The effect of gamma irradiation and vacuum packaging upon selected quality traits of refrigerated ostrich meat. Part 1. Microbial assessment

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The aim of the study was to evaluate the effects of gamma irradiation and vacuum packaging as combined process for improvement of ostrich meat shelf life. Ostrich meat was vacuum-packaged and irradiated at 0, 1 and 3.0 kGy. Bacterial growth and sensory quality were determined at 1, 7, 14 and 21 days. Microbial analysis indicated that the combined use of vacuum packaging and irradiation had a significant effect on the reduction of microbial loads. Among the analysed bacteria, *coliforms* were most sensitive to gamma radiation. Considering the total bacteria counts, vacuum-packed samples irradiated at 3.0 kGy were acceptable under refrigerated storage for at least 21 days, compared to 7 and 14 days for non-irradiated, air-packaged, and vacuum-packaged samples, respectively.

KEY WORDS: gamma irradiation / ostrich meat / refrigeration / shelf life / vacuum packaging

In recent years, increasing attention has been paid to ostrich breeding in Iran, like in other countries. One of the reasons of such interest is versatility of the use of ostriches which provide valuable products, especially meat as well as skin, feathers and eggs [Sales *et al.* 1999, Horbanczuk *et al.* 1998, 2008, Horbanczuk 2000, 2002, Cooper and Horbanczuk 2004, Kawka *et al.* 2007]. Microbial control of meat products is the major concern in the preparation of high quality foods [Jo *et al.* 2004]. The hygienic

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status of animals prior to, during and after slaughter can be critical to the finished product quality [Satin 2002]. Irradiation is known to be the best method for the control of potentially pathogenic microorganisms in meat without affecting its physical status [Gants 1998]. Food irradiation is generally defined as the process in which foods are exposed to certain forms of ionizing energy from radioactive sources, mainly gamma rays. In 1997, the FDA approved the irradiation of meat (fresh and frozen beef, lamb and pork) for controlling the disease causing microorganisms such as Escherichia Coli, Salmonella, Listeria and other pathogens borne by food [Bartlett et al. 2000]. Irradiation of food up to an overall dose of 10 kGy is accepted in several countries for commercial food processing [Lacroix and Quattara 2000]. Gamma irradiation has been employed for decontamination of animal foods [Jouki 2013, Chwla et al. 2003, Fu et al. 2000, Kamat et al. 2000, Mahrour et al. 2003, Yang et al. 1993, Jouki and Khazaei 2009] and then to prolong the storage period of irradiated food. The relatively high pH of ostrich meat creates an ideal environment for rapid microbial spoilage in some packaging conditions [Alonso-Calleja et al. 2004, Doherty et al. 1996, Sales and Mellet 1996]. Ostrich meat also contains high amount of polyunsaturated fatty acids as compared to beef and chicken making it more susceptible to oxidation [Sales and Oliver-Lyons 1996, Sales and Horbańczuk 1998, Poławska et al. 2011, 2013]. In our earlier study physical properties of ostrich meat treated with gamma irradiation was evaluated [Jouki 2012], whereas no data have been published so far on the preservation and extension of the shelf-life of fresh ostrich meat by gamma irradiation and vacuum packaging. The objective of this study was to investigate the effects of gamma irradiation combined with vacuum packaging on microbial quality of ostrich meat during refrigerated storage.

Material and methods

Sample preparation

Ostrich meat samples from six ostriches (*Struthio camelus var. domesticus*) were obtained as was previously described by Jouki [2012]. Any visible fat was removed from the muscle tissues. Measurements of microbial quality were conducted on meat samples. This study was performed in the Department of Food Science, University of Tehran, Iran, in February 2012.

