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Supercritical fluid extraction of essential oil from cryo ground ajwain seed

Sardar NR, Prasad RV, Sharma HP, Bhatt HG, Tagalpallewar GP and Fenn BN

Sardar NR
Department of Food Processing Technology, College of Food Processing Technology and Bio-Energy, Anand Agricultural University Anand, Gujarat, India

Prasad RV
Professor & Head Department of Food Quality Assurance, College of Food Processing Technology and Bio-Energy, Anand Agricultural University Anand, Gujarat, India

Sharma HP
Assistant Professor Department of Food Processing Technology, College of Food Processing Technology and Bio-Energy, Anand Agricultural University Anand, Gujarat, India

Bhatt HG
Associate Professor & Head Department of Food Safety and Quality, College of Food Processing Technology and Bio-Energy, Anand Agricultural University Anand, Gujarat, India

Tagalpallewar GP
Assistant Professor Department of Food Processing Technology, College of Food Processing Technology and Bio-Energy, Anand Agricultural University Anand, Gujarat, India

Fenn BN
Research Associate Department of Post Harvesting Engineering and Technology, College of Food Processing Technology and Bio-Energy, Anand Agricultural University Anand, Gujarat, India

Correspondence
Sardar NR
Department of Food Processing Technology, College of Food Processing Technology and Bio-Energy, Anand Agricultural University Anand, Gujarat, India

Abstract
The experiments were conducted to optimized the processing parameters i.e. temperature, pressure, dynamic time and static time for maximum essential oil yield from Ajwain seeds (*Trachyspermum ammi* L.) and shelf life evaluation of essential oil at College of Food Processing Technology and Bio-energy, Anand Agricultural University Anand Gujarat. A Response Surface methodology (RSM) was used for the determination of optimum extraction temperature (35,40,45,50,55 °C), pressure (100, 150, 200, 250, 300 bar), dynamic time (30,60,90,120,150 min) and static time (30,45,60,75,90 min) were evaluated with respect to essential oil yield from Ajwain seeds using supercritical fluid extraction process. The shelf life evaluation of the extracted essential oil from Ajwain seed was also carried out at two storage temperature-18 ± 2°C and 7 ± 2° C. The maximum essential oil of 3.9% was found by using temperature, 300 bar pressure with 65 min and 30 min dynamic time and static time respectively. The degradation of the essential oil was found less stored at -18 ± 2°C as compaired to the essential oil stored at 7 ± 2°C.

Keywords: spices, ajwain, supercritical fluid extraction, essential oil

Introduction
Seed spices have been known for ages as effective therapeutic foods. The capacity of spices to impart biological activity is now emerging as an area of interest for human health. Ajwain (*Trachyspermum ammi* L.) is an important seed spice that belongs to family Apiaceae. Seed are small, oval and ridged shape greyish brown in colour. Its characteristic odour and taste due to presence of an essential oil (2.5-5%). Spices constitute an important group of agricultural commodities, which are virtually indispensable in the culinary art. The term 'spices' applies to natural plant or vegetable products or mixtures thereof, in whole or ground form, as are used for imparting flavor, aroma and piquancy to and for seasoning of foods (Pruthi, 1974) [9].

Spices are well-known as appetizers and are considered essential oil in the culinary art. They add a tang and flavour to food. Some of them also possess antioxidant properties, while others are used as preservatives in some foods like pickles and chutneys. Some spices also possess strong anti-microbial activities. Many of them possess medicinal properties and have a profound effect on human health. Spices intensify salivary glands and helps for secreting amylase, neuraminic acid and hexosamines. They favour the cleansing of oral cavity from food adhesion and bacteria and help to check infection and cavities, and to protect the mucous membrane against thermic, mechanical and chemical irritations. Spices increase the secretion of saliva rich in ptyalin, which facilitates digestion of starch in the stomach, rendering the meals (Morrison, 2011) [7].

Ajwain (*Trachyspermum ammi* L.) belongs to family *Apiaceae* originated in Egypt and India. It is also cultivated in arid and semiarid regions of world like Iran, Pakistan and Afghanistan. It is widely distributed in northern parts of India in the states of Rajasthan, Gujarat, Uttar Pradesh, Punjab, Tamil Nadu and Andhra Pradesh. The seed of Ajwain are widely used in India and eastern Asia, for diet as well as for traditional medicine. Ajwain is an important commercial plant due to its essential oil, which is used in the flavour/food industries. Ajwain oil contributes to a wide range of medicinal applications such as antibacterial, antifungal, anti-inflammatory, antioxidant, antibacterial, cytotoxic, antilithiasis, nematicidal, anthelmintic and antifilarial activities. Its seed exhibit remarkable digestive and antiseptive properties and are used in traditional medicine, primarily to control bowel disorders such as indigestion, flatulence, colic, and diarrhea. They are also used as a stimulant, stomachic, carminative, aromatic, antispasmodic, antihypertensive, antiseptic, antiparasitic, antiscorbutic, antihistamine, vermicide, emmenagogue, and sialagogue. The ajwain oil components thymol and carvacrol have attributes for antibacterial and antifungal action against wide range of microbes. This distinctive contribution by ajwain oil components makes it a good food preservative to prevent food spoilage caused by bacteria and fungi.

