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Proteolytic Activity in Byproducts from Cod Species Caught at Three Different Fishing Grounds

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Proteolytic activity in byproducts from cod species caught at three different fishing grounds has been characterized and compared. The overall highest activity in the byproduct fractions was found in viscera at pH 3 (35 °C). Cut off and liver fractions also show maximum activity at pH 3, 35 and 50 °C, respectively. Proteolytic activity in viscera and cut off fractions are more affected by fishing ground than by species. Proteolytic activity at pH 3 in viscera is higher in samples from the south coast of Ireland, while viscera samples from the Icelandic Sea have the highest activity at pH 7. For cut off, the activity is higher in samples from the south coast of Ireland than the other two fishing grounds.

KEYWORDS: Proteolytic activity; byproducts; viscera; liver; cut off; temperature; pH

INTRODUCTION

Due to a general decline in the total landings from marine fisheries, there is an increasing demand to utilize more of the fish for human consumption. Today, only about 50−60% of the total catch is used for human consumption, and the annual discards from the world fisheries have been estimated to be approximately 20 million tons (*1*). It is, therefore, a great potential for the fishing industry to utilize more of what is landed.

At present, most of the byproducts are processed into fishmeal or other low price ingredients. In Norway, about 80−90% of the byproducts generated at sea are dumped, and the remainder is used for fishmeal. About 66% of the byproducts generated on shore are utilized, mainly for fishmeal, silage, and feed. Approximately 10% is processed into food or specialty products and accounts for almost half the value from byproducts. In Iceland, the total value of exported byproducts makes up about 8% of the total value from fisheries, and between 15% and 20% of the export value. In the UK, only about 43% of the total available fish and shellfish resources end up as products for human consumption; the rest is categorized as waste. The major outlet for this raw material is fishmeal and oil production; small quantities are used for pet food, etc. Some of the material also ends up in landfill sites (*2*).

Byproducts from fish contain protein with an excellent amino acid composition and digestibility. The byproducts are highly perishable, and enzymatic processes are important determinants of the raw material quality. The quality and freshness limit the possibilities for utilization. To stabilize and subsequently utilize byproducts, natural degradation processes need to be controlled. Enzymatic activities along with microbial degradation are the most important quality deteriorating processes. Fish proteins may be utilized as functional ingredients, for example, thickeners and/or stabilizers, in food products such as minced products, soups, and sauces. For proteins to act as functional ingredients, molecular weight is important. For this purpose, choice of storage and processing methods to minimize protein breakdown is important. Application of enzyme technology to convert byproducts into protein hydrolyzates is a promising way of utilization. Protein hydrolyzates may be produced via autolytic processes, via addition of commercial enzymes, or via a mixture of both. Independent of the process used for production of protein hydrolyzates, quantitative and qualitative knowledge of the endogenous enzymes in the different byproduct fractions is needed to control the process and obtain the wanted product. Knowledge of enzymatic activities and variations according to species, season, and fishing ground is needed. Studies of enzymes in fish have shown that proteolytic activity in fish viscera varies with species and age (*3*). The activity and stability of fish enzymes are influenced by environmental temperature (*4*−*6*), and enzymatic activity and relative concentrations in intestines are influenced by feed (*7*, *8*). Water temperature and composition of feed may vary with different geographical locations and with season.

Proteolytic enzymes from cold adapted fish have been found to exhibit higher activity at low temperatures, to exhibit an increased ability to hydrolyze native protein substrates, and to be less heat stable than proteolytic enzymes from mammals, thermophilic organisms, and plants (*9*−*11*).

Numerous studies characterize the activity of different proteases and peptidases from the digestive tract (*12*−*15*) or muscles of fish (*16*−*19*). The digestive proteolytic enzymes from aquatic organisms that are most commonly studied include pepsin, trypsin, trypsin-like, chymotrypsin, gastricin, and elastase (*20*), while the most studied muscle proteases include different cathepsins, calcium-activated proteases, heat-activated proteases, multicatalytic proteases, and metalloproteases. Most studies of

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enzymes from the digestive tract aim to characterize the digestive system or parts of this to learn more about the uptake and utilization of nutrients, or they focus on utilization of extracted enzymes. Studies of enzymes from fish muscle most often focus on the quality and shelf life of the fish meat. Proteolytic activity in the liver of fish has not been well characterized.

