Food Chemistry 230 (2017) 507–515

Contents lists available at ScienceDirect

Food Chemistry

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

Effect of ultrasound pretreatment and Maillard reaction on structure and antioxidant properties of ultrafiltrated smooth-hound viscera proteins-sucrose conjugates

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ARTICLE INFO

Article history:
Received 21 October 2016
Received in revised form 22 February 2017
Accepted 9 March 2017
Available online 11 March 2017

Keywords:
Ultrasound
Maillard reaction
Low molecular weight peptides
Glycation degree
Antioxidant activities

ABSTRACT

The effect of ultrasound (US) pre-treatment on the evolution of Maillard reaction (MR), induced between low molecular weight (LMW) peptides and sucrose, was studied. LMW peptides (<1 kDa) were obtained by the ultrafiltration of smooth hound viscera protein hydrolysates, produced by Neutrase, Esperase and Purafect. MR was induced by heating the LMW peptides in the presence of sucrose for 2 h at 90 °C, without or with US pre-treatment. During the reaction, a marked decrease in pH values, coupled to the increase in colour of the Maillard reaction products (MRPs), were recorded. In addition, after sonication, the glycation degree was significantly enhanced in Esperase-derived peptides/sucrose conjugates ($p < 0.05$). Moreover, results showed that thermal heating, particularly after US treatment, reduced the bitter taste and enhanced the antioxidant capacities of the resulting conjugates. Hence, it could be concluded that US leads to efficient mixing of sugar-protein solution and efficient heat/mass transfer, contributing to increase the MR rate.

1. Introduction

Among the numerous existing protein modification techniques, the glycation of proteins induced by sugar conjugation, via naturally-occurring Maillard reaction (MR), is commonly known as the best process on improving functional properties of food proteins (Seo, Karboune, L'Hocine, & Yaylayan, 2013). Furthermore, the MR is one of the most complex reactions in food chemistry, due to the variety of compounds generated throughout the reaction. In fact, the amine-carbonyl condensation occurring during the early stages of the MR leads to the formation of primordial intermediary products (Amadori products), which tended to be transformed during the advanced stages. These products can undergo numerous reactions giving rise to protein-sugar conjugates and then the formation of brown and polymeric products.

Different factors are involved in the MR evolution, the final glycation degree and the MRPs composition, including the protein/carbohydrate ratio, temperature, incubation time, pH, as well as the interaction rate between the free amino groups of proteins and the carbonyl groups of sugars (Lan et al., 2010). Besides conventional thermal treatment, ultrasound (US) is a new processing technology that has been recently reported to promote the interaction between proteins and reducing sugars (Corzo-Martínez et al., 2014). In addition, it was reported that high-intensity sonication improves the propensity of proteins to aggregate (Chandrapala, Zisu, Palmer, Kentish, & Ashokkumar, 2011) and can modify the secondary structure of proteins leading to increase their surface hydrophobicity (Stanic-Vucinic et al., 2012). On the other hand, US treatment was found to modify the functional properties of carbohydrates (Sun, Hayakawa, & Izumori, 2004) and to enhance the isomerization of glucose to fructose (Wang et al., 2012).

Depending on the operating conditions, different MRPs with various biological and functional properties and flavour sensory will be generated. Bioactive properties of MRPs include the anti-inflammatory (Chen & Kitts, 2015), antihypertensive (Rufia'n-Henares & Morales, 2007) and anti-oxidative (Kitts, Chen, & Jing, 2012) activities. Matmaroh, Benjakul, and Tanaka (2006) have shown that the capacity of MRPs to reduce reactive oxygen species is influenced by various factors, such as the concentration of reactants, duration of reaction, temperature, starting pH, water activity and the presence of metal ions or salts. In addition, it has been previously reported that peptides below 1000 Da showed higher reactivity than high molecular weight peptides (Zhao, Zeng, Cui, & Wang, 2007) and they are considered as a key taste enhancer

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http://dx.doi.org/10.1016/j.foodchem.2017.03.053
0308-8146/

of flavour sensory, including umami, continuity and mouthfulness (Ogasawara, Katsumata, & Egi, 2006).

Proteins and peptides from animal origin are of great importance in food formulations, such as sausages from poultry, bovine or porcine meats (Hathwar, Rai, Modi, & Narayan, 2012; Nasri et al., 2013). Particularly, proteins from fish meat or its by-products are considered as premium quality proteins for their high content in essential amino acids and their numerous nutritional characteristics.

In order to obtain new products from fish by-products, the major conventional research is still restricted to fishmeal production for animal feed. Recently MR was described to enhance the physicochemical, antioxidant and hepato-protective properties of fish byproducts protein hydrolysate-ribose conjugate (Yang et al., 2016).

Among byproducts, fish viscera represent a huge part of the total discards rejected in the local fish markets and fish processing industries. Smooth hound (*Mustelus mustelus*) is the most abundant hound shark in Tunisian coasts, with an interesting catch count of 192 tons (FAOSTAT, 2015). Regarding the huge quantities of visceral mass generated from freshwater fish marketing and the dearth of their scientific exploitation, the present investigation aims to obtain protein hydrolysates from smooth hound viscera and their fractionation using ultrafiltration (UF) process in order to produce low molecular weight peptides.

Because sucrose is the cheapest sugar and the most commonly used in food industries for the fabrication of sweet products, the fractionated peptides were thereafter conjugated to sucrose via the MR. On the other hand, the impact of US pre-treatment on the final products was also investigated by the study of the physicochemical, sensory, structural and antioxidant properties of the resulting MRPs.

