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Drying-induced protein and microstructure damages of squid fillets affected moisture distribution and rehydration ability during rehydration

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ABSTRACT

Moisture distribution and rehydration ability of squid fillets subjected to heat-pump drying (HPD), freeze-drying (FD) and hot-air drying (AD) were investigated. The impact of drying methods on protein, microstructure and physicochemical qualities of dried fillets were evaluated. Nuclear magnetic resonance analysis showed that water penetrated from periphery to central region during rehydration. Nuclear magnetic signal intensity after 60 min rehydration was high at the periphery and low in the center for FD samples, but was identical at the surface of AD and HPD samples. AD induced the severest compact structure and myosin degradation while FD resulted in the porous structure in dried fillets. The new band at 37 kD protein for AD samples was more intense than that for HPD and FD samples. FD resulted in the greatest rehydration ability but the softest texture, followed by HPD and AD. There was a good coordination among moisture distribution, rehydration ability, and hardness with the microstructure and protein damage of dried fillets.

1. Introduction

Fresh squids are rich in nutrients, but contain a high amount of water, which lead to a very short shelf-life. Drying is one of the common preservation methods that can extend shelf-life of the products (Okos et al., 2007). Dried and seasoned squid products possess palatable flavor, have been consumed as popular snack foods worldwide. The demands on dried squid products increase continuously (Mauricio et al., 1998; Zhang and Mao, 2004).

Drying process involves simultaneous heat and mass transfer. Different drying methods cause different modifications on the composition, physicochemical and structural properties of foods. Transient thermal and moisture gradients lead to the changes in the conformational stability and degradation of muscle proteins, and develop tensional and compressional stresses in the cellular structure that result in changes in microstructure, such as the formation of pores and compactness (Bai and Sun, 2011; Laopoolkit and Suwannaporn, 2011; Wang et al., 2012). Conventional hot-air drying (AD) is the most widely used method for drying of seafood (Bai and Sun, 2011). A disadvantage of AD is the substantial quality degradation including extremely shrunken, tough texture, severe browning, low rehydration rate, and low nutritive value, high energy consumption, and long drying time (Bai and Sun, 2011; Rahman, 2006; Wang et al., 2012). Freeze-drying (FD) is a modern drying technology that provides dried products with porous structure, little or no shrinkage and structure change, good rehydration capacity, and minimal loss in physicochemical and nutritional qualities (Crapo et al., 2010; Rahman, 2006). However, freeze drying is very expensive and may cause an increase in friability, thus limited its applications (Rahman, 2006; Reyes et al., 2011). Heat pump drying (HPD) is an alternative drying process that can independently control the operation parameters and has improved energy efficiency, thus suitable for seafood products that are sensitive to heat. At present, there are only a few reports on drying squid products using HPD (Deng et al., 2011a,b).

Rehydration is one of the most important quality properties for dried products. Rapid and complete rehydration can reduce labor cost and floor-space requirement, and improve production efficient (Crapo et al., 2010). Water uptake during rehydration is a complex process and is affected by various factors, such as the

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morphological structure, chemical composition, drying pretreatments, drying methods, immersion media, and rehydration temperature and time (Babic et al., 2009; Okos et al., 2007; Reyes et al., 2011). Some factors may induce irreversible modifications in structure and composition of food, which cause impaired reconstitution properties (Wang et al., 2012). Rehydration rate of dried product is associated with its water absorption capability and water holding capacity (Lewicki, 1998), which is generally estimated by measuring the change in moisture content of the products. However, water is not absorbed homogeneously by a single product, and the mobility and distribution of moisture within a product could affect the rehydration capacity, textural characteristics, physical properties of the product surface and the final product quality. Based on my best knowledge, no information is available about the moisture distribution during rehydration of dried products. Hence, a complete investigation of the rehydration kinetics and its correlations with the moisture distribution, protein denaturation degree and microstructure of the dried products are necessary. Such information is critical to optimize the balance between rehydration rate and final product texture quality, as well as to solve many product control problems during production.

Therefore, the objectives of this study were: (a) to investigate the impact of different dry methods on the moisture distribution and mobility characteristics during rehydration of dried squid fillets by means of three dimensional MRI image analysis and rehydration kinetics; (b) to evaluate the coordination of moisture distribution in rehydrated products with the microstructure and myofibrillar protein denaturation caused by drying; and (c) to analyze the influences of different drying methods on the quality of dried products including proximate compositions, total volatile basic nitrogen, texture and color.

2. Materials and methods

2.1. Materials

Frozen North Pacific squids (*Todarodes pacificus*) were obtained from Chinese Academy of Fishery Sciences, Shanghai, China, and transported with ice to our laboratory at Shanghai Jiao Tong University. Squids (660 ± 10 g per a whole squid) were first defrosted in a refrigerator at 4 °C overnight, and then thawed with running water. The internal organs, arms, and tentacles were removed by hands to obtain the mantle. The mantle muscle was cut into rectangular sheets with an average length of 4.0 ± 0.5 cm, width of 4.0 ± 0.5 cm and thickness of 3.0 ± 0.5 mm. The squid fillets were immersed in 30 g kg^{-1} sodium chloride solutions at a ratio of 4 L kg^{-1} of samples and at 4 °C for 14 h. After salting, the samples were removed from the brine solution, quickly rinsed with distilled water (ca. 30 s) to remove the excessive brine, and then gently blotted with tissue paper to remove excess water.

2.2. Drying

Hot-air drying (AD) was performed in a laboratory-scale food dryer (Shang Hai Sunny Institute of Environment and Energy Co., Ltd., Shanghai, China). Pretreated squid fillets (~450 kg m^{-2}) were spread uniformly on a mesh tray as a single layer. The dryer was set at 60 ± 2 °C, 1.5 ms^{-1}air velocity, and 20% relative humidity (Rahman, 2006). Samples were dried for about 10 h until reached the final moisture content of <10 g/100 g dry matter.

