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Oil content and fatty acid profiling of soybean (*Glycine max* L. Merrill) of Indian cultivar

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Abstract
The fatty acid (FA) composition of soybean (*Glycine max* L. Merrill) oil determines its stability. The FA composition of 40 Indian soybean genotype collected from Agriculture Research Station, Junagadh Agricultural University, Amreli, Gujarat. The concentration of palmitic acid (PA), stearic acid (SA), oleic acid (OA), linoleic acid (LA), and linolenic acid (LNA) differed significantly amongst genotype. Overall, levels of Palmitic acid (PA) and Stearic acid (SA) ranged from 8.45% (AS3) to 13.23% (PBN107) and 2.28% (J339) to 9.52% (AGS84), with means of 11.73% and 5.86%, respectively. The coefficients of variation (CVs) of PA and SA were 4.59% and 3.74%, respectively. Elaidic acid (EA) levels ranged from 19.16% (J556) to 31.85% (K166) with an average of 22.98% and a CV of 2.76%. Oleic acid (OA) levels ranged from 1.21% (JD (SH) 131) to 3.40% (EC93741), with an average of 1.89% and a CV of 3.15%. Linoleic acid (LA) and Linolenic acid (LNA) ranged from 34.15% (K166) to 53.79% (JS335) and 7.86% (K166) to 11.90% (J245) with averages of 44.97% and 9.78% and CVs of 3.95% and 3.19%, respectively. Among the 40 genotype the total oil content was range of (16.89- 19.79). Among them genotype G.Soya 2 reported maximum oil content (19.79). While lowest total oil content was observed in AGS84 (16.89) genotype. Producing oil with greater OA content and lower amounts of LA and LNA these would be ideal for particular applications in the food business. Our findings will aid in the development of high-quality soybeans to fulfill human nutritional and industrial needs.

Keywords: GC-MS, Fatty acids, oil, soybean, PUFA
Abbreviations: PA - palmitic acid, SA - stearic acid, OA -oleic acid, LA -linoleic acid, LNA- linolenic acid, GC- gas chromatography

1. Introduction
Soybeans (*Glycine max* L. Merrill) are major source for protein and oil in soybean seeds its around 35 percent to 55 percent dry weight of total seed. oil component around 18 percent to 20 percent of total dry seed weight due to this reason soybean oil mostly used for human consumption and industrial application [1]. In soybean seed there are five type of fatty acid found saturated fatty acid include palmitic acid (PA) 11 percent and stearic acid (SA) 4 percent and in unsaturated fatty acid oleic acid (OA) 23 percent Linoleic acid (LA) 55 percent and Lenolenic acid (LNA) 8 percent [2, 3].
It is worth noting that wide variations in levels of saturated and unsaturated fatty acids have been detected in several studies on crop germplasm collections [11, 12]. Such variations could offer possibilities of developing superior accessions with high quality edible and specialized industrial oils. The variability in fatty acid composition is undoubtedly due to both genetic and weather factors [13, 14], which affect their nutritional value and processing property. Basically, much more changes are observed in the preferences for soybean oil owing to the noticeable awareness towards human health care. Consequently, breeding programs designed for altering the soybean oil profile have become a priority for improving both food and industrial uses of soybean oil [15]. Therefore, many studies on soybean seed fatty acid composition have been conducted by soybean breeders in order to develop modified oils that will match the increasing needs of consumers [16, 17, 11]. Those several needs of particular uses are divided into nutritional, industrial, or pharmaceutical aspects which generally depend on the vegetable oil quality and its fatty acid composition.
In various studies it has been shown that temperature and environmental factor play role in determine composition of fatty acid. Fatty acid composition is different in different grown areas [4, 5]. As compared to unsaturated fatty acid, saturated fatty acid affect mostly. Generally in soybean oil saturated fatty acid found in very less quantity if we taken saturated fatty acid trough diet it lead to increased the risk of cardiovascular disease and it also increased blood and cholesterol level but another site saturated fatty acid also improve oxidative

Stability [18, 19]. If the amount of stearic acid in the oil is higher, the melting point will be higher, and it will be used for processing and baking. If soybean oil contains a high amount of unsaturated fatty acids, the oil's quality will improve, and it will be beneficial to human health. Fatty acid composition plays an important part in food, and there are several methods available these days, with GC-MS being the most widely employed to evaluate fatty acid variation in vegetable oil and animal fat. As a result, a fatty acid profile that meets the needs of the confectionery sector for high melting point and oxidative stability while also being non-harmful to human health would have enhanced stearic acid and high oleic acid, as well as low polyunsaturated fatty acids. The goal of this study was to look into the seed FA content in a large panel of different Indian soybeans in order to guide soybean quality improvement in terms of FA composition.

