

Effluence of thymol content by supercritical fluid extraction system from ajwain (*Trachyspermum ammi* L.)

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Abstract

Supercritical fluid extraction has emerged as a highly efficient and selective method for extracting essential oils from spices. The aim of this study was to investigate the outcomes of supercritical fluid extraction in terms of both yield and extraction of valuable essential oil from ajwain. Factors such as temperature (35 to 55°C), pressure (100 to 300 bar), dynamic duration (30 to 150 min), and static time (30 to 90 min) were evaluated to determine the concentration of thymol through the use of a central composite design for supercritical fluid extraction, and the extraction process was optimized. The optimal factors were found to be 35°C, 300 bar pressure, 65 minutes of dynamic time, and 30 minutes of static time. The essential oil yield with a thymol content of 60% was 3.9%.

Application

1. The supercritical fluid extraction technique for extracting oils and other substances has attracted considerable attention.
2. Supercritical fluid extraction (SFE) stands out as a distinctive unit operation in the realm of food processing, employing solvents to extract a diverse range of bioactive ingredients, heat-sensitive substances, and flavor components.
3. Supercritical fluid extraction-based technologies offer several notable benefits in comparison to organic solvent technology, such as simplified product fractionation and a more environmentally friendly approach.

Introduction

Thymol, a monoterpene alcohol, has a variety of medical applications, as do other volatile terpenes. Thymol, camphor, and menthol are used as cooling agents, pain relievers, anti-itch chemicals, and liniment ingredients, respectively (Ghori *et al.*, 2016). Sarkic & Stappen (2018). Many monoterpenes have been used as anthelmintic, antispasmodic, analgesic, calming, and astringent remedies. Ajwain can be used to extract thymol. Ajwain, scientifically known as *Trachyspermum ammi* L., is a member of the Apiaceae family, and its origin has been traced back to Egypt and India. This herb is cultivated in various arid and semiarid regions. Eastern Asia, specifically India, extensively utilizes ajwain seeds in both traditional medicine and cuisine (Bairwa *et al.*, 2012). Ajwain is a highly valued commercial plant due to its essential oil, which is applied in the food and flavor industries. The medicinal properties of ajwain oil, including its antibacterial, antifungal, anti-inflammatory, antioxidant, cytotoxic, antilithiasis, nematocidal, anthelmintic, and antifilarial properties, make it indispensable for various medical treatments. Seeds are extensively used in traditional medicine for the treatment of gastrointestinal issues such as indigestion, flatulence, colic, and diarrhea due to their exceptional digestive and antiseptic properties. Additionally, they are employed as antiseptics, antiparasitics, antiscorbutics, antihistamines, vermicides, emmenagogues, sialagogues, aromatics, stomachics, carminatives, antispasmodics, and antihypertensives (Anwar *et al.*, 2016). The constituents of Ajwain oil, carvacrol and thymol, possess antibacterial and antifungal properties that effectively combat various microorganisms. As a result of these unique qualities, Ajwain oil

serves as an effective food preservative, preventing bacterial and fungal deterioration (Escobar *et al.*, 2020).

Khajeh *et al.* (2004) reported that Ajwain essential oil is produced in Iran using hydrodistillation and SFE processes. The oils were analyzed using capillary gas chromatography with flame ionization and mass spectrometric detection. The impacts of various parameters on the supercritical fluid extraction of *C. copticum* oil were examined. The results showed that the process was most selective for extracting thymol at 30.4 MPa, 35°C, 0% methanol, and a dynamic extraction period of 30 minutes. Eight chemicals were found in the hydrodistilled oil. Thymol (49.0%), c-terpinene (30.8%), p-cymene (15.7%), b-pinene (2.1%), myrcene (0.8%), and limonene (0.7%) were the main components of *C. copticum*. However, under ideal circumstances, only three components made up more than 99% of the oil when employing supercritical carbon dioxide. According to hydrodistillation, the extraction yield was 2.8% (v/w). Under various circumstances, the SFE-based extraction yield varied between 1.0% and 5.8% (w/w). The findings indicated that thymol constituted a significant constituent of Iranian *C. copticum* oil.

