ABSTRACT

Proteolytic enzymes, or proteases (proteinases) are of particular importance to the food scientist and the food processing industry. Downstream processing of proteases/enzymes, in general, involves three major steps: cell disruption, initial fractionation, and high resolution fractionation. The inactivation of enzymes/proteases encountered during the different stages of downstream processing is presented in a quantitative fashion. The separation and downstream processing techniques for enzymes/proteases presented together provide a judicious framework to obtain reasonable quantitative estimates for inactivation of enzymes/proteases encountered by different workers under operating conditions.

INTRODUCTION

Proteolysis is important in digestion and in assimilation of food, in the cellular maintenance of proteins, in the manufacture of food, and other processes. Proteolytic enzymes, or proteases (proteinases) are of particular importance to the food scientist and the food processing industry. For example, the proteases are used in the production of cheese, tenderization of meat, chill-proofing of beer, and for other processes. Downstream processing is largely a matter of not losing more of the desired product than is absolutely necessary. Therefore, the reasons for such disappearance, physical loss, physical damage and irreversible chemical change are of central concern. At each stage of processing the proteases (or enzymes, in general) are subjected to inactivating influences. An integral stage in the production of enzyme preparations for industrial and other large-scale uses includes in most cases concentration and purification by such methods as vacuum evaporation, adsorption on specific carriers,
and precipitation with organic solvents or salts. These methods can expose
delicate enzymes to high temperatures or physico-chemical changes to alter
the properties of the enzyme molecule, leading to appreciable losses of
enzyme activity. This paper examines the influence of downstream process-
ing on protease inactivation.

From an economic point of view proteases are the most important indus-
trial enzymes. Alkaline serine protease of Bacillus licheniformis
utilization in detergents is the dominating commercial application of pro-
teases, followed by the Mucor protease in cheese manufacture, now firmly
established as a calf rennet substitute. The use of Aspergillus oryzae
fungal protease, particularly for modification of dough for bread and
cracker making, have made this enzyme the third most important of the
microbial proteases.

Enzymes are responsible for changes in food during growth, harvest,
storage, processing and subsequent retailing. Many enzyme-induced changes
are deleterious and the main aim of handling raw and processed foods is to
create conditions that are not favorable for those indigenous enzymes.
Thus, it is often desirable to inactivate enzymes in fluid food products
like fruit juices. This is usually accomplished by heating. However,
where destruction of micro-organisms is not necessary for the process, such
as in the production of frozen, concentrated orange juice, there may be
some advantage in protecting the properties of the material by inactivating
the enzymes without heat.

Downstream processing is largely a matter of not losing more of the
desired product than is absolutely essential. Therefore, the reasons for
such disappearance, physical loss, physical damage and irreversible
chemical change are of central concern. At every stage of downstream pro-
cessing, proteins and enzymes are subjected to physical shear forces. In
general, future development of large and medium-scale processes in biotech-
nology is likely to be increasingly dependent upon the costs of downstream
processing and product recovery operations because these costs represent a
significant proportion of the total process costs, and thereby have an
important bearing on the overall viability of processes on this scale.

Let us now examine the different steps involved in the separation of
proteins from cells, and in particular the influence of these processing
steps on the inactivation of enzymes in general, and more specifically, on
proteases. This is important since the isolation and purification of pro-
teins (and biomolecules, in general) may well become a major issue for