Extraction of Fennel Essential Oil Constituents Using a New Modified Rotating Disc Contactor

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ABSTRACT: *This paper presents a new application of the Rotating Disc Contactor as an extractor of essential oil from a medicinal plant. Its capability for a solid-liquid extraction was tested. The extraction of essential oil from fennel seeds using a 70 % (v/v) ethanol-water solution was amLomplished in different particle sizes, rotor speeds, and solvent ratios per solid. The modeling of experimental data obtained from each sample's GC analysis was implemented through the response surface methodology of two mathematical regression models. Findings indicated that a linear model is applicable for estimating the extraction yield. Also, in the optimum condition (Least size of particle; Highest solvent per solid ratio, Highest rotor speed), the extraction yield of Anethole (the major component in fennel essential oil) was 1.387.*

KEYWORDS: *RDC; Essential oil; RSM; Fennel.*

INTRODUCTION

Foeniculum Vulgare, commonly known as Fennel, is one of the most widely used medicinal and aromatic plants, which belongs to the Apiaceae (Umbelliferaceae) family [1, 2]. This plant has been grown widely in the Mediterranean and Southern Europe [3], and Iran has an appropriate climate for growth.

Fennel is well known for its Essential Oils (EOs) properties used in the food and pharmaceutical industries. Various studies have been done on the structure of essential oils in the fennel and its quantitative and qualitative components [1, 3, 4]. Evaluating the chemical composition of fennel oil seeds and their potential addition to moisturizing cream formulas, Analyzing the potential usefulness of byproducts of fennel seeds by assessing the total phenolic and flavonoid contents, as well as the antioxidant and antibacterial properties of their extracts [4].

The amount of the components in fennel's essential oil varies depending on the crop's climate and type. The major components of the Egyptian fennel essential oil were Estragole (51.04%), Limonene (11.45%), l-Fenchone (8.19%), and trans-Anethole (3.62%) by GC–MS analysis [5], while these values are different in Canadian fennel [6] or Iranian as well and Anethole is the major component in the essential oil of Iranian fennel [2].

Various research has been conducted on the specific compounds of the essential oil of fennel. Estragole

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is the subject of several studies due to being the primary compound in some fennel species and also because this volatile compound could possess potential carcinogenic properties [7, 8]. Another study focused on comparing Soxhlet and AmLelerated Solvent Extraction [9] techniques to improve the volatile characterization of fennel to evaluate the potential volatiles [10]. Also, optimizing the quantification method to assess the quantity of Estragole present in the fennel seeds can improve the oil quality [10]. In addition, optimization of Estragole quantity from fennel seeds by Supercritical Fluid Extraction [SFE] method, amLording to an experimental Box-Behnken design carried out by CO_2 as supercritical fluid and methanol as co-solvent [10]. The aforementioned studies on Estragole inspired our focus of work and led us to perform research on Iranian fennel and the extraction of Anethole.

Most essential oils are extracted from plants through different separation methods, including distillation, heating, or cold pressing. Supercritical Fluid Extraction Assisted by Pressing [SFEAP] method has been employed to improve fennel oil extraction yield. another study on SFE method compared with SFEAP and Effects of grinding time and mass of milled fennel seeds on the primary volatile oil content, namely Anethole and Fenchone were investigated. The extracted composition was evaluated by Gas Chromatography [GC] analysis [2, 11]. Also, extraction of trans-Anethole from fennel seeds by the SFE method was carried out; experiments and modeling were designed through Response Surface Methodology [RSM] in Design-Expert software [12-15].

Essential oils also have biological applications; Fennel oil is well-known worldwide and traditionally used as a curative herbal therapy to treat epileptic disease, seizures, carminative, digestive, lactagogue, diuretic, treating respiratory and gastrointestinal disorders [16]. The possible effect of fennel oil against the toxicity of Sodium-Valproic has also been investigated in albino rats [16].

