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FTIR Characterization and Physicochemical Evaluation of Cottonseed Oil

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Abstract

In present study, cottonseed (*Gossypium*) varieties (RH-112, Lankart-57, K-25, F-20 and D-9) were evaluated for possible variation in quality attributes by using conventional and instrumental techniques. Physiochemical characteristics of seed and oils varied as: weight of individual seed (0.069-0.085 g), weight of 100 seeds (5.95-7.73 g), width (0.461-0.544 cm), length (0.736-0.915 cm), moisture (6.2-8.5%), oil content (12.01 to14.55%), refractive index at 40^{o}C (1.4661-1.4665) iodine value (IV) (93.90-105.76 gI_2/100g), saponification value (SV) (181.83-190.55 mg KOH/g), peroxide value (PV) (1.0-6.0 m Eq/Kg), free fatty acids (FFA) (17.30-38.80 %) and induction period (IP) (1.95 -2.65 h), respectively. Analyzed varieties showed higher level of FFA (17.30-38.80%), while lower level of IP (1.95-2.65 h) respectively. Furthermore, GC-FID and FT-IR studies were carried out for quantitative and qualitative analysis of cottonseed oil. Analysis showed that most abundant fatty acid in each variety was linoleic acid (42.09-52.55 %) among unsaturated fatty acids, whereas palmitic acid (22.70-26.20 %) was major saturated fatty acid. Some band intensities of FTIR spectra highly correlated with the chemical properties of cottonseed oil such as IV, SV, PV, FFA and IP. In conclusion significant variation in physiochemical properties was observed among five cottonseed varieties, especially FFA and IP of cottonseed oil which is not good for the quality and edible point of view. This can be associated with the specific genetic variability and climatic conditions.

***Keywords*:** Cottonseed, Oil yield, Physiochemical parameters, GC-FID, FTIR spectroscopy.

Introduction

Cotton is a shrub that grows to about 40 cm high; belongs to Malvaceae family and its genus Gossypium has marketable significance and food value [1]. To improve the yield of crop and its productivity several varieties of cotton have been introduced to the market [2]. Gossypium *hirsutum* is one of the most significant crops (fiber/food) and native to tropical and subtropical regions in the world. After soybean, cotton is assumed as one of the most excellent source of plant (vegetable) protein and the fifth major seed oil crop after sunflower, canola, palm and soybean [3].

Cottonseed oil is obtained from the seeds of cotton plant and known as a by-product with about (12%) of the gross value of the total product. Cottonseed oil is usually utilized in cooking or frying and also used in some other industrial applications, whereas cakes after oil extraction are used in the preparation of poultry and animal feeds. Due to unique fatty acid profile, cottonseed oil is different among other vegetable oils as it holds a comparatively high level of unsaturation and considered as a healthy vegetable oil. Its fatty acid composition is distinctive of the oleic/linoleic group of vegetable oils, as these two fatty acids make up 73% of the total fatty acids (oleic acid and linoleic acid 17% and 56%, respectively) along with palmitic acid approximately (23%) [4].

Owing to the high level of antioxidant (tocopherol) and oleic acid, the oil has good oxidative stability [5]. The quality of vegetable oils, especially the fatty acids profile and physicochemical properties are an imperative feature in determining the market value and commercial uses [6]. It is usually acknowledged that the chemical composition, functional properties, nutritive value and yield of plant seed oils are notably affected by the certain factors such as geographic, agroclimatic and genetic/varietal [7].

The aim of this study was to check the variations in the physiochemical properties of selected cottonseed varieties (RH-112, Lankart-57, K-25, F-20 and D-9) collected from Seed Unit, Institute for Agricultural Research, Tandojam, Pakistan and Fourier transform infrared characterization of cottonseed oil.

Materials and Methods

Reagents and sample collection

All the chemicals and reagents used in the present work were purchased from E-Merck (Dermastd, Germany). The seed of five different cottonseed varieties (Gossypium specie) RH-112, Lankart-57, K-25, F-20 and D-9 were collected directly from the Seed Unit, Institute for Agricultural Research, Tandojam, Pakistan. Seeds were packed in clean sampling bags and classified according to their morphological characteristics. Collected samples were dried and cleaned properly to remove foreign materials and other impurities at room temperature in the absence of light.

Physical parameters

Some physical characteristics of cottonseed and oil of different varieties were analyzed such as individual seed weight, weight of 100 seeds, thickness, length, moisture and refractive index according to the American Oil Chemists Society (AOCS) method [8].

