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Research Article

Fatty acid profiling through GC-MS in oil extracted from thirty varieties of groundnut grown in Gujarat, India

V. B. Gore, P. J. Rathod, A. G. Vala, Gulla Bhavitha, D. Kumar

Abstract

Groundnut is an important crop of Saurashtra region especially for edible oil content. The current information from this experiment focused on profiling of fatty acid, oil content, O/L ratio and MUFA/PUFA ratio. Hexane solvent extracted oil from seeds was used for the fatty acid profile by using Gas chromatography mass spectrometry [GC-MS]. The data showed oil content higher in semi-spreading type varieties with the range of 40.9 to 54.92 % and difference for oil content and fatty acids was found to be statistically significant the unsaturated fatty acids were detected in large amount, whereas saturated were found in small amount in all varieties with irrespective of types. The range of fatty acids varied from 6.83-14.14% of palmitic acid, 2.27-5.25% of stearic acid, 0.97-2.11% of arachidic acid, 1.37-3.54% of behenic acid, 0.45-1.30% of lignoceric acid, 0.03-0.17% of palmitoleic acid, 39.22-82.32% of oleic acid, 0.45-1.79% of gadoleic acid,0.07-0.51% of linolenic acid and 2.26-38.73% of linoleic acid in thirty groundnut varieties comprising 10 each of spreading, semi spreading and bunch type. The oleic to linoleic ratio and MUFA/PUFA ratios found very high in variety GIRNAR-4 and GIRNAR-5 of semi spreading.

Keywords bunch, groundnut, semi spreading, spreading

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Introduction

Groundnut (*Arachis hypogaea* L.) is an important oil-rich seed leguminous plant. It also known as "The King of Oilseeds," is a member of the Leguminosae family. It is the world's fourth largest and third producer in respect to edible cooking oil and vegetable protein respectively. It is a major oilseed crop in India, holding first place in terms of area and second place in terms of production after soybean. Out of the total production of groundnut in India oil extraction accounts for 81% followed by seed 12%, for direct eating 6%, and 1% for exportation. Antioxidants are abundant in oil. It is cholesterol-free and trans-fat-free, and it helps to lower the risk of cardiovascular disease. its oil has 80% UFA constitution whereas saturated fatty acids make up 20% (SFA). Unsaturated fatty acids are composed of 42 % monounsaturated fatty acids (oleic acid) and 37 % polyunsaturated fatty acids (linoleic acid). The three decisive fatty acids oleic acid, linoleic acid, and palmitic acid complete the oil of the peanut. More linoleic acid, a source of rancidity of oil, lowering the shelf life, and off-flavours [1]. Cardiovascular disease (CVD) risk is increased by the increased concentration of Palmitic acid in oil [2]. Additionally, compared to linoleic acid, oleic acid has a 10-fold better auto-oxidative stability [3]. Peanut oil or any other material with well oleic acid amount supreme for dropping the hazards of coronary heart diseases, decreasing the hazard of CVD, sluggish atherosclerosis, and tumorigenesis [4]. Peanut oil is protected from free radicals and other harmful substances by the ideal composition of fatty acids and antioxidants [5]. For nutritional enhancement of peanuts and storage effects, fatty acids level is important [6].

High oleic acid peanut is best as compared to normal peanut for the reason that its many health benefits extended shelf life and application in industries. Fatty acid content determines the flavor, stability, shelf life, and nutritional value of peanuts and peanut products [7]. Due to increased oxidative stability and improved nutritional properties more oleic acid content germplasms of peanuts are important for the recent breeding research point of view. The part of polyunsaturated fat is one of the main determinants of quality. Many varieties of groundnut have been developed by researchers and fatty acid profiling has given very less attention. The present findings from this experiment can help in determining the O/L ratio and MUFA/PUFA ratio of varieties of semi spreading, spreading, and bunch types as well as the lipid indices of groundnut varieties.

Methodology

Seeds of thirty varieties (Table 1) with three types *viz*., spreading, semi spreading and bunch type including each group containing ten varieties were collected from Main Oilseeds Research Station, JAU, Junagadh.