On the other hand, Liquid-Liquid Extraction [8] and Solid-Liquid Extraction [SLE] are important unit operations [17]. One of the most widely used columns in LLE is Rotating Disc Contactor [RDC], which has been extensively studied. New research is continually executing on hydrodynamics [18], mass transfer [9], modeling [19], and molecular dynamic simulation [20-25], and Aspen plus [26] for various purposes [27]. For example, the influence of the presence of SiO_2 nanoparticles in Kerosene in an LLE on mass transfer performance and static holdup into the RDC extractor column was carried out [27]. Another work combined computational fluid dynamics [CFD] and droplet population balance modeling to simulate the drop size distributions and flow fields in a liquid-liquid RDC extractor [21]. Environmental strategies toward the "Green Industry" are important issues that have attracted the direction of research; a mathematical model for extraction of aromatic from aliphatic hydrocarbons in a counter-current RDC was investigated in a ternary system consisting of toluene/n-heptane/different solvents to predict the concentration of toluene [28].

Furthermore, the hydrodynamics and mass transfer performance of an RDC with sieved trays (RSDC) has been investigated for lysozyme's reversed micelle extraction [29]. Optimization of droplet coalescence in RDC [30], proposing a correlation for mean drop diameter in an RSDC [31], innovative method to prepare a stable emulsion liquid membrane for high CO_2 absorption in a natural gas feed into RDC extractor [32], influence of Cetyl Trimethyl Ammonium Bromide [CTAB] surfactant on static holdup and mother drop's size in RDC and RSDC [33], and extraction of heavy metals from aqueous solutions in a modified RDC extractor [34] are summaries of the research done via RDC.

The purpose of this work is to: (I) introduce a new modified RDC and its application in solid-liquid extraction and extracting Essential Oils, (II) evaluate the extraction yield of EOs and particularly, Anethole from fennel seeds traditionally grown in Iran, and finally, (III) compare the quantitative composition of the obtained EOs from experiments in RDC with hydro-distillation Clevenger method. It should be noted that the rotating disc contactor extractor column has never been used in Solid-Liquid Extraction. Still, it is prevalent in Liquid-Liquid Extraction (LLE) processes. In this study, by changing the design of an RDC, this extractor was prepared for solid-liquid contact operation.

EXPERIMENTAL SECTION

Materials

Fennel seeds were purchased from the Institute of Medicinal Plants in Hamadan, Iran. Ethanol (96% v/v) from Razi Alcohol Company was used as the primary solvent, and pure n-Hexane (99%) from Merck in Germany was applied as the second solvent for the extraction process.

Also, Sodium Sulfate anhydrous from Merck in Germany was used to dehydrate the final samples for analysis.

Experimental setup

The device consisted of an RDC and an electromotor used to agitate and settle the feed. Physical features of the column have been mentioned in Table 1, and the setup can be seen schematically in Fig. 1.

A glass funnel with a 45-degree of slope steepness was designed to collect fennel seeds from the bottom of the column after extraction operation, which covered the inside diameter of the column. As shown in Fig. 1, it was placed inside the column. The apertures and edge of the funnel were amLurately sealed with a silicone sheet to head all the seeds towards the hole and to prevent the seeds from passing along the edge of the funnel. Also, the funnel outlet was connected to another tube with a spring. A valve was attached to the end of the tube, collecting the fennel seeds from the bottom of the RDC by closing the valve. The end of the tube, which had the tank in place for managing the outflow seeds, was also connected to another tap to control the output of the grain or liquid by opening and closing. Since the flow direction of seeds was from the top of the column to the bottom while the path of solvent entry was from the bottom *via* a nozzle, this operation is considered counter-current.

Extraction parameters

Several parameters can influence a Solid-Liquid Extraction, especially in extracting Active Pharmaceutical Ingredients (API) and essential oil from medicinal plants, which depends on the plant and extraction method. In this study, three following parameters were selected for the testing in the laboratory at ambient temperature (25^{o}c):

1. Size of Particle (mm)
2. Solvent per Solid Ratio (Solid weight (gr) in a constant volume of solvent)
3. Rotor Speed (rpm)

Considering the constant weight of the solvent in tests, changing the solid weight can be expressed by another title: 50 grams of fennel in 2 liters of ethanol 70% is equal to the ratio of solvent to solid 1:20, 75 grams equivalent to 1:15 and 100 grams equals 1:10. Also, to prevent the amLumulation of fennel particles on the discs, the size of the fined particles tried to be smaller than the hole diameter of the discs to make a smooth flow.

