

**OPTIMISATION OF CHOLESTEROL REDUCTION IN SQUID BY
SACCHAROMYCES CEREVISIAE USING RESPONSE SURFACE
METHODOLOGY (RSM)**

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ABSTRACT

OPTIMISATION OF CHOLESTEROL REDUCTION IN SQUID BY *SACCHAROMYCES CEREVISIAE* USING RESPONSE SURFACE METHODOLOGY (RSM)

The purpose of this study was to optimise the cholesterol reduction in squid by yeast extract glucose peptone (YEGP) broth containing *Saccharomyces cerevisiae* using Response Surface Methodology (RSM) of MINITAB Software (Version 14). Experimental design was created by RSM whereby test variables; pH of YEGP broth, concentration of *S. cerevisiae* (%), incubation temperature ($^{\circ}\text{C}$) and incubation time (hours) were used. Whole part of squid were blended and mixed thoroughly in different pHs of broth containing different concentrations of *S. cerevisiae* and treated differently in terms of incubation temperature and incubation time as suggested by the experimental design of RSM. The optimal centrifugal speed was investigated and relative centrifugal field (RCF) of 1000 x g at the temperature of 2°C for 10 minutes showed the best performance. This speed was able to give 33.51% reduction in total fat content of squid. This centrifugation condition was used to determine the optimum amount of cholesterol reduced in treated squid. Cholesterol analysis was carried out according to AOAC Official Method 994.10 and measured using Gas Chromatography: with Flame Ionization detector (GC-FID). Cholesterol content in squid was reduced to 77.49% at the optimum condition; pH 3.76 of broth, 0.6% concentration of *S. cerevisiae* 35.1°C of incubation temperature and 22 hours of incubation time. Verification experiment was also carried out and it was found that there was no significantly different at the 5% level between predicted and verified values of cholesterol reduction using the optimum condition determined by RSM. In addition, the significant regression equation or model at the 5% level of confidence was also established for the estimation of the percentage reduction of cholesterol in squid treated by *S. cerevisiae*. This indicates that *S. cerevisiae* is a potential source to be employed to reduce cholesterol in squid.

CHAPTER 1

INTRODUCTION

1.1 Background and problem statements

Cholesterol is a well-known and commonly determined lipid component and an important intermediate in the synthesis of steroid hormones. It is a sterol (C₂₇H₄₅OH) that occurs notably in animal fats and oils. The determination of cholesterol in serum and foods is of significance because of the implication of cholesterol in the etiology of arteriosclerosis and coronary heart disease (CHD) (Sheppard and Pennington, 2003). Arteriosclerosis is the narrowing and blockage of the arteries by plaque, which consists of cholesterol, calcium, clotting proteins, and other substances. When this process occurs in the arteries leading to the heart, the result is coronary heart disease (CHD) (Lavon, 2002). Therefore, the recommendation of dietary cholesterol is less than 300 mg/day (The American Heart Association, 2004).

Squid is widely used in our daily dishes and foods and contains high cholesterol levels. Squid and cuttlefish come under the class *Cephalopoda* (Phylum - *Mollusca*), which form one of the major marine resources and commercially exploited for its mantle meat that fetches a high export value (Sherief *et al.*, 1980). In 2004, squid contributed about 6% of the catch in Malaysia (Department of Fisheries, 2004).