Abstract

Currently, there is an increasing demand for minimally processed or convenience food products without any quality loss. Electron Beam Irradiation (EBI) is a non-thermal processing technique used to preserve the nutrient value and shelf-life extension of food products. In the present study, the effect of electron beam irradiation on the quality of tilapia fish chunks was evaluated. Tilapia fish chunks were vacuum packed and exposed to 0, 2.0, and 4.0 kGy doses of electron beam irradiation and kept under chilled storage. Biochemical, microbiological, and sensory qualities were analysed for up to 41 days. pH (6.85 to 7.10), total volatile base nitrogen (TVB-N), peroxide value (PV) and thiobarbituric acid (TBA) content showed an increasing trend during storage. It was observed that TVB-N content was lower in the irradiated sample than control. Thiobarbituric acid reactive substances (TBARS) values were within the acceptable limits during storage. Microbiological analysis revealed that irradiated fish chunks had lower total plate count, *Pseudomonas* count and *Brochothrix thermosphacta* count compared to the control. The count of hydrogen sulphide producers and *Lactobacillus* were nil in the irradiated fish chunks. In terms of microbial and sensory qualities, it was found that electron beam irradiated samples had an extended shelf-life of 28-38 days (with respect to dose level), compared to the control which had a shelf-life of only 16 days.

Introduction

Fish is considered a highly nutritious and easily digestible animal protein. Due to the depletion of marine catch, freshwater fish farming has been increasing to meet consumer demand. Inland fish production is gaining remarkable growth in India reaching 121.21 lakh t during 2021-2022 (DOF, 2022). *Tilapia* (*Oreochromis niloticus*) is one of the most important freshwater fish having consumer preference due to its taste and availability. Generally, it is sold as whole fish or as steak. Due to changing lifestyles, ready-to-cook or ready-to-eat seafood is gaining more demand in domestic and export markets. Fish is one of the highly perishable food items, and it needs a proper cold chain till it reaches the consumer. Generally, chilling, freezing, drying, or a combination of two or more of these methods are used for the shelf-life extension of seafood (Leistner, 2000). Apart from this, packaging also plays a significant role in product appearance and quality. It has been reported that vacuum packaging can extend the shelf-life of fish and fishery products under chilled conditions (Parvathy et al., 2016; Jeyakumari et al., 2020). Consumers now prefer high-quality convenience seafood products with natural flavours and nutrients, making the industry to go for producing seafood products with improved shelf-life using appropriate technologies. Electron beam irradiation...
(EBI) is a non-thermal processing technique used to preserve the nutrient value and extend the shelf-life of food products, which gaining much attention from food processors (Lewis et al., 2002). The advantage of electron beam irradiation is that it can be applied in a bidirectional manner, in which the irradiation can come into contact with the food product from the top and bottom of the samples, thereby reduction in the microbial count of the food product is achieved with less processing time (Levanduski and Jacyznski, 2008). The World Health Organisation has approved the treatment of foodstuffs with ionising radiation up to doses of 10 kGy (WHO, 1999). So far, very few studies have reported on the quality of electron beam-irradiated marine fish, shellfish, and meat products (Joong-Ho et al., 2008; Zhen et al., 2014; Feng et al., 2019, Jeyakumari et al., 2020; Yu et al., 2022). India has been exporting radiation-hydrogenised spices and dry ingredients to several countries since 2000. Moreover, from 2007 onwards, mangoes are exported to USA after gamma irradiation treatment (APEDA, 2007). In 2015, FSSAI formulated standards for irradiated foods including fruits, vegetables, meat, and seafood (FSSAI, 2015). Currently, irradiated seafood and meat products are not exported from India and the process is still under the research and development stage. To our knowledge, there is no report on the quality and shelf-life of electron beam-irradiated tilapia fish chunks. Hence, the present study was undertaken to evaluate the biochemical, microbiological, and sensory quality of e-beam irradiated tilapia fish chunks and to assess the shelf-life under chilled storage.

