{16} Bq). Samples were separated into three different lots, each one consisting of 33 samples. Each lot was gamma irradiated with a Cobalt-60 source at 0, 1.8, and 3.3 kGy (minimum absorbed doses, Dmax/Dmin = 1.15). Doses were selected on the basis of previous studies on fish irradiation, where it was reported that doses ranging between 0 and 4 kGy were useful to extend the shelf life of different fish and seafood products (Arvanitoyannis and Stratakos, 2010; Foley, 2006; International Atomic Energy Agency [IAEA], 2000; Lescano et al., 1990). Doses were determined with Amber Perspex dosimeters (Harwell Dosimeters Ltd., Oxfordshire, U.K.). Irradiated and non-irradiated samples (control, 0 kGy) were stored at 4 ± 1°C for 20 months. Samples were analyzed before irradiation (Day 0) and on Days 1, 90, 175, 425, and 602 after irradiation. The experiment was repeated once.

Microbiological Analysis

Ten grams of fish sample in saline solution (0.85%) with 0.1% w/v peptone (International Commission on Microbiological Specifications for Foods [ICMSF], 1983) made to 100 mL were macerated in a Stomacher 400 Circulator Homogenizer (Seward, Ltd., Worthing, UK). Microbiological analyses were done in triplicate and expressed as colony forming units per gram of fish (CFU/g). The counts of total microorganisms and isolations of particular microbial groups were performed using the following culture media and culture conditions (ICMSF, 1983): psychrotrophic bacteria and aerobic mesophilic bacteria on plate count agar were incubated at 7 ± 0.5°C for 10 days and 35 ± 0.5°C for 48 h, respectively. Total coliforms on violet red bile agar were incubated at 35 ± 0.5°C for 24 h. Fecal coliforms in brilliant green lactose bile broth were incubated at 35 ± 0.5°C for 48 h. *Staphylococcus* spp. on Baird-Parker agar were incubated at 35 ± 0.5°C for 48 h. Sulfite reducing *Clostridium* on SPS agar (Merck, Darmstadt, Germany) were incubated in anaerobic jars at 35 ± 0.5 and 46 ± 0.5°C for 48 h. Molds and yeasts on Handl agar (Britania Laboratories, Buenos Aires, Argentina) were incubated at 25 ± 0.5°C for 5 days.

Physical and Chemical Analysis

Water Activity (a_w), Acidity, and pH

Acidity was determined by titration with NaOH 0.1 N (Kirk et al., 1996). Results were expressed as percentage of acetic acid (%). The value of pH was measured using a digital pH-meter Hanna HI 98150 (Hanna Instruments, Woonsocket, RI, USA) with a glass electrode, in a fish–distillated water ratio of 1:1 (AOAC, 1993, Sec. 981.12). All determinations were carried out in triplicate.

The a_w was measured using a digital hygrometer (Aqualab, Model CX-2T, Decagon, Pullman, WA, USA). Determinations were done in quadruplicate.

Lipid Content and Fatty Acid Profile

Lipids were extracted according to the method of Bligh and Dyer (1959), from 100 g of ground marinades using a solvent mixture of chloroform–methanol–distilled water ratio of 2:2:1.8 (v/v/v). The lipid content was gravimetrically determined.

The fatty acid profile was determined by gas chromatography after fatty acids methylation. A gas chromatograph (Shimadzu® GC-17A, Tokyo, Japan) equipped with a fused silica capillary column (Omegawax 320, Supelco Inc., Bellefonte, PA, USA) (30 m × 0.32 mm ID, 0.25 μm phase film) and a flame ionization detector was used to separate and quantify fatty acid methyl esters. The injected volume was 1 μL, done by duplicate, and the carrier gas was nitrogen. The temperature of the injector port and detector was held at 250°C. Column oven temperature ranged from 150 to 225°C (at 1.5°C min^{-1}). Fatty acids were identified by comparison of their retention time and peak areas with reference standards (PUFA-1, Marine Source Supelco®, Cat. No. 4-7033). Fatty acids were quantified as percentage ratio of peak areas with the total area.

Total Volatile Basic Nitrogen (TVBN)

TVBN was determined according to the method adapted from direct distillation (Giannini et al., 1979). Ten grams of ground anchovy fillets were homogenized with 300 mL of distilled water; 2 mL of antifoaming, porous plate; and 5 g of magnesium oxide.

The distillate was collected in 50 mL of boric acid (20 g L^{-1}) and 1 mL of indicator (100 mL ethanol, 0.05 g methyl red, 0.075 g bromecresol green) to a final volume of 230 mL. Then, it was titrated with sulphuric acid 0.1 N. Results were expressed in milligrams of TVBN per 100 g of wet sample. Determinations were done in duplicate.

