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Application of Fourier transform infrared spectroscopy for the comparison of genetically modified Maize from non-GM maize varieties

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Abstract
ATR-FTIR spectroscopy was applied for the comparison of GM maize with non-GM maize. The grains were also analysed for changes in the nutrient composition. From the results of FTIR analyses, GM maize was distinguished from non-GM varieties of maize based on the absorption wavelength or wave number of a particular nutrient group. To perform the study, first, we identified the main wave numbers of the FTIR spectrum. A thorough investigation was conducted to compare them individually on the fingerprint region of a specific nutrient group. It was observed that there were minor differences in the grain samples when considering the wide genetic variety of the maize under examination. The highest protein content of 12.92% was observed in GM MON 810, followed by WS3 with 10.52%. The area under the FTIR spectrum was marked in the following order GM-MON 810> WS3>WS2>WS5>WS4>WS1. It was concluded that the WS3 sample of maize was found to have similar functional groups and a larger area under the spectrum as that of GM MON 810 than the other samples. This quick and non-destructive technique can thus be used to spot suspect samples, which can be further analyzed. Hence there is a necessity to investigate the genetic modifications in the maize grains sown in India.

Keywords: Fourier transform infrared spectroscopy, Maize, Genetic modification

1. Introduction
The term "GM foods" refers to the food items made from genetically modified crops created using genetic engineering techniques. (South, Gautam, and Kushwaha 2018) [18]. The USA, Brazil, Argentina, Canada and India are the top five GM growing countries. Genetically modified maize, soybeans, canola, cottonseed oil, potatoes, and sugar beet make up most of the key GM foods with herbicide tolerance and insect resistance worldwide. (Bhawan *et al*. 2014) [5]. Maize is the world's most important grain crop in which genetic engineering has been used extensively to improve many of its features (Yadava *et al*., 2017) [19]. Genetic engineering can raise the quality of food. The availability of foods with higher mineral, vitamin, and nutritional content benefits consumers. (Brandner 2002) [6]. Customers are apprehensive about the safety of genetically modified (GM) foods because of concerns about potential health and environmental risks. So far, FSSAI has not permitted GM foods in India (Khurana, Taneja, and Khullar 2018) [13]. Monitoring GM foods is necessary for public awareness and labelling of GM foods is a significant part of monitoring. Beginning on March 1 2021, FSSAI had mandated 'GM-free certificate and non-GM origin certificate' for the import of 24 of the specified food consignments into India. (Zealand and Africa 2021). Maize (*Zea mays*) is one of the listed food crops.

MON 810, the genetically engineered corn variety, is grown worldwide from the Monsanto Corporation branded as Yield Gard. This plant was genetically altered (GMO) to stave off crop loss from insects. The DNA of MON 810 has a gene introduced from *Bacillus thuringiensis* that enables the plant to produce a protein (Bt) that is toxic to insects that attempt to consume it.

Total attenuated reflectance (ATR)-based Fourier transform infrared spectra (FTIR) has frequently been used to evaluate product composition and spot chemical alterations by various processing techniques. In actuality, it is a non-destructive technology that also saves laborious preparation steps. ATR-FTIR was used for differentiating the maize flour samples based on location in southern Brazil (Kuhnen *et al*. 2010) [14].

FTIR spectroscopy was also a tool for determining feed authentication by discriminating grain maize based on different geographical origins (Achten *et al*. 2019) [2].

2. Materials and Methods

2.1 Certified Reference material (CRM)

The standard CRM consisting of 10% MON 810 Genetically Modified maize Powder Standard was purchased from Sigma Aldrich Chemicals. The obtained CRM was used as the standard for comparing functional groups of GM maize with the collected maize samples.

2.2 Sampling

Raw maize grains (5 different varieties) were collected from farmers of four different states in India (Table 1). A laboratory electric grinder was used to grind and homogenize all samples. The obtained ground sample is stored in a deep freezer until further usage.

Fig 1: Maize grains

Table 1: Details of Maize Products

Sample code	Product category	Origin
WS1	Maize grains	Telangana
WS2	Maize grains	Kerala
WS3	Maize grains	Tamilnadu
WS4	Maize grains	Telangana
WS5	Maize grains	Karnataka

*WS code is assigned for the wild variety of maize samples

2.3 Analysis of Proximate composition

Analysis of the proximates (moisture content, crude protein, carbohydrate, crude fat, crude fibre and ash contents) of all the maize samples collected were conducted in NIFTEM-T laboratory using the standard Association of Official Analytical Chemists methods [2005].

