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Control of the potential health hazards of smoked fish by gamma irradiation

Hesham M. Badr *

Atomic Energy Authority, Nuclear Research Center, Abou Zaabal, P.O. Box, 13759 Cairo, Egypt

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ABSTRACT

This study aims to control the presence of *Listeria monocytogenes* and *Vibrio parahaemolyticus* and the formation of biogenic amines in cold-smoked salmon by gamma irradiation. Irradiation at doses of 3 and 1 kGy inactivated 6.59 and 6.05 log cfu/g of *L. monocytogenes* and *V. parahaemolyticus* in the inoculated samples, respectively. Furthermore, irradiation of the un-inoculated samples significantly decreased their microbial populations of mesophilic aerobic bacteria, anaerobic bacteria, psychrophilic bacteria, lactic acid bacteria, and molds and yeasts. The Enterobacteriaceae were almost undetectable in samples irradiated at 2 kGy dose. The concentrations of biogenic amines significantly decreased in the irradiated samples due to microbial inactivation. However, irradiation of samples had no significant effects on their moisture and salt contents as well as on their pH values, total volatile base nitrogen, and trimethylamine nitrogen contents, but significantly decreased their amounts of phenolic compounds and increased their levels of thiobarbituric acid reactive substances. Moreover, irradiation treatments at doses up to 3 kGy showed no significant effect on the sensory acceptability of samples. Therefore, gamma irradiation at dose of 3 kGy can be successfully applied to provide significant improvement in the safety of cold smoked salmon with respect to *L. monocytogenes*, *V. parahaemolyticus*, and biogenic amines without adverse effects on chemical or sensory quality attributes of the product.

1. Introduction

Smoking is one of the most old ways for making a popular fish product being of considerable economic importance worldwide. No other way of fish processing can produce such attractive characteristic color and flavor (González-Rodrígues et al., 2002; Siskos et al., 2005). The great acceptance of smoked fish is mainly based on its sensory characteristics more than on any preservation purposes (Fuentes et al., 2010). Cold smoked fish is a product subjected to only light preservation process before being purchased and is typically consumed as ready-to-eat with no heat treatment (Joffraud et al., 2006; Tomé et al., 2008). There is no point in the process of cold-smoking that can fully assure the absence of foodborne pathogens. Neither the smoking temperature nor the salt content is enough to kill them. This led to a great deal of interest in the potential for growth of foodborne pathogens in cold smoked fish. Quantitative risk assessments have identified cold-smoked fish as a high-risk product with respect to listeriosis, a severe invasive illness in humans caused by *Listeria monocytogenes* with a high fatality of approximately 30% (FDA/FSIS, 2003; FAO/WHO, 2004; Ye et al., 2008). The microorganism survives cold-smoking and the product characteristics, including pH, water activity, salt, and smoke components, are insufficient to prevent its growth in chilled and vacuum-packaged product (Mejlholm and Dalgaard, 2007), thus could represent a serious hazard especially for susceptible groups including pregnant women, infants, the elderly, and immuno-compromised people.

On the other hand, the presence of *Vibrio parahaemolyticus* in seafood is of public health concern in view of its pathogenicity to man and its wide occurrence in marine environments. The illness caused by *V. parahaemolyticus* food poisoning is characterized by watery diarrhea and abdominal cramps in nearly all cases, usually with nausea, vomiting, fever and headache. About one quarter of patients experience a dysentery-like illness with bloody or mucoid stools, high fever and high white blood cells (Heymann, 2004). It has been found that fish samples that contaminated at low levels of *V. parahaemolyticus* showed relatively high levels of this bacterium after cold-smoking, suggesting that a small population of naturally occurring organism could multiply to significant levels during the process of cold-smoking or during subsequent storage at temperature abuse. Therefore, smoked fish should be considered a potential health hazard with respect to this pathogen (Alvarez, 1982; Karunasagar et al., 1986).

Furthermore, the food safety concerns associated with cold-smoked fish are not limited to microbiological hazards. Consideration must be also given to the formed chemical hazards such as biogenic amines which were found to be formed at high levels in cold-smoked fish (Jørgensen et al., 2000). Biogenic amines constitute a potential public health concern due to their physiological and toxicological effects and as possible precursors of carcinogens, such as N-nitrosamines. Thus, control measures to prevent formation of biogenic amines or to reduce their levels once formed need to be considered (Kim et al., 2009; Naila

* Tel.: +20 101982882; fax: +20 244620791.
E-mail address: heshambadr_aea@yahoo.co.uk.

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et al., 2010). The formation of biogenic amines results from decarboxylation of free amino acids by the bacterial amino acid decarboxylase enzymes. Amino acid decarboxylation occurs through the removal of a carbonyl group to give the corresponding amine. In addition, proteolysis, either autolytic or bacterial, may play a significant role in the release of free amino acids from tissue proteins which offer a substrate for bacterial decarboxylases (Shalaby, 1996). Thus, prevention of bacterial growth would be very important to control the formation of biogenic amines.

Gamma irradiation is an effective process for inactivating foodborne pathogens and reducing microbial populations in foodstuffs. The irradiation process is one of the few technologies which address both food quality and safety due to its ability to control spoilage and foodborne pathogenic microorganisms. After many years of research and the development of national and international standards, more than 60 countries have regulations allowing food irradiation of at least one product (Blackburn, 2011). Irradiation of cold smoked fish is expected to inactivate the present harmful pathogens. Furthermore, the irradiation treatment may reduce the formation of biogenic amines through the reduction of bacterial populations to meet the growing consumer demand for a safe smoked fish product. Therefore, the objective of this study was to examine the effectiveness of low gamma irradiation doses in the inactivation of *L. monocytogenes* and *V. parahaemolyticus* and reducing the formation of biogenic amines in cold-smoked salmon, in addition to studying the effects of irradiation on chemical and sensory quality attributes of samples.

2. Materials and methods

2.1. Materials

Freshly produced cold-smoked salmon fillets were obtained from fish processing plant in Egypt. The smoked fillets were immediately transported to the laboratory in a cool box for preparation of the experimental samples.

2.2. Experiments with inoculated samples

2.2.1. Preparation of inoculum

Experiments with inoculated samples were performed with *L. monocytogenes* and *V. parahaemolyticus* as inocula. A single colony of confirmed *L. monocytogenes*, originally isolated from ground beef, was transferred from a slant of tryptic soy agar supplemented with 0.6% yeast extract (stored for 11 days at 4 °C) into 10 ml of tryptic soy broth plus 0.6% yeast extract. The culture was incubated for 24 h at 35 °C and 0.1 ml of this culture was then transferred into 10 ml of fresh tryptic soy broth plus 0.6% yeast extract and incubated for 24 h at 35 °C. Afterwards, a tenfold serial dilution was prepared in a sterile maximum recovery diluent and the appropriate dilution was used for inoculation of samples. For *V. parahaemolyticus*, a single colony of confirmed pathogenic (kanagawa-positive) *V. parahaemolyticus*, originally isolated from sardine fish, was transferred from a slant of tryptic soy agar containing 3% NaCl (stored at 4 °C for 9 days) into 10 ml of tryptic soy broth containing 3% NaCl. The culture was incubated overnight at 35 °C and 0.1 ml of the overnight culture was then transferred into 10 ml of fresh tryptic soy broth containing 3% NaCl and incubated for 18 h at 35 °C. A tenfold serial dilution was then prepared in a sterile phosphate buffered saline and the appropriate dilution was used for sample inoculation.

2.2.2. Inoculation of cold-smoked fillets

Under aseptic conditions, slices of the smoked fillets (approximately 50 g) were placed onto a piece of sterile aluminum foil and 100 μl of the appropriate dilution of *L. monocytogenes* or *V. parahaemolyticus* (samples were prepared separately for each organism) was surface-inoculated on one side of the slices, spread evenly using a sterile spreader, and left to dry for 5 min before the slices were flipped and inoculated on the other side to reach a final level of approximately 4×10^6 and 1×10^6 cfu/g for *L. monocytogenes* and *V. parahaemolyticus*, respectively. After another 5 min, for drying, the inoculated slices for each of the studied organism were cut into small pieces ($\approx$2.5 cm$\times$2 cm) in a sterile vessel using a sterile knife and carefully mixed by hand stirring with sterilized stainless-steel straight basting spoons without affecting the structure of the salmon pieces (which were about 1.5–1 cm$\times$1 cm after mixing). This step was done for obtaining a relatively homogeneous mix of the salmon muscles to minimize the possible differences between counts and types of the natural microflora contaminating the original slices and, in turn, may have some effects on the inoculated bacterium.

