A Sustainable Future:

Fish Processing Byproducts

Peter J. Bechtel and Scott Smiley, editors

Proceedings of the symposium:
A Sustainable Future:
Fish Processing Byproducts

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Introduction

The first major symposium on byproducts from Alaska seafood processing, the International Conference of Fish By-Products, was organized by the Alaska Fisheries Development Foundation (AFDF) in 1990, and the proceedings volume was published by the Alaska Sea Grant College Program (1). The symposium was held only a few years after the Bering Sea fisheries were Americanized, with the implementation of the 200 mile Exclusive Economic Zone through the Magnuson Fisheries Conservation and Management Act of 1976. By 1990, shoreside processing plants had been built to handle the fisheries resources of the eastern Bering Sea. These dwarfed other fish processing plants in Alaska, with capacities ranging up to 4.5 million pounds per day.

The Alaska Department of Environmental Conservation, through the authority of the Environmental Protection Agency (Region 10) and the Clean Water Act, set standards for the high-capacity shoreside plants. They mandated the plants to handle seafood processing waste in a way similar to that accomplished by the City of Kodiak, after it was designated a seafood processing center in 1974. Raw fish processing waste generated from human food processing lines are ground, cooked, and made into four standard coproducts: fish protein meal, fish oil, bone meal, and stickwater.

Twelve years after AFDF conducted the first byproducts symposium, leaders concluded that a second conference on byproduct advances was warranted. The rationalization of the Bering Sea fisheries, though the American Fisheries Act of 1998, reduced the at-sea fleet of factory trawlers and changed the allocation of harvests with a significantly greater percentage going to shoreside plants. World fish production from commercial aquaculture was increasing rapidly, especially in China, and there were concerns about the continued availability of fish protein meals and oils for aquaculture feeds. The 2nd International Seafood Byproduct Conference, held in 2002 in Anchorage, focused on the status of Alaska's fisheries, feeds, fertilizers, and alternative fuels as well as food supplements and pharmaceuticals, among other topics. The Alaska Sea Grant College Program published the proceedings volume (2).

Following the 2002 byproducts symposium, huge changes accompanying the exponential growth of aquaculture in China and the influence of global fish markets available through the Internet substantially altered the character of fish processing and the handling of fish processing byproducts. Additionally, seven years after the 2002 symposium, our group had published more than 50 new research papers on Alaska fisheries processing byproducts and made numerous presentations at scientific meetings. Significant changes in global marketing of seafood had occurred and the focus of the Alaska seafood industry had changed markedly. As a consequence, we decided to field another symposium, this time seeking a substantial increase in participation of the Alaska fishing industry.

This book is a result of the symposium A Sustainable Future: Fish Processing Byproducts, held February 25-26, 2009, in Portland, Oregon, immediately after the 60th Pacific Fisheries Technologists annual meeting. The byproducts symposium was well attended and discussions were both extensive and productive. We have divided the material in the book into plenary talks and four major sections: New Products and Uses; Stickwater and Wash Water; Storage, Stabilizing, and Processing; and finally Proteins, Hydrolysates, and Oils.

Acknowledgments

It is important to acknowledge contributions to the symposium and the proceedings book. Individuals who helped organize the symposium, beginning in 2008, were

Peter J. Bechtel, U.S. Department Agriculture, Agricultural Research Service, Fairbanks, Alaska

Jim Browning, Alaska Fisheries Development Foundation, Anchorage, Alaska

Robert Pawlowski, Alaska Fisheries Development Foundation, Anchorage, Alaska

Scott Smiley, University Alaska Fairbanks, Fishery Industrial Technology Center, Kodiak, Alaska

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Sue Keller copy-edited the manuscripts and managed the production of the book. The proceedings book is published by the Alaska Sea Grant College Program, University of Alaska Fairbanks.

Conclusion

At the conclusion of the conference, virtually all the participants were in agreement that the third international seafood byproduct conference was a real success and that the interactions between the national and international components of the processing sector and a diverse collection of researchers were both exhilarating and meaningful. This was truly an international conference; 85 people attended, including strong participation from the seafood industries, with 29 oral presentations and an industry forum and a poster session. Thank you for sharing information and the needs of our industries as we strive to increase the economic and responsible utilization of our fishery resources.

—Peter Bechtel, Jim Browning, and Scott Smiley, Steering Committee

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Browning, J. 2010. From Sustainability to Full Utilization. In: P.J. Bechtel and S. Smiley (eds.), A Sustainable Future: Fish Processing Byproducts. Alaska Sea Grant, University of Alaska Fairbanks, pp. 5-7. doi:10.4027/sffpb.2010.01

From Sustainability to Full Utilization

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We live in an age in which technology can revolutionize a particular industry, and the Alaska fishing industry needs a revolution. I am speaking specifically of the way we think about the portions of salmon and other fish species that don't go in the package, box, bag, or freezer, i.e., the "waste" stream. With the likelihood of carbon taxes entering the business model picture in Alaska, seafood companies should be looking ahead to every opportunity to protect their bottom line by increasing utilization and decreasing this waste stream. The more material and value extracted from the round weight processed, the better the margin will be.

In 2007, the fourth largest salmon harvest since statehood, harvesters delivered 257,000 t of the five commercial species of salmon (ADFG 2008). If this were all processed as headed and gutted, we would have discarded (ground and flushed) over 77,000 t of salmon byproducts. Because a growing proportion of the pack has been processed as fillets in recent years, the figure jumped to over 115,000 t of salmon byproduct including skins, viscera, heads, and frames. If we view this as high quality raw material for use in new products, as opposed to waste to be disposed of, both the mind-set and the economic picture change considerably. This raw material is from a certified, wild, sustainable fishery, processed for human consumption, so the quality standard is high. The material contains not only high quality fish protein, but also omega-3 fatty acids, and it is a natural source of other healthful marine compounds including astaxanthin and chondroitin sulfate. The major parts—heads, viscera, skins, and frames—are available in large quantities directly from most salmon processing lines.

The Alaska Fisheries Development Foundation (AFDF) has been involved in projects that investigate and/or support increased utilization of salmon byproducts since the mid 1980s, and in 2002 (Browning 2008) cosponsored Advances in Seafood Byproducts, the Second

International Seafood Byproducts Conference, with USDA ARS, the University of Alaska Fairbanks, and Alaska Sea Grant. This was back when the price of a ton of fish meal was less than $400, and a gallon of fish oil sold for less than $2. Today, the economic picture of increased utilization has changed considerably, as has the raw material composition and interest in more value-added processing. Our raw material stream has increased as a percentage of round weight processed with the recent success of fillets as a salmon product form, but our utilization of the raw material stream has not increased perceptibly despite a threefold increase in its value since 2002.

AFDF is currently involved in several promising projects that are aimed at increasing utilization of this raw material stream from salmon processing:

Modified silage

Modified silage is a demonstration project conducted by AFDF, with Pete Nicklason of FishTek and Peter Stitzel of Quotamax, showing the utility of a system for stabilizing and storing fish waste by adding organic compounds that reduce the breakdown of proteins and bacterial activity. The resulting stabilized material can be held and transported to a site for processing into meal and oil during the shoulder portions of the fishing season. This can be accomplished using small mobile batch plants. Many of the major processors have visited the demonstration mini-plant at the NMFS Northwest Fisheries Science Center facility on Montlake in Seattle.

Salmon peat composting

AFDF is collaborating on the salmon peat composting project with the University of Alaska Fairbanks and Jim Van Oss, of Ocean Earth in Homer, to investigate the carbon/nitrogen ratios in peat infused with salmon waste. The resulting data will allow "standardizing" the mixture of salmon and earth to produce some of the richest organic soil in the world.

Adsorption—an economic method to produce salmon oil for human consumption

The market for high quality refined salmon oil is growing dramatically, along with the wholesale value. AFDF is partnering with Subramaniam Sathivel of the Louisiana State University Agricultural Center, and the Kodiak Fishmeal Company, to investigate adsorption technology in order to partially purify high quality salmon oil. Adsorption technology, when compared to conventional refining, provides lower refining loss, less lipid oxidation, and potentially less flavor reversion in the refined product. This project looks at the methodology from a scientific, practical, and economic perspective to produce a superior product.

Other projects

AFDF is collaborating on several smaller projects as well, including the development of salmon excluder panels for the Bering Sea and Aleutian Islands pollock catcher/processor fleet to help reduce salmon bycatch in that large fishery. AFDF also conducted a session at Pacific Expo in Seattle in November 2008 on byproduct utilization, and continues to encourage the industry to support research and experimentation with the raw material that we're currently grinding and flushing, and occasionally paying fines to "dispose of"!

About AFDF

The Alaska Fisheries Development Foundation was chartered in 1978 after passage of the Magnuson Fisheries Conservation and Management Act in 1976. The act contained language allowing for the formation of fishery development corporations to help build fisheries infrastructure for the tremendous, newly claimed fisheries resources between 3 and 200 miles off our shores. AFDF was the first of these nonprofit development corporations formed, and received funding primarily from the federal Saltonstall-Kennedy Grant Program to assist in development of the first shore-based surimi plant in Alaska in the mid 1980s. AFDF has continued work in support of the commercial fishing industry for over 30 years, including projects on regional branding, the Alaska Symphony of Seafood, Steller sea lion research, and bycatch reduction for both halibut and salmon (see www.afdf.org and www.symphonyofseafood.com). We look forward to continuing our work with Alaska fishing industries.

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Alaska Seafood Byproducts: 2008 Update on Potential Products, Markets, and Competing Products

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Abstract

In 2003 a report was produced for the Juneau Economic Development Council (JEDC) Southeast Alaska Value Added Salmon Waste Study. The report, *Alaska Seafood Byproducts: Potential Products and Markets and Competing Products*, was a compendium of information on the raw materials and products that were and could be used for the production of fish meal and oil, crude and advanced silage products, hydrolysates, composts, fertilizers, and other products. It is unknown how many copies of the report were distributed, since the project is no longer on the JEDC Web site, but requests for copies were coming in until late 2008.

In early 2008 the Alaska Fisheries Development Foundation (AFDF) inquired about the possibility of updating the JEDC report since much had happened within the seafood byproducts industry in the previous five years. They requested that the report be expanded to include other regions of Alaska, since the JEDC report concentrated on the southeast region, and that the market information be somewhat expanded as well. They also requested that the report be submitted by September 30, 2008, so that there would be sufficient time to circulate it before the 2009 Pacific Fisheries Technologists Annual Meeting held in Portland, Oregon, in late February 2009. The draft report was submitted October 3, 2008, and the final version was submitted January 9, 2009. The report has 180 tables and 96 figures and can be downloaded from the AFDF Web site at http://www.afdf.org/past_research/2008_by_product_mkt_study.pdf (Bimbo 2008).

Introduction

The AFDF report is divided into 12 sections. The major sections include a statistics report that covers information on the raw materials used to produce the coproducts: fish meal, fish oil, hydrolysates, fish solubles, silage, compost, fertilizer, and other products. It also includes information on the production of these coproducts when information was available. The report deals with fish meal and fish oil as the major products simply because these coproducts are produced globally, markets are readily available, the technology is well known, pricing information is easy to acquire, and these processes can handle large volumes of processing byproducts as raw material with very little loss. However, it should be pointed out that since the 2003 report, the literature has much more information on some of the other coproducts, for example silage, compost, fish bone meal, and hydrolysates.

Sections on fish meal and fish oil markets include both the pet food and aquaculture industries. There is also a section on coproduct categories that includes legal and regulatory descriptions for the various marine coproducts. There is a section on coproduct quality parameters that includes information from various companies producing and selling these coproducts on a global scale. In all cases, information was updated from the previous report; it was interesting to see that while some products were dropped or combined and some of the companies merged, generally the information was remarkably unchanged. There is a section on geographical markets with information on feed tonnage, number of animals raised and in the case of aquaculture, volumes of fish meal and fish oil used in the dietary formulations of various fish and crustacean species. Global statistics are not published on the same time schedule, so in some cases data are current through 2008 while in other cases only through 2006.

Alaska statistics are complex, since the fisheries are regulated by both the federal and State of Alaska governments; the more specific the inquiry, by law, the more confidential the data becomes. A section on pricing includes fish meal and fish oil and where possible, some information on fertilizers, compost, and fish bone meal. Since biodiesel is becoming a popular topic, some pricing data are presented comparing feed grade fish oil to biodiesel and diesel fuel.

The final sections are Alaska specific, and were expanded to include all Alaska regions and where possible, communities within the regions. As mentioned, as you try to discover specific information, by law the statistics are held more confidentially (especially when there are fewer than three producers in the community). A goal was to make this section as community specific as possible, but that was not always possible. Finally there is a section on the advantages and disadvantages of Alaska seafood byproducts.

The remaining part of this paper highlights some of the information in the report. It is important to recall that the numbers presented here are drawn from federal and state reports and may not cover all possibilities. For example, when larger processors on the Bering Sea coast burn fish oil in their boilers, this fact is not necessarily recorded in the federal or state data.

Discharged waste may represent economic opportunity

There is a tendency throughout the global seafood industry to select options with the lowest required investment and the lowest potential penalties or consequences. Thus the conversion of seafood processing byproducts into coproducts normally would not become a high priority for processors until the penalties or consequences of disposal outweighed the benefits of recovery. For example, a company might select disposal at sea or in a landfill as long as those options had a lower cost even though there is some intrinsic value to the waste material. When the cost of disposal increases or is no longer available, such as through the closing of a landfill, the producer will evaluate other options. At this point they generally seek out the rendering industry to dispose of the waste material. Renderers generally are willing to pick up the raw material free of charge or even pay a small fee to the processor for the waste material. When fuel oil prices increased, renderers began to charge for the pickup and processing of the waste materials. At that point processors began to consider other options even if it meant simply breaking even. In Alaska, the least onerous choice is to grind and discharge the waste material into local waters according to the requirements of the Environmental Protection Agency and the Alaska Department of Environmental Conservation in their General Discharge Permit that covers much of the state. However, the new administration in Washington, D.C., with their well advertised emphasis on environmental protection and green processes, makes one wonder how long the General Discharge Permit option, as presently configured, will remain viable. The government may decide their obligation to protect and maintain the pristine Alaska environment is harmed by the current policy and invoke new, or tighter, rules.

For the purposes of this paper let's consider the following: "What if I were told that starting next season I could no longer discharge my fish waste into local waters. Do I have any viable options? Or do I simply shut down?" Table 1 shows the potential economic loss in discharged waste, the total value of fish meal and fish oil that was not recovered since our last conference in 2002. The assumption is that Alaska lands an average of 2.43 million metric tons (million t) of fish per year and that the average waste is 46% (a conservative value), which

Table 1. Potential value of fish meal and fish oil not recovered.

	2002-2007 total	Ave per year
Total fish meal value not recovered[a]	US$464 million[b]	US$77 million
Total fish oil value not recovered[a]	US$60.15 million[b]	US$10 million

[a]Calculated fish meal and oil value less actual value exported.

[b]Calculated fish meal and oil value using 5 year average data less $100 per t for freight. Actual value of exports, U.S. Customs data, from Anchorage, Seattle, and Portland combined.

Sources: NOAA 2008, Oil World 2009.

equates to 1.12 million t of seafood processing byproduct per year. At a 5:1 conversion ratio of fish waste to fish meal, that equals ~223,000 t of fish meal per year. If we assume that the fish oil yield would be an additional 2% of the total waste, then it would yield 22,400 t of fish oil per year, although clearly some commercial species harvested in Alaska have much higher values. Using a five year running average fish meal and fish oil pricing data (less an estimated US$100 per ton for freight), the fish meal would have been worth US$119 million per year and the fish oil US$16.8 million per year for the 2002-2007 time period.

If we used the all-time high prices for fish meal and fish oil those figures would be US$286 million for fish meal and US$38 million for fish oil per year over the 2002-2007 time period. The total landings for 2002-2007 at 14.6 million t would have yielded 6.7 million t of fish waste or 1.34 million t of fish meal and 134,000 t of fish oil. Using five year average prices (less US$100 per t for freight) would get us to US$714 million for fish meal and US$101 for fish oil.

According to US Customs data for exports through Anchorage, Seattle, and Portland, Oregon, a total of 340,010 t of fish meal and 41,042 t of fish oil were exported during the 2002-2007 period with a value of US$250 million and US$40.85 million for fish meal and fish oil respectively (NOAA 2008). A calculated estimate of the volume and value of fish waste, fish meal, and fish oil over the period 2002-2007 for individual communities in Alaska is shown in Table 2. These data reflect total landings of fish.

When you consider that the brochure for our symposium, A Sustainable Future: Fish Processing Byproducts, mentions that the annual volume of fish waste in Alaska can approach 1.75 million t per year (I conservatively used 1.1 million t) we are potentially talking about a lot of money. The report goes into more detail with the major communities in each statistical region and calculates the potential fish meal and oil production and their value.

The question for this industry then becomes "As an industry in Alaska, are we willing to leave an extraordinary amount of revenue on the table, or are we willing to simply dump it into the sea?"

Table 2. Potential fish meal and fish oil values for major Alaska communities.

	Average landings (t), 2002-2007[a]	46% waste (t)	5:1 waste to fish meal (t)	5 year average fish meal value (US$)[b]	Fish oil yield 2% (t)	5 year average fish oil value (US$)[c]
Dutch Harbor-Unalaska	393,339	180,936	36,187	$27,574,637	3,619	$3,133,810
Kodiak	134,069	61,672	12,334	$9,398,773	1,233	$1,068,155
Ketchikan	36,810	16,933	3,387	$2,580,528	339	$293,273
Petersburg	36,280	16,689	3,338	$2,543,373	334	$289,050
Cordova	32,100	14,766	2,953	$2,250,338	295	$255,747
Naknek-King Salmon	31,948	14,696	2,939	$2,239,683	294	$254,536
Sitka	21,085	9,699	1,940	$1,478,143	194	$127,058
Seward	20,525	9,442	1,888	$1,438,885	189	$123,684
Dillingham-Togiak	12,792	5,884	1,177	$896,770	118	$77,085
Homer	9,064	4,169	834	$635,423	83	$54,620
Kenai	7,840	3,606	721	$549,615	72	$47,244
Juneau	6,468	2,975	595	$453,433	60	$38,976
Wrangell	3,874	1,782	356	$271,583	36	$23,345
Haines	3,017	1,388	278	$211,504	28	$18,180
Yakutat	1,920	883	177	$134,600	18	$11,570
Craig	847	390	78	$59,378	8	$5,104
Anchorage	469	216	43	$32,879	4	$2,826
Alaska pollock	1,506,333	692,913	138,583	$105,599,969	13,858	$9,077,163
Total for listed locations	2,258,780	1,039,039	207,808	$158,349,513	20,781	$13,611,408
Average Alaska landings (t)	2,427,816	1,116,795	223,359	$170,199,613	22,336	$14,630,019
Average U.S. landings (t)	4,318,367	1,986,449	397,290	$302,734,800	39,729	$26,022,480

[a]Total landings, all species.
[b]5 year running average Oil World fish meal price cif Hamburg on Dec. 31, 2007 less US$100 for freight, or $862– 100 = $762.
[c]5 year running average Oil World fish oil price cif Rotterdam on Dec. 31, 2007 less US$100 for freight, or US$755–100 = $655.
t = metric tons.
Alaska pollock are listed separately because of the significant at-sea processor harvest.
Sources: Oil World 2009, NOAA 2009.

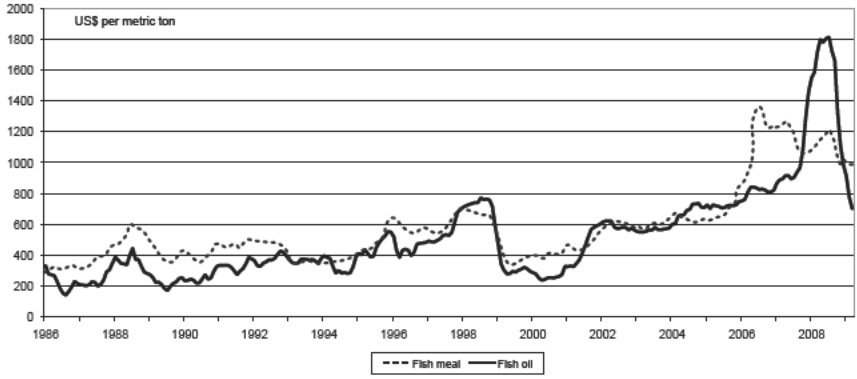

Figure 1. Historic prices of crude fish oil and fish meal delivered into Europe. Data through March 5, 2009. Source: World Oil 2009.

Recently companies that made investments or had fish meal operations during 2002-2007 were able to take advantage of the major run-up in prices for these commodities over the 2006-2008 time period, as shown in Fig. 1.

Statistics

Alaska accounted for about 56% of the U.S. landings of fish and shellfish over the 2002-2007 period (NOAA 2008; Fig. 2). Alaska pollock, Pacific cod, and Pacific salmon make up 85.53% of the Alaska catch over the period 2001-2006 (data came from a different source which lags the Alaska data) (NOAA 2009). This can be seen in Fig. 3.

Alaska is divided into 11 statistical regions with Southeast, Prince William Sound, Kodiak, Bristol Bay, Alaska Peninsula, and Cook Inlet accounting for almost 98% of the salmon catch over the period 2002-2008 (ADFG 2008), as shown in Fig. 4.

One of the disadvantages of Alaska wild capture fisheries is that a large volume of fish is landed in a relatively short period of time (ADFG 2008). The salmon statistics can be seen in Fig. 5.

Potential product concepts

There are several options available for processing seafood waste into coproducts. These range from the low value uses of mollusk shells for road construction, and fish waste for use in baits or chums, all the way up to the high value uses of mollusk shells as a calcium dietary supplement and fish processing waste for the production or extraction of bio-

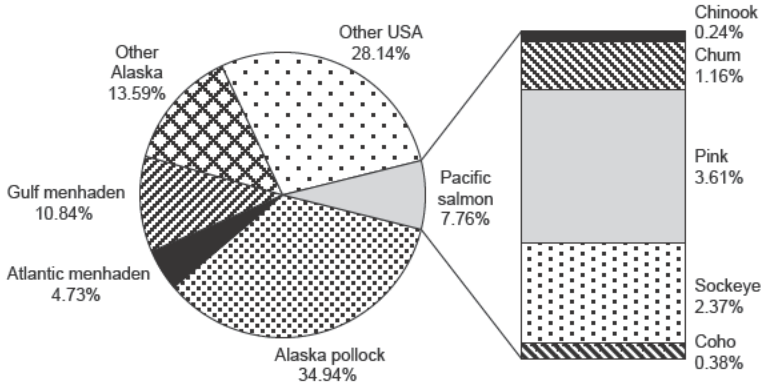

Figure 2. Composition of the U.S. fish harvest, 2002-2007 average. Source: NOAA 2008.

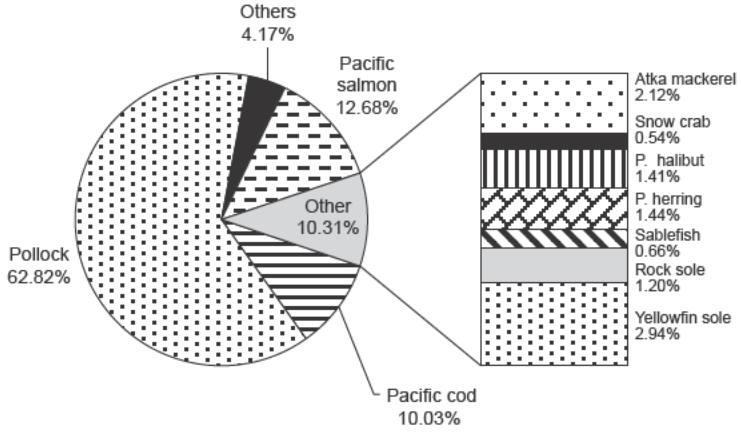

Figure 3. Species landed in Alaska, 2001-2006 average percents. Source: NOAA 2009.

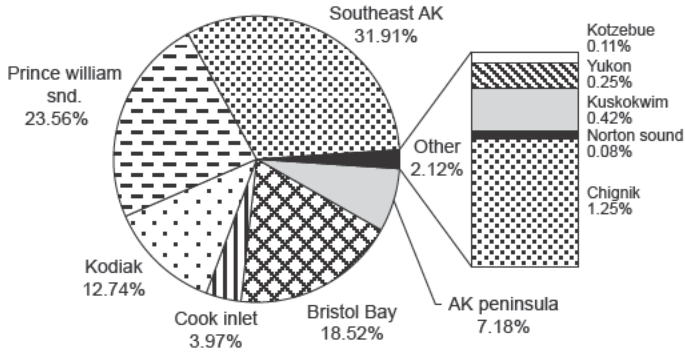

Figure 4. Average Alaska salmon landings by region, 2002-2008. Source: ADFG 2008.

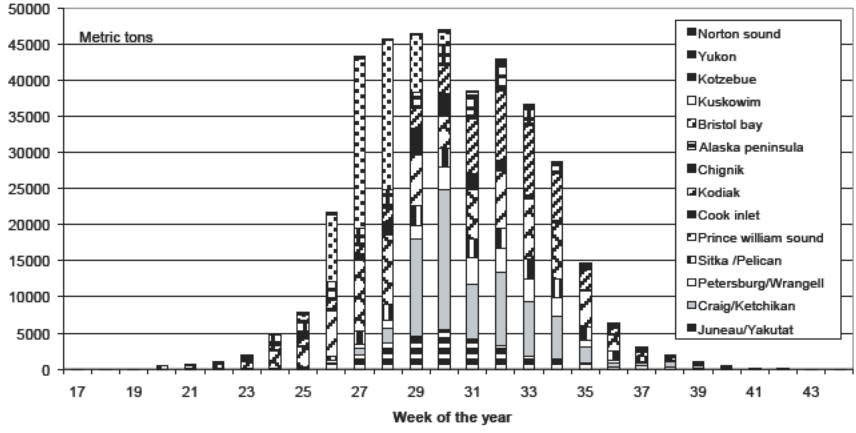

Figure 5. Alaska salmon season by statistical regions, 2002-2007 average metric tons per week. Source: ADFG 2008.

Table 3. Selected options for utilizing seafood wastes.

Extract biochemicals, color additives, and other potential pharmaceuticals
Use mollusk shells in calcium health food supplements
Chondroitin and glucosamine from fish cartilage and bones
Fish bones in water and wastewater purification processes
Leather from skins and gelatin from skin and bones
Solid byproduct material in specialty sauces and other Asian products
Shark, halibut, and cod liver oils
Various concentrated and dry hydrolysate products
Fish meal and fish oil production
Modified silage production
Advanced silage production
Cold or crude silage production, dilute hydrolysate
Composting—need vegetable waste source
Use liquid and solid wastes directly as fertilizer
Use solid waste for bait and chum fishing
Use crushed mollusk shells for roadbed construction
Landfill solid wastes send liquid waste to a sewer system
Dispose of liquid and solid wastes in the sea

chemicals and enzymes. In between these options we have the manufacture of fish meal and fish oil, the various silage and hydrolysate products, and composts (Bimbo 2003). The report covers many of these options; however, the report is not a one size fits all document. What works in one place might not be feasible in another location. While fish meal and oil might work in some areas, there may not be a sufficient amount of raw material to produce fish meal and oil in other locations. Working out what might be cost effective in the different communities will require more precise data. Table 3 lays out options for utilizing these various products.

Compost, fertilizer, and bone use

In this coproducts sector, the current buzzwords are environmental remediation, land reclamation, and pollution prevention. Some of the Alaska byproducts have a potential to go into these markets, perhaps even within Alaska.

Road and highway construction and repairs as well as any landscaping around new construction must be protected against erosion. Compost can be used in long mesh tubes to stop the flow of debris while allowing water to pass through the tubes. In addition to stopping debris from entering drainage pipes the compost can absorb heavy metals and

other unwanted compounds in the water while controlling erosion. This has been referred to as "compost in a sock." Alaska has large mining and wood product operations. Once land is cleared or the mining operation is completed, compost can be used in the reclamation process in efforts to bring the land back to its original natural state.

A newspaper article mentioned that farmers in Alaska were faced with mounting fertilizer costs, in some cases increases of 400%. Fish compost and fish silage can be used in some cases to provide the elements of nitrogen, phosphorus, and potassium for the farmland. While agriculture is not a major industry in Alaska, some region-specific areas might be able to utilize portions of the fish waste produced in those areas.

A publication and patent (US6217775) out of New Mexico State University describes how fish bones can be made to absorb heavy metals, TNT (trinitrotoluene), and a number of other compounds. Beds or columns of fish bones can be used to purify water by absorbing these toxic compounds. A possible use for fish bones recovered from seafood processing is to purify mine wastewater.

Omega-3 fatty acids

In general terms, the fish meal and fish oil industry is being seduced by headlines proclaiming that global fish oil sales into the omega-3 fatty acid supplement market will grow to US$2.6 billion by 2012 and that omega-3 fish oil sales into the food ingredient market will add an additional US$1.54 billion. Another headline proclaims that the U.S. market for omega-3 fatty acid is expected to grow to US$7 billion by 2012. These, of course, are retail sales.

Data obtained from U.S. Customs information for fish oil and fish oil fractions imported into the United States indicates that the value for these items has increased from about US$15 million in 2002 to US$95 million in 2008. Imported fish oil and fish oil fractions do not include commodity oils used for feed, aquaculture, or technical use—only food or pharmaceutical use. The imports have increased in volume from 4,000 t in 2002 to 15,000 t in 2008 (NOAA 2009).

Moving from crude fish oil to a product acceptable in the food or food supplement market is complex and requires significant processing to remove contaminants and other impurities such as color compounds, moisture, minerals, and free fatty acids. The report suggests that wild Alaska salmon oil might play a role in this market; however, it is extremely important to recognize that while salmon are caught in pristine clean cold waters, they neither feed nor live in Alaska waters for much of their lives. In addition, their food is also migrating in from other areas. To market "virgin" sockeye or wild Alaska salmon oil in its natural state directly to the consumer may require a great deal of

Table 4. Percent omega-3 fatty acids in commercial fish oils.

Species	C18:3	C18:4	C20:5	C22:5	C22:6	EPA + DHA	Total omega-3
Anchovy	1	2	22	2	9	31	36
Tuna species	1	1	6	2	22	28	32
Atlantic menhaden	1	3	14	3	12	26	33
Sardine/pilchard	1	3	16	2	9	25	31
New Zealand hoki	0	0	5-7	2	10-37	15-44	17-46
Herring	2	3	6	1	6	12	18
Sand eel	1	5	11	1	11	22	29
Gulf menhaden	2	3	13	3	8	21	29
Norway pout	1	3	9	1	14	23	28
Salmon, wild	2	1	8	4	11	19	26
Jack mackerel	1	2	7	2	12	19	24
Salmon, farmed	1	3	9	2	11	18	24
Pollock		2	16		4	20	22
Mackerel	1	4	7	1	8	15	21

Source: Bimbo 2008.

expensive testing to be sure that the oil does not contain any environmental contaminants. Such testing has been done on many of the salmon oils produced in Alaska, but some remain to be tested. Today, contaminants are identified in the parts per trillion range, not parts per million. Should a product be picked up in the marketplace by a consumer protection group and found to contain a contaminant in harmful concentrations, it could be devastating not only to the producer of the oil but also indirectly to the reputation of wild salmon.

Not all fish oils are suitable for inclusion in the omega-3 market. Some fish oils contain very high levels of saturated and monounsaturated fatty acids and less of the long chain omega-3 fatty acids. To make omega-3 concentrates, the saturated fatty acids and monounsaturated fatty acids are generally removed during processing of the fish oil. This results in reduced yields.

Table 4 shows the general omega-3 fatty acid composition of the major fish oils available on the market today. The black bar represents the threshold—above the bar are the desirable oils for the omega-3 market and below the bar are the marginal and unacceptable oils (personal observation). Wild and farmed salmon fall into the marginal/unacceptable area, but it might be possible to find seasonal batches of oil or seasonal, geographic, and species-specific batches that could move into the desirable range. That requires more data reflecting actual production batches of salmon oil over the seasons. The confusion comes when you

search the literature and find that the omega-3 composition of salmon oil is quite variable. A similar situation with New Zealand hoki supports this. Hoki liver oil is relatively low in omega-3 fatty acids while the body oil is quite high. By separating the Hoki body oil production from the liver oil production, a very suitable oil can be produced.

On the borderline, the level of omega-3 fatty acids in salmon oil is somewhat intermediate. Based on literature information the omega-3 range is extremely variable. The industry must define the oil, and this should be done by taking weekly or biweekly production samples of the various salmon species and testing them over multiple seasons. This will identify whether some of the species are better directed to the omega-3 market and if there is seasonal variability in the oil composition (author's opinion). For pollock oil, there is a need for further studies on seasonal and other sources of variation that alter oil composition.

Resource sustainability

Terms like responsible fishing, resource sustainability, environmentally friendly, and others have become very potent and consumers are keen to purchase products defined by these terms. The environmental activist groups have been very successful in promoting these issues and now are reaping the benefits via the certification process. These terms generally project that we are insuring that the living we make from the sea will be available to our children, grandchildren, and future generations. It also projects that we are making the maximum beneficial use of the resource, while protecting stocks as well as the environment, and fully utilizing the catch.

But who should regulate these "requirements"? Self-appointed nongovernment organizations, elected governments, and the United Nations through the Food and Agriculture Organization (FAO) seem to be in competition with one another. The FAO published their Technical Guidelines for Responsible Fisheries in 1996 with updates through the year 2000 (FAO 2009). It lists 15 guiding principles that most of the certifying organizations have adopted in one form or another. One of the principles mentions that post harvest losses and waste after fish are caught should be minimal and bycatch (catch that the fishermen don't want) should be utilized as much as possible. Currently, federal guidelines often penalize harvesters for bringing their bycatch to the processing plants. A goal of the Alaska salmon industry should be to increase utilization of processing waste.

Fish oil as biofuel

According to the Alaska Energy Authority (AEA) about 8 million gallons or 28,000 t of Alaska fish oil are produced each year, some of which has

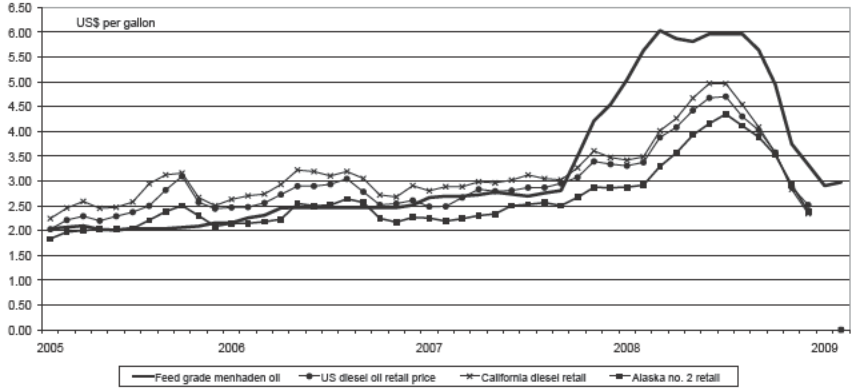

Figure 6. Comparison of California, Alaska, and U.S. average retail prices of diesel oil vs. average price of feed grade menhaden fish oil. Sources: EIA 2009a, Jacobsen 2009.

been successfully used as fuel in boilers (Steigers 2002, AEA 2009). The amount burned seems to vary from 1 to 2 million gallons (3,500-7,000 t). Companies that burn the fish oil say that the cost of storing and shipping the oil to markets is higher than the cost of fuel oil and it is more economical to burn the fish oil or mix it with diesel oil. When the report was written the price of no. 2 oil (diesel oil) was not available for Alaska, so California prices were used. Data showing the historical retail price of Alaska no. 2 oil over the period 1994-2008 is included in Fig. 6.

According to these data from the EIA, the retail price of no. 2 oil in Alaska is lower than the U.S. and California average prices and all are much lower than feed grade menhaden fish oil. Figure 7 shows the historical retail price for no. 2 oil in Alaska.

Alternative processing equipment and processes

One of the major issues advanced as the reason that Alaska seafood byproducts cannot be converted to fish meal and fish oil is that fish meal plant capital costs are very high and the payback over the short fishing season is difficult to justify. The cost of installing a fish meal plant is high, because the individual equipment items are very expensive, used plant equipment is expensive, and installing a new packaged plant is prohibitive. In addition, the cost of fuel is high. Another problem is that much of the fish processing byproducts currently not captured in reduction plants is processed at small, remote, and seasonal

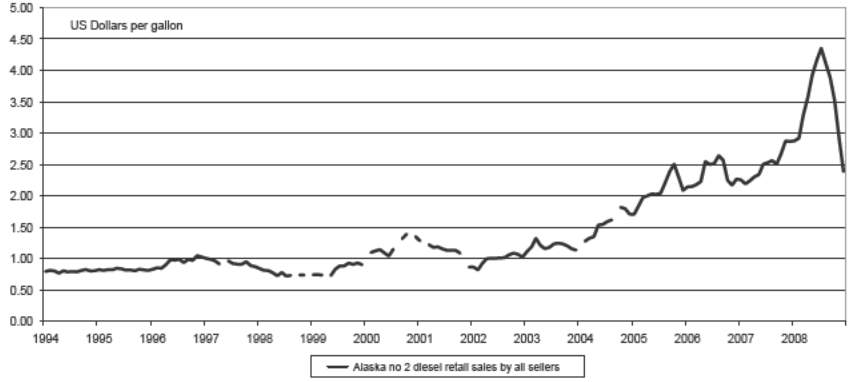

Figure 7. Alaska no. 2 diesel retail sales, all sellers. Source: EIA 2009b.

processing operations. These plants do not have the volume of fish processing byproducts to justify the capital costs of such operations.

Thinking outside the box, there are areas of Alaska where massive amounts of timber waste are allowed to decompose and in other places are composted with fish waste. Perhaps we should draw some analogies from the less developed countries in the world. In Southeast Asia, wood, coconut husks, and even cow dung are used as a fuel source to create steam. In Alaska, there are probably some areas where fish waste and wood waste can complement each other, by using wood waste to create the steam to dry fish waste. Again, within Southeast Asia, some companies initially could not afford to buy the large steam dryers and centrifuges that are used in the modern fish meal plants. Some have developed home grown small rotary steam coil dryers. These are relatively small pieces of equipment set up in series so that the discharge from dryer one enters dryer two, etc., until the fish meal is dry. They also utilize gravity separation tanks instead of expensive centrifuges and achieve a remarkable degree of high quality fish oil. One company even uses a manual press to squeeze the oil and water out of the fish. These operations are designed for small processors, and are remarkably profitable considering what they do. Perhaps smaller operators in Alaska could adopt some of these entrepreneurial ideas. Eventually, the smaller companies will be able to add more modern equipment and expand.

One company in Scotland has developed multi-sized compact silage processing plants. The smallest will handle 300 liters (about 79 gallons) of waste, while the largest was designed for large catastrophic kills in ponds or lagoons. The smaller devices are self contained, stainless steel with a large hatch and attached cutter mixer (ScanBio 2009). Silage

from such a process could be used as fertilizer. In Iceland almost every home has a greenhouse that produces fresh vegetables for the home. In smaller locations in Alaska, the silage could be used for these smaller communities.

Advantages and disadvantages of Alaska seafood byproducts

Alaska seafood byproducts and the products produced from them offer certain advantages, disadvantages, and factors that could go either way.

On the advantage side, there is a sufficiently large volume of Alaska byproduct raw material available. This material, if it comes from salmon or pollock, is Marine Stewardship Council (MSC) certified. This means that any products made from this raw material would also be MSC certified. This type of certification is becoming increasingly important in global commerce. Alaska salmon oil has been kosher certified which also is important in the food application area. Combine kosher with MSC certification and you have a potent marketing message.

Alaska seafood is associated with a pristine environment, cold waters, and a potentially contaminant-free environment. Add this to the message of kosher and MSC certification and the marketing message becomes even more potent.

Alaska fish meal and fish oil is produced from the trimmings of edible fisheries. In many parts of the world, the European Union for example, this is most desirable since it promotes the idea that the entire fish is being used. This is one of the principles of sustainable fishing operations and is usually considered during the certification process.

The Alaska seafood industry is very fortunate to have the support of a dedicated group of scientists and technical staff at the University of Alaska Fairbanks, including the Alaska Sea Grant College Program statewide, the Fishery Industrial Technology Center in Kodiak, and the USDA Agricultural Research Service fish byproduct utilization program in Fairbanks. Technological support facilities for the seafood industry, unlike agriculture, have become an endangered species. On a national basis, many fish utilization facilities have either been closed or rededicated to environmental, climate, and species protection issues. So the advice to the Alaska seafood industry is to protect, support, and nurture this group of dedicated scientists and support personnel or you could lose them.

Alaska also produces cod, pollock, and halibut liver oils. The global production of liver oils is falling, and in many cases fish body oils are supplemented with vitamins A and D to meet the monograph definitions of fish liver oils. The production of liver oils is a relatively simple process and does not require major capital investment. There are com-

panies constantly looking for liver oil sources that would profit from becoming acquainted with Alaska sources.

On the disadvantage side, Alaska seafood byproducts are generally produced in large volume over a relatively short period of time. However, within the various statistical regions of the state there are areas that could supply reasonably large volumes and when combined with other areas might be able to extend the processing season. This would require movable plants or a mechanism for stabilizing the byproduct during transport to a central plant.

The Jones Act (1924) has had a considerable effect on the cost of transportation in Alaska. Vessels hauling cargo from a U.S. port to a U.S. port must be U.S. flagged; if foreign flagged, the vessel must stop at a non-U.S. port between visits to U.S. ports. This rule has made the cost of ocean transportation extremely high in Alaska, effectively relegating the state to the status of an economic colony of Seattle.

Logistics is a major issue in Alaska. One transportation problem is that there may be too few carriers to move product. On the other hand, if there were more product to move, more carriers might be attracted. Fuel costs are also commonly cited as a major issue. Although the cost of wholesale fuel in Alaska may be comparable to that in other parts of the United States, transportation and handling to distant rural processing plants are perceived as additional costs.

To find more and better markets, Alaska fish meal and oil will need more detailed characterization. This would include proximate composition, amino acids, macro and micro minerals, and fatty acid profiles. The potent message of pristine environment and cold, clean uncontaminated waters where the salmon are caught doesn't identify where the salmon have been and where their food has been. The message is good, but from the perspective of a producer of fish meal and fish oil, it should be backed up with data. Very low levels of contaminants in fish flesh have a tendency to concentrate in the oil. When the oil is recovered, the levels could be quite large. While selling virgin, wild Alaska salmon oil in crude form directly to the consumer is benign, we should be careful about believing our own marketing message without backup data. The oil should be thoroughly tested to make sure it meets all the required contaminant specifications before it is marketed to the consumer. The last thing anyone wants is to have a consumer advocacy group pick up a bottle in a store, test it, and report that high levels of some contaminant has been found in wild Alaska salmon oil. This will then carry over to the salmon itself and the adverse publicity will create a public relations nightmare for the industry. The aim is to back up the marketing message with real data.

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Fish Gelatin: An Unmet Opportunity

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Abstract

Gelatin is a class of protein fractions that has no existence in nature, but is derived from the parent protein collagen, by procedures involving the destruction of crosslinkages between polypeptide chains of collagen and breakage of polypeptide bonds. Most commercial collagen and gelatin are obtained from bovine bone, bovine hide, and pork skin. In recent years collagen and gelatin obtained from seafood processing byproducts have drawn extensive interest, in part due to the requirements for kosher and halal food product development and consumer concern about bovine spongiform encephalopathy (BSE, "mad cow disease") in products from mammals as well as being a way to increase the economic return for the fishing industry. About 30% of the waste from fish processing after filleting consists of skin, bone, and scales with high collagen content that could be used to produce collagen and gelatin. The yield and properties of collagen and gelatin are influenced by the source of raw material, the nature of and concentration of acid or alkali used during pretreatment, the temperature and time of pretreatment and extraction, and other process variables depending on the details of the process selected. This chapter will review the various processes and properties obtained for fish gelatin.

Collagen and gelatin from mammal sources

Collagen is one of the major structural components of both vertebrates and invertebrates, and is found widely in skin, bone, and other connective tissues (Balian and Bowes 1977). Gelatin, on the other hand, is a class of protein fractions that has no existence in nature, but is derived from the parent protein collagen, by procedures involving the destruction of crosslinkages between polypeptide chains of collagen and breakage of polypeptide bonds. Most commercial collagen and gelatin are obtained from mammals, mainly from bovine bone, bovine hide, and pork skin. In recent years studies on collagen and gelatin obtained from seafood processing byproducts have drawn extensive interest, in part due to the requirements for kosher and halal food product development and consumer concern about bovine spongiform encephalopathy (BSE, "mad cow disease") in products from mammals.

At present, most of the worldwide production of collagen and gelatin is from pork skin, bovine hide, or bovine bone (Table 1; Gelatine Manufacturers of Europe 2004). Unfortunately, these sources of collagen and gelatin present religious and safety-orientated concerns for various consumer communities.

Kosher and halal certifications are important components of the food business. Kosher food, in particular, has become a major part of modern food production. Many companies in the food industry would prefer to operate all of their processing lines as kosher because of the complications and cost involved in switching back and forth between non-kosher and kosher production. On the other hand, the number of Muslims in the world is more than 1.3 billion people, and trade in halal products is currently about 150 billion dollars (Regenstein et al. 2003).

The absence of any widely accepted collagen and gelatin for both Jewish and Muslim groups is a significant factor holding up further expansion of kosher and halal food products. Although some lenient rabbis permit some types of traditional collagen and gelatin as kosher, the mainstream Orthodox kosher supervision agencies, such as the OU, OK, Star-K, and Kof-K, only certify collagen and gelatin from kosher fish (fish with fins and removable scales) and kosher slaughtered animals (Regenstein et al. 1996). Similarly, for most Muslims, pork gelatin is forbidden and gelatin from non-religiously slaughtered cattle is not desirable, but most fish collagen and gelatin can be certified as halal.

BSE, which has been spread throughout Europe but is also found in many other countries, raised a serious health concern with respect to collagen and gelatin. In many countries, such as the United States, government agencies issued specific rules to regulate the source and processing of collagen and gelatin to reduce the potential risk posed by BSE. Although the scientific evidence has not show that collagen and gelatin were BSE carrier materials, many consumers still have concerns about the safety of these products.

Table 1. World gelatin market data for 2003.

	Production (metric tons)	Percent
Pork skin	118,000	42%
Bovine hides	82,000	29%
Bones	77,000	28%
Fish and others	2,000	1%

Source: Gelatine Manufacturers of Europe 2004.

Collagen and gelatin from marine byproducts

The waste from fish processing after filleting can account for as much as 75% of the total catch weight (Shahidi 1995). It includes the heads, skin and scales, guts/internal organs, frames (bone rack with adhering meat), and trim (pieces cut from the fillets during processing) (Regenstein 2004). About 30% of such waste consists of skin, bone, and scales with high collagen content that could be used to produce collagen and gelatin (Young and Lorimer 1960, Gómez-Guillén and Montero 2001, Sadowska et al. 2003).

Thus, preparation of collagen and gelatin from marine byproducts can satisfy kosher and halal requirements as well as consumer's concern for BSE, and also can increase the economic return for the fishing industry.

Collagen and gelatin can be obtained from various marine sources. The marine species available for collagen and gelatin manufacture can be roughly divided into three categories: marine invertebrates, sea mammals, and fishes. Fish, based on their living environments, are usually subdivided into four groups: hot-water fishes, warm-water fishes, cold-water fishes (Eastoe and Leach 1977), and ice-water fishes. Cold-water fishes, such as pollock, cod, and salmon, account for a large part of commercial fish capture (FAO 2002). They are often processed into skinned and boned fillets, leaving large amounts of fish skin, scales, and bones as waste. These byproducts, especially the skin and scales, usually contain a large amount of protein, most of which is collagens (Young and Lorimer 1960). Warm-water fishes account for most fresh-water fish aquaculture production (FAO 2002), and currently many commercial fish gelatins come from fishes in this category. Fish gelatin may well be the most lucrative way to use large quantities of fishery byproducts.

Collagen and gelatin extraction

The yield and properties of collagen and gelatin are influenced by the source of raw material, the nature of and concentration of acid or alkali

used during pretreatment, the temperature and time of pretreatment and extraction, and other process variables depending on the details of the process selected. Since collagen is only one of the constituents of the raw materials, before collagen/gelatin extraction, one or several pretreatments might be applied to remove the contaminants and increase the purity of the final extract.

Non-collagenous proteins, pigments, and lipids are usually treated as contaminants during collagen extraction. Alkaline pretreatment can be used to remove non-collagenous proteins and pigments (Nagai et al. 2001), and a diluted NaOH solution is widely used. To remove lipids, the raw materials can be treated with 10% butyl alcohol, and washed with distilled water (Nagai and Suzuki 2000); however, a diluted alkaline solution can also partly satisfy this function. Some raw materials, such as fish bones (Nagai and Suzuki 2000) and fish scales (Kimura et al. 1991, Nomura et al. 1996) are highly calcified. Two different solvents have been used for demineralization: 0.05 M EDTA in neutral buffer (Nagai and Suzuki 2000) or HCl solution (Nomura et al. 1996). Both solvents can remove most of the Ca^{++} in the raw materials, but loss of protein is higher in treatments using HCl solution compared to those using 0.05 M EDTA.

Most manufacturers produce gelatin, instead of collagen, due to the wider industrial uses of gelatin. The ultimate aim in gelatin production is the conversion of collagen into gelatin with a maximum yield and good physicochemical properties. The yield and qualities of gelatin are influenced not only by the species and tissues from which it is extracted, but also by the manufacturing process.

In gelatin manufacture two methods are ordinarily used: the acid process and the alkaline process. Gelatin prepared by the acid process is called type A gelatin, while that prepared by the alkaline process is called type B gelatin (Hinterwaldner 1977). The dual processes—acid and alkaline—also apply to marine gelatin extraction, even though the properties of marine raw materials are different from mammals (the most common source of gelatin) and avian species.

For gelatin extraction, the acid process refers to a pretreatment of raw materials with an acid solution followed by an extraction that is carried out in an acid medium (Devictor et al. 1995, Gómez-Guillén and Montero 2001, Gilbert et al. 2002, Arnesen and Gildberg 2002, Muyonga et al. 2004b). During the acid pretreatment, the breakage of some interchain crosslinkages occurs, which facilities the subsequent extraction process. On the other hand, the acid pretreatment can partly preclude the degradation of collagen by endogenous proteases and minimize the enzymatic breakage of intra-chain peptide bonds of collagen during extraction (Zhou and Regenstein 2005).

The period of acid pretreatment varies with raw materials, but is usually within one day. The pretreatment temperature is critical for the

acid process; increasing the temperature may facilitate effectiveness of the pretreatment, but it will cause the loss of collagen. For warm-water fishes, the pretreatment can be done at room temperature, while for cold-water fish gelatin extractions, the optimal temperature is lower than 10ºC (Zhou and Regenstein 2005). This discovery has led to a much higher quality gelatin from cold-water fish.

The alkaline process refers to a pretreatment of raw materials with an alkaline solution, in most cases followed by the neutralization with an acid solution; the extraction may be carried out in an alkaline, neutral, or acid medium. One advantage of this process is that the pretreatment with an alkaline solution can remove considerable amounts of non-collagenous materials (Johns and Courts 1977, Zhou and Regenstein 2005). The alkaline pretreatment also breaks some inter-chain crosslinkages and precludes the effects of proteases on collagen. The neutralization with an acid solution provides an optimal weak acid extraction medium, which guarantees a high extraction yield with good gel quality.

Yang et al. (2007) applied combinations of alkaline and acid pretreatment to the extraction of gelatin from catfish skin. They concluded that pretreatments with 0.20 M NaOH for 84 min and 0.12 M acetic acid for 60 min at 4ºC, and extraction at 55ºC for 180 min gave the best results with a protein yield of 19.2%, a gel strength of 252 g, and a viscosity of 3.23 cP. With these production conditions, the extracted gelatin also showed relatively good hardness, cohesiveness, springiness, and chewiness.

During all the pretreatments, the temperature should be controlled within a particular optimal range. For cold-water fishes, this temperature is very critical, and should be kept below 10ºC to avoid the extensive loss of collagen during pretreatment processes. In the studies on pollock skin gelatin, Zhou and Regenstein (2005) also suggested that during the alkaline pretreatment, the type of alkali does not make a significant difference, but the concentration of alkali is critical. The acid type and its concentration during the acid neutralization process determines the final pH of the extraction medium, and affects both the yield and gel quality of the gelatin extracts. Processes for each species should be optimized, recognizing that biological variability may require further fine-tuning of such optimizations.

Extraction process optimization

The quality of collagen and gelatin as mentioned earlier is influenced not only by the species or tissue from which it is extracted, but also by the extraction process. For each specific source, an optimization of the extraction procedure would be necessary to improve the quality of extracts. One important step for process optimization is to determine the critical control variables. In an earlier study on cod skin gelatin extraction, Gudmundsson and Hafsteinsson (1997) suggested that the

alkaline and acid concentrations during pretreatments would affect gelatin quality. In a subsequent project, Gómez-Guillén and Montero (2001) also suggested that the type of acids used in the extraction might affect the gel properties of gelatin from megrim skin. With a fractional factorial design, Zhou and Regenstein (2004) further confirmed the importance of the pretreatment alkaline and acid concentrations on the quality of gelatin extracts. To obtain a high quality gelatin extract, the alkaline concentration should be high enough to remove the non-collagenous proteins and exclude the effect of proteases, and the acid concentration should be in a range to offer an optimal weak acid extraction medium (Zhou and Regenstein 2004, 2005). Too strong an alkaline or acid extraction medium can cause significant degradation of the peptide chains, and result in an extract with poor quality. To guarantee a reasonable yield, the pretreatment temperature and the extraction temperature should also be controlled within a proper range. The use of SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) is generally the best way to assure that the molecular weight distribution of gelatin subunits is optimized, i.e., that hydrolysis is limited. The best gelatins for any specific species/raw material component can be most easily characterized by SDS-PAGE.

Removing impurities

Extracted marine collagen and gelatin may contain some impurities, such as insoluble particles, inorganic salts, pigments, and compounds responsible for the unpleasant fish flavors. The insoluble particles can be removed by centrifugation and/or filtration. Inorganic salts are traditionally removed by ion exchange technology (Gelatin Manufacturers Institute of America 1993), but they can also be removed by ultrafiltration, which concentrates the extract at the same time (Chakravorty and Singh 1990, Simon et al. 2002). Pigments and some of the compounds causing the unpleasant fish flavors might be removed by activated charcoal. Activated charcoal has been used to improve the quality of catfish gelatin solutions, although sensory testing was not done (Yifen Wang, Auburn Univ., Auburn, Alabama, 2007, pers. comm.). These are all techniques that have received limited study, and for commercial success these must be much more extensively studied.

Properties of marine collagens and gelatins

Marine collagens and gelatins, especially those from fishes, have lower melting points than mammalian gelatins, and care is needed during preparation and storage because they are extremely susceptible to microbiological attack and thermal hydrolysis (Jones 1977). Fish collagens and gelatins are commercially available in both concentrated liquid

or solid form. The liquid form usually requires a mixture of methyl and propyl hydroxybenzoates or other preservatives to prevent bacterial attack (Norland 1990). Solid gelatins usually contain less than 15% moisture. In most situations, gelatin is dried and processed into a powder or sheet, and the quality of the final product is influenced by the methods applied. It has been reported that freeze-dried gelatins have better gel properties than air-dried gelatins (Gudmundsson and Hafsteinsson 1997, Fernández-Díaz et al. 2001). But for commercial preparations air-dried gelatin is more cost-effective.

Molecular composition

The amino acid composition of gelatin is very close to its parent collagen, and no amino acid sequence rearrangements occur during the collagen-gelatin conversion. Glycine (Gly), alanine (Ala), proline (Pro), and hydroxyproline (Hyp) are four of the most abundant amino acids in collagen and gelatin. The frequency of occurrence of Gly is about one out of every three amino acids (Eastoe and Leach 1977). The amino acid sequence is characterized by the repeating sequence of Gly-X-Y triplets, where X is mostly Pro and Y is mostly Hyp. The presence of Gly at every three residues is a critical requirement for the collagen super-helix structure. Gly contains no side chain, which allows it to come into the center of the super helix without any stearic problems to form a close packing structure (te Nijenhuis 1997). The super-helix structure is further stabilized by the stearic restrictions that are imposed by the pyrrolidine residues and the hydrogen bonds that are formed between amino acid residues. The Hyp is unique to collagen/gelatin and can be used as a marker for gelatin. However, the factor used to convert Hyp concentration into gelatin concentration must be determined for each species. This is one of the goals of the current work—to provide a set of such conversion factors. For this purpose, the highest purity gelatin that can be obtained should be used to determine the conversion factor between measured Hyp and gelatin. Using the Hyp as a marker permits gelatin concentration to be determined in solutions containing other proteins.

The conversion of collagen to gelatin yields molecules of varying mass: each is a fragment of the collagen chain from which it is cleaved. Therefore, gelatin is a mixture of fractions varying in molecular weight from 15 to 400 kDa (Gelatin Manufacturers Institute of America 1993). By optimizing the pretreatment and extraction conditions, a gelatin extract with a higher molecular weight distribution can be obtained, where certain inter-chain crosslinkages present in the collagen are destroyed but with less breakage of peptide bonds. The higher molecular weight gelatins have a higher viscosity and a stronger gel strength.

Marine collagens and gelatins vary according to the living environments of their sources, particularly with respect to water temperature.

In general, collagens and gelatins from warm-water fishes have a lower imino acid content than those from mammals, but a higher imino acid content than those from cold-water fishes and ice fishes. In addition, the amino acid composition may vary among collagens and gelatins prepared from different tissues and/or with different methods. Based on studies on a fresh water fish, Nile perch, Muyonga et al. (2004a,b) suggested that the maturation stage of the source could also influence the amino acid composition of the collagen and gelatin extracts. Clearly a better understand of the within species variability of gelatin is needed.

The formation of thermo-reversible gels in water is characteristic of gelatin. When an aqueous solution of gelatin is cooled down, it will form a gel if the concentration is high enough. The critical gelation concentration and temperature for gel setting are mainly dependent on the amino acid composition and molecular weight distribution of the gelatin. This is another important variable that needs to be determined systematically for various fish gelatins and may differ with various sources within a single fish, i.e., skin gelatin may be different from scale gelatin.

Gel strength

Gel strength is the major physical property of gelatin gels, and the commercial value of gelatins is principally based on their gel strength. Besides the influence of amino acid composition and molecular weight distribution of the gelatin itself, the strength of a gelatin gel also varies with gelatin concentration, thermal history (gel maturation temperature and time), pH, and the presence of any additives (Choi and Regenstein 2000). In addition, the size and shape of the container used to form the gelatin gel and the parameters of the instrument applied in the determination of gel strength will also affect the final value. Bloom strength, the gel strength determined by the standard bloom method at 10ºC with certain well-defined requirements (Wainewright 1977) was developed to standardize the test, and it has become the most critical standard used to commercially assess the grade and quality of a gelatin. A detailed review of gel strength and its determination can be found in Wainewright (1977). The gel strength of most commercial gelatin varies from less than 100 bloom to more than 300 bloom. Although warm-water fish gelatin gels can have a bloom strength of 300, some cold-water fish gelatin solutions remain liquid at 10ºC (Norland 1990) so that measurements at 2ºC are also required. Gel strength at both temperatures or only at 2ºC for those that do not have any gel strength at 10ºC can be obtained, but are not called bloom strength. The temperature dependence of gel strength does vary for the different gelatins.

Melting point

As a thermo-reversible product, a gelatin gel will start melting when the temperature increases above a certain point. This is called the melting

point and is usually lower than the human body temperature and higher than the setting temperature. This melt-in-the-mouth property has become one of the most important characteristics of gelatin gels, and is widely applied in the food and pharmaceutical industries. No other biopolymer has this unique property, although many efforts have been made to find a substitute for gelatin. The melting point of gelatin from beef or pork ranges from 30 to 33ºC, but fish gelatins show lower melting temperatures than mammalian gelatin, due to their lower imino acid content. The melting point for warm-water fish gelatin usually ranges from 23 to 29ºC, while cold-water fish gelatin may melt around 10ºC. There are standard methods for gelatin melting, and this parameter can be obtained relatively easily (Wainewright 1977).

Viscosity

Viscosity is the third of the major commercially important property of gelatin samples. Low viscosity gives short, brittle gelatin gels, while high viscosity gives tougher and more extensible gels. For many applications, gelatins of high viscosity are preferred and command higher prices, given that other properties are equal (Wainewright 1977). In addition to the chemical properties of the gelatin itself, the viscosity of gelatin depends on its concentration, temperature, pH, and the presence of additives. Molecular weight distribution appears to play a more important role in viscosity than it does on gel strength and melting point. The viscosity of gelatin is normally measured at 60ºC and at a 6.6% gelatin concentration in both Europe and North America. Most commercial gelatins have a viscosity between 15 mPs and 75 mPs (Gelatin Manufacturers Institute of America 1993, Poppe 1997). Obviously, this property needs to be measured when working with gelatin.

It is worth noting that no single physical property can adequately convey the texture and sensory appreciation of a gel by a consumer, and an overall evaluation of gelatin combining all the critical physical properties may be necessary.

Gelatin and collagen consumption

Collagen or gelatin completely lacks the essential amino acid Trp and is deficient in several other essential amino acids so that it has a chemical score of 0 out of 100. Gelatin alone is of very low nutritive value, but since it is most often eaten with other proteins this is not a practical impediment.

Although gelatin alone cannot serve as a major dietary protein, as a supplementary protein it may have some advantages. Gelatin hydrolysates are used widely as nutritional supplements due to their high protein content, availability, ease of preparation, ease of addition, and low cost; however, the benefits, if any, of the high Pro and especially

the unique Hyp contents, have not yet been worked out. In previous work, bone gelatin has generally yielded a hydrolysate. This may be of commercial importance in evaluating the economic potential of bone gelatins if many of them are already hydrolyzed by the time the gelatin is isolated. On the other hand, methods to extract fish bone gelatin without hydrolysis are clearly needed.

Collagen and gelatin are usually used as functional rather than nutritional ingredients in food applications. Collagen can be used to produce edible casing for sausages. Sausage casings were originally derived from the washed gastrointestinal tract of cattle, sheep, and pig. With rapid growth in demand for sausage products, collagen casings were developed, with several advantages over natural gut casings: convenience, economic efficiency, and uniformity (Hood 1987). Collagen can also be used as a clarification agent to remove the colloidal suspensions during production of alcoholic drinks and fruit juices (Courts 1977).

The largest single use for gelatin is in water gel desserts. Gelatin desserts can be traced back to 1845 when a U.S. patent was issued for "portable gelatin" in desserts. The current U.S. market for gelatin desserts exceeds 100 million pounds annually. Gelatin desserts consist of mixtures of gelatin powder, sweeteners, acids, and compounds to offer the desirable flavor and color. Other biopolymers, such as agar and carrageenan, can also form thermally reversible gels with water. However, the main difference between gelatin and the seaweed polymers is the low melting point of gelatin gels, which is usually lower than the body temperature. A study from our laboratory has suggested that gelatin dessert made from warm-water fish gelatin, which has a lower melting point than mammalian gelatin, showed a better release of aroma and gave a stronger flavor than gelatin dessert made of pork gelatin (Choi and Regenstein 2000). Furthermore, gelatins of high viscosity give chewier jellies than gelatins of low viscosity, which are more brittle (Jones 1977). Further work on characterizing the sensory properties of the various gelatins is needed. To study a phase change that has a time dependence requires that the sensory measurements must be able to capture the time dependence. Methods to do this for gelatin need to be worked out.

Gelatin is used in dairy products as a stabilizer and a texturing agent. It is widely employed in yogurt, ice cream, and other dairy products. Gelatin is added to yogurt to reduce syneresis and to increase firmness. Gelatin is an ingredient compatible with milk proteins, and gives a fat-like sensory perception because of its unique property of melting in the mouth. It also masks the product flavor less than some other gums (Jones 1977). Potentially, the use of different concentrations of gelatin would make it possible to obtain a wider range of textures, from the creamy, slightly gelled texture of yogurt to the firm, "moldable" gel of curd. In ice cream, gelatin is used to prevent the formation of coarse

ice crystals, and to give body and a firm smooth texture (Jones 1977). The gelatin concentration required for ice cream depends on its bloom strength and other factors such as melting point (Jones 1977).

Although gelatin has unique characteristics and is widely used in dairy products, there are continuing attempts to replace it with food polysaccharides. This is because the gelatin used in current dairy products is mainly from pork or non-religious slaughtered beef and is unacceptable to Jewish and Muslim consumers. Based on the gelatin status, the dairy products in the market can be divided into two main groups: those where gelatin is replaced with other gums and can meet U.S. normative kosher and halal requirements (a fairly small set of products) and those where it does contain a gelatin that is not permitted by any of the major U.S. normative kosher and halal certifying agencies and is not accepted by the normative Muslim and Jewish consumers. Gelatins from kosher and halal fish species, which include both warm-water fishes and cold-water fishes, may be a promising solution. Furthermore, fish gelatins can have a broad range of melting points, which may provide a food manufacturer with more choices in designing the texture and melting properties of dairy products. Some dairy products using fish gelatins have begun to appear in the marketplace. Most use foreign gelatin and most products are made overseas.

Gelatin has been used in confectionery products such as gummy-type products and marshmallows. Gummy-type products contain gelatin as the main gelling agent, because it offers the right texture and mouth feel. Marshmallow usually contains about 2-3% gelatin, where it serves as a stabilizer and whipping agent (Jones 1977). In recent years, marshmallows made from fish gelatin have become commercially available.

Gelatin can also be applied in wine fining and juice clarification, in meat products to absorb meat juices, and to give form and structure to products that would otherwise fall apart (Gelatin Manufacturers Institute of America 1993). Further information about food applications can be obtained from the reviews by Jones (1977), Johnston-Banks (1990), and Poppe (1997).

The non-food uses of collagen and gelatin are in the pharmaceutical industry, photographic industry, and other technical fields such as paper manufacture and printing processes (Gelatin Manufacturers Institute of America 1993). Although these are important applications, this review does not address any specific aspects of these uses.

