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Original Article

Effect of NaCl on texture modification of cuttlefish mantle (*Sepia brevimana*)

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Abstract

The mechanical properties of cleaned cuttlefish mantle (*Sepia brevimana*) before and after spinning in cold salt solution were characterized by tensile and shear test. The objective was to relate the physical changes of the cuttlefish mantle to the composition and microstructure changes mediated by spinning in 5 % (w/v) NaCl solution at 0-(-5)^{0}C for 10 min. Textural difference between outer and inner surface of the cleaned mantle was revealed by the tensile force applied to transverse direction of the longitudinal axis of the mantle. Shear test was found suitable to highlight textural modification due to the spinning. It was found that the operation increased the mantle total weight and caused mantle curvature and hardening. These changes were concomitant with an increase of the mantle moisture and salt content at specific layers in the mantle. Microstructure of the mantle obtained by scanning electron microscopy (SEM) showed both expanded and packed fibers depending on their position. The results suggested that modification of the mantle texture due to spinning in the cold NaCl solution was a complex process coupled with changes in chemical composition and microstructure.

Keyword: cuttlefish, tensile test, shear test, texture, SEM

1. Introduction

Spinning of molluscs in cold NaCl solution is a general practice both in seafood fresh market and frozen molluscs manufacturing. It is used to harden the cuttlefish mantle texture before skinning in order to facilitate skin removal. It is also used to modify the mantle texture and to increase NaCl content according to the customer requirement before using in frozen cuttlefish processing. NaCl concentration used for the spinning varies among the manufactures ranging from 3-20% w/v. The spinning condition (NaCl concentration and spinning time) is commonly decided by an experienced operator based on type, size, freshness, and initial NaCl content of the raw materials. The operation causes a loss in taste of freshness and the mantle total weight (about 3-5%). These operation drawbacks, especially weight loss, were disregarded under the previous lucrative market. Nevertheless, minimization of the loss has become a highly desirable goal recently due to the continually rising production cost. Therefore, a better understanding of factors affecting cuttlefish texture is important in developing techniques that will improve process efficiency.

The muscle of cuttlefish differs from the muscle of fish and mammals. It combines a complex, three-dimensional arrangement of muscle fibers, connective tissue fibers, and the gladius to provide shape and structural support for the mantle. The mantle can be separated into 5 tissue layers having different composite hierarchical structures namely, from outer surface to inner/visceral surface, outer lining, outer tunic, muscle tunic, inner tunic, and visceral lining (Lluch *et al.*, 2001). The muscle fibers in squid mantle are arranged primarily in two orientations: circumferential and radial. The fibrous connective tissues of the squid mantle are arranged into five networks: the inner and outer tunics, which sandwich the circumferential and radial muscles, plus three networks of intramuscular collagen fibers (Bone *et al.*, 1981). Intra-

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muscular fiber system 1 (IM-1) consists of collagen fibers that originate and insert on the inner and outer tunics (Gosline and Shadwick, 1983; MacGillivray *et al.*, 1999). Intramuscular fiber system 2 (IM-2) is composed of collagen fibers localized to the radial muscle bands. The intramuscular fiber system 3 (IM-3) are collagen fibers arranged parallel to the circumferential muscle fibers and are not attached to the tunics (Bone *et al.*, 1981; MacGillivray *et al.*, 1999).

Sodium chloride affects physicochemical properties and interactions between proteins thus it is used to manipulate the functional properties of proteins. It is a basic ingredient to solubilize myofibrillar proteins for subsequent denaturation/aggregation to give good water-fat-retention and acceptable rigidity/elasticity of the muscle gels (Gordon and Barbut, 1992). Application of salt affects texture properties, water holding capacity, isoelectric point and protein functionality (Barat *et al.*, 2002). The increase in water holding capacity is attributed to the rise in solubility of meat proteins as well as to the increase of the ionic strength (Hamm, 1994; Offer and Knight, 1988). In addition, salt affects the collagen solubility and salt precipitation is general protocol to recovery collagen isolate (Mizuta *et al.*, 2002, 2003). However, little is known about the concomitant effect of sodium chloride on cuttlefish protein together with the modification of mantle texture.

