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*Full Length Research Paper*

# Supercritical carbon dioxide extraction of oil from *Clanis bilineata* (Lepidoptera), an edible insect

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**Oil was extracted from the dry meat of *Clanis bilineata* (Lepidoptera) using supercritical carbon dioxide in a continuous flow extractor. The following optimum extraction conditions were investigated: temperature, 35°C; pressure, 25 MPa; supercritical $CO_2$ flow rate, 20 L/min and time, 60 min. Under these extraction conditions, the oil extraction rate reached up to 97% (w/w). The level of unsaturated fatty acids in the extracted oil was 63.21% (w/w). In addition, the level of linolenic acid, a functional fatty acid, was as high as 37.61% (w/w) of the total fatty acids. Results of the present work indicate that *C. bilineata* (Lepidoptera) may be a promising oil resource for humans.**

**Key words:** Supercritical $CO_2$, *Clanis bilineata*, fatty acids, oil.

## INTRODUCTION

Many studies on a variety of edible insects which grow in the wild indicate that these insects are potential sources of oil for food and medicine (Chen, 1997; Raksakantong et al., 2010; Wu et al., 2000). *Clanis bilineata* (CB) Walker, a member of the subfamily Ambulicinae (Sphingidae, Lepidoptera), is an edible insect that usually grows on soybean leaves. About 6000 tones of CB larvae (CBL) are consumed in China each year. The fat content of dried CBL is 23.68% (w/w) and the level of unsaturated fatty acids in it is 64.17% (w/w). Linolenic acid, a functional fatty acid, can reach as high as 36.53 % (w/w) of the total fatty acids (Wu et al., 2000) in CBL.

In this paper, supercritical carbon dioxide extraction of oil from CBL was developed. The optimum process conditions were investigated and the extracted oil was characterised.

## MATERIALS AND METHODS

### Extraction of oil from CBL

Fifth instar larvae were purchased from a local agricultural market. The larvae were washed with tap water, killed with hot water (90°C), and dried at 60°C to a constant weight. The dried CBL was crushed into a fine paste and was defatted using a supercritical carbon dioxide extraction method (Model: HA 420-40-96, Jiangsu Huaan Scientific Instrument Co., Ltd., Jiangsu, China) with the following parameters: dried CB meat, 20 g; approximate size of dried CB meat, 0.53 mm; supercritical $CO_2$ flow rate, 20 L/min; pressure, 25 MPa; temperature, 35°C and time, 60 min.

### Characterisation of the extracted oil

About 1 g extracted oil was refluxed using 0.5 N sodium hydroxide solution in 5 ml methanol for 10 min. Subsequently, 5 ml of 14% $BF_3$-MeOH solution was added using a pipette through the condenser. The contents were boiled for 2 min, and then 5 ml heptane was added. The solution was further boiled for 1 min and then cooled. Next, 5 ml saturated NaCl solution was added and the fatty acid methyl esters were extracted using heptane (2 × 5 ml). The organic layer was dried over anhydrous $Na_2SO_4$. The fatty acid methyl esters were recovered after solvent evaporation in vacuum (Gören et al., 2003), and then analysed using a Trace 2000 GC series gas chromatograph and a Thermo mass spectrometer. A SGE BPX70 column (60 m × 0.25 mm, 0.25 mm film thickness) was used. The carrier gas was helium flowing at a rate of 1 ml/min. Oven temperature was kept at 100°C for 5 min, programmed to 240°C at a rate of 4°C/min, and kept constant at 240°C for 5 min. Injection and source temperatures were 250 and 220°C, respectively. Mass spectroscopy (MS) interface temperature was 240°C. Injection volume was 0.5 ml with a split ratio of 1:30. Electron impact/MS was taken at 70 eV ionisation energy. Mass range was from m/z 50 to 650 amu, and scan time was 0.5 s with a 0.1 interscan delay. The library search was carried out using NIST, Wiley gas chromatography (GC)/MS library, and TÜBÍTAKUME library. SupelcoTM 37 components FAME mixture (Catalog No. 47885-U) was used for the comparison of the GC chromatograms. Relative percentage of the separated compounds was calculated from the total ion chromatography by the computerised integrator.

