Subcritical water extraction of *Foeniculum vulgare* Mill (Fennel) essential oil

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Abstract

This study extracted the essential oil from *Foeniculum vulgare* Mill seeds (Fennel) with subcritical water and compared it to the method of Hydro distillation. The important ingredient of essential oil is trans-Anethole. Identification of substances in essential oil and their amount were performed by GC and GC/MS analysis. The effect of temperature, mean particle size, flow rate, and extraction time on the amount, and quality of subcritical water extraction was studied. To facilitate the experiments and investigate the effect of the parameters in their extraction and interaction, used the method of response surface with a central composite design (CCD). The optimum conditions for trans-Anethole extraction happened at a temperature of 125 °C and a mean particle size between 0.5 and 0.71 mm in 65 min and a flow rate of 25.1 mL/min. The total maximum yield (0.02345 mg essence/g dry sample) obtained at the optimal conditions was more than that achieved by the Hydro distillation method (0.01322 mg essence/g dry sample).

Keywords: Extraction, Subcritical water, Hydro distillation, *Foeniculum vulgare* Mill, Response surface methodology.

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1. Introduction

Plants have a wide range of bioactive compounds such as Lipids, Phytochemicals, Pharmaceuticals, Pigments, Flavors, and Fragrances. Essential oils and plant extracts are generally used in the food, medicine, cosmetics, and health industries [1]. This is why the optimal extraction of these compounds is important. One of these important plants is Fennel. The Fennel plant (*Foeniculum vulgare* Mill) belongs to the Apiaceae family and the Parsley genus, and is an aromatic and medicinal plant and perennial with regular leaves that are found in Europe, North Africa, and Southwest Asia. This plant is cultivated in Iran due to its medicinal properties. In most parts of the country, we can see this plant, which is traditionally used to treat various diseases, including stomach problems and obesity [2]. Trans-Anethole, Fenchone, and Limonene are the main constituents of this plant's essential oil. According to studies, the most important ingredient of essential oil is trans-Anethole (70-80%) [3]. One of these methods is extraction with liquid water at subcritical temperature (SWE). The term liquid water with subcritical temperature is used for water at a temperature above the boiling point of 100 ºC and less than the critical temperature of 374 ºC and the pressure corresponding to the corresponding temperature. So, water at this temperature and pressure retains its liquid nature [4].

SWE is used more than traditional methods because of its advantages, for example, high speed, high efficiency, reduction of organic solvents, and compatibility with the environment [6, 7]. The pressure range needed to keep water in the liquid state at 200 °C and 300 °C are 15 bar and 85 bar, respectively. If the pressure at the mentioned temperatures is less than the above value, or in other words, if the pressure reaches below the pressures proportional to the boiling point, supersaturated steam is produced [8]. Under these conditions, water can be used as a suitable alternative to dissolve medium-polarity organic compounds and even non-polar compounds [9].

On the other hand, increasing the temperature increases the penetration rate, reduces the viscosity, and reduces the surface tension of water. If the pressure increases to such an extent that the water remains liquid, these changes will continue beyond the normal boiling point of water to the critical point range of 375 °C and 8.21 bar. In this case, the polarity of the water is significantly reduced so that it can be applied as an extraction solvent for a wide range of analytes [10]. SWE was first used in 1955 by Basil et al. to extract essential oils from the Rosemary plant [11].

This research study aimed to optimize the essential oil extraction of Fennel seeds by the subcritical water method. Due to 70-80 % of the overall essential oil of Fennel seeds being trans-Anethole, the whole essential oil was expected as a single component. Then, this constituent was measured to optimize the process of essential oil extraction. The main parameters such as flow rate, extraction time, temperature, and mean particle size were explored in the subcritical water extraction of essential oil. The results of optimized subcritical water extraction of Fennel seeds were likened to the conventional technique of Hydro distillation extraction.

