THE USE OF CHITOSAN AS BIOADHESIVE AND ITS PROPERTY IMPROVEMENT BY IRRADIATION FOR WATER-STABLE SHRIMP FEED PRODUCTION

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SUMMARY

Chitosan with small content in the feed (0.48 - 0.75%) could be selected to prepare shrimp feed-pellet having so high water-stability that met the Standard of Vietnam Ministry of Fisheries 28-TCN 102/1997. The radiation treatment at sterilization doses (20 - 30 kGy) was evaluated as the most practical technology because irradiated chitosan with reduced content of 0.34% has capacity to be prepared feed-pellets as stable as comparable to imported products. The results from feeding trials showed that the chitosan-containing feed did not affect the growth response and feed utilization efficiency such as weight gain (WG), feed conversion ratio (FCR) and productivity at harvest.

I - INTRODUCTION

For sustainable development in shrimp culture, one of the most important strategies is exchanging of extensive farms, which is popular mode at present to semi-intensive and extensive ones. The high quality feed is a very important factor in shrimp culture because it is required to meet the increased demand from farmers on quantity and nutritive quality for minimizing feed losses and for avoiding the over-feeding problem. In other side, the industrially produced feed with high water-stability also greatly contributes in reduction of pond pollution, which is one main reason for shrimp diseases [1 - 3]. The imported feeds maintain the water-stable structure in 6 - 8 hrs, while most of domestic shrimp feed from small-scale enterprises has been required only 2 hrs as based on the Standard of Ministry of Fisheries 28-TCN 102 [4].

The aim of the present study was to investigate for selecting the locally available adhesive from marine carbohydrates for shrimp feed production. Quality of adhesives was evaluated in terms of their ability for making feed pellet stable in water to meet the national and regional standards. Gamma irradiation has been used as a method contributing to reduce adhesive content in feed materials for enhancement of economic application.

II - MATERIAL AND METHOD

1. Marine polysaccharides and radiation treatment

Alginate sodium was obtained from Sigma Chemical Company. Carrageenan (type WG-115) was a product of Genugel Carrageenan, Denmark. Chitosan was provided from Institute of Chemistry, Vietnam. To use biopolymer as adhesive for feed preparation, each of selected polymers was mixed directly with feed ingredients or it was dissolved in suitable solvent before mixing. Domestic chitosan with deacetylation degree (DDA) of about 90% and viscosity-average molecular weight ($\overline{M_v}$) of 552,000 was used without further purification and was gamma-irradiated in solid state. The radiation treatment was undertaken at dose of 20, 40, 60, 75, 100, 150, and 200 kGy with dose rate of 10 kGy/h in Takasaki Radiation Chemistry Research Establishment, Japan. The $\overline{M_v}$ was calculated using Mark-Houwink equation relating to intrinsic viscosity: $[\eta] = K_m M_w^a$ where $K_m = 1.81 \times 10^{-3}$ cm^3/g and a = 0.93 [5].

2. Shrimp feed-pellet preparation

The preparation of experimental diet for its water-stability evaluation was done as those reported in detail by Thoa et al. [6]. The diets were prepared by thoroughly mixing the dry ingredients with adhesive then adding water until the whole mixture reached moisture 40-45%. The dough was pelletized through a 2-mm die, and then dried in an oven overnight at 75^{0}C. Other procedure was also utilized for feed preparation, in which the adhesive firstly has been dissolved in water or suitable solvent, and then the received solution was taken to moisturize the diet mixture. The pellet water-stability was evaluated according to the Standard 28-TCN 102 [4].

Diets for feeding trials using indoor tanks were prepared as follows: Two commercial feeds from CP company (Thailand) and KP-90 (Vietnam) were used without additional treatment for feeding control shrimps. Test diets were prepared by using two mentioned commercial feeds as the initial material for nutritive evaluation of chitosan adhesive. Commercial pellets were finely ground and passed through a 0.5-mm sieve, then well mixed with solution of irradiated chitosan to get suitable moisture. After peletizing, feed was dried overnight at 75^{o}C. By this preparation, the test feed was available from CP and KP-90, respectively. Diets for feeding trials using pond shrimp-culture were ordered for Halong canned Food Company Ltd. (Haiphong, Vietnam). The company's commercial shrimp feed (HCFC) was used as control and chitosan-added HCFC was the test feed.

3. Feeding trial for nutritive evaluation

Feeding trials using indoor tanks: Twenty *Penaeus monodon* fabrius shrimps per tank (two tanks per treatment) were randomly distributed in 2 m^3 circular plastic composite tanks (water depth of 1 m) equipped with a system supplying air and brackish water. The water temperature, pH and dissolved oxygen during 60-day culture period varied from 27 to 29^{0}C, 7.2 to 7.8, and 5.8 to 7.7 mg/l, respectively. All shrimps in each tank were initially fed 8% of total body weight daily. Shrimp with mean initial weight 5.54 ± 0.42 g were fed the experimental feed for 60 days three times per day. Every ten days shrimp from each tank were weighed and measured to evaluate the growth.