Table 1: Characteristics of the RDC.

Different parts of the column	Material and Dimensions
Body Material of the column	Pyrex
Material of the column's Bottom and trays	Teflon
Column's Height (ht)	1500 mm
Inner Diameter (di)	92 mm
Outer Diameter (do)	102 mm
Active Height (ha)	270 mm
Disc's Diameter (dd)	54 mm
Disc's Width (dw)	6 mm
Disc's Hole Diameter (hd)	2 mm
Number of Disc's holes (nh)	36
Spacer's Height (hs)	40 mm
Shaft Diameter (sd)	10 mm

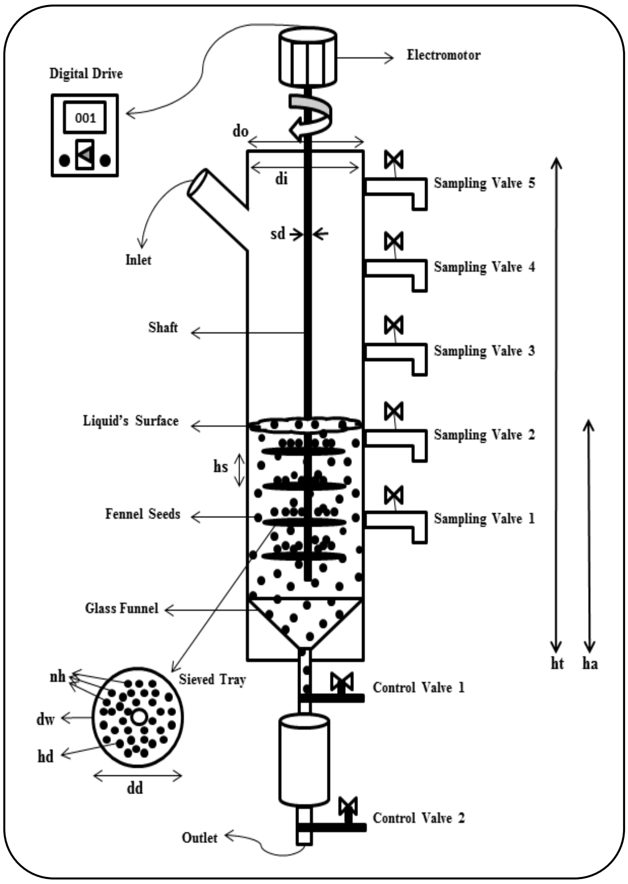

Fig. 1: Schematic of the RDC.

Table 2: Factors and their levels for RSM design.

Symbol	Factor	(Unit)	Levels		
A	Solid Weight	(gr)	50	75	100
B	Particle Size	(mm)	0.3	1	1.7
C	Rotor Speed	(rpm)	90	135	180

Design of experiments

Design-Expert software was used for the designation of Experiments, and Response Surface Methodology (RSM) was applied to model the obtained experimental data in three levels: −1, 0, and +1, representing low, middle, and high levels, respectively. Fourteen runs were chosen to estimate the extraction yield. Table 2 shows the parameters and their levels in numerical values.

Method of experiments

Fennel grinding

Fennel seeds were chopped into smaller particles by a mill. They were separated through the distinct sieves by Electrical Sieve Shaker with mesh numbers of 12, 18, and 50 in amLordance with the American Society for Testing and Material [ASTM] E11 standard and were separated into three classes with diameter sizes of 0.3, 1 and 1.7 mm to examine the effect of different particle sizes on the process.