Chemical parameters

For the chemical characterization of different varieties of cottonseed oil, official methods were used to check oil content, iodine value, saponification value, peroxide value, free fatty acids and oxidative stability as reported in AOCS method [8].

Oil content

Crude oil from cottonseed varieties were extracted by Soxhlet extraction method using n-hexane as a solvent with slight modification as reported in AOCS standard method [8]. Approximately, 20 g of ground cottonseed were transferred to cellulose thimbles and carefully placed inside the Soxhlet extractor, then 300mL of solvent was added to round bottom flask. The process continued for 5 hours at a fixed temperature of 70°C. At the end of extraction the solvent was distilled under vacuum in a rotary evaporator (Buchi, Switzerland). The extracted oil was transferred to a desiccator and allowed to cool before being weighed and stored at 5°C till further analysis.

Oxidative stability

The oxidative stability of cottonseed oil was determined by an automatically controlled Metrohm Rancimat apparatus (Model 679) to record the induction period (IP). The operating temperature of Rancimat instrument was maintained at 120 ± 0.1°C. Samples in duplicate (2.5 g) were weighed and placed in glass reaction tubes and run at the same time. The IP of cottonseed oil was noted automatically and linked with the break points of the curves plotted as reported earlier [9].

GC-FID analysis of cottonseed oil

The complete profile of fatty acid composition of different varieties of cottonseed oil was determined by GC-FID according to IUPAC standard method [10]. Fatty acid methyl esters (FAMEs) were analyzed on a Perkin Elmer gas chromatograph model 8700, fitted with a SP-2340 poly (bis-cyanopropyl siloxane) column (60 m x 0.25 mm), with 0.2 µm film thickness. Oxygen free nitrogen was used as a carrier gas at a flow rate of 3.5 mL/min. The temperature programming were as follows: initial oven temperature 130°C, ramp rate 4°C/min, final temperature 220°C followed by 15 minutes hold time, injector temperature 260°C and detector temperature 270°C. A sample volume of 1.0 µL was injected. FAMEs were identified by comparing their relative and absolute retention times to those of authentic standards of FAMEs obtained from Sigma Chemical (St. Louis, MO, USA).

Statistical analysis

The cottonseed samples were analyzed in triplicate. Data were reported as means ± Standard deviation (n=3×3).

Results and Discussion

Physical parameters of seed and oil

The physical properties of five selected varieties RH-112, Lankart-57, K-25, F-20 and D-9 of cotton (*Gossypium*) varieties collected from the Seed Unit, Institute for Agricultural Research, Tandojam, Pakistan are given in Table 1. Some significant variations in individual seed weight, width, length, moisture content and refractive index were observed among the five cottonseed varieties. This variation might be due to different environmental conditions and soil types [7].

Individually the variation in different varieties are as follow: individual seed weight of cottonseed found in the range of 0.069-0.085g, weight of 100 seeds 5.95-7.73 g, width 0.461-0.544 cm, length 0.736-0.915cm, and moisture content 6.2-8.5%. Whereas refractive index (at 40^{o}C) of cottonseed oil was found in the range of 1.4661-1.4665 as shown in Table 1. The values are comparable with the reported values of cottonseed oil grown in Bahawalpur (1.4607-1.4632) [11]. NIAB-III variety 1.4643 harvested in Faisalabad [12], and Nigerian cottonseed oils (1.458-1.466) [13]. The moisture content of cottonseed varieties harvested in Tandojam, Sindh was found in the range of 6.2-8.5%. These values were comparable to the NIAB-III variety grown on a saline (7.1%) and a non-saline (7.2%) area of Punjab [12], and Desi variety (8.44%) grown in Bahawalpur region [11]. On the other hand moisture content values of studied varieties harvested in Tandojam, Sindh were lower than that reported in the literature for cottonseeds (9.9%) [14].