Materials and methods

Sample preparation for the experiment

Fresh tilapia (Oreochromis niloticus) were procured from the fish market (Navi Mumbai, Maharashtra, India) in iced condition. Tilapia fish chunks were sliced into 3-4 cm thick size and vacuum-packed. Samples were divided into 3 lots. The first and second lots were exposed to 2.0 and 4.0 kGy electron beam irradiation dose, respectively. The third lot was kept as control without exposure to electron beam irradiation. The electron beam irradiation process was carried out at Board of Radiation and Isotope Technology (BRIT), Mumbai, using an EB RF accelerator (Beam power 40kW, 5 MeV energy). During the irradiation process, sample packs were kept under gel ice medium to reduce temperature fluctuation on the sample. After the irradiation process, all samples were kept in an insulated box, brought to the laboratory and kept under chilled (2°C) conditions. The samples were drawn at known intervals for up to 23 days, and subjected to analysis of biochemical, microbiological, and sensory qualities.

Evaluation of biochemical and sensory quality

Moisture, protein, fat, and ash content of fish chunks were analysed according to AOAC (2019). The pH was determined by dispersing fish meat in distilled water (1:10) and analysed using a digital pH meter (EcoScan pH 5, EUTECH Instrument.) Non-protein content (NPN) was evaluated as per AOAC (2019). Expressible moisture content was measured according to Treesa and Saleena (2016). Total volatile base nitrogen (TVB-N) content was determined as per Conway (1950) and peroxide value (PV) according to Yildiz et al. (2003). The thiobarbituric acid (TBA) value was estimated as per the method of Tarladgis et al. (1960). Sensory quality parameters, including appearance, colour, odour, taste and overall acceptability of tilapia fish chunks were assessed following Parvathy et al. (2016)

Microbial quality

Total plate count (TPC) was assessed according to FAO (1992); Pseudomonas count following Mead and Adams (1977) and hydrogen sulfide (H2S) producing bacterial count according to Koneman et al. (1992). Brochothrix thermosphacta count was determined as per Corry et al. (1995) while Lactobacillus count was enumerated following Downes and Ito (2001).

Statistical analysis

All the analyses were performed in triplicate. One-way ANOVA was done (p < 0.05) using SPSS software version 16.0. (SPSS Inc., Chicago, Illinois, USA).

Results and discussion

Proximate composition

Tilapia fish meat had 80.15 ± 0.15% moisture, 17.25 ± 0.20% protein, 0.42 ± 0.15% fat, and 1.05 ± 0.02% ash. Results were comparable with earlier reports for tilapia fish meat (Dhanapal et al., 2016; Parvathy et al., 2016; Murthy et al., 2017)

Changes in expressible moisture content and pH

Expressible moisture content (EMC) measurement is used to indicate the textural quality of fish meat (El Rammouz et al., 2004). In the present study, the control had the highest EMC on the 20th day (22.50%). However, 2.0 and 4.0 kGy irradiated fish chunks had an EMC of 22.9 and 26.50% on the 24th and 28th day respectively (Fig.1.a). The increase in EMC might be due to a decrease in the water-holding capacity of tissue due to changes in the microstructure of myofibrillar proteins during chilled storage (Murthy et al., 2011). Results agree with an earlier report for irradiated fish fillets and shrimp (Gu, 2013; Jeyakumari et al., 2020). The pH is an indicator of the freshness of fish. The pH of fresh tilapia fish meat had a pH of 6.71, and it showed a gradual increase during
storage (Fig. 1b). It might be due to the decomposition of the nitrogenous compounds by microbial and enzymatic activity (Mexis et al., 2009). The lower pH in the electron beam irradiated sample might be due to the inhibition of volatile base formation and microbial activity (Jeyakumari et al., 2020).