2. Materials and methods

2.1. Preparation of LMW peptides

Viscera were obtained following the processing of fresh filleted smooth-hound (*M. mustelus*) fish available in the local fish market of Sfax City, Tunisia. The biological material was brought to the research laboratory in polyethylene bags, in iced conditions, within 30 min. Upon arrival, they were immediately rinsed with tap water to remove contaminants, and then weighed and stored in plastic bags at −20 °C until being used for protein hydrolysates elaboration.

Hydrolysis was carried out as previously described in our study (Abdelhedi et al., 2016). Smooth-hound viscera were first cooked in distilled water at 95 °C for 15 min, with a solid/solvent ratio of 1:1 (w/v), to inactivate endogenous enzymes. After being well homogenized, the pH of the mixture was adjusted to the optimum value of the enzymatic activity by adding 4 N NaOH solution. Thereafter, viscera treated mass was subjected to enzymatic hydrolysis, using three different microbial enzymes, Neutrase® (pH 7.0), Esperase® (pH 9.0) and Purafect® (pH 10.0), added at an enzyme/protein ratio of 6/1 (U/mg of protein). During the reaction (50 °C), the pH of the mixture was maintained constant at the desired value by continuous addition of NaOH solution. The degree of hydrolysis (DH) was calculated based on the volume of NaOH added during the reaction, as described by Adler-Nissen (1986) using the following formula:

$$DH(\%) = \frac{h}{h_{tot}} \times 100 = \frac{B \times Nb}{MP} \times \frac{1}{\alpha} \times \frac{1}{h_{tot}} \times 100$$

where B is the amount of NaOH consumed (ml), *Nb* is the normality of the base, *MP* is the mass (g) of the protein (N = 6.25), and α represents the average degree of dissociation of the α-NH_2 groups in protein substrate expressed as:

$$\alpha = \frac{10^{pH-pK}}{1 + 10^{pH-pK}}$$

where pH and pK are the values at which the proteolysis was conducted. The total number of peptide bonds (*h*tot) in protein substrate was assumed to be 8.6 meq/g (Adler-Nissen, 1986).

After the achievement of the digestion process, the reaction was stopped by heating the different solutions for 20 min at 95 °C to inactivate endogenous enzymes. Protein hydrolysates were then centrifuged at 9500*g* for 20 min to separate soluble fraction (peptides) and insoluble fraction (non-hydrolyzed proteins). Hydrolysates obtained through the digestion of smooth-hound viscera proteins with Neutrase, Esperase and Purafect were noted NH, EH and PH, respectively.

Hydrolysates were fractionated by UF, as illustrated in Fig. 1. Two successive UF steps were applied on the liquid hydrolysates to obtain the LMW peptide fractions. First, a tangential flow filtration (Millipore, Labscale™ TFF System, USA) was performed using a 50 kDa molecular weight cut-off (MWCO) membrane to remove proteins and contaminants. Then, permeates were separated through 1 kDa MWCO membrane using a stirred cell (Amicon Mini membrane system, 1-800-Millipore, USA). The inlet and the outlet pressure were 10 psi and 5 psi, respectively. UF experiments were carried out in batch mode, where the initial volume was reduced to one-quart, *i.e.* to a volume concentration factor (VCF) = 4. After the UF processes, permeates (MW < 1 kDa) were freeze-dried (Bioblock scientific Christ ALPHA 1–2, IllKrich-Cedex, France) and then stored at −20 °C until use.

2.2. Preparation of Maillard reaction products

The conjugation between LMW peptides from smooth hound protein hydrolysates and sucrose (S) was performed by suspending the freeze-dried peptide fractions and sucrose powder (1:1; w/w) in distilled water (1:5; w/v). Then, the mixtures were homogenized at room temperature (25 ± 1 °C) using a magnetic stirrer to form a uniform dispersion. After suspension, ultrasonic treatment (10 W/cm^2) was performed at 40 °C for 30 min with a frequency of 25 kHz and a maximum nominal power of 160 W, using SCIENTZ Electronics ultrasound device (JY98-3, Ningbo, China). Thereafter, the mixtures were incubated at 90 °C during 2 h to induce MR. All samples were then cooled immediately in iced water and then kept at 4 °C. The heated peptides-sucrose mixtures were termed as MRPs. A reference of lysine-sucrose conjugate (1:1, w/w) was prepared with the same manner and heated under the same conditions.

MRPs prepared from LMW-NH, LMW-EH and LMW-PH conjugated to sucrose without US pre-treatment were named LMW-NH/S, LMW-EH/S and LMW-PH/S, respectively, while those treated by US were referred to LMW-NH/S-Us, LMW-EH/S-Us and LMW-PH/S-Us, respectively. The control groups consisted of the protein hydrolysate fractions (<1 kDa) heated alone during 2 h at 90 °C without sugar addition or US pretreatment.

During heating, products were sampled every 30 min in order to evaluate physiochemical properties, colour, free amino acid contents, taste score and structural changes produced owing to the caramelization process. Antioxidant activities of the resulting MRPs were also investigated.

2.3. pH and browning intensity measurements of the MRPs

The pH values of MRPs, obtained at 0, 30, 60, 90 and 120 min of heating, were measured using a 827-pH meter (Metrohm, Swiss made) calibrated with buffer solutions at pH 4.0 and 10.0.