

# Identification of oxidative modification of shrimp (*Metapenaeus ensis*) tropomyosin induced by malonaldehyde

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**Abstract** In this study, shrimp tropomyosin was subjected to malonaldehyde (MDA)-induced oxidative stress in aqueous situation. The in vivo cross-linking of tropomyosin was evaluated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and the structural changes were investigated by differential scanning calorimetry, circular dichroism, and Fourier transform infrared spectroscopy (FTIR). The location of the resulting protein carbonyls was determined by mass spectrometry. The SDS-PAGE of the cross-linked tropomyosin showed four new bands corresponding to two, three, four, and five-time molecular weights of tropomyosin. The conformational structures were partly destroyed because of the thermal denaturation at higher temperatures. The  $\alpha$ -helix content increased, and new chemical bonds were formed by the MDA modification. These physiochemical properties were confirmed by the identification of amino acid side-chain modifications by using liquid chromatography–tandem mass spectrometry. Lysine (Lys), glutamine (Gln), and asparagine (Asn) residues in tropomyosin were modified at four lysine (Lys-76, 168, 189, and 233), five glutamine (Gln-61, 70, 118, 147, and 247), and four asparagine (Asn-17, 107, 203, and 215) sites. Apart from these MDA modification sites, oxidized methionine was observed by data search. These results indicate that some active food ingredients, such as MDA, can react with the side chains of amino acids resulting in structural changes in the protein.

**Keywords** Shrimp tropomyosin · Malonaldehyde (MDA) · Oxidation · Liquid chromatography–tandem mass spectrometry (LC–MS/MS)

## Introduction

Shrimp is one of the most frequent and major reasons for food allergy in human [1]. The allergy symptoms include severe atopic dermatitis and other diseases [2]. Tropomyosin, identified as the major shrimp allergen, can induce more than 80 % shrimp allergy. Previous studies have focused on allergenicity reduction [3], epitope identification [4], detection [5], and structural changes [6] during shrimp processing and preservation. However, little information is available on mechanism of these character changes in shrimp tropomyosin.

Among active ingredients that easily react with the side chains of amino acids, end products of lipid oxidation have attracted much attention because of their high activity. Several studies focused on lipid oxidation affording various compounds [6–8]. The lipid fraction in shrimp is rich in polyunsaturated fatty acids (PUFAs), such as docosahexaenoic (DHA-22:6n-3) and eicosapentaenoic (EPA-20:5n-3) acids, and is highly susceptible to lipid oxidation despite its low lipid content (0.70–1.16 g/100 g) [9, 10]. Malonaldehyde (MDA), among various compounds formed during lipid oxidation, was recognized as the major compound with high reactivity. MDA can easily undergo the Michael addition by reacting with the side-chain functional groups of lysine (Lys), asparagine (Asn), and glutamine (Gln) because of its chemical structure to slowly afford the Michael-adducts. Moreover, MDA can also form Schiff bases by reacting with the  $\epsilon$ -amino group of the Lys side chain following a reversible reaction pathway [7].

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Chemical modifications resulting in property changes in the shrimp tropomyosin during preservation and processing have been reported previously. For example, Li et al. [11] reported that the immunoglobulin E (IgE)-binding capacity of tropomyosin can be increased using <5 kGy irradiation while decreased with >10 kGy irradiation. However, Byun et al. [12], reported that the ability of the IgE, of patients allergic to shrimp, to bind to irradiated shrimp decreased in a dose-dependent manner. Similar results were obtained from other processing and preservation methods [13]. These contradictory results indicate that some alternative mechanisms might exist during the processing. Because the IgE binding capacity mainly depends on the primary structure of allergens, the amino acid modification is essential to understand the changes in the IgE binding capacity during shrimp processing and preservation. During the last decade, several studies reported that the shrimp tropomyosin consists of 284 amino acids without disulfide bonds and is abundant in Lys, Arg, and some other amino acids with active side chains [12, 14–16]. Active molecules, such as MDA, can react with the amino acids containing functional groups in the side chains. Therefore, to illustrate the pathway for amino acid modification of tropomyosin by treating with MDA is necessary to gain mechanistic insight.

In this study, purified shrimp tropomyosin was exposed to MDA, and then a “bottom-up” proteomics strategy was applied, followed by LC–MS/MS and database searching for the site-specific identification of tropomyosin oxidation. The secondary structure was analyzed by circular dichroism (CD) spectroscopy. The conformational structure was evaluated by differential scanning calorimetry (DSC), and new functional groups were analyzed by Fourier transform infrared (FTIR) spectroscopy.

## Materials and methods

### Chemicals

Shrimp tropomyosin was prepared in the Food Safety Lab, Ocean University of China. 1,1,3,3-tetraethoxypropane (MDA precursor), and trypsin was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Reagents used for gel electrophoresis were obtained from Bio-Rad (Hercules, CA, USA). Unless otherwise stated, all chemicals were of analytical grade.

