Effects of High Hydrostatic Pressure Treatment on Physicochemical Characteristics of Sea Cucumber

Yuanjing Xia, Zhijun Liu and Zhiyi Li

R&D Institute of Fluid and Powder Engineering, Faculty of Chemical, Environmental and Biological Science and Technology, Dalian University of Technology, Dalian 116024, China

Received: February 24, 2012 / Published: April 20, 2012.

Abstract: The effects of high hydrostatic pressure (HHP) treatment on sea cucumber qualities, such as shelf-life, autoenzyme, total volatile basic nitrogen (TVB-N), mucopolysaccharide and protein, were investigated experimentally. The shelf-life of sea cucumber was greatly prolonged by HHP treatment. High pressure treatment of sea cucumber significantly reduced the activity of autoenzyme at 550 MPa. The TVB-N content was 8.4 mg/100 g in the HHP-treated samples after 38 days' storage at 4 °C, while it had already reached 11.2 mg/100 g in the untreated ones after 5 days' storage under the same condition. The relative mucopolysaccharide content of the HHP-treated samples was 94.3%, while that of the heat-treated ones was only 35.5%. The sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), Differential scanning calorimetric (DSC), ANS fluorescence probe method and fourier-transform infrared (FTIR) spectroscopy results indicated some changes in protein subunits, denaturation, surface hydrophobicity and secondary structure of sea cucumber protein. This study has provided complementary information of pressure-induced structural changes on both the molecular and the sub-molecular level of sea cucumber protein.

Key words: High hydrostatic pressure, sea cucumber, shelf-life, autoenzyme.

1. Introduction

Sea cucumber is one of the most valuable species in seafoods, known as ginseng in the sea, with very high economic value. It contains many active substances and possesses a variety of biological and pharmacological activities [1], such as hemolytic, antitumoral, anti-inflammatory, antifungal, antibacterial, antiviral, cytostatic and antineoplastic activities [2-5]. In recent years, with the improvement of living standard, the consumption of sea cucumber has increased constantly. Sea cucumber aquaculture has developed rapidly for the natural resources being limited. With the increase of consumption and aquaculture production, how to extend the shelf-life of sea cucumber has become the focus of attention. As a result, the sea cucumber processing technique has

become a hot research topic. Since fresh sea cucumbers would be hydrolyzed by autoenzyme under normal condition [6], heat treatment was used traditionally to inactivate the autoenzyme. The traditional thermal processing is effective to inactivate bacteria and autoenzyme. However, heating can cause unwanted side-effects on the functional properties of sea cucumber and many nutritional components are lost in the treatment process.

With growing consumer demand for foods being safe, additive-free and fresh-tasting, high hydrostatic pressure (HHP) treatment is attracting much interest. HHP treatment can inactivate microorganisms and enzymes at ambient or lower temperatures without affecting the flavour, colour and nutritional quality of food [7]. The HHP treatment process is low in energy consumption, environment-friendly and with little or no chemical additives [8]. HHP treatment is therefore particularly important for seafood which is mostly

Corresponding author: Zhijun Liu, professor, research fields: supercritical fluid and food processing. E-mail: liuzj@dlut.edu.cn.

freshly consumed or minimally cooked and has been employed for the commercial processing of oysters [9, 10], but it has not been reported for the treatment of sea cucumber yet.

The objective of this work was to study the effects of HHP treatment on the shelf-life, autoenzyme, TVB-N, mucopolysaccharide and protein of sea cucumber. The effects assessed by a number of methods in order to obtain fundamental knowledge of HHP treatment on sea cucumber. Since high pressure treatment may be considered as a “cold pasteurization” process in contrast to a thermal pasteurization process, we focused on pressure treatment at room temperature.

2. Materials and Methods

2.1 Preparation of Samples

Fresh sea cucumber cultivated in Bo sea (Northern China) were purchased from a local supplier, then were slaughtered, cleaned and scrubbed under running water to remove fouling organisms and mud, then were scrubbed twice with deionized water. After cleaning, sea cucumbers were minced and packed individually in a vacuum pouch for each using a vacuum packaging system, and heat sealed. The headspace in the sealed pack was kept to a minimum. All experimental sea cucumbers and controls were processed within 24 h of harvesting.

