

Effects of Pressure and Temperature on the Extraction of Benzyl Isothiocyanate by Supercritical Fluids from *Tropaeolum majus* L. Leaves

Espinoza S. Clara, Gamarra Q. Flor, Marianela F. Ramos Quispe S. Miguel, Flores R. Omar

Abstract—*Tropaeolum majus* L. is a native plant to South and Central America, used since ancient times by our ancestors to combat different diseases. Glucotropaeolin is one of its main components, which when hydrolyzed, forms benzyl isothiocyanate (BIT) that promotes cellular apoptosis (programmed cell death in cancer cells). Therefore, the present research aims to evaluate the effect of the pressure and temperature of BIT extraction by supercritical CO₂ from *Tropaeolum majus* L. The extraction was carried out in a supercritical fluid extractor equipment Speed SFE BASIC Brand: Poly science, the leaves of *Tropaeolum majus* L. were ground for one hour and lyophilized until obtaining a humidity of 6%. The extraction with supercritical CO₂ was carried out with pressures of 200 bar and 300 bar, temperatures of 50°C, 60°C and 70°C, obtained by the conjugation of these six treatments. BIT was identified by thin layer chromatography using 98% BIT as the standard, and as the mobile phase hexane: dichloromethane (4:2). Subsequently, BIT quantification was performed by high performance liquid chromatography (HPLC). The highest yield of oleoresin by supercritical CO₂ extraction was obtained pressure 300 bar and temperature at 60°C; and the higher content of BIT at pressure 200 bar and 70°C for 30 minutes to obtain 113.615 ± 0.03 mg BIT/100 g dry matter was obtained.

Keywords—*Tropaeolum majus* L., supercritical fluids, benzyl isothiocyanate.

I. INTRODUCTION

TROPAEOLUM *majus* L. is a native plant to South and Central America that is used worldwide to combat different diseases, because it has a component called Glucotropaeolin, which when hydrolyzed produces BIT which is a potent antibiotic for combat respiratory and urinary infections, preventing the proliferation of bacteria [1].

At the global level, concern for cancer diseases is increasing and the interest of research is growing to obtain compounds in vegetables, seeds, ornamental flowers, which help counteract the disease and prevent it.

BIT promotes apoptosis or programmed cell death in

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cancerous cells of the breast and ovaries, without affecting healthy cells contrary to chemotherapy [2].

The plants of *Tropaeolum majus* L. produce significant amounts of benzyl glucosinolate [3].

The Glucotropaeolin or benzyl glucosinolate accumulates in mature plants of *Tropaeolum majus* L. [4], this being the main active principle of *Tropaeolum majus* L. which is an essential oil glycoside called Glucotropaeolin that when ingested in therapeutic doses is attributed bacteriostatic and antimycotic properties [5].

The extraction method reported by Barroso et al. [6], who performed the extraction of BIT from seeds of *Carica papaya* L., was taken as the reference. The identification of BIT was performed by thin layer chromatography and quantification by HPLC.

For the extraction, supercritical CO₂ was used. This is considered as green technology increasingly used, in which there is no residue of solvents in the extracted product and its purity is high.

There is very little bibliography referring to the extraction of BIT by supercritical CO₂, so a screening of extraction at various pressures and temperatures was carried out prior to commencing the work and those selected reported a higher yield. The objectives were: Determination of the effect of supercritical fluid pressure and temperature on the yield of oleoresin and BIT from leaves of *Tropaeolum majus* L.

II. MATERIAL AND METHODS

A. Materials and Equipment

Leaves of *Tropaeolum majus* L. were collected from the greenhouse of the Experimental Station "El Mantaro" of the National University of the Center of Peru.

The leaves were harvested, weighed and rested for one hour to be lyophilized at 1 Pa pressure conditions, Freezing temperature of -70 °C, and final temperature of 15 °C; until obtaining a final moisture of 6%, in a freeze-dryer, brand BIOBASE. The leaves were then crushed until obtaining a modulus of average finesse. The carbon dioxide used in the supercritical extraction was 99.99% pure (PRAXAIR S.A. Lima - Peru).