Information resources

This is a limited introduction to collagen and gelatin from marine byproducts. For a more thorough understanding of collagen and gelatin, additional references should be consulted. The oldest review on

gelatin was a book entitled *Glue and Gelatin* by Alexander (1923). Half a century later, Ward and Courts (1977) edited an excellent book of contributed chapters, *The Science and Technology of Gelatin*, which covers almost every area related to gelatin and is still a very useful source of information for current collagen and gelatin producers and users, and for researchers. The book edited by Pearson et al. (1987), on the other hand, gives a solid introduction to collagen. The book by Veis (1964) mostly focuses on the chemical properties of collagen and gelatin. For those who are interested in the rheological properties of gelatin, the review article by te Nijenhuis (1997) would be very helpful. There are also several brief reviews on gelatin, including those by Johnston-Banks (1990) and Poppe (1997). In addition, the article prepared by the Gelatin Manufacturers Institute of America (1993) gives a good introduction to gelatin from the point of view of the gelatin manufactures.

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Extraction and Determination of Chondroitin Sulfate from Fish Processing Byproducts

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Abstract

Chondroitin sulfate (CS), a sulfated glycosaminoglycan, is an important structural component of connective tissues such as cartilage and the extracellular matrix. Along with glucosamine, CS has become a popular dietary supplement widely used for the treatment of osteoarthritis. Sources of CS include organs such as cow trachea, pig ears and snouts, and skeletons of sharks and skates. Fish processing byproducts, in particular fish heads, constitute a sizable source of cartilaginous material. This, coupled with kosher and halal dietary restrictions, may make fish heads a valuable source of CS. This study was conducted to determine the concentrations of chondroitin sulfate that are extractable from Alaska seafood processing byproducts. Samples of mixed fish processing waste including fish heads were sent to a commercial laboratory for analysis of CS concentration. Approximately 10 mg per g CS (1%) on a dry weight basis was found in pollock samples. High pressure liquid chromatography, and a spectrophotometric assay based on dimethylmethylene blue, were adapted to determine CS quantities in fish processing byproducts. The two analyses gave similar results. The

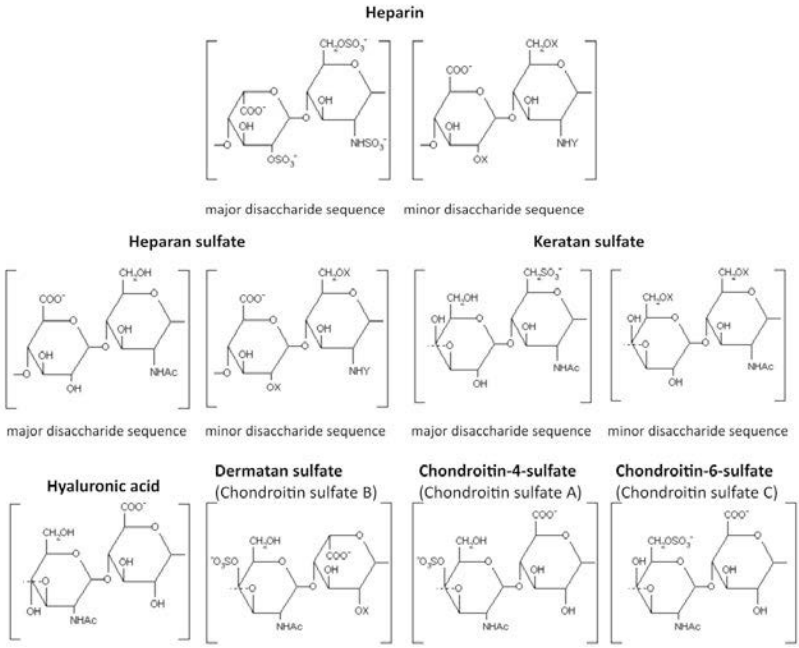


Figure 1. The structures of the major disaccharide repeating unit found in each glycosaminoglycans. Heparin, heparan sulfate, and keratan sulfate have diverse species, where X = SO_3^- or H and Y = SO_3^-, Ac, or H. From Mao et al. (2002) with permission.

HPLC results allowed determination of not only the quantity of CS but also the structure of the particular CS present in the samples. These results showed that chum salmon (*Oncorhynchus keta*) heads have a higher concentration of CS, with an average value of 19.7 mg per g, than the other salmon heads. This study indicates that fish processing byproducts, particularly salmon heads, could provide a readily available and economical source of CS.

Introduction

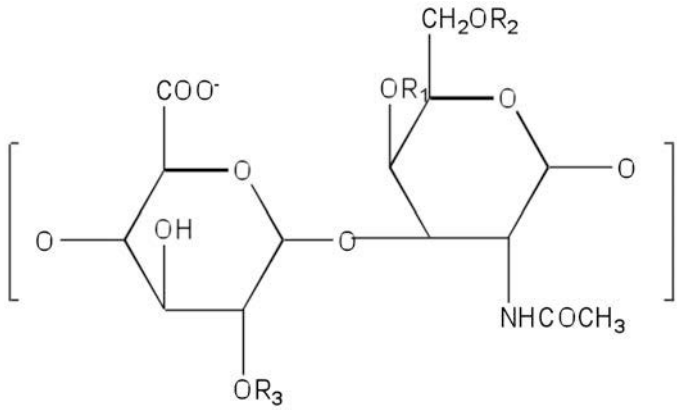
Glycosaminoglycans (GAGs) are linear, complex, polydisperse natural polysaccharides composed predominately of hyaluronic acid, keratan sulfate, chondroitin sulfate (CS), dermatan sulfate, and heparan sulfate (Fig. 1). GAGs are distributed as side chains of proteoglycans in the extracellular matrix or at the cell surface of animal tissues. With the exception of keratan sulfate, they are composed of alternating copoly-

mers of uronic acids and amino sugars, and their structures are commonly represented by typical disaccharide sequences. Although the polysaccharide backbones of these GAGs are simple, repetitive linear chains, these structures acquire a considerable degree of variability because of extensive modifications involving sulfation and uronate epimerization, which are the basis for the wide variety of domain structures with biological activities (Kjellen and Lindahl 1991).

CS is a major component of connective tissues and is partially responsible for providing their flexibility. Oral administration of CS may help treat symptoms of osteoarthritis (Pelletier et al. 1992; Malemud et al. 1995; Fawthrop et al. 1997; Uebelhart et al. 1998; Deal and Moskowitz 1999; McAlindon et al. 2000; Volpi 2004; Volpi 2006a,b) and as a result, dietary supplements containing CS that claim to promote healthy joints are readily available. Sources of CS used in nutritional supplements are the cartilaginous rings of bovine trachea, pork ears and snout, and shark cartilage. Other available sources of CS have been investigated by many researchers (Hjerpe et al. 1983, Luo et al. 2002, Jeske et al. 2007, Davies et al. 2008, Malavaki et al. 2008), with a wide variety of extraction and detection methods (Denuziere et al. 1997, Schiller et al. 1999, Sim et al. 2005, Volpi 2006a, Garnjanagoonchorn et al. 2007, Ji et al. 2007, Sakai et al. 2007, Hitchcock et al. 2008, Malavaki et al. 2008).

Quantitative analysis of CS in raw material and dietary supplements has been challenging owing to the wide molecular weight variation of CS polymers, its poor UV absorbance, and its strongly ionic nature. Other related GAGs may be present as impurities or adulterants; therefore any analytical methodology designed to quantify CS must be selective for CS in the presence of these other GAGs. The carbazole reaction (Dische 1950, Dische and Rothschild 1967), cetyl pyridinium chloride (CPC) titration (Liang et al. 2002), and size exclusion chromatography (Way et al. 2000) have been used to characterize CS. However, these methods cannot distinguish between CS and related GAGs, and are subject to interference. CPC titration, in particular, is a common method for determining CS purity; however, this method not only cannot distinguish between CS and other GAGs, but also will give false positive results for any large molecular anion, including carrageenan, proteins, and surfactants. CS is composed of alternate sequences of D-glucuronic acid and differently sulfated *N*-acetyl-D-galactosamine residues linked by b(1→3) bonds. As shown in Fig. 2, CS has a complex structure that is known to change with animal source as well as individual tissues and organs (Volpi 2007). There are three major molecular species of CS, nonsulfated chondroitin (Di-0S), chondroitin sulfate A (Di-4S), and chondroitin sulfate C (Di-6S). These individual species vary in the position of the sulfate group on the *N*-acetyl-D-galactosamine unit.

There is a general lack of literature on the CS content in fish byproducts with the exception of collagenous material from shark and skate



$R_1 = R_2 = R_3 = H$: nonsulfated chondroitin
$R_1 = SO_3^-$ and $R_2 = R_3 = H$: chondroitin-4-sulfate, CSA
$R_2 = SO_3^-$ and $R_1 = R_3 = H$: chondroitin-6-sulfate, CSC
$R_2 = R_3 = SO_3^-$ and $R_1 = H$: chondroitin-2,6-disulfate, CSD
$R_1 = R_2 = SO_3^-$ and $R_3 = H$: chondroitin-4,6-disulfate, CSE
$R_1 = R_3 = SO_3^-$ and $R_2 = H$: chondroitin-2,4-disulfate, CSB
$R_1 = R_2 = R_3 = SO_3^-$: trisulfated chondroitin

Figure 2. Structures of disaccharides forming chondroitin sulfate.

(Lignot et al. 2003, Garnjanagoonchorn et al. 2007). However, processing byproducts are available in Alaska from a number of different species in large volumes, and are a potential source for commercial CS extraction. The goal of this study was to develop procedures for determining the CS content in processing byproducts such as fish heads. Methods of extracting CS from salmon heads were developed and CS content was determined using spectrophotometric and HPLC detection. Detection using the dimethylmethylene blue assay is fast and simple, but it has the potential to give misleading results caused by interference from other GAG complexes. Therefore, the dimethylmethylene blue assay was compared to an HPLC method for CS quantitation. Previous research has shown that by employing this method, all CS species present can be identified (Ji et al. 2007).

Samples

Samples for the pilot study included pollock (*Theragra chalcogramma*) byproduct heads, viscera, skin, and frames from an automated processing line. Salmon byproducts included pink (*Oncorhynchus gorbuscha*) and red (*O. nerka*) salmon heads, pink viscera, and pink and chum (*O.*

keta) carcasses. The stickwater sample was obtained from a commercial fish meal plant processing whitefish byproducts. All samples were lyopholyzed and sent to Silliker JR Laboratories, ULC (Burnaby, B.C.) for CS analysis. Fresh red, pink, chum, and king (*O. tshawytscha*) salmon heads were obtained from salmon delivered to Kodiak during summer 2007 and were immediately placed in plastic buckets and frozen at –40ºC. Prior to grinding, heads from each species were sorted into five separate samples that were then individually ground, resulting in 20 samples (4 species and 5 samples per species). Samples were stored in a –20ºC freezer until analyzed for CS.

Methods

Sample digestion

Samples of fish byproducts were lyophilized and ground to a powder. Approximately 1 g was placed into a 10 ml test tube. To this was added 10 ml of digestion solution (100mM phosphate buffer pH 6.80, 5mM EDTA, 5mM cysteine HCl, and 1.2 mg per ml papain). Samples were incubated at 45ºC in a water bath for 24 hours with agitation. After digestion, 600 µl of quench solution (1M $NaBH_4$, 50mM NaOH) was added and samples incubated at 45ºC in a water bath for 16 hours with agitation. Samples were neutralized with acetic acid. Samples were centrifuged at 1,800 G and the supernatant passed through a Whatman 0.45 µm syringe filter. These solutions were then analyzed for chondroitin sulfate (CS) content using either the dimethylmethylene blue assay or HPLC method. All chemicals were obtained either from Sigma or ACROS.

Dimethylmethylene blue assay for CS quantitation

The dimethylmethylene blue assay was adapted from Farndale et al. (1982, 1986). The dimethylmethylene blue solution was prepared in a 1 L volumetric flask; to the flask was added 16 mg 1,9 dimethylmethylene blue, 5 ml ethanol, 2.0 g sodium formate, 2.0 ml formic acid, and then the flask was filled with deionized water and sonicated. A new standard curve was created for each measurement with bovine CS. Two mg shark and bovine CS were dissolved into 100 ml deionized water (DI) to create stock solutions, and standards were created by diluting the stock with DI. These standards, ranging in concentration from 0 to 100 µg per ml, were then analyzed using the dimethylmethylene blue assay to see if the test had any bias as to sample origin. To perform a measurement 25 µl of CS standards were loaded onto the same microplate as 25 µl of the samples. To these samples 200 µl of dimethylmethylene blue solution was added and the absorbance at 525 nm was measured within 30 seconds using a SpectraMax Plus 384 (Molecular Devices, Sunnyvale) plate reader. The assay was conducted at 21ºC.

Table 1. HPLC linear mobile phase gradient, chondroitin sulfate quantitation.

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0	80	20
0-7	35	65
7-12	35	65
12-12.5	80	20

The column was re-equilibrated at the starting mobile phase conditions for 10 min after each injection. See text for details.

HPLC method for CS quantitation

The HPLC method used was a modification of Ji et al. (2007). Briefly, 20 µl of digested sample is mixed with 20 µl TRIS buffer and 30 µl enzyme solution (chondroitinase ABC 10 U per ml, Sigma) and the mixture heated at 37ºC for 3 hours. The samples are cooled to room temperature and brought up to a 1 ml total volume with HPLC solvent A. Separation was made on a Waters Nova-Pak C18 column (4 µm particle size, 3.9 × 150 mm). Mobile phases for HPLC analysis were as follows: solvent A—340 mg tetrabutylammonium bisulfate in 1 L DI; and B—340 mg tetrabutylammonium bisulfate in 330 ml DI and 670 ml acetonitrile (ACROS). Solvents were degassed and filtered using vacuum filtration with Fisher P4 filter paper before use. Standards and samples were injected into a Beckman System Gold (Beckman Coulter, Fullerton) with 166 diode array UV detector, 508 autosampler, and 32 Karat software V8.0. The HPLC method followed the gradient elution program of mobile phases A and B shown in Table 1. A standard curve was created for the known compounds Di-0S, Di-4S and Di-6S (Sigma) as described by Ji et al. (2007).

Statistical analysis

Five replicates of each head sample were analyzed for CS using the dimethylmethylene blue assay and the HPLC method. One-way analysis of variance (ANOVA) test were used to assess statistical significance ($P < 0.05$) for CS concentration and subjected to a Tukey's post hoc test. ANOVA was run on Statistica version 6.0 (StatSoft Inc., Tulsa). Regression line and equations for CS standards were developed with Microsoft Excel (Redmond).

Table 2. Chondroitin sulfate results (mg/g, dry weight) from Silliker Labs pilot study. Red salmon heads, n = 3; all others, n = 1.

Pollock heads	9.8
Pollock viscera	13.8
Pollock skin	9.1
Pollock frames	8.7
Whitefish stickwater	15.4
Pink salmon viscera	17.3
Pink salmon heads	15.6
Pink salmon carcass	16.4
Chum salmon carcass	19.5
Red salmon heads	8.8

Results

Silliker Labs results

The results from the samples sent to Silliker Labs are presented in Table 2. Although replicate measurements were not performed on the samples, the results indicated that fish byproducts could be a viable source of chondroitin sulfate (CS), with CS concentrations ranging from approximately 9 to 19 mg per g on dry weight basis. Predicated on these results, an in-house method for the quantitative analysis of CS in fish byproducts was developed.

Dimethylmethylene blue assay

The dimethylmethylene blue method was very fast and simple. As can be seen in Fig. 3, the dimethylmethylene blue assay gave a linear response from 0 to 80 µg per ml. The method was shown to be equally responsive to bovine and shark CS. Based on the equation obtained from the standard curve the amount of CS was calculated in the unknown samples and the average of the five samples for each species was calculated. Table 3 presents the CS content for salmon heads from each of the four species. The data are presented as means and standard deviation.

HPLC method

Samples were analyzed using HPLC to determine the distribution of the three predominant CS species and to ascertain if the two assays would give consistent results. To do this standard curves of the most common

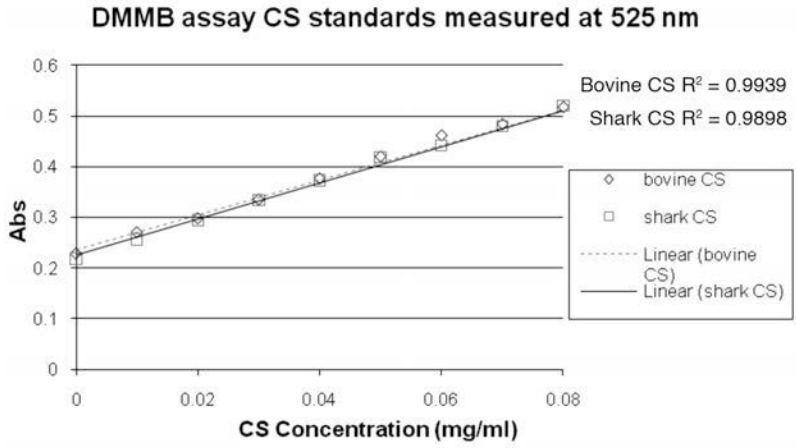


Figure 3. Graph of chondroitin sulfate concentration vs. absorbance after 30 second exposure to dimethylmethylene blue solution, measured at 525 nm at 21ºC.

Table 3. Chondroitin sulfate concentration (mg/g, dry weight) and standard deviation (n = 5) of salmon heads using dimethylmethylene blue assay.

Sample	Mean	SD
King	14.59	3.06
Chum	23.22	3.19
Red	16.58	6.17
Pink	15.18	3.29

Table 4. Average chondroitin sulfate concentration (mg/g, dry weight) and standard deviation (n = 5) of salmon heads using HPLC analysis.

Sample	Mean	SD
King	13.96	2.82
Chum	19.69	2.96
Red	14.28	4.81
Pink	14.22	2.88

CS species, Di-0S, Di-4S, and Di-6S, were generated as shown in Fig. 4. CS Di-0S gave a linear response from 0 to 20 µg ml, while Di-4S and Di-6S gave linear responses from 0 to 40 µg per ml. Based on the equation obtained from each standard curve, the amount of CS was calculated for each CS form and the sum of each gave the total CS concentration. The average for each fish species sample (n = 5) was calculated. To ensure accuracy, a known CS standard from bovine trachea (Sigma) was analyzed by the same methods. The methods were tested against the known bovine CS concentration and verified.

Shark CS standard was also analyzed using this method; however, because of the presence of another CS species, di-di(2,6)S, which was not present in our samples, the concentration could not be accurately calculated. To analyze for possible matrix interference, samples were split in two equal portions—filtered and unfiltered as described in the methods section—and analyzed. Table 4 gives the combined results for the HPLC methods.

Discussion

The dimethylmethylene blue assay demonstrated a linear standard curve for both shark and bovine chondroitin sulfate (CS) as seen in Fig. 3. The three most common forms of CS also gave linear standard curves, Fig. 4, when analyzed by HPLC and gave accurate results for known samples. Although shark CS was not quantitated by the HPLC method, it could be easily accomplished in future work by employing a standard for di-di(2,6)S. This was not pursued because the samples did not display a measureable quantity of di-di(2,6)S. The dimethylmethylene blue samples gave statistically similar results ($P = 0.05$) to both the HPLC samples.

Previous studies on CS have focused only on extracted cartilaginous material, normally trachea in the case of bovine, pork, and

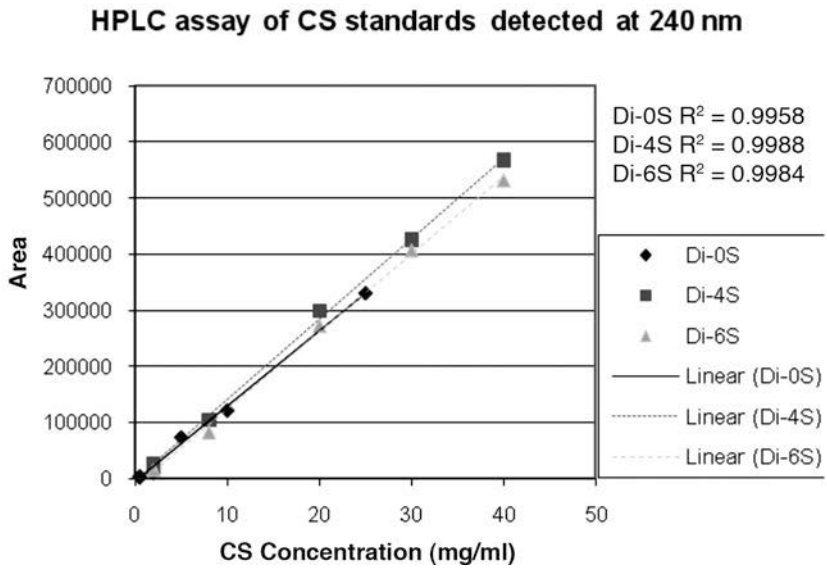


Figure 4. HPLC standard curve of the three main chondroitin sulfate species, Di-0S, Di-4S, Di-6S detected at 240 nm.

avian, and fins for shark and skate (Luo et al. 2002, Lignot et al. 2003, Garnjanagoonchorn et al. 2007, Jeske et al. 2007, Sakai et al. 2007, Xu et al. 2008). To the authors' knowledge this is the first study examining CS concentration in groundfish byproducts. The accuracy of the study was demonstrated by obtaining the expected CS concentration for a known sample (Ji et al. 2007). This study has shown that one can obtain statistically similar CS concentration using the dimethylmethylene blue assay or the HPLC method.

Conclusion

In summary, both analysis methods give the same results. Based on these results it was determined that chum salmon heads have a significantly higher concentration ($P < 0.05$) of CS than the other salmon heads analyzed, equal to an average value of 19.7 mg per g. The three other species, king, red, and pink, did not show a statistical difference in the amount of CS present ($P > 0.05$).

The extraction and quantification of chondroitin sulfate was evaluated for fish processing byproducts to determine if they could constitute a viable source of CS. The dimethylmethylene blue assay, while unable

to distinguish between CS forms, was fast and simple. The HPLC method gave the concentrations for each CS form and agreed well with dimethylmethylene blue assay data. For quantification of total CS concentration, the dimethylmethylene blue assay will give fast and accurate results. However, to determine the composition of the CS forms within a sample, the HPLC method provides more specific information.

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Characterization of Dried Heads from Five Pacific Salmon Species, Dried at Different Temperatures

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Abstract

In many cultures, fish heads are a preferred part of a traditional diet; however, this is not the case in North America or northern Europe. In Alaska, heads are removed from the fish in processing and are a major component of the waste stream with ~65,000 t produced per year. These heads are generated from human food processing lines and, therefore, can be used for the manufacture of human grade food products.

Our objective was to evaluate the effects of low temperature drying on the quality and chemical composition of the protein and oil from heads of five different species of wild Pacific salmon harvested in Alaska. Heads from each of the species were processed and dried at 40ºC or 77ºC. Temperature did not measurably affect the proximate composition, fatty acid concentrations or, for the most part, water activities of the salmon head meals. Meals dried at 40ºC had similar nutritional value, were of uniform high quality, and showed potential for commercialization as a human grade food supplement or food ingredient.

The composition of the dried salmon heads provides a product with a unique and very desirable nutritional profile derived from sustainably managed fisheries.

Introduction

In Alaska's wild salmon industry, considerable volumes of processing byproducts are discarded each year (Crapo and Bechtel 2003). This is because Alaska salmon are often processed in small, remote, seasonal operations along the Gulf of Alaska and in Bristol Bay that are allowed to "grind and dump" their processing waste into local tidal waters, as long as certain environmental restrictions are observed. However, many processors speculate that these rules may be changing and that soon smaller operators will no longer be allowed to discard processing waste. In anticipation of such changes, alternatives should be explored in terms of effectiveness (Kristinsson and Rasco 2000).

Alaska pollock, harvested along the Bering Sea, is the largest human food capture fishery on earth. Large shoreside processing plants are required by law to effectively handle their processing waste by making fish meal, bone meal, solubles, and fish oil (Smiley et al. 2003). Large wet-reduction plants, costing millions of dollars, were required to be in place before these operations could begin processing fish. Fish processing byproducts from cold-water marine species delivered to human food plants are excellent sources of high quality coproducts that can be used as food and feed ingredients with industrial applications (Sathivel and Bechtel 2006). For the smaller and seasonal salmon processors in Alaska, such wet-reduction plants are prohibitively expensive (Smiley et al. 2003). The Bering Sea pollock operations handle upwards of 1,600 metric tons (t) per day of processing waste, while Alaska's salmon processors produce 5 to 250 tons of waste per day. Drying salmon heads to make a high quality protein meal for use as food or feed ingredients offers a relatively inexpensive method to handle a large fraction of the waste volume for these processors, provided there is sufficient commercial interest in the final product. Also, there is the potential for developing export markets for dried salmon heads. Currently in Alaska, a very small percentage of Pacific cod heads are frozen and shipped to Asia, and heads from pollock, salmon, and other species are at best used as raw material for fish meal and oil. Salmon heads with their high fat content (Bechtel 2003) are a desirable food in some cultures, but they need to be dried to reduce storage and transportation costs.

This study evaluates the effects of low temperature drying on the quality and chemical composition of protein meals made from heads isolated from the five different species of wild Pacific salmon available commercially in Alaska. Drying at lower temperatures prolongs the process, all other variables being equal. This can result in higher pro-

duction costs. The question is whether the prolonged time and greater cost is made worthwhile by expanding market opportunities and/or in raising the value of the final product.

Alaska and Canada (as well as the U.S. west coast) have different common names for the five Pacific salmon species. *Oncorhynchus gorbuscha* is the pink (Alaska) or humpy (Canada) salmon. *Oncorhynchus nerka* is the red (Alaska) or sockeye (Canada) salmon. *Oncorhynchus keta* is the dog (Alaska) or chum (Canada) salmon. *Oncorhynchus kisutch* is the silver (Alaska) or coho (Canada) salmon. *Oncorhynchus tshawytscha* is the king (Alaska) or in Canada, the chinook or spring salmon.

Fish protein meals have been evaluated as a food ingredient for some time. In 1993, the Association of Danish Fish Processing Industries and Exporters commercially produced fish protein meals for use with frozen products to enhance water binding and frozen stability properties (Urch 2001). Sathivel et al. (2003) reported that protein powders made from herring and arrowtooth flounder were good sources of high quality protein with desirable functional properties. Phillips et al. (1994) reported that protein-rich seafood byproducts can have a range of dynamic properties and could potentially be useful to food manufacturers as binders, emulsifiers, and gelling agents. Sathivel et al. (2005) indicated that soluble protein powders made from salmon heads could be employed as an emulsifier or as nutritional supplements. Fish are regarded as an excellent source of high quality protein, particularly in their concentrations of the essential amino acids lysine and methionine (Sathivel and Bechtel 2006), and long-chain polyunsaturated fatty acids (PUFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). There are a number of reasons why fish proteins are not widely used in food ingredients, including the loss of protein functionality as a result of processing, and the negative sensory properties of oxidized and partially oxidized fish lipids (Sathivel and Bechtel 2006).

Marine lipids, such as those found in wild salmon, are of significant interest because of the health benefits associated with high levels of the long chain omega-3 PUFA, EPA and DHA. Heads of Pacific salmon are particularly rich in these lipids (Bower et al. 2007). Among the health benefits are decreased rates of cardiovascular disease (Shahidi and Miraliakbari 2004), and normal brain development and retina formation in infants (Hoffman and Uauy 1992).

The annual harvest of Pacific salmon from Alaska waters can be over 330,000 t (ADFG 2007). An estimated 110,000 t of processing waste was produced by Alaska salmon processors in 2005 (Bechtel 2007). The two major byproducts from the processing lines are heads (~65,000 t per year) and viscera (ADFG 2007). In Alaska, wild pink and red salmon are harvested in large quantities, and pink salmon byproducts have been reported to contain fish oil at levels over 10% by weight in the heads and about 2% in the viscera (Bechtel 2003). Alaska pink salmon oils were

analyzed by Oliveira and Bechtel (2005) and are an excellent source of omega-3 fatty acids.

Traditionally, fish heads have been a part of human diets in many cultures and dried fish heads can be found in markets places across the world. One goal of the Alaska salmon industry is to increase the number of salmon food product forms, as well as to convert processing byproducts into more valuable foods or food ingredients. Salmon heads generated on Alaska fish processing lines can be dried whole for export or processed into human grade protein powders and oil. However, much of this byproduct stream remains underutilized. Perhaps the most important reasons are economic, such as the cost of new equipment and the price of energy in remote Alaska. Drying is an expensive operation requiring roughly 1,000 BTUs to remove a pound of water (Van Arsdel and Copley 1963). The objective of this research was to evaluate the effects of two drying temperatures on the composition of protein meals made from heads of the five different species of wild Pacific salmon available commercially in Alaska, and as far as possible, to provide sufficient quality information for a cogent economic analysis of meals made from heads as a new value added salmon product.

Materials and methods

Salmon head sampling

Pink, red, chum, silver, and king salmon heads were procured from a local seafood processor in Kodiak, Alaska, and kept frozen at –40ºC until processed. For each of three experimental replicates three fish heads from each species were rinsed, hearts, gills and other attached viscera were removed, and heads were split in half and dried at a temperature of either 40ºC (104ºF) for 36 h or 77ºC (170.6ºF) for 22 h. The drying temperatures were chosen to reflect a cooking (77ºC) and noncooking (40ºC) regime for salmon flesh. Drying was performed at 100% fan speed until samples contained less than 10% moisture using an Enviro-Pak air dryer (MP-2500/CHU-150E, Clackamas). Dried heads were finely ground, vacuum packaged, and stored at –27ºC until analyzed. For each sample, three separate determinations were run, with three replicates run for each treatment. Samples were analyzed for proximate composition, water activity, amino acids, mineral composition, and fatty acid profiles using previously published methods.

Proximate composition

Nitrogen content was determined by pyrolysis using a Leco FP-2000 nitrogen analyzer (LECO Co., St. Joseph). Protein content was calculated as 6.25 times percent nitrogen. Total lipid content was determined using the method of Folch et al. (1957). After lipid extraction, the solvent

was removed at 49ºC on a rotary evaporator (Büchi Rotavapor R-205, Westbury) and lipids transferred into a pre-weighed amber screw-top vial. The remaining solvent was removed under a nitrogen gas stream until constant weight was achieved and percent lipid determined. Oils were stored in chloroform containing 0.01% BHT at –70ºC until analysis. Moisture and ash content were determined using AOAC methods (AOAC 2005) no. 952.08 and no. 938.08, respectively.

Water activity

Water activity measurements of dried head meals were determined at room temperature using an Aqualab water activity meter (Model Series 3TE, Decagon Devices Inc., Pullman).

Amino acid profiles

Amino acid profiles were determined by the AAA Service Laboratory Inc. (Boring). Samples were hydrolyzed with 6N HCL and 2% phenol at 110ºC for 22 h. Amino acids were quantified using a Beckman 6300 analyzer with post column ninhydrin derivitization.

Mineral analysis

Samples for mineral analysis were sent to the University of Alaska Fairbanks, School of Natural Resources and Agricultural Sciences, Palmer Research Center, where they were ashed overnight at 550ºC. Ashing residues were subsequently digested overnight in an aqueous solution containing 10% (v/v) hydrochloric and 10% (v/v) nitric acids. Digests were diluted as needed and analyzed for P, K, Ca, Mg, S, Na, Zn, Mn, and Fe by inductively coupled plasma optical emission spectrometry on a Perkin Elmer Optima 3000 Radial ICP-OES.

Fatty acid analysis

Fatty acid methyl esters (FAMEs) were prepared using KOH and methanol as described by Maxwell and Marmer (1983). The FAME mixture was transferred to 1.5 ml snap-cap amber GC vials and immediately analyzed on a GC model 6850 (Agilent Technologies, Wilmington). Chromatographic conditions were previously described (Bechtel and Oliveira 2006). Identification of peaks was performed using Supelco® (Bellefonte) standards Marine Oil #1, Marine Oil #3, S-37, and Bacterial Acid Methyl Esters Mix. Samples were run in triplicate and expressed as mg per g of dried head powder. Cod liver oil was used as a secondary reference standard (Ackman and Burgher 1965).

Statistical analysis

A *t*-test was used to determine whether the values for heads dried at 40ºC were different from those for heads dried at 77ºC. Analysis of the

data was done for proximates, water activity, and select FAME data. Neither the amino acid nor the mineral concentrations were analyzed because there was only one value from a composite sample for each of these measurements. The *t*-test was used to identify significant differences between the two temperature treatments with $\alpha = 0.05$. Sample number was three for each treatment and sample value was the average of three determinations. The data analysis package for Microsoft Excel (Microsoft Office 2004 for Apple Mac, Redmond) was used for these analyses.

Results and discussion

Overall, salmon head meals dried at the lower temperature of 40ºC showed results that were similar to the results obtained for the salmon head meals dried at 77ºC. There was no compelling pattern of significant differences between the 40º and the 77º for the proximate analysis, water activity, or FAME data. Clearly, whether the residual flesh or the lipids in the heads were cooked and dried at 77ºC or dried at 40ºC made little difference in the chemical makeup of the heads. A potent new sales issue among minimally processed organic foods is the processing temperature, as evidenced by the increase in the "cold-pressed" appellation applied to both food and nutraceutical oils. According to the online site Wisegeek, "Cold pressed oil is oil that has been produced with the use of a low heat technique. The introduction of heat to the process of making oil will degrade the flavor, nutritional value, and color of the oil. Heat, however, increases the yield. For this reason, cold pressed oil tends to be more expensive, although it is also of higher quality."

The marketing cachet of low temperature when applied to the drying of salmon heads may still be of value, even though the data do not robustly support it. Certainly, the materials dried at 40ºC were as good as or marginally better than those dried at 77ºC.

Proximate composition

Table 1 shows the results for the analysis of proximate composition and water activity. The protein content of the dried heads ranged from 33% to a high of 53%. None of the salmon species showed any significant difference in protein concentration between the treatments. The dried heads contained large amounts of lipid. There were no significant differences due to treatment for any of the species for either lipid or ash concentration. Moisture in pink salmon heads was significantly higher ($p < 0.02$) in the 40ºC dried heads than in the 77ºC dried heads. As a generality the dried salmon heads contained over 33% protein, over 40% lipids, and over 10% ash. The exception was the dried chum heads, which were highest in protein and ash but lowest in lipid content. The composition of the dried salmon heads provides a product with a

Table 1. Proximate analysis (percent wt/wt) and water activity of dried salmon heads.

		Pinks	Reds	Chums	Silvers	Kings
Protein	40º	41.5 ± 1.6	35.3 ± 2.2	46.3 ± 1.6	33.4 ± 0.7	40.0 ± 3.7
	77º	42.3 ± 2.1	34.6 ± 3.7	52.6 ± 1.1	33.8 ± 1.4	40.7 ± 7.2
Lipid	40º	40.3 ± 4.0	46.5 ± 8.5	28.3 ± 5.7	49.8 ± 0.3	42.4 ± 3.5
	77º	40.4 ± 7.2	48.1 ± 3.2	23.0 ± 2.0	47.3 ± 1.7	42.8 ± 6.5
Ash	40º	13.1 ± 0.5	11.9 ± 0.6	17.8 ± 4.4	11.1 ± 0.6	9.7 ± 1.3
	77º	13.9 ± 0.6	11.6 ± 1.1	19.3 ± 1.1	10.4 ± 0.6	9.6 ± 2.6
Moisture	40º	**5.2 ± 1.2[a]**	6.2 ± 1.1	**7.6 ± 1.6[a]**	**5.6 ± 0.5[a]**	7.9 ± 1.0
	77º	**3.5 ± 3.2[a]**	5.7 ± 1.6	**5.1 ± 3.1[a]**	**8.5 ± 1.7[a]**	6.8 ± 2.9
Water activity	40º	0.24 ± 0.01	**0.43 ± 0.01[a]**	0.28 ± 0.00	**0.46 ± 0.01[a]**	0.53 ± 0.01
	77º	0.25 ± 0.01	**0.64 ± 0.02[a]**	0.27 ± 0.00	**0.73 ± 0.00[a]**	0.64 ± 0.01

[a]Significant difference between values for the two temperatures ($p < 0.05$), n = 3.

unique and very desirable nutritional profile derived from sustainably managed fisheries.

Water activity values ranged from 0.24 for pink salmon to a high of 0.73 for the 77ºC silver salmon heads. As expected, higher moisture content was associated with higher water activity values for most treatments. Water activity was higher in the heads dried at 77ºC for both red ($p < 0.04$) and silver ($p < 0.02$) salmon, but there was no statistically significant difference between temperatures for pink, chum, or king salmon dried head meals.

Amino acids

Table 2 lists the amino acid composition of the heads from the five salmon species dried both at 40ºC and 77ºC as a percent of total amino acid composition. The data consists of one measurement for both 40ºC and 77ºC for each of the five species. Replicates were not sought because of the expense of each analysis. In general, the amino acid profiles were consistent with those determined for Alaska fish processing byproduct meals made by the wet-reduction method (Smiley et al. 2003) and by hydrolysis (Smiley et al. 2010, this volume). The relatively high levels of lysine (5.9% to 7.0%) and methionine (2.8% to 3.6%) indicate that the protein in the head meals is of high nutritional value. The hydroxyproline content ranged from 2.7 to 4.3, indicating substantial levels of collagen in the heads. This is expected, as collagen would be found in the cartilaginous materials and bone found in the heads.

Mineral composition

Table 3 shows the mineral content of the salmon head meals dried at 40ºC and 77ºC. Expense prevented replicate analyses. The values for mineral content in the salmon head meals are similar to those reported

Table 2. Amino acid analysis of dried salmon heads.

	ºC	Pinks	Reds	Chums	Silvers	Kings
ALA	40º	6.4	6.9	6.4	7.3	6.6
	77º	6.9	6.6	6.3	7.1	6.5
ARG	40º	6.9	7.6	7.1	7.1	6.9
	77º	7.3	7.5	7.3	7.0	6.7
ASP	40º	8.9	8.2	8.4	9.0	8.6
	77º	8.5	8.5	8.6	8.2	8.6
GLU	40º	13.3	12.4	11.2	12.9	13.1
	77º	12.8	12.7	12.7	12.4	13.3
GLY	40º	10.4	14.2	13.5	14.0	10.9
	77º	12.6	11.8	11.4	14.2	10.8
HIS	40º	2.6	2.4	2.4	2.1	2.7
	77º	2.6	2.5	2.8	2.3	2.4
HYP	40º	2.7	4.1	4.3	3.9	2.9
	77º	3.3	3.2	3.0	4.3	2.7
ILE	40º	3.8	3.1	3.2	3.4	3.8
	77º	3.5	3.5	3.7	3.1	4.0
LEU	40º	6.5	5.2	5.3	5.7	6.2
	77º	5.8	6.0	6.1	5.3	6.4
LYS	40º	6.8	5.7	5.9	6.0	7.0
	77º	6.0	6.3	6.5	5.9	6.1
MET	40º	3.6	3.3	3.4	2.4	3.1
	77º	2.9	2.8	3.1	2.8	2.8
PHE	40º	3.9	3.6	3.6	3.4	4.0
	77º	3.9	4.1	4.0	3.6	4.6
PRO	40º	6.3	7.6	7.7	7.4	6.9
	77º	6.7	7.1	7.0	8.0	7.1
SER	40º	4.4	4.6	4.5	4.4	4.3
	77º	4.4	4.3	4.4	4.4	4.3
THR	40º	4.2	3.8	4.2	3.8	4.2
	77º	3.9	4.0	4.1	3.7	4.1
TYR	40º	3.5	2.7	3.0	2.6	3.2
	77º	3.4	3.7	3.3	2.9	3.8
VAL	40º	6.0	4.7	4.6	4.1	5.8
	77º	5.5	5.4	5.8	4.9	5.8

Values are percent of total amino acids analyzed.
TRP and CYS not analyzed.

Table 3. Mineral analysis.

	ºC	Pinks	Reds	Chums	Silvers	Kings
P[a]	40º	2.1	2.3	2.8	1.6	1.8
	77º	1.5	1.8	2.8	1.6	1.6
K[a]	40º	0.5	0.5	0.6	0.4	0.5
	77º	0.5	0.4	0.5	0.4	0.5
Ca[a]	40º	3.6	4.1	4.9	2.8	2.9
	77º	2.5	3.1	5	2.7	2.7
Mg[a]	40º	0.1	0.1	0.2	0.1	0.1
	77º	0.1	0.1	0.2	0.1	0.1
S[a]	40º	0.7	0.7	0.8	0.6	0.6
	77º	0.6	0.6	0.8	0.5	0.6
Na[a]	40º	4.3	3.8	6.1	3.3	2.0
	77º	3.6	3.5	6.7	3.0	2.0
Zn[b]	40º	76.0	72.0	61.0	43.0	44.0
	77º	59.0	66.0	57.0	37.0	41.0
Mn[b]	40º	1.0	1.0	2.0	1.0	ND
	77º	BDL	1.0	2.0	1.0	1.0
Fe[b]	40º	73.0	67.0	101.0	56.0	ND
	77º	51.0	78.0	44.0	38.0	86.0

BDL = below detection level, ND = not determined.
[a]Percent of dry matter.
[b]Parts per million, ppm.

in other studies (Sathivel et al. 2005, Sathivel and Bechtel 2006). Mineral concentrations appear slightly higher in the heads dried at 40ºC and could be due to loss of minerals in the cook drippings. As expected for a product containing bone, the calcium content was 2.5% or higher (dry weight) and the Ca:P ratio was over 1.6. Also the heads were good sources of the microminerals zinc and iron.

Fatty acid analysis

Table 4 shows the nine most abundant fatty acids found in the heads (percent fatty acid of total fatty acids). Together these nine fatty acids account for about 75% of the total fatty acids for each species at each drying temperature. Myristic acid (14:0) was significantly ($p < 0.05$) higher in meals dried at 40ºC than at 77ºC for pink salmon. Among the long-chain polyunsaturated fatty acids (PUFA), EPA was significantly higher at 77ºC in both chum ($p < 0.01$) and silver ($p < 0.05$) heads. DHA was significantly more abundant ($p < 0.02$) at 77ºC for red salmon heads. In conclusion, there appears to be relatively little difference in the fatty acid content whether the meals were dried at 40ºC or 77ºC.

Table 4. Abundant fatty acids in dried salmon heads.

Fatty acid	ºC	Pinks	Reds	Chums	Silvers	Kings
14:0	40º	5.28 ± 0.24[a]	4.66 ± 0.02	7.46 ± 0.22	4.96 ± 0.19	5.56 ± 0.76
	77º	4.86 ± 0.07[a]	4.50 ± 0.23	7.08 ± 0.27	4.78 ± 0.17	4.54 ± 0.90
16:0	40º	14.20 ± 0.44	14.96 ± 0.15	12.84 ± 0.27	14.33 ± 0.81	15.24 ± 0.82
	77º	14.08 ± 0.24	14.16 ± 0.16	12.19 ± 0.37	13.54 ± 0.04	14.83 ± 0.46
16:1ω7	40º	4.45 ± 0.05	4.63 ± 0.26	4.34 ± 0.08	4.58 ± 0.14	6.10 ± 0.73
	77º	4.07 ± 0.04	4.33 ± 0.27	4.50 ± 0.22	4.66 ± 0.29	5.85 ± 1.38
18:1ω9 cis	40º	13.95 ± 1.53	16.24 ± 0.18	19.55 ± 0.82	13.16 ± 1.51	15.18 ± 0.79
	77º	12.42 ± 0.21	15.98 ± 0.73	18.26 ± 1.25	12.71 ± 0.29	16.11 ± 2.34
20:1ω9	40º	3.40 ± 0.22	4.11 ± 0.23	3.43 ± 0.24	4.29 ± 0.26	3.87 ± 0.04
	77º	3.21 ± 0.18	3.80 ± 0.11	3.25 ± 0.30	4.16 ± 0.14	3.49 ± 0.33
20:1ω11	40º	7.91 ± 0.33	8.65 ± 0.06	6.09 ± 0.41	8.65 ± 1.16	6.87 ± 0.27
	77º	7.99 ± 0.19	8.10 ± 0.60	6.93 ± 0.74	8.32 ± 0.21	6.15 ± 1.13
20:5ω3	40º	6.67 ± 0.46	7.38 ± 0.46	5.35 ± 0.11[a]	7.13 ± 0.22[a]	7.50 ± 0.86
	77º	7.37 ± 0.28	8.45 ± 0.08	6.27 ± 0.15[a]	8.09 ± 0.47[a]	8.65 ± 0.80
22:1ω11	40º	9.90 ± 0.93	9.05 ± 0.53	7.30 ± 0.42	9.66 ± 1.33	8.34 ± 0.69
	77º	10.42 ± 0.50	8.33 ± 0.26	7.93 ± 0.90	9.36 ± 0.19	7.01 ± 1.22
22:6ω3	40º	9.58 ± 1.15	7.28 ± 0.33[a]	5.00 ± 0.11	11.18 ± 0.74	8.61 ± 2.34
	77º	11.60 ± 0.20	8.92 ± 0.62[a]	5.36 ± 0.64	11.88 ± 0.52	10.59 ± 3.19

Values are percent of total fatty acids, average and standard deviation.
[a]Significant difference between values for the two temperatures ($p < 0.05$) n = 3.

Conclusion

The goal of this project was to determine whether drying salmon heads at 40ºC led to a compromise in the quality or chemistry of the finished meals. The data generated show some differences, most of which are relatively small. Therefore, should there be a desire to use the market cachet of "low temperature minimal processing" as a mechanism for market penetration, the product would be considered at least as high in quality as material dried at 77ºC. In conclusion, heads from salmon processed in Alaska, material generally destined for the waste stream, can be turned into a healthful ingredient, rich in high quality protein and lipids. The next step is to perform an economic analysis to determine a breakeven point for commercial production of a protein meal made from dried salmon heads.

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Functional Proteins from Catfish Roe

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Abstract

Channel catfish (*Ictalurus punctatus*) roe is an abundant and underutilized byproduct that can be used as a unique source of protein. The objectives of this study were to develop a functional protein powder from farmed catfish roe and evaluate the chemical, functional, and nutritional properties of the powder. Spray-dried soluble catfish roe protein powder (SCR) has a high content of essential amino acids and desirable mineral content. Nitrogen solubility, emulsion stability, and fat adsorption values for SCR are 64%, 85.1%, and 5.7 ml per g protein, respectively. Viscosity, flow behavior index (n), and consistency index (K) values of emulsion containing soluble SCR are 1.8 Pa.s, 0.14, and 89.09 (Pa.s)^n, respectively and the emulsion shows a pseudoplastic fluid characteristic. The soluble protein powder from catfish roe can be used as an emulsifier in food and feed systems. Developing functional protein from catfish roe may provide new opportunities to the catfish industry.

Introduction

Channel catfish roe (*Ictalurus punctatus*) is an underutilized and abundant byproduct that can be used to produce protein powder. In the United States, channel catfish is the fourth most popular fish consumed. Alabama, Arkansas, Louisiana, and Mississippi are the four major commercial catfish producing states in the United States. The National Agricultural Statistics Service (NASS 2006) reported that these states produced over 272,000 metric tons of catfish in 2005 with a stable monthly production of about 22,700 tons. Catfish processing generates about 55% processing byproducts and these byproducts often end up in landfills or rendering plants. Fish roe are collected during the fish filleting process. Roe obtained from fish such as salmon, cod, and pollock have commercial markets but there is no significant catfish roe market in the United States. Eun et al. (1994) reported that catfish roe contains 24.6% protein, with high concentrations of the amino acid leucine, and can be used in food ingredients for human consumption. Additionally, Mol and Turan (2008) reported that fish roe may be considered a high quality food with respect to human nutrition. Fat adsorption capacity and emulsion stability impact the potential uses of fish roe as a food or in food ingredients. Tippetts and Martini (2009) reported that factors such as oil content and homogenization condition can be influenced by emulsion stability. Egg yolk is frequently used to stabilize emulsions, playing a major role in emulsion food formulations (Kontogiorgos et al. 2004). However, there is limited information available on preparing emulsions using fish proteins as an emulsifier. Is it possible to use a catfish roe protein powder in an oil-in-water emulsion system? This paper is focused on two issues: (1) elucidating the functional and nutritional properties of catfish roe protein powder, and (2) determining the properties of emulsions made with catfish roe protein as the emulsifier.

Nutritional properties of catfish roe protein powder

Values for the proximate analysis of catfish roe have considerable variation: moisture 56-65%, protein 25-28%, ash 1.8-2.4%, and fat 8-17% (Eun et al. 1994, Sathivel et al. 2009). In general, the proximate composition of fish roe depends on the fish age, the season of the year, and the type of processing (Iwasaki and Harada 1985). Spray-dried soluble catfish roe protein powder (SCR) contained 67.1% protein (Sathivel et al. 2009), similar to values reported for herring protein powders (Sathivel et al. 2004). The fat content of SCR (18.3%) was similar to reported fat content for pollock protein powder (Sathivel and Bechtel 2006). Sathivel et al. (2009) reported that SCR contains higher levels of glutamic acid (121 mg amino acid per g protein), leucine (108.4 mg amino acid per g protein),

aspartic acid (92.2 mg amino acid per g protein), and alanine (84.8 mg amino acid per g protein). The values for essential amino acids from the SCR are generally above infant requirements (WHO 1985); however, histidine and methionine for SCR are below the recommended level. The lysine content of SCR is 76.3 mg of amino acid per gram of protein; this is similar to the pollock frame soluble protein powder (73.9 mg per g protein) (Sathivel and Bechtel 2006) but is lower than arrowtooth protein powder (Sathivel and Bechtel 2008).

Functional properties of catfish roe protein powder

Nitrogen solubility for SCR is 64% (Sathivel et al. 2009); this is higher than the value reported for herring gonad protein powder (Sathivel et al. 2004) but similar to pollock viscera protein powder (Sathivel and Bechtel 2006). It is important to have higher nitrogen solubility values to enhance product appearance and provide a smooth mouth feel (Petersen 1981).

The emulsifying stability of SCR is 85.1% (Sathivel et al. 2009). Factors such as protein solubility and hydrophobicity play major roles in emulsifying properties. Fat binding/adsorption capacity is an important functional characteristic of ingredients used in the meat and confectionery industries. Sathivel et al. (2009) reported that the adsorption capacity of SCR was 5.7 (ml oil per g protein). The mechanism of fat binding capacity is thought to be primarily due to physical entrapment of the oil. According to Sathivel et al. (2009), the particle size for SCR ranges from 1.9 to 88 microns. SCR has higher amounts of 31-44 micron particles while the least abundant (0.4%) particle size was 1.9 microns. Kuakpetoon et al. (2001) have reported that particle size is one of the important physical properties affecting the flowability of protein powders. Spray dryer operating conditions such as feed temperature, air inlet temperature, air outlet temperature, viscosity, and fluidity of the solution determine the particle size distribution of the resulting powders (Zakarian and King 1982, Zbicinski et al. 2002, AIChE 2003). By increasing feed temperature, viscosity, and the droplet size of the solution, the particle size will decrease. Water evaporation from the powder during spray drying directly depends on the air inlet temperature of the spray dryer. Low rates of water evaporation from the powder cause particle agglomeration. Thus, the functional properties of the spray-dried materials can depend on the spray dryer operation conditions and physical properties such as the viscosity of material.

An emulsion made with SCR had values for viscosity of 1.8 Pa.s, flow behavior index (n) of 0.14, and consistency index (K) value of 89.09 Pa.s^n (Sathivel et al. 2009). The flow behavior index value (n) of the emulsion was less than 1, which indicates that it is acting as a pseudoplastic fluid

(Paredes et al. 1989). The flow behavior index value for the SCR emulsion is lower than that reported for emulsions made from arrowtooth flounder soluble protein powders (0.5) (Sathivel et al. 2005). The *K* value of the SCR emulsion was higher than that for emulsions made from soluble arrowtooth protein powders (4.2 and 5.6 Pa.s^n) (Sathivel et al. 2005). A higher *K* value indicates a more viscous consistency (Paredes et al. 1989).

The dynamic rheological data of the SCR emulsions was fitted to the following equation in order to explain the emulsion viscoelastic:

$$G' = \frac{\sigma_o}{\gamma_o} \cos\delta$$

$$G'' = \frac{\sigma_o}{\gamma_o} \sin\delta$$

where G' (Pa) is the storage modulus, G" (Pa) is the loss modulus, tan δ is the loss tangent, σ is generated stress, and γ is oscillating strain. According to Sathivel et al. (2009), the emulsion shows a gradual increase in both G' and G" with increasing frequency and the emulsion had higher G' than G", which indicates a viscoelastic material with both G' and G" being independent of frequency (Ferry 1980). The G' value for the SCR emulsion was lower than that for a commercial mayonnaise, indicating that the viscoelastic characteristics of the SCR emulsion was greater than in commercial mayonnaise. This may be due to SCR's high soluble protein content, which could increase interactions between the neighboring droplets.

Conclusion

Spray-dried catfish roe soluble protein powder contains an abundance of essential amino acids and has desirable functional properties such as emulsion stability and fat finding capacity. This protein powder potentially can be used in nutritional supplements and as a functional ingredient. The soluble protein powder from catfish roe can be used as an emulsifier with its emulsions having both pseudoplastic and viscoelastic characteristics. The recovered roe protein can potentially be converted into a higher value food ingredient suitable for use as an emulsifier in food and feed systems. Value-added products can be developed from catfish roe, which is currently underutilized in the catfish industry.

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Alaska Fish Byproducts as a Feed Ingredient for Reindeer

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Abstract

A high protein ration must be fed to intensively farmed reindeer to support rapid tissue growth, in interior Alaska in the summer. The Alaska fishing industry generates fish meals, which are priced competitively with imported protein supplements. However, the palatability of diets containing fish meal, reindeer growth and performance when fed fish meal diets, and meat quality of reindeer fed a ration using fish byproducts are unknown. Several studies evaluated a milled reindeer ration supplemented with Alaska fish meals, for palatability, effects on antler and body growth, and meat quality and flavor. In a free-choice preference trial, reindeer consumed significantly more soybean meal–based ration than either a whitefish meal or salmon meal–based ration, a difference that declined during the course of the study. In performance trials, feed conversion efficiency was significantly higher for animals fed a whitefish meal–based diet than a salmon meal diet, and there was no significant difference in body weight gain or in dry matter intake. When whitefish meal was compared to soybean meal in the diet, there

was no difference in body weight or carcass and meat characteristics, and no "fish-related" flavor was identified in taste panel studies. When a whitefish meal–based diet and whitefish meal plus bone meal diet were compared, the bone meal group had numerically greater dry matter intake, antler base circumference, and antler beam length than non-supplemented animals, but the differences were not significant. In conclusion, when reindeer consumed rations based on fish byproducts as the protein source, they demonstrated good performance with no negative effects on meat quality or meat flavor.

Introduction

The red meat industry in Alaska is primarily based on cattle and reindeer (*Rangifer tarandus tarandus*) production (Alaska Agriculture Statistics 2008). Reindeer production in Alaska is currently based on an extensive range system where animals are allowed to free-range in rural Alaska over large designated grazing areas on the Seward Peninsula, St. Lawrence and Nunivak islands, and the Aleutian chain where there is limited or no access to slaughtering, processing, or transportation infrastructure. These limitations prevent production from meeting the market demand for a continuous supply of high quality, inspected meat. By expanding reindeer production to areas that have access to distribution networks and slaughtering abattoirs, the industry will be capable of reaching its production and economic potential. Therefore, some producers want to develop more intensively managed operations near transportation hubs in interior Alaska that would utilize cereal grain and forage produced in Alaska as the primary source of feed. Barley is a high yield crop grown in Alaska; it is available at a relatively low cost and is being used in a milled ration for reindeer (Finstad et al. 2007). Smooth brome grass (*Bromus inermis*) is the dominant perennial forage species grown on rotational croplands in Alaska and is readily consumed by reindeer as both pasture and hay. Chopping the hay and mixing it with barley can provide a relatively low cost, primary energy component for a milled reindeer ration.

Nutritional requirements of reindeer vary seasonally (Suttie and Webster 1995) and the composition of a summer diet needed to support growth and lactation should contain relatively high crude protein (>15%) and mineral concentrations (Ryg and Jacobsen 1982, White 1992). Barley and brome can supply the energy and fiber requirements for farmed reindeer, but they do not contain sufficient crude protein to meet the summer nutritional requirements. Soybean meal is often used as a protein supplement; however, soybeans cannot be grown in Alaska and the meal must be shipped into the state. Fish meal can be used in the beef and dairy industry as a protein source and supplement to increase animal performance (Hussein and Jordan 1991). Fish meal may further

increase animal production because of its rumen non-degradable protein properties that improve the nutritional profiles of absorbed amino acids.

The Alaska fishing industry commonly harvests over 2 million metric tons of fish for human consumption and over 70,000 metric tons of fish meal and fish bone meal from the byproducts (Crapo and Bechtel 2003). In Alaska, fish meal can often be priced competitively with soybean meal on a unit-protein basis and is readily available (Finstad et al. 2007). Fish meal is generally recognized as very digestible, an excellent source of rumen non-degradable protein (Hussein and Jordan 1991) and useful as a protein supplement in high production settings for cattle (Carroll et al. 1994). Typically bone meal from ruminants (sheep, cows, goats) was used to increase mineral and protein levels in livestock feed, but this use led to recent "mad cow" food scares in Europe, the United States, and Canada. The recent European and North American prohibition on using such bone meal in livestock feeds may be an opportunity for development of an energized market for fish bone meal.

The value of velvet antler, harvested from reindeer, has increased in Alaska due to the demands of a recently developed North American market for antler health aid supplements (Alaska Agriculture Statistics 2008). Some consumers are taking antler products as a mineral supplement and also to ease pain associated with joint trauma and arthritis (Sunwoo et al. 1995, Bucsi and Poor 1998). The growth of antlers in deer is highly dependent on adequate mineral and protein nutrition (Grasman and Hellgren 1993, National Research Council 2006) and may be increased in reindeer by the addition of a fish bone meal supplement to the diet.

The palatability of a fish byproduct to reindeer is influenced by any characteristic of the supplement affecting its acceptability, typically measured as the preference animals exhibit given a choice among two or more feeds in a cafeteria-style feeding experiment, as used in our study. If reindeer consume a ration containing fish or fish bone meal, then effects on performance such as antler and lean tissue production and meat quality can be evaluated, to assess the potential use of these ingredients in an intensively managed reindeer production system.

Materials and methods

Fish meal palatability study (Experiment 1)

Sixteen 2-year-old female reindeer were offered three rations supplemented with commercially available soybean meal, whitefish meal (WFM, Kodiak Fishmeal Company), or salmon meal (SM, Kodiak Fishmeal Company) respectively, for a period of 4 weeks in a cafeteria-style feeding experiment. At the University of Alaska Fairbanks Agricultural and Forestry Experiment Station, Reindeer Research Program, the rations

were milled with barley, smooth brome hay, and a supplemental protein source: soybean meal (SBM), WFM, or SM. Minerals were added to the diet to a concentration of 0.7% calcium, 0.5% phosphorus, and 0.7% potassium. Corn oil, molasses, and urea were used in the formulation to provide readily available energy and nitrogen sources for maintenance of rumen microflora. Vitamins and other feed components were added to achieve a balanced ruminant diet. Diets were mixed every 5-7 days in a 250 kg capacity Davis feed mixer. Ten feed samples were randomly taken from each batch of feed used and were analyzed for percent dry matter, crude protein, digestibility, neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin. All feed samples were oven-dried for 48 h at 60ºC (nutritional analysis) or for 48 h at 100ºC (dry matter content) and ground through a 20 mesh screen in a Wiley mill. Neutral detergent fiber and acid detergent fiber concentrations were determined sequentially using an Ankom fiber analyzer (Vogel et al. 1999). Hemicellulose concentrations were obtained by subtracting acid detergent fiber from neutral detergent fiber values. Nitrogen (N) and mineral analysis (K, P, Ca, Mg, S, Na, Cu, Zn, Mn, Fe, Co, Mo, Se) were performed at the University of Alaska Fairbanks Soil and Plant Analysis Laboratory in Palmer, where samples were digested in a nitric-perchloric acid mixture prior to analysis in an ICP Optima 3000 XL analyzer (PerkinElmer). In vitro true dry matter digestibility was done at the University of Alaska Fairbanks Institute of Arctic Biology, Nutritional Laboratory, in Ankom bags using the method of Goering and Van Soest (1970) with the Tilley and Terry modification (1963). Rumen inoculum was obtained from two rumen-fistulated reindeer at the University of Alaska Fairbanks Large Animal Research Station. The composition of the feed mixtures used in all trials is described in Table 1.

The cafeteria format used for the palatability trials was a 30 × 30 m pen where feed samples were placed in 21 rubberized tubs fitted into wooden stands at a height of 50 cm. The feeding stands were systematically distributed under an 8 × 15 m shelter to allow free access to all feeding tubs. The three rations were distributed randomly in the 21 feed tubs each day. Reindeer were given fresh feed daily between 10 am and 12 pm, and all feed from the previous day was removed from the feeding tub and weighed before adding fresh feed to determine proportion of diet eaten per day. Each day approximately 0.5 kg of the weigh-back was collected for estimating dry matter.

Fish meal performance and meat quality study (Experiment 2)

Eight 4-month-old reindeer steers and six 14-month-old female reindeer were randomly allocated to a control group (soybean meal, SBM diet) and a treatment group (whitefish meal, WFM diet) and put in two 25 × 25 m pens two weeks prior to the start of the study. Diets were gradu-

Table 1. Nutritional values for feed mixtures used in feeding trials: soybean meal ration, whitefish meal ration, and salmon meal ration.

	SBM ration	WFM ration	SM ration
Crude protein (%)	15.3 ± 0.2	14.9 ± 0.1	16.7 ± 0.3
Crude fat (%)	1.7 ± 0.05	2.3 ± 0.05	n.a.
Metabolizable energy (MJ/kg)	13.0 ± 0.07	13.0 ± 0.07	n.a.
Ash (%)	5.1 ± 0.1	5.1 ± 0.1	n.a.
In vitro total digestibility (%)	85.1 ± 0.3	84.6 ± 0.4	85.6 ± 1.2
Neutral detergent fiber (%)	25.8 ± 0.2	26.8 ± 0.4	24.2 ± 0.5
Acid detergent fiber (%)	9.6 ± 0.2	9.5 ± 0.2	7.2 ± 0.3
Lignin (%)	1.3 ± 0.06	1.4 ± 0.08	1.4 ± 0.03
Phosphorus (P) (%)	0.4 ± 0.01	0.4 ± 0.01	0.4 ± 0.01
Potassium (K) (%)	0.8 ± 0.02	0.7 ± 0.03	0.8 ± 0.01
Calcium (Ca) (%)	0.6 ± 0.01	0.6 ± 0.02	0.7 ± 0.02
Magnesium (Mg) (%)	0.2 ± 0.004	0.1 ± 0.004	0.2 ± 0.003
Sulfur (S) (%)	0.2 ± 0.004	0.2 ± 0.007	0.2 ± 0.007
Sodium (Na) (ppm)	810.3 ± 52.5	1010.5 ± 72.8	764.5 ± 121.0
Copper (Cu) (ppm)	22.2 ± 1.4	16.8 ± 0.4	13.9 ± 1.4
Zinc (Zn) (ppm)	124.3 ± 1.6	121.4 ± 3.9	121.4 ± 3.9
Manganese (Mn) (ppm)	106.6 ± 2.9	115.3 ± 8.7	96.3 ± 4.4
Iron (Fe) (ppm)	190.0 ± 4.3	177.9 ± 10.3	169.9 ± 10.3
Cobalt (Co) (ppm)	0.6 ± 0.02	0.6 ± 0.03	0.8 ± 0.06
Molybdenum (Mo) (ppm)	0.4 ± 0.02	0.1 ± 0.03	0.4 ± 0.07
Selenium (Se) (ppm)	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1

Values are means ± S.E.
Soybean meal (SBM; n = 10); whitefish meal (WFM; n = 7); salmon meal (SM; n = 6).
n.a. = not analyzed.

ally shifted to experimental diets over the two-week period to allow for rumen adjustment.

Diets with either SBM or WFM were formulated to produce protein equivalent rations. Soybean meal made up 3.5% and WFM was 2.1% of the total dietary protein of the two feed mixtures. Daily dry matter intake (DMI) of each diet was calculated by dividing total feed consumption (corrected for dry matter content) by the number of animals in the pen to determine mean DMI per animal per day.

Animals were weighed at the start of the trial using a squeeze chute mounted on Tru-test MP 800 load bars (Tru-test Ltd., Auckland, New Zealand) and then twice weekly throughout the experiment. Weighing occurred at the same time each day. Feed conversion efficiency was calculated from the average weekly weight gain of the animals in each

treatment group (SBM and WFM) divided by the respective groups' average weekly feed consumption.

After 8 weeks the steers were slaughtered. One steer was removed from the performance part of the experiment when he sustained a severe leg laceration that required individual handling and treatment with antibiotics. However, this animal was fed the WFM diet throughout the experiment period, and was sent to slaughter with the other steers and is included in the meat quality data. A total of twelve reindeer (10 steers and 2 females, of which 8 were the steers from the performance trial and 4 were free-ranging animals) were included in the study of meat quality parameters. All twelve animals were transported for about two hours (158 km) to a USDA-approved slaughter facility. Following the normal slaughter procedure, the weights of the carcasses were registered before they entered the chilling room.

The ultimate pH in the *M. longissimus* (LD, *longissimus dorsi*, at the last rib) was measured in all twelve carcasses at approximately 24 h post mortem. Meat samples for analysis of shear force, color, and cooking loss were collected in connection with the sensory evaluation. Sensory evaluation was performed by a six-member trained sensory panel at the University of Illinois Department of Animal Sciences. Panelists were selected based on previous experience conducting trained sensory evaluation, and had also participated in an earlier study where meat from caribou and reindeer was compared with beef (Rincker et al. 2006). A consumer test was performed in collaboration with the University of Alaska Fairbanks Cooperative Extension Service research kitchen. A triangle test (Mielgaard et al. 1999) was performed with consumers (n = 59), most of whom were university students and staff.