Table 1. List of Varieties used in this study

SN.	Name Variety	Botanical Name	Originating centre	Growth Habit	Year of Release	National Identity No.
1	GG-11	*Arachis hypogaea* L.	JAU, Junagadh	Virginia runner	1984	IC-304950
2	GG-12	*Arachis hypogaea* L.	GAU, Junagadh	Virginia runner	1992	IC-304951
3	GG-13	*Arachis hypogaea* L.	JAU, Junagadh	Virginia runner	-	-
4	GJG-17	*Arachis hypogaea* L.	JAU, Junagadh	Virginia runner	2013	IC-591739
5	GJG-19	*Arachis hypogaea* L.	JAU, Junagadh	Virginia runner	2015	IC-610391
6	GG-HPS-1	*Arachis hypogaea* L.	JAU, Junagadh	Virginia runner	2010	IC-573466
7	GG-41	*Arachis hypogaea* L.	JAU, Junagadh	Virginia runner	2020	-
8	GAUG-10	*Arachis hypogaea* L.	JAU, Junagadh	Virginia runner	1973	-
9	M-13	*Arachis hypogaea* L.	PAU, Ludhiana	Virginia runner	1972	IC-304981
10	JVR-700	*Arachis hypogaea* L.	-	Virginia runner	-	-
11	GG-21	*Arachis hypogaea* L.	JAU, Junagadh	Virginia bunch	2005	IC-445051
12	GJG-22	*Arachis hypogaea* L.	JAU, Junagadh	Virginia bunch	2011	IC- 591738
13	KDG-123	*Arachis hypogaea* L.	MPKV Rahuri	Virginia bunch	2016	-
14	KDG-128	*Arachis hypogaea* L.	MPKV Rahuri	Virginia bunch	2016	-
15	GIRNAR-4	*Arachis hypogaea* L.	ICAR-DGR Junagadh	Virginia bunch	2020	ICGV 15083
16	GIRNAR-5	*Arachis hypogaea* L.	ICAR-DGR Junagadh	Virginia bunch	2020	ICGV 15090
17	KAUSHAL	*Arachis hypogaea* L.	CSAUA&T, Manipuri	Virginia Bunch	1984	IC-304976
18	GG-HPS-2	*Arachis hypogaea* L.	JAU, Junagadh	Virginia Bunch	2018	IC- 625896
19	MALLIKA	*Arachis hypogaea* L.	RAU, Hanumangarh	Virginia Bunch	2009	IC-561239
20	SOMNATH	*Arachis hypogaea* L.	BARC, Mumbai	Virginia runner	1990	IC-113086
21	GG-2	*A. hypogaea* L. ssp. *fastigiata*	JAU, Junagadh	Spanish bunch	1983	IC-304947
22	GG-5	*A. hypogaea* L. ssp. *fastigiata*	JAU, Junagadh	Spanish bunch	1997	IC-305102
23	GG-7	*A. hypogaea* L. ssp. *fastigiata*	JAU, Junagadh	Spanish bunch	2001	-

Continued Table 1.

24	GJG -9	*A. hypogaea* L. ssp. *fastigiata*	JAU, Junagadh	Spanish bunch	2012	-
25	GJG-31	*A. hypogaea* L. ssp. *fastigiata*	JAU, Junagadh	Spanish bunch	2010	IC-583379
26	GJG-32	*A. hypogaea* L. ssp. *fastigiata*	JAU, Junagadh	Spanish bunch	2017	IC-620846
27	SB-II	*A. hypogaea* L. ssp. *fastigiata*	MPKV, Rahuri	Spanish bunch	1965	IC-113088
28	TG-26	*A. hypogaea* L. ssp. *fastigiata*	BARC, Mumbai	Spanish bunch	1995	-
29	TG-37A	*A. hypogaea* L. ssp. *fastigiata*	BARC, Mumbai	Spanish bunch	2004	IC-398406
30	TPG-41	*A. hypogaea* L. ssp. *fastigiata*	BARC, Mumbai	Spanish bunch	2004	IC-296547

