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Optimization of gelatine gel preparation from New Zealand hoki (*Macruronus novaezelandiae*) skins and the effect of transglutaminase enzyme on the gel properties

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ABSTRACT

Response Surface Methodology (RSM) was adopted to optimize the preparation of NZ hoki (*Macruronus novaezelandiae*) gelatine gels treated with Transglutaminase (TGase) and some of the rheological properties were characterized. The optimum concentration of the enzyme [X_1] was 3.33 mg/g, incubation time, [X_2] was 30 min, and incubation temperature, [X_3] was 37 °C. The calculated gel strength achieved by RSM was in very good agreement with the experimental value. The addition of TGase to hoki gelatine at the optimum concentration of increased the gel strength from 197 ± 5 g to 278.2 ± 0.19 g and the melting point from 21.4 ± 0.8 °C to 25.9 ± 0.1 °C. The increase in the *G'* values with the addition of TGase indicated the formation of firmer gels and the changes in *G'* and *G"* values with increase in temperature showed increase in melting point.

1. Introduction

Gelatine is a high molecular weight polypeptide obtained by thermal denaturation of collagen, which is the main protein component of animal connective tissues, skins, bones and tendons (Bailey & Light, 1989; Foegeding, Lanier, & Hultin, 1996). Collagen comprises approximately 30% of total animal protein with three polypeptide chains called α-chains wound around each other. Commercial gelatine is mainly derived from porcine skins and bovine bones and they are preferred as compared to those from marine sources due to their superior rheological properties especially in the gel strength and melting point (Cho, Gu, & Kim, 2005). Gel strength and gel melting point are the major physical properties of these thermoreversible gels (Karim & Bhat, 2009). The gel strength of commercial gelatins ranges from 100 to 350 g, but those with gel strength of 250–260 g are the most suitable for a wide range of applications in the food industry especially in the processing of jellies, marshmallows and yoghurts (Holzer, 1996). As a thermoreversible gel, gelatine has the melt-in-the mouth property which is widely used in the food industries. However, the occurrence of bovine spongiform encephalopathy (BSE) disease in land animals and developments in the Halal/Kosher markets has inspired researches to look for gelatine from marine sources. In our earlier study, gelatine was extracted from hoki, a species of cold water fish found in the Southern Oceans and it was observed that hoki gelatine exhibits better physico-chemical properties compared to other cold water fish species studied, but poor rheological properties (especially gel strength and melting point) in comparison to commercial mammalian gelatine (Mohtar, Perera, & Quek, 2010).

In our preliminary studies on Transglutaminase (TGase, EC 2.3.2.13) modification of hoki gelatine we reported a higher gel strength of the modified gelatine (Mohtar, Perera, & Quek, 2011). In this paper we report on the optimization of the gel preparation with TGase and rhelogical properties of the modified hoki gelatine.

2. Materials and methods

2.1. Materials

Skins of hoki fish provided by Independent Fisheries Limited (Auckland, New Zealand) were used for the preparation of gelatine. The hoki skins (approximately 80 × 200 cm) were transported in frozen state and were stored at −20 °C and used within one week. TGase enzyme was generously donated by Ajinomoto, Malaysia (Activa TG-BW-MH). All chemicals and reagents used were of analytical grade.

2.2. Purification of commercial TGase enzyme

Ammonium sulphate [$(NH_4)_2SO_4$] precipitation was used to purify TGase by removing unwanted materials such as sodium

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caseinate, maltodextrin and sodium chloride from the commercial enzyme preparation as reported in our earlier paper (Mohtar et al., 2011). Briefly, the method involves adding $(NH_4)_2SO_4$ granules to a solution of TGase up to 40% saturation and centrifuging to separate any precipitate. The supernatant was then collected in a beaker and $(NH_4)_2SO_4$ granules were added to bring the saturation level up to a final saturation level of 50%. The contents were stirred at 4 °C for 12 h and centrifuged at 10,000× *g* for 15 min at 4 °C. The pellet obtained was dissolved in a small volume of 10 mM Tris-acetate buffer (pH 7.5) and further dialysed against the same buffer in dialysis tubing with molecular weight cut-off of 50,000 Da (Medicell International Ltd., London, UK). The dialysis was done at 4 °C for 12 h and the buffer in the beaker was changed 5 times during that time. After the final dialysis, the tubes were rinsed in Milli-Q water (Millipore Corporation, Billerica, MA, USA) and the contents obtained were frozen at −80 °C and freeze dried. The highest TGase activity was obtained in the protein fraction precipitated between 40 and 50 % of $(NH_4)_2SO_4$ saturation.

