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Research on application of bromelain and alcalase for production of hydrolyzed powder from round scads (*Decaterus punctatus*)

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ABSTRACT

Round scad (*Decaterus punctatus*) is a species with high nutritional value and is used mainly as food for humans, and partly as food for aquatic animals. There are no processed products from scad fish in our country; thus, the value of scad fish products still needs to be higher. In this report, we have established the hydrolysis procedure by the enzyme mixture of bromelain and alcalase with the following conditions: the enzyme alcalase/bromelain ratio of 1/3; the enzyme and substrate ratio of 1%; hydrolysis temperature at 50oC; nature pH of fish; the incubated time of 6 h; the speed of 200 rpm; The hydrolyzed powder product is rich in protein (74.5%), and minerals (calcium, potassium, phosphorus, copper), thus has many potential applications in functional foods.

Keywords: Hydrolyzed powder, round scad, *Decaterus punctatus*, enzyme technology.

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INTRODUCTION

Round scad (scientific name: *Decapterus punctatus*) is a species of marine fish in the genus scad, widely distributed in the Pacific and Atlantic oceans. Round scad fish have few bones, lots of meat, fragrant meat, tastier than other scad species, and the fish meat is sweet and fatty. Round scads are often caught in the waters of the Gulf of Tonkin, the central coast (especially Quang Ngai), and the eastern and western waters of the South. Some studies on the chemical composition of scads show that in 100 g of fish, there are 20.2 g protein, 3.3 g fat, 85 mg Calcium, 160 mg Phosphorus, and 76.3 g water. The lipid composition of scad fish includes saturated fatty acids (SFA, 861.11 mg/100 g sample), monounsaturated fatty acids (MUFA 452.10 mg/100 g sample), and polyunsaturated fatty acids (PUFA). 977.64 mg/100 g sample). The composition of PUFA fatty acids is quite diverse, of which the two dominant types are docosahexaenoic acid (DHA 384.30 mg/100 g sample) and eicosapentaenoic acid (EPA 148.84 mg/100 g fresh sample). Each 100 g of scad will provide about 110 kcal, a relatively low-calorie level, so it is quite suitable for people who want to lose weight [1–3].

Due to the enzyme system available from the microorganisms living in the fish intestine, fish proteins will easily be hydrolyzed into amino acids and peptides, and the addition of external enzymes helps accelerate the hydrolysis of fish proteins. Research results on the use of enzymes in hydrolysis by domestic and foreign authors show that protease is the enzyme most used and gives the best hydrolysis results. This enzyme can be extracted from many different types of microorganisms, such as the filamentous fungus *Aspergillus oryzae* (Flavourzyme), or the bacterium *Bacillus licheniformis* (Alcalase). The addition of proteolytic enzymes can make the hydrolysis process more controllable. Alcalase - an endo-protease produced from *Bacillus licheniformis* is one of the best enzymes to prepare fish protein hydrolysates [4–8]. *Bromelain* is an exo-protease isolated from pineapple (extracted from the stem and fruit) that has the function of decomposing proteins, supporting the protein digestion process in the body. The proteolytic activity of bromelain is ten times higher than the activity of papain - an enzyme extracted from papaya.

In addition, using enzyme technology in scad processing in this study aims not use chemical solvents but to be safe for human health. Products manufactured using environmentally friendly processes are a priority research direction in technology solutions in the world today. In addition, using membrane filtration techniques helps isolate oligo peptides of different sizes, which have higher biological activity than directly using finished scad to create the product. Functional foods containing peptides and minerals with anti-osteoporosis activity are superior to current domestically processed products and are equivalent to imported products of the same type.

MATERIALS AND METHODS

Materials

Round scad

Round scad (*Decapterus punctatus*) is caught in the North Central Sea (Thanh Hoa). The fish has an average length of 40 cm and weighs about 300 g.

Enzyme bromelain and alcalase

Bromelain is provided by Biogreen, Vietnam with an activity of 2,000 IU/g.

Alcalase provided by Novozyme of Denmark has an activity of 2.4 AU/g.

Experimental setup

The procedure of hydrolyzing round scad using the enzyme system: bromelain and alcalase is designed with experimental steps shown in Figure 1.

