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# A comparative study on chemical and lipid composition of amaranth seeds with different origin

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A comparative study on chemical and lipid composition of amaranth seeds with different origin (Indian and Turkish) was performed. The amount of glyceride oil in the seeds was 6.2 and 6.6%, respectively. The content of proteins, total carbohydrates, fibers and ash was 19.4 – 20.0%, 60.5 – 61.4%, 1.8 – 5.3% and 2.0 – 2.4%, respectively. Starch consisted of 53.5 – 56.5% of all carbohydrates and the amount of the available sugars was 2.2 – 2.6%. The moisture of the samples varied between 10.6 and 10.9%. The main components in triacylglycerols of seed oil from India were oleic (38.7%) and palmitic acid (38.4%), while in that from Turkey – palmitic (33.1%), oleic (31.7%) and linoleic acid (22.6%). Total content of unsaponifiable substances in the oils was found to be 8.5 – 9.3%. The amount of sterols in the oils was 1.3 – 2.6% and the main component was β-sitosterol (38.1 – 41.9%), followed by stigmasterol (24.9 – 26.1%) and $\Delta^5$-avenasterol (20.1 – 23.5%). Total tocopherol content was 1015 - 1060 mg/kg and the main components were β-tocopherol (54.2 – 55.5%), δ-tocopherol (26.1 – 26.3%), and α-tocopherol (13.6 – 14.6%). The total content of phospholipids in the oils was 3.4 – 3.5%. The major representative in the oil from seeds with Indian origin was phosphatidylserine (21.5%), while in that with Turkish origin all identified phospholipid classes were present in similar quantities (10.1 – 14.9%). Overall, significant differences were observed in the fatty acid and phospholipid composition of the oils from Indian and Turkish amaranth seeds, as well as in their content of macro- and microelements.

**Keywords**: amaranth seeds, chemical composition, lipid composition, biologically active components

## INTRODUCTION

Recently, many not sufficiently examined plants which are a source of valuable nutrients can successfully replace the already established plant species in the diverse diet. A similar plant is amaranth which belongs to the family Amaranthaceae. It is an annual plant that has some agronomic advantages, including rapid growth, ability to adapt to unfavorable growing conditions such as low-nutrient soils, sand, heat resistance and irradiation. Its tolerance to different conditions is of interest for its potential use as a nutritious cereal crop in many geographic areas [1].

Chemical composition of amaranth seeds has been the subject of a number of studies and it has been found that the content of the major chemical components varies in a great extent depending on the species, variety, climatic conditions and the way the plant has been grown. The moisture content of the seeds was found to be between 7.5 (in *A. caudatus*) and 11.4% (in *A. hypochondriacus*) [2 - 4]. This is one of the indicators for the shelf-life of food products and shows how long products can be stored without development of molding processes [5, 6]. Amaranth seeds have a high nutritional value that is even higher than those of some cereals. The protein content in them reaches 13 – 18% [3, 7-11], which is comparable to corn (12%), wheat (12 – 14%) and rice (7 – 10%) [9, 11]. The oil content of the seeds is relatively low (from 4.9 to 10.0%) [3, 10-12] with predominantly unsaturated fatty acids (61.0 – 87.3%), mainly oleic (20.2 – 32.9%) and linoleic acid (37.0 – 47.8%). The content of saturated fatty acids ranges from 20.1 to 30.9%, and palmitic (12.3 – 25.9%) and stearic acid (2.7 – 4.7%) are the main representatives [3, 13-16]. Linolenic, arachidic and behenic acids are found in smaller amounts (from 0.11 to 1.54%). The lipid fraction also contains a number of biologically active substances (tocopherols, sterols and phospholipids) that define this oil as a valuable source of useful compounds for the human body [9, 16].

Although some previous studies on chemical and lipid composition of amaranth seeds and oils were performed, there was not observed results about the influences of the origin of the seeds on their proximate composition. Therefore, the aim of the present research was to be carried out a comparative study on chemical and lipid composition of amaranth seeds with different origin (from India and Turkey), and to be established the differences in the composition of the seeds that were in result of their origin.