2. Material and Method

2.1 Material

Fennel-dried seeds were purchased in March 2018 from a store in Semnan (Semnan, Iran). High-performance liquid chromatography (HPLC) grade water was employed as an extractant. In the separation of water from the aqueous extracts, n-pentene, Na_2SO_4, and NaCl, (Merck, Darmstadt, Germany, >95%, HPLC grade) were employed as an extractor, a drying agent, and an emulsion breaker, respectively.

2.2. Preparation of sample

Fennel seeds became dry in shadiness at room temperature for two days and then were kept in polyethylene bags at a -4 ºC fridge (HARRIS Co., Germany) until the analysis process. The wet content of Fennel seeds was 8% (dried basis). Before performing the experiments, the dried samples were crushed in a laboratory mill. The powder was ready by appropriate standard sieves with sizes less than 0.25 mm, between 0.25 to 0.5 mm, between 0.5 to 0.71 mm, between 0.71 to 1 mm, and larger than 1 mm. The sample preparation was done nearly before extraction to escape losses of volatiles and samples were stored in five glass containers.

2.3. Hydro distillation method

Hydro distillation of 100 g of grounded Fennel without leveling was done and poured directly into 1000 mL of deionized water into Clevenger and after 3 hours and 30 min, the distillation was completed. About 2 mL of essential oil was collected. The essential oil was dried using anhydrous Na_2SO_4 and kept in a dim cut-glass flask at 4 ºC until analysis.

2.4. Subcritical water extraction method

SWE was performed in a laboratory apparatus shown in Figure 1.

Figure 1.

To remove dissolved oxygen in water, HPLC water was first placed in an ultrasonic bath for 20 min and then deoxygenated for 30 min in the feed tank of the extraction apparatus with subcritical water by nitrogen gas flow. HPLC pump (BFRL Company, SY-8100 series, Germany) was used to transfer water into the system. A burette was also located to control the operation of the pump, and Stainless steel heating screw with a length of 3 meters for further heating. The

extraction vessel was 520 mm long and 52 mm in diameter. In each test, 2 g of ground Fennel placed inside the longitudinal cloth packs were placed inside the test vessel. 210 °C were capable of working inside the furnace, the inlet and outlet temperatures of the extraction vessel were measurable and observable, the pressure was adjustable, and after the extraction process, a heat exchanger was placed in the furnace outlet to lower the extracted temperature. It should be noted that when extracting the extract, 20 mL of liquid, which was approximately the size of the tubes of the device, was discarded, and then the output extract was collected in a sample glass container.

In all experiments, a certain volume of extract was removed to separate the essential oil. To break the colloidal bond, a 25% solution of salt extract was formed and separated by liquid-liquid extraction using normal pentane solvent and a 1 to 2 ratio, in two stages of the organic phase. The obtained organic phase was placed in a test tube for complete separation of the material from the essential oil in two stages in a centrifuge, the first stage for 15 min and, 4000 rpm and the second stage for 5 min and 4000 rpm. The essential oil obtained was completely exposed to air for 4 to 5 hours in each experiment for complete separation from normal pentane. After complete separation, 2 mL of normal pentane was added to the essential oil, immediately poured into the sample container, and kept in the refrigerator until injected into a gas chromatographic analyzer.

2.5. Central composite design

To optimize the main parameters of the subcritical water extraction of essential oils of Fennel seeds, central composition design (CCD) was performed. The CCD method uses a two-level factor design, ie central points and axial points. In this method, each factor has five different levels, which include three points inside the range and two points outside it. A quadratic model, a second-order polynomial, was used to obtain the optimal point, and its equation was presented as follows:

$$Y = \beta_o + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j$$

Where the response (yield) is Y, the interception coefficient, the linear coefficient, the quadratic coefficients, and the interactive regression coefficient are 0, i, ii and ij, respectively and Xi and Xj represent the independent variable levels [13]. Four process parameters at five different levels to evaluate the essential oil yield were studied which included temperature (90, 107, 5, 125, 142, 5, 160 °C, mean particle size (less than 0.25 mm, between 0.25 and 0.5 mm, between 0.5 to 0.75 mm, between 0.75 to 1 mm and greater than 1 mm), flow rate (0.5, 0.88, 1.25, 1.63 and 2 mL/min) and time (40, 52.5, 65, 77.5 and 90 min). In all experiments, 1 g of Fennel seeds, the ratio of sample to co-packing 1:1.5, and 20 bar pressure were applied.

