Effects of freeze-dried *Ecklonia cava* hot water extract as a gel enhancer for fried fish cakes with threadfin bream (*Nemipterus* spp.) surimi

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Abstract

In the present work, we used threadfin bream (*Nemipterus* spp.) surimi fish cakes to test the viability of freeze-dried *Ecklonia cava* hot water extract (ECWE) as a gel enhancer. The effects of freeze-dried ECWE on the gel strength and the colour of the fish cakes were investigated. The gel strength increased in fish cakes incorporated with 0.5% freeze-dried ECWE as compared to the control (no ECWE). The lightness and whiteness values of samples containing ECWE were lower than those of the control samples. Scanning electron microscopy (SEM) observations of fish cakes containing 0.5% freeze-dried ECWE exhibited a finer and more continuous matrix as compared to the control samples. Incorporating 0.5% ECWE improved the gel strength, and produced high-quality emulsions with a finely distributed gel network due to the presence of mannitol and some polyphenolic compounds in freeze-dried ECWE. The use of freeze-dried ECWE in the production of fish cakes is effective as a natural gel enhancer for surimi-based products.

Keywords

*Ecklonia cava*, fish cake, mannitol, gel strength, threadfin bream surimi

Introduction

Fish cakes (*eomuk* in Korean) are made by grinding fish into a paste, adding salt, grinding again, adding starch, and lastly food additives. After shaping, the fish cake gels are formed by heat treatment. In the Republic of Korea, the total sales of fish cakes in 2019 were US $621 million, with sales of fish cakes having increased by 25% over the past five years (MFDS, 2020). Fish cakes can be prepared by frying, boiling, or roasting, but in the Republic of Korea, approximately 80% of fish cakes are prepared by frying (FIS, 2016). The textural properties developed during surimi gelation are normally expressed in terms of gel strength, which is the basic parameter for determining the quality and price of surimi (Benjakul et al., 2004). To increase the gel strength of surimi, various food grade ingredients and protein additives have been used (Shitole and Balange, 2014). The incorporation of these ingredients, however, adversely affects the flavour and colour of the surimi gel (Rawdkuen and Benjakul, 2008). Recent attention has focused on the interactions between phenolic compounds and proteins in the processing of some food products. Several studies have described the cross-linking ability of phenolic compounds with proteins using seaweed extract (Shitole et al., 2014; Shitole and Balange, 2014).

*Ecklonia cava* is a common edible brown alga, and is found plentiful on Jeju Island in the Republic of Korea (Kim et al., 2014). Polyphenols from *E. cava* exhibit strong anti-inflammatory activities (Kim et al., 2014). Various foods possess antioxidant, anticancer, and antihypertensive properties, and are valuable as new functional materials (Nagayama et al., 2002; Kang et al., 2012; Kwon et al., 2013). Previous studies reported improvements in the sensory attributes, quality characteristics, and storage stability of fish cakes by adding various functional materials such as additives from seaweed or coconut husk (Shitole et al., 2014; Buamard and Benjakul, 2015; Phuon et al., 2015). The improvement in the physical properties of fish cakes (Park et al., 2015; Choi, 2017) and the extension of their shelf life have been achieved using gamma rays, lysozyme, green tea extract (Kim et al., 2004; Lee et al., 2004; Shin et al., 2007), and by using fish paste material from red fish meat and underutilised fish resources (Shin et al., 2014). Little information is available, however, on the effects of seaweed hot water extract as a cross-linking agent in food proteins on the characteristics of fish cakes (Shitole et al., 2014).

In the present work, we investigated the effectiveness of using *E. cava* hot water extract (ECWE) to enhance fish cake gelation, and optimised freeze-dried ECWE and water concentrations in
Materials and methods

Preparation of Ecklonia cava hot water extract

The extract was prepared from powdered *E. cava* obtained from Jeju Island (Republic of Korea). The powder was first softened in an autoclave (Model HB-506-6, Dong Il Industry, Ulsan, Republic of Korea) using high-pressure hot water (121°C for 1 h), maintained at 50°C for 24 h, and then centrifuged for 10 min at 14,300 g (Supra 22K, Hanil Scientific, Gimpo, Republic of Korea). The supernatants were then freeze-dried (LFD-24L-DW, Lee Won Freezing, Busan, Republic of Korea) to produce ECWE.

Preparation of surimi

Threadfin bream (*Nemipterus* spp.) surimi (FA grade, frozen, ~2 months old) was obtained from Qadri Noori Seafood (Karachi, Pakistan). The frozen surimi was cut into blocks (~100 g), sealed in a vacuum bag, and maintained at -18°C for approximately five months. Frozen surimi was partially thawed at room temperature for 1 h to allow the core temperature to reach approximately -5°C (Poowakanjana *et al.*, 2012).

