

## Research Article

# Detection of Artificially Water-Injected Frozen *Octopus minor* (Sasaki) Using Dielectric Properties

Dongyoung Lee <sup>1</sup>, Sangdae Lee,<sup>2</sup> Chang Joo Lee <sup>3</sup>, and Seung Hyun Lee <sup>1</sup>

<sup>1</sup>Department of Biosystems Machinery Engineering, College of Agricultural and Life Science, Chungnam National University, 99 Daehak-ro, Yuseong-gu, Daejeon 34134, Republic of Korea

<sup>2</sup>Convergence Components and Agricultural Machinery R&D Group, Korea Institute of Industrial Technology, 119 Jipyeongseonsandan 3-gil, Gimje, Jeollabuk-do 54325, Republic of Korea

<sup>3</sup>Department of Food Science and Biotechnology, College of Life Resource Science, Wonkwang University, 460 Iksandae-ro, Iksan, Jeollabuk-do 54538, Republic of Korea

Correspondence should be addressed to Seung Hyun Lee; [seunglee2@cnu.ac.kr](mailto:seunglee2@cnu.ac.kr)

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A few importers of marine products have practiced the fraud of artificially injecting water into *Octopus minor* for increasing their weights prior to the freezing process. These rampant practices have recently become a serious social issue and threaten public health. Therefore, it is necessary to develop the detection method for artificially water-injected frozen *Octopus minor*. This study was conducted to develop the nondestructive method for verifying adulterated *Octopus minor* by measuring dielectric properties using the coaxial probe method. Regardless of weight and measurement locations, a significant difference between  $\epsilon$  values from normal octopuses was not observed. The  $\epsilon$  values of *Octopus minor* were decreased in the microwave frequency range between 500 and 3000 MHz. The  $\epsilon$  values of water-injected octopuses also showed similar trend with normal octopuses; however, the dielectric loss factor ( $\epsilon''$ ) values of adulterated octopuses were much lower than normal octopuses. After thawing normal, adulterated, and imported frozen *Octopus minor*, the  $\epsilon$  values measured at the trunk part from these octopuses were compared and statistically analyzed. The  $\epsilon''$  values from normal frozen octopus were significantly different from adulterated and imported frozen octopuses. In addition, the  $\epsilon''$  values from the adulterated frozen octopus group that weight gain rate was less than 20% was significantly different from other adulterated octopus groups with higher weight gain rate than 20%. The  $\epsilon''$  values from adulterated frozen octopus groups with the range of weight gain rate between 20 and 30% were quite similar to imported frozen octopuses. Therefore, it was found that the measurement of  $\epsilon''$  values from *Octopus minor* has a great possibility to distinguish normal frozen octopuses and artificially water-injected frozen octopuses.

## 1. Introduction

*Octopus minor* (Sasaki, 1920), which is widely distributed along the coastal waters of Korea, China, and Japan, is one of commercially important marine species in East Asia countries [1]. Since octopus is recognized as a high nutrition and low calorie food that has the effects on lowering the cholesterol level in blood and preventing anemia, its consumption in Korea has been consistently increased; however, from 2006, domestic production of octopus in Korea has been annually decreased because of environmental destruction and overfishing. A tremendous amount of octopus was imported

in Korea to meet demands from consumers, and the imported volume in 2013 was approximately 38,533 tons.

Freezing method that can retain the quality of foods is the common preservation method for octopus. Because the majority of imported octopuses are in the frozen state and their price is mainly decided by the weight, a few of importers (or distributors) of marine products have practiced excessive ice glazing of frozen fish and forced water injection into octopus for increasing weights of marine products. This rampant practice threatens public health and became a social issue in Korea. Although the Ministry of Food and Drug Safety in Korea has been inspecting for imports of octopus

from Asia countries, this inspection is entirely dependent on the ability of the inspector. Skillful inspectors of marine products have strived to distinguish normal and adulterated octopuses by using thermogravimetric analysis that can determine water content in frozen octopus; however, the precise quantitation of water content in *Octopus minor* is complicated because some specific amount of water is generally absorbed into octopus during transportation and the ice glazing process. The analysis equipment that can directly examine water content in frozen octopus is not yet existed. Therefore, the determination of water content in an imported frozen octopus is time-consuming and laborious.

In microwave and radio frequency heating, dielectric properties are the most important factor that can provide information about the interaction between electromagnetic energy in applied microwave and radio frequency and dielectric material [2]. Dielectric properties are also described in terms of complex relative permittivity and are represented as the following term [3, 4]:

$$\varepsilon = \varepsilon' - j\varepsilon'' \quad (1)$$

where  $\varepsilon'$  is the dielectric constant (the real part) that indicates the ability of the material to store energy in the applied electric field,  $\varepsilon''$  is the dielectric loss factor (the imaginary part) that is associated with the ability of the material to dissipate energy in the applied electric field, and  $j$  is  $\sqrt{-1}$ .