The extraction of natural ingredients from high-quality, pure, and healthy sources is a common practice in the food industry. However, the need for more sustainable food processing, the increasing cost of organic solvents, and the desire to minimize environmental impact have driven the food processing sectors to innovate in separation methods. Traditional separation methods, such as leaching, adsorption, extraction, and distillation, often involve the use of solvents and high temperatures, and they have the greatest effect on the

volatile oil content of the extracts (Gaun *et al.*, 2007), which can lead to the degradation or instability of certain food ingredients. In response to this challenge, efforts have been made to develop separation methods that produce pure, safe, and environmentally friendly products. One such method is supercritical fluid extraction (SFE). SFE has the potential to serve as an alternative to traditional solvents in both extractive and nonextractive procedures, offering a safe, cost-effective, nonflammable, and environmentally friendly solvent option. Hrnčić *et al.*, 2018.

Materials And Methods

Ajwain

The Nathwani group of companies in Jamnagar, Gujarat, India, provided a batch of reliable ajwain (variety Gujarat Ajwain 1).

Cryogenic grinding

The ajwain seeds were ground at -60°C using a cryogen. The resulting cryoground ajwain powder was then stored for further use in research after being properly packed in aluminum laminated pouches to maintain the essential oil content. (Sardar *et al.*, 2018). Studies conducted by Saxena *et al.* revealed that cryogenic milling of spices is capable of preserving more marker compounds and volatile oils.

Chemical analysis

Using common analytical techniques, the moisture, fat, protein, fiber, ash, and essential oil contents of the ajwain seeds were ascertained (AOAC, 2012). Three trials of each analysis were performed, and the mean values were noted.

Measurement of thymol content in essential oil

After supercritical fluid extraction (SFE), important chemicals such as p-cymene, γ -terpinene, and thymol were identified via gas chromatography–mass spectrometry of the ajwain essential oil. A gas chromatograph (Clarus 680) and mass spectrometer (Clarus SQ8C) were used in the GC–MS system (Perkin Elmer Ltd.). A 30 m long, 0.25 mm ID, and 0.25 μ DF stationary phase Elite 5MS capillary column was utilized. After two minutes at 60°C, the oven temperature was increased to 160°C at a rate of 10°C/min and then to 270°C at a rate of 5°C/min. The carrier gas was helium, and the flow rate was 1 ml/min. The injector split ratio was 1:2, and the injector temperature was held constant at 230°C. The injection volume for both standards and samples was 2 μ l.

The marker compound reference standards were obtained from Dr. Ehrenstorfer GmbH, located at 86199 Augsburg, Germany. These standards were diluted to various concentrations, namely, 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 20, and 50 ppm. Subsequently, a stock solution was prepared by diluting it 1:5000, which was then utilized for estimating the thymol content. Additionally, a separate stock solution was prepared by diluting it 1:100000 to estimate the content of thymol, p-cymene, and γ -terpinene in the oil.

Experimental Design

Central composite design (CCD)

Pressure levels of 100, 150, 200, 250, and 300 bar; temperature levels of 40, 45, 50, and 55°C; dynamic time levels of 30, 60, 90, 120, and 150 min; and static time levels of 30, 45, 60, 75, and 90 min were considered in this investigation. Thymol content (%) was the dependent variable under observation. These characteristics were merged, and their combined effects were evaluated using central composite design.

Data Analysis and Optimization

The software Design Expert, version 10, was used to analyze the data using response surface methodology, while central composite design (CCD) was employed for experimentation (Stat-ease, 2016). The model was fitted using analysis of variance (ANOVA), and the statistical significance of the model terms was evaluated. To assess the model's suitability, the lack of fit and R^2 (coefficient of determination) were also examined.

Results And Discussion

Chemical Composition

The chemical composition of the ajwain seeds was analyzed, including moisture, fat, essential oil, protein, fiber, and ash content, using standard procedures outlined in A.O.A.C 2012. The moisture content was 6.45%, the fat content was 9.91%, and the essential oil content was 3.9%. Additionally, the protein, fiber, and ash contents were 19.08 ± 0.05 , 21.89 ± 0.62 , and 6.70 ± 0.49 , respectively. These results align with previous studies conducted by Bairwa *et al.* (2012), Javed *et al.* (2012), and Dashora *et al.* (2006), with minor variations possibly attributed to factors such as different varieties and environmental conditions.

