

## Determination of Optimum Conditions for the Extraction of Squalene from Olive Pomace with Supercritical CO<sub>2</sub>

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Olive pomace is a standard by-product of olive oil production containing valuable compounds recoverable by supercritical fluid extraction (SFE). The solvent/solubility characteristics of a supercritical fluid may be fine tuned by changing the external pressure-temperature conditions of the extraction. This allows for the selective extraction of certain compounds. The present study describes a series of experiments, the methodology, instrumentation, and results of supercritical extraction of olive pomace with CO<sub>2</sub>. The effects that primarily affect supercritical extraction, temperature, pressure and flow rate, have been investigated and monitored on, both, the concentration of squalene at the extract and the overall recovery of squalene from pomace. The Box-Behnken approach was used for designing the experiments. The effects are easily visualized in the resulting 3D plots, which were used for identifying the optimum extraction conditions..

*Keywords:*

Supercritical fluid extraction, carbon dioxide, olive pomace, Box-Behnken, design of experiments

### Introduction

The economic interest of by-product utilization in the olive oil production sector is well documented in the relevant literature.<sup>1,2</sup> In the Mediterranean, where olives are primarily cultivated and olive oil production is cornerstone to the local economy, by-product utilization would mean less pollution in some cases of toxic substances that can be removed from wastes and increased income in the form of select chemicals that can be extracted from them. Tabera et al. for example mention the existence in the Mediterranean area there of ca. 8 mil. ha of cultivated olive trees, how this points out the great economic and social importance of this crop and the possible benefits that can be derived from utilization of its by-products.<sup>3</sup>

Olive pomace is a standard by-product of olive oil production. Depending on the production line (two-phase or three phase decanter) the pomace may be more watery or less. Two-phase pomace is rather a cheap raw material, it amounts to ~72.5 % of the olive, of which 40 % solids, and has only been used for the extraction of the second rate oil or pomace oil it may contain. This oil may be cheaper than regular "extra virgin" olive oil, but it contains chemicals (hydrocarbons like squalene, vitamins like tocopherol, phosphatides like lecithin, and sterols like  $\beta$ -sitosterol) which, when purified, are quite valuable.<sup>3,4</sup> These compounds are known to have

many applications in the food, cosmetic and pharmaceutical industries. Wang et al. for example indicates that squalene is normally used in cosmetic preparations as a moisturizing or emollient agent.<sup>5</sup> There have also been publications on its uses in cosmetic formulations either as it is or after hydrogenation to squalane<sup>4</sup> and products with squalene are already distributed in commerce.<sup>5,6</sup>

Supercritical fluid extraction is the technology that uses a solvent in the supercritical state to extract certain compounds. Supercritical solvents are known to have properties found in liquids with viscosities comparable to gases.<sup>7</sup> So CO<sub>2</sub> was considered a viable option for the extraction of many compounds, oils being one of them. Many researchers have successfully extracted vegetable oils from different oil-bearing seeds.<sup>8–14</sup> Some have managed to establish models for this extraction that correlate well with the existing data, in order to assist in scale-up processes, an interesting aim and one the author will also be undertaking.<sup>9,11,12</sup> For the utilization of oil production by-products in the industry many researchers prefer deodorizer distillate, a refinery by-product, already highly concentrated in free fatty acids, squalene, sterols and tocopherol.<sup>15–18</sup> Others obtain squalene from shark liver,<sup>19</sup> amaranthus grain<sup>14</sup> or even olive leaves.<sup>3</sup> To the best knowledge of the author, however, data for the supercritical extraction of olive pomace is, for the moment, scarce, probably due to its very low oil content (~7–9 %).

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Quite important is also the fact that supercritical compounds may change their solvent characteristics according to the pressure-temperature conditions of the extraction.<sup>20</sup> This allows for the selective extraction of target compounds, a task already researched in a multitude of cases. From a strictly physicochemical point of view, there have been various articles on the solubility of minor lipid compounds to supercritical solvents, some even establishing the solubility of squalene itself to supercritical CO<sub>2</sub> (for example via the Chrastil equation).<sup>21</sup> Ruivo et al.<sup>22</sup> research the phase equilibria of the ternary system methyl oleate/squalene/CO<sub>2</sub>, which produces results very similar to the ones we reach. This of course should be expected since methyl oleate is the main triglyceride in olive oil.

CO<sub>2</sub> is also more environmental friendly than hexane – the current solvent of preference, inflammable, less toxic, and extractions can be conducted in low temperatures, which would protect the thermal labile components of pomace oil. Many researchers specifically point to supercritical CO<sub>2</sub> as the most desirable of solvents for the separation of natural products used in foods and medicines, because of its inertness, non-toxicity, low cost, and high volatility.<sup>5</sup> Naturally with supercritical extraction, there is no solvent residue.