Material and Methods

A bulk sample of uniform, ajwain seed (var. Gujarat Ajwain 1) were procured from the Nathwani group of Companies, Jamnagar Gujarat, India.

Cryogenic grinding

The cryogenic grinding of ajwain seed done at temperature -60°C with 8 kg/h feed rate and sieve size 0.8 mm, after completion of grinding cryo ground ajwain powder was packed in aluminum laminated pouches to prevent the loss of essential oil and stored for further studies. The standard analytical methods (AOAC, 2012) [1]. Used for determination of moisture, fat, protein, fiber, ash and essential oil content. Each analysis carried out in triplicate and the mean values were observed.

Experimental Design

Independent variables

1. Temperature (°C): 35, 40, 45, 50, 55
2. Pressure (bar): 100, 150, 200, 250, 300
3. Dynamic time (min): 30, 60, 90, 120, 150
4. Static time (mm): 30, 45, 60, 75, 90

Dependent variables

1. Essential oil yield (%)

Data analysis and optimization

Central Composite Design was employed to evaluate the combined effects of different parameters such as temperature, pressure, dynamic time and static time on essential oil yield of ajwain seed. The levels were determined using the code values of - 2, - 1, 0, + 1, + 2 as described by Nasrin, *et al.*, (2004). Central composite design (CCD) was used to conduct experiments and the response surface methodology was applied to the experimental data using a commercial statistical package, Design Expert – version 10 (Stat-ease, 2016). Analysis of variance (ANOVA) was conducted for fitting the model represented by examines the statistical significance of the model terms. Model analysis with respect to lack-of fit test and R^2 (coefficient of determination) was done for determining adequacy of model.

Shelf life study of essential oil from SFE

After the optimization of SFE parameters for production of superior quality ajwain seed oil the oil was extracted from ajwain seed at optimized condition. Eighteen samples, each of 5 ml were prepared and packing in the glass vials, sealed and stored. The essential oil packed samples were kept for the shelf life study at -18 ± 2°C and 7 ± 2°C conditions. The essential oil was evaluated at every 15 days interval up to 3 months of the storage.

Results and Discussion

1. Chemical properties

Chemical constituents moisture, fat, essential oil protein, fibre, ash content, of ajwain seed were determined using standard procedures. (A.O.A.C 2012) [1]. Moisture content of ajwain seed was 6.45%. The fat content was 9.91% and essential oil content was 3.9%. Protein, fibre and ash contents were 19.08%, 21.89% and 6.70 respectively. The chemical properties observed are similar to the observation made by Bairwa, *et al*., (2012) [2], Javed, *et al.*, (2012) [5]. and Dashora, *et al.*, (2006) and the minor variations could be due to various factors such as varieties and other environmental factors.

Table 1: Chemical Properties of ajwain seed

Parameter	Mean values (%) ± SD
Moisture	6.45±0.55
Fat	9.91±0.64
Protein	19.08±0.05
Fibre	21.89±0.62
Ash	6.70±0.49
Essential oil	3.9 ± 0.14

2. Supercritical Fluid Extraction (SFE)

The sample of cryogenically ground ajwain seed powder was taken for studies on of Supercritical fluid Extraction (SFE) for maximum yield essential oil.

2.1 Effect of supercritical fluid extraction parameters on essential oil yield

Experiments on supercritical fluid extraction of essential oil from ajwain seed were conducted as per the standard method. Optimized combinations of temperature, pressure, dynamic time and static time were selected on the basis of maximum recovery of essential oil The data obtained for all thirty experiments is shown in Table. It was observed that essential oil yield was influenced by temperature, pressure, dynamic time and static time.

Analysis of variances (ANOVA) of experimental data and the significance of temperature, pressure, dynamic time and static time as well as the interactions of parameters on essential oil yield were shown in table. Stastical significance of linear, quadratic and interaction effects were calculated for each response.