Freeze drying (FD) was carried out in a Freezone 2.5 L Triad system (Labconco Inc., USA). Pretreated samples (~450 kg m^{-2}) were spread as a single layer on the plate, frozen at −80 °C for 24 h, and then dried for 24 h under 45 Pa absolute pressure until samples reached the final moisture content of <10 g/100 g dry matter. The condenser temperature was set at 550 °C, and the temperature of the heating plate was controlled at 40 °C.

Heat pump drying was done in a self-made laboratory-scale heat pump dehumidified dryer (Deng et al., 2011c). Pretreated samples (~450 kg m^{-2}) were spread as a single layer on a mesh tray and dried at the temperature of 40 °C and ~2 ms^{-1} air velocity. Again, samples were dried for about 12 h until reached the final moisture content <10 g/100 g dry matter.

All drying experiments were carried out in triplicate. After drying, squid fillets were put into a desiccator containing silica gel desiccant overnight at 25 °C and for moisture to equilibrate. The samples were then packed in the Ziploc® bags and kept inside a desiccator for less than a week for further analysis.

2.3. Chemical component analysis

Moisture, ash, crude fat and crude protein content were determined according to the AOAC methods (1995). The moisture content of the squid samples was determined by placing 5 g of sample into an air oven at 105 °C for ~24 h until constant weight. The crude protein was calculated from total nitrogen content determined by Kjeldahl method using the conversion factor of 6.25. The crude lipids were determined by a Soxhlet extraction method using hexane as solvent. Ash was determined using a muffle furnaceat 550 °C. Total volatile basic nitrogen (mg N per 100 g squid sample) was determined by the method of Antonacopoulos and Vyncke (1989) with some modifications. Briefly, 10 g of squid muscle was homogenized with 90 mL distilled water for 30 s, and centrifugalized at 12,000*g* at 4 °C for 30 min. One gram of Magnesium Oxide was added into the supernatant, and distillated for 4 min. The distillate was collected in boric acid (2%) with 1 drop of 1% bromocresol green and 2 drops of 1% methyl red, and then titrated with 0.1 N HCl until reaching the neutral point. All measurements were done four times.

2.4. Microstructure observation of fresh and dried squids

Microstructure analysis was conducted on a JSM-7401F Scan Electric Microscope (SEM) (JEOL Ltd., Japan) at an acceleration voltage of 10 kV. Dried squid muscle were cut into pieces (3 × 3 × 1 mm) and fixed in 0.1 mol L^{-1} phosphate buffer (pH = 7.0) containing 3% (v/v) glutaraldehyde and 2% (v/v) formaldehyde (4:1) at 4 °C for 24 h. After the fixation, the samples were dehydrated stepwise after successive immersions in a graded ethanol series (50%, 70%, 80%, 90% and 100% (w/v)). The specimens were kept for 15 min per step to 90% ethanol gradient, dehydrated 30 min in absolute ethanol three times, and then further dried in vacuum-assisted desiccators overnight. Thereafter the dried specimens were attached on the stainless stubs with double sticky tabs, sputtered immediately with gold in approximately 10 nm.

2.5. Electrophoresis

Protein in the squid samples was extracted using a modified method of Hernández-Andrés et al. (2005). Briefly, 1.5 g of fresh squid paste or 0.5 g of dried squid powder were dispersed in 30 mL of solubilization buffer (20 mmol L^{-1} Tris–HCl, pH 8.0, containing 20 g kg^{-1} β-mercaptoethanol, 20 g kg^{-1} sodium dodecyl sulfate, and 8 mol L^{-1} urea) and homogenized at 15,000*g* for 30 s. The homogenates were incubated in the iced water shaken at 100*g* for 3 h and then centrifuged at 12,000*g* for 45 min at 4 °C. The supernatant containing total soluble protein of raw squid and dried squid samples were separated by 12% SDS–PAGE. All samples were boiled for 5 min prior to electrophoresis and 10 μL of every sample were added to the lanes.

2.6. Color

In order to reduce the effects of different locations of the squid sample on color and texture analysis, squid fillets were cut from the same location on the nine whole squids with almost the same shape and weight, and then dried by three different drying methods. Color of dried squid samples was measured using a Color Difference Meter (LabScan XE, HunterLab, USA). CIE color values of L (lightness), a (redness) and b (yellowness) were obtained from the reflection spectra of the samples using a D65 illuminant and the observer at 10°. Total color differences ($\Delta E = \Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$) were also calculated, where $\Delta L = L - L_0$, $\Delta a = a - a_0$, and $\Delta b = b - b_0$ L_0, a_0, and b_0 were the color values of the standard with the values of 93.37, −0.91 and 0.19, respectively, while $L, a,$ and b were the color values of the samples. Twenty individual samples were tested for each treatment.

2.7. Texture analysis

Texture properties of the dried samples were measured using a TA-XT2i texture analyzer (Stable Micro Systems Ltd., UK) with a 2 mm diameter of cylinder penetrometer probe. The probe was passed through the center of the squid fillet along its axis with the test parameters as: 5 mm/s of pre-speed and post-speed, 2 mm/s of test speed and 20 g of trigger. The hardness was the amount of maximum force required to break the sample. Eight samples were tested for each treatment.

2.8. Rehydration kinetics

Rehydration experiments were performed according to the modified method of Duan et al. (2011). Dried fillets were immersed in distilled water at 40 ± 1 °C for 1 h, taken out at 5, 10, 15, 20, 25, 30, 40, 50 and 60 min, respectively, and drained over a mesh for 30 s to eliminate the superficial water. The evolution of the sample weight was recorded. Rehydration rate (RR) was defined as the ratio of weight of rehydrated sample (M_f) to the dry weight of the sample (M_0).

$$RR = \frac{M_f}{M_0} \tag{1}$$

where M_0 and M_f were the weights of dried squid fillets and rehydrated samples, respectively. All rehydration experiments were carried out in triplicate.

