Introduction

The black cumin (Nigella Sativa Linn.), also named as Roman corian-
der, kalonji or nigella, orginate from south and southwest Asia and has
been cultivated in the tropics, subtropics and temperate regions like cen-
tral Europe on a large scale. The seeds of black cumin have length about
0.3 cm each, and have black colour. The seeds are used for their strong
smell and bitter tasting flavour in sweets, alcoholic beverages and as
whole seeds on bread. [Paarakh, 2010]. In addition, Nigella fixed oil
and its volatile oil are used in the pharmacy and cosmetics industry.
Depending on the way and the region of cultivation, Nigella seeds contain
more than hundred different volatile components. Primary component of
volatile fraction is thymoquinone [Khan, 1999]. Thymoquinone is a
biologically active component, and is used for the treatment of many
different illnesses, such as diarrhea, bronchial asthma and as a cancer
therapy [Khan, 1999; Wajs et al., 2008]. Despite the extensive studies,
there are still many components in black cumin seed that have not been
identified.

The edible oil is conventionally extracted by the mechanical cold-
pressing process or solvent extraction. The properties of oil extracted
by the mechanical press process are better, since oil is not contaminated
with any chemical, but the yield of this process is low [Brunner,1994].
The output and extraction rate are higher using solvent extraction but in
conventional solvent extraction methods, solvent is mixed with oil and
during post processing, a lot of valuable volatile components are easily
lost. Supercritical carbon dioxide (SC-CO\textsubscript{2}) extraction of the edible oil
has attracted high attention as a sustainable alternative to conventional
solvent extraction and cold-pressing process. The main reason to use
that technology is that SC-CO\textsubscript{2} not only has a higher extraction rate
but also is a non-toxic, non-explosive, non-flammable, readily available
solvent, which is easy to remove from the extracted materials. The ex-
tact quality can also be controlled, and storage capability of extract can
be extended [Weinhold et al., 2008].

Some authors have done supercritical fluid extraction of Nigella se-
eds at the conditions of 150-200 bars and 308, 318 K with gas flow
rate range of 0.6-1.2·10^{-6} m\textsuperscript{3}/s [Fullana et al., 1999; Wawrzyniak et al.,
2003].

Results of batch supercritical carbon dioxide extraction of black cu-
min seeds are presented. The influence of extraction conditions on yield
and volatile oil composition of black cumin seeds is discussed.

Materials and methods

Materials

Black cumin seeds were supplied by Sasa, Germany, and were used
without further purification. Pure CO\textsubscript{2} (99.9\%) was purchased from Air-
Liquid delivered at pressure up to 60 bar.

Batch Extraction

The solid-liquid batch extractor BRA 098/78/2002, TUHH Germany
[Zetzl et al. 2003; Parisotto et al., 2011] was used to obtain black cumin
extract. Extraction procedure was previously described in literature
[Michelin et al. 2009]. Extractor had 0.032 m long and 100 mL ca-
nomer for flow control. The extracting conditions were carried out
at temperature of 45°C and pressure of 200, 250 and 350 bar; at tem-
perature of 50°C and pressure of 300, 450 and 500 bar, at flow rates of
0.8 (±0.03) kg CO\textsubscript{2}/h.

Experimental conditions are presented at the Table 1.

<table>
<thead>
<tr>
<th>Nr</th>
<th>m</th>
<th>(d_{\text{fixed}}) g/cm\textsuperscript{3}</th>
<th>P bar</th>
<th>T °C</th>
<th>(m_{\text{fixed}}) g</th>
<th>(m_{\text{volatile}}) g</th>
<th>Errors (Lost) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>27.09</td>
<td>0.469</td>
<td>200</td>
<td>45</td>
<td>23.60</td>
<td>6.44</td>
<td>20.06%</td>
</tr>
<tr>
<td>2</td>
<td>27.06</td>
<td>0.468</td>
<td>250</td>
<td>45</td>
<td>23.00</td>
<td>6.44</td>
<td>18.23%</td>
</tr>
<tr>
<td>3</td>
<td>27.33</td>
<td>0.469</td>
<td>350</td>
<td>45</td>
<td>23.32</td>
<td>6.36</td>
<td>16.21%</td>
</tr>
<tr>
<td>4</td>
<td>50.07</td>
<td>0.469</td>
<td>300</td>
<td>55</td>
<td>42.77</td>
<td>6.44</td>
<td>11.78%</td>
</tr>
<tr>
<td>5</td>
<td>49.80</td>
<td>0.437</td>
<td>450</td>
<td>55</td>
<td>45.80</td>
<td>6.38</td>
<td>3.08%</td>
</tr>
<tr>
<td>6</td>
<td>47.90</td>
<td>0.437</td>
<td>500</td>
<td>55</td>
<td>40.50</td>
<td>7.13</td>
<td>3.65%</td>
</tr>
</tbody>
</table>

