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Research Article

Improvement in Physicochemical, Microbial, and Sensory Properties of Common Squid (*Todarodes pacificus* Steenstrup) by Superheated Steam Roasting in Combination with Smoking Treatment

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The common squid (*Todarodes pacificus*) is one of the most widely consumed food ingredients in East Asian countries, especially Korea and Japan. It is generally marketed raw, which leads to inconvenient preparation, less commercial value, and short shelf life. Therefore, in order to handle these problems, superheated steam roasting (260°C heater, 240°C steam for 4 min) in combination with smoking treatment (15 min) using sawdust from the oak tree was used to process squid before mixing with vegetables and special sauce to make a ready-to-eat squid dish in an effective packaging. The processed squid product showed favorable sensory characteristics (appearance, odor, color, flavor, and texture) and physicochemical properties including maintained pH level (pH > 6.4), low microbial activity (4.74 ± 4.27 log CFU/g), low VBN level (18.27 ± 0.52 mg%), and good texture profile during storage for 10 days at 10°C. Results were significant at the significance level of $p < 0.05$. Findings of this study suggest that the superheated steam-roasting treatment was the best method to maintain nutritional value of the common squid. It also showed favorable sensory and physicochemical properties, while smoking treatment prolonged the shelf life through enhancing antioxidant and antimicrobial activities and enriched flavor of the squid product.

1. Introduction

The common squid (*Todarodes pacificus* Steenstrup) is an important commercial cephalopod species mainly found in East Asian countries including China, Korea, and Japan [1]. This squid has a maximum mantle length of 50 cm in females and somewhat smaller in males with a maximum weight of 0.5 kg (generally 0.1–0.3 kg). Female squids normally attain sexual maturity at a 20–25 cm mantle length while males at a 17–19 cm [2]. The *T. pacificus*, well known as O-Jing-Eo

(오징어) in Korean language, is largely distributed around the North Pacific Ocean, and due to its abundant quantity and special flavor, it is considered as a favorite ingredient for making squid-based food products for domestic consumption and as an export commodity [3, 4]. South Korea and Japan are the largest consumers of these squids, consumed either raw or as a processed product [5]. Consumption of the processed frozen squid in South Korea during 2013 was recorded as more than 200-kilo metric tons [6]. In South Korea, fried and dried squid products are considered as snacks or light food, while the raw squids are purchased to be cooked into various delicious dishes (e.g., stir-fried marinated squid) that require relatively delicate effort and time-consuming cooking process. However, this process could be simplified by providing a precooked and marinated squid products in an effective packaging that can even prolong its shelf life. Roasting and smoking are two commonly used methods for cooking seafood and meat products and are generally adopted during commercial foodservice operations [7].

Utilization of superheated steam roasting in the food industry is recognized as one of the highly effective cooking methods. Superheated steam is generated by providing additional heat to the saturated steam until a slightly higher temperature than the boiling point of H_2O is reached. Superheated steam provides a nonoxygen environment during the heating and roasting process which could eliminate the oxidation phenomenon in processed foods [8]. Superheated steam is also reported to minimize energy consumption during the roasting process [9]. It has also been reported that the roasting process of the superheated steam is better than the traditional cooking methods in reducing lipid oxidation and preserving food nutrient substances, color, and texture [10–14]. These beneficial factors make superheated steam an attractive alternative cooking method with minimum nutritional destruction, especially polyunsaturated fatty acids (PUFA) content in squids. Previously, in order to improve the food quality characteristics of the pen shells, a combined superheated steam-roasting and smoking method was proposed by our group [15].

Smoking is one of the traditional methods of preserving fish and other food products as it inhibits microbial growth and delays the oxidative changes [16]. It is done by releasing incomplete burning smoke from wood or sawdust and letting the volatile chemical compounds in the smoke enhance the aroma of the processed food while inhibiting the bacterial growth on its surface and in the flesh. Factors that affect the effectiveness of smoking treatment are the type of wood, type of volatile compound, and burning temperature [17–19]. Smoked food has special flavor and color that can improve the sensory characteristics and the commercial value of a food product [20].

Squid products are a rich source of polyunsaturated fatty acids, essential amino acids, and micronutrients including essential vitamins and minerals having antioxidant and anti-inflammatory effects [21–23]. Squid is highly appreciated for its nutritional and functional properties, which encourages research on the preparation, processing, and storage methods to minimize the quality degradation of the squid-based products. The nutritional, chemical, and physical composition of a food product varies depending on the processing method. The heating process has been considered a favorable method to minimize the quality degradation of a food product; however, this method is also known to cause denaturation of myosin at ± 50°C and actin at ± 70°C [24]. Changes in protein structure would affect the texture and palatability of the squid meat. Texture has been considered a crucial parameter to evaluate the quality of a food product; thus, it is important to understand the physical changes in texture during heating. The quality and shelf life of squid differ while using different heating processes and have different acceptability by consumers [25].

In order to make sure that the consumers get the best nutritional content and savory taste of a processed food, each processing method should be compared and studied in detail. This study aimed at finding the best preparation method for processing squid that could enhance not only its sensory characteristics but also its physicochemical parameters with extended shelf life leading to the elevated commercial value. The effects of superheated steam treatment on processing squid were studied and compared with the conventional hot steam oven treatment through analyzing the sensory characteristics (i.e., appearance, odor, texture, flavor, and overall acceptance), physicochemical characteristics (i.e., pH level, VBN level, TBARS level, fatty acid content, and texture profile), and microbial activity. The effects of superheated steam roasting in combination with smoking treatment to prolong shelf life of the processed squid product during storage were also investigated.

2. Materials and Methods

2.1. Experimental Sample. The common squids (*T. pacificus*) were obtained from EBADA Fishery Co. Ltd., Busan, Republic of Korea, and were divided into three groups, namely, (i) raw sample, (ii) steam oven-roasted sample, and (iii) superheated steam-roasted sample.

2.2. Roasting Treatment of Squid. Squids were treated using two different roasting methods: (i) steam oven roasting and (ii) superheated steam roasting. The steam oven-roasting method was performed by placing 1 kg of the sample in a steam oven (SK Magic, Seoul, South Korea) at 230, 240, and 250°C and roasting for 5, 10, and 15 min. Similarly, the squid sample used for superheated steam roasting was placed in an Aero-Steam Oven (Naomoto Corporation, Osaka, Japan) at 240, 260, and 280°C and roasted for 3, 4, and 5 min. The roasted samples were placed in polyamide/low-density polyethylene bags and vacuumed using LOVERO® vacuum sealer (Sambo Tech Corporation, Gyeonggi-do, South Korea) and stored at 4°C for later use.

2.3. Smoking Treatment. Superheated steam-roasted samples were then smoked using a time-controlled electric smoker (Braai Smoker, Bradley, Canada). Briefly, the samples were placed in a drying chamber and passed through a tray (42 cm × 47 cm × 4 cm) while smoking at 65°C for 10, 15, and 20 min. The combustion of oak sawdust produced smoke for the smoking treatment, and the smoked samples were kept at 4°C for further experiments.

2.4. Preparation of Squid Product. The processed squid product was prepared by mixing the superheated steamed and smoked squid samples with vegetables (a mixture of spring onion, onion, and cabbage) and sauce at a ratio of 6 : 3 : 1 (Table 1). The prepared product was placed in vacuum plastic bags and pasteurized in a water bath at 65°C for 15 min. The final product was stored at 10°C and 15°C for shelf life analysis.

2.5. Sensory Evaluation. Sensory evaluation for appearance, flavor, odor, texture, and overall acceptance of the treated squids was performed by thirty panelists aged between 25 and 40 years old. The panelists involved in the sensory evaluation were researchers at the Industry-Academic Cooperation Foundation, Silla University, Busan, Rep. of Korea, and were trained and certified panelists for the sensory evaluation test. The sensory evaluation test was approved by the Institutional Review Board of Silla University, Busan, Republic of Korea. Before evaluation, all experimental samples were encoded. Approximately, 10 g of each squid sample group was served. Each panelist randomly evaluated all samples and was asked to give a numerical score for each group. In order to simplify the marking system, a 1- to 9-point hedonic scale was used, where 1 indicating as the extremely disliked and 9 as the extremely liked. Between these extreme values, 5 was considered as the threshold value, and a sample valued less than 5 was considered unacceptable [26].

2.6. pH Measurement. The final squid product (2 g) was mixed with 18 mL of distilled water and homogenized using a homogenizer (Scilab, Seoul, Rep. of Korea) for 3 min. The pH of the homogenate was measured at room temperature using a pH meter (Ohaus, Seoul, Rep. of Korea). Three measurements were taken for each sample.

