

1 **Effect of different drying methods on the protein and product quality from hairtail fish meat gel**

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12 **ABSTRACT**

13 Three different methods, namely hot air drying (HA), microwave vacuum drying (MV), and vacuum
14 freeze drying (FD) were employed to investigate the effect of drying method on the quality of hairtail
15 fish meat gel. Compared with HA and MV, FD samples showed a better quality in terms of moisture
16 content, water absorption index and water solubility index, and had the highest overall acceptance in
17 sensory evaluation. FD well preserved the protein from degradation and formed an ordered porous
18 microstructure. The nitrogen fraction assay revealed that protein was degraded into 40-100kDa
19 fragments during drying in HA, while which was almost not affected by MV and FD. Overall, FD was
20 the most suitable method for drying of meat gel made from hairtail, followed by MV and HA.

21 **Keywords:** hairtail, fish protein, vacuum freeze drying, hot air drying, vacuum microwave drying

22 **Running Title:** Effect of Drying Methods on Protein of fish meat gel

23

24 INTRODUCTION

25 It is well known that cereal proteins as a single protein resource are generally not complete proteins
26 because of some limited essential amino acids, while fish meat is a good protein resource that could be
27 the compensates. ^[1] Up to date, Alaska pollock, Pacific whiting, and threadfin bream have been
28 extensively utilized in the processing of fish meat paste, which are considered as nutritionally balanced
29 food and consumed all around the world. ^[2] The global decrease in high quality fish supply is an
30 initiative to the research of processing and utilization of low value but more abundant fish resources in
31 the current market which typically use low value fish as a major ingredient. ^[3] For fish meat paste
32 processing, the fish meat is collected after the fish is deboned and gutted. Water soluble proteins,
33 pigment, some enzymes, and fat in the meat are leached out and myofibrillar proteins are predominant
34 in the final product. ^[4,5]

35 The principle of fish meat paste processing is the formation of an elastic gel during the heat processing
36 which help the setting of salt soluble proteins with high water content. ^[6,7] This high water content high
37 protein matrix will be enhanced during the cold chain circulation that can avoid the matrix corruption.
38 ^[8] However, the shelf life of fish meat gel will be significantly reduced if the protein structure is
39 destroyed under an unstable temperature environment. ^[9-11]

40 Drying is a commonly used technology to converts liquid and/or wet product into a dry state.
41 Compared with wet materials, the dried counterparts have the benefits of preventing microbial growth
42 and spoilage, ease of handling due to reduction in bulk, and reduced handling costs. ^[12] Because of the
43 low moisture content and water activity, the dried products are generally more stable under adverse
44 conditions such as fluctuated temperatures. Drying technology is also widely used in traditional aquatic
45 product processing, usually combined with salting and smoking processes. ^[13]

46 However, there is lack of information on drying of fish meat gel. Particularly, the effects of drying
47 technologies on the gelling components of fish meat-proteins are not widely investigated. And few
48 reports on hairtail protein property were found, despite that the fish was widely used to produce fish
49 meat gel. The aim of this study was to compare the effect of three drying methods, e.g. hot-air drying,
50 microwave vacuum drying, and vacuum freeze-drying on the protein characteristics of hairtail fish
51 meat gel.

52

53 MATERIALS AND METHODS

54 **Materials**

55 Hairtail fish was purchased from a commercial fishing boat in Zhoushan city, China, and was carried to
56 the laboratory in ice within 30 min after its harvest. The fish was manually gutted to collect the fish
57 meat. The meat was bleached with de-ionized water (temperature and time) at 10 °C for 5 min to
58 remove pigments, fat and water soluble proteins to obtain the fish meat paste.

