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Quantitative Analysis of Fatty Acids in Pumpkin (C*ucurbita pepo* subspp *pepo* var styrica) Seed Oil

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ABSTRACT

Hull-less pumpkin (*Cucurbita pepo* subspp *pepo* var styrica) seeds have a high pharmaceutical value and consumed as snack seeds. It is newly added member in *Cucurbita* spp, as being evolved by natural single recessive mutation in the 19th century. It is notable for high seed oil content and the absence of hard seed coat have smoothened the process of oil extraction. Its seeds and seed oil are rich in fatty acid content. In present experiment, 46 advance breeding lines of hull less seeded pumpkin were characterized to assess fatty acid content. Four predominant fatty acids found in oil of 46 pumpkin genotypes in variable range were oleic acid (22.9-50.1%), stearic acid (2.2-5.5%), palmitic acid (6.6-14.70%) and linoleic acid (34.3-48.3%) and all together made (79.50-99.80%) of total fatty acid content. The pumpkin seed oil contained 15.04% saturated fatty acids (palmitic and stearic acid) and 82.93% unsaturated fatty acids (linoleic and oleic acid). Among 46 genotypes, PWT-22(50.1%), PWT 14(48.3%) and PWT-41(5.5% and 14.76%) were highest in oleic acid, linoleic acid, stearic acid and palmitic acid respectively.

Key Words: Hull-less,Fatty acid,Pumpkin, Oil Seed.

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INTRODUCTION

Cucurbitaceae is a highly diverse family that consists of at least 119 genera and over 825 species (Andres, 2003) of plants. *Cucurbita* genus (2n = 40) belongs to family cucurbitaceae and native to America (Whitaker, 1947). Pumpkin variety *Cucurbita pepo* subsp *pepo* var styriaca was emerged due to spontaneous mutation as a result of recessive gene during the 19th century in Austria. Its seed and the product prepared using seeds are widely consumed, as they have high pharmaceutical value. It is helpful to mitigate several prostrate diseases (Nitsch-Fitz, 1979). This accidental mutation resulted in huge change in morphology of seed (Fruhwirth and Hermetter, 2007). However, the seeds obtained were having very thin outer layer, which has smoothen the process of oil extraction. They are rich in their oil content as compared to other *Cucurbita pepo* spp. (Murkovic *et al*, 1996). The seed and seed oil of styrian pumpkin is mostly green in colour (Loy, 2004) and has high content of fatty acids. Mainly four fatty acids are present in considerable amount *i.e.*, palmitic, stearic, oleic, and linoleic acids (Stevenson *et al*, 2007). Linoleic acid is an essential fatty acid for humans and required for formation of cellular membranes, vitamin D and various hormones (Murkovic *et al*, 1996). Pumpkin seed oil is served as an antioxidant (Willis *et al,* 2009), antidiabetic (Pericin *et al*, 2009), antifungal (Wang and Ng, 2003), antibacterial and anti-inflammatory (Caili *et al*, 2006). The aim of this study was to find advance breeding lines of *Cucurbita pepo* with high oil content and content of unsaturated fatty acids.

MATERIALS AND METHODS

Experimental material

The experimental material consisted of 1 hulled (PCK-1) and 45 hull-less seeded genotypes of pumpkin (*Cucurbita pepo* subsp *pepo* var styriaca) grown at Vegetable Research Farm of Punjab Agricultural University, Ludhiana. The harvesting was done at full crop maturity, later after drying seeds were stored at room temperature in air tight containers. Oil used for fatty acid assessment was extracted by using method given by Folch *et al* (1957).

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Figure 1: (a) Seed of hulled genotype PCK-1 (b) Seed of hull-less genotype Lady Godiva

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Preparation of fatty acid methyl esters (FAMEs)

Fatty acid methyl easters (FAMEs) were prepared from the seed oil samples of hull-less seeded pumpkin for determination of fatty acid content. One milliliter petroleum ether was added to 50 mg oil sample, then 1.5 ml sodium ethylate (0.02 M sodium hydroxide in 99.5 % ethanol) was added. After properly shaking this mixture solution, it was kept for rest for half an hour at room temperature. Then, 1.5 ml of Nacl (8%) solution was added and shaked to mix it. After the distinction of two visible layers, upper layer containing petroleum ether was taken into other tube and was kept to evaporate. Obtained precipitates were dissolved in one milliliter ether. Three microliter from this solution was injected into GC by using Hamilton microsyringe.