Statistical experimental design is an established approach on the experimental field.<sup>23,24</sup> As a first step, the determination of the parameters that affect a given result is required. After that, however, instead of conducting the whole set of experiments and evaluating the parameters throughout their whole range along with all their combinations, with

factorial design the scientist need only conduct a fraction of the experiments and evaluate the result of the rest with statistical modeling. In our case the parameters that were estimated as the most important were three and were studied at three levels. Such a design would require at least 29 experiments. With the Box-Behnken methodology only 15 were ultimately performed.<sup>23</sup>

As mentioned, there have been numerous articles on modeling of supercritical extraction.<sup>9,11,12,25,26</sup> The present study describes a series of experiments, the methodology, instrumentation and results, of supercritical extraction of olive pomace with CO<sub>2</sub>. The extraction takes place in a pilot plant (figure 1). The extract is recovered through depressurization. The effects that primarily affect supercritical extraction, temperature, pressure and flow rate, have been calculated, both, on the concentration of squalene at the extract and on the overall recovery of squalene from pomace, and have been shown in 3D figures. The ultimate objective of this work was to identify the “optimum locus” of conditions which would allow for the highest concentration of squalene. As a secondary objective, the overall recovery of squalene through supercritical CO<sub>2</sub> (SC-CO<sub>2</sub>) extraction of olive pomace was monitored and produced its own set of results.

## Materials and methods

### Plant material, reagents and standards

Fresh olive pomace was collected from an olive oil production site, in Kalyves, Chalkidiki,

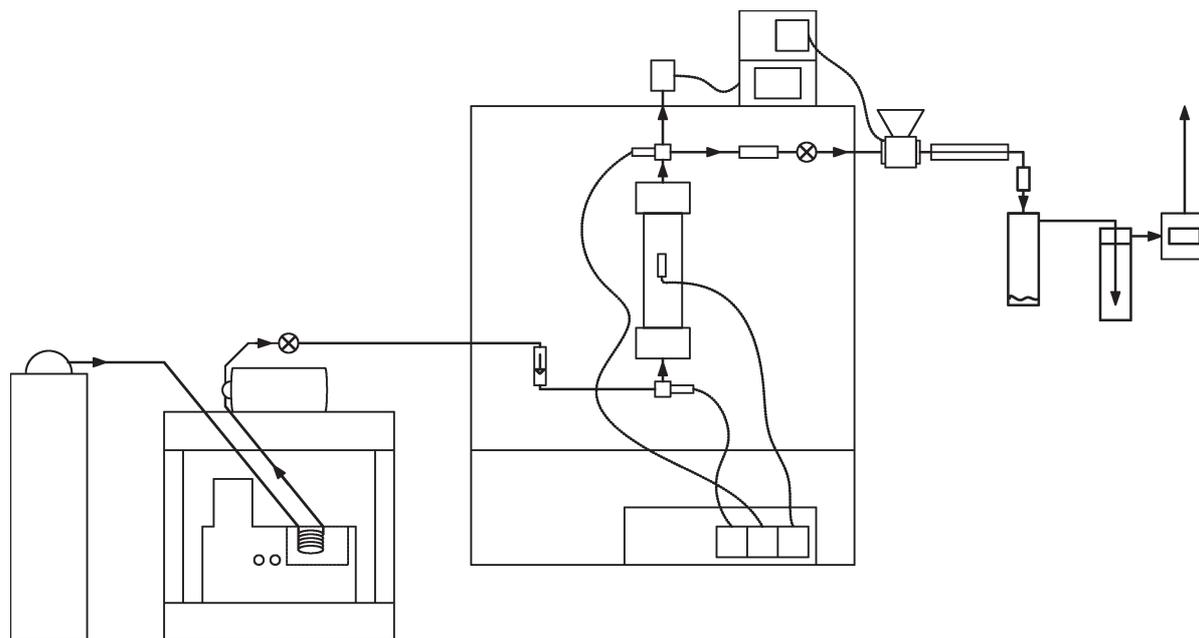


Fig. 1 – Drawing of the supercritical extraction pilot plant

Greece. The site uses a 2-phase decanter for the separation of oil. The second phase is a mixture of water and pomace. Field collections were made from October to December 2003. Squalene (2,6,10,15,19,23 – hexamethyl – 2,6,10,14,18,22 – tetracosahexaene) and 5 $\alpha$ -cholestane were purchased from Sigma Aldrich. N<sub>2</sub>, CO<sub>2</sub>, H<sub>2</sub>, He and air (Respal) were supplied from air-liquide. 2-propanol (analytical grade) was purchased from Fluka. Water (HPLC grade) was purchased from Merck. n-heptane (HPLC grade) and potassium hydroxide (analytical grade) were purchased from Riedel-de-Haën.

### Preparation of pomace for extraction

Olive pomace was collected through a three month period (8.5 kg). It was dried under a heated air current (50 °C) for 4 days until the remaining water was well below 10 %. It has been shown that at higher moisture levels, extracted oil decreases due to increased water concentration in the fluid phase. In addition high concentration of water in the solid matrix may also form a barrier for supercritical CO<sub>2</sub> to access the lipids in the muscle structure.<sup>27</sup> The water content of dried pomace was determined with TGA (Thermogravimetric Analysis), using a Shimadzu TGA-50 analyzer. The dried material was cut in a cut mill and the granulometry was determined with sieving. Glass beads were introduced to the pomace, prior to extraction, as packing material. The amount of packing was equal to the amount of sample for extraction. Both were weighed at 150 ± 0.1 g.