The result showed that quadratic effect of temperature, pressure, static time and dynamic time was significant ($p<0.05$) on essential oil yield at 5% level. The essential oil yield (%) varied from 0.77 to 4.85% during different experiments. The maximum yield was obtained at experiment number 19 i.e. 4.85% and minimum yield was noticed in experiment number 1 i.e. 0.77%. The Model F-value of 15.72 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large error could occur due to noise. R^2 and CV% values are 0.93 and 15.68 respectively for essential oil yield.

The response surface equation developed to predict the change in essential oil yield with varying levels of processing parameters is as under:

Essential Oil Yield (%)=+2.15- 0.070 * Temperature + 0.92 * Pressure +0.32 * Dynamic time +0.044 * Static Time

Table 2: Variables and responses of experiments on supercritical fluid extraction

Variables					Responses
Expt. No.	**Temperature °C**	**Pressure bar**	**Dynamic time, min**	**Static time, min**	**Essential oil yield,%**
1	45	100	90	60	0.77
2	40	150	120	75	1.49
3	45	200	90	60	1.72
4	35	200	90	60	2.27
5	45	200	90	60	1.72
6	50	250	120	45	3.01
7	40	150	60	45	0.96
8	45	200	90	60	2.51
9	50	150	60	75	1.05
10	50	250	60	75	2.11
11	50	150	60	45	0.88
12	45	200	90	30	1.83
13	55	200	90	60	2.21
14	40	250	120	75	3.18
15	45	200	90	60	2.21
16	45	200	30	60	1.38
17	50	150	120	75	1.24
18	40	250	60	45	2.60
19	45	300	90	60	4.85
20	45	200	90	90	2.04
21	45	200	150	60	2.51
22	40	150	60	75	1.13
23	50	150	120	45	1.08
24	45	200	90	60	2.51
25	40	250	60	75	2.66
26	50	250	120	75	3.99
27	45	200	90	60	2.21
28	40	250	120	45	3.54
29	40	150	120	45	1.76
30	50	250	60	45	2.39

Table 3: ANOVA for essential oil yield

Source	df	Essential oil yield		
		Sum of squares	**Mean square**	**p-value Prob > F**
Model	14	24.481	1.7486	< 0.0001
A-Temperature	1	0.1190	0.1190	0.3174
B-Pressure	1	20.258	20.258	< 0.0001
C-Dynamic Time	1	2.5155	2.5155	0.0003
D-Static Time	1	0.0459	0.0459	0.5302
AB	1	0.0232	0.0232	0.6541
AC	1	0.0045	0.0045	0.8423
AD	1	0.1278	0.1278	0.3007
BC	1	0.3630	0.3630	0.0910
BD	1	0.0018	0.0018	0.9003
CD	1	0.0095	0.0095	0.7740
A^2	1	0.0005	0.0005	0.9474
B^2	1	0.5240	0.5240	0.0464
C^2	1	0.1669	0.1669	0.2394
D^2	1	0.1778	0.1778	0.2254
Residual	15	1.6686	0.1112	
Lack of Fit	10	1.0324	0.1032	0.6371
Pure Error	5	0.6361	0.1272	
Cor Total	29	26.149		

2.2 Effect of temperature and pressure on essential oil yield

Effect of temperature and pressure on essential oil yield is shown in Figure 1. The influence of pressure on essential oil yield was more prominent than the influence of temperature. It was observed that, when there was increase in pressure, essential oil yield increased. Temperature had little effect on essential oil yield. Increase in pressure will increase the density of CO_2. The solvent strength of SC-CO_2 increases with the density of CO_2. As the density increases, the distance between the molecules decreases and therefore the interaction between the analyte and CO_2 increases, leading to greater solubility of the analyte in CO_2 (De Castro, *et al.*, 1994) [4].. The increase in pressure will also accelerate mass transfer of analysts and solvent in supercritical extractor vessel and improve the extraction yield. Minimum essential oil yield of 0.77% was obtained at 45 °C, 100 bar for the dynamic time of 90 min and static time 60 min. Maximum oil yield of 4.85% was obtained at 45 °C, 300 bar for the dynamic time 90 min and static time 60 min.

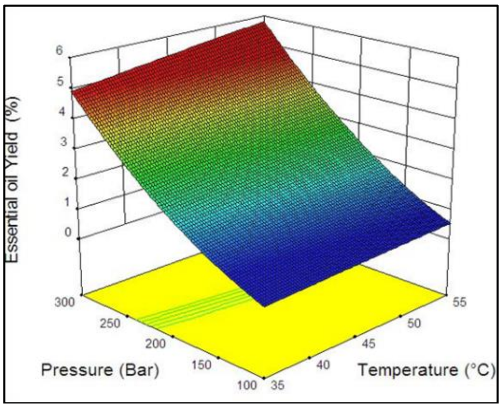

Fig 1: Effect of temperature and pressure on essential oil yield

2.3 Effect of temperature and dynamic time on essential oil yield

Influence of temperature and dynamic time on essential oil yield is shown in Figure.2 from RSM graph it can be seen that with increase in temperature, essential oil yield had linear effect. It was observed that essential oil yield increased with increase in dynamic time from 30 to 150 minute.