The essential oil yield (w/w) in both extraction methods was calculated with the following relation and based on percentage:

$$yield\ (\%) = \frac{amount\ of\ essential\ oil\ (g)}{amount\ of\ dry\ sample\ (g)} \times 100 \qquad (1)$$

2.6. Analysis by gas chromatography (GC)

The analysis was performed by gas chromatography in the analysis laboratory. The devices used had the following characteristics: GC device (Series 6100, ACME), used column TRB-WAX with a length of 60 meters, an internal diameter of 32 micrometers, and a thickness of polyethylene glycol film of 25 micrometers. The operating conditions of the device were as follows: 50-230 °C, temperature rate of 3 °C per min, helium carrier gas with a purity of 99.99% (ROHAM company, Tehran, Iran), with a separation ratio of 1: 100 and a flow rate of 5 mL/min.

2.7. Analysis by gas chromatography-mass spectrometer (GC-MS)

GC/MS analysis was achieved under the following conditions: Varian, Walnut Creek, USA, DB-5 silica column with a length of 60 m, internal diameter of 25 μm, and film thickness of 25 μm, and software. The operating conditions of the device were as follows: Ionizing energy 70 eV, mass range 40-400 amu and, EI scanning method, under temperature conditions of 40 to 200 °C with a temperature velocity of 3 °C per min and a separation ratio of 1:30.

3. Results and discussion

The maximum yield (0.02345 mg essence/g dry sample) obtained at the optimal conditions was more than that achieved by the Hydro distillation method (0.01322 mg essence/g dry sample). The GC-MS results showed that in Fennel essential oil, besides t-Anethole (73.89 %), Fenchone (9.14 %), Limonene (6.01 %), Methyl chavicol (2.96 %), Carvone (1.93 %) and α-pinene (0.8 %) were main and major components. GC-MS results are shown in Figure 2. Different substances identified for the essential oil obtained by the Hydro distillation and subcritical water extraction are given in Table 1.

Figure 2.

Table 1. Different substances were identified for the essential oil obtained by the Hydro distillation and subcritical water extraction at mean particle size between 0.5 to 0.71 mm, temperature 125 °C, flow rate 1.25 mL/min, extraction time 65 min.

Components	Hydro distillation (%)	SWE (%)
α-pinene	0.8022	-
Sabinene	0.2785	-
β-pinene	0.528	-
p-cymene	0.7345	-
Limonene	6.0115	3.8243
1,8-cineole	0.2022	-
(z)-β-ocimene	0.2688	-
γ-teripene	-	4.1274
Fenchone	9.1433	0.508
Comphor	0.1893	-
Methyl chavicol	2.9612	0.3759
Carvone	1.9295	-
p-Anis aldehyde	0.4862	-
t-Anethole	73.8921	88.8957

The experimental results by four variables in CCD are shown in Table 2.

Table 2. The experimental results used four variables in CCD.