Lipid Oxidation Assessment

Thiobarbituric acid reactive substances (TBARS) were determined as being associated to the degree of lipid oxidation. The products of lipid oxidation react with 2-thiobarbituric acid (TBA) yielding pigments that can be quantified by absorbance determination. Some of these products are malondialdehyde (MDA) and other aldehydes (ALs). MDA reacts with TBA to form a product which has a maximum absorbance at a wavelength of 532 nm (pink pigment; $TBARS_{532}$). In turn, ALs react with TBA to produce yellow pigments that have a maximum absorbance at 455 nm ($TBARS_{455}$; Kosugi et al., 1987). Extraction was done according to Tironi et al. (2007). Two grams of minced marinades were agitated with 16 mL of 5% p/v TCA. Two milliliters of 0.5% w/v TBA were added to 2 mL of filtrate and incubated at 70°C for 30 min (Botsoglou et al., 1994). Absorbance was measured in a spectrophotometer (Shimadzu® UV-1601 PC, Kyoto, Japan) at wavelengths of 532 and 455 nm. TBARS was determined according to Lambert-Beer's law. For quantification of MDA and other aldehydes, $TBARS_{532}$ and $TBARS_{455}$ were expressed as mg of MDA and mg of ALs per kg of fish, respectively. For the latter, the average molecular weight of most common aldehydes found was considered (89.6 g mol^{-1}). Determinations were done in triplicate.

Color Analysis

CIELAB color space parameters—L^* = lightness, a^* = red (+) and green (−) color intensity, and b^* = yellow (+) and blue (−) color intensity—were determined with a portable colorimeter (Lovibond, SP60, Amesbury, UK), using D65 standard illuminant and 10° standard observer. Three measurements were made on the internal surface of five fillets from each sample. Color difference, DE^*_{ab} (CIE, 1978), was calculated for each time with respect to color before irradiation, using

$$DE^*_{ab} = \left[\left(L^* - L_r{}^*\right)^2 + \left(a^* - a_r{}^*\right)^2 + \left(b^* - b_r{}^*\right)^2\right]^{1/2}.$$

Statistical Analysis

Results were analyzed by a completely aleatorized design with two main factors: radiation dose (0, 1.8, and 3.3 kGy) and storage time (before irradiation (00), 0, 3, 6, 14, and 20 months). Interaction between them was also analyzed. A two-way analysis of variance (ANOVA) test was used with a 5% significance level. In further analysis, the Tukey test was used to compare means ($p < 0.05$). The statistical analysis was carried out using the R-Project software (R Development Core Team, 2008).

RESULTS AND DISCUSSION

Microbiological Analysis

Previous to marination, initial counts of mesophilic and psychrotrophic bacteria in frozen anchovies were 7.5×10^3 and 5.0×10^3 CFU/g, respectively, similar to the results observed in frozen *Engraulis anchoita* by Fuselli et al. (1994) and in *Engraulis encrasicholus* by Günsen et al. (2011). *Staphylococcus aureus* and sulphite-reducing clostridia were not detected. After processing (thawing, beheading, gutting, cutting off the tail, filleting, and marinating), mesophilic counts were reduced to 3.8×10^2 CFU/g, and no psychrotrophic bacteria were detected (< 10 CFU/g). This was due to the preservative action of marination, as observed by various authors in fish products (Fuselli et al., 1994; Kilinc and Cakli, 2004; Sallam et al., 2007). Günsen et al. (2011) found similar results when marinating *E. encrasicholus*, where initial mesophilic counts were reduced in one logarithmic cycle, and psychrotrophic bacteria were inhibited (< 10 CFU/g) by the reduction of three logarithmic cycles, respectively, due to marination. The addition of acetic acid produces the pH drop to values below the growth range of different microbial groups. In its undissociated form, it can penetrate the cell of microorganisms denaturing proteins (Baird-Parker, 1980). Also, NaCl diminishes the water activity, inhibiting microbiol growth (Kilinc and Cakli, 2004; Sallam et al., 2007).

The results of mesophilic bacterial counts (MBC) during storage are shown in Figure 1. The initial MBC of 38.0×10^1 CFU/g after marination and previous to irradiation were similar to those found by Fuselli et al. (1994) in marinated anchovy. These initial bacterial counts were significantly ($p < 0.05$) reduced by gamma irradiation at 1.8 and 3.3 kGy to values below the detection limit (< 10 CFU/g).

MBC of nonirradiated samples significantly ($p < 0.05$) increased, reaching a maximum of 79.5×10^1 CFU/g at 3 months of refrigerated storage. According to Fuselli et al. (1998), this would correspond to the typical flora of this product. Bacteria that were not completely inactivated after the marination process would have been able to grow during storage according to their ability to adapt in the acid medium (Fuselli et al., 1998). MBC of control samples tended to decrease after 6 months and were no longer detected until the end of the storage period. This could be explained by the effect of the different hurdles used to preserve the product (addition of salt and organic acid and vacuum-packaging) on microorganism homeostasis. According to Leistner (2000), when microorganisms use their energy to repair mechanisms so that their homeostasis would overcome the hostile environment, they might become exhausted and die. Meanwhile, growth of mesophilic bacteria was inhibited on gamma irradiated samples during 20 months of storage at 4 ± 1°C. MBC of control sample was significantly ($p < 0.05$) higher than irradiated samples immediately after irradiation was applied and during 6 months of refrigerated storage. Results of this work are in agreement with

