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Original Research Article

Genetic Diversity for Fatty Acid Profiling in Cotton Genotypes across the Species

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A B S T R A C T

Cotton seed is one of the important byproduct of cotton, the use of which has not been exploited to the fullest extent. The seed collected after ginning is used for extraction of oil. The keeping quality of cottonseed oil is comparable to groundnut and safflower oil and its nutritional value is around 9 k cal/g. The average digestibility of cotton seed oil is 97 per cent and could be compared with that of soybean, safflower and sunflower oils. The current work aims to study fatty acid profiling of selected lines of four species of cotton. Cotton seeds were collected from ARS Dharwad farm, Karnataka and crushed to get fine powder and the oil was extracted with hexane in Soxhlet apparatus. Extracted oil was methyl esterified and qualitative and quantitative analysis of oil was carried out in Shimadzu Chromatopac GC-MS QP2010S. The major chemical components present in oil samples were, palmitic acid, stearic acid, oleic acid and linoleic acid.

Keywords

Cotton seed oil, Soxhlet, GC-MS, Fatty acids

Introduction

India is the largest importer of oilseeds in the world and oilseed sector occupies an important position in the agricultural economy of the country. Oilseeds are among the major crops that are grown in the country apart from cereals. India imports half of its edible oil requirement, making it the world's third-largest importer of edible oil. The country buys soya oil from Argentina and Brazil and palm oil from Malaysia and Indonesia. Total import of vegetable oil for the year 2012-13 (Nov 2012 to Oct 2013) is reported at 103.9 lakh tons compared to 99.8 lakh tons during 2011-12. The overall import of vegetable oil has increased by 4 lakh tons during the year 2012-13 over the previous year (Jha *et al.*, 2012). Cotton (*Gossypium* spp.) is a perennial plant that originated in tropical regions. Cotton, particularly Asiatic cottons *G. arboreum* and *G. herbaceum* are indigenous to India whereas new world cottons *G. hirsutum and G. barbadense* were introduced in eighteenth century. India is the only country in the world, where four species of cotton, *viz*. *G. arboreum*, *G. herbaceum*, *G. hirsutum* and *G. barbadense* along with intra and inter-specific hybrids, are being cultivated. Cotton is grown in varied or diverse agro climatic conditions, varying from 8-32° N latitude and 70-80° longitude in 3 zones, *viz.*, Northern, Central and Southern areas (Kohel and Lewis, 1984).

Cotton seed contains approximately 18-25 per cent of oil and 20-25 per cent high quality protein (Rathore, 2007). Cotton seed oil has gained importance in food preparations due to its higher smoke point (about 232 ^{0}C) compared to other cooking oils and is good for frying food articles (Brien and Walkelyn, 2005). Refined cotton seed oil has a mild taste and light golden color. It also finds a number of other non food uses in biodiesel production, in paint industry and as an environmentally accepted lubricant additive to improve the lubricating abilities of the base oil SAE 20 W50 (Ertugrul and Filiz, 2004).

Materials and Methods

The present investigation was carried out to estimate fatty acid profiling of selected cotton genotypes in all the four cultivated species. The details of material used and techniques adopted during the course of investigation are given in table 1.

Estimation of fatty acids in cotton seed oil

The study on fatty acid profile was carried out in eight genotypes including all four species of cotton. Oven dried cotton seeds were taken and the seed coat was removed by dehulling manually to get kernels, which contains oil and other constituents.
The flowing reagents were used for this test n-hexane; Methanol; Toluene; 8 % Hydrochloric acid (HCL)

The methodology adopted for this experiment includes, kernels were crushed in pestle and mortar to get a fine powder. Powder was placed in the Whatman filter paper thimble. 220 ml of n-hexane was added to all the three 500 ml bottom flasks of soxhlet apparatus**.** Thimbles were placed into the extraction tubes. The temperature was set to 70^0 C for 3 hrs and continuous flow of water was allowed to pass through the condenser to keep them cool. After 3 hrs the thimbles were removed from extraction tubes and then the remaining hexane was recovered. Extracted oil including hexane was collected from the round bottom flask and was placed in test tubes and kept in room temperature for evaporation of hexane. After evaporation of hexane the pure oil was placed in the Eppendrof tubes to avoid moisture contamination. Then fatty acid methyl esters (FAME) of extracted oil were carried out by following standard protocol. Supernatant was collected from the tubes and analysed in GCMS.

