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Gamma radiation effect on quality changes in vacuum-packed squid (*Illex argentinus*) mantle rings during refrigerated (4–5 °C) storage

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Summary The effect of gamma radiation (0, 1.8, 3.3 and 5.8 kGy) on microbiological, chemical and colour characteristics of vacuum-packed squid (*Illex argentinus*) mantle rings was studied. Total viable counts; psychrotrophic bacteria counts, *Escherichia Coli*, *Staphylococcus aureus* and *Clostridium perfringens*; total volatile basic nitrogen (TVBN) and colour differenceDE^*_{ab} were analysed during 29 days of storage at 4–5 °C. Higher doses of gamma radiation significantly reduced Total Viable, phychrotrophic counts and TVBN production ($P < 0.05$) in a dose-dependent way, delaying squid spoilage. Colour difference of non-irradiated samples with respect to first day significantly increased while it was constant in radiated samples during 22 days ($P < 0.05$). Independently from the dose, radiation avoided colour changes of squid rings. Gamma irradiation was effective in delaying deterioration reactions, improving microbiological, chemical and colour quality of vacuum-packed squid rings stored at 4–5 °C.

Keywords Colour, *Illex argentinus*, ionising radiation, microbial activity, quality, refrigeration.

Introduction

Food irradiation has been widely studied as a food preservation method for the last five decades. It has certainly proved its toxicological safety as well as it efficiency in shelf life extension by decreasing microbial counts. At present, more than 60 countries have approved irradiation of one or more foods (WHO, 1994, 1999, Diehl, 2002; Sommers & Fan, 2006).

Nutritional adequacy of irradiated food has also been largely investigated. Irradiation can induce changes in proteins, lipids, carbohydrates and vitamins due mainly to free radicals produced by water radiolysis. However, no significant losses of the nutritional quality of lipid, carbohydrate and protein constituents have been reported at irradiation doses intended for food preservation (≤10 kGy) (Josephson *et al*., 1978; Kilcast, 1995; Giroux & Lacroix, 1998; ICGFI, 1999; ADA Report, 2000).

Among lipids, polyunsaturated fatty acids (PUFAs) are more sensitive to oxidation by free radicals. The absence of oxygen can minimise this effect, as observed by Kim *et al*. (2002) in raw beef, turkey and pork meats. Erkan & Özden (2007) concluded that irradiation had only marginal effects on the lipids of fishery products, including the essential alpha-linolenic acid. Abreu *et al*. (2010) found that irradiation doses up to 6 kGy did not compromise negatively the fatty acid composition, omega three long chain PUFAs and lipid stability of frozen headed shrimps.

In proteins, irradiation can promote cleavage of peptide and disulphide bonds as well as aggregation reactions, without drastic changes in the amino acid content. According to various research works, no considerable losses were detected in essential and non-essential amino acids of different food systems (Giroux & Lacroix, 1998; Josephson *et al*., 1978; Kilcast, 1995; Urbain, 1986). Haddok fillets irradiated with 53 kGy did not show significant differences in their amino acid content (Venugopal *et al*., 1999). Erkan & Özden (2007) studied the amino acid composition of *Sparus aurata* irradiated with 2.5 and 5 kGy and observed that, in general, it was slightly increased by gamma irradiation.

Vitamins sensitivity to irradiation depends on their solubility in water or fat and the complexity of the food. Vitamins B1 (thiamin), C (ascorbic acid), A (retinol) and E (a-tocopherol) are sensitive to irradiation. Thiamin is considered the most radiation labile water-soluble vitamin. However, it is more sensitive to heat than to irradiation. Alpha-tocopherol is recognised as the most radiation sensitive fat-soluble vitamin (Giroux & Lacroix, 1998; ICGFI, 1999).

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Considering the aforementioned, the effects of irradiation on the nutritional value of foods are minimal and these observations are substantiated by the results of many feeding studies that have been undertaken to establish the wholesomeness of irradiated food (ICGFI, 1999).