Clevenger extraction

This experiment was carried out as a criterion for comparison purposes and analyzing the essential oil components with the Gas Chromatography-Mass Spectrometry (GC/MS) method as joint work in our laboratory [35]. 100 gr of the finely milled powders in size number 30 (ASTM classifications) were soaked in 96% ethanol (Merck, Germany) and was distilled for 4 hours. Fine fennel seeds were placed in a Clevenger extractor based on the hydro-distillation method. Then, the solvent was removed by evaporation, and the outgoing vapor was recycled by rotary evaporation. To remove the residual solvent, the oil was dried in an oven at 50 °C for 4 h. 0.5 mL of oil was sent to GC/MS analyzer in order to detect chemical compounds of the essential oils of Iranian fennel seeds. The yield of oil extraction by the Clevenger method was 1.84 gr oil per 100 gr seed.

$$\text{Extraction Yield}(\%) = \frac{\text{Oil weight (g)}}{\text{Seeds weight (g)}} \times 100 = 1.84\ \% \qquad (1)$$

$$\text{Extraction Yield of Anethole}(\%) = \frac{78.12}{100} \times 1.84 = 1.475\ \% \qquad (2)$$

RDC Extraction

Fennel seeds in different sizes were in contact with the ethanol 70% in a new modified Rotating Disc Contactor (RCD). Ethanol acted as the continuous phase, while fennel seeds were considered the dispersed phase in the extraction operation. In the preparation stage of Ethanol 70% (v/v), 2 liters of Ethanol 96% (v/v) were dissolved in 750 mL of distilled water using a volumetric flask. The amount of 2 Liters of Ethanol was 70% (v/v) as extraction solvent from the top of the column entered into RDC. After adjusting the rotor speed, fennel seeds with the desired mesh were poured from the top of the column. Extraction of essential oil and APIs from the solid phase into the solvent was carried out by contact operation. After 30 min., seeds were removed from the column's bottom and collected in a designed pipe attached to the bottom of the RDC. The collected seeds were poured again into the column from its top. Contact operation continued for 20 min. Thus, 50 minutes was considered the extraction time based on the time required for ethanol to destroy plant cell walls [2].

The sample was taken from the highest valve near the surface at the end of the operation. First, 10 mL of the sample containing ethanol, water, and API of fennel was taken. 3 mL of the sample were contacted with 1 mL of n-hexane in a test tube. N-Hexane 99% of Merck Company from Germany was used for the second extraction. It was necessary to ensure that the final samples were free of water. Therefore, the second extraction was the contact operation between n-Hexane and Ethanol 70% solution in a laboratory centrifuge. Consequently, it was placed in the laboratory centrifuge at 2500 rpm for 5 min. to ensure that the essential oil and its active ingredients were entirely transferred from the ethanol phase to the n-hexane phase.

There was a two-phase solution in the test tube; the heavier phase was ethanol and water, which stayed at the bottom of the test tube. The lighter phase was n-hexane

Table 3: Design matrix and the responses for RSM design.

Run	Factor A Solid Weight (gr) [Solid per Solvent]	Factor B Size of the particle (mm)	Factor C Rotor Speed (rpm)	Anethole percentage (%)	Extraction Yield (Response)
1	50 or 1:20	1	135	63.36	1.17412
2	100 or 1:10	1	135	53.59	0.815557
3	75 or 1:15	0.3	135	59.22	1.07429
4	75 or 1:15	1	90	64.76	0.886313
5	100 or 1:10	1.7	180	47.18	0.372666
6	75 or 1:15	1.7	135	26.35	0.878155
7	100 or 1:10	1.7	90	60.58	0.627494
8	50 or 1:20	1.7	180	49.52	0.705433
9	50 or 1:20	1.7	90	52.15	0.700911
10	100 or 1:10	0.3	90	33.82	0.861309
11	50 or 1:20	0.3	90	28.02	1.17412
12	100 or 1:10	0.3	180	49.98	0.842689
13	75 or 1:15	1	180	44.14	0.95033
14	50 or 1:20	0.3	180	29.57	1.38719

and dissolved oil, placed on the top of the two-phase solution in the test tube. A syringe collected the top phase.

Finally, 0.01 gr sodium anhydride sulfate was added to the n-hexane sample to ensure that water removal from the sample had been completely done. The test tube was placed again in the laboratory centrifuge at 2500 rpm for 5 min. to allow the absorbent water particles to be deposited in the powdered dough at the bottom of the test tube.