### SFE Instrumentation and extraction method

The SC-CO<sub>2</sub> extraction pilot plant, that has been used, can be seen in Figure 1. It's a pressure cell, for batch leaching, inside a temperature controlled oven. CO<sub>2</sub> passes through a spiral tube inside a cooling bath at -7 °C. The cooling bath recirculates the cooling medium through the pump heads as well, to keep the system adequately refrigerated.

The extraction general procedure is, briefly, as follows: CO<sub>2</sub> is filtered for impurities, then cooled while passing through the bath at -7 °C, and reaches the pump (Milton Roy NSI-33R) which controls the flow rate and the pressure required by the experiment. The pump's discharge leads inside the oven, in which the high pressure extraction cell resides. The oven keeps the temperature constant, another requirement of the experiment. The inlet temperature of CO<sub>2</sub> in the cell is monitored and connected to the temperature controller. Supercritical CO<sub>2</sub> then proceeds through the sample. The temperature of the pomace is monitored at a height approximately in the middle of the cell, also connected to

the controller. Oven temperature uses this temperature as a reference point. Finally CO<sub>2</sub> leaves the cell, through an intersection where, both, outlet temperature and cell pressure are monitored (temperature controller keeps inlet, center and outlet temperature constant and within ± 0.5 °C).

Having extracted a number of compounds, CO<sub>2</sub> passes through a filter, placed there to protect the backpressure regulator from blocking. This regulator is also heated and temperature-controlled at 50 °C in order to avoid the clogging of solidified CO<sub>2</sub> and lipid particles. For the same reason, a part of the metal tubing that leads out of the regulator is also heated and temperature controlled at 50 °C. CO<sub>2</sub> leaves through a side vent. Sometimes small liquid particles are washed away with the CO<sub>2</sub> flow. Such particles may damage the gas flow rate meter, so a liquid trap was placed in between. This trap is of similar design to the separator, only larger and made of glass. For increased performance it's cooled in an iced water bath.

A typical SC-CO<sub>2</sub> experiment starts with the introduction of CO<sub>2</sub> in the cell. CO<sub>2</sub> is heated and pressurized until the operating conditions are reached. The outlet valve then opens and continuous operation is maintained until the end of extraction. The whole set of experiments was designed for extractions that last a set amount of time (8 h).

It's interesting to point out here, that in a given time, for different CO<sub>2</sub> flow rates (g min<sup>-1</sup>), different amounts of CO<sub>2</sub> will pass from the cell, which would normally lead to different results for, both, solute mass and compound concentration in the solute. In experiments it has been shown that for the same conditions (pressure, temperature and flow rate) squalene concentration has been different for the 3 h extract and for the 8 h extract. Also, as the extraction continues, extract total mass changes. This fact leads to the estimation that the amount of CO<sub>2</sub> used for the extraction, is a critical factor and should be kept the same for all experiments. Besides the fact that quite a few researchers prefer the set time instead of the set CO<sub>2</sub> mass approach,<sup>3,10,28</sup> the reasoning for this decision has scale-up in mind. Time is as important as solvent cost in the industry, if not more so. Firstly olive pomace is a relatively sensitive by-product, stored in environmental conditions and it's of great importance to extract as much as possible in as little time as possible. Secondly it's a very common feature in oil extraction plants to require a set amount of time for the extraction of any raw material, for cost estimation reasons. Thirdly it's noted that time has its own effect in extraction and it will become the object of interest in future work.

Extraction conditions were designed according to a Box-Behnken experimental design for 3 factors (pressure, temperature and CO<sub>2</sub> flow rate), covering a wide range of supercritical densities and flows, and experimenting on the factors that have the most important impact on extraction efficiency,<sup>23</sup> as can be seen in Table 1. Granulometry, another important factor, was considered constant within the experimental practice, since the same material was used throughout the whole series. It should be noted however that particle diameter within each sample varies from 280 to 560  $\mu\text{m}$ . Total extraction time was also kept constant. Extractions lasted 8 h, where start is considered the moment when the extraction conditions are first met.

Table 1 – Experimental series according to Box-Behnken Design.

Expt	Conditions			CO <sub>2</sub> density $\rho/\text{kg m}^{-3}$	Extraction	
	pressure $p/\text{MPa}$	temperature $T/^\circ\text{C}$	mass flow rate $Q_m/\text{g min}^{-1}$		sample mass $m/\text{g}$	yield $Y/\%$
01	7.5	33	3.6	324.4	150.1	0.524
02	7.5	43	3.6	218.4	150.0	0.017
03	17.5	33	3.6	852.2	150.3	3.008
04	17.5	43	3.6	793.7	150.0	2.261
05	7.5	38	1.8	247.0	150.0	0.010
06	7.5	38	5.4	247.0	150.6	0.002
07	17.5	38	1.8	823.9	150.1	1.184
08	17.5	38	5.4	823.9	150.1	3.018
09	12.5	33	1.8	791.6	150.2	0.468
10	12.5	33	5.4	791.6	150.1	1.957
11	12.5	43	1.8	699.8	150.9	0.196
12	12.5	43	5.4	699.8	150.7	1.093
13	12.5	38	3.6	749.3	150.1	1.123
14	12.5	38	3.6	749.3	150.5	0.853
15	12.5	38	3.6	749.3	150.0	0.781

