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The quality alterations of rainbow trout mince treated with transglutaminase

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ABSTRACT

The use of protein restructuring enzyme transglutaminase (TGase) for extending the shelf-life of rainbow trout mince was determined during refrigerated storage. TGase added into the fish mince in the proportions of 0.0%, 0.2%, 0.5% and 1.0% and the quality alterations were evaluated in terms of chemical, microbiological and sensory parameters. The scores of TVB-N and TMA-N, fish spoilage indicators, of the fish mince treated with TGase had lower levels than control samples. It was recorded that the progress in total free amino acids was suppressed with the addition of TGase. The increase in enzyme concentration met successful results in hindering the growth of total psychrophilic bacteria and coliform bacteria. The best results were obtained with the addition of TGase in the concentration of 1.0%. Overall, utilization of TGase can be a useful tool for seafood processing technology to achieve products with extended shelf life.

1. Introduction

The degradation of proteins and lipids, microbiological spoilage, textural alterations, undesired color, flavor and odour changes takes place rapidly in marine derived organisms. Therefore, the researches dealing with quality protection and extending shelf life of seafood continues intensively. It is known that connective tissue of the fish is weaker than land animals. Less fiber bonds, holding together the muscle, lead to soften the texture of fish and allow a favorable ambient for microorganisms (Gokoglu & Yerlikaya, 2015, p. 233). Various enzymes are used to improve some functional characteristics of proteins such as straightening the structure, improving gel-forming ability, water holding capacity and thermal stability.

The enzyme transglutaminase (TGase) promotes the polymerization of proteins and catalyzes an acyl transfer between the γ-carboxamide group of glutamine and a primary amine such as the 3-amine group of lysine leading to the formation of isopeptide bonds (Grossman, Wefers, Bunzel, Weiss, & Zeeb, 2017). Chambi and Grosso (2006) reported that TGase not only used in the cross-linking of one single type of protein but also applied to the glutamines and lysine of two different types of protein, resulting in the formation of heteropolymers. By coupling two proteins with different structures, new functionalities created, but it is very difficult to ensure that two different proteins are actually coupled and not only large polymers of the separate protein are formed. Covalent cross-links between different proteins cause complex polymeric protein structures leading to changes in physical, chemical and nutritional characteristics of proteins. Usage of the enzyme leads to improvements in the solubility, thermal stability, syneresis, emulsfying properties, gel forming ability and increase water-binding capacity of food proteins (Gaspar & de Goes-Favoni, 2015).

TGase was reported to enhance protein stability and increase protein resistance to chemical and proteolytic degradation (Cui,Yuan, Wang, Sun, Fan & Wang, 2017). Also, TGase forms a coating with better oxygen barrier. Recently, TGase is utilized in order to gain restructured meats, reduced-salt and reduced-fat products (Lee, Jang, Kang, & Chin, 2017; Martelo-Vidal, Fernendez-No, Guerra-Rodriguez, & Vazquez, 2016). With these properties, TGase is predicted to have the potential to protect the initial quality and extend the shelf life of seafood. In this study, the effect of TGase on the chemical, physical, microbiological and sensory quality of fish mince was determined during refrigerated storage.

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2. Materials and methods

2.1. Materials

Rainbow trout (Oncorhynchus mykiss) was obtained from a fish farm and transferred to the laboratory in crushed ice. Fish were eviscerated, beheaded and fish bones were removed before making fish mince. MTGase was obtained from Ajinomoto Foods Europe SAS (Enzyme activity was 100u/g- Activa WM, Paris, France).

2.2. Methods

The fish mince was divided into four groups. MTGase was added into mince in the proportions of 0.2%, 0.5% and 1.0% homogenously. The mince without enzyme was regarded as control samples. Mince samples were placed in polystyrene containers covered with 8–17 μm thickness PVC stretch film (Rotopas, Kocaeli, Turkey) in portions of 50 g and stored at 4 °C for 10 days. Samples were randomly taken every 2 days for analysis. All assays were conducted on duplicate samples of the homogenates.