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## EXPERIMENTAL

### *Samples*

Amaranth seeds with Indian and Turkish origin were purchased from the local market. The seeds were stored at room temperature and after that were analyzed.

### *Chemical composition*

Protein was calculated from the nitrogen content by Kjeldahl method using factor 6.25 [17]. The carbohydrate content was calculated by the following formula: 100 – (weight in grams [protein + lipids + water + ash] in 100g of dry seeds) [18]. The soluble carbohydrates and the starch content were identified by using BS 7169:89 [19] and BS 13488:76 [20]. Crude fiber, ash content and moisture were determined according to AOAC (2016) [17].

### *Determination of macro- and microelements*

The method is based on decomposition of the samples at 180 – 200°C in a Milestone ETHOS labstation microwave oven with a high pressure HPR1000/10S segmented rotor in the presence of nitric acid and hydrogen peroxide. The element content is determined by atomic absorption spectroscopy. Multivariate standard solution with concentration of 1000 mg/dm$^3$ is used to prepare dilute working standard calibration solutions. The content of elements was calculated using standard curves of these solutions [21].

### *Isolation of glyceride oil and determination of oil content*

The oil was extracted from grounded seeds using hexane in a Soxhlet apparatus [22].

### *Fatty acid composition*

Fatty acid composition of triacylglycerols was determined by gas chromatography (GC) [23]. Fatty acid methyl esters (FAMEs) were prepared by pre-esterification of the triacylglycerols with sulfuric acid in methanol [24]. Determination of FAMEs was performed on HP 5890 gas chromatograph equipped with a 75 m × 0.18 mm × 25 μm (film thickness) capillary Supelco column and a flame ionization detector. The column temperature was programmed from 140ºC (hold 5 min), at 4ºC/min to 240ºC (hold 3 min); the injector and detector temperatures were set at 250ºC. Identification was performed by comparison of the retention times with those of a standard mixture of FAME (Supelco, USA 37 comp. FAME mix) subjected to GC under identical experimental conditions.

### *Determination of phospholipids*

Ground seeds were subjected to Folch extraction [25]. Individual phospholipid classes were isolated by two-dimensional thin-layer chromatography (TLC) [26]**.** Identification was performed by comparing the respective $R_f$ values with those of authentic standards. The quantification was carried out spectrophotometrically at 700 nm after scrapping the phospholipid spot and mineralization of the substance with a mixture of perchloric and sulphuric acid, 1:1 (v/v) [27].

### *Determination of sterols*

Unsaponifiables were determined after saponification of the glycerides oil and extraction with hexane [28]. Quantification of sterols was carried out spectrophotometrically (at 597 nm), after isolation of sterols from other unsaponifiable matter by TLC [29].

Sterol composition was determined on HP 5890 gas chromatograph equipped with 25 m × 0.25 mm DB – 5 capillary column and flame ionization detector. Temperature gradient from 90ºC (hold 3 min) up to 290ºC at a rate of change 15ºC/min and then up to 310ºC a rate of 4ºC/min (hold 10 min); detector temperature – 320ºC; injector temperature – 300ºC and carrier gas was hydrogen. Identification was confirmed by comparison of retention times with those of a standard mixture of sterols [30].

### *Determination of tocopherols*

Determination of tocopherols was carried out by high performance liquid chromatography on 250 mm × 4 mm Nucleosil Si 50-5 column and fluorescent detection at 295 nm excitement and 330 nm emission. The operating conditions were mobile phase of hexane: dioxane, 96:4 (v/v) and flow rate 1 mL/min [31].

## RESULTS AND DISCUSSION

The main chemical composition of amaranth seeds is presented in Table 1.