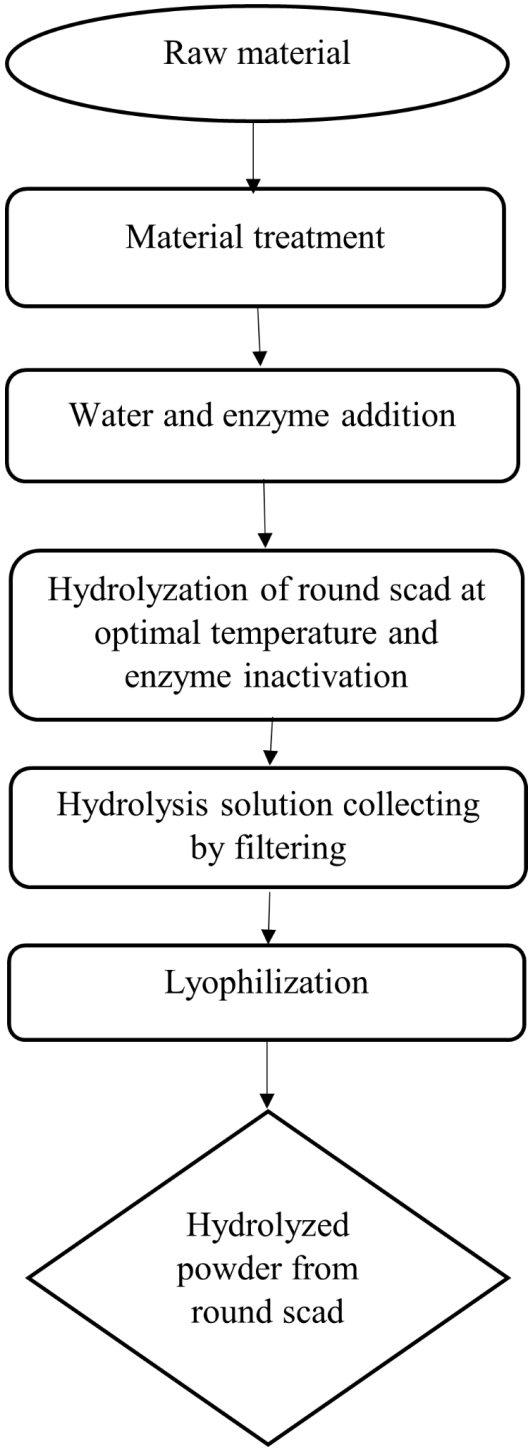

Figure 1. Schematic diagram of round scad hydrolyzing with enzyme

One factor was changed while maintaining other until almost all the main effect factors of the hydrolysis process were examined to identify optimal conditions. Table 1 shows a schematic of experimental arrangement.

Table 1. Arrangement of experiments protocol optimization

Purpose of experiment	Changeable factor	Stable factor
Experiment 1: the effect of Alcalase/Bromelain ratio (A/B ratio) determination	A/B ratio: 1:1 - 1:5 (g/g)	E/S ratio: 0,5% Material/water: 1:1 (g/mL) Temperature 50°C Time 5 h
Experiment 2: the effect of enzyme/material ratio (E/S) determination	E/S ratio (g/100 g): 0–1.5%	A/B ratio: was optimized at experiment 1 Material/water: 1:1 (g/mL) Temperature 50°C Time 5 h
Experiment 3: the effect of material/water ratio determination	Material/water ratio: 1:0.25 - 1:1.5 (g/mL)	A/B ratio: was optimized at experiment 1 E/S ratio: was optimized at experiment 2 Temperature 50°C Time 5 h
Experiment 4: the effect of temperature determination	Temperature 40–60°C	A/B ratio: was optimized at experiment 1 E/S ratio: was optimized at experiment 2 Material/water: was optimized at experiment 3 Time 5 h
Experiment 5: the effect of time determination	Time 0–8 h	A/B ratio: was optimized at experiment 1 E/S ratio: was optimized at experiment 2 Material/water: was optimized at experiment 3 Temperature: Material/water: was optimized at experiment 4

Methods

The degree of hydrolysis determination (OPA method using o-phthaldialdehyde) [9]

Regarding protein hydrolysis, the degree of hydrolysis (DH) was identified by the OPA method using an L-glutathione calibration curve (0.15–1.25 mg/mL).