Extraction of oil

Seeds of groundnut were coarsely powdered and put in a thimble with 5 g. Thimbles holding samples were inserted in the butt tubes of the soxhlet extraction device and 250 ml of Hexane was poured into the extraction chamber. Heating at 65 ° C. for 8 hours at 150 drops per minute. Before separating the extraction flask, it is allowed to cool. Remove the Hexane from the flask and then let it evaporate until the smell of Hexane is gone. Moisture is removed from the flask, and the flask is weighed. The oil was measured using the succeeding Equation:

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

Determination of fatty acid profile

As method given by Misra and Mathur [8] was used for the fatty acids profile. Methyl group ester was created for this purpose. In a 10 ml test tube take 300 µl of oil and mixed with 3 ml of hexane after that keep at room temperature for 1 hour with intermittent shaking. After 1 hour add freshly prepared 3 ml sodium methoxide and wait for 30 minutes with shaking add 3 ml of 0.8% of NaCl. It results in two layer formation one upper containing the methyl ester shifted to a tube which containing anhydrous sodium sulfate (100mg) after 5 minutes for moisture absorption, then vials were filled by a filtered syringe and kept in a sampler of GC-MS for analysis. A comparative percentage of the total highest area is the basis for the determination of the fatty acid composition. Data analysis like correlation analysis with oil % and Heatmap done by the OPSTAT and HemI 1.0, respectively.

Results and Discussion

Seed samples of 30 groundnut varieties comprising 10 each of spreading, semi spreading and bunch type were analysed aimed at fatty acid profiling, oil, MUFA/PUFA proportion, and oleic and linoleic cids ratio presented in different Tables. Oil % and fatty acid profile of spreading varieties of groundnut present in Table 2.

Oil %

Comparative analysis of oil % was presented in Figure 1. for Spreading, semi spreading, and bunch type varieties of groundnut respectively. The yield extraction of the diverse groundnut varieties which are comparatively high between 40.9-54.92%. The maximum oil % was found to be in the variety GIRNAR-5 and the KAUSHAL variety showed minimum oil content. Table 2 showed the

Table 2. oil % and fatty acid profile of spreading varieties of groundnut

		Saturated fatty acids					Unsaturated fatty acids					Ratios	
Spreading Varieties	**Oil (%)**	**C16:0**	**C18:0**	**C20:0**	**C22:0**	**C24:0**	**C16:1**	**C18:1**	**C20:1**	**C18:3**	**C18:2**	**O/L**	**MUFA /PUFA**
GG-11	47.90	10.59	3.08	1.58	3.32	1.3	0.07	56.47	1.71	0.09	21.79	2.59	2.66
GG-12	47.56	10.86	2.38	1.2	2.21	0.92	0.06	55.83	1.33	Nd	25.21	2.21	2.27
GG-13	50.33	10.39	3.02	1.51	2.93	1.19	0.07	57.57	1.32	0.09	21.91	2.63	2.68
GJG-17	47.54	9.79	2.64	1.23	3.54	0.8	0.07	58.54	1.27	0.07	22.05	2.65	2.71
GJG-19	47.37	10	2.47	1.14	2.01	0.76	Nd	58.12	1.16	0.09	24.25	2.4	2.44
GG-HPS-1	49.57	10.95	3.27	1.72	3.19	1	Nd	53.9	1.08	0.1	24.79	2.17	2.21
GG-41	48.38	11.07	2.9	1.13	1.7	0.74	0.09	56.14	0.95	Nd	25.28	2.22	2.26
GAUG-10	50.87	10.83	2.8	1.16	1.75	0.77	0.09	55.2	0.99	0.07	26.34	2.1	2.13
M-13	48.00	10.39	3.87	1.42	2.92	Nd	0.03	55.79	0.55	Nd	25.03	2.23	2.25
JVR-700	51.70	9.53	3.7	1.27	1.51	0.45	Nd	65.56	Nd	Nd	17.98	3.65	3.65
Mean	**48.92**	**10.44**	**3.01**	**1.34**	**2.51**	**0.88**	**0.07**	**57.31**	**1.15**	**0.09**	**23.46**	**2.49**	**2.53**

mean value of oil 48.92%, 48.55%, and 48.78% for spreading, semi spreading, and bunch type. These results are in line with Bishi et al., [9] who studied 41 Indian groundnut cultivars and also found a similar range for oil (44.1% to 53.8%) content also with Mandal et al., [10].

