PROMETIC BIOSCIENCES

APPLICATION NOTE

Isolation of Chymosin from Calf Stomach Rennet using Mimetic Yellow 1 A6XL.

Introduction

Chymosin otherwise known as Rennin is a proteolytic enzyme that is mainly used in the cheese making industry. It is typically used in the form of rennet, a commercial preparation made from the lining of the abomasums of calves.

Outlined below in a one step purification procedure involving the use of Mimetic Yellow 1 A6XL affinity chromatography in the isolation of chymosin.

Experimental Conditions

A 4ml Mimetic Yellow 1 A6XL affinity chromatography column (Prometic Biosciences Ltd, UK) was loaded with calf stomach rennet in 10 mM sodium phosphate buffer, pH 6.0 at a flow rate of 0.45 ml/min. Elution was undertaken using 10 mM sodium phosphate/0.2 M NaCl buffer, pH 6.0.

Results

Chymosin bound to the Mimetic Yellow 1 A6XL affinity chromatography column and was eluted using 0.2 M NaCl (Fig 1).

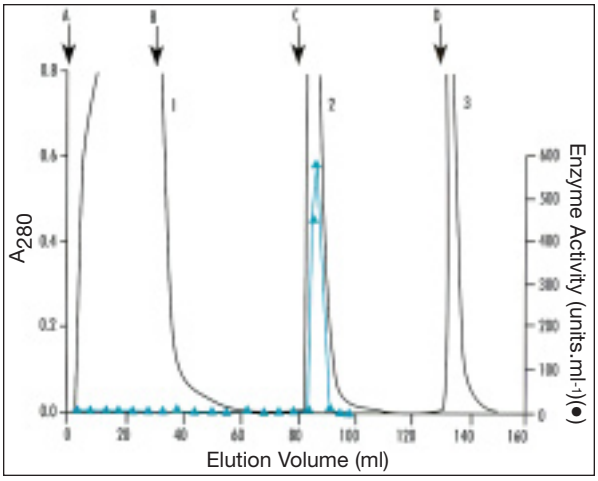

Fig 1. An FPLC trace of the capture and purification of chymosin from calf stomach rennet using Mimetic Yellow 1 A6XL. (A) Calf stomach rennet in 10 mM sodium phosphate; pH 6.0 (30ml; 0.35 mg/ml; 120 units/ml-1) (B) 10 mM sodium phosphate, pH 6.0; (C) 10 mM sodium phosphate/0.2M NaCl, pH 6.0; (D) 1 M NaOH. Eluted peaks: (1) unbound contaminants; (2) chymosin and (3) residual contaminants.

The specific activity of the chymosin eluted was 650 units/ml-1 where one unit of chymosin activity is defined as the amount of enzyme required to clot 5 ml of milk per minute at 37°C.

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