INTRODUCTION

1.0 INTRODUCTION

Today, over-exploitation of fish resources is a large problem. Only about 50 – 60 % of the catch is used for human consumption. Globally, more than 91 tonnes of fish and shellfish are caught every year. Some of the by - products are utilized but huge amounts are wasted. Annual discards from the world fisheries were estimated to be approximately 20 million tonnes (25%) per year (Rustad, 2003).

Fish industry wastes are an important source of environmental contamination. Several research works have been carried out in order to develop methods to convert these wastes into useful products (Laufenberg *et al.*, 2003).

50% of the world fish production becomes waste material, which means an expressive amount of about 65.2 million metric tons (Arruda, 2007).

A way of minimizing the environmental problems generated by the high amounts of fish waste is its transformation into a product that can be incorporated as ingredient in animal rations (Ristic *et al.*, 2002).

Fish waste proves to be a great source of minerals, proteins (58% dry matter (d.m)) and fat (19% d.m) Fatty acids (monosaturated acids, palmitic acid and oleic acid) are also abundant in fish waste, while the high ash content (22% d.m) indicates a high percentage of minerals in fish meal (Estenban *et al.*, 2006).

The conversion of waste materials with its consequent reutilization can bring economic advantages for the fish industry, besides solving a great problem with the discharge of the waste, which has pollutant materials (Borghesi, 2004).

Fish processing waste including viscera have been reported to be good sources of proteins including enzymes, fats, good substrates for lactic acid fermentation and source of protease producing bacteria. If these biological compounds can be recovered, it would serve the dual purpose of recovery of these biomolecules and reducing the pollution problems associated with them [Arnesen and Gildberg., 2007].

Fish viscera has been extensively studied as a potential source of different enzymes. It is generated during fish processing and can be used as an important source of digestive enzymes, including proteases. Proteases are present in all living forms as they are involved in various metabolic processes. They are mainly involved in hydrolysis of the peptide bonds [Gupta *et al.*, 2002].

Proteases breakdown peptide bonds to produce amino acids and other smaller peptides. It can be isolated from a variety of sources such as plants, animals and microbes [fungi and bacteria]. Its applications are very broad and have been employed in many fields for years [Yandri *et al.*, 2008].

Proteases are widely used in detergent, food, leather and tanning industries [Abidi *et al.*, 2007]. Several alkaline proteases are reported for hydrolysis of fibrous protein of horn, feather and hair and their application for various value added by-products [Giango *et al.*, 2007]. Alkaline protease has also found applications in detergent industry as a cleaning additive [Rai and Mukherjee., 2010].

In contact lens cleansing, protease plays a major role. In normal course of wearing contact lenses tear films and proteinaceous debris have a tendency to deposit on the surface of lens, which affects their optical clarity. However, in most instances they impart an unpleasant odour to the cleansing bath or develop an odour after a few hours of use. With the view of overcoming these drawbacks and to make the cleansing composition odourless and safe i.e., not producing allergic response or causing irritation to the eyes, bacterial proteases are gaining importance. Reports are available on production of protease from bacterial cultures and *Bacillus sp.* by Nilegaonkar *et al.*, (2002).

Protease treatments can modify the surface of wool and silk fibre to provide new and unique finishes. Proteases have been used in the hide dehairing process, where dehairing is carried out at a pH range of 8 to 10 [Najafi *et al.*, 2005]. As protein utilization is fundamental to growth, proteases have an important role in the larval fish as in the adult. The digestive proteases of different species show variations [Chakrabarti and Sharma, 2005], which may influence their digestive capability and feeding habits.

Fish waste can be used as a source for the isolation of enzymes, which are of commercial value. Whole viscera can also be used because these are raw by-products of the processing line. Discards from marine fisheries have been estimated at 27 million tonnes per year. Proteolytic enzymes are ubiquitous in occurrence, being found in all living organisms and are essential for cell growth and differentiation. The extracellular proteases are of commercial value and find multiple applications in various industrial sectors. (Gupta *et al.,* 2002).

In the light of the above, the present study was aimed at the following objectives:

- To collect the visceral organ wastes and head and tail wastes from the selected fish for the isolation of protease.
- To isolate the enzyme protease from the fish wastes.
- To purify the isolated protease by dialysis and Sephadex G-100 column.
- To analyse and compare the protein content, protease activity, specific activity, purification fold and recovery % in the crude, ammonium sulphate precipitated, ammonium sulphate precipitated + dialysed and ammonium sulphate precipitated + Sephadex G-100 run samples, acetone precipitated, acetone precipitated + dialysed and acetone precipitated + Sephadex G-100 run samples, ethanol precipitated, ethanol precipitated + Sephadex G-100 run samples.