where: m, g – mass of the raw dry material, \(d_{\text{fixed}}\) g/cm\textsuperscript{3} – bulk density of seeds, P, bar – pressure, T, °C – temperature, \(m_{\text{volatile}}\), g – mass of material after extraction, \(m_{\text{fixed}}\), g – the mass of the extract, Errors (Lost) – lost during process.

Weinhold et al., 2008]

The extractor was filled of 20 g/50 g grounded seeds, with bulk densi-
ty of 0.437±0.469 g/cm\textsuperscript{3}. Relative humidity of the seeds did not exceed
5±6.8%. Crushed seeds formed the
finite time intervals. At each 5±10 minutes of extraction, the flask was
changed by an empty one.

Analytical Methods

Headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography-mass spectrometry (GC-MS) were performed to
analyze volatile fraction of extract.

The SPME fibers (65 µm Stableflex DVB/CAR/PDMS) and the holder
were obtained from Supelco Ltd., (Bellefonte, PA, USA). The fi-
ers were first conditioned according to the manufacturer’s instructions.
For each extraction, 0.5 g of extract sample was immediately placed in
a glass vial with a silicone septum coated with a Teflon film. The sample
was exposed to the headspace in the vial to absorb the analytes. After
30 min exposure time, the fiber was retracted into the needle and intro-
duced into the GC injector for desorption and analysis of the volatiles.
Three SPME analyses were performed in parallel for each oily sample.

The released volatiles were analyzed by Gas Chromatographic analy-
sis GC and GC/MS. The capillary column with FID detector at Varian
3400 model gas chromatograph were used. The relative composition of
each SPME sample was calculated from the GC peak area using cor-
rection factors, retention time of volatile components were taken from
literature [Wajs et al., 2008].

Results and discussion

Volatile contents

Each experiment was performed in triplicate and analyzed with
HS-SPME sampling method, followed by GC/MS. The compo-
sition of the volatile fraction obtained from the seeds during all
runs of experiments contained around 18 different volatile compo-
nents. Extract volatile oil contents of 5 important compounds are
presented on Fig. 1, where the highest amount of thymoquinone,
\(\alpha\)-cymene, \(\alpha\)-pinene, \(\alpha\)-thujene and limonene can be observed.

In previous works, around 9 volatile components of Nigella extracts
SC-CO\textsubscript{2}, for pressure ranges of 100-250 bar and 14-37°C were identified
using Polish seeds [Wawrzyniak et al., 2003]. Using the seeds from India
Venkatachaliam et al. [2010] found around 32 volatile components at
the SC-CO₂ conditions of 280 bar/50°C and 21 volatile components at the 120 bar/40°C; conventional hydrodistillation gave more than 50% less compounds in the volatile fraction. That high gap can be explained by climate, ground and cultivation differences of Nigella seeds. For the Central European seeds, we obtained the highest value of volatiles.

**SFE kinetics**

Besides the confirmation of the high amount of volatiles in the supercritical extract from black cumin seeds, described above, the technical viability of the SFE process is important to the evaluation of economical viability. Therefore, the kinetics study was performed in order to define the region of the extraction where the highest yields were obtained. The global yield (Yield) was calculated by the ratio between extract and feed mass (1),

\[
\text{Yield} = \frac{m_{\text{ext}}}{m_{\text{fl}}} = 100 \frac{m_{\text{ext}}}{m}
\]

where: \( m_{\text{ext}} \) is the mass of the extract [g], and \( m \) – the mass of the raw dry material [g].

The overall extraction curves were obtained at 45°C and at 200, 250, 350; and 55°C and 300, 400 and 500 bar, and solvent flow rate of 8.30 and 13.30 g/min and the results are presented in Fig. 2.