### MDA preparation and reaction with tropomyosin

MDA was prepared according to Adams et al. [17]. In brief, 1,1,3,3-tetraethoxypropane (220 mg) was dissolved in 0.1 M HCl (10 mL) and heated at 40 °C for 30 min in a water bath, then pH was adjusted to 7.4 using 6 M NaOH.

The MDA solution was diluted 1,000 times with 10 mM phosphate buffer (PBS, pH 7.4), and its absorbance was measured using an UV spectrophotometer at 267 nm. The concentration of the MDA solution was calculated using the molar extinction coefficient of  $31,500 \text{ M}^{-1} \text{ cm}^{-1}$ . The MDA solution (5 mM) was freshly prepared by diluting with 10 mM PBS (pH 7.4). The samples (4 mg/mL) were dissolved in the PBS, mixed with the freshly prepared MDA solution, and incubated at 37 °C for 24 h. At last, the resulting solution was dialyzed against PBS (pH 7.4) for 10–12 h at 4 °C, and then, it was lyophilized and stored in –40 °C until use. The protein concentration was determined by using Bio-Rad with BSA as standard [18].

### SDS-PAGE analysis

Protein electrophoresis (SDS-PAGE) was performed as described by Laemmli [19]. Samples (10 µg per well) were mixed 4:1 with loading buffer (2 % SDS, 25 % glycerol, 14.4 mM β-mercaptoethanol, and 0.1 % bromophenol blue in 1 M Tris–HCl, pH 6.8), and heated at 100 °C for 6 min. The acrylamide gel was prepared as a 5 % stacking gel and a 12 % resolving gel. The electrophoresis was performed at 80 V for stacking gel and 120 V for resolving gel. Then, gels were fixed and stained with 0.125 % Coomassie brilliant blue R-250 (Bio-Rad, Richmond, CA, USA). The images were picked and analyzed with Tanon-4200SF gel imaging and analysis software (Tanon Science & Technology Co., Ltd.).

### Differential scanning calorimetry analysis

To investigate the thermal properties changes of tropomyosin with oxidative modification, DSC measurement was applied using a TA Instruments DSC 200PC [20]. In brief, the sample (~15 mg) was placed in a hermetic aluminum pan with empty pan as reference. All experiments were performed in the temperature range 293.16–423.16 K at a heating rate of 8 K/min (after cooling down at the same rate). Netzsch proteus analysis software was used to analyze the inflection point of the glass transition and the temperatures for the onset and end of the glass transition. Each experiment was performed for at least three times to ensure productivity of the results.

### Secondary structure analysis using CD

To define the secondary structure of original and oxidative modified tropomyosin, Far-UV CD spectroscopy was performed in the spectral range from 190 to 260 nm using a JASCO J-815CD spectropolarimeter (Japan Spectroscopic Co. Inc., Japan) at room temperature [21]. Protein concentrations of 1 mg/ml were used for analysis. The operation

indexes are as follows: scan speed, 50 nm/min; bandwidth, 1 nm; and response time 2 s. All samples were measured three times, and the average results were analyzed with molar residue ellipticity and expressed as  $\text{deg cm}^2 \text{dmol}^{-1}$ . Then, the contents of different secondary structures were calculated with JASCO software. Each experiment was performed for at least three times to ensure reproducible results.

### FTIR analysis

FTIR spectra analysis was performed with Nicolet iS10 spectrometer (Topology pharmaceutical & technology co., Ltd) equipped with a deuterated triglycine sulfate (DTGS) detector. The powdered sample (~1–2 mg) was mixed with spectroscopy KBr (100–200 mg) and created thin film with pressure (20 MPa). FTIR spectra were scanned from 400 to 4000 nm in the absorbance mode. Interferograms were averaged for 32 scans at  $4 \text{ cm}^{-1}$  resolution [22]. After the signal of dihydrogen monoxide and carbon dioxide were cutted, data were obtained and analyzed using OMNIC Picta software. Each sample was performed at least three times to get reproducible results.