2.3. Experiments with un-inoculated samples

Un-inoculated cold-smoked fillets were used to examine the naturally present microbial populations, the formation of biogenic amines, as well as the chemical and sensory quality attributes of the smoked fish. For microbiological and chemical studies, un-inoculated cold-smoked salmon fillets were also aseptically cut into small pieces and mixed in a sterile vessel as illustrated above. This step was also done to minimize the possible great variation between samples in their levels of the different biogenic amines due to the possible differences between types and counts of the native microflora contaminating the original slices. Meanwhile, other slices of the smoked fillets were taken as samples for sensory evaluations.

2.4. Packaging of samples

Half of the prepared samples of inoculated and un-inoculated smoked salmon were aerobically packaged (at approximately 75 g samples) in oxygen-permeable low-density polyethylene pouches, while the second half of the samples were vacuum-packaged (at the same amount) in oxygen-impermeable polyamide–polyethylene pouches. Each of the prepared half of the packages was subdivided into appropriate samples for irradiation treatments. Samples were irradiated after their preparation to minimize the increase in the counts of their initial natural microbial populations and the formation of undesirable compounds such as biogenic amines, which in turn, may affect the effectiveness of the applied irradiation doses. It is well known that the initial quality of foods intended for processing by irradiation affects the quality of the irradiated product, and therefore, foods intended for irradiation treatments must be fresh.

2.5. Irradiation and storage of samples

Aerobically and vacuum-packaged smoked salmon samples were exposed to gamma irradiation at doses of 0, 1, 2, 3, and 4 kGy. Irradiation was carried out at room temperature using an experimental Co-60 source at the National Center for Radiation Research and Technology, Nasr City, Cairo, Egypt. The control non-irradiated samples were left at room temperature during irradiation of the other samples for uniformity of conditions. Afterwards, all samples were refrigerated stored at 4 ± 1 °C, except samples of the day zero analysis, and subjected to the periodical analysis at weekly intervals during 6 weeks of storage regardless of their shelf life.

2.6. Microbiological determinations

All sample packages were disinfected with ethanol (70% w/w), aseptically opened, and sub-samples of 25 g were taken for preparation of the required dilutions using blender with sterilized jars. For *L. monocytogenes*, 25 g of samples were blended with 225 ml of buffered *Listeria* enrichment broth base CM897 and *Listeria* selective supplement SR141 (Oxoid, Basingstoke, England) and serial dilutions were prepared using maximum recovery diluent after incubation at

30 °C for 48 h. Then colony forming units for this bacterium were determined using *Listeria* selective medium (Oxford formulation, Oxoid, Basingstoke, England) after incubation at 35 °C for 24–48 h (Oxoid, 1998) and confirmed by biochemical testing according to Roberts and Greenwood (2003). For pathogenic *V. parahaemolyticus*, 25 g of samples were blended with 225 ml of alkalin peptone water (Fluka, Switzerland) and serial dilutions were prepared using maximum recovery diluent after incubation at 37 °C for 18–20 h. Then enumeration of pathogenic *V. parahaemolyticus* was carried out on TCBS Cholera medium (Oxoid, Basingstoke, England) after incubation at 35 °C for 18 h and confirmed according to the FDA manual (2004). For un-inoculated samples, 25 g of samples were blended with 225 ml of maximum recovery diluent and serially diluted with the same diluent. Then colony forming units for total aerobic mesophilic and psychrophilic bacteria were determined by plating on plate count agar medium and incubation at 30 °C for 3 days and 7 °C for 7 days, respectively, while total anaerobic bacteria were counted on plate count agar medium and anaerobic incubation at 30 °C for 3 days as described by APHA (1992). Incubation of anaerobic bacteria was carried out using AnaeroGen Compact System (Oxoid, UK). Mesophilic and psychrophilic lactic acid bacteria were enumerated on MRS agar medium after incubation at 30 °C for 2 days followed by incubation at 22 °C for a further one day (Oxoid, 1998). Enterobacteriaceae were counted on violet red bile glucose agar medium after incubation at 37 °C for 20–24 h (Roberts and Greenwood, 2003). Total molds and yeasts were enumerated on malt agar medium after incubation at 22 °C for 3–5 days (APHA, 1992).

2.7. Chemical determinations

Moisture and salt contents were determined according to AOAC's (2000) official methods, then salt contents were expressed as % in the water phase (Derrick, 2009). pH values were measured in a suspension of smoked fish in distilled water using pH meter according to Cardinal et al. (2004). The contents of total phenolic compounds were quantified according to Cardinal et al. (2004) and expressed as mg of gallic acid/100 g. Biogenic amines were extracted from samples and dansylated according to Cho et al. (2006), then identified and quantified by HPLC. The contents of thiobarbituric acid reactive substances (TBARS) were determined according to Pegg (2001).Total volatile base nitrogen (TVBN) and trimethylamine nitrogen (TMAN) contents were determined as described by Egan et al. (1981).

2.8. Sensory evaluation

Irradiated and non-irradiated cold-smoked salmon samples were subjected to sensory evaluation for their color, odor, taste, and texture on day zero only for safety precautions. The panelists consisted of 10 non expert members using the following 9-point quality scores: 9: excellent, 7: good, 5: fair, 3: poor and 1: extremely poor as described by Wierbicki (1981).

2.9. Statistical analysis

Three different replicate trials were conducted in this study and analysis was performed using duplicate pouches per each replicate trail. Data were then statistically analyzed by using the general linear models procedure of the SAS software (SAS Institute, 1998), and the differences among means (at $p<0.05$) were compared using Duncan's multiple range test.

3. Results

3.1. Microbiological properties

3.1.1. *L. monocytogenes and V. parahaemolyticus in the inoculated samples*

Table 1 summarizes the counts of *L. monocytogenes* and *V. parahaemolyticus* in the inoculated aerobically and vacuum-packaged cold-smoked salmon as affected by irradiation and refrigerated storage (4 ± 1 °C). Irradiation of samples at doses of 1 and 2 kGy significantly ($p<0.05$) decreased the counts of *L. monocytogenes*, while refrigerated storage induced significant increases in the counts of this pathogen in both irradiated and non-irradiated samples being at higher rate in the control samples. Irradiation at dose of 3 kGy was sufficient for inactivation of *L. monocytogenes* which was not detected (<10 cfu/g) in samples post treatment and during their storage. The atmosphere of packaging showed no significant ($p>0.05$) effect on this microorganism counts. Meanwhile, *V. parahaemolyticus* was not detected in all irradiated samples indicating the sufficiency

Table 1
Colony forming units of *L. monocytogenes* and *V. parahaemolyticus* in aerobically and vacuum-packaged cold-smoked salmon as affected by gamma irradiation and refrigerated storage at 4 ± 1 °C.

Storage (week)	Irradiation dose (kGy)/aerobic-packaging					Irradiation dose (kGy)/vacuum-packaging				
	0.0	1.0	2.0	3.0	4.0	0.0	1.0	2.0	3.0	4.0
Listeria monocytogenes (mean $\log_{10}$ count ± SD cfu/g)										
0	6.59 ± 2.04 g	3.92 ± 1.82 m	2.41 ± 1.11 t	ND	ND	6.60 ± 1.99 g	3.92 ± 1.78 m	2.42 ± 1.17 t	ND	ND
1	6.65 ± 1.93 f	3.94 ± 1.87 m	2.46 ± 1.25 s	ND	ND	6.66 ± 2.05 f	3.95 ± 1.74 m	2.47 ± 1.34 s	ND	ND
2	6.84 ± 2.08 e	3.96 ± 1.96 l	2.51 ± 1.20 r	ND	ND	6.85 ± 2.05 e	3.96 ± 1.84 l	2.53 ± 1.23 r	ND	ND
3	7.05 ± 2.03 d	4.13 ± 1.92 k	2.56 ± 1.32 q	ND	ND	7.08 ± 2.08 d	4.15 ± 2.04 k	2.57 ± 1.27 q	ND	ND
4	7.53 ± 2.13 c	4.57 ± 1.95 j	2.67 ± 1.30 p	ND	ND	7.55 ± 2.14 c	4.59 ± 1.97 j	2.69 ± 1.36 p	ND	ND
5	7.94 ± 2.16 b	4.86 ± 1.98 i	2.87 ± 1.28 o	ND	ND	7.95 ± 2.10 b	4.87 ± 2.06 i	2.88 ± 1.47 o	ND	ND
6	8.28 ± 2.20 a	5.06 ± 2.16 h	3.10 ± 1.55 n	ND	ND	8.32 ± 2.17 a	5.10 ± 2.14 h	3.13 ± 1.44 n	ND	ND
Vibrio parahemolyticus (mean $\log_{10}$ count ± SD cfu/g)										
0	6.05 ± 1.98 a	ND	ND	ND	ND	6.04 ± 1.96 a	ND	ND	ND	ND
1	5.58 ± 2.00 b	ND	ND	ND	ND	5.84 ± 1.91 b	ND	ND	ND	ND
2	5.63 ± 1.97 c	ND	ND	ND	ND	5.63 ± 2.01 c	ND	ND	ND	ND
3	4.99 ± 2.04 d	ND	ND	ND	ND	4.99 ± 2.08 d	ND	ND	ND	ND
4	4.79 ± 2.03 e	ND	ND	ND	ND	4.78 ± 2.05 e	ND	ND	ND	ND
5	4.74 ± 1.99 f	ND	ND	ND	ND	4.74 ± 1.97 f	ND	ND	ND	ND
6	4.59 ± 2.04 g	ND	ND	ND	ND	4.58 ± 2.08 g	ND	ND	ND	ND