Exp.	Temperature (°C)	Flow rate (ml/min)	Time (min)	P-size (mm)	Yield (%)
١	١٠٧,٥	١,٦٣	٧٧,٥	٠,٤٤	٠,٧٣٨٢
٢	١٢٥	١,٢٥	٦٥	٠,٦٣	٠,٦٨٣٢
٣	١٠٧,٥	٠,٨٨	٥٢,٥	٠,٨١	٠,٣٩١٨
٤	١٤٢,٥	٠,٨٨	٧٧,٥	٠,٨١	٠,٧٥٦٣
٥	١٢٥	١,٢٥	٤٠	٠,٦٣	٠,٦٥٢٢
٦	١٢٥	١,٢٥	٦٥	٠,٢٥	٠,٥٤٣٤
٧	١٤٢,٥	٠,٨٨	٥٢,٥	٠,٤٤	٠,٥٩٧٨
٨	١٤٢,٥	١,٦٣	٧٧,٥	٠,٤٤	٠,٩٧٧٢
٩	٩٠	١,٢٥	٦٥	٠,٦٣	٠,٤٢٣٩
١٠	١٢٥	١,٢٥	٩٠	٠,٦٣	٠,٨٣٥٢
١١	١٠٧,٥	١,٦٣	٥٢,٥	٠,٨١	٠,٥٣٢٦
١٢	١٢٥	١,٢٥	٦٥	٠,٦٣	٠,٧٠٦٥
١٣	١٤٢,٥	٠,٨٨	٥٢,٥	٠,٨١	٠,٥٦٥٢
١٤	١٠٧,٥	٠,٨٨	٧٧,٥	٠,٤٤	٠,٥١٠٨
١٥	١٢٥	٢	٦٥	٠,٦٣	٠,٧٥
١٦	١٢٥	١,٢٥	٦٥	٠,٦٣	٠,٧٠٦
١٧	١٤٢,٥	١,٦٣	٥٢,٥	٠,٨١	٠,٦١٢٧
١٨	١٢٥	١,٢٥	٦٥	٠,٦٣	٠,٧١٥٢
١٩	١٤٢,٥	٠,٨٨	٧٧,٥	٠,٤٤	٠,٧٩٣٤
٢٠	١٠٧,٥	١,٦٣	٥٢,٥	٠,٤٤	٠,٧٦٠٨
٢١	١٢٥	٠,٥	٦٥	٠,٦٣	٠,٤١٣
٢٢	١٠٧,٥	٠,٨٨	٧٧,٥	٠,٨١	٠,٣٢١٧

٢٣	١٦٠	١,٢٥	٦٥	٠,٦٣	٠,٨٦٩٨
٢٤	١٠٧,٥	٠,٨٨	٥٢,٥	٠,٤٤	٠,٤٧٨٢
٢٥	١٠٧,٥	١,٦٣	٧٧,٥	٠,٨١	٠,٥٧٣
٢٦	١٢٥	١,٢٥	٦٥	٠,٦٣	٠,٧٠٦٨
٢٧	١٢٥	١,٢٥	٦٥	١	٠,٣١٥٢
٢٨	١٤٢,٥	١,٦٣	٥٢,٥	٠,٤٤	٠,٦٢٥٦
٢٩	١٤٢,٥	١,٦٣	٧٧,٥	٠,٨١	٠,٨٥٨٦
٣٠	١٢٥	١,٢٥	٦٥	٠,٦٣	٠,٧١١

A comparison of the highest percentage of trans-Anethole was used to identify the optimal conditions in the extraction method with subcritical water, which occurred at a temperature of 125 °C, an average particle size between 0.5 to 0.71 mm, and a flow rate of 1.25 mL/min in 65 min. It should be noted that in all experimental runs, the pressure was set to 20 bar for the water to remain liquid. According to the ANOVA table, only parameters with a p-value of less than 0.0001 were considered for the model, and other parameters were removed the model was accepted. The following model was obtained based on the effective parameters:

$$\text{yield} = +0.77552 - 4.45916\text{E} - 003 \times \text{Temp} + 1.18564 \times \text{Q} - 0.045684 \times \text{t} + 1.31896 \times \text{P} - \text{size} + 2.86829\text{E} - 004 \times \text{Temp} \times \text{t} - 1.81425 \times \text{P} - \text{size}$$

Where Temp is extraction temperature (ºC), Q is the flow rate (mL/min), t is extraction time (min) and P-size (mm) is the mean particle size range. The experimental data fitted the response equation with $R^2 = 0.9804$. Also, different values of R^2_{adj} and R^2_{Pred} were 0.9621 and 0.8906, respectively. Then the effect of four process parameters on the amount of essential oil extraction was investigated. The relative standard deviation percent (%RSD) for extraction yields was considered based on the found peak areas. The %RSD values ranged from 5 to 18%.