Bone meal performance study (Experiment 3)

Twenty reindeer were used to evaluate the effect of a diet supplemented with fish bone meal (FBM) on feed intake, body weight gain, and antler growth in an 11 week performance trial conducted April through June. Yearling steers, yearling bulls, and 2-year-old steers were randomly blocked into two treatment groups and two control groups by weight, age, and sex. The control group was fed the 16% crude protein whitefish meal (WFM) diet and the treatment group was fed the same standard diet supplemented with 2.4% fish bone meal by weight. Feeding, sampling, and weighing protocols were the same as in preceding experiments. Antler growth in male reindeer is initiated from late March to early May. Base circumference (at the corona) and length along the backside of the main beam from corona to tip of right and left antler of each animal were measured with a cloth measuring tape at the beginning and end of the performance trial. Proximate analysis of the fish bone meal (pollock) was determined using AOAC (1990) methods and

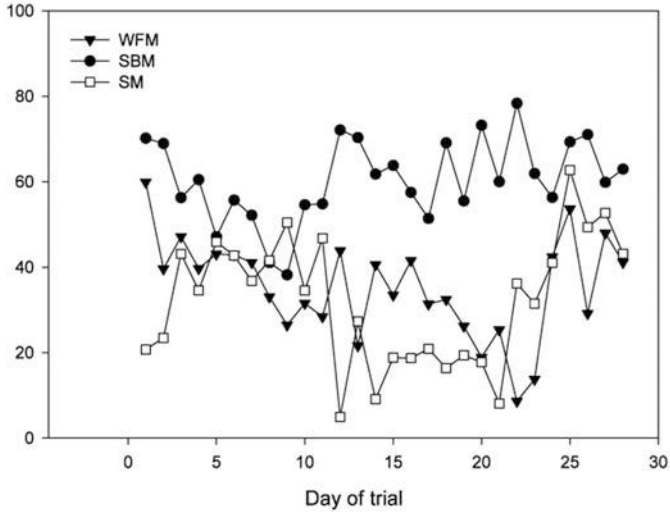

Figure 1. Daily dry matter intake (DMI) of reindeer treatment groups fed soybean meal (SBM), whitefish meal (WFM), and salmon meal (SM) based diets in a 28 day free-choice feeding trial.

mineral analysis was conducted by the University of Alaska Fairbanks Soil and Plant Analysis Laboratory in Palmer.

Statistical analysis

The statistical analyses were carried out with the Statistical Analysis System (SAS Institute 2003) and Systat (SPSS Inc. 1997) using ANOVA, GLM, and MIXED procedures. In Experiment 2, the model for comparing total weight gain, feed intake, feed conversion efficiency, meat ultimate pH, tenderness, color, and cooking loss included the fixed effect of treatment group. Significance was defined as $P \leq 0.05$. For the trained panel work, the model included the random effects (animal and panel member), as well as the fixed effect of treatment group. Statistical analysis of the data from the consumer test was performed by Creascience, Montreal, Canada.

Results and discussion

Fish meal palatability study (Experiment 1)

There was a significant difference in total dry matter intake (DMI) among the three diets ($P < 0.001$) during the course of the study (Fig. 1). The group of reindeer consumed on average 60.5 kg ± 1.8 per day of soy-

Table 2. Weight gain, dry matter intake, and feed conversion efficiency for reindeer steers and females from SBM and WFM dietary treatments, during an 8 week performance trial.

Trait	SBM group	WFM group	Degree of sign
Weight gain (kg)			
Females	14.8 ± 1.0 (n = 3)	21.5 ± 3.1 (n = 3)	n.s.
Steers	19.5 ± 1.9 (n = 4)	23.5 ± 3.5 (n = 3)	n.s.
Dry matter intake (g dry matter/day/kg body weight)			
Females + steers	29.6 ± 0.1 (n = 7)	30.1 ± 0.1 (n = 6)	n.s.
Feed conversion efficiency (g weight gain/kg feed consumed)			
Females + steers	120 ± 6.0 (n = 7)	147 ± 5.0 (n = 6)	$P \leq 0.01$

Values are means ± S.E.
SBM = soybean meal; WFM = whitefish meal.
n.s. = $P > 0.05$.
Table from Finstad et al. (2007).

bean meal (SBM) ration, 35.2 kg ± 2.2 per day of whitefish meal (WFM) ration, and 32.1 ± 2.9 per day of salmon meal (SM) ration. However, the difference in DMI among the three diets declined during the final 6 days of the trial (Fig. 1). Although reindeer that were provided with the free-choice diets consumed much more of the SBM-based ration than either of the fish byproduct–based rations, all diets were palatable. Initially, there was a significant difference in intake rates, but as reindeer became accustomed they consumed a greater proportion of a fish meal diet relative to an SBM-based diet (Fig. 1). In subsequent trials, DMI of reindeer fed exclusively SBM or WFM were similar (Table 2), suggesting reindeer will readily consume diets using fish meal as a supplementary protein source.

Growth performance and feed conversion efficiency (Experiment 2)

The nutritional profiles of the WFM and SBM rations were similar, but the fat content was significantly higher in the WFM mix than in the SBM mix (Table 1).

The SBM animals had slightly higher body weights compared with the WFM group when the feeding trial started (average group weights of SBM and WFM were 82.2 kg and 78.3 kg, respectively). There was no significant difference in overall weight gain between females and steers ($P = 0.215$); however, there was a tendency toward higher weight gains ($P = 0.068$) in the WFM animals compared with the SBM animals (groups with combined weight gain from steers and females) (Table 2).

Table 3. Carcass and meat quality characteristics in reindeer *M. longissimus* from SBM, WFM, and free range grazing animal feeding treatments.

Trait	SBM group (n = 4)	WFM group (n = 4)	Range group (n = 4)	Degree of sign
Live weight (kg)	103.0 ± 5.8	108.9 ± 5.8	n.d.	n.s.
Carcass weight (kg)	58.4 ± 3.5	59.5 ± 3.5	n.d.	n.s.
Dressing (%)	56.7 ± 0.5	54.6 ± 0.5	n.d.	$P \leq 0.05$
Ultimate pH (LD)	5.61 ± 0.02	5.60 ± 0.02	5.58 ± 0.02	n.s.
Shear force (kg)	2.4 ± 0.4	3.3 ± 0.4	2.3 ± 0.4	n.s.
Cooking loss %	232 ± 15	221 ± 15	216 ± 15	n.s.
Color L-value	22.4 ± 1.8	19.6 ± 1.8	19.3 ± 1.8	n.s.
Color a-value	9.6 ± 0.8	11.4 ± 0.8	10.3 ± 0.8	n.s.
Color b-value	11.6 ± 1.2	12.1 ± 1.2	13.3 ± 1.2	n.s.

Values are means ± S.E.
SBM = soybean meal; WFM = whitefish meal; LD = *longissimus dorsi* muscle.
n.d. = not determined.
n.s. = $P > 0.05$.
Table from Finstad et al. (2007).

The variation in dry matter intake (DMI; g dry matter per day per kg body weight) for the two treatment groups is presented in Table 2. No statistical difference was found when comparing the average DMI for the WFM and SBM groups (30.1 g and 29.6 g, respectively, $P = 0.407$). Average feed conversion efficiency (g weight gain per kg feed [dry matter] consumed) over the feeding period was significantly higher ($P = 0.003$) for the reindeer fed the WFM mix compared with the SBM animals (0.147 kg and 0.120 kg respectively).

Fish meal is considered a low rumen-degradable protein and has produced higher weight gains and feed efficiencies when replacing SBM in ruminant diets (Orskov et al. 1970, Oldham et al. 1985). Approximately 60-70% of whitefish meal (WFM) protein has been estimated to escape rumen degradation compared with only 30-35% of SBM protein (National Research Council 1996). The reindeer (both steers and females) fed the feed mixture including WFM showed a trend toward higher weight gain and significantly improved feed conversion efficiency compared with animals fed the SBM-based diet, suggesting that WFM can be used in reindeer diets across sex classes in a production setting with no negative effect on performance.

Carcass characteristics and meat quality attributes (Experiment 2)

There was a significantly higher ($P = 0.03$) dressing percentage of the carcasses from the soybean meal (SBM) group compared with the white-fish meal (WFM) animals (Table 3). No other differences were found in carcass characteristics or ultimate pH in the *longissimus dorsi* muscle (LD) between the three treatment groups (Table 3). No significant differences between the three treatment groups were found in LD samples for shear force, meat color, and cooking loss (Table 3).

No significant differences between the three treatment groups (SBM, WFM, and free-range) were found when comparing the sensory attributes of the meat (data not presented). Sensory scores from the trained panel indicated that all samples were tender and juicy, and had an intense meat flavor and low off-flavor. When summarizing the written comments from the consumers, various off-flavor attributes were mentioned (e.g., "gamey," "aftertaste," "strong," "robust," "liver," "more flavor," "iron," "not like caribou"); however, no comments were registered on any "fish-related" flavor attribute. It has been suggested that natural grazing is an important contributor to the development of various "wild" flavors in meat, possibly depending on the fatty acid composition (Wiklund et al. 2003). In this study, the trained panel did not find any differences in flavor attributes between the three treatment groups. However, the consumers certainly commented on flavor variations in meat from WFM-fed and grazing animals. The comments on "wild" flavors in the meat samples were mainly related to the group of free-range grazing reindeer and were, therefore, in good agreement with previous research.

The nutritional status and physical condition of reindeer has been demonstrated to have a considerable effect on muscle glycogen content and meat ultimate pH values (Wiklund et al. 1996). The meat pH values of reindeer in the present study indicate that the animals from all three groups (WFM, SBM, and free-range) were in good physical condition. The other meat quality attributes measured—tenderness, cooking loss, and color—were very similar in the three groups of reindeer. Earlier studies have reported equally low shear force (high tenderness) values for reindeer meat (Wiklund et al. 1997, Rincker et al. 2006). Cooking loss values in the same range as this study were reported by Wiklund et al. (1997). The Minolta color L* and a* values from this study were slightly lower and b* values higher compared with a previous study of reindeer meat (Rincker et al. 2006).

Bone meal performance study (Experiment 3)

Proximate and mineral analysis results of the fish bone meal (FBM) used as a protein and mineral supplement are reported in Table 4. Antler circumference, beam length, and weight gain were greater for reindeer

Table 4. Proximate and mineral analysis of whitefish (pollock) bone meal.

Proximate analysis	% Total
Crude protein	43.2 ± 0.4
Crude fat	6.0 ± 0.07
Moisture	1.1 ± 0.02
Ash	49.1 ± 0.4
Mineral analysis (dry matter basis)	
Phosphorus (P) (%)	5.9 ± 0.01
Potassium (K) (%)	0.7 ± 0.00
Calcium (Ca) (%)	18.2 ± 0.00
Magnesium (Mg) (%)	0.2 ± 0.00
Sulfur (S) (%)	0.6 ± 0.00
Sodium (Na) (ppm)	9400.0 ± 47.3
Copper (Cu) (ppm)	0.84 ± 0.4
Zinc (Zn) (ppm)	65.0 ± 1.2
Manganese (Mn) (ppm)	3.0 ± 0.0
Iron (Fe) (ppm)	27.3 ± 1.7

Values are means ± S.E.

Table 5. Antler growth, weight gain, and dry matter intake of reindeer fed a control or a ration supplemented with FBM after a 28 day trial.

	Antler base circumference (cm)	Antler beam length (cm	Weight gain (kg)	Dry matter intake (g consumed/g body wt)
Control	33.0 ± 2.4	112.7 ± 6.5	19.2 ± 2.7	28.6 ± 0.3
FBM supplemented	33.4 ± 1.9	117.9 ± 9.0	22.8 ± 2.1	29.0 ± 0.2

Values are means ± S.E.
FBM = fish bone meal.

fed a diet supplemented with FBM, but the differences were not significant (Table 5). There was no difference in dry matter intake (DMI) of the control diet and the FBM-supplemented diet: 28.6 g and 29.0 g eaten per kg body weight, respectively (Table 5).

After energy, phosphorus is most likely to limit growth of antlers in cervids (Moen and Pastor 1998). Fish bone meal contains relatively high concentrations of phosphorus, calcium, and crude protein (Table 4) and when added to a diet (>2.0%) should provide nutrition for maximal antler growth. Proximate and mineral analyses of the FBM diet had not yet been completed when this paper was written. No significant improvement in antler or body growth of reindeer fed an FBM-supplemented diet

was detected in this study. This may be because animals fed the control diet were already at a high optimal level of nutrition, or not enough of the FBM had been added to the base diet to adequately increase mineral levels. Supplementation with FBM may improve antler growth in a production setting where animals are not fed a balanced high quality diet, ad libitum (Finstad et al. 2007).

Conclusions

Reindeer consumed rations based on fish byproducts as the protein or mineral source and demonstrated good performance with no negative effects on either meat quality or meat flavor. In Alaska, locally produced fish meal and fish bone meal may supply an economically viable, alternate protein source to be used in rations for intensive reindeer production systems.

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Crop Nutrient Recovery from Applied Fish Coproducts

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Abstract

The Alaska fishing industry produces over 1,000,000 metric tons of fish processing byproducts annually and much of the byproduct is not utilized. Fish coproducts such as fish meal, fish protein hydrolysate, and fish bone meal, manufactured from these byproducts, are rich in plant essential nutrients and can be used as nutrient sources for crop production. The objective of the study was to quantify nutrient release in soil and crop production from the use of fish coproducts in Alaska soil and climate conditions. Laboratory incubation and field plot experiments were conducted to determine nitrogen release and nutrient recovery by barley (*Hordeum vulgare* L.) from two different Alaska soils treated with fish meal, fish bone meal, and fish protein hydrolysate. The three fish coproducts released 65 to 80% of their nitrogen during 56 days of incubation. On average, barley recovered 78% of the applied nitrogen from fish meal, 65% from fish bone meal, and 50% from fish hydrolysate at the Fairbanks site; all were higher than measured for urea treatments (42%). Biomass production for both sites followed the same trend as nitrogen uptake. Plants also recovered substantial amounts of micronutrients (e.g., Ca, Mg, Cu, and Zn) from the fish coproducts. The results indicated that all three of the fish coproducts used in this study were good nutrient sources for crop production.

Introduction

Approximately 60% of the fish harvested in the United States for human consumption come from Alaska waters. Over one million metric tons of fish processing byproducts are generated from two million tons of fish harvested annually (Bechtel and Johnson 2004). These fish byproducts, depending on species and byproduct components, vary in nutrition concentration. Bechtel (2003) showed that Alaska pollock skin contains (wet weight basis) 25% protein compared with 15.2% in the heads, whereas pink salmon heads contain 13.9% protein, and Pacific cod heads contain 16.4% protein.

Traditionally, coproducts such as fish meal, fish bone meal, fish protein hydrolysate, and fish silage made from processing byproducts are used in a variety of ways. Fish meal is used as a protein ingredient in both the aquaculture and livestock industries. Fish protein hydrolysate and silage are popular nutrient sources for organic crop production. The proportion of coproducts used for organic crop production is still small relative to other organic nutrient sources (e.g., soybean meals), and the proportion varies greatly by region in the United States. A national survey conducted in 1994 showed that only 20% of organic crop producers used fish byproducts as a nutrient source (Fernandez-Cornejo et al. 1998). In a separate survey of organic citrus and vegetable producers in Florida, 71% of the respondents had used fish protein hydrolysate as a secondary nutrient source (Swisher and Monaghan 1995). Even though fish or fish coproducts have a long history of being used as a plant nutrient source (Ceci 1975, Mariakulandai and Manickam 1975), little information is available on nutrient release from different fish coproducts in soils.

Conventionally, the ratio of carbon to nitrogen is used to estimate the tendency of nitrogen mineralization or immobilization of organic materials applied in soil. But the C:N ratio of organic nutrient sources indicates only the tendency of mineralization or immobilization, not the quantity of carbon or nitrogen that can be mineralized over a given time period. Nevertheless, the C:N ratio is still widely used as an indicator for nitrogen mineralization/immobilization in soil for organic nitrogen source application (Qian and Schoenau 2002, Loecke et al. 2004), because no other common indicators or methods can improve the estimate of available nitrogen in organic nutrient sources. Therefore, field and laboratory incubation experiments are usually conducted to determine the amount and rate of nitrogen release with a certain set of soil and environmental conditions.

Nitrogen recovery from organic sources varies with the types of organic nutrients, crops, soil types, climate conditions, and application methods. In general, nitrogen recovery by crops from organic sources is lower than that from chemical nitrogen fertilizers. Zhang et al. (2006) showed that in subarctic areas of Alaska, brome grass (*Bromus iner-*

mis Leyss.) hay recovered only 11% of available nitrogen from surface applied solid manure in contrast to 59% of nitrogen recovered from liquid cattle manure in three years after application. Kramer et al. (2002) showed that maize recovered 29% of available nitrogen from legume residues in comparison with 41% from fertilizer nitrogen. Nitrogen release from organic sources usually lasts for several growing seasons after application. Deluca and Deluca (1997) reported that release of applied nitrogen from manure was 20% per year in the first two years after application, and 10% per year in the next two years. The estimated nitrogen release by Klausner et al. (1994) from applied organic nitrogen sources was lower than that of Deluca and Deluca (1997), with a 21, 9, 3, 3, and 2% recovery in the first five years after manure application. Nevertheless, in both estimations, crops could recover only a portion of the applied nitrogen from organic sources in the year of application. The objectives of this study were to determine (1) the amount of nitrogen released from three fish coproducts applied at three levels in the year of application, and (2) the amount of nitrogen recovered by barley from these coproducts.

Materials and methods

Three fish coproducts were used in the experiments: fish meal (FM), fish bone meal (FBM), and fish hydrolysate (FH). FM and FBM were obtained from a commercial fish meal plant in Kodiak, Alaska, in spring 2006. These meals were made primarily from pollock, cod, and flatfish processing byproducts. FM consists of rendered fish heads, frames, viscera, and skins with some large bone fragments removed. The FBM was composed primarily of larger bone fragments. The FBM used in these experiments was ground to <4 mm with a Wiley mill. The FH was a commercial product (Alaska Protein Recovery, LLC, Juneau) made from salmon processing byproducts, predominantly heads and viscera. Lipid had been removed from the hydrolysate, the hydrolysate was concentrated to 25% solids, and phosphoric acid was added to stabilize the product at pH 3.6. The carbon concentration was 40.9 g C per 100 g dry matter for FM; 22.6 g C per 100 g dry matter for FBM, and 11.4 g C per 100 g wet weight basis for FH. The nitrogen concentration was 10.9 g N per 100 g dry matter for FM; 6.2 g N per 100 g dry matter for FBM; and 4.4 g N per 100 g wet weight basis for FH. Phosphorus concentration for FM was 3.6 g P per 100 g dry matter; 8.0 g P per 100 g dry matter for FBM; and 1.9 g P per 100 g wet weight basis for FH.

Incubation experiment

Soil samples were taken from the Fairbanks (64º38N) and Delta Junction (63º56N) field experiment sites in Alaska. Soil samples were dried and passed through a 2 mm sieve. Field capacity of each soil was determined

following the method described by Cassel and Nielsen (1986). Air-dried soil samples (<2 mm) were blended respectively with fish meal (FM), fish bone meal (FBM), and fish hydrolysate (FH) at a rate of 100 µg N per g soil in a liquid-solid blender (Patterson-Kelley Co.) for 20 min, followed by the addition of distilled water (90% field capacity) and blending for another 20 min. Five grams of treated moist soil samples were weighed into 20 ml glass jars (25 mm diameter by 5 mm height), which were loosely covered with lids and incubated at 15ºC, the mean growing season temperature in the region. The moisture content of the soil in the glass jars was maintained at 90% field capacity by adding water weekly. Each time water was added, the lid was opened to allow aeration of the soil in the jars. There were four replicates of each treatment at each sampling time. Destructive soil samples were taken at 0, 3, 7, 14, 28, 42, 56, 84, 182, and 364 days after incubation. Moist soil samples were then analyzed for mineral nitrogen (NH_4-N, NO_3-N) concentrations.

Field experiment

Field experiments were conducted in the Fairbanks and Delta Junction areas of Alaska. Soil at the Fairbanks experimental site has been developed from silt micaceous loess over alluvium (Tanana Series, subgelic Typic Aquiturbels). The soil usually has a pH of 5.6 or lower, and a silt loam texture (Table 1). Soil at the Delta Junction experimental site has been developed from loess overlaid on a glacial outwash. Soil at this site has an acidic pH of 4.5 and silt loam texture (Table 1). Fish coproducts (FM, FBM, FH) were applied at 50, 100, and 150 kg N per ha. For comparison, a treatment of urea applied at 100 kg N per ha, and a control treatment with no addition of nutrients were included. In all, 11 treatments were arranged in a randomized complete block design, each with four replicates. The experiment was conducted in the 2006 and 2007 growing seasons at both sites. Each year, plots were laid out in a different field having similar soil properties. In 2007, the Fairbanks site plots did not include an FH treatment due to shortage of the test material. Instead, coarse fish bones were applied as the treatment. In 2007, the plots that had received fish coproduct application in 2006 at both sites were planted with crops to study the residual nutrient release. Barley (*Hordeum vulgare* L. cv. Wooding) was grown as the test crop in each treatment to show biomass production in response to fish coproduct application, and crop nitrogen recovery from these coproducts. Urea or fish coproducts were applied on the soil surface and then rototilled into the soil to a depth of about 10 cm. Barley was seeded at a rate of 250 plants per m^2.

Benchmark soil samples were taken at each site prior to the experiment, and soil samples from each treatment were taken in the fall of 2006 and 2007. Plant samples were taken in July (growth stage = anthesis) and in August (growth stage = senescence). Plant samples

Table 1. Characteristics of soil at two experimental sites in Alaska.

Site	Soil depth	pH	NH_4-N (mg/kg soil)	NO_3-N (mg/kg soil)	M3-P (mg/kg soil)	Bulk density (mg/m^3)	Soil texture		
							Sand (%)	Silt (%)	Clay (%)
Fairbanks	0-15 cm	7.2	2.0	6.1	102.9	0.85	19	56	25
	15-30 cm	6.8	1.5	21.0	20.2	0.91	20	57	23
Delta Junction	0-15 cm	4.8	6.9	7.9	20.2	0.78	49	42	9
	15-30 cm	4.9	3.1	4.0	4.7	0.87	59	28	14

M3-P = Mehlich 3 (1.5 M NH_4F + 0.1 M EDTA) extractable P.

were dried (65ºC) and weighed. A subsample was then removed and ground with a Willey mill (<2 mm) for determination of nitrogen concentration. Soil samples from the field experiments were air-dried, and passed through a 2 mm sieve. Mineral nitrogen in air-dried samples from the field experiment, as well as the moist soil samples from the incubation experiment, was extracted with 2M KCl at a soil to solution ratio of 1:10, followed by determination of NH_4^+ and NO_3^- in a Technicon autoanalyzer (Maynard and Kalra 1993; Technicon Autoanalyzer II 1973, 1978). Total carbon and nitrogen in soil and coproduct samples, and the nitrogen content in plant samples, were determined with a LECO CNS-2000 analyzer.

The amount of nitrogen uptake by barley was calculated by multiplying tissue nitrogen concentration with biomass yield for each treatment. Percent apparent nitrogen recovered from applied nitrogen sources was calculated by first subtracting nitrogen uptake in the control treatment of each year, and dividing the result by the nitrogen application level. The apparent nitrogen recovered by barley from each fish coproduct was the average of three application levels over two years. Analysis of variance (ANOVA) was conducted for barley biomass, and the amount of nitrogen uptake using Statistix 8.0 (Analytical Software, Tallahassee). Mean comparison among treatments was made at a 5% significant level using least significant difference (LSD).

Results and discussion

Incubation experiment

In the incubation study, the nitrogen concentration in soils from both sites peaked on day 56 and subsequently leveled off (Figs. 1a, 2a). On day 56, the total mineral nitrogen released from the fish coproduct treatments (value minus control) was 80, 90, and 67 mg N per kg soil from the Fairbanks site, and 93, 80, and 62 mg N per kg from the Delta Junction soil for fish meal (FM), fish bone meal (FBM), and fish hydrolysate (FH), respectively. On average for both soils, the 56 day values accounted for 87% of added nitrogen from FM, 85% from FBM, and 65% from FH. The growing season in interior Alaska is about 110 days long, and the average growing season temperature is around 15ºC. The amount of nitrogen released in the incubation study at 15ºC indicated that the majority of applied nitrogen in the three fish coproducts could be released during the first half of the growing season. This is significant, since nutrients for crop growth such as nitrogen are most needed early in the growing season, indicating the fish coproducts can be used as a source for supplying nitrogen for crop production.

Comparing among the three coproducts, the FM and FBM were similar in total amount of nitrogen released on day 56. However, the FH released lower amounts of added nitrogen in comparison with the other

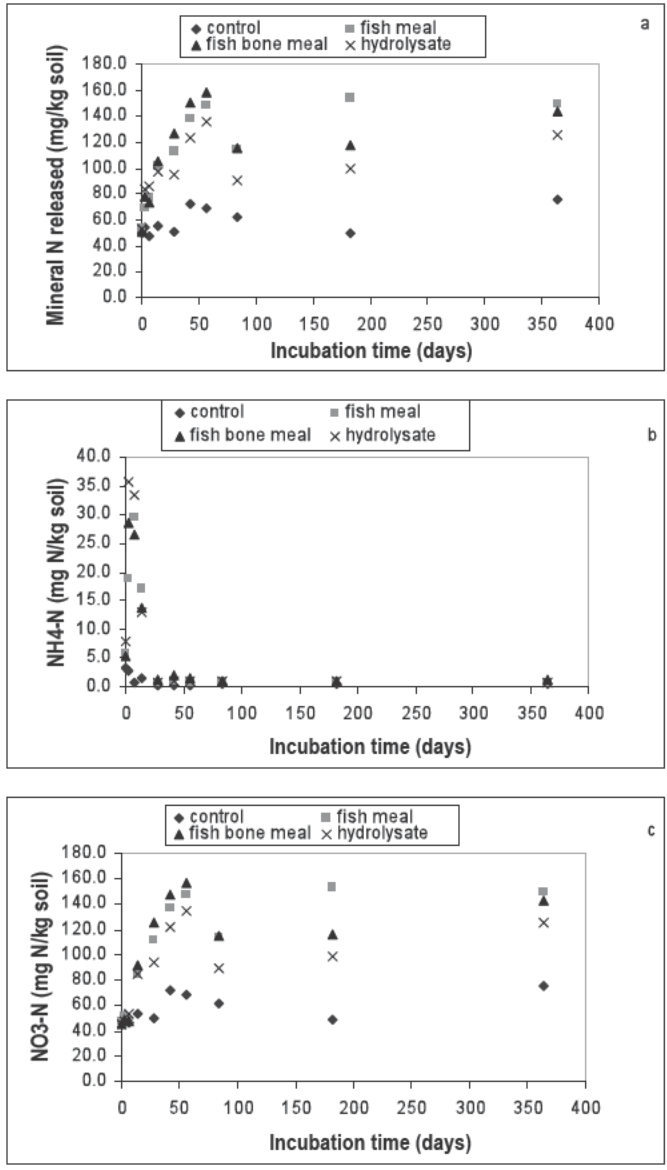

Figure 1. Nitrogen released from fish coproducts in 52-week incubation of soil from the Fairbanks, Alaska, site. (a) Total mineral nitrogen. (b) NH_4-N. (c) NO_3-N.

two coproducts. Phosphoric acid was added to stabilize the FH after it was made. The acidic condition may have contributed to further protein hydrolysis and ammonification resulting in a faster mineral nitrogen release (mostly NH_4-N, 36.0 mg N per kg soil from FH, in contrast to 18.0 mg N per kg soil for FM and 28 mg N per kg soil for FBM on average for the two soils) from the FH in the first three days of incubation (Figs. 1b, 2b). Even though the total amount of mineral nitrogen released was similar for both soils, the NH_4-N and NO_3-N dynamics for the soils were different. It appeared that a large proportion of mineral nitrogen was in the form of NH_4-N in the Delta Junction soil, corresponding to the mineral nitrogen peak on day 56 in the incubation experiment (Fig. 2). In contrast, the majority of nitrogen in the Fairbanks soil was in the form of NO_3-N at the same time period, and the NH_4-N concentration was high in the soil only in the very early stage of incubation (Fig. 1). The oxidation rate of NH_4^+ to NO_3^- was faster in the soil from the Fairbanks site than the Delta Junction site. The oxidation process transforming NH_4^+ to NO_3^- (nitrification) is controlled by a variety of factors, but the most important is soil pH (Schmidt 1982). Acidic pH inhibits nitrification (Kyveryga et al. 2004). The pH of soil from the Delta Junction site was more acidic than the Fairbanks site (Table 1), which can explain a slower nitrification process in the Delta Junction soil. Most vascular plant roots can use both NH_4-N and NO_3-N (Marschner 1998). Therefore, the difference in the proportions of NH_4-N and NO_3-N in the soil should not greatly affect plant growth.

Field experiment

At the July sampling (anthesis), barley biomass yield in 2006 at the Fairbanks site was not statistically different ($p > 0.05$) among treatments. Apparently, an increase in nitrogen application in the of fish coproducts increased barley biomass, indicating beneficial impact on plant growth from the coproducts (Table 2). For the August sampling (senescence) at the Fairbanks site, even though there was no statistical ($p > 0.05$) difference among the treatments, barley biomass yields were higher for the 150 than 50 application levels. The Fairbanks senescence biomass yield from fish coproducts was higher in some instances than biomass yield values from the control. For the Delta Junction site, the amount of barley biomass taken in anthesis was similar for all three coproducts, but all were higher than that measured for the control treatment (Table 2). For the Delta Junction senescence samples, no statistical differences ($p > 0.05$) were found among the treatments. However, apparent biomass yield from the three fish coproduct treatments at the 100 and 150 level applications were higher than the control treatment, indicating barley growth in response to fish coproduct application.

For barley biomass yield in 2007 at the Fairbanks site, fish hydrolysate (FH) was not added in the treatment regime because the supply

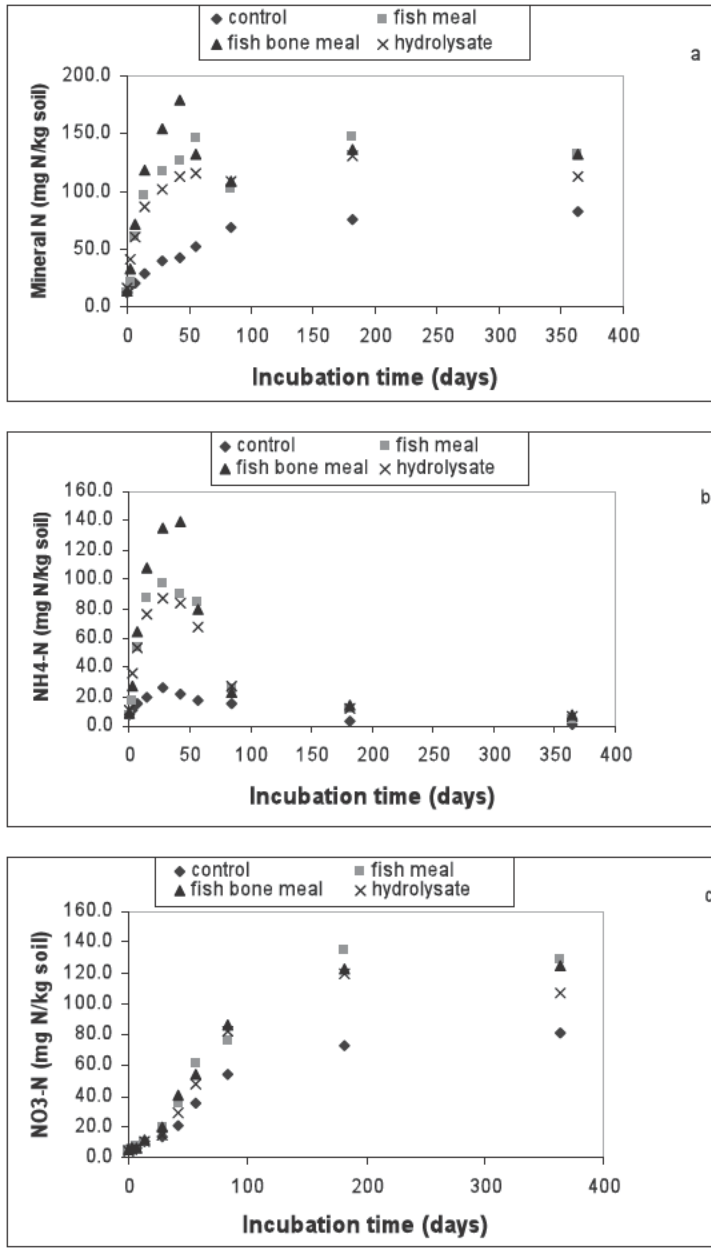

Figure 2. Nitrogen released from fish coproducts in 52-week incubation of soil from the Delta Junction, Alaska, site. (a) Total mineral nitrogen. (b) NH_4-N. (c) NO_3-N.

Table 2. Barley above-ground biomass yield at anthesis and senescence stages at two Alaska sites, after fish coproduct application.

	Biomass yield (t/ha) during year of coproduct application				Biomass yield (t/ha) in 2nd year of coproduct application			
	Fairbanks		Delta Junction		Fairbanks		Delta Junction	
Treatment[a]	Anthesis	Senescence	Anthesis	Senescence	Anthesis	Senescence	Anthesis	Senescence
2006								
Control	2.05	10.57	0.43	4.23				
Urea 100	2.25	11.51	2.00	7.30				
FM 50	1.93	13.05	0.74	3.78				
FM100	2.38	15.37	1.08	5.40				
FM 150	2.41	14.61	1.15	7.44				
FBM 50	1.91	14.05	1.03	4.89				
FBM 100	1.97	11.05	1.03	5.87				
FBM 150	2.26	16.17	1.46	7.93				
FH 50	1.73	11.29	0.92	4.91				
FH 100	2.07	11.98	1.01	6.79				
FH 150	1.90	14.81	1.56	6.18				
Probability (*F* test)	NS (0.78)	NS (0.61)	0.0008	NS (0.70)				
LSD (0.05)	NA	NA	0.58	NA				
2007								
Control	2.10	5.39	2.41	5.75	2.57	9.30	0.88	3.83
Urea 100	4.41	11.47	4.53	7.40	3.18	10.79	1.28	3.02
FM 50	3.07	9.26	3.25	6.16	2.86	8.48	0.87	3.36
FM 100	4.71	11.34	3.83	7.29	4.08	9.96	0.86	2.70
FM 150	4.07	11.05	4.11	6.19	3.04	13.61	0.98	4.04
FBM 50	3.44	9.83	2.93	6.33	2.93	9.95	1.16	4.21
FBM 100	4.32	9.89	4.41	7.38	2.81	10.62	1.32	3.88
FBM 150	4.13	11.62	4.46	7.32	4.00	10.73	1.80	3.92
FH 50	2.78[b]	7.48[b]	3.91	6.37	3.62	11.68	0.91	4.03
FH 100	3.50[b]	9.31[b]	2.85	5.40	3.47	9.86	1.13	3.26
FH 150	3.92[b]	11.01[b]	2.56	6.28	3.25	8.92	1.19	3.44
Probability (*F* test)	0.0002	0.045	0.006	NS (0.45)	NS (0.60)	NS (0.32)	0.010	NS (0.51)
LSD (0.05)	1.00	3.72	1.30	NA	NA	NA	0.47	NA

[a]Treatment, kg N per ha application. FM = fish meal, FBM = fish bone meal, FH = fish hydrolysate.
[b]Coarse fish bone meal was substituted for FH, in 2007 at Fairbanks.
NS = not significant, number in parenthesis indicated level of probability of F test.
NA = not applicable.
LSD = least significant difference.

was exhausted. Instead, coarse fish bones that had not been subjected to grinding ere substituted and applied at 100 and 150 kg N per ha rates as illustrated in Table 2. Biomass was significantly higher than the control about two months after application (anthesis). Fish meal (FM) and fish bone meal (FBM) treatment results were generally consistent with those of 2006. At the same application rate, FM and FBM were similar to the urea treatment in biomass yield for the samples taken both in anthesis and senescence. For the July samples at the Delta Junction site, the FM treatment applied at 150 kg N per ha had a similar biomass production compared with the urea treatment. Barley biomass from FBM was similar to that achieved in the urea treatment at 100 and 150 kg N per ha application levels. In general, FH generated a higher apparent biomass yield than the control treatment, but had a lower yield than the urea treatment (Table 2). For the Delta Junction samples taken at senescence, there were no statistical ($p > 0.05$) differences among treatments.

There were residual nutrients for crop growth from previous fish coproduct applications at both sites as indicated by apparently higher biomass yield compared to the control in the first sampling date (Table 2). However, the residual effect for barley biomass production was not significant ($p > 0.05$) for most sampling time and site combinations. Only FBM for both sites at the 150 kg N per ha application rate consistently generated biomass yield statistically higher than the control. Uneven distribution of soil fertility at the Fairbanks experimental site of 2006 and bird damages for the Delta Junction site in 2006 and 2007 probably caused large variations in the field experiments that contributed to the absence of statistically significant differences among treatments. Nevertheless, the trend of barley biomass yield in response to fish coproduct application can still be seen from the results in Table 2.

As shown in Table 3, barley nitrogen uptake varied with site, sampling time, and year; however, in most site-sampling time-year combinations, the amount of barley nitrogen uptake was not significantly different ($p > 0.05$) (Table 3). Generally the apparent nitrogen uptake from the treatments of fish coproducts was higher than that the control treatment, indicating plants utilizing nitrogen released from fish coproducts. In both years (2006 and 2007) at the Fairbanks site, nitrogen uptake by barley from FM and FBM applied at 100 kg nitrogen was similar to the urea treatment values at anthesis (Table 3). These data demonstrate that both fish coproducts functioned as well as commercial urea in supplying necessary nitrogen at the Fairbanks site. For senescence 2006 samples, apparent barley nitrogen uptake at the Fairbanks site from FM, FBM, and FH applied at 100 kg N per ha was higher that the comparable urea treatment. For both years at the Delta Junction site, barley nitrogen uptake from fish coproducts was lower than that from urea when applied at 100 kg N per ha at the early sampling time (anthesis) (Table 3). The trend continued at the senescence sampling

Table 3. Barley nitrogen uptake from fish coproducts.

	N uptake (kg/ha) during year of coproduct application				N uptake (kg/ha) in 2nd year of coproduct application			
	Fairbanks		Delta Junction		Fairbanks		Delta Junction	
Treatment[a]	Anthesis	Senescence	Anthesis	Senescence	Anthesis	Senescence	Anthesis	Senescence
2006								
Control	82.0	201.0	13.3	57.3				
Urea 100	87.1	221.4	80.1	111.2				
FM 50	77.3	242.6	28.5	52.2				
FM 100	94.0	295.8	41.9	81.6				
FM 150	94.5	286.6	55.5	127.8				
FBM 50	69.3	266.2	40.9	70.5				
FBM 100	76.6	220.8	48.5	82.7				
FBM 150	91.2	307.6	70.7	126.4				
FH 50	69.8	225.1	30.5	64.6				
FH 100	79.1	243.2	33.4	77.3				
FH 150	72.6	285.9	56.5	84.8				
Probability (*F* test)	NS (0.69)	NS (0.50)	<0.0001	NS (0.32)				
LSD (0.05)	NA	NA	22.4	NA				
2007								
Control	31.8	63.5	35.5	83.9	55.8	126.4	20.3	37.8
Urea 100	112.5	128.1	95.0	105.1	96.8	175.4	29.6	29.3
FM 50	70.4	112.9	50.6	86.0	79.2	121.3	20.1	38.1
FM 100	124.6	150.4	64.3	105.3	102.0	151.0	18.5	32.8
FM 150	116.8	173.4	91.7	108.9	102.1	209.4	23.7	58.7
FBM 50	58.0	125.0	53.0	82.4	64.1	166.3	24.9	50.0
FBM 100	95.8	113.1	85.0	114.4	71.7	158.2	32.2	43.6
FBM 150	111.5	150.9	95.2	117.6	126.1	177.0	41.5	48.8
FH 50	58.0[b]	82.2[b]	45.2	83.6	97.1	189.8	21.3	47.1
FH 100	80.7[b]	121.3[b]	44.2	72.9	89.6	142.7	24.8	35.3
FH 150	112.7[b]	139.3[b]	39.3	87.6	83.8	122.4	26.2	42.3
Probability (*F* test)	<0.0001	0.05	<0.0001	NS (0.28)	NS (0.46)	NS (0.59)	0.02	NS (0.13)
LSD (0.05)	20.5	61.3	24.6	NA	NA	NA	12.1	NA

[a]Treatment, kg N per ha application. FM = fish meal, FBM = fish bone meal, FH = fish hydrolysate.
[b]Coarse fish bone meal was substituted for FH, in 2007 at Fairbanks.
NS = not significant, number in parenthesis indicated level of probability of *F* test.
NA = not applicable.
LSD = least significant difference.

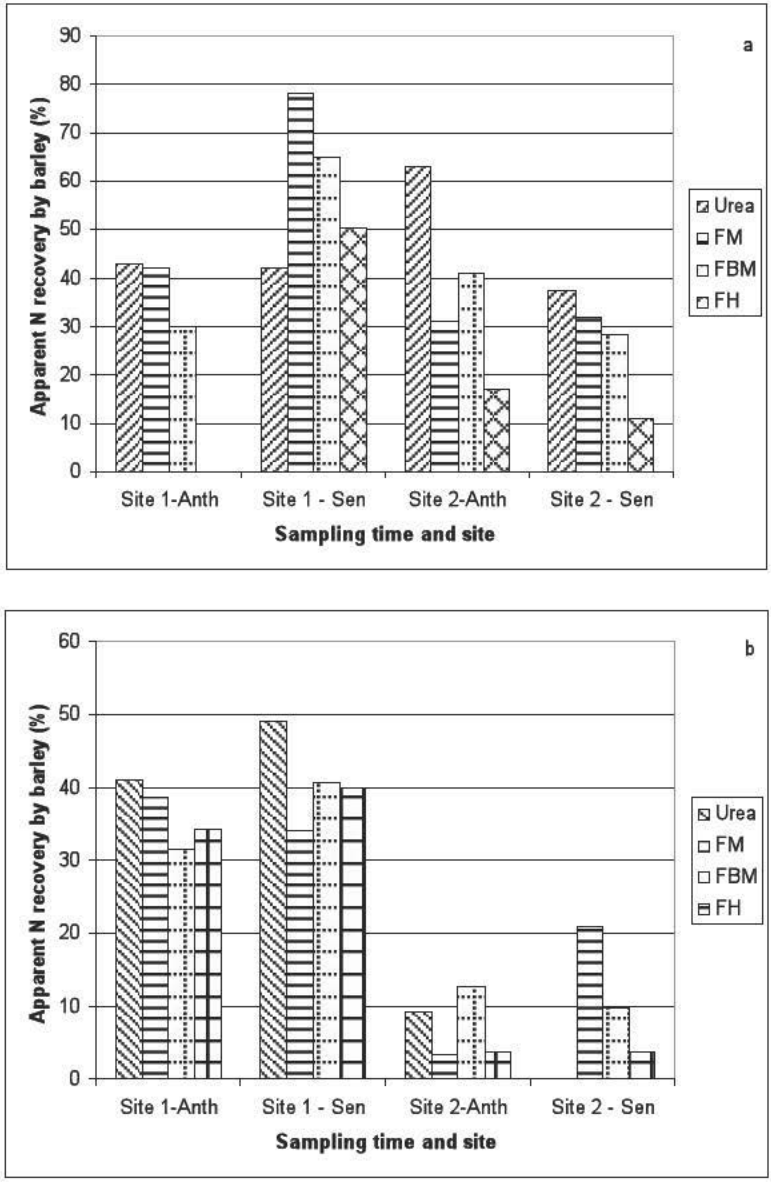

Figure 3. (a) Average (2006, 2007) nitrogen recovery during the year of fish coproduct application. (b) Residual nitrogen recovery in 2007 from fish byproduct applied in 2006. Site 1 = Fairbanks, Alaska. Site 2 = Delta Junction, Alaska. FM = fish meal. FBM = fish bone meal. FH = fish hydrolysate.

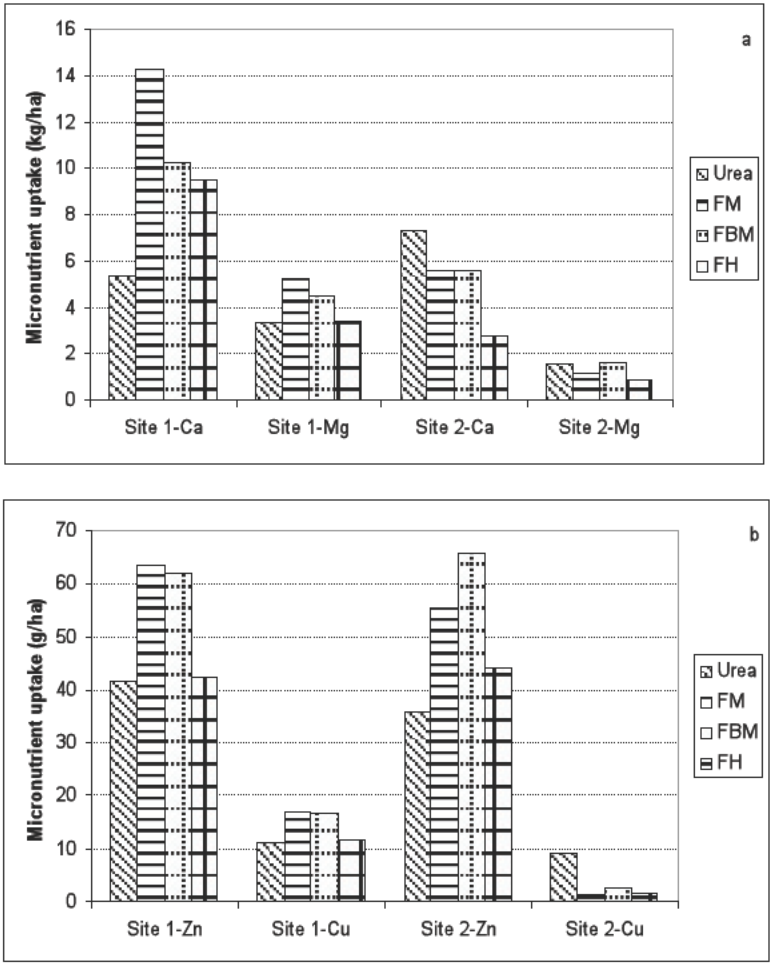

Figure 4. (a) Average net Ca and Mg. (b) Zn and Cu uptake by barley from fish coproducts and urea in 2006 and 2007 for both sites. Site 1 = Fairbanks, Alaska. Site 2 = Delta Junction, Alaska. FM = fish meal. FBM = fish bone meal. FH = fish hydrolysate.

time in 2006, but in 2007 the barley nitrogen uptake from fish meal and fish bone meal was similar to that of urea when applied at 100 kg N per ha (Table 3). One explanation for this is that residual nitrogen was released from the previous year's coproduct application, especially early in the growing season and at the high nitrogen application rate as indicated by higher barley nitrogen uptake in comparison with the control treatment (Table 3).

The apparent nitrogen recovery from fish coproducts on average from two years and three application rates was 78% for FM, 65% for FBH, and 50% for FH at the Fairbanks site in contrast to 32% for FM, 28% for FBM, and 11% for FH at the Delta Junction site (Fig. 3a). The Delta Junction site suffered from bird damage during both years, which may have contributed to the low nitrogen recovery at the site. Zhang et al. (2006) reported a 45% nitrogen recovery from liquid manure application in smooth brome grass near Palmer, Alaska. Barley also recovered some of the residual nitrogen in 2007 from the fish coproduct applied in 2006, but nitrogen recovery was lower at the Delta Junction site (Site 2) in comparison with the Fairbanks site (Site 1) due to bird damage (Fig. 3b). Using ^{15}N labeled urea, Knight and Sparrow (1993) reported an average (three years) of 40% urea nitrogen recovery by barley in the Delta Junction area. This is lower than the apparent barley nitrogen recovery (~60%) reported in this study in the Delta Junction site when non-isotopic urea nitrogen was used. This difference might be attributed to barley discrimination against isotopic ^{15}N uptake (Kolb and Evans 2003).

Barley absorbed Ca, Mg, Zn, and Cu from fish coproducts at the Fairbanks site, but only Zn at the Delta Junction site, averaging two year results (Fig. 4a,b). This site difference might have been caused by bird damage at the time of harvesting (i.e., lowering biomass production) or by soil chemical properties such as pH.

Summary

Fish coproducts released a majority of their nitrogen within 56 days of application. The three fish coproducts—fish meal, fish bone meal, and fish hydrolysate—released between 65 and 90% of added nitrogen. The field experiment demonstrated that fish meal and fish bone meal worked as well as urea fertilizer when applied at the same rate. In conclusion, fish processing coproducts can be used as a good nutrient source for crop production under conditions found in Alaska.

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Enhancing Utilization of Alaska Fish Processing Byproduct Parts

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Abstract

Over half of the total wild fish harvested and processed for human consumption in the United States comes from Alaska waters. Large volumes of pollock, salmon, cod, and flatfish are harvested annually, resulting in over 1 million metric tons of processing byproducts. Alaska fish processing byproducts have several advantages because they are derived from sustainable fisheries where all the fish are initially processed for human consumption. The major byproducts from the fish processing industry are heads, viscera, frames, and skin. There is a need to increase the utilization and value of these byproducts.

The largest volumes of individual byproduct components occur with viscera and heads, with smaller volumes of frames and skin also available. Viscera is the generic term used to describe the organs and tissue removed after the belly cavity is opened and usually includes the reproductive organs (ovary and testes), stomach, liver, kidney, and digestive tract. There are increasing uses for viscera and its components as well as heads; however, most fish meals and oils are used as animal and fish feed ingredients.

Making value-added products such as gelatins from fish skins or oils with high omega-3 fatty acids from separated fish processing byproduct is of increasing importance and interest.

Introduction

Fish processing byproducts have been utilized for many years to make protein meals and oils in Alaska and elsewhere. The establishment of the Alaska Oil and Guano Company in 1882 is considered by many to be the start of the Alaska fish meal and oil processing industry. An early

industrial fish meal industry in Alaska was the processing of whole herring into edible oil, feeds, and fertilizer. This fishery was discontinued in the late 1950s due to flagging economic viability and increasingly restrictive government regulations. Today in Alaska there is a very viable fish meal and oil industry, and all the raw material comes from what remains after fillets and other edible parts are removed from the landed fish. Many consider the making of meals and oils from fish processing byproducts to be a more sustainable model than the harvesting of whole fish for the sole purpose of making protein meals and oils to be used as feed ingredients.

The genesis of current fish meal and oil operations in Alaska was largely the result of the passage of the 1972 Clean Water Act. The subsequent Magnuson Fisheries Conservation and Management Act of 1976 led to the implementation of the 200 mile Exclusive Economic Zone (EEZ) and spurred construction of high capacity fish processing plants coincidental with the Americanization of the groundfish fisheries in the Bering Sea and Gulf of Alaska. Industrial technologies for handling the byproducts of fish processing derived from these large operations were required by the Alaska Department of Environmental Conservation as a cost of doing business. Today, there are eight modern fish meal plants in western Alaska that predominantly handle pollock, cod, and other groundfish processing byproducts. In addition, there are several fish meal and oil processing plants located in Gulf of Alaska coastal communities that process byproduct streams from salmon operations.

The production of coproducts including fish protein meal, fish oil, fish bone meal, and stickwater from fish processing byproducts may be entering a new era as there is the potential for more stringent restrictions on materials that may be discarded and an increased emphasis on full utilization of landed fish. In addition, there are opportunities to produce new or different food and feed ingredients with desirable properties such as long chain omega-3 fatty acids and the vitamins associated with seafood.

Worldwide, approximately 6.5 million metric tons of fish protein meal is made annually. Most of the meals and oils come from large-scale wholly industrial fisheries such as the anchovetta fisheries in Peru. In Alaska, fish meal and oil are produced only from processing byproducts derived from fish harvested solely as human food; however, the amount of meal and oil produced in Alaska is only an estimated 2% of world production. Nonetheless, there are advantages associated with using fish processing waste from cold water marine fisheries: (1) the assurance that the fish were from a certified sustainable fishery; (2) that the fish were processed for human consumption and initially are of very high quality; (3) cold water marine fish generally have high levels (around 30% total fatty acids) of omega-3 fatty acids; (4) separate parts such as skin, frames, viscera, and heads are readily available; and (5) viscera reproductive components such as ovaries and testes are also readily available.

Table 1. Commercial marine finfish harvest in Alaska (t).

	2000	2005	2008[a]
Alaska pollock	1,067,738	1,414,962	1,042,351
Salmon	319,472	408,014	322,470
Pacific cod	226,709	225,341	207,511
Flatfish	141,530	199,144	314,707
Atka mackerel	39,986	58,423	60,026
Pacific ocean perch	17,077	21,002	29,487
Pacific herring	32,509	37,610	35,837
Halibut	32,686	26,061	21,510
Total	1,905,249	2,447,995	2,090,379

[a]2008 data from ADFG 2008a,b and NMFS 2008a,b,c.

Methods

Total catch, by species, was calculated (ADFG 2008a,b; NMFS 2008a,b,c) and estimates made of the ratios of products and byproduct. Percent heads, frames, viscera, skin, and fillets by species were abstracted from published values (Crapo et al. 1993) and used to estimate individual byproduct values. As a generality, the waste stream from most pollock and cod fillet operations generated similar byproducts. For other species, such as salmon, heads and viscera were the major byproducts.

Discussion

The annual harvest of fish from Alaska waters is approximately 2 million metric tons (t) as shown in Table 1. In 2008, there were 2,090,179 t of fish harvested in Alaska, and walleye pollock was the most abundant species harvested with over 1 million t. The Alaska pollock fishery is the largest food fishery in the world. Salmon and Pacific cod contributed 322,470 and 207,511 t to the total landings, respectively. In 2008 more than 75% of the total fish harvest in Alaska was pollock plus cod, while salmon and flatfish contributed an additional 15%.

In 2000 approximately 70% of the fish harvest in Alaska was from the Bering Sea and Aleutian Islands, where the majority of the harvest involves groundfish (Crapo and Bechtel 2003). The Bering Sea and Aleutian Island harvest is divided between at-sea factory trawl processors and shore-based processing plants. Seven of the ten largest fish processing plants are located in this region, and the three largest processing operations are located in the number one landing port in the United States, Dutch Harbor, Alaska (NMFS 2008a,b,c).

Salmon are usually processed in smaller (often remote and seasonal) operations circumscribing the Gulf of Alaska and in the Bristol

Table 2. Estimated 2008 byproduct components from Alaska fish processing.

	Fish weight (%)[a]	Total weight (t)
Alaska pollock		
Total harvest	100	1,042,351
Total waste[b]	66	687,952
Heads	17	177,200
Viscera	21	218,894
Frames	22	229,317
Skin	6	62,541
Salmon		
Total harvest	100	322,470
Total waste	27	87,067
Heads	18	58,045
Viscera	9	29,022
Pacific cod		
Total harvest	100	207,511
Total waste[c]	46	95,455
Heads	18	37,352
Viscera	19	39,427
Frames	18	37,352
Skin	6	12,451
Flatfish		
Total harvest	100	314,707
Total waste	72	226,589
Heads	20	62,941
Viscera	14	44,059
Frames	30	94,412
Skin	8	25,177
Atka mackerel		
Total harvest	100	60,026
Total waste	32	19,208
Heads	19	11,405
Viscera	13	7,803
Perch		
Total harvest	100	29,487
Total waste	38	11,205
Heads	26	7,667
Viscera	12	3,538

Table 2. (continued)

	Fish weight (%)[a]	Total weight (t)
Pacific herring		
Total harvest[d]	100	35,837
Halibut		
Total harvest	100	21,510
Total waste	28	6,023
Heads	16	3,442
Viscera	12	2,581
Others		
Total harvest	100	56,480
Total waste[e]	33	18,638
Heads	18	10,166
Viscera	15	8,472
Totals		
Total harvest		2,033,899
Total waste		1,152,137
Total heads		330,885
Total viscera		353,797
Total frames		361,081
Total skin		100,168

[a]Crapo et al. 1993.

[b]The 66% total waste value is low, especially when surimi paste is made.

[c]Assume shoreside produce fillets (61% waste) and catcher processors headed and gutted fish (37% waste).

[d]Assume herring are frozen and shipped elsewhere for processing.

[e]General values used for calculations that may not be appropriate for species.

Bay region of the Bering Sea, although there are also significant salmon harvests on the southern side of the Alaska Peninsula southwest of Kodiak Island. Salmon processing byproducts are dominated by heads and viscera, and are geographically dispersed across Alaska, making this material more difficult to fully utilize.

Fillets and other components removed for human consumption vary in size with species and product form (Crapo et al. 1993). Headed and gutted fish retain much of the landed weight of the fish and have smaller amounts of processing waste compared to skinless fillets. The major byproduct components in large fish processing plants reflect the unit processing operations such as head removal followed by the removal of the viscera for headed and gutted processed fish, or for skinless fillets, heads removed first, followed by viscera, then frames (backbone and ribs), and finally the skin removed. These separate operations can be arranged into separate byproduct streams that could be used to make various new products or combined for fish meal or oil production.

Different byproducts can be used alone or in combinations to produce unique feed ingredients. Recoveries of food from landed weight in salmon can average 77%, producing only 23% byproducts. In 2008, a similar calculation indicated 87,067 t of which 58,045 t was salmon heads and 29,002 t was viscera (Table 2). The product mix can vary with processor, processing plant location, and a number of other variables. Crapo et al. (1993) estimated the processing waste yield for Alaska pollock to be fully 66% of landed weight or extrapolated for 2008, 687,952 t. This extrapolation showed a possible 218,894 t of viscera, 177,200 t of heads, 229,317 t of frames, and 62,541 t of skin (Table 2).

Table 2 also lists the total amount of heads, skin, frames, and viscera that are theoretically available in Alaska. The estimated amount of each component is staggering with over 300,000 t each of viscera, frames, and heads theoretically available. It is unlikely that these volumes will ever be actually achieved without some unforeseen economic opportunity arising. Currently most of the processing waste material is used to make fish meal and oil, and the prices of these commodities have increased substantially over the past decade. There are at least two opportunities to increase the value of these resources: one is to increase the utilization of byproduct that is currently being disposed of, and the second is to develop new products and/or processes from underutilized byproducts such as livers and testes.

The byproduct parts—heads, frames, viscera, and skin—each have a unique chemical composition that may be exploited to make new products for niche markets. Table 3 shows an example of the proximate composition of meals made from whole pollock, pollock fillets, heads, frames, viscera, and skin. As can be seen, there are large and significant differences in the quantities of protein, fat, and ash content in these meals. As would be expected, pollock frames and heads have a higher

Table 3. Proximate composition of meals from Alaska pollock byproduct parts.

	Water %	Protein %	Fat %	Ash %
Whole fish	8	65.7	15.5	10.8
Fillets	8	84.9	1.9	5.2
Heads	8	67.4	5.2	19.4
Frames	8	73.6	3.9	14.5
Viscera	8	41.2	47.2	3.6
Skin	8	87.5	1.7	2.8

Data adjusted for 8% moisture content from Bechtel 2003.

Table 4. Proximate composition of livers from fish harvested in Alaska.

	Protein %	Moisture %	Ash %	Fat %
Pollock	7.7	41.0	0.9	50.3
Halibut	13.5	73.3	1.3	12.0
Arrowtooth	13.7	65.3	1.5	19.4
Salmon	18.6	76.6	1.5	3.3

Data from Bechtel and Oliveira 2006.

ash content (due to greater bone content); skin is relatively higher in protein, much of which is collagen; and viscera contains a very high fat content, most of which comes from the liver. The whole fish and fillet values are included for reference.

From a food science standpoint viscera may be the most interesting material because it is composed of a variety of parts, each with different values, uses, and biological stabilities. The viscera component with the highest value is roe, which is almost always carefully collected and sold. However, in Alaska the fish's liver is seldom used even though it can be segregated easily from the waste stream and used to make high quality fish oils rich in omega-3 fatty acids and high quality protein. Table 4 shows the proximate composition of raw liver from pollock, halibut, arrowtooth flounder, and salmon (Bechtel and Oliveira 2006). Of interest is the variation in fat content of the livers and the fatty acid profiles (data not included), which differ significantly between species. As shown, the fat content of salmon liver is low at 3%, while in pollock liver the fat content is high at 50%. There is a large seasonal influence on the percent of fat in livers of pollock, cod, and other species where the liver is the major fat storage organ. The high fat content of pollock

Table 5. Proximate composition of gonad meals from Alaska pollock and salmon.

	Pollock	Salmon
Protein %	72.4	79.9
Lipids %	12.7	3
Ash %	10.1	12.8
Moisture %	5.9	5.9

Data from Plante et.al. 2007.

livers makes them an excellent raw material for producing high quality oils, and salmon livers can be made into a unique protein meal with many desirable lipid and protein characteristics.

Viscera from fish processing also contains the female and male reproductive organs (ovaries and testes). Mature roe from salmon, cod, and pollock is usually collected and processed for export markets. However, the male testes from most Pacific cod and some walleye pollock are collected and exported to Asian capitals. Salmon testes and much of the recovered pollock testes are included when making fish protein meal, or they are discarded. Table 5 shows the proximate composition of meals made from both pollock and salmon testes (Plante et al. 2007). As can be seen, both meals have high protein levels and a relatively low percentages of fat. This is a unique product with a distinctive amino acid profile including a high arginine content, and the product also has high levels of DNA.

Summary

A considerable volume of fish processing byproducts in Alaska are underutilized or discarded. There are many reasons for this including the remoteness of locations, restricted processing volumes, short seasons, shipping costs, and general economics. However, there are clearly opportunities to produce exciting new foods and ingredients from individual byproduct components in the existing fish meal plants located across Alaska and on some of the at-sea factory trawl processors. If there are appropriate economic incentives, it will be effective to collect and segregate individual byproduct parts such as skin, viscera, heads, testes, and livers and, relying on the unique individual biochemistries, use these to make new foods, ingredients, and other value-added products.

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Improving Waste Solids Quality and Recovery from Fish Processing Plants

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Abstract

The design and operation of fish processing plants can significantly impact the quality and quantity of recovered byproducts, especially if they are rendered into fish meal. Most fish processing plants are designed to maximize the recovery and quality of their food products with less consideration for the recovery and transport of non-food waste or processing byproducts. These plants generate a sizable percentage of waste as dissolved solids and very fine particles in wastewater, thereby reducing waste recovery. The recoverable fine particles that are sent to rendering will likely not be recovered as fish meal and will increase the pollutant load in the rendering operation wastewater.

A detailed inspection of a fish processing facility can reveal areas where waste solids can degrade because of inappropriate temperatures and times, and also areas where they can become contaminated. The degradation of the recovered solids has the potential to affect the protein quality of the rendered meal and oil byproducts.

Properly designed waste conveyance systems that recover waste solids before they reach floor drains or sumps are preferable. Fluming waste solids with water increases the breakdown of solids and decreases the amount of solids that can be recovered. Additionally, if saltwater is used for fluming it can increase the salt and ash content of the waste solids. If the solids are then rendered the resulting fish meal will be of a lower quality. Hence, dry conveyance of waste solids is preferred. For waste solids that circumvent the waste conveyance system, the design and operation of wastewater floor drains, sumps, and pumps can affect the quality and quantity of recovered waste solids. The same is true for the wastewater treatment equipment used for recovering waste solids. And finally, the methods by which potentially food-grade products are

rejected, and unacceptable fish are added to the waste stream, can be of concern.

Fish processing basics

In general, fish processing plants have similar inputs and outputs. Fish and water are the inputs, and food products, liquid waste, and solid waste are the outputs. Additional sources of solid waste are reject fish, solids recovered by treating the liquid waste, and reject food product. The recovered solid waste requires disposal or rendering depending on the size and location of the operation. Very large fish processing plants, especially in remote locations, are integrated operations. The recovered solid waste from the fish processing plant becomes the input for a rendering plant, which is typically a fish meal plant. The outputs of the rendering plant are coproducts such as fish meal, bone meal, fish solubles, and fish oil. Rendering plant waste is generally composed of condensate and wastewater. It should be obvious that the quality of the solids entering the rendering operation affect the quality of the rendered byproducts. What may not be as clear is how the unit operations in the fish processing plant affect the quantity and quality of waste solids recovered for rendering.

Elements for fish waste recovery

Whether fish plants use manual or mechanized butchering lines, a solid waste conveying system is used for recovering, dewatering, and transporting the solids to a central location for disposal. The liquid waste, or wastewater from the processing plant, is channeled and pumped using a liquid waste conveying system to a central location where additional solids are recovered using a wastewater solids recovery system, typically screening. Rejected unprocessed fish and food product are collected and conveyed for disposal using a rejected product handling system. Each of these operations affects the quality and quantity of recovered waste solids.

Solid waste conveying system

Waste solids from fish butchering are generally conveyed in one of two ways. Dry conveyance uses a dewatering conveyor to transport and separate any entrained wastewater from the waste solids. The wastewater is generally discharged to the plant floor and mixes with wastewater and non-captured waste solids from the butchering operation. The design of the dewatering conveyor, in particular the mesh size of the conveyor belt, can affect the moisture content of the solids recovered for rendering, and solids lost to the floor. The smaller the opening in the conveyor belt, or if a solid belt is used, the greater the amount of wastewater retained and the smaller the amount of waste solids lost to

the floor. In general, only a percentage of the solids lost to the floor can ultimately be recovered.

A second option for recovering and transporting waste solids in large plants involves the use of flumes. The waste solids from the fish butchering operation drop into open, semicircular metal or plastic channels to which water and/or recycled wastewater is added to convey the solids. The solids for rendering are then recovered by screening the flumed solids from the flume water. The operation of fluming waste solids can have several negative impacts on the recovered solids: increased moisture content, lower recovery rates, and if saltwater is used, higher salinity. In addition, a higher bacterial load, depending on the quality of the fluming, water can be expected as well as particle attrition, solubilization, and possibly solids decomposition. Some of these negative consequences can be aggravated by the temperature of the flume water, flow characteristics, and the resident time of waste solids in the flume.