Packaging

A packaging machine model A200, (HENKELMAN, Netherlands) was used for packaging. Meat samples were randomly assigned to packages (sterile polyester polyethylene (PET/Poly) bags (thickness-62lm). The samples were divided into 15 equal parts and then were packaged and sealed aseptically in polyethylene bags, each divided into two sets, one to be air-packaged and another vacuum-packaged. Each set was further divided into three groups: one kept as control (without irradiation) and the second and the third irradiated with gamma at 1 kGy and 3 kGy respectively. The packages were stored at 4°C for the entire duration of the experiment (21 days). Samples were analysed on 1, 7, 14 and 21 days post-slaughter.

Irradiation

Packaged meat samples were gamma-irradiated at the Atomic Energy Organization of Iran (AEOI, Tehran, Iran) inside a package irradiator (Gamma Cell 220, NORDION Intl. Inc., Ontario, Canada) with a 60Co source at a rate of 1.576 kGy/h. The dose was established using alanine transfer dosimeter to make sure that the dose reached the target dose.

Microbial Analyses

A sample (25 g) was drawn aseptically and transferred to 225 ml of sterile 0.1% peptone water solution. The sample was homogenised in a stomacher Lab Blender 500 for 1 min at room temperature. For microbial identifiation, 0.1 ml samples of serial dilution of meat homogenates were spread on the surface of dry media: Total plate count was performed on plate count agar (MERCK, Germany). The samples were incubated at 25°C for 72 h; lactic acid bacteria on MRS (MERCK), overlaid with the same medium and incubated at 25°C for 96 h under anaerobic conditions; Pseudomonas spp. on cetrimide-fucidin-cephaloridine (CFC) agar (OXOID, UK) incubated at 25°C for 48 h; yeast and moulds were identified using acidified potato dextrose agar (MERCK), after incubating at 30±2°C for 3 days. Enterobacteriaceae on Violet Red Bile Dextrose Agar (MERCK) incubated at 37°C for 24 h. The data (growth counts) were transformed to log 10 values. The *coliform* colony count was determined on Violet Red Bile Agar (VRBA) (OXOID, CM485, UK) at 37°C for 48h. The count of Staphylococcus aureus was accomplished in selective medium BPA (BAIRD-PARKER-AGAR) and BHI, incubated at 35°C for 48 hours [Vanderzant and Splittstroesser 1992].

Statistical

Data from microbial, chemical and sensory determination were subjected to an analysis of irradiation doses (0, 1.0 and 3.0 kGy), and storage time (1, 7, 14, and 21 days) by simple and interaction effects using two-way ANOVA. Comparisons of means were based on Tukey's post hoc multiple test. Used was the SAS [1988] statistical package.

Results and discussion

Microbial analyses

The changes in counts of *aerobic mesophilic bacteria*, *coliform*, *Staphylococcus aureus*, *Pseudomonas*, *Enterobacteriaceae* and *lactic acid bacteria* (LAB) in meat samples during storage at 4°C are shown in Figure 1. The effects of gamma irradiation

and vacuum packaging were restricted in the counts of microbial flora with the concomitant benefit of prolonging refrigerated shelf-life on the samples. During storage, these microorganisms significantly increased in vacuum and air-packaged non-irradiated, while the rate of increase was lower in samples irradiated packaged under vacuum. Initial microbial counts on day 1 were 5.23 log CFU/g for total aerobic plate counts, 4.5 log CFU/g for *Staphylococcus aureus*, 3.8 log CFU/g for lactic acid bacteria, 3.12 log CFU/g for *Pseudomonas*, 2.4 log CFU/g for *Enterobacteriaceae*



Fig. 1. Microbial changes of irradiated and non-irradiated ostrich meat under vacuum packaging during refrigerated storage. AP – air-packaged; VP – vacuum-packaged; 0 kGy – non-irradiated (control); 1 kGy – irradiated with dose 1 kGy; 3 kGy – irradiated with dose 3 kGy.

