Optimisation of Squalene Recovery from Amaranth Oil by Short Path Distillation

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We aim to determine the optimal conditions for obtaining a higher concentration of squalene ($C_{30}H_{50}$) with an increased yield from oil extracted from Amaranthus cruentus cultivated in Romania. A central composite experiment design was carried out to study the effect of operating conditions on the squalene concentration and recovery yield using short path distillation at laboratory scale. Among the three variables studied: feed flow rate, evaporator temperature and wiper speed, the most important proved to be evaporator temperature and the flow rate. Using the proposed models, we have identified three sets of values for the mentioned parameters, which ensure either a maximum squalene concentration or the best value for the squalene recovery yield, or an optimum between the maximum concentration and the best yield.

Keywords: squalene, short path distillation, Response Surface Method, Amaranthus cruentus oil

Squalene is a 30-carbon polyunsaturated, triterpenic hydrocarbon (2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene) with various, valuable nutritional, cosmetic, pharmaceutical and medical applications [1-5].

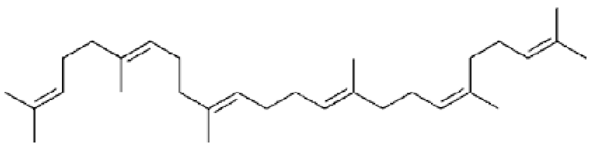

Fig. 1 Squalene ((*E*)-2,6,10,15,19,23-Hexamethyl-2,6,10,14,18,22-tetracosahexaene)

Being a crucial intermediate of phytosterol/cholesterol biosynthesis in plants/animals/humans, squalene is all-over present in nature. The richest and the oldest known source of squalene in nature is represented by the oil extracted from the liver of a deep water shark of *Squalidae* order [6]. A limitative reason in using this natural source for squalene is represented by the presence in the sea environmental of different persistent organic pollutants (POPs), what can still be found in the purified squalene, together with the concern for the preservation of marine life [7].

In recent years the microbial biosynthesis of squalene became a promising alternative source. Although the microorganisms don't accumulate as much squalene as shark liver or some plants, they grow very fast and in controlled conditions [8-16].

Most of the fractionation methods of the squalene containing oils presented in the state of the art literature, were designed for analytical purposes [17, 18]. Squalene was isolated from natural sources, by extraction with organic solvents or with supercritical CO2. One of the most commonly used methods for concentration and purification of minor components from vegetable oils both at laboratory and industrial scale is shorth path (molecular) distillation. Molecular distillation is generally accepted as the safest method to separate and purify thermally unstable compounds and substances having low volatility [19].

Squalene is thermolabile due to its unsaturated linear chain, therefore the distillation at normal pressure of vegetable oils is not an appropriate procedure for its isolation and purification. Moreover, thermal degradation of other compounds from oils such as TAGs may occur as well [20]. The boiling temperature can be reduced by applying vacuum.When reducing the pressure by one order of magnitude the boiling temperature of most liquid mixtures decreases by more than 25 degrees. If the process is carried out at pressures between 10^{-6}-10^{-4} bar, the temperature can be decreased by 100-150 degrees compared to the distillation at normal pressure.

Beside the temperature, the exposure time is another major parameter for the stability of thermo-sensitive materials. Reducing the exposure time by half may have a similar effect as decreasing the temperature by 10 degrees [21]. In a falling film molecular distillator apparatus the residence time shorter than 1 min is achieved by formation of a thin liquid film through wiping of the evaporating surface. Pietsch and Jaeger [22] investigated the process of squalene concentration from shark liver oil using short-path distillation. Starting from an initial squalene concentration of 60% in the feed, they obtained a light fraction (distillate) with more than 95% squalene. Within a relatively large range of operating conditions (200–230°C and 0.01–0.1 mbar), the yield of distillate relative to feeded oil was 56–57% of the crude oil. At temperatures below 200°C, it was possible to maintain the yield above 55% only by lowering the absolute pressure down to 0.01 mbar. On the other hand, they couldn't enhance the distillate yield above values of 58%, although squalene recovery yield was 68-98%.