### Analysis of extracts

Each sample followed the official method for Fatty Acids Methyl Esters (FAME) analysis, according to the International Olive Oil Council (IOOC – <http://www.internationaloliveoil.org>). An internal reference ( $\alpha$ -cholestane) solution in n-heptane was formed ( $500 \cdot 10^{-6}$ ). 100  $\mu\text{l}$  of this solution were

placed in a test tube about accurately. The solvent was removed via a gentle stream of N<sub>2</sub> and 0.1g of the extract was added to the tube (all extracts were diluted in n-heptane prior to addition, to protect the chromatograph from high concentrations of fatty acids). 2 ml n-heptane were consequently added and the tube was lightly shaken. After that 0.2 ml of 2 mol l<sup>-1</sup> methanolic potassium hydroxide solution were added and the tube was vigorously shaken for 30 s. Leaving some time to stratify, the upper layer, which contained the unsaponifiable material (FAMES and squalene), was removed. This material was analysed via GC-FID.

A Shimadzu GC-14A gas chromatograph was used, with a DB-1 (J & W scientific) fused silica capillary column (30 m x 0.32 mm i.d., 0.25  $\mu\text{m}$ ). The temperature program for the oven was: initial temperature  $T_i = 150^\circ\text{C}$  held for 2 min, then increased by 20  $^\circ\text{C min}^{-1}$  to 250  $^\circ\text{C}$ , held for 30 min, and finally increased by 20  $^\circ\text{C min}^{-1}$  to a final 300  $^\circ\text{C}$  for 20 min. The detector's temperature was held all along at 290  $^\circ\text{C}$ , same as the injector's temperature. The gas used was helium (He) of GC-quality at a volume flow rate of 1 ml min<sup>-1</sup>. The gas flow split/splitless valve was opened 18 s after the injection, at a 60:1 ratio.

### Statistical analysis

The analysis of the experimental data was managed with the MINITAB for Windows statistical program (MINITAB Inc.). The concentration figures and surface plots were designed with Origin 7 (OriginLab Corporation, One Roundhouse Plaza Northampton, MA 01060).

### Results and discussion

The experiments gave two sets of results, one being the amount of extract for the experimental conditions, and the other the concentration of squalene in each extract. Two sets of conclusions can be obtained from each result, one being the best set of conditions for an extract to have the highest squalene concentration possible, and the other the best set of conditions for the extraction to offer the highest amount of squalene.

Squalene measurement with the analytical procedure (GC-FID) was done in triplicate. Due to the requirement of the Box-Behnken design, to undergo the middle experiment ( $p = 125$  bar,  $T = 38^\circ\text{C}$ ,  $Q_V = 2$  l min<sup>-1</sup>) at least three times, it's possible to calculate the variance of the extraction experiment. Results can be seen in Table 2.

Table 2 – Mass fraction ( $10^{-6}$ ) of squalene for experimental extracts (measured with GC-FID) and average fraction

Expt	Squalene mass fraction			average
	$w_{sq,1}$	$w_{sq,2}$	$w_{sq,3}$	$w_{sq,a}$
01	1.004	1.248		1.126
02	419	605	573	532
03	6.251	6.724	6.307	6.427
04	6.955	8.014	8.606	7.859
05	2.634	2.684	3.781	3.033
06	9.448	6.072	3.148	6.223
07	9.351	11.663	7.126	9.380
08	10.134	9.164		9.649
09	16.849	19.100	20.657	18.869
10	13.828	12.735	20.061	15.541
11	27.107	28.750	13.105	22.987
12	10.989	11.447	14.635	12.357
13	17.628	19.123	19.226	18.659
14	15.994	21.772	23.589	20.452
15	20.226	24.710	17.233	20.723

Table 3 – Yield (mg per kg of dry sample) of squalene for experimental extracts (measured with GC-FID) and average yield

Expt	squalene yield			average
	$Y_{sq,1}$	$Y_{sq,2}$	$Y_{sq,3}$	$Y_{sq,a}$
01	6.2	7.7		6.9
02	0.1	0.1	0.1	0.1
03	221.2	238.0	223.2	227.4
04	185.0	213.2	228.9	209.0
05	0.3	0.3	0.4	0.3
06	0.2	0.1	0.1	0.1
07	130.3	162.5	99.3	130.7
08	359.8	325.3		342.5
09	92.7	105.1	113.7	103.8
10	318.4	293.3	462.0	357.7
11	62.7	66.5	30.3	53.0
12	141.9	147.8	189.0	158.8
13	233.0	252.7	254.1	246.5
14	160.3	218.2	236.4	205.2
15	185.8	227.0	158.3	190.4

In Table 3 the results focus on the yield of squalene from the initial dry pomace. These results are shown graphically in figures 2 and 3.