The aim of this study is to characterize and compare general proteolytic activity, and other quality parameters, in byproducts from cod species from different fishing grounds.

MATERIALS AND METHODS

Materials. Pooled samples of cut off, liver, and viscera from cod (*Gadus morhua*) (50/60 cm, 60/70 cm, and 70/80 cm), haddock (*Melanogrammus aeglefinus*), saithe (*Pollachius virens*), tusk (*Brosme brosme*), and ling (*Molva molva*) caught in the Barents Sea, the Icelandic Sea, and the south coast of Ireland were analyzed. Samples for each of the byproduct categories were made up of byproducts from 15 fish. Sampling took place from February to March of 2001. SINTEF Fisheries and Aquaculture, IFL (Icelandic Fisheries Laboratories), and UCC (University College Cork) were responsible for collection and sample preparation. Fish was frozen whole after bleeding onboard vessels and were stored at −24 °C. Before processing, the fish was thawed in water (1:4.5) for approximately 22 h, to a core temperature of 0−1 °C. Samples were crudely homogenized using a food processor or a Waring Blender. Handling, homogenization, and distribution of the samples were done within 14 days after catch. The samples were sent to the lab and stored at −40 °C until analyses could be performed.

Preparation of Crude Extracts. The samples were homogenized in distilled water (1:2) using an Ultra Turrax for 20 s, stirred for 10 min, and subsequently centrifuged at 10 400*g* for 20 min at 4 °C. The supernatants were filtered through glass wool, and pH values of the water extracts were recorded. Protein concentrations in extracts were determined by the BioRad method (*21*) with bovine serum albumin as standard (Sigma No. A9647). The analyses were performed in triplicate. Extracts were stored at −80 °C.

General Proteolytic Activity. Proteolytic activity was determined as described by Barret (*22*) with minor modifications. The incubation mixture consisted of 1.2 mL of phosphate-citrate buffer (*23*) and 0.4 mL of substrate (bovine hemoglobin Sigma H-2625 1% w/v), and to the zero sample 2 mL of 5% w/v TCA (trichloroacetic acid, Merck) was also added. The samples were preincubated for 10 min in water baths before 0.4 mL of suitably diluted enzyme extracts were added. Incubation time was set to 3 h at 5 °C, 2 h at 20 °C, and 1 h at 35, 50, and 65 °C. These conditions gave a linear relationship between hemoglobin breakdown and time. The reaction was stopped by addition of 5% w/v TCA. The samples were cooled for 30 min and then filtered, before the amount of short peptides in the filtrate was determined according to Lowry (*24*). Bovine serum albumin (Sigma No. A9647) was used as a standard. Activities are expressed as mg hemoglobin cut per g water-soluble protein per hour and are given in arbitrary units (U) based on the mean of three measurements. Activity was determined at three different pHs, 3, 5, and 7, and at five different temperatures, 5, 20, 35, 50, and 65 °C.

Acid-Soluble Peptides. The amount of small peptides (acid-soluble peptides) in the samples was determined as the arithmetic mean of the zero samples for pH 7 and 35 °C. The contribution of hemoglobin to the amount of acid-soluble peptides was measured and found to be small; this was, however, subtracted from the result.

Amount of Free Amino Acids. The content of free amino acids was determined in crude enzyme extracts after precipitating proteins in sulfosalicylic acid (*25*) and diluting the supernatant with deionized water. Reverse phase HPLC, by precolumn fluorescence derivatization with *o*-phthaldialdehyde, was performed using a NovaPak C18 cartridge (Waters, Milford, MA) (*26*, *27*). The amount of free amino acids is given as mg per g wet weight sample.

Total Protein. The amount of nitrogen was analyzed using a C/N analyzer, Carlo Erba NA 1500 elemental analyzer (*28*), and was determined as the mean of three or four samples. The total amount of protein was calculated using a conversion factor of 6.25.

Statistics. Data obtained in this study were analyzed using MINITAB. The distribution of the data was initially checked in the Anderson−Darling normality test. Because most parameters were found not to be normally distributed, the Kruskal−Wallis test was used to compare the byproducts from the three different fishing grounds and from the different species. The Kruskal−Wallis test is nonparametric and performs a hypothesis test of the equality of population medians. The fish were divided into three groups. Cod and saithe were treated as separate groups. Tusk, ling, and haddock were treated together, as one group, because of the small number of these fish.

