Abstract The oil of *Amaranthus cruentus* has been characterized by major and minor compounds. The series of fatty acids, triglycerides, sterols, methylsterols, terpenic and aliphatic alcohols, tocopherols, and hydrocarbons have been identified, by standards and mass spectrometry, and quantified by HRGC and HPLC. The content of these chemical compounds, together with the equivalent carbon numbers (ECN) and triglyceride carbon numbers (TCN), have been compared with the results of other edible vegetable and cereal oils. Composition of hydrocarbons is remarkable; mostly the high content of squalene (4.16 g/kg of seed) as well as concentration of *n*-alk-3-enes (C23:1–C33:1) that reaches 332 ppm, while the concentration of *n*-alkanes (C23–C33) is only 155 ppm. The high concentration of β-tocopherol (546 ppm) and the profile of fatty acids show that amaranth oil is not protected against rapid oxidation while most of the sterols are esterified. Pharmaceutical and industrial applications of the most unique chemical compounds are also given.

Keywords Amaranth oil · Triglycerides · Fatty acids · Hydrocarbons · Sterols

Introduction

The major crop among cereals is wheat that, even being the most consumed, the nutritionists recognize is deficient in essential amino acid as lysine, and has a low fat content. On the other hand, the U.S. National Foundation had identified the pseudo-cereal amaranth as one of six plants as early as the 1980s. The amaranth’s seeds and its leaves are rich in minerals [1], lysine, sulfur-containing amino acids, essential fatty acids [2], and vitamins [3]. The amaranth’s flour is used to make sweetmeat [4], breakfast cereals [5], biscuits [6], and pasta [7] among other things. Concerning the seed fatty acid profile, previous studies were mostly focused on the determination of fatty acids [8] or total content of squalene [9, 10]. Scarce studies were devoted to vitamin E – tocopherols and tocotrienols – [11] and C vitamin [12] contents while other series of chemical compounds, with a great impact in industrial and nutritional aspects, were not studied at all. This paper deals with a comprehensive study of this seed fatty profile series. Major series such as triglycerides and fatty acids have been studied together with waxes, sterols, methyl-sterols, terpenic alcohols, tocopherols, and hydrocarbons. The objective of the paper is also to analyze the potential cosmetic and pharmaceutical applications of this pseudo-cereal in the light of the qualitative and quantitative composition of its saponifiable and unsaponifiable matter.

Materials and methods

*Amaranth*. The variety *Amaranthus cruentus L.*, harvested in Austria in 1998, was used in the study of saponifiable and unsaponifiable fractions of the extracted amaranth oil. This variety is one of the most harvested inside the European Communities. Amaranth seeds were ground for 30 s in a micro mill and oil was extracted with hexane in a Soxhlet apparatus for 7 h [13].

Fatty acids. Methyl esters of fatty acids (FAME) were analyzed by high-resolution gas-liquid-chromatography (HRGC). FAME was extracted with heptane after a cold methylation with KOH (2 N) in methanol [14]. HRGC was performed with an HP-5890-II (Hewlett-Packard, Palo Alto, CA) using a fused silica capillary SP-2380 column (60 m×0.25 mm i.d., 0.2 µm film thickness). The oven temperature was held at 160 °C for 13 min, and then it was raised to 190 °C at a rate of 1.5 °C/min and held isothermally for 20 min. The injector temperature was held at 225 °C, while 250 °C was the temperature for the detector. Hydrogen (19 psi inlet pressure) was used as carrier gas while the make-up gas was nitrogen.

The determination of fatty acids in the 2-position of the triglyceride was carried out following the described methodology with a previous enzymatic hydrolysis with lipase [15].

Triglycerides. These compounds were quantified by high performance liquid chromatography (HPLC) [16]. The instrument is equipped with a low pressure quaternary pump HP-1050, a reodyne valve with a 20-µl loop, a thermostatic system of columns, and a refraction index detector HP-1074. The LiChrospher 100 RP-18 column (250×4 mm i.d., 4 µm) (Merck, Darmstadt, Germany) was
used with an acetonitrile 1:1 acetone mobile phase at 1.15 ml/min isocratic elution flow. The temperature of the oven and detector was 40 °C.

Waxes. Waxes were quantified by HRGC [17] after oil fractionation by means of a silica gel column chromatography (silica gel 60, particles size 0.063–0.2 mm) (Merck, Darmstadt, Germany). An HP-5890-II equipped with a cold on-column injector with oven track system and a flame ionization detector was used. The column was a TRB-5 (15 mm×0.32 mm i.d. 0.1 µm film thickness) (Tracer, Barcelona, Spain). The initial temperature was 70 °C which was raised to 180 °C at 45 °C/min, then raised at a rate of 5 °C/min up to 310 °C, where it was held isothermally for 7 min. Hydrogen was the carrier gas while nitrogen was the make-up gas. The detector temperature was 350 °C and the internal standard was lauryl arachidate (Sigma, St. Louis, MO).

Squalene. This hydrocarbon was isolated with the waxes by a modified process of the standard analysis of waxes [18]. A low polarity solvent (98.5 hexane: 1.5 diethyl ether) was used in a silica gel column to separate squalene from esters of aliphatic alcohols and sterol esters [19]. Squalene (Sigma, St. Louis, MO), with response factor 0.963, was the internal standard.