### Oxidation sites analysis with LC–MS/MS

The protein samples ran through SDS-PAGE electrophoresis. Objective proteins were fragmented into different peptides and put into EP tube with 50  $\mu\text{L}$  DD. Deionized  $\text{H}_2\text{O}$  was added to wash objective protein for 10 min twice. Add 50 mM solution [50 % (v/v)  $\text{NH}_4\text{HCO}_3$  and 50 % (v/v) acetonitrile] 50  $\mu\text{L}$  and bleach for 20 min at 37 °C. The samples were blotted up until the blue faded. Particles was completely dehydrated to be white by acetonitrile (50  $\mu\text{L}$ ) and blotted up in a vacuum for 10 min, then add 10 mM DTT [1 % (v/v) DTT and 99 % (v/v)  $\text{NH}_4\text{HCO}_3$ ] 20  $\mu\text{L}$  and bath 1 h at 56 °C. The samples were blotted up after cooling to room temperature, adding 55 mM IAM [5.5 % (v/v) IAM and 94.5 % (v/v)  $\text{NH}_4\text{HCO}_3$ ] 20  $\mu\text{L}$  rapidly, and then placed in a dark room for 45 min. The samples were successively washed with 25 mM  $\text{NH}_4\text{HCO}_3$  for 10 min twice, 25 mM  $\text{NH}_4\text{HCO}_3$  and 50 % acetonitrile for 10 min twice, and acetonitrile for 10 min. Particles were completely dehydrated and blotted up in the same way. The enzyme solution (0.1  $\mu\text{g}/\mu\text{L}$ ) was diluted to 15 times using 25 mM  $\text{NH}_4\text{HCO}_3$  and then made fully in contact with the particles through centrifugation slightly. The solution was placed at 4 °C for 30 min until completely absorbed by particles. Add 25 mM  $\text{NH}_4\text{HCO}_3$  to a total volume of 10–15  $\mu\text{L}$  at 37 °C and digest overnight. 1 % TFA was added to terminate the reaction, and the concentration of 0.1 % TFA was made at last. The LC–MS/MS analysis of the peptide samples was performed using ThermoFisher Q-Exactive mass

spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a nanoelectrospray ionization (ESI) source. High performance liquid chromatography (HPLC) was performed to separate the peptide samples using an Eksigent Nanoflex cHiPLC system (Eksigent, Dublin, CA, USA). Mobile phases A and B comprised deionized water containing 0.1 % (v/v) formic acid and acetonitrile with 0.1 % (v/v) formic acid, respectively. Mobile phase B was increased linearly from 5 to 30 % B in 30 min and further to 60 % B for 5 min, and finally to 5 % B for re-equilibration. All the eluted peptides from the cHiPLC column at a particular elution time were analyzed by mass spectrometry at a resolution of 60,000.

## Results and discussion

### SDS-PAGE of MDA-treated shrimp tropomyosin

The MDA-treated shrimp tropomyosin was analyzed by SDS-PAGE (Fig. 1), which showed the formation of five protein bands (a, b, c, d, and e), indicating cross-linking among proteins. The molecular weights of these bands were 2–5 times compared to that of the original tropomyosin. The intensity of the protein bands became weaker with increasing molecular weight. Previous studies reported that MDA containing two highly active aldehydes can react with the two heavy chains of myosin resulting in an aggregation [23]. Other high molecular bands may be caused by the cross-linking between the original and cross-linked tropomyosin. Further treatment with MDA may result in additional cross-linking of tropomyosin.

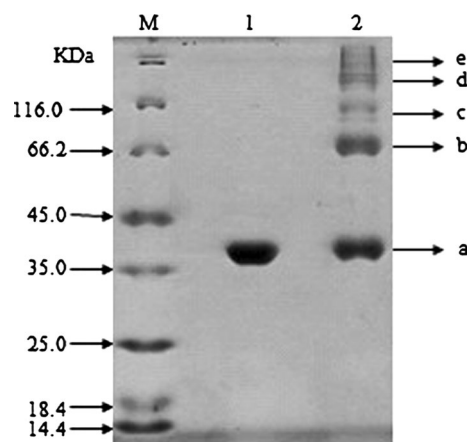


**Fig. 1** SDS-PAGE analysis of the MDA-treated shrimp tropomyosin. The molecular weight of standard protein marker ranged from 14.4 to 116.0 kDa. Lanes M Protein marker; lane 1 shrimp tropomyosin; lane 2 MDA-treated tropomyosin. a, b, c, d, and e represent new bands corresponding to one, two, three, four, and five-time molecular weights of tropomyosin, respectively

## Structural modification of shrimp tropomyosin by MDA

Tropomyosin has global conformations and its structure can be modified by heat treatment, which may affect its functional properties. The modification of the side chains of amino acids affects the structure of tropomyosin and thermal properties. The DSC thermograms obtained at  $8 \text{ K min}^{-1}$  for shrimp tropomyosin before and after the oxidation showed one endothermic peak at  $88.80^\circ\text{C}$  for the tropomyosin and another endothermic peak at  $86.14^\circ\text{C}$  for the oxidized tropomyosin as shown in Fig. 2. The thermal stability varied slightly after the oxidation. However, the oxidation of the tropomyosin resulted in inconspicuous peaks compared to the peaks in tropomyosin. The possible reason is that the oxidation of tropomyosin results in its denaturation. Previous studies reported that tropomyosin oxidation is accompanied by the destruction of the tertiary and/or secondary structures [24, 25] resulting in the modification of the functional and physiochemical properties [25]. We predicted that the degeneration occurred after the oxidation. Narayan et al. [26] reported the reduction of disulfide bonds, loss of tertiary structure, and increase in the accessibility of reactive Lys and glutamine residues in the protein substrate; thereby resulting in a difference in the thermal stability of tropomyosin.

