GAMMA IRRADIATION ON FROZEN AND PACKAGED HEADED SHRIMP

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ABSTRACT

The objective of this work was to evaluate the effects of γ irradiation (0, 2, 4 and 6 kGy doses), applied on frozen and packaged headed shrimps, on pathogenic Vibrio cholerae *O1 and* Salmonella enteritidis *bacteria, as well as on some of the physical and sensory characteristics of this kind of food. The 6 kGy dose was highly efficient in inhibiting V. cholerae O1 and S. enteritidis and in decreasing lipid oxidation in shrimps compared with the nonirradiated product. Shrimp texture was not affected by any of the irradiation doses studied, but the lightness of the surface color increased in shrimps irradiated with 6 kGy compared with those irradiated with 2 kGy. Shrimps irradiated with 6 kGy showed lower overall acceptability than those irradiated with 2 kGy or were nonirradiated. The application of γ irradiation in doses up to 6 kGy on frozen and packaged headed shrimps could improve the microbiological quality of this commodity.*

PRACTICAL APPLICATIONS

The use of γ irradiation has the potential to ensure safety effectively by inactivating bacteria, increasing shelf life and maintaining food quality without significant chemical changes in the food matrix. Besides, this process can be applied to frozen and packaged products. Thus, irradiation of frozen and packaged shrimps could benefit the local processing industry, which could

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offer the international market a high-quality product, with an additional safety treatment.

INTRODUCTION

Shrimp farming is the sector of the aquaculture that shows the highest growth potential worldwide. In the last 10 years, Brazilian-cultured shrimp production has significantly grown as an important economical activity in the northeastern areas of the country. Currently, *Penaeus vannamei*, the most intensively exploited species in the region, has reached high economic value in the local and external markets (Barbieri Júnior and Ostrensky Neto 2002; Rocha *et al.* 2004).

The international market requires products with high quality standards concerning not only their nutritional value, freshness and flavor but also the absence of microbial contamination. The presence of pathogenic bacteria such as *Vibrio cholerae*, *Salmonella*, *Staphylococcus aureus* and *Escherichia coli* in seafood has constituted one of the main barriers for shrimp world trade (Bhaskar *et al.* 1998; Cato 1998; Dalsgaard 1998; MAPA 2001).

In recent years, irradiation has proved to be a beneficial technique to control pathogens in food. This type of processing is safe when appropriately used and does not cause significant changes in product quality. Besides, the treatment can be applied to frozen and packaged products (Smith and Pillai 2004; Lawrie 2005).

In this respect, irradiation can be an alternative and appropriate technology to ensure microbiological safety and maintain sensory quality in seafoods. Moraes *et al.* (2000), using ^{60}Co ionizing irradiation reported significant reductions in the initial number of *V. cholerae* in oysters, while Ouattara *et al.* (2001), using γ irradiation in precooked shrimps (*Penaeus* spp.), did not observe any adverse effect on sensory parameters (appearance, odor, or taste) in this type of food.

The objective of this study was, therefore, to verify the effect of γ irradiation applied to frozen and packaged headed shrimps, on pathogenic *V. cholerae* O1 and *Salmonella enteritidis* bacteria and on some physical and sensorial characteristics of this food.

MATERIALS AND METHODS

Experiments and Materials

The study was conducted in two stages. The first stage intended to verify the effects of γ irradiation on *V. cholerae* O1 and *S. enteritidis* bacteria in

samples of headed shrimps inoculated before freezing and packaging. The second stage aimed to verify the effect of γirradiation on shrimp lipid stability, color, texture, aroma, flavor, juiciness and overall acceptability of the irradiated product.