2.7. Odor Intensity. Odor intensity of the samples was measured as described previously by Mohibbullah et al. [15]. The final product sample (5 g) was placed in a 50 mL conical tube and covered with Parafilm. After closing the lid, odor intensity was measured using an odor intensity meter (New Cosmos Electric Co. Ltd., Osaka, Japan). Measurements of odor intensity were sustained until the higher peak signal appeared and were expressed as an arbitrary unit.

2.8. Volatile Basic Nitrogen. To quantitatively assess the level of volatile basic nitrogen (VBN) produced in the samples, the Conway microdiffusion method with some modifications was used [27]. The final product sample (5 g) was diluted with 25 mL of distilled water in a 50 mL conical tube and vortexed for 5 min. The prepared mixture was filtered through a filter paper No. 2 (55 mm; Advantech, Tokyo, Japan). After filtration, 1 mL of the sample solution and 1 mL of potassium carbonate were added in the outer chamber of the Conway unit, and 1 mL of 0.01 M H_2SO_4 was added in the inner chamber. The Conway cell was closed, tightened with clip, and incubated at 37°C for 90 min. After incubation, 2-3 drops of Brunswick reagent were added in the inner chamber and titrated with 0.01 N NaOH until no color change was observed.

Table 1: Ingredients of sauce used in this study.

Ingredients	(%)
High-fructose corn syrup	4
Mixed seasoning	2
Seasoned red pepper powder	3
Garlic	2.3
Refined salt water	8
Onion	1
Dried red pepper powder	10
Soy sauce	20
Purified water	30
Xanthan gum	3
Paprika extract color	0.5
Monosodium glutamate	5
Black pepper	0.5
Base shielding oil	2.5
Flavor enhancer	2
White sugar	4
Soybean liquid	1.2
Oleoresin capsicum	1

2.9. Thiobarbituric Acid-Reactive Species. Thiobarbituric acid-reactive species (TBARS) values were measured as reported previously by Peiretti et al. [28], with slight modifications. The final sample (5 g) was homogenized in 12.5 mL TCA solution (20% trichloroacetic acid in 2 M phosphoric acid), and the total volume was adjusted to 25 mL with distilled water. The homogenate was then centrifuged for 10 min at 1500 rpm. The supernatant was collected and filtered through a filter paper No. 2 (55 mm; Advantech, Tokyo, Japan). Two milliliters of the supernatant was mixed with 2 mL of 0.005 M thiobarbituric acid solution followed by incubation in a water bath at 95°C for 30 min. After cooling the samples down to room temperature, 200 μL of each sample including blank group (distilled water) was added to a 96-well plate, and the absorbance was measured at 530 nm using a nano-SPECTRO star (Newtown, UK). Lipid peroxidation values were expressed in mg of malonaldehyde per kg of squid.

2.10. Total Bacterial Count and Total Coliform Count. Total bacterial count (TBC) was measured by following the methods previously reported by Chen et al. [29] and Bashir et al. [30], with slight modifications. The final product (5 g) was mixed with 45 mL of sterile saline and homogenized in sterilized plastic bags for 3 min using a Stomacher 400 Circulator (Seward Limited, West Sussex, UK). Three serial dilutions of the homogenate were prepared, spread onto Difco™ plate count agar (BD Company, New Jersey, USA),

Table 2: Sensory evaluation of steam oven-treated squid.

Temp. (°C)	Time (min)	Appearance	Odor	Flavor	Texture	Preference
230	5	6.40 ± 0.66^{a}	6.00 ± 0.00^{a}	6.15 ± 0.47^{a}	6.30 ± 0.48^{a}	6.15 ± 0.24^{a}
	10	7.45 ± 0.50^{bc}	6.05 ± 0.16^{a}	6.90 ± 0.57^{ab}	6.70 ± 0.54^{ab}	6.90 ± 0.39^{a}
	15	7.15 ± 0.24^{ab}	6.40 ± 0.52^{ab}	6.10 ± 0.88^{a}	6.40 ± 0.52^{a}	6.55 ± 0.44^{a}
240	5	6.80 ± 0.79^{ab}	6.00 ± 0.00^{a}	6.40 ± 0.52^{a}	6.25 ± 0.49^{a}	6.20 ± 0.35^{a}
	10	$\mathbf{7.90 \pm 0.32^{c}}$	$\mathbf{7.45 \pm 0.69^{c}}$	$\mathbf{7.80 \pm 0.79^{c}}$	$\mathbf{7.70 \pm 0.42^{c}}$	$\mathbf{8.50 \pm 0.28^{c}}$
	15	7.10 ± 0.52^{ab}	6.80 ± 0.35^{b}	7.45 ± 0.37^{bc}	6.75 ± 0.54^{ab}	7.40 ± 0.32^{b}
250	5	6.75 ± 0.68^{ab}	6.05 ± 0.16^{a}	6.40 ± 0.32^{a}	6.05 ± 0.16^{a}	6.20 ± 0.26^{a}
	10	7.00 ± 0.67^{ab}	6.65 ± 0.85^{ab}	7.15 ± 0.47^{ab}	7.10 ± 0.32^{bc}	7.25 ± 0.26^{ab}
	15	6.40 ± 0.21^{a}	6.15 ± 0.24^{a}	6.20 ± 0.92^{a}	6.30 ± 0.48^{a}	6.25 ± 0.35^{a}

Values are mean ± SD ($n = 30$). Different letters (a–c) indicate significant differences among means by Tukey's test at $p < 0.05$.

and incubated at 37°C for 48 h. After incubation, the observed colonies were counted and calculated according to the dilution factor. The total bacterial count was expressed as log CFU/g. For *Escherichia coli* and total coliforms count, only one serial dilution was made, and each of the homogenates was spread-plated on Difco™ EC medium (BD Company, New Jersey, USA) and incubated at 37°C for 24 h. The results were considered to be negative if no gas production was found in the medium.

2.11. Textural Analysis. Squid samples (1.0 cm^3) from each group were subjected to a texture profile analysis (TPA) to estimate the hardness, adhesiveness, cohesiveness, springiness, gumminess, and chewiness index. The TPA test involves compressing the samples to 50% of their initial height and withdrawing to the original height, followed by a second compression-withdrawal cycle. A texture analyzer (Brookfield, Massachusetts, USA) equipped with a cylinder glass probe (12.7 mm in diameter and 35 mm in length) and operated by software Texture PRO CT V1 (Middleboro, USA) was used for TPA test. Test speed was 0.5 mm/s, and there were three replicates for each experimental sample.

2.12. Fatty Acid Analysis by Gas Chromatography. Fatty acids were extracted from each squid sample by the hydrolytic method. Fatty acids were extracted by ether and then methylated to fatty acid methyl esters (FAMEs). FAMEs were quantitatively analyzed by gas chromatography (Shimadzu Corp., Kyoto, Japan) with a flame ionization detector (GC-FID). FAME samples were separated through a Supelco SP-2560 column (100 m × 0.25 mm × 0.25 μm) with an oven temperature of 240°C, which increased from 100°C to 200°C at a flow rate of 3.5°C/min. The carrier gas used was helium with a split ratio of 1 : 50. The identification and quantitation of FAME samples were performed by comparing the retention time with fatty acids standard.

2.13. Statistical Analysis. All experiments were performed in triplicate, and the data were expressed as mean ± standard deviation (SD). Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test, and results were considered statistically significant at $p < 0.05$. All statistical procedures were performed using IBM SPSS statistics version 23.0 (IBM, Corp., New York, USA).

3. Results

3.1. Optimum Conditions for Steam Oven Roasting. The optimum steam oven-roasting conditions for squid were obtained by measuring the sensory evaluation scores for appearance, odor, flavor, chewiness, and preferences under 3 different temperatures for a time duration of 5, 10, and 15 min, respectively, as shown in Table 2. The squids heated at 240°C for 10 min showed significantly ($p < 0.05$) higher scores in all test categories, and it was considered the optimum treatment condition for processing squid using a steam oven.

3.2. Optimum Conditions for Superheated Steam Roasting. The optimum conditions for superheated steam roasting were obtained by measuring the sensory evaluation scores for appearance, color, odor, flavor, and texture of squid that underwent a sequence of heating and steaming treatment for a certain amount of time. Table 3 shows the results of superheated steam treatment of squid at 240, 260, and 280°C heating sequenced with steaming at 220, 240, and 260°C for the time duration of 3, 4, and 5 min, respectively. Squids that underwent 260°C heating and 240°C steaming for 4 min showed significantly ($p < 0.05$) higher scores among all the tested parameters.