**Table 1.** Chemical composition of amaranth seeds with different origin

| Chemical composition, % | Amaranth (India) | Amaranth (Turkey) |
|---|---|---|
| Oil content | 6.2±0.2 | 6.6±0.3 |
| Proteins | 19.4±0.5 | 20.0±0.5 |
| Carbohydrates | 61.4±1.1 | 60.5±0.9 |
| - starch | 53.5±0.8 | 56.5±0.7 |
| - available sugars | 2.6±0.1 | 2.2±0.1 |
| Fibers | 5.3±0.3 | 1.8±0.1 |
| Ash | 2.4±0.1 | 2.0±0.1 |
| Moisture | 10.6±0.2 | 10.9±0.3 |

Amaranth seeds with different origin had relatively low oil content (6.2 and 6.6%), which was in agreement with the results from previous studies, according to which the oil content ranged from 4.9 to 10.0% [3, 10, 11, 12]. It was found that the amount of oil in amaranth seeds was higher than that of maize (4.5%) [11]. Despite the low oil content, the seeds were characterized with high protein content (19.4 and 20.0%, respectively). This depicts that they are products with rather high protein content. The content of total carbohydrates was relatively high (61.4 and 60.5%, respectively). Great part of the latter was starch content (53.5 and 56.5%), while the available sugars were in relatively low amount (2.2 and 2.6%) in the seeds, which content was lower than the results reported by Colmenares de Ruiz and Bressani (1990) (7.7 – 10.6, 8.0 and 8.7%) [2]. The ash content ranged from 2.0 to 2.4% and the moisture was 10.6 and 10.9%, respectively for the seeds from India and Turkey, which was higher than the results by Paredes-Lopez and Mora-Escobedo (1989) (7.5%) [4]. The most significant differences were observed in the content of fibers, which percent was much higher in the seeds from India (5.3%) than in the seeds from Turkey (1.8%). These results are in agreement with previous studies where the content of fibers is from 2.2 to 5.8% [9, 11, 32], but they are lower than the results reported by Pedersen *et al.* (1987) (8.0%) [10]. The results about the content of protein, carbohydrate, starch and ash in amaranth seeds was in agreement with these from some previous studies: protein (13 - 18%) [3, 7, 8, 9, 10, 11]; carbohydrates (57.0-72.7%) [8, 11,13]; starch (49.5 - 64.0%) [7, 10, 13]; ash (1.8-4.1%) [3, 10, 11].

**Table 2.** Element concentrations in amaranth seeds with different origin

| Elements, mg/kg | Amaranth (India) | Amaranth (Turkey) |
|---|---|---|
| Ca | 2422 | 1792 |
| Mg | 2069 | 2013 |
| K | 1658 | 1934 |
| Na | 12.8 | 4.7 |
| Ba | 2.4 | 1.8 |
| Al | 9.3 | 3.6 |
| Zn | 15.7 | 22.5 |
| Mn | 18.2 | 22.9 |
| Pb | 0.2 | 0.0 |
| Fe | 77.5 | 72.0 |
| Cu | 2.3 | 1.0 |
| Ni | 0.1 | 0.2 |

The content of macro- and microelements in the amaranth seeds is shown in Table 2.

Significant differences were observed between the concentrations of micro- and macroelements in both examined seeds. Calcium (2422 mg/kg) was in the highest amount in Indian seeds, followed by Mg (2069 mg/kg), while in the seeds from Turkey the latter macroelement predominated (2013 mg/kg), followed by K (1934 mg/kg). The concentration of Na in both seeds is rather low – 12.8 and 4.7 mg/kg, respectively. Iron was the most abundant microelements in the amaranth seeds (77.5 and 72.0 mg/kg), followed by Mn (18.2 and 22.9 mg/kg) and Zn (15.7 and 22.5 mg/kg). Lead is non-essential toxic metal [33, 34] but its content in the amaranth seeds with Indian origin is rather low (0.2 mg/kg) and is absent in these from Turkey.

The results about the content of biologically active substances in amaranth seeds and glyceride oils with different origin are presented in Table 3.