Dielectric properties are mainly altered by several factors (i.e., density, physical structure, moisture content of the target material, frequency, and temperature), and in particular, the amount of water in the target material is the dominant factor affecting these properties [5].

There are five representative methods for measuring dielectric properties of materials: transmission line, free space, resonant cavity, parallel plate, and open-ended coaxial probe methods. Among these methods, the open-ended coaxial probe method is commonly used to measure dielectric properties of food materials because it covers broad frequency bands and pretreatment is not required for preparing samples [6]. The main advantage of this method is that dielectric properties of the target material can be easily measured by making close contact between the probe and the surface. Several studies were conducted to measure dielectric properties of frozen marine products and seafood such as tuna, shrimp, salmon, and surimi by using the open-ended coaxial probe method [7–10]. It was reported that dielectric properties of aforementioned frozen marine products were significantly changed depending on frequency, temperature, composition, and measurement location. In addition, Mendes et al. [11] attempted to develop the detection method for artificially water-added *Octopus vulgaris* by measuring their electrical conductivity and dielectric properties, and they concluded that simultaneous measurement of electrical conductivity or dielectric properties could be used to distinguish artificially water added octopus among processed octopuses. However, as far as can be determined from published literatures, there was not an attempt to determine how much water were artificially

injected into octopus by measuring dielectric properties with the open-ended coaxial probe method. Therefore, this study was conducted to (1) distinguish normal and artificially water-injected *Octopus minor* (Sasaki, 1920) by measuring dielectric properties and (2) develop the nondestructive detection method verifying artificially water-injected frozen *Octopus minor* by using the open-ended coaxial probe.

## 2. Materials and Methods

**2.1. *Octopus minor*.** Live and frozen *Octopus minor* (Sasaki, 1920) imported from China were procured from a local seafood market. The live octopuses were randomly captured in a fish tank and packed in polystyrene boxes containing seawater and then transported to the laboratory. The imported frozen octopus block packed in a paper box consisted of 5 octopuses, and its weight was about 1 kg.

Convection oven drying method was employed to estimate moisture contents of live octopuses. Placing live octopus in the drying tray was difficult because it kept moving during the drying process; thus, live octopus was packaged in a plastic bag and then stored in 4°C refrigeration condition for 24 h. Octopus was exhausted and debilitated through this process. The weights of debilitated octopuses ( $n = 35$ ) were measured by digital balance with 0.01 g precision (PAG2102C, OHAUS Co., USA). The octopuses were put in the convection oven and dried at 105°C for 24 h. The initial moisture content of octopus was  $4.485 \pm 0.593$  kg of water per kg of dry matter.

**2.2. Preparation of Artificially Water-Injected Frozen *Octopus minor*.** Wells and Wells [12] reported since *Octopus vulgaris* was quite hyperosmotic compared with seawater in which it lived, osmotic uptake could occur over the general body surface. Although the species of octopus (Sasaki, 1920) used in this study was different from *Octopus vulgaris*, it was assumed that *Octopus minor* (Sasaki, 1920) was also hyperosmotic. Osmotic uptake experiment was conducted to determine the optimal concentration of sodium chloride solution for water injection into octopus to the maximum. 24 live octopuses were weighed by a digital balance and put in 24 containers (a 1 L plastic container). All containers were filled with sodium chloride solution of 700 mL with different concentrations (0% (tap water) to 2.1% (21 g/L) at increments of 0.3% (3 g/L)) and were sealed with lids. All containers were grouped by concentration of sodium chloride solution, and 8 groups of containers (3 containers for each concentration) were kept in the 4°C refrigeration condition for 24 h. Then, octopuses were removed from the solution, and their weights were measured again to determine the weight gain rate by the following equation. The experiments were conducted in triplicate:

$$\text{weight gain rate (\%)} = \frac{w_f - w_i}{w_i} \times 100 \quad (2)$$

where  $w_f$  is the weight of octopus immersed in the sodium chloride solution (kg) and  $w_i$  is the initial weight of octopus (kg).

After determining the optimal concentration of the sodium chloride solution, live octopuses were treated as the aforementioned method to prepare artificially water-injected frozen octopuses (adulterated octopuses). Then, adulterated octopuses were sorted by the weight gain rate (%) and divided into 5 groups, then frozen at in the chest freezer for 24 h.