Waste solids from the butchering operation that do not drop into the waste conveyance system can still be captured by strategically placing baskets around the butchering equipment and/or collecting the solids off the floor. Solids recovered from the floor are likely to have been on the floor in contact with wastewater for a longer time and possibly at a higher temperature than properly conveyed solids. This is likely to adversely affect moisture content, particle size, solubilization, bacterial load, and possibly salinity.

Liquid waste conveying system

Wastewater containing solid waste on the floor usually exits the plant through floor drains. The design of the floor drains can have an impact on the quality, retention, and removal of solids for rendering. The drain cover slot or hole size affects the quantity of solids that can be recovered from the floor. Smaller openings in the drain covers result in greater retention of solids for recovery, but at the risk of exposing the plant floor to flooding. Channel drains recessed into the floor are easier to clean than pipes buried under the floor. However, unless the grates covering the channel drains are secured, there is a greater temptation to remove the grates and sweep solids from the floor into the drain. The drain diameter, length, and slope combined with the flow rate of wastewater affect the resident time of solids in the channel drains or pipes. The material of construction (PVC for pipes, cement for channel drains) also affects the holdup of solids and the ease of cleaning. Solids that have a longer retention time in the drains and pipes are likely to have higher moisture, smaller particle size, greater degree of solubilization, and a higher bacteria load.

Most fish processing plants direct wastewater to a common sump. Ideally, all of the solids entrained in the wastewater should enter and exit the sump quickly and intact. This is usually not the case due to inappropriate design and inefficient operation. To minimize the holdup

of solids in the sump, the sump bottom should be sloped and self cleaning. Pumps are generally used to convey the solids and wastewater from the sump. Many plants use pumps that can grind larger solids to prevent the pump from becoming plugged. These pumps also result in particle attrition and solubilization. Preventing large solids and extraneous materials from entering the sump would allow the use of non-grinding pumps. The sump volume and the level controls for controlling the sump pump should be designed for the lowest practical retention time in the sump. Otherwise, there is a greater likelihood that entrained solids will settle in the sump with negative consequences. Regardless of how well sumps are designed, regular manual cleaning should be part of a comprehensive HACCP (Hazard Analysis and Critical Control Point) plan in order to minimize bacterial growth and the extended retention of settled solids. Sump design and operation affects the moisture content, bacterial load, particle size, and solubilization of solids.

Wastewater solids recovery system

After collecting the wastewater in a common sump, most seafood processing plants pump the wastewater through a screen to recover additional solids. The mesh size of the screen determines the fate of suspended particulates in the wastewater. Finer screens recover more solids and produce cleaner wastewater. However, finer screens tend to recover more finer particulates that do not dewater as well. This results in an increase in the water content of the recovered solids and can adversely affect a rendering operation.

Reject product handling system

Whole and damaged fish as well as reject food product can also be added to the waste solids for disposal or rendering. These solids should be kept clean and cold, and rendered promptly to minimize bacterial load.

Putting it all together: rendering

Having recovered fish waste solids from the processing plant, the next step is to process these solids into coproducts. For most processors this equates to sending the solids to an on-site or third party off-site rendering plant. The rendering process generally involves cooking the solids followed by pressing. The pressed solids are conveyed to a dryer and then milled and screened to produce fish meal and bone meal. The liquid from the press is usually centrifuged to remove more solids for drying. The remaining liquid is then further centrifuged to recover fish oil. The centrifuged aqueous liquid known as stickwater can be disposed of or further processed to recover more solids for drying as solubles.

The quantity and quality of recovered fish solids for rendering can significantly impact the rendering operation.

Impact of solids moisture content

Excessive moisture in the solids for rendering can adversely affect the operation of the cooker and the press and dryer operations. Increasing the volume of liquid that must be centrifuged can result in overloading the liquid processing and stickwater solids recovery systems and adversely affect fish oil quality and solids recovery.

Impact of bacterial load

The bacterial load in the waste solids for rendering can impact the meal quality, especially if there are any cooker temperature control issues.

Impact of particle size/solubilization

Finer particles are more likely to transfer to the liquid processing system and can adversely affect the centrifuging operation. As well, depending on the stickwater solids recovery system, finer solids could impact solids recovery. In all cases, finer solids to the rendering operation will affect the particle size distribution of the meal and can adversely affect the functionality depending on the end use of the meal. Increasing the soluble solids to the rendering operation can equate to lower solids recovery as meal or affect the soluble protein content of the meal.

Impact of solids decomposition

Solids decomposition can adversely affect solids recovery and meal and oil characteristics and quality.

Salinity

Increasing the salinity of the solids for rendering can reduce the life of the equipment due to corrosion. In addition the quality of the meal can be impacted by the higher salt and ash content, especially if the solids from the stickwater are concentrated using an evaporator and subsequently added to the meal.

The bottom line

Properly designed fish processing plant waste and wastewater handling systems yield many benefits. More recovered solids from the fish plant results in less discharge into local waters. Sending particulate fines and soluble solids to the rendering plant can reduce the discharges as well. For integrated operations, increasing the recovery of solids from the fish processing operations can result in increased recovery of rendered byproducts and profitability. Increasing the quality of the raw material entering the rendering plant increases the quality of the rendered byproducts. For integrated operations, the cost of properly designing or retrofitting the fish processing operation can be offset by higher profits from the rendering operation.

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Stickwater Processing by Membrane Filtration

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Introduction

Processing of fish byproducts by the wet-reduction method generates an aqueous stream called stickwater, which contains a substantial portion of the fish solids entering the fish meal plant. The stickwater produced by Alaska fish meal processors is often discharged into local marine environs, and the solids represent potential lost revenue and potentially a problem with environmental discharges (Pedersen 2003).

Membrane filtration represents a new technology with the potential to recover the solids solubilized in stickwater and at the same time produce wastewater with minimal environmental impact. Although membrane filtration technology has been developed successfully for many food sectors, especially the dairy industry, the viscosity, gelatinous nature, high temperature, and high fouling tendencies of stickwater represent unique problems. However, the development of low fouling polymeric membranes as well as improved ceramic membranes should aid in solving some of these problems. Testing of these new membrane configurations is the objective of this study.

Fish meal processing

The processing waste generated from converting landed fish into food contains from 80 to 85% water. Producing a dried product, such as fish protein meal, from this material will require the removal of more than 4 pounds of water per pound of finished protein meal. Removal of this much water is very costly, especially in remote Alaska. To lower the drying cost fish processing waste is cooked and then water and oil removed with a screw press, resulting in a press cake that is approximately 50% water as shown below. This press cake meal can be dried cost-effectively in steam heated dryers to roughly 6% moisture. Depending on the cooking and screw press operations, from 25 to 40% of the solids will be contained in the press water, usually called stickwater (Nissen 2003).

The traditional method for recovery of the fish solids in stickwater is to use multiple effect evaporators with high energy efficiency to concentrate the stickwater to a solids content of 40 to 50%. Subsequently the concentrate is added back to the press cake and dried to make a whole meal that contains the concentrated stickwater. This has been tried in some existing fish meal plants in western Alaska; however, the resulting whole fish meal often had a salt content that was unacceptable. The high salt content was caused by uptake of seawater by harvested fish during their transport from the harvest location to the processing plant. The high salt content also caused excessive corrosion in the evaporators and premature equipment failure. Most fish meal plants operating under similar conditions discharge the stickwater.

The chemical oxygen demand (COD) of the stickwater is quite high and discharge into local waters can be prohibited by state environmental regulations when weak offshore currents occur during certain seasons. The large volume and high concentration (COD 80,000 to 100,000 ppm) can result in significant oxygen depletion in the area of discharge. Under these conditions, the discharged material can become gelatinous when it mixes with the substantially cooler local marine waters (Fig. 1), and this gel is slow in dispersing.

Membrane filtration technology

Membrane filtration systems are built in a modular fashion with a series of individual elements. Two of the most common types of membrane element designs were tested. These were a polymeric spiral wound

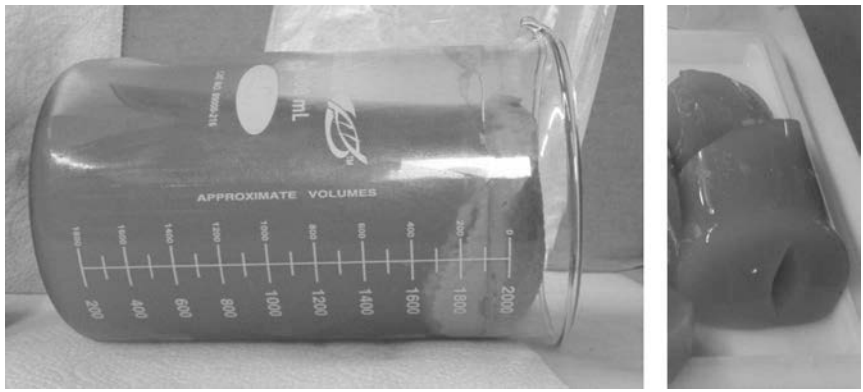

Figure 1. Stickwater. Formation of a gel when cooled to 40°F.

Figure 2. Spiral wound (left) and ceramic tubular element design (right).

design and a ceramic tubular design (Fig. 2). Among the thousands of installed membrane filtration systems, the vast majority of filtration elements are spiral wound. The advantage of the spiral wound design is lower price, reduced space requirements, and lower energy costs for circulation of fluid than a comparable ceramic tubular design. Stickwater needs to be processed at elevated temperature to have a manageable viscosity and these temperatures will require frequent replacement of the spiral wound filters. In contrast, ceramic filters better withstand both the higher operating temperatures and also the aggressive cleaning compounds required for restoring the flux rates between runs.

Figure 3. Test system. (Manufactured by Kelitek.)

Selection of membrane filter pore size

The membrane filter elements can be selected for different pore sizes. Pilot studies found that a pore size of approximately 0.01 micron would retain approximately 70% of the solids contained in the stickwater evaluated. The remaining 30% of solids passing through the membrane was primarily salts and low molecular organics with little value as feed ingredients. Membranes with yet smaller pore size (0.005 micron equivalent to a molecular weight of approximately 10,000 daltons) were also tested, but the increase in solids retention was minimal and there was a significant diminishment of the membrane flux. Membrane filters with larger pore sizes (0.1 micron or a molecular weight of approximately 100,000 daltons) had an increased flux but retained less than half of the solids contained within the stickwater.

Test setup and discussion of performance

The test system shown in Fig. 3 consisted of an 800 gallon feed tank from which stickwater is drawn though an inline filter into two 8 inch

commercial membrane modules, and then returned to the feed tank. The filtrate is continuosly removed through the white hoses in the foreground of the picture. The system can be equipped with either polymeric spiral wound or ceramic tubular membranes for testing.

A series of tests were performed to evaluate the different components, and to develop design criteria for a commercial system. Evaluations included the following:

- Prefiltering needs
- Membrane substrate and pore size
- Membrane configuration
- Operation parameters
- Cleaning parameters
- Commercial viability
- Prefiltration

The removal of suspended solids is critical to avoid plugging of the feed channels in membrane elements, and is often overlooked in system design. Several different designs were tested:

1. Organic spun filters frequently used in water filtration proved useless after being "coated" by an impermeable product layer within minutes of operation (Fig. 4a).

2. Metallic screens operated well down to 70 mesh (approximately 200 microns) but tighter screens required more frequent cleaning and caused a significant drop in system pressure (Fig. 4b).

3. The filter with the best performance was back washable. This was capable of filtering down to 20 microns (600 mesh) at high filtration rates. However, the back flush operation required high flow rates and pressures to effectively clean the filter (Fig. 4c).

Membrane substrate and element design

Two sets of membrane designs were tested:

1. Polymeric spiral wound design where the membrane substrate was modified polyacrylicnitrile (PAN) with a very high affinity to water (hydrophilic) and a low affinity toward oil. These membranes had a lower fouling rate and were easier to restore by cleaning than the more commonly used polysulfone (PS) membranes. The spiral wound configuration offers a very compact design, and the 8 by 40 inch module used in the test offered a filtration surface of 23.4 square meters (250 square feet). Membranes with two different molecular cutoffs were tested, including a membrane with a molecular cutoff of 0.05 microns (P400) and a membrane with a molecular cutoff of 0.01 microns (P200).

2. Ceramic tubular modular design where the membrane material was titanium oxide based on silicon oxide support structure. The module used in the testing contained a total of ten elements for a total of 2 square meters (20 square feet) of filtration surface.

Cleaning protocol

The cleaning was carried out using a caustic wash (pH 11) followed by an acid wash (pH 2.5). The final step in the cleaning procedure was a chlorine rinse (100 ppm) for removal of any residual oil on the membrane surface.

Test results

Fig. 5 shows a set of flux curves obtained for a 0.05 micron module (P400) as well as a 0.01 micron module (P200). The applied feed pressure was 65 psi and the back pressure 15 psi. The flux averaged approximately 11 lmh (7 gfd) for the P400 membrane and 6.5 lmh (4 gfd) for the P200 module. The flux remained stable until a concentration at approximately 16 to 18% solids was reached. Above 20% solids there was a significant increase in apparent viscosity and pumping the liquid through the membrane modules became increasingly difficult.

Fig. 6 shows an example of tests carried out with a ceramic module (0.01 microns). The system was operated at an inlet pressure of 30 psi and a back pressure of 10 psi. In test 1.0 the permeate flow was restricted to 35 lmh (20 gfd). In subsequent tests 2.0 and 3.0 the permeate flow was unrestricted (fully open permeate valves), which resulted in a higher initial flux and a rapid flux decline over time. Nevertheless, the final flux appears to stabilize at a level similar to that obtained in test 1.0 when the permeate flow was restricted.

Analytical results

Table 1 shows an example of the analytical composition for feed, concentrate, and permeate obtained using a 0.01 micron ceramic membrane. As shown, the membrane filters will retain practically all the oils found in the feed at commonly found concentrations of oil. On a weight/weight basis the concentrate contained a greater percent of oil, twice as much as in the feed. Although the ash content of the feed was relatively high, the ash content in the concentrate on a solids basis was only about one-third of the feed level. This was expected as minerals and salts (smaller molecules) passed through the membrane filter to the permeate. The chemical oxygen demand (COD) of the effluent was reduced from ~170,000 to ~15,000, a reduction of about 90%

Figure 4a. Organic spun filters before (left) and after minimal use (right).

Figure 4b. Metallic screen.

Figure 4c. Back flush prefiltration module. (Manufactured by Arkal.)

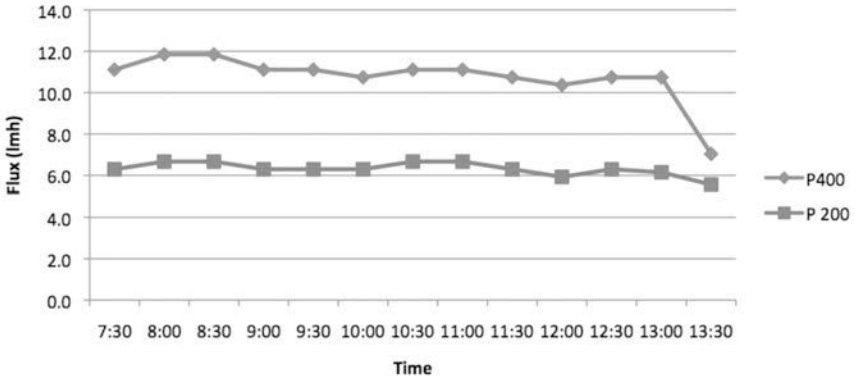

Figure 5. Examples of flux curves for two polymeric membranes. P400 = 0.05 microns. P200 = 0.01 microns.

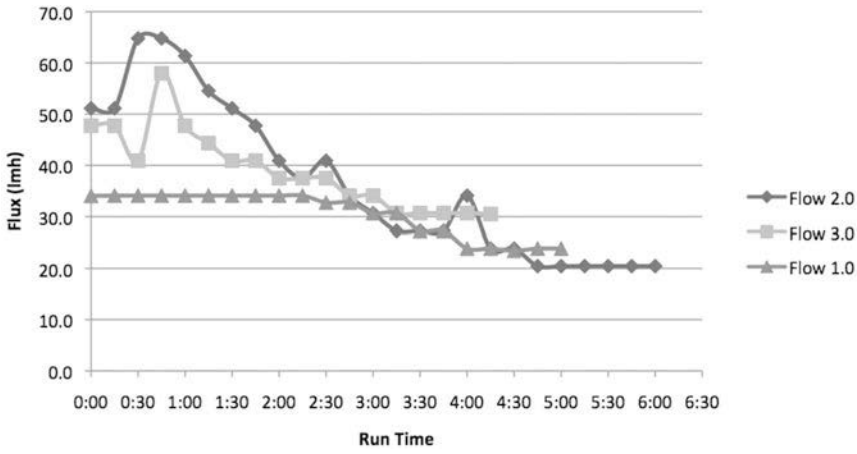

Figure 6. Examples of flux curves for ceramic membranes (0.01 microns).

Table 1. Proximate composition of feed, concentrate, and permeate from 0.01 micron ceramic membrane on a percent wet weight basis (left) and percent solids basis (right).

Sample analysis

	Feed	Conc.	Permeate
	%	%	%
Moisture	95.1	86.1	97.5
Solids	4.9	13.9	2.5
Ash	1	1	1
Oil	0.2	7.5	0
Protein	4.2	7.1	1.7
	100.5	101.7	100.2

Based on solids (dry)

	Feed	Conc.	Permeate
	%	%	%
Moisture	0.0	0.0	0.0
Solids	100.0	100.0	100.0
Ash	20.4	7.2	40.0
Oil	4.1	54.0	0.0
Protein	85.7	51.1	68.0
	110.2	112.2	108.0

Discussion

In the series of tests conducted with polymeric membranes, the flux was low at 4 to 7 lmh. Higher operating pressures resulted in higher permeation rates but caused unacceptably high fouling rates that required frequent membrane cleaning. This was especially true when there was high oil content in the processed stickwater (a season). In addition, the high processing temperature required to minimize stickwater viscosity resulted in significantly reduced membrane life. The ceramic membrane filtration systems appeared to be less susceptible to fouling due to the higher oil content of stickwater and will have a much longer operational life.

Even though the ceramic membranes are substantially more expensive and have higher operating costs, they appear to be the better choice for processing stickwater.

Summary

Stickwater, also known as fish solubles, is an aqueous liquid generated during the production of fish meal. Stickwater contain in excess of 90% water, and this very high water content makes it too costly to process directly in a fish meal dryer. The high COD levels of stickwater often restrict discarding the material close to shore. An effective solution would remove the soluble organic solids, reducing COD and allowing the stickwater to be discarded locally without penalty.

Membrane filtration technology is an alternate method for reducing the water content of materials prior to drying. For concentrating stickwater, different membrane substrates, membrane configurations, and operational parameters have been explored in pilot plant studies and design parameters derived from these tests further evaluated at plant sites.

The testing showed that spiral wound polymeric membranes show promise in this application and are less expensive than another alternative, ceramic membrane filters. However, the ruggedness and ability to tolerate harsher cleaning procedures found necessary for full flux restoration makes the ceramic membrane system better suited for plant-based operations involving stickwater concentration.

The best suited membrane for plant operation had a molecular cutoff of 300,000 daltons. This membrane pore size resulted in retention of approximately 67% of the solids contained in the stickwater. The retention based on proximate analysis were protein 80%, ash 26%, and oil 100% of feed values. The initial COD in the stickwater was 70,000, while the COD of the permeate was less than 2,000.

Membranes with higher molecular cutoff were found to have low solids retention. A lower molecular weight cutoff resulted in better retention but flux rates were too low to be economically viable.

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Methods for Drying Stickwater

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Abstract

Alaska is one of the largest fisheries in the world. More than 2 million metric tons of fish are harvested there each year. Walleye pollock (*Theragra chalcogramma*) is the most abundant fish captured, at more than 1 million t, followed by the combined species of Pacific salmon at about 250,000 t. Assuming that only 25% of the fish are used for human food, this leaves a considerable amount of byproducts to process into fish meal. Alaska's fish meal production differs because food-grade processing wastes are employed instead of whole fish. In other parts of the world, whole fish are transformed exclusively for fish meal (e.g., sardine, menhaden). Alaska's waste fish are "fresh" when received for processing. Stickwater is a byproduct of fish meal production. Derived from oil-depleted liquid fractions during processing, stickwater contains soluble molecules. Little is known about the chemical or nutritional characteristics of Alaska's stickwater. Our objective was to develop an efficient method for producing dried meals from stickwater, which has a sticky nature and unique drying challenges. We produced dried powders from pollock stickwater using different dryer technologies including lyophilization, microwave, and drum drying. We present the methods used to dry stickwater and some basic chemical analyses that were performed on each powder. Given the high quality of the waste fish used to produce Alaska fish meal, we concluded that stickwater could be a

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very good binder agent and a potential attractant or appetite enhancer for aquaculture purposes.

Introduction

Alaska is one of the largest fisheries in the world—more than 2 million metric tons (t) of fish are harvested each year (NMFS 2003). Walleye pollock (*Theragra chalcogramma*) is the most abundant fish captured (more than 1 million t) followed by the combined species of Pacific salmon (between 250,000 and 300,000 t). Assuming that only 25% of the fish end up as human food, this leaves a considerable amount of processing byproduct to convert into fish meal (Smiley et al. 2003). These fish meals, however, are very different from other fish meals because its raw material is processing waste from the fish used for human consumption, rather than whole fish harvested exclusively for fish meal (e.g., sardine, menhaden). Therefore, Alaska byproducts are of the highest quality when initially received (Pederson et al. 2003).

During standard wet-reduction fish meal manufacturing, the raw material is cooked and then pressed to remove a maximum amount of liquid (Fig. 1). This solution is called press liquor and contains a fair amount of lipids and other compounds in a watery solution. After a decantation process and a centrifugation step to separate the oil from the solution, stickwater, an aqueous product, remains (Fig. 1). The solids concentration in Alaska stickwater is typically around 5-7%, and stickwater represents about 70% of the total weight of the byproducts used for fish meal production (Pederson et al. 2003, Bechtel 2005). Stickwater contains heat-soluble proteins, such as collagen, as well as peptides, and a variety of other small, unidentified molecules (Soares et al. 1973). Additionally, stickwater is very viscous and sticky, especially when attempting to concentrate it by evaporation. Some fish meal producers will evaporate stickwater to produce a product called fish solubles. Fish solubles are usually co-dried with the press cake producing an end-product called "whole fish meal" (Windsor and Barlow 1981). Evaporating stickwater requires large and expensive equipment, so not all fish meal producers are equipped to manufacture fish solubles. In cases where fish have been exposed to refrigerated seawater for prolonged periods of time post mortem, salts are taken up by the fish. This additional salt limits fish solubles production, and in such cases stickwater has no other cost effective usage and is generally discarded directly into adjacent waters. Several studies have shown that stickwater has beneficial growth properties when added as a feed supplement (Bauersfeld and Soares 1972, Aung and Flick 1980, Gulbrandsen and Utne 1981, Campos et al. 1982). Given the sticky nature of stickwater and the unique drying challenges it offers, our objective was to develop an efficient method for producing dried meals from stickwater; these meals could potentially function as aquaculture feed supplements.

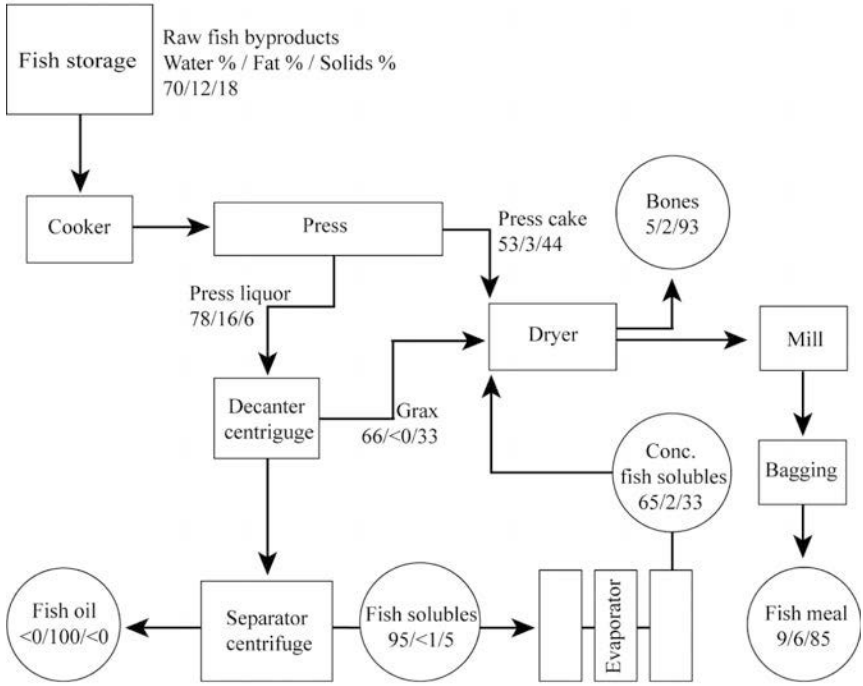

Figure 1. Stages of fish meal production in Alaska (modified from Windsor and Barlow 1981).

Material and methods

Meal preparation

Approximately 150 kg of fresh pollock stickwaters were collected on different processing days from the Kodiak Fishmeal Company in Alaska. Stickwater was stored in ten 5-gallon (22.7 L) buckets and frozen at –20ºC for less than 1 hour after collection. Before each drying trial, only the required number of buckets were thawed overnight at 4ºC. Several methods were experimented with to dry the stickwater to a stable powder, including the use of a vacuum dryer, forced-air dryer, microwave, drum dryer and freeze dryer (lyophilization). Of these methods, only the last three were successful in producing a completely dried stickwater. Once dried, the material was cooled and then milled to a powder using a Willey Mill variable speed model ED-5 (Thomas Scientific, Swedesboro, New Jersey, USA) equipped with a 1 mm mesh screen. The less successful methods will be presented briefly to describe the specific problems encountered, while the successful methods will be more

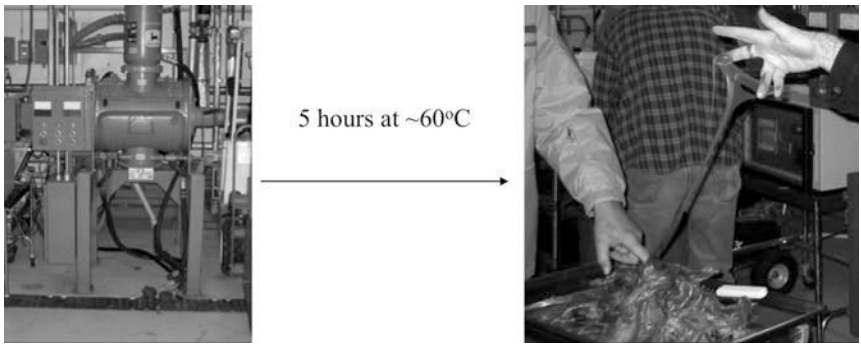

Figure 2. Littleford vacuum dryer (left) and final product after 5 hours at ~60°C (right).

fully discussed including the basic chemical characterization of the final products.

Vacuum dry method

About 45 kg (three buckets) of stickwater were transferred to a Littleford vacuum dryer model FM 130-0 (Littleford Day Inc., Florence). Product temperature quickly rose to approximately 65°C. After 45 minutes, 25 kg of water was removed. Since a minimum amount of dry matter is needed in the Littleford to operate properly during the final stage of drying, another 30 kg (two buckets) of stickwater were added and the dryer was restarted. About four hours latter, unusual noises were produced from the dryer and the product temperature climbed rapidly (>100°C). The dryer was stopped and opened to verify the product condition. The stickwater had the appearance of taffy and was so sticky it partially jammed the dryer (Fig. 2). This drying method was abandoned.

Forced-air method

Given the poor results achieved with the vacuum dryer, a second approach involving a forced-air dryer was attempted. About 30 kg of stickwater (2 buckets) were distributed onto 10 aluminum trays and placed on a rack in an Enviro-Pak smoker (model CHU-150, Clackamas). The temperature was set at 60°C and the dryer was started without injection of smoke. After 12 hours, this stickwater produced a very fine caramelized layer stuck firmly to the tray, which was very hard to scrape off (Fig. 3). The final product was dry and seemed in relatively good condition (not obviously burned), but because it could not be easily removed from the tray, this method was also abandoned.

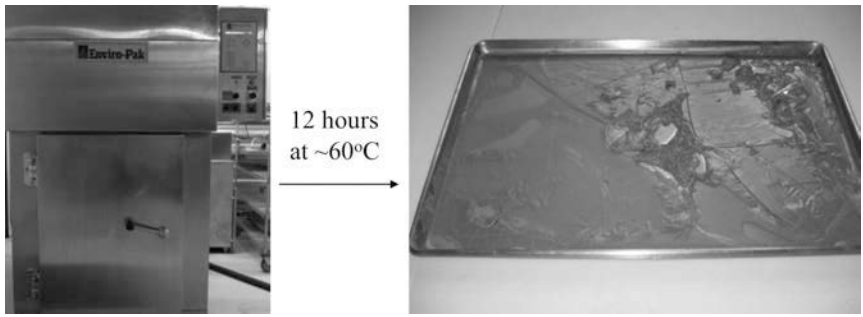

Figure 3. Enviro-Pak dryer (left) and final product after 12 hours at 60°C (right).

Figure 4. Heavy-duty microwave (left) and final product after 1 hour at >100°C (right).

Microwave method

A microwave was also tested for its ability to dry the stickwater. Without access to an industrial microwave device, a heavy-duty household microwave was employed and worked at a significantly smaller scale. Exactly 1 kg of crude stickwater was poured into a 5.0 L plastic container and placed in a Sharp Carousel 1500 W microwave at full power (Fig. 4). To monitor water loss, the weight of the container and the residual stickwater was monitored every 5 minutes (and every minute as the material became dryer) by quickly removing the container and placing it on a calibrated external balance. Unlike previous methods, the temperature of the product in the microware could not be directly controlled. Therefore, during the weighing the temperature was measured directly using a probe electronic thermometer (Fisher Scientific Dual Thermometer). Less than 10 seconds were needed to weigh the container, measure the product temperature, and return the container

to the microwave. The temperature of the stickwater stayed constant at 100ºC, except near the end of the drying when the temperature rose to around 130ºC for several minutes. It took about 1 hour to completely dry 1 kg of stickwater (Fig. 4). Unfortunately, based on the very obvious burned scent of the material, we initiated other trials to reduce this problem. The experiment was repeated several times to optimize the final drying steps by removing the product before burning, but each time, it was either wet and sticky or burned if dried. For this reason, this method was also abandoned.

Drum dryer method

After these failed attempts, we turned to another approach, using a small pilot-scaled dual drum dryer (Buflovak 6 inch diam. × 8 inch Atmospheric Double Drum Dryer). Steam pressure in the drums was set at the manufacturer's recommended pressure of ~80 psi. After several trials, we obtained a well-dried stickwater when the drums turned at 1.5 rpm with a 0.127 mm clearance between them (Fig. 5). Room-temperature stickwater was continuously poured from the top into the gap between the turning drums. It took about 45 seconds for the drying stickwater, stuck to the surface of the rotating drums, to reach the scraping knifes (dark gray area on top of the drums on Fig. 5). The drum temperatures were well above 100ºC, but the short transit time for the drying product prevented the material from reaching these high temperatures. The material was consistently around 60ºC when scraped off the drums.

Lyophilization

About 30 kg (two buckets) of stickwater were distributed into 10 rectangular plastic containers and blast frozen for about 3 hours before lyophilization. The plastic containers were then placed in a commercial VirTual freeze dryer lyophilizer (SP Industries Inc., Warminster). The stickwater was freeze-dried for about 10 days using a preset program, resulting in a high quality product as shown in Fig. 6.

Analytical procedures

Moisture and ash content were determined using AOAC methods (1990). Lipid content was determined using the method of Folch et al. (1957). Protein analyses were carried out using a nitrogen analyzer (model FP2000, LECO, St. Joseph). Due to the high quantity of collagen in stickwater, a factor of 5.714 was used instead of 6.25 for converting nitrogen values to protein concentrations (Kahn and Witnauher 1971, Gildberg et al. 2002). For amino acid analyses, meal samples were hydrolyzed in 6N HCl for 24 h under nitrogen at 110ºC followed by derivitization and HPLC amino acid determination procedures of Waters Inc. The methodology

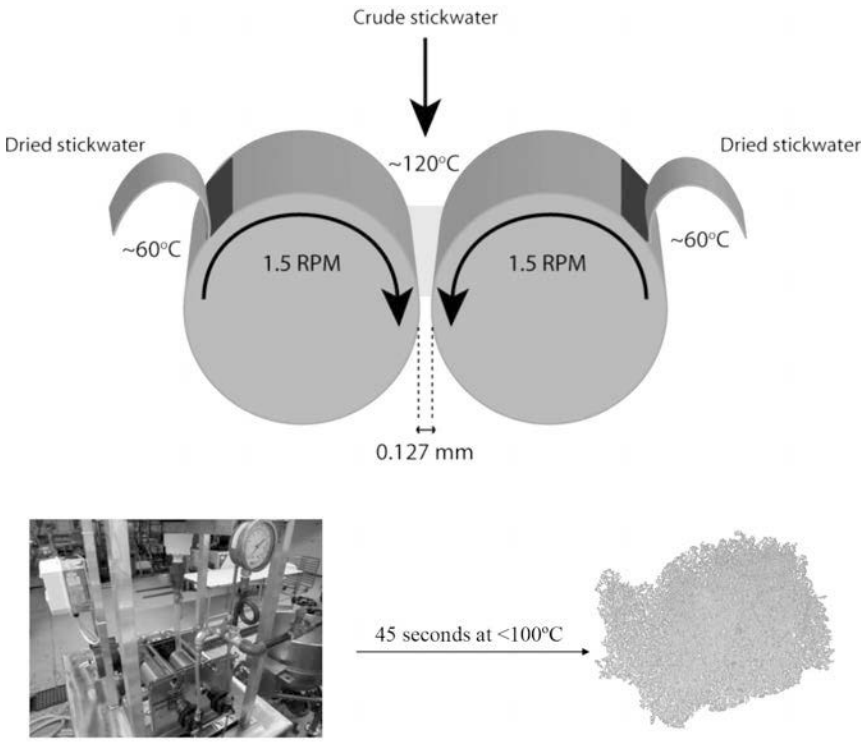

Figure 5. Schematic presentation of the drum dryer adjustments (above). Pilot-scale drum dryer setup (bottom left) and final product after 45 seconds at <100°C (bottom right).

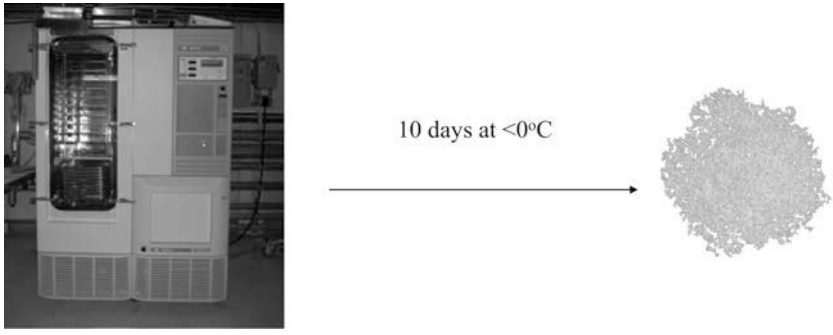

Figure 6. Freeze-dryer (left) and final product after 10 days at <0°C (right).

employed to measure amino acids did not allow measurement of either cysteine or tryptophan concentrations. Color was determined using a Minolta chromameter (model CR-300, Minolta Co, Ltd. Osaka, Japan) and reported as L*a*b* values.

Results and discussion

Stickwater dried completely in the freeze-dryer and the end product did not stick to the containers at all. On the contrary, the product took on a rough sponge-like texture. Drying stickwater using this technique is very time and energy consuming and would certainly not be a cost-effective method at a commercial scale. However, the low temperatures used during lyophilization preserve the product at its highest quality, and produce chemical analyses that become the standards against which all other drying methods could be compared.

Unsuccessful methods

The nature of stickwater makes it very difficult to dry—soluble proteins, and specifically collagen, content is very high, and the material becomes very viscous and sticky while drying. This is the main reason that fish meal processing plants add protease enzyme treatment during stickwater production. This increases the flow behavior of stickwater in the evaporators during fish solubles production (Jacobsen and Lykkerasmussen 1984, Jacobsen 1985, Curotto et al. 1986, Nilsang et al. 2005). Probably for these same reasons we also had difficulty drying crude (untreated) stickwater. Both the vacuum and forced air methods removed a large amount of water from the material, but near the end of the drying process the material became very viscous and sticky. With the vacuum dryer, a cuticle was created on the surface of the product, inhibiting any further drying. The air-forced dryer did a fair job drying the material at a constant low temperature, but the end product was not a powder and was difficult to remove from the trays. If a suitable nonstick coating on the trays could be found, this method may show greater potential.

Successful methods

Potentially, the microwave could boil the stickwater to dryness; however, the chance that the material would suffer heat damage was a concern. Even though tests showed that the microwave method could dry crude stickwater to a powder, it did produce a burned smell. This technique was abandoned because the goal was for a one-step method to quickly and economically dry stickwater while retaining a high quality product. The strong burned odor strongly suggested protein/lipid damage that could decrease palatability if included in any feed formulations (Glencross et al. 2007). Still, this method could be an interesting choice

Table 1. Proximate composition of pollock stickwater (crude, freeze-dried, and vacuum-dried) as well typical values for Alaska fish meal.

	Pollock stickwater			Typical Alaska fish meal[a]
	Crude	FD	DD	
Water (%)	92.5 ± 1.3	4.2 ± 0.6	3.4 ± 0.5	6.1 ± 1.9
Protein (%)	5.9 ± 0.5	79.7 ± 3.3	76.7 ± 2.0	69.3 ± 2.7
Lipid (%)	0.6 ± 0.1	10.7 ± 2.5	13.0 ± 2.9	7.6 ± 1.8
Ash (%)	0.8 ± 0.1	11.3 ± 0.4	10.9 ± 0.2	17.0 ± 3.9

Values are mean ± SD (g/100 g, as is).
FD: freeze-dried; DD: drum-dried.
[a]Smiley et al. 2003.

Table 2. L*, a*, and b* values for pollock stickwater (freeze-dried and drum-dried).

	FD	DD
L*	76.8 ± 6.5	73.5 ± 6.6
a*	0.8 ± 0.5	4.3 ± 1.7
b*	24.3 ± 3.9	39.5 ± 2.9

Values are mean ± SD.
FD: freeze-dried; DD: drum-dried.
L*: 0 = black, 100 = white.
a*: negative = green, 0 = gray, positive = red.
b*: negative = blue, 0 = gray, positive = yellow.

if used in tandem with another method to quickly remove a large quantity of solvent water during the first steps of the drying process.

Drum dryer technology was the only effective method tested to dry stickwater at a commercial scale; it took only seconds to completely dry the material compared to 10 days for the freeze dryer. Also, based on the similar chemical composition found for the freeze-dried and the drum-dried final products, one can argue that drum dryer technology did not negatively alter the chemical properties of the product. Protein and lipid content were similar in the freeze-dried and drum-dried stickwater (Table 1). The lyophilizer produced a very light colored (higher L* value) powder since the product was never exposed to heat during drying (Table 2). The drum dryer produced a more yellowish color (higher a* value), which is normal when the material is exposed to heat, due to Maillard reactions. Drum-dried stickwater did not exhibit the strong burned odor that was found in the microwave-dried stickwater. It seems clear that the drum-dried stickwater has been exposed to heat but not

Table 3. Common amino acids of stickwater meals (freeze-dried and drum-dried) and published values for typical Alaska fish meals. Published indispensable amino acid requirements for shrimp and coho salmon are compared.

Amino acid (%)	Pollock stickwater		Typical Alaska fish meal[a]	Indispensable amino acid requirements (g/100 g, as is)	
	FD	DD		Shrimp[b]	Coho salmon[c]
ALA	4.6 ± 0.2	4.5 ± 0.1	5.3 ± 0.3	–	–
ARG	7.3 ± 0.2	7.5 ± 0.1	6.3 ± 0.5	5.8	3.2-5.8
ASP	5.3 ± 0.3	4.4 ± 0.0	8.2 ± 0.7	–	–
GLU	7.9 ± 0.4	7.6 ± 0.1	11.5 ± 0.9	–	–
GLY	11.2 ± 0.8	11.5 ± 0.7	6.1 ± 0.8	–	–
HIS	1.2 ± 0.0	1.1 ± 0.0	1.8 ± 0.2	2.1	0.9-1.8
ILEU	1.3 ± 0.1	1.4 ± 0.1	3.0 ± 0.3	3.4	1.2
LEU	2.8 ± 0.3	3.0 ± 0.2	6.4 ± 0.7	5.4	3.4
LYS	3.3 ± 0.3	3.4 ± 0.1	6.4 ± 0.6	5.3	3.8
MET	1.5 ± 0.0	1.5 ± 0.0	1.8 ± 0.3	2.4	2.7
PHE	1.4 ± 0.1	1.5 ± 0.0	3.3 ± 0.4	4.0	4.5
PRO	4.6 ± 0.3	4.6 ± 0.6	4.0 ± 0.3	–	–
SER	3.2 ± 0.1	1.7 ± 0.0	4.5 ± 0.3	–	–
THR	1.9 ± 0.1	1.5 ± 0.0	4.1 ± 0.4	3.6	2.0
TYR	0.7 ± 0.1	0.8 ± 0.0	3.2 ± 0.4	–	–
VAL	1.7 ± 0.1	1.8 ± 0.1	3.6 ± 0.4	4.0	2.2

Values are mean ± SD (g/100 g, as is).
FD: freeze-dried; DD: drum-dried.
[a]Recalculated using data from Smiley 2003.
[b]Recommendations from Akiyama et al. 1991.
[c]Wilson 2002.

to sufficient heat to cause severe damage to either proteins or lipids. The amino acid profiles of both drum-dried and lyophilized stickwater support this conclusion. The slight reduction in the percentages of serine and threonine found in the drum-dried material was the only real difference in amino acid values between the methods (Table 3). This pilot-plant study provides sufficient information to scale-up the drying process of crude stickwater using commercial-scale drum dryers.

Stickwater: a feed supplement?

Having found an efficient method for drying stickwater, one can pose the question: can this stickwater be used as a feed supplement? The answer depends on what specific properties are being sought. The Alaska stickwater meal is higher in protein and lower in both ash and

moisture content than FAQ (fair average quality) fish meal. However, the concentration for most amino acids found in stickwater are below those found in FAQ fish meal and also below the nutritional requirements for coho salmon (*Oncorhynchus kisutch*) for essential amino acids (Table 3). Stickwater was notably high in glycine content, explained by the high concentrations of collagen in this product (Soares et al. 1973). Nutritional analyses should be performed to fully evaluate the potential of stickwater as an aquaculture feed supplement. Regardless of the outcome of those tests, dried stickwater is likely to be a very good binding agent and has potential as a feeding attractant or appetite enhancer.

Conclusions

Of all the methods tested to dry stickwater, only the drum dryer showed real potential for scaling-up to commercial application. This method quickly dried the stickwater and since the drums are equipped with knives to scrape off the product, we did not encounter the problems with excessive stickiness experienced with other methods. Spray dryer technology is another method that could present an alternative for drying stickwater. As for stickwater as a potential feed ingredient for aquaculture, due to suboptimal amino acid profiles, stickwater will not gain significant use as a fish meal replacement in feed formulations. However, onsite tests should be completed to ascertain whether dried stickwater powder could be an effective binding agent or as an appetite enhancer for aquacultured species.

Acknowledgments

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Recovery and Utilization of Protein from Surimi Processing Water

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Abstract

Developing technologies for the recovery and utilization of proteins discarded with surimi wash water (SWW) will help to ensure the future viability of the surimi industry. The low yield of surimi production, the environmental impact of discharging SWW into the environment, and the large amounts of fresh water used, about 20 times the weight of the deboned meat, are driving the need to develop technologies to treat SWW. A chitosan-alginate treatment can be used to recover SWW proteins while retaining both their functional properties and nutritional value. The optimization of the chitosan-alginate treatment, the assessment of the nutritional value of the solids recovered from SWW, and the effect of adding SWW recovered soluble protein recovered into surimi gels are presented here. Incorporating protein recovered from SWW into surimi could enhance the economic sustainability of the surimi industry, reduce environmental pollution, and increase utilization of harvested fish. In addition, the water recovered from SWW can be reused and thus lower the demand on local water supplies, an increasingly scarce resource in many coastal communities.

Introduction

Transformation of raw materials into foods, including surimi production, generates processing water discharge streams containing valuable byproducts. In the Pacific Northwest, surimi wash water (SWW) contains 0.1-2.3% protein (Lin and Park 1996, Morrissey et al. 2000), and recovering these low and variable concentrations is a technical and economic challenge. Developing technologies for the recovery and utilization of these proteins will help to ensure the future economic viability of the surimi industry. Although they can be an important source of employment to coastal communities, waste stream emissions increase oxygen demand when released into shore-side waters and raise concerns regarding the condition of local waters.

The economy-driven industrialization of fisheries has brought incredible advances and increased harvests, and has generated higher byproduct volume (Gildgerg 2002). With an appropriate technology, recovering proteins from processing streams can generate revenues for the processor, reduce effluent treatment costs, and reduce concerns about potential environmental harm. Recovered proteins can be converted into four types of products: (1) plant fertilizers, (2) agricultural feeds, (3) value-added foods, and (4) specialty ingredients. In general, value addition is lowest for conversion of byproducts into fertilizers, and is highest for value-added foods and specialty ingredients.

Two muscle types are present in aquatic foods: striated muscle characterized by transverse stripes, and smooth muscle (Torres et al. 2006). Striated muscle is the main component in fish meat and smooth muscle is typical of meat from mollusks. Fish muscle is divided into white and dark types, the latter lying alongside the fish body and under the skin. Protein from white muscle can be further classified into myofibrillar, sarcoplasmic, and connective tissue proteins (Huss 1995). Myofibrillar proteins are organized into myofibrils that are soluble in concentrated saline solutions. The myofibrils are made of two major ultramicroscopic components, thick and thin filaments containing the myosin, actin, and other contractile proteins. The thick and thin filaments form the muscle myofilaments. Myofibrils are composed of numerous myofilaments kept together by connective tissue. The amount of connective tissue in fish muscle is low (3-5% of total protein) when compared to other meat sources (e.g., 16-28% in beef) (Suzuki 1981).

Ionic strength is an important factor in the water solubility of muscle proteins. Sarcoplasmic proteins, including myoalbumins, globulins, and various enzymes, are soluble in dilute saline solutions. Myosin and actin are insoluble in water at physiological ionic strength but become soluble at extremely low ionic strength or in water containing an ionic strength of 0.6 M salt or higher. While sarcoplasmic proteins are quite water soluble, connective tissue proteins are water insoluble at normal temperatures. The water solubility of sarcoplasmic proteins decreases with increasing ionic strength (Suzuki 1981, Lanier et al. 2005).

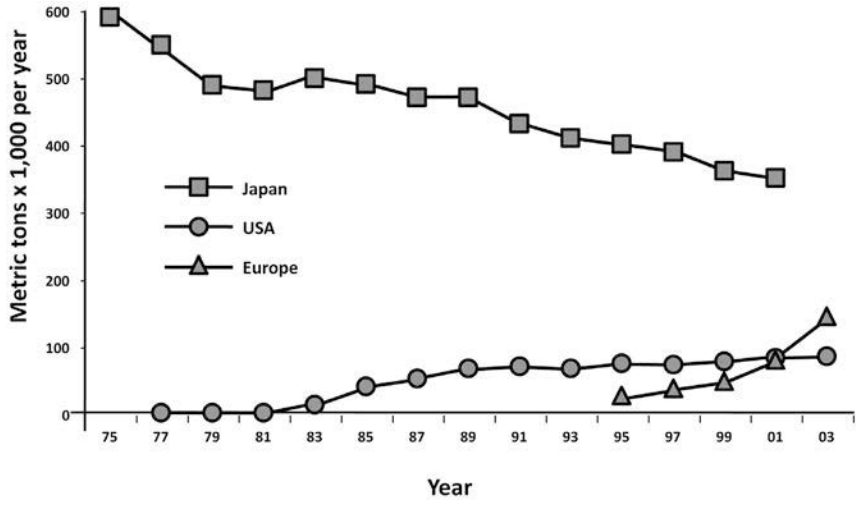

Figure 1. Surimi analog product consumption in the world. (Adapted from Park 2005.)

Protein recovery from surimi processing water

Surimi is minced fish muscle repeatedly washed and dewatered, leading to the recovery of the myofibrillar proteins (Lee 1999). Consumption of surimi-based or analog seafoods (i.e., crab-flavored seafood) has been steadily increasing in the United States from the late 1970s and in Europe from the mid 1990s. However, Japan, the world largest consumer of analog seafood products, has shown the opposite trend (Fig. 1). In 2002, the annual frozen surimi production in the United States was over 95,000 t. In 2006, surimi consumption in the European Union (EU) reached 132,000 t. In the EU, the main markets for surimi as raw material for further processing into analog products were France, Lithuania, and Spain with 2006 volumes of 15,900, 9,300, and 11,000 t, respectively, while the countries with the largest consumption of surimi products in the EU were France, Spain, and Italy with 52,200, 40,000, and 11,000 t, respectively (Catarci 2007).

In the Pacific Northwest, the most utilized fish species for surimi production are Pacific whiting (*Merluccius productus*) and Alaska pollock (*Theragra chalcogramma*). However, underutilized fish species represent potentially new resources as a cost effective raw material for surimi production. For example, two nonconventional methods have been developed to produce surimi from the jumbo squid *Dosidicus gigas*. The

first is based on protein precipitation at the isoelectric point (method A), and the second on washing with an acid solution (method B) (Campo-Deaño et al. 2009). Chemical analyses, scanning electronic microscopy, and rheological tests showed that method B more effectively preserved the functionality of the myofibrillar proteins yielding a better gel structure than method A. Another good example of an underutilized aquatic species is Antarctic krill (*Euphausia superba*), small shrimp-like crustaceans with the largest biomass of any multicellular animal species on earth. Functional proteins of high nutritional value have been recovered from whole krill by method A (Chen and Jaczynski 2007a, Tou et al. 2007, Gigliotti et al. 2008, Chen et al. 2009). Another potential biomass containing a significant amount of recoverable proteins and lipids comprises fish processing byproducts and fish bycatch (Torres et al. 2006). Method A allows efficient recovery of fish proteins and lipids from the fish processing byproducts. Recovered proteins and lipids retain functional and nutritional properties (Chen and Jaczynski 2007b, Chen et al. 2007). Carp have recently received significant attention; according to the Food and Agriculture Organization of the United Nations, carp are the least expensive and by far the most commonly consumed fish in the world (Lowther 2005). Carp have minimal growth requirements, yet rapid growth rates. Although carp are often considered unsuitable for consumption in the United States, they have rapidly started populating major bodies of fresh waters to the extent that commercial processing becomes of interest. The rapid and uncontrolled spreading of this invasive species has also caused environmental stress due to replacing native fish and endangering natural fish populations. Although typical mechanical means of muscle recovery from carp is impractical due to a bony nature of carp carcass, method A allows recovery of proteins and lipids from whole gutted carp (Taskaya et al. 2009a,b; Taskaya and Jaczynski 2009).

The relatively low protein recovery in the conventional method for surimi production means that large amounts of proteins are lost in SWW. Often regulations require that the high biological oxygen demand of SWW must be lowered before discharging it into local waters. In addition to the low recovery yield of traditional surimi production and the impact of discharging SWW, yet another industry concern is the large amount of freshwater used, about 20 times the weight of the deboned fish muscle (Lee 1999). The low process efficiency, high freshwater consumption, and impact of traditional surimi plants are creating political pressures for their shutdown (Torres et al. 2006). This chapter focuses on a chitosan-alginate treatment developed at Oregon State University as a technology alternative to lower the biological oxygen demand of SWW discharged from traditional surimi processing plants (Savant and Torres 2001, Wibowo et al. 2005a, Torres et al. 2006, Wibowo et al. 2007a). This technology has the potential for use in other dilute protein recovery applications. Chitosan-alginate complexes have been shown to

Table 1. Effect of time and chitosan-alginate concentration on the percent surimi wash water (SWW) protein adsorption.

	Chitosan-alginate, kg/ton SWW			
Time (h)	0.02[a]	0.04[a]	0.10[b]	0.15[b]
0.5[1]	66.6 ± 1.0	70.7 ± 0.2	76.2 ± 0.0	83.7 ± 0.4
1[2]	79.5 ± 0.4	79.8 ± 0.8	83.6 ± 0.2	82.7 ± 1.4
24[2]	80.6 ± 0.1	82.3 ±0.9	84.2 ± 0.3	84.4 ± 1.5

Values are means and SD.

Means with the same superscript letter or number indicate no significant concentration or time effect, respectively ($p > 0.05$). All complexes were prepared using food grade alginate (Kelgin® MV, Kelco Co., San Diego) and tested using the same SWW sample. (Adapted from Wibowo et al. 2005a.)

be effective protein coagulating agents, superior to the use of chitosan alone (Savant and Torres 2001). For example, chitosan-alginate's superior effectiveness has been confirmed in the recovery of whey proteins generated in cheddar cheese production (Savant and Torres 2000) and in SWW treatment as shown in this article.

Chitosan-alginate SWW treatment

Chitosan is an effective coagulating agent used to remove suspended solids from food processing effluents generated by shrimp, poultry, dairy, and meat processors. The suspended solids recovered as a protein-chitosan complex can be used as crude dietary supplements in animal and aquaculture feed or further processed to recover a more uniform protein fraction (Knorr 1986). In the recovery of proteins from shrimp, mussel, and crawfish processing water, the chitosan-coagulated solids retained 65, 38, and 27% crude protein from the processing waters (Torres et al. 1999). Although chitosan can remove suspended solids, its cationic nature limits it principally to the recovery of negatively charged particles. Chitosan complexed with polyanions, including alginate, pectin, carrageenan, or polyacrylic acid, has been evaluated for potential use in food processing (Mireles et al. 1992, Torres et al. 1999, Savant and Torres 2001). A chitosan-alginate complex tested for treating SWW and other food processing streams yielded a greater turbidity reduction and protein adsorption than using chitosan alone (Savant and Torres 2000, 2001; Wibowo et al. 2005a).

Optimization of the chitosan-alginate treatment

The treatment of SWW with a coagulation complex, at an optimized chitosan and alginate weight mixing ratio (MR) of 0.2 (Savant and Torres 2001), was examined as a function of coagulation time and the amount of coagulant complex added (Table 1). No further increases in protein

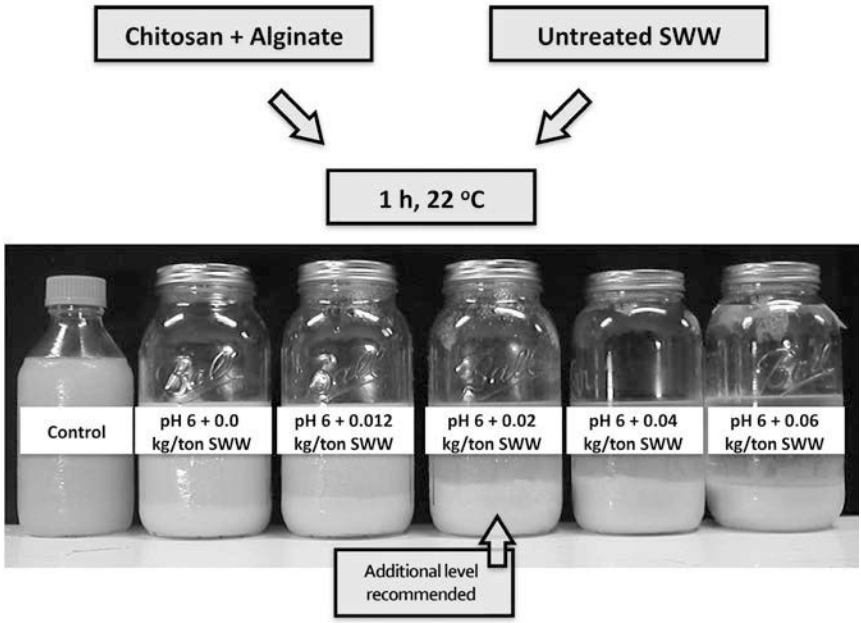

Figure 2. Pacific whiting surimi wash water (SWW) treated with chitosan and alginate at 0.2 weight mixing ratio and added after adjustment to pH 6. (Adapted from Torres et al. 2006.)

recovery were observed beyond 1 hour coagulation times, and any gains obtained by increasing the amount of complex were of little practical significance. Therefore, about 0.02 kg chitosan-alginate (0.2 MR) per ton of SWW, which yielded recovered solids containing 80% protein at 1 hour, and less than 0.1% chitosan, is recommended for the treatment of this effluent. The effectiveness of the chitosan-alginate complex as a coagulating agent can be seen in Fig. 2 showing control and pH 6 adjusted SWW, as well as samples containing increasing amounts of the complex prior to separation by centrifugation (Torres et al. 2006).

A comparison of the effectiveness of using coagulation complexes prepared from commercial and experimental chitosan that differ in their molecular weight and deacetylation degree is shown in Table 2. A 16% improvement in protein recovery was observed for the experimental sample SY 1000. Complexes prepared with commercial chitosan (CHI 84) achieved a 73.9% SWW protein recovery while the experimental sample SY 1000 yielded 86.0% when both were used at the recommended 0.02 kg per ton SWW. Further studies were conducted using commercial

Table 2. Effect of chitosan source on SWW protein adsorption.

Chitosan	Viscosity (cP)	mw (10^3 dalton)	Deacetylation degree	Protein recovery
SY 20[1]	20	22.3	94	73.2 ± 0.2^{ab}
SY 40[1]	40	47.0	93	74.0 ± 1.1^{abc}
SY 171[1]	171	225	75	74.6 ± 1.9^{bc}
SY 1000[1]	1000	1500	94	86.0 ± 0.7^{d}
SY 2150[1]	2150	3400	93	73.3 ± 1.5^{ab}
CHI 84[2]	2400	3800	84	73.9 ± 0.4^{abc}

[1]Experimental samples provided by Il-Shik Moon, Sunchon National University, Korea.

[2]Commercial sample provided by Vanson Chemicals.

Means with same letter indicate no significant concentration or time effect, respectively ($p > 0.05$). All complexes were prepared using food grade alginate (Kelgin® MV) and tested using the same SWW sample. (Adapted from Wibowo et al. 2007a.)

chitosan (Vanson Chemicals, Redmond, Washington) as the amounts of experimental chitosan SY 1000 available were insufficient for large tests.

Crude protein content of the insoluble solids recovered by the chitosan-alginate complex was over 70% (Wibowo et al. 2005a; 2007b). The amounts of recovered proteins varied with the protein concentration present in SWW, which ranged from 0.5-2.3% (Lin and Park 1996, Morrissey et al. 2000). After treatment with the coagulant complex, the protein could be recovered with a decanter centrifuge and sent to a disposal site, incorporated into surimi, or sold as an ingredient for foods and feeds. Recovering protein from SWW not only produces protein for food and feed production, but also generates treated water for potential reuse in the plant.

Nutritional value and safety of recovered SWW proteins

Flocculation using chitosan-alginate resulted in solids recovered from SWW with a 77.5% crude protein content on a dry basis, which is comparable to herring meal (77.7%), higher than menhaden meal (68.6%), and clearly superior to soybean meal (52.6%) as shown in Table 3 (Wibowo et al. 2007b). In addition, solids recovered from SWW have lower ash content and higher concentrations of essential amino acids, and compare well with other sources of high grade proteins. SWW solids contain robust concentrations of the basic amino acids lysine (9.4%), arginine (7.4%), and histidine (3%). Methionine (3.7%) and phenylalanine (5.1%) concentrations from recovered SWW proteins are higher than those usually encountered in fish and soy protein (Table 3).

Animal studies have demonstrated the nutritional value and the safety of SWW solids recovered by chitosan-alginate when tested at the levels recommended by commercial producers of animal feeds, i.e., under 15% (Wibowo et al. 2005b). These studies showed no difference in

Table 3. Percent composition and amino acid content of the solids recovered from surimi wash water (SWW) and comparison with protein sources used in commercial feeds.

Component	SWW Pacific whiting	Commercial protein meal sources		
		Herring[a]	Menhaden[b]	Soybean[c]
Ash	9.5 ± 0.1	11.5	19.1	7.4
Fat	9.1 ± 0.1	9.3	9.1	3.8
Crude protein	77.5 ± 1.8	77.7	68.5	52.6
Carbohydrate[d]	3.8	1.5[e]	3.3	36.2
	Essential amino acids (EAA), % of protein			
Arg	7.4	5.0	6.0	4.0
His	3	1.8	1.6	1.2
Ile	4.5	3.4	3.8	2.9
Leu	8.5	6.1	7.1	4.5
Lys	9.4	6.0	7.7	3.7
Met	3.7	2.5	2.5	0.8
Phe	5.1	2.9	3.8	3.1
Thr	4.5	3.4	4.3	1.8
Val	5.7	4.3	4.6	3.4
Total EAA	51.8	35.4	41.4	25.4

[a]Compiled from Cruz 1997, O'Mara et al. 1997, Ariyawansa 2000.
[b]Compiled from Piepenbrink and Schingoethe 1998.
[c]Hadjipanayiotou and Economides 2001.
[d]Calculated by difference from 100%.
[e]Calculated by difference from 100%.
(Adapted from Wibowo et al. 2005b, 2007a.)

rat feed consumption and growth rate (Fig. 3). Post-mortem examination of internal organs showed no visible signs of damage caused by feeding this experimental diet containing SWW proteins and minor amounts of the chitosan-alginate complex. Blood analysis including 20 physiological indicators supported the nutritional value and safety of SWW solids recovered by chitosan-alginate (Wibowo et al. 2005a). Subsequent studies demonstrated that 100% substitution of dietary protein by recovered SWW proteins was also safe and nutritionally equivalent to other protein sources showing higher protein efficiency ratio (PER) and net protein ratio (NPR) than the casein control (Wibowo et al. 2007b).

Incorporation of recovered SWW proteins into surimi

Many protein sources have been employed to improve the mechanical properties of surimi gels. The most frequently used are egg white and whey protein concentrates; other sources such as leguminous extracts and porcine plasma protein have also been proposed. These proteins

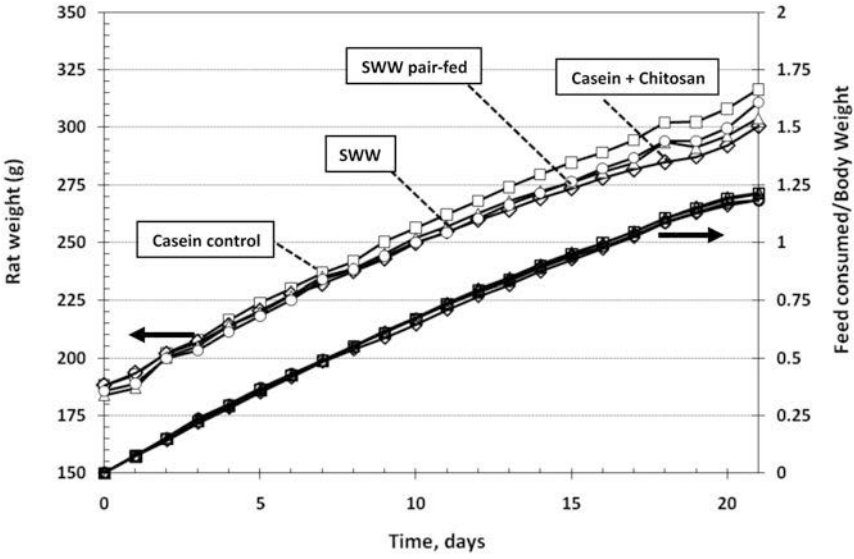

Figure 3. Evaluation of a 15% dietary protein substitution by SWW proteins recovered by the chitosan-alginate complex. The casein+chitosan control contained added chitosan in the amount present in the surimi wash water (SWW) diet. The pair fed control corresponds to rats fed the SWW feed amount consumed the day before. (See Wibowo et al. 2005b for experimental details.)

are added to inhibit the Modori phenomenon, i.e., the proteolytic degradation of fish myosin when gels are incubated at about 60ºC, or to improve the setting phenomenon associated with improved mechanical properties by the action of endogenous transglutaminases (An et al. 1996, García-Carreño 1996, Sánchez et al. 1998, Benjakul et al. 2001).