Radiation processing benefits on the preservation and microbial quality improvement of fish and seafood have been supported by more than 40 years of scientific studies. Some of these were reviewed by Foley (2006). Doses of 1–3 kGy have been used in fish and of 2–7 kGy in shellfish with satisfactory results in shelf life extension (Kilcast, 1995). Shelf life of *Sea bream* (Chouliara *et al*., 2004), *Merluccius hubbsi* (Lescano *et al*., 1990) and whole anchovies (Lakshmanan *et al*., 1999) was improved by gamma irradiation, and shelf-stable ready-to-eat shrimps were developed using this technology by Kanatt *et al*. (2006). Byun *et al*. (2000) have preserved salted and fermented squid (*Todarodes pacificus*) by gamma irradiation, but no studies on shelf life improvement of fresh minimally processed squid have been made to the moment.

In the last decades, the world market of squid has considerably risen, making of the Southwest Atlantic Ocean region one of the most important fishery zones. *Illex argentinus* is the most abundant squid species of the region, representing the second fishery in volume, after *Merluccius hubbsi*. It is frequently found between the 52°S and the 35°S over the Argentinean continental platform and slope (Brunetti *et al*., 1999). In 2006, total marine captures exceeded one million tons, of which squid represented 27.3% (MINAGRI, 2007).

Many different squid products are found in the market. Squid tubes and rings are usually treated with polyphosphates solutions that are largely used in the fishery industry to improve water-holding capacity of proteins. This fact benefits the final quality of the product by retaining natural moisture, flavour and nutrients, improving texture and reducing the cooking loss. In addition, phosphates delay lipid oxidation and stabilise colour by quelling enzyme (metal) cofactors (Lampila, 1993; Knipe, 2004; Gonçalves & Duarte Ribeiro, 2008, 2009).

After catch, quality of squid decreases because of chemical and microbiological deteriorating reactions. Research has been done on quality of fresh and spoiling squid (Melaj *et al*., 1997; Lapa-Guimarâes *et al*., 2002; Paarup *et al*., 2002a,b; Vaz-Pires *et al*., 2008). During spoilage, the main chemical change is the gradual accumulation of certain volatile amines in the flesh, which regroup mainly trimethylamine (TMA), dimethylamine (DMA) and ammonia (Huss, 1995). Spoilage is also characterised by the decrease in sensory quality because of changes in squid skin and muscle colour. These colour changes associated with quality loss have been studied by Lapa-Guimarâes *et al*. (2002) in *Loligo plei*, by Sungsri-In *et al*. (2011) in *Loligo formosana* and by Thanonkaew *et al*. (2006) in *Loligo peali*.

The objective of this work was to analyse the effect of different gamma radiation doses on microbial activity and colour changes of vacuum-packed squid (*Illex argentinus*) rings during refrigerated storage.

Materials and methods

Raw material source, treatment and storage

Peeled squid mantle rings of *Illex argentinus* specimens (width = 1.2 cm approximately.) pretreated with commercial polyphosphates solutions were acquired in the port of Mar del Plata (Argentina). They were covered in ice flakes and transported to laboratory in polystyrene containers. Samples of approximately 110 ± 2 g were vacuum packed in Cryovac bags of LDPE and nylon (125 μm) using a packaging machine MINIMAX 430M (SERVIVAC, Argentina). Samples were transported and refrigerated (4 ± 3 °C) to the semi-industrial Ezeiza Atomic Centre facility [National Atomic Energy Commission (CNEA) of Argentina; activity: 600 000 Ci]. They were gamma irradiated with a Cobalt 60 source at 1.8, 3.3 and 5.8 kGy (minimum doses absorbed). Doses were determined with Amber Persex dosimeters. Irradiated and non-irradiated samples (control, 0 kGy) were stored at 4–5 °C for 29 days. Samples were analysed days 0, 1, 5, 8, 12, 15, 19, 22, 26 and 29 after irradiation. Each sample consisted of a 110 ± 2 g bag of vacuum-packed squid rings (approximately 20 squid rings). There were three samples for each day of analysis and each radiation dose.