2.2 High Pressure Treatment

Packaged samples were treated in a HHP vessel (UHPF-750 MPa, Baotou Kefa High Pressure Technology Co., Ltd, China) equipped with temperature and pressure regulator device. Water was used as the pressure transmitting medium. Samples were pressure treated at specified pressure level (100, 200, 300, 400, 500 and 550 MPa) for a holding time of 20 min. The temperature increase due to adiabatic heating during compression was ≤ 10 K [11]. All experiments were carried out in duplicate. Pressure treated samples were stored in refrigerator for further use.

2.3 Analysis of Autoenzyme Activity

The activity of holothurian autoenzyme was determined using the method of 5 g sea cucumber slurry (homogenizing twice for 30 s) was mixed with 40 mL phosphate buffer solution (0.1 mol L^{-1} , pH 6.3) [12]. The mixtures were kept in a refrigerator at 4°C for 12 h, and then were centrifugated at $4,000 \text{ r min}^{-1}$ at 4°C for 10 min with a centrifuge (5,804 R, Eppendorf Company, Germany). Into a 10 mL sterile eppendorf tube was added 1.0 mL of supernatant (crude enzyme extract), then the tubes were equilibrated at 30°C for 5 min in a water bath and 2.0 mL 0.5% casein solution was added after 2 min. The reaction mixture was incubated in a water bath at 30°C for 10 min before the addition of the 2.0 mL 10% trichloroacetic acid solution to end the reaction. The mixtures were centrifugated at $4,000 \text{ r min}^{-1}$ at 4°C for 8 min. Into a 10 mL sterile Eppendorf tube was added 1.0 mL supernatant, 5.0 mL sodium carbonate (0.55 mol L^{-1}) and 0.5 mL folin -phenol reagent B solution, shaken and put into the water bath, incubated at 30°C for 15 min. The absorbance of the mixture solution was measured spectrophotometrically at 650 nm (UV762 spectrophotometer, Shanghai Precision Scientific Instrument Co., Ltd, China). The results are based on three replicate measurements.

The autoenzyme activity present in the sample was calculated using the standard curve of tyrosine. One unit (U) of activity is defined as the amount of tyrosine produced per min under the above assay. The relative activity of autoenzyme is defined as a percentage of autoenzyme activity of the samples to that of the untreated samples [13].

2.4 Total Volatile Basic Nitrogen

Total volatile basic nitrogen (TVB-N) was determined according to the method in the reference [14] and the distillation was performed in a UDK 152D Distillation Unit (Velp Scientifica, Milano, Italy). The TVB-N content was expressed in mg/100 g sea cucumber.

2.5 Mucopolysaccharide Content Analysis

The determination of mucopolysaccharide content was done according to the method in the paper [15]. 30 g sample with 60 mL deionized water were mashed, adjusted pH to 6.0-6.5, 1.2 g papain was added, hydrolyzed at 60 °C for 9 hours, then killed papain at 90 °C, centrifugated at 6,000 r min⁻¹ for 10 min, the supernatant was mixed with 40 mL ethanol (95%), stored in a refrigerator at 4-8 °C for 12 hours, centrifugated at 6,000 r min⁻¹ for 10 min, the deposition was scrubbed with ethanol and acetone, dried in vacuum freeze drier, then weighed.

2.6 Differential Scanning Calorimetry (DSC) Measurement

The thermal analysis for controls and pressure treated sea cucumbers were measured by a DSC (differential scanning calorimeter) (TA-DSC 910S, TA Instruments, New Castle, DE, USA). Sea cucumber samples (0.75 g) and distilled water (0.75 g) were accurately weighed into the sample and reference pans respectively. Thermal scans were performed from 20 °C to 105 °C at a heating/cooling rate of 1 °C m⁻¹ in to detect protein denaturation in sea cucumbers. The pan with water (0.75 g) was used as a reference. The DSC measurements were done more than 3 times. Thermal transitions of sea cucumbers were measured for the denaturation temperature (Td). The changes of endothermic heat flow during the heating and cooling process were recorded by computer.