When added to surimi, low concentrations of SWW proteins recovered by the chitosan-alginate can improve mechanical properties with a minimum impact on color (Ramírez et al. 2007). This finding was confirmed by Velazquez et al. (2008) by determining the effect of adding proteins recovered from Pacific whiting SWW to Alaska pollock surimi. Incorporating up to 50 g per kg recovered SWW proteins (dry weight) reduced the expressible water in the pollock surimi paste (Fig. 4a). This indicates that SWW protein complex increased the surimi water holding capacity. In addition to water holding capacity, the mechanical properties are among the most important factors defining the quality of surimi gels. Firmness and consistency revealed a similar behavior, i.e., they decreased significantly when adding SWW protein complex

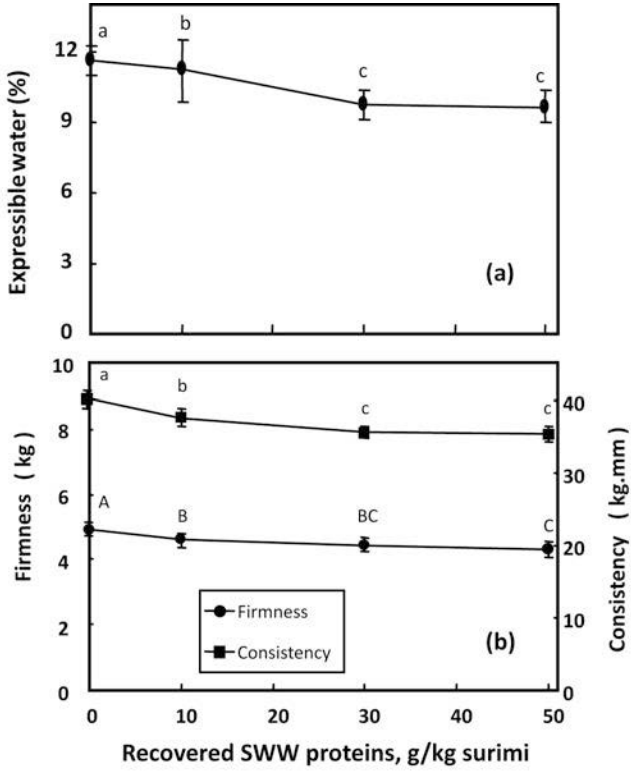

Figure 4. Effect of the added level of surimi wash water (SWW) complex protein recovered from SWW on Alaska pollock grade FA surimi pastes: (a) upper panel is percent expressible water, (b) lower panel is firmness and consistency of Alaska pollock grade FA surimi pastes. Values are means of six replicates. A TA.XT2i Texture Analyzer (Stable Micro Systems, Vienna Court, U.K.) with a back extrusion rig (model A/BE, 40 mm inner diameter, Stable Micro Systems) was used to measure the force required for the product to be extruded around a 35 mm piston disc. The maximum force and the area under the curve were defined as the firmness and consistency, respectively. Different letters indicate significant differences between treatments ($p \leq 0.05$). (Adapted from Velazquez et al. 2008.)

at 10 g per kg, but no further decrease at 30 g per kg and only a small further lowering was observed at 50 g per kg (Fig. 4b). The mechanical properties of surimi paste depend of protein-protein and protein-water interactions. The initial increase in firmness might be associated with protein-protein interactions. However, protein water interaction increased with the concentration of recovered SWW proteins added, enhancing water holding capacity (Fig. 4a). The decrease in firmness suggests that the proteins added to the surimi competed with muscle protein for water. Except for cohesiveness, all other texture parameters including springiness, chewiness, hardness, and fracturability showed similar changes (Velazquez et al. 2008). Finally, although pollock surimi gels containing the SSW protein complex showed only slight changes in color parameters at the levels used in this study, which are not likely to be perceived by consumers (Velazquez et al. 2008). These results showed that the soluble protein complex recovered from SWW could be used in surimi production and potentially enhance the financial sustainability of the surimi industry. In addition, the use of chitosan-alginate complexes to treat SWW reduces the environmental impact of the surimi industry and lowers the utilization of water resources.

Conclusions

The amounts of byproducts generated from processing aquatic and low-value fish are staggering. In the particular case of the surimi industry, the use of natural polymeric complexes for the treatment of processing water offers an opportunity to reduce environmental impacts and use of fresh water, and to increase yields. Chitosan-alginate complexes could be used in other food processing plants that face similar environmental problems including those handling shrimp and crab.

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Storage Effects on Separated Pink Salmon Processing Byproducts

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Abstract

There is growing demand for utilizing fish processing byproducts. Separation of different parts can be accomplished directly from the commercial processing line, and further processing of the separated parts can produce specialized feeds or other end products. With differences in the starting material composition, microbial content, and enzyme levels, differences in the composition of the end products as well as standard metrics of quality are to be expected. Knowledge of initial composition values and different rates of their diminishment during the process of spoilage could lead to options for storage and transport of the segregated byproducts. The objective of this study was to evaluate changes in separated pink salmon heads and viscera as a function of storage time and temperature. The results showed differences in the starting material and rates of change for oxidation, spoilage, and formation of biogenic amines between heads and viscera. Starting concentrations of thiobarbituric acid reactive substances (g malondialdehyde per g byproduct) in heads were much higher than in viscera. After 10 days of storage, total volatile base nitrogen increased only at a rate of 7 mg N per 100 g per day in heads, while in viscera the rate was five times greater at 32 mg N per 100 g per day. Formation of histamine was also higher in viscera and ended with a concentration of 1,432 ± 206 mg per kg in viscera compared to 20.8 ± 3.6 mg per kg in heads. These differences should be taken into account when examining options for storage and transport of the segregated processing byproducts.

Introduction

Renewable fish resources are being put under strain by the increasing world population. In order to meet demands and maintain sustainability, better utilization of the resource is needed. One way to maximize fish resources is by using the whole fish. After filleting, the parts of the fish remaining are often considered waste or processing byproducts and are usually discarded. These byproducts are coming directly from a food grade processing line and processors are beginning to realize the value of these byproducts. Bechtel and Johnson (2004) have characterized differences in the amino acid composition, mineral content, calculated rat protein efficiency ratio, and protein solubility in pollock, cod, and salmon processing byproducts from Alaska. While differences were noted seasonally and between species, differences were also uncovered between individual parts.

The largest harvest of wild Pacific salmon (*Oncorhynchus* spp.) in the world is in Alaska. The yearly harvest of Pacific salmon from Alaska waters is over 300,000 t (ADFG 2003). Approximately 80,000 t of salmon heads and viscera are generated from processing the catch, and much of the processing byproducts is unused (Crapo and Bechtel 2003). There is growing interest in utilizing processing byproducts such as head and viscera, which can be collected directly from commercial processing lines. These separated parts can be of value when made into specialized feeds or other end products. Oliveira and Bechtel (2005) reported that the percent of proximate lipid in pink salmon heads is 10 times higher than in viscera and there are significant differences ($P < 0.05$) in their fatty acid profile. Protein hydrolysates produced from salmon heads contain high levels of essential amino acids (Sathivel et al. 2005).

Lack of raw byproduct material is not an issue in Alaska; however, many fish processing plants have limited access to equipment to further process byproducts into potentially valuable coproducts such as fish meals and oils. Proper storage of these raw materials is required before they can be transported to a distant facility for further processing, such as rendering plants. The quality of oil extracted from aging salmon byproducts (Wu and Bechtel 2008a) and herring byproducts (Aidos et al. 2003) has been examined, but there is a lack of information on the stability of the raw material. The storage and stability properties of the individual parts have not been determined, and given the large differences in composition between heads and viscera, there will be differences during the storage. Knowledge of starting values and differential rates of decomposition changes could provide options for choosing new storage and transport schemes for these segregated byproducts. The following study was conducted to examine the changes in total volatile base nitrogen (TVB-N), biogenic amines, and thiobarbituric acid reactive substances (TBARS) occurring as a function of time and temperature in separated pink salmon heads and viscera.

Materials and methods

Sampling

Pink salmon (*Oncorhynchus gorbuscha*) were delivered on ice to a commercial processing plant in Kodiak, Alaska. The salmon were processed for the frozen market, and processing byproducts were collected for this study, including heads and viscera. Mechanically removed salmon heads were randomly collected off the processing line. Headed fish were gutted by hand, roe was removed, and the remaining viscera were randomly collected. Immediately after collection, heads and viscera were sorted and stored at 6ºC and 15ºC in new large plastic commercial trash bins, hygienically lined with food-grade polyethylene at the Fishery Industrial Technology Center in Kodiak, Alaska. Temperatures were recorded twice a day and samples were collected in triplicate on days 0, 1, 2, 3, and 4 from the 15ºC treatment and days 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 for the 6ºC treatment. Three replicate samples of approximately one kilogram each were randomly removed from the bins after mixing. Samples were then ground to a paste with a Biro grinder model 7540 (Biro Manufacturing Co., Marblehead) with a plate having 7 mm diameter holes.

Proximate analysis

Raw material samples from each storage bin were analyzed on days 0, 2, and 4 for ambient samples (15ºC) and days 0, 2, 4, 6, 8, and 10 for samples stored at ambient refrigerated temperature (6ºC) in triplicate. Moisture was determined gravimetrically by drying samples for 24 hours at 103ºC and measuring water loss. Protein was measured by drying samples and analyzing for nitrogen content on an Elementar Rapid NIII analyzer (Mt. Laurel) using Winrapid software to calculate protein values. Lipids were determined by processing dried samples on a Soxtec 2043 using methylene chloride (Fisher Scientific) extraction, after which lipid-rich solutions were evaporated to dryness to remove solvent, and then weighed. Ash content was estimated by grinding samples and placing them in a muffle furnace for 6 hours before reweighing.

Volatile amine analysis

Concentrations of ammonia, dimethylamine (DMA), and trimethylamine (TMA) in raw byproducts were determined using a capillary electrophoresis method (Timm and Jørgensen 2002) as modified by Wu and Bechtel (2008b). TVB-N values were calculated from the concentrations of the respective individual volatile amines detected in the samples. Approximately 1 g of each sample was blended in 20 ml chromatography-quality water and adjusted to pH 5.4 with HCl. The samples were placed in a 70ºC water bath with consistent shaking for 7 min and then brought back to room temperature. An Agilent capillary electropho-

resis system (Agilent Technology, Palo Alto), equipped with a diode array detector and ChemStation software for system control, was used to perform capillary electrophoresis measurements. Separations were made on an Agilent bare fused-silica capillary column with extended light path (bubble cell). The indirect mode (wavelength = 310 ± 20 nm, reference = 215 ± 10 nm) was used to detect compounds.

Biogenic amines

Biogenic amines in raw material and processed fish meal were analyzed as described by Hwang et al. (1997) using mass spectrometry. Approximately 1-5 g of sample were homogenized in 20 ml of 6% trichloroacetic acid. The samples were centrifuged at 8,000 G for 10 min and filtered. A 2 ml aliquot was transferred to a glass test tube followed by the addition of 1,7 diaminoheptane (Alfa Aesar, Ward Hill) as the internal standard, and 1 ml each of 2 M sodium hydroxide and benzoyl chloride. The mixture was vortexed and incubated at 30ºC for 40 min followed by the addition of 2 ml saturated sodium chloride. The extraction procedure was performed with the addition of 3 ml diethyl ether and vortexed for 2 min. The diethyl ether layer was transferred into a new test tube and evaporated down with nitrogen. Samples were reconstituted with 1 ml methanol and transferred into 2 ml vials for high performance liquid chromatography analysis. Seven biogenic amines (Sigma-Aldrich, St. Louis) were separated with a reverse phase C18 4 µm 3.9 × 150 mm column (Waters Corporation, Milford) and quantified with mass spectrometry. Detection was made with atmospheric chemical ionization–mass spectrometry in the positive ion mode on selective ions. The corresponding ion relates to the following derivatized biogenic amines and internal standard: putrescine = 297.1; cadaverine = 312.1; tryptamine = 265.1; 2-phenylethylamine = 226.1; spermidine = 458.2; 1,7 diaminoheptane = 339.1; spermine = 619.2; and histamine = 414.1. The setting for the mass spectrum was as follows: gas temperature = 350ºC; vaporizer = 250ºC; drying gas = 5 L per min nebulizer pressure; and capillary current at 4,000 V.

TBARS

Thiobarbituric acid reactive substances (TBARS) were measured in the raw byproducts as described by Siu and Draper (1978) with slight modification. A 2 ml aliquot of the samples extracted with 6% trichloroacetic acid was used for determining TBARS. The extracts were transferred into glass test tubes containing 2 ml of 2-thiobarbituric acid. The mixture was vortexed and incubated in a 94ºC water bath for 20 min. The tubes were then allowed to cool to room temperature and the upper layer absorbance was read at 532 and 600 nm for background correction. Quantification of TBARS was based on a calibration curve with malondialdehyde (Aldrich).

Statistical analysis

Three separate samples from each bin were used as replicates for the respective days. One-way analysis of variance (ANOVA) tests were used to assess statistical significance ($P < 0.05$) for proximate analysis and subjected to a Tukey's post hoc test. ANOVA was run on Statistica version 6.0 (StatSoft Inc., Tulsa). Regression line and equations for TVB-N and summation of biogenic amines versus storage time were developed with Sigma Plot 10. (Systat Software Inc., San Jose).

Results

Composition

The moisture, ash, lipid, and protein content of stored pink salmon heads and viscera at two temperatures for up to 4 or 10 days, wet tissue basis, are listed in Table 1. The average initial proximate starting values for combined temperature samples were 71.6% moisture, 4.3% ash, 9.9% lipid, and 13% protein for heads; and 81.6% moisture, 1.6% ash, 1% lipid, and 14.1% protein for viscera samples, respectively. Pink salmon heads and viscera exhibited similar ($P > 0.05$) moisture content for all days sampled. Lipid and ash content were inversely related in heads compared to viscera ($P < 0.05$) but there were no detectable differences for either lipids or ash content between storage days within a treatment. Protein content ranged from 12.2 to 19.2%, and higher variation was noted in the viscera samples. For the majority of the 6ºC storage samples there were no significant differences ($P > 0.05$) in protein content between heads or viscera samples, except for the day 4 viscera samples that had high protein values. Greater differences between head and viscera were seen in the samples stored at 15ºC.

Total volatile base-nitrogen

The TVB-N concentrations in heads and viscera were calculated from the summation of the individual compounds (Table 2) and graphed versus time on Fig. 1. The formation of TVB-N in viscera increased at an approximately tenfold greater rate per day than for head samples at both storage temperatures. The higher storage temperature resulted in higher concentration of TVB-N in head and viscera samples with storage time. The initial average values for TVB-N were 11.1 mg N per 100 g byproduct in head and 43.4 mg N per 100 g byproduct in viscera samples. The rate of TVB-N increase with time at both 6ºC and 15ºC temperatures approached linearity and viscera R^2 values were 0.90 and 0.86, respectively (Fig. 1).

The individual compounds that make up TVB-N values are listed in Table 2. Ammonia made up the majority of TVB-N in head and viscera samples. Average initial values of ammonia were 9.5 in heads and

Table 1. Proximate analysis of heads and viscera from pink salmon stored at two temperatures.

	Composition (%)			
Sample and day	Moisture	Ash	Lipid	Protein
Heads at 6ºC				
0	70.3 ± 2.7[a]	4.0 ± 0.5[a]	10.1 ± 1.7[a]	13.3 ± 2.4[ab]
2	72.1 ± 0.8[a]	4.7 ± 0.9[a]	9.0 ± 0.4[a]	13.2 ± 1.7[ab]
4	69.7 ± 3.5[a]	4.1 ± 0.3[a]	9.9 ± 1.1[a]	14.0 ± 0.5[abc]
6	69.9 ± 0.6[a]	4.6 ± 0.8[a]	10.2 ± 0.3[a]	13.9 ± 0.6[abc]
8	73.5 ± 1.0[a]	4.5 ± 0.2[a]	9.1 ± 0.9[a]	12.2 ± 0.4[a]
10	70.6 ± 3.0[a]	4.0 ± 0.9[a]	10.3 ± 0.8[a]	12.8 ± 1.9[ab]
Viscera at 6ºC				
0	81.5 ± 0.5[a]	1.6 ± 0.1[b]	1.0 ± 0.6[b]	14.4 ± 0.6[abc]
2	79.3 ± 0.9[a]	1.8 ± 0.1[b]	2.7 ± 2.7[b]	15.3 ± 0.7[abc]
4	78.4 ± 0.4[a]	1.7 ± 0.2[b]	2.0 ± 0.4[b]	17.1 ± 1.4[cd]
6	78.5 ± 0.8[a]	1.7 ± 0.1[b]	3.2 ± 0.2[b]	15.4 ± 0.5[abc]
8	78.1 ± 0.2[a]	1.6 ± 0.0[b]	3.6 ± 0.8[b]	15.5 ± 0.6[abc]
10	81.1 ± 1.9[a]	2.2 ± 0.2[b]	2.2 ± 0.3[b]	15.5 ± 1.2[abc]
Heads at 15ºC				
0	71.7 ± 1.4[a]	4.6 ± 0.6[a]	9.7 ± 2.6[a]	12.7 ± 1.5[ab]
2	70.0 ± 1.9[a]	4.1 ± 0.5[a]	12.5 ± 1.1[a]	14.2 ± 0.7[abc]
4	70.7 ± 1.4[a]	4.3 ± 0.7[a]	10.2 ± 1.4[a]	12.7 ± 0.9[ab]
Viscera at 15ºC				
0	81.7 ± 0.3[a]	1.6 ± 0.2[b]	1.1 ± 0.3[b]	13.7 ± 1.3[abc]
2	79.2 ± 0.5[a]	1.6 ± 0.3[b]	2.7 ± 0.2[b]	15.8 ± 0.8[bcd]
4	76.6 ± 0.6[a]	1.6 ± 0.2[b]	1.8 ± 0.4[b]	19.2 ± 0.5[d]

Values are expressed as mean (n = 3) ± standard error.
[abcd]Different letters within each column indicate a significant difference ($P < 0.05$).

Table 2. Individual volatile base nitrogen compounds in heads and viscera from pink salmon stored at two temperatures.

	Volatile base nitrogen compounds (mg N/100 g byproduct)		
Sample and day	NH_3	DMA	TMA
Viscera at 6ºC			
0	15.6 ± 8.5	0.3 ± 0.4	8.5 ± 10.7
1	51.6 ± 7.2	1.5 ± 1.9	39.4 ± 4.1
2	69.0 ± 30.9	3.9 ± 3.9	31.0 ± 14.7
3	74.7 ± 2.5	4.2 ± 0.9	40.2 ± 7.6
4	141.7 ± 16.5	9.5 ± 1.4	35.1 ± 9.1
5	153.2 ± 22.0	11.6 ± 2.4	23.4 ± 11.2
6	231.6 ± 19.0	29.5 ± 0.8	4.6 ± 0.7
7	229.7 ± 10.5	38.4 ± 1.7	6.9 ± 1.2
8	265.0 ± 18.3	53.2 ± 5.6	9.3 ± 0.4
9	276.4 ± 21.9	59.3 ± 3.5	11.6 ± 1.4
10	259.8 ± 58.5	42.0 ± 10.3	10.5 ± 2.8
Head at 6ºC			
0	10.5 ± 1.2	ND	0.1 ± 0.2
1	10.6 ± 2.5	ND	1.6 ± 1.3
2	13.2 ± 1.7	ND	2.7 ± 1.8
3	17.4 ± 2.2	ND	2.7 ± 0.6
4	26.1 ± 2.1	ND	6.5 ± 0.5
5	39.1 ± 11.1	0.9 ± 1.6	8.9 ± 0.9
6	37.7 ± 6.4	0.9 ± 1.5	10.3 ± 3.4
7	49.3 ± 6.3	2.0 ± 1.2	9.1 ± 1.8
8	48.4 ± 0.7	0.4 ± 0.7	9.4 ± 1.5
9	62.2 ± 5.7	ND	9.3 ± 1.2
10	56.4 ± 3.3	ND	11.3 ± 0.7
Viscera at 15ºC			
0	34.0 ± 4.0	3.1 ± 0.9	25.2 ± 7.5
1	74.0 ± 32.7	2.4 ± 1.5	35.3 ± 10.1
2	135.3 ± 34.1	11.3 ± 2.8	7.1 ± 2.9
3	206.0 ± 45.2	42.8 ± 8.2	5.9 ± 2.2
4	314.9 ± 38.0	27.6 ± 47.9	9.3 ± 1.2
Heads at 15ºC			
0	8.8 ± 2.1	ND	2.6 ± 1.7
1	17.9 ± 5.1	ND	5.0 ± 1.2
2	30.0 ± 2.2	ND	8.0 ± 1.5
3	62.3 ± 20.3	4.8 ± 1.3	11.2 ± 0.8
4	52.5 ± 17.7	3.4 ± 3.0	9.8 ± 1.7

Values are expressed as mean (n = 3) ± standard deviation.
DMA = dimethylamine; TMA = trimethylamine.
ND = Not detected.

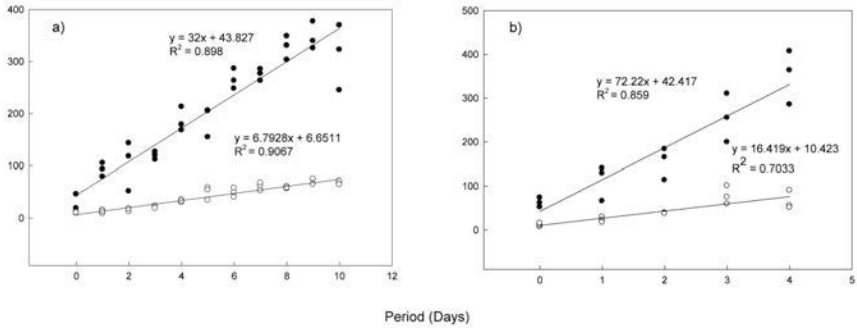

Figure 1. Total volatile base nitrogen (TVB-N) concentrations in head (open circles) and viscera (black circles) samples (n = 3) of pink salmon stored at (a) 6ºC for 10 days and (b) 15ºC for 4 days.

24.8 mg N per 100 g byproduct in viscera. Concentrations of ammonia increased with time to a final concentration on day 10 (6ºC) in head and viscera samples of 56.4 and 259.8 mg N per 100 g byproduct, respectively. A higher rate of increase was noted at 15ºC with final average concentrations on day 4 in head and viscera samples of 52.5 and 314.9 mg N per 100 g byproduct, respectively. Dimethylamine increased in viscera from 0.3 to 42 mg N per 100 g byproduct on day 10 (6ºC) and from 3.1 to 27.6 mg N per 100 g on day 4 (15ºC). In heads, dimethylamine was detected only at low amounts ranging from no detection to a high value of 4.8 mg N per 100 g byproduct on day 3 at 15ºC.

Trimethylamine levels in head samples increased with storage time and temperature to a maximum concentration of 11 mg N per 100 g of byproduct on day 4 at 15ºC. Trimethylamine levels in viscera samples were more variable with values ranging from a low of approximately 5 mg N per 100 g to a high of 40 mg N per 100 g of byproduct at 6ºC.

Biogenic amines

The summation of seven biogenic amines was calculated in head and viscera samples during storage at both temperatures (Fig. 2). Summation of biogenic amine values generally increased with time in both heads and viscera samples with average initial values on day 0 of approximately 96 ± 60 mg per kg byproduct in heads and 540 ± 228 mg per kg byproduct in viscera samples. The summation of biogenic amines was approximately tenfold greater in viscera than head samples for both temperatures. A final concentration of approximately 227 ± 33 mg per kg in heads and 2,370 ± 127 mg per kg in viscera samples were determined on day 10 at 6ºC storage. Similar patterns of approximately

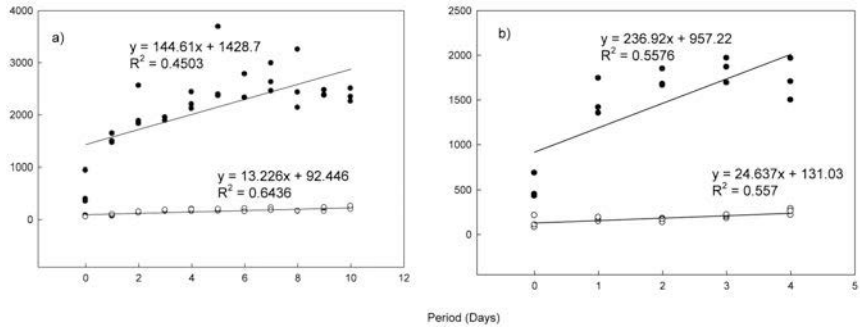

Figure 2. Summation of biogenic amines in head (open circles) and viscera (black circles) samples (n = 3) of pink salmon stored at (a) 6ºC for 10 days and (b) 15ºC for 4 days.

tenfold greater concentrations in viscera than in heads were also noted at 15ºC with a final concentration of approximately 254 ± 37 mg per kg in heads and 1,720 ± 234 mg per kg in viscera samples. Total biogenic amine values at both temperatures increased rapidly from day 0 to day 1, but then exhibited smaller increases for later samplings (Fig. 2).

The concentrations of individual biogenic amines generally increased with storage time and temperature with the exception of spermine and spermidine, which decreased in both head and viscera samples. Initial samples (day 0) of heads and viscera contained high levels of cadaverine with combined (6 and 15ºC) average concentration of 40 mg per kg in heads and 198 mg per kg in viscera. In viscera, the rate of histamine formation was greater than other biogenic amines with values on day 10 (6ºC) of 1,432 mg per kg and values on day 4 (15ºC) of 794 mg per kg. However, this was not the case in head samples, where little formation of histamine was noted on the last sampling days at either temperature.

TBARS

Levels of thiobarbituric acid reactive substances (TBARS) within head and viscera samples for the respective days and temperatures are listed in Table 5. The amount of TBARS in heads was approximately 33 times higher in heads than viscera on day 0. The difference was due in part to the approximately five times greater fat content in heads compared with viscera (Table 1). Viscera samples increased to an average concentration of 32.9 μg malondialdehyde per g byproduct on day 10 (6ºC) and 22.3 μg malondialdehyde per g on day 4 (15ºC). Head samples started off high and remained high throughout the days sampled, at both temperatures.

Table 3. Biogenic amines in heads and viscera from pink salmon stored at 6ºC.

	Biogenic amines (mg/kg byproduct)						
Sample and day	Put	Cad	Try	2-Phen	Sperm	His	Spermid
Viscera							
0	122.4 ± 15.2	170.2 ± 111.7	0.4 ± 0.7	7.6 ± 9.3	26.6 ± 3.4	124.9 ± 201.9	105.7 ± 12.8
1	107.8 ± 12.7	560.5 ± 32.4	11.3 ± 4.4	62.1 ± 11.4	20.1 ± 4.1	752.1 ± 51.8	71.2 ± 3.3
2	153.4 ± 45.8	551.9 ± 89.7	18.6 ± 4.2	80.7 ± 24.3	20.9 ± 10.0	1177.7 ± 255.0	82.1 ± 39.6
3	124.9 ± 11.4	491.7 ± 6.2	22.1 ± 4.6	89.3 ± 2.1	11.7 ± 1.0	1121.8 ± 46.9	54.2 ± 2.1
4	156.7 ± 14.9	518.1 ± 30.2	40.1 ± 5.3	108.7 ± 12.1	11.2 ± 1.4	1375.8 ± 130.6	39.0 ± 3.5
5	213.9 ± 97.8	549.2 ± 57.0	45.8 ± 19.4	93.4 ± 32.8	12.9 ± 11.5	1848.5 ± 598.4	49.2 ± 34.4
6	260.8 ± 64.2	492.9 ± 85.5	63.2 ± 10.7	112.4 ± 6.6	5.4 ± 3.7	1512.3 ± 84.4	28.1 ± 14.7
7	380.2 ± 12.1	565.0 ± 31.5	65.5 ± 5.4	108.7 ± 9.8	6.7 ± 0.3	1529.8 ± 226.8	31.9 ± 2.3
8	403.4 ± 130.3	534.1 ± 143.9	70.2 ± 21.5	94.0 ± 3.4	5.6 ± 3.7	1466.7 ± 277.3	30.0 ± 13.3
9	368.8 ± 73.4	483.2 ± 85.7	57.8 ± 7.4	96.0 ± 10.3	4.1 ± 3.2	1371.5 ± 132.4	23.4 ± 9.7
10	334.4 ± 43.4	431.6 ± 54.9	53.5 ± 9.4	90.4 ± 9.5	3.1 ± 1.5	1431.9 ± 206.1	20.8 ± 3.6
Head							
0	15.8 ± 3.9	25.3 ± 7.8	ND	ND	1.8 ± 0.8	ND	12.1 ± 2.9
1	17.8 ± 5.5	47.2 ± 10.6	ND	ND	1.1 ± 0.1	4.7 ± 8.2	12.0 ± 2.7
2	24.6 ± 3.5	72.1 ± 0.2	ND	ND	0.8 ± 0.4	30.4 ± 10.1	10.4 ± 0.7
3	39.7 ± 4.5	79.4 ± 7.3	ND	1.6 ± 0.9	1.1 ± 0.4	34.7 ± 3.5	10.4 ± 2.9
4	53.4 ± 9.3	89.0 ± 7.7	ND	2.3 ± 1.7	0.3 ± 0.4	24.0 ± 6.9	10.0 ± 3.8
5	52.8 ± 6.6	84.1 ± 8.1	ND	3.7 ± 1.4	0.0 ± 0.0	30.9 ± 5.0	7.6 ± 1.4
6	59.9 ± 8.0	89.0 ± 8.5	0.1 ± 0.0	5.5 ± 0.3	0.3 ± 0.1	16.9 ± 15.5	7.6 ± 0.6
7	68.4 ± 7.1	92.8 ± 6.9	0.1 ± 0.1	6.6 ± 0.6	0.6 ± 0.6	12.5 ± 17.4	8.6 ± 0.5
8	58.9 ± 2.7	80.4 ± 1.6	0.2 ± 0.1	7.1 ± 0.3	0.0 ± 0.0	7.7 ± 3.8	5.1 ± 0.3
9	77.7 ± 14.5	94.4 ± 18.1	0.2 ± 0.1	8.4 ± 1.0	0.0 ± 0.0	11.8 ± 17.6	7.4 ± 2.4
10	79.6 ± 2.9	97.2 ± 9.8	0.3 ± 0.2	8.8 ± 2.2	0.0 ± 0.0	34.3 ± 24.0	6.5 ± 0.3

Values are expressed as mean (n = 3) ± standard deviation.
Put = putrescine, Cad = cadaverine, Tryp = tryptamine, 2-Phen = 2-phenylethylamine, Sperm = spermine, His = histamine, Spermid = spermidine.
ND = not detected.

Table 4. Biogenic amines in heads and viscera from pink salmon stored at 15ºC.

	Biogenic amines (mg/kg byproduct)						
Sample and day	Put	Cad	Tryp	2-Phen	Sperm	His	Spermid
Viscera							
0	78.8 ± 3.8	226.2 ± 44.5	1.8 ± 0.7	19.0 ± 5.3	15.1 ± 2.8	123.9 ± 102.0	57.2 ± 8.8
1	93.0 ± 33.7	457.3 ± 115.9	31.4 ± 20.2	85.8 ± 11.8	16.4 ± 13.6	765.2 ± 128.2	52.8 ± 26.8
2	175.9 ± 47.4	407.7 ± 101.3	40.5 ± 8.0	93.2 ± 0.7	6.6 ± 4.7	971.1 ± 60.1	32.4 ± 18.5
3	344.6 ± 63.7	429.0 ± 65.5	58.6 ± 1.9	100.9 ± 4.8	5.4 ± 1.2	878.1 ± 267.2	24.4 ± 7.5
4	389.1 ± 125.3	404.5 ± 175.0	33.9 ± 11.7	60.2 ± 17.7	4.6 ± 2.8	793.9 ± 158.0	34.9 ± 17.3
Heads							
0	15.8 ± 1.7	55.3 ± 4.9	ND	ND	1.6 ± 1.2	6.9 ± 9.0	53.4 ± 74.8
1	39.6 ± 8.1	105.8 ± 16.6	ND	3.7 ± 1.4	1.7 ± 0.6	3.9 ± 6.8	15.4 ± 3.7
2	45.4 ± 5.1	93.0 ± 10.2	0.1 ± 0.2	7.5 ± 2.2	ND	8.9 ± 7.9	5.3 ± 1.1
3	59.2 ± 5.9	102.7 ± 5.9	0.2 ± 0.1	10.0 ± 1.4	ND	23.4 ± 12.4	4.5 ± 1.1
4	86.6 ± 12.9	127.8 ± 16.2	0.5 ± 0.1	16.4 ± 1.5	ND	17.4 ± 7.7	5.7 ± 0.7

Values are expressed as mean (n = 3) ± standard deviation.
Put = putrescine, Cad = cadaverine, Tryp = tryptamine, 2-Phen = 2-phenylethylamine, Sperm = spermine, His = histamine, Spermid = spermidine.
ND = not detected.

Discussion

As expected the composition of pink salmon head and viscera are uniquely different. Many bacteria have been reported in the digestive tracts of salmon (Seki 1969), while heads are considered more sterile with the exception of microorganisms on the surface of the skin, mouth, and gills. On the other hand, salmon heads contained more lipid than viscera; these highly unsaturated fatty acids are susceptible to oxidation (Kaitaranta 1992). The compositional and flora differences likely influence the initial quality and storage stability of separated byproduct parts.

The formation of TVB-N is correlated to microbial growth, and authors have described a positive relationship between the formation of these volatile amines and biogenic amines in seafood (Ruiz-Capillas and Moral 2001, Hsu et al. 2009). Separated viscera and head parts were considered of good quality with the starting TVB-N at less than 30 mg N per 100 g, which is generally the acceptable range for fresh or frozen fish (Periago et al. 2003). The rapid formation of TVB-N with increasing storage time and temperature in viscera samples is probably associated with growing microbial populations.

Biogenic amines are formed through the decarboxylation of associated free amino acids by exogenous decarboxylase enzymes released from the microbes colonizing the raw material (Rawles et al. 1996). The formation of TVB-N is related to microbial growth, and authors have described a positive relationship between the formation of the volatile amines (ammonium, dimethylamine, and trimethylamine) and the formation of biogenic amines in different fish species (Ruiz-Capillas and Moral 2001, Anderson 2007). The absence (or extremely low levels) of trimethylamine in the pink salmon heads may be attributed to the fact that microbes crucial for its formation, such as *Shewanella putrefaciens*, never reached 10^8-10^9 cfu per g, a population range considered necessary to the formation of trimethylamine (Dalgaard et al. 1993).

Histamine is a major safety concern in feeds, because at high concentrations it is toxic (Barnes et al. 2001). Histamine is formed by decarboxylation of the amino acid histidine. Bechtel and Johnson (2004) reported similar histidine concentrations in the protein of pink salmon heads and viscera, at 3.21 and 3.10 mole percent. These concentrations of histidine suggested that the low amounts of histamine detected in heads are likely due to the lack of exogenous decarboxylase enzymes from microbes or free histidine. In contrast, high levels of histamine in viscera samples suggested the opposite.

Pink salmon contains high levels of polyunsaturated fatty acids, which makes them very susceptible to lipid oxidation (Oliveira and Bechtel 2005). TBARS are used routinely in screening and monitoring lipid oxidation in fish products (Kurade and Baranowski 1986). While

Table 5. Thiobarbituric acid reactive substances in heads and viscera from pink salmon stored at two temperatures.

Temperature and day	TBARS (g MDA/g byproduct)	
	Viscera	Heads
6ºC		
0	4.8 ± 5.7	255.9 ± 5.6
1	8.3 ± 4.3	254.3 ± 23.1
2	9.6 ± 6.1	278.2 ± 12.7
3	21.4 ± 11.3	289.9 ± 7.4
4	19.3 ± 9.4	321.0 ± 22.0
5	42.5 ± 5.1	268.3 ± 94.8
6	26.8 ± 15.1	214.6 ± 98.8
7	39.4 ± 16.0	164.1 ± 2.7
8	45.3 ± 16.3	315.4 ± 3.5
9	21.8 ± 6.7	348.2 ± 17.7
10	32.9 ± 24.2	237.9 ± 83.4
15ºC		
0	8.7 ± 3.7	220.4 ± 58.0
1	22.2 ± 4.6	234.9 ± 155.6
2	18.4 ± 8.0	228.1 ± 64.0
3	14.9 ± 13.7	233.3 ± 31.2
4	22.3 ± 16.7	221.7 ± 65.0

Values are expressed as mean (n = 3) ± standard deviation.
MDA = malondialdehyde.

head samples had lower levels of TVB-N and biogenic amine formation compared to viscera samples, they contained approximately 4 to 5 times as much lipid per gram of tissue, which would be susceptible to lipid oxidation. For an unknown reason TBARS values in heads were extremely high at day 0 and remained at the elevated level throughout the storage study at both temperatures. Unlike viscera, head samples also contain gills and skin, which have pro-oxidant properties (Ke and Ackman 1976, Mohri et al. 1990). Zhong et al. (2007) reported that oil extracted from fish viscera was more stable against oxidation than oil extracted from muscle. Another explanation could be that thiobarbituric acid reacted not only with malondialdehyde but also with other compounds found in heads and not viscera (Guillen-San and Guzman-Choras 1998).

Conclusions

The major components of fish processing byproducts from pink salmon filleting operations are heads and viscera. In order to maximize their utilization, parameters associated with byproduct quality for the separated parts were evaluated. Heads remained more stable against the formation of TVB-N and biogenic amines during the storage study; however, they were more susceptible to lipid oxidation compared to viscera samples. The results suggested that if samples are to be separated, methods for microbial inhibition in viscera and protection against lipid oxidation in heads should be considered during storage.

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Converting Alaska Fish Byproducts into Compost: A Review

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Abstract

Alaska's commercial fishing industry, and sportfishing and subsistence fisheries, generate over one million metric tons of processing waste each year. Composting is a practical alternative for putting some of these discarded materials to practical use. Rural and remote coastal communities can benefit from these sources of recycled seafood materials since they already have access to other ingredients necessary for developing compost. These byproducts are rich in plant-essential nutrients, especially nitrogen. Local use of fish-based compost could promote the development of sustainable commercial greenhouses, small family farms, and home gardens. Direct application of the byproducts to the land for food production attracts wild and domestic animals and is difficult to implement. But composting the byproducts can produce a marketable product that is easy to store and use. However, arctic composting has unique challenges not found in warmer climates and requires modifications of traditional methods to be successful. This review addresses the practicality of composting fish processing waste for filling a niche in Alaska's coastal communities. Methodologies used for examining

Table 1. Organizations advocating compost (adapted and updated from Bord Iascaigh Mhara 2002, Martin 2006).

Organization	Internet address
Association for Organics Recycling (formerly The Compost Association UK)	http://www.organics-recycling.org.uk
Composting Association of Ireland Teo (Cré)	http://www.cre.ie/index.html
Composting Council of Canada	http://www.compost.org
Cornell Waste Management Institute	http://cwmi.css.cornell.edu
European Compost Network	http://compostnetwork.info
Maine Compost School	http://www.composting.org
Solid Waste Association of North America	http://swana.org
U.S. Composting Council	http://www.compostingcouncil.org
Woods End Research Laboratory (Maine)	http://www.woodsend.org

compost development, evaluation of demonstration projects, and availability of commercial products are discussed.

Literature and Web site review

Fishery consultants (Bimbo 2009), seafood industry groups (Bord Iascaigh Mhara 2002, Archer and Baldwin 2006, Seafish 2009), several university extension departments (Frederick et al. 1989, White et al. 1989, Frederick 1991, Nelson 1992, Carney et al. 2000, Vandre and Stirrup 2000, Rader 2008), and government agencies (Averill 1988, King 1996, Henry and Mellish 2001) have distilled the available information on seafood composting along with specific considerations depending on location and availability of resources. Numerous publications are available from several organizations on Web sites (Table 1) for instructing individuals and communities in how to create composts, although fish and shellfish composts constitute only a small fraction. Several organizations in other countries aim to make compost a significant part of urban and rural recycling.

The first large-scale U.S. fish waste compost study (Brinton and Seekins 1988) was conducted in Maine and the organization based on that study thrives today (see http://www.woodsend.org). The founder provided a brief review of the seafood composting process at the 1990 International Conference on Fish By-Products (Brinton 1990). In Australia, aquacultured fish mortalities and fish processing wastes are composted as a best practice for environmental management (DPI 2008). Their information is practical and provides the necessary steps for successfully dealing with an unpleasant, smelly, and unsightly material that may attract unwanted scavenging animals. Therein lies the basis

for recovering a useful component desired by vegetable growers and ornamental landscapers (MacLeod et al. 2006), mushroom producers (Green and Mattick 1979, Onokpise et al. 2008), and others (Martin and Chintalapati 1989). A novel application has been the use of fish compost in concoctions to decontaminate diesel oil in subantarctic soils (Delille et al. 2003, 2008). This compost review encompasses an update of publications relative to fish processing waste for recycling with a vision toward developing composting projects in Alaska coastal communities.

Advantages and disadvantages of fish composting

Some of the advantages of fish compost (Martin 2006) are the employment of simple "green" technology, low capital cost, transformation of waste into a hygienic and useful soil amendment, and potential commercial products. In contrast, the fish compost disadvantages (Martin 2006) are potential nitrogen loss through leachates, management requirements of the process for large-scale production, and the lower capital return compared to fish meal, hydrolysates, or silage. Fish compost is often relegated to a miscellaneous use category for fish industry waste (Correa and Martin 2000, Arvanitoyannis and Kassaveti 2008) and the topic was not presented at the Second International Seafood Byproduct Conference in 2002 (Bechtel 2003).

Compost ingredients

Bulking agents are necessary for developing fish and shellfish composts and these may include peat, wood chips, wood shavings, tree bark, and sawdust (Mathur et al. 1988; Martin 1999, 2006; Hayes et al. 1994,), seaweeds (Mathur et al. 1986), and animal manure (Martin et al. 1993). Lake fish were composted with peat, straw, or reed as a potential soil amendment for agricultural purposes in Finland (Roinila 1998). Canadian sphagnum moss has been used to reduce odor and to control air and water levels in compost piles (Canadian Sphagnum Peat Moss Association 2009). Sawdust, wood shavings, and yard trimmings composted with offal from farmed rainbow trout was applied as an organic soil amendment for Argentina's agriculture industry (Laos et al. 1988, 2002). Salmon farm mortalities in British Columbia, Canada, were composted with wood byproducts and allowed to reach temperature for the time necessary to destroy pathogenic microorganisms presumed to be present (Lo and Liao 1992; Liao et al. 1994a,b, 1995a,b, 1997; Liao 1997). The forestry industry in Alaska is keenly interested in utilizing lumber byproducts for developing composts prepared from fish and wood waste (CES 2001, Nicholls et al. 2002, Rapp 2003, JEDC 2005).

Table 2. Laboratory analyses for compost (adapted from USCC 2002).

Tests or properties	Examples
Biological	Biological assays, color, enzyme activities, odor, organic matter, volatile fatty acids
Chemical	Carbon (inorganic and organic), heavy metals, micronutrients, nitrogen, pH, phosphorus, potassium, soluble salts
Pathogens	Enterococci, parasitic worms, *Salmonella*, viruses
Physical	Air capacity, ash, bulk density, water-holding capacity, wettability
Synthetic organics	Herbicides, pesticides, polychlorinated biphenyls (PCBs), volatiles

Compost parameters and analysis

The primary compost parameters (Martin 2006) to consider for fish and shellfish composts are aeration or the amount of oxygen necessary for microbial activities, carbon (C) and nitrogen (N) content, the C:N ratio for optimum microbial growth, and moisture content for providing microbial viability. Aeration has been shown to affect the moisture content of in-vessel composting of shellfish waste (Seymour et al. 2001). No pH control is normally required for composting but temperature affects the fish byproduct degradation rate during composting, which influences the microflora before the material becomes warm enough to prevent microbial activity (Martin 2006). Compost analyses employ standard protocols starting with field sample collection and laboratory preparations. Properties measured can include physical parameters, biological, chemical, and organic properties, testing of pathogens, and synthetic organic compounds (Table 2).

Alaska compost

There are many challenges to overcome in Alaska before seafood composting can be sustainable. Potential scavengers may include rats, birds, and bears. Temperatures in Alaska coastal communities can fluctuate by location but each is typically cooler than lower latitude climates. Rain can be a major factor at Alaska fish processing sites. Occasional high winds can damage composting facilities and threaten the maintenance of sustained temperatures required for compost maturation.

The economics of transporting Alaska compost to markets likely results in the preference for local use in community and individual gardens rather than for growers in the "lower 48." Some manufacturers of fish and shellfish composts are located in Canada (Table 3). However, the "Made in Alaska" trademark (ADCED 2009) has resulted in niche marketing for Alaska compost products. Markets for fish compost outside of Alaska may capitalize on the natural allure and exotic image of Alaska.

Table 3. Commercial seafood compost manufacturers in the United States and Canada.[a]

Product and/or company	Location	Internet address
Land and Sea Compost	Rockport, Maine	http://www.mainemade.com/members/profile.asp?ID=2739
Winterwood Farm Premium Shellfish Compost	Lyman, Maine	http://shellfishcompost.com/shellfish.aspx
Oly Mountain Fish Compost	Belfair, Washington	http://www.northmasonfiber.com (Yepsen 2009)
Hoover Aquatic Farms	Balsam Grove, North Carolina	http://www.p2pays.org/ref/12/11510.pdf
Lighthouse Premium Fish Compost	Courtenay, British Columbia, Canada	http://www.lighthousesoils.com
Ocean Plus Earth, Cinnabar Valley Farms	Nanaimo, British Columbia, Canada	http://cinnabarfarms.com/detail.php?pro_ID=26
Earthbank Resource Systems Fish Compost	Parksville, British Columbia, Canada	http://www.fishcompost.com
Sea Soil, Foenix Forest Technology Inc.	Port McNeill, British Columbia, Canada	http://seasoil.com/SeaSoil-OMRIlistedOriginalSeaSoil.html
Meeker's Magic Mix All Natural Fish Compost	Evansville, Ontario, Canada	http://www.meekersmagicmix.com/index.html
SEAGRO, Shigawake Organics Ltd.	Shigawake, Quebec, Canada	http://www.seagro.com/index.htm

[a]Not a complete list.

Table 4. Compost trial studies and commercialized operations in Alaska.

Study or product	Organization	Project report or commercial product
Trial	Sitka Tribe of Alaska	http://www.sitkatribe.org/environment/compost_project.html (Sitka Tribal Enterprises 1998, Farrell 2000)
Trial and "Alaska Thunder Dirt"	Kake Tribal Corporation	http://www.allbusiness.com/north-america/united-states-alaska/1129718-1.html
"Totem Soil"	Kake Tribal Corporation	http://www.jgpress.com/inbusiness/archives/_free/000613.html (Musick 2004)
"Fishy Peat," Alaska organic potting soil	Fish and Chips Composting, Inc., Kenai	http://www.peninsulaclarion.com/stories/030507/news_0305new001.shtml

Legal requirements for managing commercial fish processing waste to comply with clean water mandates are regulated by the U.S. Environmental Protection Agency (EPA 2008) through the Alaska Department of Environmental Conservation (ADEC). ADEC regulates the disposal of commercial fish waste on land including landfills, direct land applications, and composting (ADEC 2009). A recent court settlement resulted in requiring all future local fish waste to be delivered to an Alaska compost facility established on the Kenai Peninsula (Baumann 2007, Kenai Peninsula Borough 2007, Hermanek 2007).

The potential for small-scale Alaska seafood compost (Table 4; JEDC 2005, Tarnai and Helfferich 2008) was enhanced after several trials were conducted using fish processing waste. Community and home gardeners can enjoy the public relations advantage of using compost from wild and sustainable Alaska fisheries by applying fish meal, fish compost, or liquid fish fertilizer to their crops. Last year, a community gardener established a successful business providing fresh summer vegetables in a remote Alaska location employing fish compost as nutrient source for his garden soils (Stevenson 2009). These successes promise increased use of fish byproducts as a crop fertilizer in Alaska.

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Chemical and Quality Changes When Seeking Full Utilization of Seafood Resources through Pressure Processing Technologies

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Abstract

Pressure processing technologies offer new possibilities for producing high-quality products from underutilized seafood resources. High pressure processing (HPP) increases shelf-life and microbial safety while often improving both product texture and color. However, since every fish species has different textural characteristics, fatty acid composition, and oxidative stability, it is necessary to optimize HPP treatment for each species to increase the extent of microbial inactivation and minimize any negative effects on quality. This task is challenging and greater effort needs to be focused on defining effective HPP treatments. The application of pressure-assisted thermal processing (PATP) to seafood, in spite of already being approved by the U.S. Food and Drug Administration for commercial sterilization of low-acid foods, remains only a potential alternative for producing high quality seafood products such as shelf-stable restructured meats or products enriched with recovered salmon oil. To date no studies have been reported on the effects of PATP treatments on nutrients in seafoods, including omega-3 fatty acids.

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Introduction

Resource limits and increasing public pressure on seafood processors have triggered an urgent need for improved resource utilization and a concomitant reduction in the environmental impact of seafood production and processing operations. The seafood industry is facing growing consumer demand for increased microbial safety, healthier processed foods, and greater eating pleasure—all delivered through products containing fewer synthetic additives (Velazquez et al. 2008). Consumer demands for quality and safety improvements are being satisfied by novel processing alternatives that have already been commercially adopted or are under development in research laboratories. Therefore, the multiple challenges to improve resource utilization, lessen environmental impacts, reduce food pathogen outbreaks, and improve processed seafood quality all need to be considered when implementing new technologies. Novel processing alternatives are needed because prevailing stabilization technologies, such as thermal processing, cause extensive textural and chemical changes in the product, while available minimal processing strategies do not fully eliminate some microbial risks. We should also consider the economic feasibility of the implementation of novel strategies to increase the utilization of seafood resources. The development of products with significant eating pleasure while retaining a high concentration of life-enhancing compounds should help meet this requirement.

Pressure processing technologies

High pressure processing (HPP), which causes minimum chemical changes to foods, has seen exponential growth in the United States, where more than half of the world's HPP installations are located. More than15% of these installations are involved in processing fish and other seafood. Studies show consumers prefer HPP products because they retain more nutrients, deliver high sensory quality, and contain fewer synthetic additives. Equipment advances, many examples of successful product commercialization, and a demand for minimally processed, high quality safer foods have led to considerable industrial interest in HPP technology (Torres and Rios 2006; Torres and Velazquez 2008; Torres et al. 2009a,b). A new generation of equipment is allowing the development of pressure-assisted thermal processing (PATP), i.e., the simultaneous application of pressures elevated to ~600-700 MPa and temperatures to ~90-120ºC to foods preheated to between ~60 and 90ºC) (Rajan et al. 2006; Pérez Lamela and Torres 2008a,b).

Moderate-temperature PATP (<100ºC) can be used when HPP pasteurization is not commercially feasible because of protracted processing times. High-temperature PATP (>100ºC) can be used to sterilize low-acid foods because it can kill bacterial spores (Reddy et al. 1999, 2003; Meyer

et al. 2000; Margosch et al. 2004, 2006; Rajan et al. 2006; Paredes-Sabja et al. 2007; Torres and Velazquez 2008; Torres et al. 2009b). The temperature point that distinguishes between PATP pasteurization and sterilization will decrease in the future due to increases in the maximum pressure produced by PATP units and as knowledge increases about the mechanisms of spore resistance and germination.

Applications of HPP technology to reduce losses and increase utilization of seafood

Seafood spoils faster than many other foods and its shelf-life is limited by both enzymatic activity and the growth of spoilage bacteria. This is particularly true for byproducts because the additional handling and processing steps required for their recovery provide further opportunities for microbial and enzymatic degradation of quality. High pressure processing (HPP) does not produce shelf-stable fish products; however, it is effective in reducing the counts of spoilage bacteria and minimizing the risk from pathogens such as *Vibrio parahaemolyticus* and *Listeria monocytogenes* to meet the zero tolerance levels required by current food regulations (Styles et al. 1991, Berlin et al. 1999, Amanatidou et al. 2000, Cook 2003, Kural and Chen 2008). Fish and shellfish are generally spoiled by gram-negative bacteria, which are relatively sensitive to HPP and thus the treatment can extend shelf-life and reduce seafood losses (Lopez-Caballero et al. 2000a, Hurtado et al. 2002). In addition to the elimination of spoilage organisms and food-borne pathogens (Yuste et al. 2001), HPP inactivates those endogenous enzymes causing quality deterioration (e.g., Hurtado et al. 2002). Lowering the amount of seafood spoilage and extending product shelf-life is an effective means to improve resource utilization.

Consumption of bivalve shellfish may be associated with a higher risk of food-borne disease (Potasman et al. 2002) since these species accumulate both bacteria and viruses during their filter-feeding, are normally eaten whole including the intestinal tract, and are often consumed raw or following only a very mild heat treatment. HPP is being increasingly employed in the commercial processing of oysters and clams (An et al. 2000, Cook 2003, Calci et al. 2005, Torres and Velazquez 2005, Kural and Chen 2008). Pressures above 240 MPa reduce *V. parahaemolyticus* in Pacific oysters (*Crassostrea gigas*) from 6.7×10^4 CFU per g to non-detectable levels (Raghubeer 2007). This treatment has a positive effect on quality and apparent yield because oysters appear more voluminous and juicy, characteristics attributable to an increased moisture content (Lopez-Caballero et al. 2000a,b; Cruz-Romero et al. 2008). Most important, HPP reduces losses by extending the short shelf life of fresh oysters. When compared to fresh oysters, HPP induced no significant change in their fatty acid profile; however, differences in headspace volatiles have been detected (Cruz-Romero et al. 2008). HPP-

treated oysters had higher concentrations of dimethyl sulfide, 1-penten-3-one, phenol and 1,2,4-trimethylbenzene relative to untreated samples. On the other hand, HPP-treated oysters had lower concentrations of 1-penten-3-ol, 2,3-pentanedione, (*E,E,Z*)-1,3,5-octatriene and 1,3-octadiene than untreated ones.

One interesting HPP application is the inactivation of proteolytic enzymes that limit the wider utilization of many seafood resources. For example, the large-scale commercialization of cephalopods has been challenging because of intense muscle autolysis caused by high levels of proteolytic activity (Stanley and Hultin 1984). HPP inactivates the enzymes responsible for both lipid degradation and textural changes, i.e., those quality factors that are primarily responsible for the consumer rejection of cephalopods (Ohshima et al. 1993). Pressurization was shown to reduce the proteolysis of myofibrillar proteins and thus lowered the softening of the octopus muscle (Hurtado et al. 2002).

Another example of a seafood application for HPP is pressure coagulation of proteins, which has been shown to increase the recovery of lobster, crab, and shrimp meat, and increase meat quality (Jabbour and Hognason 2007). The average percentage recovered in heat-treated Maine lobster is 25% of total body weight compared to an average of 43% for HPP shucked lobster (Raghubeer 2007). As much as 10% of the increase in yield came from the hydration of proteins by HPP. A more significant yield increase was observed in soft-shelled lobster (recent molts) showing a 45 and 22% recovery for HPP versus heat-treated lobster, respectively. HPP shucking at pressures between 250 to 400 MPa for 1 to 3 min has provided the lobster and crab industries with an opportunity to market fresh, shucked lobster and crab meats without the use of heat. In addition to the increased meat recovery, HPP shucking improves product quality by inactivating enzymes involved in the spoilage process and spoilage microorganisms. This technology also allows reductions in both direct and indirect manual labor costs exceeding 50% (Raghubeer 2007).

HPP protein coagulation can be used in the development of restructured fish products. Vacuum tumbling and massaging with salt extracts solubilizes myofibrillar proteins, resulting in a sticky exudate that can be used to bind meat pieces. However, the use of minced raw fish carries a risk of food-borne diseases due to microbial contamination during post-capture manipulation and processing, particularly during deboning, filleting, mincing, protein solubilizing, and product forming operations. Thermal treatments lower microbial risks but they can also cause undesirable chemical changes to proteins, lipids, vitamins, and other nutrients. HPP used for fish mince restructuring would reduce or eliminate microbial risks while yielding a high quality product with enhanced mechanical properties.

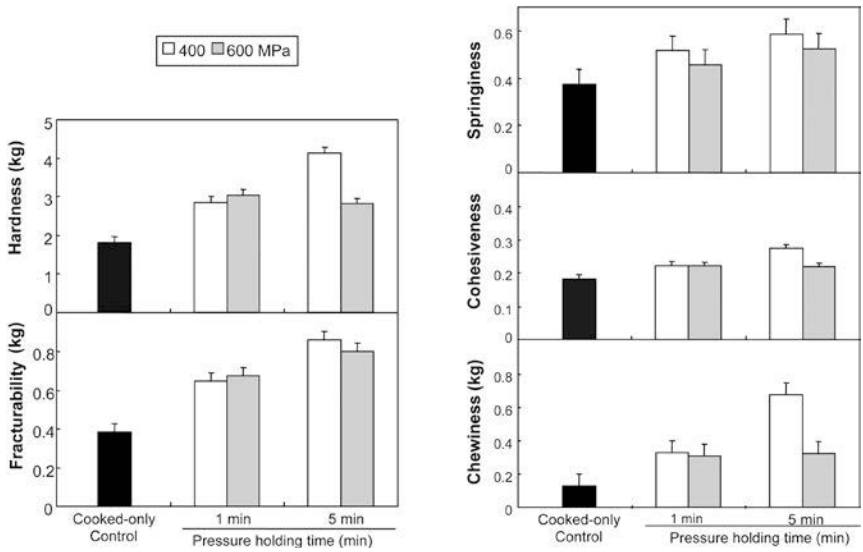

Figure 1. Mechanical properties of control and HPP-treated gels of minced arrowtooth flounder meat cooked at 90°C for 15 min (adapted from Uresti et al. 2004). HPP = high pressure processing.

An application example of HPP mince restructuring technology with much industrial interest is the manufacture of restructured fish products from noncommercial trim and flesh from species such as arrowtooth flounder (*Atheresthes stomias*), which has a reduced commercial value due to its high concentration of endogenous heat-activated proteolytic enzyme content (Wasson et al. 1993) (Fig. 1). HPP reduces the microbial risks inherent in using minced fish while inducing the gelation of myofibrillar proteins without employing heat, yet yielding products similar to raw fish in texture and gels of better quality after cooking.

Myosin seems to be the main protein affected by HPP treatments. While heat and pressure treatments both promote protein denaturation and gelation, HPP has a less severe effect when compared to thermal treatments (Fig. 2). Also, a protective effect of HPP against subsequent thermal denaturation in myosin has been observed. Textural characteristics of HPP restructured products are improved because the HPP protects the proteins against heat induced denaturation and aggregation resulting in products with undesirable texture (Uresti et al. 2005a,b). The differences in the structure of heat- and HPP-treated gels reflects differences in the mechanisms of induced gelation (Angsupanich et al. 1999, Gudmundsson and Hafsteinsson 2002). HPP-induced fish gels are

described as glossy and soft, with a smoother more uniform texture than gels produced by heat treatment. During pressure treatment the conformational arrangement of a protein is physically disturbed and intramolecular bonds stabilizing the native structure are disrupted. Thus the protein denatures and this exposes new intramolecular reactive groups that can interact by cross-linking with adjacent proteins. HPP induces myofibrillar protein aggregation through interactions of partially denatured proteins and with fewer changes in molecular conformation (Uresti et al. 2005b). Support for the protein-protein interaction model is provided by comparing the effect of freezing and pressure on myofibrillar proteins. Freezing myofibrillar proteins followed by frozen storage induces proteins to denature/aggregate with a disorganized arrangement resulting in gels with less acceptable mechanical properties (Matsumoto and Noguchi 1992). However, heat-induced gels obtained from HPP-treated samples have better mechanical properties than gels obtained only by heat. In summary, HPP induces well-structured protein systems with lower levels of protein conformational change, resulting in improved mechanical properties of these gels when cooked.

Myofibrillar protein aggregation caused by HPP treatments may limit the use of this technology in the pasteurization of high-risk products such as cold smoked salmon (Torres and Velazquez 2005) due to textural changes caused by these protein conformation modifications (Lakshmanan et al. 2003). In the case of restructured byproducts, this effect can be controlled by the addition of baroprotectants (Fig. 2). For example, sorbitol (8-12%) alone, or in combination with trehalose and sucrose, can reduce protein aggregation of arrowtooth flounder muscle paste (Uresti et al. 2005a). The mechanisms explaining this phenomenon are not completely understood but one hypothesis is that baroprotectants such as sorbitol could inhibit protein aggregation of myofibrillar proteins (Fig. 2).

Fish texture is an important characteristic that consumers use in determining product acceptability. Among factors influencing texture are the rate and extent that proteolysis causes the breakdown of myofibrils (Cheret et al. 2005). Although HPP can inactivate enzymes involved in texture deterioration (Ashie and Simpson 1996), it can also cause protein denaturation and thus modify fish texture (e.g., Uresti et al. 2004; Uresti et al. 2005a,b, 2006). In HPP treated seafood, it is not necessary to use chemicals such as tripolyphosphates (TTP) to improve product texture, since pressure-treated seafood has firmer textures than untreated controls (Uresti et al. 2004, 2005b). These textural changes caused by protein denaturation can be very desirable for seafood, as post-mortem softening occurs more rapidly in fish and shellfish when compared to other muscle foods (Ashie and Simpson 1996). Additionally, some authors have suggested that these HPP induced changes in texture

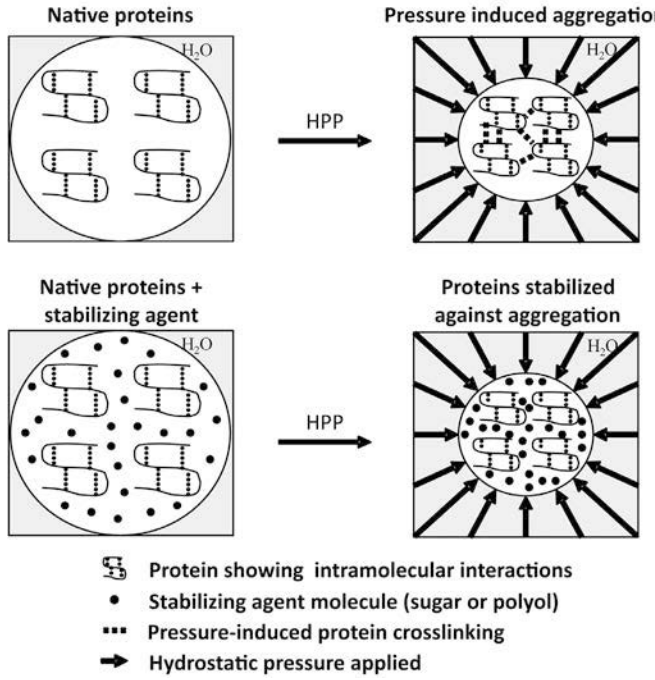

Figure 2. Mechanism of gel formation by HPP treatment of minced arrowtooth flounder meat with or without protein stabilizing agents (adapted from Uresti et al. 2005a,b). HPP = high pressure processing.

could be reversed during cooking (Gudmundsson and Hafsteinsson 2002), suggesting that protein denaturation causing undesirable texture changes may be less important in fish that will be cooked after pressure treatment.

Chemical changes induced by high pressure processing

Although there is increasing interest in the application of HPP to seafood, limited research has been conducted on the chemical changes HPP may induce in seafood. Color degradation, lipid oxidation and other quality changes are dependent not only on the intensity of the treatment applied but also on the seafood species (Yagiz et al. 2007). Lipid oxidation is an important factor reducing the quality of fish and fish products (Kolanowski et al. 2007). The acceleration of oxidation reactions by high pressure treatment in seafood could be caused by denaturing of heme proteins releasing molecules and metal ions, that can promote autooxidation of lipids (Frankel 1986).

Color is another important factor that needs to be maintained until the product reaches the consumer. Color changes can be caused by the denaturing of protein pigments such as myoglobin or hemoglobin. Studies show that HPP increases the L-value in several fish species including cod, salmon, sheephead, bluefish, hake, carp, plaice, pollock, trout, turbot, and mackerel muscle (Lakshmanan et al. 2003). These changes make the fish appear to have been cooked, which can cause confusion in consumers and potential rejection of the fish product. This color change is caused by the denaturing of myofibrillar and sarcoplasmic proteins (Ledward 1998). Although high pressure also induces a cooked appearance in fish, octopus (*Octopus vulgaris*) retains its raw appearance when treated at 400-800 MPa (Lakshmanan et al. 2003). Lower pressures, between 100 and 200 MPa, have been evaluated as a means to extend the shelf-life of salmon while keeping its raw appearance (Amanatidou et al. 2000).

Lipid oxidation generates compounds such as malondialdehyde and hydroperoxides, reducing the nutritional value through losses of essential fatty acids (Jeong-Ho et al. 2005). The overall mechanism of lipid oxidation is a free radical chain reaction requiring three steps: initiation, propagation, and termination (Frankel 1986). Initiation involves the abstraction of a hydrogen from a methylene carbon in an unsaturated fatty acid (RH) to form fatty acyl ($R^{\cdot}$) and peroxy radicals ($RO_2^{\cdot}$). The generation of the primary radicals is facilitated by the presence of oxidation initiators such as the transition metals iron and copper (Gray et al. 1996). Once a free radical is generated, a chain reaction of lipid oxidation is initiated, new free radicals are formed, and the process is easily propagated to other fatty acids which form new fatty acyl radicals as well as peroxy radicals and lipid hydroperoxides (Morrissey et al. 1998). The latter two undergo carbon-carbon cleavage to form small molecular weight–stable breakdown products including aldehydes, ketones, alcohols, hydrocarbons, esters, furans, and lactones (Frankel 1986, Morrissey et al. 1998). The fatty acid composition of muscle lipids affects their sensitivity to oxidative reactions. One of the main causes of the oxidative susceptibility of fish lipids is that some species are rich in long-chain polyunsaturated fatty acids (PUFA), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The fatty acid profile is species dependant, and for this reason, the extent of lipid oxidation differs significantly between species (Ackman 2008).

Lipolytic enzymes such as lipases, esterases, phospholipases, and lysophospholipases are involved in the hydrolytic cleavage of muscle lipids during refrigeration (Nilsson and Ekstrand 1993). Lipolysis releases free fatty acids from both triacylglycerols and phospholipids (Ishikawa 2007). This facilitates lipid oxidation because free fatty acids are more sensitive to lipid oxidation than the parent lipid molecules (Ishikawa 2007). The inactivation of these enzymes by HPP treatment

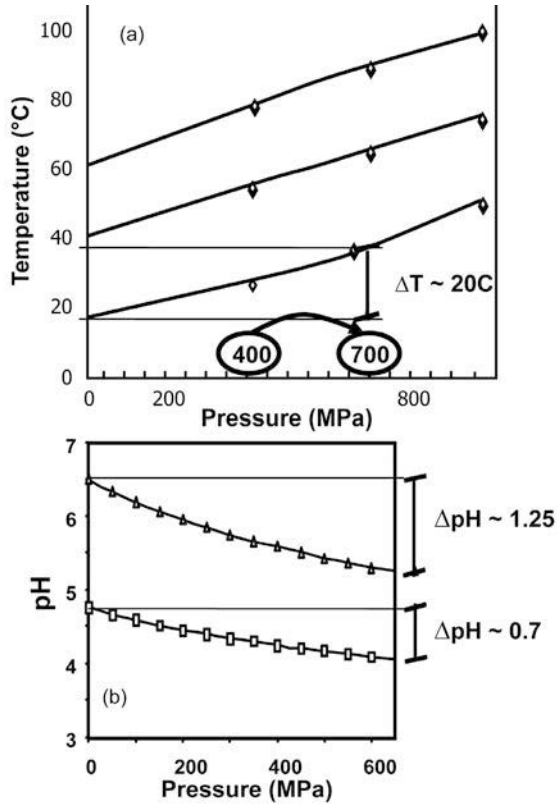

Figure 3. Experimental data limitations. (a) Compression heating of water and maximum vessel pressure increase with technology advances. (b) Pressure-induced pH shift of citric acid buffer (adapted from Torres and Velazquez 2008, Torres et al. 2009b). HPP = high pressure processing.

would decrease the deterioration of seafood and may extend shelf-life. However, HPP can also enhance the action of other enzymes leading to potentially detrimental changes in foods. This is exemplified by the increased head blackening of prawns following treatment at pressures between 200 and 500 MPa (Lopez-Caballero et al. 2000a,b; Linton et al. 2003).