In the present paper, the cuttlefish mantle composition was analyzed and its mechanical properties were evaluated by tensile and shear test. In addition, the effects of spinning cuttlefish in cold NaCl solution on chemical compositions, microstructure, and texture modification were investigated in order to verify the type of structural and/or chemical composition changes which were related to texture alteration.

2. Material and Methods

2.1 Cuttlefish

Cuttlefish (*Sepia brevimana*) in size range of 3 cuttlefish/kg, caught in the Gulf of Thailand, were taken from the dock in Songkhla, Thailand. The sample was placed on ice with a cuttlefish/ice ratio 1:2 (w/w) and transported to the Department of Food Technology, Prince of Songkla University within 2 hr. It was separated into mantle and head portion. The mantle was deskinned, eviscerated and cleaned. The cleaned mantle was kept on ice until analysis.

2.2 Spinning the cuttlefish mantle in NaCl solution

Hardening the cuttlefish mantle was performed by spinning the mantle in 5 times of 5 % (w/v) NaCl solution at 0-(-5)^{o}C for 10 min. The sample was then used for SEM and texture analysis. The spun mantle was sliced along either parallel or transverse to the mantle longitudinal axis into a strip of 2 mm thickness from inner or outer surface. It was used for texture analysis and some chemical analysis.

2.3 Analysis

1) Chemical composition

The cleaned mantle was determined for moisture, fat, ash, salt and protein according to the method of AOAC (1999). Total collagen content was measured on the basis of hydroxyproline content according to the method of Woessner (1961). The converting factor for calculating collagen content was 11.11.

2) Texture analysis

The mechanical properties of cuttlefish mantle (shear force and tensile force) were evaluated by using the texture analyzer (Stable Micro System, TA-XT2I, England). The samples for Warner-Bratzler (WB) shear test were obtained by cutting the mantle into at least 6 rectangles of 2x3 cm^2 (Figure 1A). They were completely cut from outer or inner surface by using a WB shear blade with a triangular slot cutting edge at a crosshead speed of 5 mm/s and the maximum shear forces (g) of at least 6 specimens were measured.

Samples for tensile test were obtained by cutting the mantle into a rectangle of 1.5x10 cm^2 either parallel or transverse to the mantle longitudinal axis. The rectangle was subsequently sliced into a thin strip (0.2 cm thickness) from

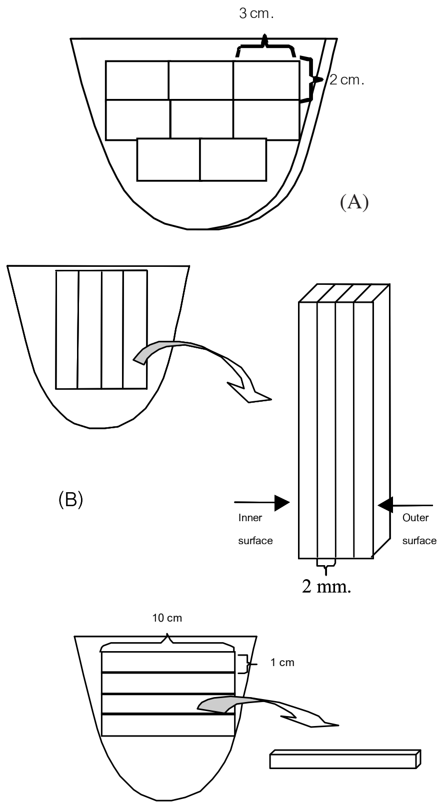


Figure 1. Sampling diagram for excising the cuttlefish mantle for shear force (A) and tensile force test (B)

outer or inner of the mantle surface (Figure 1B). A single strip was held by tensile roller grip and extended at 5 mm/s until failure. The maximum tensile forces (g) at break of at least 6 specimens obtained from each cutting direction and the mantle surface were recorded.