Microbiological analysis

The following analyses were performed to monitor bacterial flora changes during refrigerated storage. Ten grams of sample in saline solution (0.85%) with 0.1% w/v peptone (ICMSF, 1983) made to 100 mL were macerated in a Stomacher 400 Circulator Homogenizer. Microbiological analyses were done in triplicate and expressed as log CFU per gram. The counts of total microorganisms and isolations of particular microbial groups were performed using the following culture media and culture conditions.

Psychrotrophic bacteria on plate count agar incubated at 7 ± 0.5 °C for 10 days, total viable counts aerobic mesophilic bacteria (TVC) incubating at 35 ± 0.5 °C during 48 h (ICMSF, 1983), coliforms on violet red bile agar incubated at 35 ± 0.5 °C for 24 h (ICMSF, 1983), faecal coliforms in brilliant green bile broth incubated at 44 ± 0.5 °C for 48 h (ICMSF, 1983), and *Staphylococcus* spp. on Baird-Parker agar incubated at 35 ± 0.5 °C for 48 h (ICMSF, 1983). Coagulase test and manitol salt agar were used for

identification of *S. aureus* (ICMSF, 1983). Sulphite-reducing *Clostridium* on SPS Agar (Merck) was incubated in anaerobic jars at 35 ± 0.5 °C and 45 ± 0.5 °C for 48 h (ICMSF, 1983; Pascual & del Rosario, 2000). Colonies were confirmed by motility-nitrate test (ICMSF, 1983).

Total volatile basic nitrogen

It was determined by the commercial method for TVBN adapted from the direct distillation method (Giannini *et al.*, 1979). Ten grams of processed squid rings were homogenised with 300 mL of distilled water, 2 mL of antifoaming, porous plate and 5 g of Magnesium oxide. Distillate was collected in 50 mL of boric acid 2% w/v and 1 mL of indicator (100 mL ethanol, 0.05 g methyl red and 0.075 g bromocresol green) to a final volume of 230 mL. Then, it was titrated with sulphuric acid 0.1 N. TVBN was determined by duplicate. Results were expressed in milligrams of total volatile basic nitrogen per 100 g of wet sample.

Colour analysis

CIELAB colour space system parameters, (L^* = lightness, a^* = red (+) and green (-) colour intensity, and b^* = yellow (+) and blue (-) colour intensity) were determined with a portable colorimeter (NR-3000; Nipon Denshoku Kogyo Co. Ltd., Tokiyo, Japan). Measurements were made on five rings of each sample. These values were used to calculate colour differences DE^*_{ab} (C.I.E., 1978) for each storage time analysed with respect to reference (day 1) using

$$DE^*_{ab} = [(L^* - L^*_r)^2 + (a^* - a^*_r)^2 + (b^* - b^*_r)^2]^{1/2}$$

Statistical analysis

Results were analysed by a completely aleatorised design with two main factors: Radiation Dose (0, 1.8, 3.3 and 5.8 kGy) and Days of Storage (0, 1, 5, 8, 12, 15, 19, 22, 26 and 29 days). Interaction between them was also analysed. A two-ways ANOVA test was used with 5% significance level. Tukey test was used to compare means ($P < 0.05$). The R-Project software was used (R Development Core Team, 2008).

Results and discussion

Microbiological analysis

Total viable aerobic counts of mesophilic bacteria evolution during storage at 4–5 °C for different doses are shown in Fig. 1. Before irradiation was applied, initial TVC of squid rings was $2.12 \times 10^4 \pm 6.5 \times 10^2$ CFU g^{-1}. In Fig. 1, it can be seen that with doses

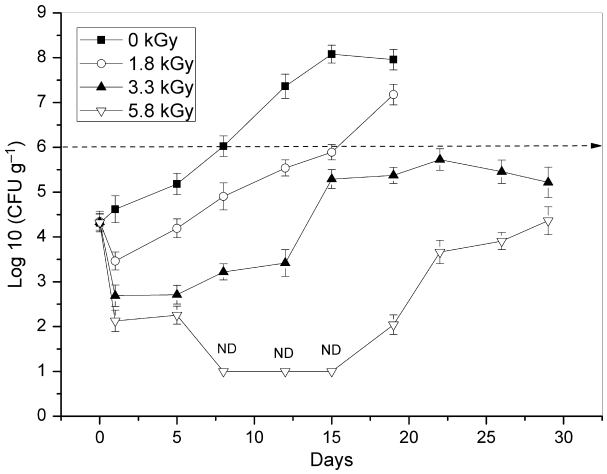

Figure 1 Total Viable Mesophilic Counts (log CFU g^{-1}) evolution in vacuum-packed *Illex argentinus* rings during storage at 4–5 °C. Standard error represented by bars ($n = 3$). ND, Not detectable (< 10 UFC g^{-1}).