HPP research limitations

Several problems with high pressure processing findings from previous research should be noted (Figs. 3 and 4). First, since HPP technology has

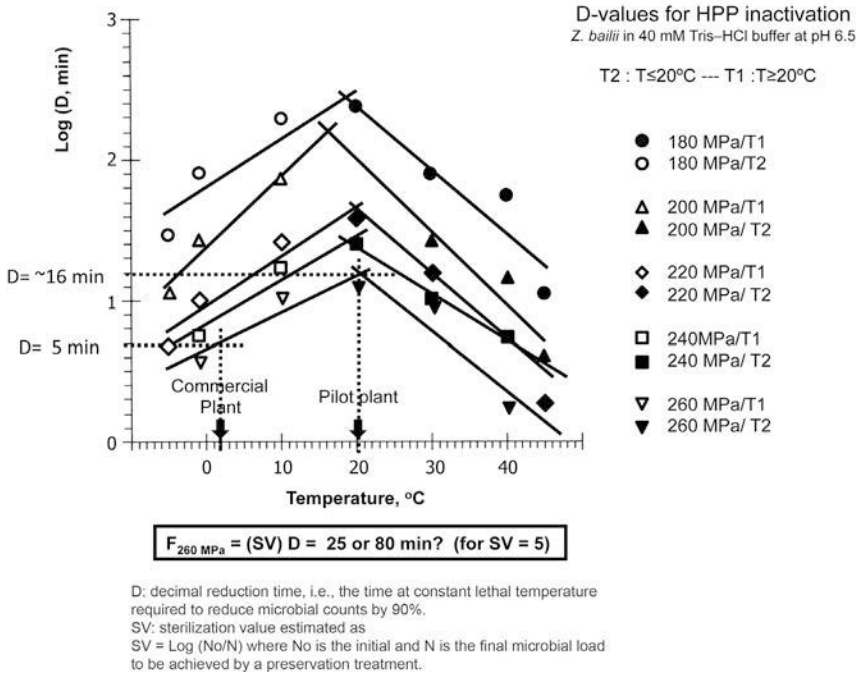

Figure 4. Experimental equipment limitations. Many commercial HPP plants operate at refrigeration temperature while pilot plant HPP vessels operate at ~20°C and thus generate incorrect process recommendations (adapted from Reyns et al. 2000). HPP = high pressure processing.

evolved very rapidly; the maximum pressure of experimental and commercial vessels has increased from about 400 to 700 MPa. Therefore, many of the earlier literature data are not directly applicable to current commercial needs. Furthermore, the increase in the maximum operating pressure has allowed processing times to be reduced from 15-30 to 0-3 min pressure holding time treatments (Torres et al. 2009b). Another limitation of earlier HPP research work is that the first experimental pressure vessels most often lacked thermometers. Consequently, the temperature increase due to the adiabatic heat of compression was not considered when interpreting experimental measurements (Fig. 3a). Still unresolved is the lack of pH measuring devices capable of operating at high pressures. Therefore the temporary pressure-induced pH shift cannot be measured during the experiment (Fig. 3b) to help the interpretation of results. Finally, it is unfortunate that most experimental HPP

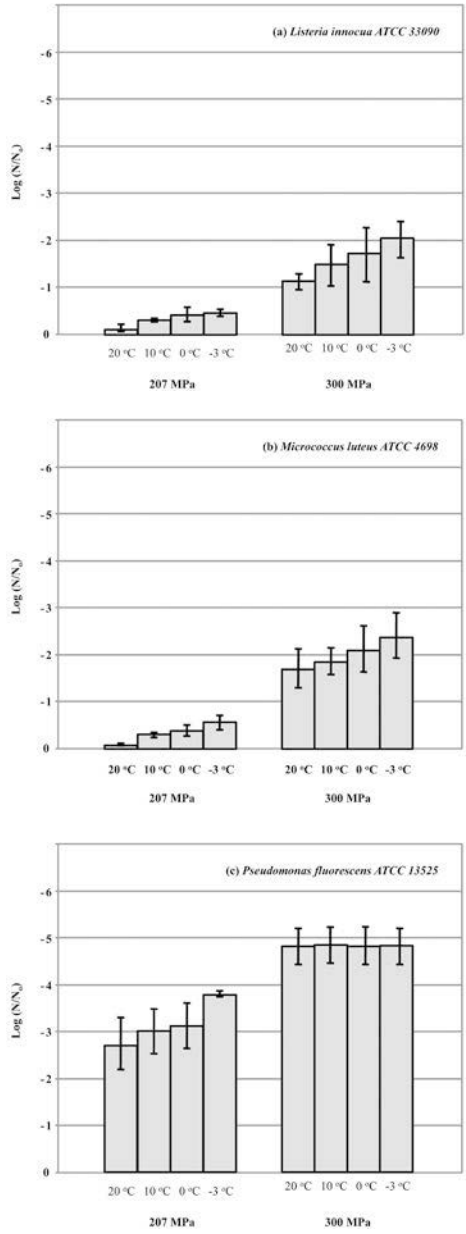

Figure 5. Microbial inactivation by different pressure treatments of smoked salmon mince (pH 6.0) inoculated with a pathogen cocktail containing (a) *Listeria innocua* ATCC 33090, (b) *Micrococcus luteus* ATCC 4698, and (c) Pseudomonas fluorescens ATCC 13525 (adapted from Picart et al. 2004).

vessels are located in research pilot plants operating at room temperature while in many commercial applications, particularly in the seafood industry, they operate at refrigeration temperatures. These limitations have led to process recommendations for microbial inactivation that tend to overestimate the required processing times (Figs. 4 and 5). As shown in the work by Reyns et al. (2000) and Picart et al. (2004), higher microbial inactivations are possible at or near refrigeration temperatures while the highest resistance to pressure inactivation seen in most microorganisms coincides with room temperature.

Applications of PATP technology to increase utilization of seafood

Industrial implementation of pressure-assisted thermal processing (PATP) is expected in the near future because the first request for the approval of a shelf-stable low-acid food has been submitted to the U.S. Food and Drug Administration (Ramírez et al. 2009) and approved (IFT 2009, IIT 2009). Noncommercial trim and meat from underutilized species could be used to formulate new PATP-treated products including shelf-stable restructured fish salads with vegetables and rice, couscous, or pasta. In the development of these added-value products, PATP offers two key advantages: (1) the combination of high pressure and elevated temperature accelerates the inactivation of bacterial spores resulting in lower temperature and/or shorter time sterilization processes; (2) compression heating and decompression cooling allows nearly instantaneous temperature changes which is particularly advantageous for solid foods due to their slow heating and cooling when subjected to conventional heat sterilization.

Although PATP is expected to yield shelf-stable foods of superior quality, use of this new technology will require reaction kinetics data to optimize product quality. Equally important is the retention of the high-value but extremely labile nutrients that are naturally abundant in fish byproducts such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Urgently needed research is the demonstration of less damage to the nutritional composition of PATP-treated seafood, showing that it is possible to manufacture fish products without reducing the healthful properties found in nature. The beneficial health effect of omega-3 fatty acids, especially long-chain EPA and DHA, are well demonstrated in the prevention of cardiovascular diseases, some types of cancer, and rheumatoid arthritis and are important factors ensuring proper development and function of the brain and vision system (Banerji 2008). Unfortunately, a recently completed review found no information on the effects of PATP treatments on seafood products (Ramírez et al. 2009). Only one publication was found reporting a direct

comparison of PATP and conventional thermal sterilization of a seafood product (salmon in pouches). These authors found that pouched salmon produced by thermal sterilization had a faded pink color, the flesh was soft, there was evidence of oil exudation, and there was a general loss of the "flaky" texture associated with properly cooked salmon. On the other hand, PATP-treated salmon had a bright pink color, was not oily, and had both the desirable moisture level and a flaky texture (Lau and Turek 2007). Unfortunately, this study provided no information on levels of the desirable nutrients in salmon, including fatty acids and vitamins.

Conclusions

Pressure processing technologies offer new possibilities for the production of high-quality products from seafood resources, including recovered byproducts and raw materials obtained from underutilized resources. Increases in shelf-life, elimination of microbial safety risks, improvements in product texture and color, and yield increases are also possible. However, since different seafood species have different textural characteristics, fatty acid composition, myoglobin content, and oxidative stability, it is necessary to optimize the pressure treatment for species individually. For instance, a study reported that 300 MPa and 450 MPa were the optimum pressure conditions for controlling microbial load, lipid oxidation, and color changes in rainbow trout and mahi mahi, respectively (Yagiz et al. 2007). Much effort needs to be focused on defining the best pressure treatment that reduces microbial loads while maintaining seafood quality. Finally, the application of PATP treatments to seafood byproducts could be a future alternative in the production of high quality, shelf-stable products. However, no studies have yet investigated the effect of PATP on chemical composition, particularly nutrients such as omega fatty acids that consumers seek out when purchasing seafood.

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Stabilizing Pink Salmon (*Oncorhynchus gorbuscha*) Byproducts through Modified Silage Processes

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Abstract

Fish byproducts (such as heads, viscera, and frames) can create disposal issues for processors in Alaska. The most common method of preservation for these high-protein byproducts is through production of fish meal; however, less energy-intensive forms of stabilization exist. Acidification by lactic acid bacteria (LAB) is a relatively simple process for stabilizing fish flesh, although a source of fermentable carbohydrate must be added. Potatoes are an agricultural product that might serve as a source of fermentable carbohydrate for the preservation of fish flesh. More than 18 million pounds of potatoes were grown in Alaska in 2006, with an estimated 20% loss. A cocktail of homofermentative LAB successfully grew in ground potato pulp without any additional nutrients, while sustaining a pH less than 4.4 for 60 days. When LAB were added to a 1:1 mixture of potato and ground salmon heads, the pH dropped from 6.5 to 5.7 within 24 hours, but steadily increased above pH 7.5 over 60 days. Increasing the potato content of the potato-salmon mixture to 70% resulted in more acid production and was most effective (pH 5.9) when the salmon had been smoke-processed. Mixtures containing lower amounts of potato did not produce sufficient acidity to inhibit spoilage bacteria. Discarded agricultural products such as potatoes represent a potential source of fermentable carbohydrate for preservation of fish byproducts. These fermented fish products could serve as inexpensive feed sources for agricultural animals, supplements in aquaculture

feeds, or as a local compost source for vegetable gardening or energy production.

Introduction

Alaska commercial fisheries are responsible for producing over one million metric tons of byproducts (e.g., heads, viscera, and frames) each year, and an estimated 110,000 metric tons of this waste comes from salmon (Crapo and Bechtel 2003). These byproducts can be processed into fish oils and meals if a fish meal plant is located nearby. However, fish meal plants are not always economical for Alaska fisheries such as salmon, since the catch is seasonal (June through September) and processing operations are often remote. This can result in local disposal of byproducts by grinding the waste material and pumping it back into the ocean according to the EPA–Alaska Department of Environmental Conservation (ADEC) General Discharge Permit. While fish meal production is the most common method for preserving these potentially valuable byproducts, alternative methods of preservation exist and could be adopted to decrease the loss of marine proteins and oils at relatively low cost.

Acidification of animal and plant tissues is a well-known method of preservation. Acids increase protein autolysis and inhibit spoilage bacteria that can produce off flavors and odors. Acidification can be achieved by using organic acids such as formic acid, inorganic (or mineral) acids such as sulfuric and phosphoric acids, or microbially produced acids such as lactic acid, which is naturally generated through the breakdown of carbohydrates during bacterial fermentation by lactic acid bacteria (LAB) (Raa and Gildberg 1982). The target pH that ensures preservation will vary, depending upon the method of acidification used. For example, a reduction to pH 2.0 may be necessary when using inorganic acids, whereas a pH of 4.0 may be sufficient when formic acid is added.

Utilizing microbially produced acids is a common method for producing silage from animal feeds such as alfalfa and grass crops, since these acids are less dangerous to handle than concentrated organic and inorganic acids. A broad range of agricultural waste has successfully served as a fermentable carbohydrate source for ensiling nonfermentable products, including fruit waste (Ash and Elliott 1991, Bello and Fernández 1995), sugar cane (Evers and Carroll 1998), and beets (Fagbenro and Jauncey 1998). The soluble levels of carbohydrate in these waste products are very high, thereby allowing LAB to produce sufficiently high levels of acid during fermentation. Non-fermentable, low carbohydrate-containing waste products, such as fish byproducts (Bello and Fernández 1995, Ahmed et al. 1996, Fagbenro and Jauncey 1998, Bower and Hietala 2008) and shrimp heads (Le-Van-Lien et al. 1996, Evers and Carroll 1998), could not be preserved without co-fer-

mentation of a carbohydrate-containing product. Although effectively used by microorganisms to produce acids, these fermentable waste products are not accessible in many areas including Alaska, and likely would not be cost-effective to ship. However, other carbohydrate-rich crops could potentially be utilized to ensile the tons of salmon byproducts that are produced in Alaska annually. For example, Alaska's potato industry produced over 18 million pounds of potatoes in 2006 with an estimated 20% loss. This waste product may serve as a source of fermentable carbohydrate.

Potato waste consists of a liquid portion and pulp. The liquid contains a large amount of proteins, free amino acids, and salts and is often utilized as a fertilizer due to its high nitrogen content (Mayer and Hillebrandt 1997, Mayer 1998). The pulp contains starch, cellulose, hemicelluloses, pectin, proteins, free amino acids, and salts and has been used as a source for cattle feed, alcohol fermentation, vitamin B_{12} production, and biogas feedstock, depending on the treatment of the pulp (Mayer 1998). Endogenous lactic acid-producing bacteria and fungi have also been isolated from potato pulp (10^7-10^9 cells per g wet pulp) in the lower anaerobic layers (Mayer and Hillebrandt 1997). This suggests that potato waste currently serves as a growth medium for LAB and might be readily adapted to preserving low-carbohydrate byproducts such as salmon. The objective of this study was to evaluate Alaska-grown potatoes as a fermentable carbohydrate source for LAB, thereby creating a low-cost method for preserving underutilized byproducts from salmon.

Materials and methods

Fish byproducts and potatoes

Pink salmon (*Oncorhynchus gorbuscha*) heads were collected from a commercial processor in Kodiak, Alaska, in 2007. All heads were shipped frozen to Fairbanks for processing. Alaska-grown potatoes (NorDonna cultivar) were from Vanderweele Farms, LLC, in Palmer. Pink salmon heads were smoked (95ºC, 5 hours) in a Bradley smoker (Bradley Technologies Canada Inc., Richmond, British Columbia) using hickory bisquettes. Raw salmon heads, smoked salmon heads, and potatoes were each ground using a Tor Rey F12-FS electric meat grinder (Tor Rey USA, Inc., Houston, Texas) with a 0.3175 cm (⅛ inch) plate. Oils were extracted from smoked salmon tissues by centrifugation in a Beckman J2-HS centrifuge equipped with a JA-14 rotor (Beckman Coulter, Inc., Palo Alto, California), then stored at –80ºC for later use. Ground salmon and potato mixtures were prepared at different ratios in separate beakers, and then transferred in triplicate to 50 ml tubes. All samples contained a final concentration of 2.5% (w/w) NaCl to minimize spoilage bacteria (Ahmed et al. 1996). Control samples received sucrose at a final con-

centration of 5% (w/w). Twelve treatment groups were compared: (i) raw salmon; (ii) raw:potato (1:1); (iii) raw:potato (7:3); (iv) raw:potato (3:7); (v) raw with 5% sucrose; (vi) smoked salmon; (vii) smoked:potato (1:1); (viii) smoked:potato (7:3); (ix) smoked:potato (3:7); (x) smoked with 5% sucrose; (xi) potato; and (xii) potato with 5% sucrose. After inoculation with lactic acid bacteria (LAB), samples were incubated at 37ºC for up to 60 days while maintaining a reduced-oxygen environment. All sampling was performed in triplicate.

Lactic acid bacteria

Three strains of lactic acid bacteria were used to prepare a cocktail for inoculation of silage mixtures. Only homofermentative strains (bacteria that only produce lactic acid from glucose) were chosen to maximize lactic acid production. Strains included *Lactobacillus curvatus* (NRRL B-4562) or *Lactobacillus casei* (NRRL B-1922), *Lactococcus lactis* (NRRL B-1821), and *Pediococcus pentosaceus* (NRRL B-14009). All LAB were obtained from A.P. Rooney of the USDA ARS Microbial Genomics and Bioprocessing Research Unit in Peoria, Illinois. Cultures were individually grown in 10 ml BHI broth (Oxoid, Ltd., Basingstoke, Hampshire, England) for 24 hours at 37ºC prior to preparing the LAB cocktail. Cultures were briefly centrifuged to concentrate cells and introduce minimal liquid to the silage. Starting inoculum was 10^5 CFU per g tissue. Prior to use, each culture was individually tested for ability to grow in homogenized potatoes. Inoculations of each strain grew well and rapidly reduced the pH to 3.9-4.4 within 24 hours.

Bacterial enumeration

Total bacterial counts were achieved by plating serial dilutions from Butterfield's Buffer (Hardy Diagnostics, Santa Maria, California) onto BHI agar. Coliforms were enumerated on MacConkey agar (Oxoid, Ltd.) and presumptive LAB were enumerated on MRS agar (Oxoid, Ltd.). All plates were incubated at 37ºC for one week to ensure that mold was not present. Microbial populations were enumerated at days 0, 15, 30, and 60.

Acidity measurements

All pH determinations were made using an Accumet pH meter (Fisher Scientific). Measurements were taken daily for the first 5 days and weekly thereafter, including 0, 15, 30, and 60 days. Lactic acid production was analyzed using a procedure by Taylor (1996), where hot sulfuric acid was used to cleave acetaldehyde from lactic acid molecules. The acetaldehyde reacted with copper and *p*-phenylphenol to produce a chromogen, which was read spectrophotometrically at 570 nm using a SpectraMax Plus microplate spectrophotometer (Molecular Devices, Union City, California). All samples were run in triplicate and were

diluted to fall within the standard graph range of 10^{-2} to 10^{-3} g per L. The assay was linear to a sensitivity of 0.4 g per L. Lactic acid production was determined at days 0, 15, 30, and 60.

Compositional analyses

Three samples from each treatment method were analyzed for moisture, ash, lipids, and protein. Moisture was determined by drying samples at 103ºC for 24 hours and measuring water loss (method 952.08, AOAC 1990). Ash content was determined by placing samples in a muffle furnace at 550ºC for 24 hours and weighing the remaining material (method 938.08, AOAC 1990). Lipids were determined by processing dried samples on a Soxtec Model 2043 (Foss Analytical, Denmark) using dichloromethane as an extraction solvent, after which lipid-rich solutions were evaporated to dryness and then weighed. Protein content was determined by measuring nitrogen content on an Elementar Rapid NIII analyzer (Mt. Laurel, New Jersey) using WINRAPID™ software to calculate protein values. Compositional analyses were determined at days 0, 15, 30, and 60. Carbohydrate concentrations were estimated by subtracting the percent moisture, ash, lipids and protein quantities from 100.

Statistical analyses

The effect of treatment was investigated using one way analysis of variance (ANOVA) conducted with the Statistica v. 7.1 software package (Statsoft, Tulsa, Oklahoma). The ANOVA *P*-value was set to 0.05, and differences between treatments were examined using the post-hoc test Tukey's equal *N* honestly significant differences ($P < 0.05$).

Results and discussion

Acidification by LAB fermentation

Oil was extracted from pink salmon heads (smoked only) and the remaining tissues were individually inoculated with lactic acid bacteria (LAB) and supplemented with homogenized potatoes to serve as a carbohydrate source for bacterial fermentation. Prior to fermentation with LAB, the difference in pH between raw (pH 6.4) and smoked (pH 5.9) salmon was statistically significant. Wood smoke is a preservation method and has been shown to introduce compounds such as phenols, organic acids, alcohols, carbonyls, hydrocarbons, and nitrogen compounds (Pearson and Gillett 1996), which likely contributed to the pH difference between smoked and raw samples. Raw potatoes initially showed a pH of 5.9. During 60 days of storage, only potato silage and salmon tissues (raw or smoked) containing sucrose decreased below a pH of 4.8 within 24 hrs of the initiation of fermentation and maintained the low pH (Fig. 1). Raw salmon samples that contained different per-

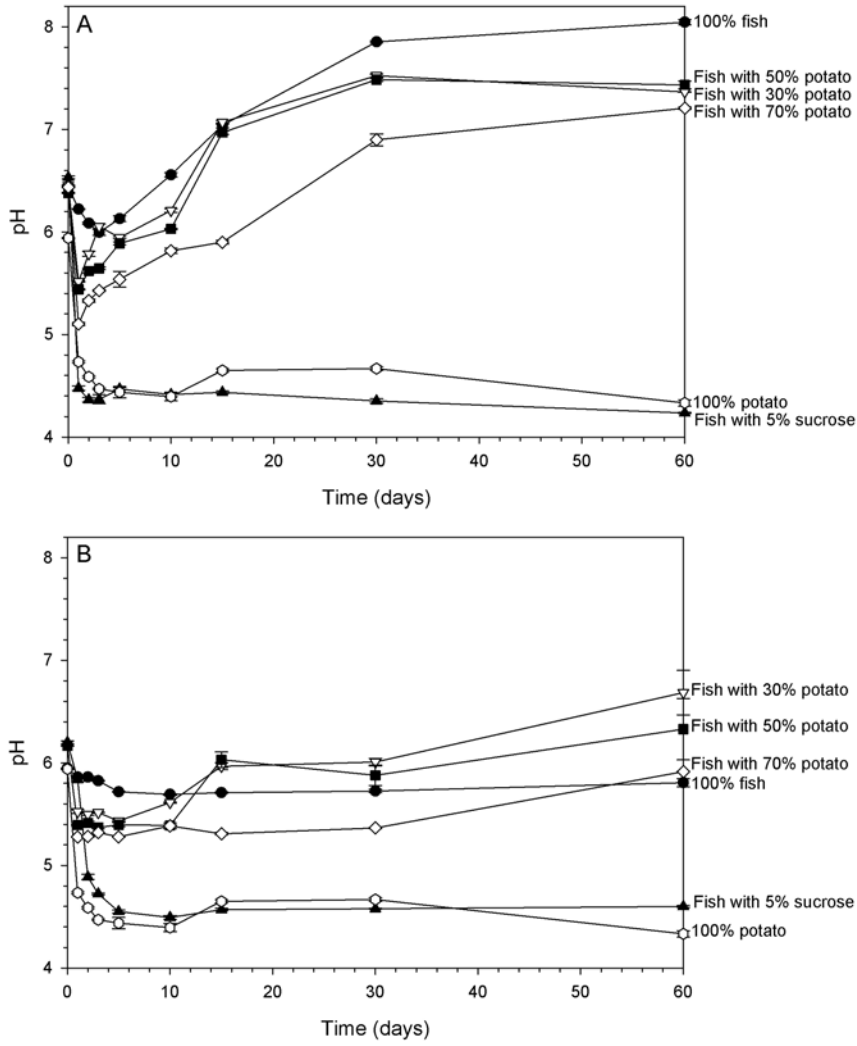

Figure 1. Change in pH over 60 days for (A) raw pink salmon heads, and (B) smoked pink salmon heads containing different percentages of potato as a carbohydrate source for the lactic acid bacteria inoculum.

centages of potato all showed an initial decrease in pH (as low as 5.1 when 70% potato was added), but this value increased over time (Fig. 1A). Smoked salmon samples containing different percentages of potato also experienced an initial decrease in pH at the start of fermentation, but the pH value increased more slowly over 60 days than in their raw salmon counterparts (Fig. 1B). This may be due to compounds imparted during smoke processing that enhanced LAB growth and/or inhibited competitive organisms such as spoilage bacteria (Bower et al. 2009).

Because a marked decrease in the pH of potato silage (as low as 4.3) was observed, it can be assumed that the LAB cocktail successfully fermented potato pulp when this was its sole carbohydrate source. However, when potato pulp was mixed with raw salmon tissue, even in ratios as high as 70% potato, a similar decrease in pH did not occur, suggesting that something in raw salmon is interfering with lactic acid production or LAB survival. Smoke-processing appears to mitigate the interfering action of raw salmon to some degree, but not completely. This may indicate that a heat-labile compound, perhaps an enzyme found in raw salmon, is responsible for inhibiting the growth of LAB.

Bacterial enumeration

To verify that the decreased pH observed in treatment groups was due to growth of lactic acid producing bacteria, all samples were cultured for bacterial identification. Although total bacterial counts and the presence of coliforms were also determined, only results for presumptive LAB (designated as colonies growing on MRS agar) are shown in Fig. 2. All inoculated samples maintained growth of presumptive LAB within the range of 10^4-10^8 CFU per g tissue over 60 days of storage. For samples containing raw salmon, any addition of potatoes allowed the LAB to grow near (or above) the original inoculum level for the entire 60 days (Fig. 2A). However, samples prepared with 100% salmon did not support growth to the same extent. A salmon composition of less than 50% appears to enhance growth of LAB when compared to samples prepared with 100% potatoes. As the percentage of raw salmon increased, the growth-sustaining ability of the potato-salmon mixture decreased. The compositional analysis of each sample (Table 1) suggests that the decreased growth might be due to depletion of fermentable carbohydrates, rather than being caused by inhibitory factors present in raw fish. When smoked salmon, instead of raw salmon, was combined with potatoes, the results were very different. Samples containing high percentages of fish were superior in supporting bacterial growth, whereas samples containing no fish resulted in a lower level of LAB survival. In all samples, cell counts peaked within the first few weeks and then slowly decreased over 60 days. These bacterial enumeration results suggest that smoke-processing may impart compounds that enhance LAB growth, compared to raw salmon samples. Alternately, the smok-

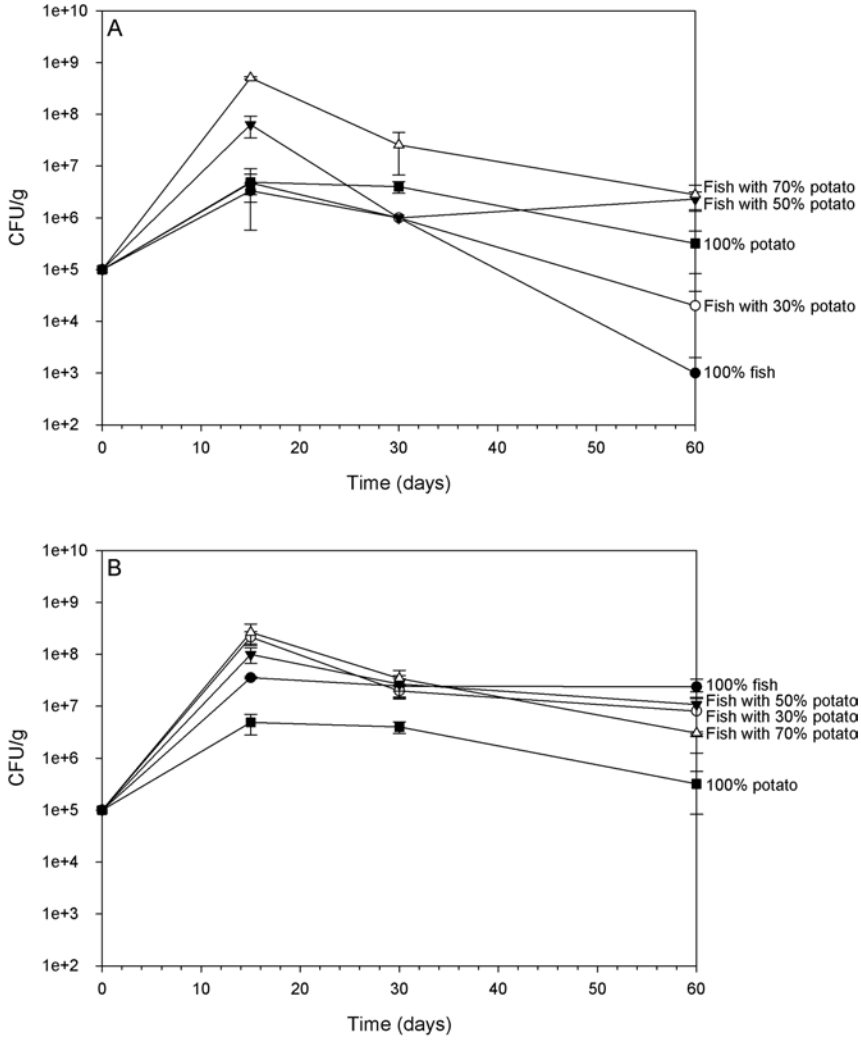

Figure 2. Enumeration of lactic acid bacteria (CFU per g tissue) over 60 days for (A) raw pink salmon heads, and (B) smoked pink salmon heads containing different percentages of potato as a carbohydrate source for the lactic acid bacteria inoculum.

Table 1. Compositional analysis of ensiled salmon byproducts mixed with different percentages of potato and sampled at 0, 15, 30, and 60 days.

	Raw salmon mixed with potato												Smoked salmon mixed with potato														
	100% salmon			30% potato			50% potato			70% potato			100% salmon			30% potato			50% potato			70% potato			100% potato		
	Mean	SE	p^a	Mean	SE	p^a	Mean	SE	p^a	Mean	SE	p^a	Mean	SE	p^a	Mean	SE	p^a	Mean	SE	p^a	Mean	SE	p^a	Mean	SE	p^a
Moisture																											
0	74.5	0.2	cd	75.0	0.1	cd	75.4	0.1	cde	75.9	0.4	cde	67.6	0.8	ab	71.2	0.0	bc	72.9	0.3	bc	74.3	0.4	cd	72.8	0.1	bcd
15	84.1	0.4	f	80.3	0.2	ef	78.9	0.4	de	77.6	0.2	de	68.5	1.4	ab	70.9	0.1	bc	74.3	0.3	cd	76.1	0.1	cde	70.1	0.7	abc
30	86.0	0.1	f	80.5	0.4	ef	79.5	0.3	de	79.8	0.6	ef	66.0	0.6	a	72.0	0.3	bc	74.9	0.5	cd	76.3	1.0	cde	67.1	0.0	ab
60	86.8	0.5	f	81.8	0.1	ef	79.6	0.2	def	78.5	0.1	de	65.7	0.4	a	74.1	3.1	cd	77.2	2.1	cde	75.9	0.1	cde	67.7	0.6	ab
Lipid																											
0	2.7	0.0	bc	1.8	0.3	abc	1.2	0.1	ab	0.7	0.1	a	5.5	0.4	d	3.3	0.3	c	2.7	0.0	bc	1.4	0.3	ab	0.0	0.0	a
15	3.3	0.2	c	2.1	0.0	abc	1.1	0.0	ab	0.7	0.0	a	5.7	0.0	d	3.7	0.2	c	2.9	0.1	bc	1.4	0.3	ab	0.1	0.0	a
30	4.2	0.0	cd	1.8	0.1	abc	1.1	0.0	ab	0.8	0.1	a	6.0	0.1	d	3.4	1.1	c	3.0	0.3	bc	0.9	0.2	a	0.1	0.0	a
60	2.1	0.2	abc	1.0	0.1	ab	0.7	0.0	a	0.5	0.8	a	6.0	0.4	d	1.9	0.7	abc	1.9	0.5	abc	0.6	0.2	a	0.1	0.0	a
Protein																											
0	16.6	0.9	d	12.3	0.5	cd	9.6	0.3	c	5.7	0.3	b	19.9	0.3	e	15.9	0.7	d	12.2	0.3	cd	8.5	0.5	bc	1.9	0.1	a
15	15.6	0.6	d	11.7	0.5	c	9.6	0.2	c	6.3	0.3	b	21.3	0.8	e	15.6	0.6	d	12.2	0.1	c	7.8	0.5	bc	2.3	0.5	a
30	14.4	0.1	d	11.3	0.1	c	9.5	0.1	c	6.5	0.4	b	21.5	0.3	e	15.7	0.0	d	12.6	0.1	cd	8.1	0.3	bc	1.6	0.1	a
60	14.7	0.1	d	11.4	0.3	c	9.5	0.2	c	6.6	0.1	b	22.8	0.1	e	16.4	0.3	d	12.5	0.2	cd	8.8	0.1	bc	2.0	0.1	a
Ash																											
0	5.5	0.0	c	4.6	0.1	c	4.4	0.1	c	4.2	0.0	bc	3.9	0.5	bc	3.4	0.1	ab	2.7	0.0	a	2.3	0.2	a	2.4	0.0	a
15	4.1	0.1	bc	4.7	0.2	c	4.5	0.0	c	4.5	0.2	c	4.2	0.3	bc	4.0	0.1	bc	2.9	0.1	a	2.0	0.1	a	3.1	0.0	ab
30	4.3	0.0	bc	5.1	0.1	c	4.6	0.1	c	4.5	0.1	c	5.2	0.1	c	3.4	0.0	ab	2.8	0.1	a	2.2	0.0	a	2.8	0.1	a
60	4.5	0.1	c	4.9	0.0	c	4.7	0.0	c	4.6	0.2	c	4.8	0.0	c	3.4	0.4	ab	2.8	0.2	a	2.4	0.3	a	3.1	0.0	ab
CHO[b]																											
0	1.0	0.8	a	6.3	0.6	bc	9.5	0.3	cd	13.5	0.5	d	3.2	0.7	ab	6.3	0.8	bc	9.5	0.6	cd	13.4	0.5	d	22.9	0.2	e
15	0.0	0.0	a	1.2	0.5	ab	6.0	0.5	bc	10.9	0.5	cd	0.7	0.4	a	5.8	0.7	bc	7.7	0.4	bc	12.8	0.5	d	24.4	0.2	e
30	0.0	0.0	a	1.2	0.4	ab	5.4	0.3	b	8.4	0.7	bc	1.2	0.4	ab	5.4	0.9	b	6.8	0.7	bc	12.6	0.7	d	28.4	0.2	e
60	0.0	0.0	a	0.9	0.1	a	5.5	0.1	bc	9.8	0.1	cd	0.8	0.8	ab	4.0	2.1	ab	5.6	1.6	b	12.3	0.5	cd	27.1	0.6	e

[a]Letters represent significant differences ($p < 0.05$) within each individual compositional parameters, not the whole table.

[b]CHO (carbohydrate) was estimated by subtraction from all other proximate parameters, assuming 100%

ing process may inactivate antagonistic enzymes or inhibit competitive organisms such as spoilage bacteria.

Lactic acid concentrations

Lactic acid levels were measured to confirm the growth of lactic acid bacteria in inoculated samples. Although LAB are generally considered normal flora of fish (Gram and Huss 1996), the concentration of lactic acid in uninoculated controls was less than 1 g per L suggesting that lactic acid bacteria were present, but not abundant in the raw salmon heads used for this study. Raw salmon tissue inoculated with LAB, but not provided an additional carbohydrate source, displayed low levels of lactic acid over 60 days (Fig. 3A). When raw salmon was mixed with potatoes, lactic acid levels increased with increasing potato concentrations. The highest quantity of lactic acid was produced by samples containing salmon with 70% potatoes, although these mixtures were unable to maintain the high lactic acid levels seen in 100% potato controls over 60 days of storage. When smoked salmon was substituted for raw salmon, lactic acid levels also increased as larger percentages of potatoes were added (Fig. 3B). These high lactic acid values correlate with low pH levels detected in Fig. 1, as well as with the fluctuations in LAB cell counts graphed in Fig. 2. The concentration of lactic acid is considered a good indicator of fermentation stability of silage (Madrid et al. 1999).

Changes in silage composition

Generally there were no significant changes in percent moisture of either raw or smoked salmon when samples were mixed with potatoes and stored for 60 days (Table 1). Lipid levels also did not experience significant differences within treatment groups, but were generally higher for smoked salmon than for raw samples, which correlates with the lower moisture levels observed after the heat treatment that occurred during smoke processing. Similarly, protein values did not significantly decrease in any samples over time. High protein values found in smoked salmon, but not raw samples, corresponded to the lower moisture levels that resulted after heat was applied during smoke processing. As expected, raw and smoked samples each contained more protein individually than when they were combined with low-protein potatoes. The ash component of each sample generally varied according to the percentage of low-ash potatoes added to the higher-ash fish. The carbohydrate component (potato starch) appeared to decrease over time in all samples, except for the 100% potato treatment group, which may have released previously inaccessible carbohydrates during decomposition.

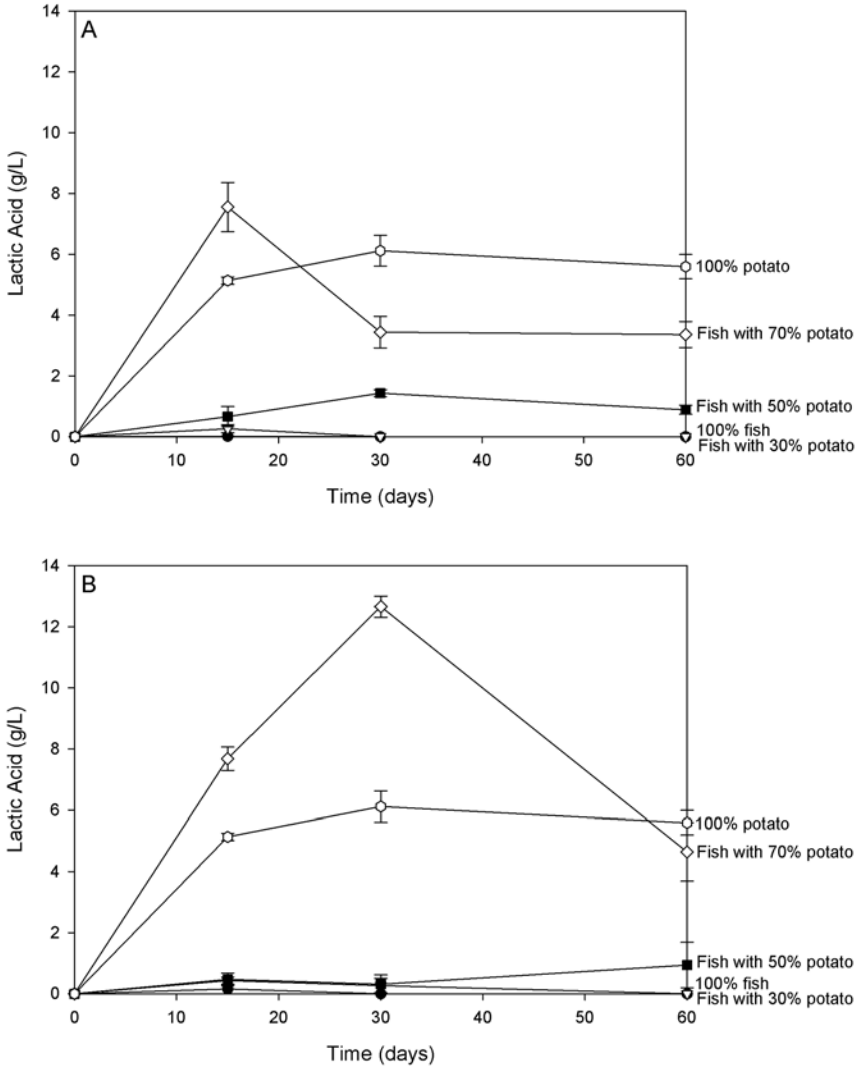

Figure 3. Lactic acid production during lactic acid bacteria fermentation over 60 days for (A) raw pink salmon heads, and (B) smoked pink salmon heads containing different percentages of potato as a carbohydrate source for the lactic acid bacteria inoculum.

Conclusion

Agricultural waste products represent a convenient source of carbohydrate to promote fermentation during silage production. Fish byproducts can be co-fermented with agriculture waste materials containing sufficient quantity of fermentable carbohydrate is present. Potatoes were able to decrease the pH of salmon byproducts, but would require the addition of sucrose or another LAB metabolizable carbohydrate to maintain a satisfactorily acidic pH during extended storage. However, other crop wastes, such as barley that is also grown in Alaska, may give better results, thereby allowing preservation of currently discarded high-protein fish byproducts.

Acknowledgment

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Montlake Process for Utilization of Salmon Processing Waste in Alaska

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Abstract

Short seasons and inconsistent volume of waste material make it difficult to use existing technology to recover salmon and other fish processing waste in many areas of Alaska. A new process was developed that combines wet reduction technology, acid stabilization, and drum drying of fish waste. Products are high protein–low ash meal, oil, and gelatin. Addition of 0.8% formic acid to deboned and semi-processed fish meat and viscera allows short-term storage at ambient temperatures. Stored acidified material can be dried when capacity is available, allowing more hours of operation that will accommodate smaller production lines. Muscle-containing fish cuts such as heads, trim, and frames are first mechanically deboned. The recovered muscle tissue is cooked and decanted to produce meal cake and oil. Raw wet viscera are ground and combined with the decanter cake to form a blended meal. The blended meal can be immediately dried on a drum dryer or stabilized with 0.8% formic acid and stored for later drying. The finished meal is high in protein and low in ash, and has good binding properties that allow a rugged feed particle to be extruded with no added gelatinized starch as is the case with conventional fish meal–based feeds. In a separate

processing line, a crude gelatin fraction is extracted from the bones by wet heat and straining. The crude gelatin is also dried on a drum dryer. Short-term storage of material reduces equipment requirements and increases working days for a given amount of material. This, in turn, allows the design of portable process units that can service more than one fishery and further extend operating days. The Montlake process and Montlake meal are named after NOAA's Northwest Fisheries Science Center Montlake lab in Seattle where the work was initiated and conducted.

Introduction

It is well established that fish protein and oil are nutritious and valuable feed commodities (Deuel et al. 1946, Folador 2006). These commodities are not recovered from waste produced from fish processed for human consumption in many areas of the world due to a lack of appropriate technology. A major unused resource is fish processing waste in Alaska. In the salmon canneries of Alaska, salmon production has traditionally been based on standard bone-in and skin-on canned products. The development of new markets is increasing the demand for more skinless/boneless (SB) frozen and canned salmon products. Demand for these new convenience salmon products has resulted in a substantial increase in the volume of material in waste streams discharged from the plants. Whereas traditional canned salmon results in waste of approximately 35% to 40% of the round fish weight, these new processes result in a waste of 50% and higher of the landed round weight due to more skin, trim, and frames in the waste stream. The increase of waste material from the expansion of SB production is estimated to add 20,000 to 30,000 t of salmon waste in the next three years. This is in addition to an annual average of over 100,000 t of salmon processing waste produced annually in Alaska. This added waste burden increases problems of meeting discharge limitations set by Environmental Protection Agency regulations in many salmon processing areas in Alaska.

The problem of remotely located processing plants, the brevity of the canning seasons, and the expense of waste processing technology have favored the current practices, i.e., grind and discharge to local waters. Recent developments in international commodities markets have seen prices for fish meals and oils increase substantially, which makes the case for utilizing Alaska's salmon processing waste as a recoverable commodity. As a result, there is a need to develop new process technologies for capturing salmon processing waste and turning it into profitable, high quality fish meal, oil, and specialty products for the marketplace. This project is a continuation of previous research based on the modified silage process at the Northwest Fisheries Science Center (NWFSC) (Stone and Hardy 1986, Nicklason et al. 2003). Other

workers have demonstrated that preservation and ensiling of fish waste can be controlled with temperature, acid type, and acid concentration (Tatterson 1982, Lo et al. 1993). The objectives of the Montlake process are threefold: (1) minimize use of imported chemicals, (2) support short term storage with limited autolysis, and (3) design a process that is economical, using existing technology.

Materials and methods

Raw material

Whole round late season male pink salmon (*Oncorhynchus gorbuscha*) from Prince William Sound, Alaska, were blast frozen, glazed, and delivered to Seattle cold storage in good condition. Heads of chum salmon (*Oncorhynchus keta)* obtained from Puget Sound, Washington, were frozen and held at NWFSC in good condition in cold storage until use.

Experimental design

Whole Montlake meal

Determination of low acid stabilization was done using whole ground pink salmon. The fish were thawed and ground through a 0.5 inch plate using a Hobart grinder (Model 4146, Troy). The ground fish was deboned in a Beehive deboner (Beehive Corp., Sandy) with progressive hole sizes of 2.0 mm, 1.0 mm, and 0.5 mm. The deboned tissue was blended with 50% formic acid to a final concentration of 0.8% formic acid to fish w/w. Three five gallon chemical buckets, containing approximately 16 kg of formic acid stabilized fish tissue each, were stored at 14ºC for three weeks. Temperature measurements and observations on the material were recorded for three weeks. A 25% solution of NaOH was mixed with the samples to a final concentration of 0.55% w/w at the conclusion of three weeks to neutralize the formic acid stabilized fish material. The material was dried on a double drum dryer with 20 cm diameter × 28 cm length drums using 80 PSIG (pounds per square inch gauge) steam to produce the final dried meal.

Blended Montlake meal

To produce a salable product, dried meal with a consistent composition derived from acid stabilized fish processing waste was combined with commercially available wet rendered fish meal to adjust the proximate composition of the final fish meal product.

Portions of the fish containing higher concentrations of lipid (primarily the head and trim) were first deboned and then wet rendered. Continuous cooking of deboned meat to >80ºC was done with a Votator scraped surface heat exchanger (Waukesha Cherry-Burrell, Delavan) using 80 PSIG steam. The cooked fish was decanted using a Sharples

P660 zonal centrifuge (Hutchinson-Hayes, Houston) at 2200 × G to produce a high protein cake, a liquid stream, and an oil stream. In the case of salmon where the eggs are removed from the raw viscera, the result is a low fat product. The rendered cake and raw viscera are combined and stabilized with 0.8% formic acid for storage (Fig. 2). A 40 kg sample was prepared that consisted of 60% raw viscera and 40% decanter cake. A 5 kg portion was held at 3ºC and the remaining was held at 11-13ºC. One hundred gram samples were taken daily and heated to >70ºC in a microwave oven. Two 20 g aliquots of the cooked material from each sample were centrifuged at 14,000 × G for five minutes and the supernatant was measured as weight percent of initial material. On day 8, 2 L samples of material at each temperature were stored under refrigeration and the remainder neutralized with NaOH and dried on the double drum dryer, as above.

Gelatin and chondroitin sulfate

Chum salmon heads were cooked in water for >30 min at 80ºC. Upon cooling, the heads were easily broken and the cartilage separated. Clean cartilage samples were weighed and freeze dried for later chondroitin sulfate analysis. Gelatin recovery was also quantified, identified by its 20ºC melting point and high glycine concentration. Application of wet heat, >80ºC for 1.5-3 hours, to deboner waste was sufficient to extract both gelatin and chondroitin as a liquid mixture. Addition of formic acid at 1.0%, 1.5%, and 2.0% w/w to deboner waste was done to measure the effect of pH on extraction. Bones and connective tissue from pink salmon heads were also used as raw material for gelatin extraction as above. Gelatin solutions were drum dried prior to analysis.

Analysis

Moisture and ash were determined according to standard methods (AOAC 1980). Protein was measured by determining nitrogen concentration in a 0.25 g sample by Dumas combustion methodology with a LECO FP-2000 nitrogen analyzer (LECO Corp., St. Joseph), and multiplying the result by 6.25. Fat content was determined by Soxhlet extraction. The Experiment Station Chemical Laboratory (ESCL) at the University of Missouri-Columbia performed amino acid analysis on the meals. Chondroitin sulfate was analyzed by Analytical Laboratories, Anaheim, as described by Ji et al. (2007). Private lab fees limited analysis to one replicate.

Results and discussion

One key goal of the Montlake process is to provide short-term storage of fish waste for better process control. Another is to limit liquefaction and autolysis common to acidified silage processes. The initial study

used 0.8% formic acid to stabilize whole deboned male pink salmon against microbial degradation and autolysis. The temperature during the three weeks of storage ranged from 12.8 to 13.5ºC. The experiment was terminated when the first visible mold colonies appeared. There was no apparent odor or visual indication of bacterial decomposition of the mixture during the three-week holding time. Liquefaction was limited and the mixture had a thick consistency. There was no separation of liquid or oil at the surface and no obvious settling of particulates to the bottom. The final pH was 4.8. Addition of 0.55% w/w NaOH raised the pH to 6.9. A pump was used to apply the final product to the drum dryer for a finished dry fish meal.

Whole deboned pink salmon meal that was stabilized with 0.8% formic acid and stored for seven days before being neutralized and dried was used in a preliminary feeding study with Hawaiian moi (*Polydactylus sexfilis*) at the Northwest Fisheries Science Center (NWFSC). Graded levels of Montlake meal or anchovy were fed, and although feeding response was not quantified the technician identified the Montlake meal blends by the active feeding response toward the salmon formulas (Gregory Oliver, University of Maryland Eastern Shore, 2008, pers. comm.). Other feed formulations have been developed with fish meal made from fish waste that has been stabilized with 1.1% to 0.8% formic acid. These have exhibited good acceptability and promoted reasonable fish growth (Nicklason et al. 2003; Frederic Barrows, U.S. Fish and Wildlife Service, Bozeman Fish Technology Center, Bozeman, Montana, pers. comm.; Matt Cook and Mark Tagal, NWFSC, Seattle, 2008, pers. comm.). Although a feeding trial has not been reported in the literature, these preliminary studies support the concept that the quality of the raw material can be maintained for up to three weeks by using 0.8% w/w formic acid and controlling the storage temperature. In the Alaska coastal environment ambient temperatures during the summer salmon harvest is below 20ºC where this storage strategy can be applied. This work was done with frozen fish; studies are needed to establish the effectiveness of this process with fresh unfrozen material.

Table 1 shows the proximate analysis and amino acid composition of the whole late season male pink salmon meal using the process outlined above. Amino acid composition of the meal reflects the composition of the starting material. A goal of this study was to construct a pilot process using a commercial equipment unit that could provide an alternative technology to the current practice of "grind and dump" as allowed by the Alaska Department of Environmental Conservation in almost any location. In the case of whole late season pink salmon, proximate composition of the resulting dried meal meets or exceeds the criteria for acceptable fish meal used in dietary formulations for aquacultured species. However, the volume of this material is not sufficient to support a recovery effort. The major salmon waste volume is heads,

Table 1. Proximate composition amino acid analysis of whole deboned pink salmon meal.

Proximate analysis	
Protein[ab]	78.8%
Fat[b]	8.8%
Ash[b]	12.9%
Amino acid analysis	
Taurine	0.57
Hydroxyproline	0.63
Aspartic acid	5.95
Threonine	2.59
Serine	2.22
Glutamic acid	8.19
Proline	2.83
Glycine	4.87
Alanine	4.02
Cysteine	0.54
Valine	3.29
Methionine	1.82
Isoleucine	2.85
Leucine	4.66
Tyrosine	2.26
Phenylalanine	2.45
Hydroxylysine	0.16
Ornithine	0.11
Lysine	4.99
Histidine	1.49
Arginine	4.57
Tryptophan	0.71

Amino acids are w/w percent = grams per 100 grams of sample "as received" with estimated 6.8% moisture).

[a]6.25 × nitrogen percent.

[b]Dry matter basis.

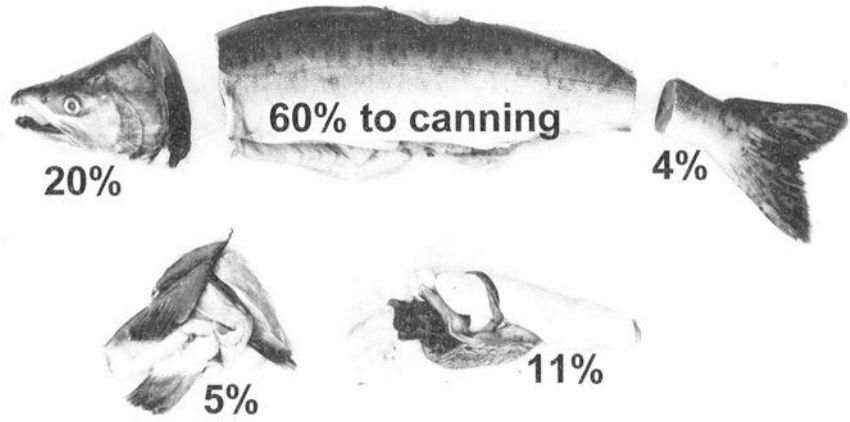

Figure 1. Estimated yields of portions of salmon prepared for traditional bone in and skin on canned salmon. Parts include head 20%, body 60%, tail 4%, trim 5%, and viscera 11%.

viscera, and trim. When skinless boneless product forms were being produced there would be more backbones, skins, and trim in the offal stream. Figure 1 shows a distribution of salmon parts from a canning operation of where approximately 60% is canned, 20% is the head, 10% is viscera without roe, and 10% trim. Deboning the mixed salmon waste stream and processing to meal would cause difficulty in controlling the resulting proximate composition. Additionally, salmon heads have a high oil content that may not be acceptable.

To solve these problems a hybrid process was developed that begins by rendering the high fat tissue from the head and trim to produce meal cake, stickwater, and oil. The low fat salmon viscera and other tissues can be combined with the meal cake to form a pumpable mixture that can be dried immediately, or the viscera and other low fat tissues mixed with formic acid and stored for drying at a later time. Figure 2 shows the mass balance of a hybrid process for the salmon waste values shown in Fig. 1. Deboning and rendering the head, and other muscle tissue from tails, trim, and backbones from filleting, reduces the volume of material to be dried, allows for oil recovery, and concentrates the protein as meal cake. A 40 kg batch of blended meal from pink salmon was produced from 60% viscera and 40% meal cake from heads. This blend was stabilized with 0.8% formic acid. Samples held at either an average temperatures of 3ºC or 12ºC were taken daily and centrifuged to measure liquefaction. The results are shown in Fig. 3 and the general trend, as shown by the polynomial fit to the data, is that the soluble fraction increases with time and temperature. This is consistent with

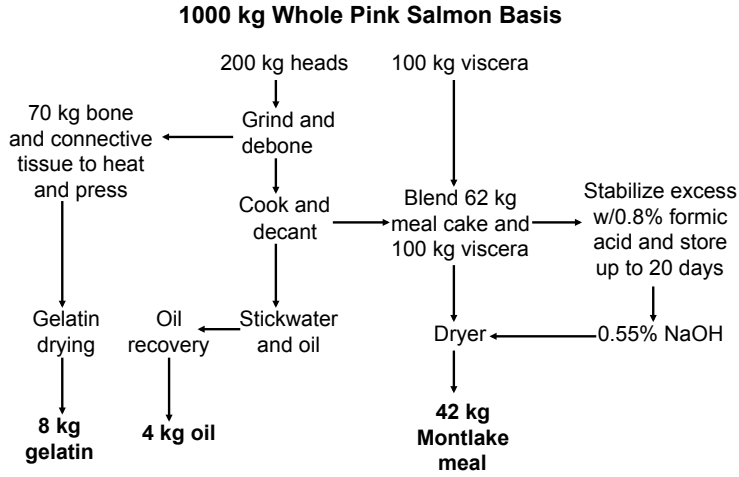

Figure 2. Estimated material flow for salmon cannery waste processing using the Montlake process.

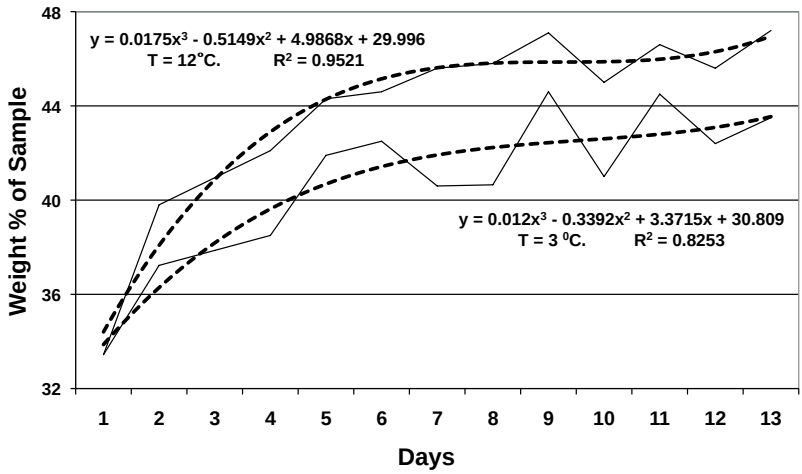

Figure 3. Supernatant from stabilized fish stored for different amounts of time at 3ºC and 12ºC. Supernatant used as an index for liquefaction during storage. See methods for assay details.

Table 2. Proximate composition and amino acid analysis of stabilized blended meal.

Proximate analysis	
Protein[ab]	77.1%
Fat[b]	9.9%
Ash[b]	8.7%
Amino acid analysis	
Taurine	0.69
Hydroxyproline	0.32
Aspartic acid	5.50
Threonine	2.57
Serine	2.52
Glutamic acid	7.29
Proline	2.47
Glycine	3.98
Alanine	3.55
Cysteine	0.57
Valine	3.32
Methionine	1.66
Isoleucine	2.74
Leucine	4.53
Tyrosine	2.03
Phenylalanine	2.26
Hydroxylysine	0.09
Ornithine	0.09
Lysine	4.92
Histidine	1.39
Arginine	6.68
Tryptophan	0.61
Percentage of protein digested by pepsin	92.40

Amino acids expressed as w/w percent = grams per 100 grams of sample on an "as is" basis with 5.8% moisture content.

[a]6.25 × nitrogen percent.

[b]Dry matter basis.

Table 3. Effect of formic acid concentration on liquid yield and percent solids recovered from heated chum salmon head deboner waste.[a]

Treatment	Liquid yield	Liquid solids
2.0% formic acid	26.0%	19.1%
1.5% formic acid	25.3%	19.0%
1.0% formic acid	18.9%	16.2%

[a]Liquid recovery enhanced by pressing with 23 kg weight. Yield is waste plus 20%.

the results of Tatterson (1982). In our study the solids content of the supernatant averaged 12.2% and 13% for 3ºC and 12ºC, respectively. On day 8, a large aliquot of blended meal held at 12ºC was neutralized with NaOH and dried. The proximate composition and amino acid analysis are shown in Table 2. Controlling the rate of liquefaction is important and increased temperature, time, and mixing will increase autolysis. Limited autolysis of fish offal has been demonstrated to produce better feed performance (Stone and Hardy 1986). In addition, the handling and drying of stabilized blended meal is more efficient with less liquefied and higher viscosity material.

In Alaska and other remote areas producing a bone meal from the deboning operation and drying of stickwater to produce high protein solids is prohibitive due to operational costs. At this time these materials would also be discharged as waste under the hybrid processing presented here. However, before discharging the bone, gelatin and oil can be extracted in the Montlake process. In conventional wet rendering, large bone fragments are often removed by screen separation of the dry finished meal. Standard fish bone meal has been cooked, pressed, and dried resulting in loss of gelatin into the stickwater stream (Bimbo 2000). To recover gelatin from chum salmon heads, deboner waste with 20% added water was heated to >80ºC overnight. Simple straining and draining yielded 30% of the initial bone waste weight as a gelatin-rich liquid whose solids content was 13%. When cooled to 4ºC this formed a semisolid gelatinous mass. To increase extraction the deboner waste was extracted with 1.0%, 1.5%, and 2.0% formic acid treatments. After 10 hours in a lab convection oven at 90ºC the samples were drained in a colander and weighed. Liquid yields were 29.3%, 35.8%, and 35.7% respectively for increasing acid amounts. In another test the same acid-treated material was heated to >90ºC for 1.5 hours. The samples were drained through a colander but this time a 23 kg weight was placed on each sample during draining. The yield of liquid and the percent solids in the liquid are shown in Table 3. Pressing cooked deboner waste also increased the yield. The liquid from pressing contained oil and some

Table 4. Liquid yields and composition of liquids and solids from pink salmon head deboner waste heated for two hours at 75°C with 20% added water and 1.5% formic acid based on bone waste weight.

Material	Solids	Protein[b]	Fat[b]	Ash[b]	% Yield[a]
Drained liquid	0.136	0.78	0.024	0.15	31.5%
Pressed yield	0.321	0.62	0.217	0.14	31.5%
Bones	0.446	0.46	0.133	0.346	37.0%

[a]Based on weight of sample divided by total weight of deboner waste and 20% added liquid.
[b]Dry matter basis.

Table 5. Proximate composition and amino acid analysis of pink salmon gelatin fraction.

Proximate analysis	
Protein[ab]	83.9%
Fat[b]	4.3%
Ash[b]	10.3%
Amino acid analysis	
Aspartic acid	6.48
Threonine	2.37
Serine	3.62
Glutamic acid	9.90
Proline	9.61
Glycine	22.00
Alanine	8.16
Valine	1.91
Methionine	2.22
Isoleucine	1.55
Leucine	2.74
Tyrosine	0.90
Phenylalanine	2.16
Lysine	3.51
Histidine	1.40
Arginine	7.45

Amino acids expressed as w/w percent = grams per 100 grams of sample, on an "as received" basis with an estimated 7% moisture content.
[a]6.25 × nitrogen percent.
[b]Dry matter basis.

Table 6. Analysis of dry salmon snout cartilage.

Free chondroitin sulfate (CS)[a]	10.9%
CS "A"	3.39%
CS "C"	6.36%
CS nonsulfated	1.11%
CS "D" (disulfated)	0.052%
CS "E" (disulfated)	ND
CS trisulfated	ND

Testing was performed at Analytical Laboratories, Anaheim, using HPLC-UV methodology.

[a]Sample tested as-is, with no protein hydrolysis.

ND = not detected

particulates. The oil and particulates must be decanted before the gelatin-rich liquid can be dried.

These extraction techniques were applied to pink salmon heads run through the deboner. The drained bone material (pressed recovery) was further strained through 2 mm holes in a food mill. Deboner waste yields and composition from pink salmon heads that was heated to 75ºC for 2 hours with 20% water and a 1.5% formic acid are shown in Table 4. The proximate and amino acid analysis of dried "pink salmon gelatin" product is shown in Table 5. The liquid gelatin stream from this source can be drum dried to a flake product. Sale of the dried gelatin-containing product as a functional additive to human, pet, or fish food manufacturers has the potential to enhance revenue. The protein and solids content of the liquid gelatin stream are also high enough to mix with the blended meal stream to increase protein meal yield.

Chondroitin sulfate, a carbohydrate polymer found in cartilage, forms a considerable portion of the head and skull cartilage of salmon. To examine recovery of head cartilage, chum salmon heads were cooked in hot water (80ºC) for >30 minutes. The cooked heads easily broke apart and the large pieces of cartilage in the snout and the skull were extracted. Cleaned cartilage accounted for 7.5% of the total head dry weight and total cartilage with attached skull bones was 14.7%. The solids content of the clean snout cartilage was 20.5% and solids content of bony skull was 29.7%. A sample of clean cartilage was freeze-dried before being sent for analysis. The chondroitin sulfate concentration was 10.9% on a dry weight basis with the majority being marine type C chondroitin sulfate as shown in Table 6. There is an existing market for salmon chondroitin sulfate, and recovery of the salmon cartilage containing significant concentrations of chondroitin sulfate is a potential separate income source for salmon processors.

Conclusion

The main feature of the Montlake process is combining wet rendering with low acid ensiling. This process results in lower chemical use, limited autolysis, and the ability for short-term storage of fish waste without microbial degradation. Less equipment is needed to process volumes of fish waste versus conventional wet rendering. The lower equipment requirements allow the Montlake processing equipment to fit into shipping containers that can operate in multiple locations and sequential fisheries to increase days of operation. Additionally, extraction of high quality meal, oil, and gelatin have the potential to maximize the value of these secondary materials. The end result is a new process option that can address recovery of valuable fish products in areas that cannot be served with existing technology.

Acknowledgments

This work was supported by the Alaska Fisheries Development Foundation. Dr. Frederic T. Barrows, U.S. Department of Agriculture, Agricultural Research Service, conducted preliminary feeding trials to establish acceptability and performance of Montlake meal–based feeds. The Montlake process and Montlake meal are named after the National Oceanic and Atmospheric Administration's Northwest Fisheries Science Center Montlake lab in Seattle, where the work was conducted.

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Hydrolysates from Scottish Salmon: Look before You Leap

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Abstract

Rossyew Ltd utilizes Scottish Atlantic salmon processing byproducts and converts them into nutritious omega-3 fatty acid–rich ingredients for the animal feed and pet food industries. A growing awareness of the importance of omega-3s in human nutrition has been mirrored in the poultry, pig, and ruminant sectors. Niche poultry market segments include healthier omega-3 enriched eggs and improved welfare of broiler chickens. Fertility improvements in sows and cows are becoming accepted and the biochemical mechanisms involved are better understood. Opportunities for fish hydrolysates are increasing, primarily in piglet and premium dry pet food diets. In particular, fish hydrolysate is highly palatable and ideal for pets with a sensitive dietary (allergenic) disposition.

Economical manufacturing techniques for a successful entry into these specialist markets are not for the faint hearted, as superb product quality, convincing trial data, and product application knowledge are major barriers to entry. Rossyew has implemented a successful salmon byproduct utilization strategy and is keen to cooperate in new commercial byproduct ventures wherever these may arise.

Introduction

This article looks at the advantages of fish hydrolysate manufacturing, as well as some of the pitfalls, and gives examples of product applications that the Scottish company, Rossyew Ltd, has developed from Scottish Atlantic salmon processing byproducts. Most fish byproducts are processed using the wet-reduction method into dried fish meal and liquid oil and these are traded internationally into various markets, particularly in the Far East. Wet-reduction fish meal in particular has the advantage of been easy to use in animal feed factories. Some of

these factories handle many hundreds of thousands of tons each year and are designed to efficiently handle powder or granule processing. However, other fish byproduct technologies exist including hydrolysis of the fish processing byproducts and subsequent separation into liquid protein and oil fractions, creating products with specific and functional applications.