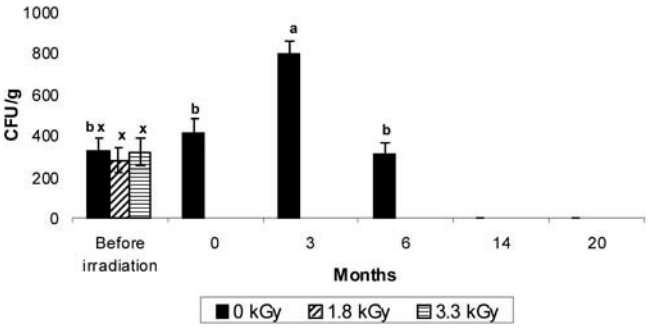

FIGURE 1 Total mesophilic bacteria counts (MBC) evolution in marinated anchovy fillets during refrigerated storage. Same letters (x) indicate nonsignificant differences of MBC between radiation doses. Different letters (a, b) indicate significant differences of MBC due to time. Standard error represented by bars ($n = 2$).

studies on fish and fish product preservation by ionizing radiation. Narvaiz et al. (1989) found that gamma irradiation satisfactorily increased shelf life of *Merluccius hubbsi* by reducing mesophilic and psychrotrophic bacterial counts. Other authors also found bacterial count reductions due to ionizing radiation in fish such as sea bream (*Sparus aurata*), threadfin bream (*Nemipterus japonicus*), sardine (*Sardina pilchardus*), sea bass (*Dicentrarchus labrax*), and anchovies (*Stolephorus commersonii*; Chouliara et al., 2004; Jeevanandam et al., 2001; Kasimoglu et al., 2003; Özden et al., 2007; Lakshmanan et al., 1999).

Psychrotrophic bacteria, sulfite reducing *Clostridium*, total and fecal coliforms, *Staphylococcus* spp., and yeasts and molds were not detected (< 10 CFU/g) in irradiated or nonirradiated marinated anchovy fillets during 20 months of refrigerated storage.

The low MBC counts found in this work show a good stability of the nonirradiated product, which might be the consequence of the preservative action of marination. Marination with 7% acetic acid solution gave the product stability by decreasing the pH of the flesh to 3.97 ± 0.03. Fuselli et al. (1994) worked with frozen *Engraulis anchoita* and found that mesophilic and psychrotrophic bacteria were not detected after marination process with a 3% acetic acid and 10% NaCl solution. Kilinc and Cakli (2004) determined that initial total viable microorganisms, lactic acid bacteria, psychrotrophic bacteria, and mold and yeasts in raw sardines were inhibited by marination with 7% acetic acid and 14% NaCl solution. Moreover, vacuum-packaging by itself could have improved microbial quality, as determined by Özogul et al. (2004), who found a threefold increase in sardines' shelf life due to vacuum-packaging. Mbarki et al. (2009) found an improvement in shelf life of chub mackerel (*Scomber japonicus*) due to the combined action of vacuum-packaging and gamma irradiation. In this work, gamma irradiation at 1.8 kGy was enough to inhibit mesophilic bacteria growth during 20 months of refrigerated storage, improving microbiological quality of marinated anchovy fillets.

Physical and Chemical Analyses

Water Activity, Acidity (% of Acetic Acid), pH

Water activity of anchovy fillets before irradiation was 0.967 ± 0.04 and was not affected by gamma irradiation after its application ($p > 0.05$). This is in accordance with results obtained by Cabrer et al. (2002) for marinated fillets of *E. anchoita*. The reduction of a_w in comparison with the raw material (0.989 ± 0.02) could be explained because NaCl tends to diffuse inside the muscle in

an inverse flow than that of water, until equilibrium concentration is reached, decreasing a_w (Cabrer et al., 2002; Capaccioni et al., 2011; Meyer, 1965).

Previous to irradiation, acidity was 2.52 ± 0.08, and 2.65 ± 0.07% after its application. There was no significant ($p > 0.05$) effect of irradiation on acidity. Similar values were found in marinated fish products, such as sardine *Sardina pilchardus* (Kilinc and Cakli, 2004). This increase of acidity in comparison with nonmarinated anchovy is explained by the diffusion of acetic acid inside fish muscle, similar to that of NaCl (Cabrer et al., 2002; Meyer, 1965).

The initial average pH value for all samples was 3.97 ± 0.03 and did not significantly change during 20 months of refrigerated storage. The pH reduction is in accordance with the increase of acidity. These results are in accordance with Gökoglu et al. (2004) who found no significant changes in pH during 5 months of refrigerated storage in marinated sardines. In this work, the effect of gamma irradiation on the pH of marinated anchovies was not significant ($p > 0.05$).