Experiment: 50 µL of each standard or analyzed sample was taken and put into the test tube. 2 mL o-phthaldialdehyde (OPA 0.8 mg/mL) was added, mixed, and left in 2 minutes exactly. OD absorbance was measured at 340 nm by UV-V is multimode microplate reader (UV-1650PC, Shimadu, Japan). Calibration curve is shown in Figure 2 and Table 2.

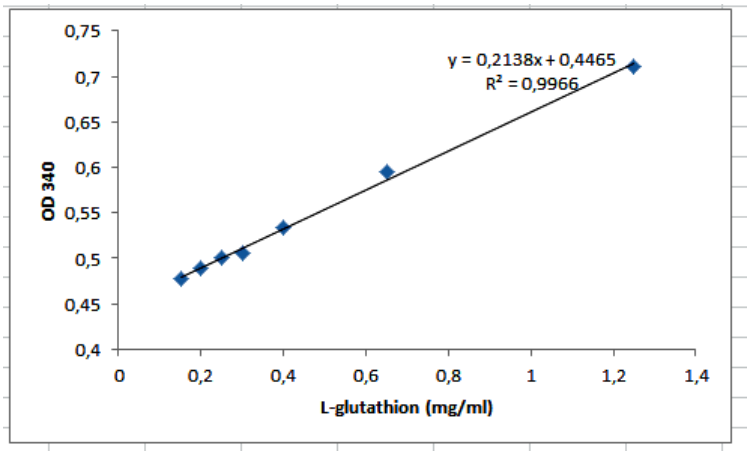

Figure 2. L-glutathione calibration curve

Table 2. Absorbance of L-glutathione at different concentrations

L-glutathion (mg/mL)	0,15	0,2	0,25	0,3	0,4	0,65	1,25
OD (340 nm)	0,477	0,489	0,501	0,505	0,533	0,595	0,710

Calculation:

Degree of hydrolysis *DH* was calculated by formula: $DH = (h/h_{tot})*100\%$

h (mol/kg) is the mol value of primary *α*-amino released during hydrolysis of 1 kg of protein in raw material. h_{tot} (mol/kg) is the maximum mol value of primary α-amino that was released during complete hydrolysis of 1 kg of protein in raw material. h was calibrated from h', which is the result that identified the content of primary α-amino: $h = (h'/\alpha) - \beta$. Regarding seafood protein source, $\alpha = 1$, $\beta = 0,4$ [9].

Total lipid content identification: according to the method of Bligh and Dyer, 1959 [10]

The total lipid extraction method was processed following the modified method of Bligh and Dyer. Samples (10 g) were crushed and added to 30 mL methanol-chloroform (MeOH–CHCl3; 2:1 v/v). Then, the mixture was continuously supersonicated for 30 minutes. After filtration, the solution was added to 8 mL water (H_2O), shaked strongly, and moved to the separating funnel. Total lipid was dissolved in $CHCl_3$ and preserved at -5°C. Total lipid content was examined as a percent ratio of the weight of collected lipids on the weight of the sample.

Determination of soluble protein content: according to Bradford method [11].

Bradford's method was based on the ability to form complexes of Coomassie Blue G250 with protein. The standard reagent was albumin BSA at concentration from 0,05 to 5 (mg/mL). Standard line equation: $y = 0.0001x + 0.2457$ ($R^2 = 0,9926$). Bradford reagents included 20 mg of G250, 50 mL of ethanol, and 100 mL H_3PO_4 85% in total 100 L. An amount of sample (1 mL) was added to 4 mL of reagent, incubated in the dark for about 20 minutes, and then the absorbance was measured at 595 nm using a spectrophotometer. Based on the linear equation between protein concentration and optical density OD at A595, our study can calculate the protein content in the sample.

Axit amin analysis was carried out by HPLC [12]

50 g of sample was hydrolyzed with HCl at 110°C for 16 h. Then, the hydrolyzate was recovered and filtered through a 0.45 µm Whatman membrane before analysis. Amino acid analysis was conducted on an Agilent 1200 HPLC with dual pump G1311A and detector G1315B Diode Array Detector (DAD), 10 mm chamber, wavelength 338 nm.

