LWT - Food Science and Technology xxx (2014) 1–7

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Study of the combined effects of a gelatin-derived cryoprotective peptide and a non-peptide antioxidant in a fish mince model system

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ARTICLE INFO

Article history:
Received 15 February 2014
Received in revised form
27 July 2014
Accepted 30 July 2014
Available online xxx

Keywords:
Pro–Ala–Gly–Tyr
Non-peptide antioxidant
Cryoprotection
Fish mince
Freeze-thaw processing

ABSTRACT

The combined effect of a gelatin-derived cryoprotective peptide (Pro–Ala–Gly–Tyr) and a non-peptide antioxidant (caffeic acid) in a fish mince model system was investigated. The physicochemical properties (peroxide value, thiobarbituric acid-reactive substances, total sulfhydryl group content and protein carbonyl content), water distribution and thermal properties of Japanese sea bass mince were determined before and after 6 freeze-thaw cycles. Mince supplemented with a combination of a cryoprotective tetrapeptide (12.5 ppm) and 50 ppm caffeic acid had the lowest peroxide value and comparable thiobarbituric acid-reactive substances to 100 ppm caffeic acid. The cryoprotective tetrapeptide at 12.5 ppm also showed the greatest effect when combined with caffeic acid in lowering protein oxidation, in protecting the total sulfhydryl group content and in having the lowest protein carbonylation, which led to least myosin heavy chain denaturation. The state of water was studied using low-field nuclear magnetic resonance. The tetrapeptide reduced the formation of free water in the mince, especially in conjunction with caffeic acid. Thus, the cryoprotective tetrapeptide worked with caffeic acid in retarding the quality losses in the mince induced by freezing and thawing.

1. Introduction

Freezing is the most widely used preservation method to maintain fish quality, accounting for a 50% share of the total processed fish for human consumption and 21% of total fish production (Gonçalves, Nielsen, & Jessen, 2012). The state and mobility of the water in the muscle tissue affects the structure and texture of muscle (Andersen & Rinnan, 2002). To ensure optimal quality, it is necessary that water be frozen quickly to assure small ice crystals and that subsequent storage minimize fluctuations so as to not provide opportunities for water molecules to aggregate which causes the destruction of muscle proteins and loss of functional properties such as the ability to retain water (Andersen & Jørgensen, 2004). Therefore, during storage cryoprotectants have sometimes been added to reduce the denaturation of muscle proteins from ice crystal formation and the increased salt concentration in the unfrozen phase (Benjakul & Visessanguan, 2011).

Beside commercial cryoprotectants (such as a mixture of sucrose and sorbitol) (Liu, Kong, Han, Chen, & He, 2014), some protein hydrolysates have been shown to exhibit a cryoprotective effect without increasing the sweetness of seafood (Cheung, Liceaga, & Li-Chan, 2009; Hossain et al., 2004; Kittipatthanabawon, Benjakul, Visessanguan, & Shahidi, 2012). However, the study of the cryoprotective effect of a pure peptide with a known amino acid sequence, especially in seafood, is very limited. A recent study from this laboratory indicated that the tetrapeptide Pro–Ala–Gly–Tyr isolated from an Amur sturgeon skin gelatin hydrolysate showed a cryoprotective effect in fish mince subjected to different freeze-thaw cycles using low-field nuclear magnetic resonance (LF ^{1}H NMR) (Nikoo et al., 2014). Damodaran (2007) suggested that the unique tripeptide repeating structure of –Gly–Pro–X– or –Gly–Z–Hyp– in gelatin peptides might play a role in their ice crystal growth inhibitory characteristic. The tetrapeptide also reduced the formation of thiobarbituric acid-reactive substances (TBARS) in the mince. However, caffeic acid showed a greater effect in preventing lipid oxidation (Nikoo et al., 2014).

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http://dx.doi.org/10.1016/j.lwt.2014.07.051
0023-6438/

The use of peptides with antioxidant activity in foods because of their "presumed" safety may reduce the need for synthetic antioxidants which may lead to a cleaner ingredient label (Harnedy & FitzGerald, 2012; Mills, Stanton, Hill, & Ross, 2011; Shahidi & Zhong, 2010). As the antioxidant activity of peptides to inhibit lipid oxidation in fish muscle, and specifically the cryoprotective tetrapeptide Pro–Ala–Gly–Tyr is weaker than that of the phenolic compounds (Nikoo et al., 2014), it is not known whether a better antioxidant activity could be achieved if the cryoprotective tetrapeptide is combined with a non-peptide antioxidant. Furthermore, the use of low-field ^{1}H NMR to determine the state of water has not been used to date with fish muscle supplemented with both a cryoprotective peptide and a non-peptide antioxidant. The objective of this study was to investigate the combined effects of a gelatin-derived cryoprotective tetrapeptide and a non-peptide antioxidant in a fish mince model system.

2. Materials and methods

2.1. Chemicals

5,5-Dithiobis(2-nitrobenzoic acid) (DTNB), sodium acetate, caffeic acid, cumene hydroperoxide and streptomycin sulphate were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Other chemicals were purchased from Sinopharm Chemical Reagents Co., Ltd. (Shanghai, China) and were of analytical grade unless otherwise noted.

2.2. Peptide synthesis

The peptide sequence identified as Pro–Ala–Gly–Tyr in a previous study (Nikoo et al., 2014) was synthesized by Shanghai Mocell Biotech Co., Ltd. (Shanghai, China) with a purity of 99.5% as verified by them using RP-HPLC (Xbridge BEH130C18, 4.6 × 250 mm column) using a SHIMADZU LCMS-2020 instrument (Shimadzu, Kyoto, Japan).