59 **Preparation of Fish Meat Gel Samples**

60 To prepare the fish gel, NaCl was added to the fish meat paste to a final concentration of 2.5%, and the
61 final moisture was adjusted to 80%. The meat was ground at 4°C for 30 min using a mortar and a pestle
62 to form a fish paste, and then stuffed into stainless steel rings (diameter 3.0 cm) for shaping. ^[1] As two
63 step heating could result in a better gel strength than one step heating, two step heating was preferred in
64 this study. After incubating at a two-stage heating process of 50°C for 40min followed by 90°C for 20
65 min, the obtained fish meat gel was cooled down with iced water and was sliced into 1-2cm thickness
66 discs.

67 **Freeze Drying (FD)**

68 The fish gel discs were frozen in a freezer at a temperature of -70°C and then freeze dried in a freeze
69 dryer (Labconco freezon 6Plus, America) operating at -50°C and 1.65Pa for 26 hours. The water
70 content of the samples was in situ monitored with an infrared auto-moisture analyzer (DHS20A,
71 company, China). The freeze dried samples had a water content of 4-5% (w/w). The samples were
72 either analysed immediately or stored in desiccators for further analysis. ^[14]

73 **Hot Air Drying (HA)**

74 Hot air drying was followed the procedure of Krokida, Karathanos, Maroulis, and Marinos-Kouris. ^[15]
75 Briefly, fish meat gel discs were placed in metal trays with a sample thickness of 1-2 cm. The samples
76 were dried at 65 °C and air velocity of 1.5m/s for approximately 20 h (V-33 convection cabinet dryer,
77 Despatch Oven Co, Minneapolis, MN).

78 **Microwave Vacuum Drying (MV)**

79 The fish gel discs were dried as described by Therdtthai and Northongkom ^[16], using a microwave
80 vacuum drier (WZD2S, Nanking Sanle, China) consisting of three pairs of magnetrons with a rotating
81 plate. The oven was operated for 40min at a microwave power of 1300 W, pressure of -90 kPa and
82 frequency of 2540 MHz. All experiments were conducted with three independent triplications.

83 **Water Content Determination**

84 The water content of the samples was determined using a vacuum oven (AOAC, 1990).^[17]

85 **Water Absorption Index and Water Solubility Index Determination**

86 Water absorption index (WAI) and water solubility index (WSI) were determined according to the
87 method reported by Lee and Rhee with some modification.^[14] Briefly, about 0.2g dried samples were
88 immersed in 20 ml of distilled water in 50 ml centrifuge tubes and mixed on a vortex mixer, then the
89 tubes were kept in a water bath at 25°C for 1 h with shaking at regular intervals. The tubes were
90 centrifuged at 3000 rpm for 15 min. The supernatants were separated and their solid contents were
91 determined respectively. The solid sediments were further dried and weighed. The WAI and WSI were
92 calculated by the following equations:

93 $WAI = \text{weight of sediment} / \text{weight of dried sediment}$

94 $WSI (\%) = (\text{weight of dissolved solid in supernatant} / \text{weight of dry solids}) * 100$

95 **Measurement of whiteness**

96 The whiteness value of the hairtail meat gel was tested by a color difference meter (WSC-S80,
97 Shanghai, China) and calculated according to the following formula: $W = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}$.

98^[18, 19] Hue-different angle value was calculated by: $h^* = \arctan b^* / a^*$.^[20]

99 **Protein Degradation determination**

100 The protein concentration was determined by the Biuret method using bovine serum albumin (BSA) as
101 standard.^[21] Protein degradation of hairtail meat gels were evaluated by SDS-polyacrylamide gel
102 electrophoresis analysis. The fish meat gel was extracted following the method of Bechtel and Parrish.
103^[22] An aliquot of 20μL extracted sample was subjected to SDS-PAGE electrophoresis under reducing
104 conditions. After electrophoretic running (EPS-300 Tanon vertical electrophoresis apparatus, Shanghai,
105 China), proteins in the gel was stained with Coomassie Brilliant Blue R-250 for further analysis.^[23]

106

107 **Nitrogen Fraction Determination**

108 Aliquots (10 ml) of extracts were mixed with the same volume of 4% trichloroacetic and 10%
109 phosphotungstic acid solutions to obtain non-protein nitrogen (NPN) and 5%
110 phosphotungstic-acid-soluble nitrogen (PTN), respectively. Mixtures were kept at 4°C for 60 min, and
111 the insoluble material was removed by filtration through Whatman no. 4 paper. Total nitrogen, NPN
112 and PTN were determined by the Kjeldahl method.^[17] The total nitrogen was considered as total
113 water-soluble nitrogen (WSN) because it was determined in the aqueous phase of the fish meat gel

114 after removal of the insoluble material.