The analysis of the rehydration kinetics can be very useful for optimizing process condition. Peleg (1998) proposed a two-parameter, non-exponential equation to adequately describe the rehydration of various foodstuffs (Peleg, 1998; Reyes et al., 2011).

$$X = X_0 + \frac{t}{k_1 + k_2 t} \tag{2}$$

When $t \to \infty$, Eq. (2) gives the equilibrium moisture content:

$$X_e = X_0 \pm \frac{1}{k_2} \tag{3}$$

where X was the moisture content (g g^{-1} in dry basis) at a known process time t (min); X_o was the initial moisture content (g g^{-1} in dry basis); X_e was the equilibrium moisture content (g g^{-1} in dry basis); t was process time (min); k_1 was the Peleg rate constant; and k_2 was the Peleg capacity constant.

Water absorption capacity (WAC), dry matter holding capacity (DHC) and rehydration ability (RA) have been used to evaluate the influence of absorbed water fluxes and lixiviated solids on material mass increase during rehydration.

$$\mathrm{WAC} = \frac{m_r(100 - s_r) - m_d(100 - s_d)}{m_0(100 - s_0) - m_d(100 - s_d)} \tag{4}$$

$$\mathrm{DHC} = \frac{m_r \cdot s_r}{m_d \cdot s_d} \tag{5}$$

$$\mathrm{RA} = \mathrm{WAC} \cdot \mathrm{DHC} \tag{6}$$

where m was the sample mass, s was the dried solid content (g g^{-1} in dry basis), and r, d and 0 were subscripts meaning rehydrated, dried and initial sample, respectively. WAC indicated how drying decreased the capacity of the product to absorb water, varying from 0 to 1. DHC, in the range of 0 to 1, indicated the capacity of the material to retain soluble solids, as well as the damages to the tissues and their permeability to solute. RA evaluated the damages resulted from the drying and rehydration processes and the rehydration ability of the dried product, ranging from 0 to 1. The greater the RA was, the smaller the damage to the muscle tissue.

2.9. Moisture distribution analyzed by NMR

Moisture distribution during rehydration was analyzed at approximately 32 °C on a microimaging nuclear magnetic resonance (NMR) system (NMI20, Shanghai Niumag Corporation, China) with a vertical 0.5 T magnet. Two dimensional (2D) images were acquired with the imaging software (ver.1.06, Niumag). In order to control the effects of different squid locations on the moisture distribution, squid fillets (40 × 40 × 3 mm) were cut from the same location from the nine whole squids with almost the same shape and weight. After drying, a rectangular slice (18 × 8 × 2 mm) was cut from the same location from the dried fillets. Thereafter a rectangular slice was loosely held in a 15 mm (o.d.) NMR tube. Magnetic resonance imaging (MRI) measurements were conducted at 5, 10, 15, 20, 25, 30, 40, 50 and 60 min, respectively, during 1 h of rehydration period. Magnetic resonance images of squid piece were acquired by a spin-echo sequence, and the acquisition parameters were as follows: repetition time = 250 ms, echo time = 11 ms, flip angle = 90°, field of view = 26 × 26 mm^2, and matrix size = 256 × 256 mm. Consequently, the acquisition time was 5 min for each piece, and the spatial resolution was 0.1 × 0.1 mm^2. The degree of porosity of dried sample was quantified as the average values of the signal intensity profiles based on NMR test. NMR signal intensity is proportional to the proton density, i.e., to the water content. In dried samples, water intake is directly associated with moisture diffusion which depends on the microstructure and compositional properties of porous materials. For the same porous material, a high degree of porosity resulted in great moisture intake, reflected in a high NMR signal intensity.

2.10. Data analysis

Statistical analysis of variance (ANOVA) was performed using SAS (SAS Inst., Inc., Cary, N.C., USA). The significant differences between means were determined by a least significant difference (LSD) test procedure at $p < 0.05$. In this study, several different statistical parameters were used to select the best fitting models, including the coefficient of determination (R^2), chi-square (χ^2), the root mean square error (RMSE), and the percent mean relative deviation modulus (E%), and were described below (Peleg, 1998; Deng et al., 2011b).

$$\chi^2 = \sum_{i=1}^{n} \frac{(V_{\mathrm{exp}} - V_{\mathrm{pre}})^2}{V_{\mathrm{pre}}} \tag{7}$$

$$\mathrm{RMSE} = \frac{1}{n}\left[\sum_{i=1}^{n}(V_{\mathrm{exp}} - V_{\mathrm{pre}})^2\right]^{0.5} \tag{8}$$

$$E\% = \frac{100}{n}\sum_{i=1}^{n}\frac{|V_{exp} - V_{pre}|}{V_{exp}} \quad (9)$$

where V_{exp} and V_{pre} were the experimental and predicted values, respectively, n was the number of experimental data points. The R^2 was the primary criterion for choosing the best equation, the RMSE is required to reach zero, and the $E\%$ value was below 10% indicating a good fit for practical purposes (Deng et al., 2011b). In general, a best fitting model occurred when R^2 was the highest and χ^2, RMSE and $E\%$ were the lowest.

3. Results and discussion

3.1. Proximate compositions and total volatile basic nitrogen

The moisture, crude protein, lipid and ash content of raw and dried squid fillets are reported in Table 1. Compared with the initial proximate compositions, the reduction of moisture content was the most prominent in squid fillets after drying and there were no significant differences among all dried samples (Table 1). After drying, there was a significant decrease in protein content compared with the raw fillets ($p < 0.05$), probably due to water soluble protein (such as sarcoplasmic protein) loss during long time soaking and washing process prior to drying (Wu et al., 1985). No significant differences in protein contents among all dried samples are a consequence of no protein nitrogen loss during drying. Salting pretreatment before drying led to an increase in the ash levels in dried samples ($p < 0.05$), but there were no significant differences in the ash contents among three drying methods. In addition, no significant variations in fat contents were found among all samples (Table 1).