![Fig. 2. Accumulated Yield of Extract, Batch SFE Pressure, Temperature SFE curves for black cumin at different conditions of pressure and temperature](image)

We observed the extraction curves, presented a similar behavior, with a constant extraction rate period (CER), followed by a decreasing extraction rate period and ended by a diffusional period, typical for different seeds [Brunner, 1994; Ferreira et al., 2002]. Additionally, the increase in flow rate \( Q_{\text{CO₂}} \) (from 0.5 to 0.8 kg/h) enhanced the process yield, probably due to the enlargement in the concentration driving force between solvent and substrate phase, as also discussed by several authors [Wawrzyniak et al., 2003, Ferreira et al., 2002]. The overall extraction curves, extract mass versus extraction time, were performed for the experimental conditions studied in order to evaluate the process yield and the mass transfer behavior.

In Table 2 are presented determined parameters for the extraction curves.

<table>
<thead>
<tr>
<th>Nr exp</th>
<th>( P/\text{CO}_2 ) bar (g/min)</th>
<th>( t_{\text{CER}} ) min</th>
<th>( M_{\text{CER}} ) g/min</th>
<th>Yield</th>
<th>( \rho_{\text{CO}_2} ) g/cm³</th>
<th>( D ) m²/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>200 8.30</td>
<td>90.2</td>
<td>0.033</td>
<td>10.3</td>
<td>0.814</td>
<td>1.552·10⁻¹²</td>
</tr>
<tr>
<td>2</td>
<td>250 8.30</td>
<td>75.0</td>
<td>0.039</td>
<td>12.3</td>
<td>0.830</td>
<td>4.038·10⁻¹²</td>
</tr>
<tr>
<td>3</td>
<td>350 13.30</td>
<td>67.3</td>
<td>0.046</td>
<td>12.4</td>
<td>0.858</td>
<td>1.889·10⁻¹²</td>
</tr>
<tr>
<td>4</td>
<td>300 13.30</td>
<td>89.0</td>
<td>0.038</td>
<td>12.9</td>
<td>0.723</td>
<td>1.245·10⁻¹²</td>
</tr>
<tr>
<td>5</td>
<td>450 8.30</td>
<td>64.0</td>
<td>0.092</td>
<td>7.8</td>
<td>0.850</td>
<td>2.922·10⁻¹²</td>
</tr>
<tr>
<td>6</td>
<td>500 13.30</td>
<td>73.8</td>
<td>0.096</td>
<td>14.9</td>
<td>0.948</td>
<td>9.580·10⁻¹⁴</td>
</tr>
</tbody>
</table>

Where: \( P/\text{CO}_2 \) – operation pressure and flow rate of CO₂ [bar/g(min)]; \( t_{\text{CER}} \) – time of the CER [min]; \( M_{\text{CER}} \) – mass extraction rate at CER[g/min]; Yield – global yield [w/w%]; \( \rho_{\text{CO}_2} \) – density of CO₂ [g/cm³] and \( D \) – the diffusivity [m²/s].

The time (\( t_{\text{CER}} \)) and mass extraction rate (\( M_{\text{CER}} \)) of CER (constant extraction period) were calculated by the tool for simulation [BATCHSFETUHH, 2012]. The diffusivity \( D_{\text{CER}} \) was determined by Simple Single Plate (SSP) model [Gaspar et al., 2003], where \( D_{\text{CER}} = f(t_{\text{CER}},M_{\text{CER}}) \). Modified SSP model is neglecting dispersion, were assumed a constant substrate flow with permanent mass transfer to the pore surface; substrate is easy accessible to solvent during CER section; substrate concentration at surface is equivalent to saturation concentration (solubility) [Gaspar et al., 2003].

The kinetics study of the SFE of black cumin showed that the operating parameters such as temperature, pressure and solvent flow rate, did affect the mass transfer and the process yield and must be carefully determined combining the quality aspects of the product (extract) and the process efficiency.

**Conclusions**

This study has clearly brought out the possibilities to obtain higher percentage of valuable volatiles such as thymoquinone and \( \rho \)-cymene through high-pressure SC-CO₂ technology. The sustainable conditions of 300 bar and 45°C allowed us to obtain the highest amount of volatile oil yield with maximum volatile content.

REFERENCES


Parisotto E, Michielin E, Biscaro F, Ferreira S, Filho D, Pedrosa R., 2012. The antimicrobial activity of extracts of Cordia verbeneaceae obtained by supercritical fluid extraction. J. Supercritical Fluids, 61, 101-107, DOI: 10.1016/j.supflu.2011.08.016


DOI: 10.1016/j.supflu.2011.08.016