The significance level was set at 90% ($p = 0.1$) because of the high standard deviations in the raw material. The Pearson correlation test, significance level of 95% ($p = 0.05$), was used to determine the extent to which the variables were correlated.

RESULTS AND DISCUSSION

Comparing Proteolytic Activity in Viscera, Cut Off, and Liver. Highest median proteolytic activity for all viscera samples is found at pH 3, 35 °C. The highest activity in cut off and liver samples is also found at pH 3, 35 and 50 °C, respectively. This is partly supported by earlier findings of Stoknes and others (*29*) who found maximum proteolytic activity at pH 3−4.5 and temperatures of 45−60 °C in four different fractions (liver, intestine, muscle and head, skin and bones) of herring (*Clupea harengus*) and cod (*Gadhus morhua*). Maximum median proteolytic activity in viscera is approximately 20 times higher than in liver and 250 times higher than in cut off. Stoknes and others (*29*) found the activity in intestinal fractions to be 100 times greater than those observed for muscle.

All three fractions show highest median proteolytic activity at 50 °C at pH 5, while the highest activity at pH 7 is at 50 °C in viscera and at 65 °C in liver and cut off.

Figure 1 shows the relative activity at different pHs and temperatures for viscera, cut off, and liver, respectively. Activity at pH 3, 35 °C is set to 100%. The figures show that activity at pH 3 is more dominating in the viscera fraction than in cut off and liver fractions. The relative contribution to proteolytic activity of enzymes active at the different pHs is different in the three fractions. Enzymes active at pH 5 contribute to more of the general proteolytic activity in liver and cut off fractions as compared to viscera fractions. The enzymes active at pH 5 and 7 in liver and cut off respond to temperature in the same way, suggesting similar families of enzymes active at these pHs in both fractions.

The activity at pH 3 is still significant when the temperature is as low as 5 °C, 25%, 11%, and 12% of maximum in viscera, cut off, and liver, respectively. Enzymes active at pH 5 and 7 show 5% and 7% of the maximum activity in viscera at 5 °C. In cut off and liver, enzymes active at pH 5 and 7 show less than 2% of maximum activity at 5 °C.

Proteolytic Activity in Viscera. It is well documented that protein digestion in fish of different species occur both in the acidic region in the stomach and in the alkaline region of the intestines (*8*, *30*, *31*). The main proteolytic enzyme for the acid activity of the stomach is pepsin, with optimum pH between 2 and 4 and optimum temperature between 35 and 40 °C (*14*, *31*, *32*). In this study, the proteolytic activity measured at pH 3 in viscera has maximal activity at 35 °C and is predominantly caused by pepsin. It can also be assumed that the activity at pH 7 is mostly due to the alkaline proteases from the intestines, while both acidic proteases from the stomach and alkaline proteases from the intestine contribute to the activity at pH 5. Alkaline proteases from the intestines of fish have been