of 1.8, 3.3 and 5.8 kGy, logarithmic cycle's reductions of 0.9, 1.6 and 2.2 were achieved in initial TVC, respectively, 1 day after irradiation. This fact shows the significant effect of radiation to reduce initial TVC values compared with control, in which TVC increased on day 1 with respect to day 0 ($P < 0.05$).

Initial TVC of control significantly increased up to $9.1 \times 10^7 \pm 2.1 \times 10^6$ CFU g^{-1} after 19 days of refrigerated storage. It was significantly higher than TVC of radiated samples during the whole storage period because its increment was faster than in radiated samples ($P < 0.05$). TVC of samples radiated with 1.8 kGy significantly increased during 19 days but in a lower rate than it did in control ($P < 0.05$). Total viable counts of samples radiated with 3.3 kGy significantly increased during 22 days but tended to decrease on days 26 and 29 due possibly to nutrient depletion. At day 19 after radiation, a reduction of one logarithmic cycle was achieved with a dose of 1.8 kGy. With 3.3 and 5.8 kGy, TVC counts were reduced in three and six logarithmic cycles compared with control, respectively. Samples radiated with higher doses reached lower viable counts. During storage, samples radiated with 1.8 and 3.3 kGy had a similar tendency to control, showing exponential growth (lower counts with higher dose, as aforementioned). However, samples radiated with 5.8 kGy presented a different behaviour, with decreasing mesophilic counts during storage, reaching a reduction under TVC values of day 1. In samples radiated with 5.8 kGy, TVC values of days 8, 12 and 15 were significantly lower than TVC of days 1 and 5 ($P < 0.05$). This behaviour was also commented by Kodo (1990) with a dose of 3 kGy in mackerel fillets.

Different microbiological counts limits have been suggested for different fish and mollusc species, and they vary

according to many factors, such as, species, sample treatment and storage conditions, among others. Moragas Encuentra & De Pablo Busto (1991) mentioned a limit of 10^6 CFU g^{-1} for fresh fishery products (dotted line in Fig. 1). In this work, TVC of 10^6 CFU g^{-1} was found in control at day 8, while with a dose of 1.8 kGy that value was reached after 15 days of storage. It was not reached during 29 days of refrigerated storage with doses of 3.3 and 5.8 kGy, which highest TVC were 5.3×10^5 and 2.3×10^4 CFU g^{-1}, at days 22 and 29 after irradiation, respectively. Considering these values, gamma irradiation extended microbiological shelf life of squid rings in seven and in more than 21 days with respect to control, using doses of 1.8 and 3.3 or 5.8 kGy, respectively.

In control *Staphylococcus* spp. colonies developed on day 8 and after 12, 15 and 19 days in samples radiated with 1.8, 3.3 and 5.8 kGy, respectively. Coliforms were detected on day 5 in control, on day 12 in samples irradiated with 1.8 kGy and on day 19 in samples irradiated with 3.3 kGy. Coliforms were not detected in samples irradiated with 5.8 kGy during 29 days of storage. Gamma radiation reduced the rate of growth of *Staphylococcus* spp. and coliforms.

Pathogens microorganisms investigated (*Staphylococcus aureus*, *Clostridium perfringens* and *Escherichia coli*) were not found during the whole storage period, neither in control nor in radiated samples.

Gamma radiation significantly reduced TVC counts in a dose-dependent way. During the whole storage period, TVC of control was higher than radiated samples. The higher dose applied the lower bacterial counts found in vacuum-packed *Illex argentinus* rings ($P < 0.05$).