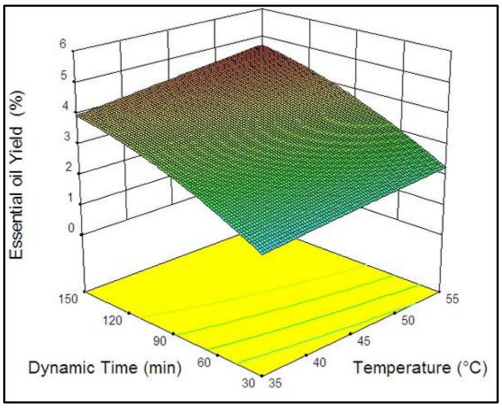

Fig 2: Effect of temperature and dynamic time on essential oil yield

2.4 Effect of temperature and static time on essential oil yield

Effect of temperature and static time on essential oil yield is shown in figure 3. It was observed that essential oil yield increased slightly when temperature of the ajwain seed powder increased. When static time increased from 30 to 90 minute, essential oil yield increased at certain point. This phenomenon can be explained taking into account the mass transfer mechanism involved in the extraction process (Reverchon, 1992) [10].

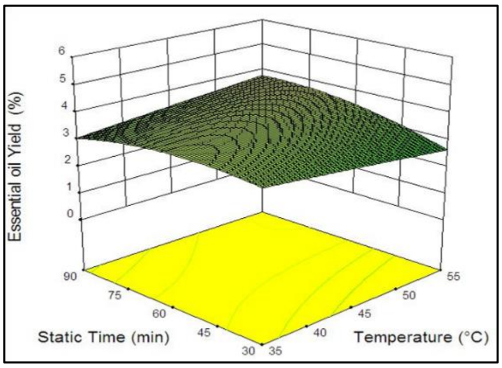

Fig 3: Effect of temperature and Static time on essential oil yield

2.5 Effect of pressure and dynamic time on essential oil yield

Effect of pressure and dynamic time on essential yield is shown in Figure 4. As increasing pressure from 100 to 300 bar and dynamic time from 30 to 150 minute, the essential oil yield increases and hence increase in essential oil yield is proportional to pressure and dynamic time.

Maximum essential oil yield was observed at pressure of 300 bar and dynamic time of 90 min. From the RSM graph it can be observed that essential oil yield obtained at dynamic time from 60 min to120 min is not significantly different. The observations made by Liza, *et al.*, (2010) [6]. Are quite similar to the present observations.

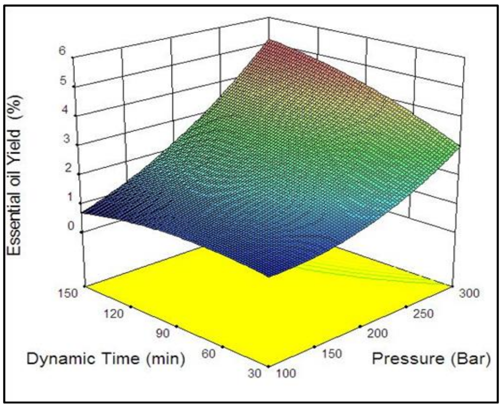

Fig 4: Effect of pressure and dynamic time on essential oil yield

2.6 Effect of pressure and static time on essential oil yield

The effect pressure and static time on essential oil is shown in Figure. 5

It can be seen that increase in essential oil yield was recorded when there was increase in pressure from 100 to 300 bar whereas, there was slightly increases in essential oil yield with increase in static time from 30 to 90 minute. This can be attributed to the fact that due to increase in pressure which increase of the solubility of the oil components.