2.2.1. pH value

The pH value was determined by dipping a pH electrode into homogenates of fish mince in distilled water (1/1) (Manthey, Karnop, & Rehbein, 1988). All measurements were performed at room temperature using pH-meter (WTW Inolab Level 2, Weilheim, Germany).

2.2.2. Total volatile basic nitrogen (TVB-N)

Total volatile bases of homogenized fish mince were obtained by steam distillation and these bases were gathered into a flask containing 0.1 N HCL. Distillate was titrated with 0.1 N NaOH in the presence of Tashiro's indicator. TVB-N was calculated and expressed as mg TVB-N/100 g sample (Schormuller, 1968, pp. 1482–1537).

2.2.3. Trimethylamine nitrogen (TMA-N)

The TMA content of the fish mince was extracted with trichloracetic acid and formaldehyde used to block the primary and secondary amines. Anhydrous toluene and 3 mL KOH solutions were added to the mixture. The toluene layer was dyed with picric acid at a 1:1 ratio. The absorbance of solutions was measured at 410 nm using a spectrophotometer (Evolution 160 UV–visible; Thermo Scientific, Dreieich, Germany) (Schormuller, 1968, pp. 1482–1537).

2.2.4. Total free amino acids

The sample (2 g) was mixed with 17 ml of 0.2 mol/l perchloric acid and placed in an ultrasonic bath for 15 min. The samples were centrifuged at 3250 rpm for 30 min and 1 mL of the supernatant was transferred to a test tube. The extract was kept in a boiling water bath for 15 min after addition of 2 ml of 0.5 mol/l sodium citrate buffer (pH 5.0) and 1 ml ninhidrin reagent. One ml of 60% ethanol was added to the cooled mixture. The absorbance measurement was made against blank at 570 nm (Evolution 160 UV–visible). Total free amino acid content was expressed as mg glutamic acid equivalents/kg sample (Yokoyama & Hiramatsu, 2003).

2.2.5. Microbiological analyses

Fish mince samples of 10 g were taken aseptically and homogenized in stomacher containing 90 ml pre-chilled sterile ringer solution. Decimal serial dilutions were prepared from this homogenate. Appropriate dilutions were used for enumeration of total psychrophilic bacteria by plating on Plate Count Agar (PCA) and incubated at 8–10 °C for 7 days. Yeast and mould were investigated by using Potato Dextrose Agar (PDA) adjusted to pH 3,5 and incubated at 25 °C for 36–48 h. Total coliform bacteria were investigated by pour plating using Violet Red Bile Agar (VRBA) after an incubation period of 24–48 h at 37 °C (Andrews & Hammack, 2017; Feng, Weagant, Grant, & Burkhardt, 2017; Maturin & Peeler, 2017; Tournas, Stack, Mislivec, Koch, & Bandler, 2017). The results were transformed into log cfu/g sample.

2.2.6. Sensory analyses

Sensory analyses performed on raw fish fleshes. Panelists were chosen from the staff members of the fisheries faculty who are familiar with fresh fish and fish products. Panellists (4 female and 5 male) aged between 25 and 48 had experience in evaluating seafood. Descriptive terminology generated by consensus of the panellists in previous trials. The intensity of each attribute was rated for odour (from strong seaweed, fishy odour to amine and ammonia),

Table 1
The effect of treatment and storage time on pH, TVB-N, TMA-N and total free amino acid contents.