Live octopuses were also frozen and were used as normal frozen octopuses (the control group) for the comparison with imported frozen octopus blocks and artificially water-injected octopus groups.

**2.3. Dielectric Properties Measurement System.** Dielectric probe (85070E option 050, Agilent Technologies, Santa Clara, CA) coupled with a vector network analyzer (E8361C, Agilent Technologies, Santa Clara, CA) was used for measuring dielectric properties ( $\epsilon$ ) of an octopus. The type of the dielectric probe was the performance probe operated in the frequency range between 500 MHz and 50 GHz and temperature range from  $-40$  to  $200^\circ\text{C}$ . The probe was calibrated for the measurement using air, a standard shorting block, and distilled water at  $10^\circ\text{C}$ . The reflection coefficient ( $S_{11}$ ) at the interface between the probe and the surface of octopus was measured by the network analyzer. Dielectric probe kit software (85070E software, Agilent Technologies, Santa Clara, CA) was used for the calculation of dielectric properties based on the measured reflection coefficient. As a preliminary experiment, the dielectric properties ( $\epsilon$ ) of normal octopuses and artificially water-injected octopuses were measured in the frequency range from 500 MHz to 20 GHz. Dielectric constant ( $\epsilon'$ ) values from both octopus groups were constantly decreased with an increase in frequency. However, dielectric loss factor ( $\epsilon''$ ) values were sharply decreased below 3 GHz and afterward remained constant. Over 3 GHz, there was not a significant difference between dielectric loss factor ( $\epsilon''$ ) values from both octopus groups. Ionic conduction generally occurs at low frequencies, and dipole relaxation occurred at higher frequencies. Traffano-Schiffo et al. [13] reported that the ionic conductivity effect on meat can be observed below 2 GHz. In addition, Castro-Giráldez et al. [14] reported that the dielectric loss factor ( $\epsilon''$ ) measured at low frequencies (0.5 GHz), which can determine the effect of ionic conductivity, was useful in distinguishing the quality of pork meat. Therefore, the dielectric properties of octopus were measured within the frequency range between 500 MHz to 3 GHz at 101 frequency sample points. In addition, all data points ( $\epsilon'$  and  $\epsilon''$ ) were monitored, recorded, and graphically plotted.

**2.4. Dielectric Properties Measurement Procedure.** The weight of live octopuses was measured, and their dielectric properties were measured at three locations, namely, mantle, trunk, and the thickest arm, as shown in Figure 1(a). Octopus secretes mucus from the skin when there is an external threat. Therefore, the mucus from the skin at the measurement location was clearly removed using paper wipers. After the measurements, live octopuses were exhausted and

debilitated by using the early mentioned method and were put in the aluminum trays and frozen at  $-30^\circ\text{C}$  for 24 h. Then, normal frozen octopuses were thawed at room temperature for 1 h, and their weights and dielectric properties were measured again. In this measurement, dielectric properties were measured only at the trunk after all water on their skin was clearly removed.

In the case of adulterated octopuses, they were sorted by weight gain rate after water injection, and all measurement procedure was the same with the aforementioned measurement procedure. Imported frozen octopuses were also thawed at room temperature for 1 h, and then, the measurement for weight and dielectric properties was performed. The experimental protocol is clearly represented in Figure 1(b).

Based on the dielectric properties measured at different stages, the penetration depth of microwave was calculated by the following equation [3]:

$$d_p = \frac{c}{2\pi f \sqrt{2\epsilon' \left[ \sqrt{1 + (\epsilon''/\epsilon')^2} - 1 \right]}}, \quad (3)$$

where  $d_p$  is the penetration depth (m),  $f$  is the frequency (Hz),  $c$  is the speed of light in the free space ( $2.9979 \times 10^8$  m/s), and  $\epsilon'$  and  $\epsilon''$  are the measured values of dielectric constant and dielectric loss factor of octopus, respectively.

**2.5. Statistical Analysis.** The result from the osmotic uptake experiment was statistically evaluated by one-way analysis of variance (ANOVA). Tukey's HSD multiple range test ( $p$  value  $< 0.05$ ) was carried out using SPSS 24.0 software (SPSS Inc, Chicago, IL, USA).  $T$ -tests were conducted to compare the means of dielectric constant ( $\epsilon'$ ) and loss factor ( $\epsilon''$ ) measured from normal and adulterated *Octopus minor* at different measurement locations and frequencies. Furthermore, dielectric properties measured from the normal frozen octopus group, artificially water-injected octopus groups, and imported frozen octopus group were analyzed and compared according to the procure of least squares means by using SAS (SAS 9.1; SAS Institute, Cary, NC, USA).

