Changes in physicochemical properties of water-soluble proteins from crucian carp (*Carassius auratus*) during heat treatment

Kaifeng Li · Huixing Shen · Bo Li · Hang Wang ·
Yongkang Luo

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Abstract In order to understand physicochemical properties of water-soluble proteins obtained from crucian carp, turbidity, total sulfhydryl content, hydrophobicity and SDS-PAGE of crucian carp water-soluble proteins during heat treatment were investigated. Turbidity remained unchanged up to 44°C and considerably increased from 46°C to 54°C, one peak of increase rate was found at 50°C; total SH content decreased rapidly when heated from 50°C to 55°C; hydrophobicity increased sharply when heated up to 45°C, indicating the conformation of water-soluble proteins from crucian carp began to unfold and expose the buried nonpolar amino acids at temperatures above 45°C; analysis of SDS-PAGE indicating the formation of disulfide linkage of creatine kinase and glyceraldehy-3-phosphate dehydrogenase when the temperature reached 65°C and 80°C, respectively.

Keyword Water-soluble proteins · Heat treatment · Crucian carp · Physicochemical properties

Introduction

Surimi wash-water recovers 20–35% of fish muscle protein, which is frequently discarded as waste (Krasaechol et al. 2008). There are lots of reports related to sarcoplasmic

proteins, such as high pressure treatment on bovine sarcoplasmic proteins (Marcos et al. 2010), proteolytic activity and kinetic parameters of *C. striatus* muscle sarcoplasmic protein hydrolysates (Ghassem et al. 2011), many researchers found that addition of sarcoplasmic proteins improved the gel properties of surimi (Jafarpour and Gorczyca 2009; Kim et al. 2005; Yongsawatdigul and Piyadhamviboon 2007), besides, Japanese researchers developed films based on sarcoplasmic proteins obtained from Blue Marlin (*Makaira mazara*) muscle (Iwata et al. 2000).

Heat treatment is an important procedure during meat and seafood processing (Van Der Plancken et al. 2006), which is important to make the food safe by killing contaminating bacteria, and also to inactivate several heat-labile anti-nutritional factors present in many foods (Bains et al. 2011). There are many researches related to thermal studies on proteins from meat and fish, such as differential scanning calorimetric studies of rabbit muscle (Wright et al. 1977), thermal study of washed minced tilapia surimi (Barreto et al. 2000), thermal denaturation of myosins from three kinds of fish (Chan et al. 1992), thermal studies of actomyosin from threadfin bream (Yongsawatdigul and Park 2003) and seer fish (Vinay Kumar and Srikar 1997), thermal studies on myofibrillar protein from Indian major carps (Sankar and Ramachandran 2005) and silver carp (Pan et al. 2011). However, information about heat treatment of sarcoplasmic proteins obtained from crucian carp was limited. Research about changes in physicochemical properties of water-soluble proteins during heat treatment can help to understand the thermal denaturation and provide practical information for crucian carp muscle processing, so as to promote the utilization of huge amount of freshwater fish resources in China.

Crucian carp (*Carassius auratus*) is a main freshwater fish species in China and has been widely used as a raw material for food productions, the output of crucian carp

K. Li · B. Li · H. Wang · Y. Luo (✉)
College of Food Science and Nutritional Engineering,
China Agricultural University, Beijing Higher
Institution Engineering Research Center of Animal Product,
Beijing 100083, China
e-mail: luoyongkang@cau.edu.cn

H. Shen
College of Science, China Agricultural University,
Beijing 100083, People's Republic of China

reached 2,055,478 t during 2009 in China according to the Yearbook of Fishery Statistics 2010. In order to make good use of crucian carp muscle protein, changes in physico-chemical properties of it during heat treatment should be fully studied. The paper aimed to study the mechanism of thermal denaturation and aggregation of water-soluble proteins obtained from crucian carp. Turbidity, hydrophobicity, total sulfhydryl content and SDS-PAGE of water-soluble proteins from crucian carp were determined during heat treatment from 30°C to 80°C, so as to provide reliable information for processing of crucian carp water-soluble proteins.

Materials and methods

Materials and chemicals

Crucian carp were collected in aquatic products markets of Sidaokou in Beijing, China, immediately after the catch, the samples were transported to laboratory alive.

Tris (hydroxymethyl aminomethane) was purchased from Ameresco Company (St. Louis, MO). ANS (1-anilinonaphthalene-8-sulfonic acid), EDTA (ethylenediamine tetraacetic acid) and DTNB(2-nitrobenzoic acid) from Sigma-Aldrich (Shanghai Trading Co., Ltd.).