Means with a different letter within each determination are different significantly ($p<0.05$).
ND: Not detected.

of the lowest applied dose (1 kGy) for keeping it below the detection limit (<10 cfu/g). Refrigerated storage at 4 ± 1 °C significantly decreased the counts of this organism in the control samples, but the atmosphere in packages had no significant effect (Table 1).

3.1.2. Microbial populations in the un-inoculated samples

The effects of irradiation and refrigerated storage (4 ± 1 °C) on microbial counts in the un-inoculated aerobically and vacuum-packaged smoked salmon are presented in Fig. 1(a & b). Irradiation of the un-inoculated smoked salmon samples significantly ($p<0.05$) decreased their initial counts for mesophilic aerobic bacteria, anaerobic bacteria, psychrophilic bacteria, lactic acid bacteria, and molds and yeasts, proportionally to the applied dose. Meanwhile, Enterobacteriaceae were not detected in samples exposed to 2 kGy dose or higher. During subsequent refrigerated storage, further significant ($p<0.05$) increases were observed for the counts of microbial populations in the non-irradiated samples as well as those survived irradiation treatments, however, the rate of increase was higher in the control samples. The increase in counts was significantly higher for lactic acid bacteria and lower for molds and yeasts in vacuum refrigerated samples as compared with aerobic refrigerated ones.

3.2. Chemical properties

3.2.1. Moisture, salt, phenolic compounds and pH

As shown in Table 2, irradiation treatments and refrigerated storage of cold-smoked salmon samples had no significant ($p>0.05$) effects on their moisture and salt contents as well as on their pH values under both aerobic and vacuum-packaging conditions. However, significant ($p<0.05$) reductions in the amounts of phenolic compounds were observed due to irradiation treatments and during refrigerated storage of both irradiated and non-irradiated aerobically and vacuum-packaged samples.

3.2.2. Biogenic amines

Fig. 2(a & b) represents the levels of biogenic amines in aerobically and vacuum-packaged smoked salmon as affected by irradiation treatments and refrigerated storage (4 ± 1 °C). Irradiation of cold-smoked salmon samples at dose of 4 kGy significantly ($p<0.05$) decreased their agmatine levels, while the levels of putrescine, tyramine, and β-phenylethylamine showed a gradual significant decrease in samples with increased applied dose. Cadaverine and histamine, however, showed no significant ($p>0.05$) changes in

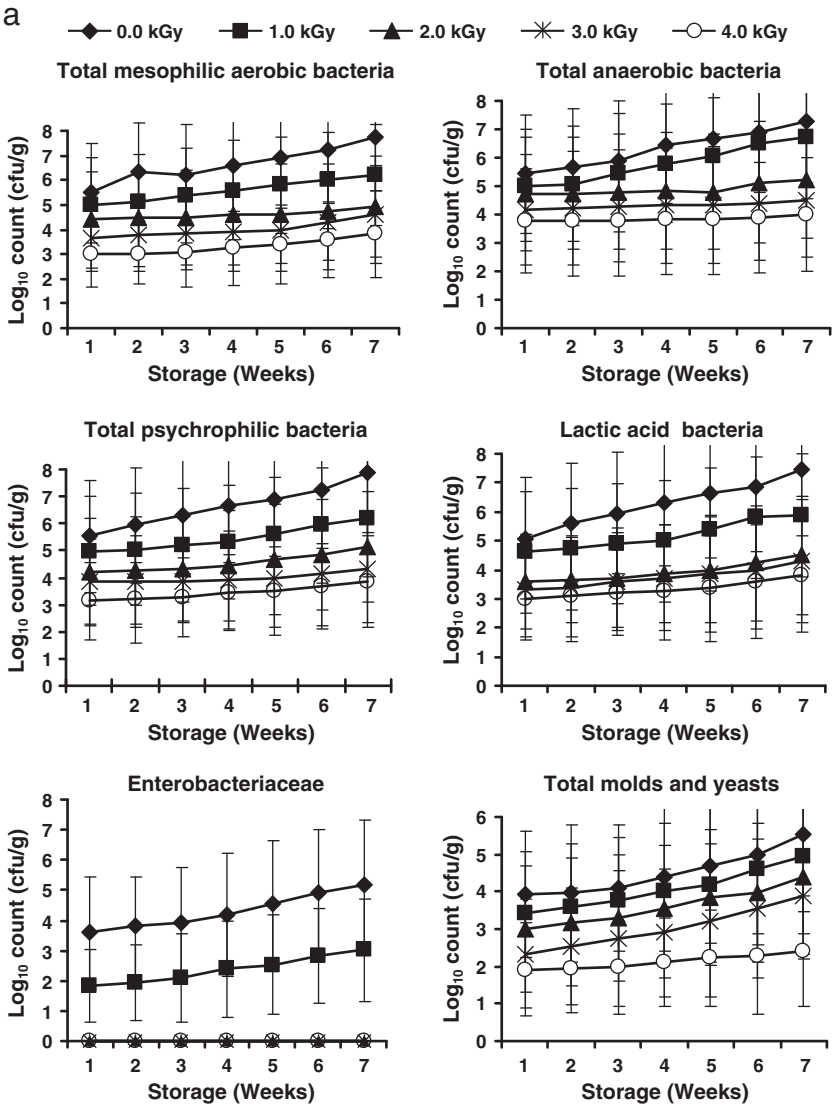

Fig. 1. a. Counts of microbial populations in aerobically-packaged cold-smoked salmon as affected by gamma irradiation and refrigerated storage at 4 ± 1 °C. b. Counts of microbial populations in vacuum-packaged cold-smoked salmon as affected by gamma irradiation and refrigerated storage at 4 ± 1 °C.

