# EVALUATION OF THE EXTRACTION OF TACAMAHACO (*PROTIUM HEPTAPHILLUM)* RESIN'S ESSENTIAL OIL USING SUPERCRITICAL CARBON DIOXIDE

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**Abstract.** Protium heptaphillum (Aubl.) March. Burseraceae (PH) is a medicinal plant widely encountered in the Amazon region and Suriname, Colombia, Venezuela and Paraguay. The resin collected from the trunk wood of PH is used in skin diseases, healing of ulcers and as an analgesic. Studies have reported pharmacological effects of its essential oils. An experimental design $2^3$ was developed to investigate the effect of operating pressure, operating temperature and extraction time on essential oil yield and on its quality during supercritical carbon dioxide extraction from the resin. The equipment used was a Spe-ed SFE (Applied Separations). The variables evaluated were: pressure (80-100 bar), temperature (40-60°C) and static time (30-35 min). The response variables were yield and quality of the essential oil measured as the concentration of p-cymen-8-ol. The statistical analysis of the experiment showed that individual factors such as pressure, temperature and static time and the interactive effect of pressure with static time had significant effects on essential oil yield and on the quality. The central composite design followed produced a surface response which gave the optimal condition for essential oil extraction within the experimental range of the variables studied which was for 33°C a pressure of 105.6 bar and a static time of 28 min; for the percentage of p-cymene-8-ol for 43°C, a pressure of 106.8 bar and a static time of 36.63 min. A perfume was made with the essence oil obtained by the extraction supercritical and a market survey to find its level of acceptance from the public.

**Keywords**: Protium heptaphillum, supercritical fluid extraction, experimental design.

## 1. Introduction

Production of essential oil is one of the activities which countries with a high biological diversity can take economic advantage. In our country, an oil producer, the industry of essential oil production does not exist. However, Venezuela imported essential oils in 2011 to the value of more than 476 million U.S. dollars [1]. The species of Burseraceae are known to exude resins which are rich in essential oils [2] terpenes and other compounds [3]. The high amount of essential oil produced for some of the species in this family could be used in the perfumery industry with all the economic potential this implies [2]. The Burseraceae family contains 18 genera with 700 species divided in three tribes: Protieae (three genera), Canarieae (eight genera) and Bursereae (seven genera). The Protium genus (Tribe Protieae) is the main family member with 150 species [4]. All these species are represented in South America, Africa and Indo-Asian Tropics with the higher diversity found in the Southern Hemisphere [5]. The genus Protium makes up 80% of the Burseraceae in the Amazon Region of Brazil and can be found in countries as Surinam, Colombia, Paraguay and Venezuela and is known as an excellent source of oleoresin, which is used in medicine as an anti-inflammatory, analgesic, expectorant and insect repellent [4] it was also used in colonial times as incense in churches [6].

Protium Heptaphillum (Aubl.) March is known in Venezuela as tacamahaco and in Brazil as breu branco or almecega, its resin has a high percentage of volatile compounds and several terpenes. The essential oil obtained from leaves and resin have being found to have anti-inflamatory, antitumoural an anti-leishmaniasis activities [7,8]. The major constituents described from the essential oil hydrodistilled were the monoterpenes: limonene and cineol (44%); p-cymene (27%) and phellandrene (9%) [7].Other specimens collected in Ceara, Brazil, had reported 86.4 % monoterpenes with terpinolene the main constituent (28.5 %) followed by alfa-pinene (10.5%) and phellandrene (16.7%). Other researchers have studied the essential oil in fruits and leaves [9]. Two subspecies have been studied in Acre state, Brazil and reported terpinolene (42.31%) and p-cymene (39.93 %) as the main components [4].

This paper reports the evaluation of the effect of pressure, temperature and static time in the extraction of the essential oil from the oleoresin in order to find out which one produces the one with highest quality to be used in perfume production.

## 2. Materials and methods

The resin of Protium Heptaphillum was collected by the indigenous community of Maniapure in the Cedeño District in Bolivar State of Venezuela. The resin was dried and manually triturated and five grams were used in every extraction.