**Changes in total volatile base nitrogen (TVB-N) and non-protein nitrogen (NPN)**

Total volatile base nitrogen (TVB-N) is generally used to measure fish spoilage. In marine fish the formation of TVB-N is due to the degradation of ammonia, trimethylamine, and dimethylamine, while in the case of freshwater fish, TVB-N forms from the degradation of ammonia (Bahar et al., 2004). In the present study, fresh tilapia had a TVB-N content of 9.8±0.5 mg%, indicating that the fish used for the analysis was of good quality. It has been reported that TVB-N content of less than 30 mg% is considered fresh (Thanachan et al., 2010). Several authors reported that the acceptability levels of TVB-N (30-35 mg%) vary from species to species in freshwater fish (Al-Kahtani et al., 1996; Lakshmanan, 2000; Siddaiah et al., 2001). Accordingly, all the samples had an acceptable level of TVB-N during storage (Fig. 2a). NPN showed decreasing trend during storage (Fig. 2b). It might be due to the discharge of soluble compounds from fish muscle during storage. It was observed that the control lot had a lower NPN value than the irradiated lots. Previous researchers also observed similar results for irradiated fish and shrimp (Zhen et al., 2014; Jeyakumari et al., 2020).
Changes in peroxide value (PV) and thiobarbituric acid reactive substances (TBARS)

The PV assay is generally used to evaluate the primary lipid oxidation in seafood (Antolovich et al., 2002). Lipid oxidation in fish is mainly influenced by fat content and storage condition. PV showed an increasing trend during storage (Fig. 3a). Control had a PV of 7.74 meq. O₂ kg⁻¹ on 16th day. PV crossed the permissible level of 20 meq. O₂ kg⁻¹ on the 24th day for 2.0 kGy (22.8 meq. O₂ kg⁻¹) and 4.0 kGy (23.5 meq. O₂ kg⁻¹) irradiated fish chunks respectively. In the present study, the peroxide value in fish chunks failed to indicate the fish chunk spoilage. Zhen et al. (2014) and Jeyakumari et al. (2020) also reported similar results for shrimp and salmon fillets processed under electron beam irradiation. They also reported that samples packed under vacuum conditions formed lower peroxide values in fish chunks during storage. Thiobarbituric acid reactive substances (TBARS) value indicates the secondary lipid oxidation that occurs in fish and fishery products during storage. Fresh tilapia fish chunks had a TBARS value of 0.12±0.02 mg malonaldehyde kg⁻¹ of fish meat, and it showed a gradual increase during the storage period (Fig. 3b). Jeyakumari et al. (2020) observed higher TBA values for 5.0, 7.5, 10 kGy irradiated sample than 2.5 kGy irradiated sample and control. Park et al. (2010) also reported higher TBA values for beef sausages irradiated at 5, 10, and 15 kGy, compared to control. Hocaoglu et al. (2012) observed similar results for irradiated shrimp and reported that irradiation could induce changes in lipid oxidation. It has been reported that irradiation causes electrons to become free radicals, which results in lipid oxidation. Higher irradiation doses cause the formation of more free radicals and the cholesterol oxides, leading to higher TBA and peroxide values (Lee et al., 2001). Generally, a TBARS value of 2 mg malonaldehyde kg⁻¹ is considered the limit for the acceptability of fish (Goulas Kontominas, 2007; Parvathy et al., 2016). Accordingly, all the samples had a TBARS value within the permissible level during storage. Results are in accordance with earlier findings for irradiated meat and seafood (Park et al., 2010; Hocaoglu et al., 2012; Jeyakumari et al., 2020).

Changes in organoleptic quality

The sensory quality of fish chunks revealed that the overall acceptability of control and irradiated fish chunk varied significantly (p<0.05) during chilled storage (Fig. 4). The overall acceptability scores below 5 was set as the rejection level for fish chunks for consumption. Parvathy et al. (2016) reported 19 days shelf-life for vacuum-packed tilapia steak under iced condition. In the present study, the control reached a score of 6.0 on the 16th day, and was rejected on the 20th day with a score of 4.5. However, 2.0 and 4.0 kGy irradiated fish chunks were rejected in 33 days (score of 4.0), and 41 days (score of 4.1), respectively. It has been reported that the combined effect of vacuum packing and electron beam irradiation treatment could extend the shelf-life of meat and seafood (Al-Bachir, 2013; Jeyakumari et al., 2020; Du et al., 2002).