### 3. Results and Discussion

**3.1. Osmotic Water Uptake of *Octopus minor*.** Figure 2 shows the weight gain rate (%) of water-injected octopus by NaCl solutions with different concentrations. The amount of absorbed water by octopuses immersed in tap water was close to 60% of their body weight and was much larger than it by other octopuses. There was no significant difference ( $p < 0.05$ ) in weight gain rate of octopuses immersed in NaCl solutions with different concentrations; however, the weight gain rate of octopuses was between 40% and 50%. An artificial injection of water into *Octopus minor* could be easily practiced in the environment that has lower salt concentration than seawater in which it lives. It was found that *Octopus minor* could be considered as a hyperosmotic animal. An immersion of octopus in tap water was employed to artificially inject water into octopus as much as possible.

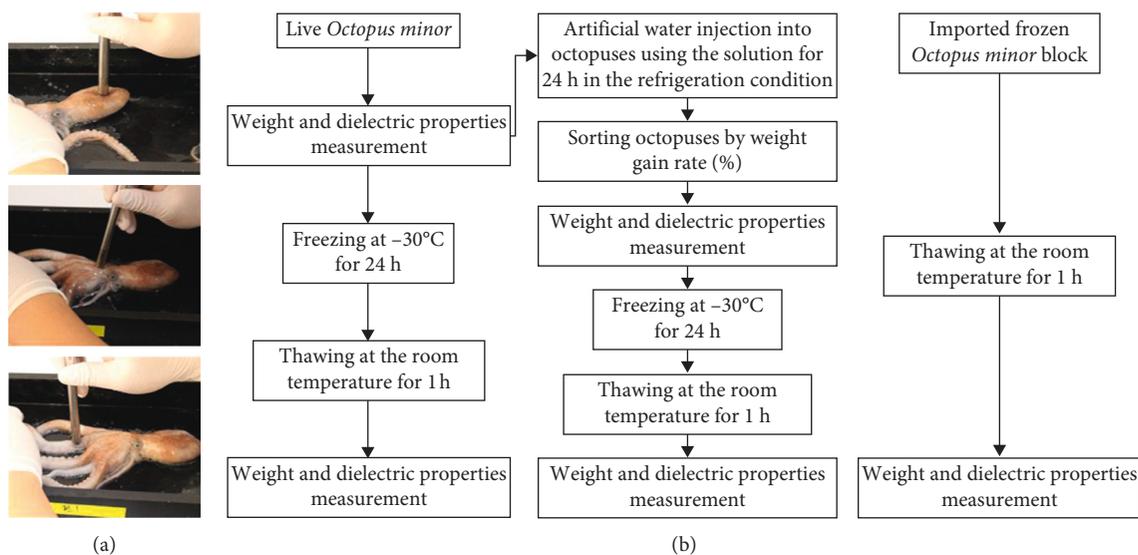


FIGURE 1: Dielectric properties measurement: (a) measuring location; (b) experimental protocol.

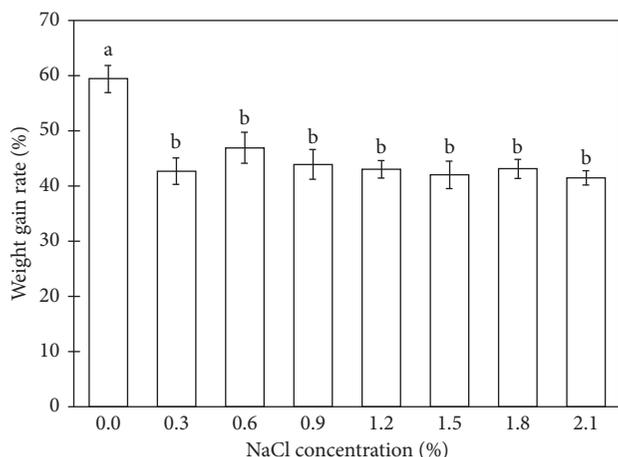


FIGURE 2: Weight gain rate of *Octopus minor* immersed in NaCl solutions with different concentrations.

