Food Chemistry 254 (2018) 64–69

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Effects of electron beam irradiation on the biochemical properties and structure of myofibrillar protein from *Tegillarca granosa* meat

Mingchun Lv[a,b], Kalin Mei[a], Han Zhang[a], Dalun Xu[a], Wenge Yang[a,b,*]

[a] School of Marine Sciences, Ningbo University, 315211, China
[b] Key Laboratory of Animal Protein Food Deep Processing Technology of Zhejiang Province, Ningbo University, Ningbo 315211, China

ARTICLE INFO

Keywords:
Tegillarca granosa
Electron beam irradiation
Myofibrillar protein
Biochemical property
Protein structure

ABSTRACT

The effect of electron beam irradiation at doses of 0–9 kGy on the biochemical properties and structure of myofibrillar protein from *Tegillarca granosa* (*T. granosa*) was investigated. There was no effect of irradiation on the surface hydrophobicity of the myofibrillar protein, however total sulfhydryl content and Ca^{2+}-ATPase activity both declined to varying extents with increasing irradiation dose. Irradiation also affected the composition of the myofibrillar protein; with increasing dose of irradiation, the actin, paramyosin and myosin heavy chain contents of the myofibrillar protein were partially degraded. Irradiation reduced the α-helix content and increased the β-sheet content but these effects were less dependent on the dose of irradiation. The 'hardness', 'gumminess', 'chewiness' and 'resilience' of *T. granosa* meat were improved at irradiation doses of 3–5 kGy. Collectively, our results support an optimum irradiation dose of 3–5 kGy for the preservation of *T. granosa* meat.

1. Introduction

Shellfish are vulnerable to microbial contamination from a variety of sources (Oliveira, Cunha, Castilho, Romalde, & Pereira, 2012). New preservation technologies are being sought that maintain the original edible quality of food whilst providing effective sterilization in order to extend shelf life.

Electron bean irradition (EBI), sourced from accelerators that produce controlled amounts of X-rays and high-energy electron beams, generates free radicals, e.g. H· and OH·, that have the capacity to inactivate microorganisms by damaging critical components of cells, mostly the chromosomal DNA (Acheson & Steele, 2001). The free radicals can also cause cross-linking or polymerization of proteins, that can disrupt the molecular structure and composition of proteins. These effects can kill parasites present, inactivate endogenous enzymes and inhibit microbial growth, ultimately extending the shelf-life of food (Lin, Yang, Xu, & Wang, 2015). As a cold sterilization method, EBI is highly effective, safe and hygienic. It does not increase the internal temperature of the food and can maintain the original color, flavor and visual appearance of food (Ehlermann, 2005). Irradiation of food with doses of up to 10 kGy has been shown to produce no adverse effects in humans (Campo & Tovar, 2008), and the method has gradually been adopted by food industries for the preservation of aquatic food products.

Tegillarca granosa (Linnaeus) (*T. granosa*) is a saltwater shellfish that is widely farmed along the southeast coast of China. *T. granosa* meat is delicious and often consumed raw. Several previous researchers have used election beam irradiation to treat *T. granosa* (at doses of 0–9 kGy), and have studied its effect on microbial content (total plate count and coliform count), as well as on the sensory quality and nutritional composition of *T. granosa* meat (Li, Yang, Xu, Ou, & Shi, 2011; Li, Yang, Xu, Zhang, & Shi, 2009). They reported that 3–5 kGy was the optimal irradiation dose and the shelf life of *T. granosa* was extended from 5 days to between 15 and 19 days. A further study, by Yang, Li, Xu, Zhang, and Mao (2012), concluded that irradiation at 3–5 kGy did not adversely affect the original aroma profile of *T. granosa*, and may even improve the flavor to some extent. However, currently there is little information available regarding the effects of irradiation on the microstructure and biochemical properties of myofibrillar protein from *T. granosa* meat.

In terms of its nutritional composition, protein (10.6%) is the second most dominant component of *T. granosa* meat, after water (82.7%) (Li et al., 2011). The main component of shellfish meat protein is myofibrillar protein, whose biochemical characteristics and structure are closely related to quality of the meat. Thus, any effects of irradiation on biochemical properties and microstructure of myofibrillar protein could indirectly affect the edible quality of *T. granosa* meat.

