Gel Quality and In Vitro Digestion Characteristics of Celery *Nemipterus virgatus* Fish Sausages

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**Abstract.** This study evaluated gel properties, flavor characteristics, and in vitro digestion characteristics of fish sausages with 10% celery petioles and leaves. The results indicated that the breaking force, water holding capacity, saltiness, astringency and sensory evaluation of fish sausage significantly increased, whereas whiteness, cooking loss and umami significantly decreased, and the microstructure of the fish sausages showed higher density and uniformity. After in vitro digestion, the digestibility, degree of hydrolysis and Fe²⁺ chelating capacity of the fish sausages significantly increased, and protein aggregate size and thiobarbituric acid number significantly decreased and protein aggregates decreased in number and were dispersed. The breaking force, saltiness, and protein digestibility of the sausages with celery petioles and leaves were 13.91%, 67.06%, and 0.55% and 10.75%, 55.62%, and 3.9% higher than the control group respectively. Celery significantly improved gel properties, flavor characteristics, and in vitro digestion characteristics. The effect of celery leaves on in vitro digestion was significantly greater than celery petioles.

1. **Introduction**

Surimi-based products are aquatic foods that mainly consist of surimi as the main raw material and mixed other raw materials for molding and heating. Gel properties are important physicochemical properties in evaluating surimi quality [1]. Surimi forms into a gel during heating, which then goes through gelation, gel cracking, and fish cake stages to ultimately develop three-dimensional network structures. The protein conformation and chemical forces undergo changes during gel formation [2]. A well-formed gel has a uniform and dense three-dimensional network structure that increases gel strength and retains a specific amount of water [3].

Celery belongs to the subfamily *Umbellales*, (*APIOIDEAE* Drude), and includes *Apium graveolens* L. and *Oenanthe javanica* (Blume) DC. *A. graveolens* L. has the highest medicinal value and known as “medicinal celery”, which is grows around the world [4]. Celery is regarded as a source of biologically active compounds, which include a variety of volatile components, such as monoterpenoids, sesquiterpenoids, phenylpropanol, isoocetane and limonene, antioxidants, such as vitamin C, folic acid, carotene, flavonoids, phenolic acids, and various trace elements, such as calcium, phosphorus, iron, selenium and can prevent cardiovascular diseases [5-6]. Dietary fiber (DF), which is abundant in
celery is a nutrient that cannot be digested by the human digestive system and consists of soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) [7]. SDF is produced by the fermentation of intestinal microflora, which is related to carbohydrates and lipid metabolism; IDF can promote intestinal function and play an important role in preventing colonic diverticulum and constipation [8]. It has been suggested that celery may be utilized as a nutritional supplement and improve the flavor of rinsed surimi [9].

In vitro digestion can mimic the physiological conditions of in vivo digestion and it is a useful tool for understanding the changes, interactions, and bioavailabilities of nutrients, medicines, and non-nutritive compounds. Therefore, In vitro digestion is widely used in nutrition, pharmacology, and food chemistry, with advantages of being inexpensive, rapid, and highly repeatable [10]. In recent years, digestion models have been enriched and optimized such as the in vitro static model, in vitro dynamic model, single-chamber digestion model, multi-chamber digestion model, single-enzyme system digestion model, multi-enzyme system digestion model, and the conditions of in vitro simulated digestion are closer to the real conditions in the body and more accurately reflect the true state [11]. In vitro simulated digestion is extensively used in the food industry, and is often employed to investigate the mechanism of protein digestion, absorption and the transport of ingested in the body [12].

The nutrient composition of celery and Nemipterus virgatus surimi, the effects of sausage with celery on gel quality, flavor and antioxidation, and its digestion by the method of in vitro simulated digestion were investigated and the effects of celery petioles and leaves were compared. To improve the nutritional content of surimi-based products such as DF, this study aimed to increase the nutritional composition of surimi-based products.

