STUDY ON APPLICATIONS OF CARRAGEENAN OBTAINED FROM *Kappaphycus alvarezii* ALGA TO MINIMIZE DRY MATTER LOSS DURING PRESERVATION OF FROZEN SHRIMP

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Received: 23 September 2019; Accepted: 5 December 2019

ABSTRACT

Carrageenan was extracted from *Kappaphycus alvarezii* alga for application in shrimp preservation to reduce the loss of dry matter during preserving frozen shrimp. The main material in this study is dried alga. The solution 3% NaOH was used to remove color compounds and lipids. The selected extraction conditions include distilled water, pH 7, the ratio of material/solvent 1/40 (w/v) at 80 °C for 2 hours, 1% $CaCl_2$ for the precipitation. The semi-refined carrageenan powder was ivory white, uniform smoothness, 9.21% moisture content. The recovery yield of the extraction was 65.96%. The solution used for shrimp coating was a mixture of 0.3 g carrageenan, 0.075 g glycerol, 0.075g polyethylene glycol 6000, the ratio of the mixture and distilled water 1/40 (w/w). The mixture was stirred in a heating condition at 80 °C for 30 minutes, then cooled down. Next, the shrimp were dipped in solution before freezing, shrimp samples were frozen to investigate the loss of dry matter between the control sample and the shrimp immersed in the preserved solution for 12 weeks.

Keywords: Carrageenan, dry matter, *Kappaphycus alvarezii* alga, shrimp preservation.

1. INTRODUCTION

Carrageenan is a kind of the colloid phycocolloid group with agar, alginate. The structure of the carrageenan extracted from *Kappaphycus alvarezii* is a polysaccharide. Carrageenan is widely used in food, dairy, and pharmaceutical industries as colloids, emulsifiers, and stabilizers [1]. Carrageenan, glycerol and polyethylene glycol can help to create a film to limit the growth of ice crystals in shrimp muscle tissue. Polyethylene glycol 6000 (PEG 6000) is non-toxic, odorless, inert, non-volatile. When combining with glycerol, it can create food packaging materials. Glycerol is colorless, odorless, non-toxic, sweet taste, mainly prepared from triglycerides extracted from plants and animals (soy, palm, beef fat ...). In the food scale, glycerol is used as a humectant, solvent, sweetener as well as a preservative. It is used in some low-fat processed foods and thickeners in alcohol. It is considered as an alternative of sugar, but it contains more energy than sugar and is sweeter by 60% of sucrose. At the same time, it is also an additive. In medicine, glycerol is used as a smoothing and slimming agent, and a moisturizer.

Litopenaeus vannamei is known as a great resource in Vietnam's marine economy, which is mostly exported. However, the fresh shrimp are susceptible to spoilage due to bacterial invasion, resulting in the rapid reduction of storage time. Freezing is one of the measures to preserve shrimps and their related products longer because it can inhibit the growth of microorganisms and active enzymes to maintain shrimps' flavor and nutritional values [2, 3]. Besides, some unexpected changes such as protein denaturation, ice sublimation, growth or

crystallization of ice crystals, or impaired tissue structure reduced water retention and tissue rupture in the process of freezing/thawing, caused adverse affects on nutrition quality and consumer health [4]. Among these changes, the formation and development of ice crystals during freezing is the most important quality issue in frozen seafood. However, these processes promote the link among free water molecules in muscle tissues, and create the polycrystalline state of ice crystals (crystallization) [5]. Freezing and thawing cause great damage to muscle tissue. Large ice crystals form inside and outside the cells and disrupt the connective tissue of shrimp, resulting in broken tissue and nutrient loss during the defrosting process. The ice crystals continue to grow and gradually form larger ice crystals that can cause serious physical damages to muscle tissues [6]. In addition, recrystallization also occurs rapidly at temperatures below freezing and during warming from the crystallization state [7]. Even when the temperature below 0 °C, migration recrystallization occurs, also known as melt recrystallization. It is classified by diffuse-diffuse recrystallization and recombines melting. Small crystals have stronger thermodynamics in shrinking or melting. Thus, large ice crystals grow rapidly to minimize surface area and free energy. Finally, the total number of ice crystals decreases, and the average size of crystals increases. Therefore, the mechanical strength of muscle connective tissue is significantly weak, and the damage caused by the formation of large ice crystals. Moreover, the proteins in shrimp muscle are affected by freezing which leads to rearrangements and changes in shape, cross-linking, and denaturation of muscle protein, resulting in soft shrimp [8]. In this study, we applied carrageenan obtained from *Kappaphycus alvarezii* to create a solution to preserve *Litopenaeus vannamei* in order to limit the loss of dry matter during cryopreservation.