This study aimed to investigate the effect of EBI on structure and

* Corresponding author at: School of Marine Sciences, Ningbo University, Ningbo 315211, China.
E-mail addresses: lvmingchun93@126.com (M. Lv), mklwater@126.com (K. Mei), echoecho26@126.com (H. Zhang), xudalun@nbu.edu.cn (D. Xu), yangwenge@nbu.edu.cn (W. Yang).

https://doi.org/10.1016/j.foodchem.2018.01.165
Received 25 October 2017; Received in revised form 6 January 2018; Accepted 24 January 2018

biochemical properties of *T. granosa* myofibrillar protein. It was anticipated that the outcomes of this research would provide a better understanding of the effects of irradiation on shellfish myofibrillar protein composition and structure, and would wider the application of EBI in shellfish preservation.

2. Materials and methods

2.1. Handing of T. granosa

Live *T. granosa* of commercial size, i.e. measuring 3.1 ± 0.4 cm in length and weighing 8.8 ± 1.8 g, were purchased from a local supplier in Ningbo, Zhejiang Province, China. The shellfish were scrubbed to remove mud and other contaminants adhering to the shell, and then immediately transported to the laboratory and refrigerated at 4 °C prior to use. All experimental *T. granosa* were processed within 24 h of harvest.

2.2. Irradiation treatment of samples

A total of 360 *T. granosa* were vacuum packed in polyethylene plastic bags, with 20 shellfish per bag and 3 bags per treatment group, and then irradiated with doses of 0, 1, 3, 5, 7 or 9 kGy, respectively, at a rate of 1 kGy/s, using an electron linear accelerator (NBL-1010, 10 MeV, Ningbo Superpower High-Tech. Ltd., Ningbo, China). The absorption dose was determined using a cellulose triacetate dosimeter. Experimental verification confirmed that the absorbed dose was within 3% of the target dose in all cases.

2.3. Preparation of myofibrillar protein solution (MPS)

Myofibrillar protein solution was prepared according to the method of Rowe, Maddock, Lonergan, and Huff-Lonergan (2004). *T. granosa* meat was homogenized with 10 volumes (w/v) Tris-maleate buffer (20 mM, pH 7.0, containing 0.05 M KCl), at 4 °C. The homogenate was centrifuged at 10,000 rpm for 10 min at 4 °C and the deposit was collected. The deposit was homogenized with 10 volumes (w/v) Tris-maleate buffer (20 mM, pH 7.0, containing 0.6 M KCl) and extracted for 1 h at 4 °C. The homogenate was again centrifuged at 10,000 rpm for 10 min at 4 °C and the supernatant comprising the MPS was collected.

The Lowry method was used to determine the concentration of myofibrillar protein in the *T. granosa* meat, using bovine serum albumin as a standard. The MPS was diluted ten-fold with water and then a 1 mL sample of the resulting solution was mixed with 5 mL of reagent X (this comprising a mixture of solution A (0.2 M NaOH, 0.19 M Na_2CO_3) and solution B (1% sodium tartrate, 0.02 M $CuSO_4{\cdot}5H_2O$) in a rate of 50: 1), and incubated at 20–25 °C for 10 min. Then, 0.5 mL folin-phenol was added and the sample was immediately homogenized and incubated at 20–25 °C for 30 min, after which the absorbance was recorded at 500 nm. Water was used as a blank control. The protein concentrations of the tested MPS samples were all within the range of 2.58 ± 0.12 mg/mL and did not differ significantly from one another ($P > .05$).

