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# Antioxidant activity of Maillard reaction products derived from stingray (*Himantura signifier*) non-protein nitrogenous fraction and sugar model systems

Jutaporn Limsuwanmanee [a], Manat Chaijan [a,*], Supranee Manurakchinakorn [a], Worawan Panpipat [a], Sappasith Klomklao [b], Soottawat Benjakul [c]

[a] Department of Food Technology, School of Agricultural Technology, Walailak University, Thasala, Nakhon Si Thammarat 80160, Thailand
[b] Department of Food Science and Technology, Faculty of Technology and Community Development, Thaksin University, Phattalung Campus, Phattalung 93110, Thailand
[c] Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand

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ABSTRACT

This study aimed to evaluate the antioxidant activity of the Maillard reaction products (MRPs) derived from stingray non-protein nitrogenous (NPN) fraction and sugar (glucose, galactose and fructose) model systems. MRPs were prepared by heating the solution containing NPN (2 g nitrogen/100 ml) and sugar (2 g/100 ml) in 0.05 M sodium hydrogen carbonate buffer, pH 12 for 120 min at 100 °C. Reducing power and capacity to scavenge hydrogen peroxide ($H_2O_2$), hydroxyl radical ($OH^{\bullet}$), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) radical ($ABTS^{\bullet+}$) and 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\bullet}$) of all MRPs were greater than those of original NPN solution ($P < 0.05$). The MRPs derived from NPN–fructose tended to have the highest reducing capacity and free radical scavenging ability. However, the ferrous ($Fe^{2+}$) chelating activity of all MRPs was lower than the original NPN solution ($P < 0.05$). Therefore, reducing power and scavenging activity toward free radicals and reactive oxygen species of NPN from stingray muscle can be improved significantly by reacting NPN with sugar particularly fructose via the Maillard reaction. However, the Maillard reaction seemed to show a negative impact on the $Fe^{2+}$ chelating activity of stingray NPN.

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## 1. Introduction

Nitrogenous compounds are very important components of fish meat and affect both nutritional value and sensory properties of fish products. The major nitrogenous compounds of fish flesh are protein and NPN compounds. The contents of NPN compounds in fish depend on the species, the habitat, life cycle and state of freshness after catch (Sikorski, 1994). NPN makes up generally 9–15 g/100 g of the total nitrogen in the meat of marine white fish, 16–18 g/100 g in clupeides and up to 55 g/100 g in sharks (Finne, 1992; Shahidi, 1995; Sikorski, 1994). About 95% of the total amount of NPN in the muscle of marine fish is composed of free amino acids, imidazole dipeptides, trimethylamineoxide (TMAO) and its degradation products, urea, guanidine compounds, nucleotides and the products of their postmortem changes and betaines (Ikeda, 1979; Sikorski, 1994). The main peptides found in fish meat are carnosine, anserine and balenine (ophidine) (Sikorski, 1994). Numerous studies indicated that low-molecular weight water-soluble NPN compounds such as polyamines (e.g. spermine and spermidine), histidine-containing dipeptides (particularly carnosine, anserine, balenine), nucleotides, and glutathione tripeptide have excellent potential for use as natural food antioxidants (Kanner, German, & Kinsella, 1987; LØvaas, 1991; Reische, Lillard, & Eitenmiller, 1997; Sasaki, Ohta, & Decker, 1996). Liwa, Chaijan, and Manurakchinakorn (2011) examined the NPN content in whole muscle of 4 fish species including Indian mackerel (*Rastrelliger kanagurta*), threadfin bream (*Nemipterus bleekeri*), stingray (*Himantura signifier*) and tilapia (*Oreochromis niloticus*) at the same postmortem period. These fish were selected for representing marine teleost dark-fleshed fish, marine teleost white-fleshed fish, marine cartilaginous (elasmobranch) fish and fresh water teleost fish, respectively. It was found that stingray muscle contained the highest amount of NPN. At the equal nitrogen content, stingray NPN exhibited the highest $OH^{\bullet}$ and $H_2O_2$ scavenging activities and

* Corresponding author. Tel.: +66 7567 2384; fax: +66 7567 2302.
*E-mail addresses:* cmanat@wu.ac.th, mchaijan@yahoo.com (M. Chaijan).