2.7 FTIR (Fourier Transforms Infrared) Spectroscopy

The FTIR spectra were recorded using a FT/IR-430 spectrometer (Jasco, Japan) with detector at 4 cm⁻¹ resolution from 1,500 cm⁻¹ to 1,700 cm⁻¹, and 1,024 scans per sample.

2.8 ANS Fluorescence Probe Method

Untreated and HHP treated sea cucumber slurry were diluted with phosphate buffer (0.1 M, pH 6.8) to obtain sea cucumber protein solutions with

concentrations of 1.0 mg/mL. 20 µL of ANS solutions (100 µM in 0.1 phosphate buffer, pH 6.8) were added to 4 mL protein solutions. After mixing, extrinsic ANS fluorescence was determined with the spectrophotofluorometer (FP-6300, JASCO Company, Japan) using an excitation wavelength of 390 nm and observing emission at a wavelength of 470 nm.

2.9 Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Electrophoresis was performed using a vertical slab-gel unit (DYY-III28A, Beijing Liuyi Instrument Factory, China). The stacking gel and resolving gel were 4% and 12% acrylamide (w/v) for SDS-PAGE, respectively. Slab gels (0.75 mm thickness) were run at a constant current of 20 mA. Electrophoresis was terminated when the tracking dye front (bromophenol blue) reached the 1 cm mark at the bottom of the slab. After electrophoresis, the gels were removed from glass plates and placed for 2 h in a fixing solution, which contained 50% ethanol, 10% glacial acetic acid and 40% water. Protein bands were stained by immersion of the gel for 18 h in 0.25% Coomassie Brilliant Blue R250 staining solution. Destaining was carried out by storing of the gel in the solution, which contained 20% ethanol and 7% glacial acetic acid, until the background color was completely removed. The desired gels were stored in 7% glacial acetic acid solution until they are photographed.

3. Results and Discussion

3.1 Shelf-life

The fresh sea cucumbers were cut open and the viscera were removed. The samples were then washed with deionized water and packed separately in a vacuum pouch for each. Some vacuum-sealed samples were treated at the pressure of 550 MPa for 15 min, the others were untreated. The treated and untreated samples were kept in same refrigerator at temperature between 4 °C and 8 °C, and the changes of their appearances were observed. Fig. 1 shows the

appearances of the treated and untreated samples after the storage in the refrigerator for 7 days and 20 days respectively.

It can be seen from Fig. 1, the untreated sample had begun to melt after 7 days, while treated sample still kept its shape and appearance as fresh after 20 days. This observation confirms that HHP treatment can greatly prolong the shelf-life of sea cucumbers and effectively prevent the occurrence of autolysis.

The melting and decaying of sea cucumber are due to the autoenzyme and microorganism in the body of sea cucumber, especially the autoenzyme. Sea cucumber would be melted entirely by the autoenzyme under normal condition after a few hours. The extending of the shelf-life of HHP-treated sea cucumber suggests that HHP treatment can inactivate the autoenzyme and microorganism.

3.2 Inactivation of Autoenzyme

Fig. 2 shows the changes in the relative activity of autoenzyme of control sample and pressure treated samples in the pressure range 0.1-550 MPa, holding time of 20 min. The autoenzyme was highly susceptible to high pressures. A gradual decrease in the activity was observed with increasing pressure when the treatment pressure level was less than 200

MPa. However the autoenzyme was activated in the 250 MPa treatment, with the highest activity of 106.8%. After that the activity decreased significantly with pressurization, showing the lowest relative activity (29.8%) at 550 MPa. These results are in agreement with other researchers [13, 16] who reported such a tendency of polyphenoloxidase in pear juices and proteolytic enzymes in meat by high pressure treatment.