2.4. Surface hydrophobicity of myofibrillar protein

Due to being water-insoluble, hydrophobicity of myofibrillar protein cannot be determined by the method which use 1-anilinonaphthalene-8-sulphonic acid (ANS) as a probe. Therefore, surface hydrophobicity was determined as described by Chelh, Gatellier, and Santé-Lhoutellier (2006), using bromophenol blue (BPB) as a hydrophobic chromophore. The MPS (1 mL), extracted from irradiated and control samples, was mixed with 200 μL of 1 mg/mL BPB. The MPS was replaced by 20 mM Tris-maleate buffer (pH 7.0, containing 0.6 M KCl) as an internal control. All samples were agitated for 10 min at room temperature, then centrifuged at 7000 rpm for 15 min. The supernatants (0.5 mL) were diluted in 4.5 mL distilled water and their absorbance measured at 595 nm against a blank of 20 mM Tris-maleate buffer (pH 7.0, containing 0.6 M KCl). The amount of bound BPB in the sample, calculated according to the following formula, was used as an index of hydrophobicity:

$$\text{BPB bound } (\mu g) = 200 \times (A_0 - A)/A_0,$$

where A_0 refers to the absorbance of the blank sample and A refers to the absorbance of the experimental sample.

2.5. Total sulfhydryl (SH) content of myofibrillar protein

Determination of total SH content was performed according to the method of Benjakul, Seymour, Morrissey, and Haejung (1997), as modified by Thanonkaew, Soottawat, Wonnop, Wonnop, and Decker (2006). A 1 mL sample of MPS was mixed with 9 mL of 0.2 M Tris-HCl buffer (pH 6.8, containing 8 M urea, 2% sodium dodecyl sulfate (SDS), and 10 mM ethylenediaminetetraacetic acid (EDTA)). The resulting mixture (4 mL) was further mixed with 0.4 mL of 0.1% 5,5′-Dithio bis-(2-nitrobenzoic acid) (DTNB) and incubated at 40 °C for 25 min. Absorbance was measured at 412 nm using a solution of 0.6 M KCl as a blank. Total SH content was calculated using the extinction coefficient of 13,500 M^{-1} cm^{-1}. Percent solubility was calculated as follows:

$$\text{Total SH content } (\mu\text{mol/g pro}) = (A - A_0)/13{,}600/\text{MPS concentration}(\text{mg/ml}) \times 11 \times 10^6,$$

where pro refers to protein, A refers to the absorbance of the experimental sample and A_0 refers to the absorbance of the blank sample.

2.6. Ca^{2+}-ATPase activity of myofibrillar protein

The Ca^{2+}-ATPase activity of the irradiated and control samples was determined according to the method of Xiong et al. (2009). Briefly, a solution containing 0.25 mL 0.5 M Tris-Maleate buffer (pH 7.0), 0.5 mL 1 M KCl, 0.25 mL 0.1 M $CaCl_2$ and 3.25 mL distilled water, was maintained in a water bath at 25 °C for 10 min. A 0.5 mL sample of the MPS, together with 0.25 mL adenosine triphosphate (ATP) solution (20 mM, pH 7.0) was then added, thoroughly mixed, and the resulting solution maintained in a water bath at 25 °C for 5 min. After this time, 2.5 mL of 15% trichloroacetic acid (TCA) was immediately added in order to stop the reaction. A blank solution was prepared as described for the experimental samples, but 2.5 mL of 15% TCA was added before the water bath stage. The reaction solutions were centrifuged at 5000 rpm for 10 min and the supernatants were collected to determine their inorganic phosphoric acid content using the ammonium molybdate spectrophotometric method. The Ca^{2+}-ATPase activity of MPS was expressed as μmol (pi) mg^{-1}(pro) min^{-1} (where pi refers to inorganic phosphorous).

2.7. SDS-PAGE of myofibrillar protein

SDS-PAGE was performed according to the method of Laemmli (1970). The MPS from samples was respectively mixed at a 4:1 ratio with bromophenol blue buffer and heated at 100 °C for 5 min. Subsequently, 10 μL aliquots were applied to individual wells of a 10-well gel. The acrylamide gel was prepared as a 5% stacking gel and a 15% resolving gel. Electrophoresis was performed at 80 V for the stacking gel and 120 V for the resolving gel. Gels were stained with Coomassie Brilliant Blue R-250, then destained with aqueous solution containing 30% methyl alcohol and 10% glacial acetic acid until the solution was clear. Images were captured and analyzed using the Bio-Rad Gel Doc XR + system (Bio-Rad, Richmond, CA, USA).