In Fig. 2, it is shown psychrotrophic bacteria counts evolution during refrigerated storage of control and radiated *Illex argentinus* rings.

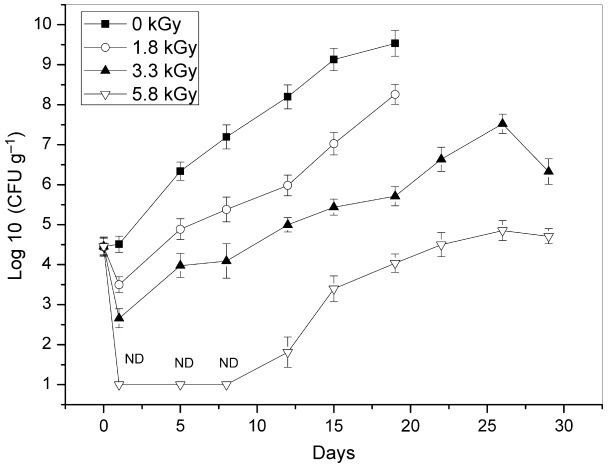

Figure 2 Psychrotrophic bacteria counts (log CFU g^{-1}) evolution in vacuum-packed *Illex argentinus* rings during storage at 4–5 °C. Standard error represented by bars ($n = 3$). ND, Not detectable (< 10 UFC g^{-1}).

Initial psychrotrophic counts were $2.8 \times 10^4 \pm 4.6 \times 10^2$ CFU g^{-1}. One day after radiation, logarithmic cycle's reductions of 1, 1.8 and 3.4 were achieved with 1.8, 3.3 and 5.8 kGy, respectively; meanwhile, psychrotrophic counts of control increased in the same period. After radiation induced initial counts reduction, psychrotrophic counts of samples 0, 1.8 and 3.3 kGy significantly increased up to $3.9 \times 10^9 \pm 1.3 \times 10^8$, $1.8 \times 10^8 \pm 2.1 \times 10^6$ and $5.1 \times 10^5 \pm 2.1 \times 10^4$ CFU g^{-1}, respectively, during 19 days of refrigerated storage ($P < 0.05$). In samples radiated with 5.8 kGy, colonies were not detected at days 1, 5 and 8 after radiation, but after that period counts significantly increased up to $1.4 \times 10^4 \pm 1.5 \times 10^2$ CFU g^{-1} on day 19.

Psychrotrophic counts for control were significantly higher than 1.8, 3.3 and 5.8 kGy during the whole storage period after irradiation was applied. Psychrotrophic counts of samples radiated with 1.8 kGy were significantly higher than 3.3 kGy and 5.8 kGy. Samples radiated with 3.3 kGy had significantly higher counts than 5.8 kGy during 29 days of refrigerated storage ($P < 0.05$). Statistical results indicated that gamma radiation significantly reduced psychrotrophic counts in a dose-dependent way ($P < 0.05$).

Even when a dose of 1.8 kGy was enough to reduce microbial counts compared with control, with doses of 3.3 and 5.8 kGy, this effect was more important. At day 19 a 1, 3.8 and 5.5 logarithmic cycle reduction in psychrotrophic bacteria counts were achieved with 1.8, 3.3 and 5.8 kGy, respectively. With a dose of 1.8 kGy, bacterial counts were lowered but no more than in one logarithmic cycle; therefore, higher doses could be more useful to extend squid rings microbiological shelf life.

Psychrotrophic bacteria counts after 19 days of refrigerated storage were higher than mesophilic counts in all samples. According to Huss (1994), psychrotrophic bacteria are particularly the major group of microorganisms responsible for spoilage of fresh seafood. Refrigeration temperatures favour their growth, and so they were at an adequate temperature range to growth during storage conditions of this work.

Initial TVC and psychrotrophic counts reductions because of gamma radiation found in this work are in accordance with Kodo (1990), who explains the radiation induced initial counts reduction by the inhibition of predominant bacteria and a subsequent growth of more radio resistant species that were initially limitated by the others. Similar results were found by Byun *et al.* (2000) who found that increasing radiation doses lowered initial viable cell populations in fermented *Todarodes pacificus* and that a dose of 5 kGy helped to improve microbiological quality of 10% NaCl salted squid.