**Fig. 2** DSC heating curves ( $8 \text{ K/min}$ ) of shrimp tropomyosin (a) and the MDA-induced tropomyosin (b). Peak a:  $88.80^\circ\text{C}$ , onset:  $59.52^\circ\text{C}$ , end:  $104.4^\circ\text{C}$ ; Peak b:  $86.14^\circ\text{C}$ , onset:  $54.43^\circ\text{C}$ , end:  $110.81^\circ\text{C}$

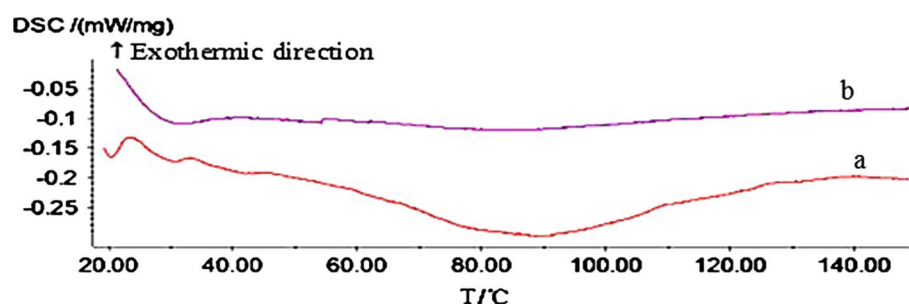
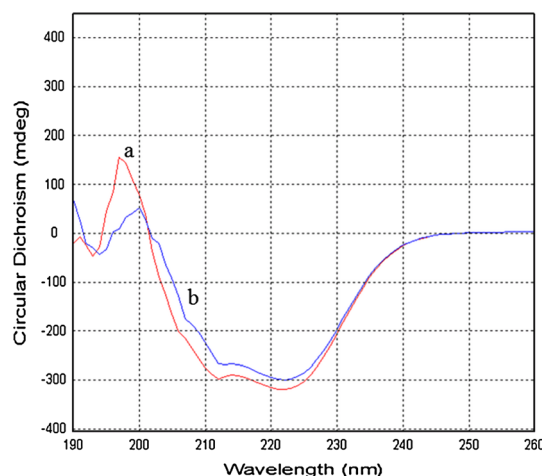


**Fig. 3** Effect of MDA modification on the secondary structure of shrimp tropomyosin measured by Far-UV CD spectroscopy. a secondary structure of shrimp tropomyosin; b secondary structure of the MDA-induced tropomyosin. The chart shows the proportion of different secondary structures



|              | 190–260 nm(a) | 190–260 nm(b) |
|--------------|---------------|---------------|
| Helix        | 64.1%         | 50.6%         |
| Antiparallel | 1.0%          | 2.3%          |
| Parallel     | 3.5%          | 5.2%          |
| Beta-Turn    | 12.5%         | 13.9%         |
| Rndm. Coil   | 12.0%         | 20.0%         |
| Total Sum    | 93.1%         | 91.9%         |

The changes in the secondary structure in shrimp tropomyosin induced by MDA oxidation were investigated by CD spectroscopy. As shown in Fig. 3, when tropomyosin was treated with MDA, the negative peaks at about 212 and 223 nm and the positive peak at 200 nm were slightly decreased, indicating a decrease in the amounts of  $\alpha$ -helix and  $\beta$ -sheet. These results were in agreement with the data calculated by the JASCO secondary structure software, explaining that the ratio of the  $\alpha$ -helix decreased. Interestingly, the proportion of the  $\alpha$ -helix in the secondary structure increased, indicating that the structure of the tropomyosin became more out of order as compared to the original sample. This is because when MDA is cross-linked with the side chains of the amino acids in tropomyosin, the original van der Waals interaction maintaining the secondary structure is destroyed, and the secondary structure partly rearranges; this is in agreement with the previous studies [27, 28].

FTIR spectroscopy was used to characterize the effect of MDA on the functional groups of the shrimp tropomyosin. The FTIR spectrum of the shrimp tropomyosin shows new peaks at  $3,010.06$ ,  $1,647.72$ ,  $1,363.48$ , and  $948.24 \text{ cm}^{-1}$  (Fig. 4). Based on the protein structure and FTIR analysis, the new peaks at  $\sim 1,647.72$  and  $3,395.94 \text{ cm}^{-1}$  were assigned to the stretching vibrations of  $\text{C}=\text{O}$  and  $\text{N}-\text{H}$ , respectively. The peak at  $3,010.06 \text{ cm}^{-1}$  indicates the