3.3. Comparison of Sensory, Physicochemical, and Microbial Properties of Steam Oven-Roasted, Superheated Steam-Roasted, and Raw Squid. Sensory, physicochemical, and microbial properties of the processed and raw squids were studied to compare the quality parameters of each roasting treatment against the raw state. Results of the sensory evaluation, demonstrating the preference criteria for appearance, odor, taste, texture, and overall preference are shown in Table 4. The squids treated with superheated steam-roasting treatment showed significantly ($p < 0.05$) higher scores of 8.45 ± 0.55, 8.40 ± 0.46, 8.55 ± 0.44, 8.20 ± 0.42, and 8.65 ± 0.34 for appearance, odor, taste, texture, and overall preference, respectively.

Roasting treatment on squid affected its physicochemical properties including pH value as shown in Figure 1(a). The

Table 3: Sensory evaluation of superheated steam-treated squid.

Heater (°C)	Steam (°C)	Time (min)	Appearance	Odor	Flavor	Texture	Preference
240	220	3	7.50 ± 0.53^{b}	7.10 ± 0.21^{ab}	6.75 ± 0.49^{a}	6.65 ± 0.34^{a}	6.82 ± 0.37^{ab}
	240		7.40 ± 0.52^{b}	7.45 ± 0.50^{b}	6.65 ± 0.53^{a}	6.60 ± 0.52^{a}	6.90 ± 0.32^{ab}
	260		7.60 ± 0.52^{b}	7.15 ± 0.82^{ab}	7.05 ± 0.50^{ab}	7.00 ± 0.41^{b}	7.05 ± 0.28^{ab}
	220	4	7.65 ± 0.47^{bc}	6.99 ± 0.32^{ab}	7.07 ± 0.29^{ab}	6.93 ± 0.29^{ab}	7.05 ± 0.28^{ab}
	240		7.50 ± 0.58^{b}	6.90 ± 0.46^{ab}	7.30 ± 0.35^{ab}	6.97 ± 0.34^{ab}	7.08 ± 0.16^{ab}
	260		7.30 ± 0.54^{ab}	7.47 ± 0.47^{b}	7.30 ± 0.48^{ab}	7.15 ± 0.53^{b}	7.40 ± 0.39^{b}
	220	5	7.25 ± 0.35^{ab}	7.37 ± 0.40^{b}	7.45 ± 0.48^{b}	7.35 ± 0.41^{b}	7.45 ± 0.37^{b}
	240		7.60 ± 0.57^{b}	7.45 ± 0.32^{b}	7.70 ± 0.59^{b}	7.65 ± 0.39^{b}	7.69 ± 0.27^{b}
	260		7.50 ± 0.58^{b}	7.76 ± 0.33^{bc}	7.83 ± 0.47^{bc}	7.82 ± 0.23^{b}	7.88 ± 0.21^{bc}
260	220	3	7.76 ± 0.31^{bc}	7.74 ± 0.19^{bc}	7.89 ± 0.12^{bc}	7.89 ± 0.12^{bc}	7.90 ± 0.13^{bc}
	240		7.79 ± 0.30^{bc}	7.82 ± 0.15^{bc}	7.85 ± 0.22^{bc}	7.93 ± 0.17^{bc}	7.94 ± 0.10^{bc}
	260		7.54 ± 0.47^{b}	7.88 ± 0.39^{bc}	8.10 ± 0.32^{bc}	8.07 ± 0.29^{bc}	7.97 ± 0.07^{bc}
	220	**4**	8.01 ± 0.20^{bc}	8.65 ± 0.47^{c}	8.67 ± 0.30^{c}	8.50 ± 0.33^{c}	8.40 ± 0.21^{c}
	240		$\mathbf{8.78 \pm 0.34^{c}}$	$\mathbf{9.00 \pm 0.00^{c}}$	$\mathbf{8.91 \pm 0.10^{c}}$	$\mathbf{8.80 \pm 0.22^{c}}$	$\mathbf{8.87 \pm 0.09^{c}}$
	260		8.25 ± 0.27^{c}	8.35 ± 0.25^{c}	8.30 ± 0.26^{c}	8.27 ± 0.25^{c}	8.12 ± 0.21^{c}
	220	5	7.71 ± 0.48^{bc}	8.15 ± 0.24^{bc}	8.37 ± 0.26^{c}	7.99 ± 0.19^{bc}	7.97 ± 0.18^{bc}
	240		7.66 ± 0.46^{b}	7.87 ± 0.20^{bc}	7.70 ± 0.42^{b}	7.84 ± 0.20^{bc}	7.80 ± 0.26^{b}
	260		7.40 ± 0.52^{b}	7.73 ± 0.29^{bc}	7.45 ± 0.34^{b}	7.68 ± 0.20^{b}	7.65 ± 0.16^{b}
280	220	3	7.32 ± 0.35^{ab}	7.14 ± 0.21^{ab}	7.10 ± 0.18^{ab}	7.03 ± 0.07^{b}	7.08 ± 0.10^{ab}
	240		7.22 ± 0.29^{ab}	7.20 ± 0.26^{ab}	7.13 ± 0.16^{ab}	7.05 ± 0.11^{b}	7.06 ± 0.10^{ab}
	260		7.08 ± 0.18^{a}	7.06 ± 0.13^{ab}	7.01 ± 0.11^{ab}	6.87 ± 0.31^{ab}	6.97 ± 0.05^{ab}
	220	4	6.92 ± 0.15^{a}	6.95 ± 0.05^{ab}	6.94 ± 0.05^{ab}	6.92 ± 0.06^{ab}	6.93 ± 0.07^{ab}
	240		6.96 ± 0.05^{a}	6.75 ± 0.26^{a}	6.64 ± 0.15^{a}	6.61 ± 0.14^{a}	6.66 ± 0.14^{a}
	260		6.95 ± 0.07^{a}	6.58 ± 0.13^{a}	6.57 ± 0.12^{a}	6.52 ± 0.06^{a}	6.62 ± 0.13^{a}
	220	5	6.76 ± 0.33^{a}	6.53 ± 0.23^{a}	6.50 ± 0.21^{a}	6.50 ± 0.19^{a}	6.43 ± 0.24^{a}
	240		6.63 ± 0.32^{a}	6.25 ± 0.26^{a}	6.30 ± 0.26^{a}	6.20 ± 0.26^{a}	6.25 ± 0.26^{a}
	260		6.25 ± 0.26^{a}	6.16 ± 0.24^{a}	6.12 ± 0.21^{a}	6.07 ± 0.16^{a}	6.05 ± 0.16^{a}

Values are mean ± SD ($n = 30$). Different letters (a–c) indicate significant differences among means by Tukey's test at $p < 0.05$.

Table 4: Sensory evaluation of raw, superheated steam-treated, and steam oven-treated squid.

Group	Appearance	Odor	Flavor	Texture	Preference
Raw squid	7.45 ± 0.28^{a}	7.40 ± 0.32^{a}	—	7.45 ± 0.37^{a}	7.45 ± 0.28^{a}
Superheated steam-treated squid	$\mathbf{8.45 \pm 0.55^{b}}$	$\mathbf{8.40 \pm 0.46^{c}}$	$\mathbf{8.55 \pm 0.44}$	$\mathbf{8.20 \pm 0.42^{b}}$	$\mathbf{8.65 \pm 0.34^{c}}$
Steam oven-treated squid	7.65 ± 0.47^{a}	7.90 ± 0.21^{b}	7.70 ± 0.42	7.86 ± 0.21^{b}	7.80 ± 0.26^{b}

Values are mean ± SD ($n = 30$). Different letters (a–c) indicate significant differences among means by Tukey's test at $p < 0.05$.

pH level of raw squid was 6.36 ± 0.03, while the superheated steam and steam oven-treated squids showed pH levels of 6.83 ± 0.02 and 6.72 ± 0.05, respectively. Superheated steam treatment significantly ($p < 0.05$) increased the pH of squids to a normal pH level (pH = 7) compared to the steam oven-treated squids. Roasting treatment also affected odor intensity as shown in Figure 1(b). The odor intensity of raw squid was 286.50 ± 32.13, while the superheated steam-treated and steam oven-treated squids showed an odor intensity of 204.50 ± 21.66 and 120.50 ± 25.97, respectively. The steam oven treatment reduced the odor intensity more effectively than superheated steam treatment.

VBN is one of the important parameters to determine freshness of the seafood products. The effect of roasting treatment on VBN is shown in Figure 1(c). Raw squids exhibited a significantly ($p < 0.05$) higher VBN level of 4.89 ± 0.35 mg% compared to superheated steam (2.21 ± 0.40 mg%) and steam oven-treated (4.04 ± 0.63 mg%) squids. Superheated steam significantly ($p < 0.05$) reduced the VBN levels in squid samples.

