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Variability of fatty acid content in pumpkin seeds (*Cucurbita pepo* L.)

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Abstract Pumpkin (*Cucurbita pepo* L.) seed oil is a common salad oil which is produced in Slovenia, Hungary and the southern parts of Austria. It is dark green and has a high content of free fatty acids. The seed itself can be eaten. Due to its colour and the foam formation, the oil cannot be used for cooking. The content of vitamin E, especially γ-tocopherol, is very high. The oil content of the pumpkin seed is about 50%. The variability in the oil content is very high resulting from a broad genetic diversity. Thus a breeding programme for increasing the oil productivity is very promising. The four dominant fatty acids are palmitic, stearic, oleic and linoleic acids. These four fatty acids make up 98 $\pm$ 0.13% of the total amount of fatty acids, others being found at levels well below 0.5%.

Key words Fatty acid composition · Pumpkin seed oil · *Cucurbita pepo*

Introduction

Pumpkin (*Cucurbita pepo* L.) seed oil is a common salad oil which is produced in the southern parts of Austria (Styria, Carinthia), Slovenia and Hungary [1]. It is dark green and has a high content of free fatty acids. The pumpkin which is cultivated in Styria has a high content of green seeds without husks. The colour of the oil which is pressed from the seeds is dark green to red ochre and has a strong red fluorescence. The production of the oil is very labour intensive. After crushing, the seeds are roasted and pressed at elevated temperatures. Due to its colour the oil cannot be used for cooking. The content of vitamin E, especially γ-tocopherol, is very high [2]. The oil content of the pumpkin seed is about 50%. The seed itself can be eaten and shows good results in alleviating several prostrate diseases [3]. Therefore a pumpkin variety with high vitamin E content that can be used as a nutraceutical is desirable. The development of seeds for production of pumpkin seed oil with a high vitamin E content is part of a project dealing with pumpkin seed oil production.

The composition of fatty acids varies depending on several factors (variety, area in which the plants are grown, climate, state of ripeness) [4, 5]. Due to the temperature dependence of the microsomal oleoyl phosphatidylcholine desaturase (ODS) in sunflower seeds, more linoleic acid is produced at lower temperatures [6]. This desaturation is a special feature of plant systems. There are two desaturating enzymes in plants: one which attacks the fatty acids in thiol linkages and produces a double bond at the 9-position, or, in some cases, in the 7-position from the carboxyl end (Δ9-desaturase), and another which attacks the fatty acid bound covalently to the glycerol moiety of phosphatidylcholine (oleoyl desaturase, linoleoyl desaturase) [7]. For *Cucurbita pepo* no such data are available. The dominant fatty acids that are found in pumpkin seed oil are palmitic (C_{16}), stearic (C_{18}), oleic ($C_{18:1}$) and linoleic acids ($C_{18:2}$). Older data show that in different varieties which are used for oil production, palmitic acid occurs in the range of 10.3% to 11.7%, stearic acid 4.1% to 5.4%, oleic acid 30.5% to 40.8% and linoleic acid 42.1% to 51.5% [4].

The aim of this study was to find breeding lines of *Cucurbita pepo* with a high oil production capacity and a high content of unsaturated fatty acids.

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Materials and methods

Cultivation of pumpkins. Lines of *Cucurbita pepo* L. convar. *citrullina* var. *styriaca* have dark green naked seeds and long shoots. These