Fish cakes

The fish cakes were prepared using frozen surimi from threadfin bream. The other ingredients were starch, salt, sugar, D-xylose, potassium sorbate, glucono-δ-lactone, monosodium L-glutamate, and ECWE or water (for the control) as shown in Table 1.

The frozen surimi was first cut into appropriate sizes, then chopped again using a silent cutter (UMC 5, Stephan Machinery, Hameln, Germany) at 15°C. Salt (1.5%) was then added. After mixing for 3 min up to 1,500 rpm, ECWE (0.5 - 1.5%), starch (15%), sugar (0.3%), D-xylose (0.3%), potassium sorbate (0.3%), glucono-δ-lactone (0.35%), monosodium L-glutamate (0.8%), and water (8.5 - 10%) were added, and the mixing continued for an additional 3 min. The fish cakes were shaped into hemispheres with a diameter of 4 cm, and height of 2 cm, and then fried in soybean oil at 170 - 180°C for 2.5 min. These fish cakes were used for the storage comparison experiment.

Gel strength

A 7-mm diameter spherical probe was used to penetrate the fish cakes at a speed of 60 mm/min using a Compac-100 Rheometer (Sun Scientific, Tokyo, Japan). The peak force [breaking strength (g)], and the distance from the starting point to the peak force [deformation (cm)] was measured. The gel strength (g•cm) was calculated using Eq. 1:

\[
\text{Gel strength} = \text{Breaking force (g)} \times \text{deformation (cm)}
\]  
(Eq. 1)

Colour

Colorimetric measurements were made using a CR-400 photoelectric colorimeter (Konica Minolta, Tokyo, Japan). The lightness (*L*), redness (*a*), and yellowness (*b*) values of the fish cakes incorporated with freeze-dried ECWE or with water (control) were measured. The whiteness values were calculated using Eq. 2 (Lanier, 1992):

\[
\text{Whiteness} = 100 - [(100 - L^*)^2 + a^*^2 + b^*^2]^{1/2}
\]  
(Eq. 2)

<table>
<thead>
<tr>
<th>Material</th>
<th>Control</th>
<th>ECWE-0.5</th>
<th>ECWE-0.75</th>
<th>ECWE-1.0</th>
<th>ECWE-1.25</th>
<th>ECWE-1.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surimi</td>
<td>71.45</td>
<td>71.45</td>
<td>71.45</td>
<td>71.45</td>
<td>71.45</td>
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<tr>
<td>Freeze-dried ECWE</td>
<td>0</td>
<td>0.5</td>
<td>0.75</td>
<td>1.0</td>
<td>1.25</td>
<td>1.50</td>
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<tr>
<td>Starch</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
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<td>1.5</td>
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<tr>
<td>Sugar</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
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<tr>
<td>D-xylose</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
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<td>Potassium sorbate</td>
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<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Glucono-δ-lactone</td>
<td>0.35</td>
<td>0.35</td>
<td>0.35</td>
<td>0.35</td>
<td>0.35</td>
<td>0.35</td>
</tr>
<tr>
<td>Monosodium L-glutamate</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>Water</td>
<td>10</td>
<td>9.5</td>
<td>9.25</td>
<td>9.0</td>
<td>8.75</td>
<td>8.5</td>
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<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

ECWE: *Ecklonia cava* hot water extract.
Experimental design

A central composite design with central points was employed to analyse the effects of two different parameters [ECWE (X₁, %) and water (X₂, %)] on gel strength and overall acceptance (Table 2). The response surface plots were considered for optimisation parameters to prepare a sample with the highest gel strength (Y₁) and overall acceptance (Y₂).

The effect of two independent responses (Ŷ) was modelled with a polynomial response surface using Minitab version 14.0 (Harrisburg, PA, USA). Table 3 shows the actual design of the experiments, which contained 11 randomised experimental runs, including four replicates at the centre point for evaluating the experimental error and the suitability of the mathematical model. The following second-order equation or its reduced form was used to fit the model:

\[
Y = \beta_0 + \sum_{i=1}^{p} \beta_i X_i + \sum_{i<j=1}^{p} \beta_{ij} X_i X_j
\]

(Eq. 3)

Where, \(Y\) = dependent variable (gel strength and overall acceptance); \(\beta_0\) = constant; \(\beta_i\) and \(\beta_{ij}\) = regression coefficients; and \(X_i\) and \(X_j\) = levels of the independent variables. The dependent variables, \(Y_1\) (gel strength) and \(Y_2\) (overall acceptance), were considered as parameters for optimisation to prepare a sample with the highest gel strength and overall acceptance. The response surface plots were developed using Maple software version 7 (Waterloo Maple Inc., Ontario, Canada), and represented a function of two independent variables.