Pressure will enhance the processes and reactions which tend to decrease in volume, and inhibit those which are associated with increases in volume [17]. The chemical essence of enzyme is protein. The biologic activity of enzyme is produced from the activity center which is produced by tertiary structure of molecule. The change of tertiary structure is related to the change of volume. High pressure can lead to the collapse of tertiary structure and therefore make enzyme lose its activity [18]. The increase of relative activity of autoenzyme observed in the present experiment was not due to an increase in enzymic activity but to an increase in its content in the extract. Generally, the enzyme exists in the lysosome. Therefore, increase of enzyme content was derived from the leakage of the enzyme from the disruption of the lysosome membrane by the pressure treatment [16].

Fig. 1 Appearance of HHP-treated and untreated sea cucumbers for different storage time.

Fig. 2 Effect of pressure on holothurians autoenzyme activity.

3.3 TVB-N

During the decaying of seafood like sea cucumbers, due to the growth of bacteria and the effect of enzyme, the protein is decomposed and produces ammonia and amines which are called TVB-N. TVB-N is often used as an indicator in assessing the shelf life and storage quality of seafood products [19]. The spoilage pattern of fresh seafood generally shows an increase in TVB-N content, which closely parallels the bacterial population [20, 21].

Changes of TVB-N content in untreated and HHP-treated samples after different storage time at 4 °C are shown in Fig. 3. TVB-N content of untreated sample increased significantly from 3.4 (day 0) to 43.4 (day 13), and the samples had a terrible smell. While that of HHP-treated increased insignificantly. TVB-N content of samples at 500 MPa was 8.4 mg/100 g after 37 days' storage, still remained below the acceptable limit. In the present study, the TVB-N content of about 10 mg/100 g could be regarded as the limit of acceptability for sea cucumber according to the sensory evaluation.

3.4 Mucopolysaccharide

Mucopolysaccharide is one of the important active

ingredients of sea cucumber, having many pharmacological functions, such as immunity enhancing, anticoagulant, anticancer and anti-aging [22]. In order to examine the effect of processing methods on mucopolysaccharide of sea cucumber, the mucopolysaccharide contents of untreated, heat-treated (100 °C for 20 min) and HHP-treated (500 MPa for 20 min) samples were analysed. The results are shown in Fig. 4. The relative content is defined as the ratio of mucopolysaccharide content of the samples to the one of the untreated samples.

From Fig. 4 it can be seen that the relative content of the HHP-treated samples was 94.3%, while it was only 35.5% for the heat-treated samples, with a reduction of 64.5% compared with untreated samples. During the traditional heating process the indican bond of mucopolysaccharide is disrupted, the holothurians mucopolysaccharide is broken down into monosaccharide, oligosaccharides and saccharide derivatives, the molecular structure of mucopolysaccharide is changed, and then the activity function is lost. HHP treatment does not destroy the molecular structure of mucopolysaccharide, so that the content and activity function are kept after HHP processing.

Fig. 3 Effect of HHP treatment on TVB-N content of sea cucumber.

3.5 Effect of HHP Treatment on Protein

High pressure treatment changes the conformation and coagulation of proteins by opening the native structures [23, 24]. To determine the structural changes of proteins upon pressurization, FTIR (Fourier transform infrared) spectroscopy, differential scanning calorimetry (DSC), ANS fluorescence spectra and SDS-PAGE electrophoresis are commonly used tools.

3.5.1 Protein Denaturation Study by DSC

DSC is a powerful technique that has been used to study the structural and thermal properties of natural polymers such as proteins. The information obtained by DSC is on a macroscopic level, it enables to assess the overall structure of the protein molecule. In order to investigate the effect of high pressure on sea cucumber protein, samples were scanned immediately after pressure treatment. The DSC thermogram of untreated (0.1 MPa) and pressure treated sea cucumber samples are shown in Fig. 5 respectively.

Three distinct endothermic peaks ($Td1 = 22\text{ }^{\circ}\text{C}$, $Td2 = 51\text{ }^{\circ}\text{C}$, $Td3 = 85\text{ }^{\circ}\text{C}$) were observed in the DSC thermogram for the untreated sea cucumber sample (0.1 MPa). The melting enthalpies (ΔH), i.e. the areas

of the peaks on the DSC thermograms, decreased as pressure increased demonstrating progressive denaturation as the pressure levels increased. The sea cucumber protein subjected to 400 MPa for 20 min showed no peak and hence no ΔH value. Complete denaturation resulted in the disappearance of the enthalpy of the endotherm peaks. Results indicated that the sea cucumber protein was significantly affected by the HHP treatment, complete denaturation occurred when the treatment pressure level increased to 400 MPa. The decrease of enthalpy with pressure level has been reported in the literature for various proteins [25-27].