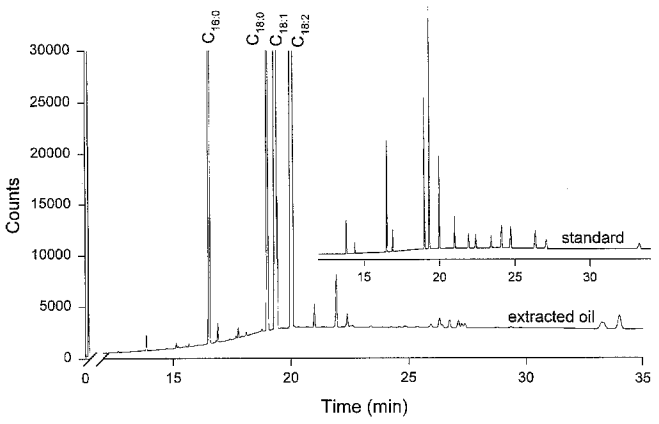

Fig. 1 Chromatogram of the derivatised fatty acids and the standard mixture

lines have been crossed with pale-seeded, long-shoot and bushy lines from Russia, Germany, Hungary and the USA, resulting in much higher genetic and phenotypic variation in comparison to the original form. The 100 breeding lines (F_4 to F_6) used in this study were selected from 400 lines grown in 1993. The sowing was done in 1994 by hand with three seeds in each planting hole. After emergence, only one seedling was left. Each plot contained four plants. To prevent the plants from growing into one another, the space for each plant was 6.7 m^2 for plants with long shoots and 2.1 m^2 for plants with short shoots. For production purposes the space assigned per plant would be 1 m^2.

Chemicals. All chemicals and solvents (e.g. hexane) used were of analytical grade and were purchased from Merck (Darmstadt, Germany). The reference materials (fatty acids) were purchased from Nu Check Prep, (USA). The boron trifluoride-methanol complex (solution 20% in methanol) which was used for derivatisation of the fatty acids was purchased from Merck.

Determination of oil content. The determination of oil content was done after freeze-drying of the milled seeds (Waring Blendor, Netherlands) overnight. Of the seeds, 5 g was extracted in a Soxhlet apparatus for 4 h with petroleum benzine (boiling range 40–60°C). After evaporation of the solvent (2 h, RT, 2.5 kPa), the oil content was determined gravimetrically.

Derivatisation of fatty acids. The derivatisation was done according to [8]. To 150 mg of the extracted oil, 4 ml of 0.5 M NaOH in MeOH was added and heated in a steam bath for ≈5 min until the lipids had dissolved. Then 5 ml of the boron trifluoride-methanol complex was added and refluxed for 2 min. For extraction with petroleum benzine, saturated salt solution was added. The organic phase was used for GC analysis.

GC analysis. The gas chromatograph was an HP 5890 with a split/splitless (split ratio of 1:30) injector and a flame ionisation detector (FID). It was equipped with a PC and the Chemstation software running under Windows (Microsoft). An HP Innowax (30 m × 0.32 mm I.D.) capillary column with a film thickness of 50 µm was used. The temperature programme was as follows: 1 min isothermal at 100°C, 8°C/min to 240°C followed by an isothermal period of 18 min at 240°C. The sample volume injected was 1 µl. The injector temperature was 250°C and the temperature of the detector was 280°C. As carrier gas He 5.0 was used at a constant pressure of 100 kPa.

Identification and quantification. For identification of the peaks in the chromatogram, relative retention times, with palmitic acid methyl ester being 1, were used. For calculation of the fatty acid content, the method of DGF [8] was used.

Calculation of correlation. The correlation is calculated according to the following equations [9]:

$$\rho_{x,y} = \frac{Cov(X, Y)}{\sigma_x \cdot \sigma_y} \quad (1)$$

where $-1 \leq \sigma_{xy} \leq 1$ and $Cov(X, Y) = \frac{1}{n}\sum_{i=1}^{n}(x_i - \mu_x)(y_i - \mu_y)$ (2)

Results and discussion

The dominant fatty acids that are found in the seeds of the 100 breeding lines are palmitic ($C_{16:0}$, 9.5–14.5%), stearic ($C_{18:0}$, 3.1–7.4%), oleic ($C_{18:1}$, 21.0–46.9%) and linoleic ($C_{18:2}$, 35.6–60.8%) acids. The content of the four fatty acids ranges from 98.1 to 98.7%. The oil content varies from 41.8% to 54.9%. The distribution of all fatty acids found is shown in Fig. 1. The fatty

