IMMOBILISATION OF COCOA ASPARTIC ENDOPROTEASE

S.V. Shinde, S. Jinap, N. Saari and H.M. Ghazali

Faculty of Food Science and Biotechnology
Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia

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Introduction

Accumulation of cocoa aroma precursors during fermentation is a synergistic effect of the co-operative degradation of storage 7S globulin by endogenous aspartic endoprotease and carboxypeptidase enzymes (Voigt et al. 1993). These aroma precursors undergo Maillard reactions during roasting to produce typical cocoa aroma compounds. The specificity of cocoa aspartic endoprotease is a prerequisite for the formation of cocoa aroma precursors. Voigt et al. (1993) indicate the possibilities of *in vitro* production of cocoa aroma precursors using partially purified fractions of aspartic endoprotease, carboxypeptidase and 7S globulin from cocoa seeds. The advantages of enzyme immobilisation include the ability to recover and reuse enzymes, frequent improvement in enzyme stability and prevention of autolysis in proteases by restricting intermolecular contact. (Trevan, 1980) However there are no attempts towards immobilisation of the potentially important cocoa endoprotease enzymes. The aim of the present study was to produce an immobilised form of cocoa aspartic endoprotease with improved catalytic properties, and to explore the possibilities of *in vitro* production of cocoa aroma precursors using these immobilised enzymes.

Materials and Methods

The ripe, ungerminated cocoa pods were obtained from Golden Hope Plantations Sdn bhd, Perak. The acetone dry powders of the cocoa cotyledons were prepared as per the method of Kirchoff et al. (1989). Endoproteases were extracted in a chilled Mcilvaine buffer and further purified by ammonium sulphate precipitation. The partially purified endoproteases were ionically bound to DEAE-cellulose and covalently bound to crab shell Chitin according to the method described by Ge et al. (1996). The immobilised enzymes were characterised in terms of pH, temperature, operational and storage stability.

Results and Discussion

The endoprotease was only extracted under alkaline conditions (pH 7.5) whereas the enzyme activity was assayed in acidic conditions (pH 3.5) using bovine haemoglobin as a substrate. The ammonium sulphate precipitation of crude enzyme extract resulted in several proteolytically active fractions. The 80% salt saturated fraction was most sensitive to pepstatin A inhibitor compared to other fractions, indicating that it comprises exclusively of the aspartic endoproteases. Cocoa aspartic endoprotease (EC 3.4.23) was immobilised on DEAE-cellulose and chitin by adsorption and covalent coupling respectively. A significant shift in the optimum pH and temperature was observed in the immobilised enzyme. The apparent optimum temperature for the DEAE- cellulose and chitin bound enzymes was 50°C and 65°C respectively, compared to 45°C of soluble enzyme. Both the immobilised enzymes exhibited maximum activity at pH 2.5 compared to 3.5 in case of the soluble enzyme. The highest immobilised activity (418U) was obtained in chitin bound enzyme. The DEAE-cellulose and chitin bound enzymes retained 60% and 90% of their original activities respectively after one month storage at5°C. The DEAE-cellulose and chitin bound endoproteases lost 37 and 18% of their initial activities respectively after 48 hr continuous haemoglobin hydrolysis at ambient temperature. Both the immobilised enzymes exhibited enhanced thermal stability as a consequence of enhanced conformational stability.

Conclusions

The cocoa aspartic endoprotease was successfully immobilised on DEAE-cellulose and chitin by ionic and covalent cross-linking respectively. The covalent coupling to chitin is a better method of immobilisation on account of high immobilisation yield, high protein binding and flexibility during application conditions. The DEAE-cellulose bound enzyme is susceptible to leaching upon minute changes in pH, ionic strength reducing its appeal for integration in continuous reactor system.

References

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