2. MATERIALS AND METHODS

2.1. Materials

Dried *Kappaphycus alvarezii* alga was bought at Dac San Ngon Company Limited, Ho Chi Minh city (serving size 500 g).

Litopenaeus vannamei shrimp (alive), size 3 (30-40 individuals per kilogram) were bought at Hung Hoa hamlet, Tan Khanh Trung commune, Lap Vo district, Dong Thap province, Vietnam.

Glycerol 99.95%, polyethyleneglycol 6000 99.95% (Merk), sorbitol 99% (France).

2.2. Methods

2.2.1. Carrageenan extraction from Kappaphycus alvarezii alga [9]

The protocol for carrageenan extraction was refered from study of Vu Ngoc Boi *et al.* (2008) with some adjustment. 200 g of dried *Kappaphycus alvarezii* alga (calculated by dry matter) was soaked in 3% NaOH to remove color compounds and lipids with the ratio of material and NaOH 1/30 (w/v), 30 minutes. Then, it was neutralized with 1% HCl to pH 7, filtered the residue, dried and extracted in water with a substrate/solvent ratio of 1/40 (w/v). Next, carrageenan was extracted for 2 hours at 80 °C (once time). The hot extract was filtered in hot status, precipitated with 1% $CaCl_2$, frozen overnight, defrosted and dried the gel at 60 °C to gain carrageenan pieces. Finally, the carrageenan pieces were ground to obtain carrageenan powder with ivory white and uniform fineness. The recovery yield was calculated, and the carrageenan spectrum was determined by the FT-IR method.

2.2.2. Determination of carrageenan recovery yield [10]

Principle: the carrageenan sample was dried at 60 °C to completely dry, weighed and

determined the moisture content of the carrageenan sample obtained.

Procedure: The weight and moisture content of the material and the extract (from experiment 2.2.1) were determined. The recovery yield was calculated by the followed formula:

$$X = \frac{A \times (100 - W_2)}{P \times (100 - W_1)} \times 100\%$$

Where: A and P are the weight of carrageenan and material, respectively (g), W_1 and W_2: The moisture content of in material and in carrageenan, respectively (%).

2.2.3. Pre-treatment of shrimp samples

L. vannamei shrimp were alive. They were kept at 5 ºC for 30 minutes to kill shrimp simultaneously. Then, shrimp were peeled, resined well and weighted.

2.2.4. Investigation effects of carrageenan, glycerol, polyethylene glycol 6000 on the coating solution

A coating solution was prepared by dispersing glycerol (investigated at 0.025 g, 0.05 g, 0.075g, 0.1g, 0.125g), polyethylene glycol 6000 (investigated at 0.025 g, 0.05 g, 0.075 g, 0.1 g, 0.125 g), and carrageenan (investigated at 0.1 g, 0.2 g, 0.3 g, 0.4 g, 0.5 g) in distilled water with the ratio of substrate/solvent was 1/40 (w/v) at 80 °C for 30 minutes under magnetic stirring. The solution was cooled at room temperature. Then, shrimp were immersed into the carrageenan film-forming solution in 10 ± 1 minutes and checked the capacity of film-forming of the solution.

2.2.5. Investigation of dry matter loss during shrimp storage

A coating solution was prepared according to the results of experiment 2.3.4, and then cooling to room temperature. Besides 12 control shrimp samples, 12 samples were immersed in the above solution. Weekly, the samples were thawed, washed the coating solution, dried naturally (in a cool place for about 15±1 minutes) and weighed exactly the samples. The comparison between the control and samples in terms of dry matter of shrimp samples and the appearance was conducted. The experiment was repeated three times.