B. Methods

For extraction of BIT from *Tropaeolum majus* L. leaves, the Spe-ed SFE BASIC supercritical fluid extraction system, Spe-ed SFE - PolyScience, was used. Crushed leaves of 25 g were used and the experiments were performed under 200 bar and

300 bar conditions for pressure; and 50, 60 and 70 °C for the temperature, with an extraction time of 30 minutes, the extract obtained was weighed to determine the yield of oleoresin. The experiment was performed in duplicate.

C. Identification of BITC

It was performed by TLC (thin layer chromatography), 7 cm x 6 cm silica gel plates were used, each treatment being identified. The plates were divided into four equidistant points, at one point the standard 98% BIT standard was sowed and at the other three points the BIT was extracted at temperatures of 50, 60 and 70 °C for the two pressures studied. Dichloromethane: hexane (2: 4) was used as the mobile phase and each plate was allowed to develop in a glass chamber for chromatography for about 5 minutes. It was visualized in a UV light chamber at 254 nm, then sprayed with ammoniacal silver nitrate until dark brown spots appeared, then sprayed with 0.5 N nitric acid. Finally, the Rf was determined.

After revealing the presence of BIT by the spots present in the chromatograms, the quantification was performed by HPLC.

D. Quantification of Benzylisothiocyanate

HPLC equipment was used. Brand: Knauer, Model: SMARTLINE, consisting of: Quaternary Analytical Pump, Variable Wave UV-VIS Detector, Automated Autosampler, Column Oven. ChromGATE software was used, using the Rodriguez method [7]. This method was validated by the proficiency tests mentioned in USP [8], with five times the same standard injection at a concentration of 53.9 µg/mL, obtaining RSD of 1.21%. The HPLC reading was developed with the following chromatographic conditions:

Column: Knauer Eurospher II L7 (C8) 50 mm x 4.6 mm x 5 µm. Mobile Phase: Mixture filtered and degassed Acetonitrile: Methanol (50:50). Detector: UV, 246 nm. Flow rate: 1 mL/minute. Injection volume: 20 µL. Temperature: 30°C. Run time: about 5.00 min.

III. RESULTS AND DISCUSSION

A. Identification of BIT

By thin layer chromatography, the presence of BIT was detected in all extracted samples (Figs. 1 and 2). The BIT pattern stain was at the same height as the other applied treatments, obtained as Rf values between 0.5 to 0.6. Csáky and Martinez [4] mentions that if a component elutes an Rf of less than 0.2 or greater than 0.7, it may happen that what looks like a single compound is actually a mixture of several, in these cases it must be changed with another more or less polar solvent, respectively. The Rf obtained is within the standards mentioned above.

The hexane: dichloromethane solution in 4:2 ratio was used as the eluent, the visualization was performed in a UV light chamber at 254 nm and used ammoniacal silver nitrate as a developer until the appearance of dark brown spots, then sprayed with acid 0.5 N nitric acid, thus identifying the BIT,

then performing the quantification by HPLC.

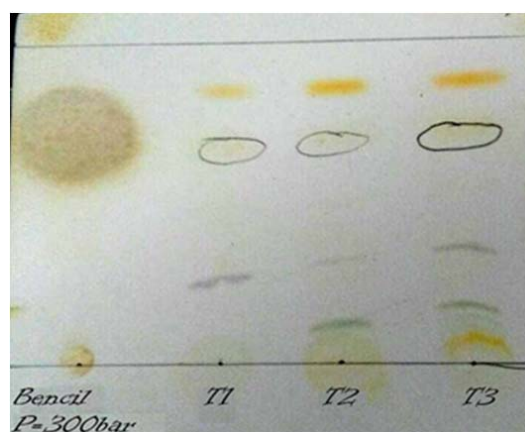


Fig. 1 Plate chromatography pressure conditions 200 bar and temperatures 50, 60 and 70 °C