Analysis of FAMEs

FAMEs were determined with a gas chromatograph (Varian CP 3800, USA). Gas chromatograph consisted of a flame ionisation detector (FID) with a fused silica capillary column (50 m -0.25 mm i.d.), coated with CP-SIL 88 as the stationary phase. Temperature of the oven was kept at 200˚ C for 13 min. The injector and FID were at temperature of 250˚ C. For identification of peak, a reference standard FAME mix (Supelco Inc.) was run under the alike circumstances. The samples were analysed for Palmitic acid (C16:0), Stearic acid (C18:0), Oleic acid (C18:1) and Linoleic acid (C18:2) the FAMEs were represented as relative area percentage.

Statistical data

Data analysis was done by using SPSS software and Duncan's multiple range tests were used to compare means for each trait and significance was accepted at $p \leq 0.05$ to find out the variation among genotypes.

RESULTS AND DISCUSSION

The fatty acid profile predicted four predominant fatty acids in oil of 46 genotypes of hull-less seeded pumpkin *i.e.,* oleic acid (22.9-50.1%), stearic acid (2.2-5.5%), palmitic acid (6.6-14.70%) and linoleic acid (34.3-48.3%) and all together made (79.50-99.80%) of total fatty acid content (Table 1). The pumpkin seed oil contained 15.04% saturated fatty acids (palmitic and stearic acid) and 82.93% unsaturated fatty acids (linoleic and oleic acid). On an average, linoleic acid (41.64%) was higher among all, followed by oleic (41.29%), palmitic (10.9%) and stearic acid (4.07%), respectively.

The average fatty acid content results revealed that linoleic acid (41.64%) was highest followed by oleic acid (41.29%), palmitic acid (10.97%) and stearic acid (4.07%) (Figure 2). Among 46 genotypes, PWT-22 (50.1%) has showed maximum oleic acid content followed by

Table 1. Fatty acid profile of seed oil from hull-less seeded pumpkin.

