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Combined effect of gamma irradiation and cold temperature storage on the sensory properties of edible estuarine crab *Scylla serrata*

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Abstract

The present study evaluates the combined effect of dose-dependent gamma irradiation and cold storage on sensory characteristics of mud crab, *Scylla serrata*. The samples were irradiated at 0.5, 1.0 and 2.0 kGy and stored at 4°C and -20°C along with non-irradiated control. The sensory properties of the both irradiated and cold stored samples were assessed at weekly intervals up to maximum of 28 days. Though extended storage at 4°C (up to 14 days) resulted reduction in sensory quality of both irradiated and non-irradiated control samples, irradiated samples scored significantly higher value which was concomitant with the irradiation doses applied. Gamma irradiation at any applied dosage (0.5, 1.0 and 2.0 kGy) has no significant advantage on sensory characteristics of crab during their storage at -20°C until 14 days compared to control; further extended storage (up to 28 days), however, enhanced the degree of sensory quality in the samples received 1.0 and 2.0 kGy gamma irradiation compared to the samples irradiated with the low dose (0.5 kGy) and control. The overall experimental results indicated that gamma irradiation at the dose range of 1.0-2.0 kGy and the subsequent cold (4°C) and frozen storage (-20°C) would be helpful to extend the shelf life of *Scylla serrata* by maintaining fairly good sensory quality for maximum duration of 14 and 28 days respectively.

Introduction

Seafoods including finfishes and shellfishes are known for the major source of dietary protein (Skonberg and Perkins, 2002; Sudhakar *et al*., 2009). However, the rapid quality deterioration of these food resources occurred during handling, transportation and storage limit their shelf life (Azam *et al*., 1998; Sallam, 2007). Like other seafood resources, the mud crab, Scylla serrata, one of the immense global market demanding shellfish, also have highly perishable nature influenced by various factors such as high a_w, neutral pH, and autolytic enzymes (Jeyasekaran *et al*., 2006; Begum *et al.*, 2009; Lalitha and Thampuran, 2012).

Growing consumer demands for safe and durable food products with high nutritional and sensory value lead modern food preservation strategies through technological developments as well as new packaging system (Stone and Sidel, 2004; Walkling-Ribeiro *et al*., 2009: Giménez *et al*., 2012). The application of low-dose irradiation has been proven as an effective method for the preservation of food products (Nortjé *et al*., 2006; Özden *et al.*, 2007; Rivera *et al*., 2011). Low- dose irradiation in the range of 1.0–3.0 kGy has been applied to extend the shelflife of fishery products (Venugopal *et al.*, 1999). Cleemput *et al.* (1980) reported the shelflife of 8 days for 5.0 kGy irradiated shrimp stored at +6°C in contrast to the control sample, that was unacceptable after 4 days of storage. Further, 3.0 kGy irradiation has facilitated the improvement in the shelflife to 14 days in crab and to 11 days in precooked shrimp (Chen *et al.*, 1996; Ouattara *et al.*, 2001).

The shelflife of food products, which is a function of time, environmental factors, and susceptibility of product to quality change, is limited mainly by changes in their sensory characteristics (Labuza and Szybist, 2001; Hough, 2010). Nowadays, sensory shelflife estimation of foods has become an issue of continuous and extensive research on both the deteriorative mechanisms occurring in food systems and the development and application of methodologies for shelflife estimation (Stone and Sidel, 2004; Manzocco and Lagazio, 2009).

Irradiation effects on the sensory characteristics depend on type of the food that is being irradiated. Dose-dependent gamma irradiation with doses