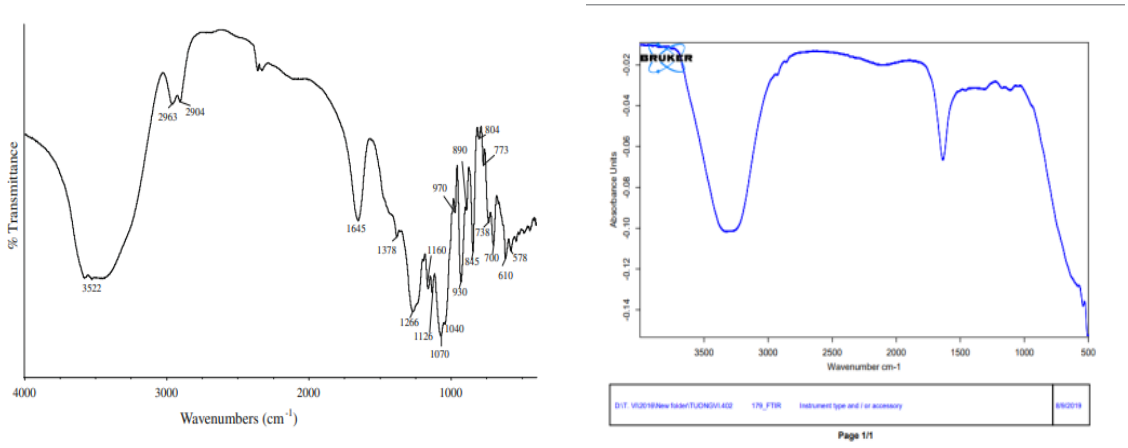
2.3. Statistical analysis

Statistical analysis of the experimental data was conducted using Microsoft Excel 2013. Results were expressed as means ± SD and statistical differences among experiments were compared by IBM SPSS Statistics 20. Differences between the experiments were considered significant when $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Carrageenan extraction from *Kappaphycus alvarezii* alga

The dried *Kappaphycus alvarezii* alga has been common on the market. The carrageenan was extracted as the protocol of section 2.2.1. As a result, the carrageenan powder was ivory white, homogeneous with a recovery yield of 65.96%. The spectroscopy of carrageenan via FT-IR method was shown in Figure 1.



(a) (b)

Figure 1. FT-IR spectroscopy of standard carrageenan (a),
FT-IR spectroscopy of carrageenan from *Kappaphycus alvarezii* alga (b)

There were similar in terms of wavelength absorption of major functional groups and bonds in the carrageenan molecule of the standard and sample. Thus, it can be confirmed that the extracts from *Kappaphycus alvarezii* alga contain carrageenan (Figure 1).

3.2. Effects of carrageenan, glycerol, polyethylene glycol on forming coating solution

3.2.1. Effects of carrageenan

The effects of carrageenan content on the dip-coating capacity of the research solution were shown in Table 1.

Table 1. The effects of carrageenan content on forming coating solution

No.	Carrageenan content (g)	Viscosity (cP)	Coating solution characteristics (room temperature)	Coated shrimp sample chacteristics
1	0.1	150.333 ± 0.58^{a}	The dilute, poorly coagulated, and un-adhesive solution	Shrimp samples have not been coated with the solution
2	0.2	178.000 ± 1.00^{b}	The slightly viscous, less stick solution	Shrimp samples were coated with a thin layer of the solution
3	0.3	268.667 ± 0.58^{c}	The solution was justified thick, well adhesive at room temperature as well as tightly linked, durable molecules	Shrimp samples were coated with a homogeneous solution
4	0.4	585.000 ± 1.00^{d}	The solution was slightly thick	Shrimp samples were coated with the less homogeneous solution
5	0.5	755.333 ± 0.58^{e}	The solution was too thick. It was required to heat for coating, so it could affect the product properties	Shrimp samples did not coat because of a too thick solution

Reported data were average values ± standard deviations. Data in the same columns with different superscripts were significantly different ($p < 0.05$).

As observed, in terms of 0.1 g carrageenan (experiment 1), 0.2 g carrageenan (experiment 2), the solution was diluted, easy to dip but the less and inhomogeneous remains on shrimp samples. The more carrageenan of 0.4 g (experiment 4), 0.5 g (experiment 5), the solution became too thick, not suitable for dipping. Compared to other experiments, the coating solution with 0.3 g carrageenan (experiment 3) had justified viscosity, homogeneous remains on shrimp samples. Thus, 0.3 g carrageenan was chosen for further experiments.