***Table 1.* Physical properties of cottonseed and oil.**

Parameters	RH-112 Mean±SD	Lankart-57 Mean±SD	K-25 Mean±SD	F-20 Mean±SD	D-9 Mean±SD
Weight of individual Seed	0.071±0.015^{a}	0.069±0.006^{a}	0.077±0.001^{b}	0.085±0.011^{c}	0.077±0.017^{b}
Weight of 100 seeds	6.61±0.005^{b}	5.95±0.045^{a}	7.32±0.01^{c}	7.73±0.005^{d}	7.32±0.060^{c}
Width (cm)	0.544±0.099^{c}	0.467±0.001^{a}	0.464±0.002^{a}	0.472±0.003^{b}	0.461±0.004^{a}
Length (cm)	0.787±0.002^{b}	0.915±0.004^{d}	0.802±0.001^{c}	0.801±0.003^{c}	0.736±0.005^{a}
Moisture (%)	7.5±0.97^{b}	8.5±0.99^{c}	8.0±0.98^{c}	6.2±1.16^{a}	6.8±1.01^{a}
Refractive Index (40^{o}C)	1.4664±0.00025^{a}	1.4662±0.00011^{a}	1.4665±0.00030^{a}	1.4661±0.00020^{a}	1.4663±0.00029^{a}

Chemical parameters of oil

Chemical properties are the main quality characteristics of any vegetable oil. These parameters are very important for the industrial uses as well as edible point of view. The hexane extracted oil content of five selected cotton (*Gossypium*) varieties is given in Table 2. The oil content of different varieties of cottonseed ranged from 12.01 to 14.55 %. A noteworthy variation among the varieties was observed for oil content. The oil yield was highest (14.55%) in variety F-20, whereas lowest (12.01%) in D-9. The oil content present in analyzed varieties is much lower than the cotton varieties harvested in other regions of Pakistan such as Bahawalpur N-121 (18.35%) [11], Faisalabad NIAB-III (18.60%) [12], and Peshawar SLH-279 (30.15%) [15]. The possible variation in oil content among local cottonseed varieties might be due to genetics of cotton plant and change in different agro-climatic conditions. On the other hand oil content present in studied varieties were somehow comparable to few cotton species G. *arboretum* (14.4%) and G. *hirsutum* (15.8%) grown in India [16], and Nigeria (15%) [17]. According to Ashokkumar & Ravikesavan seed oil yield in cotton is controlled by the environmental factors and highly influenced by gene multiple [18].

Other chemical properties of cottonseed oil were determined such as IV, SV, PV, FFA and IP. Among the analyzed parameters maximum IV was noted for RH-112 cottonseed variety 105.76 g I_2/100g, while Lankart-57 shows minimum 93.90 g I_2/100g. These values are comparable with the reported IV of cottonseed grown in Bahawalpur (100.54-108.73 g I2/100g) [11], and Faisalabad region (102.2-103.0 gI2/100g) [12]. Whereas much lower than Nigerian cottonseed oil (119.78 g I_2/100g). The IV is a key indicator for evaluating the unsaturation of any oil. According to Pritchard the IV of cottonseed oil falls in between (99- 119 g I_2/100g) [14]. In current study SV of cottonseed oil was determined in the range of 181.83-190.55 mgKOH/g. The SV of the examined cottonseed oil are in line with those given within the literature for cottonseed oils [11]. However, SV of Nigerian cottonseed variety 199.42 mg of KOH/g was higher than the values reported in present study [19]. Formation of hydroperoxide products in fats and oils reflects the peroxide value. In comparison to literature reported values, examined cottonseed varieties showed relatively higher PV, except variety D-9 which was lower than the cottonseed varieties grown in Bahawalpur region (1.81-1.98 mEq/kg of oil) [11]. FFA gives information regarding oxidative deterioration of seed oil by chemical or enzymatic reactions. The quality of oil depends on the presence of FFA. In current study all cottonseed varieties showed higher FFA content in the oils and found in the range of 17.30-38.80%. These FFA values are much higher than the FFA values reported in the Bahawalpur region 0.71-1.24% [11]. The high level of FFA in edible oil is not recommended for edible purpose. According to Rossel, oxidative stability of oils is directly linked with the induction period. The IP (Rancimat, 20 L/ h, 120°C) of cottonseed oil was determined in the range of 1.95-2.65 h [20]. The maximum and minimum values of IP were recorded for Lankart-57 and F-20 varieties. These values are comparatively lower than the IP values of cottonseed oil grown in Bahawalpur region 3.19-3.61 h [11].