Sensory evaluation

The fish cakes incorporated with ECWE, and the control sample were evaluated by scoring on attribute scales with values ranging from 1 to 7 points (1 = very poor, 2 = poor, 3 = slightly worse than normal, 4 = normal, 5 = slightly better than normal, 6 = good, and 7 = very good) using a 28-person panel. The scoring attributes were taste, colour, flavour, chewiness, and overall acceptance.

Scanning electron microscopy

Scanning electron microscopy was used to examine the inner surface of the fish cakes incorporated with ECWE, and the control sample. The fish cake samples were lyophilised and then

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**Table 2. Experimental range and value of independent variables on the central composite design for fish cakes incorporated with freeze-dried ECWE.**

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Factor</th>
<th>Range level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>-1.414 -1 0 1 1.414</td>
</tr>
<tr>
<td>ECWE (%)</td>
<td>X1</td>
<td>0.28 0.3 0.5 0.7 0.78</td>
</tr>
<tr>
<td>Water (%)</td>
<td>X2</td>
<td>5.7 7 10 13 14.2</td>
</tr>
</tbody>
</table>

**Table 3. Central composite design and responses of the dependent variables for the preparation of fish cakes incorporated with freeze-dried ECWE.**

<table>
<thead>
<tr>
<th>Run no.</th>
<th>X1 ECWE (%)</th>
<th>X2 Water (%)</th>
<th>Y1 Gel strength (g•cm)</th>
<th>Y2 Sensory evaluation (score)</th>
<th>Coefficients assessed by</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-1</td>
<td>-1</td>
<td>1060.69</td>
<td>5.142857</td>
<td>Fractional factorial design (4 points)</td>
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<tr>
<td>2</td>
<td>1</td>
<td>-1</td>
<td>1078.93</td>
<td>4.214286</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>-1</td>
<td>1</td>
<td>719.16</td>
<td>5.714286</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>1</td>
<td>959.88</td>
<td>4.928571</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>-1.414</td>
<td>0</td>
<td>949.43</td>
<td>5.5</td>
<td>Star points (4 points)</td>
</tr>
<tr>
<td>6</td>
<td>1.414</td>
<td>0</td>
<td>932.22</td>
<td>4.571429</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>-1.414</td>
<td>1044.12</td>
<td>4.642857</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>1.414</td>
<td>882.33</td>
<td>4.714286</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>0</td>
<td>1096.99</td>
<td>4.69231</td>
<td>Central points (3 points)</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>0</td>
<td>1096.99</td>
<td>4.69231</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>0</td>
<td>0</td>
<td>1096.99</td>
<td>4.69231</td>
<td></td>
</tr>
</tbody>
</table>
coated with platinum by carbon ion sputter (E-1010, Hitachi, Tokyo, Japan). The SEM images were obtained using a Hitachi SEM (S-2400, Hitachi).

**Proximate composition**

The moisture, crude protein, crude lipid, and ash contents of the samples were determined following the methods of the AOAC (1995). The total carbohydrate content in the sample was calculated by subtracting the combined contents of crude protein, lipid, moisture, and ash from 100.

**Mannitol content**

Approximately, 2 g of the sample was weighed and added to 50 mL of 30% ethanol, and extracted by shaking for 10 min. This extract was centrifuged at 3,700 g for 10 min, and the resulting supernatant was diluted with 30% ethanol up to 50 mL. Next, 1 mL aliquot of this solution was concentrated under nitrogen flow before 2 mL of p-nitrobenzoyl chloride was added, and the mixture reacted for 50 min. Five or six drops of methanol were added to stop the reaction, and then the solution was concentrated under a nitrogen stream. The residue was dissolved in 5 mL of chloroform, and the solution was activated with n-hexane, passed through a Sep-Pak silica cartridge (Waters, Milford, MA, USA), and eluted with 25 mL ethyl acetate. The solution was evaporated to dryness at 40°C using a vacuum evaporator. The dried residue was dissolved in 10 mL of acetonitrile, and filtered through a membrane filter (pore size, 0.2 µm) prior to analysis. A high-performance liquid chromatograph (Surveyor Plus HPLC system, Thermo Fisher Scientific, Waltham, MA, USA) equipped with a photodiode array detector was used. A Capcell Pak C18 (4.6 x 250 mm, 5 µm; Shiseido, Tokyo, Japan) column with isocratic elution (acetonitrile:water; 77:23) was used to separate the analytes. The flow rate was 1.0 mL/min, and the injection volume was 10 µL. The photodiode array detector wavelength was 260 nm.