Feeding trials using pond shrimp-culture: The experiment for evaluation of feed-pellets and adhesive quality was implemented using 4 earthen ponds with 800 m^2 per each (2 ponds per treatment). Density at the release was 8 shrimps/m^2 with shrimp size P45. The control shrimps fed commercial feed obtained from Halong Can Food Company Ltd. (HCFC). The test shrimps fed the feed that produced by HCFC with the same materials supplemented with 0.75% (w/w) irradiated chitosan.

III - RESULTS AND DISCUSSION

1. Selection of suitable polysaccharide as bioadhesive for water-stable feed production

Sodium alginate and carrageenan in powder were used as bioadhesive at content of 2-5% to prepare feed pellets. These polysaccharides were added to the feed material in two ways, e.g. in powder by well mixing with feed ingredients before water added, and in liquid state by polymer solution. All of received feed dissolved quickly in water after several minutes only. Thus, alginate and carrageenan can not be used as adhesive for producing the water-soluble feed.

In contrast to alginate and carrageenan, chitosan provided high water-stability when it was added to the feed in solution, even at low chitosan content of 0.5%. Content of 0.5% made feed pellets water-stable exceeding the Standard 28 TCN 102, and content of 0.75% chitosan provided the feed meets the parameter equal to the regional standard. Hence, among marine polysaccharides, only chitosan was selected due to its suitable adhesive properties such as its low content in the feed is required and its raw main resource for extraction is available in shrimp shell.

2. Effect of radiation treatment on chitosan

a) Improvement of solubility of chitosan in acid solvents by irradiation treatment

To dissolve chitosan for food/feed preparation the organic acids, especially acetic acid is commonly used. However, the content and taste of the selected acid can affect the palatability of the animals, so increasing of chitosan solubility with reduction of acid content could be useful in some chitosan utilization including the supplement to shrimp feed. In this part the potential of radiation treatment was investigated to clarify how much its effectiveness to increase chitosan solubility. Results of the solubility in radiation dose dependence treating in solid state are shown in Fig 1. The solubility time of 1% (w/v) chitosan in acetic and oxalic acid reduced quickly in a range from 10-75 kGy. Solubility time of original chitosan in acetic acid, for example, from 80 min was reduced to 38 min by 60 kGy-irradiation. The time reduction expressed at lower rate with increasing radiation doses from 75 kGy to 200 kGy.

Table 1: Effect of radiation treatment on solubility of chitosan at different concentrations using 0.125 M (0.65% w/v) acetic acid as solvent

CTS % (w/v)	Unirradiated		Irradiated at 60kGy	
	Solubility time, min	pH	Solubility time, min	pH
1	80	4.45	39	4.43
2	111	5.32	64	5.22
3	158	5.81	85	5.66
4	-	-	153	6.06

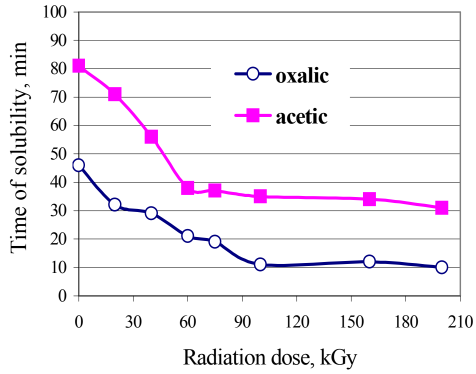

Fig 1: Effect of irradiation on the solubility of chitosan in acetic and oxalic acid

In laboratory practice, chitosan solution at high concentration can be prepared by using very high acid content. However, 10% chitosan in 5% acetic acid probably is the most optimal way according to our experience. From this stock chitosan paste, 1% chitosan solution in 0.5% acetic acid can be received by water dilution. This technique can not be used to prepare solution of 1% chitosan in acetic acid with concentration less than 0.5%. For this reason, we used 0.125M (0.65%) acetic acid to prepare solution chitosan at 1, 2, 3 and 4%. Two chitosan samples of unirradiated and irradiated at 60 kGy were taken to experiment to compare the effectiveness of radiation treatment on preparation of 1% chitosan in acetic acid with lower than 0.5% concentration. Table 1 showed that it takes 158 min to prepare a 3% chitosan solution in 0.65% acetic acid. But it takes very long time for a 4% chitosan solution in the same condition. It was required shorter time (85 min) for completing 3% 60 kGy-irradiated chitosan and not so difficult to prepare a 4% (153 min). From the latter solution, the 1% chitosan can be received by water dilution and its acid concentration was 0.16% only. Thus, radiation treatment can be use to degrade chitosan making it easier to dissolve in diluted acid, by which no side-effect can be received from acid content and taste.