Table 2. **Chemical properties of cottonseed oil.**

Chemical Parameters	RH-112 Mean±SD	Lankart-57 Mean±SD	K-25 Mean±SD	F-20 Mean±SD	D-9 Mean±SD
Oil Content (%)	14.52±1.06^c	13.53±0.99^b	12.02±1.00^a	14.55±0.56^c	12.01±0.99^a
Iodine Value (gI_2/100g of oil)	105.76±0.82^d	93.90±0.71^a	105.75±0.77^d	97.29±0.94^c	96.44±1.22^b
Saponification Value (mgKOH/g)	181.83±1.08^a	183.91±0.89^c	182.83±1.15^b	183.55±1.00^c	190.55±1.20^d
Peroxide Value (mEq/kg)	5.0±1.04^c	6.0±0.98^c	5.5±0.10^c	4.0±1.0^b	1.0±0.90^a
Free Fatty Acid (%)	23.70±0.86^b	38.80±1.10^d	35.60±1.05^c	17.30±0.91^a	18.60±1.02^a
Induction Period Rancimat Method (h)	2.41±0.032^b	1.95 ± 0.015^a	2.11±0.029^a	2.65±0.089^b	2.61±0.076^b

Fatty acid composition

Table 3 shows the fatty acid composition of extracted oil of cottonseed varieties. Analysis showed that most abundant fatty acid in each variety was linoleic acid (42.09-52.55%), followed by oleic acid (16.14-19.37%) among the unsaturated fatty acids. On the other hand palmitic acid (22.70-26.20%) was the major saturated fatty acid followed by stearic acid (2.14- 14.72%) and myristic acid (0.22-3.17%), respectively. In comparison to the literature values, except Lankart-57 and D-9, rest of the varieties showed higher content of linoleic acid than the varieties grown in Bahawalpur region 48.96- 50.46% [11]. Whereas D-9, Lankart-57 and F-20 were relatively lower in linoleic content, RH-112 showed almost similar results and K-25 were higher in the linoleic content (50.6%) as compared to the cottonseed grown in the Faisalabad [12]. Highest and lowest palmitic acid was determined in RH-112 and Lankart-57. Evaluated cottonseed varieties showed lower palmitic acid values as compared to the cottonseed variety of Faisalabad (27.0%) [12], while in comparison to the cottonseed of Bahawalpur region except Lankart-57 and D-9, rest of the varieties revealed higher content of palmitic acid. Previously, myristic and palmitoleic acid in Pakistani cottonseed varieties has not been reported in the literature, whereas in present study both fatty acids were detected in considerable amounts. In comparison to the world literature, both fatty acids have been reported in some Turkish cottonseed varieties (0.67-1.08% and 0.82-1.23%) although reported values were relatively lower than the current analyzed cottonseed varieties [21].

Table 4 represents the comparison of fatty acid profile of indigenous Pakistani cottonseed varieties with Turkish and Indian cottonseed varieties. High content of oleic acid in cottonseed oil represents the good quality of oil. The unique oleic acid in indigenous varieties was higher than Turkish (13.96-17.60%) varieties [21]. Also high content of stearic acid was determined in local varieties in comparison to the Indian (1.1-4.5%) [22] and Turkish (1.87-2.37%) varieties. The present work has detected almost all the fatty acids as reported previously.

Table 3. **Fatty acids composition of cottonseed oil %.**

Fatty Acids (%)	RH-112 Mean±SD	Lankart-57 Mean±SD	K-25 Mean±SD	F-20 Mean±SD	D-9 Mean±SD
C14:0	1.33±0.11^{c}	0.22±0.03^{a}	3.45±0.13^{d}	3.17±0.900^{d}	0.33±0.08^{b}
C16:0	26.20±1.01^{d}	22.70±0.94^{a}	24.73±0.84^{c}	24.64±1.01^{c}	23.34±0.13^{b}
C16:1	0.42±0.07^{a}	4.05±0.05^{d}	0.57±0.02^{a}	0.87±0.04^{b}	3.38±1.02^{c}
C18:0	2.14±0.09^{a}	8.83±0.88^{c}	2.49±0.08^{a}	4.09±0.92^{b}	14.72±0.90^{d}
C18:1	19.37±0.99^{d}	18.50±1.27^{c}	16.21±0.74^{a}	17.96±0.93^{b}	16.14±0.93^{a}
C18:2	50.54±1.01^{c}	45.70±1.09^{b}	52.55±0.02^{d}	49.80±0.80^{c}	42.09±0.10^{a}
ΣSFA	29.67	31.75	30.67	31.39	38.39
ΣMUFA	19.79	22.64	16.78	18.83	19.52
ΣPUFA	50.54	45.70	52.55	49.80	42.09

Table 4. **Comparison of fatty acid composition of cottonseed oil % with other countries.**