2. Materials and Methods

2.1. Raw materials

N. virgatus surimi (degree of AAA) was purchased from Qingdao Xinjinyun International Trade Co., Ltd, (Qingdao, Shandong, China). Celery (A. graveolens L.) and salt were purchased from the local market (Jinzhou, Liaoning China). α-Amylase (CAS: 9000-90-2, 300-1500 units mg-1 protein), pepsin (CAS: 9001-75-6, ≥2500 units mg-1 protein), and pancreatin (CAS: 8049-47-6, powder, suitable for cell culture, 4xUSP specifications) were purchased from Shanghai Sigma-Aldrich Trading Co., Ltd. (Shanghai, China). Bile salt (CAS: 8008-63-7, purity: BR) and ferrozine (CAS: 69898-45-9, purity: 97%) were purchased from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). 2-Thiobarbituric acid (CAS: 504-17-6, purity: ≥98.5%, grade: AR) was purchased from Nanjing Dulai Biotechnology Co., Ltd. (Nanjing, Jiangsu, China). Ferrous chloride [CAS: 7758-94-3, purity: anhydrous, 99.5% (metals basis)] was obtained from Energy Chemical of Saen Chemical Technology Co., Ltd. (Shanghai, China). Standard liquid NaOH (0.1 M, 0.01 M, grade: AR) and Nile Blue A (CAS: 3625-57-8, biological stain) were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Petroleum ether, potassium chloride, copper sulfate, glutaraldehyde, sodium dihydrogen phosphate, sodium phosphate, absolute ethanol, potassium chloride, tartaric acid, ethylene diamine tetraacetic acid (EDTA), trichloroacetic acid (TCA), and formaldehyde solution were of analytical grade and purchased from Tianjin Zhiyuan Chemical Reagent Co., Ltd. (Tianjin, China).

2.2 Analysis of composition and pH

N. virgatus surimi and celery petioles and leaves were measured respectively according to the National Standards of GBT 22906.3-2008, GB 5009.4-2016, GB 5009.5-2016, GB 5009.6-2016 and GB 5009.92-2016 (National Food Safety Standards, China). The total dietary fiber (TDF), SDF, IDF and total acid content of the celery petioles and leaves were each measured according to the National Standards of GBT 5009.88-2014 and GBT 12456-2008. The pH of the N. virgatus surimi and the celery petioles and leaves were measured according to the National Standards of GB 5009.237-2016. Each sample was measured thrice [13-20].
2.3 Gel properties

2.3.1. Preparation of fish sausages

*N. virgatus* surimi was thawed in running water to a semi-defrosted state, cut into small pieces, placed in a vacuum chopping machine (UMC 5, Stephan Mannebeck Co. Ltd, Gronau, Germany) and mixed at 750 rpm for 2 min. Then, 2.5% salt was added to the surimi and mixed at 1,500 rpm for 2 min under vacuum. Then, 10% celery petioles or leaves (minced with a chopping machine) was then added and mixed at 3,000 rpm for 3 min under vacuum (-0.6 Pa). The material was taken out, and filled into a sausage casing (2.5 cm-diameter). During the chopping process, the temperature of the material was lower than 10°C, and the moisture of the surimi gel was adjusted to 78%. The fish sausages were heated at 40°C for 30 min, and then at 90°C for 20 min in a water bath. These were then immediately cooled in ice water and stored in a refrigerator at 4°C.

2.3.2. Sausage color determination

The sausages were placed at 25°C, cut into 2.0-cm-high cylindrical specimens, and the L*, a*, and b* were measured by using a colorimeter (CR-400, Konica Minolta Holdings Inc., Tokyo, Japan), and each sample was measured six times. The formula for calculating whiteness was as described by Park [21] as follows:

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

2.3.3. Puncture test

The sausage was placed at 25°C, cut into 2.0-cm-high cylindrical specimens, and puncture test was measured by a texture analyzer (TA-XT-Plus, Stable Micro Systems Ltd, Surrey, UK) as described by Pita-calvo et al. [22]. The measuring parameters were as follows: the speed of pre-test, test, and post-test was 1 mm s^{-1}; trigger force was 5 g; probe model was P/5S; and the strain of the shape was 75%.

Each sample was measured for 10 times.

2.3.4. Cooking loss and water holding capacity

The sausages (approximate 1.00 ± 0.02 g) were placed at 25°C and cut into slices of 1 × 1 × 0.5 cm³ for measuring. Cooking loss (CL) was expressed as the weight change of the sample before and after cooking, as described by Yang et al. [23]. The sample was wrapped in three sheets of filter paper, placed in a centrifuge tube, and centrifuged at 4°C, 5,000 g for 15 min as described by Cao et al. [24]. Each sample was measured thrice.

2.3.5 Scanning electron microscope (SEM)

The sausages were cut into cubes of 3 × 3 × 2 mm³, fixed with 2.5% glutaraldehyde (containing 50%, pH 7.2 phosphate buffer solution) for 24 h, rinsed in the phosphate buffer solution (thrice, 15 min each time), rinsed in deionized water for 1 h to remove the glutaraldehyde solution, dehydrated through across gradient ethanol series of 50%, 70%, 90% (once, 15 min), 100% (thrice, 10 min each time), and air-dried. The microstructures of the samples were observed under an SEM (S4800, Konica Minolta Holdings, Inc., Tokyo, Japan) at an acceleration voltage of 3.0 kV.