Sr No	Genotype	Oleic acid (18:1) (Mean±SD)[A]	Stearic acid (18:0) (Mean±SD)[A]	Palmitic acid (16:0) (Mean±SD)[A]	Linoleic acid (18:2) (Mean±SD)[A]
1	PWT 1	41.6±2.91^{defghijkl}	4±0.26^{bcdefghij}	11.00±0.66^{efghijkl}	43.2±2.51^{bcdefgh}
2	PWT 2	40.2±0.13 ^{hijklm}	4.2±0.40^{abcdefghij}	10.70±0.26^{fghijklm}	38.7±1.98^{jklmn}
3	PWT 3	43.5±1.06^{bcdefghi}	4.8±0.13^{abcde}	11.90±1.72^{defgh}	39.6±0.93^{hijklm}
4	PWT 4	45.2±1.19^{bcd}	3.5±0.40^{efghijkl}	8.80±0.26^{no}	42.3±0.93^{cdefghij}
5	PWT 5	39.7±0.53^{ijklm}	4.7±0.26^{abcdefg}	11.80±1.19^{defghi}	43.4±2.51^{bcdefgh}
6	PWT 6	43.8±1.59^{bcdefgh}	4.3±0.40^{abcdefghij}	11.90±0.13^{defgh}	38.2±1.06^{klmn}
7	PWT 7	44.1±0.66^{bcdefg}	3.8±0.40^{cdefghij}	10.50±0.79^{ghijklmn}	40.9±1.59^{efghijklm}
8	PWT 8	39±1.45^{klm}	4.3±0.26^{abcdefghij}	10.70±1.06^{fghijklm}	43.3±2.12^{bcdefgh}
9	PWT 9	41.5±1.85^{defghijkl}	4±0.79^{bcdefghij}	11.10±0.79^{efghijkl}	43.1±0.66^{bcdefghi}
10	PWT 10	43.4±0.66^{bcdefghi}	2.4±0.79^{klm}	11.00±0.93^{efghijkl}	41.4±1.72^{defghijkl}
11	PWT 11	43.1±0.79^{cdefghij}	3.5±0.53^{ghijklm}	9.60±0.66^{klmn}	43.5±2.12^{bcdefg}
12	PWT 12	44.9±1.59^{bcde}	3.1±0.40^{jklm}	6.60±0.66^{p}	41.3±2.25^{efghijkl}
13	PWT 13	42.4±2.51^{defghijk}	4±0.66^{bcdefghij}	10.70±0.66^{fghijklm}	42.4±1.98^{cefghlj}
14	PWT 14	35±3.18^{no}	3.8±0.40^{cdefghij}	11.80±1.46^{defghi}	48.3±2.12^{a}
15	PWT 15	37.4±2.38^{mn}	3.9±0.79^{cdefghij}	11.40±1.59^{efghij}	46.6±1.19^{ab}
16	PWT 16	33.9±2.91^{o}	2.4±0.26^{lm}	7.60±0.66^{op}	35.6±1.19^{no}
17	PWT 17	42.1±1.72^{defghijkl}	3.4±0.53^{hljklm}	9.00±0.66^{mno}	45.2±1.72^{abcd}
18	PWT 18	41.3±1.59^{efghijkl}	3.6±0.66^{efghijkl}	10.60±0.79^{fghijklm}	43.7±0.26^{bcdef}
19	PWT 19	22.9±3.04^{q}	3.5±0.79^{ghijklm}	11.50±1.06^{efghi}	41.6±1.98^{defghijkl}
20	PWT 20	46.9±3.18^{b}	3.9±0.93^{cdefghij}	11.30±0.53^{efghijk}	37.2±2.91 ^{mno}
21	PWT 21	42.1±1.59^{defghijkl}	3.4±0.40^{fghijklm}	10.40±0.93^{ghijklmn}	42.9±3.04^{bcdefghi}
22	PWT 22	50.1±3.18^{a}	4.1±0.53^{bcdefghij}	10.40±0.93^{ghijklmn}	34.3±3.57^{o}
23	PWT 23	45±0.13^{bcde}	5.0±0.93^{abcd}	11.10±0.26^{efghijkl}	37.9±3.44^{lmn}
24	PWT 25	46.3±1.98^{bc}	4.4±0.40^{abcdefghij}	9.70±0.66^{jklmn}	39.3±2.38^{ijklm}
25	PWT 26	46.2±1.19^{bc}	4.4±0.00^{abcdefghij}	10.30±0.79^{hijklmn}	38.8±1.95^{jklmn}
26	PWT 27	40.3±2.51 ^{ghijklm}	4.5±0.79^{abcdefghij}	12.40±0.93 ^{bcdef}	42.3±0.53^{cdefghij}
27	PWT 28	42±0.66^{defghijkl}	4.1±0.26^{abcdefghij}	**13.70±1.72^{abc}**	39.7±1.72^{ghijklm}
28	PWT 29	43.4±1.06^{bcdefghi}	4.7±0.66^{abcdefg}	11.00±0.66^{efghijkl}	40.1±1.19^{fghijklm}
29	PWT 30	41.6±1.98^{defghijkl}	3.9±0.26^{cdefghij}	10.90±0.40^{efghijkl}	43.3±0.53^{bcdefgh}
30	PWT 31	41±0.27^{fghijklm}	3.8±0.13^{cdefghij}	12.50±1.19^{bcde}	41.9±2.25^{defghijk}
31	PWT 32	44.8±0.53^{bcdef}	5.3±1.06^{ab}	10.10±0.40^{ijklmn}	39.6±3.17^{hijklm}
32	PWT 33	39.4±1.32^{jklm}	4.5±0.26^{abcdefgh}	12.10±0.93^{cdefg}	43.7±1.46^{bcdef}
33	PWT 34	38.5±1.19^{lm}	3.6±0.40^{defghijk}	10.50±0.13^{ghijklmn}	44.1±1.59^{bcde}
34	PWT 35	41.7±1.19^{defghijkl}	4.3±0.26^{abcdefghij}	11.10±0.40^{efghijkl}	42.7±1.19^{cdefghi}
35	PWT 36	43.4±0.93^{bcdefghi}	4.8±0.66^{abcdef}	10.40±0.40^{ghijklmn}	41.2±1.72^{efghijkl}
36	PWT 37	39.8±1.06^{ijklm}	4.3±0.40^{abcdefghij}	11.70±1.06^{defghi}	42.3±0.93^{cdefghlj}
37	PWT 38	41.6±1.19^{defghijkl}	4.5±0.53^{abcdefghi}	11.30±0.40^{efghijk}	42.3±2.12^{cdefghlj}
38	PWT 39	41.3±1.19^{efghijkl}	4.9±0.93^{abcde}	11.20±0.79^{efghijk}	42.5±3.44^{cdefghlj}
39	PWT 40	33.5±3.18^{o}	5.1±0.40^{abc}	13.80±1.46^{ab}	46.1±0.53^{abc}
40	PWT 41	39.1±1.59^{klm}	5.5±1.32^{a}	14.70±1.72^{a}	37.9±1.59^{lmn}
41	PWT 42	39.9±3.04^{ijklm}	4.3±0.26^{abcdefghij}	13.30±0.26^{abcd}	42.1±0.66^{defghij}
42	PWT 43	43.4±0.93^{bcdefghi}	4±0.40^{bcdefghij}	9.40±0.93^{lmn}	42.9±1.19^{bcdefghi}
43	PWT-44	30.2±3.84^{p}	4.6±0.66^{abcdefgh}	13.30±0.40^{abcd}	41.5±1.46^{defghijkl}
44	PWT-45	43±1.72^{cdefghij}	4.4±0.26^{abcdefghi}	10.10±0.53^{ijklmn}	42±1.72^{defghij}
45	Lady Godiva	45.1±2.25^{bcde}	3.3±0.66^{ijklm}	10.30±0.53^{hijklmn}	41.2±1.72^{efghijkl}
46	PCK-1	44.8±0.93^{bcdef}	2.2±0.53^{m}	9.40±0.13^{lmn}	43.4±0.26^{bcdefgh}
	Mean	41.29	4.07	10.97	41.64