**Statistical analysis**

Data were analysed using analysis of variance (ANOVA) with a general linear model procedure. Multiple comparisons of the differences between significant factors on the ANOVA were identified using Duncan’s multiple-range test. All statistical analyses were performed using SAS for windows (SAS enterprise guide ver. 4.3).

**Results**

**Gel strength**

The textures of fish cakes incorporated with 0, 0.5, 0.75, 1.0, 1.25, and 1.5% freeze-dried ECWE are shown in Table 1. Gel strength significantly increased in all fish cakes incorporated with ECWE (1,026.7 – 1,110.6 g•cm) as compared to the control sample (919.1 g•cm; p < 0.05). Fish cakes incorporated with 0.5% freeze-dried ECWE had the highest gel strength (1,110.6 g•cm), and the gel strength did not significantly increase at ECWE concentrations greater than 0.75%.

**Colour**

The colour measurements of fish cakes incorporated with freeze-dried ECWE are shown in Table 4. The control exhibited a higher lightness value (61.67) than the fish cakes incorporated with freeze-dried ECWE (48.86 - 54.82). The redness and

<table>
<thead>
<tr>
<th>Sample</th>
<th>Gel strength (g•cm)</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>Whiteness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>919.1 ± 97.3b</td>
<td>61.78 ± 0.63a</td>
<td>-1.56 ± 0.14f</td>
<td>2.46 ± 0.19e</td>
<td>61.67 ± 0.63a</td>
</tr>
<tr>
<td>ECWE-0.5</td>
<td>1110.6 ± 89.4a</td>
<td>54.20 ± 0.09c</td>
<td>1.19 ± 0.03e</td>
<td>7.65 ± 0.03d</td>
<td>53.55 ± 0.09c</td>
</tr>
<tr>
<td>ECWE-0.75</td>
<td>1069.9 ± 50.3a</td>
<td>55.50 ± 1.12b</td>
<td>1.35 ± 0.04d</td>
<td>7.71 ± 0.03d</td>
<td>54.82 ± 1.10b</td>
</tr>
<tr>
<td>ECWE-1.0</td>
<td>1047.1 ± 79.6a</td>
<td>51.79 ± 0.61d</td>
<td>2.03 ± 0.09c</td>
<td>8.51 ± 0.14c</td>
<td>51.00 ± 0.62d</td>
</tr>
<tr>
<td>ECWE-1.25</td>
<td>1026.7 ± 27.6a</td>
<td>49.79 ± 0.15e</td>
<td>2.80 ± 0.01b</td>
<td>9.32 ± 0.07b</td>
<td>48.86 ± 0.15c</td>
</tr>
<tr>
<td>ECWE-1.5</td>
<td>1038.9 ± 34.9a</td>
<td>50.23 ± 0.16c</td>
<td>3.01 ± 0.07a</td>
<td>9.70 ± 0.03a</td>
<td>49.20 ± 0.16c</td>
</tr>
</tbody>
</table>

Different lowercase superscripts in same column indicate significant differences based on Duncan’s multiple range test at p < 0.05.
yellowness values of the control were -1.56 and 2.46, respectively, and those of fish cakes incorporated with freeze-dried ECWE ranged from 1.19 - 3.01 and 7.65 - 9.70, respectively. The fish cakes incorporated with freeze-dried ECWE had significantly lower lightness, and higher redness and yellowness values than the control \((p < 0.05)\).

**Optimisation of multiple responses**

In the present work, important factors influencing the quality of fish cakes – gel strength \((Y_1)\) and sensory attributes \((Y_2)\) – were affected by the amount of freeze-dried ECWE \((X_1)\) and water \((X_2)\) added to the fish cakes. The estimated response function and the effects of adding freeze-dried ECWE and water are shown in Figure 1. \(X_1\) increased from 0.28% (-1.414) to 0.78% (+1.414), and \(X_2\) increased from 5.7% (-1.414) to 14.2% (+1.414). As the coded values, \(X_1\) and \(X_2\), reached 0.57% (+1) and 4.78% (-0.5), respectively, and the coded values \(Y_1\) (1137 g·cm, gel strength) and \(Y_2\) (5.71, overall acceptance) also increased. These results indicated that an ECWE content up to 0.5% increased gel strength and overall acceptance.