2.4. Flavor characteristics

2.4.1 Analysis of electronic tongue

The sausages (> 50 g) were crushed in a chopping machine and heated until the temperature of the centre of the sample reached 40°C. The sample (50 g) was transferred into a beaker, mixed with deionized water at 40°C at a mass ratio of 1:5, and homogenized. Then, the mixture was centrifuged at room temperature at 5,000 g for 15 min. The supernatant was filtered twice with a 0.45-μm filters, and then evaluated by using a taste analysis system (SA402B, Nikon Corp, Tokyo, Japan), using 30 mM
potassium chloride and 0.3 mM tartaric acid solution as the reference liquids. Each same sample was measured thrice.

2.4.2 Sensory evaluation
The color, texture, taste and acceptability of the samples were evaluated according to Table 1 by a sensory evaluation group consisting of five boys and five girls. The scores of each item and total scores of all items were calculated.

| Sensory evaluation standards of sausages |
|----------------------------------------|
| Sensory evaluation (score) | 1-5 | 6-10 | 11-15 |
| Colour | Too dark or light, nonuniform | Suitable, more uniform | Bright and full, uniform |
| Texture | No springiness, nonuniform, more large holes | More springiness, uniform, small holes | Better springiness, uniform, few holes |
| Taste | Too salty or light, strong fishy odor | Suitable, a little fishy odor, a little vegetable flavor | Suitable, little fishy odor, rich vegetable flavor |
| Acceptability | Unacceptable | Acceptable | Especially acceptable |

2.5. In vitro digestion characteristics

2.5.1 Preparation of the in vitro digestion products
The stock solutions of simulated salivary fluid (SSF), simulated gastric fluid (SGF), and simulated intestinal fluid (SIF) were prepared according to Minekus et al [25], and presented in Table 2. Each 60 mL of SSF consisted of 48 mL of the SSF stock solution, 300 μL of CaCl₂(H₂O)₂, 11.7 mL deionized water and 152.8 mg α-amylase in pH 7. Each 210 mL of SGF comprised of 168 mL the SGF stock solution, 105 μL of CaCl₂(H₂O)₂, 41.23 mL deionized water and 1,292.34 mg pepsin in pH 1.6. Each 350 mL of SIF was made up of 280 mL of the SIF stock solution, 600 μL of CaCl₂(H₂O)₂, 69.30 mL deionized water, 1,090.30 mg pancreatin and 2.80 g of bile salt in pH 7.00. The simulated digestion fluids were all freshly prepared.

| Composition/Concentration | SSF (mm/L) | SGF (mm/L) | SIF (mm/L) |
|---------------------------|------------|------------|------------|
| KCl                       | 15.1       | 6.9        | 6.8        |
| KH₂PO₄                   | 3.7        | 0.9        | 0.8        |
| NaHCO₃                   | 13.6       | 25         | 85         |
| NaCl                     | —          | 47.2       | 38.4       |
| MgCl₂(H₂O)₆             | 0.15       | 0.1        | 0.33       |
| (NH₄)₂CO₃               | 0.06       | 0.5        | —          |
Approximately 150 g of sausage was mixed with 60 mL SSF in 25°C and minced together in a chopping machine at 10 speed for 15 s to simulate oral digestion. Then, the mixture was placed in a stomach model of an *in vitro* simulated digestion system (DIVHS-I, Xiao Dong Yi Jian Instruments and Equipment Co., Ltd. Suzhou, Jiangsu, China) for 2 h to simulate gastrointestinal continuous digestion. The SGF was flowed into the empty stomach model initially using 10 mL, and then using a simulated gastric fluid flow acceleration, 230 mL of SGF was flowed into stomach model by shifting speed during digestion (Figure 1). The 336 mL of SIF was flowed in at a constant speed of 2.8 mL min⁻¹. The operating parameters of the equipment were as follows: the speed of the gastric rollers was 12 rpm; the extrusion frequency and depth of the extrusion plate were 5 times min⁻¹ and 25 mm, respectively; the right inclination was 30° at 0-30 min, 22.5° at 30-60 min, 15° at 60-90 min, and 7.5° at 60-120 min; the extrusion frequency of the pylorus was 2 times·min⁻¹, the position of the extrusion end was 25 mm, and the position of extrusion opening was 10 mm; and the chyme after digestion in the stomach was directly sent into the duodenum for digestion, and the speed of the intestinal rollers was 12 rpm. All samples after digestion were collected, centrifuged (4°C) at 8,000g for 15 min, separated into precipitate and supernatant, and immediately stored in a freezer at -80°C.

