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# Potential application of static hydrothermal processing to produce the protein hydrolysates from porcine skin by-products

Sang-Gi Min [a], Yeon-Ji Jo [a, b], Sung Hee Park [c, *]

[a] Department of Bioindustrial Technologies, Konkuk University, Seoul 05029, South Korea
[b] Institute of Process Engineering in Life Science, Section I: Food Process Engineering, Karlsruhe Institute of Technology, 76131 Karlsruhe, Germany
[c] Department of Marine Food Science and Technology, Gangneung-Wonju National University, Gangneung-si, Gangwon-do 25457, South Korea

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ABSTRACT

Protein hydrolysates from animal by-products have good bioavailability and high nutritional value; thus, they could be used for value added foods, cosmetics and pharmaceutical products. This study investigated the potential of hydrothermal treatment for protein hydrolysis of porcine skin by-product at high temperature (150–250 °C) and pressure (350–3900 kPa). The control showed free amino acid content of 1.4 mg/ml and then increased to 9.4 mg/ml after hydrothermal treatment at 250 °C & 3900 kPa which was named porcine skin hydrolysate PSH-VI. In empirical model fitting, the linear combination coefficient of pressure and temperature ($\beta_5$) showed the positive values with statistical significance ($P < 0.05$). Those results suggest that both elevated temperature and pressure are required for protein hydrolysis as compared to single use of elevated temperature or pressure only. The synergistic effect of temperature and pressure on protein hydrolysis was confirmed in the hydrothermal treatment. On protein gel electrophoresis, no obvious peptide band of >15 kDa was observed after treatment above 190 °C & 1100 kPa treatment. This study showed the potential of hydrothermal processing to produce protein hydrolysates used for food ingredients, cosmetics or pharmaceutical products from animal by-products as a green and sustainable technology.



## 1. Introduction

In meat industry, around 35% of whole carcass is classified for animal by products and pork skin charges 6% of those animal by products (Bae et al., 2015). Production of useful or value added substances from animal by-products will minimize the disposal cost and ecological issues of animal by-products (Toldrá, Aristoy, Mora, & Reig, 2012). Protein hydrolysates from porcine skin by-products can be used for manufacture of value added foods, cosmetics and pharmaceutical products. Porcine skin contains high-molecular-weight collagen and other proteins; however, high-molecular-weight substances must be hydrolyzed to low-molecular-weight hydrolysates to enhance the process suitability and bioavailability. The nutritional value of collagen is quite low since essential amino acids are not available; however, once it is hydrolyzed, collagen hydrolysates are very useful source of bioactive peptides (Herregods et al., 2011; Saiga et al., 2008; Toldrá et al., 2012). Processes including protein hydrolysis have been studied in relation to production of bioactive peptides. Bioactive peptides can be defined as protein hydrolysates where specific protein fractions contain amino acid sequences (peptides) that can exert certain desirable and physiological actions (Ryan, Ross, Bolton, Fitzgerald, & Stanton, 2011; Vermeirssen, Camp, & Verstraete, 2007). Protein hydrolysates have good bioavailability and high nutritional value; thus, their bioactive properties make them potential sources of functional food ingredients (Jain & Kumar Anal, 2016). Hydrolysis can be implemented by several methods, including chemical, enzymatic, and hydrothermal treatment (Orozco et al., 2012). In general, a dilute acid or strong alkali is incubated with a raw animal material to achieve partial cleavage of crosslinks and to break the structure of high-molecular-weight proteins (Karim & Bhat, 2009; Yilmaz et al., 2013). For degradation of high-molecular-weight substances, strong acid or alkali have been used (Costa, Barbosa, & Sousa, 2012; Forgács, Alinezhad, Mirabdollah, Feuk-Lagerstedt, & Horváth, 2011). Alkaline and acidic hydrolysis using solvent extractions are harmful not only to the environment but also for

* Corresponding author. Department of Marine Food Science and Technology, Gangneung-Wonju National University, 7 Jukheon-gil, Gangneung-si, Gangwon-do, 25457, South Korea.
*E-mail address:* park.912@gmail.com (S.H. Park).

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humans who consume the resulting products. This danger is due to composition changes in the materials extracted at high pH; these changes reduce nutrient levels, benefits, and functionalities and produce toxic compounds (Abdul Rahim, Azian Morad, & Long, 2015; Kinsella, 1981). An alternative green and sustainable extraction technology is thus needed. In recent years, the ability of hydrothermal processing to hydrolyze or enhance the solubility of high-molecular-weight substances has been explored. Hydrothermal processing involves a combination of high temperature (>150 °C) and pressure up to 25,000 kPa (Daorattanachai, Viriya-Empikul, Laosiripojana, & Faungnawakij, 2013). During hydrothermal processing, water properties including water density, ionic products, and the relative dielectric constant are evidently changed to favor decomposition of organics with lower energy requirements and synergy of elevated pressure and temperature (Kruse & Dinjus, 2007; Tian et al., 2012). Under hydrothermal condition, water could interact with non-polar compounds since its dielectric constant decreases (Guo, Yang, Fan, & Qiu, 2014). The use of water as a solvent has an almost negligible environmental impact considering production and transportation (Plaza & Turner, 2015). In the hydrothermal processing technique, the autoclave batch reactor is the most common research tool since it does not require pumping system with easy operation although heating and cooling times can be relatively long depending on the size and weight of the reactor (Déniel, Haarlemamer, Roubaud, Weiss-Hortala, & Fages, 2016).