TBARS value is an index of the lipid oxidation measuring MDA content that is formed through hydroperoxides, the initial reaction product of PUFA with oxygen [31, 32]. Lipid oxidation is an important factor that causes spoilage in fishery products and has become a major quality problem due to the development of disagreeable odor and taste of fatty marine species. A significant ($p < 0.05$) reduction in TBARS values of the samples treated using a superheated steam oven (0.518 ± 0.02) and a slight reduction in samples treated by steam oven treatment (0.714 ± 0.018) compared with the raw squid (0.722 ± 0.027) were observed (Figure 1(d)).

The bacterial count in raw squids was extremely high (3.72 ± 0.1 log CFU/g), while the superheated steam (1.77 ± 0.09 log CFU/g) and steam oven-treated (2.46 ± 0.04 log CFU/g) squids showed a significant ($p < 0.05$) reduction in bacterial count (Figure 1(e)). *E. coli* and coliform bacteria were not detected in any of the tested squid samples. Superheated steam treatment showed a lower bacterial count compared with other groups, thus it was

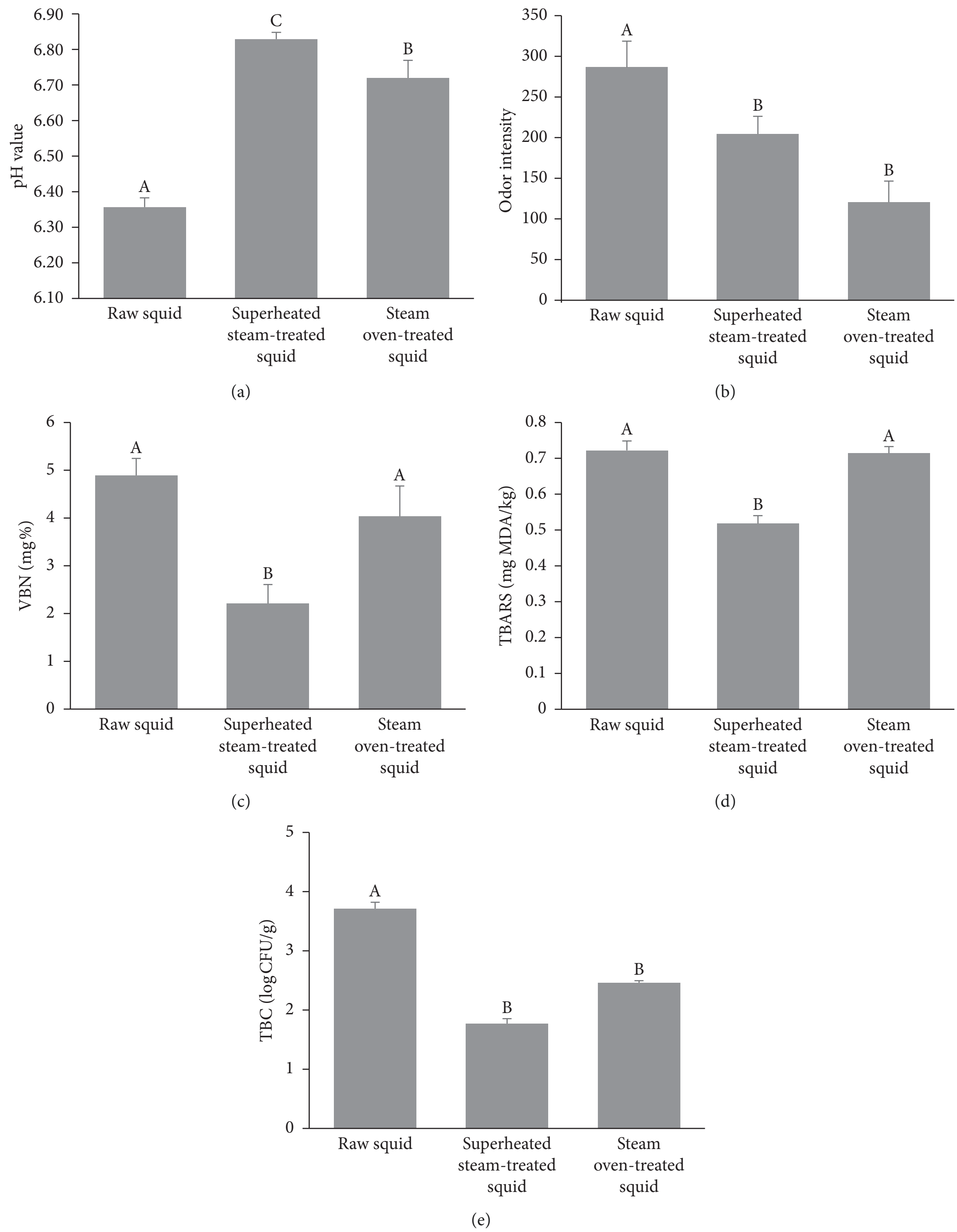

FIGURE 1: Physicochemical and microbial properties of raw, superheated steam-treated, and steam oven-treated squid. (a) pH value, (b) odor intensity, (c) VBN value, (d) TBARS value, and (e) TBC. Different letters (A–C) indicate significant differences among means by Tukey's test at $p < 0.05$. Values are mean ± SD ($n = 10$).

suggested to be the most effective treatment to reduce the bacterial count in the processed squids.

The textural profile of the squid processed using superheated steam-roasting treatment is shown in Table 5. The hardness level for the raw squid sample was recorded as 20.22 ± 3.10 g, while for the superheated steam-treated and steam oven-treated squids, it was 2.13 ± 0.59 g and 2.70 ± 0.39 g, respectively. Interestingly, adhesiveness of the raw squid samples was recorded as 0.08 ± 0.04 g/cm. However, the superheated steam-treated squid samples showed a slightly higher adhesiveness of 0.10 ± 0.00 g/cm, and the steam oven-treated squid samples showed a

Table 5: Textural properties of raw, superheated steam-treated, and steam oven-treated squid.

Group	Hardness (g)	Adhesiveness (g/cm)	Cohesiveness	Springiness (mm)	Gumminess (g)	Chewiness (g/cm)
Raw squid	20.22 ± 3.10^a	0.08 ± 0.04^a	0.60 ± 0.18^a	1.14 ± 0.18^a	10.58 ± 3.42^a	13.44 ± 3.20^a
Superheated steam-treated squid	$\mathbf{2.13 \pm 0.59^b}$	$\mathbf{0.10 \pm 0.00^a}$	$\mathbf{0.74 \pm 0.07^a}$	$\mathbf{1.56 \pm 0.11^b}$	$\mathbf{1.62 \pm 0.55^b}$	$\mathbf{2.54 \pm 0.95^b}$
Steam oven-treated squid	2.70 ± 0.39^b	0.02 ± 0.04^b	0.74 ± 0.07^a	1.65 ± 0.03^b	2.00 ± 0.27^b	3.32 ± 0.44^b

Values are mean ± SD ($n = 10$). Different letters (a-b) indicate significant differences among means by Tukey's test at $p < 0.05$.

Table 6: Fatty acids composition of raw, superheated steam-treated, and steam oven-treated squid.

Fatty acids		Raw squid (g/100 g)	Superheated steam (g/100 g)	Steam oven (g/100 g)
Myristic acid	C14 : 0	0.01	0.02	0.03
Pentadecanoic acid	C15 : 0	0.00	0.01	0.01
Palmitic acid	C16 : 0	0.23	0.48	0.51
Palmitoleic acid	C16 : 1	0.00	0.01	0.01
Margaric acid	C17 : 0	0.01	0.01	0.01
Stearic acid	C18 : 0	0.03	0.05	0.06
Tricosanoic acid	C23 : 0	0.00	0.00	0.03
$\sum$ *SFA*		**0.28**	**0.58**	**0.66**
Oleic acid	C18 : 1 *n*-9c	0.03	0.06	0.05
cis-11-Eicosenoic acid	C20 : 1 *n*-9	0.03	0.06	0.07
Erucic acid	C22 : 1 *n*-9	0.01	0.02	0.02
Nervonic acid	C24 : 1 *n*-9	0.00	0.01	0.01
$\sum$ *MUFA*		**0.07**	**0.15**	**0.15**
Linolelaidic acid	C18 : 2 *n*-6t	0.00	0.01	0.00
Linoleic acid	C18 : 2 *n*-6c	0.00	0.01	0.01
cis-11,14-Eicosadienoic acid	C20 : 2 *n*-6	0.00	0.00	0.01
Arachidonic acid	C20 : 4 *n*-6	0.01	0.02	0.00
$\sum n-6$		0.01	0.04	0.02
cis-11,14,17-Eicosatrienoic acid	C20 : 3 *n*-3	0.00	0.01	0.01
Eicosapentaenoic acid	C20 : 5 *n*-3	0.19	0.40	0.43
Docosahexaenoic acid	C22 : 6 *n*-3	0.62	1.26	1.39
$\sum n-3$		0.81	1.67	1.83
$\sum$ *PUFA*		**0.82**	**1.71**	**1.85**
n-3/n-6		**81**	**41.75**	**91.5**