With the use of the MINITAB 13 statistical program a second rate regression analysis was performed. The equations that connect concentration and yield to the various quantities can be seen right below:

$$w_{sq}(p, T, Q_m) = 19,945 + 2,799 \cdot p + 220 \cdot T - 1,312 \cdot Q_m - 13,164 \cdot p^2 - 2,797 \cdot T^2 + 291 \cdot Q_m^2 + 504 \cdot P \cdot T - 730 \cdot P \cdot Q_m - 1,826 \cdot T \cdot Q_m \quad (1)$$

$$Y_{sq}(p, T, Q_m) = 214 + 113 \cdot p - 34,4 \cdot T + 71,4 \cdot Q_m - 76,6 \cdot p^2 - 26,6 \cdot T^2 - 19,1 \cdot Q_m^2 - 2,9 \cdot pT + 53,0 \cdot P \cdot Q_m - 37,0 \cdot T \cdot Q_m \quad (2)$$

$p$ ,  $T$  and  $Q_m$  is pressure in MPa, temperature in °C, mass flow rate in  $\text{g min}^{-1}$  and  $w_{sq}$  and  $Y_{sq}$  are squalene mass fraction in  $10^{-6}$  and squalene yield in

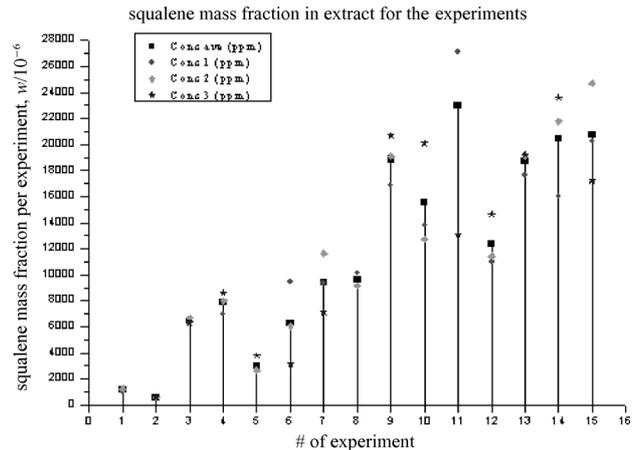


Fig. 2 – Mass fraction ( $10^{-6}$ ) of squalene in the extracts measured by GC-FID in triplicate. The squares show the average mass fraction

mg of squalene per g of dry pomace, respectively. The quantities are expressed in reduced values (i.e., for  $p$ ,  $-1$  is 7.5 MPa,  $0$  is 12.5 MPa and  $+1$  is 17.5 MPa). These equations allow the estimation of

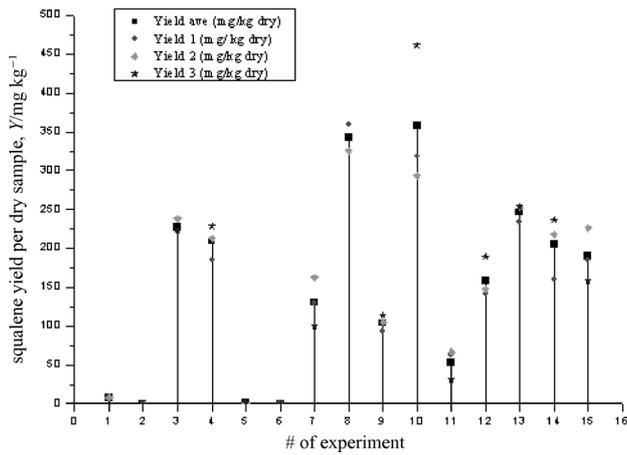


Fig. 3 – Overall yield of squalene (mg/m of initial dry sample) from the sample calculated from the concentration of squalene in the extracts, in triplicate. The squares show the average yield

squalene fraction and yield for extractions with conditions between the ones used for the design of this series of experiments (e.g. 13.5 MPa pressure, 41 °C temperature and 4.9 g min<sup>-1</sup> solvent flow rate).

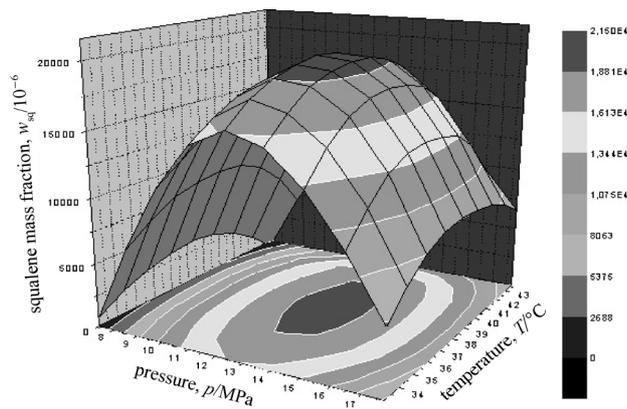


Fig. 4 – 3D and Contour Plot of the mass fraction of squalene in the extract for different pressures and temperatures