3) Scanning Electron Microscopy (SEM)

The cuttlefish mantle was excised to obtain a mantle piece of 0.5x1.0 cm^2. They were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 24 h at 4^oC. The solution was discarded and the dehydration was repeated in 25% (v/v) ethanol for 1 h with increasing strength of solution from 25, 50, 70, 95 and 100% (v/v) ethanol, consecutively. The specimens were dehydrated in 100% (v/v) ethanol twice. The dried specimen samples were then cut in liquid nitrogen and the critical point drying was done by using liquid carbon dioxide. The fragments of dried tissue were mounted on holders with silver cement and coated twice with AuPd. The SEM was conducted using a SEM JOEL JSM-5200 with an accelerating voltage of 10 kV and a working distance of 15 mm. The micrographs were taken at magnification of x 5,000.

3. Results and Discussion

3.1 Chemical composition and texture of the cleaned cuttlefish mantle

The chemical compositions of cuttlefish mantle are shown in Table 1. The reported value of fat content was lower than the value of 0.47±0.01 g/100 g of the same specie reported by Thanonkaew *et al.* (2006). They reported a protein content of 14.91±0.61 g/100 g, which was lower than the value of this study. This discrepancy might stem from the difference in size and catching season of the cuttlefish used for the experiments. The cuttlefish size of this study was 3 cuttlefish/kg, which is much larger than the size of 8-10 cuttlefish/kg of their study. The result was, however, in agreement with the chemical compositions of Giant squid (*Dosidicus gigas*) (Gomez-Guillen *et al.*, 1997).

The collagen content of cuttlefish mantle of this study was higher than the value (0.64±0.22 g/100 g) reported by Thanonkaew *et al.* (2006). This difference could be explained according to the above mentioned reason. The collagen accounted for 7.25% of crude protein. This was slightly higher and lower than the collagen content of squid mantle (*Todarodes pacificus*) and octopus, which contained 5.4 % and 14% of crude protein, respectively (Mizuta *et al.*, 1994; Mizuta *et al.*, 2003). Collagen content was proposed to be a principal contributor to the texture of raw or cooked meat of seafood (Hatae *et al.*, 1986; Kuo *et al.*, 1990; Sato *et al.*, 1997; Sato *et al.*, 1986). The collagen content of the slices obtained from various layers of the mantle is shown in Table 2. The second slices would include mainly the muscle tunic whereas the first slices would account primarily for the outer/inner tunic. The collagen contents of the second slice either from inner or outer mantle surface are significantly lower ($p<0.05$) than that of the first slice. This would suggest that contribution of muscle fiber in each tissue layers on texture of the intact mantle is different.

Table 1. Chemical compositions of cuttlefish mantle

Compositions	Percent (w/w)
Moisture	82.02±0.43*
Protein	16.56±0.09
Fat	0.01±0.01
Ash	0.77±0.02

* Mean ± SD from triplicate determinations

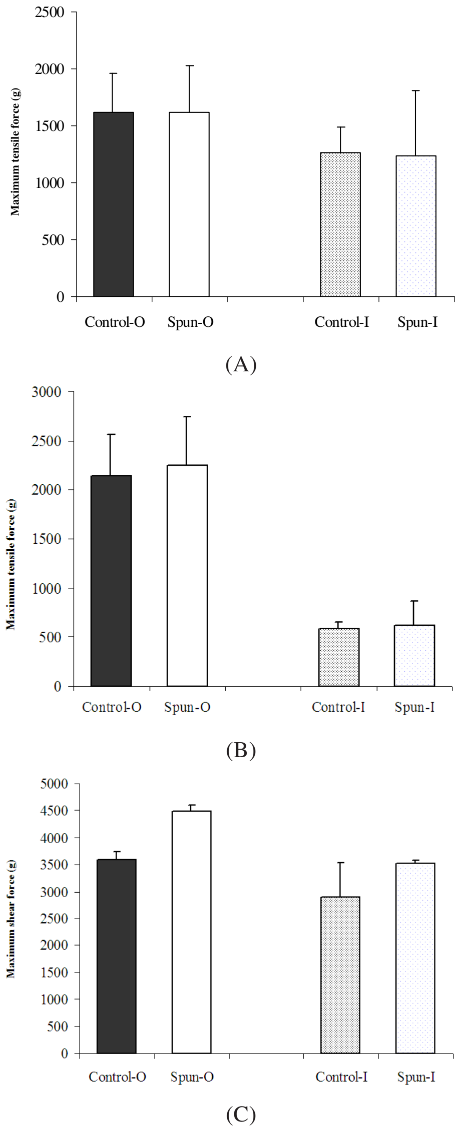
Table 2. Collagen contained in various positions of the cuttlefish mantle