### 2.1 Extraction with supercritical $CO_2$

The extraction was carried out with a supercritical equipment type Spe-ed SFE-2, model 701, Applied Separations. To determine the most statistically significant variables to obtain the best quality essential oil, a $2^3$ experimental design was used, 2 meaning the levels under study, (low and high) and 3 the evaluated factors: pressure (80-100 bar); temperature ($40^0$ C) and static time (30 - 35min). The response variables were yield and quality of the essential oil measured by the amount of p-cymen-8-ol responsible for the aroma. The factorial design gives a combination of 8 treatments plus the replicas made a total of 16 experiments. Table 1 shows the first 8 experiments for the design.

### 2.2 Chromatographic analysis

To identify p-cymen-8-ol the essential oil was diluted in ethanol and analysed in a GC HP Agilent 7890 a coupled with a mass detector 5975VL with a HP5 capillary column (30m x0.25mm i.d x 0.25µm f.t) using helium as carrier gas and a temperature program with a initial temperature of 50ºC for 2 min increasing 4ºC/min to 180ºC and then to 250ºC at 20ºC/min. The individual components were identified comparing the mass spectra with the Wiley library.

## 3. Results and discussion

The chromatograms of the essential oil extracted from the resin of Protium heptaphillum were studied to look for the compounds responsible for the aroma and which were in a major proportion. P-cymen-8-ol was the compound which was chosen to measure the quality of the essential oil.

A variance analysis showed how the response is affected by the variables under study, pressure being the most statistically significant variable for extraction yield and for quality of the essential oil followed by temperature and for static time, so the three factors had to be taken in consideration. A central composite design was chosen to extend the range of the levels of the variables. Table1 shows these factors.

Table 2 shows the results of the experiments for the extraction yield and the quality of the essential oil to introduce in the software program Statgraphics Centurion XV.II to get the response surfaces for the central rotable design both for yield of the extraction and for quality. As there are three statistically significant factors for each response variable, it is not possible to make a graph in three dimensions, so, to make a response surface one of the factors should be left constant to analyse the figures for each factor in its low, high and optimal level.

**Table 1.** Experimental results for the supercritical fluid extraction of the essential oil from the Protium Heptaphillum 'resin.

| Nº | Extraction conditions | | | Results | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **P ($P \pm 0,1$) bar** | **T ($T \pm 1$) ºC** | **Static time ($t_E \pm 0,01$) min** | **Sample weight ($m_m \pm 0,001$) g** | **Weight container ($m_{ev} \pm 0,001$)g** | **Container plus oil ($m_{ea} \pm 0,001$) g** | **Empty weight ($m_{ae} \pm 0,002$) g** | **($R \pm 0,003$) %** | **Yield of p-cymen-8-ol ($p \pm 0,01$)%** |
| **1** | 100,0 | 60 | 35,00 | 5,010 | 16,550 | 16,621 | 0,071 | 1,417 | 57,22 |
| **2** | 80,0 | 60 | 35,00 | 5,013 | 16,606 | 16,659 | 0,053 | 1,057 | 17,09 |
| **3** | 100,0 | 40 | 35,00 | 5,007 | 16,614 | 16,680 | 0,066 | 1,318 | 67,09 |
| **4** | 100,0 | 60 | 30,00 | 5,001 | 16,561 | 16,626 | 0,065 | 1,299 | 54,53 |
| **5** | 100,0 | 40 | 30,00 | 5,005 | 16,428 | 16,492 | 0,063 | 1,278 | 66,42 |
| **6** | 80,0 | 40 | 35,00 | 5,088 | 16,623 | 16,672 | 0,049 | 0,963 | 48,08 |
| **7** | 80,0 | 60 | 30,00 | 5,017 | 16,577 | 16,620 | 0,043 | 0,857 | 38,95 |
| **8** | 80,0 | 40 | 30,00 | 5,016 | 16,529 | 16,563 | 0,034 | 0,677 | 47,47 |

**Room Pressure**: (720.45 ± 0.05) mmHg, **$CO_2$ Flow**: (2.5 ± 0.1) L/min, **Dynamic Time:** (60.0 ± 0.1) min, **Room Temperature:** (28. 0 ± 0.5) °C