Fatty Acid (%)	Turkish[21]	India[22]	Pakistan[11]	Current Study
Myristic (C14:0)	0.67-1.08	-	-	0.22-3.45
Palmitic acid (C16:0)	20.11-26.77	8.83-28.0	24.42-25.8	22.70-26.20
Palmitoleic acid (C16:1)	0.82-1.23	-	-	0.42-4.05
Stearic acid (C18:0)	1.87-2.37	1.1-4.5	2.49-2.81	2.14-14.72
Oleic acid (C18:1)	13.96-17.60	10.3-30.7	17.81-23.15	16.14-19.37
Linoleic acid (C18:2)	51.19-59.15	20.6-59.3	48.96-50.46	42.09-52.55

FTIR characterization of cottonseed oil

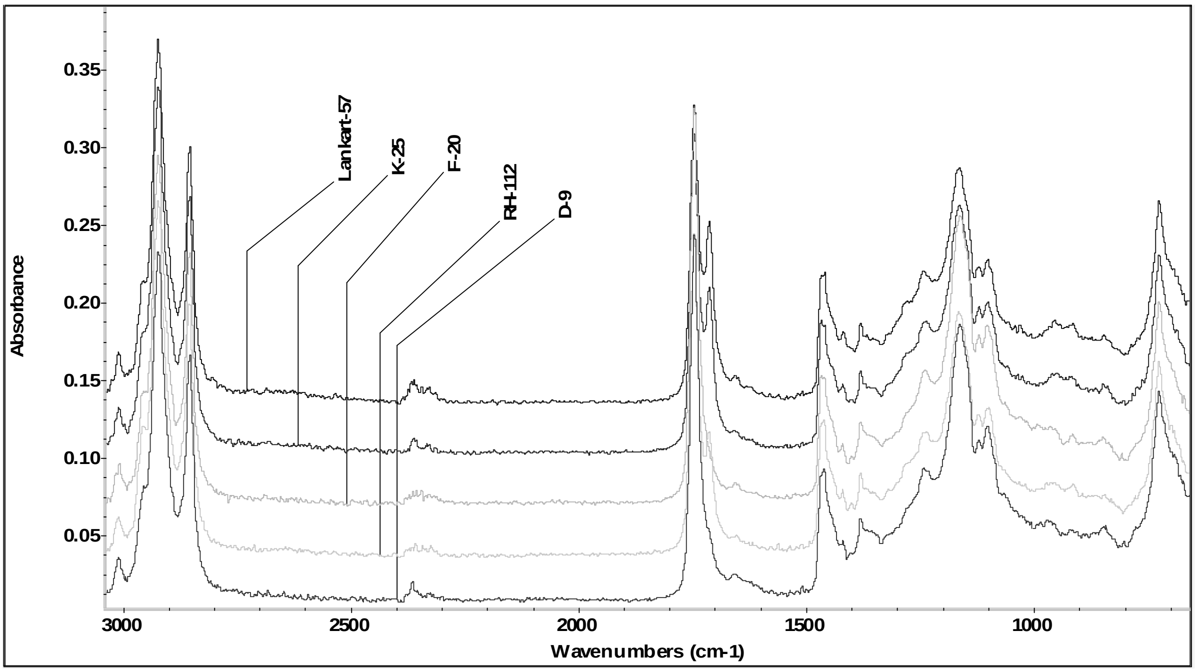
FTIR spectroscopy is a versatile analytical instrument and has been used for the qualitative and quantitative determination of many noteworthy parameters of vegetable oils [23]. The FTIR spectrum in the mid IR region consists of various basic and feature bands. The specific region of FTIR spectrum indicates presence or absence of particular functional groups. The FTIR study was carried out to check the variation in the intensity of common bands typically present in the edible oils. (Fig. 1) shows the FTIR spectra of five analyzed cottonseed oil varieties. The intensity of each functional groups present in cottonseed oil is given in Table 5. All varieties showed nearly similar spectra except some bands, which can be witnessed in the Table. On the basis of variations in some functional groups, the intensities were correlated with some chemical parameters of cottonseed oil.