US Patent No. 7161055B2 [23] describes a process for the separation and recovery of minor components from vegetable oils, especially palm oil. Bondiolli et al [24] obtained squalene from olive oil deodorization distillate (10-30% squalene) by supercritical carbon dioxide extraction with a 90% yield.The same method of SC CO2 extraction was also used for the extraction of squalene from from palm oil mesocarp.

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Experimental part

Materials and methods

Raw materials

Amaranthus cruentus seed oil was obtained by extraction with hexane of milled seeds and subsequent concentration thereof under low pressure (Rotavapor Bucchi R215). The relevant oil characteristics are presented in table 1.

Analytical Methods

Squalene concentration was determined by GC method. The proposed method uses a GC - 6890N Agilent Technologies with split-splitless injector and FID detector and chromatographic conditions as: capillary column - HP88 (88% cyanopropyl - methyl polysiloxane) (60m x 0.25mm, 0.20μm), 2 gradient steps (the first step: T_i: 150⁰C, 10 min; T_f: 175⁰C, 1 min; rate: 1⁰/min.; the second step: T_f: 220⁰C, 5 min; rate: 10⁰/min.), split ratio 20:1, carrier gas: N_2 with a flow rate of 1.5 mL/min. For the evaluation, the external standard method was used, with Sigma squalene 442784 as standard. The total time for analysis was 45.5 min with a retention time for squalene 39.63 min.

Free Fatty Acids (FFA), Peroxide Value (PV) and phosphorous concentrations were determined by standard methods (table 1).

Short path distillation

Distillation of amaranth oil was carried out using a short-path distillation unit VKL-70 provided by VTA Verfahrenstechnische Anlagen GmbH & Co. KG (Germany) with an evaporating surface area of 0.043 m² and condenser area of 0.022 m². The main operation characteristics were: feed rate 0.1-1.5 kg/h, t_{max} 300°C, P_{min} 2.3-2.4 x10^{-3} mbar, wiper system speed 200-1000 rpm. A schematic flow diagram of short distillation unit is depicted in figure 2.

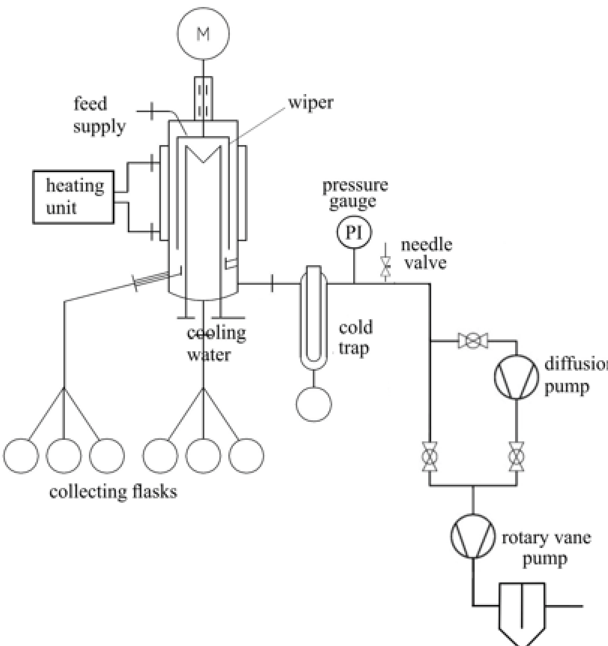

Fig. 2. Short path distillation unit VKL 70 (VTA)

The significant operating conditions of the short-path distillation are represented by feed temperature, evaporator temperature, system pressure, feed flow and condenser temperature. These parameters need to be optimized to obtain the highest possible recovery yield and/ or highest concentrations of squalene. The range of investigated parameters is presented in table 2. The other parameters were maintained at constant values: feed temperature 80-82°C, condenser temperature 60°C, pressure (2.3 ÷2.4) × 10^{-3}mbar.