Parameter	Treatment	Storage Time (Days)					
		0	2	4	6	8	10
pH	A	6.52 ± 0.01^{Xa}	6.53 ± 0.01^{Xa}	6.47 ± 0.01^{Zb}	6.42 ± 0.01^{Zc}	6.40 ± 0.04^{XYc}	6.40 ± 0.04^{Xc}
	B	6.52 ± 0.01^{Xb}	6.53 ± 0.01^{Xab}	6.54 ± 0.01^{Xab}	6.52 ± 0.01^{Xab}	6.56 ± 0.05^{Xa}	6.43 ± 0.01^{Xc}
	C	6.52 ± 0.01^{Xa}	6.50 ± 0.01^{Yb}	6.50 ± 0.01^{Yb}	6.43 ± 0.01^{Zc}	6.31 ± 0.01^{Yd}	6.00 ± 0.01^{Ze}
	D	6.52 ± 0.01^{Xa}	6.49 ± 0.00^{Yb}	6.51 ± 0.01^{Yab}	6.46 ± 0.01^{Yc}	6.45 ± 0.01^{XYc}	6.24 ± 0.02^{Yd}
TVB-N (mg/100g)	A	16.01 ± 0.95^{Xd}	20.88 ± 0.03^{Xc}	23.03 ± 1.05^{Xc}	29.31 ± 0.12^{Xb}	30.66 ± 0.19^{Xb}	34.23 ± 1.08^{Xa}
	B	16.01 ± 0.95^{Xc}	18.15 ± 0.06^{Yc}	19.55 ± 1.96^{Yc}	23.66 ± 0.03^{Yb}	27.08 ± 1.02^{Yb}	31.38 ± 1.07^{Ya}
	C	16.01 ± 0.95^{Xd}	18.16 ± 0.06^{Yd}	18.79 ± 1.01^{Yd}	23.67 ± 0.01^{Yc}	27.07 ± 1.00^{Yb}	30.71 ± 2.06^{Ya}
	D	16.01 ± 0.95^{Xd}	17.54 ± 0.93^{Ydc}	18.17 ± 0.04^{Ydc}	19.51 ± 0.12^{Yc}	25.72 ± 0.95^{Yb}	29.25 ± 1.93^{Ya}
TMA-N (mg/100g)	A	0.22 ± 0.08^{Xd}	0.85 ± 0.13^{Xc}	1.27 ± 0.22^{Xb}	2.04 ± 0.20^{Xa}	2.10 ± 0.12^{Xa}	2.37 ± 0.24^{Xa}
	B	0.22 ± 0.08^{Xd}	0.71 ± 0.15^{Xc}	1.18 ± 0.19^{Xb}	1.53 ± 0.13^{Xa}	1.90 ± 0.11^{Xa}	2.06 ± 0.20^{Xb}
	C	0.22 ± 0.08^{Xd}	0.75 ± 0.19^{Xdc}	1.17 ± 0.17^{Xbc}	$1.88 \pm 0\ .024^{Xba}$	2.03 ± 0.18^{Xbc}	2.19 ± 0.20^{Xa}
	D	0.22 ± 0.08^{Xc}	0.76 ± 0.14^{Xb}	1.05 ± 0.06^{Xb}	1.68 ± 0.14^{Xa}	2.03 ± 0.10^{Xa}	2.18 ± 0.04^{Xc}
Total Free Amino Acid (mg/kg glutamic acid eq)	A	99.13 ± 0.53^{Xa}	86.38 ± 1.24^{Xc}	88.25 ± 0.71^{Xc}	93.00 ± 1.06^{Xb}	98.13 ± 2.30^{Xa}	99.25 ± 1.77^{Xa}
	B	99.13 ± 0.53^{Xa}	85.13 ± 2.30^{Xdc}	81.88 ± 1.24^{Yd}	94.63 ± 1.59^{Xb}	91.50 ± 1.06^{Yb}	86.38 ± 2.30^{Yc}
	C	99.13 ± 0.53^{Xa}	96.13 ± 0.18^{Yb}	78.75 ± 0.35^{Yf}	88.13 ± 1.59^{Yd}	90.13 ± 0.53^{Yc}	86.25 ± 0.35^{Ye}
	D	99.13 ± 0.53^{Xa}	90.63 ± 0.18^{Yb}	76.13 ± 2.30^{Yd}	86.50 ± 1.06^{Yc}	85.75 ± 2.47^{Zc}	76.38 ± 1.59^{Zd}

Values are mean ± standard deviation.
Means within the same row (a,b,c,d,e) and the same column (X,Y,Z, W) with different letters are different ($P < 0.01$).
A:Control, B: %0.2 enzyme, C: %0.5 enzyme, D:%1.0 enzyme treated fish mince.