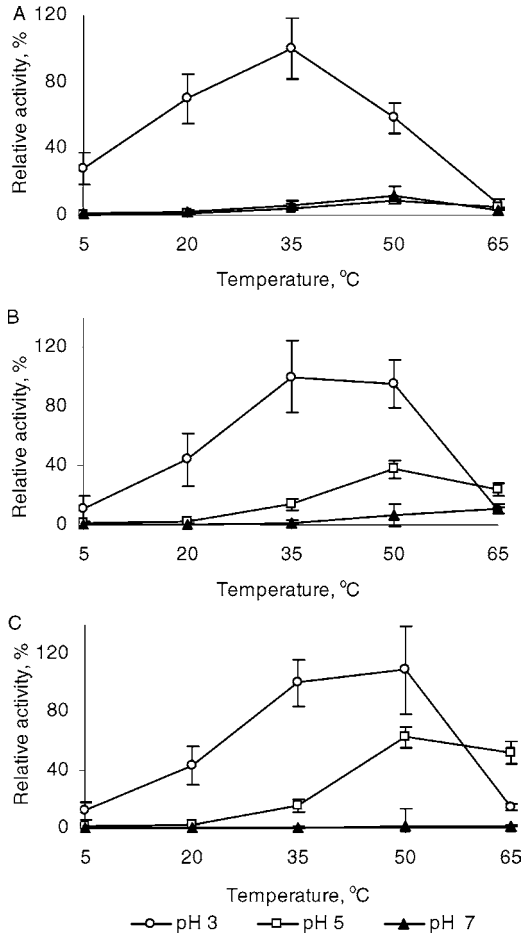

Figure 1. Relative median activity as a function of pH and temperature in 16 samples of viscera (**A**), 14 samples of cut off (**B**), and 16 samples of liver (**C**) from fish of the cod species caught at different fishing grounds. Each sample (*n*) is made up of byproducts from 15 fish. Activity at pH 3 35 °C is defined as 100%. Standard error of the mean is indicated as bars.

thoroughly characterized through numerous studies. Trypsins, chymotrypsin, elastase, and collagenolytic serine proteinase purified from the intestines of Atlantic cod have an optimum pH above 7.8 and optimum temperatures above 40 °C (*13*, *33*−*35*). Proteolytic activity in viscera at pH 5 and 7 show maximum activity at 50 °C, as compared to 35 °C for the activity at pH 3. This is in accordance with the literature where the optimum temperature for enzymes in intestinal extracts has been reported to be about 10 °C higher than the optimum temperature for enzymes in the stomach, that is, 45−50 °C (*31*).

Proteolytic Activity in Cut Off. To our knowledge, no studies of enzymes in cut off from fish have been reported in the literature. However, it is reasonable to assume that enzymes active in fish muscle will also be active in cut off. The most studied proteases in fish muscle are the cathepsins. The activity of cathepsin B, D, H, and L has been described (*36*), cathepsin D (*17*, *37*), and cathepsin B-like enzymes (*16*, *38*). The enzymes most likely responsible for the activity measured at pH 3 in cut off are the acid cathepsins D and E. Cathepsin B, C, and L have all been found to show optimum at pH values between 5 and 6.5 and could therefore be the dominating proteases measured at pH 5.

Alkaline proteases may contribute to activity measured at pH 7. Two alkaline proteases isolated from Atlantic menhaden muscle retained 41% and 65%, respectively, of the maximal activity at pH 7 and 55 °C (*39*). Low activity is detected in cut off at pH 7 and temperatures below 50 °C; this is in accordance with findings in the literature. Makinodan and others (*19*) reported an alkaline protease from white croaker muscle that also showed very low or no activity below 50 °C, but considerable activity at around 65 °C. **Figure 1** shows that the activity in cut off at pH 7 increases up to 65 °C. Calcium-activated proteases are known to have optimum pH at 6.9−7.5 (*40*, *41*), and calpain I has been found to be heat stable up to 50−60 °C (*42*).

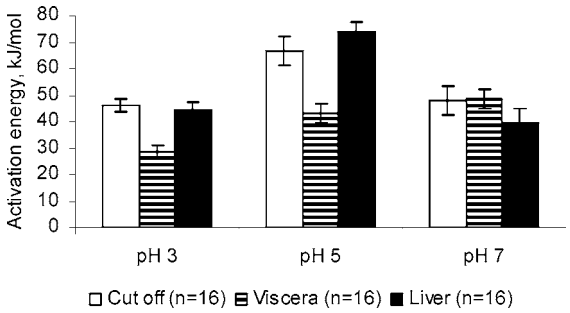

Figure 2. Median activation energies for proteolytic enzymes active at pH 3, pH 5, and pH 7 in cut off, viscera, and liver from fish of the cod species. Each sample (*n*) is made up of byproducts from 15 fish. Standard error of the mean is indicated as bars.

Proteolytic Activity in Liver. Few studies have looked at proteolytic activity in fish liver. The influence of diet on the metabolism in liver has, however, been reported for eel (*43*, *44*), juvenile tilapia (*45*), and cod (*46*). Increased protein synthesis and level of tissue protein in liver has been found in cold-acclimated trout (*47*).