GC Analyzer

In the end, 1 mL of the final sample containing n-hexane and dissolved oil (Essential oil + API) was sent to the GC analyzer. The extract and essential oil were analyzed using an Agilent Technologies 7890A Gas chromatograph DB-5 and FID as the mass selective detector.

RESULTS AND DISCUSSION

Statistics result

Table 3 presents the designed matrix of variables and responses; meanwhile, Table 4 lists the sum of squares, mean square, F value, and P-value (probability). The F-value of 20.26 and P-value of 0.0009 (<0.05) approve the significance of the model. The proposed model is "Linear" based on a higher F-value and lower amount in Lack of Fit [LOF]. Prediction Error Sum of Squares (PRESS) was selected to fit the experimental data. R^2 is the percentage of variation of the dependent variable to be explained by the independent variables in the model. It is used to evaluate the goodness of fit. As indicated in Table 5, the value of R^2 was found to be equal to 0.9594, while a value close to 1 theoretically shows a good fit for the model. In addition, the Adj. R^2 is 0.9121, and the predicted R^2 value of 0.7590 is in reasonable agreement with Adj. R^2 value. In addition, the statistical significance of the model was validated by both the F-value (20.26) and the small matter of probability (<0.05).

Based on statistical parameters validated by the software, the mathematical model representing the extraction yield based on actual data is given in Equation (3):

$$Y^{1.48} = -0.098741 - 0.005485 \times A - 0.547448 \times B + 0.030929 \times C + 0.006139 \times AB - 0.000073 \times AC - 0.00235519 \times BC - 0.000084 \times C^2 \quad (3)$$

GC/MS results

Overall, 18 compounds had been identified in the extracted essential oil by hydro-distillation Clevenger apparatus and listed in Table 6 [35]. The primary constituent of fennel's essential oil was Anethole, which composed 78.12 % of the total essential oil. Anethole is the main active pharmaceutical ingredient (API) for many pharmaceutical products. Furthermore, other important compounds identified by the GC-MS analyzer are Fenchone (8.81%), Estragole (4.52%), and Limonene (4.39%) [35].

Table 4: Analysis of variance (ANOVA) table of the linear response surface method.

Source	Sum of Squares	Degree of freedom	Mean Square	F-value	p-value
Model	1.61	7	0.2295	20.26	0.0009*
A-Solid Weight	0.5271	1	0.5271	46.54	0.0005*
B-Size of particle	0.8036	1	0.8036	70.95	0.0002*
C-Rotor Speed	0.0024	1	0.0024	0.2159	0.6586
AB	0.0923	1	0.0923	8.15	0.0290*
AC	0.0538	1	0.0538	4.75	0.0722
BC	0.0440	1	0.0440	3.89	0.0961
C^2	0.0833	1	0.0833	7.35	0.0350*
Residual	0.0680	6	0.0113		
Cor Total	1.67	13			

** significant values*

Table 5: Statistical parameters for the model.

Std. Dev.	0.1064	R^2	0.9594
Mean	0.8642	Adjusted R^2	0.9121
C.V. %	12.31	Predicted R^2	0.7590
PRESS	0.4035	Adequate Precision	16.6371

Table 6: Identified compounds in fennel essential oil via GC/MS [35].

No.	Compound	RT	%
1	3-Methyl pentane	1.85	0.088
2	2-Methyl butyraldehyde	1.94	0.268
3	Methyl cyclopentane	2.17	0.107
4	Alpha.Pinene	9.45	0.779
5	Sabinen	11.16	0.256
6	Myrcene	12.01	0.424
7	Limonene	13.70	4.389
8	Terpan	13.82	0.288
9	Alpha-Ocimene	14.16	0.636
10	Fenchone	16.6	8.810
11	d-Camphor	19.11	0.181
12	Estragole	21.74	4.523
13	Fenchyl acetate	23.25	0.087
14	Carvone	23.8	0.146
15	trans-Anethole	24.17	0.133
16	p-Anisaldehyde	24.5	0.248
17	Anethole	26.26	78.125
18	Dillapiole	39.49	0.511