Experimental design

The Response Surface Method (RSM) was used to study the optimization of three selected factors: flow rate, temperature and wiper speed. The authors' choice for the experimental design was chose a central composite one with 6 axial points and 4 repetitions in central point (table 3). Minitab17 software was used to build up the experimental design and to analyze the results. The general model proposed was a quadratic one:

$$f_i=b_0+b_1x+b_2y+b_3z+b_{11}x^2+b_{22}y^2+b_{33}z^2+b_{12}xy+b_{13}xz+b_{23}yz$$

i=1 -squalene concentration, wt%; *i=2* -squalene recovery yield, %

The complete RSM equation describes the contributions of the various factors on the concentration of squalene (f_1) and on the squalene recovery yield (f_2).

Results and discussions

The raw material used in our experiments was the amaranth oil obtained by hexane extraction. The crude oil was first degummed and then neutralized. The main purpose of degumming process is to produce an oil that does not deposit a residue on the condenser wall during short path distillation process. Before introducing in short path distillation installation apparatus, the oil was dried and degassed at la 90°C, 100 mm Hg for 30 min (Rotavapor Bucchi R215).

The influence of the operation parameters of the short path distillation installation on the squalene concentration and recovery yield was studied using the response surface method (RSM). The independent variables and their variation range are presented in table 2. These levels of independent variables were selected based on the values obtained in preliminary experiments. Response variables were squalene concentration (f_1), squalene recovery yield (f_2):

$$\eta_{sq} = \frac{d \times c_D}{c_F} \times 100 \qquad (1)$$

d =D/(D+R) - distillate weight fraction

D - distillate, (g)

R - residue, (g)

c_D- qualene concentration (weight fraction) in distillate

c_R - squalene concentration (weight fraction) in residue

c_F- squalene concentration (weight fraction) in feed

η_{sq}- squalene recovery yield.

After calculating the model coefficients and their standard errors (table 4), the quality of the data fitting model

Table 1

CHARACTERISTICS OF *A. CRUENTUS* SEED OIL

Characteristics	Value	Method
Squalene (%, wt/wt)	4.98±0.079	
Free fatty acids (FFA), %	1.97±0.13	ISO 660:2009
Peroxide Value (PV), meq/kg	2.13±0.81	ISO 3960:2007
P (mg/kg)	280±32	ISO 10540-1:2003

			Coded levels				
Variables	Units	Symbol	-1.682	-1.000	0	1.000	1.682
Flow rate	g/h	x	150	180.5	225	269.5	300
Temperature	^{0}C	y	190	202	220	238	250
Wiper speed	rotation/min	z	200	300	450	600	700
Squalene concentration	% (w/w)	f_1					
Squalene recovery yield	% (w/w)	f_2					

Table 2
REAL AND CODED VALUES OF VARIABLES USED IN OPTIMIZATION

Standard order	Run order	x (Flow rate)	y (Temperature)	z (Wiper speed)
11	1	1.00000	-1.00000	1.00000
4	2	-1.00000	1.00000	-1.00000
2	3	0.00000	0.00000	0.00000
1	4	-1.68179	0.00000	0.00000
9	5	1.00000	1.00000	-1.00000
12	6	-1.00000	1.00000	1.00000
3	7	1.00000	-1.00000	-1.00000
8	8	1.00000	1.00000	1.00000
14	9	-1.00000	-1.00000	1.00000
6	10	-1.00000	-1.00000	-1.00000
10	11	1.68179	0.00000	0.00000
17	12	0.00000	1.68179	0.00000
20	13	0.00000	0.00000	0.00000
15	14	0.00000	-1.68179	0.00000
13	15	0.00000	0.00000	-1.68179
7	16	0.00000	0.00000	1.68179
16	17	0.00000	0.00000	0.00000
18	18	0.00000	0.00000	0.00000

Table 3
EXPERIMENTAL POINTS AND RUN ORDER USED IN RESPONSE SURFACE METHOD

	Squalene concentration (f_1)			Squalene recovery yield (f_2)		
Term	Effect	Coefficient	S.E. Coefficient	Effect	Coefficient	S.E. Coefficient
b_0		81.601	0.559		79.660	1.10
b_1	29.775	14.888	0.510	24.156	12.078	0.998
b_2	-2.781	-1.391	0.510	50.926	25.463	0.998
b_3	15.882	7.941	0.510	8.554	4.227	0.998
b_{11}	-20.190	-10.095	0.891	-52.670	-26.340	1.740
b_{22}	-15.870	-7.935	0.891	-65.42	-32.710	1.740
b_{33}	-11.770	-5.885	0.891	-28.87	-14.440	1.740
b_{12}	-9.670	-4.840	1.120	-32.19	-16.10	2.190
b_{13}	-23.350	-11.67	1.120	31.49	15.740	2.190
b_{23}	-9.770	-4.890	1.120	-54.53	-27.260	2.190