The maximum proteolytic activity in liver is found at pH 3 and 50 °C. This is in contrast to findings in liver fractions of carp and tench where the proteolytic activity was very low at acidic pH as compared to neutral and alkaline pH. Proteolytic activities in liver fractions of goldfish, sea bream, trout, and eel, using casein as substrate, were not detectable (*48*). Our results are, however, in accordance with findings of Stoknes and others (*29*) who found proteolytic activity in cod liver to be remarkably high at acidic pHs. Cod liver samples showed two clear optimal temperatures in the acidic range, 45 °C at pH 3.2 and 60 °C at pH 4.5 (*29*). Capasso and others (*49*) found cathepsin D from the liver of the Antarctic icefish (*Chionodraco hamatus*) to have maximum activity at pH 3 and an unexpectedly high-temperature maximum of 45 °C. It is reasonable to assume that proteolytic activities at pH 3 in liver samples are mostly due to acidic cathepsins such as cathepsin D and E, while the activities at pH 5 are caused by other cathepsins. Heat-activated and heat-stable proteases, and calcium-activated, multicatalytic, alkaline, and neutral proteases may contribute to proteolytic activity at pH 7.

Activation Energies. Figure 2 illustrates the activation energies for proteolytic enzymes active at different pHs in the three byproduct fractions. The activation energies are calculated using the Arrhenius equation in the temperature range from 5 to 35−50 °C, depending on the maximum activity of the samples. The activation energies for proteolytic enzymes active at pH 3 and pH 5 are significantly lower in viscera samples as compared to cut off and liver samples. This indicates that proteolytic enzymes active at pH 3 and pH 5 in cut off and liver are more sensitive to temperature changes as compared to those in viscera. There are no significant differences in activation energies for proteolytic enzymes active at pH 7 in the different fractions. The activation energies for proteolytic enzymes in cut off (pH 3) and liver (pH 5) are significantly

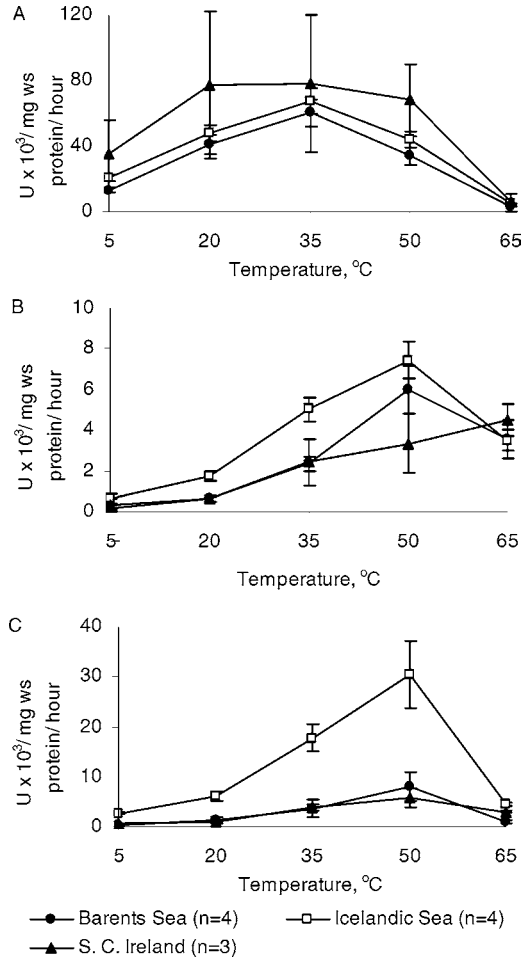

Figure 3. Median proteolytic activity at pH 3 (**A**), pH 5 (**B**), and pH 7 (**C**) as a function of temperature in viscera from cod and saithe caught in different fishing grounds. Each sample (*n*) is made up of viscera from 15 fish. Standard error of the mean is indicated as bars.