The change of protein structure during pressure treatment was explained on the basis of increase in water density [28]. Water density increases with pressure levels and subsequently the number of water molecules per lattice increases significantly from 4.3 molecules per lattice at atmospheric pressure and at $4\text{ }^{\circ}\text{C}$ to 10 molecules per lattice at 1,000 MPa at $25\text{ }^{\circ}\text{C}$. These changes could be attributed by cleavage of hydrogen bonds between surface of the protein and the surroundings, protecting water molecules. The residual denaturation enthalpy provides a net value from a combination of endothermic reactions like the

Fig. 4 Effect of processing methods on mucopolysaccharide of sea cucumber.

Fig. 5 Differential scanning calorimetry thermograms of proteins in sea cucumbers by different pressures.

disruption of hydrogen bonds, and exothermic processes, including the break-up of hydrophobic interactions and protein aggregation [27, 29]. As a result, DSC-thermograms of the pressure-treated sea cucumber exhibited subsequent thermal denaturation of the proteins remaining in a nativelike conformation.

3.5.2 Surface Hydrophobicity Study by Fluorescence Spectra

Protein hydrophobicity has been receiving much attention since the hydrophobic interactions are

considered to play important roles in the functional properties of food proteins. Especially surface hydrophobicity has much greater significance for elucidating the functionality of food proteins. The surface hydrophobicity showed close relationship between emulsion capacity and emulsion stability of proteins [30]. The more hydrophobic the protein is, the better the functional properties become. Increased fat binding capacity was associated with an increase in hydrophobicity of the protein [31].

The fluorescence method using 1-anilino-naphthalene-8-sulfonate (ANS) as a probe was applied to determine the surface hydrophobicity of proteins [32, 33]. ANS fluorescence is very weak in aqueous solutions, but it is greatly enhanced when bound to β -lactoglobulin [34, 35]. Thus, ANS probes are widely used to assay hydrophobicity of food proteins [30, 36, 37].

The ANS extrinsic fluorescence intensity increased significantly with the increasing of treatment pressure level, especially above 300 MPa, as shown in Fig. 6. The result indicates that the surface hydrophobicities of sea cucumber protein were increased by high

pressure treatment. Most proteins probably increase their surface hydrophobicity as denaturation proceeds, because the hydrophobic residues buried in the interior of proteins are exposed at the molecular surface. Therefore, protein functionality, especially surface properties, would be improved by partial denaturation if no coagulum occurs. The effects of partial denaturation on protein functionality are important for food applications.

3.5.3 SDS-PAGE Electrophoresis

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to demonstrate the effects of high pressure on the protein components

Fig. 6 Fluorescence spectra of ANS in sea cucumbers by different pressures.

1: control sample, 2: 200 MPa, 3: 300 MPa, 4: 400 MPa and 5: 500 MPa.

Fig. 7 SDS-PAGE patterns of untreated and HHP treated sea cucumber protein solutions.

of sea cucumber. SDS-PAGE electrophoretograms of control and pressurized sea cucumber samples are shown in Fig. 7.

The protein patterns showed that sea cucumber proteins were significantly affected by HHP treatment after pressure level of 400 MPa. Bands a, b, c and d were pressure labile. The intensity of band “c” and “d” decreased in 500 MPa-treated samples compared with control and other pressurized samples. New band “b” appeared in samples pressurised at 400 and 500 MPa. On the contrary, band “a” disappeared in 500 MPa-treated samples. Changes of band intensities could be related to: (a) dissociation of oligomeric structures into their subunits, (b) partial unfolding and denaturation of monomeric structures, (c) protein aggregation [23]. The results suggest that protein structures could be changed by HHP treatment.