Total volatile basic nitrogen (TVBN) is the most common chemical quality indicator in seafood products (Deng et al., 2011a) and the changes in TVBN values are presented in Table 1. The initial TVBN level of untreated squid fillet samples was 8.18 mg/100 g w.b., indicating the freshness and high quality of raw squid material. The TVBN values of all dried fillets were higher than that of raw fresh squids. Increased levels of the TVBN were associated with the formation of volatile basic components, such as trimethylamine and ammonia, by enzymatic degradation and bacterial spoilage (Vega-Gálvez et al., 2011). The levels of TVBN in AD or HPD-dried samples were significantly higher than those prepared from FD method ($p < 0.05$). These results are in agreement with a previous study reporting that the TVBN contents of cured fish using hot-air drying (30–70 °C) were higher than that of using freeze-drying (Zhang et al., 2010). In this study, the TVBN was the highest for AD, followed by HPD and FD, the exact reasons for the differences are unknown and require further investigations.

3.2. Microstructure

The microstructures of raw, AD, FD and HPD squid fillets are illustrated in Fig. 1. From Fig. 1 R, the raw, untreated squid muscles were composed primarily of muscle fibers which were clearly visible and formed network structure. Compared to raw squid muscles, the muscle fibers in AD and HPD samples became very dense and firm after drying (Fig. 1A and B), which was due to the fact that thermal treatments led to denaturation of myosin and shrinkage of myofibrils (Chang et al., 2011). Similar changes were observed by Dewi et al. (2010) on shard Dendeng using different drying methods. The AD samples presented the most compact and coherent structure with hardly any spaces among the fibers (Fig. 1A). Higher temperature induced faster denaturation of proteins and subsequently more reduction in dimension of myofibrils and collagen, resulting in shrinkage of muscle fiber diameter and sacromere length (Kong et al., 2007; Lepetit et al., 2000). Fig. 1C shows a porous structure with large and irregular cavities or pores resulted from ice sublimation during FA processes. A similar behavior was observed on freeze-dried loco (Reyes et al., 2011). The degree of porosity (Fig. 1) also influenced the texture (Table 1) and rehydration ability (Table 2) of the dried products. Barret and Peleg (1992) reported a negative coordination between mechanical resistance and the size of air cells in porous materials: the larger the cells, the less the material resistance. Hence, more porous space inside the freeze-dried tissue resulted in a softer texture that was more favorable for rehydration. In a word, SEM photograph showed that different drying methods affect the compactness of the muscle structure.

3.3. Electrophoretic patterns of myofibrillar proteins

Proteins components of fresh and dried samples from *T. pacificus* squid were analyzed by SDS–PAGE and the results are displayed in Fig. 2. Lane R was proteins extracted from fresh squid muscles, mainly composed of 205, 172, 102, 90, 57, 48 and 43 kDa. The 205 kDa protein (arrowhead ①) was myosin heavy chain. As shown in Fig. 2, AD caused myosin degradated extemsively, followed by HPD and FD treaments. Furthermore, 102 kDa (arrowhead ③) and 57 kDa (arrowhead ⑤) proteins followed the same pattern as myosin. Protein at 172 kDa (arrowhead ②) was probably composed of the degraded myosin, which was disassociated with a light chain or a subunit during the processes. The changed pattern of 172 kDa protein was conversely to that of myosin. Also, the changes of 90 (arrowhead ④) and 43 (arrowhead ⑥) kDa proteins might be attributed to the fragments or subunits disassociated from other proteins with high molecular weight. Based on these results, it could be concluded that the degree of protein denaturation and degradation of squid muscle depended on both drying methods and drying conditions (such as temperature and time). However, FD could effectively maintain the protein integrity,

Table 1
Moisture, protein, lipid, ash, TVBN, texture and color of raw and dried squid samples.

Drying methods[A]	Moisture[*] (g/100 g d.b.)	Protein[*] (g/100 g d.b.)	Fat[*] (g/100 g d.b.)	Ash[*] (g/100 g d.b.)	TVBN[*] (mg/100 g w.b.)	Hardness[*] (N)	Color[**]			
							L	*a*	*b*	Δ*E*
Raw	325.27 ± 2.4^{b}	86.78 ± 1.80^{b}	3.36 ± 0.31^{a}	6.47 ± 0.05^{a}	8.18 ± 0.33^{a}					
AD	8.71 ± 0.12^{a}	84.16 ± 1.66^{a}	3.13 ± 0.15^{a}	10.49 ± 0.60^{b}	44.03 ± 0.22^{d}	20.91 ± 0.44^{b}	39.17 ± 0.64^{a}	4.12 ± 0.51^{b}	17.06 ± 0.91^{b}	56.97 ± 0.34^{c}
FD	8.88 ± 0.07^{a}	82.77 ± 0.68^{a}	3.28 ± 0.17^{a}	11.26 ± 0.89^{b}	14.29 ± 0.48^{b}	15.91 ± 1.10^{a}	87.48 ± 1.34^{c}	−0.29 ± 0.17^{a}	8.72 ± 0.31^{a}	10.43 ± 0.64^{a}
HPD	8.54 ± 0.06^{a}	82.87 ± 1.56^{a}	3.31 ± 0.25^{a}	11.20 ± 0.41^{b}	34.19 ± 0.63^{c}	20.21 ± 0.58^{b}	45.36 ± 2.10^{b}	−0.76 ± 0.80^{a}	10.15 ± 1.86^{a}	49.05 ± 2.02^{b}

[A] AD: hot air drying; FD: freeze-drying; HPD: heat pump drying.
[*] TVBN: total volatile basic nitrogen. Each value is expressed as an average ± standard error ($n = 4$). Means in same column with same lowercase letters are not significantly different ($p < 0.05$).
[**] Each value is expressed as an average ± standard error ($n = 8$). Means in same column with same lowercase letters are not significantly different ($p < 0.05$).

