Project Final Completion Report

Assessment of potential value of sea scallop viscera hydrolysate as specialty aquaculture feed and marine nutraceutical ingredients

NOAA Award Number: NA08NMF4720595

To
Commercial Fisheries Research Foundation

Submitted by

Chong Lee
University of Rhode Island

December 26, 2013

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Part I: Final Report Summary

Title: Assessment of potential value of sea scallop viscera hydrolysate as specialty aquaculture feed and marine nutraceutical ingredients

NOAA Award Number: NA08NMF4720595

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Period of Project : July 1, 2011 – June 30, 2013 (extended to December 31, 2013)

Supporting institution/organization: University of Rhode Island and Eastern New England Scallop Association

Total amount of sub-award : $82,953

I-1. Summary of tasks scheduled:

Based on the landing figure reported by Northeast Fisheries Science Center (NEFSC), a large volume of scallop viscera can be realized at around 164 million pounds (1.2 million in RI) based on the 23 viscera to 7 edible meat ratio with seasonal variations. At present, all these viscera are thrown overboard for the lack of commercial use other than baits along with a concern that the viscera may contain marine biotoxins, primarily saxitoxin, the cause of paralytic shellfish poisoning (PSP). The viscera (a high volume-low value product) are largely ignored although there have been some efforts to add values without much commercial success. We proposed to produce scallop viscera hydrolysate and assess its potential value as specialty aquaculture feed additives for feeding attractant and growth stimulant and marine nutraceuticals with the following tasks scheduled.

1. Optimization of hydrolysis process using combination of scallop viscera and squid processing byproduct (SPB) where SPB provides enzymes needed for proteolysis
2. Chemical characterization and biotoxin assay of scallop viscera hydrolysate (SVH)
3. Fish feeding trials to test feeding attractant and growth enhancing properties of SVH
4. Assessment of nutraceutical properties of SVH
5. Production cost and market potential analysis

SPB was included not only to aid hydrolysis, but also to serve as a reference for comparison.

I-2. Summary of tasks accomplished

The analysis of scallop viscera collected 7 times (2011-2013) showed saxitoxin (STX) level far below the FDA limit and variations in composition with season. The lipid content in March was the lowest and peaked in summer months, while no significant variations in the protein content. The scallop viscera (SV) oil contains relatively high amounts of EPA and DHA, which is a highly desirable compositional characteristic for an aquafeed ingredient. The hydrolysis of SV was optimized with aid of squid processing byproduct (SPB). For practicality, a 60 SV-40 SPB combination appears to be workable since SPB is available in large quantities. Therefore, in our fish feeding trials, we tested SV100-0 (scallop only), SV60-SPB40 and SPB100-0 (squid only). The general hydrolysis process starts with homogenization of viscera followed by incubation for 1.5 h at 55^{o}C and pasteurization for 0.5 h at 75^{o}C while stirring, and filtration to yield residue-free hydrolysate.

Feeding trial of scallop viscera hydrolysate in summer flounder and European seabass revealed a clear trend that the scallop hydrolysate diet outperformed the rest in feed consumption, weight gain, feed conversion ratio and feeding attractability with however no statistical differences noted due to large individual variations. Regarding feed attractability, European seabass actively consumed Scal 100, 60-40 and Sq 100 diets which all received higher scores of around 7 (1-9 points scale). In view of the preference for a dry powder form by aquaculture feed manufacturers, a powder hydrolysate was prepared by plate-coat drying and tested for feeding performance. The dry hydrolysate diet outperformed the wet hydrolysate diet in both feed consumption and weight gain suggesting that the heat applied during drying did not adversely affect the nutritional quality of hydrolysate. Instead, the drying process may have enhanced the palatability of hydrolysate.

The assessment of nutraceutical properties indicated that SV and SV-SPB hydrolysates have appreciable antioxidant and anti-hypertension activities, while SV hydrolysate possesses lipase (lipid digesting enzyme) enhancing activity suggesting potential as a digestion aid. Results of our investigation on the nutraceutical properties of scallop hydrolysates were presented at the Annual Institute of Food Technologists (IFT) in Las Vegas (June 25-28. 2012) - “The *in vitro* potential of scallop viscera and squid processing byproduct hydrolysates for type 2 diabetes and cardiovascular disease management".

Estimated production cost based on energy and labor is 6.91 ¢/ lb or 58 ¢/gal hydrolysate excluding raw material, packaging, management fee, maintenance, depreciation and others.

Initial discussions with scallop vessel owners indicate a strong willingness to further explore the possibility of harvesting viscera in large quantities, however all participants explained that substantial vessel modifications would have to be made to bring about the lowest possible landed price, and all participants agreed that they would be interested in participating with further research efforts to explore engineering and practice modifications necessary. All expressed an opinion that the experimental figure of $ 1.00 per landed pound named of clean, frozen and dewatered viscera could be a reasonable target, however anything further than speculation would require sufficient research, engineering, infrastructure and regulatory changes for at least one working vessel to prove out the concept. Quantities necessary to meet aquafeed demand would require multiple working vessels to be similarly equipped, along with the construction of one or several specialty combined storage and processing facilities purpose-built for the conversion of scallop viscera into aquafeed ingredient. No estimates of costs necessary were discussed as industry would have to see a substantial number of vessels sufficiently equipped before they could begin to estimate the size and cost of such a facility.

I-3. Explanation of any problems encountered or differences between the scheduled and accomplished tasks

No problems were encountered in completing the scheduled tasks except for market potential analysis. The market potential is determined by the product performing quality and uniqueness. Two markets are expected from scallop viscera hydrolysate, namely, aquaculture feed ingredients for feed markets and nutraceutical ingredients for health food markets on a global scale. If the analysis points to strong market demand and profitability, commercial production of scallop viscera hydrolysate can be encouraged. As a result, the value addition to unused scallop viscera will be realized benefiting scallop harvesters in the long run.

I-4. Summary of major project results

Scallop viscera offers commercial potential as a high-value specialty aquaculture feed ingredient based on our feeding trials on summer flounder and European seabass, and shows early promise as a nutraceutical to improve lipid digestion and moderate anti-hypertension and antioxidant activities. Although there is a sufficient resource available based on the landing figures, at present viscera landing is not allowed through regulatory oversight and industry common practice. Viscera from western half of the resource can be landed due to no concern for potential contamination of marine toxins. For the eastern half, recent changes in 2011 FDA / ISSC regulations indicate the Abraxis ELISA test kit, used in conjunction with an on-board screening protocol, can be used to facilitate safe landing of scallop viscera from areas closed due to concerns for paralytic shellfish poisoning. Once viscera landing is allowed, one has to determine if the economics justify the landing of viscera under the state and federal regulations.

I-5. Program Area: Part I. Extension of SNECRI project

Subject area: Socioeconomics of the Southern New England Industry – Added value: creating new business opportunities and enhancing existing ones for wild shellfish harvesters.

New business opportunities depends both supply and demand to be viable. Scallop viscera has proven to be useful as a feeding attractant and growth stimulant, with further possible values as a marine nutraceutical. Scallop viscera can supply a specialty aquaculture feed additive market provided the demand exits, however that demand is extremely large, requiring high volume of low cost material. It is conceivable that scallop viscera can help to meet that demand, and while suppliers and manufacturers have expressed interest in taking the next steps, none have committed the financial resources necessary to explore or fully develop the opportunity.