115 The amino acidic nitrogen (AN) was determined as described by Cambero (1998).^[24] The protein
116 nitrogen (PN) was estimated from the difference between WSN and NPN ($WSN \pm NPN$) and the
117 non-protein non-amino-acid nitrogen (NPAN) was calculated as $NPN \pm AN$. Likewise, the
118 concentrations of peptides with a molecular weight greater than 600 Da (HPPN) were calculated
119 according to the expression $HPPN = NPN \pm PTN$ and non-amino-acid nitrogen substances of less than
120 600 Da (small non-amino-acid nitrogen compounds) were calculated as $SNAN = PTN \pm AN$.

121 **Scanning electron microscopy (SEM)**

122 Microstructures of the hairtail meat gels were determined using a Model XL 30 SEM (Philips, Holland).
123 The gel samples were first fixed with 2.5 % glutaraldehyde in phosphate buffer (pH 7.0) and
124 dehydrated in Hitachi Model HCP-2 critical point dryer with liquid CO₂. After coated with
125 gold-palladium, the microstructures were observed in the SEM and took the scanned image
126 when amplified to 1800 times.

127 **Sensory Evaluation**

128 Overall sensory quality of the fish gel was evaluated on the parameters including color, odor and
129 texture. Samples were assessed by a panel of 6 experienced food sensory evaluation members on the
130 basis of 10-point scale (10-9 excellent, 8-7 good, 6-5 fair and acceptable, 4-3 poor and 2-1 very poor).
131 ^[25]

132 **Statistical Analysis**

133 The experiments were run in triplicates and the data were presented as mean with standard
134 deviation. Statistical analysis was performed using ORIGIN (version 8.0; Microcal Software Inc.,
135 Northampton, MA). The results were analyzed using one-way analysis of variance (ANOVA).
136 Comparison of treatment means was based on Duncan's multiple range test. Differences were
137 considered significant at the $p < 0.05$ level.

138 **RESULTS AND DISSCUSIONS**

139 **Water content, color, WAI and WSI of dried fish meat gel**

140 The water content of the heated fish meat gel was 78-83%, suggesting it is susceptible to microbial
141 contamination and should be maintained in a cold chain environment during circulation. Removing the
142 moisture content through drying could effectively improve the product stability. All the drying
143 conditions of each drying method were accepted as the most popular methods currently used in aquatic

144 food product processing. Under the drying conditions of this experiment, the FD, HA and MV methods
145 had reduced the water content of the fish meat gel to 4.0, 11.8 and 8.1%, respectively (Table 1). The
146 hue-different angle value of FD and MV was within the range of 80-90, suggesting the color change
147 from green to yellow was negligible. ^[26] But the HA sample had the lowest whiteness among all the
148 tested samples. Possibly, the longest heating process of HA was beneficial to the Maillard reaction
149 which generated more black/brown compounds and consequently decreased the whiteness value. ^[27]
150 Hot air drying (HA) was a convective drying process employing heated air as the heat and humidity
151 carrier. Drying conditions such as temperature, velocity, relative humidity, as well as various
152 contamination sources could be well controlled. Compared with naturally drying method (e.g. sun
153 drying) HA result in better quality products. ^[28] Another advantage of this method is that it is easy to
154 implement because of its low investment costs and simple operation. Therefore, hot air drying has been
155 widely used to dry aquatic products as well. ^[29] In this study, fish meat gel was dried at 65°C for
156 approximately 20 h. The obtained sample had a water content of 11.8%, which could be applied in food
157 drying processing, though much higher than that of FD and MV.