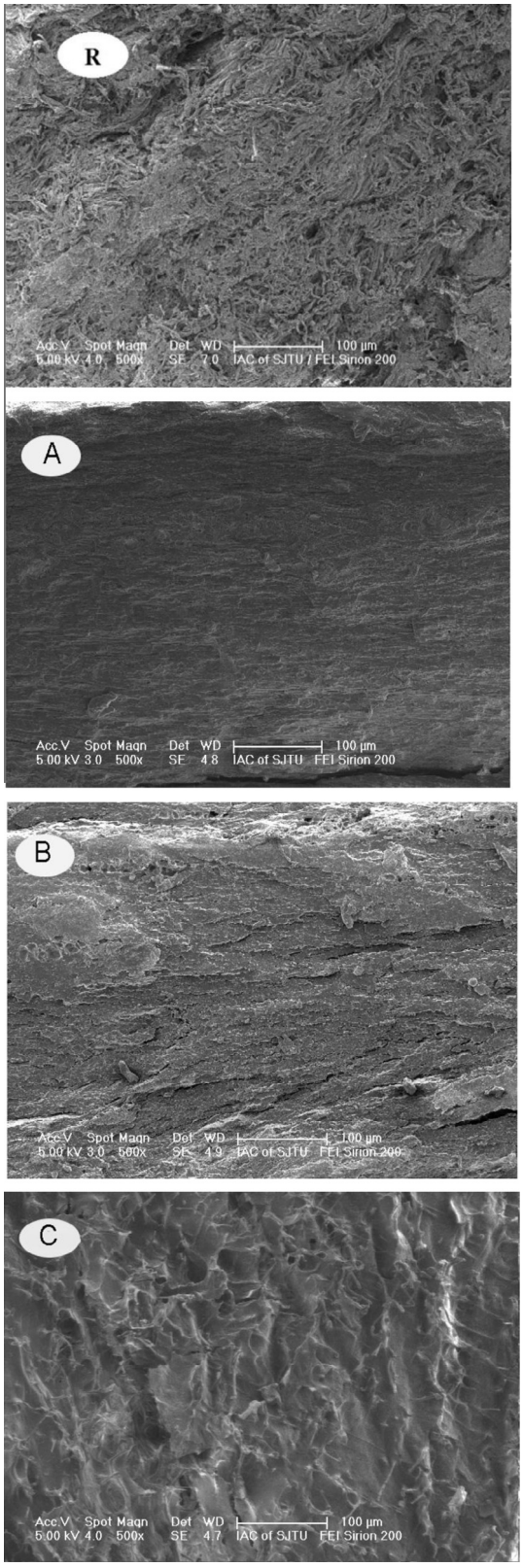

Fig. 1. Scanning electron micrograph of dried squid fillets. (A) hot-air dried samples; (B) heat pump dried samples; (C) freeze-dried samples.

which improved rehydration ability of FD muslces (Table 2). Compared to fresh sample, a new protein fragement with molecular weight of about 37 kDa was produced with AD, FD and HPD treatments (arrowhead ⑦). The amount of the newly formed 37 kDa protein in AD sample was higher than that in HPD and FD samples. This result also confirmed that the drying method affects the protein quality and quantity in certain degrees. Konishi et al. (2003) detected many fragments of lower molecular weight proteins in thesquid dried at 40 °C with an air velocity of 3 ms^{-1} for 72 h. compared with that of the fresh squid Hernández-Andrés et al. (2005) also reported that soluble proteins at 45 and 15 kDa from squid muscle showed weaker bands along with an increase in drying temperature from 40 to 50 °C.

3.4. Color

The color of dried seafood is one of the important criteria for its acceptability. The color changes of squid filets are presented in Table 1. The L values of all dried squid fillets varied with different drying methods ($p < 0.05$), where FD samples had significantly higher L values, while AD ones had the lowest L values (Table 1). AD samples had obviously higher a and b values than those of HPD and FD ones. No significant differences in a or b values were observed between HPD and FD samples. Moreover, FD gave the lowest ΔE value, and AD led to the highest value ($p < 0.05$). A decrease in L value and increase in a value indicated the increase of browning discoloration. Squid is rich in proteins and free amino acids, browning is a serious quality problem of squid products and dependent on species, processing and storage conditions. The highest L value and the lowest ΔE value in FD sample was probably due to the absence of Maillard reaction at the lower temperatures as well as dehydration of almost all water by sublimation during freeze-drying, resulting in increasing in lightness. Moreover, the lower L value, higher a and b values of AD samples meant that the samples became redder and more yellow after AD drying process which was probably due to myoglobin denaturation and pigments oxidization in the muscle caused by high drying temperature and long drying time (Kong et al., 2007).

3.5. Texture

Hardness is a mostly considered textural quality attribute of dried products by consumers (Dewi et al., 2010; Reyes et al., 2011). Compared with the initial tissue texture of raw squid, there were significant increases in hardness values ($p < 0.05$) in dried fillets. Similar results were reported by Kong et al. (2007) that heating increased the mechanical strength of salmon muscle fibers. The texture quality of the dried squids relies on collagen network, aggregated and denatured myofibrillar proteins and myofibrillar structure (Chang et al., 2011; Valencia-Pérez et al., 2008; Vega-Gálvez et al., 2011). Protein denaturation decreased water-holding capacity and contracted muscle fibers, subsequently resulting in a harder tissue texture. It was also found that FD fillets had significantly ($p < 0.05$) lower hardness (15.91 N) than AD and HPD samples, 20.91 N and 20.21 N, respectively (Table 1). Compared with FD and HPD, AD (at 60 °C) led to higher muscle toughness (Table 1) and more compact microstructure (Fig. 1A), which was due to the fact that higher temperature may aggravate the denaturation and aggregation of proteins, contraction of myofibrils and connective tissue, and the reduction of extracellular space and intracellular cavities and canals (Chang et al., 2011; Valencia-Pérez et al., 2008). It was reported that protein denaturation increased about 600-fold for every 10 °C increase in temperature (Anglemier and Montgomery, 1976). On the other hand, the surface moisture lost rapidly, leading to a stiff surface described by the case-hardening phenomenon, and resulted in obvious tissue-toughening in AD samples. In freeze drying, ice sublimation increased the formation of pores and cavities (Fig. 1 C), thus freeze-dried products had more fragile structure and lower hardness than those of other dried products. Sigurgisladottir et al. (2000) also suggested that