Lipid Content and Fatty Acid (FA) Profile

Lipid content was 6.32 ± 0.81 g/100 g of sample. This result is within the normal values found for the species caught in this season (Moreno Aizpún et al., 1979; Yeannes and Casales, 1995). The fatty acid profile indicated that of these lipids, 33.25% were saturated FA. The monounsaturated FA constituted 39.38% and the polyunsaturated FA (PUFA) represented 26.70% of total FA. The *n*-3 and *n*-6 FA content was 25.07 and 1.32% of total FA, respectively. The *n*-3 FA corresponded mainly to docosahexaenoic acid (DHA, C22:6*n*-3) and eicosapentaenoic acid (EPA, C20:5*n*-3) These results are in agreement with the findings of other authors in the same species (Massa et al., 2012).

Total Volatile Basic Nitrogen

The evolution of TVBN of marinated fillets during refrigerated storage is shown in Figure 2. Before irradiation, TVBN mean value was 15.4 ± 0.3 mg/100 g. It significantly ($p < 0.05$) increased in all samples, reaching values of 50.9 ± 1.6, 44.5 ± 1.7, and 41.8 ± 1.7 mg/100 g for samples irradiated at 0, 1.8, and 3.3 kGy, respectively, after 20 months. However, the rate of TVBN production was faster in control sample, being significantly ($p < 0.05$) higher compared with irradiated samples after 3 months of storage until the end of the storage period. This fact could be explained in part by the higher MBC found in control samples, since one cause of TVBN increase is the trimethylamine produced by bacterial activity. Meanwhile, irradiation induced bacterial reduction in radiated samples seems to have slowed TVBN production. The increase of TVBN in

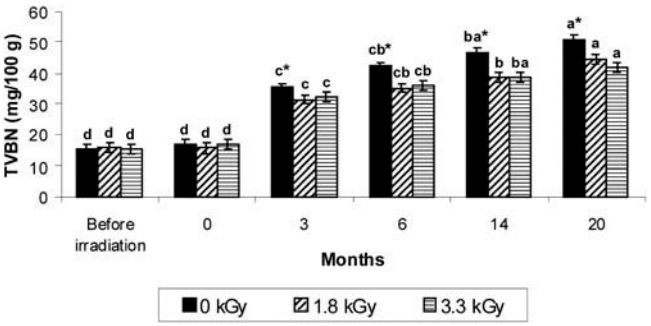

FIGURE 2 TVBN evolution in anchovy marinades during storage at 4 ± 1°C. Different letters indicate significant differences of TVBN due to storage time. *Indicates significant higher TVBN due to radiation dose. Standard error represented by bars ($n = 2$).

irradiated samples (as well as in control) could be explained by the production of dimethylamine by enzymatic reactions during refrigerated storage, considering that irradiation has little effect on enzymatic systems (Urbain, 1986).

In agreement with results of this work, other researchers found lower TVBN production in irradiated fish products compared with nonirradiated samples, related to the microbial reduction caused by gamma irradiation (Lakshmanan et al., 1999; Jeevanandam et al., 2001; Mbarki et al., 2009; Paari et al., 2012; Tomac and Yeannes, 2012).

Similar results regarding TVBN increase during storage of marinated fish have been reported by Gökoglu et al. (2004), Sallam et al. (2007), and Günsen et al. (2011) with differences in initial and final TVBN values found, which can be attributed to variations of raw material and marination process. According to Günsen et al. (2011), TVBN of marinated vacuum-packed anchovies reached a value of 15.78 ± 0.02 mg/100 g after 7 months of refrigerated storage. Meanwhile, Chouliara et al. (2004) found that TVBN of salted and vacuum-packed sea bream increased from 25.3 to 60.5 mg/100 g during 42 days of refrigerated storage. Gamma irradiation slowed TVBN production in marinades of anchovy fillets during refrigerated storage.

Thiobarbituric Acid Reactive Substances Assay

Figure 3 shows the evolution of $TBARS_{532}$ and $TBARS_{455}$ in anchovy marinades during storage. Before irradiation was applied, $TBARS_{532}$ and $TBARS_{455}$ average values for all samples were 1.4 ± 0.1 mg MDA/kg and 2.0 ± 0.1 mg ALs/kg, respectively. There were no significant changes