**Table 3.** Content of biologically active components of amaranth seed oils with different origin

| Biologically active components | Amaranth (India) | Amaranth (Turkey) |
|---|---|---|
| Unsaponifiable matter, % | | |
| - in the oil | 8.5±0.2 | 9.3±0.2 |
| - in the seeds | 0.5±0.01 | 0.6±0.01 |
| Sterols, % | | |
| - in unsaponifiable matter | 15.7±1.2 | 27.9±2.1 |
| - in the oil | 1.3±0.1 | 2.6±0.2 |
| - in the seeds | 0.08±0.01 | 0.17±0.01 |
| Tocopherols, mg/kg | | |
| - in the oil | 1015±52 | 1060±49 |
| - in the seeds | 62.9±3.2 | 70.0±3.2 |
| Phospholipids, % | | |
| - in the oil | 3.4±0.3 | 3.5±0.5 |
| - in the seeds | 0.2±0.02 | 0.2±0.03 |

Total content of unsaponifiable matters in both amaranth seed oils was similar (8.5 and 9.3%). Considerable differences were observed in total sterols of the oils with Indian and Turkish origin. Their content was two times higher in the oil from Turkey (2.6%), while in the oil from Indian seeds they were 1.3%. The latter result was in agreement with that reported by previous study about the sterol content of some common used vegetable oils (from 0.1 to 1.3%) [35]. Both oils contained relatively high amount of total tocopherols (1015 and 1060 mg/kg in the oils and 62.9 and 70.0 mg/kg in the seeds) and this was in agreement with the results reported by Bruni *et al.* (2001) [14] (51.81 and 116.02 mg/kg total tocopherols in the seeds). On the other hand, this content was much higher than those by Tang *et al.* (2016) (7.28 – 27.90 μg/g in the seeds) [16]. Total phospholipid

content in the oils ranged from 3.4 to 3.5%, which was lower than the results by Gamel *et al.* (2007) (9.1 – 10.2%) [12].

Fatty acid composition of amaranth seed oils with different origin is presented in Table 4.

**Table 4.** Fatty acid composition of triacylglycerols of amaranth seed oils with different origin

| Fatty acids, % | Amaranth (India) | Amaranth (Turkey) |
|---|---|---|
| Caprylic ($C_{8:0}$) | 0.2±0.02 | 0.1±0.02 |
| Capric ($C_{10:0}$) | 0.1±0.03 | -* |
| Myristic ($C_{14:0}$) | 0.1±0.01 | 0.1±0.03 |
| Pentadecanoic ($C_{15:0}$) | 0.2±0.03 | - |
| Palmitic ($C_{16:0}$) | 38.4±0.2 | 33.1±0.1 |
| Palmitoleic ($C_{16:1}$) | 0.9±0.05 | 0.7±0.1 |
| Margaric ($C_{17:0}$) | 0.2±0.02 | 0.2±0.03 |
| Stearic ($C_{18:0}$) | 8.6±0.1 | 6.3±0.1 |
| Oleic ($C_{18:1}$) | 38.7±0.4 | 31.7±0.3 |
| Linoleic ($C_{18:2}$) | 6.8±0.1 | 22.6±0.2 |
| Trans Linoleic ($C_{18:2}$) | 0.7±0.1 | 0.5±0.1 |
| Linolenic ($C_{18:3}$) | 0.4±0.1 | 0.5±0.1 |
| Arachidic ($C_{20:0}$) | 1.8±0.1 | 1.2±0.1 |
| Gadoleic ($C_{20:1}$) | 0.2±0.03 | 0.2±0.02 |
| Eicosadienoic ($C_{20:2}$) | 0.1±0.01 | 0.1±0.02 |
| Behenic ($C_{22:0}$) | 0.6±0.1 | 0.5±0.1 |
| Eicosatrienoic ($C_{20:3}$) | - | 0.3±0.02 |
| Arachidonic ($C_{20:4}$) | 0.4±0.1 | 0.1±0.01 |
| Tricosylic ($C_{23:0}$) | - | 0.1±0.02 |
| Lignoceric ($C_{24:0}$) | 1.1±0.1 | 1.1±0.1 |
| Eicosapentaenoic ($C_{20:5}$) | 0.5±0.1 | 0.6±0.2 |

* - Not identified

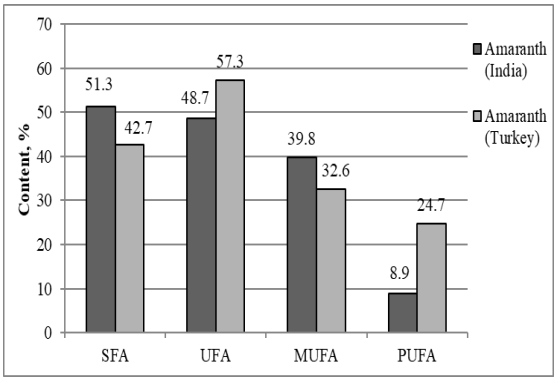


**Figure 1.** Content of saturated (SFA), unsaturated (UFA), mono- (MUFA) and polyunsaturated (PUFA) fatty acids in amaranth seed oils.