**3.2. Dielectric Properties of *Octopus minor*.** The dielectric properties measured from live octopuses were listed in Table 1. The average weight of octopuses ( $n = 300$ ) for dielectric properties measurement was  $168.43 (\pm 80.33)$  g. Dielectric constant ( $\epsilon'$ ) and dielectric loss factor ( $\epsilon''$ ) of octopus measured at three locations were decreased as frequency increased. Microwave penetration depth ( $d_p$ ) was also decreased with an increase in frequency. When dielectric properties of octopus arm muscle were measured *in vivo* over low frequency range between 5 Hz and 1 MHz,  $\epsilon'$  was decreased with an increase in frequency [15]. Dielectric properties (permittivity) describe the ability of a material to polarize in response to an applied electric field [16]. Generally, as frequency increases, the net polarization of the material drops because each polarization mechanism ceases to contribute, and consequently,  $\epsilon'$  decreased [17]. There was not a significant difference in  $\epsilon'$  and of octopus measured at the same frequency depending on measurement locations. According to research done by Tanaka et al. [7], the effect of

shrimp's measurement locations on dielectric properties was not significant. Furthermore, microwave penetration depth ( $d_p$ ) from the mantle, trunk, and arm at 500 MHz was  $0.0099 (\pm 0.001)$  m,  $0.0093 (\pm 0.001)$  m, and  $0.0099 (\pm 0.001)$  m, respectively. Therefore, it can be considered *Octopus minor* is composed with a certain structure that uniformly absorbs microwave power.

### 3.3. Dielectric Properties of Adulterated *Octopus minor*.

Dielectric constant ( $\epsilon'$ ) of artificially water-injected octopuses ( $n = 50$ ) was decreased with an increase in frequency as similar to it of normal octopus as summarized in Table 1. A significant difference between  $\epsilon'$  and  $\epsilon''$  values measured from normal and adulterated octopuses was observed regardless of measurement locations at the same frequency ( $p < 0.05$  in all cases). Despite the statistical result, it was not possible to clearly distinguish normal and adulterated octopuses using  $\epsilon'$ . On the contrary,  $\epsilon''$  of adulterated octopuses was decreased in the frequency range from 500 MHz to 1000 MHz and increased afterward. In addition, there was a considerable change of  $\epsilon''$  values between normal and adulterated octopuses than  $\epsilon'$  values. Regardless of measurement locations, the microwave penetration depths from adulterated octopuses at 500 MHz were longer than normal octopuses by at least 2 cm. In general,  $\epsilon'$  and  $\epsilon''$  of foods are increased with an increase in water amount of foods because water (the dipolar molecule) dominantly affects change in dielectric properties of food. The positive correlation between  $\epsilon'$  and water was observed; however, the correlation between  $\epsilon''$  and water was not clear [18]. Even though the water was artificially injected into octopuses, the uncertain correlation between  $\epsilon''$  and water in octopuses was observed. Salt content in food also affects the change in  $\epsilon''$  owing to an increase in ionic loss [19]. Salt concentration of adulterated octopuses *in vivo* might be decreased, finally resulting in decrease in  $\epsilon''$  of adulterated octopuses.

TABLE 1: Dielectric properties of normal and adulterated *Octopus minor* at different parts and frequencies.