2. Materials and Methods

2.1 Plant material

The experimental materials will comprise of 40 soybean genotypes collected from Agriculture Research Station, Junagadh Agricultural University, Amreli, Gujarat.

Table 1: List of Genotype selected for fatty acid profiling

Sr. No	Genotype name	Remarks	Sr. No	Genotype name	Remarks
1	G.Soy 1	Released variety	21	AGS 93	Black seeded
2	G.Soy 2	Released variety	22	PBN 107	Black seeded
3	GJS 3	Released variety	23	DS 64-6	Green seeded
4	KB 74	Black seeded	24	AGS 84	Green seeded
5	AS 3	LSVT entry	25	PK 781	Brown seeded
6	AS 15	LSVT entry	26	JB 5-2	Black seeded
7	PS 1634	Yellow seeded	27	PK 942	Green seeded
8	AMRS 258	LSVT entry	28	DS 178	Black seeded
9	JS 335	Released variety	29	KS 166	Brown seeded
10	AS 16	LSVT entry	30	AS 15	LSVT entry
11	DS 83-12	Black seeded	31	AS 14	SSVT entry
12	J 606	Black seeded	32	AUKS 176	Yellow seeded
13	AGS 112	Green seeded	33	J 15-20-2	Black seeded
14	JS 81-1619	Yellow seeded	34	JS 20-29	Released variety
15	DS 84-3	Green seeded	35	EC 93318	Green seeded
16	JS 72-128	Brown seeded	36	BR 7B	Brown seeded
17	J 556	Brown seeded	37	J 245	Brown seeded
18	JD(SH) 131	Black seeded	38	NRC 138	Yellow seeded
19	J 222	Black seeded	39	J 339	Black seeded
20	EC 93741	Yellow seeded	40	EC 1933	Brown seeded

2.2 Total Oil Content

2.3 Sample Preparation

Seeds of Soybean genotype (12 gm) was finely powdered in mortal and pestle. Packed in whatman No filter paper packet and label with pencil.

2.4 Determination of Total Oil Content by Soxhlet method

Sample packet was placed in butt tubes of Soxhlet Extraction apparatus and poured 250 ml of hexane to extraction chamber. Gently heating at 65 ° C for 8 hrs at 150 drops per minute. After extraction, the extraction flask is allowed to cooled and dismantled. Evaporate the hexane remains in water bath until no odour of hexane. moisture outside the flask is removed and weighed it. After that total oil content were estimated as per formula and expressed as percent of total oil (A.O.A.C, 1965) [20].

$$\text{Total oil (\%)} = \frac{\text{Weight of oil flask after extration -Weight of empty oil flask}}{\text{Weight of the dried material (Sample)}} \times 100$$

2.5 Preparation of FAME samples from the soybean matrices

The fatty acid composition was determined by the conversion of oil to fatty acid methyl esters prepared by adding 3.0 mL of n-hexane to 12 gm of seed pawder leave it overnight, followed by 1.0 mL of sodium methoxide (0.4 mol), according to the method of Fan *et al.* (2015) with some modification [21]. The mixtures were vortexed for 30 seconds and were allowed to settle for 30 minute. The upper phase containing the FAME was recovered and analyzed by gas chromatography (GC-MS).

2.6 GC-MS equipment and operating conditions

GC-Parameters

Capillary column	DB-Wax (30m ×0.25mm×0.25µM)
Injector temperature	250°C
Injector split	1 µl
Column Oven Program	60°C 12°C/min 150°C(1min) 5°C/min 240°C (5 min)
Column flow	1 ml/min (He)

MS-Parameters

Iron source temp	230°C
Interface temp	240°C
Detector Voltage	0.84 kV

Known quantity of standard individual fatty acids were methyl estered and run in to GC prior to sample analysis to identify and quantify the individual fatty acids in oil samples. Each peak of individual fatty acid of oil sample was identified

by comparing the peak of standard fatty acid which was obtained at similar retention time. The area under each peak was calculated by measuring peak height and base width. From the area under each peak of the oil sample, the percentage fatty acid composition was calculated as per cent distribution or g per 100g total fatty acids.

Fig 1: Determination of Total Oil Content by Sorblet method and Preparation of FAME samples from the soybean matrices A) Soyabean seeds B) Soyabean seed powder C) Soxlet appratus D) Oil extracted by Soxlet method E) Derivatization of oil for GCMS F) GC-MS used for fatty acid Profiling

2.7 Statistical analysis

The statistical analysis on following aspects was carried out:
Analysis of variance for Completely Randomized Design was computed as per the method of Gomez and Gomez (1984). The six fatty acids *viz*., palmitic (C16:0), stearic (C18:0), oleic (C18:1 n-9), linoleic (C18:2 n-6), and linolenic acids (C18:3 n-3) all the samples were analyzed by Completely Randomized Design.