3.2.2. Effects of glycerol

The effects of glycerol content on the dip-coating capacity of the research solution were shown in Table 2.

Table 2. The effects of glycerol content on forming coating solution

No.	Carrageenan content (g)	Viscosity (cP)	Coating solution characteristics (room temperature)	Coated shrimp sample chacteristics
1	0.025	237.333 ± 0.58^{a}	The less thick, poorly coagulated, less adhesive solution	Shrimp samples were covered with a very thin and homogeneous layer of the solution
2	0.05	241.667 ± 0.58^{b}	The less thick, coagulated, and adhesive solution	Shrimp samples were covered with a thin and homogeneous layer of the solution
3	0.075	266.667 ± 0.58^{c}	The moderately thick, coagulated, and well-adhesive solution as well as well linked, durable molecules	Shrimp samples were covered with a homogeneous layer of the solution
4	0.1	265.000 ± 1.00^{c}	The moderately thick, coagulated, and well-adhesive solution as well as well linked, durable molecules	Shrimp samples were covered with a homogeneous layer of the solution
5	0.125	265.000 ± 1.00^{c}	The moderately thick, coagulated, and well-adhesive solution as well as well linked, durable molecules	Shrimp samples were covered with a homogeneous layer of the solution

Reported data were average values ± standard deviations. Data in the same columns with different superscripts were significantly different ($p < 0.05$).

The glycerol content has affected the viscosity of the solution (Table 2). The solution viscosity increased with the increase in the amount of glycerol from 0.025 g to 0.075 g. With the amount of glycerol 0.075 g, 0.1 g, 0.125 g, the coating solution was moderately thick, coagulated, and well-adhesive. This led to a moderate, homogeneous, highly adhesive coating layer on shrimp samples without melting. Thus, 0.075 g glycerol was the most appropriate because of saving materials and creating favorable conditions for dipping shrimp. This condition was chosen for further experiments.

3.2.3. Effects of polyethylene glycol 6000

The effects of polyethylene glycol 6000 content on the dip-coating capacity of the research solution were shown in Table 3.

Table 3. The effects of polyethylene glycol 6000 on forming coating solution

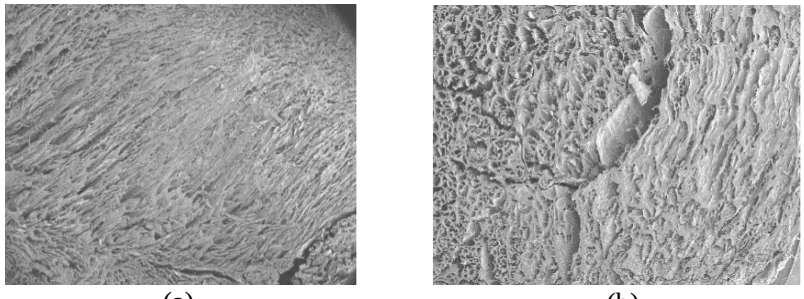
No.	Carrageenan content (g)	Viscosity (cP)	Coating solution characteristics (room temperature)	Coated shrimp sample chacteristics
1	0.025	226.333 ± 0.58^{a}	The dilute, poorly coagulated, less-adhesive solution	Shrimp samples were coated with a very thin layer of the solution
2	0.05	249.333 ± 0.58^{b}	The dilute, poorly coagulated, and un-adhesive solution	Shrimp samples were coated with a very thin layer of the solution
3	0.075	268.667 ± 0.58^{d}	The moderately thick, and well-adhesive solution as well as well linked, durable molecules	Shrimp samples were coated with a homogeneous layer of the solution
4	0.1	266.333 ± 0.58^{c}	The moderately thick, and well-adhesive solution as well as well linked, durable molecules	Shrimp samples were covered with a homogeneous layer of the solution
5	0.125	266.667 ± 0.58^{c}	The quite thick solution	Shrimp samples were coated with a homogeneous, quite thick layer of the solution

Reported data were average values ± standard deviations. Data in the same columns with different superscripts were significantly different ($p < 0.05$).

