PRODUCTION OF FLAVOUR ESTERS USING BIOTECHNOLOGICAL TECHNIQUES

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Keywords: flavour esters, microorganisms, enzymes, fermentation and optimization.

Introduction

Limited number of bacteria, yeasts and molds are involved in the production of specific flavour compounds such as lactones, terpenes, pyrazines and esters. Chemical synthesis of esters led to formation of undesirable racemic mixtures during the process and consumers do not like chemicals added to their food, therefore, search for naturally produced flavours and microbial production of esters become extremely important for the flavour industries. Research showed that amount of esters derived from production using microorganisms is comparatively low. Hence, possibility of increasing ester production either by genetic improvement of the microorganisms or optimisation of the cultural and environmental conditions during fermentation. Microorganisms are also economical source of enzymes, which can be utilised to enhance or alter the flavour of many food systems, which include enhancement of naturally occurring flavours from precursors already present in food, or elimination of undesirable flavours. This study aims to determine and evaluate the different cultural conditions for maximum ester production by *Geotrichum penicillatum* and *Hansenula anomala* and assess the flavour characteristics and apply to daily use in food products.

Materials and Methods

Microorganisms were obtained from the Food Microbiology Laboratory of the Food Science and Biotechnology, Universiti Putra Malaysia, Serdang. Culture conditions of the fungi were prepared in shake flasks and fermentor under batch systems using ten percent inoculum in 1.5 liter fermentation medium at a speed of 90 rpm and constant temperature of 30 C. Ten ml samples were withdrawn at every 24 h intervals for analyses of biomass, esters, alcohol, pH and glucose utilization. Determination of biomass was carried out using the dry weight method. Ethanol and component of esters (ethyl acetate, ethyl decanoate, isobutyl acetate, tributyl acetate, and isopentyl acetate) were determined by Gas Chromatography (GC). Efficiency of the conversion of glucose and biomass and ethyl acetate at different initial glucose concentration was calculated according to Jinap et al. (1996). Studies on effect of different initial concentration of carbon sources such as glucose, ethanol, fructose and sucrose were studied. Besides effect of different types of nitrogen sources, initial pH, aeration and temperature were also carried out to determine optimum ester production.

Results and Discussion

Geotrichum penicillatum produced esters at all the temperature and pH studied. Three major ester components of ethyl acetate, isobutyl acetate and isopentyl acetate were detected at all temperature (25C, 30C and 35C) and pH (3.0, 3.5, 4.0, 4.5 and 5.0) studied. Ethyl acetate could be detected as early as 24 h after fermentation whilst the others were detected after second day of fermentation. Maximum ethyl acetate detected 25 C and pH 3.0. Ethyl acetate was produced at day 6 or fermentation with a concentration of 820 ppm. Maximum isobutyl acetate was detected at 35 C and pH 4.5 with a concentration of 260 ppm at day 7 of fermentation (Fatimah et al, 1998). Another type of ester ie hexyl acetate was also being produced when sucrose was used as the substrate by the same fungus (Yusmiza, 1995). Production of biomass during fermentation reflected the growth of *G. penicillatum*. This was measured by the dry weight of the cell. At 25 C, maximum biomass was produced at pH 4.5 with a cell dry weight at 0.027 g/ml after 5 days of fermentation. Fermentation at 30 C showed maximum biomass production at pH 4.5 with a dry weight of 0.029 g/ml at day 7. Production of dry weight at 30 C did not indicate any significant difference between the different pH used. However, cell dry weight measured at temperature 35 C showed significant differences between the different pH used (Fatimah et al. 1998). Glucose utilisation by *G. penicillatum* showed maximum usage at 30 C and pH 4.5 with a percentage of 96.3% whilst for temperature 25 C and 35 C, percentage utilisation of glucose were at 90.5% and 91.9% respectively and both recorded at pH 4.0. Glucose was the major carbon source used by *G. penicillatum* (Yusmiza, 1995). Glucose concentration will be reduced with the growth of cells and ester production. Control of ester production could be carried out with the increase or decrease in glucose content (Jinap et al. 1996). They also reported that ester production in glucose media was high as compared to other carbon sources due to the absence of metabolic inhibition from pyruvic acid to acetyl Co A.

Conclusions

Production of esters by *G. penicillatum* is affected by temperature and pH. Ester components of ethyl acetate, isobutyl acetate and isopentyl acetate were produced at all temperature and pH studied. Maximum ethyl acetate was produced at 25 C and pH 3.0 with a concentration of 820 ppm. Isopentyl and isobutyl acetate were maximum at 35 C with a pH at 3.5 after day 7. Whilst glucose utilisation by fungus showed good usage of the substrate (above 90%) there was no correlation between biomass and the types and amounts of esters produced.

References

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Supported by IRPA Grant 03-02-04-0028