Like in this work, Lakshmanan *et al.* (1999) observed a reduction in TVC because of gamma radiation in whole anchovies irradiated at 2 kGy. Also Kanatt *et al.* (2006) found lower counts at higher doses in marinated

precooked shrimps, using gamma irradiation at doses of 1, 2.5 and 5 kGy. Goldblith (1971) has explained that ionising radiation inactivates microorganisms by direct and indirect action. In direct action, it damages cells' DNA in living organisms by an ionising particle or ray. In indirect action, the products of radiolysis, usually of water in most foods, affect the cell. Brewer (2009) also explains that free radicals generated by radiation can damage the DNA of growing cells, accomplishing a 90% reduction in spoilage bacteria with 1–1.5 kGy.

According to microbiological analyses carried out in this work on vacuum-packed *Illex argentinus* mantle rings during refrigerated storage, gamma radiation was useful for reducing mesophilic, psychrotrophic, coliforms and *Staphylococcus* spp. bacterial counts in a dose-dependent way. Higher doses extended the storage period in which squid rings reached suggested microbial counts limits. The effect of 3.3 and 5.8 kGy of shelf life extension was more important than the one achieved with 1.8 kGy.

Figures aforementioned support the feasibility of extending microbiological shelf life of vacuum-packed squid rings by gamma radiation.

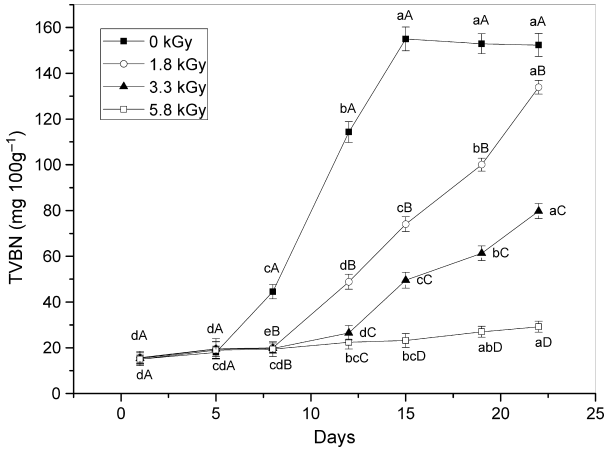

Figure 3 Total volatile basic nitrogen (TVBN) (mg per 100 g squid rings) evolution during storage at 4–5 °C for the different radiation doses (kGy). Same letters (a, b, c, d, e, f) indicate non-significant differences in time for the same dose. TVBN values with same Capital letters are not significantly different between doses (Tukey Test, $P < 0.05$). Bars represent standard error.

Total volatile basic nitrogen

One of the most widely used parameters to evaluate fish freshness is total volatile basic nitrogen (TVBN) that includes the measurement of TMA, DMA and ammonia (Huss, 1995). Woyewoda & Ke (1980) recommended the use of TVBN as a laboratory test for squid quality assessment. In this work, it was used as a freshness indicator of squid rings. In Fig. 3, it is shown its evolution in squid rings during storage at 4–5 °C for different doses applied. TVBN initial mean value was 15.16 ± 0.16 mg per 100 g. TVBN values of control and radiated squid rings showed an increasing behaviour during refrigerated storage, but the rate of TVBN accumulation was different depending on the radiation dose and the storage time. It was inverse to the dose applied (i.e. with higher doses, lower TVBN values were reached), and it was considerably accelerated after 5 days of storage in non-radiated samples, increasing exponentially. Meanwhile, it took 8 and 12 days to reach the TVBN accelerated accumulation phase in samples radiated with 1.8 and 3.3 kGy, respectively. In samples radiated with the higher dose (5.8 kGy), TVBN increased only linearly with time and did not reach an exponential phase during 22 days. TVBN values were significantly higher for samples radiated with 0, 1.8, 3.3 and 5.8 kGy, respectively, from day 8 after irradiation until day 22 ($P < 0.05$).