Gas chromatographic-mass spectrometric analysis

GC–MS analysis of ajwain essential oil was carried out to determine its marker compound thymol. The total ion chromatograms (TICs) of the marker compounds in the cryogenically ground Ajwain seed powder are shown in Figure 1. The red lines in the chromatogram represent the standards, and the green lines represent the samples evaluated. The retention time of thymol was 10.55 min.

Supercritical Fluid Extraction (SFE)

A cryogenically ground sample of ajwain seed powder was subjected to supercritical fluid extraction (SFE) to achieve the highest possible yield of essential oil and thymol content.

Effects of SFE factors on thymol content

The supercritical fluid extraction method was used to extract essential oil from ajwain seeds according to the standard protocol described in the previous section. For the best recovery of essential oil and thymol content, the optimal values for temperature, pressure, dynamic time, and static time were found. The results of the investigation showed that changes in temperature, pressure, dynamic time, and static time had a substantial impact on the yield of essential oils and the thymol content.

The effects of temperature, pressure, dynamic time, static time, and their interactions on the thymol concentration were examined via ANOVA on the experimental data (Table 1). We fitted a quadratic model to the experimental data. For the response variable, the statistical significance of the linear, quadratic, and interaction effects was ascertained. Myers (1999) described the use of design and analysis techniques for situations where randomization is restricted to nature and reported the various responses utilized by practitioners in the twenty-first century across different RSMs.

The results indicated that the interaction effect of pressure, temperature, dynamic time, and static time had a significant impact on the thymol content. A quadratic effect was also observed to have a significant influence on the thymol content ($p < 0.05$) at the 5% significance level. The thymol content ranged from 5.92 to 60.78%. The lowest thymol content was recorded in experiment number 29, while the highest was in experiment number 22. With a Model F value of 3.67, the model is considered significant. There is only a 0.72% probability that a Model F Value of this magnitude could be attributed to random error. The R^2 and CV% values for thymol were 0.65 and 34.95, respectively. Mohamed & Mansoori (2002) reported that SFE has selectivity for isolating desired compounds while ensuring that no toxic residues are left behind in the extracts and eliminating the possibility of thermal degradation in processed products.

The following is the response surface equation that was created to forecast the change in thymol with different processing parameter levels:

$$\text{Thymol content} = +30.97 + 1.14 * \text{Temperature} - 8.13 * \text{Pressure} - 6.64 * \text{Dynamic time} + 1.88 * \text{Static time}$$

Table 1: ANOVA for thymol content

Source	df	thymol content		
		Sum of squares	Mean square	p value Prob > F
Model	14	4299.99	429.999	0.0072
A-Temperature	1	31.2132	31.2132	0.6117
B-Pressure	1	1584.53	1584.537	0.0016
C-Dynamic Time	1	1058.28	1058.283	0.0073
D-Static Time	1	84.9384	84.9384	0.4051
AB	1	949.718	949.718	0.0103
AC	1	242.1914	242.191	0.1668
AD	1	28.1695	28.1695	0.6295
BC	1	42.3475	42.3475	0.5548
BD	1	277.972	277.972	0.1400
CD	1	0.62015	0.62015	0.9428
A^2	1	2226.14	117.165	
B^2	1	2135.77	152.555	0.0139
C^2	1	90.3681	18.0736	
D^2	1	6526.13		
Residual	15			
Lack of Fit	10			
Pure Error	5			
Cor Total	29			

The impact of temperature and pressure on the thymol content was analyzed, as depicted in Figure 2 (a). These findings indicate that as the pressure increased, the thymol content decreased. Conversely,

there was a gradual increase in the thymol content as the temperature decreased from 55 to 35°C. The lowest thymol content of 5.92% was recorded at 40°C and 150 bar pressure, with a dynamic time of 120 minutes and a static time of 45 minutes. On the other hand, the highest thymol content of 60.50% was achieved at 40°C and 150 bar pressure, with a dynamic time of 60 minutes and a static time of 75 minutes. This trend is attributed to the decrease in CO₂ density with increasing temperature (*Lou, et al., 2003*).