For the first stage, fresh headed shrimps, *P. vannamei*, medium size (80–100 pieces/kg) were obtained from a local industry operating in accordance with the Brazilian Federal Inspection Service (MAPA 2001) and transported to the laboratory in a thermal container (shrimp and ice at the 1 : 1 ratio). After being separated from the ice, the shrimps were divided into 300 g portions (experimental units [EUs]), packaged in polyethylene (0.15 mm thick) bags and added to 200 mL of water. At this point, samples of shrimps and water were collected to verify the presence of *V. cholerae* and *Salmonella* sp. bacteria. Inoculation of shrimps was proceeded by the addition of 20 mL *V. cholerae* suspension (10^8 most probable number [MPN]/mL) or 10 mL *S. enteritidis* suspension (10^7 MPN/mL) into the bagged shrimps followed by mechanical homogenization. Bags containing inocula or equivalent volume of water were then sealed, packaged into cardboard boxes ($14.5 \times 11.0 \times 4.5$ cm, internally lined with polyethylene film) and frozen at −30C for 12 h. These packages were considered the EUs for the study. Nine EUs (three inoculated with *V. cholerae* O1, three inoculated with *S. enteritidis* and three without inoculation) were packaged into each of four cardboard master boxes ($18.0 \times 27.0 \times 36.0$ cm) and stored at −20C until irradiation. Each box was then irradiated with a ^{60}Cobalt source with doses of 0, 2, 4 or 6 kGy at a dose rate of 1,500 Gy/h.

In the second stage, four master boxes containing five noninoculated EUs were packaged, frozen and irradiated, as described in the first stage. EUs were then analyzed for lipid oxidation, color and texture (instrumental measures), and sensory analysis.

Samples were kept in the frozen state (−20C), and analyses were performed 5 days after irradiation treatment.

Preparation of Inocula

Inocula were prepared from cultures of *V. cholerae* O1 (CT-AB IOC 18287) and *S. enteritidis* (ATCC 12228) that were reactivated in yeast extract tryptone agar by holding at 35C for 24 h. After this period, colonies of *V. cholerae* O1 and *S. enteritidis* were transferred, respectively, to alkaline peptone water (APW) and buffered peptone water (BPW) mediums and incubated at 35C for 24 h with agitation (170 rpm). After this period, these culture suspensions, containing approximately 10^8 MPN/mL of *V. cholerae* O1 and 10^7 MPN/mL of *S. enteritidis*, were used to inoculate shrimp samples.

Enumeration of *V. cholerae* O1 and *S. enteritidis* after Irradiation

For microbiological analysis, the EUs were thawed at 5C for 18 h. Then, 25 g of each EU was mixed with 225 mL APW for *V. cholerae* or 225 mL BPW for *S. enteritidis*. Further serial dilutions were prepared, and 1 mL aliquot of each dilution was transferred to test tubes (three tubes per dilution) containing APW for *V. cholera* or BPW for *S. enteritidis*. Test tubes were incubated at 35C for 24 h. After incubation, the tubes with visible growth were streak-plated to thiosulfate citrate bile sucrose agar and to xylose lysine desoxycholate agar for *V. cholerae* and *S. enteritidis*, respectively. The plates were inverted and incubated at 35C for 24 h. Five typical colonies from each plate were picked up for confirmatory tests. The results were expressed as MPN per gram of sample (American Public Health Association 2001).

Lipid Oxidation, Color, Instrumental Texture and Sensory Analysis

Lipid oxidation was measured by determining the thiobarbituric acid reactive substances (TBARS) in an acidic extract from defrosted shrimps by using the method described by Raharjo *et al.* (1992) and modified by Facco (2002).

Color parameters, determined by using a Minolta CR300 colorimeter (Tokyo, Japan) operating in the Commission Internationale D'Eclairage (CIE) system, were L^* for lightness, a^* for redness (red $\pm$ green) and b^* for yellowness (yellow $\pm$ blue). The colorimeter was standardized by using an illuminant D65, and measurements were made through a 8-mm port/viewing area (Minolta 1998) on the surface of cooked (boiled in a 3% NaCl solution for 2 min) and peeled shrimps.