Frequency (MHz)	Octopus	$\epsilon'$			$\epsilon''$		
		Mantle	Trunk	Arm	Mantle	Trunk	Arm
500	Normal	76.11 <sup>a</sup> ( $\pm 1.94$ )	76.48 <sup>a</sup> ( $\pm 1.84$ )	77.38 <sup>a</sup> ( $\pm 1.41$ )	101.8 <sup>c</sup> ( $\pm 16.36$ )	105.91 <sup>c</sup> ( $\pm 18.24$ )	97.77 <sup>c</sup> ( $\pm 8.88$ )
	Adulterated	77.03 <sup>b</sup> ( $\pm 2.60$ )	77.35 <sup>b</sup> ( $\pm 2.31$ )	76.96 <sup>b</sup> ( $\pm 2.43$ )	24.17 <sup>d</sup> ( $\pm 9.22$ )	21.28 <sup>d</sup> ( $\pm 8.80$ )	20.51 <sup>d</sup> ( $\pm 6.73$ )
1000	Normal	68.93 <sup>a</sup> ( $\pm 1.84$ )	69.51 <sup>a</sup> ( $\pm 2.24$ )	69.57 <sup>a</sup> ( $\pm 1.54$ )	55.39 <sup>c</sup> ( $\pm 7.63$ )	57.47 <sup>c</sup> ( $\pm 8.27$ )	54.00 <sup>c</sup> ( $\pm 4.28$ )
	Adulterated	76.09 <sup>b</sup> ( $\pm 2.50$ )	77.35 <sup>b</sup> ( $\pm 2.27$ )	76.79 <sup>b</sup> ( $\pm 2.38$ )	16.18 <sup>d</sup> ( $\pm 4.64$ )	15.08 <sup>d</sup> ( $\pm 4.48$ )	15.03 <sup>d</sup> ( $\pm 3.42$ )
1500	Normal	66.11 <sup>a</sup> ( $\pm 2.18$ )	66.84 <sup>a</sup> ( $\pm 2.81$ )	66.64 <sup>a</sup> ( $\pm 1.65$ )	42.05 <sup>c</sup> ( $\pm 5.03$ )	43.58 <sup>c</sup> ( $\pm 5.55$ )	41.38 <sup>c</sup> ( $\pm 3.10$ )
	Adulterated	75.09 <sup>b</sup> ( $\pm 2.57$ )	76.43 <sup>b</sup> ( $\pm 2.37$ )	75.79 <sup>b</sup> ( $\pm 2.52$ )	15.44 <sup>d</sup> ( $\pm 3.16$ )	15.16 <sup>d</sup> ( $\pm 3.12$ )	15.30 <sup>d</sup> ( $\pm 2.41$ )
2000	Normal	64.45 <sup>a</sup> ( $\pm 2.35$ )	65.19 <sup>a</sup> ( $\pm 3.08$ )	64.94 <sup>a</sup> ( $\pm 1.65$ )	35.64 <sup>c</sup> ( $\pm 3.88$ )	36.77 <sup>c</sup> ( $\pm 4.36$ )	35.21 <sup>c</sup> ( $\pm 2.74$ )
	Adulterated	73.78 <sup>b</sup> ( $\pm 2.68$ )	74.12 <sup>b</sup> ( $\pm 2.37$ )	73.11 <sup>b</sup> ( $\pm 2.58$ )	15.88 <sup>d</sup> ( $\pm 2.43$ )	16.01 <sup>d</sup> ( $\pm 2.43$ )	16.28 <sup>d</sup> ( $\pm 1.93$ )
2500	Normal	62.90 <sup>a</sup> ( $\pm 2.46$ )	63.64 <sup>a</sup> ( $\pm 3.26$ )	63.37 <sup>a</sup> ( $\pm 1.79$ )	32.15 <sup>c</sup> ( $\pm 3.06$ )	33.08 <sup>c</sup> ( $\pm 3.66$ )	31.95 <sup>c</sup> ( $\pm 2.44$ )
	Adulterated	72.96 <sup>b</sup> ( $\pm 2.46$ )	74.12 <sup>b</sup> ( $\pm 2.32$ )	73.11 <sup>b</sup> ( $\pm 2.50$ )	17.26 <sup>d</sup> ( $\pm 2.00$ )	17.78 <sup>d</sup> ( $\pm 2.04$ )	18.05 <sup>d</sup> ( $\pm 1.69$ )
3000	Normal	61.27 <sup>a</sup> ( $\pm 2.57$ )	62.05 <sup>a</sup> ( $\pm 3.35$ )	61.79 <sup>a</sup> ( $\pm 1.84$ )	30.19 <sup>c</sup> ( $\pm 2.65$ )	31.04 <sup>c</sup> ( $\pm 3.33$ )	30.13 <sup>c</sup> ( $\pm 2.34$ )
	Adulterated	71.73 <sup>b</sup> ( $\pm 2.43$ )	72.75 <sup>b</sup> ( $\pm 2.31$ )	71.57 <sup>b</sup> ( $\pm 2.43$ )	18.54 <sup>d</sup> ( $\pm 1.77$ )	19.29 <sup>d</sup> ( $\pm 1.82$ )	19.54 <sup>d</sup> ( $\pm 1.61$ )

The values having different letters are significantly different ( $p < 0.05$ ).

There was a clear difference between  $\epsilon''$  values from normal and adulterated octopuses. Therefore, among dielectric properties, the  $\epsilon''$  value could be used as the discrimination factor to detect adulterated octopuses. Furthermore, uniform osmotic water uptake over the general surface of an octopus would be possible because there was not a significant difference in dielectric properties of adulterated octopuses regardless of measurement locations.

### 3.4. Dielectric Properties of Thawed *Octopus minor*.

Figure 3 illustrates the dielectric properties of normal ( $n = 100$ ), adulterated ( $n = 140$ ), and imported ( $n = 140$ ) frozen octopuses measured at the trunk location after thawing. As a preliminary experiment, dielectric properties of three different frozen octopuses were measured at three locations in the freezing range; however, the values of  $\epsilon'$  and  $\epsilon''$  were very small, and the difference between dielectric properties of three different octopus groups was not observed. Mao et al. [8] and Liu and Sakai [20] reported that, below the freezing point, dielectric properties of frozen tuna and surimi were very small and there was not a significant difference depending on measurement locations.