![Figure 1](image.png)

**Fig. 1.** Variation tendency of simulation gastric fluid flow acceleration.

2.5.2 Determination of protein digestibility, degree of hydrolysis and protein size

Protein digestibility in vitro was expressed as protein content in the sample before (M₁) and after (M₂) digestion and was calculated by using the following equation:

\[
\text{Protein digestion (\%)} = \left( \frac{M_1 - M_2}{M_1} \right) \times 100
\]  

(2)

The protein content of each sample was measured according to the National Standards of GB 5009.5-2016 [15].

Approximately 50 mL of the formaldehyde solution was mixed with 3 mL of 0.5% phenolphthalein indicator and titrated to slightly pink with 0.1 M NaOH on a magnetic stirrer to prepare a neutral formaldehyde solution. Then, mixed 2 mL of the diluted 20 times digested sausage supernatant with 5 mL of deionized water, 200 μL of 0.5% phenolphthalein indicator, and 2 mL neutral formaldehyde, and titrated to slightly pink with the 0.01 M or 0.05 M NaOH on a magnetic stirrer. The amount of NaOH solution consumed during titration were recorded, and deionized water was used as blank. The degree of hydrolysis (DH) was expressed as the ratio of the amino acid nitrogen content (N₁) in the supernatant after digestion to the total nitrogen content (N₂) in the sample, using the following equation:

\[
\text{Degree of hydrolysis (\%)} = \frac{N_1}{N_2} \times 100
\]  

(3)

N₁ and total N₂ were respectively measured using National Standards GB 5009.235-2016 and GB 5009.5-2016 [26,15].
The supernatant of the digested sausage was diluted 20 times, and the average size was determined in the size analyzer (NanoBrook 90 Plus, Bruker Corp, N Y, USA). Each sample was measured thrice.

2.5.3 Laser scanning confocal microscopy

One milliliter of the supernatant of the digested sausage was mixed evenly with 40 μL of 0.1% Nile Blue A to dye the protein in the supernatant, and 1 drop of the mixture was placed in the center of a slide and observed on a laser scanning confocal microscope (LSM 510, Carl Zeiss AG, Jena, Germany) at a magnification of 40×. Helium neon laser at a wavelength of 633 nm was used in sample imaging, emission spectra at a wavelength of 680 nm were collected, and the resulting images were overlaid as described by Liu and Lanier [27].

2.5.4 Atomic force microscopy

The supernatant of the digested sausage was diluted 20 times, and one drop of the mixture was placed in the center of a coverslip. The coverslip was dried naturally and scanned within a range of 5 μm × 5 μm by using an atomic force microscope (XE-70, Park Systems, Cheonan, Korea).

2.5.5 Determination of thiobarbituric acid and Fe²⁺ chelating capacity

Approximately 5 g of sausage or 5 mL of the supernatant of the digested sausage were mixed with 15 mL of a mixture (7.5% TCA and 0.1% EDTA), homogenized, left to stand and filtered, and the supernatant was used in analysis. A 2.5-mL aliquot of the supernatant was mixed with 2.5 mL of 0.02 M thiobarbituric acid (TBA) and then placed in the 10-mL centrifuge tube, shaken, allowed to react in a 90°C water bath for 50 min, cooled immediately, and then the absorbance was measured at a wavelength of 532 nm using a UV-vis spectrophotometer (UV-2550, Shimadzu Inc, Kyoto, Japan), which was recorded as A_sample. A 2.5-mL aliquot of the TCA-EDTA mixture instead of the supernatant was mixed with 2.5 mL of TBA to adjust zero. Approximately 2.5 mL of deionized water instead of the TBA were mixed with 2.5 mL of the supernatant and used as blank A_blank. The TBA represented the number of milligrams of the malondialdehyde (MAD) per kilogram of sample and was calculated by using the following equations:

\[
\text{TBA/(mg·kg}^{-1}) = A_{\text{sample}} \times V_1 \times M/\left(\varepsilon \times l \times m\right) \tag{4}
\]

\[
\text{TBA/(mg·L}^{-1}) = (A_{\text{sample}} - A_{\text{blank}}) \times V_1 \times M/\left(\varepsilon \times l \times V_2\right) \tag{5}
\]

where A is the absorbance; \(V_1\) is the volume of sample reaction (mL); \(M\) is the molecular mass of malondialdehyde (72.063, g·mol\(^{-1}\)); \(\varepsilon\) is the molar absorptivity (156,000, L·(mol·cm)\(^{-1}\)); \(l\) is the optical path (1 cm); \(m\) is the weight of the sample (kg); and \(V_2\) is the total volume of the sample (L).