Table 2
Water absorption capacity; dry matter holding capacity; rehydration ability, rehydration rate, Peleg rate and capacity constants and statistical parameters of Peleg model for rehydration curves of dried squid fillets.

Drying methods*	WHC †	DHC†	RA†	Rehydration rate† (g/g)	Regression coefficients‡			Statistical parameters**			
					k_1	k_2	X_e (g/g d.b.)	R^2	χ^2	RMSE	E%
AD	0.361 ± 0.023[a]	0.859 ± 0.012[a]	0.310 ± 0.019[a]	2.12 ± 0.11[a]	15.51	0.73	1.46	0.9892	0.016	0.0194	0.319
HPD	0.348 ± 0.028[a]	0.856 ± 0.026[a]	0.299 ± 0.031[a]	2.34 ± 0.10[a]	8.33	0.60	1.76	0.9997	2.27e−004	0.0025	0.034
FD	0.409 ± 0.038[b]	0.970 ± 0.008[b]	0.396 ± 0.035[b]	2.88 ± 0.23[b]	2.10	0.56	1.87	0.9415	0.026	0.0290	0.239

† WHC: water absorption capacity; DHC: dry matter holding capacity; RA: rehydration ability. Each value was expressed as an average ± standard error ($n = 3$). Means in same column with same lowercase letters were not significantly different ($p < 0.05$).
* AD: hot air drying; FD: freeze-drying; HPD: heat pump drying.
‡ k_1: the Peleg rate constant; k_2: the Peleg capacity constant; X_e: the equilibrium moisture content (g/g d.b.).

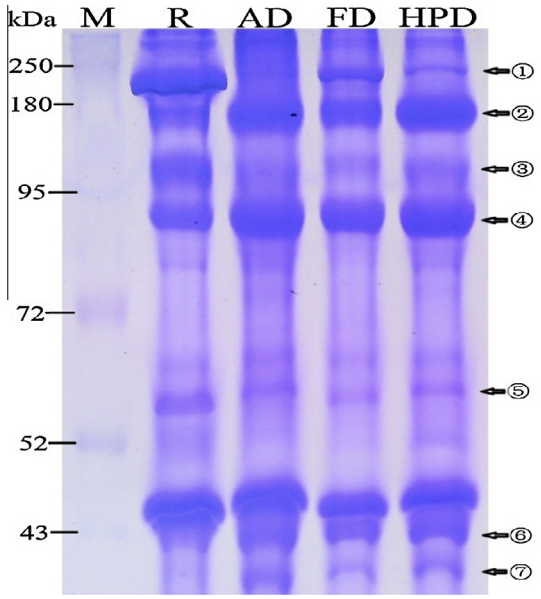

Fig. 2. SDS-PAGE analysis of proteins in squid fillets subjected to different drying treatments. Lane M, protein molecular weight markers (SM1851, Fermentas, USA); Lane R, protein of raw squid; Lane AD, protein of squid dried with heat air; Lane FD, protein of squid dried in a freezon system; Lane HPD, protein of squid dried with heat pump drying.

freezing-induced protein denaturation could cause the smaller diameter muscle fibers to shrink to a lesser extent than larger diameter fibers, leading to soft texture of dried muscles.

3.6. Moisture distribution in rehydrated squid fillets

MRI_S presenting the changes of moisture distributions during rehydration of dried squid fillets are illustrated in Fig. 3. In all MRIs, moisture distribution within each squid fillet was displayed after eliminating the NMR signals originated from water surrounding the squid fillet. Water gradually migrated from squid fillet periphery (arrow 1) to the internal region along with the increase of rehydrating time for all dried samples (Fig. 3). Individual fillets had slight variations in the first penetration site due to the different microstructures (Fig. 1). From Fig. 3, the status of water penetration in all samples can be roughly divided into three stages during 60 min rehydration: ST1: 0–15 min, ST2: 15–30 min, and ST3: 30–60 min. The water penetration rate was the greatest during ST1, followed by ST2, while ST3 showed the smallest rate. The overall patterns of water penetrations were associated with drying treatments. At 5 min, water penetration in the periphery of the anterior side of FD fillet was very evident (Fig. 3 FD), the outline of water penetration loomed up in the HPD samples (Fig. 3 HPD), but it was barely visible for AD samples (Fig. 3 AD). At the same rehydration stage, the amount of water penetration was the highest in FD squid fillets, followed by HPD and AD samples. These results indicated that different drying methods induced different microstructures of the squid fillets and thus resulted in different rehydration ability (Fig. 1).

3.7. NMR signal intensity profile of individual squid fillets

NMR signal intensity profile was obtained from the central slice in anterior view at 60 min of rehydration (Fig. 4). The rectangular region in anterior view image was selected, which was composed of a 256 × 256 mm (longitudinal × lateral axis) pixel array (Fig. 4 A). NMR signal intensities in the selected region (32 rows × 100 columns) were averaged for each column. The average signal intensity was plotted against distance from the vertical center on the lateral axis to gain the signal intensity profile for each fillet (Fig. 4).