b) Change in viscosity and molecular weight of chitosan by radiation treatment

A Brookfield viscometer (Model DV-II)

was used for viscosity measurement. Solid-state radiation treatment was undertaken at dose of 20, 40, 60, 75, 100, 150, and 200 kGy with dose rate of 10 kGy/h. The result from investigating the radiation dose dependence in viscosity of 0.75% chitosan solutions in 0.0625M acetic acid was shown in Fig 2. The change tendency has a correspondence to the reduction of solubility time with increasing radiation dose. The viscosity was decreased quickly at the doses lower than 100 kGy, after that its decrease was slow.

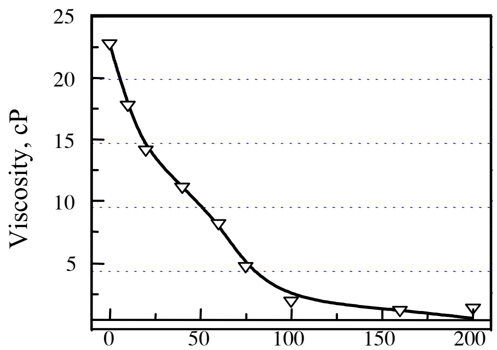

Fig 2: Change in viscosity of chitosan solution by solid state radiation treatment

The $\overline{M_v}$ was measured by Ubbelohde viscometer using 0.1M CH_3COOH/0.2M NaCl as solvent and calculated using Mark-Houwink equation relating to intrinsic viscosity: $[\eta] = K_m M_w^a$, where $K_m = 1.81 \times 10^{-3}$ cm^3/g and a = 0.93 at 25^0C. This solvent system was recommended to measure intrinsic viscosity avoiding chitosan with high DDA from the aggregation [5]. Fig 3 showed the $\overline{M_v}$ of chitosan in dependence on the radiation dose. The $\overline{M_v}$ sharply decreased in a dose range up to 100 kGy, then slowly to 200 kGy. The polysaccharides including chitosan are typical degradable materials due to ionizing radiation. The effect of irradiation on chitosan has been reported earlier with the break of glycosidic linkage to produce low molecular-weight fragments [7]. Our result was very well in agreement with the referential data. The original chitosan has $\overline{M_v}$ = 552,000. It was reduced to *ca.* 300,000 and 200,000 when was irradiated at 25 and 50 kGy, respectively. Increasing of absorbed doses to those of higher than 150 kGy did not reduced more significantly the molecular weight.

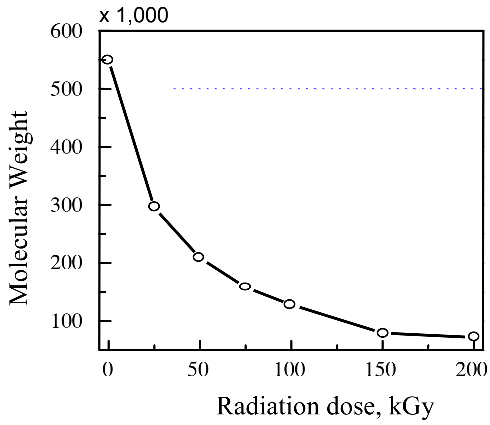

Fig 3: Change in $\overline{M_v}$ of chitosan by solid state irradiation

c) Improvement of water-stability of feed by using irradiated chitosan as bioadhesive

The water-stability of feed pellets using chitosan that has been irradiated in solid state at different doses is shown in table 2. All of chitosan solutions were prepared with the same concentration of 0.75% in 0.0635M acetic acid. Each solution then was used to moisturize feed material to get chitosan content 0.48% of feed. Three other samples were used as the control ones; they were the unirradiated chitosan, carboxymethylcellulose (CMC), and sample without adhesive addition. The result showed that the radiation treatment clearly increased the water-stability of feed pellets. In addition, the activity was increasing as observed with increase of radiation dose. Dose of 20 kGy could modify chitosan into the six hours water-stable feed, which is corresponded to that of imported ones. Dose of 60 kGy and higher showed a very high water-stability which may cause hard feed, so it is no need to irradiate chitosan at dose higher than 40 kGy because high dose requires high cost and time of irradiation. For these reasons, 20 - 30 kGy as known as sterilization dose, can be recommended to degrade chitosan for adhesive property enhancement.

The received results also showed that to reach 5 hours of water-stability, content of 0.45% is the lowest required for unirradiated chitosan, while only 0.34 - 0.38% of 20 kGy-irradiated chitosan can be used for 6 - 7 hr-water-stability. Thus, radiation treatment of chitosan not only increases the water-stability of feed pellets, but also reduces chitosan content. When chitosan is irradiated at the sterilization dose, content 0.34% is evaluated as optimal lowest level giving the feed water-stability reaching the regional level.