158 In recent years, microwave drying has gained popularity as an alternative drying method in the food
159 industry, owing to its ability to rapidly heat dielectric materials through volumetric dissipation of
160 microwave energy. ^[30] On the other hand, microwave drying is also effective in color preservation. ^[31]
161 In this study, water content was much lower in the MV sample, being 8.1%, with the whiteness (84.0)
162 close to the fish meat gel (89.9) before drying, although significant difference was still detected.

163 FD is one of the best method of water removal for most of the foods because the primary flavor, color,
164 structure, and the nutrient compositions are maintained to a great extent and final products have better
165 rehydration capacity than those produced using other drying methods. ^[32] In this study, FD samples had
166 the best whiteness of 88.4 and the lowest water content of 4.0%.

167 Water absorption index (WAI) and water solubility index (WSI) of a dried material depend on several
168 factors such as original nutrition components, enzyme content and thermal history. ^[33, 34] It was
169 observed that the WAI and WSI of fish meat gel were dependent on the drying methods (Table 1). FD
170 as well as MV products showed higher WAI and WSI values, while HA product had the lowest,
171 suggesting FD and MV meat gels have better consuming characteristics.

172 **Sensory Evaluation**

173 For all food resources, sensory evaluation on appearance, odor, taste and texture is very important as it

174 reflects direct potential consumer preference. ^[35] In this study, FD fish meat gel obtained the highest
175 overall acceptance for sensory score, followed by MV. The FD fish meat gel was crisp with a pleasant
176 fresh fishy smell. The overall appearance of FD sample was almost the same as the heated fish meat gel,
177 suggesting FD had preserved the shape very well. Samples dried by HA had the lowest score of overall
178 acceptance because of its bad appearance and texture. Appearance, especially the whiteness reflects the
179 color of the fish meat, is one of the most important attributes in the food industry, affecting the
180 acceptability by consumers. The surface of HA samples collapsed into a curve with a brown color. The
181 HA samples were also hard during chewing, resulting in lower texture and taste scores.

182 **Nitrogen Fraction Determination**

183 Effect of drying methods on the release of nitrogen compounds from the fish meat gel was studied. The
184 ratios of TCA-SN, WSN and PTA-SN/WSN could reveal the degradation depth of proteins. ^[36] Table 3
185 shows the concentrations (g/100 ml) of water-soluble nitrogen (WSN), protein nitrogen (PN), nitrogen
186 in peptides of molecular weight greater than 600 Da (HPPN), nitrogen in non-amino-acid substances
187 smaller than 600 Da (SNAN) and amino acid nitrogen (AN) of fish meat gel after different drying
188 method. The nitrogen was mainly present in peptides of less than 600 Da with the ratio about 50% of
189 the WSN, followed by amino acidic fraction (AN). FD drying had the highest protein nitrogen content,
190 which showed a better protein nitrogen preserving capacity than that of HA and MV. High protein
191 nitrogen content might contribute to flavor or indirectly contribute as flavor precursors of the fish
192 products. ^[37]

193 **Protein Degradation Assay**

194 The fish meat was mainly composed of myriad myofibrils, which contained two predominant proteins
195 of myosin and actin. Myosin comprises approximately 55-60% of the total myofibrillar proteins. Each
196 myosin molecule is composed of two 220KDa heavy amino acid chains and two pairs of light chains.
197 The myosin heavy chains (MHC) could be released and revealed in the SDS-PAGE profile. Actin (AC)
198 comprised 15-30% of the myofibrillar protein. ^[38] In this study, heated fish meat gel without drying
199 process was used as control. The protein profile of control was almost the same as that of the unheated
200 fish meat paste. After drying, actin was remained almost intact by either FD, HA or MV method,
201 suggesting no degradation was occurred (Fig. 1). In case of MHC, the density of MHC band of FD and
202 MV samples did not changed. However, the band of MHC in HA sample was obviously narrowed and
203 new bands between MHC and AC were generated, suggesting that MHC was degraded during the HA