3.5.4 Protein Denaturation Studied by FTIR Spectroscopy

FTIR spectroscopy—a vibrational spectroscopic technique has provided better understanding of secondary structure change of proteins after the influence of high pressure. The amide I absorption region ($1,600\text{--}1,700\text{ cm}^{-1}$) and the amide II absorption region ($1,500\text{--}1,550\text{ cm}^{-1}$) in the infrared spectrum of a

protein has been considered to be one of the most useful for secondary structure elucidation since the amide bands are the sum of overlapping component bands (α -helix, β -sheet, β -turn and randomly coiled conformation), which are mainly related to the C = O stretching of the peptide bonds influenced by their various environment in the different kinds of secondary structure [38]. FTIR measurement has considered a complementary approach to DSC because it provides direct structural information about the protein at a sub-molecular level [39].

The spectra of untreated (0.1 MPa) and pressurized sea cucumber samples in the spectral range of amide I and II band are shown in Fig. 8. The spectral shape of amide I ($1,600\text{--}1,700\text{ cm}^{-1}$) and amide II ($1,500\text{--}1,550\text{ cm}^{-1}$) do not change considerably when treatment pressure level is less than 400 MPa. Significant changes were observed at 500 MPa. The changes in the secondary structure (loss of intensity) of pressurized sea cucumber proteins at 500 MPa was observed at around $1,630\text{ cm}^{-1}$ and $1,512\text{ cm}^{-1}$ (Fig. 9). This frequency corresponds to β -structure which was significantly affected by HHP treatment. In addition, obvious decreases in the intensities of other two bands at $1,655\text{ cm}^{-1}$ and $1,540\text{ cm}^{-1}$ suggest a denaturation of

Fig. 8 FTIR spectra for sea cucumber proteins by different pressures.

Fig. 9 FTIR spectra for untreated and pressure treated(500 MPa, 20 min) sea cucumber samples.

the α -helix. The FTIR study indicated that sea cucumber protein secondary structure could be destroyed by high pressure.

4. Conclusions

HHP treatment influenced the physicochemical characteristics of sea cucumbers, resulting in significant changes in quality attributes compared to untreated sea cucumbers. The shelf-life of sea cucumber was extended significantly, and HHP treatment was also effective in decreasing the TVB-N content and inactivating autoenzyme. As for functional composition, HHP treatment could effectively protect the mucopolysaccharide of sea cucumber compared with traditional thermal treatment. In terms of protein denaturation, DSC and SDS-PAGE analysis indicated that pressure induced considerable protein denaturation which was observed obviously at pressures above 400 MPa. FTIR spectroscopy confirmed significant change in amide band of pressure treated sea cucumber. The results of fluorescence method indicated that the surface hydrophobicities of sea cucumber protein were increased by high pressure treatment. The results presented here give further

support for the high potential of HHP application as a sea cucumber technique.

References

- [1] M. Guo, F. Song, Z. Liu, S. Liu, Characterization of triterpenoid saponin mixture in crude extracts from leaves of *Acanthopanax senticosus* harms by saponin structural correlation and mass spectrometry, *Analytica Chimica Acta*. 557 (2006) 198-203.
- [2] R.G. Kerr, Z. Chen, *In vivo* and *in vitro* biosynthesis of saponins in sea cucumbers, *J. Nat. Prod.* 58 (1995) 172-176.
- [3] V.I. Kalinin, M.M. Anisimov, N.G. Prokofieva, S.A. Avilov, S.H. Afiyatullo, V.A. Stonik, Biological activities and biological role of triterpene glycosides from holothuroids (Echinodermata), in: M. Jangoux, L.M. Lawrence (Eds.), *Echinoderm Studies*, Balkema, Rotterdam, 1995, pp. 139-181.
- [4] V.I. Kalinin, N.G. Prokofieva, G.N. Likhatskaya, E.B. Schentsova, I.G. Agafonova, S.A. Avilov, et al., Hemolytic activities of triterpene glycosides from the holothurian order, *Dendrochirotida*: Some trends in the evolution of this group of toxins, *Toxicon* 34 (1996) 475-483.
- [5] N.G. Prokofieva, E.L. Chaikina, A.A. Kicha, N.V. Ivanchina, Biological activities of steroid glycosides from starfish, *Comp. Bioch. Physiol. B* 134 (2003) 695-701.
- [6] B.W. Zhu, B. Han, Characterization and purification of holothurians autoenzyme, *Food and Fermentation Industries* 4 (2004) 132-137.