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and 2.2 for Coliforms. The initial load of aerobic bacteria in air-packaged meat samples was 5.23 log CFU/g and reached 8.47 log CFU/g after 21 days of storage. Microbial loads showed differences ($P \le 0.05$) during storage and between packaging conditions. The aerobic bacteria population was inhibited by both vacuum packaging and irradiation. The high microbial load found in ostrich meat in relation to other red meats has been attributed to high pH of this meat which creates an ideal environment for rapid microbial spoilage under some packaging conditions [Alonso-Calleja et al. 2004, Fernandez-Lopez et al. 2008, Sales and Mellet 1996, Seydim et al. 2006]. According to Capita et al. [2006] storage temperature and time both affect the microbial count of meat. The exclusion of oxygen affects total aerobic bacteria count (psychrotropic, *Pseudomonas*). In the cited study, the time of storage influenced all the microbial groups of meat. Total aerobic counts after irradiation decreased as irradiation dose increased up to 3 kGy (Fig. 1). Irradiation at 1 and 3 kGy resulted in up to a 2 and 3 log reduction in total microbial counts resulting in a final count of about 3-log at day 21. The total aerobic bacteria count increased during storage, but it was lower in samples irradiated at higher doses (Fig. 1). The total count on day 21 in all meat samples irradiated at 3 kGy was lower than the count in non-irradiated samples on day 1, while the count on day 7 in samples irradiated at 1 kGy was similar to the count in non-irradiated samples on day 1 (Fig. 1). Thayer et al. [1995] found that the total bacteria count of chicken wings was reduced by about 2 log cycles with irradiation at 1.4 kGy. In the present experiment, the doubling time was higher in vacuum-packaged than in air-packaged samples, hinting that the aerobic bacteria were not able to start the growth in vacuum as they grew more easily in air. We reported a rapid growth of viable bacteria in air-packaged samples, reaching a population of 7 log CFU/g after four to five days of storage at 4°C; the use of vacuum packaging extended the time required for the total count of bacteria to reach 7 log CFU/g to 3 or 4 days of storage. In this study, shelf-life extension was mostly due to the irradiationinduced prolongation of the lag phase, found to be higher for the samples treated with 3.0 kGy. Lactic acid bacteria counts were different (P<0.05) between air and vacuum packaged samples. In accordance with the present study, Fernandez-Lopez et al. [2008] reported that the evolution of LAB counts in air packaged ostrich steaks was 1 log cycle lower than in vacuum and MAP packs. Irradiated packaged samples had lower (P<0.05) counts than non-irradiated packaged samples. *Staphylococcus aureus* population showed a general increase during storage time in all packages (P<0.05) and remained about 7.5 CFU/g in the meat samples packaged under air, while samples irradiated at 1 and 3 kGy showed Staphylococcus aureus population about 5 and 3 log CFU/g at the end of storage time (21 days). In contrast, vacuum packaging markedly inhibited the growth of *Staphylococcus aureus* on meat. Of the psychrotrophic bacteria, Pseudomonas spp. are gram- negative bacteria that dominated at refrigeration temperatures and are considered as one of the main spoilage microorganisms in meat and poultry [Jay 2000]. Pseudomonas began to increase in all groups and reaching 8.1 log CFU/g and 7.3 log CFU/g, respectively in air and vacuum packages at the end of storage (day 21). Irradiation dose of 1 kGy reduced the counts of Enterobacteriaceae by 2 log units, but for 3 kGy no growth of *Enterobacteriaceae* was observed (Fig.1). Chouliara et al. [2004] reported that using an irradiation dose of 2 kGy resulted in a reduction of Enterobacteriaceae by approximately 2 log CFU/g in aerobically packaged chicken breast stored at 4°C, while a dose of 4 kGy eliminated the mentioned microorganisms during 25 days of storage. Among the microflora of meat, LAB were the most resistant to irradiation process. Irradiation doses of 1 and 3 kGy produced immediate LAB reduction of 1 and 1.9 log