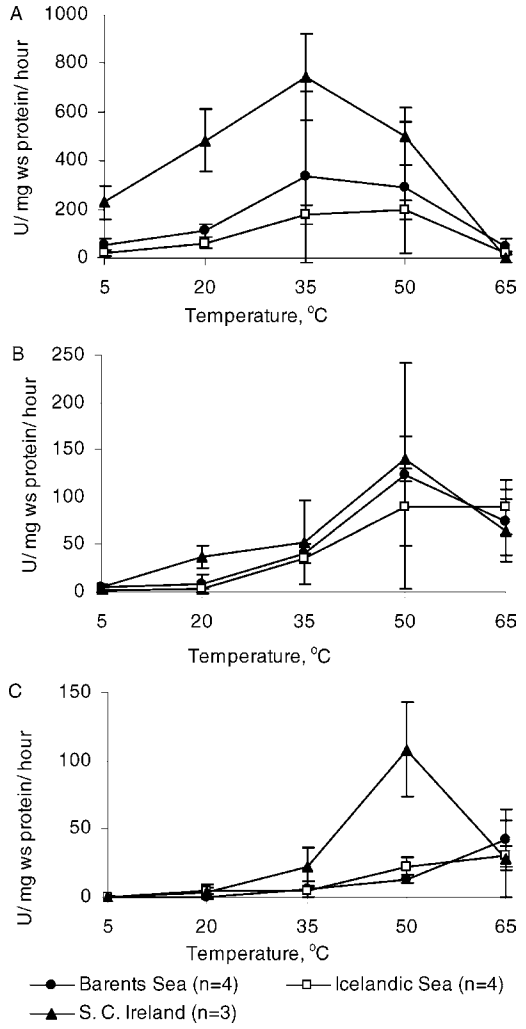

Figure 4. Median proteolytic activity at pH 3 (**A**), pH 5 (**B**), and pH 7 (**C**) as a function of temperature in cut off from cod and saithe caught in different fishing grounds. Each sample (*n*) is made up of cut off from 15 fish. Standard error of the mean is indicated as bars.

higher in samples from the Icelandic Sea. Cut off and liver samples from the south Coast of Ireland have the lowest values. Viscera samples from the south coast of Ireland have significantly lower activation energies (pH 7) as compared to the two other fishing grounds. No significant differences in activation energies for proteolytic enzymes were found between species.

Effect of Fishing Ground. Cod and saithe were sampled from all three fishing grounds and were used to compare proteolytic activity in the byproduct fractions from different fishing grounds.

Comparing viscera samples shows significant differences in proteolytic activity at pH 3 (35 °C), pH 5 (20 and 35 °C), and pH 7 (5, 20, 35, 50, and 65 °C) and in the amount of acid-soluble peptides. **Figure 3** shows the median proteolytic activity at pH 3, 5, and 7 in viscera samples from the three fishing grounds. Samples from the Icelandic Sea have the highest amount of acid-soluble peptides and proteolytic activity at pH 5 and 7, while samples from the south coast of Ireland have the highest proteolytic activity at pH 3.

Proteolytic activity at pH 3 (5, 20, 35, and 50 °C), pH 5 (20 °C), and pH 7 (50 °C) was significantly higher in cut off samples from the south coast of Ireland as compared to the other fishing grounds. The amount of dry matter, acid-soluble peptides, and pH in the extracts was also higher in these samples. **Figure 4** shows the median proteolytic activity at pH 3, 5, and 7 in cut off samples from the three fishing grounds.

Significant differences in the amount of acid-soluble peptides and proteolytic activity at pH 5 (50 °C) were found in liver samples from the three fishing grounds. Samples from the south coast of Ireland had the highest amounts of acid-soluble peptides, and samples from the Barents Sea had the highest activity at pH 5 (50 °C).

Endogenous proteins in different organs are depleted to different extents during starvation. An increase in proteolytic activity with starvation has been found in liver, kidney, spleen, and red and white muscle. White muscle experiences the largest increase in proteolytic activity. In some species, including cod (*Gadus morhua*), muscle protein breakdown increases in the early stages of starvation and maximum values have been found 3−4 weeks into a fasting period (*50*). The high proteolytic activity in cut off samples from the south coast of Ireland may suggest that this fish has recently experienced starvation. However, the nutritional state also affects the digestion, and results from turbot show lower activity of digestive enzymes in nutritionally deficient fish (*51*). This is not the case for viscera samples from the south coast of Ireland, which show the highest proteolytic activity at pH 3 as compared to the two other fishing grounds. Another possible explanation for the differences in proteolytic activity in cut off from fish caught at the different fishing grounds is different activity levels of the fish. Tagged cod from the Irish Sea and the North Sea showed marked

differences in swimming activity of fish in different regions, Irish Sea cod being extremely active at all times (*52*). No studies have been found on cod from the Barents Sea and/or the Icelandic Sea regarding this, but a high activity level in the fish from the south coast of Ireland does offer a possible explanation for the higher proteolytic activity in cut off samples from this fish.