Approximately 3.7 mL of the supernatant of the digested sausage were mixed with 100 μL of 2 mM ferrous chloride and 200 μL of 5 mM ferrozine, left to stand at 25°C for 10 min, and then absorbance was measured at a wavelength of 562 nm using a UV-vis spectrophotometer and recorded as A_sample. Approximately 300 μL of deionized water were mixed with 3.7 mL of the sample supernatant and used as blank.

| Composition           | Celery petiole | Celery leaf | Nemipterus virgatus surimi |
|-----------------------|---------------|-------------|---------------------------|
| Moisture (g/100g)     | 95.99±0.04    | 89.03±0.18  | 76.04±0.21                |
| Ash (g/100g)          | 1.08±0.01     | 2.92±0.01   | 0.71±0.01                 |
| Protein (g/100g)      | 0.40±0.00     | 2.29±0.00   | 18.32±0.34                |
| Fat (g/100g)          | 0.37±0.02     | 1.65±0.10   | 2.48±0.72                 |
| SDF (g/100g)          | 0.41±0.00     | 0.71±0.03   | ——                        |
| IDF (g/100g)          | 1.52±0.02     | 2.64±0.07   | ——                        |
| TDF (g/100g)          | 2.02±0.07     | 3.23±0.00   | ——                        |
| Calcium ion (mg/100g) | 51.40±0.60    | 457.40±0.60 | 60.00±0.50                |
Different letters indicate significant differences in pH of celery petioles and leaves and N. virgatus Surimi (p<0.05).

Fe$^{2+}$ chelating capacity was calculated using the following equation:

$$Fe^{2+} \text{ chelating capacity (\%)} = \frac{[A_{\text{control}} - (A_{\text{sample}} - A_{\text{blank}})]}{A_{\text{control}}} \times 100 \quad (6)$$

Each sample was measured thrice.

2.6. Statistical analysis

The results were expressed as the mean ± standard error (SE). The data were analyzed by Duncan’s method using SPSS 9.0 software, and the significance level was set at p<0.05. Images were processed using ImageJ, and Gwyddion 2.53.

3. Results and discussion

3.1. Composition and pH of celery and N. virgatus surimi

The compositions and pH of celery and N. virgatus surimi are shown in Table 3. The moisture content in the celery petioles was higher than that in the leaves, and ash, protein, fat, TDF, calcium ion, and total acid contents were lower than in the leaves. Among the compositions of celery petioles and leaves, except for moisture, the TDF content was the highest, and the content of TDF in celery leaves was 1.74 times that in the petioles. The ratio of SDF to IDF in the petioles and leaves was 1:3.7. Calcium ion content in celery leaves was 8.9 times that in the petioles. Total acid content in celery leaves was 1.74 times that in the petioles. Overall, the nutrients in celery leaves were higher than the petioles, and thus, are more beneficial for a healthy diet.

Except for moisture, N. virgatus mainly consists of protein (18.32 g·100 g$^{-1}$). The pH of the celery leaves was 5.86 and significantly lower than the petioles (6.11; p< 0.05) because of the higher total acid content in the leaves. The pH of N. virgatus was 7.68, which is within the neutral range, and is conducive to the formation of gel networks. Its moisture content was 76.04%, which met the grade standard of surimi-based products.

| Table 4. Effect of celery on sausage color |
|-------------------------------------------|
|                | L*     | a*     | b*     | Whiteness     |
| Control        | 81.12±0.14$^a$ | -6.27±0.02$^a$ | 12.33±0.11$^c$ | 76.59±0.06$^a$ |
| Celery petiole | 78.33±0.22$^b$ | -9.57±0.04$^b$ | 16.20±0.19$^b$ | 71.30±0.16$^b$ |
| Celery leaf    | 59.43±0.43$^c$ | -19.84±0.10$^c$ | 30.00±0.27$^a$ | 45.78±0.50$^c$ |

Different letters indicate significant differences among samples (p<0.05). CP, sausages with celery petiole; CL, sausages with celery leaves.

| Table 5. Effect of celery on sausage gel properties |
|-----------------------------------------------|
|                | Breaking force (g) | Deformation (mm) | Cooking loss (%) | Water-holding capacity (%) |
| Control        | 413.34±15.03$^b$ | 11.24±0.21$^a$ | 11.71±0.80$^a$ | 71.08±0.69$^b$ |
| Celery petiole | 470.84±3.87$^a$ | 11.43±0.45$^a$ | 10.25±0.76$^b$ | 76.59±0.86$^a$ |
| Celery leaf    | 457.79±12.20$^a$ | 11.66±0.37$^a$ | 8.88±0.05$^c$ | 77.23±0.00$^a$ |