[A] *Mean± SD (Standard deviation); values labeled with different letters are significantly different from the control level by Duncan test at 95.0% confidence.*

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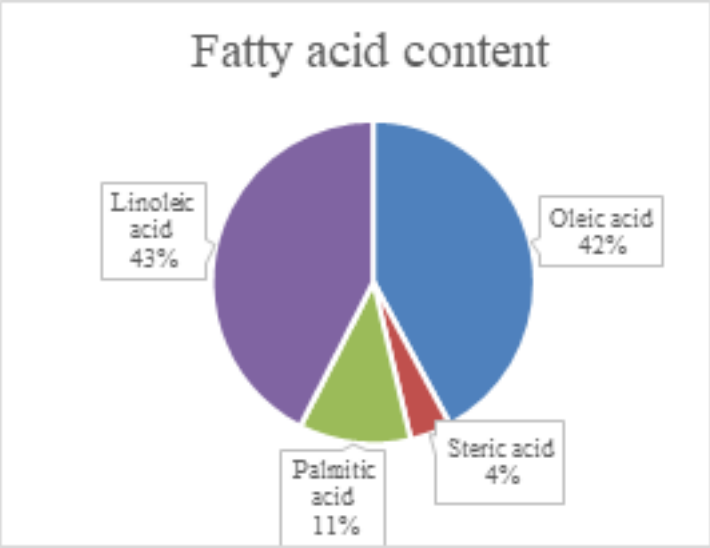


Figure 2: Average fatty acid content in oil samples of 46 pumpkin genotypes

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PWT-20 (46.9%), PWT-25 (46.3%) and PWT-26 (46.2%), whilst, PWT-19(22.9%) was having the least value for oleic acid content followed by PWT-44 (30.2%) and PWT-40(33.5%). However, PWT-41 (5.5%) was having highest stearic acid content followed by PWT-32 (5.3%) and PWT-40 (5.1%). On the other hand, PWT-46 (2.2%) has developed minimum value for stearic acid content followed by PWT-16 (2.4%) and PWT-10 (2.4%). In palmitic acid, PWT-41 (14.70%) was on the top among all 46 genotypes followed by PWT-40 (13.80%) and PWT-28 (13.70%), whereas, PWT-12 (6.6%) was lowest yielder of palmitic acid followed by PWT-16 (7.6%) and PWT-4 (8.8%). PWT-14 (48.3%) developed maximum value for linoleic acid followed by PWT-15 (46.6%) and PWT-40 (46.1%). Nevertheless, PWT-22 (34.3%) was at the bottom among all the genotypes followed by PWT-16 (35.6%) and PWT-20 (37.2%) for linoleic acid content.

Hull-less seed cultivars were enriched with oil having abundance of fatty acids. Its fatty acid composition was affected by many aspects such as the genotype, growth conditions (area and climate) and the harvesting stage (Schuster *et al*, 1983). The total saturated and unsaturated fatty acid content was nearly similar to Ardabili *et al* (2011) reported 19.4% saturated and 10.7% unsaturated fatty acid content and the result pattern was resembling to Stevenson *et al (*2007). Earlier analysis of various hull-less pumpkin seed oils reported that unsaturated fatty acids, linoleic acid (43.1-55.6%) percentage was more than oleic acid (20.4-37.8%) (Lazos, 1986), but Ardabili *et al* (2007) has reported linoleic acid and oleic acid at negligible difference similar to present study outcomes.

CONCLUSION

Hull-less pumpkin seeds contain many nutritional components and they are rich source of oil content, which contain four major fatty acids (oleic, stearic, palmitic and linoleic acid). In this experiment, results revealed that the unsaturated fatty acids (82.93% linoleic and oleic acid) are significantly higher in quantity than saturated fatty acids (15.04% palmitic and stearic acid). On an average basis, they have higher linoleic acid content as compared to oleic acid.

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