Saturated fatty acid

An average total of nine fatty acids were commonly found in all thirty varieties of groundnut viz., palmitic acid, stearic acid, gadoleic acid, linolenic acid behenic acid, palmitoleic acid, arachidic acid, oleic acid, and linoleic acid. The range of saturated fatty acids 13.19% to 22.57% was noted. Results from data for the fatty acid profile showed agreement with Sharma et al., [11] their study analyzed seed quality traits in 58 groundnut germplasm. Bishi et al., [9] and Chaudhary et al., [12] they studied five varieties of fatty acid composition by gas liquid chromatography method, and their results showed range for saturated fatty acid content was 10.92 to 17.47%. The data discovered that saturated fatty acids composition in semi spreading type varieties were lower as compared to the composition of bunch type and spreading type (Table 2 and 3). The ranges of fatty acids varied 6.83-14.14% of palmitic acid (C16:0), 2.27-5.25% of stearic acid (C18:0), 0.97-2.11% of arachidic acid (C20:0), 1.37-3.54% of behenic acid (C22:0) and 0.45-1.30% of lignoceric acid (C24:0) in thirty groundnut varieties comprising 10 each of spreading, semi spreading and bunch type (Table-2). Highest palmitic acid among the saturated fatty acids while the lowest percentage of lignoceric acid (C24:0) which was not found in all ten bunch type varieties of peanut varieties was found (Table 4). Among the 30 varieties, palmitic acid was found to be maximum in variety GG-2(14.14%) of bunch type and the minimum was observed in GIRNAR-5 (6.83%) of semi spreading type (Table 2). Among the thirty varieties of groundnut seeds of bunch, type showed higher saturated fatty acids were whereas semi spreading type found to be a lower percentage (Table 4).

Unsaturated fatty acid

There was a variance in unsaturated fatty acid content in three types of groundnuts i.e., more in both semi spreading and spreading when compared to bunch palmitoleic acid (C16:1), oleic acid (C18:1), gadoleic acid (C20:1), linolenic acid (C18:3) and oleic acid(C18:2) were found to be in the range of 0.03-0.17%, 39.22-82.32%, 0.45-1.79%, 0.07-0.51% and 2.26-38.73% respectively. Residues of Polyunsaturated fatty acyl are additionally prone to oxidation, due to this negative impact on stability, and the development of rancidity-related off tastes in stored oil is accelerated. These results are also in line with Nawade et al., [13] and Shin et al., [14]. Three monounsaturated fatty acids were recorded among that oleic acid found to be the maximum percentage while palmitoleic acid was lower in percentage or not detected in some varieties. With a mean value of 23.46%, 21.67%, and 35.72% in spreading semi spreading and bunch type varieties respectively of major Polysaturated