Thus, our hypothesis was that the synergy between the elevated temperature (150–250 °C) and pressure (350–3900 kPa) during hydrothermal treatment would enhance the efficacy of protein hydrolysis of porcine skin byproducts. Accordingly, the aims of this study were investigate the potential of hydrothermal processing for protein hydrolysis and evaluate the optimal processing condition to produce the porcine skin hydrolysates (PSHs) through high temperature (150–250 °C) and pressure (350–3900 kPa). Those efforts could resolve the ecological issues and minimize disposal cost of animal by-products and waste in the meat industry.

## 2. Materials and methods

### 2.1. Porcine skin pretreatment

Porcine skin was purchased from a local supplier (Korea), and all the visible fat and connective tissues were removed using a razor blade. The porcine skin used in this study was obtained from one porcine skin to minimize the biological variation. Trimmed porcine skin was stored at the deep freezer of −80 °C until processing. The frozen porcine skin was thawed at 4 °C overnight and then washed in water at 90 °C for 1 min four times to remove fat and residual materials. The porcine skin was cut into 1-cm squares and then pulverized in distilled water for 3 min using a four-wing blade blender (CNHR-26, Bosch, Hong Kong). Finally, the pulverized porcine skin was homogenized at high-speed (25,000 rpm) for 5 min using an Ultra Turrax® (T25, IKA Labotechnik, Germany). Approximately 100 g of the porcine skin mixture (50% final solid contents) was vacuum-packaged and stored at the deep freezer of −80 °C until experiments (within 1 month). All reagents were of analytical grade and purchased from Sigma-Aldrich Corp. (USA).

### 2.2. Sample package

The pretreated porcine skin was mixed with distilled water (porcine skin: distilled water = 0.05: 0.95). This dilution ratio was selected to ensure flow behavior with low viscosity which will be important consideration for continuous industrial processes. A 100-mL aliquot of the sample solution was placed into a high-temperature-resistant high-pressure-resistant glass bottle.

### 2.3. Hydrothermal processing system

Fig. 1 shows the custom-designed laboratory-scale batch type static hydrothermal treatment reactor (R 101, Rexo Engineering, Korea). While continuous type hydrothermal equipment is relevant for the development of full scale plants, the use of batch reactors prevails due to low cost, simple constructs, and easy handling (Mørup et al., 2015). It consists of a pressure chamber (1-L capacity, maximum temperature: 400 °C, maximum pressure: 30,000 kPa), a movable electric heater, cooling jacket, and data acquisition system. The pressure chamber was filled with previously heated distilled water at 95 °C and then three sample bottles were placed into the pressure chamber as triplicate treatments. In an extraction process, water as a solvent has advantages of non-flammable, non-toxic, and cheap, and can be recycled with minimal environmental impact (Vazquez-Roig & Picó, 2015). The pressure chamber was heated to 150 °C, 170 °C, 190 °C, 210 °C, 230 °C, or 250 °C using a movable electric heater. In our static hydrothermal equipment, pressure was naturally generated by volume expansion of distilled water due to the temperature increase. In a static batch type hydrothermal system, a pump is not equipped; instead, water is added manually to the pressure chamber and then chamber is heated to build-up pressure for saturation pressure of the system (Plaza & Turner, 2015). Pressure build was correspondent to 350 kPa, 660 kPa, 1100 kPa, 2100 kPa, 2600 kPa, and 3900 kPa with temperature increment at 150 °C, 170 °C, 190 °C, 210 °C, 230 °C, and 250 °C, respectively. Thus, in this study, the temperature-pressure combinations were 150 °C & 350 kPa, 170 °C & 660 kPa, 190 °C & 1100 kPa, 210 °C & 2100 kPa, 230 °C & 2600 kPa, and 250 °C & 3900 kPa. Target temperature and pressure were maintained for 10 min to ensure the holding time for protein hydrolysis. The movable electric heater was then vertically removed from the pressure chamber and the cooling jacket was manually tightened. Ethanol cooled at 0 °C was circulated through the cooling jacket coupled to a cryostat (FP-80, Julabo, Berlin, Germany) for rapid cooling. A K-type thermocouple and pressure transducer were installed in the pressure chamber to monitor the temperature and pressure changes during processing. Temperature and pressure were recorded with a data logger (34970A, Agilent Technologies, USA) for process intensity analysis. After the hydrothermal treatment, samples were immediately removed from the pressure chamber and cooled to 0 °C using ice water. The samples (porcine skin hydrolysates, PSHs) were named PSH-I (150 °C & 350 kPa), PSH-II (170 °C & 660 kPa), PSH-III (190 °C & 1100 kPa), PSH-IV (210 °C & 2100 kPa), PSH-V (230 °C & 2600 kPa), and PSH-VI (250 °C & 3900 kPa).

### 2.4. pH measurement

The pH levels of the samples (control and PSHs) were determined using a pH meter (Model S220, Mettler Toledo GmbH; Switzerland).

### 2.5. Color measurement

The color of the samples (control and PSHs) was determined using a colorimeter (Minolta Chroma meter CR-210; Japan) calibrated with a white standard (CIE $L^* = +97.83$, CIE $a^* = -0.43$, CIE $b^* = +1.96$). The color values of $L^*$, $a^*$, and $b^*$ were defined as indicators of lightness, redness, and yellowness, respectively. The total color difference ($\Delta E$) between the control and PSHs was