2.8 Completely Randomized Design

Analysis of variance for Completely Randomized Design was computed as per the method of Gomez and Gomez (1984), which is based on the following mathematical model:

$Y_{ij} = \mu + t_i + e_{ij}$

Where

Y_{ij} = Fatty acid expression of i^{th} treatment and j^{th} unit,
μ = Population mean,
t_i = Effect of the i^{th} treatment and
e_{ij} = Random error associated with the j^{th} unit receiving i^{th} treatment.

The form of analysis of variance as presented in the Table 3.1 was constructed for individual fatty acid palmitic (C16:0), stearic (C18:0), oleic (C18:1 n-9), linoleic (C18:2 n-6), and linolenic acids (C18:3 n-3) all the samples were analyzed by Completely Randomized Design.

Table 2: The structure of ANOVA for CRD

Source of variation	d.f	S.S	M.S	Cal F
Treatment	t-1	TrSS	TrMS	TrMS/ EMS
Error (E)	n-t	ESS	EMS	
Total	n-1	TSS		

Where

t = Number of treatments,
TrSS = Sum of square due to treatments,
ESS = Sum of square due to error,
TSS = Total sum of square,
TrMS = Mean sum of square due to treatment,
EMS = Mean square due to error and
n = Number of observation.

Mean squares due to treatment are tested against error mean square (EMS) by calculating 'F' values.
The standard error of mean (S. Em.) was calculated using following formula

$$S.Em = \sqrt{\frac{E\,M.S}{r}}$$

The critical difference (C.D.) to compare the mean of any two genotypes was calculated using following formula.

$C.D. = S.Em \times \sqrt{2} \times t$

Where

't' = Table value of 't' at 5% level of significant at error degree of freedom

The coefficient of variation (C.V.) was determined according to the following formula

$$C.V.(\%) = \sqrt{\frac{EMS}{\bar{X}}} \times 100$$

Where,

$\bar{X}$ = General mean

3. Results and Discussion

3.1 Fatty acid profiling of different Indian soybean cultivars

3.1.1 Variation in seed FA composition in soybean genotype

The detected fatty acids were classified into four types: saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA), Six fatty acids *viz*., palmitic (C16:0), stearic (C18:0), oleic (C18:1 n-9), linoleic (C18:2 n-6), and linolenic acids (C18:3 n-3) were detected in all the samples (Tables 3).

Overall, levels of Palmitic acid (PA) and Stearic acid (SA) ranged from 8.45% (AS3) to 13.23% (PBN107) and 2.28% (J339) to 9.52% (AGS84), with means of 11.73% and 5.86%, respectively. The coefficients of variation (CVs) of PA and SA were 4.59% and 3.74%, respectively (Table 3). Elaidic acid (EA) levels ranged from 19.16% (J556) to 31.85% (K166) with an average of 22.98% and a CV of 2.76%. Oleic acid (OA) levels ranged from 1.21% (JD (SH) 131) to 3.40% (EC93741), with an average of 1.89% and a CV of 3.15% (Table 3). Linoleic acid (LA) and Linolenic acid (LNA) ranged from 34.15% (K166) to 53.79% (JS335) and 7.86% (K166) to 11.90% (J245) with averages of 44.97% and 9.78% and CVs of 3.95% and 3.19%, respectively (Table 3).

Table 3: Variation in seed FA composition in soybean genotype

Fatty acid	C.D	C.V (%)	SD	G.M	Highest	Lowest
Palmitic acid (PA)	0.88	4.59	0.79	11.73	13.23 (PBN107)	8.45 (AS3)
Stearic acid (SA)	0.36	3.74	2.40	5.86	9.52 (AGS84)	2.28 (J339)
Elaidic acid (EA)	1.04	2.76	2.59	22.98	31.85 (K166)	19.16 (J556)
Oleic acid (OA)	0.10	3.15	0.55	1.89	3.40 (EC93741)	1.21 (JD(SH)131)
Linoleic acid (LA)	2.90	3.95	5.46	44.97	53.79 (JS335)	34.15 (K166)
Linolenic acid (LNA)	0.51	3.19	1.05	9.78	11.90 (J245)	7.86 (K166)