Different letters indicate significant differences among samples (p<0.05). CP, sausages with celery petiole; CL, sausages with celery leaves.
Table 6. Effect of celery on sensory evaluation of sausages

|                | Colour          | Texture         | Taste            | Acceptability | Total score |
|----------------|-----------------|-----------------|------------------|---------------|-------------|
| Control        | 10.67±0.82b     | 10.17±0.75b     | 5.50±0.55c       | 8.67±0.82c    | 35.00±1.55c |
| Celery petiole | 11.50±0.55b     | 11.67±0.82a     | 7.17±0.98b       | 10.83±0.75b   | 41.17±1.47b |
| Celery leaf    | 13.17±0.75a     | 12.33±0.52a     | 11.33±1.21a      | 12.17±0.75a   | 49.00±1.79a |

Different letters indicate significant differences among samples (p<0.05). CP, sausages with celery petioles; CL, sausages with celery leaves.

3.2. Gel properties

3.2.1. Color analysis

Generally, whiteness is expressed by L*, a*, and b*, where L* is the brightness, and a* is the gradient between red and green, and b* is the gradient between yellow and blue [28]. Table 4 shows that the whiteness of sausage, and the control was 76.59, which was significantly higher by 7.42% and 67.30% than the celery petioles and leaves, respectively (p<0.05). The L* and a* of the celery leaves was significantly lower than petioles and the control, whereas b* was the opposite (p<0.05). These results indicated that celery could increase the degree of green and yellow colors and reduce the brightness, thereby reducing the whiteness of the sausage, and the effect of the celery leaves on the sausages was significantly superior than that of the petioles. Celery contains a variety of pigments such as chlorophyll a, chlorophyll b, and carotenoid, and thus, the pigment content of celery leaves are higher [29].

3.2.2. Puncture test, CL, and water holding capacity analysis

Gel properties is one of the most important for evaluating the quality of surimi gels [30]. The breaking force of the sausage with the control, celery leaves, and petioles was 413.34 g, 457.79 g, and 470.84 g (Table 5). The breaking force of the celery group was significantly higher than the control group (p<0.05), but the distance had no significant difference between groups (p>0.05). Table 5 indicated that the addition of celery increased the breaking force of the sausage, and the effect of celery leaves is superior than that of the petioles. These results are concordant with the findings of Yin et al. [31], who discovered that a low concentration nanosized okara dietary fiber markedly improves gelation properties of silver carp surimi. Celery may be rich in DF and calcium ions, and these contents are higher in the leaves. DF in celery can increase the hardness of bologna sausages to a certain degree [34]. Calcium ions could increase the breaking force of tilapia surimi gel, have a better network structure, and induce the formation of more disulfide and ionic bonds [35]. These results agreed with the findings of the present study.

CL can reflect the ability of the gel system in binding water after denaturation and protein aggregation [36]. The water holding capacity, which is determined by the uniformity and density of the three-dimensional network of the surimi gel, indicated that the gel can physically surround a large amount of water using external or gravitational force, reduce water outflow, and reveal the binding of protein to water, which indirectly reflects the gel properties of surimi-based products [37]. The CL of sausage with the control was 11.65%, which was significantly higher by 13.66% and 31.19% than the celery petioles and leaves, respectively (p<0.05). Water holding capacity was 71.08%, 76.59%, and 77.23%, respectively. The water holding capacity of the celery leaves was higher than the petioles,
than the control group significantly (p<0.05). The results indicated that celery can reduce CL and increase water holding capacity, which reflect that the ability to bind water was stronger in the gel network structure, and the effects of celery leaves on the sausages were better than the petioles, which was consistent with the results of puncture test. It is possible that the DF in celery had strong water absorption, water holding capacity, and the ability to form combined water, which are beneficial to the formation of a denser network structure for the surimi gel, and enhanced the water-binding ability, thereby decreasing water loss [38].

3.2.3. Microstructure analysis

Figure 2 and 3 shows that the control group gel network structure was loose, with distinct fractures that formed larger holes and exhibited unevenness. Compared with the control group, the structure of celery petioles was complete, the pores were small, although gel breakage was also observed. The structure of the celery leaves was dense and uniform, without any detectable gel breaks, and the pores were smaller and fewer. The average hole area of the sausage with control was 727.36 μm², which was larger by 79.43% and 171.44% than the celery petiole and leaf samples, respectively. Polyphenols could modify the secondary structure of proteins and increase the breaking force and ability to bind water of the surimi gel, thereby forming a dense and uniform gel structure [39].