Instrumental texture (shear force) was determined on shrimps treated as described for color measurement. Shear force necessary to cut transversely the second abdominal segment of each shrimp tail was measured by using a texture analyzer TA-XT2i (Stable Micro System, Surrey, Goldaming, England) equipped with a Warner-Bratzler blade operating at a speed of 3.3 mm/s.

Sensory evaluation of irradiated shrimps was performed by 64 untrained panelists, consumers of the product, in one session conducted in individual booths under controlled conditions. The shrimps were cooked, as described, for color measurements, coded, placed randomly in plastic cups (100 mL) and served warm (50C) to panelists. Samples were presented to panelists orderly arranged, so as to avoid biased marking (Macfie *et al.* 1989).

The panel assessed the cooked shrimps for overall, color and texture acceptance, using a 9-point hedonic scale, ranging from 1 (dislike extremely) to 9 (like extremely). The attributes of aroma, flavor and juiciness were measured as a diagnosis of attributes (Meilgaard *et al.* 1987). Aroma and flavor

were evaluated by using a 5-point intensity scale raging from 1 (imperceptible) to 5 (very strong). For juiciness, the scale ranged from 1 (not juicy) to 5 (extremely juicy). For overall, color and texture acceptances were also calculated approval percentages, consisting of the sum of % frequency values comprised between 7 and 9 in the hedonic scale.

Experimental Design and Statistical Analysis

Based on the independent character of the shrimp inoculation modalities, microbiological data from the first stage followed a complete randomized (4×3) design with four irradiation doses (0, 2, 4 and 6 kGy) and three replicates per treatment.

Data from the second stage (lipid oxidation, color and texture) were analyzed as a complete randomized (4×5) design with four irradiation doses (0, 2, 4 and 6 kGy) and five replicates. The analysis of sensory data followed a whole randomized, balanced complete blocks design, where each panelist was considered as a block.

Results were submitted to analysis of variance (ANOVA) by the procedure PROC ANOVA (SAS Institute 2000), and the Student–Newman–Keuls test was used to compare mean values (5% of probability).

RESULTS AND DISCUSSION

Microbiological analysis of fresh headed shrimps and covering water used before inoculation showed the absence of *V. cholerae* and *Salmonella* sp., indicating an appropriate sanitary treatment of such food at the local industry. Inoculated and nonirradiated samples (0 kGy dose) showed viable colonies of these bacteria, indicating that packaging and freezing do not cause complete loss of viability of these pathogenic strains. However, values of 10^6 MPN/g for *V. cholerae* O1 indicated a decrease of this population concerning initial contamination (10^8 MPN/g).

The 4 kGy irradiation dose was able to eliminate *V. cholerae* O1 in frozen shrimps (Table 1). This observation is consistent with reports by several authors (Nouchpramoul 1984; Ito *et al.* 1989; Hau *et al.* 1992) who found doses of up to 4 kGy efficient in the control of pathogens in frozen seafoods including shrimps. *S. enteritidis*, however, was completely eliminated only in frozen shrimp samples irradiated with 6 kGy. This result confirms that *Salmonella* is one of the most resistant gram-negative pathogens found in many foods. Similar observations were reported by Spoto *et al.* (2000) in a study that evaluated the effect of γ irradiation in the control of pathogenic bacteria in refrigerated (5C) ground chicken meat. These authors did not detect *Salmonella typhimurium* in samples irradiated with 6 kGy.

TABLE 1.
SURVIVOR POPULATIONS OF *VIBRIO CHOLERAE* AND *SALMONELLA ENTERITIDIS* BACTERIA ON HEADED SHRIMPS (*PENAEUS VANNAMEI*) AFTER INOCULATION, FREEZING, PACKAGING AND IRRADIATION (^{60}CO) WITH 0, 2, 4 AND 6 kGy DOSES

Irradiation dose (kGy)	*V. cholerae* O1 (MPN/g)	*S. enteritidis* (MPN/g)
0	4.30×10^6a	2.30×10^7a
2	2.97×10^1b	4.63×10^3b
4	<3.00c	3.00×10^1b
6	<3.00c	<3.00b

Means values with different letters within a column are significantly different ($P < 0.05$) by Student–Newman–Keuls test.
MPN, most probable number.

