EXPERIMENTAL METHODS IN MODERN BIOTECHNOLOGY

Editors

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CONTENTS

Chapter 3. Methods for Screening the Potential Natural Compound for Treating Metabolic Disorder Diseases: Anti-Inflammatory and Griess Assay [Nitric Oxide (NO) Measurement]

Azura Amid, Sulawatie Semail, Wan Dalila Wan Chik and Hammed Ademola Monsur

Chapter 4. Factors Affecting Enzyme Assays

Hamzah Mohd Salleh

Chapter 5. Techniques of Extraction and Purification of Fucoxanthin from Brown Seaweeds

Irwandi Jaswir, Dedi Noviendri, Hamzah Mohd Salleh, Muhammad Taher and Kazuo Miyashita

Chapter 6. Fundamentals of Proximate Analysis in Food Products

Irwandi Jaswir and Asiyanbi-Hammed Tawakalit Tope

Chapter 7. Fish Gelatin Production: Extraction Method and Quality Analysis

Irwandi Jaswir, Hammed A. Monsur and Hamzah M. Salleh

Chapter 8. Application of Fourier Transform Infrared Spectroscopy Edible Fats and Oils Analysis

Mohamed Elwathig Saeed Mirghani

Chapter 12. Response Surface Methodology (RSM) Design for Bioreactor Operation

Maizirwan Mel and Najiah Nadir

Chapter 13. Screening Natural Compounds for Antibacterial Activity by Disc Diffusion Method

Raha Ahmad Raus

CHAPTER 4

Factors Affecting Enzyme Assays

Hamzah Mohd Salleh

1. Introduction to Enzyme Assay

Enzymes are proteins that have been termed "agents of life" since life processes are so dependent on them. Enzymes play key functions in controlling rate of reaction, coupling reactions, and sensing the momentary metabolic needs of the cell. Nowadays, many enzymes have been identified for diagnostic, medical, therapeutic and industrial uses.

An enzyme assay is used as a measure of the amount of a particular enzyme in a sample. Enzyme assays measure either the consumption of substrate or accumulation of product in a specific time. The depletion of substrates or the accumulation of products in enzyme-catalyzed reactions is commonly conducted in initial rate experiments where rates are measured for a short and specific time and under specific conditions after all components are added into the enzyme assay mixture.

Determination of substrate depletion or product accumulation can be divided into 2 types of sampling method during the enzyme assay:

a) Continuous assays - where the complete enzyme assay mixture gives a continuous reading of activity (giving direct measure of substrate depletion or product formation), and
b) Discontinuous assays - where aliquots are taken from the complete enzyme assay mixture at specified time intervals, the reaction stopped (by boiling or bringing the assay mixture to extreme pH) and then the concentration of substrates/products determined.

Continuous assays can be performed by spectrophotometric, fluorometric, calorimetric, and chemluminescent methods, whereas radiometric and chromatographic techniques are more common in discontinuous assays.

In certain continuous assay cases where neither the substrate(s) nor the product(s) are easily measurable, a coupled assay can circumvent this problem. In coupled assay, the product of one reaction is used as the substrate of another in a carefully designed and easily detectable reaction.

A discontinuous assay is also sometime referred to as stop time assay and it is the easiest way to do many assays at one time provided that the assays are linear as well as no major depletion of substrate and no inhibition by product, and that the compound to be measured is stable in the duration of measurement after stopping the enzyme reaction.

1.1 Factors That Affect Enzyme Activity

There are several factors that affect enzyme activity including temperature, pH and concentration of small compounds (substrates, inhibitors, salts). In this chapter, the effects of temperature, pH

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