

# CHARACTERIZATION OF THE ENZIMATIC ACTIVITY IN THE GASTRIC JUICE AND DIGESTIVE GLAND OF OCTOPUS (*Octopus vulgaris*) AND CUTTLFISH (*Sepia officinalis*) AT DIFFERENT pH AND TEMPERATURES

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## INTRODUCTION

*O. vulgaris* and *S. officinalis* are high price valued species, with great potential for aquaculture diversification, due to their fast growth and high conversion rates (Domingues *et al.* 2006; Sykes *et al.* 2006). The understanding of digestive physiology mechanisms, and particularly digestive enzymes function, is of extreme importance for the development of adequate artificial feeds. Due to the protein metabolism of cephalopods (Domingues *et al.* 2006), proteolytic enzymes play a key role in the digestion, and nutrient availability. Enzyme activity is affected by pH and temperature, since these determine enzyme structure and capacity to transform substrates according to energetic needs (Murray *et al.* 2001).

The objective of the present research was to determine the most important protease activity of the gastric juice (GJ) and digestive gland (DG) of *O. vulgaris* and *S. officinalis* in pH ranging between 2 and 12, and temperatures between 20° and 70°C.

## MATERIAL & METHODS

### Animals



10 octopus (1102.3±198.1 g)



10 cuttlefish (96.5±45.6 g)

Samples of GJ and DG were taken 24 h after the last feeding. In order to obtain enough volume of GJ, the food was shown to the animals inside a transparent trap, preventing capture.



### Enzyme activity assays



Acid proteases  
Anson (1938)  
hemoglobin (0.5%)

Alkaline proteases  
Kunitz (1947) - Walter (1984)  
azocasein (0.5%)

Total Soluble Protein  
Bradford (1976)

Trypsin  
Charney & Tomarelli (1947)  
BAPNA

Chymotrypsin  
Delmar *et al.* (1979)  
SAAPNA

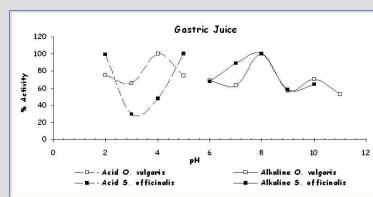
Effect of pH → The optimal pH for protease activities was determined using Universal Buffer (Stauffer 1989) ranging from 1 to 5 (acid proteases) and from 6 to 12 (alkaline proteases)

Effect of the temperature → The optimal temperatures for acid and alkaline proteases were determined by incubating enzyme extracts at their optimal pH, pre-equilibrated at temperatures ranging from 10-70°C

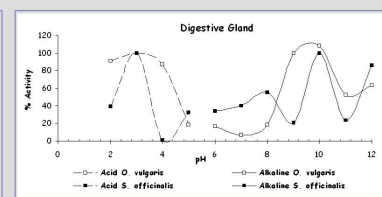
## ACKNOWLEDGMENTS

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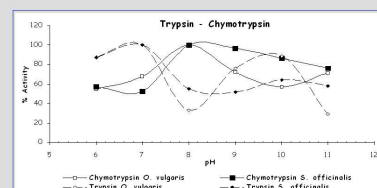
## RESULTS



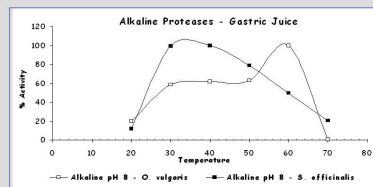
Effect of pH on protease activity (%) in the GASTRIC JUICE extracts



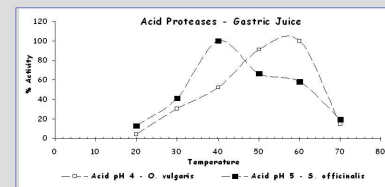
Effect of pH on protease activity (%) in the DIGESTIVE GLAND extracts



Effect of pH on Trypsin and Chymotrypsin activity (%) in the GASTRIC JUICE extracts



Effect of temperature (°C) on alkaline proteases activity (%) in GASTRIC JUICE extracts



Effect of temperature (°C) on acid proteases activity (%) in GASTRIC JUICE extracts

All data are presented as mean of triplicate determinations

## CONCLUSIONS

- There are marked differences between the enzyme activity in the gastric juice and DG for both species. Nevertheless, differences between the two species were small and, when applicable, to a maximum of +/- 1 pH unit.
- Higher differences were found for total acid proteases.
- For temperature, well defined values of higher activity were obtained at 40°C for cuttlefish and 60°C for octopus, both for total acid or alkaline proteases.
- It appears that enzyme thermostability is higher in octopus, compared to cuttlefish. Total acid proteases remain stable for longer, at higher temperatures for octopus, compared to cuttlefish. For octopus, acid proteases present higher stability than alkaline proteases.