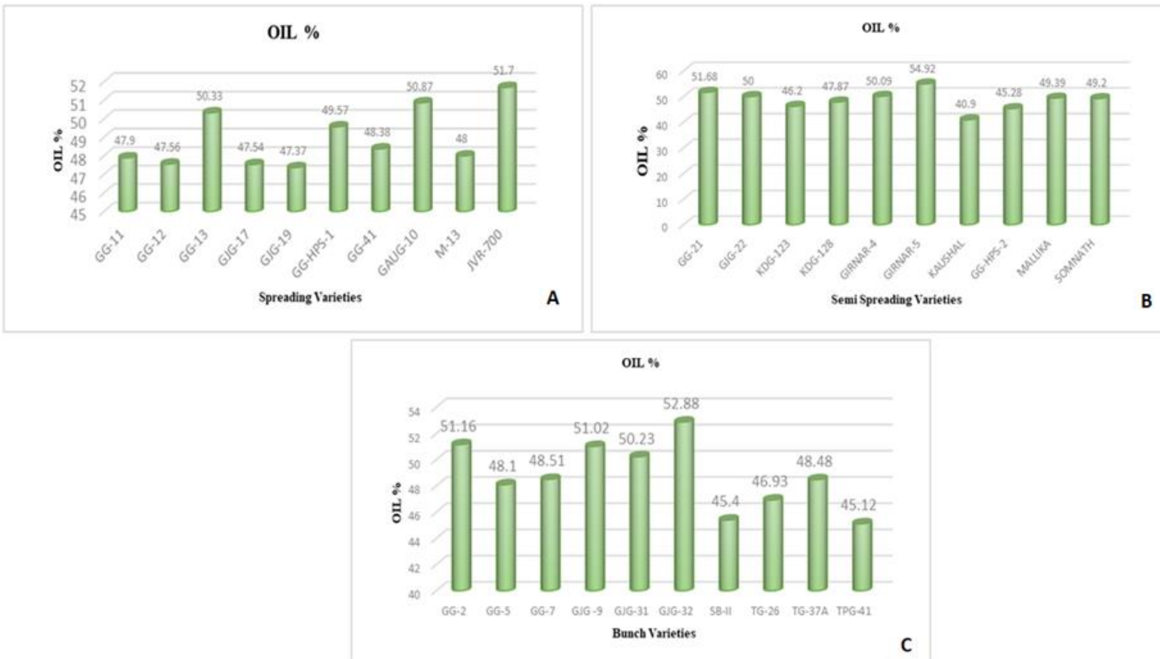

Figure 1. Oil % (A) Spreading (B) Semi spreading (C) Bunch type varieties of Groundnut

Table 3. Oil % and fatty acid profile of Semi spreading varieties of groundnut

Semi spreading varieties	Oil (%)	C16:0	C18:0	C20:0	C22:0	C24:0	C16:1	C18:1	C20:1	C18:3	C18:2	O/L	MUFA /PUFA
GG-21	51.68	9.19	3.37	1.14	1.37	Nd	Nd	65.74	0.64	Nd	18.55	3.54	3.58
GJG-22	50.00	10.77	3.52	1.31	1.91	0.6	0.07	61.09	0.85	Nd	19.88	3.07	3.12
KDG-123	46.20	12.22	3.4	1.37	2.43	0.57	0.13	48.19	0.64	Nd	31.05	1.55	1.58
KDG-128	47.87	12.07	3.27	1.25	2.22	0.49	0.14	47.53	Nd	Nd	33.03	1.43	1.44
GIRNAR-4	50.09	7.03	2.94	1.29	2.81	0.52	0.08	81.5	1.57	Nd	2.26	36.06	36.79
GIRNAR-5	54.92	6.83	2.87	1.21	2.28	Nd	0.06	82.32	1.79	Nd	2.64	31.18	31.88
KAUSHAL	40.90	10.69	2.27	0.97	2.06	Nd	0.07	54.61	1.68	0.07	27.58	1.98	2.04
GG-HPS-2	45.28	10.16	4.99	1.46	Nd	Nd	Nd	58.03	Nd	Nd	25.36	2.28	2.29
MALLIKA	49.39	10.65	4.49	1.71	2.29	Nd	Nd	54.6	0.68	Nd	25.58	2.13	2.16
SOMNATH	49.20	11.95	3.07	1.4	2.08	Nd	Nd	49.3	1.44	Nd	30.76	1.6	1.65
Mean	**48.55**	**10.16**	**3.42**	**1.31**	**2.16**	**0.55**	**0.09**	**60.29**	**1.16**	**0.07**	**21.67**	**8.48**	**8.65**

fatty acids (linoleic acid). Peanut seed oils have important comparative concentrations of linoleic acid (C18:2). Linoleic acid contains anticarcinogenic, antiatherogenic, and antidiabetic modifying qualities that may have positive impacts on health. By decreasing body fat mass and boosting lean mass are some effects of linoleic acid. Leading and recessive alterations in sunflower and groundnut respectively are caused for high oleate traits [15]. Lower temperatures (22–29°C) are often linked to higher linoleic acid production [16]. O/L and MUFA/PUFA ratios were recorded highest in the GIRNAR-4 and GIRNAR-5 varieties of semi spreading type. In all thirty varieties of groundnut, the oleic to linoleic ratio and MUFA/PUFA ratio were found to be ranged from