Several studies have concluded that enzymatic activity in fish intestines and gut content is affected by feed (*7*, *8*, *53*). Studies of stomach content of haddock from the North Sea and cod from the Celtic Sea have shown that the type of feed differed significantly between season and area (*54*, *55*).

Water temperature is also likely to differ between fishing grounds, and relationships between activity/stability of fish enzymes and environmental temperature have been observed (*4*−*6*). It is difficult to predict the temperature experienced by cod, because they show both vertical and horizontally migration patterns. Vertical profiles for cod and haddock have shown that the highest concentration of fish is below 100 m where the vertical temperature gradient is low (*56*), and the seasonal variation has a minor influence as compared to the geographical differences (*57*). It is documented that changes in temperature in different parts of the Atlantic have opposite effects on cod recruitment. In the cold, arctic regions of the North Atlantic, such as the Icelandic Sea and the Barents Sea, a positive relationship is generally observed between cod recruitment and water temperature, while negative relationships are observed in warm-temperate regions, such as the south coast of Ireland (*58*). Differences in environmental temperatures between the fishing grounds cannot be the sole explanation for the differences in proteolytic activity found in this study, because the activity at pH 3 in viscera is highest in samples from the south coast of Ireland while the activity at pH 5 and 7 is highest in samples from the Icelandic Sea.

Recent research supports genetic differentiation between stocks. Jonsdottir and others (*59*) collected Atlantic cod from six locations across the North Atlantic and revealed substructure of the species. In particular, the results indicated a clear genetic differentiation between the Barents Sea population and other populations in the east Atlantic. The differences in enzymatic activity cannot be explained by any variable alone and are likely to be influenced by multiple factors, and in different manners in the different byproduct fractions.

Effect of Species. Comparing viscera samples from different species of cod shows that proteolytic activity in cod samples is significantly higher than for the other species at pH 5 (50 and 65 °C) and pH 7 (5 and 20 °C) and lower for ling, tusk, and haddock at pH 7 (65 °C). The ling, tusk, and haddock group had significantly higher values for water-soluble protein as compared to the cod and saithe groups. Cod had the lowest values. **Figure 5** shows the proteolytic activity in viscera from fish of different species. There are no significant differences in pepsin activity between species, but significant differences in activity of enzymes active mainly at pH 7, but also at pH 5. This is in accordance with findings of Munilla−Morãn and Saborido−Rey (*31*) who found the enzymatic profile of fish intestine to be more species specific than for the stomach.

For the cut off fraction, a significant difference in activity at pH 5 (35 and 65 °C) and pH 7 (35 °C) was found between the different species. Also in cut off, the activity was highest in the cod group. Saithe liver showed significantly higher activity at pH 5 (5 °C) and pH 7 (5 and 50 °C) than the other two groups.

Effect of Size in Cod. A study of ambient temperature and distribution of northeast Arctic cod indicated that older fish were found in warmer water than were younger fish (*60*). This, together with the fact that cod is sexually mature when it reaches 70−90 cm and that the spawning season is in March−April (*61*), might cause differences in enzymatic activity between size categories. To study the effect of size on the enzymatic activity, the cod were divided into three size-categories, 50−60, 60−70, and 70−80 cm. No significant differences were, however, found between samples of cut off, liver, and viscera from the different size categories. This is in accordance with findings from the Bouri fish which had no differences in proteolytic activity in viscera according to age or size within the same species (*3*). Studies of trypsin and chymotrypsin in crude enzyme preparations from cod viscera done in our laboratory also concluded that there were no significant differences in activity between the three size categories (*62*).