204 drying process. The endogenous proteinase in the heated gel might still active and degraded the MHC
205 during HA drying. It was reported that some proteinase is heat insensitive, for example, the optimum
206 temperature of cathepsin L, one of the gel dis-integration involved proteinases, was reported to be
207 about 50-60°C. ^[39] The HA drying was undertaken at 65°C for 20h. The longtime heating at this
208 temperature might have caused severe protein degradation. The results of nitrogen fraction assay
209 revealed that most of the protein of HA sample degraded into peptides with molecular above 600Da.
210 By SDS-PAGE assay, the peptides should be within the range of 200-40KDa, also suggesting the
211 protein degradation during HA drying.

212 **Microstructures of the dried fish meat gels from different drying methods**

213 Although hot air drying has the advantages of low investment costs and simple operation, it generally
214 involves high energy consumption and long drying times. The size of the food material and the drying
215 temperature significantly affect all quality attributes of the dried products. It was reported that an air
216 temperature above 100°C was positive to the quality of shrimp, with high percentage of rehydration,
217 low maximum shear force and high value of redness. ^[40, 41] However, Kowalski and Pawłowski
218 observed that although higher drying temperature (100 °C) resulted in a higher drying rate and reduced
219 drying time, the quality of the dried product was worse than that dried at a lower temperature (65°C).
220 ^[42] In the present study, the HA fish meat gel was dried at 65°C. As shown in figure 2b, the HA dried
221 matrix collapsed entirely from the surface to the center to form a very tight compact structure. This
222 tight microstructure may have contributed to the low WAI and WSI values (table 1) as mentioned
223 above, and also negatively affect the overall acceptance during the sensory evaluation (Table 2).

224 Although microwave drying is getting more and more acceptance in both industry and home
225 applications, the texture of the products might be damaged due to the rapid mass transfer during the
226 drying process. In addition, non-uniformity of electromagnetic field could create hot spots during
227 microwave drying and result in burn points on the product surface. ^[43, 44] In this study, large spots and
228 holes were observed on the surface and inside of the MV samples (Figure 2 f). Under vacuum condition,
229 the water is transferred in a rapid way from the inside of the sample and evaporated from the surface,
230 which could cause a number of burning spots inside the matrix in a very short time. ^[45] A collapsed
231 surface was formed because it could not stand the high speed evaporation in such high water content
232 (80%) from both inside and the surface of the sample in a very short drying time (40 min) (Figure 2 e).
233 Because of the spotted structures inside, the MV sample had better WSI, WAI and overall acceptance

234 than the HA sample as described above.
235 FD under vacuum conditions was first developed for protein pharmaceutical manufacturing. [46]
236 Currently, it was applied in drying of heat sensitive food and other biological materials. It was
237 considered to be one of the best water removal method for most kinds of foods because the primary
238 flavor, color, structure, and the nutrient compositions can be maintained to a great extent and final
239 products have a better rehydration capacity than those produced using other drying methods. [29] As
240 described above, the FD sample had the highest sensory scores, best WSI, WAI and highest protein
241 nitrogen fraction. The myofibrillar proteins were well preserved after vacuum freeze drying. The SEM
242 micrographs revealed that the FD generated bigger size pores of homogeneity and a more ordered
243 structure (Fig. 2), which was consist with the reports of other food matrix. [47, 48] An amplified
244 microscopy by 5000 times clearly showed that the wall of pores was homogenously arranged, assuring
245 the FD dried samples recover water when put into water again. This could be one of the factors that
246 attributed to the best quality of the fish meat protein gel by FD.

247 Different from other matrix, such as rice, [49, 50] fruits [51] and maize, [52] etc., drying matrix of protein
248 showed to be quite sensitive to the vacuum. Under common pressure, although in low temperature of
249 HA at 65°C, the protein in hairtail fish meat was degraded and a collapsed microstructure was formed
250 (Fig. 2, b). Under vacuum condition, in case of MV and FD, the less degradation of protein and an
251 ordered microstructure was observed. This might partly be due to prevent of oxidation from the protein
252 matrix by the vacuum condition. Further studies need be undertaken yet.