3.1 Effect of temperature

Temperature is the most important parameter in extraction with subcritical water and a change in water polarity is the most important effect on the extraction process [14]. Water at temperatures above 200 °C can be a solvent for non-polar compounds and Reduces tissue adhesion between particles. By reducing the viscosity, the solvent penetrates better into the plant tissue [15]. Increasing the temperature due to increasing solubility causes an increase in the amount of essential oil extraction. However, at high temperatures, unwanted paraffin compounds in the plant may be added to the essential oil [16]. Increasing the temperature destroys the bioactive compounds and darkens the extract. The opacity of the extract is attributed to the disintegration of plant tissue and its dispersion in the extract [17]. Therefore, the temperature should be high enough to facilitate the extraction of the target compound, but to prevent the extraction of unwanted compounds [18]. In Figure 3, the effect of the extraction time and temperature on the extraction yield at a constant flow rate of 1.25 mL/min and a mean particle size of 0.63 (between 0.5 to 0.75 mm) is presented. It demonstrates that the extraction yield improved with increasing temperature up to 142.5 ^{o}C. Due to device restrictions, tests were not possible at higher temperatures.

Figure 3.

3.2. Effect of mean particle size

The mean particle size of the plant is one of the important parameters in the extraction rate. Different plant tissues differ in chemical and physical properties, type of composition, or particle diameter. These factors affect the retention and adsorption properties of the target material. For example, by reducing the particle size, the extraction efficiency is improved because of better mass

transfer. But if the mean particle size is too small, the adhesion of the particles to each other reduces the extraction efficiency. Also, reducing the particle size is time-consuming and there is a possibility of destroying the compounds during crushing [19]. If it is too large, the process will not be able to extract the essential oils fully. Figure 4 demonstrates that until the mean particle size was 0.44 mm (particle size range was between 0.5 and 0.71 mm), the essential oil extracted augmented with declining particle size. This indicates that the extraction process is controlled by the mass transfer of the essential oil components in the plant seed.

Figure 4.

3.3. Effect of flow rate

One of the factors affecting the amount of essential oil extraction in the SWE process is the flow rate. Usually, the flow rate is selected between 1-4 ml/min [20]. The effect of the mean particle size and flow rate on the extraction yield at a constant temperature of 125 ºC and time of 65 min is shown in Figure 5. As displayed in Figure 4, the extraction yield improved with the increasing water flow rate. Therefore, the mass transfer of the essential oil from the solid plant surface to the water phase controls most of the extraction process. At low flow rates, the extraction rate is low, but the extract is more concentrated, and also, at high flow rates the extraction rate is high but is more diluted [21, 22].

Figure 5.

3.4. Effect of extraction time

Another influential parameter in extraction with subcritical water is time. In general, the extraction of essential oil increases with increasing time. But it should be borne in mind that if the time is too short, the essential oils may not be able to be extracted. On the other hand, if the reaction time is too long, substances may be added to the extract to form a new combination with the important substances in the essential oil. Figure 6 shows the effect of two parameters of time and mean particle size. The extraction time powerfully depends on the nature of the network and analytes and the extraction temperature [23]. For extracting materials from plant samples or examining environmental samples, it takes 5 min to 2 h in dynamic mode and 5 to 10 min in the static method has good efficiency. As the extraction time increases, the efficiency increases and tends to be constant [24].

Figure 6.

4. Conclusion

The water extraction method with subcritical temperature is a new method that has been considered today due to its fastness, less need for organic solvents, high selectivity, and environmental compatibility. This study aimed to extract the essential oil of the Fennel plant by liquid water extraction with subcritical temperature, which is a new method. The extraction method with liquid water with subcritical temperature was compared with the water distillation method and the optimal conditions for trans-Anethole extraction were identified. Also, the parameters affecting the extraction at subcritical water were investigated. The results show that the extraction method at subcritical water is faster than Hydro distillation, and its essential oil has a higher quality. Also, by changing the operating conditions, the quality of the essential oil can be changed, while this is impossible in Hydro distillation. It is also clear from the procedures that the

temperature and time parameters of extraction, as well as flow rate and temperature, had the most significant effect of interaction in this experiment and the least effect of interaction was related to two parameters of time and mean particle size.