Changes in microbial quality

Total plate count (TPC), Pseudomonas and Brochothrix thermosphacta count.

Total plate count (TPC) showed a gradual increase in control and irradiated fish chunk during chilled storage (Fig. 5a). In the present study, TPC reached 6.9 log cfu g⁻¹ on the 16th day for control, and it crossed the permissible level of 7 log cfu g⁻¹ (ICMSF,1998) on the 20th day. In the case of 2.0 kGy treated...
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Fish chunk, TPC count of 6.8 log cfu g⁻¹ was recorded on the 28th day which crossed the permissible limit on the 33rd day. However, the 4.0 kGy irradiated fish chunk had a TPC of 7.5 log cfu g⁻¹ on the 41st day. A similar trend was also observed for *Pseudomonas* sp. during storage (Fig. 5b). Results showed a significant (p<0.05) reduction in TPC and *Pseudomonas* sp. count in the irradiated sample. Earlier researchers also observed a similar result for irradiated seafood (Hocaoglu et al., 2012; Jeyakumari et al., 2020). *Pseudomonas* sp. is a common specific spoilage organism (SSO) of iced or refrigerated freshwater fish (Gram and Dalgaard, 2002). SSOs often cause sensory spoilage, with off-odours and off-flavours, when they reach concentrations of about 7 log cfu g⁻¹ in fresh fish (FAO, 2014). It was observed that TPC and *Pseudomonas* count coincided with overall acceptability. 

*B. thermosphacta* is recognised as a common spoilage organism and can grow in both aerobic and anaerobic conditions. Moreover, it is responsible for the spoilage of fish and meat stored in modified atmosphere packaging/vacuum-packed conditions (Casaburi et al., 2015). In the present study, *B. thermosphacta* increased from 3.83 to 6.2 log cfu g⁻¹ in control (Fig. 5c). However, irradiated fish chunks had a one-log reduction in *B. thermosphacta* count. Results revealed that electron beam irradiation significantly inhibits *B. thermosphacta* growth. Our results agree with previous reports on electron beam-irradiated seafood (Zhuet et al., 2004; Jeyakumari et al., 2020).

**H₂S producers and Lactobacillus count**

H₂S producers increased from 2.65 to 5.9 log cfu g⁻¹ in control and was found to be absent in irradiated fish chunks throughout storage. The presence of H₂S producers in fish indicates the formation of volatile sulphur compounds from sulphur containing amino acids in fish meat, and it occurs mainly due to microbial action. They are usually very foul-smelling and even minimal quantities substantially affect the quality of fish. Results indicated that electron beam irradiation could prevent microbial activity, thereby extending the shelf-life of fish chunks. Lactic acid bacteria can grow in anaerobic conditions and at less than 4ºC. In the present study, *Lactobacillus* count increased from 2.17 to 3.14 log cfu g⁻¹ in control, and it was found to be nil throughout storage.
in irradiated fish chunks. It has been reported that electron beam irradiation can delay or eliminate *Lactobacillus* growth in seafood (Zhu et al., 2004; Jeyakumari et al., 2020).

It can be concluded that the electron beam irradiated tilapia fish chunks had a lower TVB-N content than the control. TBARS values were within the acceptable limits during storage. A significant (p<0.05) reduction in TPC, *Pseudomonas* and *B. thermosphacta* counts was achieved in electron beam irradiated samples. *Lactobacillus* and *H. S* formers were found to be nil throughout the storage in the irradiated fish chunks. The sensory quality of fish chunks indicated that flavoured change in irradiated meat was not reflected in biochemical and microbial quality. Results of the present study suggest that vacuum packing and electron beam irradiation treatment at a 2-4 kGy dose level, would help to extend the shelf-life of tilapia fish chunks up to 28-38 days without affecting their quality.

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