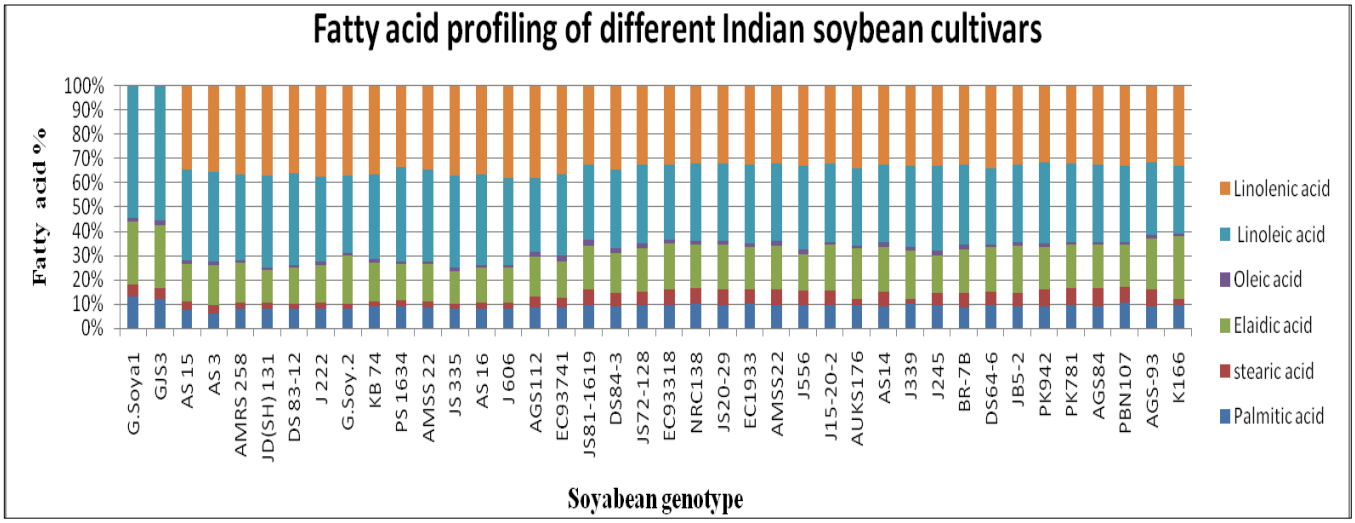

Fig 2: Fatty acid profiling of different Indian soybean cultivars

The considerable changes in FA composition seen across cultivars, accession types, and ecoregions underscored the importance of genetic variables in developing desired FA profiles in soybeans. Similar findings have been seen in recent investigations [6, 7]. We compared the differences in seed FA composition across landraces and cultivars in our work to earlier research on lupine (*Lupinus albus* L.) [8], *Brassica napus* and B. *rapa* [9], and common bean (*Phaseolus vulgaris* L.) [10].

3.2 Total Oil

The data for oil content in soyabean seed of 40 genotype are represented in Figure 3