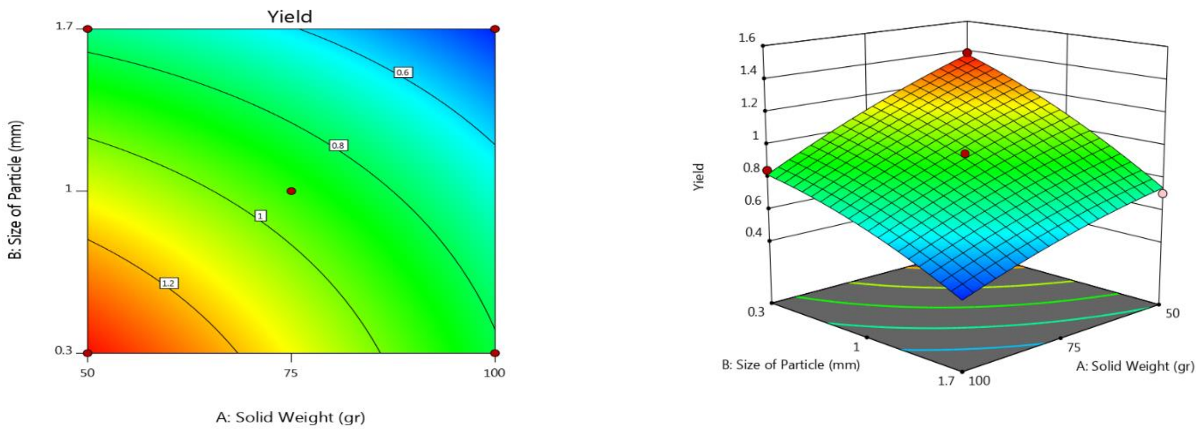

Fig. 2: Contour plot (A), Response Surface plot (B); Effect of A and B on extraction yield.

The findings of similar published research done by students in our lab-the Surface Phenomena Laboratory -in which the same approach in methods, apparatus, and procedures have been used as well with only a difference which is about the type of the extraction process and a solid-liquid extraction has been done in the present work. For example, Jafari investigated the mass recovery of hydro-distillation wastewater of spearmint in a Rotating Disc Contactor [RDC] [36], and *Najafipour* studied the mass recovery of hydro-distillation wastewater of dill seeds in a pulsed column [37]. In addition, *Eyvazkhani* researched the extraction of fennel essential oil in the effluent of hydro-distillated by using an RDC [35]. As a comparison between our findings and his work, it can be concluded that while the percentages of Anethole are in the range of 26% (run 6) to 64% (run 4) in the solid-liquid extraction done in our study, these figures are lower in *Eyvazkhani* [35] work (18% to 52%), in which a Liquid-Liquid Extraction has been applied. Also, the RSM could demonstrate the effect of the operational parameters very well in these similar studies.

Software results

In the Contour plot, which is shown in Fig. 2-A, a wide range of extraction yields based on solid weight (A) and size of the particle (B) has been demonstrated. The minor efficiency results in higher particle weight and bigger sizes of fennel seeds. Also, the Response surface plot in Fig. 2-B illustrates the effect of solid weight (solvent per stable ratio) and particle size on the extraction yield at a constant rotor speed (C) of 180 rpm. Plots indicated that increasing the solvent per solid ratio -which means decreasing substantial weight- and decreasing particle size led to the enhancement of extraction yield. Based on the linear model, it is concluded that the optimum conditions for obtaining the highest extraction yield omLurred at the rotor speed of 155 rpm (Shown in Fig. 3), the proportion of solvent per solid ratio of 1:20 (or 50 gr fennel in 2 liters of solvent), and the smallest size of particles (0.3 mm). The predicted yield in these amounts was 1.39032%, while the actual work was 1.38719%.

The two-dimensional interaction of parameters is shown in Fig. 4. Identified points approach the highest yield of extraction. The perturbation Diagram in Fig. 5 Compares how parameters can affect the work amLording to their sharpness. Obviously, the size of particles is the most influential parameter, and then the solvent per solid ratio is the second important factor. A parabolic behavior was observed for the rotor speed, where 155 rpm is the optimal mode for maximum efficiency.