Part II, Final Report

Title: Assessment of potential value of sea scallop viscera hydrolysate as specialty aquaculture feed and marine nutraceutical ingredients

Project Team:

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Date of report: December 26, 2013

II-1. Abstract

With an estimated potential generation of scallop viscera annually at around 164 million pounds (1.2 million in RI), we proposed to produce scallop viscera hydrolysate and assess its potential value as specialty aquaculture feed additives for feeding attractant and growth stimulant and marine nutraceuticals. Our feeding trials on summer flounder and European seabass indicated that scallop viscera offers commercial potential as a high-value specialty aquaculture feed ingredient. Our biochemical assays showed early promise as a nutraceutical which possesses moderate anti-hypertension and antioxidant activities and improves lipid digestion. Recent changes in 2011 FDA / ISSC regulations indicate the Abraxis ELISA test kit, used in conjunction with an on-board screening protocol, can be used to facilitate safe landing of scallop viscera from areas closed due to concerns for paralytic shellfish poisoning. One has to determine if the economics justify the landing of viscera under the state and federal regulations.

II-2. Introduction

According to Northeast Fisheries Science Center (NEFSC), the sea scallop (*Placopecten magellanicus*) resource off New England is currently at historic high levels. Each of the last five years (2004-2009) has produced scallop landings in excess 50 million pounds (22,700 MT) annually of which a total of 164 MT was landed during March 2009 – February 2010 in Rhode Island. The catch volume in the different areas were: 54 MT (33%) from Georges Bank North, 49 MT (30%) Georges Bank South, 31 MT (19%) Southern New England, 19 MT (12%) New York Bight, and 11 MT (7%) Delmarva. Upon shucking, about 70% shell, 23% viscera and 7% edible meat is generated (Myer et al., 1987). This translates into a large volume of viscera at around 164 million pounds of viscera (1.2 million in RI) with seasonal variations. An issue of concern with utilization of scallop viscera for human consumption is the occurrence of saxitoxin (causing paralytic shellfish poisoning, PSP) in gonad and viscera. Day et al. (2007, 2009) surveyed the occurrence in the New England ocean collecting over 6000 samples from 500 locations. In 2004, the occurrence of saxitoxin in roe samples was localized in the Nantucket Lightship closed area. In early 2005, an unusually large bloom of the toxic dinoflagellate *Alexandrium fundyense* caused sharp increases in PSP toxins in scallop viscera in the Great South Channel and Nantucket Shoals area. In both years, the saxitoxin levels in the Mid-Atlantic and Georges Bank roes were well below the FDA limit (80 μg/100 g tissue). All Amnesic Shellfish Poisoning (ASP) results were less than half of the 20 μg/g federal regulatory action levels in all areas at all times. All sample groups west and south of Block Island were well below the federal limits for marine biotoxins (PSP and ASP) using all testing methods, namely, Jellett Rapid Test (JRT), Receptor Binding Assay (RBA) and Mouse Bioassay (MBA). The report (Day et al., 2009) stated that 'While it is clear that a short term study cannot be considered definitive, these test results give us a clear indication that the presence of toxins in scallop roe is not as widespread as previously thought'. Further, commercial harvest of viscera in the western half of the scallop resource is considered reasonably safe. Although limited by management reasons, biotoxins restrictions of any type are not a limiting factor west of 71° W longitude as the area is not restricted due to concerns for PSP, unlike the areas east of 71° W. The 71°

line, which runs approximately through New Bedford MA, conveniently splits the scallop fishery neatly into 2 halves, the eastern half closed to viscera harvest due to PSP, the western half without similar regulation. (Day, personal communication)

At present, other than use as baits all these viscera and roe are thrown overboard for the lack of commercial use coupled with federal regulations preventing landing from areas closed for PSP and poor understanding of biotoxins absence in waters south and west of Rhode Island. There are some potential uses for fresh roe in overseas market which is yet to be explored due to a 2010 European ban on importation of whole molluscan shellfish which includes whole or roe-on scallops – source: http://www.seafood.nmfs.noaa.gov/News_Notices_10.html - See 6/29/2010). Other than potential value of roe as a low volume-high value product harvested from legal areas and sold as a niche product to seafood consumers, the viscera (a high volume-low value product) consisting of hepatopancreas, roe, mantle and gill, are largely ignored although there have been some efforts to add values without much success. An earlier investigation by Myer et al. (1987) focused on the use of scallop viscera in the form of silage as feedstuff for swine. The viscera silage supported good growth when included at levels of 4, 8 or 12% in corn-soybean meal-based diets without adversely affecting the meat quality. Later in Japan, scallop viscera was incorporated into fish feed which was fed to Japanese flounder with good results (Shingo et al., 2000). This was followed by use of squid liver and scallop viscera combination in specialty feed development (Seki and Kawabe, 2010). Takaoka et al. (1995) identified feeding stimulants in scallop extract for pufferfish, *Takifugu rubripes*, including glutamic acid, adenosine monophosphate (AMP), alanine, arginine, betaine, taurine, and glycine in increasing order.

In regard to potential nutraceutical properties of scallop viscera, recently Okada et al. (2011) found that scallop viscera phospholipids exhibited antiobesity effects in mice when used together with seaweed (*Undaria pinnatifida*) lipid. Specialty feed additives have been sought for application in larval feeds to improve growth and health in an effort to replace live feed with artificial micro-diets. They include microalgae and krill and squid hydrolysates. While the majority of nutraceutical products in the current market are of botanical origin, the marine-based nutraceuticals are gaining attention due to their unique features which are not found in the terrestrial-based resources. In recent years, a series of promising new marine nutraceutical products have been introduced to the nutraceuticals and functional foods markets (Lee et al., 2011, photo right).

II-3. Statement of research question or problem investigated

The present study seeks opportunities for Rhode Island wild shellfish harvesters who can make use of scallop viscera which is estimated to be 164 million pounds in New England (including 1.2 million in RI and 0.2 million pounds in southern NE) based on the 23 viscera to 7 edible meat ratio with seasonal variations. This large volume of scallop viscera can be converted into value-added products such as specialty aquaculture feed additives and human and animal nutraceutical products. We proposed to produce scallop viscera hydrolysate through bioconversion and assess its potential value as specialty aquaculture feed additives for feeding attractant and growth stimulant and marine nutraceuticals. The viscera hydrolysate was subjected to fish feeding trials and *in vitro* biochemical assays for nutraceutical properties (antioxidant, anti-hypertension and lipase enhancing activities).

II-4. Goals and objectives of research project

The goal of our study was to develop a bioconversion process to enzymatically convert scallop viscera into value-added hydrolysate for specialty aquaculture feed additives for feeding attractant and growth stimulant and marine nutraceuticals.

Specific objectives

1. Optimization of hydrolysis process using combination of scallop viscera and squid processing byproduct (SPB) where SPB will provide enzymes needed for proteolysis
2. Chemical characterization and biotoxin assay of scallop viscera hydrolysate (SVH)
3. Fish feeding trial to test feeding attractant and growth enhancing properties of SVH
4. Assessment of nutraceutical properties of SVH
5. Production cost and market potential analysis

II-5. Methodology

II-5-1. Optimization of hydrolysis process and production of scallop viscera hydrolysate

Our previous study (Lian et al., 2005) demonstrated that squid processing byproduct (SPB) can be readily hydrolyzed with endogenous enzymes by converting high molecular weight proteins to low molecular weight peptides and amino acids which render feeding attractant and growth stimulant properties in the larval fish feed of summer flounder, *Paralichthys dentatus* (Lian et al., 2008). Because of the lack of such endogenous proteolytic enzymes typically in bivalve mollusks such as clam and scallop viscera (SV) (Lee and Lian, 2005), SPB was used as a source of hydrolyzing enzyme and the optimum ratio of SPB to SV for hydrolysis was determined using an experimental design as follows.

SPB	0	2	4	6	8	10
SV	10	8	6	4	2	0

Residues remained after hydrolysis at varying times

0 0.5 1.0 1.5 h

Changes in the degree of hydrolysis (DH) was measured as a function of varying ratios of SPB to SV. The degree of hydrolysis (DH) was determined as follows.