253 **CONCLUSIONS**

254 The effects of 3 different drying methods, including hot air drying (HA), microwave vacuum drying
255 (MV) and freeze drying (FD), were investigated on the drying of hairtail fish meat gel. The FD method
256 could effectively preserve the protein from degradation during the drying process and obtained the best
257 sensory scores for appearance, odor, texture and overall acceptance. The FD sample also had the
258 highest WAI, WSI and protein nitrogen fraction. SEM results revealed that FD could preserve the fish
259 meat gel microstructure by forming homogenous ordered structure. Compared with HA, MV showed
260 advantages in preservation of protein with a porous structure inside, although not so effective to that of
261 FD. For drying of the high protein and high moisture contents fish meat gel, FD was recommended as
262 the most suitable method.

263

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386

387 **Captions of Figures**

388 FIG.1. SDS-PAGE profile of proteins of fish meat gel from different drying methods

389 M: marker; 1: unheated fish meat paste; 2: control (heated fish meat gel); 3: FD sample (heated fish
390 meat gel dried by FD); 4: HA sample (heated fish meat gel dried by HA); 5: MV sample (heated fish
391 meat gel dried by MV)

392

393 FIG.2. Micro-structure of fish meat gel obtained by different drying methods

394 a: unheated fish meat paste ($\times 1800$); b: HA sample (heated fish meat gel dried by HA, $\times 1800$); c:
395 FD sample (heated fish meat gel dried by FD, $\times 1800$); d: FD sample (heated fish meat gel dried by
396 FD, $\times 5000$); e: Inside of MV sample (heated fish meat gel dried by MV, $\times 1800$); f: Surface of
397 MV sample (heated fish meat gel dried by MV, $\times 1800$)

398

399 **Captions of Tables**

400 TABLE 1 Water content, color, WAI and WSI of hairtail fish meat gel dried from different methods

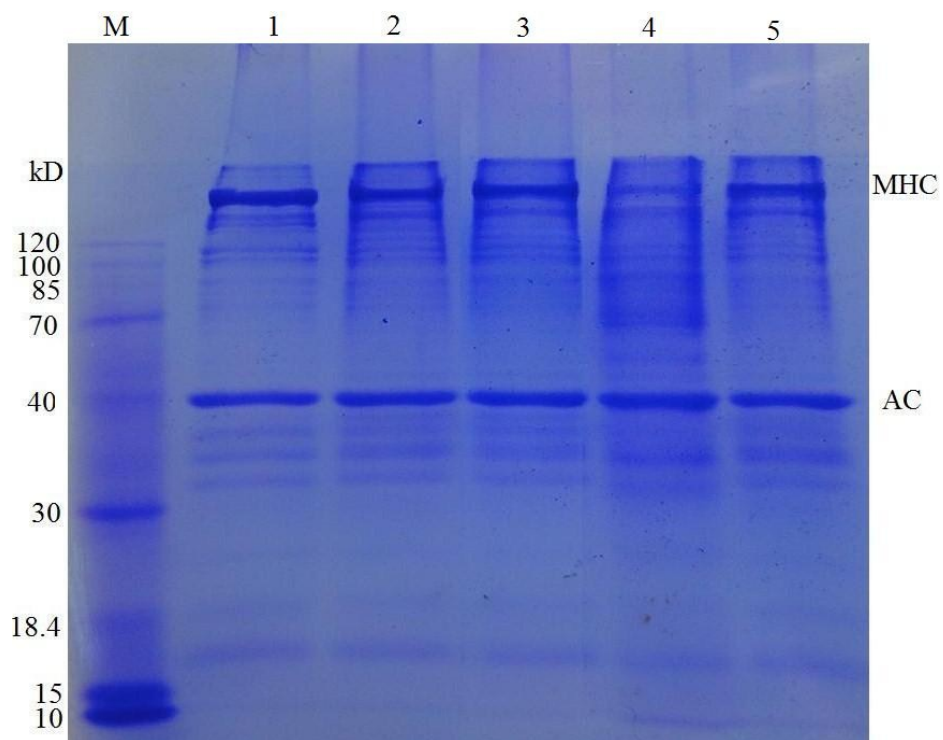
401 TABLE 2 Sensory evaluation of hairtail fish meat obtained by different drying methods