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metal chelating ability compared with the others (Liwa et al., 2011).

The Maillard reaction is generally regarded as an efficient and safe method to improve functional properties of proteins such as solubility, heat stability, emulsifying properties, anti-allergenicity and antioxidant activity (Guo & Xiong, 2013). It has been reported that MRPs pronounced antioxidative effects *in vitro*, *in vivo* and in food systems (Chuyen, Ijichi, Umetsu, & Moteki, 1998). MRPs, especially melanoidins, have antioxidant activity through scavenging oxygen radicals or chelating metals. However, compounds in the MRPs with amino reductone structures may have both antioxidant and pro-oxidant activities depending on the reaction conditions (Pischetsrieder, Rinaldi, Gross, & Severin, 1998). MRPs have been used to prevent lipid oxidation in many products. Lipid foods have been relatively stable when the Maillard reaction was involved (Chuyen et al., 1998). The antioxidative property of MRPs was found in potato chips (Kato, Chuyen, Utsunomiya, & Okitani, 1986), biscuit and cookies (Yamaguchi, Koyama, & Fujimaki, 1981), sausages (Lingnert & Ericksson, 1981), ground pork patties (Bedinghaus & Ockerman, 1995), cooked ground beef (Alfawaz, Smith, & Jeon, 1994; Bailey, 1988) and cooked beef patties (Fernandez, Sturla, Doval, Romero, & Judis, 2012). Furthermore, MRPs can prevent the oxidation of sardine lipid (Chiu, Tanaka, Nagashima, & Tagushi, 1991) and can be used as soaking agent to retard the oxidation of lipid and myoglobin in mackerel fillet prior to keep refrigeration (Chaijan, Kewmanee, Hirunkan, Aryamuang, & Panpipat, 2009).

Any nitrogenous compound can be used as a substrate for the Maillard reaction. Thus, NPN fraction can be used as an alternative substrate for the Maillard reaction. However, NPN fraction originally showed the antioxidant activity. Positive or negative impact of the Maillard reaction on antioxidant capacity of NPN has not been reported. In addition, no information regarding the antioxidant activity of MRPs derived of fish NPN, particularly from stingray muscle, and sugar has been reported. Therefore, the objective of this study was to evaluate the antioxidant activity of MRPs produced from stingray NPN and sugar (glucose, fructose and galactose) model system. Analyses of free radical and reactive oxygen species scavenging activities, reducing power and metal chelation of produced MRPs were done in comparison with those of original NPN solution.

## 2. Materials and methods

### 2.1. Chemicals

DPPH, ABTS, deoxyribose, potassium ferricyanide ($K_3Fe(CN_6)$) and 3-(2-pyridyl)-5,6-bis(4-phenyl-sulfonic acid)-1,2,4-triazine (ferrozine) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Trichloroacetic acid (TCA) was obtained from Riedel-deHaen (Seelze, Germany). Glucose, galactose, fructose, ferric chloride, $H_2O_2$ and thiobarbituric acid (TBA) were obtained from Fluka (Buchs, Switzerland).

### 2.2. Fish samples

Stingrays with an average weight of 0.5–1 kg were caught from Thasala–Nakhon Si Thammarat Coast along the Gulf of Thailand. The fish, off-loaded approximately 12 h after capture, were placed in ice with a fish/ice ratio of 1:2 (w/w) and transported to the School of Agricultural Technology, Walailak University, Thasala, Nakhon Si Thammarat, Thailand within 15 min. The fish were immediately washed and filleted. The whole muscle were collected and used for NPN preparation. The muscle was kept on ice during preparation.