The polyethylene glycol 6000 content has affected the viscosity of the solution (Table 3). The solution viscosity increased with the increase of the amount of polyethylene glycol 6000 from 0.025 g to 0.075 g. With the amount of glycerol 0.075 g, 0.1 g, 0.125 g, the coating solution was moderately thick, coagulated, and well-adhesive. This led to a moderate, homogeneous, highly adhesive coating layer on shrimp samples without melting. Thus, 0.075 g polyethylene glycol 6000 was the most appropriate because of saving materials and creating favorable conditions for dipping shrimp. The condition was chosen for further experiments.

3.3. Scanning electron microscopy (SEM)

From the observation, the carrageenan restricted the growth of ice-crystal in shrimp muscle tissue. The control and shrimp with coating carrageenan were scanned electron microscopy (Center for German-Vietnamese Technology Academy, Ho Chi Minh University of Food Industry). The technological conditions of SEO 3.0 kW, WO 10.3 mm, Std. PC 30.0, magnification of 500 μm. The results were shown in Figure 2.



(a) (b)

Figure 2. The shrimp muscle tissue of the samples (a), and shrimp control stored for 12 weeks (b)

3.4. Dry matter loss during shrimp storage

The loss of dry matter was investigated for 12 weeks. Weekly, the shrimp control and the samples were weighted, and the results were shown in Table 4.

Table 4. The loss of dry matter of shrimp during storage

Time (weeks)	Shrimp samples		Control	
	Dry matter (g)	% loss of weight	Dry matter (g)	% loss of weight
1	14.465 ± 0.090^{ns}	0.000 ± 0.000^{a}	14.705 ± 0.085^{a}	0.000 ± 0.000^{i}
2	14.458 ± 0.090^{ns}	0.045 ± 0.001^{b}	14.656 ± 0.084^{b}	0.334 ± 0.001^{hi}
3	14.455 ± 0.089^{ns}	0.070 ± 0.001^{c}	14.575 ± 0.084^{c}	0.883 ± 0.001^{ghi}
4	14.452 ± 0.089^{ns}	0.088 ± 0.001^{d}	14.462 ± 0.083^{d}	1.650 ± 0.001^{gh}
5	14.449 ± 0.090^{ns}	0.108 ± 0.001^{e}	14.377 ± 0.083^{e}	2.230 ± 0.001^{fg}
6	14.445 ± 0.089^{ns}	0.135 ± 0.001^{f}	14.201 ± 0.082^{f}	3.421 ± 0.001^{f}
7	14.434 ± 0.089^{ns}	0.209 ± 0.001^{g}	13.962 ± 0.080^{g}	5.051 ± 0.001^{e}
8	14.427 ± 0.089^{ns}	0.262 ± 0.001^{h}	13.760 ± 0.079^{h}	6.421 ± 0.001^{de}
9	14.405 ± 0.090^{ns}	0.415 ± 0.002^{i}	13.650 ± 0.079^{i}	7.173 ± 0.001^{cd}
10	14.376 ± 0.089^{ns}	0.614 ± 0.002^{j}	13.519 ± 0.078^{j}	8.062 ± 0.001^{bc}
11	14.351 ± 0.089^{ns}	0.783 ± 0.001^{k}	13.381 ± 0.077^{k}	9.001 ± 0.001^{ab}
12	14.318 ± 0.089^{ns}	1.013 ± 0.001^{l}	13.227 ± 0.076^{l}	10.047 ± 0.001^{a}

Reported data were average values ± standard deviations. Data in the same columns with different superscripts were significantly different ($p < 0.05$).