402 TABLE 3 Concentration of nitrogen fractions in the dried samples

403

404

405 FIG. 1.



406

407

408 M: marker; 1: unheated fish meat paste; 2: control (heated fish meat gel); 3: FD sample (heated fish

409 meat gel dried by FD); 4: HA sample (heated fish meat gel dried by HA); 5: MV sample (heated fish

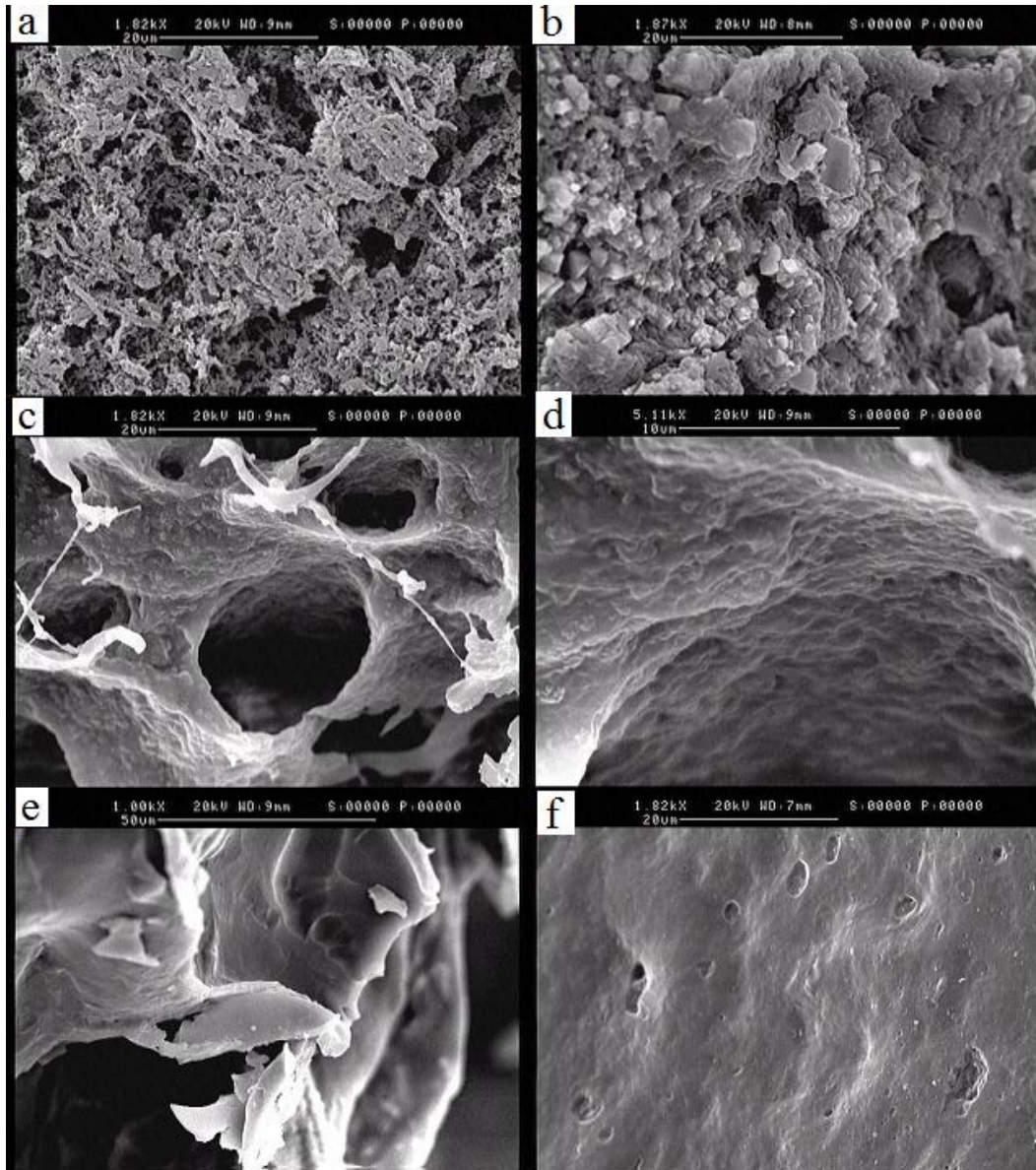
410 meat gel dried by MV)