2.3. Preparation of fish mince

Japanese sea bass (*Lateolabrax japonicus*) (231.84 ± 23.56 g) ($N = 10$) was bought from a supermarket (Wuxi, Jiangsu, China). The fish were placed in ice with a fish/ice ratio of approximately 1:2 (w/w) and transported to the laboratory within 1 h. Upon arrival, the fish were eviscerated, beheaded, filleted, skinned and the red muscle removed by hand in a cold room (3 °C). The white muscle obtained was minced through a 3 mm plate using a grinder. In total, 600 g white muscle mince was obtained. Streptomycin sulphate (200 ppm) was added to the obtained mince to inhibit microbial growth. To ensure even distribution of the streptomycin sulphate throughout the mince, the streptomycin sulphate was dissolved in 2 mL of distilled water and added to the mince, and then thoroughly mixed using a fork for 5 min. Mince was then divided into 100 g portions, to which PAGT or CA was added. Because PAGT is hydrophilic, it was dissolved in distilled water (0.5 mL in 100 g mince) and added into the mince at 10 and 25 ppm. Caffeic acid (CA) was dissolved in ethanol according to Larsson and Undeland (2010) (0.5 mL in 100 g mince) and used at a level of 100 ppm. In two additional treatments, mince was treated with PAGT and CA to give a final concentration of 50 ppm for CA and 5 and 12.5 ppm for PAGT, respectively. An appropriate amount of PAGT, CA or the combination of PAGT and CA was mixed well with the mince using a spatula for 1 min at 3 °C to ensure even distribution. Mince without addition of PAGT or CA was used as a control. Since ethanol was used to dissolve caffeic acid, 0.5 mL of either distilled water or ethanol was added to the control. However, there was no difference between two controls and thus the data from control with added distilled water is presented. Each mince sample (100 g) was further divided into 9 portions (10 g), placed into high density polyethylene bags (100 mm × 150 mm) (Shanghai Rihong Packaging Products Co., Ltd., Shanghai, China) and covered using aluminium foil to protect from light. Unfrozen minces (fresh/0 freeze-thaw cycle) were analyzed on the same day, while the others were subjected to 6 freeze–thaw cycles (20 h freezing at −18 °C and 4 h thawing at 4 °C for each cycle) before analysis. At each time point (freeze-thaw cycle 3 and cycle 6), the thawed minces were mixed well using a fork for 1 min to ensure homogeneity and then analyzed.

2.4. Chemical analyses

2.4.1. Peroxide value

Peroxide value was determined using the method of Richards and Hultin (2002) in unfrozen (fresh) mince and in mince after being freeze-thawed for 3 and 6 cycles. Mince (1 g) was homogenised at a speed of 13,400 rpm using an Ultra Turrax (IKA® T25 digital Ultra Turrax®, Staufen, Germany) for 2 min in 11 mL of cold chloroform/methanol (2:1, v/v). The homogenate was then filtered using Whatman No. 4 filter paper (Hangzhou Wohua Filter Paper Co., Hangzhou, Zhejiang, China). Two mL of 0.5% NaCl was then added to 7 mL of the filtrate. The mixture was vortexed for 30 s and then centrifuged at 5000 *g* for 5 min to separate the sample into two phases. Two mL of chloroform/methanol (2:1, v/v) were added to 3 mL of the lower phase. Twenty-five μL of ammonium thiocyanate and 25 μL of iron (II) chloride were added with 2–4 s vortexing between each addition (Shantha & Decker, 1994). The reaction mixture was allowed to stand for 20 min at room temperature prior to reading the absorbance at 500 nm. Cumene hydroperoxide (at a concentration range of 0–20 μmol kg^{-1}) was used to obtain a standard curve for quantification and results were expressed as μmol hydroperoxide kg^{-1} sample. The percentage inhibition of oxidation was calculated according to Sekhon-Loodu, Warnakulasuriya, Vasantha Rupasinghe, and Shahidi (2013) using the following equation:

$$\%\ \text{Inhibition of oxidation by PV} = [1 - (\text{sample absorbance}/\text{control absorbance})] \times 100.$$

2.4.2. Thiobarbituric acid-reactive substances (TBARS)

Thiobarbituric acid-reactive substances (TBARS) in unfrozen (fresh) mince and in mince after being freeze-thawed for 3 and 6 cycles were determined using the method described by Buege and Aust (1978). One g of mince was mixed with 5 mL of TBA reagent (15% TCA (w/v), 0.375% TBA (w/v) in 0.25 M HCl). The mixture was heated in a boiling water bath (97–99 °C) for 10 min until a pink colour developed. The mixture was cooled with iced water and centrifuged at 5000 *g* at 25 °C for 10 min. The absorbance of the supernatant was read at 532 nm. Results were expressed as mg MDA equivalents kg^{-1} sample. The percentage inhibition of oxidation was calculated using the following equation:

$$\%\ \text{Inhibition of oxidation by TBARS} = [1 - (\text{sample absorbance}/\text{control absorbance})] \times 100.$$

2.4.3. Total sulfhydryl group content

Total sulfhydryl groups content in unfrozen (fresh) mince and in mince after being freeze-thawed for 6 cycles was determined using

Please cite this article in press as: Nikoo, M., et al., Study of the combined effects of a gelatin-derived cryoprotective peptide and a non-peptide antioxidant in a fish mince model system, *LWT - Food Science and Technology* (2014), http://dx.doi.org/10.1016/j.lwt.2014.07.051