### 2.3. Preparation of NPN fraction from stingray muscle

NPN was fractionated from stingray muscle using the method of Hashimoto, Watabe, Kono, and Shiro (1979). Whole fresh stingray meat was manually chopped and homogenized with 3 volumes of chilled distilled water (4 °C) at 13,500 rpm using an IKA Labortechnik homonenizer (Selangor, Malaysia) for 2 min in iced bath. The homogenate was centrifuged at 5000×*g* for 30 min at 4 °C using a Sorvall Model RC-B Plus centrifuge (Newtown, CT, USA). The resulting supernatant (water-soluble fraction) was added with TCA to obtain the final concentration of 5 g/100 ml. The dispersion was centrifuged at 5000×*g* for 30 min at 4 °C. The precipitate containing sarcoplasmic proteins was discarded and the supernatant was filtered through Whatman No. 1 filter paper. The resulting filtrate was collected and referred to as "NPN". The pH of NPN solution was adjusted to 7.0 using 6 mol/L NaOH and subjected to total nitrogen determination by Kjeldahl method (AOAC, 2000). The NPN content in stingray muscle was 11.23 ± 0.07 mg/kg wet muscle. The antioxidant activity of the original NPN (2 g nitrogen/100 ml, pH 7.0) was determined by measuring reducing power, scavenging activities of $H_2O_2$, $OH^{\bullet}$, $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ and $Fe^{2+}$ chelating activity and used as references.

### 2.4. Preparation of MRPs

The NPN previously adjusted pH to 12 using 6 mol/L NaOH was mixed with sugar (glucose, galactose or fructose) in 0.05 mol/L sodium hydrogen carbonate buffer, pH 12 to obtain the final concentrations of 2 g/100 ml for both NPN (as total nitrogen) and sugar (Lertittikul, Benjakul, & Tanaka, 2007). The final volume of the mixture was brought to 100 ml using the same buffer. The solution was then transferred to screw-sealed tubes, tightly capped and heated in an oil bath (Buchi Labortechnik AG, Flawil, Switzerland) at 100 °C for 120 min. After cooling down with running tab water and filtering with Whatman No. 1 filter paper, the MRPs obtained were subjected to analysis for antioxidant activity.

### 2.5. Reducing power

The reducing power of MRPs was determined according to the method of Oyaizu (1986) with a slight modification. One ml of MRPs sample was mixed with 1 ml of 0.2 mol/L sodium phosphate buffer (pH 6.6) and 1 ml of potassium ferricyanide (1 g/100 ml). The reaction mixture was incubated in a temperature controlled water bath (Memmert, Schwabach, Germany) at 50 °C for 20 min, followed by addition of 1 ml of TCA (10 g/100 ml). The mixture was then centrifuged at 750×*g* for 10 min at 25 °C. The supernatant obtained (1 ml) was added with 1 ml of distilled water and 200 μl of ferric chloride ($FeCl_3$) (0.1 g/100 ml). The blank was prepared in the same manner as the samples except that potassium ferricyanide (1 g/100 ml) was replaced by distilled water. The absorbance of the reaction mixture was measured at 700 nm using a UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan). The reducing power was expressed as an increase in $A_{700}$ after blank subtraction.

### 2.6. $H_2O_2$ scavenging activity

$H_2O_2$ scavenging activity of MRPs was determined according to the method of Ruch, Cheng, and Klaunig (1989) as described by Wang, Yuan, Jin, Tian, and Song (2007). A solution (2 mmol/L) of $H_2O_2$ was prepared in 0.1 mmol/L phosphate buffer (pH 7.4). MRPs sample (3.4 ml) was mixed with 600 μl of a $H_2O_2$ solution. The absorbance of the $H_2O_2$ at 230 nm was determined after 10 min incubation at room temperature (25 °C) against a blank solution containing MRPs in phosphate buffer without $H_2O_2$. The scavenging

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