With 1.8, 3.3 and 5.8 kGy, TVBN reductions of 52%, 69% and 86% were achieved with respect to control on storage day 15, respectively. Alur *et al.* (1994) found a 50% to 60% reduction in total volatile bases in irradiated fish flesh compared with non-irradiated samples.

Gamma irradiation delayed squid spoilage with higher radiation doses decreasing TVBN production (Fig. 3) in accordance with microbiological results.

Gamma radiation has been reported to have little effect on enzymatic systems but to have a considerable microcidal effect (Kodo, 1990; Ahn & Lee, 2006). Hwang & Hau (1995) studied the residual activity of proteolytic enzymes [Ca^{2+} dependent proteases (CDP) and Catepsyn D] in irradiated chicken. These proteases are considered responsible of meat deterioration. They found that irradiation doses up to 50 kGy did not affect the activity of Catepsyn D. CDP activity decreased with increasing irradiation dose, but it was not fully inactivated. According to Urbain (1986), lipolytic enzymes involved in the endogenous hydrolysis of phospholipids and neutral lipids were not fully inactivated by an irradiation dose of 50 kGy.

The microcidal effect was observed in this work (3.1). Lower microorganism counts would imply a decrease in TMA production leading to a reduction in the rate of TVBN accumulation, also detected in this study. TVC and psychrotrophic counts evolution correlated in a good way with TVBN results, because exponential TVBN production was related to high bacterial counts. In samples radiated with 5.8 kGy (which presented better microbial and chemical parameters), TVC and psychrotrophic counts of 9.5×10^1 and 1.0×10^4 CFU g^{-1}were found, respectively, at day 19 and this samples never reached exponential TVBN production phase. Higher doses implied lower microor-

ganisms counts that lead to a retard in TMA accumulation and hence a slowed down TVBN production rate.

The last day of analysis, day 22, TVBN of control, 1.8, 3.3 and 5.8 kGy was 152.3 ± 5.0; 133.9 ± 3.0; 79.8 ± 3.3 and 29.2 ± 2.4 mg per 100 g, respectively. According to the scale established by Woyewoda & Ke (1980) for squid (*Illex illecebrosus*), the quality of squid can be considered excellent when TVBN is below 30 mg per 100 g; meanwhile, it is considered unacceptable when TVBN is higher than 45 mg per 100 g. Also, 30 mg per 100 g is the value established as limit of acceptance in most of commercial transactions. In this study, higher radiation doses permitted to maintain an excellent quality during a longer storage period, by increasing the time at which TVBN reached a value of 30 mg per 100 g. Quality of control sample was considered excellent during 6 days, while samples radiated with 1.8, 3.3 and 5.8 kGy during 9, 12 and 22 days, respectively.

This results would show the efficiency of gamma radiation to delay squid spoilage and thus to increase its shelf life.

Colour

Colour changes were analysed for being associated with spoilage reactions. In Fig. 4, it is shown the evolution of colour difference DE^*_{ab} of vacuum-packed *Illex argentinus* mantle rings during storage at 4–5 °C. Colour difference of control significantly ($P < 0.05$) increased during storage, reaching a value of 14.67 on day 22. For all treated samples, there were no significant differences of DE^*_{ab} during the 22 days of storage at 4–5 °C ($P > 0.05$). Values of DE^*_{ab} were practically constant ranging between 1.96 and 3.81 for radiated samples during the whole storage period. After day 8, DE^*_{ab} of control was significantly higher than all radiated samples until the end of the analyses period ($P < 0.05$).

