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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.

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

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.*

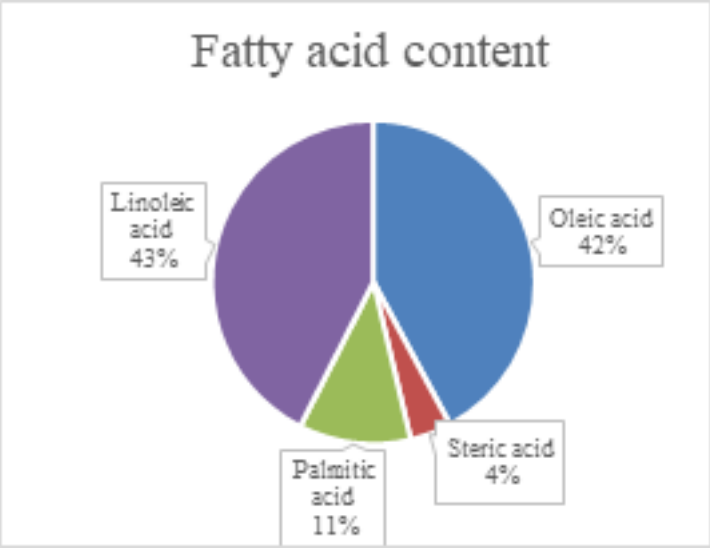

Figure 2: Average fatty acid content in oil samples of 46 pumpkin genotypes

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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