Preparation of water-soluble proteins

Crucian carp were killed by blowing its head, eviscerated and then washed with cold water. White muscle obtained from three fishes was used. The water-soluble proteins were extracted as described by Tadpitchayangkoon (Tadpitchayangkoon et al. 2010) with some modifications: the fish mince was homogenized using a homogenizer (FM 200, Fluko Co. Ltd., Shanghai, China) with cold deionized water at a ratio of 1:3 (w/v) at speed 5 for 1 min. The mixture was put into 4°C refrigerator for 30 min, then centrifuged for 20 min at 14000×g, 4°C. The supernatant was a water-soluble proteins' fraction with concentration of 7.62 mg/mL.

Heat treatment of water-soluble proteins

Water-soluble proteins was diluted to a certain concentration with deionized water. Samples in tube were heated in a temperature controller water bath at a heating rate of 1°C/min. After reaching each studied temperature, the tube was taken out and immediately cooled in ice water for 1 min, then used for the determination of turbidity and hydrophobicity. The cooled sample still needed to be centrifuged at 1800 × g for 5 min and the supernatant (the protein concentration was determined after centrifuge) was used for total sulfhydryl content and SDS-PAGE determination

Measurement of turbidity

The turbidity was determined according to Pan (Pan et al. 2011) with some modifications: The solution of water-soluble proteins was diluted to 2 mg/mL with deionized water, then heated from 30°C to 80°C. After reaching the studied temperature, absorbance of sample at 350 nm was determined using a spectrophotometer (UV-2600, Shanghai UNICO Instrument Co., Ltd., Shanghai, China). Each experiment was conducted three times.

Measurement of hydrophobicity

The hydrophobicity was determined according to Thanonkaew (Thanonkaew et al. 2006) with some modifications: The solution of water-soluble proteins was diluted to 0.125 mg/mL, 0.25 mg/mL, 0.5 mg/mL and 1 mg/mL with 10 mM phosphate buffer, pH 6.0. After reaching the studied temperature, 10 µL 8 mM ANS, 0.1 M phosphate buffer was added into the heated water-soluble proteins' solution, then the fluorescent intensity was determined using a fluorospectrophotometer. The excitation and emission slit widths were 10 nm and 10 nm when the solution was measured. Fluorescent intensity against protein concentration (mg/mL) was plotted and the surface protein hydrophobicity was calculated as the initial gradient of the curve.

Measurement of total sulfhydryl content (SH)

The total sulfhydryl content was determined using 5, 5'-dithio-bis (2-nitrobenzoic acid) (DTNB) according to Benjakul (Benjakul et al. 1997) with some modifications: 0.5 mL solution of water-soluble proteins (4 mg/mL) was mixed with 4.5 mL of 0.2 M Tris-HCL buffer pH 6.8, containing 8 M urea, 2% SDS, 10 mM EDTA. 4 mL of mixture was mixed with 0.5 mL 0.1% DTNB, 0.2 M Tris-HCL buffer, pH 8.0, then incubated in dark at 40°C for

Fig. 1 Changes in turbidity, total sulfhydryl content and hydrophobicity of water-soluble proteins' solution from crucian carp muscle during heating. Each observation is a mean ± SD of triplicate experiments ($n=3$). ANS: 1-anilinonaphthalene-8-sulfonic acid

Fig. 2 Rate of change in turbidity (dA/dT) of water-soluble proteins' solution (2.0 mg/mL) from crucian carp muscle during heating measured at 350 nm. Each observation is a mean $\pm$ SD of triplicate experiments ($n=3$)

25 min. The absorbance at 412 nm was determined. The total sulfhydryl content was calculated from the absorbance using the molar extinction of $13,600 \text{ M}^{-1} \cdot \text{cm}^{-1}$ and was expressed as $\text{mol}/10^5 \text{ g}$ protein.

Measurement of SDS-PAGE

A discontinuous SDS-PAGE with 4% stacking gel and 12.5% running gel was used to determine the protein pattern of water-soluble proteins. The concentration of protein was diluted to 2 mg/mL with deionized water, then mixed with sample buffer (with or without β -mercaptoethanol) at ratio of 1:1, the mixture was boiled for 4 min. Aliquot of 10 μL of samples was loaded into the sample wells in the stacking gel, so did the marker with standard molecular weight from 14.4 kDa to 97.0 kDa.

Statistical analysis

All the data were subjected to one-way analysis of variant (ANOVA). Analyses were performed with the SPSS computer program (SPSS 17, SPSS INC., Chicago, Illinois,

USA). LSD procedure (significant was defined at $p < 0.05$), descriptive statistic including mean value and standard deviation was used, each observation is a mean $\pm$ SD of triplicate experiments.