Table 4. oil % and fatty acid profile of Bunch varieties of groundnut

Bunch Varieties	Oil (%)	C16:0	C18:0	C20:0	C22:0	C24:0	C16:1	C18:1	C20:1	C18:3	C18:2	O/L	MUFA /PUFA
GG-2	51.16	14.14	4.29	1.64	2.46	Nd	Nd	39.22	0.8	Nd	37.45	1.05	1.07
GG-5	48.10	12.87	3.91	1.42	3.2	Nd	Nd	40.23	0.45	0.12	37.8	1.06	1.07
GG-7	48.51	12.94	5.25	2.11	Nd	Nd	Nd	40.97	Nd	Nd	38.73	1.06	1.06
GJG -9	51.02	13.29	5.19	1.79	2.3	Nd	Nd	40.42	0.53	Nd	36.48	1.11	1.12
GJG-31	50.23	12.74	3.32	1.34	2.76	Nd	Nd	43.05	0.8	Nd	35.99	1.2	1.22
GJG-32	52.88	12.92	2.55	1.24	Nd	Nd	0.17	47	Nd	0.36	35.76	1.31	1.31
SB-II	45.40	12.4	3.79	1.87	3.19	Nd	Nd	43.78	Nd	Nd	34.97	1.25	1.25
TG-26	46.93	12.43	3.39	1.67	2.25	Nd	Nd	42.5	Nd	Nd	37.76	1.13	1.13
TG-37A	48.48	12.38	2.9	1.24	1.55	Nd	Nd	43.3	Nd	0.51	38.12	1.14	1.12
TPG-41	45.12	10.41	2.76	1.55	3.53	Nd	Nd	56.23	1.39	Nd	24.13	2.33	2.39
Mean	48.78	12.65	3.74	1.59	2.66	Nd	0.17	43.67	0.79	0.33	35.72	1.26	1.27

Table 5. Correlation matrix of oil and fatty acids for spreading, semi spreading and bunch type varieties of groundnut seed

	Oil	Palmitic acids	Stearic acid	Arachidic acid	Behenic acid	Lignoce-ric acid	Palmitoleic acid	Oleic acid	Gadoleic acid	Linolenic acid	Linoleic acid
Oil	1.000										
Palmitic acids	-0.159	1.000									
Stearic acid	0.089	0.356	1.000								
Arachidic acid	-0.019	0.428*	0.719**	1.000							
Behenic acid	-0.169	-0.123	-0.270	0.075	1.000						
Lignoceric acid	0.021	-0.275	-0.411*	-0.184	0.303	1.000					
Palmitoleic acid	0.074	-0.108	-0.463**	-0.456*	-0.107	0.392*	1.000				
Oleic acid	0.273	-0.977**	-0.357	-0.457*	0.060	0.285	0.198	1.000			
Gadoleic acid	-0.013	-0.527**	-0.535**	-0.323	0.497**	0.430*	0.138	0.531**	1.000		
Linolenic acid	0.091	0.21	-0.312	-0.210	-0.221	-0.019	0.165	-0.224	-0.214	1.000	
Linoleic acid	-0.278	0.964**	0.330	0.389*	-0.172	-0.347	-0.180	-0.989**	-0.598**	0.277	1.000

* Significant, ** Highly Significant

1.05-36.06 and 1.06-36.79, respectively (table 2). These outcomes are also promising with Gulluoglu et al., [6]. In the present study, oleic acid was for GIRNAR-5 (82.32%) and GG-2(39.22 %) while linoleic acid was for GIRNAR-4 (22%) and GG-7 (38.73 %). Jonnala et al., [17] the peanut seeds contained significantly higher 80% (w/w) oleic acid.

Correlation analysis

Table 5 displays the data on the correlation among fatty acids. A strong but negative correlation showed between oleic acid and linoleic acid ($r = --0.989$, $P < 0.001$) [18-19]. As the experimental results of Shasidhar et al., [20] shows the strong negative correlation between oleic and linoleic acid studied. These incompatible collaboration outcomes from the metabolic processes that palmitoyl CoA through in the peanut germplasm to become stearoyl CoA, which is then desaturated to generate oleic acid. Oleoyl-phosphatidylcholine desaturase enzyme was responsible for converting oleic to linoleic acid [21]. With a correlation value of $r=0.964$ Palmitic acid is positively correlated to linoleic acid and negatively correlate to oleic having a value $r = -0.977$, and gondoic acids of value $r = -0.527$. Same negative correlation pattern between palmitic acid with oleic and gondoic acid contents was found by some researchers [22]. But according to the finding of Andersen and Gorbet [18] palmitic acid only negatively correlated with oleic acids.