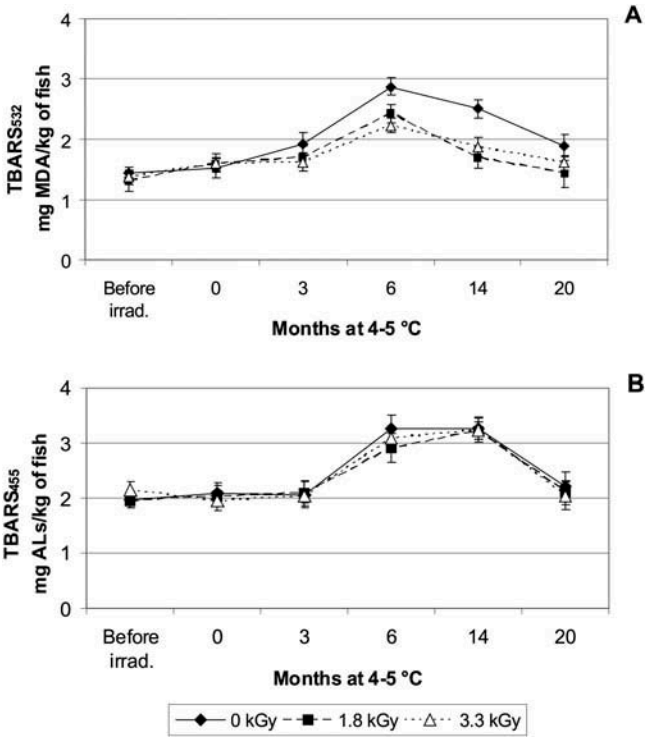

FIGURE 3 $TBARS_{532}$ (A) and $TBARS_{455}$ (B) evolution in gamma irradiated anchovy marinades during storage at 4 ± 1°C. Standard error represented by bars ($n = 2$).

($p > 0.05$) in $TBARS_{532}$ and $TBARS_{455}$ due to gamma irradiation, immediately after it was applied. Both remained unchanged during 3 months of refrigerated storage. After that, $TBARS_{532}$ significantly increased ($p < 0.05$) in all samples. This increase was slightly but significantly higher ($p < 0.05$) in control compared to irradiated samples. From that period until the end of the storage time, $TBARS_{532}$ tended to decrease, probably due to further reactions of lipid oxidation products as reported by Chouliara et al. (2004) in irradiated sea bream, Özden et al. (2007) in sea bass, and Lakshmanan et al. (1999) in irradiated anchovies.

$TBARS_{455}$ remained unchanged until the sixth storage month, when it increased in all samples up to an average value of 3.1 ± 0.2 mg ALs/kg and then decreased toward the end of the storage period. There were no significant differences in $TBARS_{455}$ between nonirradiated and irradiated samples for 20 months. The fatty acid analysis of these marinades indicated that 26.7% of total lipids corresponded to PUFA, which are the most oxidation sensitive FA due to their high degree of unsaturation. Ionizing radiation can generate free radicals that could easily oxidize polyunsaturated FA. However, the application of gamma irradiation in this product did not produce an important increase of lipid oxidation. It has been reported that the exclusion of oxygen in the package, as in this work, can reduce lipid oxidation by avoiding aldehyde generation (Brewer, 2009; Urbain, 1986). Other authors found similar results in irradiated and vacuum-packed sardines (Kasimoglu et al., 2003). Mbarki et al. (2009) found a synergic effect between irradiation and vacuum-packaging on slowing TBARS formation in gamma irradiated (1.5 kGy) vacuum-packed chub mackerel.

It has been suggested that fish can be considered of good quality up to a TBARS value of 5 mg MDA/kg fish and that it can be consumed up to 8 mg MDA/kg fish (Schormüller, 1969; Sallam et al., 2007). In this work, that first value was not reached by any sample, control or irradiated, during 20 months. Unlike this work, Günsen et al. (2011) found that TBARS together with the sensorial test were the main factors determining the shelf life of marinated (4% acetic) vacuum-packed anchovies. They found that TBARS increased from 1.04 ± 0.02 to 6.74 ± 0.03 mg MDA/kg fish during 7 months of storage at $2 \pm 2°C$. Sallam et al. (2007), working with vacuum-packed Pacific saury marinades (3% acetic acid and 12% NaCl), found that TBA increased from 0.63 to 2.13 mg MDA/kg fish after 70 days of storage at 4°C and then tended to decrease until the end of the analyses period (90 days).

In control samples, the maximum value of TBARS found was 3.25 mg ALs/kg fish. This would indicate that the preservative effect of marination and vacuum-packaging gave the product stability against lipid oxidation. In turn, gamma irradiation was effective in improving stability and did not induce rancidity due to the action of free radicals induced by irradiation, probably due to vacuum-packaging.

Color

Deteriorating and oxidative reactions produce changes in color of fish that can be measured by the parameters of the CIELAB color space system. Figure 4 shows the evolution of a^*, b^*, and DE^*_{ab} in marinated anchovy fillets during 20 months of refrigerated storage. Values of b^* (yellowness) significantly ($p < 0.05$) increased in all samples from a mean value of 13.1 ± 1.3 to 18.1 ± 0.9 during storage. Gamma irradiation did not produce changes in b^* immediately after it was applied. This slight increase in yellowness can be related to the small increase in $TBARS_{455}$ value in either control or irradiated samples. Increases in b^* have been associated with deteriorating and lipid oxidation product reactions in squid (Thanonkaew et al., 2006). Positive values of a^* represent red color intensity. Behavior of a^* (redness) was similar for control and irradiated samples throughout 20 months of refrigerated storage. A slight decrease in a^* from 1.0 ± 0.5 to 0.2 ± 0.3 was observed (mean value of control and irradiated samples) after 6 months of storage. Parameter a^* was not affected by radiation dose ($p > 0.05$).