**Microstructure of fish cakes incorporated with freeze-dried ECWE**

The microstructures of the control fish cakes without freeze-dried ECWE, and those incorporated with 0.5% freeze-dried ECWE are shown in Figures 2A and 2B, respectively. Fish cakes incorporated with 0.5% freeze-dried ECWE had a smooth surface, uniformly porous structure, and compact gel network with a fibrillar structure.

**Discussion**

The rheological properties of surimi depend on the fish species, its quality, salt content, additives, and processing methods used (Kong et al., 1999a, 1999b; Eom et al., 2013). To provide the textural
properties of surimi-based products such as fish cakes that consumers prefer, additives such as starch, beef plasma protein, gum, egg white, and soy protein are often used (Khan et al., 2003; Jafarpour et al., 2012). Previous studies reported that the gel strength of surimi could also be enhanced by adding phenolic compounds such as those in coconut husk extract, seaweed extract, aqueous extracts of ginger, kiw wood extract, carrageenan, and microbial transglutaminase (Balange and Benjakul, 2009; Eom et al., 2013; Kaewudom et al., 2013; Shitole et al., 2014). Melanin-free ink also improves the textural properties of sardine surimi and prevents lipid oxidation during refrigerated storage (Vate et al., 2015).

In the present work, fried fish cakes incorporated with 0.5% ECWE exhibited improved gel strength. The proximate composition of freeze-dried ECWE was: moisture, 3.85 g/100 g; crude protein, 4.73 g/100 g; crude lipids, 0.42 g/100 g; ash, 23.4 g/100 g; and carbohydrate, 67.6 g/100 g. The mannitol content in freeze-dried ECWE was 38.2 g/100 g.

Three minor extracts derived from brown seaweeds are also worthy of mention: laminarin, fucoidan, and mannitol. Mannitol is an important sugar alcohol present as a cell sap in a number of brown algae, especially certain species of Laminaria and Ecklonia (Løtze and Hoffman, 2016). Gel-forming ability is a direct indicator of the quality of fish proteins. Salt linkages, hydrogen bonds, disulphide bonds, and hydrophobic interactions are the main types of bonds that contribute to the network structure during gelation (Sen, 2005). It is likely that intermolecular protein cross-linking by S–S bonds affects mannitol during enhanced gelation.

The major component of ECWE extracted by ethanol is phlorotannin, which has physiological, antioxidative, 2,2-diphenyl-1-picrylhydrazyl-radical scavenging, and superoxide dismutase-like activities (Kim et al., 2013). Incorporating ECWE to seafoods enhances their shelf-life, quality, and health-related beneficial properties (Roohinejad et al., 2017). Freeze-dried methanol extract from E. cava contains 18 – 1,953 mg/g extract of polyphenolic compounds, and the phenolic content of Sargassum spp. extract is 0.2 - 17 mg/g (Zahra et al., 2007; Zubia et al., 2008; Hwang et al., 2010). In the present work, the extract from E. cava contained 18 – 1,953 mg/g extract polyphenolic compound, but its phenolic content was 9.3 mg equivalents phloroglucinol per gram and 11.3 mg equivalents gallic acid per gram.

Shitole et al. (2014) reported that seaweed extract can be used as a gel enhancer in lesser sardine surimi with a corresponding increase in the acceptability of its texture because of the phenolic compounds in the seaweed. Also, Shitole and Balange (2014) reported that aqueous brown seaweed (Sargassum tenerrimum) extract strengthened the gel of Japanese threadfin bream surimi at the optimum level (0.02%) with no detrimental effect on the sensory properties of the surimi gel. Seaweed extract can be used as a natural gel enhancer for the fish surimi industry.

The increase in gel strength is attributed to the cross-linking activity of phenolic compounds in the seaweed extract, which induce the formation of both covalent and non-covalent bonds in the gel matrix (Prigent et al., 2003). The multidentate mechanism requires a much lower phenolic compound/protein molar ratio, thus allowing for a lower concentration of phenolic compound to be used. The decrease in gel strength of surimi with higher concentrations of freeze-dried ECWE (> 0.5%) in the present work may be associated with self-aggregation of phenolic compounds, leading to impaired protein cross-linking (Shitole and Balange, 2014).

**Conclusion**

To develop gel-enhanced fried fish cakes, we used seaweed hot water extract from brown algae, Ecklonia cava, which is a healthy food ingredient. Freeze-dried ECWE significantly improved the texture of the fish cakes, but reduced their whiteness. Adding 0.5% freeze-dried ECWE improved the gel strength to help produce high-quality emulsions that were finely distributed over the gel network due to the presence of mannitol and some polyphenolic compounds in the freeze-dried ECWE.

**Acknowledgement**

The present work was financially supported by the National Institute of Fisheries Science, Korea (grant no.: R2021062).

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