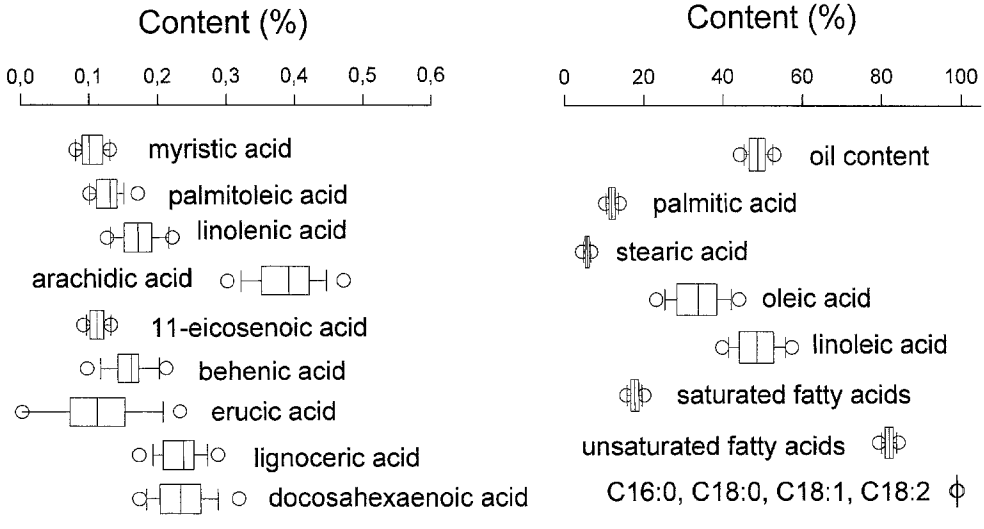

Fig. 2 Box-Plot of distribution of oil content and fatty acids content in the seeds of 100 breeding lines of Cucurbita pepo. The data are graphed as a box whose extents indicate the 25th and 75th percentiles. The line inside the box marks the value of the 50th percentile. The capped bars indicate the 10th and 90th percentiles, and the circles mark either the 5th and 95th percentiles or all data outside the 10th and 95th percentiles

Table 1 Matrix showing the correlation of oil content, sum of saturated and sum of unsaturated fatty acids, and single species of fatty acids to each other

	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	22:0	22:1	24:0	22:6	Saturated	Unsaturated
Oil content	0.197	0.194	−0.163	−0.107	−0.330	0.364	−0.383	−0.281	−0.308	0.037	0.088	0.013	0.188	0.104	−0.089
14:0	–	0.571	−0.301	0.160	−0.480	0.411	−0.028	0.168	−0.129	0.363	0.271	0.309	−0.079	0.569	−0.582
16:0		–	0.064	−0.013	−0.771	0.686	0.092	0.023	−0.575	0.353	0.388	0.342	−0.057	–	−0.836
16:1			–	−0.571	0.325	−0.310	−0.013	−0.375	−0.193	−0.220	−0.133	−0.153	−0.147	−0.261	0.273
18:0				–	−0.128	0.010	0.058	0.797	−0.093	0.246	−0.235	−0.007	0.125	–	−0.532
18:1					–	−0.984	−0.041	0.014	0.624	−0.378	−0.549	−0.452	−0.021	−0.719	–
18:2						–	0.012	−0.142	−0.595	0.323	0.584	0.444	0.014	0.583	
18:3							–	0.220	−0.011	0.130	−0.041	0.039	−0.001	0.109	−0.136
20:0								–	0.057	0.456	−0.323	0.011	0.157	0.460	−0.473
20:1									–	−0.248	−0.253	−0.187	−0.011	−0.535	0.527
22:0										–	0.053	0.271	−0.127	0.432	−0.458
22:1											–	0.493	−0.242	0.197	−0.235
24:0												–	0.053	0.284	−0.333
22:6													–	0.021	−0.041
Saturated														–	−0.997
Unsaturated															–

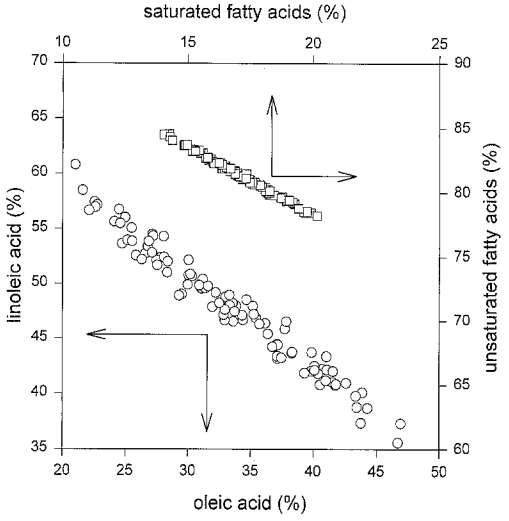

Fig. 3 Correlation of oleic to linoleic acid and saturated to unsaturated fatty acids