Unsaponifiable matters. The method for determining the unsaponifiable matter involves saponification, isolation, and purification. After saponification, the unsaponifiable matter was isolated by an extraction method but using two different solvents: (i) diethyl ether for the isolation of sterols and alcohols (aliphatic and terpenic) and (ii) petroleum ether for the isolation of hydrocarbons [19]. Sterol and alcohol compounds were purified by thin-layer chromatography [20] while hydrocarbons were purified by column chromatography [21]. All these series of compounds were quantified by HRGC excepting tocopherols which were analyzed by HPLC. The oven temperature programming varied, as described below, from one series to another.

Alcohols (aliphatic and terpenic). The initial oven temperature was 220 °C which was then raised to 280 °C at a rate of 3 °C/min and held isothermally for 23.7 min. The injector temperature was held at 280 °C and the detector at 300 °C. The internal standard was arachidic alcohol (Sigma, St. Louis, MO).

Sterols. The oven temperature was isothermally held at 265 °C for 30 min. The injector temperature was 280 °C while the detector was held at 300 °C. The internal standard was α-cholestanol (Fluka, Buchs, Switzerland).

Hydrocarbons. The initial oven temperature was 110 °C which was isothermally held for 4 min, and then raised to 310 °C at a rate of 4.0 °C/min where it was held isothermally for 11 min. The injector temperature was 280 °C while the detector was held at 320 °C. The internal standard was eneicosane (Fluka, Buchs, Switzerland).

Tocopherols. These compounds were quantified by an HPLC instrument composed of a low pressure quaternary pump HP-1050, a red- dyne valve with a 20-µl loop, a thermostatic system of columns, and a fluorescence detector RF-235 (Shimadzu, Kyoto, Japan). The column LiCrospher Si-60 (250×4 mm, 5 µm thickness) (Merck, Darmstadt, Germany) was used with n-hexanol 99:1 iso-propanol mobile phase at 1 ml/min isocratic elution flow. Quantification was carried out by a calibration system based on standards [22].

HRGC-mass spectrometry (MS). The MS system MAT 95S (Finnigan, Bremen, Germany) was used in combination with a GC HP-5890 series II equipped with DBS-5MS fused capillary column (30 m×0.25 mm i.d., 0.25 µm film thickness) (J&W Scientific, Folsom, CA). The oven temperature programming has already been described above for each series. Helium was the carrier gas, for all the analyses, at a flow rate of 1 ml/min. The end of the fused-silica column was inserted directly into the ion source block. Mass spectra in the chemical ionization mode (MS-CI) were recorded at 70 eV, the ion source temperature being 250 °C.

Mathematical analysis. The HP-ChemStation software, version 5.01, carried out the quantification of the chemical compounds. Statistica release 5.5 (Statsoft, Tulsa, OK) was used in the statistical analysis. Euclidean distance and Ward algorithms were used to compute the cluster analysis.

Results and discussion

The crude fat content of the amaranth seeds was 6.34% which is consistent with previous findings that reported a crude fat range of 4.9–8.1% [23]. Table 1 shows that amaranth oil contains considerable amounts of linoleic (38.2%) and oleic (33.3%) acids, about 20% palmitic and lower quantities of stearic (4%) and linolenic (1%). Compared with other amaranth species, this variety cultivated in Austria has a fatty acid composition similar to some *Amaranthus caudatus* described in the literature [11]. However, the profile of the main fatty acids (palmitic, stearic, oleic, linoleic, and linolenic) contains multivariate information, and hence we applied the multivariate statistical procedure of cluster analysis to determine the similarity among amaranth fatty acid profiles of a set of edible oils [11, 24]. Figure 1 shows the tree structure

![Fig. 1 Cluster analysis of the major fatty acids of edible oils](Image 312x167 to 538x316)

### Table 1 Relative percentages of fatty acids and fatty acids at the second position of triglycerides

<table>
<thead>
<tr>
<th></th>
<th>C12:0</th>
<th>C14:0</th>
<th>C16:0</th>
<th>C16:1</th>
<th>C17:0</th>
<th>C17:1</th>
<th>C18:0</th>
<th>C18:1 w9</th>
<th>C18:1 w7</th>
<th>C18:2</th>
<th>C18:3</th>
<th>C20:0</th>
<th>C20:1</th>
<th>C22:0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (%)</td>
<td>0.7</td>
<td>0.2</td>
<td>20.4</td>
<td>0.4</td>
<td>0.1</td>
<td>0.7</td>
<td>3.9</td>
<td>32.1</td>
<td>1.2</td>
<td>38.2</td>
<td>0.7</td>
<td>0.8</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>2-position (%)</td>
<td>0.1</td>
<td>1.1</td>
<td>0.2</td>
<td>0.1</td>
<td>0.3</td>
<td>35.9</td>
<td>0.2</td>
<td>61.3</td>
<td>0.9</td>
<td>4.0</td>
<td>0.4</td>
<td>3.9</td>
<td>0.3</td>
<td>0.3</td>
</tr>
</tbody>
</table>