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

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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

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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 × 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 × 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