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

significantly ($p < 0.05$) lower adhesiveness of 0.02 ± 0.04 g/cm. Raw squids showed a nonsignificant cohesiveness value of 0.60 ± 0.18 points as compared with the treated squids (0.74 ± 0.07 points). A significantly ($p < 0.05$) lower springiness level of 1.14 ± 0.18 mm was observed for raw squids compared to superheated steam-treated (1.56 ± 0.11 mm) and steam oven-treated (1.65 ± 0.03 mm) squids. Higher gumminess levels of 10.58 ± 3.42 g were recorded for raw squids, while the superheated steam-treated squids showed significantly ($p < 0.05$) lower levels of 1.62 ± 0.55 g. Significantly ($p < 0.05$) higher chewiness values of 13.44 ± 3.20 g/cm were observed in raw squids than in the superheated steam-treated squids (2.54 ± 0.95 g/cm) and the steam oven-treated squids (3.32 ± 0.44 g/cm). The superheated steam-treated squids showed better textural properties compared to steam oven-treated squids.

Changes in the fatty acid composition of squid roasted using different methods are shown in Table 6. From the overall fatty acid content of squid samples, PUFA had the highest value, followed by saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA). Palmitic acid, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) were dominant fatty acids in squid. Roasting treatment affected the fatty acid content of the squid with a consistently increasing value of $\sum$ SFA, $\sum$ MUFA, and $\sum$ PUFA compared to the raw squid samples.

All of the above results suggest that superheated steam roasting can be considered the best treatment that could exhibit outstanding performance in improving physicochemical, microbial and sensory properties of squid.

3.4. Optimum Condition for Smoking Treatment. The optimum conditions for smoking treatment were obtained by measuring the sensory evaluation scores for appearance, odor, flavor, and texture of the squids that underwent smoking treatment after superheated steam-roasting treatment for a certain amount of time. A smoking treatment for 15 min significantly ($p < 0.05$) increased all sensory evaluation points, thus it was concluded as the optimum condition for smoking treatment (Table 7).

3.5. Sensory Evaluation of the Processed Squid. The sensory evaluation results of superheated steam-roasting and

Table 7: Sensory evaluation of oak sawdust-smoke-treated squid at different time durations.

Smoking time (min)	Appearance	Odor	Flavor	Texture	Preference
0	7.65 ± 0.58^{a}	7.30 ± 0.67^{a}	7.45 ± 0.90^{a}	7.45 ± 0.69^{a}	7.55 ± 0.69^{a}
10	8.00 ± 0.62^{ab}	7.80 ± 0.92^{ab}	7.90 ± 0.84^{a}	7.70 ± 0.67^{a}	8.00 ± 0.33^{a}
15	$\mathbf{8.65 \pm 0.47^{b}}$	$\mathbf{8.60 \pm 0.52^{b}}$	$\mathbf{8.85 \pm 0.34^{b}}$	$\mathbf{8.65 \pm 0.41^{b}}$	$\mathbf{8.90 \pm 0.21^{b}}$
20	7.85 ± 0.58^{a}	7.50 ± 0.47^{a}	7.45 ± 0.44^{a}	7.50 ± 0.47^{a}	7.60 ± 0.32^{a}

Values are mean ± SD ($n = 30$). Different letters (a-b) indicate significant differences among means by Tukey's test at $p < 0.05$.

Table 8: Sensory evaluation of the processed squid stored at different temperatures.

Storage temperature (°C)	Parameters	Days						
		0	2	6	8	10	12	14
10	Appearance	8.95 ± 0.16^{c}	8.90 ± 0.21^{c}	8.35 ± 0.41^{c}	8.00 ± 0.33^{c}	6.75 ± 0.26^{b}	5.25 ± 0.35^{b}	4.25 ± 0.35^{a}
	Smell	8.90 ± 0.21^{c}	8.90 ± 0.21^{c}	8.25 ± 0.26^{c}	7.90 ± 0.32^{c}	6.85 ± 0.47^{b}	5.15 ± 0.24^{b}	4.45 ± 0.37^{a}
	Taste	9.00 ± 0.00^{c}	8.95 ± 0.16^{c}	8.35 ± 0.34^{c}	7.95 ± 0.37^{b}	7.20 ± 0.63^{b}	—	—
	Texture	8.90 ± 0.21^{c}	8.90 ± 0.21^{c}	8.35 ± 0.41^{c}	7.80 ± 0.26^{c}	6.75 ± 0.42^{b}	5.25 ± 0.35^{b}	4.30 ± 0.26^{a}
	Preference	8.95 ± 0.16^{c}	8.90 ± 0.21^{c}	8.30 ± 0.42^{c}	7.80 ± 0.26^{c}	6.90 ± 0.39^{b}	5.35 ± 0.24^{b}	4.25 ± 0.26^{a}
15	Appearance	8.95 ± 0.16^{c}	8.90 ± 0.21^{c}	5.40 ± 0.46^{b}	4.60 ± 0.39^{a}	4.20 ± 0.26^{a}	3.90 ± 0.21^{a}	3.80 ± 0.26^{a}
	Smell	8.90 ± 0.21^{c}	8.90 ± 0.21^{c}	5.30 ± 0.35^{b}	4.25 ± 0.35^{a}	4.10 ± 0.21^{a}	3.80 ± 0.26^{a}	3.65 ± 0.24^{a}
	Taste	8.95 ± 0.16^{c}	8.95 ± 0.16^{c}	5.45 ± 0.37^{a}	—	—	—	—
	Texture	8.90 ± 0.21^{c}	8.90 ± 0.21^{c}	5.20 ± 0.35^{b}	4.40 ± 0.46^{a}	4.15 ± 0.24^{a}	3.80 ± 0.26^{a}	3.75 ± 0.26^{a}
	Preference	8.95 ± 0.16^{c}	8.75 ± 0.26^{c}	5.45 ± 0.37^{b}	4.35 ± 0.34^{a}	4.10 ± 0.21^{a}	3.85 ± 0.24^{a}	3.70 ± 0.26^{a}

Values are mean ± SD ($n = 30$). Different letters (a–c) indicate significant differences among means by Tukey's test at $p < 0.05$.

smoking treatment of the squid processed product are shown in Table 8. Five main parameters including appearance, odor, flavor, texture, and overall preference were measured at 10°C and 15°C storage temperatures for 2, 6, 8, 10, 12, and 14 days. All parameters showed a downward trend in evaluation values from day 0 through day 14 with 5 as the threshold value to determine the acceptability of the squid condition. At 10°C storage temperature, samples were managed to maintain their condition up to day 10, while at 15°C, squid's preserving capability was shortened to day 6.

3.6. Effect of Superheated Steam Roasting and Smoking Combined Treatment on pH Value. The pH values of superheated steam roasting and smoking combined treated squids are shown in Figure 2(a). The treated squids stored at 10°C showed better performance in maintaining pH above 6.4 for a maximum of 12 days, while the samples stored at 15°C showed a significant ($p < 0.05$) drop in pH value (pH 6.0) at day 10 and continued to decrease until it reached pH 5.56 at day 14.

3.7. Effect of Superheated Steam Roasting and Smoking Combined Treatment on Odor Intensity. Odor is considered an important factor that could significantly stimulate the appetite. The squids stored at 10°C showed significant ($p < 0.05$) decrease in odor intensity (396.67 ± 7.51) after day 14, while the odor intensity of samples stored at 15°C significantly ($p < 0.05$) increased to 686.67 ± 13.05 (Figure 2(b)).

3.8. Effect of Superheated Steam Roasting and Smoking Combined Treatment on VBN. VBN is considered as the indicator of measuring the degree of product's spoilage primarily due to ammonia production after deamination of amino acids and trimethylamine by spoilage bacteria and autolytic enzymes during frozen storage [33]. At a storage temperature of 15°C, the VBN value significantly ($p < 0.05$) increased from day 0 (5.85 ± 0.49) to day 14 (27.82 ± 1.22), while at 10°C, the increment decreased to 23.35 ± 0.52 (Figure 2(c)). This indicates that storing the processed squid product at 10°C could extend its shelf life.