Sample position	Collagen (%)
First slice from inner surface	1.08±0.39c*
Second slice from inner surface	0.80±0.62d
First slice from outer surface	1.78±0.93a
Second slice from outer surface	0.82±0.74d
Whole cleaned mantle	1.25±0.98b

* Mean ± SD from triplicate determinations
The means followed by same letters are non-significantly different ($p\leq0.05$).

Cuttlefish mantle is soft tissue. However it is accepted that cuttlefish mantle has a much stiffer and stronger texture than that of most fish fillet. To gain qualitative information of the mechanical properties, the mantle was evaluated by tensile and shear forces (Figure 2). It was clear that the outer lining and visceral lining, the collagen-rich skin, which were removed at the cleaning step, would not be relevant to the texture of this cleaned mantle. A major problem of performing a tensile test on a thick and soft specimen as the cuttlefish mantle was the fixation of the sample. A pressure applied on the sample by the tensile roller grip led to damage or breakage out of the sample in this high stressed region and caused faulty interpretation of the real mechanical properties of the material. It was found that using a thin strip reduced the damage at the grip successfully, although one might speculate that the mechanical information obtained on such thin samples is not relevant for understanding the mechanical properties of the whole structure.

The tensile forces used to break the longitudinal mantle strips obtained from either inner or outer surface of the untreated cuttlefish were not significantly different ($p>0.05$) (Figure 2A). The difference in mechanical properties between inner and outer mantle surface was exhibited by tensile test of strips obtained from transverse orientation of the mantle longitudinal axis (Figure 2B) ($p\leq0.05$). This finding underlined the crucial difference in composition, type of fibers and their arrangement in these two specimens. As a



(A)

(B)

(C)

Figure 2. Maximum tensile forces of transverse strip (A) and longitudinal strip (B) and maximum shear force (C) of the cuttlefish before and after spinning in cold salt solution
Remark: Control-O and Control-I were the slices obtained from outer and inner mantle surface, respectively. Spun-O and Spun-I were the slices obtained from outer and inner mantle surface after the spinning, respectively.

matter of fact, the strips used for the tensile test would contain primarily outer or inner tunic layer and at least some portion of muscle tunic. The outer/inner tunic layers contained collagen fiber of IM-1 and IM-2 and circumferential muscle fibers of muscle tunic (Gosline and Shadwick, 1983; MacGillivray *et al.*, 1999). The IM-1 collagen fibers are arranged at a low angle relative to the long axis of the mantle (Kuo *et al.*, 1990; Ward and Wainwright, 1972) whereas IM-2 collagen fibers oriented along the thickness of the cuttlefish mantle. In general cases, the tensile force of any direction would have the greatest value if the force direction were identical to the fiber orientation. Thus, it would appear that the tensile attributes of the transverse strips are contributed to primarily by the circumferential muscle fibers. Moreover, the collagen fibers in the inner tunic were smaller and arranged in less order than that of the outer tunic (Macgillivray *et al.*, 1999; Otwell and Hamann, 1979; Thompson and Kier, 2001). In addition, it was observed that the collagen content in the outer surface of the cuttlefish strip was higher than that in the inner surface (Table 2), however, these surfaces show no significant difference in their mechanical properties ($p \leq 0.05$). Thus, it seems likely that all these facts, at least partially, accounted for the disparity between tensile strength of the strips obtained from different mantle side. In contrast, it was found that cutting the rectangle of cuttlefish mantle from either inner or outer surface had no significant effect on the maximum shear forces ($p > 0.05$) (Figure 2C). Thus, the tensile force is likely a prime illustration of the mantle mechanical properties.