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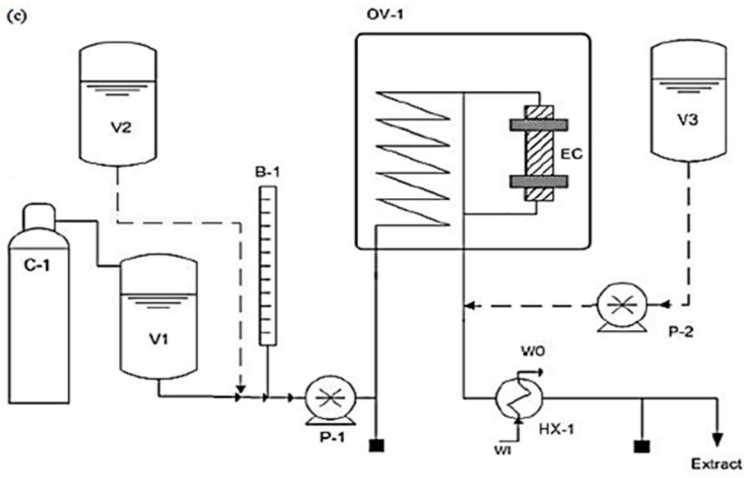

Figure 1: The extraction process diagram with subcritical water,

Includes Burette (B), nitrogen container (C), extraction chamber (EC), a heat exchanger (HX-1), Oven (OV-1), pumps (P-1, P-2), water reservoir (V-1), solvent reservoir (V-2), solvent wash reservoir (V-3), Water input (WI) and water output (WO) [12].

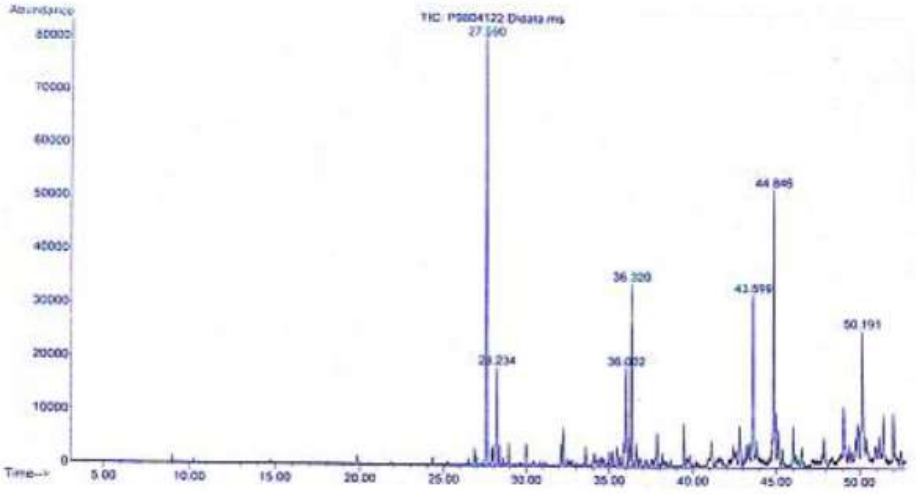

Figure 2: GC/MS analysis for subcritical water extraction for 2 g of grounded Fennel.

(Mean particle size between 0.5 to 0.71 mm, temperature 125 °C, flow rate 1.25 ml/min, extraction time 65 min).