**Table 2.** Experimental design for the elaboration of response surface

| Nº | Extraction conditions | | | Results | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | P ($P \pm 0.1$) bar | T ($T \pm 1$) ºC | Static time ($t_E \pm 0.01$) min | Sample weight ($m_m \pm 0.001$) g | Weight container ($m_{ev} \pm 0.001$) g | Container plus oil ($m_{ea} \pm 0.001$) g | Empty weight ($m_{ae} \pm 0.002$) g | ($R \pm 0.003$) % | Yield of p-cymen-8-ol ($p \pm 0.01$)% |
| **C1** | 90,0 | 50 | 32,50 | 5,006 | 16,522 | 16,571 | 0,049 | 0,979 | 65,81 |
| **C2** | 90,0 | 50 | 32,50 | 4,999 | 16,588 | 16,633 | 0,045 | 0,900 | 56,46 |
| **C3** | 90,0 | 50 | 32,50 | 5,005 | 16,503 | 16,556 | 0,053 | 1,058 | 49,84 |
| **C4** | 90,0 | 50 | 32,50 | 5,003 | 16,608 | 16,659 | 0,051 | 1,019 | 53,74 |
| **C5** | 90,0 | 50 | 32,50 | 5,003 | 16,540 | 16,593 | 0,053 | 1,059 | 58,70 |
| **A1** | 106,8 | 50 | 32,50 | 5,001 | 16,558 | 16,626 | 0,068 | 1,360 | 60,60 |
| **A2** | 73,2 | 50 | 32,50 | 5,010 | 16,535 | 16,568 | 0,033 | 0,658 | 62,75 |
| **A3** | 90,0 | 67 | 32,50 | 5,007 | 16,600 | 16,654 | 0,054 | 1,078 | 51,83 |
| **A4** | 90,0 | 33 | 32,50 | 5,014 | 16,562 | 16,631 | 0,069 | 1,376 | 52,88 |
| **A5** | 90,0 | 50 | 37,00 | 5,010 | 16,484 | 16,533 | 0,049 | 0,978 | 58,31 |
| **A6** | 90,0 | 50 | 28,00 | 5,001 | 16,512 | 16,566 | 0,054 | 1,079 | 51,96 |

**Room Pressure**: (720.45 ± 0.05) mmHg, **$CO_2$ Flow**: (2.5 ± 0,1) L/min, **Dynamic Time:** (60.0 ± 0.1) min, **Room Temperature:** (28.0 ± 0.5) °C

The following Equation 1 for yield of the extraction was achieved:

$$\boldsymbol{YIELD} = -1.99462 + 0.072013P - 0.0694841T + 0.0156511(ST) + 0.0000539932P^2 - 0.000027P * T - 0.00141P * (ST) + 0.000806315T^2 + 0.00038T * (ST) + 0.00165729(ST)^2 \quad (1)$$

**P**= Pressure, **ST**= Static Time,**T**= Temperature

In the following figures some of the response surfaces for yield of extraction are shown:

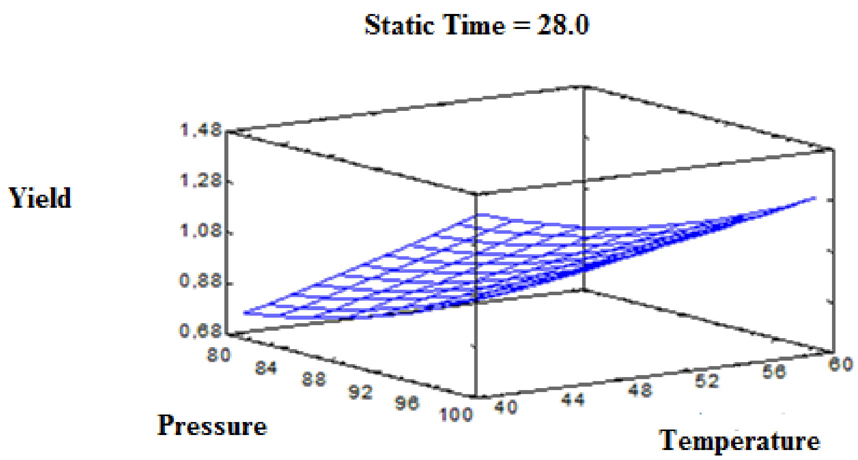


**Figure 1.** Estimated response surface for yield in the supercritical extraction process

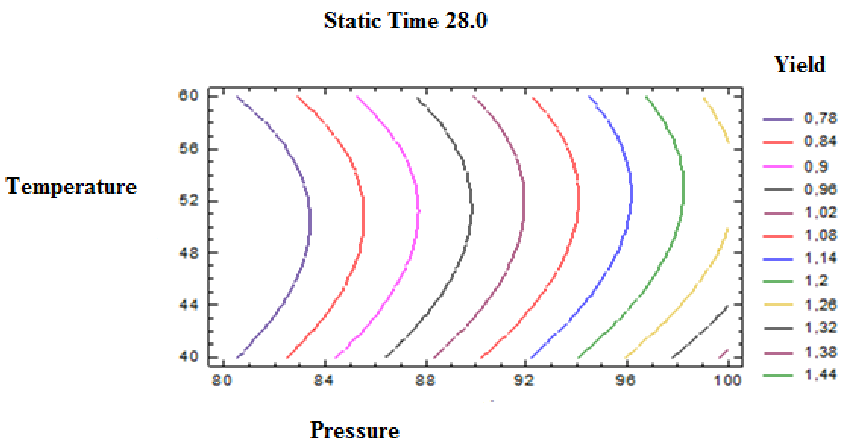


**Figure 2.** Contour for the estimated response surface for yield with static time: 28 min

It's observed from figures 1 and 2 that the higher the pressure the higher the yield and it is possible to estimate the operation conditions to maximize yield which are the following:

**Table 3.** Optimal values for yield

| Factor | Low | High | Optimal |
|---|---|---|---|
| **Pressure (bar)** | 73.2 | 106.8 | 105.61 |
| **Temperature (°C)** | 33.0 | 67.0 | 33.0 |
| **Static Time (min)** | 28.0 | 37.0 | 28.0 |
| **Optimal values for yield** = 1.776 % | | | |

Results for the quality (% of p-cymen-8-ol) were analysed by the software program and the following regression equation for quality was produced;

$$\boldsymbol{Quality} = 26.006 - 4.75623P + 2.87111T + 9.85041(ST) + 0.00398484P^2 + 0.0234375P * T + 0.10815P * (ST) - 0.0280675T^2 - 0.08015T * (ST) - 0.244606(ST)^2 \qquad (2)$$

**P**= Pressure, **ST**= Static Time, **T**= Temperature

In the following figures some of the response surfaces for quality are shown:

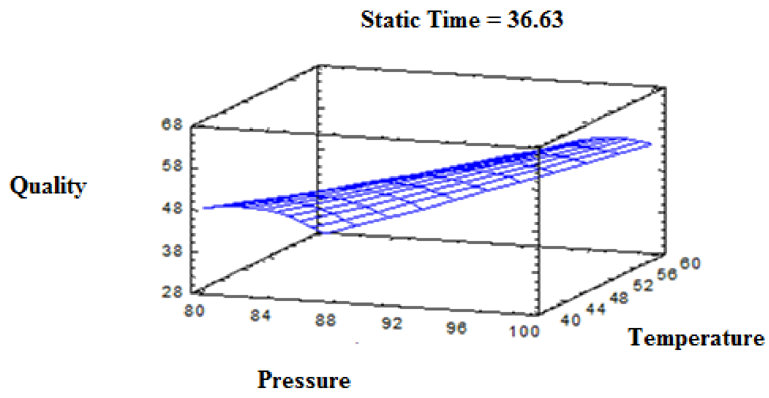


**Figure 3.** Estimated response surface for % of p-cymen-8-ol in the supercritical extraction process with static time: 36.63 min