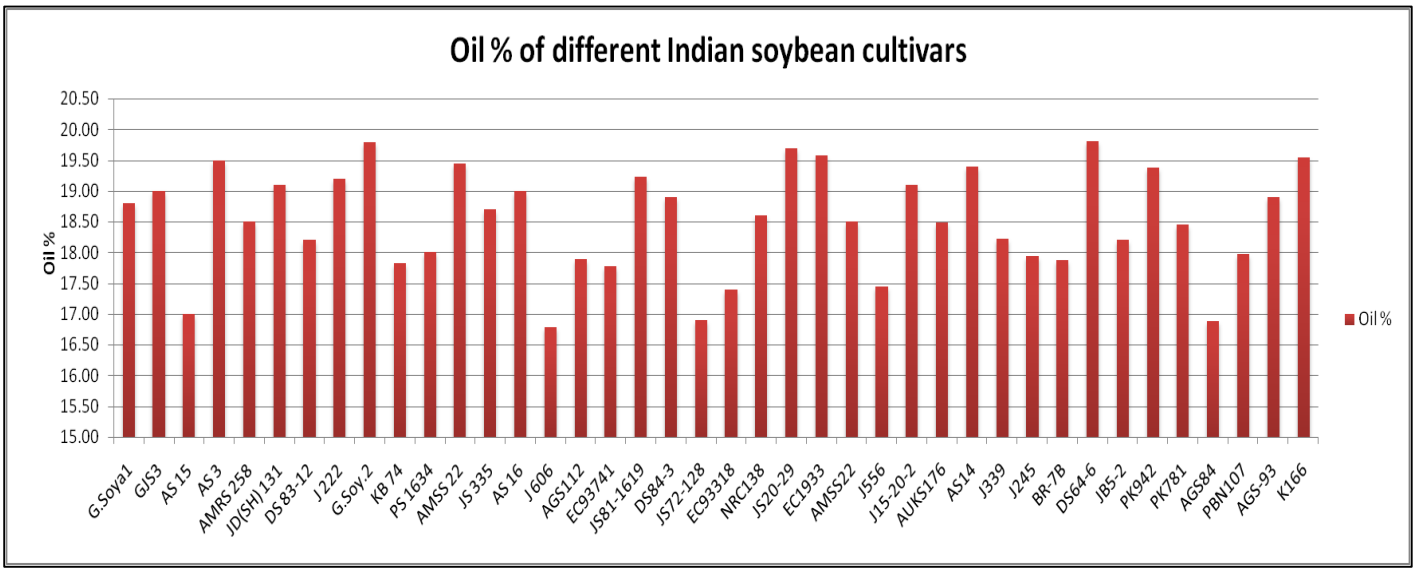

Fig 3: Total oil (%) among different Indian soybean cultivars

The Result was shown significant difference among the 40 genotype the total oil content was range of (16.89- 19.79). Among them genotype G. Soya 2 reported maximum oil content (19.79). While lowest total oil content was observed in AGS84 (16.89) genotype

3.3 Oleic and Linoleic acid (O/L) ratio in soybean

The data for O/L ratio in the soybean seeds of 40 genotype are represented in figure 4

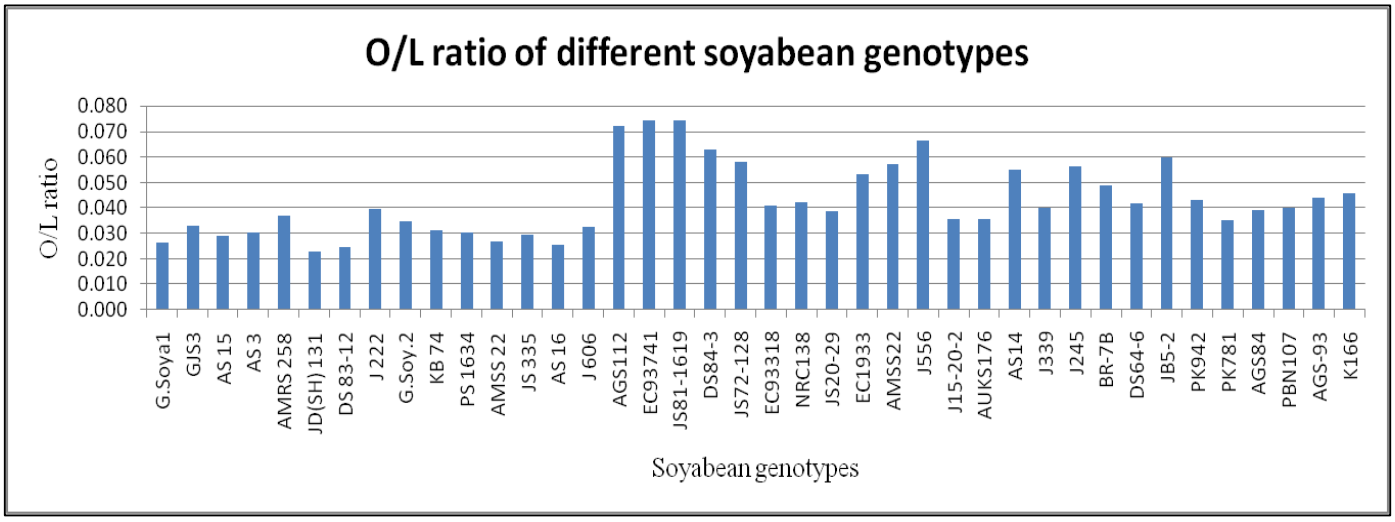

Fig 4: O/L ratio of different soyabean genotype

The result was shown significant difference among 40 genotype the O/L range of (0.025-0.074) among them the cultivars EC9374 (0.074), JS81-1619 (0.074), and AGS112 showing the most variation (0.072). The O/L ratio was found to be quite low in the DS 83-12 (0.025), AS 16 (0.026), and AMSS 22 cultivars (0.027)

4. Conclusion

Soybean genotype origins have an impact on soybean seed FA composition, demonstrating a tendency for genotypes from different ecoregions to have different FA compositions. The results of our correlation coefficients among soybean seed FA components might assist breeders in selecting accessions with better profiles. Our data indicate that the domestication process and recent breeding initiatives have had a significant impact on seed FA composition, resulting in a significant improvement in seed FA composition in promising current cultivars. In general, different geographical origins can give distinct collections of accessions with desirable seed FA composition to suit the nutritional demands of a wide variety of customers with various dietary needs.

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