2.4 FTIR analysis

ATR-FTIR (Shimadzu IR tracer-100) spectrophotometer was used to measure the spectra of all the chosen samples. In the transmittance mode, all spectra were captured. For each sample, 45 scans in the spectral region of 4000-400 cm^{-1} were performed using the transmission technique. Background spectra was measured before proceeding for sample analysis. Then the powdered samples are placed directly on the ATR-diamond crystal of FTIR equipment for measurement. The ATR diamond crystal surface was cleaned with chloroform after each sample measurement with lint-free tissues. All the samples were measured in triplicates

Since it is ATR-FTIR, there is very little sample preparation required. To avoid moisture from the air, powdered samples were stored in a hot air oven at 50 °C until the start of the FTIR analysis.

2.5 Statistical analysis

The proximate parameters were analyzed in triplicate, and the results were expressed as mean ± SD. The data were statistically evaluated by one-way analysis of variance followed by the Tukey method using Minitab Statistical Software. $P< 0.05$ was used to define the statistical level of significance.

3. Results and Discussion

Safety assessment of maize grains is an important topic of concern related to public health. Knowledge of safety and traceability is a must as per the present scenario. It is crucial to determine whether the genetically modified maize composition is identical to conventional maize or differs noticeably from it. FTIR is used as a basic, non-destructive approach which promises reliable results in the least time for the same.

3.1 Proximate analysis

Proximate composition is a crucial factor in evaluating the general composition and nutritional content of any component utilized for use in food. The results of the proximate composition of maize grain samples and the standard GM maize powder (MON 810) are presented in table 2. Moisture is an important parameter that decides the shelf life of the product as well as the growth of microbes and also attributes to the germination of seed. The moisture content of the grains in the present study was between 10% and 12%. The moisture variation is due to the hygroscopic nature of the seed and surrounding environment and sample handling. Proteins, also known as a major building block, is chosen as one of the important parameters for food and feed. Proteins pose a unique challenge when it comes to analysing nutrition because they have the potential to behave as toxins (like phytohemagglutinin), anti-nutrients (like protease inhibitors), or allergies, and they may also be involved in the synthesis of these substances. In addition, in contrast to the genome, which remains unchanged throughout the life of an organism, the proteome is highly dynamic and changes in response to the cell cycle, external factors, and the different types of tissue or cells (Huang 2017) [11]. The protein content ranged from 8.28-12.92%. The protein content was highest in MON 810 with 12.92%, followed by WS3 with 10.52%. The least protein content of 8.10% was observed in the WS1 variety of maize. Thus, protein content varied significantly among the samples. (Zhang, Ao, and Kim 2019) [21] observed a similar trend of greater protein content in GM soybean and less variation in maize. This may be due to the change in geographical location, variety and type of modification in maize. The crude fat content of the samples ranged from 3.4-3.7%. Similar results can be observed from (Jiménez *et al*. 2009) [12] for the same MON-810 variety, and the author observed that the profile of major and minor fatty acids in the isogenic and

transgenic counterparts is identical; no unique isomer (cis/trans, double bond position) or molecule that signifies the genetic origin of the crop. Trans fatty acids were not found in the sample. The crude fibre content ranged from 2.3-2.6%. The carbohydrate content of the maize grains was between 80 and 85%. All the results were as per the literature range (Rayan and Abbott 2015) [16]. There were only minor variations among the samples, and standard GM maize and a very less significant difference was observed.

Table 2: Results of proximate analysis of maize grains

Sample	Moisture (%)	Dry Matter (%)	Crude Protein (%)	Crude fat (%)	Crude fibre (%)	Ash (%)	Carbohydrate (%)
MON 810	10.23 ± 0.02^{e}	89.77 ± 0.021^{a}	12.92 ± 0.006^{a}	3.69 ± 0.006^{a}	2.35 ± 0.05^{a}	0.99 ± 0.00^{f}	80.04 ± 0.017^{d}
WS1	11.59 ± 0.006^{c}	88.41 ± 0.005^{c}	8.10 ± 0.006^{f}	3.49 ± 0.006^{de}	2.60 ± 0.05^{a}	1.52 ± 0.006^{b}	84.29 ± 0.015^{ab}
WS2	12.11 ± 0.015^{a}	87.89 ± 0.015^{e}	8.71 ± 0.006^{c}	3.51 ± 0.005^{cd}	2.55 ± 0.06^{a}	1.12 ± 0.004^{d}	84.10 ± 0.015^{abc}
WS3	11.25 ± 0.021^{d}	88.75 ± 0.021^{b}	10.52 ± 0.0058^{b}	3.54 ± 0.027^{c}	2.51 ± 0.07^{a}	1.25 ± 0.005^{c}	82.18 ± 0.021^{c}
WS4	11.91 ± 0.01^{b}	88.09 ± 0.01^{d}	8.54 ± 0.006^{d}	3.58 ± 0.005^{b}	2.50 ± 0.04^{a}	1.91 ± 0.01^{a}	82.46 ± 1.723^{bc}
WS5	11.89 ± 0.006^{b}	88.11 ± 0.005^{d}	8.28 ± 0.005^{e}	3.47 ± 0.006^{e}	2.56 ± 0.01^{a}	1.01 ± 0.003^{e}	84.67 ± 0.021^{a}