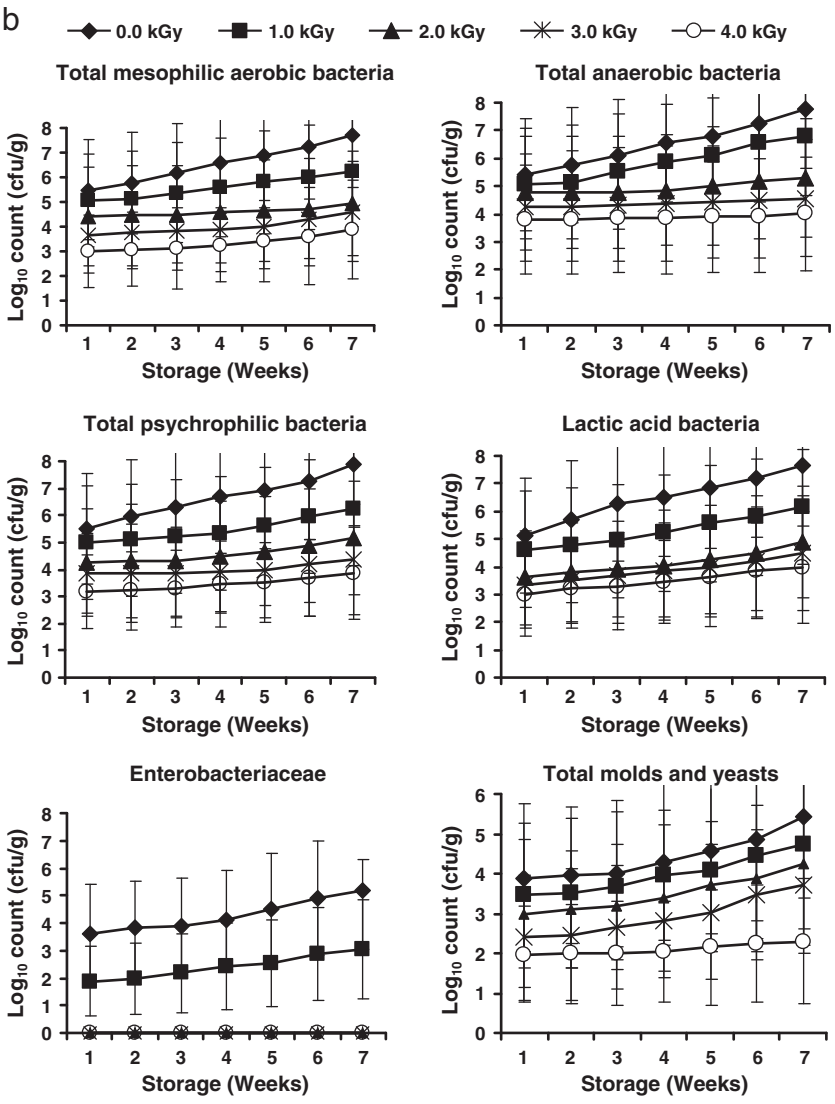

Fig. 1 (*continued*).

their amounts due to irradiation treatments. During refrigerated storage at 4 ± 1 °C, the formation of biogenic amines significantly ($p<0.05$) increased in both non-irradiated and irradiated samples, but the increase was much lower in the irradiated samples and proportionally to the applied dose. The atmosphere in packages, however, had no significant ($p>0.05$) effects on the levels of biogenic amines in samples neither during irradiation nor during refrigerated storage.

3.2.3. TBARS, TVBN, and TMAN

As illustrated in Fig. 3, irradiation of cold-smoked salmon samples significantly increased the amounts of TBARS in samples (proportionally to the applied dose), while refrigerated storage significantly increased the amounts of TBARS in both irradiated and non-irradiated samples. The observed increases in the levels of TBARS were significantly ($p<0.05$) lower in vacuum-packages than in aerobically-packaged samples. Irradiation, however, had no significant effect ($p>0.05$) on the concentrations of TVBN and TMAN in samples, meanwhile, significant increases in the concentrations of these compounds were observed during refrigerated storage of irradiated and non-irradiated samples, being significantly lower in the irradiated ones and proportional to the applied dose. The atmosphere in packages showed no significant effect on the concentrations of TVBN and TMAN neither during irradiation treatments of samples nor during their refrigerated storage.

3.3. Sensory properties

Irradiated and non-irradiated cold-smoked salmon samples were subjected to sensory evaluation and the mean of scores is presented in Fig. 4. Irradiation of samples at doses up to 3 kGy had no significant effect ($p>0.05$) on their color acceptability, while significantly reduced scores were recorded by the panelists for samples exposed to 4 kGy dose due to discoloration which was lower in vacuum-packaged samples. Irradiation treatments at all applied doses, however, showed no significant ($p>0.05$) effects on the acceptability of samples for their odor, taste, and texture (Fig. 4).

4. Discussion

From both public health and economic standpoints, it is extremely important to control potential human pathogens in smoked fish products. In the present study, samples of non-irradiated inoculated cold-smoked salmon showed that *L. monocytogenes* could grow during refrigerated storage, which was in agreement with the results of other investigators that showed the ability of *L. monocytogenes* to grow in

Table 2
Contents of moisture, salt and total phenolic compounds and pH values of aerobically and vacuum-packaged cold-smoked salmon as affected by gamma irradiation and refrigerated storage at 4 ± 1 °C.