Venn diagram

As shown in Figure 2 Venn diagram of seven fatty acids was commonly detected in spreading type, semi spreading and bunch type varieties of groundnut specifically palmitic acid, stearic acid, arachidic acid, oleic acid, gadoleic acid, linolenic acid and linoleic acid. Spreading type and semi spreading type share single common saturated fatty acid i.e., lignoceric acid whereas palmitoleic acid and behenic acid are part of both spreading and bunch types. Palmitoleic and behenic acid are only seen in the case of semi spreading type of varieties.

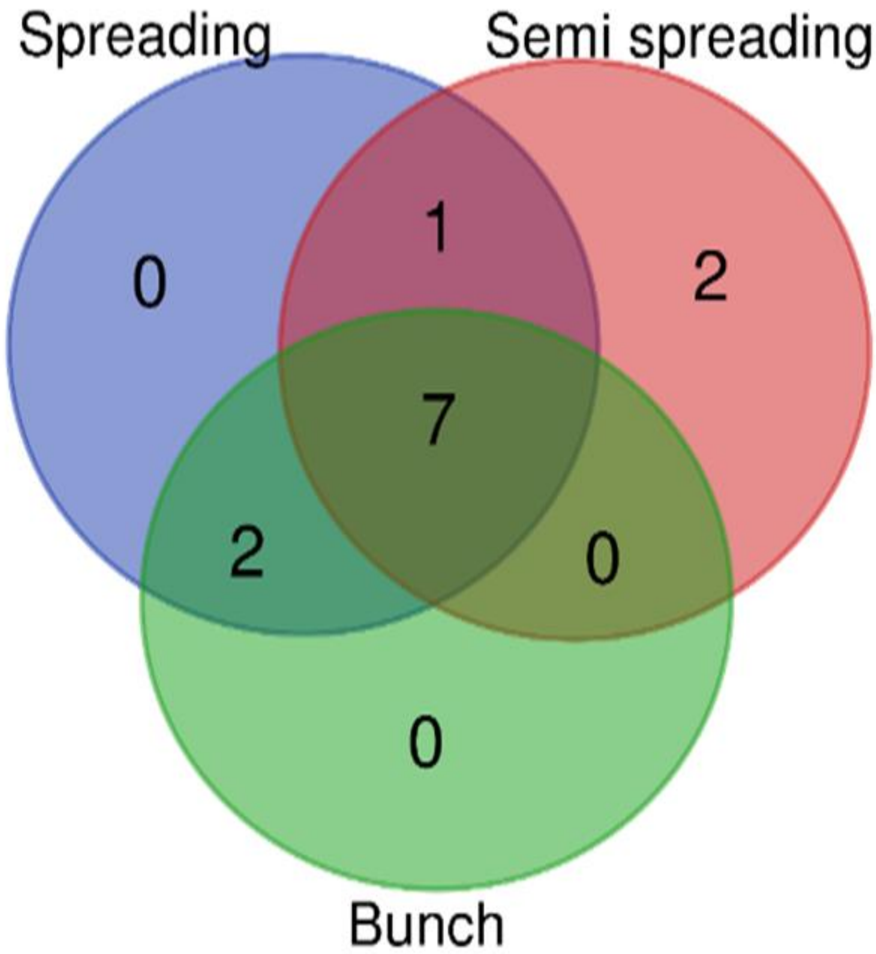

Figure 2. Venn diagram

Heat map analysis

The heat map shows similarities matrix of fatty acids contents where coloring showed intensities as well as cluster wise hierarchical clustering showed Figure 3. Heat map analysis of fatty acids and ratios in oil samples of thirty groundnut varieties revealed a high accumulation of O/L and MUFA/PUFA ratio in varieties such as GIRNAR-4 and GIRNAR -5. Fatty acids such as linolenic acid were detected highest only TPG-37A oil sample.