3.2 Effect of NaCl on texture and microstructure of cuttlefish mantle

It was found that spinning of cuttlefish mantle in cold NaCl solution caused changes in the 3 macroscopic physical parameters; mantle total weight, mantle curvature, and mantle texture. In contrast, if the mantle was wrapped with a plastic sheet before the spinning as well as if the unwrapped mantle was spun at other higher temperatures (data not shown), non-significant change of the mantle occurred. The observation thus pointed out the consequence of salt adsorption and solution temperature on the changes.

Preliminary investigation revealed that if concentration of the salt solution increased (3-15%), the spinning time to harden the mantle was reduced dramatically. By contrast, the total weight loss would increase markedly (data not

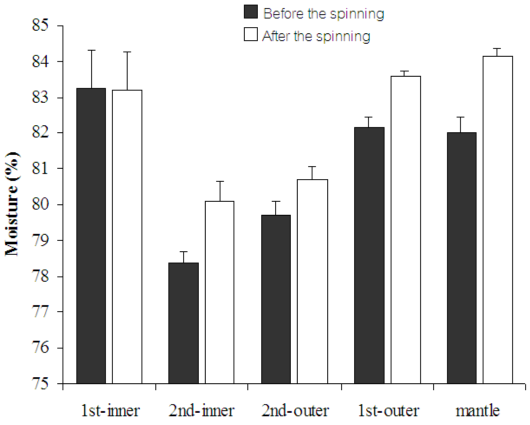


Figure 3. Distribution of moisture content in cuttlefish mantle before and after spinning in cold salt solution
Remark: 1st-inner and 2nd-inner were first and second slice obtained from inner mantle surface, respectively. 1st-outer and 2nd-outer were first and second slice obtained from outer mantle surface, respectively. Mantle was the intact cuttlefish mantle.