Production of wet-reduction fish meal by cooking, pressing, and drying is a tried and tested process. Equipment is readily available and the technology well understood. The capital start-up cost is high because fish meal factories need to have sufficient drying capacity to cope with peak incoming demand. The amount of energy needed to cook and dry is also high and there is a risk of fishy malodors in pre-processing storage and during the cooking process.

By contrast fish hydrolysate production is less capital intensive, requires less energy input, and can dramatically reduce fishy odors. By mincing and acidifying the fish (called ensiling) prior to hydrolysis, the material is effectively "pickled in acid." As a result, fish byproduct at peak production can be stored and then processed over an extended period provided there are sufficient storage tanks and reduction equipment. This means that the hydrolysis process developed by Rossyew Ltd can be financially rewarding, even in locations where fish byproduct volumes are relatively modest.

Unlike wet-reduction, the processing equipment needed to make fish hydrolysates and oil is not available off the shelf, and our company has spent considerable time and money adapting alternative technologies to develop its proprietary process. The sales of fish hydrolysate into animal feed markets often depend on product application skills.

EC regulatory considerations

Within the European Commission, specific regulations govern the processing of fish byproduct manufacturing to ensure food safety and traceability. There is tough European legislation preventing *Salmonella* bacteria and undesirable pollutants (persistent organic pollutants, POPs) from entering the food chain. Fortunately the formic acid used in the ensiling and hydrolysis process is very efficient in killing any *Salmonella*, and our company has a proprietary micro-filtration system designed for the removal of PCBs and dioxins from the oil.

Rossyew's efforts start at the salmon processor where byproducts are macerated to a thick soup consistency and sufficient formic acid is added to reduce the pH to less than 4, thereby pickling and preserving the fish. Bulk tankers collect the ensiled material and deliver it to a dedicated site in Greenock, on the west coast of Scotland. In an overview of the process at our Greenock factory, the material undergoes hydrolysis at optimal pH, temperature, and time and then is fractionated by

centrifugation into salmon protein and oil fractions. The process has the advantage of using the endogenous enzymes present within the fish tissues, so no further enzyme addition is required. The dilute protein hydrolysate is then vacuum evaporated at low temperature into a thick syrupy consistency. Because the pH of the hydrolysate is still less than 4 it remains stable for up to one year. Rossyew salmon oil undergoes an activated carbon micro-filtration process to minimize dioxins and PCBs, giving additional assurance to our customers of European Commission legislative compliance.

Fish oil—in the media spotlight

Of the two end products, fish oil is probably the best known as a health supplement and has gained the most news media attention. In August 2009, the UK *Daily Express* newspaper had a front page headline that proclaimed, "Omega-3 is the secret of a long life," reporting a compelling American study showing that regular fish oil consumption can reduce cardiovascular death by 30% (Gloger 2009, Lavie et al. 2009).

Fish oil is an excellent source of the long chain omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). In humans, it is becoming clear that too much saturated fat and omega-6 fatty acids in the diet are correlated with many of the "life style" diseases. In addition to reducing both heart disease and arthritis, several inflammatory gut disorders are also ameliorated by the omega-3 fatty acids in fish oil (Yoshikawa et al. 2000).

The increasing importance of long chain omega-3 fatty acid supplementation for human health is mirrored in an increasing awareness of their benefit in feeds designed for intensively raised livestock as well as in premium pet foods. Premature death from heart disease is not the issue for livestock, but ill health and sub-optimal growth during early rearing certainly is. Rossyew has collaborated with the Roslin Institute in Scotland on a 3 year study into improving broiler chicken leg strength and general welfare. Chickens fed diets high in omega-6 fatty acids were shown to have weaker legs than those from broilers raised on salmon oil–enriched diets. The chickens fed salmon oil also had significantly improved feed conversion ratios (Whitehead 2007). Laying chickens are also efficient at transferring omega-3 fatty acids from their diet into their eggs, enabling the production of so called "omega-3 enriched" eggs (Moghadasian 2008). These enriched eggs have no fishy taste and appeal to a segment of the public averse to eating fish regularly.

Fish oil also improves pig and cow fertility. Fish oil has been shown to ameliorate age-related sperm number decline in older boars. Pregnant sows fed salmon oil at a 1% level show a significant increase in the numbers of live piglets reared (Rooke et al. 2003). Intensification has reduced fertility rates in high-yielding dairy cows. The problem is acute because

unless a cow becomes pregnant and gives birth, no milk is produced the following year and the animal has to be culled. Santos et al. (2004) reported that in high yielding dairy cows some 40% or more of viable embryos are lost within the first 3 weeks of pregnancy. This is because the cow uterus fails to biochemically recognize pregnancy, a condition called "early embryo loss." Pro-inflammatory omega-6 and anti-inflammatory omega-3 fatty acids have an opposing effect because they are precursor molecules of pro- and anti-inflammatory prostaglandin hormones, respectively. When the cow's dietary fats are adjusted, the prostaglandin hormone levels are rebalanced and the cow's biochemical recognition of pregnancy is improved, and more embryos survive to full term (Thatcher et al. 2004).

Fish hydrolysate—more than protein

The large fish proteins are cleaved during hydrolysis, into shorter proteins, peptides, and amino acids. These are in effect pre-digested proteins; therefore they are potentially more easily absorbed in the digestive tract. In addition, some of these peptides may have additional functional effects such as improving palatability and modifying metabolic regulation. Young piglets in particular benefit from the inclusion of a small amount of fish hydrolysate in the diet. Feed intake is improved, leading to improved growth rates and feed efficiency (I.D. Wright, pers. observation).

Fish hydrolysate also makes an ideal ingredient in premium dog and cat foods. The move to dry extruded diets is more convenient than raw or home-cooked pet foods; however, this move has led to an increase in allergic diseases (Quintavalla et al. 2002). Dogs in particular can become allergic to wheat and dried meat products, resulting in an irritable digestion system and flaky skin. By replacing wheat with dried potato or ground rice and substituting meat with salmon hydrolysate, tasty premium "hypoallergenic" diets can be manufactured.

It is known that fish hydrolysates contain certain bioactive peptides that can be considered medicinal (Yoshikawa et al. 2000). The proteolytic hydrolysis of larger proteins creates smaller protein fragments or peptides. Some of these peptides have the same composition and structure as naturally occurring peptide hormones such as calcitonin and gastrin. Methods are available for the separation of theses peptides from other proteins and protein fragments. No doubt other peptide hormones are also present and are waiting to be identified.

Turning hydrolysate potential into economic success?

Conversion of fish byproducts into high quality hydrolysates and oil holds great potential, but to realize this potential is not without difficulties. In developing the process on a green field site in Scotland, Rossyew discovered just how much money, time, and fortitude is needed to make the hydrolysate process economical and efficient. Not least are the engineering requirements for automation and efficient extraction, both of which can be real barriers to profitability. In addition, animal feeding trials needed to gain entry into niche animal feed applications and premium pet food sectors took longer and cost far more than initially anticipated. Having successfully implemented a Scottish salmon byproduct utilization strategy, Rossyew Ltd is keen to share its technology with processing partners in parts of the world where salmon, or other fish processing byproducts are available.

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The Chemical Composition and Oxidative Stability of Alaska Commercial Salmon Oils

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Abstract

A number of different bulk and encapsulated commercial salmon oil samples from Alaska were chemically characterized to determine their composition and oxidative stability. Compositional analysis included the determination of fatty acid profiles, distribution of lipid classes, fat-soluble vitamin content, and levels of environmental contaminants. Oxidative stability was determined by measuring free fatty acid values, peroxide values, p-anisidine values, and 2-thiobarbituric acid values. Overall, results indicate that salmon oils commercially produced in Alaska were primarily composed of triacylglycerides, being rich in long chain omega-3 fatty acids and in the fat soluble vitamins A, D, and E. Moreover, the level of the 38 environmental contaminants analyzed was either low or below detectable limits. Oxidative stability analysis of most samples found levels of free fatty acids, peroxide values, and

p-anisidine values were below limits established for human grade and nutraceutical grade fish oils.

Introduction

Over the past decades research reporting on the importance of omega-3 fatty acids in human nutrition has flourished. Hoffman and Uauy (1992) reported the essentiality of DHA addition to preterm infant formula to ensure proper brain development and retina formation. Logas et al. (1992) pointed out clinical benefits of marine oils and particularly omega-3 fatty acids in the treatment of diseases such as hyperlipidemia, autoimmune disease, arthritis, cancer, and thromboses. Additionally, many independent studies have shown that increasing the daily intake of dietary long chain omega-3 fatty acids, while reducing the intake of omega-6 fatty acids, decreases the risk of coronary heart disease (Nettleton 1987, Sargent and Henderson 1995, Ruxton et al. 2005, Harris et al. 2008). As pointed out by Ackman (1989), fish oils are generally rich in long chain omega-3 fatty acids, specifically 20:5ω3 (eicosapentaenoic acid, EPA) and 22:6ω3 (docosahexaenoic acid, DHA). Among fish oils, salmon oil is of great interest regarding human nutrition due to its high ratio of DHA to EPA (Oliveira and Bechtel 2005, Oliveira et al. 2008).

Alaska is the largest producer of wild salmon in the world (Knapp 2008). From 2000 to 2004, Alaska's wild salmon harvest averaged 340,000 t (ADFG 2007). Crapo and Bechtel (2003) estimated that in 2000, about 90,000 t of salmon byproducts were generated in Alaska. On a six-year average (2002-2007), Alaska produced 3,447 t sockeye salmon oil annually (Bimbo 2009). Despite the evident growth in the nutraceutical market for fish oils, few published data exist on the composition of Alaska salmon oil (Bimbo 2009). It is important to determine quality parameters and identity standards for salmon oil produced in Alaska so these products can be more competitive globally. Furthermore, identity standards will ensure that Alaska salmon oil products have consistent quality and composition (Bimbo 2009).

Quality standards for human grade fish oils include the assessment of lipid oxidation products, and this is especially important for oils having high levels of polyunsaturated fatty acids such as salmon oil. Lipid oxidation products not only impart unpleasant tastes and smells to the oils but they can also exert cytotoxic and genotoxic effects (Halliwell and Chirico 1993, Esterbauer 1993). Ingestion of lipid oxidation products may cause low-density lipoprotein cytotoxicity (Morel et al. 1983), atherosclerosis (Kubow 1993), and liver enlargement indicating nutrition-induced toxicity (Nwanguma et al. 1998). It is important to monitor the quality and oxidative stability of fish oils and to prevent product oxidation (Shahidi 2007). This survey was conducted to determine the chemical composition and oxidative stability of nine commercial bulk and encapsulated Alaska salmon oil samples.

Table 1. Information and codes for Alaska salmon oil samples.

Sample code	Type of oil	Sample age at the time of analyses (years)	Procurement year	Expiration year	Year analysis was conducted
BSK0	Bulk sockeye oil	0	2009	na	2009
ESK0	Encapsulated sockeye oil	0	2009	2013	2009
ESK2	Encapsulated sockeye oil	2	2006	2008	2008[a]
ESK3	Encapsulated sockeye oil	3	2006	2008	2009[b]
ES0	Encapsulated Alaska salmon oil (species unknown)	0	2009	2010	2009
BS0	Bulk Alaska salmon oil (species unknown)	0	2008	na	2008
BS1	Bulk Alaska salmon oil (species unknown)	1	2008	na	2009
B1S1	Bulk Alaska salmon oil (species unknown)	1	2007	na	2008
B1S2	Bulk Alaska salmon oil (species unknown)	2	2007	na	2009

na = information not available.
[a]Analysis conducted immediately before expiration date.
[b]Analysis conducted after expiration date.

Materials and methods

Sampling and chemical analyses

Samples of salmon oil were procured from Alaska salmon processors as indicated in Table 1. Information regarding sample identities and time of analyses can also be found in Table 1. Five species of Pacific salmon, all in the genus *Oncorhynchus*, are commercially harvested in Alaska. The scientific names are clear, but there are different common names for the species in Alaska and Canada and most of the United States follows the Canadian scheme. King (Canadian: chinook or spring) salmon are *Oncorhynchus tshawytscha*. Silver (Canadian: coho) salmon are *O. kisutch*. Red (Canadian: sockeye) salmon are *O. nerka*. Dog (Canadian: chum) salmon are *O. keta.* Pink (Canadian: humpy) salmon are *O. gorbuscha*. Salmon oils in Alaska can be derived from any combination of these species unless the source is explicitly identified.

Chemical analysis conducted included the determination of fatty acid profiles, distribution of lipid classes, quantification of fat-soluble vitamins, and abundance of environmental contaminants. The degree of lipid oxidation was estimated by determining peroxide values, thiobarbituric acid (TBA) values, and p-anisidine values (p-AV). An important quality parameter determined was the abundance of free fatty acids.

Sample results were reported as the averages of either duplicate or triplicate determinations for each analysis.

Analysis of lipid classes

An Iatroscan TLC/FID analyzer model MK-6s (Iatron Laboratories Inc., Tokyo) was used to determine the abundance of triacylglycerols, 1,2-diacylglycerols, monoacylglycerols, free fatty acids (FFA), 1,3-diacylglycerols, sterols, and phospholipids in the oil samples. Materials and methods used for lipid class analysis were adapted from Whitsett et al. (1986), Parrish (1987), and Ackman et al. (1990) as previously described (Oliveira and Bechtel 2006). Lipid classes were reported as percent triacylglycerols, 1,2-diacylglycerols, monoacylglycerols, free fatty acids, phospholipids, and the combined percentages of sterols and 1,3-diacylglycerols (these two lipid classes coeluted in this system).

Fatty acid profiles

Fatty acid methyl esters (FAME) were prepared using KOH and methanol as described by Maxwell and Marmer (1983). FAMEs were transferred into 1.5 ml snap-cap amber GC vials (Agilent Technologies, Wilmington) and immediately analyzed. Fatty acid profiles were determined with a GC model 6850 (Agilent Technologies) fitted with a DB-23 (60 m × 0.25 mm i.d., 0.25 µm film) capillary column (Agilent Technologies). An autosampler performed the GC injections and injection volume was 1 µl. The chromatographic conditions were described by Bechtel and Oliveira (2006).

Fat-soluble vitamin content

Quantification of the fat soluble vitamins A, D, and E was contracted out to Warren Analytical Laboratories, Inc. (Greeley). Salmon oil samples with codes B1S1 and BS0 were sent to Warren Laboratories during summer 2008, while samples with codes BSK0, ES0, and ESK0 were sent to that same laboratory during summer 2009. The methods used for analyses were NOVIT 3.1 (Kraft USA Analytical Test Procedure), AOAC Official Method 995.05 (AOAC 1994) and the Journal of Lipid Research 24652656 (Kayden et al. 1983) for vitamins A, D, and E, respectively.

Environmental contaminant analyses

Quantification of 38 environmental contaminants was contracted out to Minnesota Valley Testing Laboratories, Inc. (MVTL, New Ulm). Salmon oil samples with codes B1S1 and BS0 were sent to MVTL during summer 2008, while samples with codes BSK0, ES0, and ESK0 were sent to that same laboratory during summer 2009. The official method EPA 8082/3545 was used for the analysis (U.S. EPA 2007a,b).

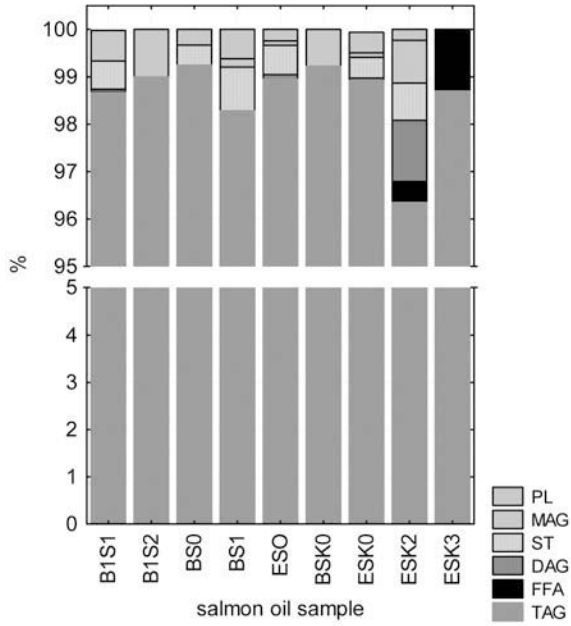

Figure 1. The lipid classes (percent) in Alaska salmon oil samples. PL = phospholipids; MAG = monoacylglycerols; ST = sterols; DAG = diacylglycerides; FFA = free fatty acids; TAG = triacylglycerides.

Rancidity and oxidative stability analyses

The free fatty acid values reported as percent oleic acid), peroxide values, p-anisidine values (p-AV), and the 2-thiobarbituric acid values (TBA) were determined using AOCS official methods Ca 5a-40, Cd 8-53, Cd 18-90, and Cd 19-90, respectively (Firestone 2004).

Results and discussion

Lipid classes

Figure 1 shows the levels of different lipid classes in salmon oil samples. Results showed that the amount of triacylglycerols, the most abundant lipid class in all samples, ranged from 98% to 99%, except ESK2 which was lower at 96%. Garcia et al. (2005) stated that triacylglycerols are the main components of lipid depots in animal cells, and triacylglycerols are also known to serve as the most concentrated form of energy storage (Zhong et al. 2007). Salmon accumulates large amounts of lipid in the head, which is the main source of salmon fish oil. Phospholipids

Table 2. Fatty acid profiles (mg per g oil) of bulk and encapsulated Alaska salmon oil samples.

Fatty Acids	B1S1	B1S2	BS0	BS1	ES0	BSK0	ESK0	ESK2	ESK3
14:0	37.63	35.23	44.82	39.35	41.78	36.84	29.85	37.64	39.00
16:0	104.27	98.20	116.83	103.10	110.60	141.78	98.95	108.88	112.56
16:1ω7	30.54	28.65	37.25	32.71	32.79	46.89	36.41	42.03	41.98
18:0	17.13	16.53	24.03	21.16	19.80	21.61	16.01	21.10	22.94
18:1ω9 *cis*	94.87	89.81	119.80	106.06	98.14	151.72	117.75	142.30	150.35
18:1ω7	17.65	16.74	18.29	16.24	16.92	36.48	24.67	28.35	28.42
18:2ω6 *cis*	13.84	13.23	13.36	11.87	14.44	15.46	11.90	12.31	12.26
18:3ω3	10.82	10.37	12.32	11.09	13.92	10.06	7.07	9.48	9.53
18:4ω3	29.01	27.78	23.16	20.60	0.53	0.78	13.59	16.78	0.66
20:1ω11	63.57	60.83	42.43	38.11	54.80	63.51	65.74	51.02	50.63
20:1ω9	33.18	31.62	21.42	19.15	29.42	33.10	32.85	24.11	23.81
20:4ω3	15.08	14.50	15.63	13.93	18.15	12.94	9.24	11.29	11.34
20:5ω3 EPA	73.38	70.38	92.68	82.34	84.77	90.28	64.81	75.15	73.32
22:1ω11	112.56	107.46	57.86	51.62	49.95	70.18	69.08	49.95	48.62
22:1ω9	11.64	11.03	13.32	11.83	55.57	9.38	8.41	9.32	9.10
22:5ω3	17.29	16.85	24.19	21.79	21.00	20.00	13.87	16.81	16.59
22:6ω3 DHA	88.99	86.08	99.22	88.68	101.94	73.63	62.69	76.13	73.92
24:1ω9	10.27	9.88	6.18	5.56	9.93	6.61	6.32	5.23	5.32
DHA/EPA	1.21	1.22	1.07	1.08	1.20	0.82	0.97	1.01	1.01
Label claim for EPA	NA	NA	NA	NA	80-110	NA	80	80	80
Label claim for DHA	NA	NA	NA	NA	90-120	NA	70	70	70
Label claim for ω-3 FA	NA	NA	NA	NA	250-340	NA	200	200	200

If a given FA value was less than 10 mg per g oil for all samples, the FA was omitted from this table. NA = information not available.

are the major component of cell membranes (Zhong et al. 2007), and are present in the salmon oil samples at a level below 1%. It is noteworthy that the levels of free fatty acids determined for ESK3 was high at about 1.3%. Also, the majority of the sterols found in fish oil will be in the form of cholesterol, which was found at relatively low levels in all salmon oils investigated. Other components such as diacylglycerols and monoacylglycerols were present at low levels in the salmon oil samples indicating little breakdown of the triacylglycerols.

Fatty acid profiles

The fatty acid profiles of the samples are shown in Table 2. Among the saturated fatty acids, palmitic acid (16:0) was found to be the most abundant in all of the salmon oil samples, with BS0 having the highest and B1S2 the lowest levels. On the other hand, oleic acid (18:1ω9 *cis*) was the most abundant monounsaturated fatty acid, except in the samples B1S1 and B1S2. In these two samples, cetoleic acid (22:1ω11) was the most abundant monounsaturated fatty acid. This difference likely can be attributed to the differences in the salmon feeding regime, since cetoleic acid, as well as gadoleic acid (20:1ω11), are exogenous in origin (Ackman 1999); hence their concentration reflects abundance in their diets. The two most abundant polyunsaturated fatty acids were the long chain omega-3 fatty acids, eicosapentaenoic acid (EPA, 20:5ω3), and docosahexaenoic acid (DHA, 22:6ω3). DHA to EPA ratios were similar for similar samples regardless of the age of the fish oil; the ratios were found to be ≥1 in all samples except BSK0 and ESK0. The ratio of DHA to EPA of B1S1, B1S2, BS1, and BS0 were similar to that found by Wu and Bechtel (2008) for pink salmon byproduct oil (1.22) but slightly lower than those reported by Oliveira and Bechtel (2005) for pink salmon head and viscera oil (1.56 and 1.58, respectively). It is noteworthy to point out that in the work of Oliveira and Bechtel (2005) the lipids were extracted from raw tissues in a laboratory using solvent extraction methods, which generally yield higher lipid recoveries compared to industrial fish oil rendering processes. Most important is the fact that solvent extraction methods, combining both polar and apolar solvents, tend to provide significantly higher recovery of polar lipids such as phospholipids. Phospholipids are richer in long-chain omega-3 fatty acids such as DHA and EPA than neutral lipids such as triacylglycerols. Therefore, it is not surprising that Oliveira and Bechtel (2005) report higher DHA to EPA ratios for pink salmon byproducts than the values determined for salmon oils investigated during this survey. The results also indicated that the label claims for the capsules analyzed were accurate except for ESK0, where EPA, DHA, and total omega-3 contents were below the label claims.

Table 3 shows the summary of the fatty acid profile analyses of the nine salmon oil samples. Monounsaturated fatty acids were the major

Table 3. Summary of results of fatty acid profiles (mg/g oil) of Alaska salmon oil samples.

Fatty acids	B1S1	B1S2	BS0	BS1	ES0	BSK0	ESK0	ESK2	ESK3
Total known FA	853.60	817.78	856.66	766.89	864.80	917.92	745.98	799.51	796.47
Total unknown FA	7.01	17.31	9.98	13.63	56.88	37.54	14.68	6.58	33.49
Σ SAT FA (S)	177.72	168.99	206.22	183.58	194.39	220.56	158.48	182.47	195.19
Σ MUFA	408.32	388.71	348.98	311.95	390.57	452.46	387.61	382.48	382.96
Σ PUFA (P)	267.56	260.09	301.46	271.35	279.84	244.90	199.88	234.56	218.32
Σ ω-3	236.35	227.93	269.35	240.57	242.80	209.45	172.96	207.29	187.23
Σ ω-6	31.21	32.16	32.11	30.78	35.36	33.74	26.92	27.27	29.71
P/S	1.51	1.54	1.46	1.48	1.44	1.11	1.26	1.29	1.12
ω-3/ω-6	7.57	7.09	8.39	7.82	6.87	6.21	6.42	7.60	6.30

SAT = saturated; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acids.

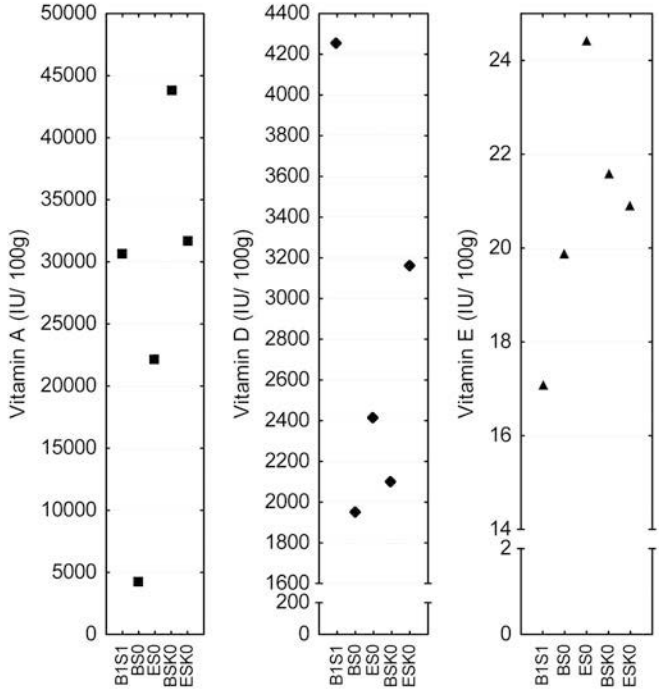

Figure 2. Fat-soluble vitamin contents in Alaska salmon oil samples.

fraction accounting for 40-51% of the fatty acids, and polyunsaturated fatty acids were the second-most abundant class, followed by saturated fatty acids. Omega-3 to omega-6 ratios of the salmon oil samples were high, similar to that found in pink salmon viscera oil (7.74) (Oliveira and Bechtel 2005). Katz and Nettleton (2003) suggested a recommended daily intake (RDI) of 650 mg of omega-3 fatty acids. Considering the abundance of the omega-3 fatty acids in the encapsulated salmon oils, it will take about three capsules of 1,000 mg each to meet the 650 mg target if the salmon oil capsules are to be the sole sources of omega-3 fatty acids in the daily diet. The samples ESK0 and ES0 would require a dose of 2 capsules, while ESK2 and ESK3 would require a dose of 1 capsule.

Fat-soluble vitamins and environmental contaminants

Fat-soluble vitamin levels are shown in Fig. 2. Results showed that B1S1, which was aged for one year, had very high vitamin A content compared with the values determined for BS0, a fresh sample. This variation could

be attributed to differences in the raw material, because these oils were produced in different years. There are no thorough studies of the vitamin variation during a season, between seasons, or due to other factors such as the fish diet composition. Wu and Bechtel (2009) also recorded a significant increase in vitamin A concentrations in the lipid fraction of aging walleye pollock (*Theragra chalcogramma*) byproducts. On the other hand, BSK0 had a higher vitamin A level than ESK0 and there is little basis on which explain the observed difference, except for potentially intrinsic differences that may occur in the composition of the raw material from season to season. There were also some variations in concentration observed in the vitamin D levels, while the range of vitamin E levels was less than for either vitamin A or D. There is a growing interest in vitamin D rich foods due to studies showing that people living in areas where winter is accompanied by light deprivation do not ingest sufficient levels of this fat soluble vitamin. In this context, salmon oil has an added nutraceutical benefit as a natural source of vitamin D.

Environmental contaminants

All salmon oil samples had similar contaminant levels, which were at or below the laboratory detection level. This included organophosphates, chlorinated hydrocarbons, and polychlorinated biphenyls as reported by the commercial laboratory (Table 4).

Rancidity and oxidative stability

Free fatty acid values (FFA)

Free fatty acid values are used as an indicator for rancidity in edible oils. Results of FFA value analyses (percent oleic acid) determined for the nine salmon oil samples are presented in Fig. 3. The highest FFA values were obtained from aged encapsulated sockeye oil samples (ESK2 and ESK3). The remaining salmon oil samples had FFA values of <1%, which is below the recommended level of 1-7% for food grade fish oils (Bimbo 2009). Bimbo (2009) reported that the standards from Global Organization for EPA and DHA omega-3s for acid values (GOED-AV) for nutraceutical grade fish oils has a maximum of 3. Converting the results of FFA from this study to acid values (GOED-AV) by multiplying the FFA values by 1.99, results in values for ESK2 and ESK3 over the limit of 3; however, one must take into consideration that these capsules were past their expiration date.

Peroxide values

Peroxide values reflect the abundance of the primary products of lipid oxidation. Peroxide values determined for the nine salmon oil samples are shown in Fig. 4. Bulk salmon oils and bulk sockeye salmon oil samples had concentrations in the range of 6-10 meq per kg while

Table 4. Environmental contaminant levels (ppm) of salmon oil samples surveyed in Alaska.

Contaminant	B1S1	BS0	ES0	BSK0	ESK0
Diazinon	nd	nd	<0.14	<0.14	nd
Ethion	nd	nd	<0.14	<0.14	nd
Malathion	nd	nd	<0.14	<0.14	nd
Methyl parathion	nd	nd	<0.14	<0.14	nd
Parathion	nd	nd	<0.12	<0.12	nd
Ronnel	nd	nd	<0.13	<0.13	nd
Carbophenothion (Trithion)	nd	nd	<0.15	<0.15	nd
Disulfoton	nd	nd	<0.15	<0.15	nd
Phorate (Thimet)	nd	nd	<0.15	<0.15	nd
PCB-1016	<0.05	<0.05	<0.05	<0.05	<0.05
PCB-1221	<0.05	<0.05	<0.05	<0.05	<0.05
PCB-1232	<0.05	<0.05	<0.05	<0.05	<0.05
PCB-1242	<0.05	<0.05	<0.05	<0.05	<0.05
PCB-1248	<0.05	<0.05	<0.05	<0.05	<0.05
PCB-1254	<0.05	<0.05	<0.05	<0.05	<0.05
PCB-1260	<0.05	<0.05	<0.05	<0.05	<0.05
Aldrin	<0.01	<0.01	<0.01	<0.01	<0.01
Alpha-BHC	<0.01	<0.01	<0.01	<0.01	<0.01
2,4'-DDD (O, P-DDD)	<0.02	<0.02	<0.02	<0.02	<0.02
4,4'-DDD	<0.02	<0.02	<0.02	<0.02	<0.02
2,4'-DDE (O, P-DDE)	<0.02	<0.02	<0.02	<0.02	<0.02
4,4'- DDE	<0.02	<0.02	<0.02	<0.02	<0.02
2,4'-DDT (O, P-DDT)	<0.03	<0.03	<0.03	<0.03	<0.03
4,4'-DDT	<0.03	<0.03	<0.03	<0.03	<0.03
Dieldrin	<0.02	<0.02	<0.02	<0.03	<0.02
Endrin	<0.02	<0.02	<0.02	<0.03	<0.02
Heptachlor	<0.01	<0.01	<0.01	<0.01	<0.01
Heptachlor epoxide	<0.01	<0.01	<0.01	<0.01	<0.01
Hexachlorobenzene	<0.01	<0.01	<0.01	<0.01	<0.01
Gamma-BHC (Lindane)	<0.01	<0.01	<0.01	<0.01	<0.01
Methoxychlor	<0.05	<0.05	<0.05	<0.05	<0.05
Mirex	<0.01	<0.01	<0.01	<0.01	<0.01
Toxaphene	<0.10	<0.10	<0.10	<0.10	<0.10
Endosulfan (Thiodan)	<0.02	<0.02	<0.02	<0.02	<0.02
Beta-BHC	<0.01	<0.01	<0.01	<0.01	<0.01
Delta-BHC	<0.01	<0.01	<0.01	<0.01	<0.01
Alpha chlordane	<0.01	<0.01	<0.01	<0.01	<0.01
Gamma chlordane	<0.01	<0.01	<0.01	<0.01	<0.01

nd = not determined.

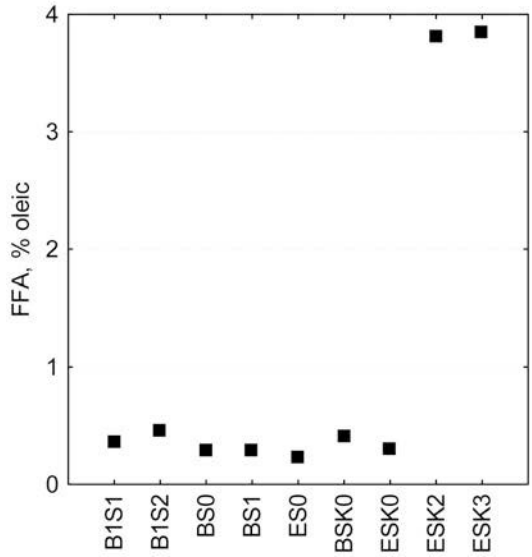

Figure 3. Free fatty acid values of Alaska salmon oil samples. FFA data expressed as percent oleic acid.

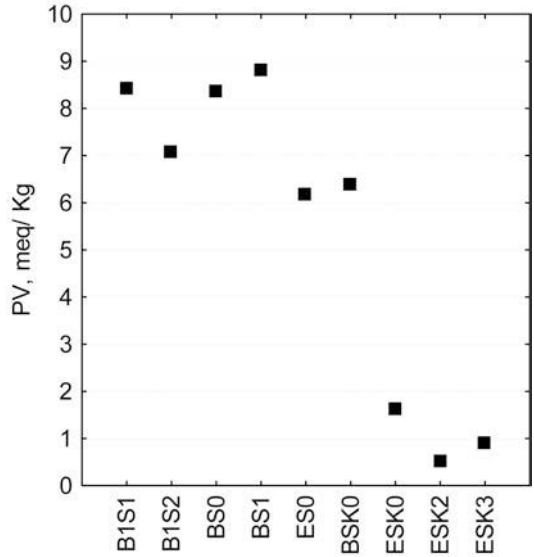

Figure 4. Peroxide values of Alaska salmon oil samples, expressed as meq per kg oil.

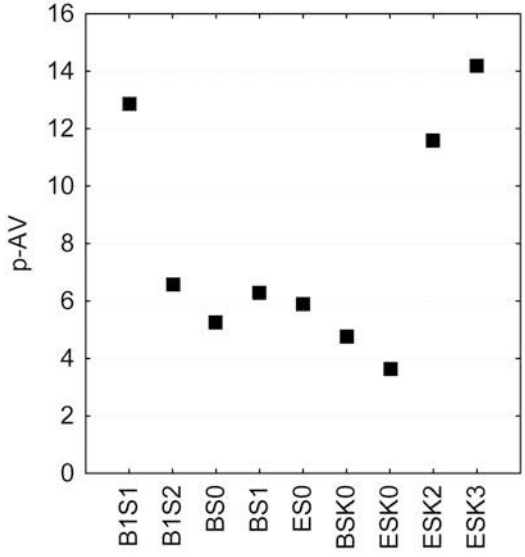

Figure 5. The p-anisidine values of Alaska salmon oil samples. See methods for p-anisidine determination.

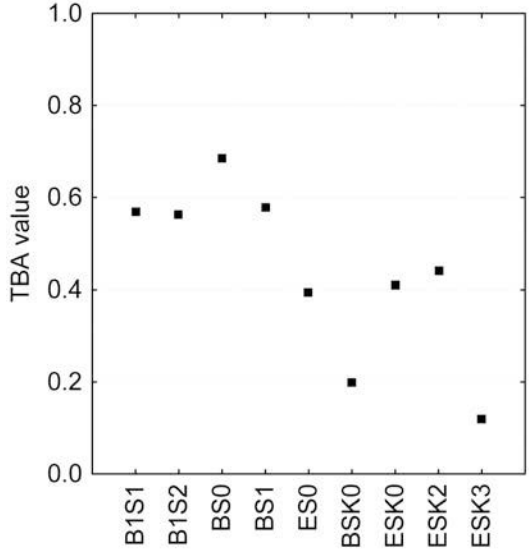

Figure 6. Thiobarbituric acid values of Alaska salmon oil samples. See methods for TBA determination.

encapsulated sockeye salmon oil had values <2 meq per kg. All salmon oil samples were within the recommended range for food grade fish oils of 3 to 20 meq per kg (Bimbo 2009). Note that the recommended peroxide value for nutraceutical grade fish oils is 5 meq per kg maximum (Bimbo 2009).

p-Anisidine values (p-AV)

Results of the p-anisidine analyses are shown in Fig. 5. The p-AV in ESK3 was found to be the highest; this is not surprising because this sample had been 3 years in storage. The p-AV determined for ESK0 was lowest. Overall the p-AV for these samples were within the recommended levels for food grade fish oils (p-AV = 4-60) as well as for nutraceutical grade fish oils (p-AV max = 20) (Bimbo 2009).

2-thiobarbituric acid (TBA) values

This method measures the secondary products of lipid oxidation. Results of TBA analysis are shown in Fig. 6. Note that salmon oil samples had low TBA values in a narrow range, results that are not consistent with the age differences between the samples. The TBA values determined for walleye pollock crude oils were 16 µg MDA (malonaldehyde) per g crude oil (Wu and Bechtel 2009), 63 mg MDA per kg oil for pink salmon head oil and about 12 mg MDA per kg oil for smoked pink salmon head oil (Bower et al. 2009). It is difficult to compare TBA values with data in the literature due to the many methods used to measure this parameter; moreover, there is a lack of any guidelines or established standards for TBA values for feed or food grade fish oils.

Conclusion

Surveyed salmon oil samples were rich in omega-3s fatty acid, and had a DHA to EPA ratio higher than 1. Both of these characteristics are desirable in fish oils from a nutraceutical point of view. Small differences in fatty acid profiles between bulk and encapsulated oils were detected. Contaminant analyses showed levels of persistent organic pollutants to be below detection levels, suggesting that salmon oils recovered from wild Alaska salmon do not require removal of harmful compounds such as PCBs and dioxins. Rancidity and oxidative stability indices, such as free fatty acids and peroxide values, showed that salmon oil samples were in good condition, except for the expired salmon oil capsules which had elevated concentrations of FFA. These data showed the importance of determining shelf life for fish oils. Data from this survey can serve as a baseline for establishing specific standards for salmon oils produced in Alaska to ensure consumers will be consistently offered the highest product quality.

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Effects of Extraction and Purification Processes on the Quality of Fish Oil

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Abstract

Fish oil can be extracted using a number of methods, including rendering, enzymatic hydrolysis, chemical extraction, and mechanical pressing. Increasing the value of fish oil is a critical concern for the fish oil industry. Finding more lucrative markets for unrefined fish oil requires well-designed purification steps to reduce impurities such as free fatty acids, oxidative components, and moisture. It is important to develop cost-effective methods to extract, clarify, and stabilize fish oil for small processors. Conventional fish oil refining steps include degumming, neutralizing, bleaching, and deodorizing. The adsorption process is an alternative process for further refining edible oil, which removes non-triglyceride materials in a cost-effective way. However, information related to quality of fish oil prepared from the different extraction and purification methods are not readily available. The main focus is (1) fish oil extraction methods and their effects on the quality of the fish oil, and (2) effects of the adsorption process on the quality of the purified fish oil.

Introduction

Producing and purifying fish oil from fish processing byproducts for the growing fish oil market can benefit the fishing industry in the United States and around the world. Interest in the dietary effects of marine omega-3 fatty acids has increased because of their major role in human health (Kronhout et al. 1985, Haglund et al. 1998). Producing fish oil from fish processing byproducts is a growing business. Entrepreneurs and small fish oil processors are interested in establishing small-scale,

cost effective extraction methods for fish oil destined for human consumption. A number of methods including rendering, enzymatic hydrolysis, chemical extraction, mechanical pressing, and use of centrifugal force can be used in the extraction of fish oil. In addition to significant volumes of triglycerides, extracted fish oils contain various amounts of free fatty acids, primary oxidation products, minerals, pigments, moisture, phospholipids, and insoluble impurities that reduce oil quality. Sathivel et al. (2009) has reported that the amount of these impurities present in fish oil may depend on the extraction methods employed. It is important to find the most suitable method to extract oil from fish processing byproducts to reduce the impurities in the oil and diminish fish oil purification processes to one or two steps.

Fish oil extraction methods

Fish oil can be produced from fish processing byproducts by different processing methods: (1) ground fish homogenized with water, the homogenized mixture is centrifuged, and fish oil is separated; (2) ground fish (no added water) is heated and centrifuged, and fish oil is separated; (3) fish oil is extracted by rendering; and (4) ground fish is hydrolyzed using commercial enzymes and the oil collected by centrifugation.

Virtually all animal oils are extracted by rendering, whereas vegetable oils are obtained by crushing, pressing, or solvent extraction or a combination of these. Rendering is a process in which high heat is used to extract fat or oil primarily from animal tissues. Rendering can be performed wet or dry. Wet rendering is conducted with large amounts of water and for fish the ground raw material is homogenized with water and heat is supplied to the homogenized mixture. During heating, fat cell membranes are broken down and the released fat floats to the water's surface. Separated fat may be removed by decanting or by centrifugal methods. Wet rendering is used in the majority of the fish oil industries around the world both on land and in ships (Bimbo 1990).

Oil extraction method comparisons

In experiments conducted by S. Sathivel and coworkers, ground fish byproduct was enzymatically hydrolyzed (no added water), centrifuged, and the fish oil separated. The enzyme alcalase with an activity of 2.4 Anson units per gram (AU per g) (Novo Nordisk, Franklinton) was added to ground catfish viscera at 0.5% w/w. The mixture was stirred for 75 min at 50ºC and then the temperature was increased to above 85ºC for 15 min to inactivate the enzyme. The hydrolysate was centrifuged and the fish oil fraction collected. Sathivel et al. (2009) reported higher amount of crude catfish oil recovered from catfish viscera by

the hydrolysis procedure, compared to a process involving a mixture of ground catfish viscera and water, no heat treatment, and centrifugation; a process involving ground catfish viscera (no added water), heat treatment, and centrifugation; or a process involving a mixture of ground catfish viscera and water, heat treatment, and centrifugation. However, oil produced using the enzymatic hydrolysis procedure had a higher value of free fatty acid (FFA) and peroxide value (PV) than oils made using other procedures.

The PV value for an oil sample indicates the amount of primary oxidation elements produced during the oil extraction. In these experiments the oil extraction process involving a mixture of ground catfish viscera and water, no heat treatment, and centrifugation recovered a lower amount of oil from catfish viscera, but with a lowest FFA and PV. Extraction methods clearly affected recovery of the catfish oil from viscera, as well as the FFA and PV of the oil. In addition, Sathivel et al. (2009) reported that all these oil samples had similar fatty acid compositions, which demonstrated, as expected, that extraction procedures had little effect on fatty acid composition.

Purification of extracted fish oil

The impurities present in fish oil need to be removed before the oil will be considered acceptable for many markets. Conventional refining steps of extracted oils include degumming, neutralizing, bleaching, and deodorizing. Degumming of plant and animal oils removes impurities such as phospholipids, FFA, and trace metals, while minimizing damage to the natural oil. The presence of gums in the oil affects the final oil color and the smoke stability of the oil. However, fish oils generally have low amounts of phospholipids; therefore, in general, degumming is not practiced in fish oil processing. However, the treatment of fish oils with phosphoric acid prior to caustic refining has been a practice in Europe (Brekke 1980).

Free fatty acids can be removed from oils in two ways, either by chemical (neutralization) or physical methods. Neutralization removes non-acylglycerol impurities such as FFA in the oil. Caustic soda is most commonly employed for neutralization of oils. Use of high-temperature steam distillation is also possible to remove FFA from oils (Sullivan 1976), in the process known as physical refining. In general, fish oils are not usually physically refined because they are easily oxidized and the process significantly increases production costs. During the distillation, the highly unsaturated triacylglycerols (TAG) could polymerize and result in flavor deterioration after refining (Bimbo 1990).

Bleaching removes off-flavors and oxidation products (Taylor 2005). Bleaching also removes trace and heavy metals, soap, and sulfur compounds. During bleaching, oxidation products such as alde-

hydes and ketones are adsorbed onto the surface of a matrix such as activated earth. After bleaching, the oils have lower peroxide values. Deodorization is a distillation process that removes undesirable odors and volatile components, leaving the oil flavor and odor characteristics that are most readily recognized by the consumers (Gavin 1978).

An alternative process for further refining edible oils is the adsorption process, which removes non-triglyceride materials cost effectively (Taylor and Ungermann 1984, Proctor and Palaniappan 1990, Sathivel and Prinyawiwatkul 2004). Advantages of adsorption technology are lower refining losses, less lipid oxidation, and less flavor reversion in the refined oil. Adsorption technology can potentially provide a simplified process for refining salmon oil for human consumption.

Huang and Sathivel (2010) reported that combined neutralization and adsorption steps can be an alternative for removing most of the impurities that are generally present in unpurified fish oil. The adsorption process involves the mass transfer of adsorbates from the liquid phase to the adsorbent surface until an equilibrium is reached (McCabe et al. 1993). Huang and Sathivel (2010) evaluated the performance of three fish oil purification methods, including activated earth adsorption, neutralization, and the combination of neutralization and activated earth adsorption steps. They reported that free fatty acid content of unpurified salmon oil can be reduced from 3.5% to 0.12% by neutralization. They also reported that during the neutralization, the peroxide value of unpurified salmon oil increased from 2.35 to 4.75 mmole per kg, confirming an increase in lipid oxidation as a result of the process. Neutralization is conducted at a higher temperature with a prolonged duration, which can be a factor contributing to lipid oxidation in the neutralized oil. Sathivel et al. (2008) has reported changes in PV of the unpurified fish oil as a function of time and temperature. Huang and Sathivel (2010) found that an activated earth adsorption step on the neutralized salmon oil decreased PV with increased adsorption time. This indicated that activated earth was effective in adsorbing primary oxidation products.

Conclusion

Oil extraction methods can affect the final quality of fish oil, especially peroxide value and free fatty acids. Information on methods of fish oil extraction is very important for the design of a purification process to produce fish oil being marketed for human consumption. Free fatty acids can be significantly reduced by neutralization; however, the process itself can increase peroxide value. The combination of neutralization and adsorption steps can reduce both peroxide and free fatty acids in fish oil. The combined methods can potentially provide a simplified process for purifying fish oil for human consumption.

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Composition of Hydrolysate Meals Made from Alaska Pollock, Salmon, and Flatfish Processing Byproducts: Comparisons with Traditional Alaska Fish Meals

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Abstract

Alaska annually processes roughly 2.2 million metric tons of fish harvested for human food, generating some 1.5 million t of fish waste, depending on season, species composition, and product form. In the western Gulf of Alaska and along the Bering Sea, larger seafood processing operations are mandated by regulation to effectively handle the byproducts of seafood processing. These large processors employ wet reduction processing to manufacture fish meal, bone meal, fish oil, and stick water. Other seafood processing operations in Alaska, although smaller and often seasonally operated, could potentially employ less capital-intensive methods such as enzymatic hydrolysis, to cost effectively handle processing byproducts. In this project, dried hydrolysate

meals were made from the byproducts of seafood processing, derived from commonly encountered Alaska commercial species, collected during the different harvesting seasons. To establish their utility, these hydrolysate meals were chemically characterized as to proximate composition, mineral content, lipid class, fatty acid, and amino acid analyses. The data from this study may be useful in developing markets for hydrolysates made from Alaska fishery byproducts.

Introduction

Roughly 2.2 million metric tons of fish are harvested for human food annually in Alaska. The majority of these fish are caught in the U.S. exclusive economic zone of the Bering Sea and either processed on shore or at sea on factory trawl vessels (NOAA 2005). Alaska walleye pollock (*Theragra chalcogramma*) constitutes the largest portion of this harvest at about 1.2 million t per year, but statewide some 300,000 t of salmon are also harvested annually, including the most abundant species, pink salmon (*Oncorhynchus gorbuscha*) and sockeye or red salmon (*Oncorhynchus nerka*) (ADFG 2009). Flatfish harvests, including arrowtooth flounder, amount to roughly 200,000 t per year.

In the Gulf of Alaska, significant amounts of pollock are also landed during the two major seasons, winter and fall. This pelagic trawl fishery is limited to the central and western parts of the Gulf of Alaska by statute. Other trawl fisheries in the Gulf of Alaska include Pacific cod (*Gadus macrocephalus*) and the shallow and deepwater flatfish assemblages. Across the Gulf of Alaska, salmon are also harvested in large quantities. Kodiak and communities westward generally have larger processing operations than are found in Bristol Bay and southcentral and southeast Alaska.

In 1974, the Alaska Department of Environmental Conservation and U.S Environmental Protection Agency declared Kodiak a Seafood Processing Center for the purposes of the Clean Water Act (ADEC 2009, EPA 2009). In part, this meant that processors in Kodiak were required to develop methods and processes to effectively handle the byproducts of seafood processing on shore. With the Americanization of the Bering Sea fisheries, new seafood processing facilities built along the Alaska Peninsula and the Aleutian chain were also required to effectively handle the byproducts of seafood processing on shore. In southcentral and southeast Alaska, smaller processing operations operate under a suite of regulations called the General Discharge Permit. This allows processors to discharge the byproducts of seafood processing directly into local marine waters, provided that a number of specific conditions are met.

Larger scale seafood processors generally employ a wet reduction method to handle their processing waste. Most of the operations in Alaska use technology purchased from Atlas-Stord Denmark A/S. These

plants are somewhat scalable in throughput, but in Alaska the average capacity is around 1,200 t of fish waste per day. These plants are expensive, representing a capital investment of several tens of millions of dollars. For smaller, seasonal processing operations, the volume of seafood processing byproducts is usually less than 25 t per day, often less than 5 t, and the capital requirements for implementing large scale wet reduction plants is prohibitive. This is the case for many of the seasonal processors located around the north and eastern Gulf of Alaska. It is also true for the smaller salmon and herring processing communities around Bristol Bay. For these operations, enzymatic hydrolysis of the byproducts of seafood processing offers a way to reduce dumping into local waters, and makes available new potential revenue streams.

The volume of processing byproduct is contingent not only on the volume of fish processed but also on species and product form. For salmon, the total food recovered for red salmon skinless fillets is about 46% of round weight, while if the salmon is canned, approximately 65% of the round weight is recovered. When walleye pollock is made into surimi, the fraction recovered as human food is between 15 to 20% of round weight. This rises to 34% of pollock round weight for skinless fillets (Crapo et al. 1993). Given these inherent variations, 1-1.5 million t of processing byproduct is generated annually by the Alaska seafood industry. Fish flesh is approximately 75% water by weight. Applying this factor suggests that something upwards of 350,000 t of protein, ash, bone, and lipid are available from the seafood processing byproducts generated each year in Alaska.

Species are harvested in different seasons in Alaska. While the five species of Pacific salmon are all harvested during their summer and fall return to natal rivers to spawn, pollock, cod, and flatfish can be harvested either in the spring prior to spawning or in the fall prior to overwintering. For these species harvested in different seasons, the chemical composition of the fish can vary with season (Aidos et al. 2002, Shirai et al. 2002). Additionally, there is evidence of differences in endogenous protease amounts in some Pacific salmon species as they get closer to the time of ultimate spawning (Yamashita and Konagaya 1990, Martin et al. 2001).

Hydrolysis of the byproducts of food processing has a long history (Adler-Nissen 1986, Flick and Martin 2000). Some operations in Alaska have used phosphoric acid hydrolysis of salmon processing byproducts to make fish fertilizer or fish silage (Hall et al. 1985, Arason et al. 1990, Pigott and Tucker 1990), but given high transportation costs and low unit values for the product, most have ceased operation. Commercially available hydrolytic enzymes cleave peptide bonds linking specific amino acids within the protein chains (Haard et al. 1982, Adler-Nissen 1986, Hall and Ahmad 1992, Shahidi 1994). In this method, proteins are cleaved, under relatively specific conditions of time, temperature, pH,

and enzyme concentration, and the fish processing byproduct liquefied. Bones and undigested tissues can then be sieved out or separated using a decanter centrifuge and the liquid divided into three fractions: proteinaceous solids, a lipid phase, and an aqueous phase containing soluble protein fragments and smaller organic molecules. When the liquid is dried it is often used as an ingredient in agriculture and aquaculture feeds (Tacon 1994). However, there is no literature on the similarities and differences between dried hydrolysates made from the byproducts of processing the most abundant commercial fish in Alaska.

This research was embarked upon to investigate the utility of employing enzymatic hydrolysis as a method to effectively handle seafood processing waste for smaller and seasonal fish processors in Alaska. The goal was to investigate the likely kinds of secondary products that could be generated should smaller processing operations wish to produce saleable products from their processing waste stream (Hardy and Masumoto 1990). The outcome sought was to characterize hydrolysates that are commercially made from byproducts of abundant species harvested in Alaska.

Materials and methods

Preliminary experiments

Using an ASTA hydrolysis unit, salmon processing waste was ground through a plate with 0.5 cm holes. Papain (Corolase L 10, AB enzymes) was discharged into the ground raw material at the rate of 3.5 ml per min, and the temperature of the material was raised from 18º to 60ºC using a tubular scraped surface heat exchanger. The enzyme reaction mixture was pumped through the integral reactor where the hydrolysis continued at 60º to 65ºC. The reaction was terminated by raising the mixture temperature above 85ºC with live steam. Bone was separated from hydrolysate by passing over a shaking screen. Bone-free hydrolysate was concentrated in a scraped surface vacuum evaporator and further dried to powder as needed. In this process, endogenous proteolytic enzymes were not denatured prior to the addition of exogenous commercial enzyme (Yamashita and Konagaya 1990, Martin et al. 2001), and the reproducibility of peptide fragment size may have been affected. In order to compare hydrolysates it was deemed prudent to heat-inactivate endogenous enzymes prior to the addition of commercial enzymes as was the practice at BioOregon, in Warrenton.

Raw material

Between 4,500 and 5,000 kg of seafood waste from each harvest, derived from freshly processed fish, was collected from local processing plants in Kodiak. Five separate sample lots were collected: (1) spring

harvest walleye pollock processing byproduct, March 2003; (2) red salmon processing byproduct, June 2003; (3) late harvest pink salmon, August 2003; (4) fall pollock, August 2003; and (5) flatfish processing byproducts, August 2003. The raw material was frozen in totes and stored until shipment.

When received at BioOregon, the byproducts were stored frozen until processed. Prior to hydrolyzing, fish byproducts were thawed, ground to a uniform size, and cooked at 93-96ºC to kill endogenous enzyme activities. The material was then deboned and the excess liquid containing soluble proteins, oil, and other soluble organic molecules was removed by decantation and discarded. The water content of the mince recovered from the decantation was 72%. The remaining mince was treated with the addition of 0.2% Corolase L 10 enzyme and incubated at 57ºC for 60 min to promote efficient hydrolysis. The temperature of the mixture was subsequently increased above 88ºC for 10 min to inactivate the enzyme. The finished hydrolysate was concentrated by evaporation to 60% water content and then dried to about 5% water content. The hydrolysate was stored in 25 kg fish meal paper bags in a 5-10ºC refrigerated cold room. The cost for the commercial production of hydrolysates and shipping limited the number of replicates to one large sample for each of the five byproduct assemblages. Samples were taken from three separate bags for each of the five lots and analyzed in this study.

Proximate analysis

Proximate composition was determined in triplicate for each sample and the data reported as the average percent of total. Moisture and ash content were measured using AOAC (Helrich 1990) methods no. 952.08 and no. 938.08, respectively. Analyses of protein content were carried out using an automated protein analyzer (FP2000 LECO, St. Joseph). Lipid content was determined as previously described (Oliveira et al. 2006) using an accelerated solvent extractor (ASE200 Dionex, Sunnyvale). Lipids were extracted with dichloromethane under a nitrogen atmosphere. Solvent was removed using an automated evaporator (TurboVap LV Zymark, Hopkinton) operated under a nitrogen atmosphere at 45ºC. Lipids were quantified gravimetrically and oils stored in hexane with 0.01% BHT in amber screw-cap vials at –70ºC until analysis.

Percent TCA-soluble nitrogen

Percent trichloroacetic acid–soluble nitrogen measurements and calculations were performed using the method of Hoyle and Merritt (1994). Briefly, 50 g water was added to 50 g dried hydrolysates, and the mixture was adjusted to pH 8.0 and subsequently warmed to 50ºC. An aliquot was removed and mixed with 50 ml 20% trichloroacetic acid (TCA), and the mixture was centrifuged at 2,560 × g for 15 min to obtain

TCA-soluble and insoluble fractions. These were analyzed for nitrogen by the combustion method using a nitrogen analyzer (FP200 LECO) calibrated with EDTA (ethylenediaminetetraacetic acid).

Color determination

A Hunter ColorFlex spectrocolorimeter was used to determine color. The data are the average of three measurements and is reported as Hunter L*, a*, and b* values. The L* value describes whiteness on a scale of 0 (black) to 100 (white). The a* value describes the red-green color axis, with negative numbers indicating a greener hue and positive numbers more reddish. The b* value describes the blue-yellow color axis, with negative numbers indicating a bluer hue and positive more yellowish.

Mineral analysis

Samples of each hydrolysate lot, taken for mineral analysis, were sent to the University of Alaska Fairbanks, School of Natural Resources and Agricultural Sciences, Palmer Research Center, where they were ashed overnight at 550ºC. Ashing residues were digested overnight in an aqueous solution containing 10% (v/v) hydrochloric acid and 10% (v/v) nitric acid. Digested solutions were diluted as needed and analyzed for Ag, Ca, Cd, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, Sr, and Zn by inductively coupled plasma optical emission spectroscopy on a Perkin Elmer Optima 3000 Radial ICP-OES.

Amino acid analysis

Amino acid profiles determined in this study, except the whitefish meal values from Smiley et al. 2003, were determined by the AAA Service Laboratory Inc., Boring. Samples were hydrolyzed with 6N HCl and 2% phenol at 110ºC for 22 hours. Amino acids were quantified using a Beckman 6300 analyzer with post column ninhydrin derivatization.

Lipid analysis

An Iatroscan TLC/FID analyzer model MK-6s (Iatron Laboratories Inc., Tokyo) was used to determine the distribution of the main lipid classes in the extracted lipids as described by Oliveira and Bechtel (2005). Seven standards obtained from Sigma (St. Louis) were used to identify the lipid classes and included cholesterol (ST), tripalmitin (TAG), palmitic acid (FFA), L-a-phosphatidylcholine (PL), 1,2-dipalmitoyl-sn-glycerol (1,2 DAG), 1,3 dipalmitoyl-sn-glycerol (1,3 DAG), and DL-a-monopalmitoylglycerol (MAG) (see p. 245). The solvent system used was a mixture of hexane:ethyl ether:formic acid, at the ratio of 80:25:1.2. In this system 1,3 DAG co-elutes with the sterol peak, which was separated from the 1,2 DAG peak.

Table 1. Proximate composition of Alaska fish meals and hydrolysates.

	WFM	SPH	FPH	FlatsH	SalmM	RSH	PSH
Protein	69.3 ± 2.7	79.7 ± 0.2	79.6 ± 0.1	71.7 ± 0.2	70.3 ± 1.5	69.4 ± 0.1	73.9 ± 0.1
Lipid	17.0 ± 3.9	9.6 ± 0.6	12.1 ± 0.1	13.8 ± 0.1	10.1 ± 1.2	15.8 ± 0.1	13.8 ± 0.3
Ash	7.6 ± 1.8	6.8 ± 0.1	4.4 ± 0.1	8.6 ± 0.2	8.1 ± 1.2	4.5 ± 0.1	5.5 ± 0.0
Moisture	6.1 ± 1.9	5.7 ± 0.3	5.0 ± 0.1	6.7 ± 0.1	10.1 ± 1.1	11.2 ± 0.0	8.6 ± 0.0

Data for salmon meal and hydrolysates are means of three measurements (percent wt/wt) with SD. Whitefish meal data are means of eight meals with SD (Smiley et al. 2003).

WFM: whitefish meal. SPH: spring pollock hydrolysate. FPH: fall pollock hydrolysate. FlatsH: flatfish hydrolysate. SalmM: salmon fish meal. RSH: red salmon hydrolysate. PSH: pink salmon hydrolysate.

Fatty acid methyl esters were prepared in duplicate according to the methods of Maxwell and Marmer (1983) and analyzed on a GC/FID (GC6850 Agilent Technologies, Wilmington) as described by Oliveira and Bechtel (2005). An autosampler performed the GC injections of standards and samples, and the injection volume was 1 µl. The ChemStation enhanced integrator program was used to integrate chromatogram peaks. All standards were purchased from Supelco® (Bellefonte). The standards used were Supelco 37, Bacterial Acid Methyl Esters Mix, Marine Oil #1, and Marine Oil #3. Commercial cod liver oil was used as a secondary reference standard (Ackman and Burgher 1965). The data are reported as percent weight per weight.

Comparisons

Data from the analysis of the hydrolysates will have greater interpretability when compared to standard fair to average quality (FAQ) wet reduction commercial fish meals. The whitefish meal data are from Smiley et al. (2003), and a different method was employed for analysis of amino acids. Salmon meal data are more problematic, because multiple salmon species are harvested concurrently during the summer salmon season in Alaska. The commercial salmon meal used for comparative purposes was pink salmon, red salmon, and chum salmon.

Results and discussion

Proximate composition

The protein proximate values of the salmon hydrolysates, shown in Table 1, are consistent with the protein content for standard wet reduction salmon meal with protein content of about 70% by weight. The results for the flatfish hydrolysates as well as the spring and fall pollock hydrolysates and the standard wet reduction whitefish meal are quite similar. Some seasonal variation is to be expected (Shirai et al. 2002). These values show that even without adding back the soluble proteins lost in the initial 93-96ºC cooking step, protein values from commercial hydrolysis are roughly equal to those derived from the wet reduction

method. The whitefish meal data are from Smiley et al. (2003), while the salmon meal data are derived from a commercial salmon fish meal made in Alaska.

Ash levels are dependent on several variables. The ash level in the whitefish meal may be higher because the producer has added bone meal back to the fish meal, reducing the protein concentration but still meeting the standard 65% protein concentration required by many customers. There are significantly larger markets for protein fish meal than for bone meal. Bone meal, while containing on average 45% protein, has relatively low value. However, it is useful to note that recent work has shown bone meal to be an effective soil emolument when applied appropriately (Johnson et al. 2003).

Small differences in the ash values of spring and fall pollock may be due to factors intrinsic to the fish themselves, such as differences in oil content. The relatively larger difference between red and pink salmon hydrolysates may reflect differences in product forms being processed into human food at the time. If headed and gutted product is being produced, the ash levels of byproduct meals will be relatively lower than if fillets are being made, due to the increase in ash in the backbone. Moisture levels are relatively consistent with the exception of the red salmon hydrolysate. There is no ready explanation for this higher value.

Lipid levels were in a fairly narrow range between 9.6% for spring pollock and 15.8% for red salmon (Table 1). These differences probably reflect the nascent lipid composition of the fish flesh at the times they were harvested. However, the raw material for hydrolysis was cooked and decanted prior to introduction of the hydrolytic enzyme. Some species, including gadoids such as Alaska pollock, have significant concentrations of lipid in their livers during the fall harvest, while salmon have high concentrations of lipid in their heads. Within reason, lipid levels in commercially made seafood fish meals and hydrolysates can be adjusted by application of the processing system employed in wet reduction operations.

Percent TCA-soluble nitrogen

The percent TCA-soluble nitrogen results varied among all the hydrolysate samples, from a low of 1.6 to a high of 5.3. Since neither the whitefish nor the salmon meals had been hydrolyzed, they are not included. Spring pollock hydrolysates had 2.9% TCA-soluble nitrogen, fall pollock hydrolysate 5.3%, flatfish hydrolysate 4.1%, red salmon hydrolysate 2.2%, and pink salmon hydrolysate 1.6%. Although all the percent TCA-soluble nitrogen values are a small percent of total nitrogen, there is a 3.3 fold difference between the highest and the lowest values. It is possible that proteins from the different species have different susceptibility to the action of the Corolase enzyme; however, further experiments would be required to determine the source of the variation.

Table 2. Color analysis (L*a*b*) for hydrolysates.

	SPH	FPH	FlatH	RSH	PSH
Avg L*	39.3 ± 2.0	46.6 ± 0.4	51.2 ± 0.4	35.1 ± 0.7	34.7 ± 0.8
Avg a*	4.4 ± 0.3	4.5 ± 0.1	4.2 ± 0.1	4.7 ± 0.1	5.4 ± 0.1
Avg b*	17.1 ± 1.0	20.2 ± 0.1	21.4 ± 0.1	17.7 ± 0.5	18.2 ± 0.4

Data are means of three measurements with SD.

SPH: spring pollock hydrolysate. FPH: fall pollock hydrolysate. FlatsH: flatfish hydrolysate. RSH: red salmon hydrolysate. PSH: pink salmon hydrolysate.

L* lightness (100 white, 0 black). a* [red (+) and green (–)]. b* [yellow (+) and blue (–)].

Hunter color

Color analysis results are listed in Table 2. Measurements made in daylight color mode show the two salmon hydrolysates were considerably darker than those for whitefish measuring only about 75% of the average L* value for the two pollock and flatfish hydrolysates. The whitefish showed about 85% of the salmon value for a* consistent with the slightly redder color of the salmon hydrolysate. These color values suggest that the addition of dried hydrolysates from any of these species will have only marginal effects on the color of the final feed formulation.

Mineral composition

Table 3 shows the mineral content of the meals. Measurements of calcium, potassium, magnesium, sodium and phosphorus are reported as percent by weight. Measurements of silver, cadmium, copper, iron, manganese, nickel, lead, strontium and zinc are reported as parts per million. The concentration data represent the averages of triplicate samples of each hydrolysate meal.

The hydrolysates were made from byproduct that had been mechanically deboned and would thus have lower calcium and phosphorus levels than standard fish meals where bones were not removed. Whitefish meal and salmon meal more closely resemble each other in the concentrations of calcium, phosphorus, and strontium, while the hydrolysates resemble one another in many of the other minerals. Two other anomalies stand out. One is the relatively high levels of iron in pink salmon hydrolysate. This is more than twice the concentration of iron found in red salmon hydrolysate. Interestingly, spring pollock hydrolysate has almost twice the level of iron as fall pollock, and red salmon show levels of copper from 5 to 20 times higher than those in other hydrolyzed species. We have no ready explanation for this. In conclusion, the levels of minerals found in these hydrolysates are broadly similar to those found in the two wet reduction fish meals, absent the augmentation of those meals with bone meal or the spraying of stickwater onto the drying presscake.

Table 3. Mineral content of hydrolysates and Alaska fish meals.