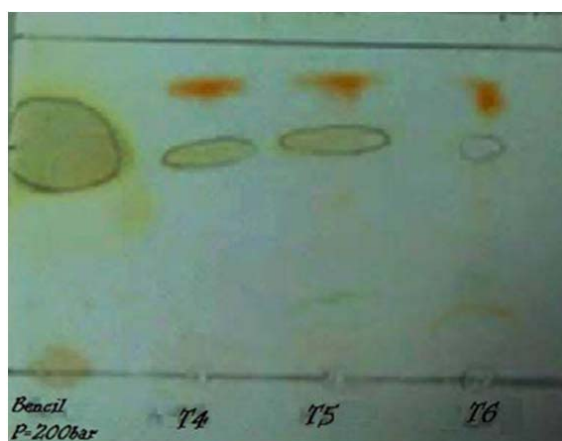


Fig. 2 Plate chromatography pressure conditions 300 bar and temperatures 50, 60 and 70 °C

B. Quantification of BIT

The amount of BIT that each treatment presented was quantified by HPLC, the highest amount in the treatment was obtained at pressure conditions 200 bar and temperature of 70 °C with 113.615 ± 0.03 mg of BIT per 100 g of dry sample. BIT amounts were calculated from the areas of the peaks obtained by performing HPLC.

The chromatographic profile of the BIT standard (Fig. 3) is shown, which depicts the well-defined chromatographic peak, and solved at a retention time of 1.367 min corresponding to BIT.

The chromatographic profiles of the six treatments applied for the extraction of BIT from *Tropaeolum majus* L. by supercritical CO₂ (Figs. 4 and 5) were used, where the chromatographic peaks are well defined and resolved in a time of 1.367 minutes, when compared with the standard appear in an approximate retention time indicating the presence of the compound (BIT) in the oleoresins obtained by supercritical CO₂.

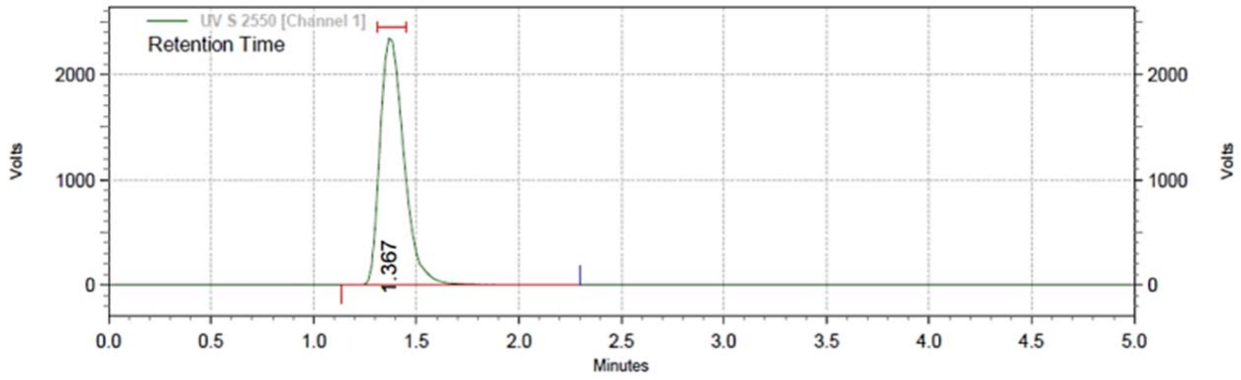
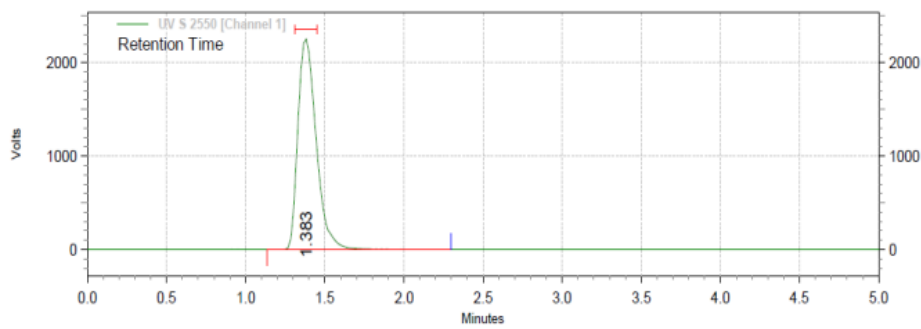
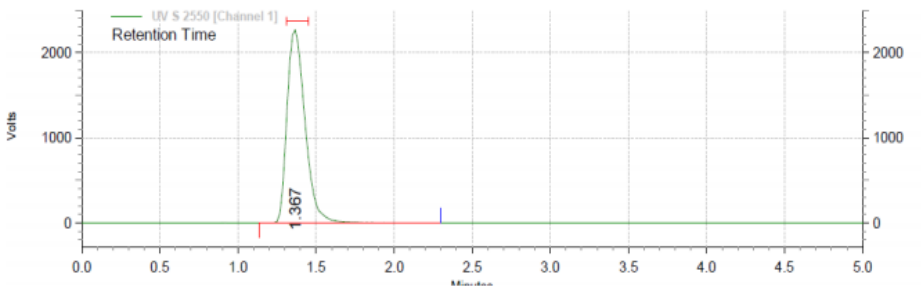