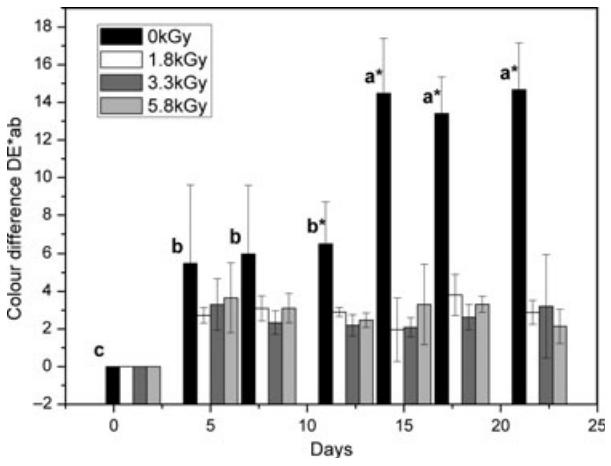

Figure 4 Squid rings Color Difference with respect to day 1 during storage at 4–5 °C. Standard error represented by bars. Different letters (a, b, c) indicate significative differences in time for control. Values with * are significantly different between doses. No significant differences during storage time were found for all radiated samples (Tukey Test, $P < 0.05$, $n = 3$).

Mean value of L^* on the first day was 52.7 ± 0.6. L^* significantly increased to 63.2 ± 1.9 in control sample until day 22. Meanwhile, in irradiated samples, L^* only reached a mean value 54.7 ± 0.7 for doses 1.8, 3.3 and 5.8 kGy. L^* of non-irradiated samples was significantly higher than irradiated samples since day 5 ($P < 0.05$). There were no significant differences in L^* on day 22 between irradiated samples ($P > 0.05$). Sugiyama *et al.* (1989) informed that after capture of squid, the transparency of its meat is gradually lost and squid meat becomes tinged white (related with L^* increase).

The first day, b^* mean value was −6.5 ± 0.9. In control sample, b^* significantly increased to −3.3 ± 1.2 (day 22), while it remained almost unchanged in irradiated samples during the whole storage period, reaching a mean value of −6.2 ± 0.2 on day 22. There were no significant differences between irradiated samples ($P > 0.05$) at the end of the storage period. Increases in b^* have been informed during squid spoilage. Thanonkaew *et al.* (2006) mentioned an increase in b^* in *Loligo peali*, associated with an increase in yellowness because of deterioration and lipid oxidation products reactions. They suggested that yellow pigmentation in squid mantle could be due to non-enzymatic browning reactions between products of aldehydic lipid oxidation and the amines of phospholipids head groups.

Mean value of a^* was −2.7 ± 0.8 on the first day of storage. Values of a^* decreased in control to −4.8 ± 0.7 (day 22), but there were not significant differences during storage time for all irradiated samples ($P > 0.05$). Mean value for samples irradiated with 1.8, 3.3 and 5.8 kGy was −2.3 ± 0.4 on day 22, without significant differences among them ($P > 0.05$). During spoilage of squid, an increase in a^* has been informed by Sungsri-In *et al.* (2011) because of pink spots formation. In this work, a^* tended to decrease in control due probably to the previous skinning of squid and the use of polyphosphates that have been reported to improve colour characteristics of fish products.

Gamma irradiation avoided colour changes of squid rings. It permitted to keep colour parameters almost unchanged during 22 days of storage at 4–5 °C in squid rings. Meanwhile, colour parameters of control significantly changed during storage, with an increase in b^* and L^* and a decrease in a^* values. These changes in control can be explained by the loss of quality because of deteriorating reactions. According to results found in this work, gamma irradiation would avoid colour

changes because of deteriorating reactions in squid rings, without changing their characteristic colour.

Conclusions

Gamma irradiation permitted to improve microbiological quality of vacuum-packed squid rings, lowering bacterial populations, reducing immediately after radiation the TVC and psychrotrophic counts and slowing down coliforms and *Staphylococcus spp*. growth. A dose-dependent reduction was observed, reaching lower counts with higher doses. TVBN production during storage at 4–5 °C was lower at higher doses, indicating a deterioration delay, in accordance with microbiological results. With higher doses, linear phase of TVBN evolution was extended, delaying exponential phase related to bacterial growth. Gamma radiation avoided colour changes in vacuum-packed squid mantle rings during 22 days of storage at 4–5 °C, while colour significantly changed in non-irradiated sample. Gamma irradiation helped to delay deterioration reactions that lead to quality loss of minimally processed vacuum-packed *Illex argentinus* rings, extending microbiological shelf life.

Acknowledgments

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