Note: Means that do not share a similar alphabet indicate they are significantly different ($p< 0.05$)

3.2 ATR-FTIR visual analysis

The ATR-FTIR spectra of the five varieties of grain maize samples and standard GM maize (MON 810) are presented in fig 2. The infrared in the intermediate range (400-4000 cm^{-1}) allows for the identification of functional groups in chemical substances. It identifies similarities and differences in the biochemical composition of samples, such as carbohydrate, protein, lipid, and nucleic acid content. The spectra between 3500-500 cm^{-1} had major peaks. In the spectra of the samples, different peaks in the region depict different parameters. The regions of 2800-2980 cm^{-1} and 1700-1770 cm^{-1} have C=O stretching, representing the fingerprint region of fat. This region can also arise due to the stretching in C-H and methyl groups. Amino acids, fatty acids, lipids, proteins, and peptides have peaks at 2860 and 2930 cm$^{-1,}$ and the greater peaks at 2924 cm-1 indicate the presence of alkanes and carboxylic acids (Andrade, Medeiros Coelho, and Uarrota 2020) [4]. But by careful visual examination, no such changes were found except for the changes in the transmittance.

The greater stretch in GM-MON-810 might be due to the methylation of DNA in the transgene. Peaks for moisture contents can be observed near 1640 and 3300 cm^{-1}. Water's OH stretching and H bending vibrations are absorbed strongly in infrared regions (Amir *et al*., 2013) [3]. Analysing the data indicates the presence of moisture in the samples, which can be correlated with proximate analysis. The fingerprint of the Carbohydrate region was assigned at a wave number of 1200-800 cm^{-1}. The strong presence of peaks near the range of 1743-1745 cm^{-1} clearly indicates the vibration arising from lipids, which is associated with the stretching of the ester carbonyl group. The presence of peaks in the area of 1645 cm^{-1} indicates the characteristic absorption in fatty acids due to the carbonyl group. A similar absorption can support these results in non-granular maize starch for fatty acid profiling using FTIR (Marinopoulou, Papastergiadis, and Raphaelides 2019) [15]. Anomeric regions of carbohydrates in the region 950 and 750 cm^{-1} arise due to the vibration of the pyranoid and furanoid rings due to their presence in both mono and polysaccharides. The present findings showed clear peaks in the areas of 927 and 850 cm^{-1} that can be attributed to their presence. It is generally agreed that starch groups are responsible for the frequencies in the range of 1000–1100 cm^{-1} (Cueto *et al*. 2018) [7]. The fingerprint of the protein region was observed at a wavelength between 1650-1500 cm^{-1}. Amide-I (1600 and 1700 cm^{-1}) and Amide-II (1550-1570 cm^{-1}) are the primary bands indicating the main features of protein in the bove two regions. Amide I originate from the C=O stretch of the protein's peptide group. The amide II band results primarily from NH bending and secondly from CN stretch. Strong absorption of the 1537 cm^{-1} stretch in the present spectrum indicates NH bending, which strongly correlates with the maize high protein content (Ghasemi *et al*. 2022) [10]. As maize is the important source of protein which is grown as the third major cereal crop, this strongly supports our findings. The stronger transmittance was observed in the case of MON-810, which had a higher protein content. The attributable peak at 1645 cm^{-1} is due to the intermolecular β-sheet in protein (Duodu *et al*. 2001) [8].