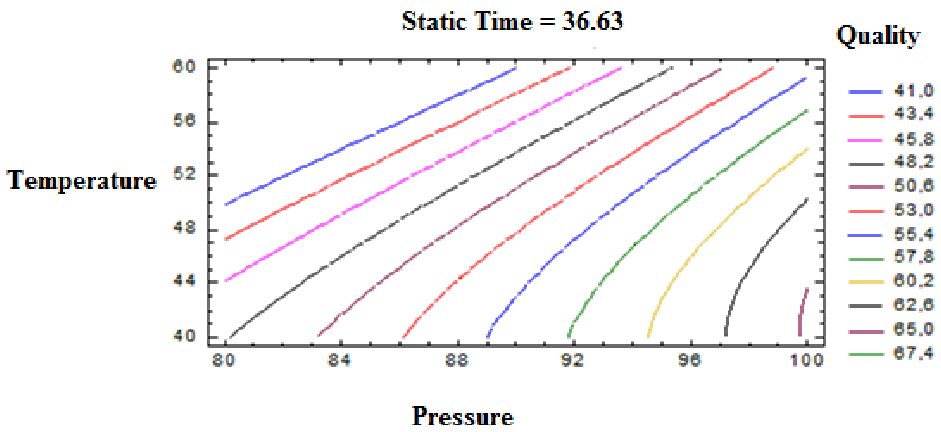


**Figure 4.** Contour for the estimated response surface for % p-cymen-8-ol with static time: 36.63 min.

From these figures it can be seen that at high pressure and low temperature the highest quality is achieved.

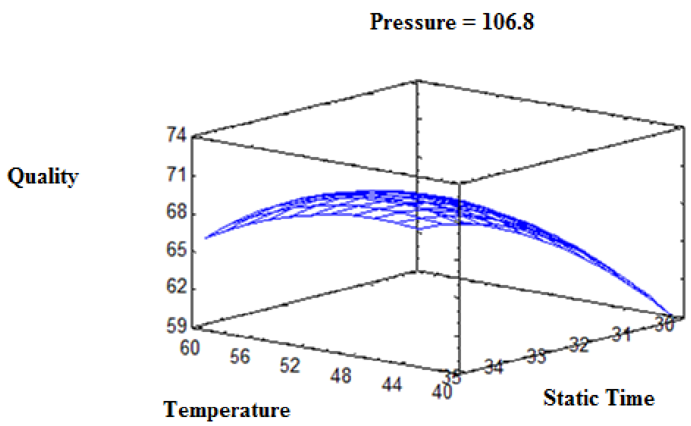


**Figure 5.** Estimated response surface for % of p-cymen-8-ol in the supercritical extraction with pressure = 106.8 bar.

This Figure 5 shows the effect of temperature and static time on quality when pressure is kept constant. So the optimal values for quality are as shown in Table 4.

**Table 4.** Optimal values for quality

| Factor | Low | High | Optimal |
|---|---|---|---|
| **Pressure (bar)** | 73.2 | 106.8 | 106.8 |
| **Temperature (°C)** | 33 | 67 | 43 |
| **Static Time (min)** | 28.00 | 37.00 | 36.63 |
| **Optimal value for quality: % of p-cymen-8-ol = 72.16 %** | | | |

An exhaustive run was carried out with the optimal values for quality (pressure 106.8 bar, temperature, 43°C and an static time of 36.63 min), which would give an essential oil with the best quality to made the perfume; additionally these values were introduced in the equation 1 to evaluate the response surface for yield to determine the expected yield which was 1.455%. The exhaustive run was made repeating the static and the dynamic time with the same sample changing the collector vessel until the total mass remained the same. The results are shown in the Table 5:

**Table 5.** Exhaustive run results

| Nº | Empty Container weight ($m_{ev}$±0.001) g | Container plus oil ($m_{ea}$±0.001) g | Oil weight ($m_{ae}$ ± 0.002) g | Yield (R ± 0.003) % | Percentage of p-cimen-8-ol (p ± 0.01)% |
|---|---|---|---|---|---|
| **1** | 16.553 | 16.626 | 0.073 | 1.459 | **70.60** |
| **2** | 16.541 | 16.545 | 0.004 | 0.080 | |
| **3** | 16.559 | 16.561 | 0.002 | 0.040 | |
| **4** | 16.549 | 16.550 | 0.001 | 0.020 | |
| **Total yield of the extraction** | | | | 1.598 | |

This essential oil with a 70% of p-cymen-8-ol was used to formulate a perfume which was evaluated by a selected sample of people.

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