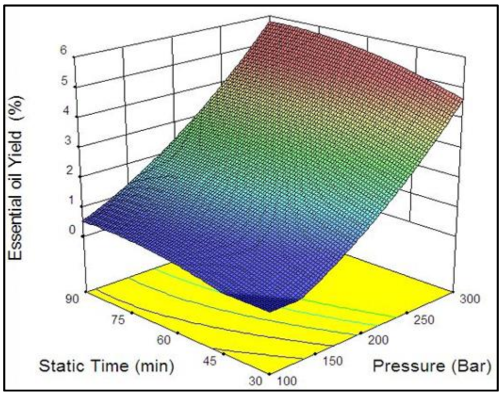

Fig 5: Effect of pressure and static time on essential oil yield and

2.7 Effect of dynamic time and static time on essential oil yield

Effect of dynamic time and static time on percent essential oil yield is shown in Figure.6. Essential oil yield slightly increased with increased in static time. On other hand essential oil yield found to increase with increased dynamic time. This phenomenon is because higher dynamic time will increased the more extract was obtained.

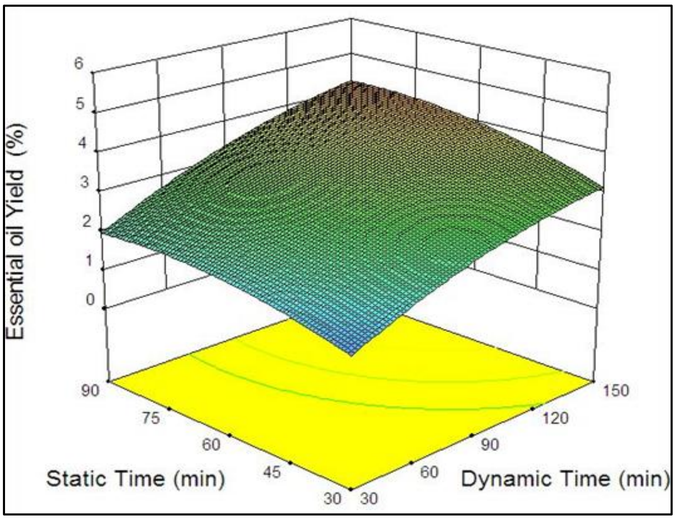

Fig 6: Effect of dynamic time and static time on essential oil yield

2.8 Shelf life evaluation of essential from SFE

Essential oil obtained from the optimized values and the samples were packed in glass vials which were kept for the shelf life study at temperature-18 ± 2°C and 7 ± 2°C conditions and evaluated for the degradation of essential oil content at an interval of every 15 days up to 3 months of storage. The results obtained are given in Table 4.

The variation in oil content during the storage study is shown in Figure 7. Essential oil degradation was faster in case of ambient storage than that of sample stored under refrigerated (-18 ± 2°C), and (7 ± 2°C) conditions. At -18±2°C the shelf life of essential oil content decreased slowly compared to 7± 2°C condition. Ogzewalla and Willins (1962) [11]. Reported that essential oil slowly lost during storage.

Table 4: Effect of storage conditions on essential oil of SFE

Days	Essential oil	
	-18±2°C	7 ± 2°C
0	3.7	3.7
15	3.7	3.7
30	3.7	3.6
45	3.7	3.6
60	3.7	3.5
75	3.7	3.5
90	3.6	3.2

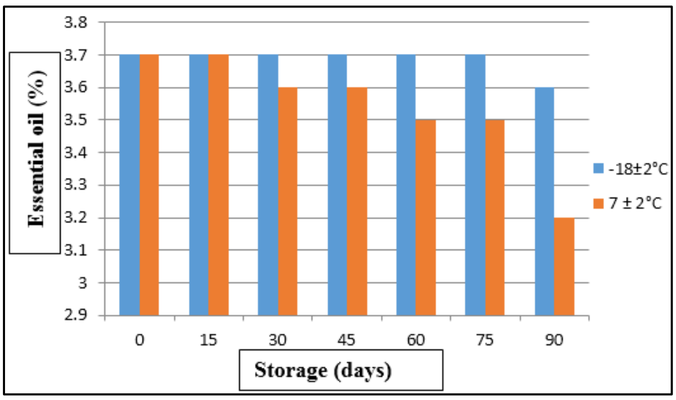

Fig 7: Effect of storage conditions on essential oil content from SFE

Conclusion

Optimization of parameters for supercritical fluid extraction of essential oil was done based on analysis of various parameters and statistical data. As explained earlier, Design Expert- version 10 was used for optimization of responses. The suitable combination of temperature, pressure, dynamic time and static time was calculated on the basis of experimental results obtained and statistical analysis. Numerical optimization found a point that maximizes the desirability function and the best combination was selected having higher desirability function. From the numerical optimization, solutions were found out of which best combination having desirability of 0.875 was selected. SFE process was done on the basis of percent essential oil yield. The optimized parameters were 35 °C temperature, 300 bar pressure, 65 min dynamic time and 30 min static time and during storage study degradation of essential oil of SFE were relatively low at 7 ± 2°C as compared to -18 ± 2°C.

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