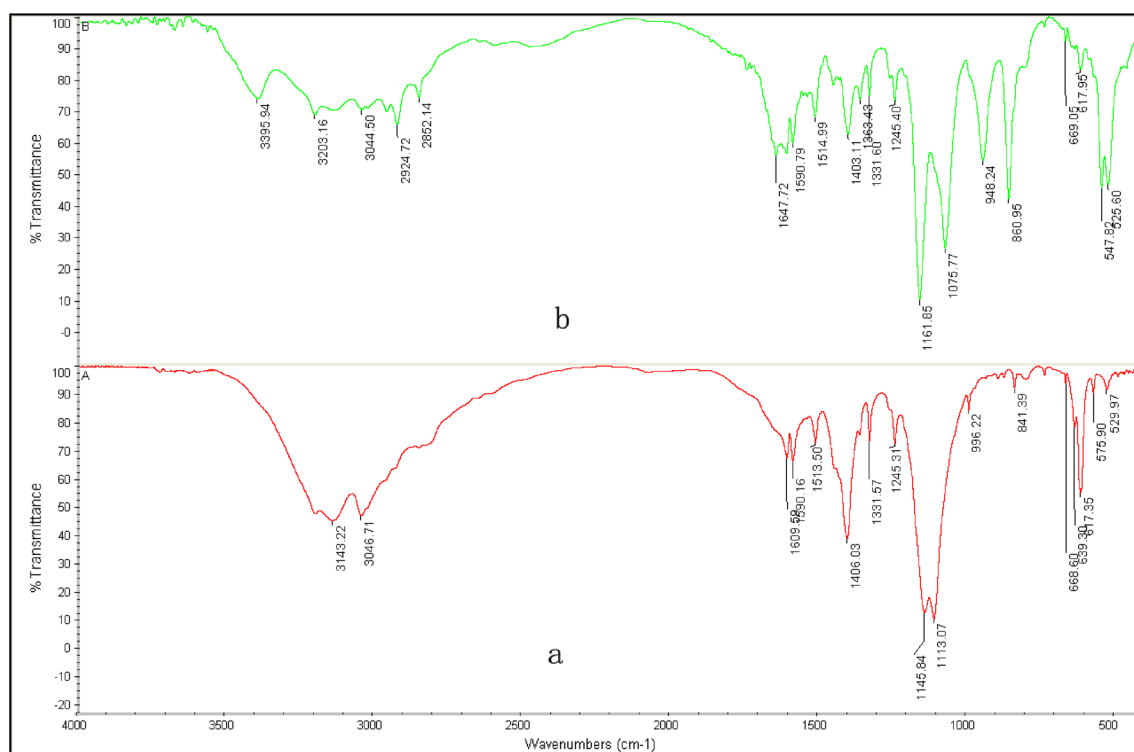


**Fig. 4** Effect of MDA modification on the structure of shrimp tropomyosin measured by FTIR spectroscopy. **a** shrimp tropomyosin; **b** MDA-induced shrimp tropomyosin

presence of olefins. Similar results were also observed in a previous study on the structural changes of Lys and glutamine residues in tropomyosin treated with MDA [6], indicating that MDA can bind with the active side chains of the protein and result in new carbonyl groups or some other functional group change [29].

#### Identification of MDA adduction sites by LC–MS/MS

Reactions between MDA and amino acid residues in peptides and proteins mainly resulted in the formation of Schiff bases at the nucleophilic amino groups, and some of the amino groups reacted via the Michael addition [6]. These reactive nucleophilic amino groups are mainly present in Lys, Asn, Gln, and the N-termini of the peptide chains. Moreover, the reaction of MDA with the nucleophilic amino groups in the side chains of amino acids forms adduct that results in the cross-linking of proteins [6], which was confirmed by the SDS-PAGE of oxidized tropomyosin (Fig. 1). All of the new protein bands (a, b, c, d, and e) were sliced and analyzed by LC–MS/MS.

Mascot search with a mass increment of 54 was used to identify the oxidized side-chain groups of Lys, Gln, and Asn in the form of Schiff bases, which were formed by the reaction with MDA. Moreover, when a mass increment of 16 was applied to identify the [O] addition through

a reactive oxygen species (ROS), the methionine (Met) in multiple peptides were found to be oxidized (Table 1). This phenomenon was previously reported. Moreover, certain amino acids, such as Met, can be oxidized during LC–MS/MS analysis [30]. In this study, the control experiments with tropomyosin by LC–MS/MS analysis showed the oxidized Met. As shown in Table 1, 49 peptides were identified to react with MDA by the formation of Schiff bases and mainly detected at the side chains of Lys, Gln, and Asn. Interestingly, oxidized Lys and Met are found in all of the five protein bands, whereas oxidized Gln exists in “a” and “b” bands, and oxidized Asn is found in “d” and “e” bands. The possible reason was that Lys is abundant (28/284) and easily oxidized in shrimp tropomyosin, whereas Gln (17/284) and Asn (17/284) are less abundant in shrimp tropomyosin and not enough to be oxidized. Another possible reason is that some of the information was lost when the sample was sliced and analyzed by LC–MS/MS.