![Fig. 2. Effect of celery on SEM microstructure (×2,500)](image)

![Fig. 3. Effect of celery on average hole area of the sausages. Different letters indicate significant differences among samples (p<0.05).](image)

C, control; CP, sausage with celery petioles; CL, sausage with celery leaves.

3.3. Flavor characteristics

3.3.1. Flavor analysis

The electronic tongue is a bionics instrument based on the biological taste system with specific responsiveness to umami, bitterness, saltiness, richness, and astringency. Figure 4 shows that the flavor values of the sausages consisted of umami, bitterness, saltiness, richness, and astringency that ranged from large to small, with significant differences and is readily distinguishable. The umami of the sausages was higher, with that of the celery leaf group was lower than the celery petiole group and significantly lower than the control group (p<0.05). The results indicated that umami is the main
flavor of surimi-based products, because the sausages have more delicious amino acids and celery has a negative effect on umami. The saltiness value of the celery petiole group was higher than the celery leaf group and significantly higher than the control group (p<0.05). Celery contains nitrite, which could reduce salt addition. A high-sodium diet is closely related to hypertension, gastric cancer, osteoporosis, and other diseases [40]. The astringency value of the celery petiole group was higher than the celery leaf group and significantly higher than the control group (p<0.05). Tannic acid in celery also influenced the flavor. Furthermore, celery has different effects on bitterness and richness.

Fig. 4. Effect of celery on umami, bitterness, saltiness, richness, and astringency radar maps of the sausages. C, control; CP, sausages with celery petioles; CL, sausages with celery leaves.

3.3.2. Sensory evaluation analysis

Sensory evaluation can reflect food traits such as color, flavor, and taste, which are difficult to express using equipment with human senses and can directly reflect consumer acceptability for the product and product quality and has the advantages of accuracy, simplicity, and rapidity. Table 6 shows that the color, texture, taste, acceptability, and total score values of the sausages with the celery leaves group were higher than the celery petiole group and significantly higher than the control group (p<0.05), which coincide with the results of gel strength and texture characteristics.

3.4. In vitro digestion characteristics

3.4.1. Protein digestibility, degree of hydrolysis and protein size analysis

Protein digestibility is an indicator that can directly quantitatively reflect the disintegration degree of food proteins in the gastrointestinal tract by digestive enzymes. The protein digestibility of the sausages with celery leaves was 43.61%, which was significantly higher by 3.32% and 3.9% than the celery petiole and control samples, respectively (p<0.05), whereas the celery petiole group showed no significant difference from the control group (p>0.05; Figure 5). These results were attributable to the DF in celery, which can increase the hydrophobic interactions in the secondary structure of the protein [23]. The main enzyme sites of pepsin are hydrophobic amino acids. The DF in celery can increase the exposure of hydrophobic amino acids in the protein during digestion, which is beneficial to the enzymatic cleavage of pepsin and in turn promotes digestion [41]. The positive charge of metal ions such as Ca$^{2+}$, Zn$^{2+}$, and Fe$^{2+}$ in celery can increase the electrostatic repulsion among proteins, promote protein disintegration, and enhance protein digestion under the action of pepsin [42]. Celery leaves has higher DF and Ca$^{2+}$ contents and thus its inclusion in surimi-based products enhances its digestion.

The degree of hydrolysis reflects protein digestion efficiency, producing small peptides and free amino acids when extensively hydrolyzed. The degree of hydrolysis of sausages with the control and celery leaves and petioles was 3.64%, 5.28%, and 3.98%, respectively. The degree of hydrolysis of sausages with celery leaves was 45.05% higher than the control samples. Figure 5 illustrates the celery could promote hydrolysis of proteins, disintegrating them into smaller aggregates such as peptides and amino acids, and the degree of hydrolysis of celery leaves is higher than the petioles. Protein
hydrolysis can produce digestible peptides, bioactive peptides, and specific amino acids (such as glutamic acid) that can provide nutrients and play an important role in physiological regulation. Hydrolysis of proteins facilitates their digestion and absorption, and thus celery is beneficial for the disintegration of proteins into small molecular peptides, which is beneficial to human health [43]. Muscle proteins are extensively hydrolyzed during dry-cured ham processing, producing a large number of essential amino acids [44].

Analysis of protein size is an important physical and chemical index for evaluating digestion products, which can indirectly reflect the degree of digestion. The average protein size of sausages with celery leaves was 664.87 nm, which was respectively lower by 9.09% and 24.25% than the celery petiole and significantly lower than the control groups (p<0.05), and showing the opposite trend to protein digestibility and degree of hydrolysis. These results indicated that celery can disintegrate proteins into aggregates with smaller particle sizes and that of celery leaves are better. These findings coincided with the results of protein digestibility and degree of hydrolysis.