Fig. 3 Pico standard chromatographic BIT

T1



T2



T3

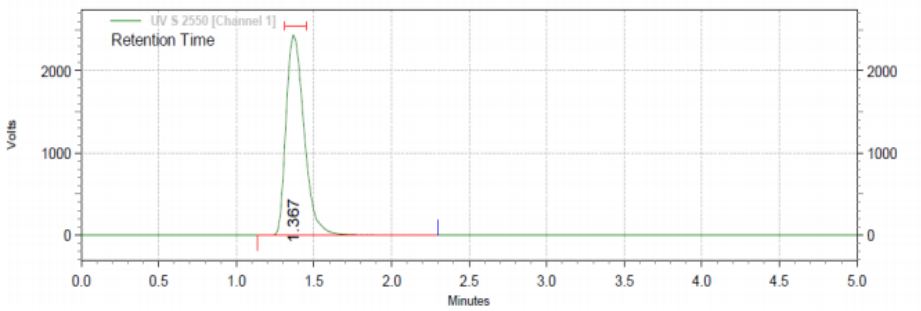
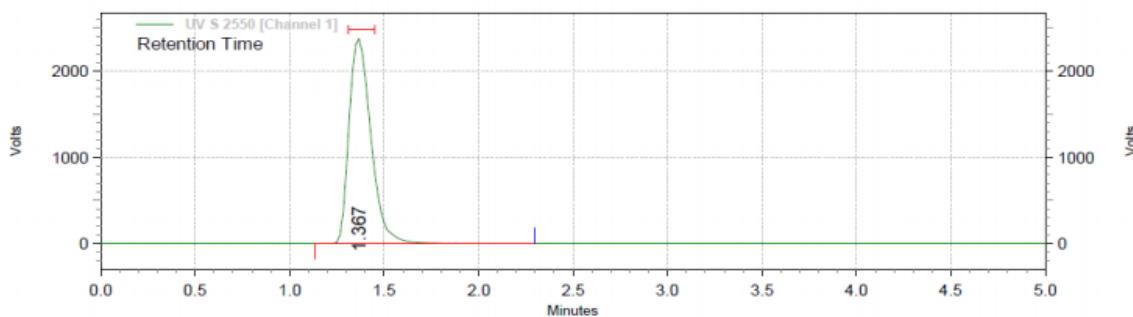
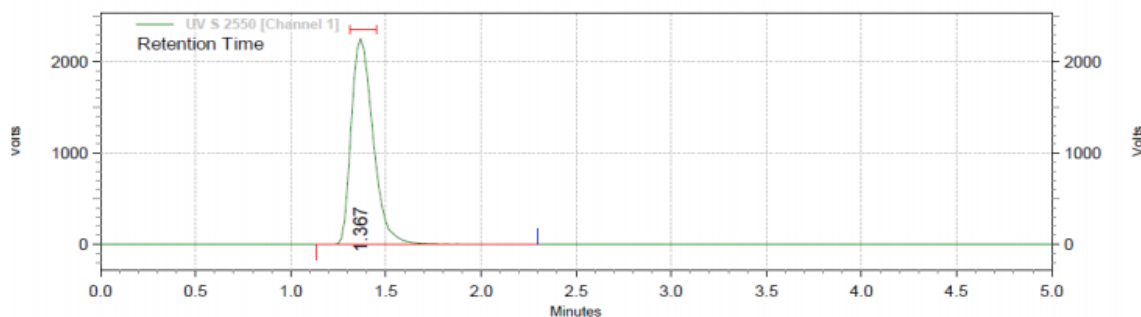