Storage (week)	Irradiation dose (kGy)/aerobic-packaging					Irradiation dose (kGy)/vacuum-packaging				
	0.0	1.0	2.0	3.0	4.0	0.0	1.0	2.0	3.0	4.0
Moisture %										
0	60.31 ± 0.13 a	60.30 ± 0.12 a	60.34 ± 0.15 a	60.28 ± 0.17 a	60.31 ± 0.13 a	60.29 ± 0.17 a	60.32 ± 0.14 a	60.31 ± 0.16 a	60.33 ± 0.10 a	60.32 ± 0.13 a
1	60.28 ± 0.10 a	60.30 ± 0.11 a	60.27 ± 0.13 a	60.32 ± 0.10 a	60.31 ± 0.11 a	60.30 ± 0.09 a	60.32 ± 0.11 a	60.33 ± 0.10 a	60.28 ± 0.16 a	60.31 ± 0.12 a
2	60.30 ± 0.11 a	60.31 ± 0.10 a	60.32 ± 0.09 a	60.27 ± 0.14 a	60.30 ± 0.16 a	60.32 ± 0.14 a	60.28 ± 0.13 a	60.31 ± 0.13 a	60.31 ± 0.11 a	60.29 ± 0.14 a
3	60.33 ± 0.08 a	60.29 ± 0.13 a	60.29 ± 0.11 a	60.32 ± 0.09 a	60.31 ± 0.12 a	60.32 ± 0.10 a	60.30 ± 0.09 a	60.33 ± 0.08 a	60.29 ± 0.15 a	60.29 ± 0.13 a
4	60.27 ± 0.16 a	60.30 ± 0.07 a	60.30 ± 0.11 a	60.31 ± 0.08 a	60.29 ± 0.11 a	60.28 ± 0.13 a	60.31 ± 0.08 a	60.28 ± 0.09 a	60.30 ± 0.14 a	60.31 ± 0.10 a
5	60.30 ± 0.12 a	60.27 ± 0.08 a	60.33 ± 0.14 a	60.29 ± 0.13 a	60.29 ± 0.10 a	60.30 ± 0.07 a	60.30 ± 0.12 a	60.27 ± 0.10 a	60.31 ± 0.12 a	60.28 ± 0.09 a
6	60.31 ± 0.10 a	60.33 ± 0.11 a	60.31 ± 0.08 a	60.27 ± 0.13 a	60.31 ± 0.10 a	60.32 ± 0.09 a	60.30 ± 0.07 a	60.29 ± 0.13 a	60.27 ± 0.09 a	60.33 ± 0.12 a
Salt % (in water phase)										
0	3.11 ± 0.02 a	3.11 ± 0.01 a	3.08 ± 0.03 a	3.10 ± 0.01a	3.11 ± 0.02 a	3.10 ± 0.01 a	3.11 ± 0.02 a	3.09 ± 0.02 a	3.10 ± 0.01 a	3.11 ± 0.01 a
1	3.10 ± 0.01 a	3.08 ± 0.03 a	3.11 ± 0.01 a	3.09 ± 0.02 a	3.09 ± 0.03 a	3.11 ± 0.01 a	3.08 ± 0.03 a	3.10 ± 0.01 a	3.11 ± 0.02 a	3.09 ± 0.02 a
2	3.10 ± 0.01 a	3.08 ± 0.02 a	3.10 ± 0.02 a	3.10 ± 0.01 a	3.10 ± 0.01 a	3.08 ± 0.03 a	3.09 ± 0.02 a	3.11 ± 0.01 a	3.09 ± 0.03 a	3.10 ± 0.01 a
3	3.08 ± 0.03 a	3.09 ± 0.01 a	3.09 ± 0.03 a	3.11 ± 0.01 a	3.09 ± 0.02 a	3.11 ± 0.01 a	3.10 ± 0.01 a	3.10 ± 0.02 a	3.10 ± 0.01 a	3.10 ± 0.02 a
4	3.09 ± 0.02 a	3.07 ± 0.03 a	3.11 ± 0.01 a	3.10 ± 0.02 a	3.08 ± 0.03 a	3.10 ± 0.02 a	3.11 ± 0.01 a	3.08 ± 0.03 a	3.11 ± 0.01 a	3.08 ± 0.03 a
5	3.07 ± 0.03 a	3.11 ± 0.01 a	3.10 ± 0.01 a	3.10 ± 0.01 a	3.07 ± 0.02 a	3.11 ± 0.01 a	3.08 ± 0.03 a	3.09 ± 0.02 a	3.08 ± 0.02 a	3.10 ± 0.01 a
6	3.10 ± 0.01 a	3.08 ± 0.02 a	3.11 ± 0.01 a	3.07 ± 0.03 a	3.09 ± 0.01 a	3.10 ± 0.02 a	3.07 ± 0.03 a	3.11 ± 0.01 a	3.10 ± 0.01 a	3.07 ± 0.03 a
Total phenol compounds (as mg of gallic acid/100 g)										
0	11.7 ± 0.9 a	11.5 ± 1.1 b	11.1 ± 1.2 cd	10.7 ± 1.3 e	9.5 ± 1.0 j	11.8 ± 1.1 a	11.6 ± 1.3 a	11.3 ± 1.1 bc	10.9 ± 1.2 de	9.9 ± 1.1 hi
1	10.9 ± 1.3 de	10.4 ± 1.2 f	10.3 ± 1.0 fg	9.8 ± 1.2 i	8.7 ± 1.0 no	10.9 ± 1.1 de	10.7 ± 1.1 e	10.4 ± 1.2 f	9.9 ± 1.2 hi	9.0 ± 1.3 lm
2	10.1 ± 1.1 gh	9.8 ± 1.0 i	9.1 ± 1.1 kl	8.8 ± 1.0 mno	7.9 ± 0.9 qr	10.1 ± 1.2 gh	9.9 ± 1.0 hi	9.4 ± 1.1 j	8.9 ± 1.0 lmn	8.1 ± 0.9 q
3	9.4 ± 1.1 j	9.0 ± 1.1 lm	8.7 ± 1.0 no	8.4 ± 1.2 p	7.5 ± 1.1 s	9.5 ± 0.9 j	9.3 ± 1.0 jk	8.9 ± 1.2 lmn	8.4 ± 1.3 p	7.9 ± 1.1 qr
4	8.6 ± 1.0 op	8.6 ± 1.1 op	8.1 ± 0.9 q	7.7 ± 1.1 rs	7.0 ± 0.8 t	8.8 ± 1.0 mno	8.7 ± 1.1 no	8.4 ± 1.2 p	7.9 ± 1.0 qr	7.5 ± 1.2 s
5	6.8 ± 1.2 t	6.8 ± 1.0 t	6.4 ± 1.1 u	5.2 ± 1.0 w	5.4 ± 0.9 vw	7.0 ± 1.1 t	6.8 ± 0.8 t	6.5 ± 1.0 u	6.3 ± 0.9 u	5.6 ± 1.0 v
6	5.2 ± 1.0 w	5.2 ± 0.9 w	4.5 ± 0.8 x	3.5 ± 0.7 yz	3.3 ± 0.7 z	5.4 ± 0.8 vw	5.2 ± 0.8 w	4.6 ± 0.9 x	4.4 ± 0.7 x	3.7 ± 0.8 y
pH-value										
0	6.01 ± 0.04 a	6.03 ± 0.02 a	6.00 ± 0.04 a	6.02 ± 0.04 a	6.03 ± 0.02 a	6.03 ± 0.03 a	6.01 ± 0.01 a	6.04 ± 0.03 a	6.02 ± 0.04 a	6.03 ± 0.02 a
1	6.11 ± 0.07 a	6.08 ± 0.04 a	6.08 ± 0.05 a	6.05 ± 0.03 a	6.10 ± 0.05 a	6.09 ± 0.03 a	6.08 ± 0.05 a	6.07 ± 0.04 a	6.10 ± 0.06 a	6.11 ± 0.04 a
2	6.08 ± 0.06 a	6.07 ± 0.04 a	6.10 ± 0.03 a	6.09 ± 0.06 a	6.11 ± 0.04 a	6.10 ± 0.05 a	6.11 ± 0.06 a	6.08 ± 0.04 a	6.07 ± 0.03 a	6.10 ± 0.03 a
3	6.14 ± 0.05 a	6.20 ± 0.02 a	6.14 ± 0.03 a	6.10 ± 0.02 a	6.10 ± 0.04 a	6.11 ± 0.06 a	6.08 ± 0.04 a	6.08 ± 0.04 a	6.10 ± 0.06 a	6.09 ± 0.03 a
4	6.12 ± 0.04 a	6.18 ± 0.06 a	6.15 ± 0.04 a	6.16 ± 0.03 a	6.15 ± 0.04 a	6.13 ± 0.03 a	6.11 ± 0.05 a	6.15 ± 0.05 a	6.15 ± 0.04 a	6.14 ± 0.05 a
5	6.23 ± 0.05 a	6.27 ± 0.03 a	6.27 ± 0.05 a	6.23 ± 0.06 a	6.19 ± 0.04 a	6.21 ± 0.03 a	6.18 ± 0.04 a	6.17 ± 0.03 a	6.20 ± 0.06 a	6.19 ± 0.05 a
6	6.32 ± 0.06 a	6.30 ± 0.07 a	6.25 ± 0.04 a	6.20 ± 0.06 a	6.21 ± 0.05 a	6.29 ± 0.07 a	6.31 ± 0.06 a	6.28 ± 0.07 a	6.29 ± 0.06 a	6.24 ± 0.06 a

Means with a different letter within each parameter are different significantly ($p<0.05$).

cold smoked salmon during refrigerated storage under both aerobic and vacuum-packaging (Hudson and Mott, 1993; Rørvike et al., 1991; Hwang and Sheen, 2009). The obtained results further show that, although refrigerated storage significantly ($p<0.05$) reduced the counts of *V. parahaemolyticus* in non-irradiated inoculated samples, the complete inactivation of the organism was not achieved. Similar results were observed by Johnson et al. (1973) who reported that refrigerated storage at 4 °C for 3 weeks induced little or no apparent decrease in the counts of *V. parahaemolyticus* naturally contaminated oyster shellstock. On the other hand, the results of microbiological determinations showed that non-irradiated samples of un-inoculated cold-smoked salmon retained a mixed bacterial population as well as molds and yeasts which also showed a significant count increase during aerobic and vacuum refrigerated storage. The observed initial microbial populations may be a function of the indigenous fish flora and the microflora of the processing environment. Hwang and Sheen (2009) also showed that the growth of native microflora in cold-smoked salmon significantly increased during refrigerated storage at 4 °C. In the present study, it is apparent from the chemical profile of the studied cold-smoked salmon that the product had high moisture and low salt contents and neutral pH values. These properties, which generally characterize cold-smoked fish products and indicate lightly preserved and perishable products, are capable of supporting the growth of the native microflora and pathogenic bacteria. Although *V. parahaemolyticus* grow best at pH values slightly above neutrality, its growth has been demonstrated down to pH 4.5–5 (Adams and Moss, 2008). The studied samples had also considerable concentrations of phenolic compounds (11.7 mg/100 g), but showed no inhibitory effects on the examined microorganisms. The growth behavior of microorganisms in cold-smoked salmon has been studied extensively. It has been reported that salt, moisture content, pH of the smoked salmon, and the prevailing storage temperature were supportive to the growth of *L. monocytogenes* (Dalgaard and Jorgensen, 1998) and the simultaneous growth of *L. monocytogenes* and native microflora in cold-smoked salmon were similar during refrigerated storage (Gimenez and Dalgaard, 2004; Hwang and Sheen, 2009). Recommendations for the acceptable levels of *L. monocytogenes* vary throughout the world. Most countries and organizations currently require the absence of the organism in a 25 g food sample as in FDA's policy of "zero-tolerance" on ready-to-eat products. This means that ready-to-eat smoked fish must have no detectable *L. monocytogenes* per 25 g throughout the shelf life (Hong et al., 2008). Whereas the current regulations in Health Canada and the European Community permit a quantitative limit of 100 cfu/g for *L. monocytogenes* in ready to eat foods