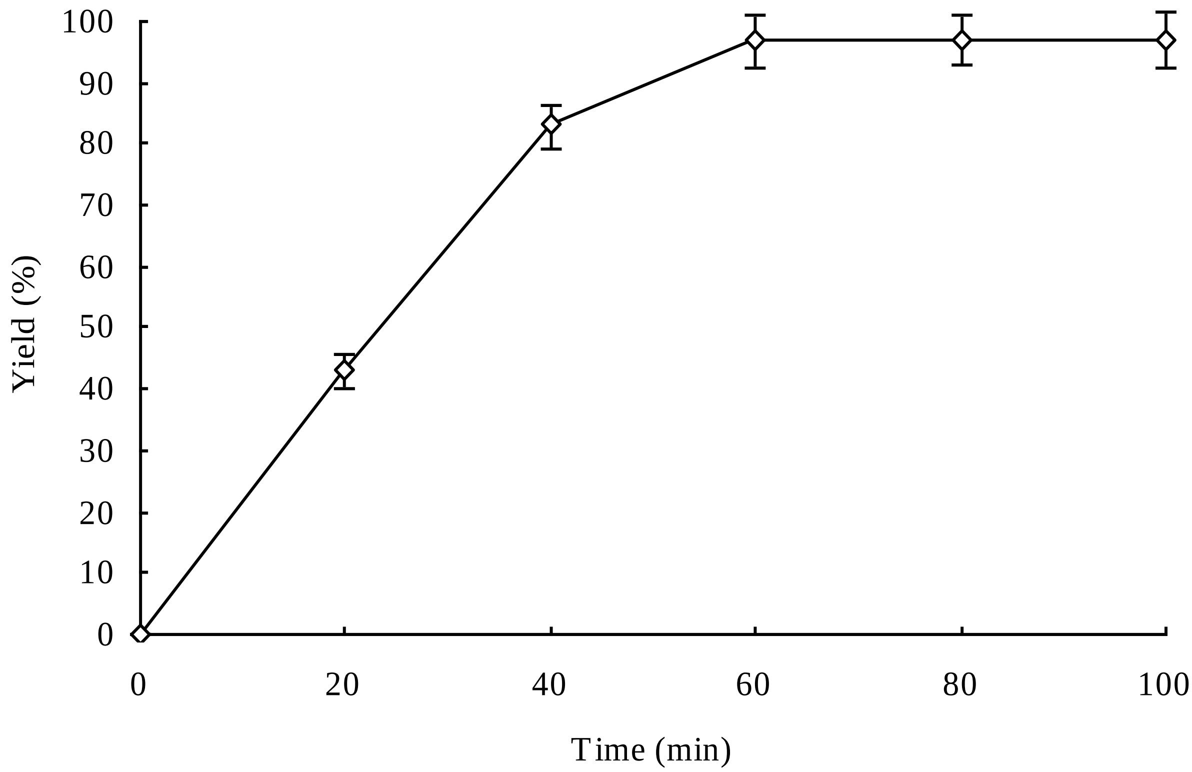


**Figure 1.** Effect of time on extraction of the oil from *C. bilineata* larvae.

## RESULTS

A sharp increase in yield occurred within 40 min, a slow increase from 40 to 60 min, and no further increase after 60 min (Figure 1). Therefore, optimum extraction time was 60 min.

Extract yield of the oil from CBL increased initially (<25 MPa), and then did not increase further with pressure at a given temperature (35°C) (Figure 2). Figure 3 shows the effect of temperature on the extract yield of the oil from CBL. The yield increased sharply with the increase in temperature ranging from 20 to 35°C. However, an additional increase in temperature did not improve the yield further, but decreased it instead.

The effect of supercritical $CO_2$ flow rate on the extraction of oil from CBL is summarised in Figure 4. Extraction yield increased sharply with an increase in supercritical $CO_2$ flow rate, reaching an optimum yield of 91% at 20 L/min, and then did not increase further.

The proportions of unsaturated fatty acids of CBL (63.21%, w/w) and linolenic acid content (37.61%, w/w) were comparable to those determined from previous research (Wu et al., 2000).

## DISCUSSION

The effects of time are important for the efficient extraction of oil from CBL. Therefore, time course studies on the extraction of oil from CBL were performed.

Generally, higher pressure is necessary to recover the oil completely from CB because increasing the pressure at a given temperature results in higher fluid density, which increases the extract solubility. The levelling off of the extract yield of the oil from CBL could be ascribed to the fact that most of the oil was already extracted.

The initial increase in yield was due to the enhanced solubility of oil in the fluid at higher temperature. However, the decline in yield at higher temperature may be attributed to the decrease in fluid density, which reduced the extraction efficiency.

Higher supercritical $CO_2$ flow rate could, of course, be advantageous to obtaining sufficient extract. However, the levelling off of the extract yield of the oil from CBL could also be ascribed to the fact that most of the oil was already extracted.

Jensen et al. (1997) reported that infants who receive formula with 3.2% (w/w) α-linolenic acid have higher plasma concentrations of phospholipid docosahexaenoic acid, but lower concentrations of arachidonic acid at 21, 60, and 120 days of age. Peterson and Calder (1999) observed that the spleen lymphocyte proliferation of rats in response to concanavalin A is significantly reduced (by 60%) by feeding them a diet containing high levels of γ-linolenic acid. Therefore, the high content of linolenic acid in CBL may have some physiological effects on humans.