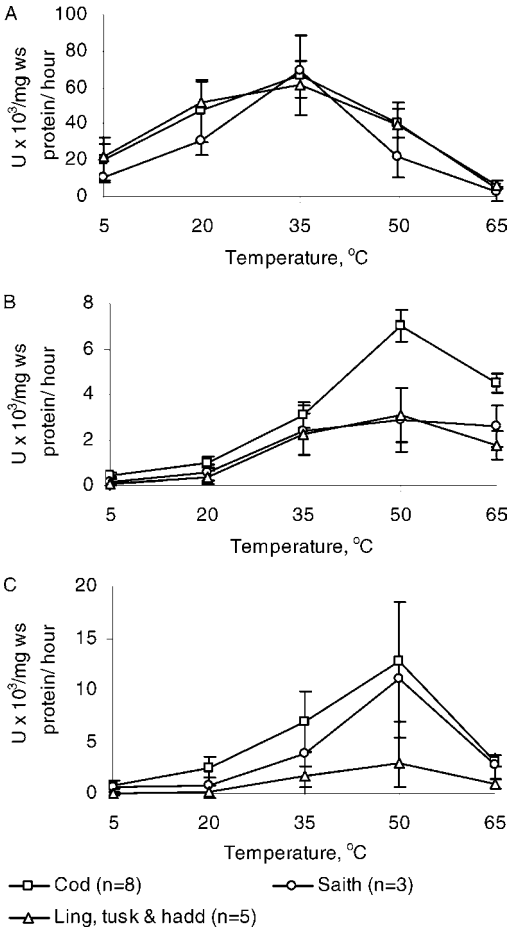

Figure 5. Proteolytic activity in viscera at pH 3 (**A**), pH 5 (**B**), and pH 7 (**C**) for different species. Each sample (*n*) is made up of byproducts from 15 fish. Standard error of the mean is indicated as bars.

Correlations. The Pearson correlation test revealed linear relationships ($p < 0.05$) between proteolytic activity at pH 5 and 7, and the amount of free amino acids, acid-soluble peptides, and water-soluble protein in viscera (**Table 1**). In general, high proteolytic activity leads to increased amounts of free amino acids and acid-soluble peptides, and decreased amounts of water-soluble protein. The amount of acid-soluble peptides also includes free amino acids such as tryptophan, tyrosine, cystine, cystein, and histidine, because they contribute to the color measured by the Lowry method (*63*). This suggests that the activity is due to exoproteases. Possible exoproteases present are carboxypeptidases and aminopeptidases (*64*, *65*). High amounts of free amino acids and acid-soluble peptides indicate that more of the proteins in the samples are degraded.

Table 1. Correlations Found between Free Amino Acids, Acid-Soluble Peptides, Water-Soluble Proteins, and Proteolytic Activity at Different pH Values and Temperatures in Viscera

	proteolytic activity	*p* value
free amino acids	pH 5 and 20 °C	0.004
	pH 7 and 20 °C	0.015
	pH 5 and 35 °C	0.045
	pH 5 and 50 °C	0.002
	pH 5 and 65 °C	0.012
acid-soluble peptides	pH 7 and 5 °C	0.006
	pH 5 and 20 °C	0.008
	pH 7 and 20 °C	0.000
	pH 5 and 35 °C	0.009
	pH 7 and 35 °C	0.036
	pH 5 and 50 °C	0.037
	pH 7 and 50 °C	0.000
	pH 7 and 65 °C	0.003
water-soluble protein	pH 5 and 35 °C	0.021
	pH 5 and 50 °C	0.006
	pH 5 and 65 °C	0.002
	pH 7 and 65 °C	0.006

In liver samples, a positive linear correlation was also found between proteolytic activity at pH 3 and 5 (65 °C) and free amino acids, and between free amino acids and water-soluble protein.

Conclusion. As expected, the overall highest activity in the byproduct fractions was found in viscera at pH 3 (35 °C). Cut off and liver fractions also show maximum activity at pH 3, 35 and 50 °C, respectively. Maximum median proteolytic activity in viscera is approximately 20 times higher than in liver and 250 times higher than in cut off. Enzymes active at pH 3 are less affected by temperature than enzymes active at pH 5 and 7. Viscera, cut off, and liver show 25%, 11%, and 12% of maximum activity at pH 3 when temperature is lowered to 5 °C, respectively. Proteolytic activity in viscera and cut off fractions is more affected by fishing ground than by species. Proteolytic activity at pH 3 in viscera is higher in samples from the south coast of Ireland, while viscera samples from the Icelandic Sea have highest activity at pH 7. For cut off, the activity is higher in samples from the south coast of Ireland. Fishing ground affects proteolytic activity in viscera and cut off to a larger extent than in liver, and species affects proteolytic activity in viscera to a larger extent than in liver and cut off.

Results from this study also suggest that viscera from cod should be sorted and stored frozen. Contamination of liver and cut off fractions with viscera fractions should be avoided.

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