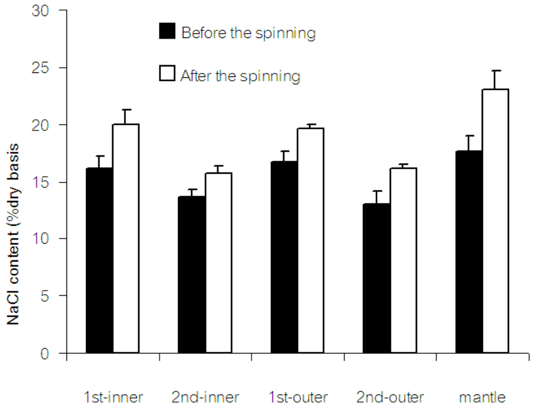


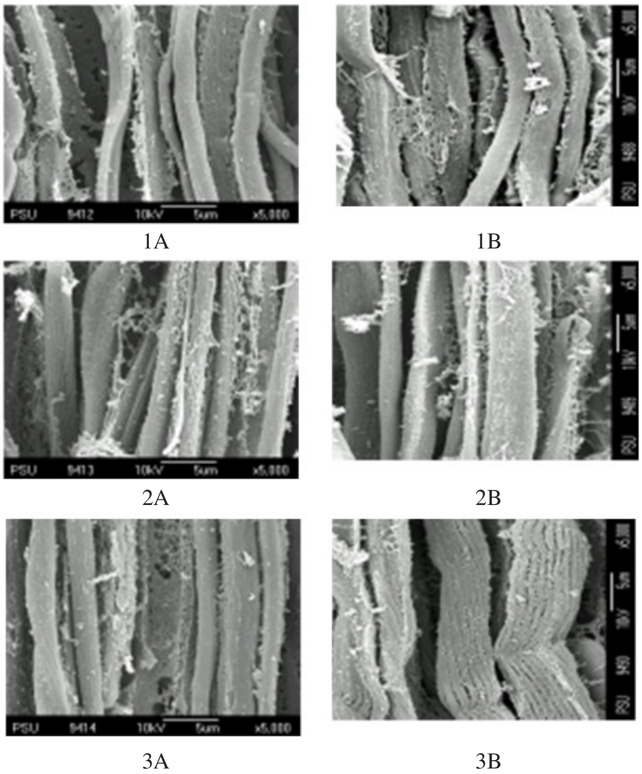
Figure 4. Distribution of salt content in cuttlefish mantle before and after spinning in cold salt solution
Remark: 1st-inner and 2nd-inner were first and second slice obtained from inner mantle surface, respectively. 1st-outer and 2nd-outer were first and second slice obtained from outer mantle surface, respectively. Mantle was the intact cuttlefish mantle.

shown). The salt concentration at 5% w/w, which gave a good balance of these consequences, was thus selected for this study. It was found that the mantle total weight was increased about 3% after spinning. This obviously originated from a significant increase in moisture content (Figure 3). Increasing of moisture content of was found associated with an increase of salt content of the spun mantle (Figure 4). It is likely that the spinning established the salt concentration in the mantle to the salting in range causing an increase of water holding capacity of the muscle proteins. It was expected initially that the spinning would establish a salt concentration gradient with the highest concentration in thin layers beneath outer and inner layers of the mantle. According to this hypothesis the moisture content gradient in the spun mantle would be consequently established in a fashion that refracted the solubility/water holding capacity of the muscle protein. For instance, salt concentration within the salting in range would enhance solubility or water holding capacity of muscle proteins resulting in increased moisture content. The hypothesis of NaCl concentration gradient was valid regarding to the observation shown in Figure 4. Although non-significant difference in NaCl content of the first slices obtained from inner and outer mantle surface was noted, the moisture content of the first slice of the outer mantle surface was, however, significantly higher than that of slice of the inner surface. The distinct effect of the salt content (~19% w/w dry basis) on water holding of each parts of the cuttlefish mantle may be associated with the difference in their composition.

Curling of the mantle due to an increase of the outer mantle curvature after the spinning was likely a consequence of alteration of several structural elements. Nevertheless, it may be useful to devise a simple model to reflect the experimental results. Toward this end, we propose that curling occurred was based solely on the expansion of the outer tunic and the first few consecutive layers of muscle tunic due to adsorbed water. Kier and Smith (1985) reported that the muscle fibers and connective tissue fibers of the mantle are packed into a dense, three-dimensional array. Water contained within the muscle fibers and the connective tissue fibers themselves serves as the incompressible fluid. The term “muscular hydrostat” was coined for such a structural system in which the volume of the mantle remains constant. Thus, a change in one dimension must result in a change in at least one of the other dimensions of the mantle. The water adsorbed by the mantle after the spinning, especially in fibers closed to the outer surface, would consequently exert an additional pressure on the mantle fibers causing them to expand. Since the intramuscular fiber systems, especially IM-2, resist effectively the substantial increase in mantle thickness, the mantle fibers thus readily extend in circumferential orientation. Under the assumption, the circumferential muscle fibers would be expected to make their greatest contribution in an outer surface extension. The curling may be favored at least partially by non-expansion of muscle fiber in the inner surface based on its non-significant increase of moisture content.

The effects of the spinning on mechanical properties were evaluated by performing tensile and shear tests and the results are shown in Figure 2. It was found that the operation did not significantly alter the tensile characteristics of the strips obtained from outer and inner mantle surface (Figure 2A, 2B). According to the previous explanation applied to the mechanical properties of the cleaned mantle, it would appear that the operation causes non-significant effect on the strength of IM-1 collagen fibers and of the circumferential muscle fibers. In contrast, the operation increased the mantle shear strength significantly (Figure 2C). This is likely due to the adsorbed water that exerted the additional expansion pressure in either intramuscular or intermuscular fibers resulting in an increase resistance to shear force.