The analysis of the effects of pressure and temperature on the thymol content is shown in Figure 2 (a). The results show that the thymol content decreased with increasing pressure. In contrast, the thymol level gradually increased as the temperature decreased from 55 to 35°C. At 40°C and 150 bar of pressure, with a dynamic period of 120 minutes and a static time of 45 minutes, the lowest thymol content of 5.92% was reported. Conversely, a maximum thymol content of 60.50% was attained at 40°C, 150 bar of pressure, and 60 minutes of dynamic and 75 minutes of static phases. This pattern is explained by the fact that CO₂ density decreases as temperature increases (*Khajeh, et al., (2004)*).

Figure 2 (b) illustrates the impact of temperature and dynamic time on the thymol content. As the temperature and dynamic time increased, there was a noticeable change in the thymol content. Specifically, when the dynamic time was extended from 30 to 150 minutes, the thymol content decreased. Similarly, when the temperature increased from 35 to 55°C, there was a slight decrease in the thymol content. Figure 2 (c) shows the effect of temperature and static time on the thymol content. As the temperature increased, the thymol content decreased. Additionally, an increase in static time also resulted in a decrease in the thymol content. The findings of this study are comparable to the work presented by Chattare (2011) for basil leaves and Belsare (2012) for ginger and turmeric oil extraction.

It is clear from the response surface plot in Figure 3(a) that a rise in pressure was followed by a decrease in thymol concentration. Moreover, the thymol content decreased in tandem with increasing dynamic time, suggesting that pressure and dynamic time had comparable impacts on the thymol content. Furthermore, an increase in pressure led to a significant improvement in the extraction yield, which can be explained by the increased solubility of the oil components as a result of the increased density and dissolving power of CO₂.

The high-molecular-weight compounds were found to be coextracted as the pressure increased. Consequently, the highest marker compound content was noted at lower pressure levels, as shown in the RSM graph in Figure 3 (b). This graph illustrates the impact of pressure and static time on the thymol content, indicating that the thymol content increases with increasing pressure and exhibits a linear relationship with the static time. Figure 3(c) displays the influence of dynamic time and static time on the thymol content. An increase in the dynamic time led to a decrease in the thymol content, while an increase in the static time also resulted in a decrease in the thymol content.

Standardization of Supercritical Fluid Extraction

The analysis of factors and statistical data revealed that the supercritical fluid essential oil extraction process was optimized. The program Design Expert, version 10, was used to accomplish this. The best possible combination of temperature, pressure, dynamic duration, and particle size was identified by

statistically evaluating the experimental data. The combination with the highest desirability function was chosen after a point that maximized the desirability function was found through numerical optimization. Out of all the options found by numerical optimization, the combination that had the highest attractiveness (0.875) was selected.

Conclusion

SFE provides efficient and selective extraction, minimizing the extraction of undesirable components while preserving the delicate flavor and aroma components of spices. Its environmentally favorable qualities, along with the ability to customize extraction parameters, ensure premium essential oils. SFE systems are also more suitable for large-scale commercial manufacturing as well as small-scale research due to their scalability. This research study revealed the significance of the active component thymol and the process of supercritical fluid extraction. The SFE method was fine-tuned by considering the thymol percentage in the essential oil. The optimal parameters included a pressure of 300 bar, a temperature of 35°C, a dynamic time of 65 minutes, and a static time of 30 minutes.

Declarations

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Conflict of interest

There is no conflict of interest with regard to the research, writing, or publication of this research article, according to the authors.