This study investigated the extraction of essential oil and active pharmaceutical ingredients from fennel seeds by ethanol 70% as a solvent in the modified RDC. Considering the wide application of RDC in several industries, this research has also sumLeeded in demonstrating the new application of the RDC in the food and pharmaceutical field for extracting essential oil and APIs from aromatic and medicinal plants. A comparative analysis between our work and *Eyvazkhani* [35] reveals that higher values in extraction yield of essential fennel oil can be achieved in a direct operation through Solid-Liquid Extraction in an RDC instead of indirect function using hydro-distillation wastewater of the fennel in the same RDC based on a Liquid-Liquid Extraction.

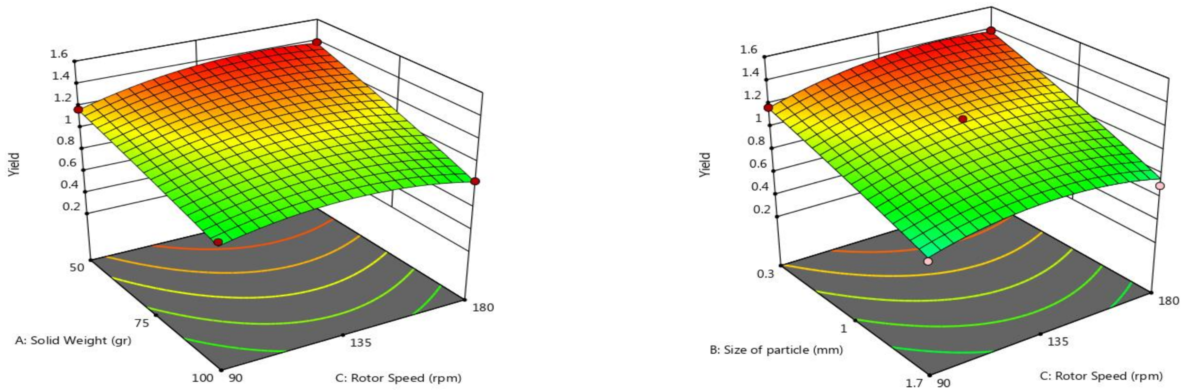

Fig. 3: Response Surface plots; optimum model at the rotor speed of 155 rpm.