Nineteen fatty acids were identified in the oils. Oleic (38.7%) and palmitic (38.4%) acids predominated in the oil from Indian seeds, while in the oil from Turkey the main fatty acids were palmitic (33.1%) and oleic (31.7%). Stearic (8.6%) and linoleic (6.8%) acids were observed in lower amount in the oil from India. The most significant difference was observed in the quantity of linoleic acid which amount was much higher in the oil from Turkish seeds (22.6%).

The obtained results about the fatty acid composition of amaranth seed oils distinguished from the data from previous authors where the main fatty acid was linoleic and its amount varies from 37.0 to 47.8%, followed by oleic acid (20.2 – 32.9%). On the other hand, the content of palmitic acid (12.3 - 25.9%) [3, 13-16] were much lower than those found in the present study (38.4%).

The content of saturated (SFA), unsaturated (UFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids in the amaranth seed oils is shown on Figure 1.

The oil from Turkish seeds contained higher amount of unsaturated fatty acids (57.3%), while in the oil from Indian seeds predominated saturated fatty acids (51.3%). Monounsaturated fatty acids (39.8 and 32.6%) were in higher quantity than polyunsaturated fatty acids (8.9 and 24.7%) in both oils. On the other hand, the content of the latters were much higher in the oil from Turkish seeds.

The differences in the fatty acid composition of the examined seeds were probably due to the different geographical areas where the plants had been grown. Triacylglycerols which contain predominantly unsaturated fatty acids are synthesized in the oil-bearing plants that have been grown in countries with moderate and cool climates (e. g. the northern part of Turkey), while triacylglycerols containing saturated fatty acids are mostly synthesized in countries with warm climate [36]. This is most likely the reason why the oil from Turkish seeds is richer in unsaturated fatty acids (57.3%).

The results about individual sterol composition of amaranth seed oils are shown in Table 5.

**Table 5.** Individual sterol composition of amaranth seed oils with different origin

| Sterols, % | Amaranth (India) | Amaranth (Turkey) |
|---|---|---|
| Cholesterol | 0.3±0.01 | 0.2±0.02 |
| Campesterol | 2.8±0.2 | 2.0±0.1 |
| Stigmasterol | 26.1±1.2 | 24.9±1.0 |
| β-Sitosterol | 38.1±1.3 | 41.9±1.2 |
| Fucosterol | 1.2±0.02 | 0.3±0.02 |
| $\Delta^5$-Avenasterol | 23.5±0.7 | 20.1±0.5 |
| $\Delta^7$-Stigmasterol | 7.7±0.1 | 10.3±0.2 |
| $\Delta^7$-Avenasterol | 0.3±0.01 | 0.3±0.01 |

No considerable differences were observed in the sterol composition of the examined oils. The main component was β-sitosterol (38.1 and 41.9%),

followed by stigmasterol (26.1 and 24.9%) and $\Delta^5$-avenasterol (23.5 and 20.1%). $\Delta^7$-Stigmasterol (7.7 and 10.3%) and campesterol (2.8 and 2.0%) were also detected in relatively high amounts. The results about the sterol composition of the examined seeds were completely different from the data reported in a previous study [3]. Tocopherol composition of amaranth seed oils is presented in Table 6.