Trace elements and heavy metals determination: according to the ICP-OES method [13]

The determined content of heavy metals consisted of lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg) by atomic absorption spectroscopy (AAS). The equipment used and accompanying parts including Thermo Elemental-Model Solaar M6 Dual Zeeman Atomic Absorption Spectrometer come with VP 90-hydride generation hydride generation system, hollow cathode lamp and HCl data Coded.

Each experiment was performed in parallel, with three samples each time. Data were processed and calculated on Microsoft Office Excel software ($p < 0.05$ is considered statistically significant).

RESULTS AND DISCUSSION

Nutritional composition of round scads

The results of analyzing the nutritional composition of round scads in Tables 3, 4 show that round scads have a relatively high percentage of meat (60.1%); Total protein content (19.2%) is equivalent to that of some marine fish species such as scads (18.2%), squid

(17–21%), much higher than some other aquatic species such as oysters (8–9%), moisture (13–16%), snails (11–12%) and crabs (6–7%); low lipid content (3.6%), favorable for the hydrolysis protein [14–18].

Table 3. Body parts of round scads

Fish meat	Head	Bone	Fin	Viscera
60.1 ± 0.1	15.8 ± 0.2	12.1 ± 0.3	5.3 ± 0.2	6.7 ± 0.1

Table 4. Nutritional composition of round scads

Nutritious composition	Content (percent ratio compared to wet weight)
Water	70.5 ± 0.2
Protein	19.2 ± 0.1
Lipid	3.6 ± 0.3
Ashes	3.4 ± 0.2

Round scad hydrolysis using a mixture of alcalase and bromelain optimization

The appropriate alcalase/bromelain ratio for hydrolysis determination

The results showed that the ratio of alcalase and bromelain enzymes (A/B ratio) significantly affected on the hydrolysis of round scad. Combining an endo-protease enzyme and an exo-protease helps speed up the hydrolysis process. Among scad hydrolyzed samples with A/B ratio from 1:1 to 1:5, hydrolyzed samples with 1:3 ratio showed the highest hydrolysis efficiency with DH reaching 25.4% (Figure 3). Therefore, the A/B ratio of 1:3 was chosen for the next experiments.

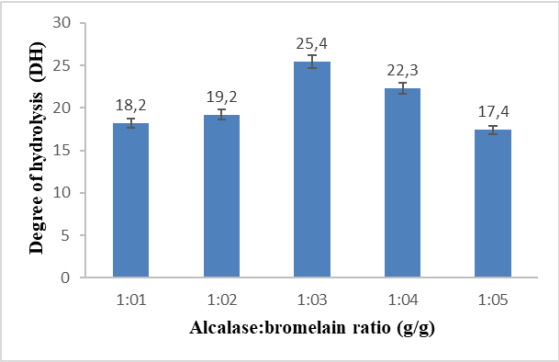

Figure 3. Effect of alcalase/bromelain enzyme ratio on hydrolysis efficiency

The effect of the E/S ratio on hydrolysis efficiency

The enzyme mixture with an A/B ratio of 1:3 was added to the hydrolysis sample with an enzyme/material (E/S) ratio of 0–1.5%, 0.25% step. The results shown in Figure 4 show that when increasing the E/S ratio from 0–1%, the hydrolysis efficiency increases with the DH value increasing from 5.2–31.5%. When increase the enzyme content from 1% to 1.5%, the hydrolysis efficiency increased insignificantly (DH increased from 31.5% to 32.4%). Thus, an E/S ratio of 1% was chosen for the following experiments.