The band at 721 cm^{-1} is correlated with the SV, as it can be seen from the Table 2, that highest SV was observed in D-9 and lower in RH-112, similarly higher intensity was observed in D-9 and lower in RH-112. IP was correlated with two bands of spectrum at 1161, 1743 cm^{-1}, as it can be seen from the Table 2, that highest IP was observed in RH-112 and lower in Lankart-57. Similar trend in higher and lower intensity was observed in RH-112 and Lankart-57, respectively. FFA is the main quality parameter of edible oil. Many have reported the band of carbonyl at ~1709 cm^{-1} for the determination of FFA [23]. In this study carbonyl band was associated with the FFA present in cottonseed oil. Highest FFA was observed in Lankart-57 and lower in F-20, same trend was also observed in the intensity of Lankart-57 and F-20 as shown in Table 5. Highest and lowest PV was observed in Lankart-57 and D-9, respectively. The band at 2852 cm^{-1} was linked with the PV of cottonseed oil. As it can be seen from the Table 5, the highest and lowest intensity value was observed in same cottonseed varieties. The band at 3009 cm^{-1} showed association with the IV of cottonseed oil. It was clear from Table 2, that highest IV was determined in RH-112 and lower in Lankart-57. Similarly higher intensity was observed in RH-112 and lower in Lankart-57.

***Table 5*. FTIR spectral intensity of different functional groups of cottonseed oil.**

Frequency (cm^{-1})	Intensity (RH-112)	Intensity (Lankart-57)	Intensity (K-25)	Intensity (F-20)	Intensity (D-9)	Observation
721	0.129^{a}	0.138^{b}	0.135^{b}	0.137^{b}	0.142^{c}	–(CH2)n– Rocking bending (out-of-plane)
841	0.0442^{a}	0.0519^{c}	0.0519^{c}	0.0491^{b}	0.0555^{d}	=CH2 Wagging
909	0.0507^{a}	0.0591^{d}	0.0565^{c}	0.0514^{a}	0.0532^{b}	–HC = CH– (cis) Bending (out of plane)
951	0.0516^{a}	0.0611^{d}	0.0590^{c}	0.0560^{b}	0.0594^{c}	–HC = CH– (trans) Bending (out of plane
1095	0.100^{a}	0.100^{a}	0.104^{b}	0.122^{c}	0.120^{c}	–C – O, –CH2– Stretching and Bending
1118	0.0955^{a}	0.0956^{a}	0.102^{b}	0.115^{c}	0.111^{c}	
1161	0.1670^{d}	0.159^{a}	0.162^{a}	0.192^{c}	0.185^{b}	
1233	0.0842^{a}	0.0929^{b}	0.0923^{b}	0.0924^{b}	0.0925^{b}	
1377	0.0580^{a}	0.0590^{b}	0.0598^{c}	0.0591^{b}	0.0603^{c}	–C – H (CH3) Bending (sym)
1416	0.0382^{a}	0.0539^{d}	0.0515^{c}	0.0451^{b}	0.0460^{b}	=C – H (cis) Bending (rocking)
1456	0.0920^{b}	0.0926^{c}	0.0927^{c}	0.0888^{a}	0.0920^{b}	–C – H (CH3) Bending (asym)
1653	0.0173^{a}	0.0258^{d}	0.0208^{b}	0.0198^{b}	0.0243^{c}	–C = C (cis) Stretching
1709	0.0845^{c}	0.126^{d}	0.114^{d}	0.040^{a}	0.052^{b}	–C = O (acid) Stretching
1743	0.215^{b}	0.200^{a}	0.212^{b}	0.262^{d}	0.244^{c}	–C = O (ester) Stretching
2852	0.168^{a}	0.173^{b}	0.171^{b}	0.167^{a}	0.166^{a}	CH2 Stretching (sy)
2922	0.234^{a}	0.243^{b}	0.244^{b}	0.231^{a}	0.232^{a}	CH2 Stretching (asy)
3009	0.0371^{d}	0.0294^{a}	0.0370^{d}	0.0353^{c}	0.0325^{b}	C-H stretching vibration of the cis double bond (=C-H)

Figure 1. **FTIR group spectra of cottonseed oil varieties in the region of 3030-650 cm^{-1}.**



Conclusion

The results of the current study showed that most of the physicochemical characteristics of cottonseed oil collected from Institute for Agricultural Research, Tandojam were quite identical with local or international varieties except FFA and IP. Analyzed verities showed higher level of FFA (17.30-38.80%), while lower level of IP (1.95 -2.65 h) respectively, which is not good for the edible and quality point of view. First time FTIR has been used for the correlation of chemical properties of cottonseed oil with the intensities of different functional groups. Among the chemical properties SV, IV, PV, FFA and IP values of cottonseed oil varieties were matched with the respective intensities of 721 cm^{-1}, 3009 cm^{-1}, 2852 cm^{-1}, 1709 cm^{-1} and 1161, 1743 cm^{-1}. It could be concluded that in future FTIR spectroscopy may be utilized as a quality check for other vegetable oils.

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