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ranging from 1.0 to 5.0 kGy was not induced any unacceptable flavor, texture and odor in Sweetlip *(Lethrinus miniatus)*, red emperor *(Lutjanus sebae)*, mackerel *(Scomberomorus commerson)*, whiting *(Sillago ciliate),* mullet *(Mugil cephalus)*, barramundi *(Lates calcalifer)* and sand crab *(Portunus pelagicus)* (Poole *et al*., 1994). But, in the case of chilled saucer scallops *(Amusium balloti)* doses in the order of 1.5 kGy or above resulted in a soft, spongy, and mushy texture (Poole *et al*., 1990). For food samples with higher fat content and the associated specific fine flavor like that of ocean perch *(Sebastes marinus)*, doses higher than 1.0 kGy are not recommended because of sensory changes (Ronsivally and Slavin, 1965; Reinacher and Ehlermann, 1978). The maximum acceptable dose for irradiation of Bombay duck *(Harpodon nehereus)* was determined to be 5.0 kGy at which there develops an off-flavor, but will subside after 4 days (Gore and Kumta, 1970; Kumta *et al*., 1970). Cho *et al*. (1992) reported that the sensory quality of irradiated dried fish powders with doses 5.0-10 kGy had greater acceptability than the controls for 3 months post-irradiation without any alteration in the sensory characteristics of the samples. Gardner and Watts (1957) observed the development of undesirable odours in oyster meats *(Crassostrea virginica, Crassostrea pacificus)* after treatment with 0.63, 0.83, and 3.5 kGy doses of ionizing irradiation. These aforesaid reports signify the importance of sensory evaluation of each post irradiated sample specifically to determine the duration of their shelflife. At this juncture, the present paper reports the assessment on the sensory characteristic changes occurred in the mud crab, *Scylla serrata* subjected for dose-dependent gamma irradiation and subsequent cold (4^0C) or frozen (-20^0C) storage for the duration of maximum 28 days.

Materials and Methods

Sampling

Live fresh adult mud crabs, *Scylla serrata* with an average carapace width (8.00 cm) and body weight (200 g) were collected from Cherukunnu estuary (Kannur, Kerala, India) (120 0' N, 750 18' S). Live crabs were immediately brought to the laboratory and thoroughly examined for the presence of any disease or wound condition. Moult stage of the crabs was characterized through the microscopic observation of setogenesis of maxilliped (Sudha and Anilkumar, 2007; Sudha *et al*., 2012) healthy intermoult male crabs were selected for the present study.

A total of 36 crabs were selected and divided into nine groups each containing 4 crab samples. Subsequently, body surface of each crab from all subgroups was delicately washed with sterile distilled water (prepared by autoclaving distilled water at 121^0C for 20 minutes), vacuum packed individually in pre-sterilized polyethylene bags (with thickness 60 ± 3.6 µm) and sealed using electrical sealing machine (SEPACK, Sevana, India).

Gamma irradiation

Three crabs from each group were subjected to dose-dependent gamma irradiation (^{60}Co) at the dosage of 0.5, 1.0 and 2.0 kGy respectively with a dose rate of 3.8 kGy per hour by adopting the gamma irradiation facility (GC - 5000, BRIT, India) available at Meat Technology Unit, Kerala Agricultural University, Thrissur, Kerala, India; and the remaining one group (or one non-irradiated crab in all the group) was taken as control sample.

Immediately after irradiation, four groups were kept at 4^0C and other four groups were kept at -20^0C for sensory evaluation at the time interval of seven days for maximum 28 days of storage period. The remaining one group was immediately subjected to sensory evaluation these samples were considered as common samples for 0 day cold (4^0C) and frozen storage (-20^0C).

Sensory evaluation

Sensory evaluation was performed by the panel of five non-professionally trained research scholars, in order to imitate the consumer behavior (Boziaris *et al*., 2011). The modified 10 point score sheet developed by Reilly *et al.* (1987) and Jeyasekaran *et al.* (2006) was used for the purpose. The overall acceptability of the crab samples after cold storage (4^oC) was determined after careful evaluation of their sensory characteristics including general appearance, colour, odour, and texture of shell and tissues (muscle, hepatopancreas and gill). The average of the sensory score assigned by panelist were taken and those samples which received sensory score ≤5 were considered as unacceptable (Jeyasekaran *et al*., 2006).

Results

Sensory evaluation of samples of S. serrata *kept at 4^oC*

The sensory scores of irradiated and non-irradiated samples of the crab *(S. serrata)* under the cold storage at 4^oC for different duration are represented in Figure 1. Live, healthy, fresh, intermoult and non-irradiated crab on 0 day cold storage appears with dark green hard carapace and creamy white sternum.

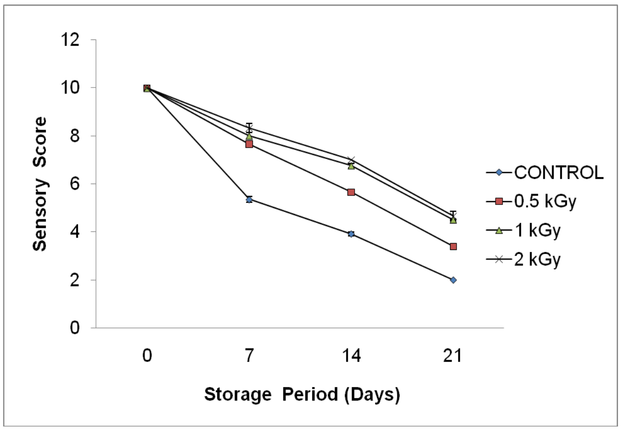

Figure 1. Changes in the sensory quality of dose-depended gamma irradiated and non-irradiated *S. serrata* during cold storage at 4^{o}C