3.9. Effect of Superheated Steam Roasting and Smoking Treatment on TBC. TBC is the main indicator for the level of consumption safety of a food product. A 10°C storage temperature was capable to maintain the bacterial growth from day 0 (3.18 ± 0.88 log CFU/g) until day 14 (5.01 ± 3.60 log CFU/g), while at the 15°C, the TBC rapidly increased at day 6 (>log 6 CFU/g) as shown in Table 9. The optimum temperature to store processed squid product with favorable bacterial count was less than 10°C.

3.10. Effect of Superheated Steam Roasting and Smoking Combined Treatment on Texture. The processed squids stored at 10°C showed significant differences in textural properties during a storage period of 14 days compared to the squids stored at 15°C (Table 10). The textural parameters that maintained their values through the evaluation period include adhesiveness, cohesiveness, and springiness, while hardness, gumminess, and chewiness showed an upward trend from day 0 to day 10 and a downward trend from day 10 to day 14.

4. Discussion

Seafood products are easily subjected to spoilage which leads to poor meat quality characteristics including deteriorated

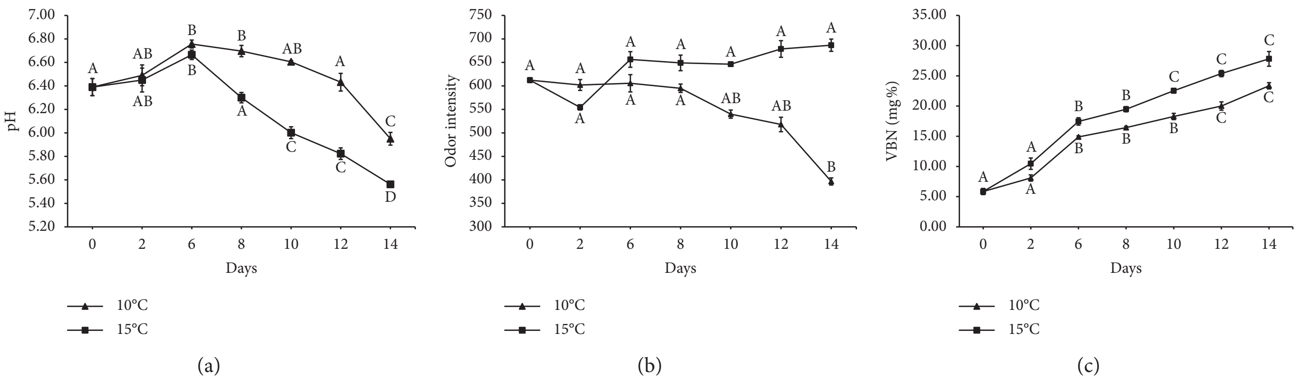

Figure 2: pH value (a), odor intensity (b), and VBN level (c) of the processed squid stored at different temperatures. Values are mean ± SD ($n = 10$). Different letters (A–C) indicate significant differences among means by Tukey's test at $p < 0.05$.

Table 9: Total bacterial count of processed squid stored at different temperatures.

Storage temperature (°C)	Days						
	0	2	6	8	10	12	14
10	3.18 ± 0.88	3.61 ± 2.32	4.35 ± 3.68	4.71 ± 3.56	4.74 ± 4.27	4.94 ± 4.01	5.01 ± 3.60
15	3.18 ± 0.88	3.81 ± 2.79	>6	>6	>6	>6	>6

Values are mean ± SD ($n = 10$); unit = log CFU/g.

flesh, bad odor, and inedibility of the whole product [33]. Thus, there is a need to explore new preservation methods to prolong the shelf life and to improve the seafood product value. In this study, the common preservation methods including steam oven roasting and superheated steam roasting were tested on squids. Furthermore, the effects of superheated steam roasting and smoking combined treatment to enhance the shelf life of the processed squid product were also investigated.

Freshness is one of the key factors for consumers in selecting squid due to its direct correlation to taste. The quickest, easiest, and most common way to determine the quality of squid is by sensory assessment that could be done by human senses such as sight (to determine shape and color of the squid), tactile (to test the flesh elasticity), and olfaction (to evaluate the odor level) [10]. The sensory assessment in this study was conducted to understand the consumer preference between raw, superheated oven-treated, and steam oven-treated squids. The superheated steam oven treatment showed better preference scores compared to other treatments. This was consistent with the experiment of Prachayawarakorn et al. [34]. They reported that superheated steam treatment improved textural properties of shrimp and showed a lower degree of shrinkage compared to the conventional hot air oven treatment. Other studies also suggest that superheated steamed partially cooked food products bring beneficial changes in texture, color, and overall acceptability [11, 35]. Seo et al. [36] reported a higher preference of samgyetang treated by superheated steam compared with conventional cooking methods.

Odor and pH are important parameters for assessing the quality of a seafood product. The pH of raw squid was significantly ($p < 0.05$) lower than both superheated steam-treated and steam oven-treated squids that were close to neutral pH (pH 7). This complies with the study of Yildiz [37] who reported that the pH level in the fresh fish flesh is almost neutral. It has been reported that in the postmortem period, the decomposition of nitrogenous compounds leads to a pH increase in fish flesh, indicating deterioration of quality. Seafood is famous for its fishy smell, thus in this study, the odor intensity of raw, superheated steam-treated, and steam oven-treated squid samples was compared. Raw squids showed significantly ($p < 0.05$) higher odor intensity compared to the treated squids. This likely happened due to the heating treatment on the squid samples that encouraged evaporation of volatile compounds which usually occurs along with the fish spoilage process [38].

Other than acidity and odor, VBN is also considered as an important parameter to determine the quality of the seafood products. An increment of the VBN level indicates the yield of nitrogenous materials produced by proteolytic bacteria and endogenous enzymes [37]. VBN that primarily includes nitrogen from ammonia, dimethylamine, and trimethylamine indicates the extent of protein degradation and nonprotein nitrogenous compounds. High VBN values are associated with unpleasant smell [39]. Raw squids showed higher VBN levels, while the superheated steam-treated squids showed significantly ($p < 0.05$) lower VBN values. This suggests that superheated steam treatment helped to slow down the spoilage process more effectively compared to steam oven treatment. This was associated with decreased TBC values on the squid body and supported the theory that superheated steam treatment contributes in maintaining squid freshness by eliminating contaminating microorganisms and protecting against enzymatic degradation [37]. This result was in accordance with a study by Seo et al. [36], which reported lower VBN values in superheated steamed groups than conventionally cooked samgyetang.

The TBARS level is another measurement of spoilage in the seafood products. The higher the value of TBARS, the

Table 10: Textural properties of the processed squid stored at different temperatures.

Storage temperature (°C)	Parameters	Days						
		0	2	6	8	10	12	14
10	Hardness (g)	6.60 ± 0.54^{ab}	4.30 ± 0.08^{a}	10.73 ± 1.66^{c}	10.15 ± 1.39^{c}	9.45 ± 1.45^{c}	6.89 ± 1.08^{b}	4.48 ± 1.11^{ab}
	Adhesiveness (g/cm)	0.04 ± 0.05^{a}	0.04 ± 0.05^{a}	0.04 ± 0.05^{a}	0.04 ± 0.05^{a}	0.08 ± 0.13^{a}	0.10 ± 0.10^{a}	0.54 ± 0.82^{a}
	Cohesiveness	0.79 ± 0.01^{ab}	0.78 ± 0.05^{ab}	0.75 ± 0.04^{b}	0.76 ± 0.02^{ab}	0.78 ± 0.04^{ab}	0.80 ± 0.02^{ab}	0.86 ± 0.08^{c}
	Springiness (mm)	2.73 ± 0.07^{ab}	2.72 ± 0.21^{ab}	2.70 ± 0.12^{ab}	2.79 ± 0.13^{ab}	2.71 ± 0.06^{ab}	2.68 ± 0.04^{ab}	2.58 ± 0.14^{a}
	Gumminess (g)	5.52 ± 0.46^{abc}	3.52 ± 0.26^{a}	8.72 ± 0.83^{d}	7.74 ± 0.39^{cd}	6.65 ± 1.55^{bcd}	6.85 ± 4.26^{bcd}	3.51 ± 1.09^{a}
	Chewiness (g/cm)	153.28 ± 16.38^{abcd}	96.06 ± 13.31^{ab}	219.05 ± 50.32^{d}	180.30 ± 30.68^{bcd}	181.24 ± 43.46^{bcd}	187.88 ± 116.68^{cd}	91.69 ± 32.03^{ab}
15	Hardness (g)	6.60 ± 0.54^{ab}	6.82 ± 0.87^{b}	5.17 ± 1.58^{ab}	6.35 ± 0.74^{ab}	6.48 ± 0.50^{ab}	6.21 ± 0.64^{ab}	4.88 ± 1.52^{ab}
	Adhesiveness (g/cm)	0.04 ± 0.05^{a}	0.06 ± 0.05^{a}	0.04 ± 0.05^{a}	0.04 ± 0.05^{a}	0.14 ± 0.05^{a}	0.28 ± 0.08^{a}	0.42 ± 0.26^{a}
	Cohesiveness	0.79 ± 0.01^{ab}	0.82 ± 0.02^{ab}	0.79 ± 0.04^{ab}	0.80 ± 0.04^{ab}	0.82 ± 0.04^{ab}	0.65 ± 0.05^{a}	0.59 ± 0.06^{a}
	Springiness (mm)	2.73 ± 0.07^{ab}	2.69 ± 0.10^{ab}	3.02 ± 0.44^{b}	2.96 ± 0.15^{b}	2.89 ± 0.08^{ab}	2.52 ± 0.15^{a}	2.04 ± 0.24^{a}
	Gumminess (g)	5.52 ± 0.46^{abc}	5.62 ± 0.85^{abcd}	4.08 ± 0.97^{ab}	4.24 ± 0.56^{ab}	4.17 ± 0.66^{ab}	4.30 ± 0.49^{ab}	3.27 ± 0.83^{a}
	Chewiness (g/cm)	153.28 ± 16.38^{abcd}	152.46 ± 22.42^{abcd}	120.49 ± 18.99^{abc}	108.68 ± 16.51^{abc}	107.86 ± 11.92^{abc}	$101..22 \pm 12.66^{abc}$	71.63 ± 16.95^{a}