References

1. A.O.A.C. (2012). Official methods of analysis. Association of Official Analytical Chemists. Washington, D. C., USA.
2. Anwar, S., Ahmed, N., Habibatni, S., and Abusamra, Y. (2016). Ajwain (*Trachyspermum ammi* L.) oils. In "Essential Oils in Food Preservation," ed. by Flavor and Safety Academic Press. pp. 181-192.
3. Bairwa, R., Sodha, R.S. and Rajawat, B.S. (2012). *Trachyspermum ammi*: A Review *Pharmacognosy reviews*,6,56-60.doi:10.4103/0973-7847.95871.
4. Belsare, A. (2012). Supercritical fluid extraction of essential oil from ginger and turmeric. M. Tech. Thesis, Anand Agricultural University, India.
5. Chhatre, A. (2011). Supercritical fluid extraction of volatile oil from basil leaves. M. Tech. Thesis, Anand Agricultural University, India.

6. Dashora, L. K., Dashora, A. and Lakhawat, S. S. (2006). "Production technology of plantation crops, spices, aromatic and medicinal plants." Agrotech Publishing Academy. Escobar, A., Perez, M., Romanelli, G., and Blustein, G. (2020). Thymol bioactivity: A review focusing on practical applications. *Arabian Journal of Chemistry*, 13,9243-9269. doi:10.1016/j.arabjc.2020.11.009.
7. Ghori, S. S., Ahmed, M. I., Arifuddin, M., and Khateeb, M. S. (2016). Evaluation of analgesic and anti-inflammatory activities of formulation containing camphor, menthol and thymol. *International Journal Pharmaceutical Science*, **8**, 271-274.
8. Guan, W., Li, S., Yan, R., Tang, S. and Quan, C. (2007). Comparison of essential oils of clove buds extracted with supercritical carbon dioxide and other three traditional extraction methods. *Food Chemistry*, **101**, 1558-1564. doi: 10.1016/j.foodchem.2006.04.009.
9. Hrnčić, M. K., Cor, D., Verboten, M. T., and Knez, Z. (2018). Application of supercritical and subcritical fluids in food processing. *Food Quality and Safety*, **2**, 59-67. doi: 10.1093/fqsafe/fyy008
10. Javed, S., Shahid, A., Muhammad, H., Umeera¹, A., Rauf, A. and Sobia, M. (2012). Nutritional, phytochemical potential and pharmacological evaluation of *Nigella Sativa* (Kalonji) and *Trachyspermum Ammi* (Ajwain). *Journal of Medicinal. Plant Research*, **6**,768-775. doi: 10.5897/JMPR11.1341
11. Khajeh, M., Yadollah, Ya., Fatemeh, S. and Naader, B. (2004). Comparison of essential oil composition of *Carum copticum* obtained by supercritical carbon dioxide extraction and hydro distillation methods. *Food Chemistry*, **86**, 587-591. doi: 10.1016/j.foodchem.2003.09.041
12. Lou, V., Folas, G., Voutasa, E. and Magoulas, K. (2003). Extraction of parsley seed oil by supercritical CO₂. *J. Supercrit. Fluids*. **7**,1-12. doi: 10.1016/j.supflu.2003.07.003
13. Mohamed, R. S., & Mansoori, G. A. (2002). The use of supercritical fluid extraction technology in food processing. *Food Technology. Magazine*, **20**, 134-139.
14. Sardar, N. R., Prasad, R. V., Sharma, H. P., Bhatt, H. G., Tagalpallewar, G. P., and Fenn, B. N. (2018). Supercritical fluid extraction of essential oil from cryo ground ajwain seed. *International. Journal of Chemical Studies*, **6**,27-31. <https://www.chemijournal.com/archives/?year=2018&vol=6&issue=6&ArticleId=4139&si=false>
15. Sarkic, A., & Stappen, I. (2018). Essential oils and their single compounds in cosmetics. A critical review. *Cosmetics*, **5**, 11. doi:10.3390/cosmetics5010011.
16. Saxena, R. Saxena, S.N. Barnwal, P. Rathore, S. S. Sharma Y. K. & Soni A. (2012). Estimation of antioxidant activity, phenolic and flavonoid content of cryo and conventionally ground seeds of coriander (*Coriandrum sativum* L.) and fenugreek (*Trigonella foenum-graecum* L.). *International Journal of Seed Spices*, **2**, 83-86.
17. Sharma, L. K., Agarwal, D., Meena, S. K., Rathore, S. S. and Saxena, S. N. (2015). Effect of cryogenic grinding on oil yield, phenolics and antioxidant properties of ajwain (*Trachyspermum ammi* L.). *Int Journal of Seed Spices*, **5**, 82-85. <http://iss.ind.in/pdf/Volume5No2/14.pdf>