The area under the peaks of the entire FTIR spectrum of the samples and standard was evaluated. To conclude, it is plausible to believe that the GM maize's conformational and compositional changes are due to the pleiotropic impact of inserting foreign genes into the parent genome, causing NIR spectra to vary and genotypes to differ in the NIR spectrum (Feng *et al*. 2017) [9]. The area percentage observed is as follows GM-MON 810> WS3>WS2>WS5>WS4>WS1. This indicates that the maize grain sample WS3 is very similar in the composition, functional groups, and area percentage to that of GM-MON 810. WS1 is completely different and had the highest degree of variability compared to the genetically modified maize standard. In all the cases, a separable form of spectra was observed, indicating that FTIR can be used to differentiate between GMO and non-GMO products.

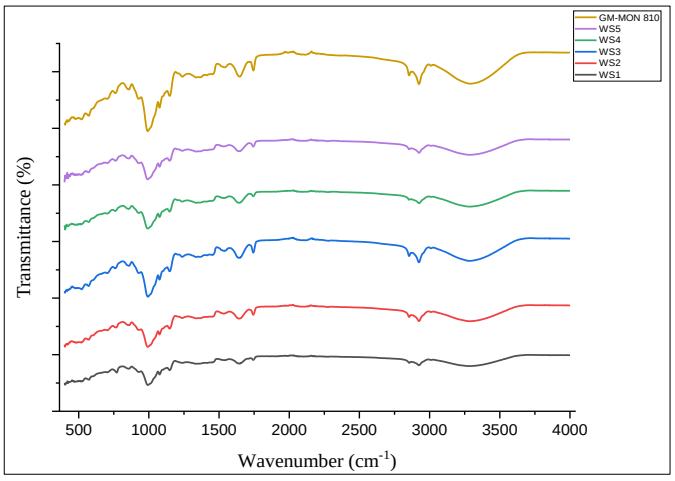


Fig 2: Comparison of FTIR spectra of different maize grains and Standard GM maize