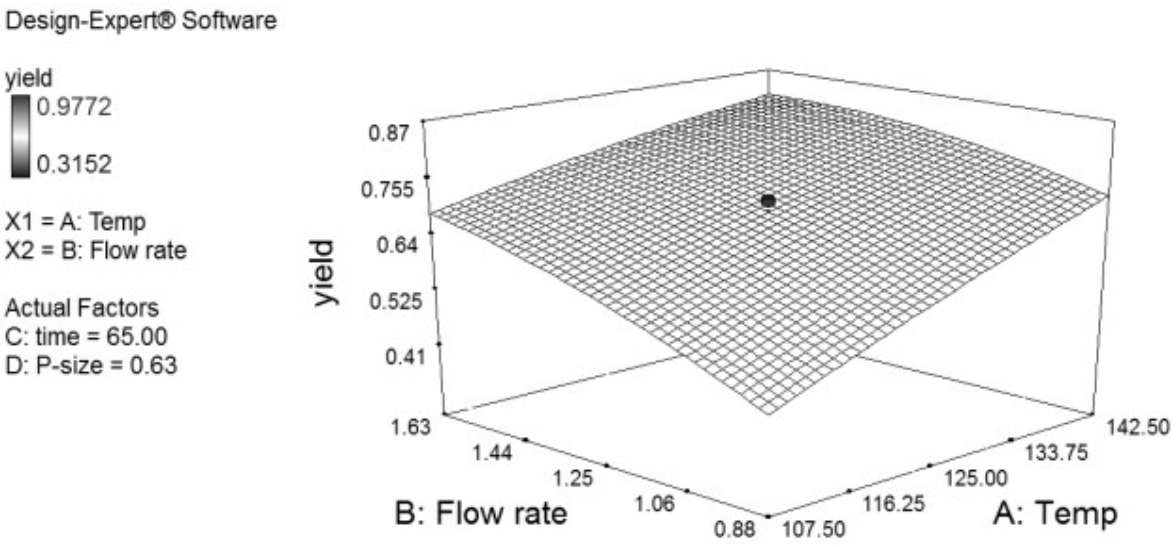

Figure 3: The extraction yield versus the simultaneous effect of temperature (A) and flow rate (B).

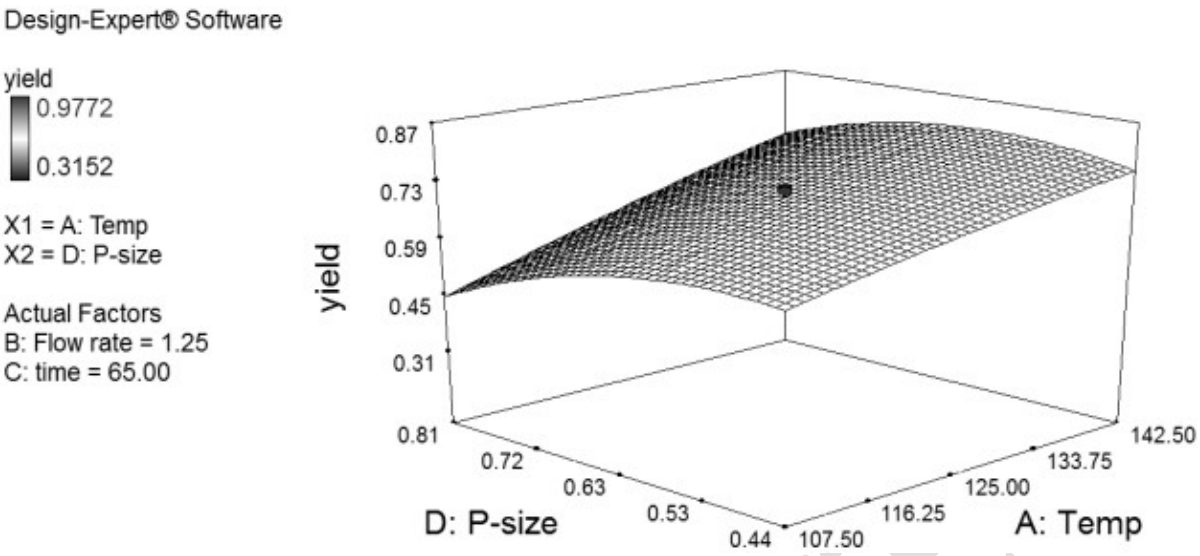

Figure 4: The extraction yield versus the simultaneous effect of temperature (A) and particle size (D).