- [7] D.G. Hoover, C. Metrick, A.M. Papineau, D.F. Farkas, D. Knorr, Biological effects of high hydrostatic pressure on food microorganisms, *Food Technol.* 43 (1989) 99-107.
- [8] M.R.A. Gomes, D.A. Ledward, Effect of high pressure treatment on the activity of some polyphenoloxidases, *Food Chem.* 56 (1996) 1-5.
- [9] L.W. Murchie, M. Cruz-Romero, J.P. Kerry, M. Linton, M.F. Patterson, M. Smiddy, et al., High pressure processing of shellfish: A review of microbiological and other quality aspects, *Innovative Food Sci. and Emerging Technol.* 6 (2005) 257-270.
- [10] M. Cruz-Romero, J.P. Kerry, A.L. Kelly, Changes in the microbiological and physicochemical quality of high-pressure-treated oysters (*Crassostrea gigas*) during chilled storage, *Food Control.* 19 (2008) 1139-1147.
- [11] J. Hinrichs, B. Rademacher, Kinetics of combined thermal and pressure-induced whey protein denaturation in bovine skim milk, *International Dairy Journal* 15 (2005) 315-323.
- [12] B.W. Zhu, B. Han, Characterization and purification of holothurians Autoenzyme, *Food Ferment. Ind.* 30 (2004) 132-137.
- [13] Q.M. Zeng, J. Pan, H.M. Xie, et al, Effect of high pressure (HP) treatments on polyphenoloxidase (PPO) activity in pear juices, *Chinese Journal of High Pressure Physics* 18 (2004) 144-148. (in Chinese)
- [14] A.C. Zheng, J. Chen, C.Y. Peng, Extraction and purification of acidic mucopolysaccharide from holothuria scabra Jaeger, *Modern Food Science and Technology* 23 (2007) 65-67.
- [15] P.J. Chen, L.H. Li, L.D. Li, et.al., Improvement on determination of total volatile basic nitrogen in fishery products, *Journal of Fishery Sciences of China* 1 (2006) 146-150.
- [16] N. Homma, Y. Ikechi, A. Suzuki, Effects of high-pressure treatment on the proteolytic enzymes in meat, *Meat Science* 38 (1994) 219-228.
- [17] M. Hendrickx, L. Ludikhuyze, I. Van den Broek, C. Weenanes, Effect of high pressure on enzymes related to food quality, *Food Sci. Technol.* 9 (1998) 197-203.
- [18] E. Morild, The theory of pressure effect on enzymes, *Adv. Protein Chem.* 34 (1981) 93-166.
- [19] J.R. Botta, J.T. Lauder, M.A. Jewer, Effect of methodology on total volatile basic nitrogen (TVB-N) determination as an index of quality of fresh Atlantic cod (*Gadus morhua*), *Journal of Food Science* 49 (1984) 734-736, 750.
- [20] K. Mitsubayashi, Y. Kubotera, K. Yano, Y. Hashimoto, T. Kon, S. Nakakura, et al., Trimethylamine biosensor with flavin-containing monooxygenase type 3 (FMO3) for fish-freshness analysis, *Sensors and Actuators B: Chemical* 103 (2004) 463-467.
- [21] P.J. Chen, L.H. Li, L.D. Li, X.Q. Yang, S.Q. Diao, Y.Y. Wu, Improvement on determination of total volatile basic nitrogen in fishery products, *J. Fishery Sci. China.* 13 (2006) 146-150.
- [22] J. Jiang, L.B. Yang, Y. Tai, Studies on resources and bioactive substances of sea cucumber, *Lett. in Biotechnol* 15 (2004) 537-540.
- [23] J.C. Cheftel, Effects of high hydrostatic pressure on food constituents: An overview, in: C. Balny, R. Hayashi, K. Heremans, P. Masson (Eds.), *High Pressure and Biotechnology*, John Libbey Eurotext, Montrouge, 1992, pp. 195-209.
- [24] R. Lopez-Fandino, High pressure-induced changes in milk proteins and possible applications in dairy technology, *International Dairy Journal* 16 (2006) 1119-1131.
- [25] I. Van der Planchen, A. Van Loey, M.E. Hendrickx, Kinetic study on the combined effect of high pressure and temperature on the physico-chemical properties of egg white proteins, *Journal of Food Engineering* 78 (2007) 206-216.
- [26] J. Ahmed, H.S. Ramaswamy, A. Ayad, I. Alli, P. Alvarez, Effect of highpressure treatment on rheological, thermal and structural changes of basmati rice flour slurry, *Journal of Cereal Science* 46 (2007) 148-156.
- [27] C.Y. Ma, V.R. Harwalkar, Effects of medium and chemical modification on thermal characteristics of b-lactoglobulin, *Journal of Thermal Analysis and Calorimetry* 47 (1991) 1513-1525.
- [28] I. Hayakawa, Y. Linko, P. Linko, Mechanism of high pressure denaturation of proteins, *Lebensmittel-wissenschaft Und-technologie* 29 (1996) 756-762.
- [29] P.L. Privalov, N.N. Khechinashvili, A thermodynamic approach to the problem of stabilization of globular protein structure: A calorimetry study, *Journal of Molecular Biology* 86 (1974) 665-684.
- [30] S. Nakai, Structure-function relationships of food proteins with an emphasis on the importance of protein hydrophobicity, *Journal of Agricultural and Food Chemistry* 31 (1983) 676-683.
- [31] L.P. Voutsinas, S. Nakai, A simple turbidimetric method for determining the fat binding capacity of proteins, *Journal of Agricultural and Food Chemistry* 31 (1983) 58-63.
- [32] A. Kato, N. Tsutsui, N. Matsudomi, K. Kobayashi, S. Nakai, Effects of partial denaturation on surface properties of ovalbumin and lysozyme, *Agricultural and Biological Chemistry* 45 (1981) 2755-2760.
- [33] A. Obata, M. Matsuura, Decrease in the gel strength of tofu caused by an enzyme reaction during soybean grinding and its control, *Bioscience, Biotechnology, and*