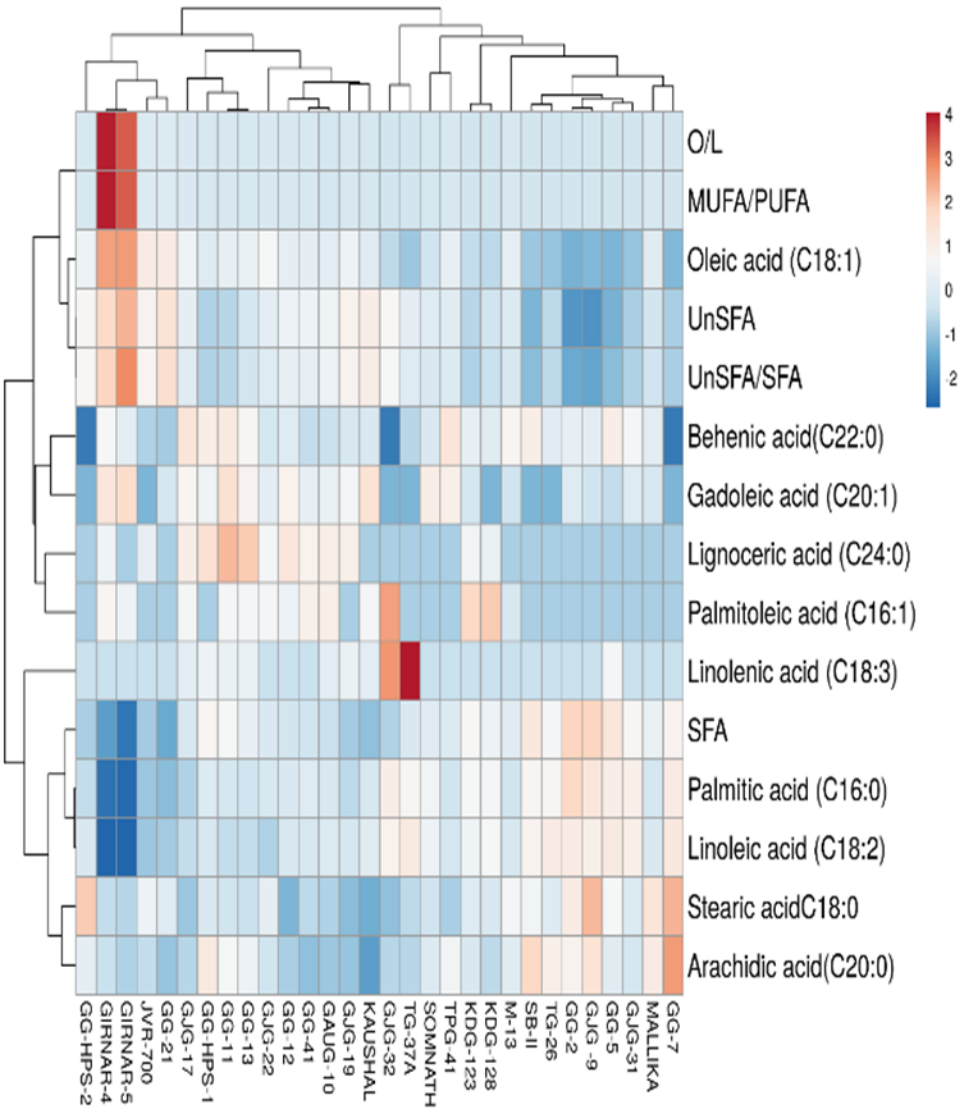

Figure 1. Heat map analysis showing the fatty acids O/L, MUFA/PUFA ratios of thirty groundnut varieties

Conclusion

The findings of this study will encouragement for groundnut food processors, breeders, and geneticists in deciding varying oil % as well as changes in the ratio of oleic to linoleic acid in groundnut fully-fledged programs. However, heterogeneity in fatty acid profiling in distinct types can be useful in identifying quality traits in groundnut genotypes from this study. Groundnut oils desire health advantages to humans and longer shelf life with more oleic/linoleic acid ratios.

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