Table 4
CODED COEFFICIENTS OF REGRESSION MODELS AND THEIR STANDARD ERRORS

was verified by ANOVA (table 5). Both models fit very well the experimental data (table 6), all the coefficients being statistically significant.

Squalene concentration is mostly influenced by the feed rate. Temperature has a lower influence on its concentration in distillate, high squalene concentrations being achieved throughout the whole investigated temperature range. As the linear component is responsible for more than 75% of the observed variance, the response surface is only slightly curved (fig 3, 4). The response surface presents a maximum (x=1.342, y= 66 0.56 z= 0.05; 285 g/h, 210^{o}C, 457 rpm) for which the predicted squalene concentration is 87.92%. Experimental verification for that point (285 mL/h, 210^{o}C, 450 rpm) resulted in a squalene concentration of 87.40±1.06. The value obtained experimentally for the recovery yield was 66.85±1.20 versus a predicted value of 65.49%. As can be seen in figure 3, the increase of feed rate has a positive influence on the squalene concentration in distillate up to 285g/h, after this value, the mentioned concentration tending to slow down. The influence of the wiper speed on the squalene concentration can be noticed especially at low feed rates (fig. 4). These two observations can be explained if we admit that the liquid film does not cover uniformly the evaporator surface at low feed rates. Increasing the wiper speed could result in a better

Source	Squalene concentration model (f_1)				Squalene recovery yield model (f_2)		
	DF	Adj SS	Adj MS	F-Value	Adj SS	Adj MS	F-Value
Model	9	1797.73	199.75	159.15	7523.45	835.94	173.74
Linear	3	1383.99	461.33	367.57	3923.34	1307.78	271.80
x	1	1070.16	1070.16	852.67	704.38	704.38	146.39
y	1	9.34	9.34	7.44	3130.64	3130.64	650.66
z	1	304.49	304.49	242.6	88.33	88.33	18.36
Square	3	230.19	76.73	61.14	2349.87	783.29	162.80
x^2	1	161.14	161.14	128.39	1096.62	1096.62	227.92
y^2	1	99.56	99.56	79.33	1691.79	1691.01	351.61
z^2	1	54.76	54.76	43.63	329.49	329.49	68.48
2-Way Interaction	3	183.56	61.19	48.75	1250.23	416.74	86.61
$x \times y$	1	23.39	23.39	18.64	259.12	259.12	53.85
$x \times z$	1	136.29	136.29	108.59	247.87	247.87	51.52
$y \times z$	1	23.87	23.87	19.02	743.24	743.24	154.47
Error	8	10.04	1.26		38.49	4.81	
Lack-of-Fit	5	3.91	0.78	0.38	27.74	5.55	1.55
Pure Error	3	6.13	2.04		10.75	3.58	
Total	17	1807.77			7561.94		

Table 5
ANALYSIS OF VARIANCE (ANOVA) FOR SQUALENE CONCENTRATION MODEL (f_1)

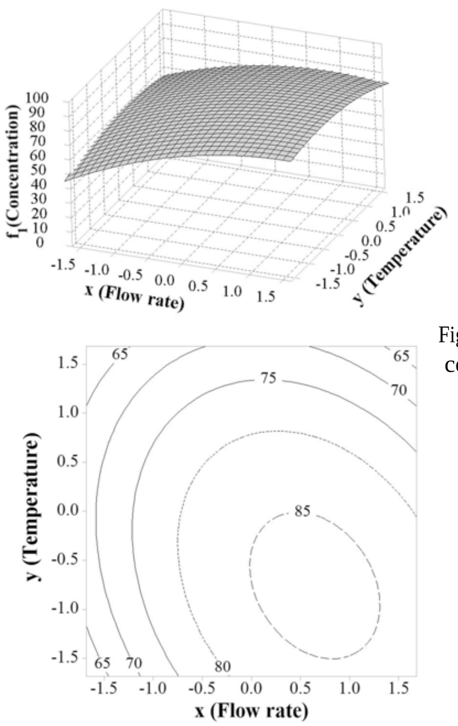

Fig.3 Surface (up) and contour (down) plots for f_1(x, y, 0.05)