Fatty acid profiling in Gas Chromatography – Mass spectrometry (GC-MS)

The chemical composition of oil was determined by chromatography technique. GC-MS analysis was carried out using Shimadzu Chromatopac GC-MS QP2010S, in USIC, Karnataka University Dharwad. GC-MS system was equipped with Rtx - 5MS column (30 m x 0.25 mm i.d.). Column temperature was 100 to 280^0 C at the rate of 20^0 C per min. Oven temperature was 40^0 C increasing to 250^0 C at a rate of 4 ^{0}C, transfer line temperature 260^0 C. The carrier gas was helium with a linear velocity of 31.5 cm/s, split ratio 1/60, Ionization energy 70 eV, scan time 1 sec and mass range of 40-300 atomic mass unit (1 Atomic mass unit = 1.66 x 10^{-27}). The compounds were quantified by the area normalization method without considering response factors. The components of the oils were identified by comparison of their mass spectra with those of NIST library, and confirmed by comparison of their retention indices with those of data published in the literature.

Results and Discussion

The fatty acid components of volatile (methyl esterified) oil were determined by

the percentage peak area normalization method. The presence of several overlapping peaks reveals the complexity of the mixture. The major chemical components present in samples were, palmitic acid, stearic acid, oleic acid and linoleic acid. The fatty acids in area percentage are presented in Table 2.

Myristic acid (14:0)

Significantly highest content of myristic acid was observed in ARBH-813 (1.02 per cent) followed by Sahana (0.90 per cent). Both are genotypes of *G. hirsutum* followed by *G. barbadense* and *G. herbaceum* genotypes SB (YF)-425 (0.8 per cent) and Jayadhar (0.8 per cent), respectively and both SB (YF)-425 and Jayadhar were at par with each other. While significant least content of myristic acid was observed in *G. barbadense spp.* BCS-23-18-7 (0.45 per cent) followed by DDhC-11 (0.5 per cent) and DLSa-17 (0.6 per cent) genotypes of *G. herbaceum* and *G. arboreum* respectively.

Palmitic acid (16:0)

Sahana (25.63 per cent) a genotype of *G. hirsutum* and SB (YF)-425 (25.63 per cent) a genotype of *G. barbadense* exhibited significantly high content of palmitic acid followed by *G. arboreum* genotypes DLSa-17 (25.21 per cent) and ARBHA-35 (24.29 per cent). While significantly least content of palmitic acid was observed in BCS-23-18-7 (17.92 per cent) (*G. barbadense*) followed by Jayadhar (19.49 per cent) and DDhC-11 (21.22 per cent), genotypes of *G. herbaceum spp.*

Palmitoleic acid (16:1)

Palmitoleic acid was observed only in five genotypes, out of which DLSa-17 (1.63 per cent) and ARBHA-35 (1.58 per cent) genotypes of *G. arboreum* had significantly high content of palmitoleic acid, while SB (YF)-425 (0.86 per cent) genotype of *G. barbadense* exhibited significantly least content of palmitoleic acid followed by Jayadhar (1.14 per cent) of *G. herbaceum*.

Stearic acid (18:0)

The range of stearic acid content was 1.27 to 10.86 per cent. A genotype of *G. barbadense* BCS-23-18-7 (10.86 per cent) showed significantly high stearic acid content as compared to other genotypes followed by ARBHA-35 (3.70 per cent) a genotype of *G. arboreum.* SB (YF)-425 (2.63 per cent) a genotype of *G. barbadense spp.*, while significantly low content was observed in ARBH-813 (1.27 per cent) a genotype of *G. hirsutum* followed by DDhC-11 (1.50 per cent) a genotype of *G. herbaceum* and Sahana (1.56 per cent) a genotype of *G. hirsutum.*

Oleic acid (18:1)

The range of oleic acid varied greatly from 2.68 per cent to 24.36 per cent. BCS-23-18-7 (24.36 per cent) a genotype of *G. barbadense* reported high content of oleic acid followed by Jayadhar (4.90 per cent) a genotype of *G. herbaceum spp.* and Sahana (4.86 per cent) a genotype of *G. hirsutum*, while ARBH-813 (2.68 per cent) a genotype of *G. hirsutum* exhibited significantly least content in oleic acid followed by ARBHA-35 (3.22 per cent) and DDhC-11 (3.25 per cent) genotypes of *G. arboreum* and *G. herbaceum spp.*, respectively.