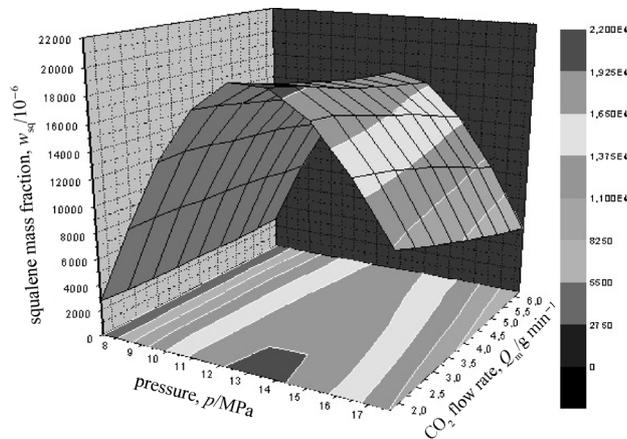


Fig. 5 – 3D and Contour Plot of the mass fraction of squalene in the extract for different pressures and flow rates

Figures 4 to 7 are surface response plots, used in literature<sup>3,30</sup> to help show the best area of conditions, for an extract to have a high squalene concentration: pressure ca. 13.0 MPa, temperature between 38 and 43 °C, and CO<sub>2</sub> flow rate at 1.8 g min<sup>-1</sup>. On the other hand, in order to have as much squalene as possible at the product, greater total amount of extract is preferred. So the best conditions were: pressure 17.5 MPa, temperature 43 °C, and CO<sub>2</sub> mass flow rate at 5.4 g min<sup>-1</sup>. Since the series of experiments was designed in order to find the highest fraction of squalene at the extract possible via SC-CO<sub>2</sub> extraction, a definite answer on the best conditions for yield cannot be offered. It's evident that the limits of the experiment should be different for higher yield experimentation.

A study of the above equations will show that the parameters with the highest absolute value multipliers are the most significant. In equation 1 for example it can be easily seen that the most important parameters are pressure and flow, but also pressure squared, temperature squared and the combined effect of temperature with flow. Similar con-

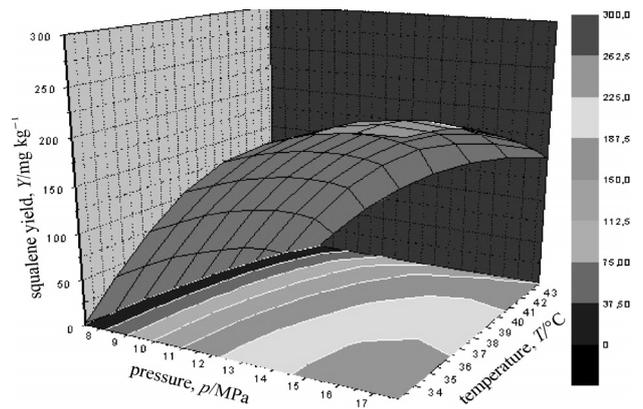


Fig. 6 – 3D and Contour Plot of the overall squalene yield from the sample for different pressures and temperatures

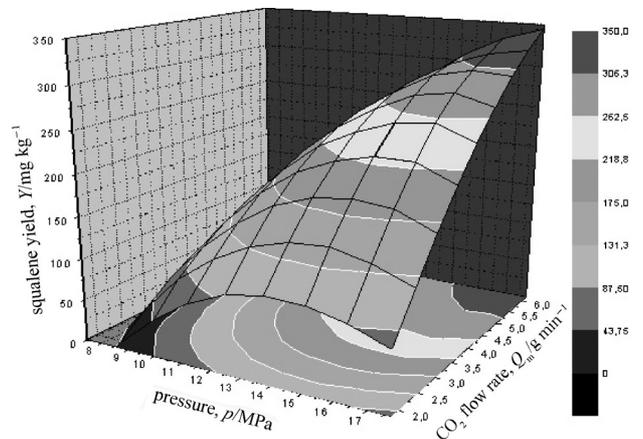


Fig. 7 – 3D and Contour Plot of the overall squalene yield from the sample for different pressures and flow rates

clusions can be drawn for equation 2. Another conclusion can be drawn from the fact that, although, concentration is related positively to pressure, it's also related negatively to pressure squared. Since it isn't obvious whether an increase in pressure would have either a positive or a negative effect on squalene mass fraction, the need for response surface plots was presented. These plots are constructed from the empirical models shown above. In the plot, the surface response is the connection of the values in the Z axis (values of response), corresponding to each pair of values in the X and Y axes<sup>3</sup>. The third parameter is considered fixed at the central value of its experimental range (i.e. for figure 4 pressure is X, temperature is Y, concentration is Z and CO<sub>2</sub> flow is considered constant at 3.6 g min<sup>-1</sup>).

If the amount of CO<sub>2</sub> that was used in the extraction is taken into consideration a new set of results can be seen in Table 4 where  $p$  and  $T$  are the experimental conditions in bar and °C, respectively,  $m_{\text{CO}_2}$  is the total amount of CO<sub>2</sub> that was used during the 8 h limit of the extraction, in g, reduced  $m_{\text{CO}_2}$  is the amount of CO<sub>2</sub> used, divided by the amount of the initial sample mass (g g<sup>-1</sup>),  $m_{\text{SQUALENE}}$  is the total amount of squalene extracted in that experiment from the corresponding initial sample mass (mg). Oil index is the amount of oil (extract) that was produced divided by the reduced amount of CO<sub>2</sub> used and squalene index is the same for squalene. Experiments 13, 14, and 15 were con-

ducted at the same conditions, so their mean value was calculated instead.