units, respectively. Lacroix et al. [2004] reported that LAB and *Brochothrix thermosphacta* are more resistant to irradiation than Enterobacteriaceae and Pseudomonas. The initial counts of Pseudomonas demonstrated a considerable number (3.12 log CFU/g) of these organisms in nonirradiated ostrich meat samples. Irradiation dose of 1 kGy reduced the initial counts of *Pseudomonas* by 3 log units, while at doses of 3 kGy these organisms were below the detection level during refrigerated storage (Fig. 1). The number of *aerobic bacteria*, Coliform and St. aureus decreases with increase of irradiation, therefore irradiation significantly (P < 0.05) reduced them (Fig. 1). The 3 kGy dose reduced the counts of St. *aureus* by more than 2 log units in ostrich meat. The results of this research concern that the reduction of St. aureus population in irradiated chicken meat was similar to those found by other authors. Nouchpramool et al. [1985] observed that the dose of radiation of 3.0 kGy was able to eliminate St aureus in frozen shrimp. The dose of 2.5 kGy was able to eliminate St. aureus from smoked fish [Research... 1978]. Thayer et al. [1997] concluded that St. aureus can be eliminated or greatly reduced in number from bison, alligator and caiman meats by doses of gamma radiation between 1.5 and 3.0 kGy and storage at 5°C. According to Thayer [1995] low doses of ionizing radiation (<3.0 kGy) may eliminate or significantly reduce the population of the most common enteric pathogens such as Campylobacter jejuni, Escherichia coli, Staphylococcus aureus, Salmonella spp., Listeria monocytogenes and Aeromonas hydrophila. Ionizing radiation can be an effective step in a program to kill enteric pathogens associated with meat and poultry products. The total coliform group did not show detectable growth in the samples irradiated at 3.0 kGy (Fig. 1). According to the data obtained, that group was eliminated from the ostrich meat irradiated to 3.0 kGy, and had difficulty to start growing after irradiation with 1.0 kGy. Abu-Tarboush et al. [1997] also found that irradiation with 2.5 kGy and storage at 4°C for 21 days was sufficient to eliminate total *coliforms* from chicken meat. In another experiment, gamma irradiation of chicken meat with 1 and 1.8 kGy was sufficient to eliminate total coliforms [Lewis et al. 2002]. The results of this study indicate that irradiation doses of 1.0 and 3 kGy were effective in eliminating bacteria from ostrich meat. Gamma irradiation treatment at 1.0 and 3 kGy reduced coliforms, Enterobacteriaceae, aerobic bacteria and psychrotrophs when compared to controls receiving no irradiation treatment. Although coliforms, Enterobacteriaceae, and psychrotrophs were eliminated using 3 kGy of irradiation, aerobic bacteria populations were greatly reduced but not completely eliminated. Counts of 7 log CFU/g is the approximate point at which meat

would be unacceptable [Dainty and Mackey 1992]. Therefore, the shelf life of ostrich meat stored under aerobic conditions would be about a week, while irradiated and packaged under vacuum would be 21 days. Irradiation in both packaging systems increased the microbial shelf life of ostrich meat.

Microbial loads showed differences (P<0.05) during storage and between packaging conditions. The aerobic bacteria population was inhibited by both vacuum packaging and irradiation. Irradiation at 1 and 3 kGy resulted in up to 2 and 3 log reduction in total microbial counts, resulting in a final count of about 3-log at the day 1. The total aerobic bacteria count increased during storage, but it was lower in samples irradiated at higher doses. Irradiation dose of 1 kGy reduced the counts of *Enterobacteriaceae* by 1 log units, but for 3 kGy no growth of *Enterobacteriaceae* were observed. Among the microflora in ostrich meat, LAB were the most resistant of them to irradiation reduced the microbial populations better than air-packaging and irradiation reduced the microbial populations better than air-packaging and irradiation. Considering the total bacteria counts as a whole, vacuum-packaged samples irradiated at 3.0 kGy were acceptable for 21 days under refrigerated storage.

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