% DH (based on residue solids before and after hydrolysis)
= 100*(residue wt before – after hydrolysis/ residue wt before hydrolysis)

<u>Preparation of hydrolysate</u>:

Viscera → homogenized in a food processor → hydrolysis for 1.5 h at 55^oC → followed by pasteurization at 75^oC for 20 min → filter through 25 mesh sieve (710 um) → finished hydrolysate (residue collected upon filtration with washing; residue also collected from raw homogenate)

Based on the optimum ratio of SPB to SV and hydrolysis condition, a large quantity of scallop viscera hydrolysate was produced for fish feed preparation and chemical characterization.

II-5-2. Chemical characterization and biotoxin assay of scallop viscera hydrolysate

Chemical characterization

It was based on the proximate analysis (moisture, protein, lipid and ash) and omega-3 fatty acids, mainly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Determination of moisture, protein and ash followed the AOAC methods, while the lipid determination was done by a method developed by Lee et al. (1996). The fatty acid analysis was carried out using our lab procedure (Lian et al., 2005).

Assay of marine biotoxins

Paralytic Shellfish Poisoning (PSP) is of major concern when dealing with any mollusks, including scallops. *Alexandrium fundyense*, a single celled marine algae, is the causative organism for PSP, due to the production of saxitoxin (SXT) (Schollin et al., 2002; Day et al., 2007). The mechanism for PSP involves binding to Na channels in nerve cell membrane, thereby blocking nerve transmission). The pathway is dinoflagellate (aka red tide)→ shellfish → human.

For food safety, toxin retention and measurement is of critical concern when designing a product for human consumption. While the scallop meats do not retain toxins, roe and viscera can become highly toxic when the infrequent *Alexandrium* blooms occur. Although the areas of harvest for this proposed study (west and south of Block Island, RI) are clean in terms of PSP and ASP (Day et al., 2007), for potential commercialization of the scallop viscera, periodic monitoring before utilization is prudent.

For toxin-absence verification, we used an ELISA-based assay (Abraxis LLC, Warminster, PA) that is shown to be more accurate than the Jellett rapid bioassay, but less accurate than the standard Mouse Bioassay (MBA). The different forms of saxitoxin and their tendency for spontaneous transformation are major factors hindering development of a simple field test kit for measuring PSP toxins (Sullivan and Wekell 1988). Currently, the mouse bioassay and Abraxis ELISA tests are approved by FDA. The mouse bioassay measures the saxitoxin level by timing the death of an 18-20 gram mouse following injection of fluid extracted from shellfish tissue. The Abraxis test, on the other hand, is an enzyme-linked immunosorbent assay (ELISA) that uses saxitoxin-specific antibodies and color change to identify saxitoxin quantitatively.

II-5-3. Fish feeding trial to evaluate feeding attractant and growth stimulant properties

Two feeding trials were carried out on juvenile summer flounder (*Paralichthys dentatus*) and juvenile European sea bass (*Dicentrarchus labrax*), and the additional trial to compare between wet and dry hydrolysate for their feeding performance.

<u>Summer flounder (*Paralichthys dentatus*):</u> A feeding trial was conducted to assess feeding attractant and growth stimulant properties of scallop viscera hydrolysate in comparison with squid hydrolysate and fishmeal and soybean meal diets as reference. Juvenile summer flounder (29 g on average) were obtained from Great Bay Aquaculture (Portsmouth, NH) and held in the tanks for two weeks prior to the start of the trials in order to acclimate to the experimental conditions. Seawater was provided in flow-through mode using filtered (20-30 μm) Narragansett Bay seawater with supplemental aeration at water temperature between 18 and 20°C. The fish was reared under a controlled light regime (12L:12D) maintained using halogen lights suspended above the water surface and controlled by a timer. After inventory for measurements of initial weight and length of each individual fish following a standard procedure, fish were graded to keep those having weights within relatively close range while those weighing too high or low were excluded, and the rest were randomly stocked in triplicate tanks per dietary treatment at a density of 10 fish per tank in total of 150 fish in 15 tanks at the URI-Blount Aquaculture Lab (Narragansett, RI). Summer flounder is a carnivorous marine fish and prefers fishmeal-based feed. The current trend is to reduce use of fishmeal and fish oil by substituting plant protein and oil for sustainability and feed cost reduction. The experimental design was based on fishmeal (FM) as reference diet and soybean meal (SBM) diets as test diet as follows.

FM
SBM
Scal 100: SBM + 100% SV hydrolysate
60-40: SBM + (60 SV-40 SPB) hydrolysate
Sq 100: SBM + 100% SPB hydrolysate

The SBM diets were prepared with 60% FM replacement with 5% (on a dry weight basis) scallop viscera hydrolysate (SVH), 60-40 hydrolysate (60-40), or squid hydrolysate (SH). In our previous study investigating replacement level and feeding stimulant and growth enhancing effect of SH in SBM diets, 60% FM replacement and 5% SH incorporation were found to be the underperforming replacement level and a minimum effective level for growth, respectively.

Table 1. Diet formulas (1 kg) for the summer flounder and European seabass feeding trials.

	FM-	60%	Hydrolysate			
Ingredients	**control**	**SBM**	**dry**	**wet**		
Hydrolysate		0	5%			
FM [1]	735	300	250			
SBM [2]	0	450	450			
Corn gluten	0	130.6	126.5	**scallop**	**60-40**	**Squid** [9]
scallop/SH	**0**	**0**	**50**	**282**	**294**	**349**
Oil [3]	25	63	63			
W flour	210	8	8			
Starch [4]	10	6	6			
Mineral [5]	10	10	10			
Vitamin [5]	10	10	10			
Arg [6]	0	0	0			
LysHCl [6]	0	5.1	7.3			
Met [6]	0	2.6	3.4			
Thr [6]	0	0.6	1.6			
Taurine [7]	0	13.8	13.9			
Phytase [8]	0	0.3	0.3			
	1000	1000	1000			
Protein (%)	47.1	40.7		46.4	46.8	48.5
Lipid (%)	9.8	9.2		10.1	10.7	10.2

All ingredients except those specified were purchased from Zeigler Bros Inc., Gardners, PA
[1] Menhaden, 68% protein; [2] Solvent extracted without hulls, 48% protein
[3] Menhaden (Virginia Prime, Omega Protein Health & Science, Reedville, VA)
[4] Pregelatinized corn starch (Instant Tender-Jel, Tate and Lyle)
[5] Mineral and vitamins were premix for marine fish.
[6] Amino acids wete supplemented to match those in fishmeal.
[7] The added level is to bring the dietary level to 1.5% as an optimum (Park et al., 2002).
[8] Ronozyme P5000 CT; The level was recommended by the manufacturer ((DSM Nutritional Products, Basel, Switzerland)
[9] The different amounts of wet hydrolysates were used to adjust the solid content on an equal basis as their moisture contents varied.

Diet preparation

According to respective formula, all ingredients were weighed into three groups (major – FM, SBM, corn gluten meal, wheat flour; minor – minerals, vitamins and amino acids; and fish oil). First, the small portions of FM and SBM were mixed with fish oil and combined with the remainder, while the minor group was mixed separately. Both were then placed and mixed to uniformity in an industrial bowl mixer and an appropriated amount of water added until it yielded a proper consistency for extrusion. Pelletized feeds of appropriate size (1.6 - 2.4 mm) were prepared via extrusion of mix using an extruder (Pre-Center, VD52, Brabender Instruments) with a cutting device. The resulting feeds were dried to 8-10 % moisture in a forced-air cabinet dryer at 80°C.