Fig. 4 Chromatogram of extracts of BIT under 300 bar, T1 =50°C, T2 = 60°C and T3 = 70°C

T4



T5



T6

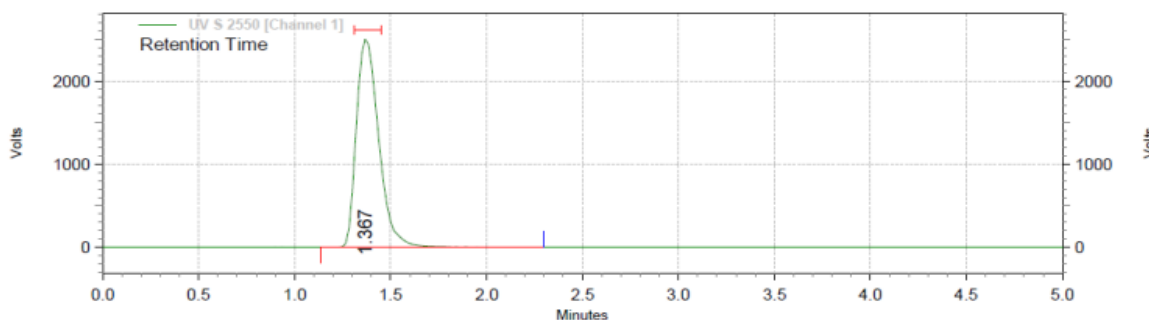


Fig. 5 Chromatogram of extracts of BIT pressure 200 bar, T1 = 50°C, T2 = 60°C, T3 = 70°C

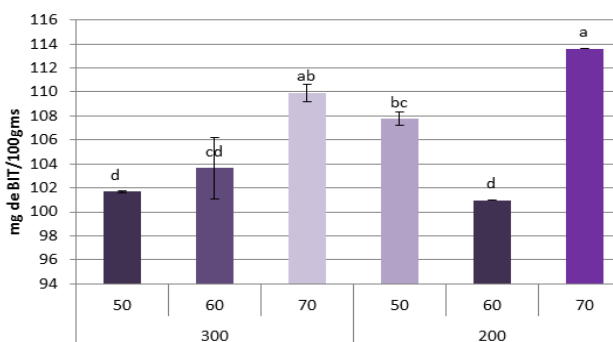


Fig. 6 Test comparison of means by Tukey Note: T1 (P = 300, T = 50), T2 (P = 300, T = 60), T3 (P = 300, T = 70), T4 (P = 200, T = 50), T5 (P = 200, T = 60) and T6 (P = 200, T = 70)

Quantification was performed by the reported areas whose mean comparison is shown in Fig. 6.