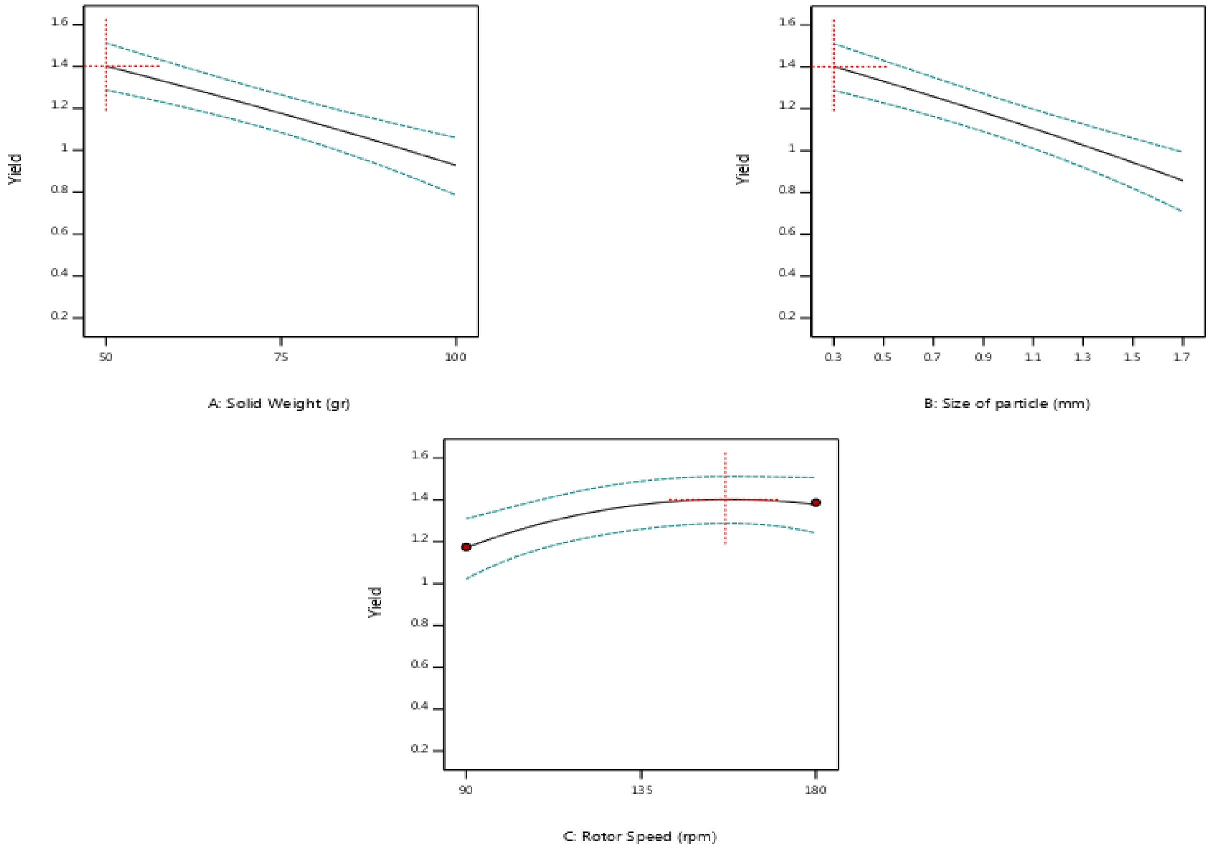

Fig. 4: Parameter ranges approach the most extraction efficiency.

Furthermore, this research proves that the amount of organic solvent and heating or electrical energy used in the RDC is dramatically less than water supplied in traditional methods such as distillation. Hence, it is cost-effective. More importantly, the desired amount of API can be achieved with less heat energy and time consumption. Another benefit of this method is that the extraction process is carried out at an ambient temperature of 25 centigrade. Thus, the probability of degradation of the active ingredients is more minor than thermal methods. At the same time, many volatile compounds might be destroyed at high operating temperatures in the traditional way of distillation with hot water or steam. It is also possible to determine the desired extraction yield or

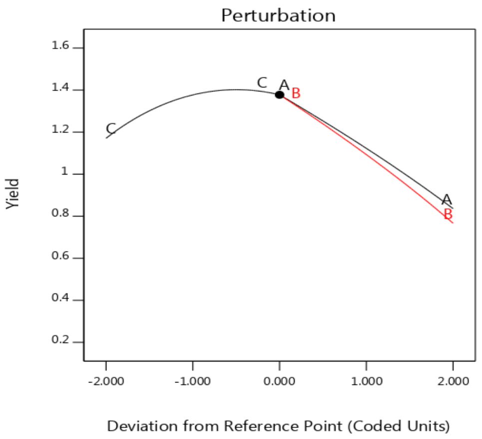

Fig. 5: ***Perturbation Diagram; Compare how parameters affect the yield.***

the required concentration of Anethole in RDC by adjusting the specific ranges for rotor speeds, size of fennel seeds, and the ratio of solvent per solid.

CONCLUSIONS

Analysis of the statistical findings in the extraction of essential oil and APIs through contact of fennel seeds with ethanol 70% as a solvent in the new modified RDC indicated that as the ratio of solvent per solid increases, extraction yield enhances; In other words, the less solid weight is used, the higher efficiency of the extraction is obtained. Also, the smaller the size of fennel particles, the higher extraction efficiency, and the extraction efficiency increases by increasing rotor speed into RDC. It should be noted that the size of fennel seeds had the most significant impact on operation yield among the studied parameters. Next, the solvent to solid ratio considerably affected the yield. Then, the rotor speed influence was considered the third most effective parameter due to its nonlinear impact at high speed. As a result, in the optimum condition (Least size of particle; Highest solvent per solid ratio, Highest rotor speed), the extraction yield was 1.387.

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