Radiation resistance of microorganisms can be expressed as D_{10} value, defined as the necessary dose to eliminate 90% of the population. Different D_{10} values for *V. cholerae* and *Salmonella* have been reported in the literature. Hau *et al.* (1992), irradiating inoculated frozen prawns, observed D_{10} value of 0.11 kGy for *V. cholerae* O1. Kamat and Thomas (1998) observed D_{10} values between 0.10 and 0.15 kGy for *S. typhimurium* in shrimps homogenate irradiated at refrigeration temperature. Differences in D_{10} values found in the literature, for an identical microorganism, are caused by the parameter influenced by the food matrix, as well as by the temperature and packaging atmosphere of the food at the time of irradiation (Loaharanu 1996).

Table 1 shows a reduction of *V. cholerae* O1 and *S. enteritidis* populations on inoculated shrimps with increasing irradiation doses. The 2 kGy dose reduced ($P < 0.05$) *V. cholerae* O1 population in approximately five logarithmic cycles, while the 4 and 6 kGy doses caused reductions ($P < 0.05$) in 6 logarithmic cycles. *S. enteritidis* population was reduced in 4, 6 and 7 logarithmic cycles with 2, 4 and 6 kGy doses, respectively.

TBARS analysis is an important food quality index indicating fat oxidation. In perfect-quality products, TBARS values should be less than 3 mg malonaldeyde/kg (Cadun *et al.* 2005). Thus, the results reported in this study are close to the acceptability limits for consumption of foods (Table 2). The low TBARS values observed could be a result of the frozen state of the product, which may reduce chemical changes caused by irradiation. According to Murano (1995), the freezing process may inhibit the formation of radicals because of the interaction of ionizing energy with water molecules.

In this study, as opposed to what was expected, a significant ($P < 0.05$) decrease was observed in lipid oxidation in samples irradiated with 6 kGy,

TABLE 2.
TBARS VALUES, COLOR COMPONENTS *L**, *a** AND *b** AND INSTRUMENTAL TEXTURE (SHEAR FORCE) OF HEADED SHRIMPS (*PENAEUS VANNAMEI*) MEASURED AFTER FREEZING, PACKAGING AND IRRADIATING (^{60}CO) WITH 0, 2, 4 AND 6 kGy DOSES†

Dose (kGy)	TBARS‡	Color component *L**	Color component *a**	Color component *b**	Shear force (kg-f)
0	0.32a ± 0.04	68.12ab ± 1.53	6.68a ± 1.22	18.34a ± 1.18	2.03a ± 0.20
2	0.26ab ± 0.03	66.88b ± 1.67	7.00a ± 2.57	19.52a ± 1.60	1.91a ± 0.18
4	0.28ab ± 0.04	69.15ab ± 2.18	7.59a ± 1.95	20.45a ± 1.63	2.04a ± 0.14
6	0.23^{b} ± 0.03	71.00a ± 2.42	6.50a ± 1.96	19.46a ± 1.63	1.86a ± 0.05

Means values with different letters within a column are significantly different ($P < 0.05$) by Student–Newman–Keuls test.
† TBARS determined on raw shrimp samples. Color and instrumental texture measured on cooked samples.
‡ mg malondialdehyde/kg of raw headed shrimp.
TBARS, thiobarbituric acid reactive substances.

compared with the nonirradiated ones (Table 2). Some of the reasons for this inconsistent effect of irradiation on TBARS values may be the low levels found for this variable and the narrow precision of this determination. According to Fernandez *et al.* (1997), methods of TBA tests have been criticized as being nonspecific and of limited sensibility for the detection of low levels of malondialdehyde.