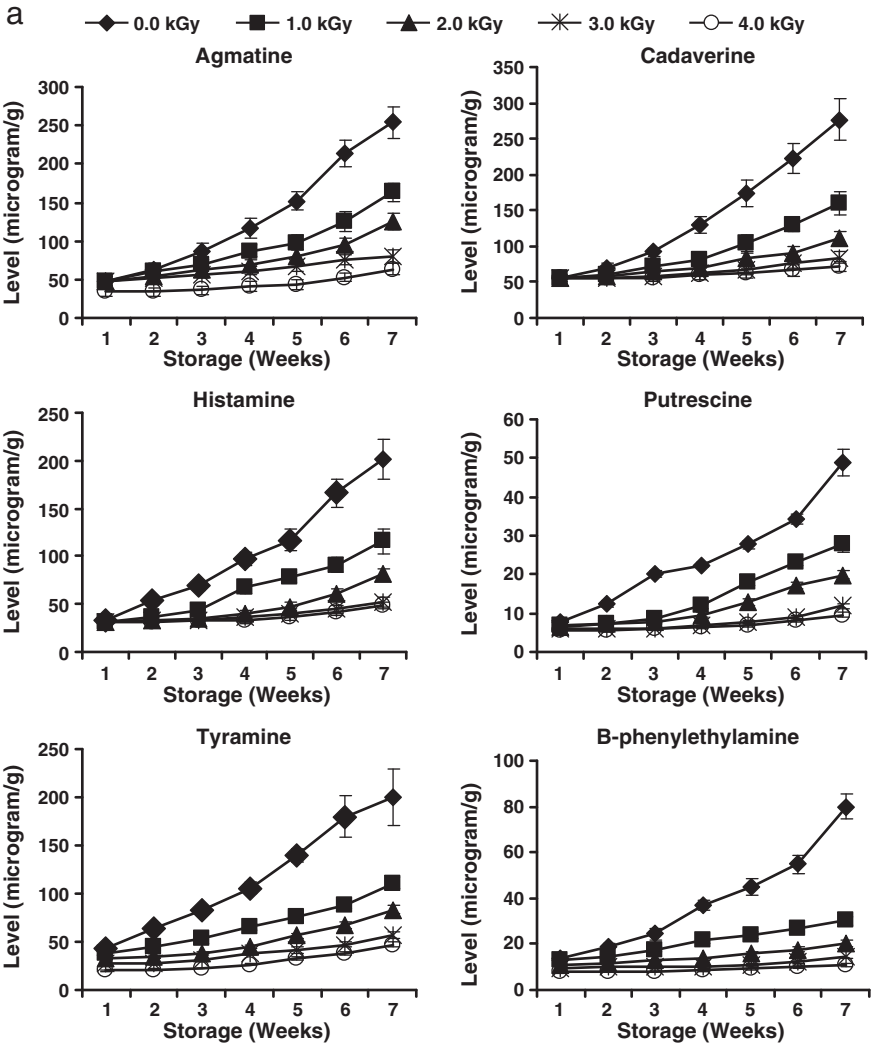

Fig. 2. a. Contents of biogenic amines in aerobically-packaged cold-smoked salmon as affected by gamma irradiation and refrigerated storage at 4 ± 1 °C. b. Contents of biogenic amines in vacuum-packaged cold-smoked salmon as affected by gamma irradiation and refrigerated storage at 4 ± 1 °C.

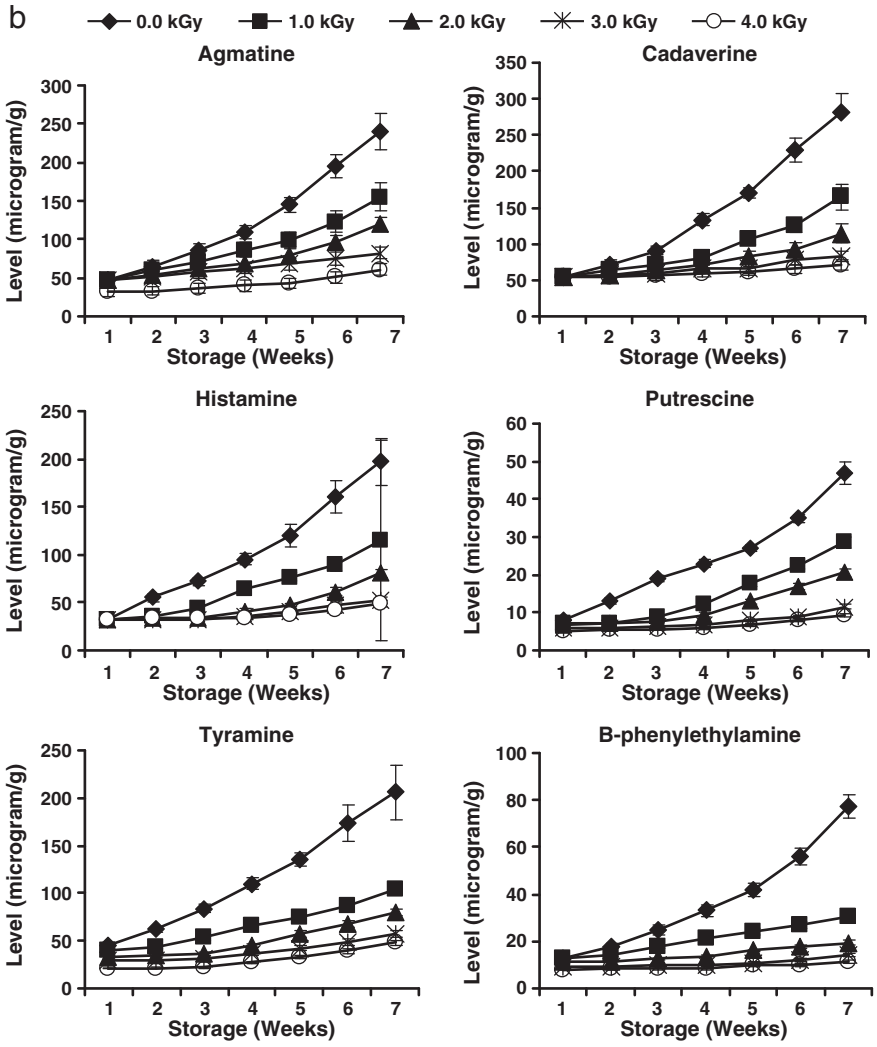

Fig. 2 (*continued*).

unable to support the growth of this organism during their shelf-life (Luber et al., 2011).

As expected, it is obvious from the present study that irradiation of cold-smoked salmon samples can provide significant improvement in their microbial safety with respect to *L. monocytogenes* and *V. parahaemolyticus*. No viable colony forming units of *L. monocytogenes* were detected in cold-smoked salmon samples that inoculated with 3.98×10^6 cfu/g and treated with 3 kGy dose of gamma radiation, meanwhile, samples inoculated with 1.13×10^6 cfu/g of *V. parahaemolyticus* showed no viable colony counts for this bacterium after irradiation with dose as low as 1 kGy. The applied doses were effective in keeping the counts of these bacteria below the detection limit (<10 cfu/g) during refrigerated storage of the cold-smoked salmon samples. Furthermore, irradiation of the un-inoculated cold-smoked samples improved their microbiological quality through inducing significant reduction in their microbial counts of the native microflora, in a dose-dependent manner, with keeping the Enterobacteriaceae undetectable after irradiation at dose of 2 kGy. Several investigators reported the effectiveness of irradiation in the destruction of microorganisms. Huhtanen et al. (1989) showed that a dose of 2 kGy was sufficient to destroy 1×10^4 cells of *L. monocytogenes*, while Patterson (2005) reported that *V. parahaemolyticus* was quite radiation sensitive, in which a dose of 1 kGy would give at least a 10^{15} reduction in the count of this organism. The observed results also agreed with the findings of other workers that indicated the effectiveness of irradiation in reducing the counts of microbial populations naturally contaminated smoked fish products (Hammad and El-Mongy, 1992; Hammad et al., 1995).

The obtained results further show that freshly produced cold-smoked salmon contained different levels of biogenic amines which may be formed in fish before and during cold-smoking. It is known that fish muscle is capable of supporting the bacterial formation of a wide variety of biogenic amines (Özogul and Özogul, 2006). The formation of biogenic amines also significantly increased during refrigerated storage of non-irradiated samples and vacuum-packaging did not show any beneficial effect in controlling their formation which was in agreement with the results of Wei et al. (1990). In addition to the observed initial significant degradation of agmatine, putrescine, tyramine and β-phenylethylamine due to irradiation treatments, the formation of all determined biogenic amines could be significantly controlled during refrigerated storage of the irradiated samples. In agreement with Min et al. (2007), the microbial control by irradiation treatment has an impact on the production of biogenic amines.