The molecular weight of chitosan is a very important property because a minimum molecular weight is most often needed to achieve desired properties. Radiation treatment has potential to degrade chitosan polymers by breaking them into low molecular fraction [7]. Their solution also become lower viscosity, and therefore easy to flow into the crevices and asperities found in solid surfaces of material like feed particles [8].

Table 2: Influence of irradiated chitosan on the water-stability of shrimp feed pellets

No.	Treatment	Water-stability, hrs	Level of standard
1	Unirrad. Chitosan	4	> VNS (VN Standard)
2	10 kGy	5	> VNS
3	20 kGy	6	RS (Regional Standard)
4	40 kGy	7	RS
5	60 kGy	8	RS
6	100 kGy	> 8	RS
7	No adhesive added	0.5	< VNS
8	CMC 2%	1	< VNS

Radiation treatment in solid state, chitosan content in feed = 4.8/1000 (w/w)

3. Feeding trial for evaluating nutritive quality by shrimp-culture experiment

The growth response and feed utilization efficiency of feeding trial using in-house tanks are presented in table 3. CP- an imported diet produced greater weight-gain than that of domestic KP90. The CP diets also produced better feed conversion ratios (FCR) compared to the KP90 ones. The shrimp survival remained 100% at both of diet throughout the 40-day period. The total feed intake reversibly reflected the weight gain. The feed intake for shrimp fed CP diets was lower than those of shrimp fed KP90 diets. The growth, FCR and total feed intake were not significantly different from the shrimp fed diet with no added chitosan and diets containing chitosan.

Table 4 shows result of the pilot-scale shrimp-culture experiment that was carried out using four 800 m^2 earthen ponds to evaluate two kinds of feed: HCFC (without chitosan) and HCFC+CTS (contained 0.5% chitosan). The difference in the average weight at harvest between the shrimp fed diet containing chitosan (HCFC+CTS) and shrimp fed HCFC was observed (28.7 and 25.5 g/shrimp, respectively). The productivity at harvest of shrimp also was different that reflecting the higher weigh gain of shrimp fed diet which containing chitosan. The FCR was 2.5 and 2.4 respectively. This proved that chitosan as a bioadhesive causing no side effect on the normal growth of shrimp culture. The chitosan composition also did not affect the feed palatability of animals.

Table 3: Results of the 40-day feeding trial on tank-scale for *P. monodon* fed CP and KP90 diets containing 0.5% irradiated chitosan

Index	Diet			
	CP	CP + CTS	KP90	KP90+CTS
Initial weight (g)	5.4	5.7	5.6	5.6
Final weight (g)	16.5	16.7	15.8	15.7
Final weight gain (%)	205.6^{b}	193.0^{b}	182.1^{a}	180.4^{a}
Feed conversion rate	2.1^{b}	2.2^{b}	2.6^{a}	2.5^{a}
Survival (%)	100	100	100	100
Total feed intake (g/shrimp)	23.3^{b}	22.1^{b}	26.8^{a}	25.1^{a}

Mean within the same row having different superscript is significantly different (P < 0.05).
Weight gain (%) = final weight-initial weight / initial weight × 100.

Table 4: Results of the 50-day feeding trial on pond-scale for *P. monodon* fed HCFC diets containing 0.5% irradiated chitosan

Index	Diet	
	HCFC without chitosan	HCFC with chitosan
Initial weight (g)	5.7	5.6
Final weight (g)	25.7	27.7
Final weight gain (%)	350.9^{b}	394.6^{a}
Feed conversion rate	2.5^{a}	2.4^{a}
Dry matter feed intake (g/shrimp)	50.3^{a}	52.2^{a}
Productivity (kg/ha)	1450^{b}	1520^{a}

IV - CONCLUSION

By experiment on various marine polysaccharides, we have selected chitosan to be used as a bioadhesive for recycling in the production of water-stable shrimp feed-pellets. The shrimp feed containing *ca*. 0.5% chitosan could be met the Standard of Ministry of Fisheries in water-stability, while feed containing 0.75% provides the stability corresponding to that of imported feed. The solid-state radiation treatment at sterilization dose (20 - 30 kGy) markedly increases their adhesive property. The radiation treatment of chitosan not only increases the water-stability of feed pellets, but also reduces the content of feed chitosan. When chitosan is irradiated at the sterilization dose, its content of 0.34% is evaluated as the optimal lowest content giving the water-stable feed reaching the regional criteria.

The use of liquid adhesive to moisturize feed materials is evaluated as an improved procedure since feed becomes higher water-stability at lower content of added adhesive. Chitosan adhesive does not influence the growth and palatability during shrimp culture.

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