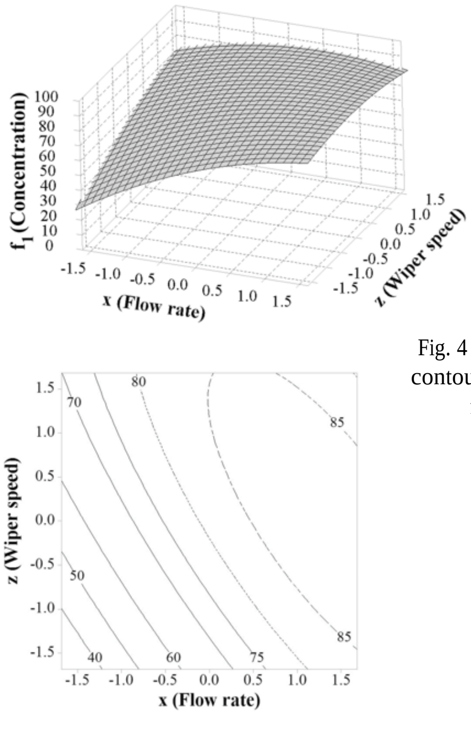

Fig. 4 Surface (up) and contour plots (down) for f_1(x, -0.56, z)

distribution, more uniform, of the liquid on the evaporator surface. During the evaporation, the growth of liquid film surface leads to the increase of the number of squalene molecules leaving the liquid phase and consequently, to the growth of the squalene concentration in distillate. Once the feed rate grows, the liquid covers the evaporator surface in a more uniform way, determining a lower influence of the wiper speed on squalene concentration.

Squalene recovery yield is mostly influenced by the temperature and only secondary by the feed rate. It is noted that in this case, the quadratic component represents 50% of the observed variability. The best squalene recovery yield was obtained at x= 6 0.39, *y*=1.27, *z*= 6 1.27 (208 g/h, 243°C, 260 rpm). As it can be seen, the maximum was moved to a higher temperature (243°C) (fig 5, 6). This observation could be explained by the positive influence of the temperature increase on the amount of resulted distillate.

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If the squalene concentration is high enough for the intended utilization, the operating parameters corresponding to maximum of the f_1 model can be used, and if further distillation is required, the the operating parameters for maximum of f_2 model can be used. Considering that the operating parameters corresponding to the two maximum values are quite different, we could not find an operating area to allow simultaneous achieving