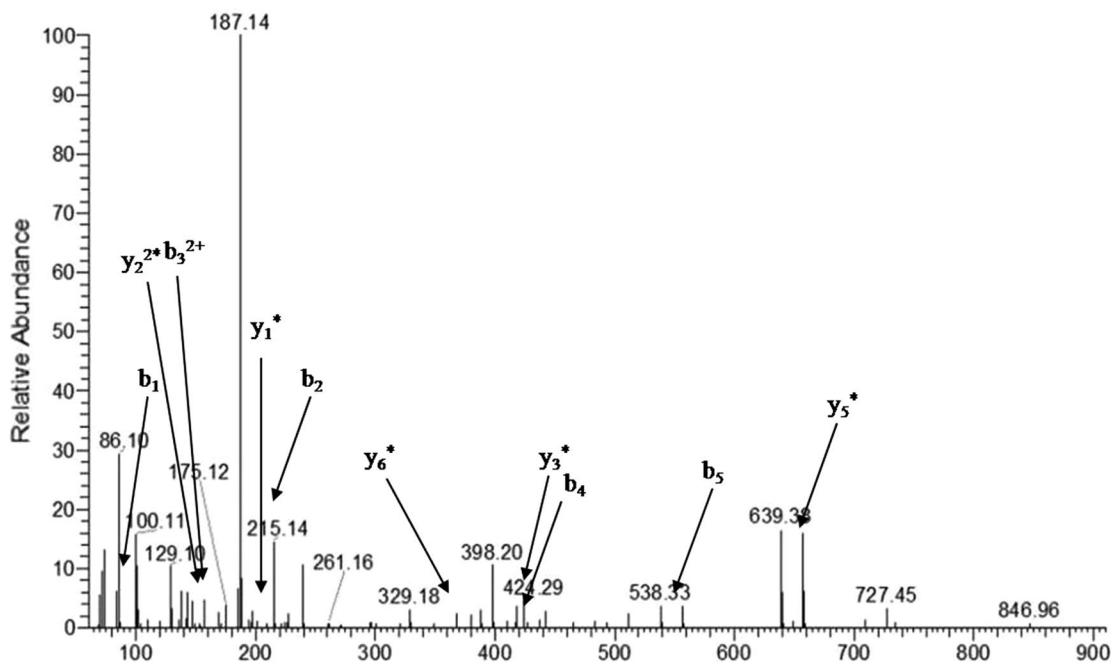
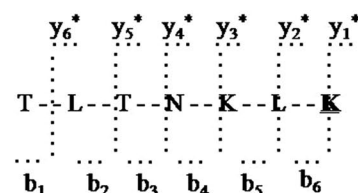
The MS/MS results for some of the MDA adducts and [O] adduct are shown in Figs. 5, 6 and 7. These adducts were observed on peptides with two or more nucleophilic sites. In this study, typical MS/MS analysis is listed. As shown in Fig. 5,  $^{227}\text{Thr-Leu-Thr-Asn-Lys-Leu-Lys}^{233}$  containing side-chain amino group in the Lys residue was oxidized by the adduct formation with MDA, and the major peptide identified in the fraction was Lys.

**Table 1** List of MDA-modified tryptic peptides of shrimp tropomyosin identified after date-dependent MS/MS acquisition

|        | MDA-modified peptides                            | Modification site(s) |     | z | m/z      | Observed mass | Theoretical mass |
|--------|--------------------------------------------------|----------------------|-----|---|----------|---------------|------------------|
| 1,989  | ANIQLVEKD <u>K</u> (+54)                         | 76                   |     | 2 | 606.336  | 1210.658      | 1210.656         |
| 2,857  | <u>K</u> LAMVEADLER (+54)                        | 168                  |     | 2 | 664.851  | 1327.687      | 1327.681         |
| 7,220  | GES <u>K</u> IVELEEELR (+54)                     | 189                  |     | 2 | 943.485  | 1844.954      | 1844.932         |
| 519    | TLTNKL <u>K</u> (+54)                            | 233                  |     | 2 | 436.267  | 870.520       | 870.517          |
| 11,825 | MQQLENDLDQVQESLL <u>K</u> ANIQLVEK (+54)         | 61                   | 66  | 3 | 1021.189 | 3033.544      | 3033.533         |
| 13,029 | MQQLENDLDQVQESLLKANI <u>Q</u> LVE <u>K</u> (+54) | 70                   | 74  | 3 | 1012.183 | 3033.526      | 3033.533         |
| 8,596  | LNTATT <u>K</u> LAEASQAADER(+54)                 | 112                  | 118 | 3 | 738.693  | 2213.056      | 2213.045         |
| 4,858  | MDALEN <u>Q</u> L <u>K</u> EAR (+54)             | 147                  | 149 | 3 | 509.252  | 1524.733      | 1524.724         |
| 736    | SV <u>Q</u> KLQK (+54)                           | 247                  | 248 | 2 | 469.769  | 937.523       | 937.523          |
| 1,930  | LE <u>K</u> DNAMDR (+54)                         | 15                   | 17  | 2 | 600.277  | 1198.539      | 1198.529         |
| 8,917  | L <u>N</u> TATT <u>K</u> LAEASQAADER (+54)       | 107                  | 112 | 3 | 738.691  | 2213.051      | 2213.045         |
| 6,345  | VVGNNL <u>K</u> SLEVSEEK (+54)                   | 203                  | 205 | 3 | 584.975  | 1751.902      | 1751.894         |
| 4,688  | SLEVSEE <u>K</u> ANQR (+54)                      | 213                  | 215 | 3 | 499.912  | 1496.714      | 1496.710         |
| 1,194  | <u>M</u> QAMKLEK (+16)                           | 8                    | 11  | 2 | 505.755  | 1009.495      | 1009.494         |
| 1,779  | LEKD <u>N</u> AMDR (+16)                         |                      | 19  | 2 | 554.259  | 1106.504      | 1106.503         |
| 8,877  | RMQQLENDLDQVQESLLK (+16)                         |                      | 50  | 2 | 1102.058 | 2202.102      | 2202.095         |
| 5,883  | LAEASQAADER <u>M</u> R (+16)                     |                      | 126 | 2 | 840.387  | 1678.759      | 1678.758         |
| 7,271  | SLSDEERMDALENQLK (+16)                           |                      | 141 | 2 | 947.447  | 1892.880      | 1892.878         |
| 1,686  | LAMVEADLER (+16)                                 |                      | 171 | 2 | 581.793  | 1161.572      | 1161.570         |