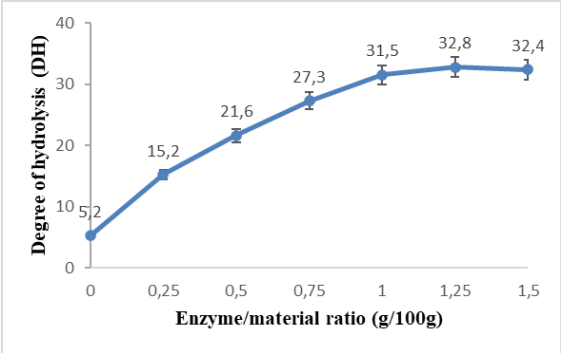

Figure 4. Effect of enzyme/material ratio on hydrolysis efficiency

The effect of material/water ratio on hydrolysis efficiency

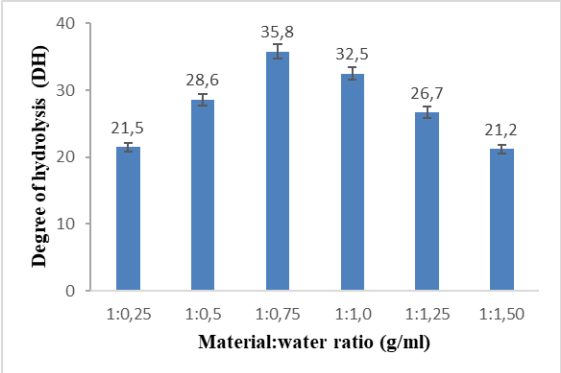

Figure 5. Effect of material/water ratio on hydrolysis efficiency

The results of investigating the effects of the material/water ratio (Figure 5) from 1:0.25

to 1:1.5 (g/mL) showed that the material/water ratio is 1:0.75 for effective results. The highest hydrolysis rate with DH reached 35.8%; this rate was chosen for the following experiments.

The effect of temperature on hydrolysis efficiency

The incubation temperature range for the hydrolysis process was investigated from 40–60°C based on the common optimal temperature range of both enzymes. The results shown in Figure 6 show that at a temperature of 50°C, the hydrolysis efficiency reached the highest with DH of 35.6%, while at temperatures of 40 and 60°C the hydrolysis efficiency decreased sharply, with DH of 16.8 and 19.6%. Thus, the most appropriate hydrolysis temperature of 50°C was chosen for the next experiment.

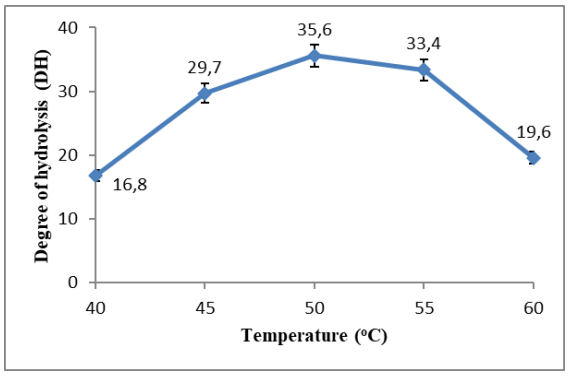

Figure 6. Effect of temperature on hydrolysis efficiency

The effect of time on hydrolysis efficiency

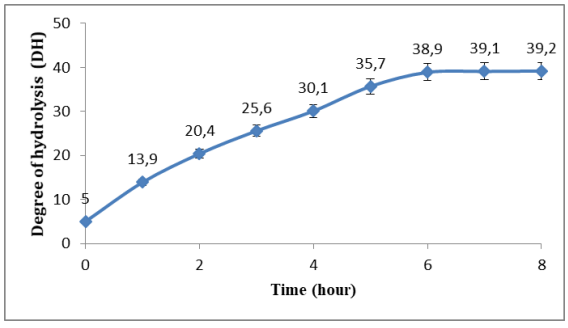

Figure 7. Effect of time on hydrolysis efficiency

The results of surveying the degree of hydrolysis at different times from 0–8 h (Fig. 7) showed that when increasing the hydrolysis time from 0–6 h, the degree of hydrolysis gradually increased over time. However, from 6–8 h, when increasing the time, the hydrolysis efficiency increased insignificantly. Thus, the most appropriate hydrolysis time was 6 h.

Suggestion for the hydrolysis procedure of round scad

After optimizing the hydrolysis condition, the hydrolysis procedure of round scad is established, as showed in Figure 8.