Table 2. Fish tank arrangement

Diet	Tank	Tank	Diet
60-40	**8**		
Scal 100	**7**	**15**	Sq 100
SBM	**6**	**14**	Scal 100
FM	**5**	**13**	Sq 100
SBM	**4**	**12**	60-40
FM	**3**	**11**	Sq 100
SBM	**2**	**10**	60-40
FM	**1**	**9**	Scal 100

<u>European sea bass (*Dicentrarchus labrax*):</u> As a follow-up study of the previous feeding trial on summer flounder, juvenile European sea bass (*Dicentrarchus labrax*) obtained from Great Bay Aquaculture, was subjected to 8-week feeding to assess feeding attractant and growth stimulant properties of scallop viscera hydrolysate in comparison with squid hydrolysate, fishmeal and soybean meal diets as reference. Twenty fish weighing 16.7 g on average were housed in each 75 L glass tank in triplicate for each diet in total of 300 fish in 15 tanks. The rearing conditions, and diet formulas (Table 1), and assessment of feeding performance were same as described in the summer flounder feeding trial.

Table 3. Fish feeding behavior monitoring sheet for the measurement of feed attractability and waste

Tank	very slow response	slow	moderately	active	exceptionally active	Waste
	1	3	5	7	9	1-5
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						

Feed attractability: 1: very slow response; 5: moderate; 9: very active
Feed waste: 1: almost none; 3: moderate; 5: high

Assessment of feeding performance

Fish were hand-fed to apparent satiation twice daily (0800, 1400). Body weight and length gains, feed consumption and feeding behavior were monitored.
The feeding performance and the extent of feed assimilation were assessed using weight gain (measured every 4 weeks), feed consumption (daily measured), and feed conversion ratio (FCR).

$$\text{Feed conversion ratio (FCR)} = \frac{DWc}{Wf - Wi}$$

where DWc = dry weight of feed consumed; Wf - Wi = weight gain

Feeding attractant property of scallop viscera hydrolysate was quantified by measuring feed attractability and consumption in comparison with fishmeal and squid hydrolysate diets. Feeding behavior in terms of feed attractability was scored on a 1-9 point scale (1: very slow response; 5: moderate; 9: very active) (Table 3). Growth enhancing property was quantified by measuring growth rate of fish fed scallop viscera hydrolysate in comparison with those fed fishmeal and squid hydrolysate diets.

II-5-4. Assessment of nutraceutical properties

A wide variety of bioactive peptides deriving from hydrolyzed fish products have been shown to have beneficial effects on human health (Fujita and Yoshikawa, 2008). Such peptides have been shown to have anti-hypertensive effect (in terms of Angiotensin Converting Enzyme, ACE inhibition) (Kawasaki et al., 2000; Ohta et al., 1997), anti-oxidative effect (Suetsuna, 2000) and anti-hypocholesterolemic effect (Hagino, 2002). In addition, peptides are known to be compounds with potentially high lipase inhibitory activity (Lunder et al., 2005; Wojdemann et al., 1998). Our laboratory has developed a hydrolyzing method for squid processing byproduct as raw material that yielded hydrolysate with unique protein/peptide profiles (Lian et al., 2005; Lian et al., 2008). We have shown that hydrolyzed squid byproduct have strong antioxidant and ACE inhibitory activity (Apostolidis et al., 2011).

Antioxidant activity assay

The 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) inhibition assay was carried out following the method of Vattem and Shetty (2002).

Lipase Enhancement Assay

The enzymatic activity of lipase (Lipase *Candida rugosa*, Ref. Sigma L-1754 at concentration of 1000 Units/ml) was determined using pure olive oil as substrate and the released fatty acid was quantified by a titrimetric method adapted from Sigma (EC 3.1.1.3; Reagent Chemicals ACS Specification, 8th ed.) (1993). The enhancement effect of hydrolysate on the release of fatty acid was determined and the percentage of enzyme activity enhancement was expressed as:

% enhancement
=100 [(V with hydrolysate – V without hydrolysate) / V without hydrolysate)]

where V: mL of NaOH used to neutralize fatty acids released from olive oil with or without hydrolysate sample at 50 mg/L

Angiotensin Converting Enzyme (ACE) Inhibition Assay

ACE inhibition was assayed by modifying a method developed by Cushman and Cheung (1973). The substrate, hippuryl-histidyl-leucine (HHL) and ACE enzyme from rabbit lung (EC 3.4.15.1) were used. The degree of inhibition was expressed as the half maximal inhibitory concentration (IC_{50}) which is a measure of the effectiveness or potency of a compound in inhibiting biological or biochemical function.

II-5-5. Data analysis

A one-way analysis of variance (ANOVA) was employed to evaluate the effect of different formulations on growth performance using a completely randomized block design using a Statistica software package (Version 9.1, Series 1009; Tulsa, OK). If ANOVA indicates significant effect of diet treatments, Tukey's HSD test was employed for comparison of various diets for the significance of difference at $P \leq 0.05$. The individual weights and lengths (for summer flounder only) were recorded at the beginning (month 0) and end (month 2) inventories.

II-5-6. Cost of production of scallop viscera hydrolysate

Cost-related variables include raw material transportation (if not processed at premises), equipment depreciation, energy and utilities, supplies, labor, and packaging. Based on known variables, the cost structure is constructed and the unit product cost is determined with increment of production scale. Fixed variables such as building lease and taxes were excluded in this cost analysis. One of the requirements for the commercial success is volume production in order for the product to be placed in the market with steady supply. In view of the size of global aquafeed and ingredient market, all the viscera output needs to be processed into hydrolysate. The minimum production for business sustainability with profit generation is yet to be determined.

II-5-7. Review of project planning

The project team members (Lee, Apostolidis, Day and Marchetti) reviewed the project planning and both Day and Marchetti provided technical advice to help move the project forward in the right direction. Day expressed a concern over the inclusion of digestive gland which concentrates saxitoxin as scallop consume toxic algae.

II-6. Results and Discussion

Scallop viscera were removed and collected on board by Marchetti and his crew at six different times, approximately 100 lb each time. Location of harvest: Buoy, South of Block Island (proximate coordinates: 41°10′11″N 71°34′48″W)

II-6-1. Seasonal changes in appearance of scallop viscera

July

August

October 2011

5/31/2011	small rim, gonad
6/23/2011	many roe, gonad
8/12/2011	many large roe *
10/24/2011	less roe
3/15/2012	thick rim, not much roe, dark (many black glands)
7/2/2012	bright, moderate number of roe and gonad

*****Roe/gonad to viscera ratio of 8-12-11 harvest: roe/viscera = 45%; gonad/viscera = 28% suggesting that roe and gonad are in full development during this period.

Table 4. Seasonal compositional and fatty acid analysis of scallop viscera

Time of harvest	Moisture	Protein	Lipid** (wet hydro)	Lipid (dry)	Ash
5/31/2011	**79.98** ± 0.43	**13.34** ± 0.17	**4.42**^{b} ± 0.24	**22.10**^{b}	**2.84**^{a} ± 0.24
6/23/2011	**79.70** ± 0.01	**10.12** ± 3.04	**4.35**^{b} ± 0.18	**21.44**^{b}	**3.52**^{a} ± 0.23
10/24/2011	**80.43** ± 0.19	**11.00** ± 1.04	**3.90**^{b} ± 0.29	**19.94**^{b}	**4.25**^{b} ± 0.15
3/15/2012	**81.31** ± 0.22	**11.92** ± 2.57	**2.44**^{a} ± 0.13	**13.05**^{a}	**3.58**^{a} ± 0.41
7/2/2012	**81.98** ± 0.05	**10.62** ± 0.32	**3.50**^{b} ± 0.27	**19.39**^{b}	**3.18**^{a} ± 0.19

*Significant differences between means with different superscripts within the same column.