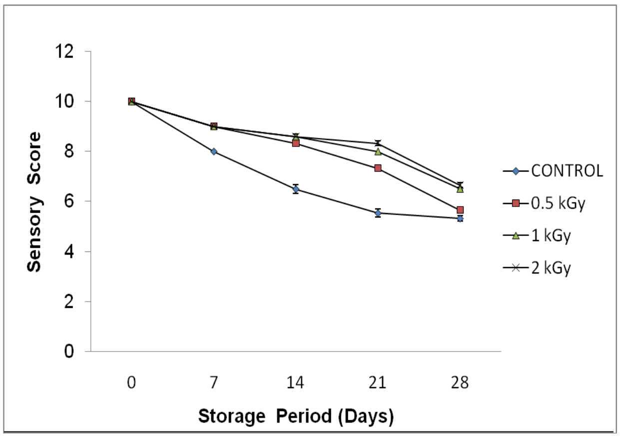

Figure 2. Changes in the sensory quality of dose-depended gamma irradiated and non-irradiated *S. serrata* during cold storage at -20^{o}C

Chelate legs are greenish orange; ventral side of the leg has a dull white shade; the texture of the muscle and gill is solid. Hepatopancreas appears as bunch of creamy yellow or yellowish orange tubules. The crabs exhibited their characteristic muddy odour and scored a sensory score of above 9.9 (Figure 1). The aforementioned characteristics were found to remain unchanged in the dose-dependent (0.5, 1.0 and 2.0 kGy) gamma irradiated crabs before they kept for cold storage (0 day cold storage).

On seventh day, the non-irradiated (control) sample showed mild ammoniacal odour, coupled with decolouration and softening of both carapace and sternum; the sensory score was declined to the level of 5.35. By 14 days non-irradiated control samples kept at 4^{o}C was completely spoiled and rejected with a score of 3.92. By this period (14 days) the 0.5 kGy irradiated and 4^{o}C stored sample exhibited fouling fishy smell and their carapace was faded with considerable reduction in sensory score (5.6). However, those samples, which received irradiation doses 1.0 kGy and 2.0 kGy and stored at 4^{o}C showed organoleptic features up to 14 days with relatively good score of 6.76 and 7.00 respectively. Significantly, all the irradiated samples irrespective of the irradiation doses, stored at 4^{o}C, were rejected by 3 weeks on account of their putrid off-odour and the complete loss of original texture of all observed tissues.

Sensory evaluation of samples of S. serrata *kept at -20^{o}C*

Both non-irradiated and dose-depended (0.5, 1.0, and 2.0 kGy) irradiated samples stored at -20^{o}C did not show any significant changes in their sensory quality for 14 days (Figure 2). During this period neither foul smell (ammoniacal odour) nor decolouration on shell (carapace and sternum) was noticed nor all tissues appeared in its intact form and texture; the sensory scores of 0.5, 1.0 and 2.0 kGy treated samples were 8.33, 8.59 and 8.59 respectively. By the completion of 3 weeks, 1.0 and 2.0 kGy irradiated samples have been shown to maintain a relatively higher sensory score (8 and 8.33 respectively), compared to that of 0.5 kGy irradiated sample, which scored 7.33. Though the non-irradiated control sample, appeared fair and acceptable with sensory score of 5.5, by this time (21st day), the original texture and colour of both carapace and hepatopancreas were found to be lost. Significantly, the samples that received irradiation doses 1.0 and 2.0 kGy have been shown to maintain its freshness and original characteristics throughout the storage period (28 days) with sensory score above 6.5. The sample irradiated with 0.5 kGy, however, had relatively low sensory score of 5.66.