Figures

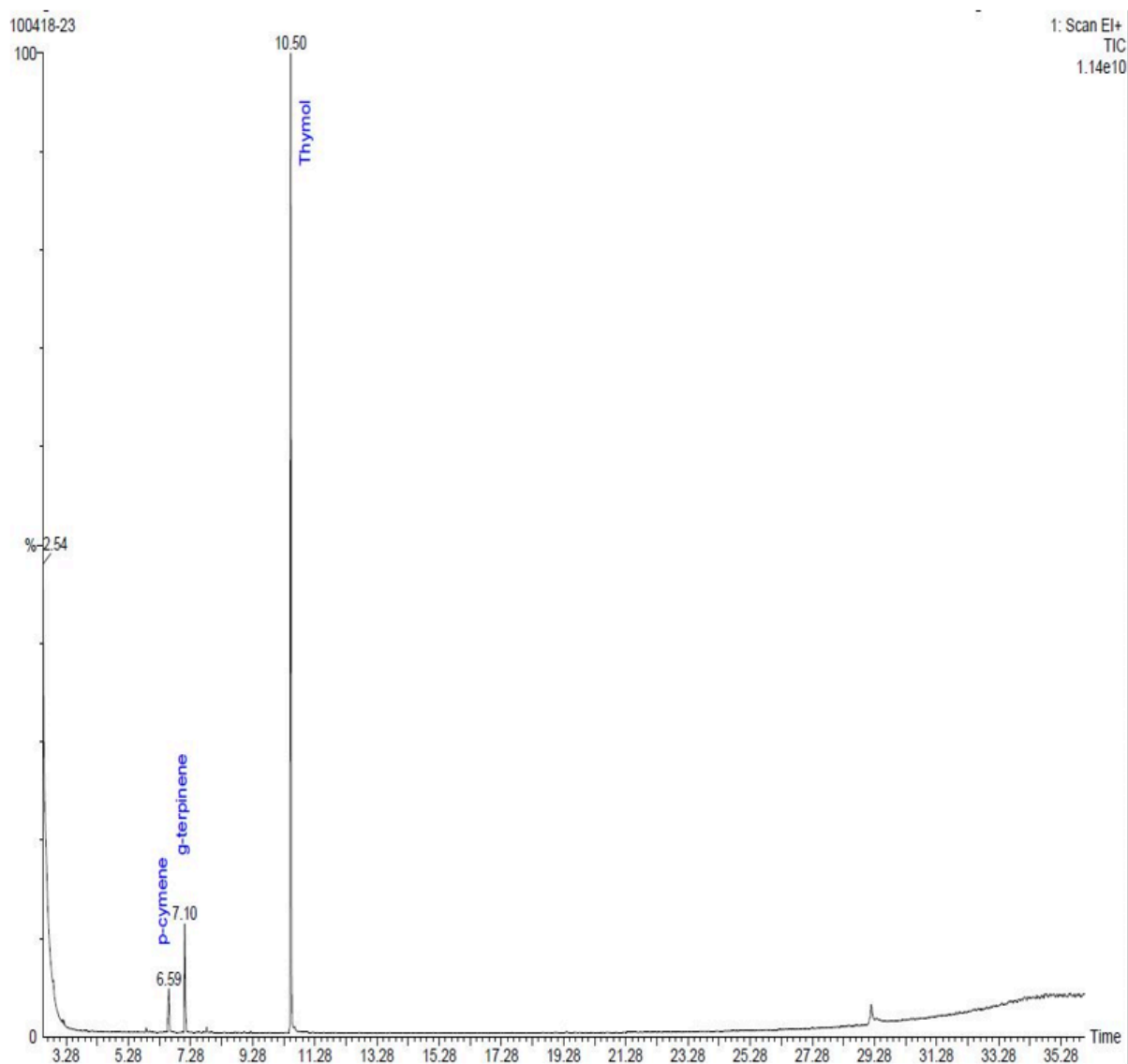


Figure 1

GC–MS chromatogram of the essential oil of ajwain seed powder

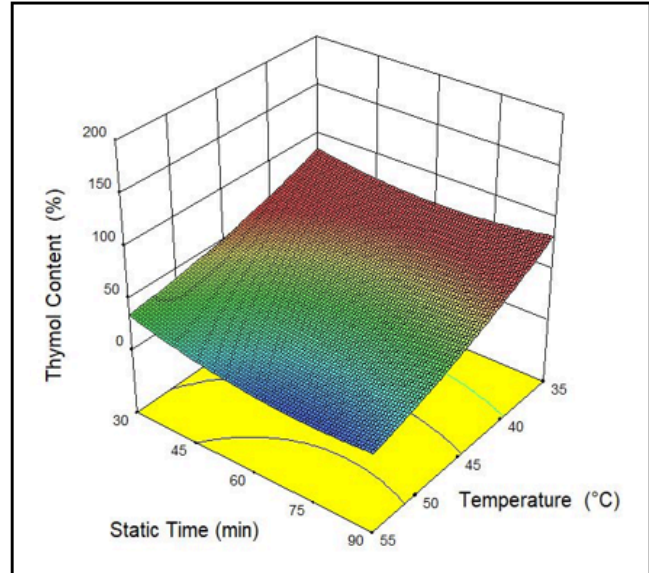