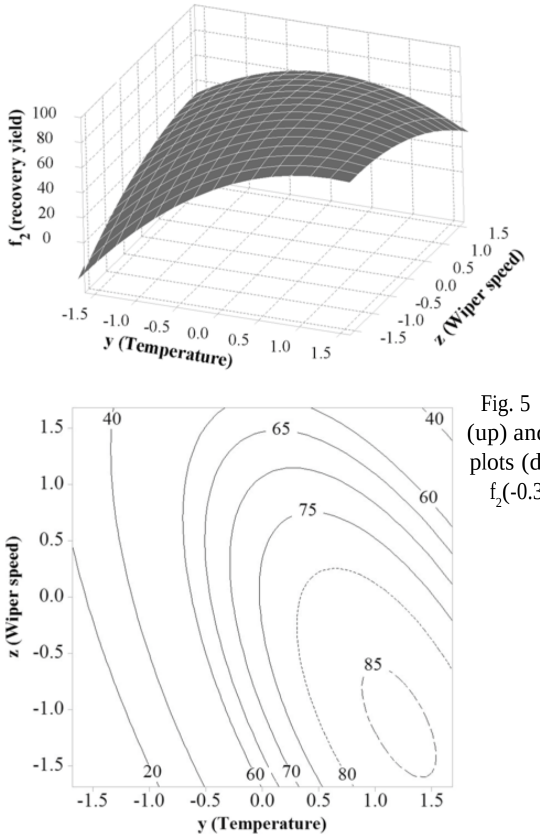

Fig. 5 Surface (up) and contour plots (down) for $f_2(-0.39, y, z)$

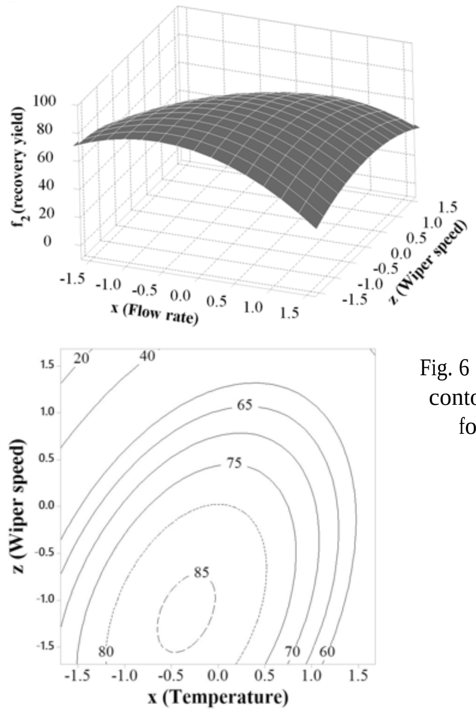

Fig. 6 Surface (up) and contour plots (down) for $f_2(x, 1.27, z)$

of a maximum squalene concentration and a maximum recovery yield of squalene.

The combination of the two models results in the following regression equation (coded coefficients):

$$f_3 = \frac{f_1+f_2}{2} = 80.63 + 13.483x + 12.036y + 6.109z - 18.22x^2 - 20.32y^2 - 10.16z^2 - 10.47xy - 16.07yz \quad (2)$$

It is very interesting to note that the combination of the two models allows for a global maximum that ensures a squalene concentration of 85,48% and a recovery yield of 83.33%. The coded coordinates for this global maximum are: x=0.561, y=0.221, z=0.323 (250 g/h, 224°C, 500 rpm) (fig.7).

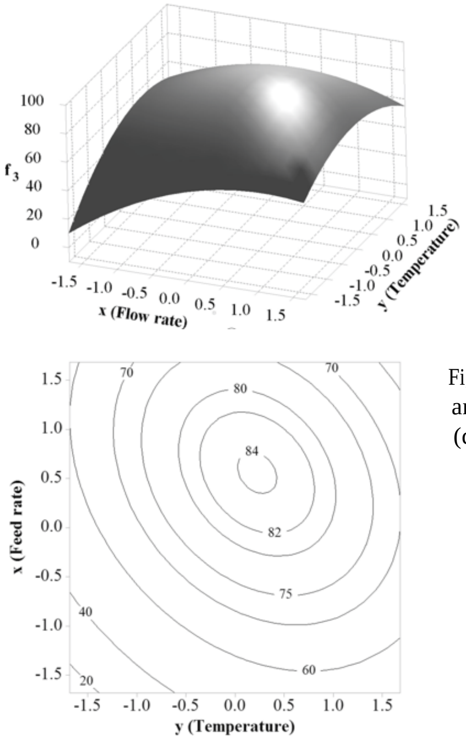

Fig.7 Surface (up) and contour plots (down) for $f_3(x, y, 0.323)$

Conclusions

We obtained squalene from amaranth oil (seeds of *Amaranthus cruentus* cultivated in Romania) by short path distillation with maximum concentration and yield. We used the Response Surface Method to optimize three important parameters of the short path distillation process: flow rate, temperature and wiper speed. We succeeded to determine the operating conditions allowing to obtain either the maximum squalene concentration or the maximum recovery yield of squalene. The combination of the two models allowed to be specified the values for the operating parameters which ensure a high squalene concentration (85.48%) in the same time with an increased recovery yield (83.33%) for the same compound.

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