Values are mean ± SD (n = 10). Different letters (a–c) indicate significant differences among means by Tukey's test at $p < 0.05$.

more rancid the seafood product becomes due to lipid oxidation. Yildiz [37] stated that the seafood products are considered as perfect material if the TBARS value is less than 3 mg MDA/kg, as good material if the TBARS value is less than 5 mg MDA/kg, and the consumable limit of the TBARS content is suggested between 7 and 8 mg/MDA kg. Raw squid samples showed significantly ($p < 0.05$) higher TBARS values compared with superheated steam-treated samples. This was in accordance with a study on rainbow trout which showed lower TBARS values in essential oil-treated rainbow trout compared with the control group [37]. Lower TBARS values in rice samples roasted by superheated steam than the hot air were reported by Yodkaew et al. [40]. Another experiment that compared superheated steam treatment and conventional pan-frying treatment on pork bundle conducted by Huang et al. [41] showed suppressed content of peroxide values (POV), TBARS, and volatile compounds. In the development of lipid oxidation, oxygen contact with meat is considered as the most important factor, thus to minimize the occurrence of autoxidation of lipid, the exclusion of molecular oxygen is expected. The elimination of oxygen using superheated steam at the beginning of the roasting process could inhibit the initiation of lipid peroxidation, which leads to minimized lipid oxidation in pork meat. The decrease in POV and TBARS values of superheated steam-treated products suggests that these may be related to less oxygen present in the process, thus resulted in higher inhibition of lipid oxidation [41].

Superheated steam oven is a drying system which performs steam heating at a temperature higher than the boiling point while corresponding to the pressure value [9]. It has many advantages compared to the conventional drying methods including lower energy consumption, oxidation-reduction on the product, elimination of fire or explosion hazards, and improved product sanitation [42]. In order to maximize the effectiveness of microbial inactivation during the roasting process, the temperature should be high enough to maximize the moisture content. Superheated steam is capable to achieve these conditions by providing a constant high temperature for a constant period of time. Therefore, the superheated steam oven is considered as an effective option for microbial inactivation than hot-air drying due to a higher temperature and a higher initial moisture content. The results of the present study confirm the effectiveness of superheated steam treatment compared with steam oven treatment. Superheated steam showed significantly ($p < 0.05$) lower microbial count compared with raw squid. Although there was no significant difference with steam oven treatment, superheated steam treatment still showed lower TBC levels compared to steam oven treatment. *E. coli* and coliform were not detected in the tested samples. The underlying cause may be due to no secondary contamination that is supported by hygienic treatment, packaging, and storage method. This corresponds with the study of Nygard and Hostmark [43] who tried to compare microbial inactivation effects during superheated steam drying and hot air drying of fish meal at a constant temperature. They reported that the microbial inactivation using superheated steam was better than hot air oven to eliminate *E. coli*, *Clostridium sporogenes*, and *Geobacillus stearothermophilus* in fish meal. The experiments of Seo et al. [36] also confirmed that superheated steam treatment in combination with high hydrostatic pressure shows lower total bacterial count compared with conventional cooking with high hydrostatic pressure treatment in samgyetang.

Textural profile of a seafood product significantly affects the overall quality assessment. It is perceived as an assessment of product freshness including hardness, springiness, cohesiveness, chewiness, adhesiveness, and gumminess. Hardness is defined as the peak force of the first compression of the product, while springiness is the measurement of how well a product physically springs back after its deformation during the first compression. Measurement of how well a product withstands a second deformation relative to how it behaved under the first deformation is called cohesiveness [44]. Chewiness is defined as the energy required chewing a solid food product to a state where it is ready for swallowing [45]. Chewiness Index (CI) can be simply calculated as the product of hardness × cohesiveness × springiness. Adhesiveness is a measurement of the force needed to remove the food that sticks to the mouth during the normal eating process. The most common technique to measure adhesiveness of a squid is to place a squid sample in the panel's mouth, press it against the palate area (the "roof" of the mouth), and evaluate the force needed to remove it with tongue. Gumminess is defined as the denseness that remains during the chewing process. The simplest technique to measure gumminess of squid is to place the squid sample between the palate and the tongue and evaluate the manipulation force needed before the food disintegrates into the state ready for swallowing [44]. In the present study, superheated steam-treated squids showed significant differences for hardness, springiness, gumminess, and chewiness, while the adhesiveness and cohesiveness properties were relatively similar with raw squids. In superheated steam oven-treated squid, hardness, gumminess, and chewiness properties significantly decreased, which confirm the results of a study by Choi et al. [46] that reported a significant decrease in hardness, gumminess, and chewiness of superheated steam-treated marinated chicken steak. Similarly, superheated steam-treated chicken dishes showed lower hardness, gumminess, and chewiness than the boiled chicken [36, 47]. Abdulhameed et al. [13] reported a significant decrease in hardness, gumminess, and chewiness values of chicken sausages cooked using superheated steam.

Previous studies have confirmed that the reduction in textural parameters is considered as the result of an increase in denaturation of myosin and collagen [48, 49]. It has also been stated that the reduction in textural parameters is caused by the denaturation of proteins induced by cooking and due to the uncoiling of polypeptide chains [50]. The three main fractions of muscle proteins in squid mantle include myofibrillar proteins (65%), sarcoplasmic proteins (15%), and collagen (16%). Myofibrillar proteins are the largest fraction of the total muscle proteins in squid, thus making myofibrillar proteins the major protein in the squid body [24]. Heating treatment causes temperature-dependent reactions that could result in quality deterioration, nutrient loss, and thermal denaturation of muscle proteins which leads to textural changes in squid body. There are two main mechanisms that could be associated with the textural changes such as thermal dissociation and enzymatic hydrolysis of muscle proteins. After cooking, the muscle fibrils denature, coagulate, shrink, and density of tissue increases but the structure of muscle fibers does not rupture and remains intact. These microstructural changes in the fibers resulting from cooking cause softening of the squid muscle [51]. Protein denaturation can be suggested as the main cause of textural changes in the squid body and was supported by the existence of VBN value. Heat treatment could improve some physiochemical and microbial properties. However, heat treatment could result in protein changes including decreased solubility and microstructural changes. Additional processing such as smoking might result in moisture changes, thus also causing the abovementioned changes [52].

Fatty acids are considered important due to their nutritional value and health benefits [53]. The fatty acid profiles of food products have been investigated under various cooking methods. Yu et al. [10] reported that both superheated steam cooking and oven cooking of catfish have a little influence on fatty acid profile for SFA, MUFA, and PUFA. Mahdy and Yang [54] confirmed that superheated steam maintained favorable fatty acid (SFA, MUFA, and PUFA) levels of omega-3 plus milk powder. Superheated steam treatment offers an advantage by lowering the percentage of oxidation and nutrient loss due to oxygen-free environment. The results confirm that there was no oxidation or degradation that occurred during the superheated steam process. However, the fatty acid content in squid body increased for SFA, MUFA, and PUFA. A similar phenomenon of increasing SFA, MUFA, and PUFA values in food processing has been reported previously by Juárez et al. [55] and Cardinal et al. [55]. The increase in fatty acids might be due to several mechanisms generated during the heating processes such as water loss, lipid oxidation, thermal hydrolysis, fatty acid migration or transformation, loss of volatile fatty acid, and the deactivation of enzymes. Therefore, further research to study the changes in the fatty acid profile of squid is highly encouraged.