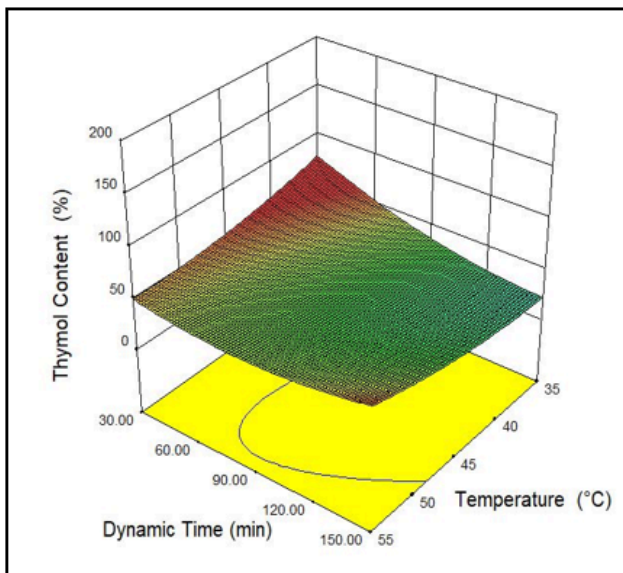
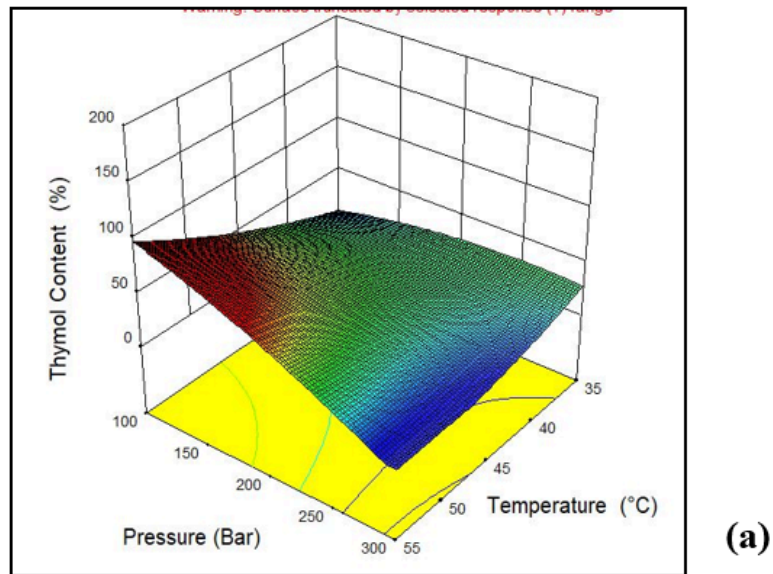
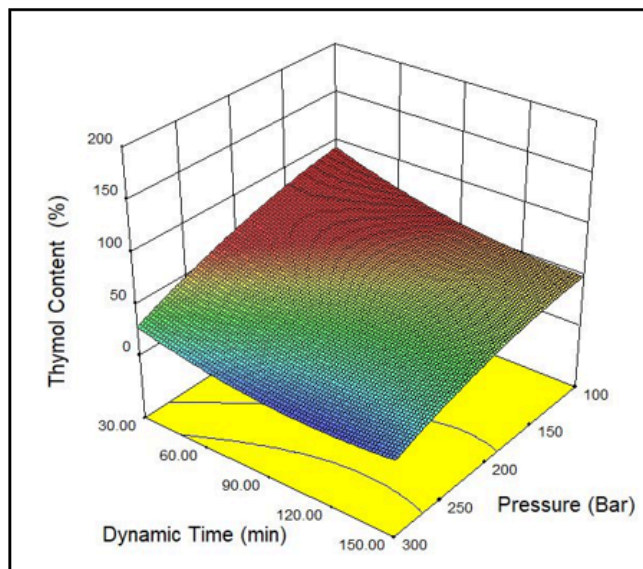
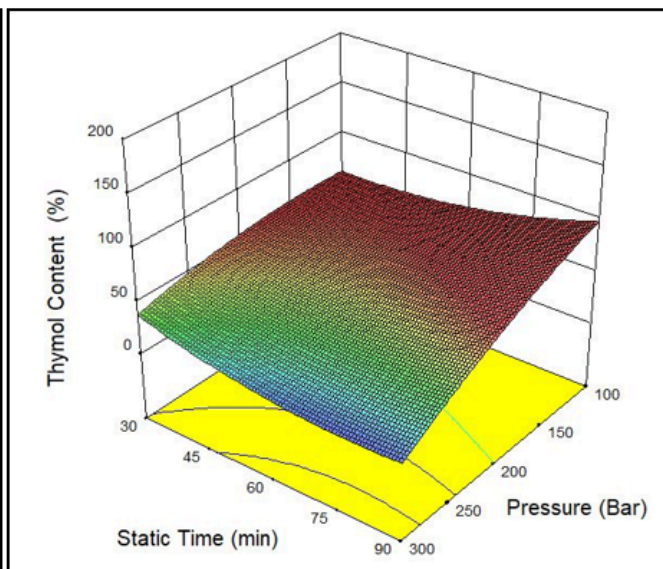