** **Scallop** **EPA: 24.52 ± 0.40; DHA: 10.84 ± 0.10 (g/100 g oil)**
Fish oil from anchovies/sardines: 18 EPA and 12 DHA (g/100 g oil)
Salmon: 9 EPA and 10 DHA*
Squid oil: **14 EPA and 26 DHA**
Typical fish oil in softgel capsule: 30 EPA and 20 DHA
* http://www.nordicnaturals.com/en/FAQ%27s/FAQs/390

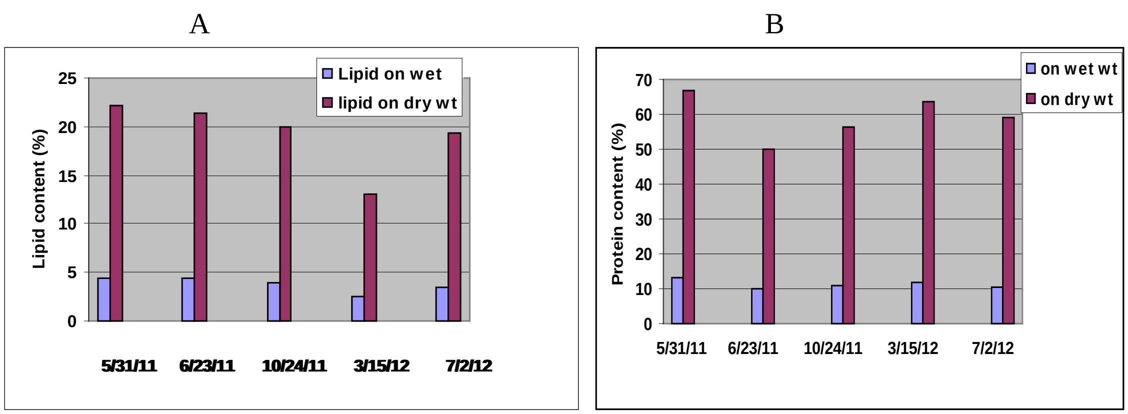


Figure 1. Seasonal variations in the lipid content of scallop viscera (A: lipid; B: protein)

One can see that the viscera composition varied with season. The lipid content in March was the lowest and peaked in summer months, while no significant variations in the protein content were observed although it appeared to peak in May. As for the omega-3 fatty acids, SV oil contains relatively high amounts of EPA and DHA, EPA being higher than DHA, whereas squid oil has higher DHA than EPA. Having high amounts of EPA and DHA is a highly desirable compositional characteristic for an aquafeed ingredient.

II-6-2. Saxitoxin (STX) assay

We used the Abraxis Saxitoxin ELISA-based assay (Abraxis LLC, Warminster, PA), which is an immunoassay for the quantitative and sensitive detection of saxitoxin. Its detection limit for saxitoxin is 0.015 ng/mL or ppm. This assay was chosen because it allows quantitative measurements and is more accurate than the Jellett rapid test.

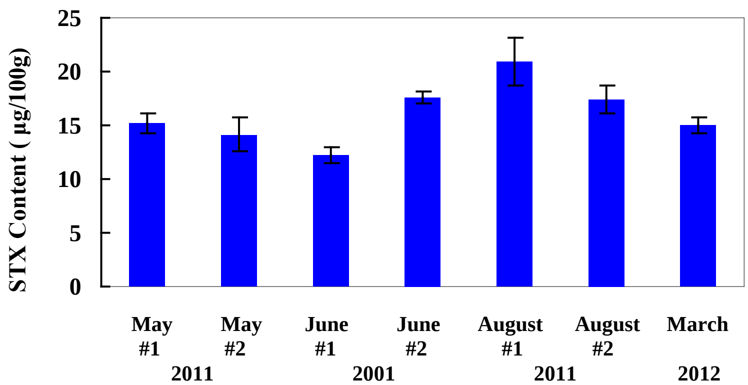


Figure 2. Saxitoxin levels in the scallop viscera in different seasons

As shown in the results above, all scallops harvested in the south of Block Island from May through August in 2011 and March in 2012 yielded viscera (including digestive glands) which were found to be negative (12 – 20 ug/100 g tissue), far below the FDA limit (80 ug/100 g tissue). The results indicate that the inclusion of digestive glands will not pose a toxin problem contrary to what we previously suspected. In reality, removal of individual glands is impractical. We then decided to proceed with hydrolysis trials using the whole viscera.

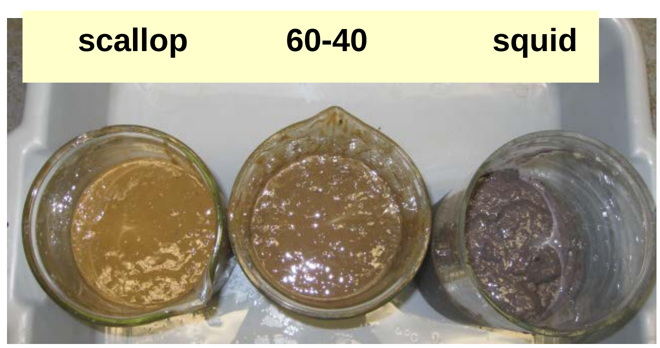
II-6-3. Optimization of hydrolysis of scallop viscera (SV) with aid of squid processing byproduct (SPB)

General hydrolysis procedure: Viscera → homogenized in a food processor → hydrolysis for 1.5 h at 55°C → filter through 25 mesh sieve (710 μm) → residue collected upon filtration with washing (residue also collected from raw homogenate)

Table 5. Extent of hydrolysis in different hydrolysates during 1.5 h incubation

	Raw homo	Res 1	Hydrolysate	Res 2	% solid R1	% solid R2	solid R1 (g)	solid R2 (g)	%DH
SV100-0	100	32.05	100	10.01	12.23	14.74	3.92	1.48	62.24
SV80-20	100	26.34	100	5.82	11.33	17.23	2.85	0.894	68.63
SV60-40	100	22.62	100	3.6	10.88	14.73	2.21	0.318	85.61
SPB100-0	100	33.45	100	3.62	12.34	17.45	3.6	0.1	97.22

SV100-0: 100 SV: 0 SPB (squid)
SV80-20: 80 SV: 20 SPB
SV60-40: 60 SV: 40 SPB
SPB100-0: 100 SPB: 0 SV



Raw homo: raw homogenate of whole viscera
Res 1: Residue weight from raw homogenate
Res 2: Residue weight from hydrolysate
% solid R1: solid wt of raw homogenate residue
% soild R2: solid wt of hydrolysate residue

% DH (based on residue solids before and after hydrolysis)
= 100*(residue wt before – after hydrolysis/ residue wt before hydrolysis

As seen in the above results, the degree of hydrolysis increased with an increase in squid viscera. For practicality, 60 SV-40 SPB combination appears to be workable since SPB is available in large quantities. Therefore, in our fish feeding trials, we tested SV100-0 (scallop only), SV60-40 and SPB100-0 (squid only).

Table 6. Proximate composition of hydrolysates

Type of hydrolysate		%Moisture (solids)	%Protein	%Lipid (dry basis)	%Ash
Scallop 100	w/o	81.09 ± 0.13	10.81 ± 0.24	5.57 ± 0.14	1.96 ± 0.02
	w/ black glands	(18.91)	**10.86 ± 0.03**	(29.45)	**1.93 ± 0.03**
60 Scal – 40 Sq	w/o	82.84 ± 0.69	11.81 ± 0.04	6.28 ± 0.30	1.20 ± 0.28
	w/ black glands	(17.16)	**11.24 ± 0.22**	(36.59)	**1.46 ± 0.01**
Squid 100		85.57 ± 0.60 (14.43)	12.46 ± 0.22	4.18 ± 0.67 (28.96)	0.94 ± 0.01

* Scallop harvested June 23, 2011

As shown in the above Table 6, the proximate composition did not change with or without black digestive glands. Squid hydrolysate tended to have more moisture than scallop viscera hydrolysate, and no apparent differences in protein and lipid contents.