2.3. Fish gelatine extraction

Hoki gelatine was extracted as per the previously described optimised procedure by Mohtar et al. (2010). Briefly, the method involves thawing the frozen skins overnight at room temperature (approximately 15 °C) followed by the removal of the flesh and scales from the skins, washing and mincing the skins in tap water. The minced skins were treated with 0.75 M NaCl solution and extracted with Milli-Q water at 49.3 °C for 60 min and centrifuged at 10,000× *g* at 15 °C for 30 min. The clear supernatant obtained was filtered using a Whatman filter paper No. 5 (Whatman International Ltd., Kent, UK) and was freeze dried and stored at −80 °C in N_2 flushed containers for further analysis.

2.4. Experimental design for optimization of the gel preparations with TGase

A Box-Behnken Design (BBD) of RSM was adopted to investigate the interactive effects of three independent parameters on the gel strength (dependent parameter) to generate optimum conditions in order to achieve maximum gel strength in gelatine gel preparations. Three independent parameters namely, enzyme concentration (mg/g gelatine), incubation temperature (°C) and incubation time (min) were coded to three different ranges of −1, 0, +1. The actual values of the variables are shown in Table 1. The experiment was conducted as 15 experimental runs in triplicate as shown in Table 2.

2.5. Gelatine gel preparations

2.5.1. Gelatine gel preparations for optimization studies

Gelatine gels were prepared according to the method published earlier (Mohtar et al., 2010) by dissolving 6.67% (w/v) of dry gelatine in Milli-Q water and heating at 45 °C in a water bath for 30 min until gelatine was completely dissolved. The effect of TGase on the gel strength of gelatine gels was performed by adding different enzyme concentrations (mg of enzyme per g of gelatine) to gelatine solutions as per the experimental design (Table 1). Gelatine solutions with different enzyme concentrations were incubated at the different temperatures for different time intervals as shown in Table 1 and coded in Table 2, and then cooled to room temperature before maturation at 10 °C for 18 h. The gel strength was measured immediately after being removed from the fridge using a TA.XT2 Texture Analyser (Stable Micro Systems, Surrey, UK) fitted with a 1.27 cm diameter cylindrical probe at a speed of 1 mm/s with a force of 0.1 N. The force (g) was recorded as gel strength when the probe had penetrated 4 mm into the gelatine gels.

2.5.2. Gelatine gel preparations under optimized conditions

A 6.67% (w/v) of dry gelatine was dissolved in Milli-Q water and heated at 45 °C in a water bath for 30 min until gelatine was completely dissolved. TGase at the optimum concentration of 3.33 mg/g was added to the gelatine solutions and were incubated

Table 1
Experimental design of independent parameters in the Box-Behnken design for gelatin gels preparation.

Independent parameters	Symbol	Range and level		
		−1	0	+1
Enzyme concentration (mg/g)	X_1	0	3	6
Incubation time (min)	X_2	10	20	30
Incubation temperature (°C)	X_3	25	37.5	50

Table 2
Responses of the gel strength (*Y*, *g*) of gelatin gels to changes in the independent variables.

Exp. No	Variable levels			Response
	X_1	X_2	X_3	*Y*, *g*
1	0	−	−	216.6
2	−	0	+	195.2
3	−	0	−	196.8
4	+	−	0	210.8
5	0	+	+	274.3
6	+	+	0	209.2
7	+	−	0	210.2
8	0	0	0	274.6
9	−	+	0	196.2
10	0	+	−	277.2
11	0	−	−	217.1
12	−	0	−	196.5
13	−	+	0	196.6
14	0	+	−	276.1
15	+	0	−	210.9
16	0	−	−	216.9
17	−	0	−	195.1
18	0	0	0	276.1
19	−	0	+	195.1
20	0	0	0	277.5
21	+	0	−	211.1
22	+	0	+	210.9
23	−	+	0	195.7
24	+	+	0	198.7
25	0	−	+	276.6
26	0	−	+	275.9
27	+	0	−	210.9
28	0	0	0	276.7
29	+	0	+	208.6
30	0	0	0	243.6
31	0	0	0	277.9
32	+	−	0	210.1
33	−	−	0	195.5
34	0	+	−	276.6
35	+	+	0	199.9
36	+	0	+	211.4
37	0	+	+	275.9
38	0	0	0	243.2
39	0	0	0	244.3
40	−	−	0	196.1
41	0	0	0	277.9
42	−	−	0	195.7
43	−	0	+	194.9
44	0	−	+	276.9
45	0	+	+	276.4

X_1: concentration of enzyme (mg TGase/g of gelatin), X_2: incubation time (min), X_3: incubation temperature (°C). The different values for the variables are denoted by (−), (0) and (+) and the actual values they represent are given in Table 1.