Figure 2

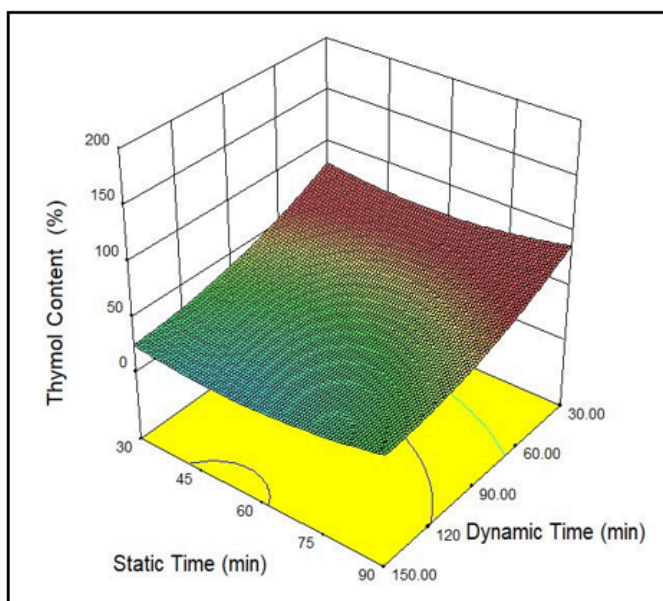
Effect of the pressure–temperature dynamic time and static time on the thymol content



(a)



(b)



(c)

Figure 3

Effect of the dynamic time pressure and static time thymol content