II-6-4. Feeding trial to evaluate feeding attractant and growth stimulant properties of scallop viscera hydrolysate in summer flounder and European seabass

Summer flounder

Table 7. Growth and feed consumption of summer flounder

	Weight gain (g)	Body length gain (mm)	Individual feed intake (g)	Feed conversion ratio (FCR)	Feeding attractability
FM	31.30±2.75	37.92±4.64	35.94±1.91	1.12±0.04	5.77±0.60
SBM	31.26±5.05	32.74±9.46	35.06±2.45	1.15±0.25	5.61±0.88
Scal 100	37.56±6.57	38.88±9.31	40.10±2.65	1.09±0.18	6.20±1.28
60-40	33.02±3.27	39.04±2.78	34.54±0.26	1.06±0.11	5.43±0.78
Sq100	31.09±1.26	30.84±4.66	33.83±3.44	1.16±0.09	5.33±0.70

The above results show a clear trend that the scallop hydrolysate diet outperformed the rest in feed consumption, weight gain, feed conversion ratio and feeding attractability.
No statistical differences were noted due to large individual variations. The level of feed uneaten waste was the highest (3 out of 1-5 scale) with FM, followed by 2 with SBM, suggesting that FM and SBM diets were not as well accepted as Scal 100, 60-40 and Sq 100.

European seabass

0 8 wk

Results of feeding trial are given below in Figures 3 - 5.

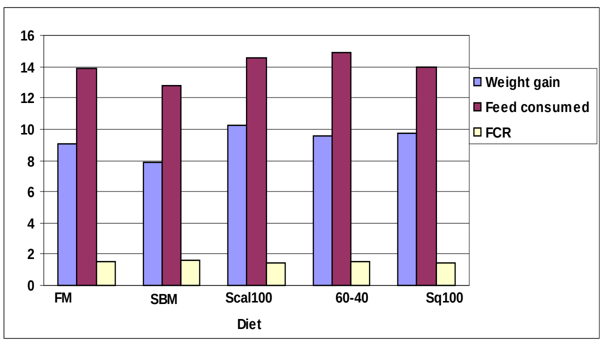


Fig. 3. Feeding performance

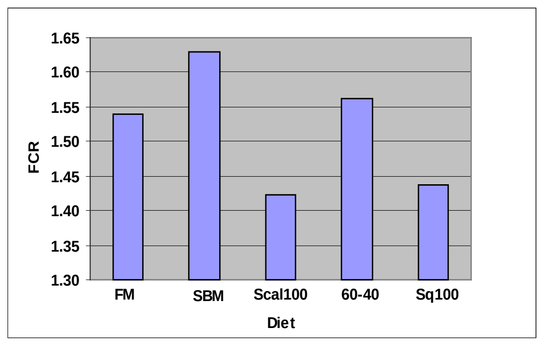


Fig. 4. Feed conversion ratio

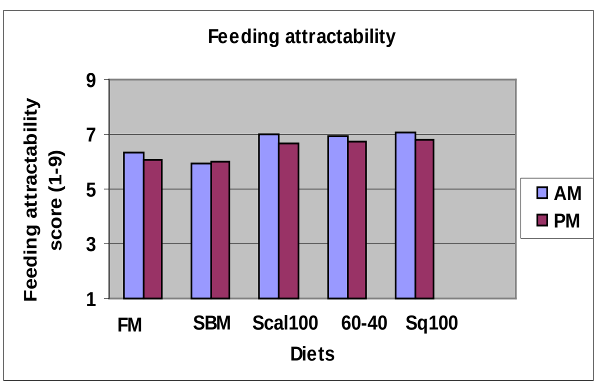


Fig. 5. Feeding attractability

The above results show a clear trend that the scallop hydrolysate diet outperformed the rest in terms of weight gain and feed conversion ratio (FCR). Regarding feed attractability, fish actively consumed Scal 100, 60-40 and Sq 100 diets which all received higher scores of around 7. Regarding feed waste, FM and SBM groups left feed waste at the level of 3 (out of 1-5) which reflected Lower feed attractability (Figure 5). We also observed cloudiness in the Scal 100 tanks indicating possible leaching or high fecal matter.

Table 8. Proximate **tissue** composition of **summer flounder** after 8 wk feeding

	% Moisture	% Protein	% Lipid	% Ash
FM	72.38 ± 0.97	17.91	2.81 ± 0.46	6.90 ± 1.57
SBM	74.05 ± 0.72	17.36	2.51 ± 0.34	6.08 ± 0.43
60-40	74.00 ± 1.42	17.07	3.03 ± 0.68	5.90 ± 1.24
Scal 100	71.80 ± 0.39	18.19	2.80 ± 0.78	7.21 ± 0.29
Sq 100	75.11 ± 0.91	16.88	2.96 ± 0.64	5.05 ± 0.86

Table 9. Proximate **tissue** composition of **European seabass** after 8 wk feeding

	% Moisture	% Protein	% Lipid	% Ash
FM	72.37 ± 0.51	16.66 ± 0.35	5.30 ± 0.43	4.15 ± 0.17
SBM	72.52 ± 0.68	16.72 ± 0.15	5.64 ± 0.73	4.05 ± 0.21
Scal 100	71.81 ± 1.83	16.83 ± 0.28	5.27 ± 0.26	3.84 ± 0.31
60-40	71.42 ± 1.33	16.84 ± 0.82	6.24 ± 0.49	4.21 ± 0.95
Sq 100	70.40 ± 0.58	16.83 ± 0.41	6.22 ± 0.55	3.70 ± 0.42

The above results indicate that diets did not induce any appreciable changes in the proximate composition of tissue except for slightly higher lipid contents observed in fish fed 60-40 and squid hydrolysate diets with no statistical significance. Similar results were observed in our previous feeding trials (Anterria et al.,2011).

II-6-5. A drying process to produce powder form of hydrolysate

During communications, the aquaculture feed manufacturers indicated their preference for a dry powder form for storage and handling. The current industrial practice calls for spray or drum drying. Both spray and drum drying are costly and not designed for production of aquafeed ingredients. So we decided to look into an inexpensive, simple drying process. The proposed process is based on plate-coat drying in which the slurry of hydrolysate is spread over the heating plate to dry, scraped off, ground, and sifted to powder of specified particle size.

A sample of plate-coat dried hydrolysate powder sifted through No. 18 sieve (mesh) (1 mm)

II-6-6. Feeding trial to compare wet and dry hydrolysate for their feeding performance using European seabass

Fish (juvenile, 4-5 g) were housed in 16 glass tanks (75L, 22 fish/tank) with flow-through seawater at 19-20°C under a controlled light regime (12L:12D). Fish were fed six different diets – scallop viscera hydrolysate wet (SVHW) and dry (SVHD), squid hydroysate wet (SHW) and dry (SHD), fish meal (FM), and soybean meal (SBM), twice a day to satiation (three tanks for

SVHW, SVHD, SHW and SHD each, and two tanks for FM and SBM each). Feed consumption, feeding behavior and weight gain were recorded to assess feeding attraction and growth stimulation for an eight-week feeding period.