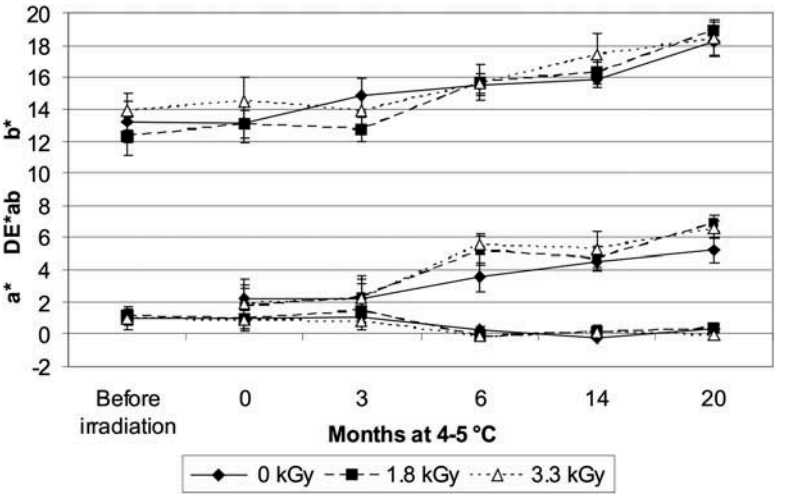

FIGURE 4 Evolution of color parameters a^* and b^* and color difference (DE^*_{ab}) in gamma irradiated anchovy fillets marinades during storage at 4 ± 1°C. Standard error represented by bars ($n = 2$).

Color difference (DE^*_{ab}) was calculated taking as reference values of L^*, a^*, and b^* before irradiation was applied. DE^*_{ab} significantly increased in all samples during storage, with no significant ($p > 0.05$) effect of gamma irradiation on it. Color varied with the same pattern in control and irradiated samples. Deteriorating reactions during storage would be responsible for color changes. These results would indicate that the effect of storage time on color characteristics of anchovy marinades was more significant that of radiation dose.

CONCLUSIONS

Gamma irradiation reduced mesophilic bacterial counts and delayed the production of TVBN without inducing modifications in the characteristic color of anchovy marinades. Lipid oxidation was lower in irradiated samples in comparison with control. Marination combined with vacuum-packaging gave the product higher stability in comparison with the traditional marinated anchovy product, but gamma irradiation delayed deteriorating reactions improving microbiological and chemical quality of marinated anchovy fillets. Results found in this work indicate that low doses of gamma irradiation could be used to extend marinade shelf life and would also make possible the reduction of the intensity of the marination hurdles in future studies.

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REFERENCES

AOAC. 1993. Official Methods of Analysis of the Association of Official Analytical Chemists (16 ed.). Washington, DC: Author.

Arvanitoyannis, I. S., and Stratakos, A. C. 2010. Effect of irradiation on fish and seafood. In: Irradiation of Food Commodities. Arvanitoyannis, I. S. (Ed.). London, UK: Academic Press. Pp. 287–365.

Baird-Parker, A. C. 1980. Organic acids. In: Microbial Ecology of Foods. International Commission for the Microbiological Specifications of Foods (Ed.). New York, NY: Academic Press. Pp. 126–135.

Barbosa-Cánovas, G. B., Pathakamury, U. R., Palou, E., and Swanson, B. G. 1999. Conservación no Térmica de Alimentos. Zaragosa, Spain: Acribia.

Bligh, E. G., and Dyer, W. J. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. 37: 911–917.

Botsoglou, N. A., Fletouris, D. J., Papageorgiou, G. E., Vassilopoulos, V. N., Mantis, A. J., and Trakatellis, A. G. 1994. Rapid, sensitive, and specific thiobarbituric acid method for measuring lipid peroxidation in animal tissue, food, and feedstuff samples. J. Agr. Food Chem. 42(9): 1931–1937.

Brewer, M. S. 2009. Irradiation effects on meat flavour: A review. Meat Sci. 81: 1–14.

Cabrer, A. I., Casales, M. R., and Yeannes, M. I. 2002. Physical and chemical changes in anchovy (*Engraulis anchoita*) flesh during marination. J. Aquat. Food Prod. T. 11(1): 19–30.

Capaccioni, M. E., Casales, M. R., and Yeannes, M. I. 2011. Acid and salt uptake during the marinating process of *Engraulis anchoita* fillets. Influence of the solution: fishratio and agitation. Ciênc. Tecnol. Aliment. Campinas 31(4): 884–890.

Chouliara, I., Savvaidis, I. N., Panagiotakis, N., and Kontominas, M. G. 2004. Preservation of salted, vacuum-packed, refrigerated sea bream (*Sparus aurata*) fillets by irradiation: Microbiological, chemical and sensory attributes. Food Microbiol. 21: 351–359.