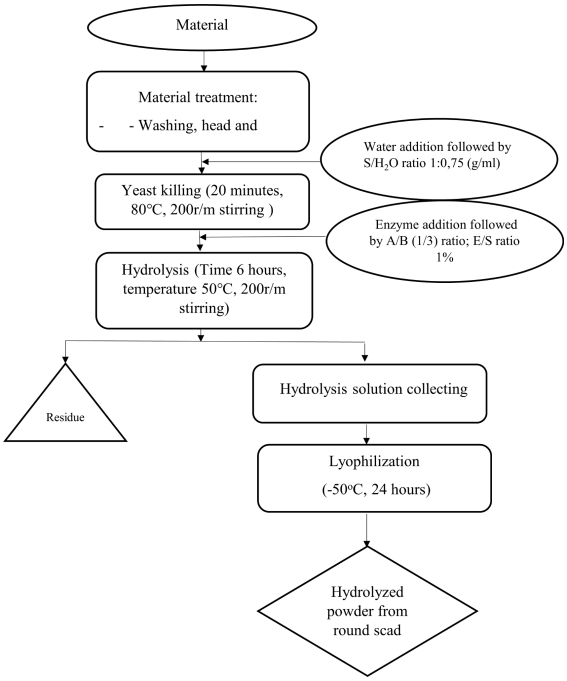

Figure 8. Hydrolysis process of round scad

Process explanation:

Step 1: Processing ingredients: the raw round scads were washed with vinegar and salt water, then crushed with sterilized tools.

Step 2: Yeast killing: Water was added to the ingredients at a 1:0.75 g/mL ratio in a hydrolysis tank, then incubated at 80°C for 10 minutes, then cooled to 50°C.

Step 3: Hydrolysis: the alcalase/bromelain enzyme ratio was 1/3, and the enzyme and

substrate mixture ratio was 1%. The hydrolysis temperature was 50°C and the stirring speed was 200 rpm for 6 h. Finally, the hydrolysis vessel was heated to 80°C for 5 minutes to inactivate the enzyme.

Step 4: Solution filtration: The hydrolyzed solution was filtered through a filter to remove residue, then lyophilized to collect the hydrolyzed powder, which was stored at -20°C.

Step 5: Lyophilization: the hydrolyzed solution was cooled to -20°C, then put into a lyophilization machine to remove water and collect hydrolyzed round scad powder.

With the above hydrolysis process, the hydrolysis efficiency was optimal. The recovery of hydrolyzed scad meal reaching an average content of 21.2 ± 0.3 g per 100 g of fresh material, equivalent to the recovery efficiency. Recovery reached 71.9% (compared to total dry matter mass).

Evaluating the quality of hydrolyzed scad fish meal products

Hydrolyzed scad fish powder was obtained from round scad fish. It was light brown color, loose and smooth, and had the aroma characteristic of sea fish. Hydrolyzed scad meal was determined to have basic nutritional components showing a moisture content of 7.2%, protein content of 75.4%, lipid content of 2.2%, and ash of 6.7%. Thus, hydrolyzed scad meal products had high protein content.

The amino acid content analysis results, as shown in Table 5, showed that the scad fish meal, after hydrolysis, had a high content of 54% non-replaceable amino acids, especially histidine and lysine.

With such high protein and amino acid content, the hydrolyzed powder round scad obtained could be applied entirely in the food fields.

Table 5. Amino acid composition of hydrolyzed powder from round scad

Amino acid	Content (g/100 g)	Amino acid	Content (g/100 g)
Aspartic	6,33	Tyrosine	3,78
Serine	5,6	Valine*	4,83
Glutamine	2,8	Methionine*	2,66
Glycine	0,98	Lysine*	9,66
Histidine*	5,74	Isoleucine*	2,45
Arginine	7,24	Leucine*	6,3
Threonine*	3,5	Phenylalanine*	4,06
Alanine	4,48	TAA	72,37
Proline	0,63	TEAA	39,2
Cysteine	1,33	TEAA/TAA	54

Note: (*): Essential amino acids, TAA (Total amino acids), TEAA (Total essential amino acids).

CONCLUSION

We have built a process to hydrolyze round scads using a mixture of two enzymes, alcalase and bromelain, with optimal conditions, including the enzyme ratio of alcalase/bromelain being 1/3, the ratio of enzyme mixture and raw materials being 1%, the Hydrolysis temperature being 50°C, pH 6, 6 hours, and stirring speed being 200 rpm.

Hydrolyzed round scad powder was extremely rich in protein (75.4%) compared to dry matter content, 1.25 times higher than the protein content in the original material.

RECOMMENDATIONS

Hydrolyzed powder from round scad must be researched to evaluate its biological activities to evaluate its potential application.

It is necessary to continue researching the application of hydrolyzed round scad powder in health protection food products

and dietary supplements for children and grown people.

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