**b<sup>+</sup>ions:**102.06, 215.14, 158.60(++), 430.23, 558.32, 336.21  
**y<sup>+</sup>ions:**201.12, 157.61(++), 422.30, 556.35, 657.39, 385.74(++)

**m/z:**871.48 **change:**+2

**Fig. 5** MS/MS spectrum for a peptide of Lys from the MDA-induced shrimp tropomyosin. MDA Schiff base adduction (\*, +54) on lysine side-chain group in Thr–Leu–Thr–Asn–Lys–Leu–Lys. MDA-modified residues are underlined

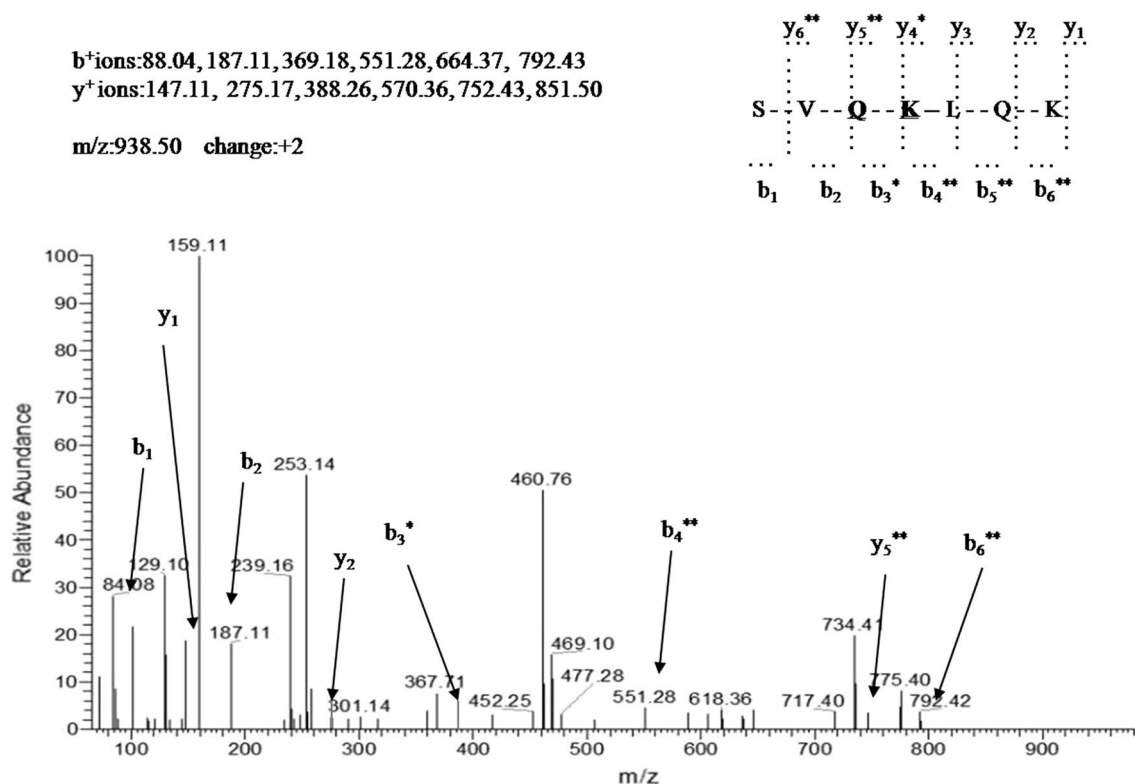


**Fig. 6** MS/MS spectrum for a peptide of Gln from the MDA-induced shrimp tropomyosin. MDA Schiff base adduction (\*, +54) on lysine and glutamine side-chain groups in Ser–Val–Gln–Lys–Leu–Gln–Lys. MDA-modified residues are *underlined*