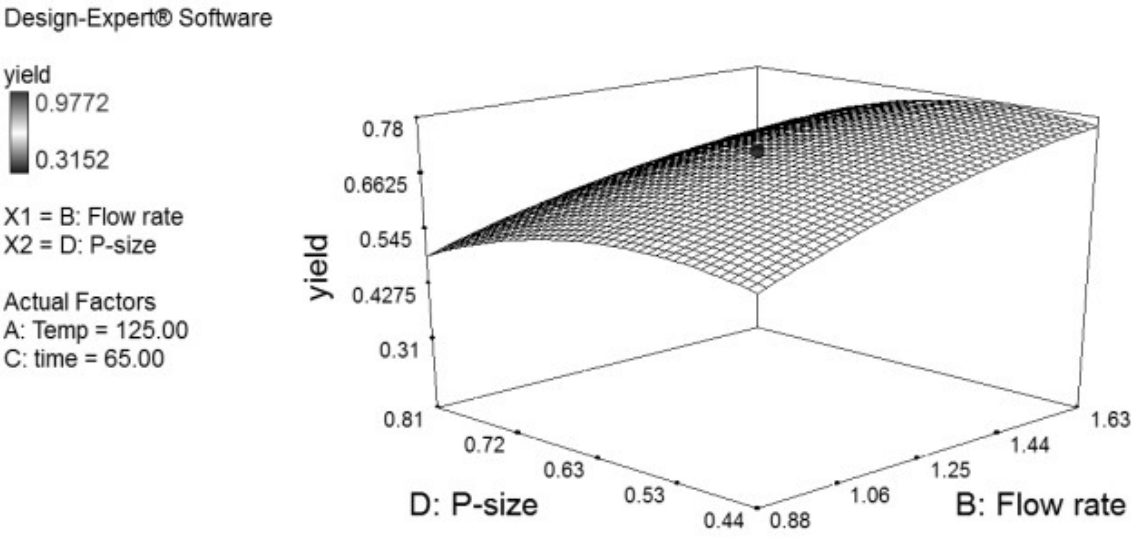

Figure 5: The extraction yield versus the simultaneous effect of flow rate (B) and particle size (D).

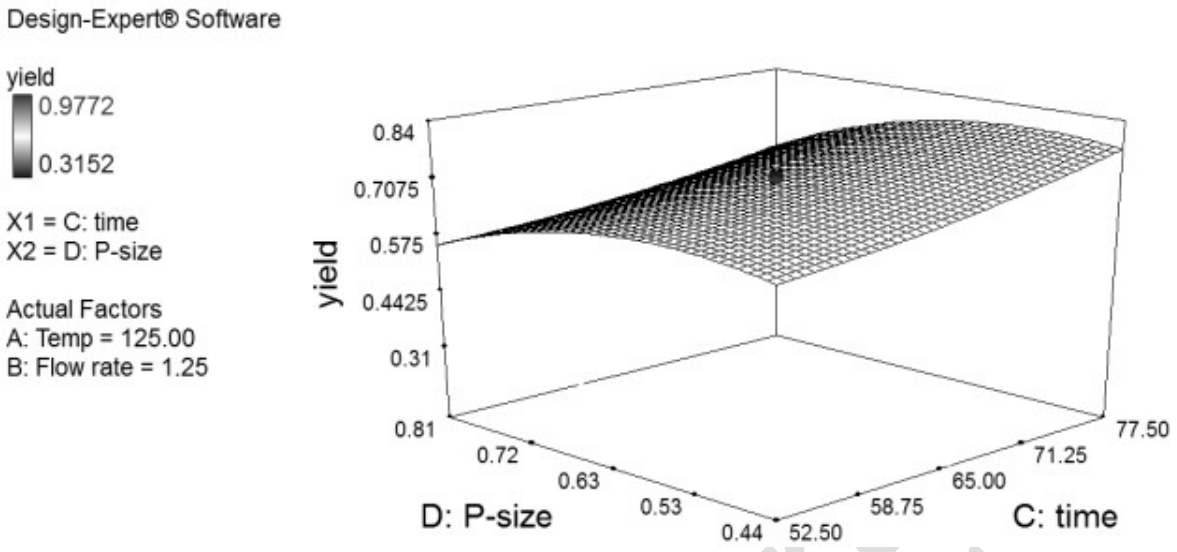

Figure 6: The extraction yield versus the simultaneous effect of time and particle size (D).