Dielectric properties of normal and adulterated frozen octopuses were similar to the values in the freezing range. After thawing, the  $\epsilon'$  values of adulterated frozen octopuses were slightly greater than normal frozen octopuses in all frequency ranges; however, there was not a significant difference between the two octopus groups. Even after thawing, the  $\epsilon''$  values of normal frozen octopuses were much greater than adulterated frozen octopus. The gap between the  $\epsilon''$  values of two octopus groups was narrow as frequency increased; however, the evident difference between the  $\epsilon''$  values of normal and adulterated frozen octopuses was observed at any specific frequency. Although it was not technically feasible to distinguish normal and adulterated frozen octopuses in the freezing range, it was possible to do two groups after thawing by using the  $\epsilon''$  values as the discrimination factor at low frequency. In addition, the dielectric properties ( $\epsilon'$  and  $\epsilon''$ ) of imported frozen octopuses after thawing were quite similar to the values of adulterated frozen octopuses. It would be that

*Octopus minor* absorbed water during the transport for their freezing process or water was added into octopuses to compensate for water loss during frozen storage. Therefore, the further statistical analysis was conducted to estimate the water amount added into imported frozen octopuses.

### 3.5. Determination of Water Amount Injected into Frozen *Octopus minor* after Thawing.

Since there was not a significant difference between  $\epsilon'$  values measured from adulterated and imported frozen octopuses, the  $\epsilon''$  values measured at the trunk part from adulterated octopuses were used as the discrimination factor. The  $\epsilon''$  values measured from adulterated octopuses were divided by weight gain rate (%), and the  $\epsilon''$  values from adulterated octopuses were classified into 5 groups for the comparison with  $\epsilon''$  values from imported frozen octopuses. 5 groups were less than 20% (the weight gain rate was less than 20%), 20% (20~29%), 30% (30~39%), 40% (40~49%), and 50% (over 50%). As shown in Figure 4, after 2 GHz, the  $\epsilon''$  values from 5 groups and the imported frozen octopus group were similar to each other group. Less than 1.5 GHz, the clear difference between 6 groups was observed. As listed in Table 2, at 500 MHz, the less 20% group was statistically quite different from other adulterated groups and the imported frozen octopus group. The 20% and 30% groups were significantly similar to the imported frozen octopus group. There was not a difference between 20% and 30% groups. Furthermore, there was no significant difference between adulterated groups when the difference in weight gain rate was at 10%. Although the clear estimation of water amount injected into octopuses was not determined by using  $\epsilon''$  values from adulterated and imported frozen octopuses, it was possible to clearly distinguish normal and adulterated frozen groups. In addition, the octopuses in which water was excessively injected could be distinguished from them with low weight gain rate. Therefore, the coaxial probe method has a high potential to detect an adulterated *Octopus minor*.

## 4. Conclusion

The detection method using a vector network analyzer and open-ended coaxial probe that can measure the sample's

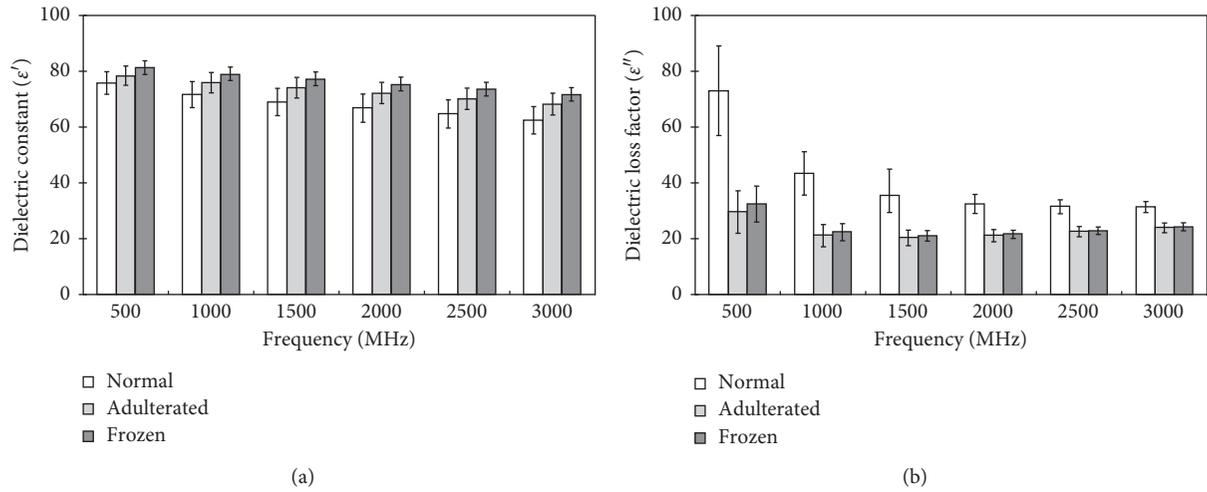


FIGURE 3: Dielectric properties of normal, adulterated, and imported frozen octopuses measured at the trunk part after thawing: (a) dielectric constant; (b) dielectric loss factor.