As chemical quality parameters, the levels of TBARS, TVBN, and TMAN in irradiated and non-irradiated cold-smoked salmon were also examined. Aerobic packaging is known to increase the rate of oxidation, however, all TBARS values were relatively acceptable. The highest TBARS value was 2.21 mg malonaldehyde/kg after 6 weeks of aerobic refrigerated storage for samples irradiated at 4 kGy dose. According to Connell (1990), the TBA value should be less than

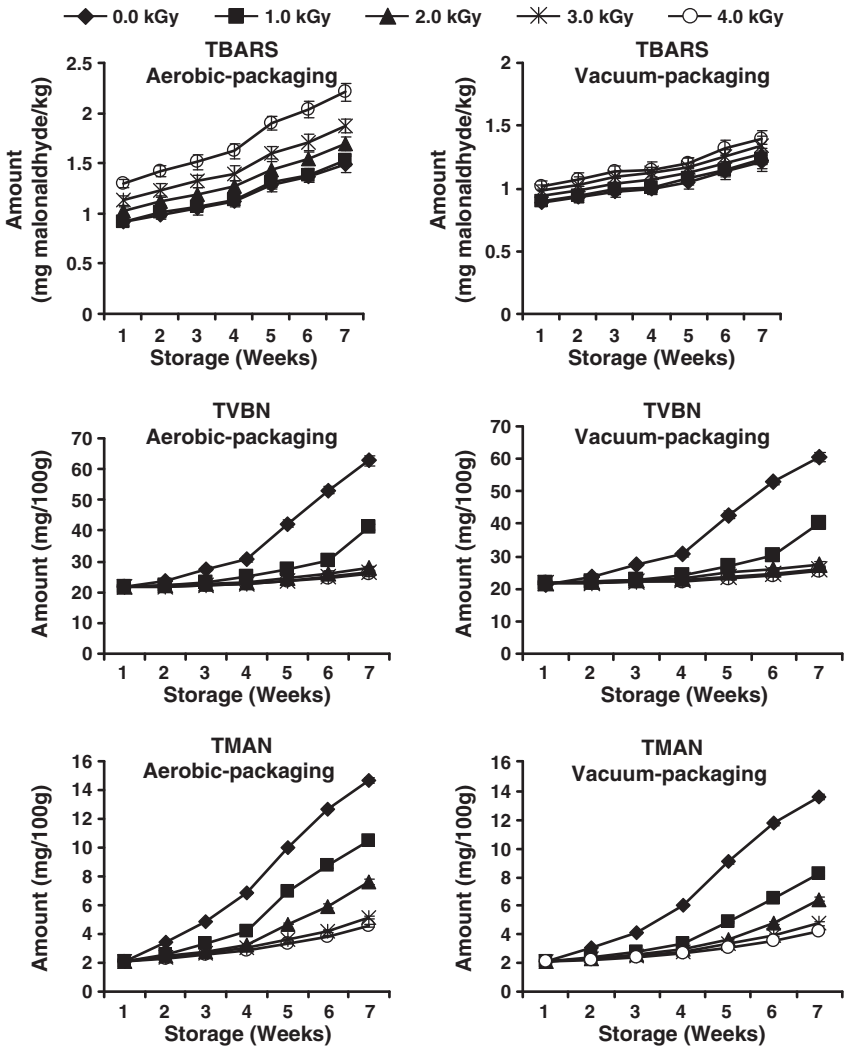

Fig. 3. Levels of thiobarbituric acid reactive substances, total volatile basic nitrogen, and trimethylamine nitrogen in aerobically and vacuum-packaged cold-smoked salmon as affected by gamma irradiation and refrigerated storage at 4 ± 1 °C.

3 mg malonaldehyde/kg in perfect material. The absorbed phenolic compounds may have an antioxidant effect in smoked fish as reported by Marc et al. (1997). On the other hand, irradiated samples of cold-smoked salmon had significantly much lower concentrations of TVBN and TMAN during their refrigerated storage as compared with the controls, which may be attributed to the reduction of microbial populations. The amounts of these compounds are correlated with increasing the viable microbial counts. Enterobacteriaceae and *Lactobacillus* spp. were reported as higher producers of TVB in cold-smoked salmon (Leroi et al., 1999).

None of the evaluated sensory properties showed a significant change due to irradiation of cold-smoked salmon at doses up to 3 kGy. Only significant degradation of the normal cherry red color was observed in samples exposed to 4 kGy dose, but samples were still acceptable. Similar findings were observed by Slusar and Vaisey (1970) and Hammad and El-Mongy (1992).

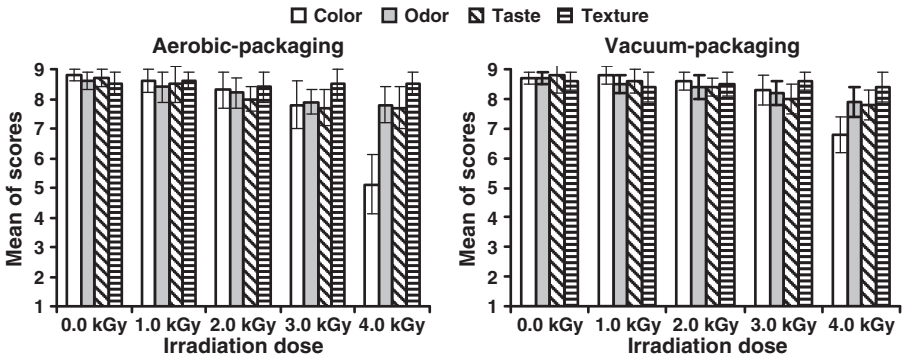

Fig. 4. Sensory scores for aerobically and vacuum packaged cold-smoked salmon as affected by gamma irradiation.

From a microbiological view point, the results of the present work clearly show that irradiation of cold-smoked salmon at dose of 3 kGy was effective in the reduction of 6.59 and 6.05 logs of *L. monocytogenes* and *V. parahaeomlyticus* in the inoculated samples to undetectable levels, respectively, and induced significant great reduction in the counts of microbial populations in the un-inoculated ones. This, in turn, significantly decreased the formation of biogenic amines during refrigerated storage of samples at 4 ± 1 °C with no significant differences being observed between aerobic and vacuum-packaging and without adverse effects on the chemical or sensory quality of samples. Therefore, it could be concluded that gamma irradiation at dose of 3 kGy can be successfully applied under aerobic packaging conditions to improve the hygienic quality and safety of cold-smoked salmon samples with regards to *L. monocytogenes*, *V. parahaemolyticus*, and biogenic amines without significant adverse effects on their chemical or sensory quality attributes.

References

Adams, M.R., Moss, M.O., 2008. Food Microbiology. RSC Publishing, UK.

Alvarez, R.J., 1982. Growth of *Vibrio parahaemolyticus*, *Escherichia coli* and *Staphylococcus aureus* on smoked mullet (*Mugil cephalus*). Zbl. Bakt. Hyg. I. Abt. Org. C 3, 416–420.

AOAC, 2000. Association of Official Analytical Chemists, Official Methods of Analysis, seventeenth ed. AOAC International, Maryland, USA.

APHA, 1992. Compendium of Methods for the Microbiological Examination of Foods, third ed. American Public Health Association, Washington, D.C.

Blackburn, C., 2011. Irradiated foods for immuno-compromised patients and other potential target groups. Food and Environmental Protection Newsletter 14, 4.

Cardinal, M., Gunnlaugsdottir, H., Bjoernevik, M., Ouisse, A., Vallet, J.L., Leroi, F., 2004. Sensory characteristics of cold-smoked Atlantic salmon (*Salmo salar*) from European market and relationships with chemical, physical and microbiological measurements. Food Research International 37, 181–193.

Cho, T.Y., Han, G.H., Bahn, K.N., Son, Y.W., Jang, M.R., Lee, C.H., Kim, S.H., Kim, D.B., Kim, S.B., 2006. Evaluation of biogenic amines in Korean commercial fermented foods. Korean Journal of Food Science and Technology 38, 730–737.

Connell, J.J., 1990. Methods of Assessing and Selecting for Quality. In: Connell, J.J. (Ed.), Control of Fish Quality. Fishing News Books, Oxford, UK, pp. 122–150.

Dalgaard, P., Jorgensen, L.V., 1998. Predicted and observed growth of *Listeria monocytogenes* in seafood challenge tests and in naturally contaminated cold-smoked salmon. International Journal of Food Microbiology 40, 105–115.

Derrick, S., 2009. Cured, Smoked and Dried Fish. In: Fernandes, R. (Ed.), Microbiology Handbook — Fish and Seafood. Leatherhead Food International LTD, UK, pp. 93–121.

Egan, H., Kirk, R.S., Sawyer, R., 1981. Pearson's Chemical Analysis of Foods. Churchill Livingstone, Edinburgh.