411

412

413

414 FIG. 2.



415

416

417 a: unheated fish meat paste ($\times 1800$); b: HA sample (heated fish meat gel dried by HA, $\times 1800$); c: FD

418 sample (heated fish meat gel dried by FD, $\times 1800$); d: FD sample (heated fish meat gel dried by FD,

419 $\times 5000$); e: Inside of MV sample (heated fish meat gel dried by MV, $\times 1800$); f: Surface of MV sample

420 (heated fish meat gel dried by MV, $\times 1800$)

421

422
423
424

TABLE 1
Water content, color, WAI and WSI of hairtail fish meat gel dried from different methods

Drying Methods	Wh	θ ($^{\circ}$)	Water content (%)	WAI	WSI (%)
Control	89.9 ± 1.5^a	88.9 ± 0.8^a	80.5 ± 1.2^a	-	-
FD	89.4 ± 1.8^a	88.4 ± 0.9^a	4.0 ± 0.6^a	5.0 ± 0.8^a	18.5 ± 0.9^a
HA	76.4 ± 1.2^b	78.8 ± 0.8^a	11.8 ± 0.9^b	2.2 ± 0.7^b	12.4 ± 0.8^b
MW	84.0 ± 1.9^c	88.9 ± 0.7^a	8.1 ± 0.8^c	5.4 ± 0.8^a	16.7 ± 0.6^c

425 ^{a-c} Different letters in the same column are significantly different (p<0.05).
426

427

TABLE 2

428

Sensory evaluation of hairtail fish meat obtained by different drying methods

Drying Methods	Appearance	Odor	Taste	Texture	Overall acceptability
FD	9.8±0.8 ^a	8.9±0.5 ^a	9.4±0.5 ^a	9.2±0.6 ^a	9.5±0.5 ^a
HA	6.4±0.7 ^b	6.8±0.4 ^b	5.8±0.3 ^b	4.8±0.2 ^b	6.4±0.5 ^b
MW	7.8±0.6 ^c	7.7±0.6 ^c	6.9±0.4 ^c	5.8±0.5 ^c	7.2±0.4 ^c

429

^{a-c} Means with different superscripts in the same column are significantly different

430

(p<0.05).

431

432

TABLE 3

433

Concentration of nitrogen fractions in the dried samples

Drying Methods	WSN	PN	HPPN	SNAN	AN
Control	0.85 ± 0.5^a	0.05 ± 0.8^a	0.04 ± 0.8^a	0.48 ± 1.8^a	0.29 ± 1.1^a
FD	0.87 ± 0.8^a	0.07 ± 0.9^b	0.04 ± 0.6^a	0.48 ± 1.6^a	0.27 ± 0.9^a
HA	0.86 ± 0.9^a	0.02 ± 0.8^c	0.07 ± 0.9^b	0.40 ± 1.7^b	0.33 ± 1.4^b
MW	0.84 ± 0.9^a	0.05 ± 0.7^a	0.03 ± 0.8^c	0.43 ± 1.5^c	0.30 ± 1.6^a

434 ^{a-c} Means with different superscripts in the same column are significantly different
 435 (p<0.05).