The results of this study suggest that superheated steam treatment could provide many beneficial effects, and it would be great if all these positive effects could be preserved for a certain amount of time. Therefore, further experiments on the superheated steam and smoking combined treatment to prolong the shelf life of the processed squid product were performed.

Smoking treatment tends to elevate the whole overall sensory characteristics, thus the sensory evaluation of the appearance, odor, flavor, and texture of the processed squid product was performed. The processed squid product stored at 10°C showed higher preference scores up to 6 days of storage. Lower preference scores were observed until day 10, and after that, the product was considered as unacceptable for consumption. The product stored at 15°C only lasted for 6 days, and then it was considered unacceptable for consumption.

The pH levels in processed squid products were measured to determine the acidity of the product corresponding to its storage time. The increase in pH during storage is presumably due to the production of basic nitrogen compounds during the bacterial growth [39]. All treated samples showed a gradual increase in pH during storage. The results showed increment at both temperatures until day 6. However, after day 8, the product stored at 15°C showed a steep drop in pH below the initial value indicating the initial phase of the spoilage process. This was consistent with a previous observation by our group that stated day 6 as the last day for the pen shell product to be considered as acceptable [15]. While for the samples that were stored at 10°C storage temperature, the pH was maintained up to day 10, but after that, it gradually decreased.

Smoke provides an appetizing aroma to the seafood products. It also enhances the preference point during sensory evaluation, inhibits bacterial growth, and extends shelf life of squid products. These effects are determined by the type of wood and the substances in the wood used for smoking process [18]. According to the results of a previous study [56], diverse materials used for hot smoking had a significant impact on the sensorial properties of the smoked fish including odor properties. In the present study, the smoked squid samples stored at 10°C showed significantly ($p < 0.05$) lower odor intensity compared with the samples stored at 15°C. This was consistent with a previous study by Yildiz [37], which showed a lowering odor intensity of the rainbow trout treated with thyme and rosemary essential oil and stored at 4°C.

The VBN levels observed at both 10°C and 15°C storage temperatures showed progressive rises. Higher VBN levels were observed at 15°C compared to 10°C storage temperature. A similar pattern of VBN levels was observed by Castro et al. [57], where fish storage under cold conditions showed a progressive rise in VBN values, mainly due to the bacterial decarboxylation of fish flesh. Later, Castro and colleagues [58] reported two main causes of fish spoilage including decomposition of trimethylamine N-oxide (TMAO) into trimethylamine (TMA) and non-TMAO reducing flora. These factors showed a slow rise in TVBN during storage which was probably caused by deamination of amino acids. According to the study of Dunn and Stabb [59], squid body contains 750–2500 mg of TMAO-N kg^{-1} wet weight, which justifies that the main cause of squid spoilage is due to the depletion of TMAO supplies. This makes TMA reach its maximum level during storage causing fishy odor and spoiled flesh. VBN levels are classified as very good, good, marketable, and spoilage if the value is up to 25, 30, 35 mg/100 g, and more than 35 mg/100 g, respectively [37]. The VBN levels of the processed squids showed values less than 30 mg/100 g until day 14. Therefore, the superheated steam and smoke combined treated squid product was categorized as "good" quality product.

The total bacterial count that resulted from superheated steam and smoke combined treatment showed that 10°C is the best temperature for squid storage compared with 15°C, which showed too many bacteria after day 6. The threshold value that differentiates inedibility of a food product is equal to 6 log CFU/g [60]. The results of this study were similar with a previous report by our group [15], where storage of pen shells at 10°C successfully and significantly inhibited the TBC up to 12 days. These results confirm that superheated steam along with smoke treatment inhibited the total bacterial count during storage.

The hardness of a squid decreases gradually with the increasing storage time, as the muscle texture of squid depends on the biological factors such as muscle fiber that eventually degrade and soften after cooking. Textural properties of the processed squids during 14 days storage at 10°C showed a rising trend since day 0 to day 8 and a slightly decreasing trend afterward, while at the 15°C storage temperature, there was no significant difference in hardness levels. Similar to this study, Cheng et al. [61] reported a reduction in hardness of fish muscle after 14 days of cold storage.

5. Conclusion

This study demonstrated an alternative method for processing squid using superheated steam in combination with smoking which showed better sensory, physiochemical, and microbial characteristics. Among the different tested methods, superheated steam (260°C heater and 240°C steam for 4 min) and smoking (oak sawdust, 65°C for 15 min) combined treatment enhanced the overall sensory, physiochemical, and microbial properties of the prepared squid product up to 10 days at a storage temperature of 10°C. The developed superheated steam and smoking combined method could provide a better alternative for processing and preserving seafood products, especially the common squid, under favorable and healthy conditions for consumers.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Authors' Contributions

Lisa Andriani Sutikno and Khawaja Muhammad Imran Bashir contributed equally to this work.

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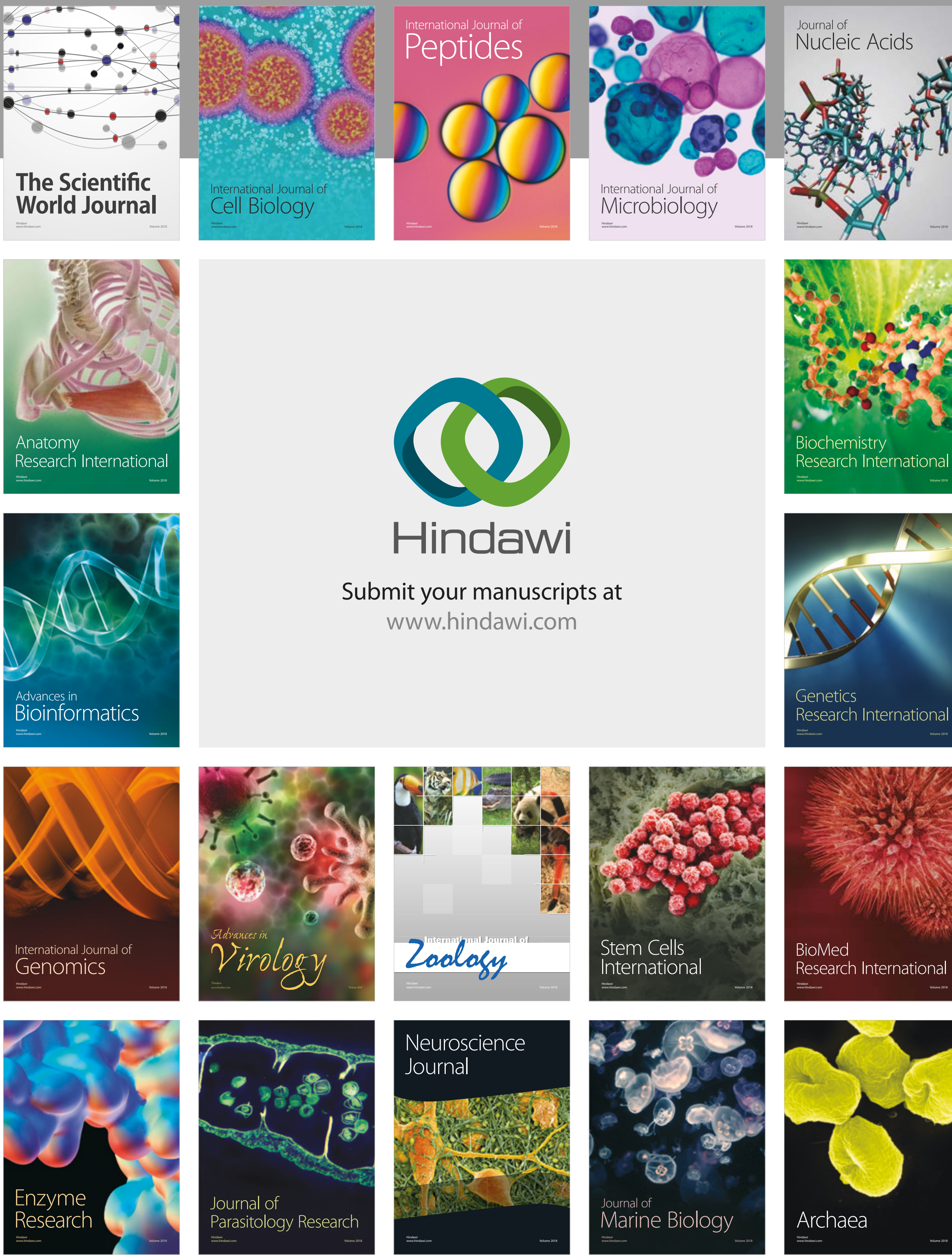
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