FAO/WHO, 2004. Microbiological risk assessment series 4: risk assessment of *Listeria monocytogenes* in ready-to-eat foods. http://www.fao.org.

FDA, 2004. Bacteriological analytical manual(Ch.9) http://www.cfsan.fda.gov/~ebam/bam-9.html.

FDA/FSIS, 2003. Interpretive summary: quantitative assessment of the relative risk to public health from foodborne *Listeria monocytogenes* among selected categories of ready-to-eat foods. http://www.%20foodsafety.gov/~dms/lmr2-toc.html.

Fuentes, A., Fernández-Segovia, I., Barat, J.M., Serra, J.A., 2010. Physicochemical characterization of some smoked and marinated fish products. Journal of Food Processing and Preservation 34, 83–103.

Gimenez, B., Dalgaard, P., 2004. Modeling and predicting the simultaneous growth of *Listeria monocytogenes* and spoilage microorganisms in cold-smoked salmon. Journal of Applied Microbiology 96, 96–109.

González-Rodrígues, M.-N., Sanz, J.-J., Santos, J.-À., Otero, A., García-López, M.-L., 2002. Numbers and types of microorganisms in vacuum-packaged cold-smoked freshwater fish at the retail level. International Journal of Food Microbiology 77, 161–168.

Hammad, A.A.I., El-Mongy, T.M., 1992. Shelf-life extention and improvement of the microbiological quality of smoked salmon by irradiation. Journal of Food Processing and Preservation 16, 361–370.

Hammad, A.A.I., Nassef, Ashga El-A, El-Mongy, T.M., 1995. Microbiological, chemical and sensory changes during storage of irradiated smoked mackerel. Egyptian Journal of Radiation Science and Applications 8, 225–235.

Heymann, D.L., 2004. Cholerae and Other Vibrioses. Control of Communicable Diseases Manual. American Public Health Association, Washington DC, pp. 113–127.

Hong, L., Jie, J., Donghua, L., 2008. Potential hazards in smoke-flavored fish. Journal of Ocean University of China 7, 294–298.

Hudson, J.A., Mott, S.J., 1993. Growth of *Listeria monocytogenes*, *Aeromonas hydrophila* and *Yersinia enterocolitica* on cold smoked salmon under refrigeration and mild temperature abuse. Food Microbiology 10, 61–68.

Huhtanen, C.N., Jenkins, R.K., Thayer, D.W., 1989. Gamma radiation sensitivity of *Listeria monocytogenes*. Journal of Food Protection 52, 610–613.

Hwang, C.A., Sheen, S., 2009. Modeling the growth characteristics of *Listeria monocytogenes* and native microflora in smoked salmon. Journal of Food Science 74, M125–M130.

Joffraud, J.-J., Cardinal, M., Cornet, J., Chasles, J.-S., Lóen, S., Gigout, F., Leroi, F., 2006. Effect of bacterial interactions on the spoilage of cold-smoked salmon. International Journal of Food Microbiology 112, 51–61.

Johnson, W.C., Salinger Jr., A.C., King, W.C., 1973. Survival of *Vibrio parahaemolyticus* on oyster shellstock at two different storage temperatures. Applied Microbiology 26, 122–123.

Jørgensen, L.V., Dalgaard, P., Huss, H.H., 2000. Multiple compound quality index for cold-smoked salmon (*Salmo salar*) developed by multivariate regression of biogenic amines and pH. Journal of Agricultural and Food Chemistry 48, 2448–2453.

Karunasagar, I., Venugopal, M.N., Segar, K., Indrani, Karunasagar, 1986. Survival of *Vibrio parahaemolyticus* in cold smoked fish. Antonie Van Leeuwenhoek 52, 145–152.

Kim, M.K., Mah, J.H., Hwang, H.J., 2009. Biogenic amine formation and bacterial contribution in fish, squid and shellfish. Food Chemistry 116, 87–95.

Leroi, F., Joffraud, J., Stohr, V., Roy, C., Cardinal, M., Berdagué, J., 1999. Characterization of the Spoilage Microflora of Refrigerated Cold-smoked Salmon. Proceedings of the 29th WEFTA Meeting, 10–14 October. A. Georgakis, Greece, S, pp. 116–123.

Luber, P., Crerar, S., Dufour, C., Farber, J., Datta, A., Todd, E.C.D., 2011. Controlling *Listeria monocytogenes* in ready-to-eat foods: working towards global scientific consensus and harmonization — recommendations for improved prevention and control. Food Control 22, 1535–1549.

Marc, C., Kaakeh, R., Mbofung, C.M.F., 1997. Effect of salting and smoking method on the stability of lipid and microbiological quality of Nile perch (*Lates niloticus*). Journal of Food Quality 22, 517–528.

Mejlholm, O., Dalgaard, P., 2007. Modeling and predicting the growth boundary of *Listeria monocytogenes* in lightly preserved seafood. Journal of Food Protection 70, 70–84.

Min, J.-S., Lee, S.-O., Jang, A., Jo, C., Lee, M., 2007. Irradiation and organic acid treatment for microbiological control and the production of biogenic amines in beef and pork. Food Chemistry 104, 791–799.

Naila, A., Flint, S., Fletcher, G., Bremer, P., Meerdink, G., 2010. Control of biogenic amines in food — existing and emerging approaches. Journal of Food Science 75, R193–R.

Oxoid, 1998. The Oxoid Manual. Oxoid Ltd., Wade Road, Basingstoke, Hampshire, R G24 8PW, England.

Özogul, F., Özogul, Y., 2006. Biogenic amine content and biogenic amine quality indices of sardines (*Sardina pilchardus*) stored in modified atmosphere packaging and vacuum packaging. Food Chemistry 99, 574–578.

Patterson, M., 2005. Food Irradiation: Microbiological Safety and Disinfestations. International Symposium "New Frontier of Irradiated Food and Non-feed Products" 2–23 September. KMUTT, Bangkok, Thailand.

Pegg, R.B., 2001. Spectrophotometric Measurement of Secondary Lipid Oxidation Products. In: Wrolstad, R.E., Acree, T.E., Decker, E.A., Penner, M.H., Reid, D.S., Shwartz, S.J., Shoemaker, C.F., Smith, D.M., Sporns, P. (Eds.), Current Protocols in Food Analytical Chemistry. John Wiley & Sons, Inc. (Unit D 2.4).

Roberts, D., Greenwood, M., 2003. Practical Food Microbiology, Methods for the Examination of Food for Micro-organisms of Public Health Significance, third ed. Blackwell Publishing Ltd, USA.

Rørvike, L.M., Yndestd, M., Skjerve, E., 1991. Growth of *Listeria monocytogenes* in vacuum-packaged smoked salmon during storage at 4 °C. International Journal of Food Microbiology 14, 111–117.

S.A.S. Institute, 1998. SAS/STAT User's Guide. Statistical Analysis Institute, Cary, NC, USA.

Shalaby, A.R., 1996. Significance of biogenic amines to food safety and human health. Food Research International 11, 675–690.

Siskos, I., Zotos, A., Taylor, K.A., 2005. The effect of drying, pressure and processing time on the quality of liquid-smoked trout (*Salmo gairdnerii*) fillets. Journal of the Science of Food and Agriculture 85, 2054–2060.

Slusar, M., Vaisey, M., 1970. Effects of irradiation on the sensory quality of refrigerated smoked white fish. Canadian Institute of Food Science and Technology 3, 162–166.

Tomé, E., Gibbs, P.A., Teixeira, P.C., 2008. Growth control of *Listeria innocua* 2030c on vacuum-packaged cold-smoked salmon by lactic acid bacteria. International Journal of Food Microbiology 121, 285–294.

Wei, C.I., Chen, C.-M., Koburger, J.A., Otwell, W.S., Marshall, M.R., 1990. Bacterial growth and histamine production on vacuum packaged tuna. Journal of Food Science 55, 59–63.

Wierbicki, E., 1981. Technological Feasibility of Preserving Meat, Poultry, and Fish Products by Using a Combination of Conventional Additives, Mild Heat Treatment and Irradiation. Proceedings of an International Symposium of Combination Processes in Food Irradiation. IAEA, Vienna, pp. 181–203.

Ye, M., Neetoo, H., Chen, H., 2008. Effectiveness of chitosan-coated plastic films incorporating antimicrobials in inhibition of *Listeria monocytogenes* on cold-smoked salmon. International Journal of Food Microbiology 127, 235–240.