The range of the amount of BIT extracted by supercritical CO₂ ranged from 100.963 ± 0.02 mg of BIT/100 g of dry matter to 113, 615 ± 0.03 mg of BIT/100 g of dry matter (Fig. 6). When comparing only the pressure of 300 bar, it is observed that the amount of BIT increases as the temperature increases from 101.684 mg to 109.905 mg of BIT per 100 g of dry matter, this does not happen with the pressure of 200 bar, since the temperature of 70 °C has a higher quantity of BIT, with respect to the temperatures of 50°C and 60°C, when the temperature drops by 10 °C, the BIT decreases significantly and by decreasing by 20 °C, the BIT decreases also but presents more quantity than that of 60 °C. The highest amount of BIT was obtained in the treatment T6 (P = 200 and T = 70)

with 113.615 mg of BIT per 100 g of dry sample, followed by T6 (P = 300 and T = 70), both presented the same temperature with the difference being that as pressure decreases, more BIT is obtained.

Barroso et al. [6] evaluated the efficiency and composition of the oil extracted from seeds of *Carica papaya* L with supercritical carbon dioxide, concluding that the best treatment was found at 40 °C and 150 bar followed by 80°C and 200 bar, Ahluwalia [9] mentions that the composition of the extract can be controlled by the regulation of the pressure; with the increase in temperature there are improvements in mass transfer as it causes the increase in the vapor pressure of the extractable compounds which is more significant than the reduction in the density of the solvent, thereby increasing the overall extraction yield.

Statistical analysis was performed using analysis of variance ANOVA with $\alpha = 0.05$ of the amount of BIT obtained in the six treatments performed with supercritical CO₂ in *Tropaeolum majus* L. leaves, to examine the variability, and to verify if the means differ significantly, two operational variables such as pressure at two levels (200 bar and 300 bar) and temperature at three levels (50, 60 and 70 °C) were tested, six treatments with two replications were applied. We used the completely randomized design (DCA) with a 2 x 3 factorial arrangement. The Tukey multiple comparisons test was performed, where T1 (P = 300 bar ^ T = 50 °C) and T5 (P = 200 bar ^ T = 60 °C) treatments were found to have no statistically significant difference yield of the BIT present in the leaves of *Tropaeolum majus* L., also indicating that they have the lowest yield of the treatments performed, and so it was assigned the subgroup d; also, it is observed that treatments T1 and T5 present a significant difference compared to treatments T3, T4 and T6. The treatments T2 (P = 300 bar and T = 60°C) and T4 (P = 200 bar and T = 60 °C) showed no significant difference with respect to BIT yield, and so was assigned group c; it is also observed that T2 treatment does not present a significant difference with treatments T1 and T5, but it is statistically significant with treatments T3 and T6. The treatments T4 (P = 200 bar and T = 60 °C) and T3 (P = 300 bar and T = 70 °C) did not present a statistically significant difference for the BIT yield present in the leaves of *Tropaeolum majus* L., it was assigned the group b, since it presents an intermediate yield between the other treatments; it is also observed that treatment T4 does not present significant difference with treatment T2, but it is statistically significant with treatments T1, T5 and T6.

Finally, in the subgroup of treatments T3 (P = 300 bar and T = 70 °C) and T6 (P = 200 bar and T = 70 °C) are the treatments with the highest amount of BIT, which are 109,905 mg of BIT/100 g of dry matter and 113,615 mg of BIT/100 g of dry matter, respectively. These treatments do not present statistically significant difference with respect to the yield of BIT. It is also observed that treatment T3 presents a significant difference with treatments T1, T2 and T5; it is also observed that treatment T6 presents a statistically significant difference with treatments T1, T2, T4 and T5. Then we can state that the best treatment applied for the extraction of BIT by

supercritical CO₂ is the treatment T6 that was performed under the conditions of: pressure (200 bar) and temperature (70 °C); followed by treatment T3 which was performed with the following conditions: pressure (300 bar) and temperature (70 °C).

IV. CONCLUSION

The highest yield of oleoresin leaves (*Tropaeolum majus* L) extracted with supercritical CO₂ was 35.67% at Pressure = 300 Bar and Temperature = 60 °C and the highest amount of BIT extracted with supercritical CO₂ at (*Tropaeolum majus* L.) was 113.615 ± 0.03 mg BIT/100 g dry matter under the conditions of: Pressure = 200 bar and Temperature = 70°C.

RECOGNITION

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