Linoleic acid (18:2)

Generally cotton seed oil comprises 55 per cent of linoleic acid. The range of linoleic acid varied from 44.07 to 66.72 per cent. DDhC-11 (67.72 per cent) and Jayadhar (66.22 per cent) genotypes of *G. herbaceum*

reported significantly higher content of linoleic acid followed by ARBH-813 (66.51 per cent) a genotype of *G. hirsutum*, while BCS-23-18-7 (44.07 per cent) genotype of *G. barbadense* significantly exhibited least content in linoleic acid followed by ARBHA-35 (58.57 per cent) genotype of *G. arboreum*.

Behenic acid (22:0)

ARBHA-35 (7.94 per cent) a genotype of *G. arboreum spp.* exhibited high content of behenic acid followed by Jayadhar (5.487 per cent) and DDhC-11 (5.47 per cent) genotypes of *G. herbaceum*, while significant least content of behenic acid was observed in BCS-23-18-7 (2.35 per cent) genotype of *G. barbadense spp.* followed by DLSa-17 (3.35 per cent) and SB (YF)-425 (3.49 per cent) genotypes of *G. arboreum* and *G. barbadense* spp., respectively.

Saturated fatty acids

High amount of saturated fatty acids were present in ARBHA-35 (36.63 per cent) followed by SB (YF)-425 (32.55 per cent), Sahana (31.96 per cent) and BCS23-18-7 (31.58 per cent), while low amount of saturated fatty acids were present in Jayadhar (27.74 per cent) followed by DDhC-11 (28.69 per cent), ARBH-813 (30.81 per cent) and DLSa-17 (31 per cent). Table 3

Unsaturated fatty acids

Jayadhar (72.26 per cent) exhibited high content of unsaturated fatty acids followed by DDhC-11 (71.31 per cent), ARBH-813 (69.19 per cent) and DLSa-17 (69 per cent). While low amount of unsaturated fatty acids were present in ARBHA-35 (63.37 per cent) followed by SB (YF)-425 (67.45 per cent), Sahana (68.05 per cent) and BCS-28-18-7 (68.4 per cent) (Table 3).

Khamar and Jasrai (2014) biochemical profile, fatty acid profile and phytochemical analysis of some selected plant oils including cotton seed oil in GC, fatty acid composition in cotton seed oil was saturated fatty acid (22-26 per cent), monounsaturated fatty acid (16-21 per cent) and polyunsaturated fatty acid (50-55 per cent).

Cotton seed oil contains 27 per cent of saturated fatty acid. High and low amounts of saturated fatty acids were present in ARBHA-35 (36.63 per cent) and Jayadhar (27.74 per cent), respectively.

Table.1 Genotypes used for fatty acid profiling

Sr. No.	Species	Genotypes
1.	*G. arboreum*	DLSa-17 and ARBHA-35
2.	*G. barbadense*	SB(YF)-425 and BCS23-18-7
3.	*G. herbaceum*	DDhC-11 and Jayadhar
4.	*G. hirsutum*	Sahana and ARBH-813

Table.2 Fatty acids present in cotton seed oil content across the species, in area percentage obtained from GCMS chromatograms