As the pressure increases and the temperature stays near the critical region, CO<sub>2</sub> density increases. However, oil solubility,  $y_r$ , in supercritical CO<sub>2</sub> follows with good accuracy the *Sovova et al.* equation<sup>30</sup>

$$y_r = \frac{(0.001 \cdot \rho_f)^{10.724} \cdot \left( e^{40.361 - \frac{18708}{T} + \frac{2186840}{T^2}} \right) \pm 2.7}{\rho_f}$$

where  $\rho_f$  is CO<sub>2</sub> density and  $T$  the temperature. From the above equation it can be shown (Figure 8) that oil solubility in CO<sub>2</sub> is inversely proportional to temperature at low pressures, but changes proportionally to temperature in pressures higher than ca. 29 MPa. These experiments were performed with highest pressure of 17.5 MPa, so this phenomenon was not met. However, in the range that they were conducted, the above equation is shown to be a good first estimate of conditions which would provide the most extract. Indeed, the highest CO<sub>2</sub> density experiment (04) provided the highest yield (3 %), amongst the ones with the same solvent flow rate.

Squalene in supercritical carbon dioxide has been shown to follow the Chrastil equation.<sup>21</sup> It shows (Figure 9) an increase in squalene solubility in supercritical CO<sub>2</sub> with an increase in solvent density (or pressure for fixed temperatures). The same can be seen in this series of experiments. Squalene

Table 4 – Extracted squalene by reduced CO<sub>2</sub> mass per unit sample mass (g g<sup>-1</sup>) – Extraction Index

Expt	$p$ /bar	$T$ /°C	$m_{\text{CO}_2}$ /g	reduced CO <sub>2</sub> , $\zeta_{\text{CO}_2}$ /g g <sup>-1</sup>	$m_{\text{sq}}$ /mg	Oil index	squalene index
1	7.5	33	2,322	15.46	1.04	0.034	0.067
2	7.5	43	1,336	8.90	0.01	0.002	0.002
3	17.5	33	2,257	15.04	34.14	0.200	2.270
4	17.5	43	2,407	16.05	31.35	0.141	1.953
5	7.5	38	1,394	9.29	0.05	0.001	0.005
6	7.5	38	2,946	19.56	0.02	0.000	0.001
7	17.5	38	1,300	8.67	19.61	0.137	2.264
8	17.5	38	3,096	20.63	51.41	0.146	2.492
9	12.5	33	904	6.02	15.59	0.078	2.590
10	12.5	33	2,774	18.49	53.68	0.106	2.904
11	12.5	43	889	5.89	8.00	0.033	1.357
12	12.5	43	3,007	19.96	23.92	0.055	1.199
13 to 15	12.5	38	1,894	12.61	32.15	0.074	2.577

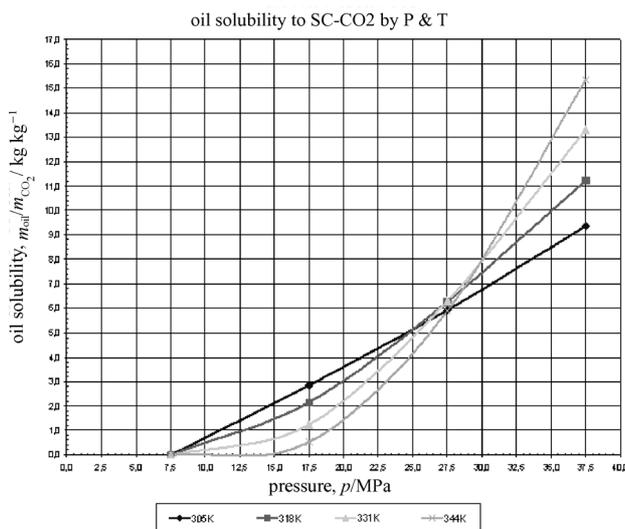


Fig. 8 – Overall oil solubility in supercritical  $\text{CO}_2$  ( $\zeta_{\text{oil/solvent}}$ ), for different pressures and temperatures, according to the Sovova et al. equation<sup>29</sup>

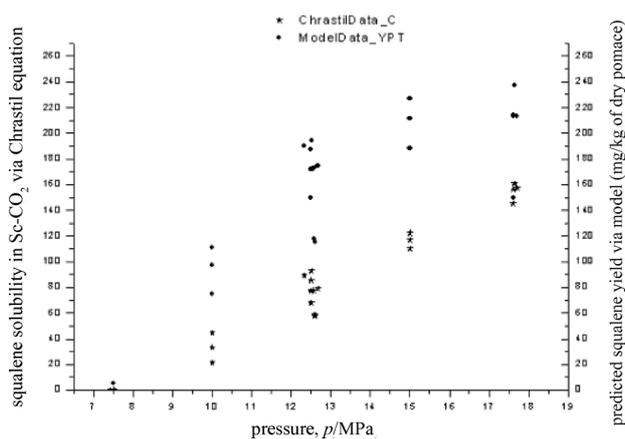


Fig. 9 – Squalene solubility as predicted via the Chrastil equation<sup>21</sup> and experimental squalene yield. The trend is increase with increase of pressure (hence density) in both cases