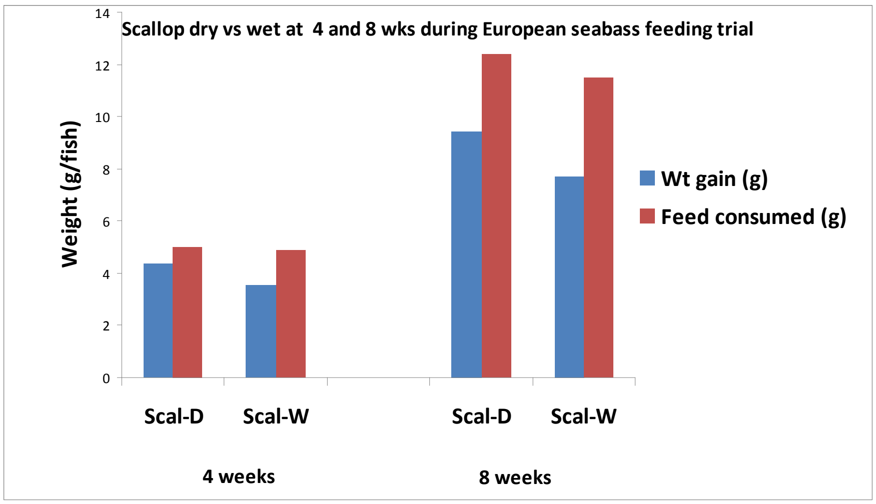


Figure 6. Feed consumption and weight gain of European seabass fed dry and wet scallop viscera hydrolysate for 4 and 8 week periods.

Figure 6 shows that the dry hydrolysate diet outperformed in both feed consumption and weight gain. This result suggests that the heat applied during drying did not adversely affect the nutritional quality of hydrolysate. Instead, the drying process may have enhanced the palatability of hydrolysate as we expected. This reflects in the result of feed conversion ratio (FCR, feed consumed/weight gain) as shown in Figure 7. A low FCR indicates the feed being more efficient.

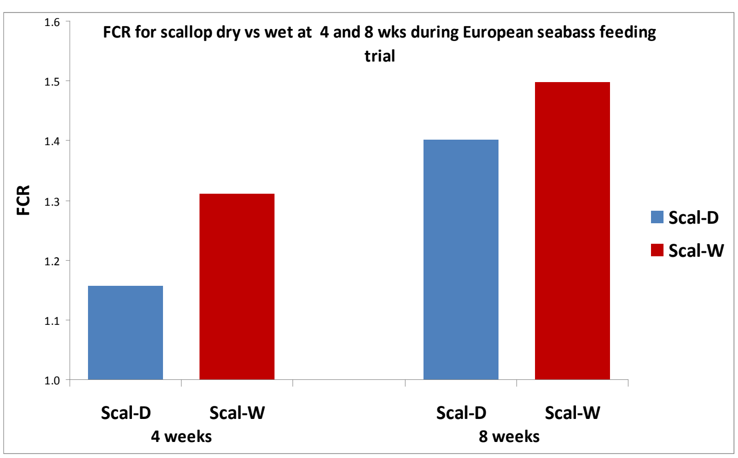


Figure 7. Feed conversion ratio of European seabass fed dry and wet scallop viscera hydrolysate for 4 and 8 week periods.

II-6-7. Assessment of nutraceutical properties of scallop viscera-squid hydrolysates

<u>Anti-type 2 diabetes, antioxidant and anti-hypertension activities</u>

The present study investigated if hydrolysates have bioactivities in relation to type 2 diabetes and cardiovascular disease management potential. The bioactivity of the 60/40 hydrolysate was compared with 100% SV hydrolysate (SVH) and 100% SPB hydrolyaste (SH). To determine the type 2 diabetes management potential, the dose-dependent inhibition of the carbohydrate-hydrolyzing enzymes, α-glucosidase and α-amylase, was measured. The highest α-glucosidase inhibitory activity was observed by SVH (62%) followed by the 60/40 hydrolysate (42%), while α-amylase inhibitory activity of SVH and 60/40 hydrolysate had a similar inhibitory effect at around 55%. To determine the cardiovascular disease management potential, the Angiotensin Converting Enzyme-I (ACE-I, relevant to hypertension management) and lipase enhancing activities (relevant to lipid digestion) were evaluated at different doses. The 60/40 hydrolysate and SH had the highest ACE-I inhibitory activity (around 85%). Results suggest that the 60/40 hydroylsate offers better industrial prospects by utilizing both marine resources with *in vitro* potential for type 2 diabetes and cardiovascular disease management.

The focus of our assessment of nutraceutical properties was on dose-dependent antioxidant, ACE inhibitory (anti-hypertension), and lipase activities. Each hydrolysate was appropriately diluted for dose-dependency and centrifuged at 10,000 g for 20 min prior to assay. The supernatant was used for the assay. Results are shown in Figures 8 and 9.

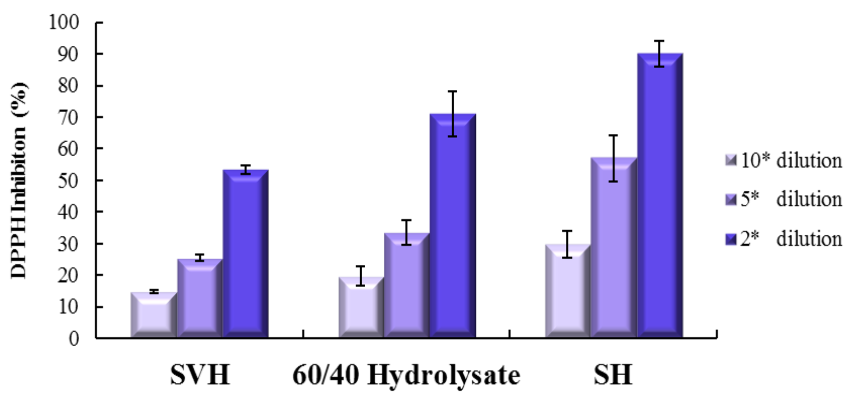


Fig. 8. Dose-dependent antioxidant activity by DPPH free radical scavenging

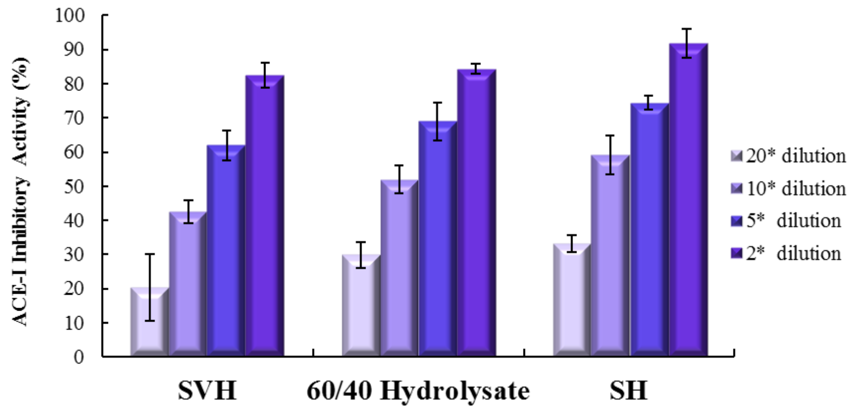


Fig. 9. Dose-dependent ACE-inhibitory activity

All samples tested had antioxidant activity in terms of DPPH free radical scavenging. SH(90%) had the highest activity followed by 60/40 hydrolysate (71%) and SVH (53%) (Fig. 8).
The ACE inhibitory activity related to hypertension management was observed with all hydrolysate samples in a dose-dependent manner. All samples had similar inhibitory activities (90 %, 84% and 82 % at the highest dose for Sq 100, 60/40 and Scal 100, respectively (Fig. 9). All samples displayed sufficient inhibitory activity for hypertension management potential as indicated by IC_{50} values 1.45, 1.54 and 1.37 mg (solids) for Scal 100, 60/40 and Sq 100, respectively. IC_{50} is the concentration that inhibits 50% of activity. The lower the IC_{50}, the more effective (potent) it is