However, similar observations were reported by Ozden *et al.* (2007), who evaluated the effects of different doses of γ radiation on refrigerated (4C) aqua-cultured sea bass (*Dicentrarchus labrax*). These authors observed lower lipid oxidation in samples irradiated with 2.5 and 5.0 kGy doses than in those nonirradiated when analyzed during a 5-day postirradiation storage period. The decrease in TBA values can be a result of interactions of malonaldeyde with proteins and amino acids (Gokalp *et al.* 1983).

The lightness (color component *L**) of the cooked shrimps increased ($P < 0.05$) in samples irradiated with the 6 kGy dose compared with those irradiated with 2 kGy dose (Table 2). Similar observations have been reported by McKenna *et al.* (2003) with salmon fillets kept at 4C after irradiation. These authors registered higher *L** values for samples irradiated with 6 kGy than those of nonirradiated samples.

No effect of irradiation ($P > 0.05$) was found on color components *a** and *b** or on shear force values (Table 2). Kanatt *et al.* (2006) investigated the effects of ionizing radiation on marinated shrimps (*Penaeus indicus*) irradiated (2.5 kGy) at ambient temperature (25C) and failed to observe significant changes on textural properties in this type of food.

Color and texture acceptability of the cooked shrimps was not significantly ($P > 0.05$) affected by irradiation (Table 3). However, shrimp overall

TABLE 3.
MEAN HEDONIC VALUES AND APPROVAL PERCENTAGE (%A) FOR OVERALL, COLOR AND TEXTURE ACCEPTANCES OF HEADED SHRIMPS (*PENAEUS VANNAMEI*) AFTER FREEZING, PACKAGING AND IRRADIATING (^{60}CO) WITH 0, 2, 4 AND 6 kGy DOSES, AND COOKING

Acceptance sensory trait	Irradiation dose (kGy)							
	0		2		4		6	
	Mean	%A*	Mean	%A*	Mean	%A*	Mean	%A*
Overall	7.39a	81.26	7.39a	82.81	7.05ab	76.57	6.95b	67.19
Color	7.23a	79.69	7.06a	75.00	7.03a	78.13	6.87a	71.87
Texture	7.47a	85.94	7.44a	76.57	7.41a	81.25	7.33[a]	81.26

Means values with different letters within a row are significantly different ($P < 0.05$) by Student–Newman–Keuls test.
* %A, sum of % frequency of hedonic values from 7 to 9.

acceptance showed a significant reduction ($P < 0.05$) when the irradiation dose was 6 kGy compared with shrimps irradiated with 0 kGy or 2 kGy. The low overall acceptance value registered with 6 kGy may be associated to the high L^* value, which means high surface lightness and low water-holding capacity of the meat. Poole *et al.* (1994) also observed a decline in the overall acceptability of cooked prawns that had been irradiated at 2C, when irradiation doses increased from 3 to 5 kGy.

The approval percentage indicated a good overall acceptability for the cooked shrimps mainly for the product irradiated with the 2 kGy dose compared with the other irradiation doses (Table 3).

The intensity of aroma, flavor and juiciness attributes was not affected ($P > 0.05$) by irradiation. These results are in agreement with those of Kanatt *et al.* (2006), who did not observe significant changes in flavor of irradiated marinated shrimps (*P. indicus*). They used 12 trained panelists to evaluate such characteristic after irradiation (2.5 kGy) at ambient temperature (25C). In a review on irradiated food, Venugopal *et al.* (1999) concluded that sensory changes may occur at lower doses and are similar to those associated with thermal food processing.

CONCLUSIONS

γ irradiation with ^{60}Co applied to frozen and packaged headed shrimps in doses of 6 kGy inhibited the pathogenic microorganisms *V. cholerae* O1 and *S. enteritidis* without affecting the lipid stability of the product. Irradiation

increased the lightness of the surface color of cooked shrimps but did not alter their instrumental texture. Doses up to 6 kGy reduced sensory overall acceptability but did not impair the color and the texture acceptance, nor the intensity of juiciness and the characteristic aroma and flavor of the product.

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