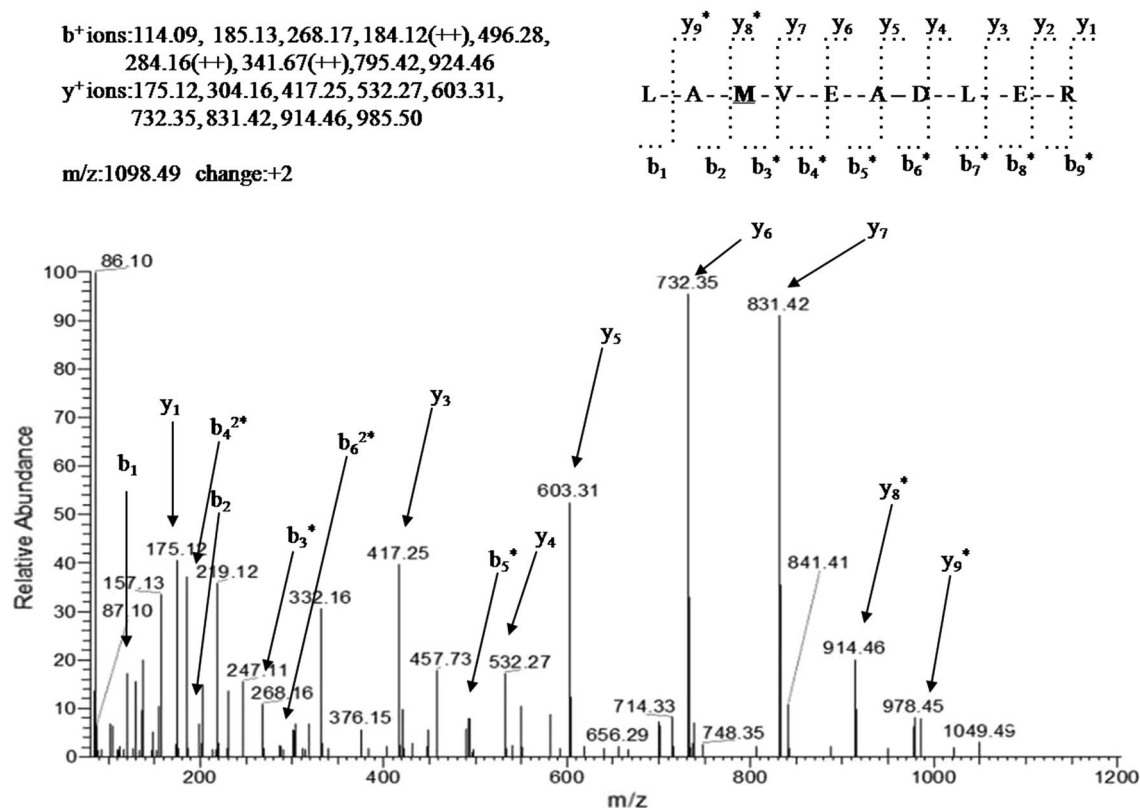


**Fig. 7** MS/MS spectrum for a peptide of Met from the MDA-induced shrimp tropomyosin. ROS adduction (\*, +16) on methionine side-chain group in Leu–Ala–Met–Val–Glu–Ala–Asp–Leu–Glu–Arg. MDA-modified residues are *underlined*

<sup>245</sup>Ser–Val–**Gln–Lys**–Leu–Gln–Lys<sup>251</sup> showed simultaneous MDA adduct formation on Gln<sup>247</sup> and Lys residues<sup>248</sup> as shown in Fig. 6. In this study, the mass spectrum of Asn is not listed because of the relatively weak signal in the MS/MS search. We predicted that aspartate probably existed in the hydrophobic part, and the structure shrunked easily. Figure 7 shows the ROS adduction on the Met side-chain amino group of <sup>169</sup>Leu–Ala–**Met**–Val–Glu–Ala–Asp–Leu–Glu–Arg<sup>178</sup>. These oxidized modifications of certain amino acids can result in secondary or tertiary structural changes and consequently in protein functions [31]. The details of the oxidized site together with epitope analysis will be helpful to understand the immunogenicity changes of shrimp tropomyosin during food processing and preservation.

In conclusion, some active food ingredients, such as MDA, can react with the side chains of amino acids in proteins, thus significantly changing their tertiary and secondary structures. The chemical modification of tropomyosin may explain the effect of different food processing conditions on tropomyosin reported previously. Moreover, the chemical reactions can only occur at the side chains of amino acids, such as Lys and Gln containing amino group. However, these amino acids cannot react with MDA. The chemical modification cannot be neglected when studying the effect of processing on the food allergens; however, further study should be conducted.

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**Conflict of interest** None.

**Compliance with Ethics Requirements** This article does not contain any studies with human or animal subjects.

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