- Biochemistry 57 (1993) 542-545.
- [34] E. Dufour, G.H. Hoa, T. Haertle, High-pressure effects of h-lactoglobulin interactions with ligands studied by fluorescence, *Biochimica et Biophysica Acta* 1206 (1994) 166-172.
- [35] M. Hamdan, O. Curcuruto, H. Molinari, L. Zetta, L. Ragona, Electrospray mass spectrometry: Complexation between 1-anilinonaphthalene-8-sulfonate and proteins, *Journal of Mass Spectrometry* 31 (1996) 1261-1264.
- [36] E. Li-Chan, S. Nakai, Effect of molecular changes (SH groups and hydrophobicity) of food proteins on their functionality, in: J.E. Kinsella, W.G. Soucie (Eds.), *Food Proteins*, Champaign' American Oil Chemists' Society, 1989, pp. 232-241.
- [37] J. Yang, A.K. Dunker, J.R. Powers, S. Clark, B.G. Swanson, h-Lactoglobulin molten globule induced by high pressure, *Journal of Agricultural and Food Chemistry* 49 (2001) 3236-3243.
- [38] P. Schwinte, J.C. Voegel, C. Picart, Y. Haikel, P. Schaaf, B. Szalontai, Stabilizing effects of various polyelectrolyte multilayer films on the structure of adsorbed/embedded fibrinogen molecules: An ATRFTIR study, *Journal of Physical Chemistry B* 105 (2001) 11906-11916.
- [39] N. Brandes, P.B. Welzel, C. Werner, L.W. Kroh, Adsorption-induced conformational changes of proteins onto ceramic particles: Differential scanning calorimetry and FTIR analysis, *Journal of Colloid and Interface Science* 299 (2006) 56-69.