![Fig. 5. Effect of celery on protein digestibility, degree of hydrolysis, protein size of the sausages.](image)

Different letters indicate significant differences among samples (p<0.05).

3.4.2. **Microstructure analysis**

The red fluorescent highlights are protein aggregates dyed by Nile Blue A. The red fluorescent highlights in SDF group were fewer and weaker in intensity, whereas those in the control group were higher in number and dense, those in the celery petiole and leaf groups were fewer and dispersed than the control group, and those in the celery leaf group were significantly reduced (Figure 6). These results indicated that the protein content of SDF is very low, and the digestive enzymes cannot affect the results of the sample. Most of the proteins in the control group were not completely digested, and there were a large number of aggregates. Celery can promote digestion and disintegration of proteins and reduce protein content, and the effect of celery leaves in the sausages was superior than petioles, which coincides with the above results.

When the color is darker, the protein size is smaller; when the peak is higher, the degree of aggregation is greater [45]. Figure 6 shows that the color of the celery leaf group was the darkest. The sausages with celery petioles and leaves had fewer and lower peaks than the control group, which were flatter, and the celery leaf group had obvious effects. The results indicated the protein aggregates in the control group were higher in number and larger, but protein digestibility was higher and protein macromolecules were fewer in the celery petiole and leaf groups, and the large protein aggregates in the gel had disintegrated into small particles. DFs in celery can expose hydrophobic amino acids and increase enzymatic cleavage, thereby enhancing protein digestion. However, the DF content of celery leaves was higher than the petioles, so the effects was more significant.
3.4.3. TBA and Fe$^{2+}$ chelating capacity analysis before and after digestion

The TBA is an important indicator of the degree of lipid oxidation and is widely used to determine the oxidation of lipids in aquatic and meat products. Figure 7 shows that the TBA values of the control and celery leaf and petiole groups were 38 μg·L$^{-1}$, 33 μg·L$^{-1}$, and 36 μg·L$^{-1}$ after digestion and 76 μg·L$^{-1}$, 54 μg·L$^{-1}$, and 61 μg·L$^{-1}$, respectively, before digestion. The TBA of the control group was higher than the celery petiole group and significantly higher than the celery leaf group ($p<0.05$), regardless of whether this was before or after digestion. The TBA values of the groups before digestion were higher than after digestion. The Fe$^{2+}$ chelating capacity of the control and the celery leaf and petiole groups was 2%, 10.95%, and 4.89%, respectively. The Fe$^{2+}$ chelating capacity of the celery leaf group was higher than the celery petiole group and significantly higher than the control group ($p<0.05$). These results indicate that celery can reduce the TBA value, increase the Fe$^{2+}$ chelating capacity, and inhibit the oxidation of surimi-based products. The effects of celery leaves in the sausage were superior than the petioles. The flavonoids and polyphenols in celery can scavenge free radicals, block free radical chain reactions, and prevent oxidation [46]. The low TBA values after digestion are likely due to the fact that gastric and intestinal digestion can increase total polyphenol and flavonoid content in celery. Chlorogenic acid is an organic acid in celery. Polyphenols in young apples could effectively delay lipid oxidation, and its main ingredient is chlorogenic acid [47]. DF also has antioxidants, and the addition of DF to the salami improves its antioxidant capacity [48].

Fig. 7. Effect of celery on TBA and Fe$^{2+}$ chelating capacity before and after digestion of the sausages. Different letters indicate significant differences among samples ($p<0.05$).
4. Conclusions
We investigated the composition of the celery and *N. virgatus* surimi; the gel properties, flavor characteristics, and *in vitro* digestion characteristics of fish sausages with 10% celery petioles and leaves. The ratio of SDF to IDF in celery is 1:3.7, and the contents of ash, protein, fat, total dietary fiber, calcium ion and total acid in celery petioles are lower than the leaves. The gel properties of sausage with celery leaves and petioles had no significant differences, except for color. The breaking force of the sausage with the celery leaves and petioles was respectively 10.75% and 13.91% higher than the control and the gel structure was dense and uniform. Celery increased saltiness, astringency, and richness and decreased umami and bitterness of the sausages. The total score of sensory evaluation of the sausage with the celery leaves was the highest. Celery could promote *in vitro* digestion of proteins, disintegrating these into free amino acids. Sausage with celery leaves increased protein digestibility and Fe$^{3+}$ chelating capacity by 3.9% and 447.5%, respectively. Celery could significantly improve the antioxidant capacity of fish sausages. The effects of celery leaves on sausages were superior than petioles.

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