CIE. 1978. Recommendations on Uniform Color Spaces, Color Difference Equations, Psychometric Color Terms (CIE Publication No. 15, Suppl. No. 2). Vienna, Austria: Author.

Diehl, J. F. 2002. Food irradiation: Past, present and future. Radiat. Phys. Chem. 63: 211–215.

Foley, D. M. 2006. Irradiation of seafood with a particular emphasis on *Listeria mocytogenes* in ready-to-eat products. In: Food Irradiation Research and Technology. Sommers, C. H., and Xuetong, F. (Eds.). Oxford, UK: IFT Press and Blackwell Publishing.

Fuselli, S. R., Casales, M. R., Fritz, R., and Yeannes, M. I. 1994. Microbiology of the marination process used in anchovy (*Engraulis anchoita*) production. LWT-Food Sci. Technol. 27: 214–218.

Fuselli, S. R., Casales, M. R., Fritz, R., and Yeannes, M. I. 1998. Isolation and characterization of microorganisms associated with marinated anchovy (*Engraulis anchoíta*). J. Aquat. Food Prod. T. 7(3): 29–38.

Giannini, D. H., Davidovich, L. A., and Lupín, H. M. 1979. Adaptación del método comercial para la determinación de nitrógeno básico volátil en merluza (*Merluccius hubbsi*). Rev. Agroquím. Tecnol. 19(1): 55–60.

Gökoglu, N., Cengiz, E., and Yerlikaya, P. 2004. Determination of the shelf life of marinated sardine (*Sardina pilchardus*) stored at 4°C. Food Control 15: 1–4.

Gökoglu, N., Topuz, O., and Yerlikaya, P. 2009. Effects of pomegranate sauce on quality of marinated anchovy during refrigerated storage. LWT-Food Sci. Technol. 42: 113–118.

Günsen, U., Özkan, A., and Aydm, A. 2011. Determination of some quality criteria of cold storaged marinated anchovy under vacuum and modified atmosphere conditions. Turk. J. Fish Aquat. Sc. 11: 233–242.

IAEA. 2000. Irradiation of Fish, Shellfish and Frog Legs: A Compilation of Technical Data for Authorization and Control. Vienna, Austria: Author.

ICGFI. 1999. Facts about food irradiation. International Consultative Group on Food Irradiation. Joint FAO/ IAEA Division of Nuclear Techniques in Food and Agriculture. International Atomic Energy Agency. Pp. 48. http://www.iaea.org/Publications/Booklets/foodirradiation.pdf.

ICMSF. 1983. Microorganismos de los Alimentos 1. Técnicas de Análisis Microbiológico. Zaragosa, Spain: Acribia.

Jeevanandam, K., Kakatkar, A., Doke, S. N., Bongirwar, D. R., and Venugopal, V. 2001. Influence of salting and gamma irradiation on the shelf life of threadfin bream in ice. Food Res. Int. 34: 739–746.

Kasimoglu, A., Denli, E., and Erhan, I. 2003. The extension of the shelf life of sardine which were packaged in a vacuum stored under refrigeration, and treated by gamma irradiation. Int. J. Food Sci. Tech. 38: 529–535.

Kilinc, B., and Cakli, S. 2004. Chemical, microbiological and sensory changes in thawed frozen fillets of sardine (*Sardina pilchardus*) during marination. Food Chem. 88: 275–280.

Kirk, R. S., Sawyer, R., and Egan, H. 1996. Composición y análisis químico de los alimentos de Pearson. Mexico, DF, Mexico: Compañía Editorial Continental S.A de C.V.

Kosugi, H., Kato, T., and Kikugawa, K. 1987. Formation of yellow, orange, and red pigments in the reaction of alk-2-enals with 2-thiobarbituric acid. Anal. Biochem. 165: 456–464.