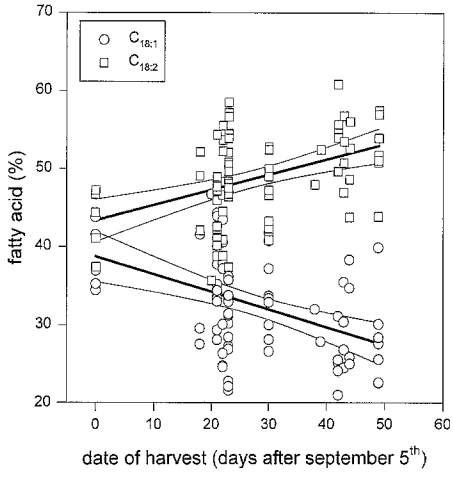

Fig. 4 Correlation of oleic and linoleic acid in relation to date of harvest with confidence interval (95%)

acids myristoleic acid ($C_{14:1}$), 11,14-eicosadienoic acid ($C_{20:2}$), homogamma linoleic acid ($C_{20:3}$), arachidonic acid ($C_{20:4}$) and nervonic acid ($C_{24:1}$) were not present in any sample.

The correlations of the oil content with the content of fatty acids and the correlations between the fatty acids are shown in Table 1. The correlation of the oil content with any type with fatty acid is very low. This means that the fatty acid composition is independent of the oil productivity.

Figure 3 shows the good correlation of linoleic acid to oleic acid ($r = 0.984$) and of saturated to unsaturated fatty acids ($r = 0.997$). This shows that the formation of linoleic acid is achieved directly by dehydrogenation of oleic acid, and that the formation of oleic acid is possibly limited by kinetic parameters [7]. This high correlation plausibly reflects different activities of desaturases in the breeding lines.

Table 2 Fatty acid and oil content of parent and progeny breeding lines

Source	Fatty acid content				Oil content (%)
	C_{16} (%)	C_{18} (%)	$C_{18:1}$ (%)	$C_{18:2}$ (%)	
Parent female	10.4	5.7	46.7	35.6	45.1
Parent male	12.1	4.7	27.2	54.5	49.0
Progeny	9.9	6.2	41.0	41.2	47.0

The harvest started on September 5th 1994 and was finished on October 24th 1994. Those pumpkins that need a long time for ripening and which are harvested very late show a higher content of linoleic acid, as can be seen in Fig. 4. This may be a result of the colder climate later in the year which normally leads to an oil with a higher content of polyunsaturated fatty acids [10] and probably reflects the higher activity of ODS at lower temperatures.

Within the 100 breeding lines, one set of parent and progeny lines was included; this should show if there are any dominant traits. The comparison of the results is summarised in Table 2. As can be seen, the palmitic acid content is lower and the stearic acid content is higher than in the parent lines. The contents of oil, oleic acid and linoleic acid are at values in between those of the parent lines.

Since these data were obtained from only one set of parents and progeny, no general conclusions about the heritage of the fatty acid content can be drawn. However, this single result shows that, for a high content of linoleic acid, both parent partners should have a high linoleic acid content. In contrast with the results which were found for sunflower seeds, in which the fatty acid distribution is dominated by the pollen, in this example no dominance can be seen [10].

For nutritional evaluation, the ratio of polyunsaturated to saturated fatty acids (P/S ratio) is 2.81 ± 0.05 for all breeding lines. Various agencies (American Heart Association, 1988 [11], National Research Council, 1989 [12], US Department of Health and Human Nutrition, 1990 [13], Health and Welfare Canada, 1990 [14]) have recommended that the current total fat consumption be reduced to 30% of calories, with the contribution of saturated fat not exceeding 10% of calories. Furthermore, a P/S ratio of 1.0 is recommended. This provides for an intake of polyunsaturated fat at 10% of calories with at least 10% of fat calories supplied by monounsaturated fat. It is emphasised that these recommendations are for adults and children over 2 years of age [15]. The high P/S ratio of pumpkin seed oil means that it should make a good contribution to a healthier nutrition.

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