The average values of the signal intensity profiles of dried squid fillets after 1 h of rehydration are presented in Fig. 4B–D, which reflected both the degree of porosity of dried sample and the moisture distributions in the lateral direction of the squid fillets.

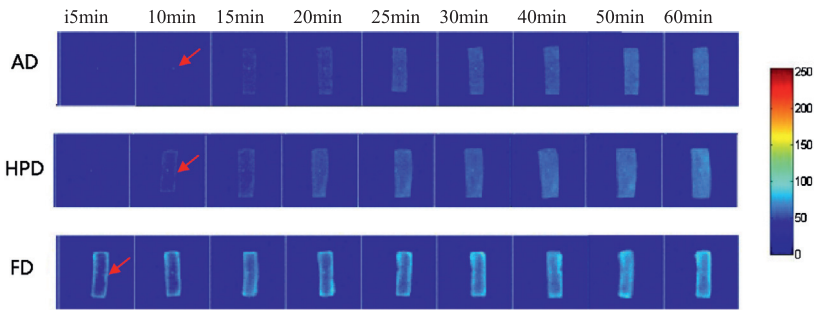

Fig. 3. NMR images of dried squid fillets during one hour of rehydration. AD: hot air drying; HPD: heat pump drying; FD: freeze-drying.

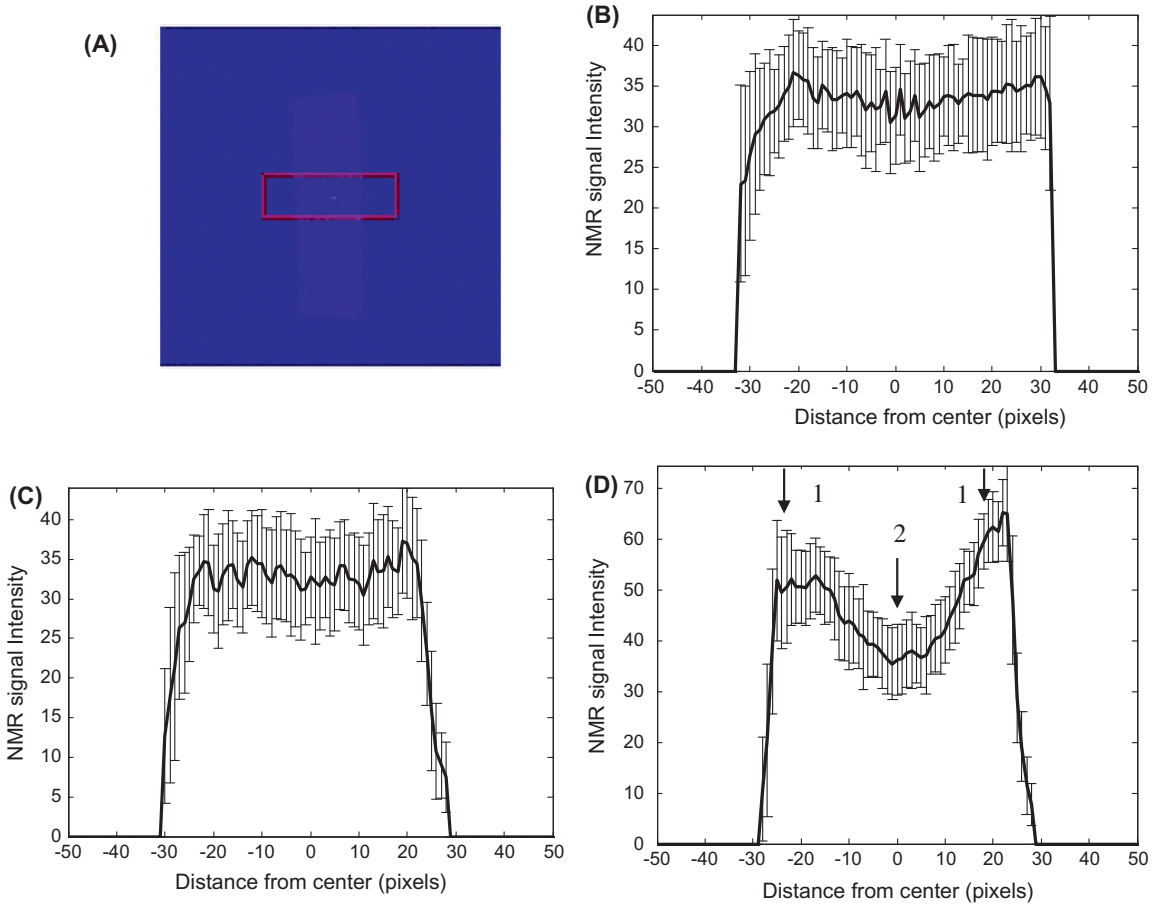

Fig. 4. NMR image of an anterior view for generating one signal intensity (A), and average signal intensity profiles (B and C) of three slices after 1 h of rehydration.

Generally, the shape of each signal intensity profile was almost symmetrical on the lateral axis for all dried squid fillets. The vertical bars in signal intensity profiles represented standard deviations of NMR signal intensities of the fillets (Fig. 4B–D). The NMR signal intensity differed discernibly between FD and AD/HPD samples. The signal intensity for FD squid fillets was high at the squid fillet periphery (arrow1) and low in the center region (arrow 2) (Fig. 4D), which suggested that more water was distributed around the fillet periphery. These results were probably ascribed to the fact that porosity, cavities, and capillaries close to the squid fillet surface caused by FD improved water ingress; while a large amount of air bubbles within the porous structures hindered innerwater penetration (Fig. 1C). However, NMR average signal intensity for both AD and HPD squid fillets were basically identical along the lateral axis (Fig. 4B–C), which was due to the less porosity degree, compact and coherent tissue structure resulted from AD and HPD treatment(Fig. 1A and B).