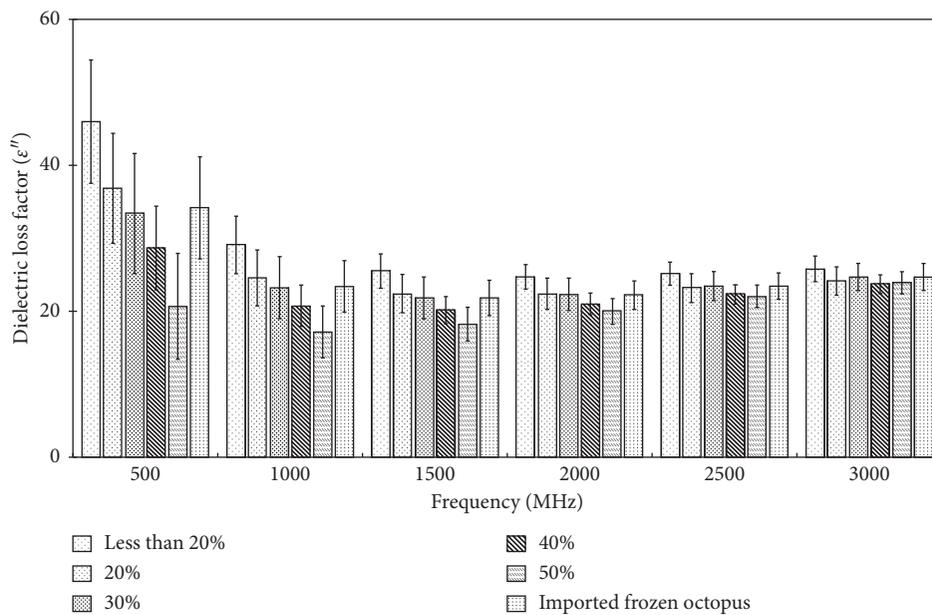


FIGURE 4: Dielectric loss factor adulterated and imported frozen octopuses measured at the trunk part after thawing.

TABLE 2: Statistical analysis of dielectric properties measured at 500 MHz and the trunk part from adulterated and imported frozen *Octopus minor* groups.

	Least squares means for the effect class					
	Less 20	20	30	40	50	Imported frozen octopus
Less than 20		4.3728* (0.0002)	5.8549* (<0.0001)	6.9993* (<0.0001)	10.8249* (<0.0001)	6.1482* (<0.0001)
20			2.2569 (0.2151)	4.1751* (0.0005)	9.2707* (<0.0001)	2.2614 (<0.0001)
30				2.3335 (0.2151)	6.5617* (<0.0001)	0.5754 (0.9926)
40					2.8037 (0.0594)	-3.0648
50						-8.8594*
Imported frozen octopus						

\*  $p$  value < 0.05; the  $p$  value is indicated in parentheses.

dielectric properties by a direct contact was developed to distinguish normal octopuses and artificially water-injected octopuses. Regardless of weight and measurement locations, the dielectric properties ( $\epsilon'$ ) of octopuses were decreased in the frequency range between 500 and 3000 MHz ( $\epsilon'$ : 76~61 and  $\epsilon''$ : 101~30). In addition, artificially water-injected octopuses showed the same trend with normal octopuses ( $\epsilon'$ : 78~72 and  $\epsilon''$ : 22~19). Among dielectric properties, the dielectric loss factor ( $\epsilon''$ ) was effective to distinguish normal and adulterated octopuses. After thawing normal, adulterated, and imported frozen octopuses, these octopus groups were easily distinguished by using the measured dielectric loss factor ( $\epsilon''$ ). At 500 MHz, the dielectric loss factor ( $\epsilon''$ ) from imported frozen octopuses was similar to the octopuses in which the weight gain rate was between 20 and 39%. The octopus group with less than 10% weight gain rate was significantly different from other adulterated frozen octopus groups. The statistical analysis of the dielectric loss factor of adulterated and imported frozen samples determined that it was possible to detect extremely water-injected octopus. Therefore, by measuring the dielectric loss factor of frozen *Octopus minor* after thawing, the adulterated frozen *Octopus minor* could be detected in a short time.

### 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.

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