To determine which structures were changing at the ultrastructural level after the spinning, the cuttlefish mantles before and after the treatment were examined by SEM. Observation of a longitudinal section of the cuttlefish mantle disclosed that only the mantle fibers positioned near the inner surface were noticeably expanded after the spinning (Figure 5). The SEM micrographs obtained from transverse orientation of the mantle longitudinal axis revealed an apparent expansion of the muscle fiber located in the outer and middle portion of the mantle, whereas the mantle muscle fiber in the inner part was packed into a thick fiber with an increase of void volume (Figure 6). The results thus indicated that the microstructure of each position in the cuttlefish mantle was altered differently by the treatment. However, it is difficult to draw a satisfactory explanation as a consequence only of the microstructure alteration and the mantle macroscopic changes. This is partially due to the fault treat artifacts caused by reliably specimen preparation, which cannot be excluded especially during the fixation. Salt content of the specimen could be leached out during the

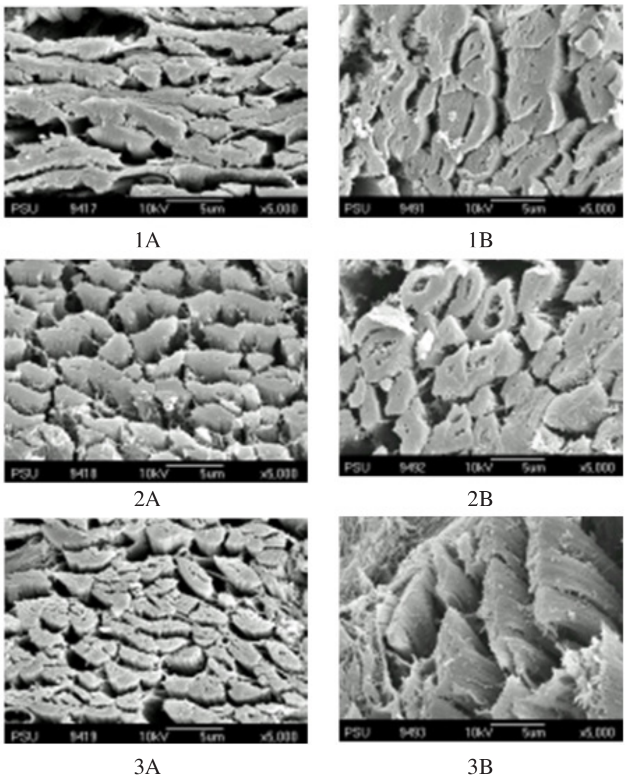


1A 1B

2A 2B

3A 3B

Figure 5. SEM micrographs of longitudinal orientation in outer (1), middle (2) and inner (3) portion of the cuttlefish mantle before (A) and after spinning in cold salt solution (B)



1A 1B

2A 2B

3A 3B

Figure 6. SEM micrographs of transverse orientation in outer (1), middle (2) and inner (3) portion of the cuttlefish mantle before (A) and after spinning in cold salt solution (B)

lengthy treatment by the fixing solution.

4. Conclusions

Maximum tensile and shear force appropriately described the mechanical properties of the cuttlefish mantle. The tensile force could be used to differentiate the strips obtained from each side of the clean mantle surfaces, whereas the shear test was an appropriate mean to describe the modification of the mantle texture due to the spinning in cold salt solution. Spinning the clean cuttlefish mantle in cold salt solution caused alteration of the mantle physical properties including an increase in the mantle weight, hardening of the mantle texture and an increase in the mantle curvature. These changes were associated with changes in chemical composition, mechanical properties and microstructure. The precise relationship of these factors is not clear. The unique modification in the mantle physical properties thus might prove of particular interest if it can be related to chemical or structural differences between the mantle before and after the spinning.

Acknowledgements

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