3.8. Rehydration kinetics and rehydration ability

Fig. 5 shows the rehydration curves of different dried samples. Moisture uptake increased with increasing rehydration time, and the rate was higher in the initial period of rehydration, but decreased up to constant level, agreed with the MRI results of rehydrated samples (Fig. 3). Similar results were reported in the previous studies (Crapo et al., 2010; Reyes et al., 2011; Wang et al., 2012).

Peleg models fitted the experimental data very well with R^2 values equal or higher than 0.94, χ^2, RMSE and $E\%$ values equal or lower than 0.026, 0.029 and 0.319, respectively (Table 2). All the

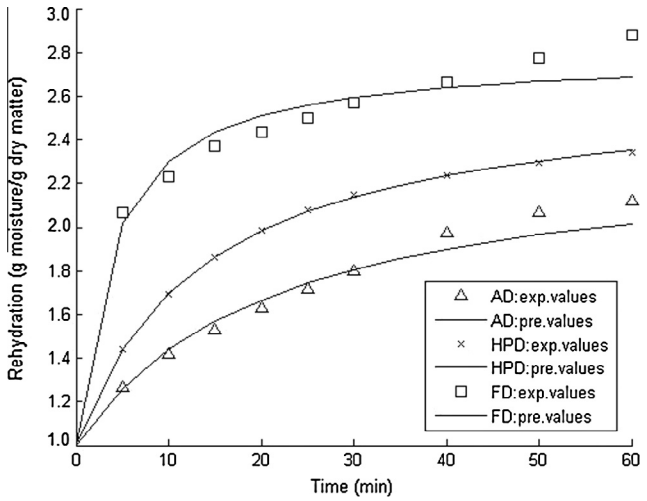

Fig. 5. Rehydration curves of dried squid fillets at distinct time. AD: hot air drying; FD: freeze-drying; HPD: heat pump drying.

parameters in the Peleg models were affected by drying modes. The Peleg constant k_1 was related to mass transfer rate and its reciprocal can be taken as a diffusion coefficient, e.g., the lower the k_1, the higher the initial moisture absorption. The k_1 value of AD-squid fillets was about 1.86, 7.39 times higher than those of HPD and FD samples (Table 2), indicating that AD samples had poorer rehydration ability than HPD- and FD products. HPD samples had

higher k_1 value than that of AD ones, which was probably due to the low degree of protein denaturation and formation of crack in tissues, contributing to water uptake. The k_2 values slightly varied among the three drying methods (Table 2), which was similar to the values of k_2 of the dried loco cubes and plates (Reyes et al., 2011). The Peleg constant k_2 is related to rehydration temperature and chemical composition of a material (Okos et al., 2007; Peleg, 1998). The equilibrium moisture content X_e showed the opposite trends as k_2. In all experiments, the X_e values at the saturation did not reach the moisture content of the fresh material (Table 2), indicating that the drying procedure is irreversible (Okos et al., 2007). The freeze-dried samples (1.87 g g^{-1}d.b.) had higher X_e values than air-dried ones (1.46∼1.76 g g^{-1}d.b.), probably owning to the fact that significant structure damage (Fig. 4) during HPD and AD led to more loss of rehydration capacity. The changes in the chemical compositions and chemical or physical structures of the materials may promote variations in k_2 and X_e during the rehydration of dried products (Okos et al., 2007; Peleg, 1998). How exactly the FD, HPD and AD affect k_2 and X_e values during rehydration needs to be further studied in the future. In conclusion, the rehydration ability of biological materials depended on the type of material, the tissue microstructures, the chemical composition of cells, and the processing conditions.

Freeze-dried squid fillets had significantly higher WHC, DHC, RA and rehydration rates than those of air-dried samples ($p < 0.05$) (Table 2 and Fig. 5), which was mainly due to their porous and less shrunken structure with a lot of voids (Fig. 1) that facilitated better and faster water uptake. The lower RA values in AD and HPD samples showed severer damage in their muscle tissues (Fig. 1A and B), leading to irreversible physical and chemical changes, which was in concert with the smaller rehydration rates. These results were consistent with NMR data (Figs. 3 and 4), showing that the more compact structure had lower capacity to absorb water when reconstituted. Furthermore, Marabi et al. (2003) explained rehydration process using Weibull distribution, and concluded that for freeze-dried products with high porosity, capillarity is the primary mode of water transport, while for air-dried products with low porosity, diffusion is predominant for mass transfer. No significant alterations in WHC, DHC, RA and rehydration rates between AD and HPD samples were found.

4. Conclusion

This study revealed that the water penetration pattern during rehydrating showed the similar trend among squid fillets dried by different methods: from the periphery to the center region, and could be generally classified into three stages: increasing, falling and constant rehydration with increased rehydration time. Moisture distribution during rehydration indicated that signal intensity at the completion of water absorption was high at periphery and low in the intermediate region for FD squid fillets, while almost identical along the lateral axis for AD and HPD samples. Drying led to muscle porteins denaturation and degradation followed by the order of AD > HPD > FD, where FD effectively maintained the proteins integrity. Results showed that moisture distribution is highly related to the microstructure of dried squid fillets. FD increased the porosity, rehydration ability, and lowered the TVBN and firmness compared AD and HPD treatments. AD samples had the highest TVBN value, the severest structure compactness, the least rehydration rate in comparison to HPD. In general, FD gave the best quality of samples, but this method was the most expensive among the three drying methods. Although drying time of HPD is longer than that of AD, HPD-treated samples showed less damages in microstructure and protein, slightly higher rehydration ability and color. Therefore, HPD demonstrated the potential for industrial application of producing squid fillets with high quality and processing efficiency. This study also provided valuable insights into the moisture migration mechanics and chemical and physicochemical properties of dried squid fillets processed by different drying methods. Such information may benefit the processed food industry for developing economical feasible drying system that provides high quality of dried products.

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