	WFM	SPH	FPH	FlatsH	SalmM	RSH	PSH
Ca[a]	5.48	1.46	0.79	2.23	3.0	0.6	0.88
K[a]	0.37	0.62	0.5	0.54	0.78	0.4	0.45
Mg[a]	0.27	0.13	0.11	0.19	0.20	0.11	0.12
Na[a]	0.89	0.89	0.68	0.80	0.68	0.78	0.58
P[a]	3.06	0.94	0.67	1.00	2.08	0.81	1.07
Ag[b]	0.67	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Cd[b]	<0.01	0.09	0.13	0.33	0.25	0.42	0.27
Cu[b]	3.5	4.4	18.5	4.2	10.7	92.5	13.1
Fe[b]	67	455	255	371	130	347	750
Mn[b]	5	4	2	10	3.5	2	7
Ni[b]	0.8	2.07	1.23	2.35	0.20	0.89	2.0
Pb[b]	<0.01	0.01	<0.01	0.19	0.23	0.02	0.29
Sr[b]	297.0	102.9	48.8	141.2	138.0	33.8	49.3
Zn[b]	99	70	106	90	183	317	329

[a]Percent by weight.

[b]Parts per million, ppm.

Data for hydrolysates are dry weight, mean of three measurements. Whitefish meal data are from Smiley et al. 2003.

SPH: spring pollock hydrolysate. FPH: fall pollock hydrolysate. FlatsH: flatfish hydrolysate. RSH: red salmon hydrolysate. PSH: pink salmon hydrolysate. SalmM: salmon meal. WFM: whitefish meal.

Amino acid composition

In Table 4 the amino acid data for the hydrolysates are reported as percentages of total amino acids analyzed, and represent averages of triplicate measurements for each sample. The methodology employed did not measure tryptophan or cysteine levels. In addition, the values for methionine were not determined for the whitefish meal (Smiley et al. 2003). There is considerable similarity between hydrolysates in the amounts of each of the amino acids present. However, there are differences between the flatfish and spring and fall pollock hydrolysates when compared to whitefish meal values for a number of different amino acids. Most significant is the value for glycine in whitefish meal. This value is probably due to the addition of concentrated solubles back into meal during the drying operation. The solubles were removed during the processing of hydrolysates. Solubles contain high levels of gelatins derived from fish collagen (Bechtel 2005), and since about one-third the collagen residues are glycine residues, the addition of solubles elevates the percent of glycine in the whitefish meal. The value for salmon meal glycine is also elevated when compared to the salmon hydrolysates, although not as much as is found in the whitefish meal.

Table 4. Amino acid composition of Alaska fish meals and hydrolysates.

	WFM	SPH	FPH	FlatsH	SalmM	RSH	PSH
ALA	8.20	5.74	5.57	5.58	6.38	5.72	5.59
ARG	5.30	7.24	7.31	6.99	7.60	7.61	9.10
ASP	14.60	10.52	10.24	10.56	9.46	10.22	9.97
GLU	17.70	14.62	14.45	14.23	13.10	12.42	12.50
GLY	11.20	5.06	4.63	5.01	7.25	5.50	5.18
HIS	1.80	2.25	2.32	2.59	2.73	2.83	2.71
ILE	3.90	4.68	4.70	4.85	4.55	4.80	4.81
LEU	6.10	8.34	8.22	8.27	8.00	8.31	8.38
LYS	6.20	9.03	9.19	8.96	8.20	8.42	8.66
MET	ND	3.37	3.70	2.76	3.38	3.62	3.43
PHE	3.00	4.97	5.84	5.52	4.80	5.10	4.64
PRO	4.70	4.32	4.11	4.26	5.15	4.62	4.61
SER	4.80	4.70	4.43	4.72	4.45	4.58	4.68
THR	4.70	5.04	4.89	5.11	4.66	5.34	5.22
TYR	2.10	4.65	4.81	4.89	4.10	4.91	4.67
VAL	5.00	5.46	5.59	5.68	5.63	5.99	5.84

Data for hydrolysates are percent total amino acids analyzed, dry weight, mean of three measurements. Whitefish meal data from Smiley et al. 2003.

Values for cysteine and tryptophan were not ascertained.

SPH: spring pollock hydrolysate. FPH: fall pollock hydrolysate. FlatsH: flatfish hydrolysate. RSH: red salmon hydrolysate. PSH: pink salmon hydrolysate. SalmM: salmon meal. WFM: whitefish meal.

The hydrolysates all have relatively high amounts of each of the essential amino acids. Lysine levels in hydrolysates ranged from 8.4 to 9.2%, which were higher that the whitefish meal or salmon meal values. Lysine values are important because of the relatively restricted value in soy-based ingredients often used in aquaculture feed formulations (Hardy 2008). Methionine concentrations ranged from 2.8% for flatfish hydrolysate to 3.7% for fall pollock hydrolysate, indicating the hydrolysates were good sources of this important amino acid. As a generality values for essential amino acids were similar for the hydrolysates.

Lipid analysis

Table 5 shows the distribution of lipid classes in hydrolysate meals, and Table 1 shows the percent lipid in each meal. Lipid classes are percent for triacylglycerides, 1,2-diacylglycerides, monoacylglycerides, free fatty acids, phospholipids, and the combined percentages of sterols and 1,3-diacylglycerides. Triglycerides in hydrolysate meals were approximately 60% except for the flatfish hydrolysate, which was substantially higher at 76%. Conversely, levels of phospholipids in the

Table 5. Lipid class distribution of Alaska hydrolysates.

	SPH	FPH	FlatsH	RSH	PSH
Triglycerides	58.3 ± 1.9	59.7 ± 1.5	75.8 ± 1.0	62.3 ± 3.5	59.0 ± 1.4
Phospholipids	18.8 ± 2.5	19.8 ± 2.0	7.2 ± 1.6	20.4 ± 2.5	23.1 ± 1.4
Free fatty acids	11.9 ± 2.7	13.4 ± 0.7	10.4 ± 1.9	9.0 ± 0.6	11.2 ± 0.4
Sterols	8.8 ± 0.9	5.2 ± 0.3	4.6 ± 0.3	6.3 ± 0.9	5.3 ± 1.3
Diglycerides	1.6 ± 0.4	1.4 ± 0.3	1.6 ± 0.3	1.5 ± 0.2	0.9 ± 0.1
Monoglycerides	0.6 ± 0.1	0.6 ± 0.1	0.4 ± 0.1	0.7 ± 0.1	0.4 ± 0.1

Data are means and SD of percent of total lipid analyzed.

SPH: spring pollock hydrolysate. FPH: fall pollock hydrolysate. FlatsH: flatfish hydrolysate. RSH: red salmon hydrolysate. PSH: pink salmon hydrolysate.

Table 6. Common fatty acids from Alaska fish meals and hydrolysates.

	WFM	SPH	FPH	FlatsH	SalmM	RSH	PSH
C14:0	3.4 ± 0.4	4.5 ± 0.3	4.0 ± 0.1	4.3 ± 0.3	4.7 ± 0.1	4.0 ± 0.0	3.8 ± 0.2
C16:0	16.7 ± 3.0	14.2 ± 0.7	13.9 ± 1.8	14.8 ± 2.3	15.2 ± 0.1	16.9 ± 3.1	17.7 ± 2.5
C16:1ω7	5.3 ± 0.7	7.5 ± 1.0	4.7 ± 0.2	6.4 ± 0.9	4.3 ± 0.0	6.0 ± 0.4	4.8 ± 0.3
C17:1ω9	1.7 ± 0.5	1.9 ± 0.5	0.7 ± 0.3	1.5 ± 0.6	0.3 ± 0.0	2.7 ± 0.6	1.7 ± 0.2
C18:0	2.7 ± 0.7	3.2 ± 0.2	3.3 ± 0.3	3.2 ± 0.6	3.6 ± 0.0	3.5 ± 0.5	3.7 ± 0.5
C18:1ω7	7.6 ± 1.5	5.6 ± 0.8	3.1 ± 0.2	4.3 ± 1.0	3.0 ± 0.0	6.9 ± 0.2	5.6 ± 0.8
C18:1ω9c	13.6 ± 3.0	16.7 ± 1.0	13.2 ± 0.9	15.4 ± 0.7	16.5 ± 0.1	18.2 ± 0.4	17.0 ± 0.1
C18:2ω6c	0.8 ± 0.2	1.4 ± 0.3	1.7 ± 0.0	1.7 ± 0.1	1.5 ± 0.0	1.0 ± 0.2	1.3 ± 0.4
C18:3ω3	0.4 ± 0.1	0.7 ± 0.2	0.9 ± 0.0	0.8 ± 0.2	1.1 ± 0.0	0.7 ± 0.1	0.8 ± 0.3
C18:4ω3	1.4 ± 0.4	2.0 ± 0.4	2.3 ± 0.0	1.8 ± 0.1	1.9 ± 0.0	1.9 ± 0.2	1.8 ± 0.4
C20:1ω7	0.4 ± 0.2	0.8 ± 0.4	0.5 ± 0.0	0.8 ± 0.5	0.8 ± 0.0	0.4 ± 0.1	0.4 ± 0.1
C20:1ω9	1.8 ± 0.9	2.1 ± 0.4	2.7 ± 0.1	2.1 ± 0.3	2.8 ± 0.0	1.8 ± 0.8	1.8 ± 0.9
C20:1ω11	3.6 ± 2.6	3.1 ± 1.9	4.9 ± 0.2	3.0 ± 1.7	5.6 ± 0.0	2.8 ± 2.1	2.8 ± 2.2
C20:4ω3	0.4 ± 0.1	0.5 ± 0.1	1.2 ± 0.0	0.9 ± 0.5	1.5 ± 0.0	0.5 ± 0.1	0.9 ± 0.6
C20:4ω6	0.8 ± 0.1	0.5 ± 0.7	0.5 ± 0.5	0.5 ± 0.5	0.8 ± 0.0	0.3 ± 0.4	0.7 ± 0.1
C20:5ω3	15.5 ± 1.3	12.6 ± 2.4	9.7 ± 0.2	12.4 ± 3.6	8.6 ± 0.1	12.8 ± 3.3	12.5 ± 3.4
C22:1ω9	0.5 ± 0.2	0.2 ± 0.3	0.8 ± 0.0	0.6 ± 0.1	1.9 ± 0.0	0.5 ± 0.2	0.5 ± 0.2
C22:1ω11	4.2 ± 3.2	3.1 ± 2.5	6.9 ± 0.2	3.0 ± 2.2	6.1 ± 0.0	3.2 ± 2.0	3.2 ± 2.2
C22:5ω3	1.0 ± 0.3	1.9 ± 0.2	2.5 ± 0.1	2.7 ± 0.2	2.5 ± 0.0	1.0 ± 0.2	1.7 ± 0.1
C22:6ω3	9.9 ± 1.9	8.4 ± 0.4	13.2 ± 0.2	10.9 ± 1.7	12.8 ± 0.1	9.0 ± 0.6	11.2 ± 1.1
Sum SFA	23.6 ± 3.4	24.2 ± 0.2	24.0 ± 0.5	25.0 ± 0.6	24.5 ± 0.1	25.6 ± 0.9	26.5 ± 0.7
Sum MUFA	39.8 ± 2.5	45.3 ± 0.7	41.5 ± 0.2	40.0 ± 0.6	44.0 ± 0.1	44.5 ± 0.6	40.8 ± 0.7
Sum PUFA	32.5 ± 2.3	29.2 ± 0.6	33.3 ± 0.1	33.2 ± 1.0	30.6 ± 0.0	28.1 ± 0.9	31.8 ± 0.9
PUFA/SFA	1.4	1.2	1.4	1.3	1.3	1.1	1.2
SUM Ω 3	29.3 ± 2.1	26.1 ± 0.8	30.0 ± 0.1	29.7 ± 1.3	28.4 ± 0.0	25.8 ± 1.2	29.1 ± 1.2
SUM Ω 6	1.9 ± 0.3	2.3 ± 0.3	2.7 ± 0.3	2.5 ± 0.2	2.3 ± 0.0	1.5 ± 0.2	2.3 ± 0.1
Ω 3/Ω 6	15.7	11.5	11.2	11.7	12.5	17.2	12.9

All values are percent w/w. Data are means and SD of total lipid analyzed. Whitefish meal values from Smiley et al. 2003.

WFM: whitefish meal. SPH: spring pollock hydrolysate. FPH: fall pollock hydrolysate. FlatsH: flatfish hydrolysate. SalmM: salmon fish meal. RSH: red salmon hydrolysate. PSH: pink salmon hydrolysate. SFA: saturated fatty acids. MUFA: monounsaturated fatty acids. PUFA: polyunsaturated fatty acids. Ω 3: omega 3 fatty acids. Ω 6: omega 6 fatty acids.

flatfish hydrolysate was lower than the other hydrolysates. The high percent of phospholipid in the hydrolysate meals was probably due to processing protocol, in which much of the triglyceride was removed after the heat treatment step. However, this step would be much less efficient in removal of membrane phospholipids from the hydrolysates. Free fatty acid values ranged from 9% to over 13%, indicating lipolysis of the samples. Although fish flesh tolerates frozen storage with minimal degradation of triglycerides, viscera, even when frozen, often exhibits significant lipolytic activity. It is interesting to note that the levels of diglycerides and monoglycerides were quite similar and relatively low.

Table 6 presents the values for the 20 most abundant fatty acids that were identified in the hydrolysate meals. While there are small-scale differences between them, the levels of each of the fatty acids are relatively consistent across processing methodology as well as species type. Perhaps most important, the levels of the long chain omega-3 fatty acids, especially EPA (C22 5w3) and DHA (C22 6w3) were consistently high no matter which sample was analyzed.

Our summary analyses based on the fatty acid composition of extracted oils from the various meals are also shown in at the bottom of Table 6. Results indicate that the wet reduction and hydrolyzed meals have relatively similar levels of saturated, monounsaturated, and polyunsaturated fatty acids. Also the levels of total omega-3 fatty acids ranged from a low of 26% to a high of 30%, while the total omega-6 levels were in the 2 to 3% range. Of importance is the ratio of polyunsaturated fatty acids to saturated fatty acids for these meals, which is relatively high for all the hydrolysates. Another interesting value is the ratio of omega-3 fatty acids to omega-6 fatty acids in the fish meals, averaging around 11:17.

Conclusions

This work has shown that employing enzymatic hydrolysis, after heating and deboning the byproduct, allowed the production of high quality fish protein meals that have many chemical characteristics similar to fish meals made using the classic wet reduction methodology. The hydrolysate meals were high in protein and low in ash. If very high quality fish oil is desired, initially heating the sample to inactivate lipolytic and hydrolytic enzymes and then removing lipid prior to the introduction of commercial enzyme could be an effective set of processes.

Hydrolysis may well prove to be an economically effective method of transforming the byproducts of seafood processing into commodities with value. Given the cost of wet reduction systems currently available, a waste handling operation employing hydrolysis could be cost effective when the volumes of available raw materials are less than 25 tons per day. In small seasonal processing plants where the waste streams are

often less than 5 tons per day, hydrolysis seems the only viable alternative allowing for sufficient value in the products (oil and protein meals) to offset transportation and processing costs.

There is increasing interest in the availability of high quality fish oils derived from cold-water species. Given that the fish harvested in Alaska are processed into human food, the initial high quality of raw materials for making fish oil is largely insured. Unfortunately, producing high quality oil from seafood processing wastes often involves the use of expensive equipment. The value of high quality salmon oil currently seen in the marketplace indicates that these costs can be amortized over only a relatively short time. However, much of this equation is predicated on the volume of seafood processing byproducts available.

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Physical and Chemical Properties of Pollock and Salmon Skin Gelatin Films

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Abstract

This study evaluated the barrier and mechanical properties of Alaska pollock (*Theragra chalcogramma*) and Alaska pink salmon (*Oncorhynchus gorbuscha*) gelatin films and related these to amino acid composition and gel properties.

Gelatin was made by extracting heat-soluble proteins from the skins of pollock and pink salmon with water, evaporating this to dryness, and rehydrating it under controlled conditions. Dynamic rheology was used to characterize the fish gelatins' gelation and melting behavior. Films were made from the gelatins and water vapor permeability was determined at 25ºC and in an 80% to 0% relative humidity (RH) gradient using a gravimetric method. Oxygen permeability was determined at

23ºC and 55% RH through a 100% oxygen gradient using a Coulox detector. Tensile strength and elongation were measured on film strips after equilibration at different percent RH. Oxygen permeability of pollock gelatin films was significantly lower than for salmon gelatin, and tensile strength was significantly higher as well. Tensile strength decreased and elongation increased at higher percent RH for each film. Pollock gelatin proteins had lower proline and hydroxyproline content than those from salmon gelatin.

This study demonstrated significant differences in barrier and mechanical properties between pollock and salmon gelatin films. Differences in amino acid composition of these gelatins affected their physical properties and potential applications. The lower oxygen permeability and higher tensile strength of pollock gelatin film could be useful, particularly for applications related to reducing water loss and oxidative degradation of foods and drugs.

Introduction

Gelatin is composed of heat soluble connective tissue proteins, principally collagen. Its application in foods is derived largely from its gelation and film-forming properties, which are a consequence of an extended, fibrous tertiary structure and a triple helical cross-linked quaternary structure (Simon-Lukasik and Ludescher 2004). In gelatin, there are virtually no covalent linkages between the protein chains, and the triple-helical configuration can involve segments of several different collagen chains. As a result, a protein network is formed with interstitial space for water. As gelatin gels age, water is excluded and the protein matrix condenses into a rubbery film that vitrifies upon drying (Simon-Lukasik and Ludescher 2004).

Gelatin from marine sources (warm- and cold-water fish skins, bones, and fins) is a possible alternative to mammalian gelatin. One advantage of marine gelatins is that they are not carriers of the causative agents for bovine spongiform encephalopathy. In addition, marine gelatins can be considered for kosher and halal labeling (Regenstein and Chaudry 2002), depending on source fish and manufacturing practices. Potential sources of gelatin that have been underutilized include the skins from Alaska pollock and pink salmon. Pollock and salmon made up approximately 73% of the annual marine finfish catch of Alaska (Crapo and Bechtel 2003). It has been estimated that over a million tons of fish processing byproducts are generated each year from the fishing industry in Alaska (Crapo and Bechtel 2003). Some of these byproducts are converted into fish meal; however, much of this material is underutilized and dumped back into the ocean (Crapo and Bechtel 2003). Underutilized byproducts include fish skin, which is an excellent source of gelatin.

The amino acid compositions of mammalian gelatins are remarkably consistent when compared to those from fish species. Glycine, the simplest amino acid, accounts for approximately one-third of the total amino acid residues in mammalian gelatins, proline and hydroxyproline for approximately one-fifth, and alanine for approximately one-ninth. In all, these four amino acids account for approximately two out of every three amino acid residues in mammalian collagen used in gelatin manufacturing (Balian and Bowes 1977). Fish collagens show a wider variation in amino acid composition, resulting in different physical behaviors from fish gelatins. Their hydroxyproline and, to a lesser extent, proline contents are lower than those of mammalian collagens and this is compensated by higher concentrations of both serine and threonine (Balian and Bowes 1977). Consequently, cold-water fish gelatin solutions behave as viscous liquids at room temperature, which could make them desirable in some specific applications, such as ice cream, yogurt, dessert gels, confections, and imitation margarine (Regenstein and Chaudry 2002). Choi and Regenstein (2000) reported that fish gelatin had less undesirable off-flavor and off-odor and better release of aroma. This resulted in stronger flavors and offered new opportunities for food product development. Antoniewski et al. (2007) reported that bovine gelatin reduced water loss and color deterioration in gelatin-coated beef. Sensory analysis of beef tenderloins confirmed that color deterioration was reduced, and flavor was not affected by the application of the gelatin coating. They concluded that the gelatin coating reduced color deterioration by acting as a barrier to oxygen.

Hydrocolloid films have good barrier properties with regard to oxygen, carbon dioxide, and lipids but not to water vapor (Bourtoom et al. 2006). It is expected that gelatin, like other protein films, has sufficiently low oxygen permeability to serve as an effective barrier, especially at low water activities (Krochta and De Mulder-Johnston 1997, Krochta 1998, Sothornvit and Krochta 2000, Perez-Gago and Krochta 2001, Carvalho and Grosso 2004). Most hydrocolloid films also possess superb mechanical properties, which are quite useful for stabilizing fragile food products. The viscoelastic properties of gelatins, including gel strength, melting or setting temperatures, and viscosity are also important parameters for product development in the food industry (Choi and Regenstein 2000). Tensile strength and the elasticity of fish skin gelatin films are critical parameters for defining potential food packaging applications (Sobral et al. 2001).

The objective of this study was to evaluate the oxygen permeability and the mechanical and viscoelastic properties of both pollock and salmon skin gelatin films, and to correlate these results with amino acid composition.

Materials and methods

Gelatins

Alaska pollock (*Theragra chalcogramma*) and pink salmon (*Oncorhynchus gorbuscha*) skin gelatins were prepared using an extraction procedure reported by Avena-Bustillos et al. (2006).

Amino acid composition

Dry gelatin (1-2 g) was hydrolyzed (200 μL, 0.1% phenol in 6 N HCl) at 110ºC for 24 h and dissolved in dilution buffer to a final volume of 10 ml. After the sample was spun down, 50 μL was injected into a Beckman 6300 amino acid analyzer (Beckman Instruments Inc., Fullerton, California). Concentrations of amino acids (nmoles per injection) were obtained and mole percent calculated. Cysteine is destroyed during hydrolysis with 6N HCl. Cysteine was determined by oxidation with performic acid, yielding the acid stable form cysteic acid, prior to the standard acid hydrolysis. The conversion of cysteine is >90%.

Gel strength

Gel strength (Irish standard 1953) was determined on a 6.66% gel (w/w) by first adding 7.5 g gelatin to 105 ml distilled water and mixing with a stir bar for 5 min at 500 rpm. Then the solution was heated in a water bath at 60ºC for one hour with occasional stirring. The gelatin solution was degassed by vacuum and 25 g of the solution was poured into 30 ml beakers, which were refrigerated at 2ºC (maturation temperature) for 48 h. Each sample was 3.3 cm in diameter and 6 cm in height. Gel strength was determined at 2ºC on an Instron model 5500R Universal Testing Machine (Instron Corp., Canton, Massachusetts) using a 100 N load cell. A 1.27 cm diameter flat-faced cylindrical stainless steel plunger was used with the cross-head speed set at 10 mm per min. Measurements were taken as quickly as possible to reduce temperature change and experimental error. The maximum force (in N) was recorded when the plunger penetrated 4 mm into the gelatin gels and there were four replications for each treatment.

pH

The pH of 6.66% (w/w) gelatin solutions at room temperature was measured with a Beckman model 390 pH meter (Beckman Instruments Inc.). Eight readings were made for each sample.

Gel clarity

Clarity was determined by measuring transmittance at 620 nm in a Varian spectrophotometer through 6.66% (w/w) gelatin solutions heated at 60ºC for one hour. Eight readings were made for each sample.

Viscoelastic properties

Dynamic viscoelastic measurements were determined with a Brookfield digital rheometer (model DV-III+) set up with a TC-500 refrigerated bath/circulator using a model 107 programmable temperature controller running Rheocalc for Windows (Brookfield Engineering Laboratories Inc., Middleboro, Massachusetts). A small sample adapter along with a spindle SC4-21 (0.66 mm diameter, 1.23 mm long) was used to measure the gel set point of each gelatin. For the experiments, 8.5 ± 0.1 g of a 6.66% (w/w) gelatin solution was added to the small sample adapter. The initial testing temperature was 35ºC for mammalian gelatin and 25ºC for fish gelatin. Samples were allowed to rest in the small sample adapter for 30 minutes to equilibrate to the initial test temperature. The temperature was then lowered at a rate of 1ºC per min to 2ºC or until the gel set point was reached. Eight readings were made for each sample.

Gelatin film casting

Gelatin solutions (Irish standard 1953) were prepared by dissolving 7.5 g of gelatin in 105 ml distilled water (6.66% w/w) and mixed for 5 min at 500 rpm. These solutions were then heated in a water bath at 60ºC for 60 min, degassed, and 20 ml of it was poured onto a flat Mylar film on a glass plate. Stainless steel bar spreaders with 0.06 or 0.08 mil gaps were used to obtain mammalian and fish gelatin films with the same thicknesses. The films were dried overnight at ambient temperature and peeled from the Mylar surface. Circular (65 mm diameter) pieces of the film were then cut using a round watch glass and a razor blade. Film thickness was measured with a micrometer at five random positions before testing water vapor permeability.

Water vapor permeability of gelatin films

The ASTM E96-80 (ASTM 1989) "water method," modified to estimate the percent relative humidity at the film underside (McHugh et al. 1993), was used to measure water vapor permeability of gelatin films. Eight 65 mm diameter gelatin films were mounted on polymethylmethacrylate test cells (50.8 mm diameter opening, 9.525 mm height) filled with 6 ml distilled water. A top cover smeared with a thin layer of silicone high vacuum grease (Dow Corning, Midland, Michigan) was placed over the film. The top and bottom parts of the test cells were then fitted together tightly using screws to avoid vapor leaks through cell joints. Test cells were placed inside a cabinet containing anhydrous calcium sulfate (W.A. Hammond Drierite Co., Xenia, Ohio). The cabinet was equipped with a fan to provide sufficient air movement to ensure 0% RH throughout the chamber. The films were equilibrated for an hour prior to the initial weighing of the test cells. Subsequently, at least nine weighings were done every two hours over the next two days. The cabinet was inside an incubator set at 25ºC.

The water vapor transmission rate (g per h) was calculated by simple linear regression analysis of the slope of weight loss vs. time. Water vapor flux, permeance, and water vapor permeability were calculated according to values of test cell mouth area, percent RH difference from the film underside, and mean film thickness, respectively (ASTM 1989, McHugh et al. 1993, Avena-Bustillos and Krochta 1993). Eight replicates were made for each sample.

Oxygen permeability of gelatin films

The oxygen permeability of gelatin films was determined using a Coulox detector at 55% RH through a 100% oxygen differential. An Ox-Tran® 2/20 modular system (Modern Controls Inc., Minneapolis, Minnesota) was utilized to measure oxygen transmission rates through the films according to standard method D3985 (ASTM 1995). Oxygen transmission rates were determined at 23ºC and 55 ± 1% RH. The films were prepared by cutting circular pieces using a round watch glass and razor blade. Film thickness was measured with a micrometer at five random positions before testing oxygen permeability. Each film was placed on a stainless steel mask with an open testing area of 5 cm^2. Masked films were placed into the test cell and exposed to 98% N_2 + 2% H_2 flow on one side and pure oxygen flow on the other. The system was programmed to have a 2 h waiting period and up to 20 cycles of readings every 2 h to allow the films to achieve equilibrium. Oxygen permeability was calculated by dividing oxygen transmission rate by the difference in oxygen partial pressure between both sides of the film (1 atm) and multiplying by the average film thickness. The oxygen permeability was reported in $cm^3 \cdot \mu m$ per $m^2 \cdot day \cdot kPa$ units. Four replicates of each film were evaluated.

Tensile tests

Samples were prepared on a Carver press (Carver, Inc., Wabash, Indiana) using a die (The Right Image Die Co., Sacramento, California) made according to the dimensions found in the ASTM method D638-02a, Type 1 (ASTM 2002). Samples were placed in a chamber at humidities of 0% (anhydrous calcium sulfate), 33% (magnesium chloride), 54% (magnesium nitrate), or 75% (sodium chloride) for 72 hours at 20ºC. Tensile tests were performed on the Instron 5500R using a 100 N load cell. Samples were placed in pneumatic grips (30 N) with an initial grip separation of 100 mm and that increased at a rate of 10 mm per minute. The tensile strength (TS) and elongation (percent E) were determined using Series IX software (Instron Corp.).

Statistical analyses

Data were analyzed by *t*-tests, one-way analysis of variance, and Tukey's multiple comparison tests at 95% confidence level using Minitab version 14.12.0 statistical software (Minitab Inc., State College, Pennsylvania).

Results and discussion

Amino acid composition

In general, the collagens from warm-water fish gelatins have higher concentrations of proline and hydroxyproline than cold-water fish gelatins, but lower concentrations than mammalian gelatins (Eastoe and Leach 1977). Proline and hydroxyproline stabilize the ordered triple helical conformation when the renatured soluble protein forms a gel network (Gomez-Guillen et al. 2002, Haug et al. 2004). In particular, hydroxyproline is believed to play an important role in the stabilization of the triple-stranded collagen helix due to the hydrogen bonding of its hydroxyl group. The higher content of proline, hydroxyproline, and alanine in mammalian gelatins has been reported as one of the major reasons for its higher viscosity properties when compared to fish gelatins (Sarabia et al. 2000). As indicated in Table 1, salmon gelatin showed higher values of proline, hydroxyproline, and alanine than pollock gelatin.

Physical properties of solutions and gels

As shown in Table 2, 6.66% (w/w) pollock and salmon gelatin solutions had similar low pHs, most likely due to the similar method of extraction. Stainsby (1977) indicated that small variations in pH could give rise to quite marked changes in a gelatin's intrinsic viscosity, especially at low ionic strength (0.2% gelatin concentration). As indicated in Table 2, the salmon gelatin solution was very opaque resulting in low percent transmittance values.

Salmon gelatin had a numerically, but not significantly, higher gel set temperature than pollock gelatin, as shown in Table 2. In a previous study (Chiou et al. 2006), both fish gelatin solutions showed large increases in their elastic modulus at around 5ºC. This large modulus increase resulted from changes in the molecular conformation of the gelatin during the cooling process. At higher temperatures, molecules in the gelatin solution behave as random coils giving these gelatins a low elastic modulus. As the temperature is lowered, the molecules begin to form triple helical junction zones. The triple helix is the structural unit characteristic of collagens, which largely make up gelatin. Thus, gelatin molecules in solution partly revert to this characteristic collagen structure at lower temperatures. As more physical cross-linking occurs, a network structure eventually develops and the elastic modulus rapidly increases in value. Gelatin samples with larger elastic modulus values,

Table 1. Amino acid composition of cold-water fish skin gelatins.

Amino acid	Pollock (mole %)	Salmon (mole %)
Alanine	10.88	12.49
Arginine	5.18	5.06
Aspartic acid	5.21	5.12
Cystine	0.14	0.08
Glutamic acid	7.17	7.25
Glycine	35.74	35.54
Histidine	0.80	0.87
Homocystine	0.16	0.12
Hydroxylysine	0.61	0.76
Hydroxyproline	5.30	5.56
Isoleucine	1.07	0.97
Leucine	2.10	1.83
Lysine	2.78	2.47
Methionine	1.13	1.00
Phenylalanine	1.20	1.27
Proline	10.09	10.79
Serine	5.85	4.73
Threonine	2.68	2.55
Tyrosine	0.24	0.13
Valine	1.67	1.41

Table 2. Physical properties of cold-water fish gelatin solutions and gels.

Measurement	Pollock	Salmon
pH	3.25 ± 0.13^{NS}	3.24 ± 0.04
Transmittance, %	92.59 ± 0.62^{b}	44.00 ± 0.30^{a}
Gel set temperature, °C	4.58 ± 0.63^{NS}	5.33 ± 0.29
Gel strength, N	1.81 ± 0.41^{NS}	2.38 ± 0.26

Values are means with SD.

[a,b]Significant differences within row at $p < 0.05$.

[NS]No significant difference.

Table 3. Barrier properties of cold-water fish skin gelatin films.

Gelatin film	Water vapor permeability ($g \cdot mm/m^2 \cdot h \cdot kPa$)	Oxygen permeability ($cm^3 \cdot \mu m/m^2 \cdot day \cdot kPa$)
Pollock	0.56 ± 0.03^{NS}	2.50 ± 0.43^{a}
Salmon	0.59 ± 0.04	3.47 ± 0.46^{b}

Values are means with SD.
^{a,b}Significant differences within column at $p < 0.05$.
^{NS}No significant difference.

as indicated by higher gelation temperatures, contain higher concentrations of helical structures (Gomez-Guillen et al. 2002, Joly-Duhamel et al. 2002, Simon et al. 2003). As shown in Table 2, salmon gelatin set at a higher temperature than pollock gelatin, although a significant difference was not detected, suggesting that salmon gelatin had a higher concentration of helical structures.

Gelatin is a polymeric mixture that forms thermoreversible gels. Even after formation, the structure of the gel is not static and continues to develop due to the instability of low energy interactions within the gel network (Tosh et al. 2003). As shown in Table 2, pollock gelatin had lower gel strength than salmon gelatin. The difference in thermostability between pollock and salmon gelatins can be attributed to differences in proline and hydroxyproline content. The lower content of these two amino acids compared with salmon gelatin (Table 1) probably gives pollock gelatin its lower gel modulus, as well as lower gelation and melting temperatures (Haug et al. 2004). A higher proline and hydroxyproline concentration has been shown to result in higher gelation temperatures, consistent with results shown in Table 2 for salmon gelatin.

Barrier properties of cold-water fish skin gelatin film

As shown in Table 3, the water vapor permeabilities of the cold-water fish gelatin films were not significantly different and averaged 0.55 $g \cdot mm$ per $m^2 \cdot h \cdot kPa$. This low water vapor permeability could be related to the temperature (23ºC) used for casting the aqueous gelatin solutions. This casting method should result in amorphous films, which have lower water vapor permeability values compared to films containing helical structures (Chiou et al. 2009).

Hydrophilic films, such as gelatin films, have been shown to exhibit a positive slope for the relationships between thickness and water vapor permeability resulting from variations in the water vapor partial pressure at the underside of films during testing (McHugh et al. 1993). Therefore, it is essential for practical comparison purposes of water

vapor permeability values to get similar film thicknesses. There was no statistical difference between film thicknesses among the different gelatins tested and the mean thickness of gelatin films and pooled standard deviation were 0.0496 mm and 0.0041 mm, respectively. The average relative humidity at the underside of the film was estimated at 84% for these experiments using the water vapor correction method (McHugh et al. 1993).

Hydrophilic edible films are very prone to plasticization, with water tending to cluster within the polymer matrix. It is recognized that an increase in plasticizer concentration is directly proportional to an increase in water vapor permeability (McHugh et al. 1993; Arvanitoyannis et al. 1998a,b; Carvalho and Grosso 2004).

Oxygen permeability of pollock gelatin films was significantly lower than that of salmon gelatin films as indicated in Table 3. This behavior is related to the amino acid composition of the collagen, with pollock skin gelatin having lower proline and hydroxyproline concentrations than salmon skin gelatin. In general, the amino acid profile of salmon gelatin is intermediate between those of pollock and mammalian gelatins (Avena-Bustillos et al. 2006). Bourtoom et al. (2006) reported oxygen permeability values on the order of 351-624 $cm^3 \cdot \mu m$ per $m^2 \cdot day \cdot kPa$ at 25ºC and 50% RH for edible films made from water-soluble fish proteins derived from surimi wash-water that are unlikely to contain significant amounts of collagen. Those oxygen permeability values are 150-270 times higher than the values of cold-water fish skin gelatin films (Table 3). Lim et al. (1999) reported that oxygen permeability of mammalian gelatin films remained constant in the range of 45-60% RH while oxygen permeability increased exponentially with higher percent RH.

The oxygen permeability of the gelatin films studied was lower than polymeric and most biopolymer films. For instance, oxygen permeability values of gelatins were lower than the value of 8.4 $cm^3 \cdot \mu m$ per $m^2 \cdot day \cdot kPa$ at 23ºC and 50% RH reported by McHugh and Krochta (1994) for edible films formulated with a 1:1 ratio of whey protein isolate and sorbitol. Sorbitol is a plasticizer that increases molecular mobility (McHugh and Krochta 1994). The gelatin films tested in this study did not require plasticizers. This probably contributed to the lower oxygen permeability values. The lower oxygen permeability of pollock gelatin films (2.5 $cm^3 \cdot \mu m$ per $m^2 \cdot day \cdot kPa$) can be considered superior when compared with several other edible films (Mehyar and Han 2004). The lower oxygen permeability of pollock skin gelatin could lead to new applications, such as films, coatings, and capsules for use in the food and drug industries to reduce oxidation and increase shelf life. Villegas et al. (1999) also reported a beneficial oxidative and color stability effect of gelatin coating on cooked ham and bacon pieces during frozen storage.

Table 4. Mechanical properties of cold-water fish skin gelatin films at different relative humidities.

	Tensile strength (MPa)		Elongation (%)	
% RH	Pollock	Salmon	Pollock	Salmon
0	36.8 ± 6.9	NA	1.73 ± 0.46	NA
33	64.7 ± 6.4[b]	46.7 ± 7.7[a]	3.06 ± 0.30[NS]	2.31 ± 0.33
53	61.2 ± 5.4[b]	38.3 ± 11.5[a]	2.61 ± 0.37[NS]	1.81 ± 0.58
75	51.3 ± 8.6[b]	34.5 ± 8.1[a]	3.98 ± 0.77[NS]	4.45 ± 2.89

Values are means with SD.
[a,b]Significant differences within row at $p < 0.05$.
[NS]No significant difference.
NA: Data not available.

Mechanical properties

Changes in relative humidity imparted some significant effects on the mechanical properties of gelatin films. As humidity increased from 0% to 33% RH, tensile strength (TS) and percent elongation (E) increased significantly (Table 4). Tensile strength decreased as RH increased from 33 to 75%, while elongation increased as RH increased in both pollock and salmon gelatin films (Table 4). Water can act as a plasticizer in films, reducing the strength of intermolecular bonds while allowing for greater molecular mobility. As a result, decreases in TS and increases in percent E are commonly observed with increasing RH (Gontard et al. 1993). Pollock gelatin films showed higher tensile strength than salmon gelatin (Table 4). In contrast to this study, Haug et al. (2004) reported that improved mechanical behavior is related to higher concentrations of proline and hydroxyproline, which allows for greater physical cross-linking in films. As the gelatin dries, water evaporates and the polymers become entangled, reducing molecular mobility of films (Lukasik and Ludescher 2006).

Conclusion

This study demonstrated significant differences in oxygen permeability, and mechanical and viscoelastic properties between pollock and salmon gelatin samples. These are most likely due to differences in their amino acid composition. The lower oxygen permeability and higher mechanical properties of pollock gelatin films can be useful for a number of applications in the drug and food industries.

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Bioactivities Found in Sardine (*Sardinops sagax caerulea*) Byproduct Hydrolysates

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Abstract

The sardine canning industry uses approximately 40-45% of the sardine body, leaving the rest (heads, viscera, and tails) as processing byproducts that are subjected to reduction. However, these byproducts have high protein content that can be used to produce bioactive peptides. The objective was to produce bioactive peptides through the autolysis of sardine (*Sardinops sagax caerulea*) processing byproducts. Hydrolysis conditions of the homogenate at different pH, temperature, and reaction times were monitored by measuring absorbance at 540 nm (Biuret reaction) and tricine SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis). The autolytic generation of low molecular weight (<30 kDa) peptides was best at the pH 8.5, 37ºC and 45 min reaction time, and physiological pH (6.5) was used as a control. Fractions (<1, 1-5, 5-10, 10-30 kDa) were collected by ultrafiltration. Antihypertensive (inhibition of angiotensin-converting enzyme [ACE] and by IC_{50}), antimicrobial (well diffusion assay), and antioxidant bioactivity (TBARS) of fractions were evaluated. Fractions of 1-5 kDa (pH 8.5) and <1 kDa (physiological

pH) gave the highest ACE inhibition values of 68.4 and 62.9%, respectively. Fractions that gave the lowest IC_{50} values were the <1 and 1-5 kDa (physiological pH), with 0.48 and 0.81 mg per ml, respectively. Only the 10-30 kDa fraction (physiological pH) showed microorganism inhibition. However, the nature of the inhibition remains to be elucidated. All fractions showed antioxidant activity. Sardine processing byproducts can be used to obtain bioactive peptides without using commercial enzymes.

Introduction

Sardines are an abundant and very important fish resource in the Mexican Republic, and represented around 42% of total Mexican fish catch in 2007 (CONAPESCA 2009). Most of the harvest (near 100%) occurs in the Gulf of California, where the state of Sonora has almost 80% of the national production. In Sonora, the sardine harvest has fluctuated between 300,000 and 700,000 metric tons in the past; approximately 62.5% of the harvest is used for fish meal production, 30% is canned, and the remaining 7.5% is directed toward the frozen fish market.

The canning industry uses only the fillet muscle portion of the whole sardine, representing approximately 40-45% yield; the remainder, with a relatively high protein content, is subjected to reduction. To exploit thus underutilized protein source, processing byproducts can be treated by new or existing technology to make value added coproducts. The production of low molecular weight (MW) polypeptides with biological activity made from protein hydrolysis is one of the value adding technologies. Protein hydrolysis can be accomplished with the use of either exogenous or endogenous enzymes, although endogenous approach makes the process potentially more cost effective because hydrolysis can be accomplished with the fish's own digestive enzymes. Several approaches have been proposed for the production of bioactive peptides from different protein sources. These include the use of gastrointestinal proteolytic enzymes (Kitts and Weiler 2003), fermentative processes (Gobbetti et al. 2002), or a combination of both techniques (Pihlanto-Leppälä et al. 1998). Studies have identified bioactive peptides from marine resources (e.g., muscle, algae) and their byproducts (fish skin). Among the bioactivities that have been found in these studies are antihypertensive (Yokoyama 1992, Suetsuna and Nakano 2000), antioxidant (Mendis et al. 2005), and antimicrobial (Fernandes and Smith 2002) activities. The objective of the present study was to obtain bioactive peptides from the autolysis of homogenates from Monterey sardine byproducts (heads, viscera, and tails) generated during processing for the canning industry.

Materials and methods

Raw material

Monterey sardine byproducts (heads, viscera, and tails) were obtained during processing for canning at Productos Pesqueros de Guaymas S.A., located in Guaymas, Sonora, Mexico. Byproducts were collected in ziplock freezer bags with heads and tails stored separately from viscera. All reactants used in this study were obtained from Sigma Chemical Co. (St. Louis).

Monterey sardine byproduct homogenate production

A basic homogenate from these byproducts was made as follows: heads, tails:viscera (ratio 2:1) were placed on a C-35 STP Talsa cutter and processed for 1 min at maximum speed. The material was then placed in ziplock freezer bags in 2.5 kg portions and stored at –20ºC for later use.

Homogenate chemical composition

To determine the chemical composition of the basic homogenate, a proximate analysis (moisture, protein, lipid, and ash composition) was conducted following the methods of AOAC (2000).

Homogenate autolytic activity

In order to find optimal autolytic activity conditions for peptide production from the basic homogenate, the effects of temperature (0, 10, 20, 30, 40, 50, and 60ºC), reaction times (0, 45, and 120 min), and pH (2.5 to 10.5) were studied.

To evaluate the effects of temperature and reaction times, 100 g of the basic homogenate was further homogenized with 200 ml of HPLC water for 3 min at 50,000 rpm using a model SDT 1810 Tizzumer Tekman tissue homogenizer (Tekmar Co.). This material was then incubated at the established temperatures and reaction times. Reactions were stopped by heating the material at 80ºC for 45 min. To obtain the soluble peptides, samples were centrifuged at 35,000 G for 30 min at 0-2ºC, using a model J2-21 Beckman refrigerated centrifuge (Beckman Instruments Inc., Palo Alto). The supernatants were filtered through No. 4 Whatman paper and the filtrate was stored at –80ºC for later analysis.

After optimal reaction times and temperatures for peptide production had been determined, analysis of pH effect analysis was conducted. The effect of pH on peptide production was evaluated by incubating the material at pH between 2.5 and 10.5. The buffers used for this evaluation were citric acid 0.4 M + NaOH (for pH 2.35, 3.35, and 4.35), malic acid 0.4 M + NaOH (for pH 5.35 and 6.35), TRIS 0.4 M + HCl (for pH 7.55 and 8.55), and glycine + NaOH 0.4 M (for pH 9.55 and 10.9). For this study, 50 g of the basic homogenate was processed with 250 ml of the respective buffer solution for 3 min at 50,000 rpm, using a model SDT

1810 Tizzumer Tekman tissue homogenizer. This material was then incubated at the corresponding pH. Halting the reaction, extraction of soluble peptides, and sample storing were accomplished as previously described.

Hydrolysis evaluation with the Biuret reaction

Peptide production under the different hydrolysis conditions was evaluated by monitoring absorbance at 540 nm for the Biuret reaction using a Cary BIO 50 spectrophotometer (Varian Instruments, La Jolla).

SDS-PAGE and tricine SDS-PAGE

Analysis of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and tricine SDS-PAGE using 17% and 16.5% polyacrylamide on separating gels, respectively, allowed evaluation of the effectiveness of different hydrolysis conditions. SDS-PAGE was carried out as described by Laemmli (1970) with a constant voltage of 120 V for 2 h. Tricine SDS-PAGE was carried out as described by Schägger and von Jagow (1987) with a voltage of 30 V for the first hour and a voltage of 105 V for the second hour. Gels were stained with Coomassie blue and destaining with acetic acid solution allowed the protein bands to be visualized. Broad range MW standards (Bio-Rad Laboratories, Hercules) were included in both electrophoresis gel systems.

Monterey sardine byproduct hydrolysate production

After evaluation of the different hydrolysis conditions, Monterey sardine byproduct hydrolysates were produced as follows: the basic homogenate (150 g) was further homogenized with 750 ml of buffer (TRIS + HCl 0.4 M, pH 8.55) for 8 min at 50,000 rpm in a water-ice bath, with a model SDT 1810 Tizzumer Tekman tissue homogenizer. Physiological pH (6.3) and pH 8.5 at t = 0 min were used as controls. Although results suggested that a 40ºC incubation temperature resulted in higher absorbencies on the autolytic activity of sardine byproducts (see Fig. 1), this last mixture was incubated at 37ºC for 45 min, since it is a commonly used temperature for enzymatic reactions. Reactions were stopped by heating the sample to 80ºC for 45 min. To obtain soluble peptides, samples were centrifuged at 16,000 G for 30 min at 0-2ºC, using model J2-21 Beckman refrigerated centrifuge. The supernatant was then vacuum filtered using no. 4 Whatman paper and the filtrate stored at –80ºC for later analysis.

Ultrafiltration of hydrolysates produced and lipid extraction of fractions

Fractionation of hydrolysates was performed by ultrafiltration as follows: two liters of each hydrolysate were sequentially passed through Millipore membranes of 30, 10, 5, and 1 kDa (Millipore Corporation,

Billerica), producing 4 fractions (<1 kDa, 1-5 kDa, 5-10 kDa, and 10-30 kDa). This ultrafiltration pressure was 1-1.3 bars with a flow rate of 16-20 ml per min using a model #72-370-000 Manostat Preston peristaltic pump (Barnant Co.). Lipid in the ultrafiltration fractions was removed by extraction to avoid possible lipid masking of activities in the fractions. Extraction was performed following the methodology described by Folch et al. (1957).

Angiotensin II–converting enzyme (ACE) inhibitory activity assay

The ACE inhibition has been used to determine potential antihypertensive activity in proteinaceous samples (Suetsuna and Nakano 2000, Miguel et al. 2004). ACE inhibition was assayed following the method of Cushman and Cheung (1971) with some modifications: 150 µL of substrate solution (5 mM Hip-His-Leu in 50 mM sodium borate buffer containing 0.3 M NaCl, pH 8.3) was mixed with 50 µL of the different ultrafiltration fractions (containing the peptide fractions of interest) and 40 µL of an ACE solution (0.1 U per ml) prepared from rabbit lung powder containing ACE (2 U). After incubation at 37ºC for 60 min, the reaction was stopped by the addition of 250 µL of 1 M HCl. The released hippuric acid was then extracted with 1.5 ml of ethyl acetate. After 5 min of vigorous vortexing, samples were centrifuged at 1500 G for 15 min at room temperature and then 1 ml of the organic phase (upper layer) was transferred to a glass tube for ethyl acetate evaporation at 95ºC for 30 min. The residue was then dissolved in 1 ml of HPLC water and its absorbance was read at 228 nm.

The ACE inhibitory activity was calculated using the following formula:

$$\% \text{ inhibitory activity} = [(B - A) / (B - C)] \times 100$$

where:

A = optical density of both ACE and ACE inhibitory component
B = optical density without the ACE inhibitory component
C = optical density without the ACE

The ACE inhibitory activity was also expressed as the concentration of peptide (mg per ml) required to inhibit 50% of ACE activity (IC_{50}).

Antioxidant activity assay

Fractions (≤1, 1-5, 5-10, 10-30 kDa) obtained by ultrafiltration of sardine byproduct hydrolysates were assayed for antioxidant activity following the methodology of Osawa and Namiki (1985) with modifications in the type of oil used. Each fraction (1.3 mg) was dissolved in 10 ml of 50 mM (pH 7.0) phosphate buffer, 0.13 ml of cod liver oil, and 10 ml of 99.5% methanol in a glass test tube. The mixture was diluted to 25 ml with distilled water and incubated in the dark at 40 ± 1ºC in a water bath.

Oxidation progress was evaluated every day for a week with the thiobarbituric acid reactive substances (TBARS) assay as described by Ohkawa et al. (1979) as follows: samples to be incubated (50 µL) were diluted with 0.8 ml of distilled water, 0.2 ml of 8.1% sodium dodecylsulphate, and 1.5 ml of 0.8% TBA solution. The mixture was incubated at 5ºC for 1 h and then at 95ºC for 1 h in the dark. Absorbances were read at 532 nm and the data was plotted vs. incubation days. Two controls were included in the study: tocopherol as a natural antioxidant and butylated hydroxytoluene (BHT) as a chemical antioxidant.

Antimicrobial activity assay

Antimicrobial activity was measured by the well diffusion assay following the methodology described by Schillinger and Lücke (1989). Hydrolysate fractions were dissolved in phosphate buffer (10 mM, pH 7) at 0, 50, and 100 mg per ml. Wells were filled with 30 µL of each fraction. Plates were previously inoculated with one of the tested microorganisms at a logarithmic phase (10^6-10^7 cfu per ml). Antimicrobial activity was evaluated by the formation of an inhibition halo when plates were incubated at 37ºC for 24 h. Microorganisms tested were *Listeria innocua* ATCC 33090, *Enterococcus faecium* MXVK29, *Staphylococcus aureus* NCTC 8325, *Brochothrix thermosphacta* NCIB-10018, and *E. coli* JM P101.

Tricine SDS-PAGE analysis

Fractions that presented antimicrobial activity were further analyzed by tricine SDS-PAGE following the procedure of Shägger and von Jagow (1987) using a separating gel of 3% C [(grams crosslinker × 100)/(grams monomer + grams crosslinker)] and 49.5% T [total monomer in solution (w/v)]. Duplicate samples (to generate two lines) were injected and run at 40 mA for 2 h at 5ºC. One lane, stained with Coomassie blue, was used to develop the different peptides contained in the sample; the second lane was set on an agar plate previously inoculated with the sensitive microorganism (*Listeria innocua* in this case) in order to verify the presence of inhibition halos produced by a protein band after incubation at 36ºC for 24 h.

Protease treatments

In order to probe the inhibitory nature generated by hydrolyzed fractions, a sample at (100 mg per ml in 10 mM phosphate buffer, pH 7) was treated with the following exogenous proteases: chymotrypsin, trypsin, lysozyme, proteinase K, and pepsin. After incubation at 37ºC for 6 h, the enzyme reaction was stopped by increasing the temperature to 70ºC for 15 min. Antimicrobial analysis (well diffusion assay) was then conducted on samples following the recommendations of Schillinger and Lücke (1989). Two controls were used; both were without protease

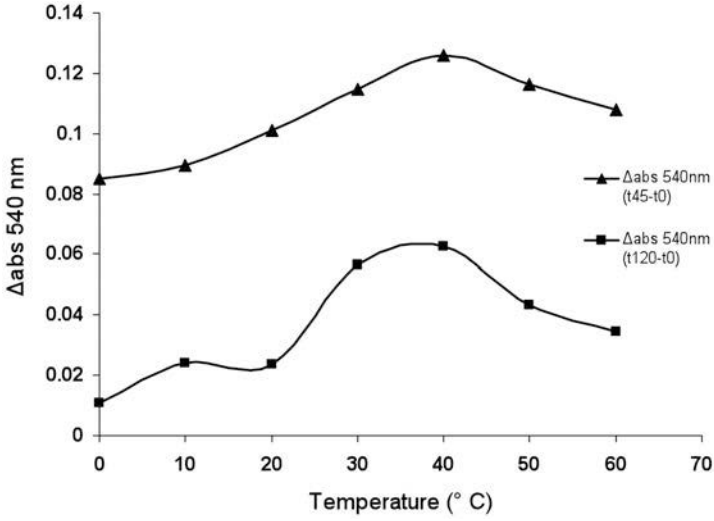

Figure 1. Effect of temperature and incubation time (t = 45 and 120 min) on the autolytic activity of the Monterey sardine (*Sardinops sagax caerulea*) byproduct homogenate; abs = absorbance.

addition, and one was non-thermally treated sample (control 1) while the other was a thermally treated sample (control 2). All analyses were carried out in triplicate.

Results and discussion

Chemical composition of homogenate

The homogenate made from the sardine byproducts (heads, tails:viscera, 2:1 ratio) had a chemical composition of 58.4 ± 1.6% moisture, 24.7 ± 0.6% lipid, 10.8 ± 0.4% protein, and 4.3 ± 0.9% ash. Monterey sardine has been characterized in the literature as being a relatively fatty species with a lipid content fluctuating between 1.1 and 8.4% depending on the season (Pacheco-Aguilar et al. 2000). In the present study, the homogenate had a significantly higher lipid content possibly due to the raw material being a processing byproduct that may possess a higher lipid content. In contrast, the protein content of the byproduct homogenate was lower than is found in Monterey sardine muscle (14.0-20.3%) (Pacheco-Aguilar et al. 2000); however, the processing byproduct still has a sufficient protein content to make it a good resource for these inhibitory peptides.

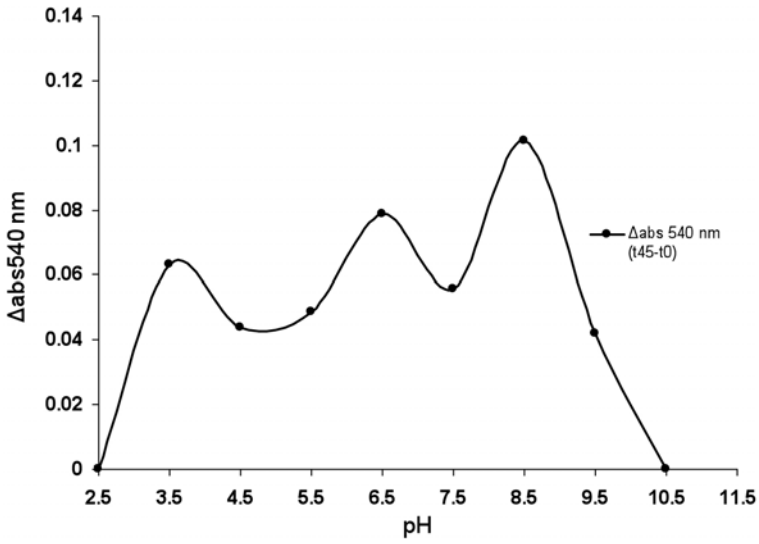

Figure 2. Effect of pH on the autolytic activity of Monterey sardine (*Sardinops sagax caerulea*) byproduct homogenate. t = 45 min; abs = absorbance

Endogenous proteolytic activity

Effect of temperature and reaction time

As shown in Fig. 1, the incubation temperature that had a major influence over the endogenous proteolytic activity of homogenate was in the 40ºC range. This result was corroborated by the SDS-PAGE analysis (data not shown) in which major hydrolysis was observed at this temperature (40ºC). The hydrolysis temperature subsequently used in this study was set at 37ºC because this is a commonly used temperature in analyzing enzymatic reactions and well within the optimal hydrolysis range (Fig. 1). Similar to our results, Castillo-Yañez et al. (2004) found that 45ºC was the optimum proteolytic activity temperature of Monterey sardine viscera extract.

The incubation time of 45 min was sufficient for peptide and polypeptide production (Fig. 1). Additionally, SDS-PAGE analysis showed no major differences in peptide production from 45 to 120 min of incubation (data not shown).

pH effect on autolytic activity

Optimal endogenous proteolytic activity occurred with a pH of 8.5 at 37ºC (Fig. 2), indicative of alkaline proteolytic enzyme action. This last

Table 1. Protein concentration and angiotensin-converting enzyme (ACE) inhibitory activity from defatted fractions.

Treatment	Fraction (kDa)	Peptide concentration (mg/ml)	ACE inhibition (%)
Physiological pH (1st repetition)	10-30	0.76	47.4
	5-10	0.82	54.3
	1-5	0.41	33.2
	<1	0.02	0.00
Physiological pH (2nd repetition)	10-30	0.66	34.5
	5-10	1.24	48.4
	1-5	1.09	56.1
	<1	0.67	62.9
pH 8.5, t = 0	10-30	2.13	47.7
	5-10	1.09	30.8
	1-5	0.54	15.2
	<1	0.05	1.5
pH 8.5, t = 45 (1st repetition)	10-30	1.71	22.2
	5-10	1.77	43.7
	1-5	1.15	42.9
	<1	1.79	57.7
pH 8.5, t = 45 (2nd repetition)	10-30	2.34	56.1
	5-10	2.86	52.2
	1-5	1.57	68.4
	<1	1.17	29.3

result was corroborated by tricine SDS-PAGE analysis, where higher rates of hydrolytic changes were observed under these conditions (data not shown). Similar results were found by Castillo-Yañez et al. (2005, 2006), who characterized trypsin and chymotrypsin from Monterey sardine viscera and found higher activities at pH 8.0.

ACE inhibition of fractions

Preliminary studies of the different fractions obtained from ultrafiltration showed high ACE inhibition activity from practically all fractions due to excess lipid in the fractions. After the lipid was extracted the angiotensin-converting enzyme (ACE) inhibitory activities were determined (Table 1). The highest antihypertensive activities were found in the 1-5 kDa, pH 8.5, t = 45 min (2nd repetition) and <1 kDa, physiological pH (2nd repetition) fractions with 68.4 and 62.9%, inhibition respectively. A study by Kim et al. (2003) on squid ink crude extract showed

Table 2. Fraction peptide concentration required for angiotensin-converting enzyme (ACE) activity inhibition at 50%.

	IC_{50} (mg/ml)		
Fraction (kDa)	pH 6.5[a], 45 min (2nd repetition)	pH 8.5, 45 min (1st repetition)	pH 8.5, 45 min (2nd repetition)
10-30	1.18	3.32	2.04
5-10	1.26	1.80	2.71
1-5	0.81	1.43	1.32
< 1	0.48	1.43	1.54

[a]Physiological pH.

three different fractions with ACE inhibitory activity, with the highest having a 32% inhibition. Peptide/polypeptide fractions from the sardine byproduct hydrolysates showed higher antihypertensive activities than reported for the squid ink crude extract. Most active ACE inhibitory peptides have been found to be no larger than 5 kDa MW (Cheng et al. 2008), similar to this study. A better way to evaluate the bioactivity of peptides from hydrolysates can be shown by the peptide concentration required to inhibit the ACE activity at 50% (IC_{50}). Table 2 shows the IC_{50} values for each fraction under different hydrolysis conditions. Fractions that had the lowest values, therefore the best ACE inhibition per unit protein, were the <1 and 1-5 kDa at physiological 6.5 pH, with values of 0.48 and 0.81 mg per ml respectively. In a study by Hyun and Shin (2000), the authors reported IC_{50} values ranging from 0.26 to 246.7 mg per ml from bovine blood plasma hydrolysates. In the present study, the values ranged from 0.48 to 3.32 mg per ml indicating a high antihypertensive activity for peptides produced from autolysis of sardine byproducts.

A review by Byun and Kim (2002) has found that ACE has a preference for substrates (or competitive inhibitors) having hydrophobic amino acid residues such as proline, phenylalanine, and tyrosine at three positions from the *C*-terminal, with most natural inhibitors containing proline at their *C*-terminal. Another review by Kitts and Weiler (2003) suggests that for this type of inhibitor, mostly lysine, arginine, or proline are located at this end of the peptide. These results can give us an idea of the type of amino acids that could possess the inhibitors in the present study.

Antioxidant activity

Lipid oxidation occurring in food products is a problem, causing the deterioration of quality as rancid flavors and unacceptable tastes are developed, reducing food shelf life. In the present study, the different fractions obtained by autolysis of the sardine byproduct homogenates were tested for this activity using the TBARS analysis. Fig. 3 shows that

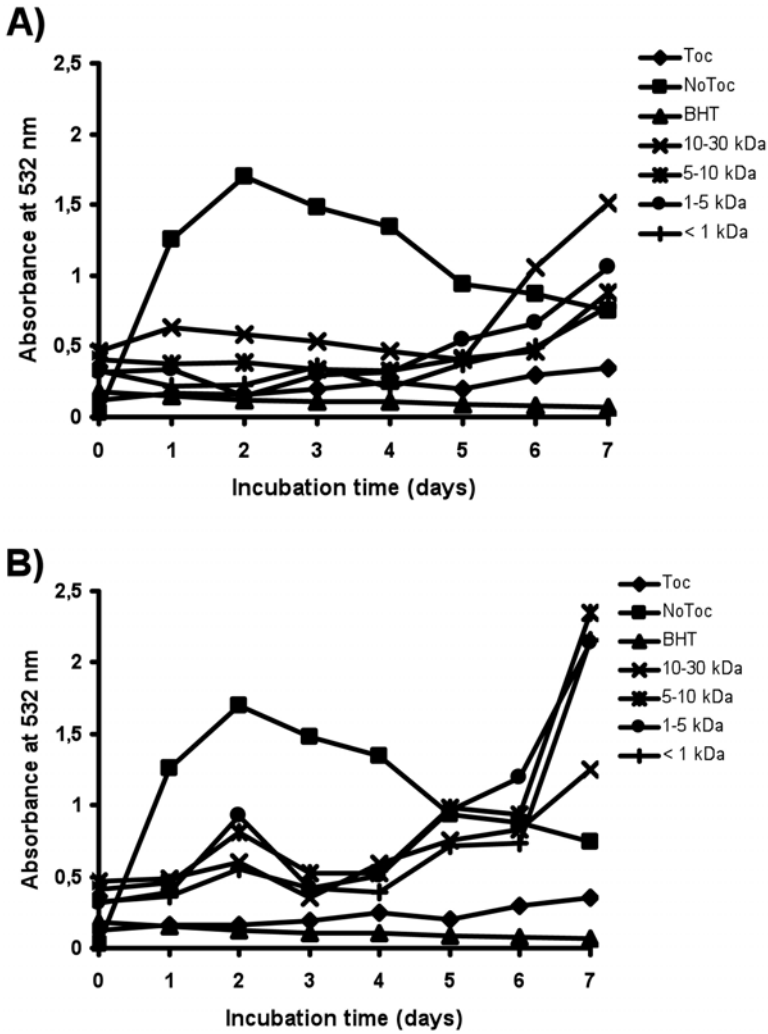

Figure 3. Antioxidant activity of different fractions obtained by ultrafiltration of hydrolysates of Monterey sardine (*Sardinops sagax caerulea*) byproducts (heads, tails, and viscera). (A) Antioxidant activity of hydrolysates produced at physiological pH (6.5). (B) Antioxidant activity of hydrolysates produced at pH 8.5. Toc = control with tocopherol, NoToc = control without tocopherol; BHT = butylated hydroxytoluene.

Table 3. Inhibition halos generated by the different microorganisms tested on the study. (1) Physiological pH, 5-10 kDa fraction. (2) Physiological pH, 10-30 kDa fraction, 45 min. (3) pH 8.5, 10-30 kDa fraction, 45 min.

Fraction	*L. innocua* ATCC 33090	*E. faecium* MXVK29	*S. aureus* NCTC 8325	*B. thermosphacta* NCIB-10018	*E. coli* JM P101
1	–	–	–	–	–
2	+	++	+	+	+
3	–	–	–	–	–

all fractions produced by the pH 8.5 and physiological treatment exhibited antioxidant activity with values lower than the control. However, this activity diminished toward the end of the incubation times at days 6 and 7 (Fig 3). Peptides produced under physiological pH presented higher and more stable antioxidant activity, especially the lower MW fractions (<5 kDa). These fractions presented activity similar to the antioxidant controls during the first two days, but subsequently the activity of the 1-5 kDa fraction started to diminish. Results in the present study agree with Saiga et al. (2003), who found antioxidant peptides in <1 kDa MW fractions from porcine myofibrillar protein hydrolysates. Further characterization of these peptides is needed, including determination of the relevant amino acid sequences.

Antimicrobial activity

Table 3 shows the antimicrobial activity of fractions produced from the sardine byproducts. Only the 10-30 kDa fraction produced under physiological pH conditions generated an inhibition halo at the tested concentrations. Robinette et al. (1998) also isolated three antibacterial proteins from acid extracts of channel catfish (*Ictalurus punctatus*) skin with MW ranging from 15.5 and 30 kDa, which behaved similarly to the present study. To further elucidate the nature of this antimicrobial activity, the fraction was analyzed in duplicate by electrophoresis and one of the lanes excised and set on an *L. innocua* inoculated agar. Electrophoretic analysis showed the presence of large amounts of peptides greater than 10 kDa MW and less than 22 kDa, with an intense band at approximately 13 kDa (data not shown). However, none of these bands produced inhibition halos when incubated on *L. innocua* inoculated agar. In other experiments protease hydrolysis of peptide fractions, heat treatment, and lysozyme caused a reduction or loss of the halos. It has been shown that antimicrobial activity can be obtained from sardine byproducts, but the understanding of the antimicrobial activity needs further study.

Conclusions

The present study showed the feasibility of producing bioactive peptides from the autolysis of Monterey sardine processing byproducts. After autolysis ultrafiltration methodology was used to fractionate samples. Greater ACE inhibitory activity (IC_{50}) was present in low MW fractions. All fractions produced showed antioxidant activity, with low MW fractions showing activity levels similar to that measured for the natural and chemical antioxidants tested. Of all the fractions, only the 10-30 kDa fraction obtained at physiological pH showed significant antimicrobial activity. Results indicate that autolysates from Monterey sardine canning byproducts (heads, viscera, and tails) is a very promising source of bioactive peptides.

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