yield increases as the pressure increases, since more squalene was removed from the pomace and dissolved in SC- $\text{CO}_2$  as the pressure increased for fixed temperatures. However, squalene concentration in the extract didn't increase in the same manner, as with the increase in SC- $\text{CO}_2$  density the solubilities of other compounds found in the pomace also increased, faster than squalene's. In fact *Ruivo et al.*<sup>22</sup> have concluded that in the ternary system methyl oleate/squalene/supercritical  $\text{CO}_2$  this happens and pinpoints the optimum conditions for squalene enrichment at 110 bar and 313 K (40 °C). Their extraction was supercritical  $\text{CO}_2$  from a liquid model mix based on olive oil deodorizer distillate; ours was SC- $\text{CO}_2$  extraction from a solid phase (olive pomace). The results are very similar. The optimum conditions according to our model are ~130 bar and temperature between 36 and 40 °C.

Pressure has been shown to play a very important role in the efficiency of the extraction. The “optimum locus” is quite narrow on the pressure axis. This leads to the conclusion that a small change in pressure (~1 MPa) can have serious impact on squalene fraction (even as much as  $4.0 \cdot 10^{-3}$  depending on the area of pressures). Pressure is also very important in the overall recovery of squalene from pomace, albeit to a lesser degree than in the case of squalene enrichment in the extract.

Temperature doesn't seem to affect concentration much in the studied temperature range. Temperature could not exceed very much the 43 °C limit because of the very liability of the nutraceutical extract. The “optimum locus” is quite wide on the temperature axis. This is a direct consequence of the fact that, within the investigated limits, a temperature change of 10 °C may affect  $\text{CO}_2$  density as little as 7 % near the higher pressures. In contrast, pressure effect on density, alters density as much as 72 % (for a change of 100 bar). So for high squalene concentration at the extract temperature may be between 36 and 40 °C. On the other hand, temperature plays a much more important role in the recovery of squalene, pinpointing the preferred temperature at 33 °C. As already mentioned this preference for low temperatures is true for pressures lower than 29 MPa.

$\text{CO}_2$  flow rate is essentially equivalent to the time interval the  $\text{CO}_2$  is allowed to interact with the solid pomace matrix. This is a most important factor and depicts plainly that the SF extractor cannot have, both, high squalene concentration and high recovery: low  $\text{CO}_2$  flow rates ( $1.8 \text{ g min}^{-1}$  lowest) produce an extract rich in the sought after compound, whereas high  $\text{CO}_2$  flow rates ( $5.4 \text{ g min}^{-1}$ ) produce more of it, although less pure. It is also shown that flow is a delicate condition, allowing for great changes of, both, concentration and recovery with small changes in flow. In this set of experiments the flow boundaries were set according to the experimental possibilities and with economic scale-up in mind. Other experiments may overcome these barriers and produce a wider investigation of  $\text{CO}_2$  flow.

## Conclusions

The aim for this series of experiments was to determine the optimum conditions for the extraction of pomace oil with supercritical  $\text{CO}_2$ , with the maximum enrichment of squalene in the extract possible in mind. The conditions that were optimized during extraction were pressure, temperature and SC- $\text{CO}_2$  flow. The optimum conditions were found

to be at 1.3 MPa, 36–40 °C and 1.8 g min<sup>-1</sup>, respectively. With these extractions it was shown that squalene can reach concentrations as high as 23.000 10<sup>-6</sup> in the extract, about 10 times the concentration of squalene in a hexane extract of the same pomace. This enrichment however was accomplished with the simultaneous drop in overall extracted squalene quantities. The optimum conditions for total squalene extraction were at 17.5 MPa, 33 °C and 5.4 g min<sup>-1</sup> for this set of extractions.

With the contour plot analysis, besides locating the optimum experimental conditions, the graphical representation allowed for the extraction of conclusions on the effect the conditions have to the final result, as well as a guideline for future experimentation. The application of Sovova's solubility equation for vegetable oils in supercritical CO<sub>2</sub> was verified. The application of Chrastil's equation for squalene in supercritical CO<sub>2</sub> was also verified. Finally, it became evident that the mass of solvent used in supercritical extraction is not as important a factor in extraction yield as the experimental conditions. More conclusions could be extracted on these facts if more experiments were performed, instead of only the ones necessary by the statistical approach. Extraction yield was however a secondary objective, whereas squalene enrichment was the primary and the experimental procedure was adequate for it.

The results lead to the conclusion that the next step in optimizing SC-CO<sub>2</sub> extraction of squalene from pomace is the mass transfer study of the process where quantities like extraction time and pomace granulometry will also show up. Also, the conditions for the new series of experiments should be wider. Since lower pressures and temperatures would lead to subcritical CO<sub>2</sub>, higher pressures and temperatures should be included in the experimental design.

#### List of symbols

$m$	– mass, g
$p$	– pressure, bar MPa
$Q_m$	– mass flow rate, g min <sup>-1</sup>
$Q_v$	– volume flow rate, ml min <sup>-1</sup>
$T$	– temperature, °C
$w$	– mass fraction
$Y$	– yield
$\gamma$	– mass concentration
$\zeta$	– mass ratio
$\rho$	– density, kg m <sup>-3</sup>

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