Results and discussions

Turbidity

Changes in turbidity of water-soluble proteins solution from crucian carp muscle during heating was shown in Fig. 1. No significant ($p > 0.05$) change was observed between 30°C to 44°C, however, it went up considerably from 46°C to 54°C and a top of increase rate was observed at 50°C (Fig. 2), the turbidity remained unchanged when heated from 56°C to 80°C. The increase of turbidity was derived from the denaturation and aggregation of protein during heating (Pan et al. 2011). This might indicate that water-soluble proteins aggregated to form insoluble particles when temperature was higher than 46°C. Three peaks at 48°C, 64°C and 69°C of turbidity increase rate of myofibrillar protein from silver carp were found according to Pan (Pan et al. 2011), which was caused by myosin, actomyosin and actin, respectively. Water-soluble proteins are composed of proteins that exist in the cytoplasm, the proteases of metabolism, pigments and so on (Lin et al. 2011), only one peak of increase rate of turbidity of water-soluble proteins in crucian carp muscle might indicate that the main component of water-soluble proteins aggregated around 50°C, this result was confirmed by the total sulfhydryl content (Fig. 1).

Total sulfhydryl content (SH)

Changes in total sulfhydryl content of water-soluble proteins obtained from crucian carp muscle during heating was shown in Fig. 1. There was no significant ($p > 0.05$) change in total sulfhydryl content when temperature is

Fig. 3 SDS-PAGE patterns of solution of water-soluble proteins after heat treatment. Lane 1, 3, 5, 7 were water-soluble solution heated to 35°C, 50°C, 65°C and 80°C respectively, Lane 2, 4, 6, 8 were water-soluble proteins' solution heated to 35°C, 50°C, 65°C and 80°C with addition of β -mercaptoethanol

lower than 50°C, the total sulfhydryl content decreased from 2.72 mol/10⁵ g to 2.14 mol/10⁵ g when heated from 50°C to 55°C and then gradually decreased from 55°C to 80°C, suggesting the formation of disulfide linkage upon heating. The analysis of SDS-PAGE (Fig. 3.) indicated disulfide linkage of creatine kinase and glyceraldehyde-3-phosphate dehydrogenase when the temperature reached 65°C and 80°C, respectively.

Hydrophobicity

Changes in hydrophobicity of water-soluble proteins solution from crucian carp muscle during heating was shown in Fig. 1. There was a continually increase at temperature higher than 45°C and reached a maximum at 55°C. Hydrophobicity indicates an exposure of nonpolar amino acids that unfold in a polar environment (Yongsawatdigul and Park 2003). This might mean that the conformation of water-soluble proteins from crucian carp began to unfold and expose the buried nonpolar amino acids above 45°C.

SDS-PAGE

SDS-PAGE patterns of water-soluble proteins solution during heating were shown in Fig. 3. The SDS-PAGE patterns were similar to those sea bass (Ladrat et al. 2003) and threadfin breams (Yongsawatdigul and Hemung 2010) sarcoplasmic proteins. The most abundant proteins band were as followed at the range of average molecular weights from Lane 1: 97 kDa, 80 kDa, 60 kDa, 51 kDa, 41–39 kDa huge doublet band, 36 kDa band, other supplementary bands were observed at 34, 27, 25, 22, 15 and 14 kDa. The 41–39 kDa doublet band was assumed to be creatine kinase and aldolase and the 36 kDa band was assumed to be glyceraldehyde-3-phosphate dehydrogenase with comparison to the patterns described by Nakagawa (Nakagawa et al. 1988). There was no significant change in the SDS-PAGE patterns when heated to 50°C, however, most proteins band disappeared or lightened especially creatine kinase and aldolase when heated to 65°C, the glyceraldehyde-3-phosphate dehydrogenase band disappeared when heated to 80°C, indicating aggregation and denaturation of proteins at different temperature. Lane 6 and 8 showed the patterns of water-soluble proteins solution with addition of β -mercaptoethanol heated to 65°C, the creatine kinase band was much darker than that in lane 5 and 7 which without addition of β -mercaptoethanol, this might indicate formation of disulfide linkage of creatine kinase or aldolase upon heating to 65°C, the increased binding of aldolase under thermal injury of muscle is established by Stabrovskaja (Stabrovskaja and Braun 1977). The glyceraldehyde-3-phosphate dehydrogenase band appeared again in Lane 8 indicating disulfide

linkage was formed in glyceraldehyde-3-phosphate dehydrogenase upon heating to 80°C.

Conclusions

Water-soluble proteins of crucian carp suffered denaturation and aggregation during heat treatment, leading to increase in turbidity and hydrophobicity, and decrease in total SH content. SDS-PAGE indicated formation of disulfide linkage of creatine kinase or aldolase and glyceraldehyde-3-phosphate dehydrogenase when the temperature reached 65°C and 80°C, respectively. Further works may focus on the detailed mechanism of the denaturation and aggregation of each component of water-soluble proteins during heat treatment.

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