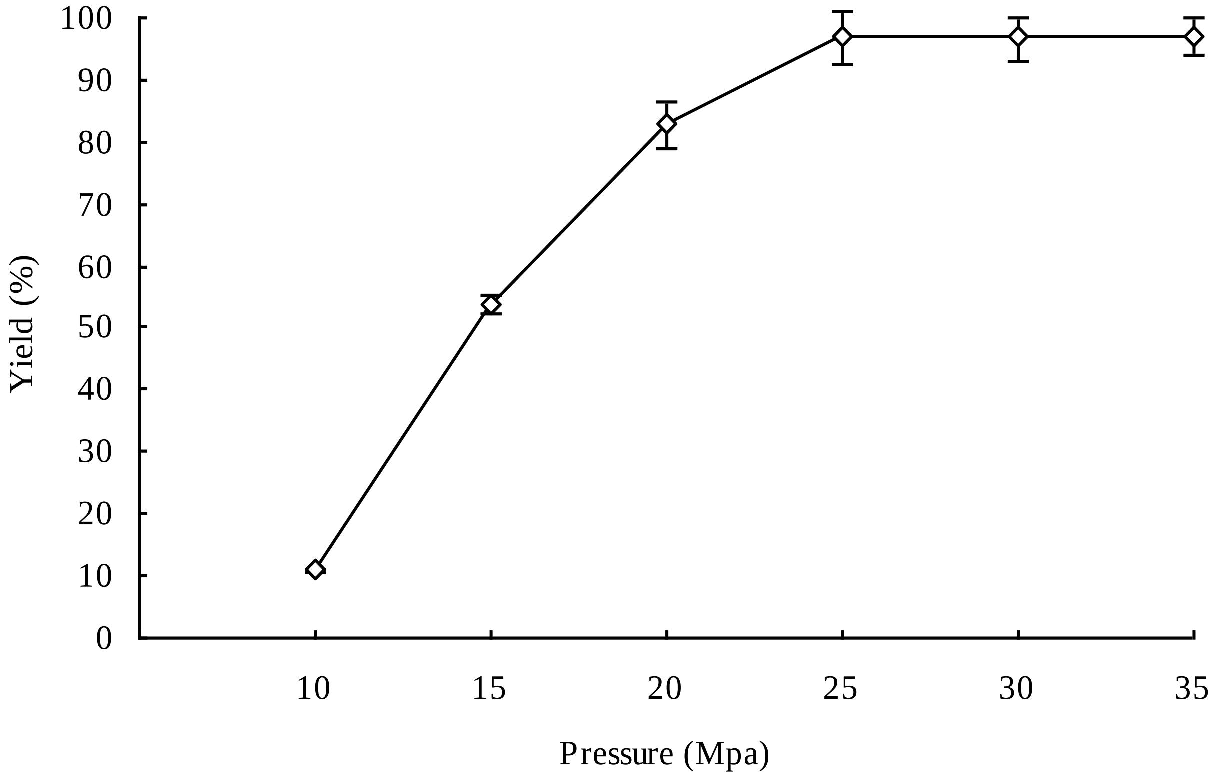


**Figure 2.** Effect of pressure on extraction of the oil from *C. bilineata* larvae.

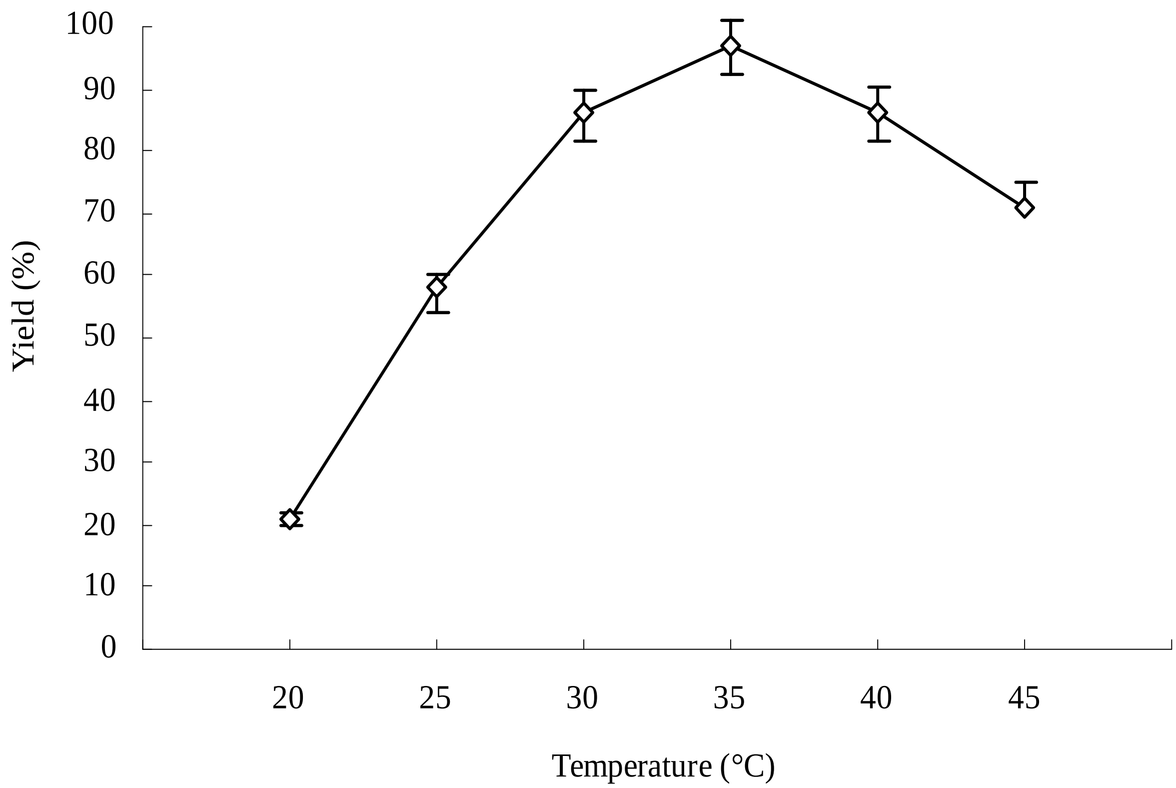


**Figure 3.** Effect of temperature on extraction of the oil from *C. bilineata* larvae.

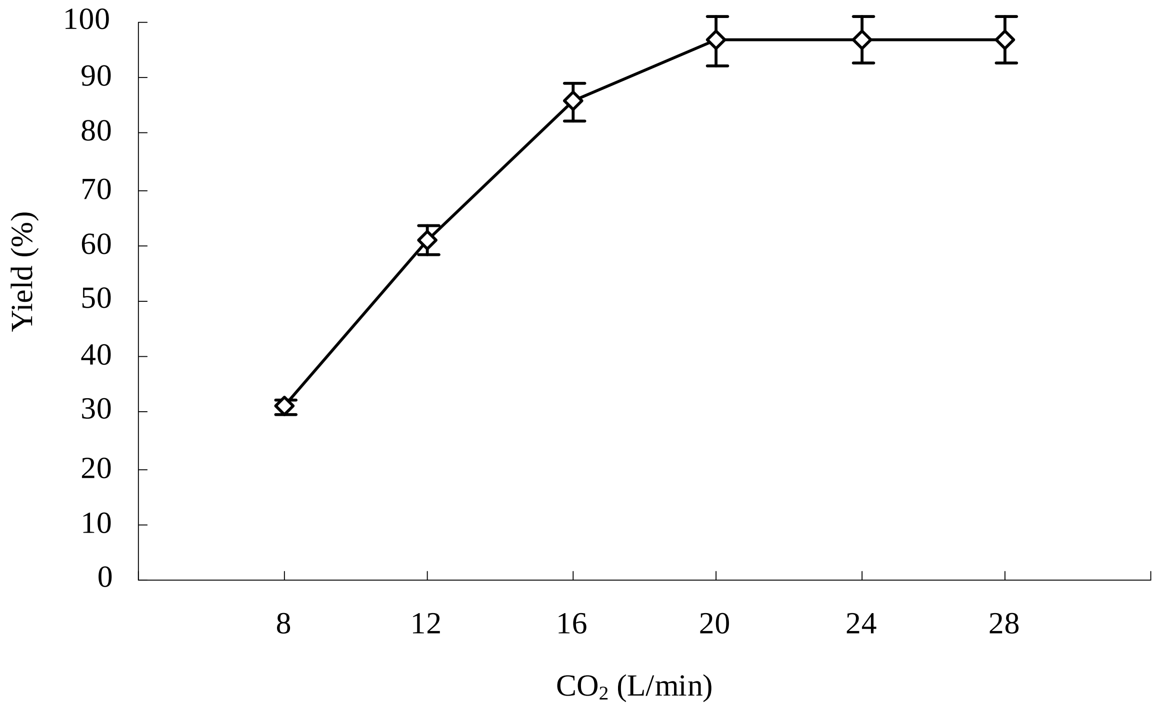


**Figure 4.** Effect of supercritical $CO_2$ flow rate on extraction of the oil from *C. bilineata* larvae.

## Conclusions

Supercritical $CO_2$ is effective in the extraction of oil from CBL. The highest yield (97%, w/w) was obtained at the following optimised extraction conditions: temperature, 35°C; pressure, 25 MPa; supercritical $CO_2$ flow rate, 20 L/min and time, 60 min. The composition of the oil extracted from CBL was comparable to that determined from previous research (Wu et al., 2000). Results indicate that oil from CBL may be a potential oil source for humans.

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