**Table 6.** Tocopherol composition of amaranth seed oils with different origin.

| Tocopherols, % | Amaranth (India) | Amaranth (Turkey) |
|---|---|---|
| α-Tocopherol | 14.6±0.2 | 13.6±0.5 |
| β-Tocopherol | 54.2±1.5 | 55.5±1.0 |
| γ-Tocopherol | 5.2±0.4 | 4.7±0.6 |
| δ-Tocopherol | 26.1±0.8 | 26.3±1.1 |

β-Tocopherol predominated in the tocopherol fraction of both oils (54.2 and 55.5%), followed by δ-tocopherol (26.1 and 26.3%) and α-tocopherol (13.6 and 14.6%). The amount of γ-tocopherol was significantly low (4.7 and 5.2%). These results were in agreement with the reported by Ogrodowska *et al.* (2014) [3], who also found that the main tocopherol was β-tocopherol (38.4%), followed by δ-tocopherol (31.4%). Bruni *et al.* (2001) [14] also reported that β-tocopherol (43.0-61.0%) was in the highest quantity, but the amount of α-tocopherol was considerably higher (29.8-42.7%) than in the examined amaranth seed oils.

The results about phospholipid composition of the investigated amaranth seed oils are shown in Figure 2.

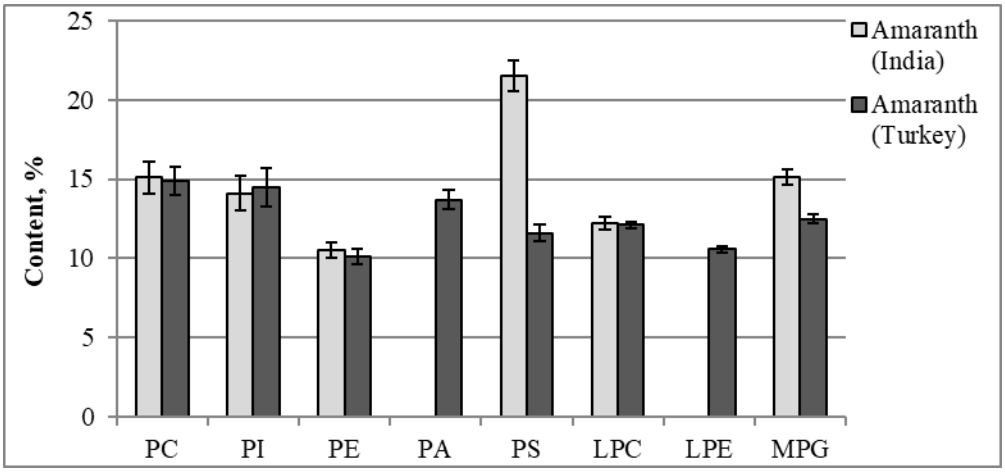


**Figure 2**. Phospholipid composition of amaranth seed oils with different origin. PC – Phosphatidylcholine; PI – Phosphatidylinositol; PE – Phosphatidylethanolamine; PA - Phosphatidic acids; PS – Phosphatidylserine; LPC - Lysophosphatidylcholine; LPE - Lysophosphatidylethanolamine; MPG – Monophosphatidylglycerol

Phosphatidylserine (21.5%) was the main component in the phospholipid fraction of the oil from Indian seeds, followed by phosphatidylcholine (15.1%) and monophosphatidylglycerol (15.1%), while the phospholipid classes of the oil with Turkish origin were present in similar quantities from 10.1 to 14.9%. Phosphatidic acids and lysophosphatidylethanolamine were identified only the oil from Indian seeds (13.7 and 10.6%, respectively).

## CONCLUSIONS

Detailed examinations on the chemical and lipid composition of amaranth seeds and seed oils with different origins (Indian and Turkish) were performed for the first time. No significant differences were observed in the chemical composition of the two examined seeds. The seeds were characterized with low oil content, but were abundant in proteins and carbohydrates. No considerable differences were observed in the tocopherol and sterol composition of the two examined seed oils. They were characterized with relatively higher sterol and tocopherol content than other common seed oils (sunflower, soybean, sesame, etc.). β-Tocopherol predominated in the oils, while β-sitosterol – in the sterol fraction. Significant differences were observed in the fatty acid and phospholipid composition of the oils from Indian and Turkish amaranth seeds. Finally, can be concluded that the origin of the plants have the greatest impact on fatty acid and phospholipid composition of their seed oils as well as on the content of macro- and microelements of the seeds. Overall, despite the different chemical and lipid composition of the amaranth seeds with Indian and Turkish origin, they both are a source of valuable nutrients and can fully participate in the diverse human nutrition.

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