





# Byproduct utilization for increased profitability, part 1

2 May 2013 By George J. Flick, Jr., Ph.D.

Protease enzymes have diverse applications



Proteases have been purified from the processing waste of mackerel and various other fish and shellfish species.

Fish processing generates large amounts of solid and liquid wastes in the forms of heads, tails, skin, bones and intestines. The utilization of these wastes is a major problem for fishermen and the fish-processing industry. As profit margins continue to decrease and waste disposal becomes more expensive with fewer options, the ability to convert waste into a useful product has considerable interest.

#### **Proteases**

Protease enzymes constitute one of the most important groups of industrial enzymes and account for at least 50 percent of all global enzyme sales. They have diverse applications in detergents, food, leather, silk and the agrichemical and pharmaceutical industries.

Fish are regarded as one of the richest sources of proteolytic enzymes. It is possible to recover about 1 g of the enzyme from 1 kg of viscera.

Proteases have been purified from the processing waste of various fish and shellfish species, including tambaqui, Nile tilapia, several salmon species, carp, catfish, Monterey sardines, Japanese anchovies, spotted goatfish, rainbow trout, true sardines, arabesque greenlings, jacopever, elkhorn sculpin, crawfish, mackerel, cod, albacore and yellowfin tuna, lane snappers, shrimp and cuttlefish.



(https://www.grantthornton.ca/insights/state-of-inflation-how-canadian-businesses-are-reacting/).

Digestive enzymes from cold-adapted fish are active catalysts at relatively lower temperatures than similar enzymes from mammals, thermophylic organisms and plant sources. Isolated fish proteases vary significantly in their optimal pH activity and temperature, but have high activity over a wide range of pH, temperature and chemical solutions (Table 1).

## Flick, Optimal temperatures and enzyme activities, Table 1

Fish Species	Optimal pH	Temperature (°C)	Activity Retention
Sardinella	8.0	60	50% at 30 minutes at 50° C
Common carp	11.0	50	60% in ionic surfactants 50% in 5% hydrogen peroxide
Hybrid catfish	9.0	50	120 minutes at pH 7-11 at 40° C
Nile tilapia*	8.0	50	30 minutes at 50° C
Sea bream*	8.0-10.0	50	50% after 30 days at 30° C
Tuna**	9.0	55	-
Surf clams	2.5-3.0	44-46	-

<sup>\*</sup> Alkaline proteinase

Table 1. Optimal temperatures and enzyme activities for several fish and shellfish proteases.

The enzymes exhibit high catalytic activity at relatively low concentrations, which makes them suitable for many different applications.

#### Alkaline proteases

Alkaline proteases, mainly trypsin and subtilisin, are the most important group of industrial enzymes, with applications in the leather, food and pharmaceutical industries, as well as in bioremediation processes. However, about 60 percent of all proteases sold are used in the detergent industry. Biological detergents are commonly used in domestic laundry soaps because the enzymes provide the benefit of low-temperature washes with improved cleaning performance.

The addition of proteases to detergents considerably increases the cleaning effect by removing protein stains and consuming surface-active substances, thereby decreasing the pollution load. Although most of the research on fish enzymes has primarily focused on saltwater fish (Table 2), the excellent activity exhibited by enzymes recovered from Nile tilapia (*Oreochromis niloticus*) and hybrid tilapia demonstrates that freshwater fish can also be important sources of enzymes.

## Flick, Optimal temperatures and enzyme activities, Table 2

Fish Species	Optimal pH	Temperature (°C)	Activity Retention
Pacific cod	8.0	8.0	Unstable above 30° C and below pH 5.0

<sup>\*\*</sup> Yellowfin, Thunnus albacores; skipjack, Katsuwonus pelamis; tongol, Thunnus tonggol

Saffron cod	8.0	8.0	Unstable above 30° C and below pH 5.0
Lane snapper	9.0	9.0	100% at 30 minutes
Red snapper	8.5	8.5	Stable 25-55° C and pH 7.0-10.0
Skipjack tuna	9.0	9.0	Stable below 50° C and pH 6.0-11.0
Skipjack tuna	9.0	9.0	Stable below 50° C and pH 6.0-11.0
Walleye pollock	8.0	8.0	Unstable above 30° C and below pH 5.0
Pirarucu	9.0	9.0	30 minutes at 55° C and pH 6.0-11.5
Cuttlefish	8.5	8.5	Stable 1 hour at 50° C and pH 7.0-10.0
Smooth hound	8.5	8.5	Stable 1 hour at 40° C and pH 7.0-9.0
Sardine	8.0	8.0	Stable at pH 6.0-9.0
Catfish	8.0	8.0	Stable at pH 6.0-11.0
Freshwater shrimp	8.0	8.0	Stable at pH 7.0-11.0

Table 2. Optimal temperatures and enzyme activities for several fish and shellfish trypsins.

The high activity of fish trypsins at low temperatures is interesting for several industrial applications, such as in certain food-processing operations that require low processing temperatures. Furthermore, trypsins from cold-adapted fish are inactivated at relatively low temperatures, which make such enzymes potentially useful in food applications where ready and rapid denaturation is desirable.

The ability to obtain a commercial product from tuna is of particular interest. Approximately two-thirds of the whole fish are ensiled, and the rest (including the viscera) becomes the waste. Use of the processing waste will minimize economic loss and ecological hazards sometimes caused by waste.

Tripsins have also been obtained from both pink and Atlantic salmon, but specific optimum temperatures and pH values were not stated.

## **Perspectives**

The market for various enzymes is expected to expand, and new applications for their use will continue to develop. Significant research on the isolations and applications of fish and shellfish proteases have been performed and reported in the literature. Consequently, the technology to produce the enzymes exists, large volumes of processing wastes are available, and markets for the enzymes exist.

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#### **Author**



GEORGE J. FLICK, JR., PH.D.

Food Science and Technology Department Virginia Tech/Virginia Cooperative Extension (0418) Blacksburg, Virginia 24061 USA

flickg@vt.edu (mailto:flickg@vt.edu)

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