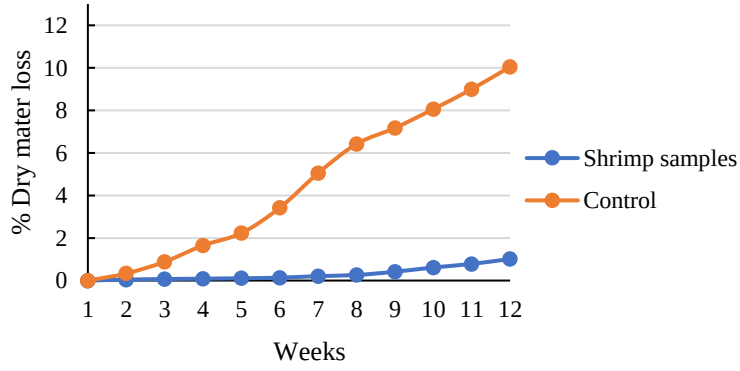


Figure 3. The loss of dry matter of shrimp during storage

It was found that shrimp samples with coating solution experienced the negligible loss of weight for the first 8 weeks (0.262%) while it was a significant loss (0.334%) of the control in the second week. By 12th week, the loss of weight of shrimp samples was 1.013%, whereas the control was 10 times higher than the samples. Thus, dipping in a coating solution helps the shrimp samples to reduce dry matter loss during cryopreservation. In detail, the shrimp samples maintained the initial content for more than 5 weeks in comparison with the control.

This leads to a significant change in the amount of control and the samples during 12 research weeks. The characteristics and appearance of samples were tested and calculated the mass loss. The results showed that the coating shrimp samples were cited as less mass loss. On the contrary, the control was muscle softness and significant loss of dry matter of 0.334% and 6.421% after 2 and 8 weeks, respectively. So, the shelf life of the control was a maximum of 8 weeks.

The coating solution of carrageenan, glycerol and polyethylene glycol 6000 could inhibit the growth of ice crystals in peeled shrimp. The tissue microstructure demonstrated that the shrimp muscle tissue of the samples appeared more rigid and has a smoother physical appearance compared to the samples. This means that the coating solution help to reduce significantly the damage of large ice crystals, resulting in a decrease of dry matter loss and quality maintenance.

4. CONCLUSION

The carrageenan extraction conditions were found, namely distilled water solvent, the ratio of material and solvent 1/40 (w/v), temperature 80 °C, in 2 hours. The solution 1% $CaCl_2$ 1% was used for precipitating, and the recovery yield was 65.96%. The present study has demonstrated that shrimp samples immersed in coating solution (a mixture of carrageenan, glycerol and polyethylene glycol 6000) could prohibit the growth of ice crystals, thus muscle tissue would be protected from damage. This is one of the initial researches to exploit and create a coating solution for shrimp preservation in freezing, which is aimed at the great economic and ecological value of marine resources.

Acknowledgment: This work was funded by Ho Chi Minh City University of Food Industry (Contract No.137 QD/DCT dated November 7, 2018).

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TÓM TẮT

NGHIÊN CỨU ỨNG DỤNG CARRAGEENAN THU NHẬN TỪ RONG SỤN ĐỂ GIẢM SỰ HAO HỤT CHẤT KHÔ TRONG QUÁ TRÌNH BẢO QUẢN TÔM LẠNH ĐÔNG

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Nghiên cứu này thực hiện trích ly carrageenan từ rong sụn (*Kappaphycus alvarezii*) và ứng dụng trong bảo quản tôm nhằm giảm sự hao hụt chất khô trong quá trình bảo quản tôm lạnh đông. Rong sụn sử dụng trong nghiên cứu này là loại rong khô. Sử dụng NaOH 3% để loại hợp chất màu và lipid. Carrageenan từ rong sụn thu được bằng phương pháp trích ly với dung môi nước cất, tỷ lệ nguyên liệu/dung môi 1/40 (w/v), nhiệt độ trích ly 80 °C, thời gian trích ly 2 giờ và số lần trích ly là 1, tủa dung dịch thu được bằng $CaCl_2$ 1%. Quy trình sản xuất carrageenan bán tinh chế thu được carrageenan dạng bột, màu trắng ngà, độ mịn đồng nhất, hiệu suất thu hồi carragenaan đạt 65,96%. Dung dịch bảo quản tôm là hỗn hợp gồm 0,3 g carrageenan, 0,075 g glycerol, 0,075 g polyethylene glycol 6000, tỷ lệ hỗn hợp/ dung môi nước cất là 1/40. Hỗn hợp được khuấy từ gia nhiệt ở 80 °C trong 30 phút, để nguội và tôm được nhúng vào dung dịch trước khi cấp đông, mẫu tôm được cấp đông để khảo sát sự hao hụt chất khô giữa mẫu đối chứng và mẫu có nhúng dung dịch bảo quản trong 12 tuần.

Từ khóa: Bảo quản tôm, carrageenan, chất khô, rong sụn.