Lipase enhancing effect of scallop viscera hydrolysate (SVH)

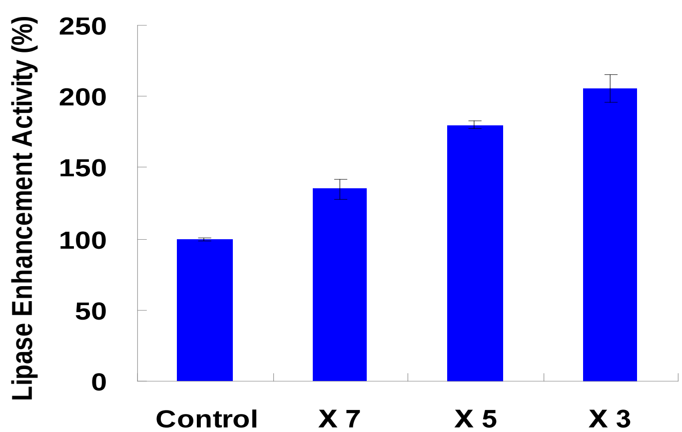


Figure 10. Does-dependent lipase activity enhancement of scallop viscera hydrolysate (SVH)

The figure above shows the lipase activity enhancing effect of SVH when the hydrolysate was diluted at varying levels (3, 5 and 7 times with distilled water) and olive oil used as a substrate. This result suggests that SVH offers potential as a digestion aid.

II-6-8. Estimated production cost for scallop viscera hydrolysate

This estimate is based on a typical commercial production scale.

• Raw material input	250 gal (2,100 lb) per hydrolysis vat		
	2 vat operation	4 h	
	4 vat	7- 8 h/day (8,200 lb); 2 h cleaning	
• Yield	90 % (based on our pilot plant trial); 7,380 lb hydrolysate/day		
• Energy cost	Gas	$100/day ($140 incl. concentration)	
	Electricity	$60/day ($ 80 incl. concentration)	
	Est. total	$160/day	
		2.17 ¢ /lb product	
• Labor	Principal technician	$150/day	
	Assistants (2 x 100)	$200/day	
	Est. total labor	$350/day	
		4.74 ¢/lb product	

Estimated production cost based on energy and labor only: 6.91 ¢/ lb or 58 ¢/gal product
(Excluding: raw material, packaging, management fee, maintenance, depreciation and others)

II-7. Technical presentation and introduction of products

Results of our investigation on the nutraceutical properties of scallop hydrolysates were presented at the Annual meeting of Institute of Food Technologists in Las Vegas (June 25-28. 2012).The title of the paper was "The *in vitro* potential of scallop viscera and squid processing byproduct hydrolysates for type 2 diabetes and cardiovascular disease management" The scallop viscera hydrolysate as an aquaculture feed ingredient was introduced to and its commercial potential was discussed with representatives from feed manufacturers including Skretting at Northeast Aquaculture & Exposition (NACE) (Groton, CT, December 14, 2012).

II-8. Market potential

The market potential is determined by the product performing quality and uniqueness. Two markets are expected from scallop viscera hydrolysate, namely, aquaculture feed ingredients for feed markets and nutraceutical ingredients for health food markets on a global scale. The test results indicate scallop hydrolysate offers a strong potential as a specialty aquafeed ingredient, while it shows early promise as a nutraceutical to improve lipid digestion. Should the analysis point to strong market demand and profitability, commercial production of scallop viscera hydrolysate can be encouraged. As a result, in the long run the value addition to unused scallop viscera will be realized benefiting scallop harvesters.

II-9. Future work needed

- Determine realistic estimate of viscera that the New England scallop industry can produce under the state and federal regulations.
- Assess the feasibility of reengineering their vessels to enable cheap and effective freezing and landing large quantities of scallop viscera with a simple onsite testing kit for toxin screening.
- More feeding trials on additional fish species including Atlantic cod, salmon and yellow tail (*Seriola lalandi*) at the early stage of growth.
- Produce a sufficient quantity of ‘dry’ hydrolysate for aquafeed manufacturers’ in-house trials.
- For nutraceutical properties, the lipid digestion enhancing activity of scallop viscera hydrolysate needs an in-depth investigation on its mechanism.
- Determine the minimum production of hydrolysate for business sustainability with profit generation.

II-10. Summary of conclusions

- The 60 scallop-40 squid viscera combination appears to be the most effective in hydrolyzing scallop viscera in terms of process and production cost since squid viscera is available in large quantities at no cost.
- The inclusion of digestive black glands will not pose a toxin problem contrary to what we previously suspected. In reality, removal of individual glands is impractical. We then decided to proceed with hydrolysis trials using the whole viscera.
- Scallop viscera hydrolysate has a good compositional characteristic including high omega-3 fatty acids suitable for a high grade aquafeed ingredient.
- Scallop viscera offers commercial potential as a high-value specialty aquaculture feed ingredient due to its strong feeding attractability and high omega-3 fatty acids, and shows early promise as a nutraceutical which possesses moderate anti-hypertension and antioxidant activities and improves lipid digestion.
- Although there is a sufficient resource available based on the landing figures, at present viscera landing is not allowed due regulatory oversight and industry standard practice partially due to potential contamination of marine toxins.
  To overcome this, a simple onsite testing kit for toxin detection (Abraxis) can be utilized for on-board screening.
- In the last couple of years, there has been no occurrence of marine biotoxin in the southern New England ocean.
- Substantial vessel engineering and practice modifications would have to be made to cost-effectively freeze and land large quantities of scallop viscera, bringing about the lowest possible landed price.
- Sufficient research, engineering, infrastructure and regulatory changes are required for at least one working vessel to prove the concept.

- Should the analysis point to strong market demand and profitability, commercial production of scallop viscera hydrolysate can be encouraged. As a result, in the long run the value addition to unused scallop viscera will be realized benefiting scallop harvesters.

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Roles of project personnel

Project Team:

- Chong Lee (main contact person), Dept. of Nutrition and Food Sciences-Food Science-Nutrition Research Center, University of Rhode Island
  Oversaw the project planning and execution; secured raw materials; carried out production of hydrolysate and composition analysis; and feed preparation and fish feeding trials with URI food science and aquaculture students.
- Emmanouil Apostolidis (Research Associate, Dept. of Nutrition and Food Sciences, University of Rhode Island) Carried out all assays of saxitoxin and nutraceutical properties with Kang.
- Geoffrey Day (Seafood Research Associates) Provided current information and issues on scallop fisheries and regulations and advice on research directions and planning, and assisted with project report preparation.
- Michael Marchetti (President, Eastern New England Scallop Association) provided scallop viscera Provided scallop viscera samples in a timely manner throughout the project.

Postdoctoral associate

- Barry Volson (fish rearing and feeding performance analysis)

Graduate assistants

- Bouhee Kang (nutraceutical properties)
- Dan Ward (diet formulation and fish rearing)
- Rachel Bone (diet formulation and fish rearing)

Undergraduate assistants (fish diet preparation, composition analysis and fish rearing)

- Christopher Andrikos
- Elizabeth Gomez
- Aaron Attwater

Parties involved in the project

- Dept. of Nutrition and Food Sciences, Food Science and Nutrition Research Center, University of Rhode Island (research conducted)
- Commercial Fisheries Research Foundation (main financial support)
- R.I. Sea Grant Program (partial financial support)
- Eastern New England Scallop Association (scallop viscera)
- Seafood Research Associates, Newburyport, MA (consulting on resources and regulations)
- Sea Fresh USA (squid processing byproduct)
- Great Bay Aquaculture (summer flounder and European sea bass supply)