Cotton spp.	Genotypes	Myristic acid	Palmitic acid	Palmitoleic acid	Stearic acid	Oleic acid	Linoleic acid	Behenic acid
G. arboreum	ARBHA-35	0.70	24.29	1.58	3.70	3.22	58.57	7.94
	RT	**7.64**	**8.71**	**8.61**	**8.93**	**9.80**	**9.59**	**9.67**
	DLSa-17	0.60	25.21	1.63	1.84	3.95	63.42	3.35
	RT	**7.64**	**8.71**	**8.61**	**8.94**	**9.80**	**9.59**	**9.67**
G. barbadense	BCS-23-18-7	0.45	17.92	-	10.86	24.36	44.07	2.35
	RT	**7.64**	**8.70**	**-**	**8.93**	**9.81**	**9.57**	**9.67**
	SB (YF) 425	0.8	25.63	0.86	2.63	4.23	62.36	3.49
	RT	**7.64**	**8.72**	**8.62**	**8.95**	**9.81**	**9.60**	**9.68**
G. herbaceum	DDhC11	0.5	21.22	1.34	1.50	3.25	66.72	5.47
	RT	**7.64**	**8.73**	**8.62**	**8.95**	**9.80**	**9.62**	**9.68**
	Jayadhar	0.8	19.49	1.14	1.97	4.90	66.22	5.48
	RT	**7.64**	**8.73**	**8.62**	**8.96**	**9.82**	**9.62**	**9.69**
G. hirsutum	Sahana	0.90	25.63	-	1.56	4.86	63.19	3.87
	RT	**7.64**	**8.72**	**-**	**8.94**	**9.80**	**9.59**	**9.68**
	ARBH-813	1.02	23.97	-	1.27	2.68	66.51	4.55
	RT	**7.64**	**8.73**	**-**	**8.95**	**9.81**	**9.62**	**9.68**

Note: RT- retention time

Table.3 Saturated and unsaturated fatty acid composition of cotton genotypes

Cotton spp.	Genotypes	Saturated fatty acids (%)	unsaturated fatty acids	
			MUFA (%)	PUFA (%)
G. arboreum	ARBHA-35	36.63	3.22	58.57
	DLSa-17	31.00	3.95	63.42
G. barbadense	BCS-23-18-7	31.58	24.36	44.07
	SB (YF)-425	32.55	4.23	62.36
G. herbaceum	DDhC-11	28.69	3.25	66.72
	Jayadhar	27.74	4.90	66.22
G. hirsutum	Sahana	31.96	4.86	63.19
	ARBH-813	30.81	2.68	66.51

Note: MUFA-monounsaturated fatty acid, PUFA-polyunsaturated fatty acid

The range for palmitic acid in cotton seed oil in present study was between (17.92 to 25.63 per cent) and the range for stearic acid was between (1.27 to 10.86 per cent). Myristic acid in cotton seed oil was below 1 per cent, while behenic acid was below 8 per

cent.

Ory *et al.,* (1992) in groundnut oil reported 55-65 per cent of monounsaturated fatty acids (MUFA) and 26 to 28 per cent polyunsaturated fatty acids (PUFA).

Rodriguez *et al.,* (2002) studied fatty acid composition in sunflower and reported oleic acid (17.8 per cent) and linoleic acid (69.2 per cent). Jokic *et al.,* (2013) studied soybean oil and the results indicated that soybean oil is rich in polyunsaturated fatty acids (PUFA).

Agarwal *et al.,* (2003) in cotton seed oil reported (oleic acid 17.2 per cent), (linoleic acid 55 per cent) and (linolenic acid 0.3 per cent), while in cotton seed oil the range for unsaturated fatty acid in present study was between 63.37 to 72.26 per cent which is at par with that of groundnut oil.

Cotton seed oil when compared to that of soybean oil, unsaturated fatty acid mostly contained was linoleic and was found to be in higher amounts than soybean oil but the oleic acid in cotton seed oil was less than 20 per cent except in genotype BCS-23-18-7 (24.36 per cent) which recorded the highest oleic acid content and the oil containing high amount of oleic acid is good for frying purpose as it helps to increase the shelf life of fried products, so the oil of genotype BCS-23-18-7 can be considered as good oil compared to other genotypes.

In plants, oleate Δ^{12} desaturase (*FAD2*) is a key enzyme involved in the conversion of oleic acid (C18:1) into lin oleic acid (C18:2). In *G. barbadense* genotype BCS-23-18-7 the activity of oleate Δ^{12} desaturase enzyme may be less and hence the oleic acid has accumulated and correspondingly stearic acid has also accumulated more as compared to the other genotypes studied, and simultaneously the linoleic acid has drastically decreased to 44.07 per cent. Hence the genotype BCS-23-18-7 is of interest for studying the mechanism of regulation of fatty acid biosynthesis and oil of BCS-23-18-7 is more suitable for deep frying purpose since the thermostable oleic acid content is high.

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