Discussion

Based on overall evaluation on the sensory characteristics made in the present study, it is assumed that 1.0 and 2.0 kGy irradiated vacuum packed crab, S. serrata stored at 4^{o}C could be acceptable up to 14 days with relatively high sensory score (6.72 and 7.00 respectively) compared to 0.5 kGy irradiated (5.66) and non-irradiated control samples (3.92). Our present observations support the previous study on the shelf life extension of the refrigerated crab meat products at 4^{o}C for maximum 14 days under irradiated condition (Chen *et al.*, 1996). Sensory evaluation studies conducted in horse mackerel (Mendes *et al.*, 2005) revealed that 1.0 kGy irradiated and non-irradiated horse mackerel were acceptable up to 12th and 4th day of cold storage respectively. Similarly 1.5 kGy irradiated fresh water carp was reported to be acceptable at sensorial level up to 31 days (Icekson *et al.*, 1996). These observations indicate the significance of optimization of irradiation dose for each species of seafoods for the long term storage under refrigerated conditions.

In the present study, 0.5 kGy irradiated sample *(S. serrata)* refrigerated at 4°C for 14 days exhibited low sensory score of 5.6. On 7th day itself the non-irradiated (control) crabs refrigerated at 4°C begin to exhibit the sign of spoilage evidenced by the presence of decolouration and softening of shell with reduced score of 5.35. Cleemput *et al.* (1980) reported that 5.0 kGy irradiated shrimp and the non-irradiated control sample stored at +6°C was found to be unacceptable after 8 and 4 days respectively. In crustaceans, various post mortem activities of autolytic enzymes brings about tremendous changes including enzymatic browning leading to the loss of original colour of the shell (Ashie *et al*., 1996). By 14th day, the non-irradiated control crabs kept at 4°C showed strong ammoniacal and putrid off-odours with very low sensory score (3.92), which is far below the point of rejection (5.00). Similar observations were reported in some other edible crustaceans such as *Cancer pagurus* and *Nephropes norvegicus* and the reason for this is suggested to be the changes in the microflora (Anacleto *et al*., 2011; Gornik *et al*., 2011). The degree of spoilage potential depends on the composition of the microflora (Gornik *et al*., 2011) and the main spoilage mechanism is the metabolic activity of spoilage organisms causing the production of off-odours (Dainty, 1996; Gram and Huss, 1996). Various species of *Bacillus* is found to be dominated both in the irradiated and non- irradiated tissues of S. serrata, caught from Cherukunnu estuary (unpublished observation). *Bacillus*, in general, produces a range of exoenzymes such as amylases, proteases and lipases that could attack major nutrients in food such as carbohydrates, proteins and lipids (Maya *et al*., 2011). In Norway lobster, *Nephropes norvegicus*, microbial spoilage is mainly caused by *Pseudomonas* sp. *Enterobacteriaceae* and H_2S producing bacteria (López-Caballero *et al*., 2000; Boziaris *et al.*, 2011)

It is significant to note that, the variation in sensory properties of the non-irradiated and irradiated (0.5, 1.0, and 2.0) samples stored at -20°C is relatively quite less throughout 14 days. Absence of microbial growth at freezing temperature (Finegold, 1996; Rivkina *et al.*, 2000) and there by halting microbial spoilage (Archer, 2004) might be the root cause for the present observation. The temperature limit of bacterial growth was reported to be from about -5°C to about -8°C in frozen food (Geiges, 1996).

Decay of hepatopancreas derived from non-irradiated crab stored at -20°C was begun quite earlier compared that of irradiated samples. This high rate of deterioration in the reduced level is apparently due to the activity of enzymes particularly lipases and proteases, extruded from microorganisms. These enzymes, responsible for the decomposing of large molecules into their fundamental elements, remain active at freezing temperature, even below -15°C (Hall and Alcock, 1987; Makarios-Laham and Lee, 1993; Geiges, 1996). Being a rich resource of lipid and protein (Arshad *et al.*, 2014), hepatopancreas is more prone to these enzymes resulting the fast rate of spoilage. Certain strains of bacteria that harboured the tissues at low temperature have been shown to produce enzymes at high rate likely to overcome the greatly reduced activity of these enzymes at low temperature (Peterson and Gunderson, 1960). In the present study possible occurrence of rich bacterial strains causing quick deterioration of hepatopancreas under non-irradiated condition through elevated enzyme secretion at cold temperature cannot be ruled out.

Conclusion

From the observations on sensory characteristics made in the present study, it may be concluded that the irradiation dose of 1.0-2.0 kGy is the reliable dosage to improve sensory properties and thereby shelf life of *S. serrata* (whole crab) by 14 days and 28 days for storage at 4°C and -20°C respectively. Gamma irradiation has no significant advantage on sensory characteristics of crab during storage at -20°C for short period of time (14 days). It is also indicated that sensory properties of 1.0 and 2.0 kGy gamma irradiated *S. serrata* during the storage at 4°C and -20°C are almost comparable.

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