Lakshmanan, R., Venugopal, V., Venketashvaran, K., and Bongirwar, D. R. 1999. Bulk preservation of small pelagic fish by gamma irradiation: Studies on a model storage system using anchovies. Food Res. Int. 32: 707–713.
Leistner, L. 2000. Basic aspects of food preservation by hurdle technology. Int. J. Food Microbiol. 55: 181–186.
Lescano, G., Kairiyama, E., Narvaiz, P. and Kaupert, N. 1990. Studies on quality of radurized (refrigerated) and non-radurized (frozen) Hake (Merluccius merluccius hubbsi). Lebensm Wiss u Technol. 23: 317–321.
Massa, A. E., Manca, E., and Yeannes, M. I. 2012. Development of quality index method for anchovy (*Engraulis anchoita*) stored in ice: Assessment of its shelf life by chemical and sensory methods. Food Sci. Technol. Int. 18(4): 339–351.
Mbarki, R., Miloud, N. B., Selmmi, S., Dhib, S., and Sadok, S. 2009. Effect of vacuum packaging and low-dose irradiation on the microbial, chemical and sensory characteristics of chub mackerel (*Scomber japonicus*). Food Microbiol. 26: 821–826.
Meyer, V. 1965. Marinades. In: Fish as Food (Vol. 3, Pt. I). Borgstrom, G. (Ed.). New York, NY: Academic Press. 221 p.
Moreno Aizpún, J. E., Moreno, V. J., and Malaspina, A. M. 1979. Variaciones en la composición bioquímica proximal de la anchoíta durante tres temporadas de pesca (1975–1977). Rev. Invest. Desarr. Pesq. 1(1): 44–53.
Moseley, B. E. B. 1989. Ionizing irradiation: Action and repair. In: Mechanisms of Action of Food Preservation Procedures. Gould, G. W. (Ed.). London, UK: Elsevier Applied Science. Pp 43–70.
Narvaiz, P., Kairiyama, E., Lescano, H. G., and Kaupert, N. L. 1989. Prolongación del tiempo de comercialización de filete de merluza (*Merluccius merluccius hubbsi*). La Alimentación Latinoamericana 177: 26–33.
Özden, Ö., Inugur, M., and Erkan, N. 2007. Effect of different dose gamma radiation and refrigeration on the chemical and sensory properties and microbiological status of aqua cultured sea bass (*Dicentrarchus labrax*). Radiat. Phys. Chem. 76: 1169–1178.
Özogul, F., Polat, A., and Özogul, Y. 2004. The effects of modified atmosphere packaging and vacuum packaging on chemical, sensory and microbiological changes of sardines (*Sardina pilchardus*). Food Chem. 85: 49–57.
Paari, A., Kanmani, P., Satishkumar, R., Yuvaraj, N., Pattukumar, V., Agrawal, M., and Arul, V. 2012. The combined effect of irradiation and antioxidant packaging on shelf life extension of goat fish (*Parupeneus indicus*): Microbial, chemical and EPR spectral assessment. J. Food Process. Pres. 36(2): 152–160.
Pastous-Madureira, L. S., Castello, J. P., Prentice-Hernández, C., Queiroz, M. I., Espírito Santo, M. L., Ruiz, W. A., Raggi Abdallah, P., Hansen, J., Bertolotti, M. I., Manca, E., Yeannes, M. I., Avdalov, N., and Fernández Amorín, S. 2009. Current and potential alternate food uses of the Argentine anchoita (*Engraulis anchoita*) in Argentina, Uruguay and Brazil. In: Fish as Feed Inputs for Aquaculture: Practices, Sustainability and Implications (FAO Fisheries and Aquaculture Technical Paper No. 518). Hasan, M. R., and Halwart, M. (Eds.). Rome, Italy: FAO. Pp. 269–289.
Phillips, C. A. 1996. Modified atmosphere packaging and its effects on the microbiological quality and safety of produce. Int. J. Food Sci. Tech. 31(6): 463–479.
R Development Core Team. 2008. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from http://www.R-project.org
Sallam, Kh. I., Ahmed, A. M., Elgazzar, M. M., and Eldaly, E. A. 2007. Chemical quality and sensory attributes of marinated Pacific saury (*Cololabis saira*) during vacuum-packaged storage at 4 °C. Food Chem. 102: 1061–1070.
Schormüller, J. 1969. Handbuch der lebensmittelchemie (Band IV), Springer Verlag, Berlin, Heidelberg, New York.
Stamatis, N. and Arkoudelos, J., 2007. Quality assessment of Scomber colias japonicus under modified atmosphere and vacuum packaging. Food Control 18(4): 292–300.
Thanonkaew, A., Benjakul, Z., Visessanguan, W., and Decker, E. A. 2006. Development of yellow pigmentation in squid (*Loligo peali*) as a result of lipid oxidation. J. Agr. Food Chem. 54: 956–962.
Tironi, V. A., Tomás, M. C., and Añón, M. C. 2007. Lipid and protein deterioration during the chilled storage minced of sea salmon (*Pseudopercis semifasciata*). J. Sci. Food Agr. 87: 2239–2246.
Tomac, A., and Yeannes, M. I. 2012. Gamma radiation effect on quality changes in vacuum packed squid (*Illex argentinus*) mantle rings during refrigerated (4–5°C) storage. Int. J. Food Sci. Tech. 47(7), 1550–1557. doi:10.1111/j.1365-2621.2012.03005.x
Urbain, W. M. 1986. Food Irradiation: Food Science and Technology Monographs. Orlando, FL: Academic Press.
WHO. 1994. Safety and Nutritional Adequacy of Irradiated Food. Geneva, Switzerland: Author. Pp. 81–107.
Yeannes, M.I. and Casales, M.R. 1995. Estudio de las variables de proceso de marinados de anchoíta (E. anchoita). Alimentraria, Mayo 95: 87–91.
Yeannes, M.I. and Casales, M.R. 2008. Modifications in the chemical compounds and sensorial attributes of Engraulis anchoita fillet during marinating process. Food Science and Technology, Campinas 28(4): 798–803.