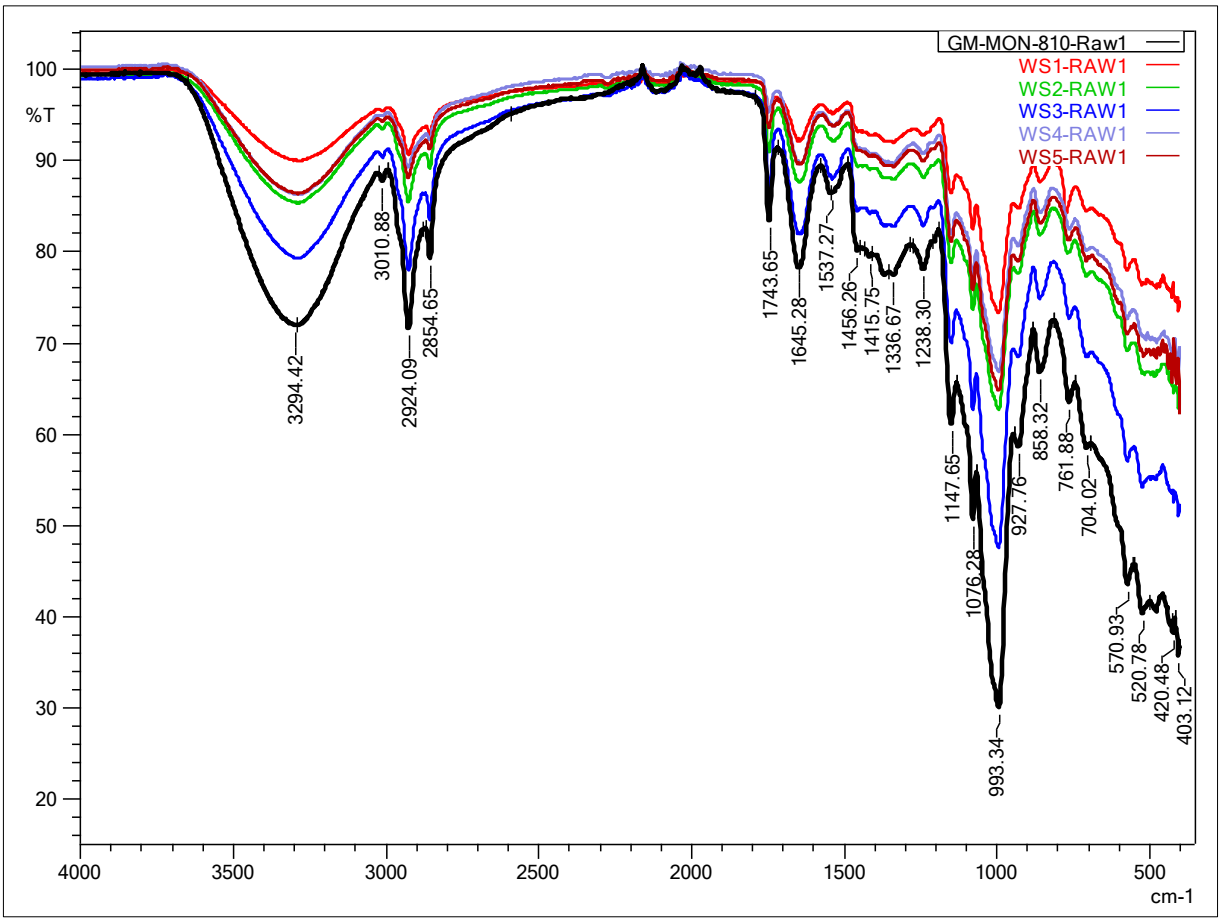
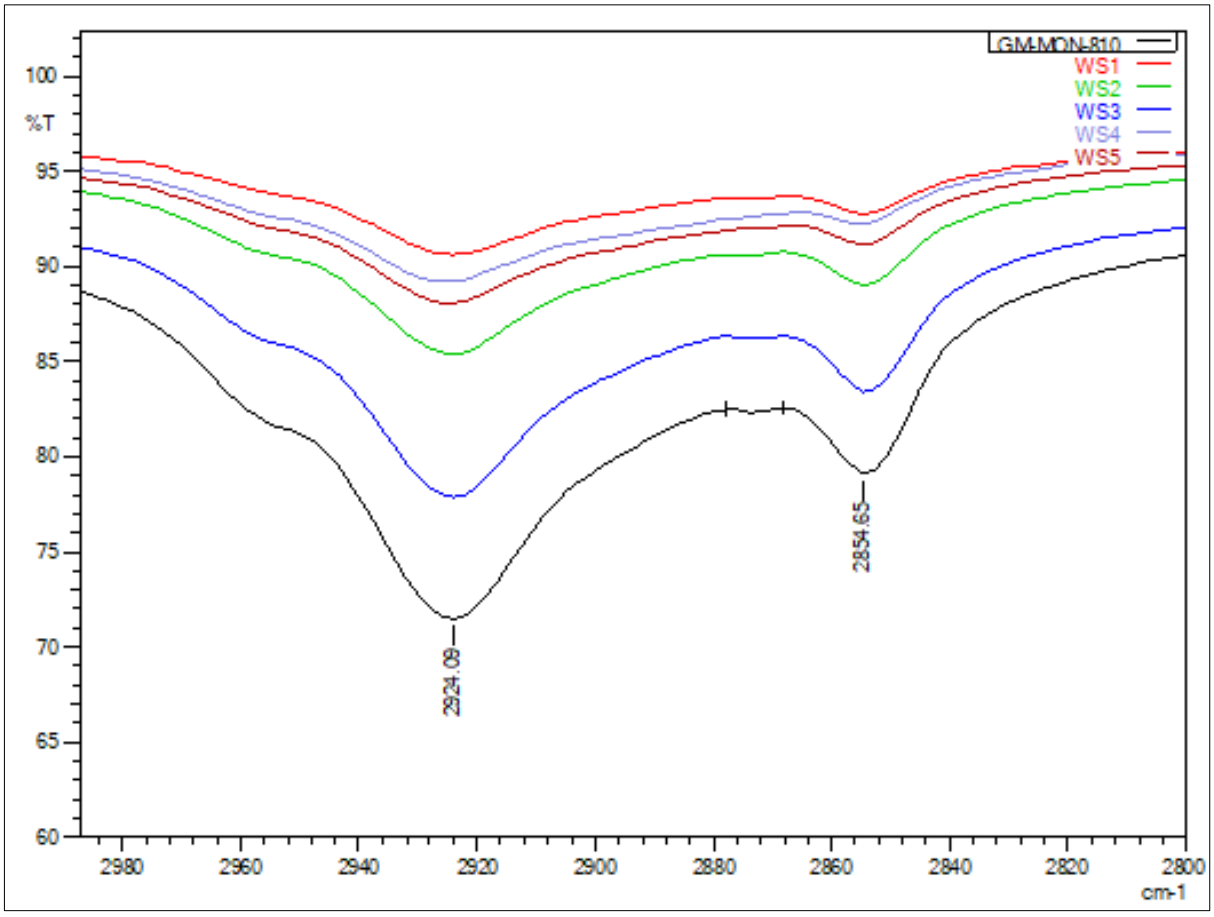
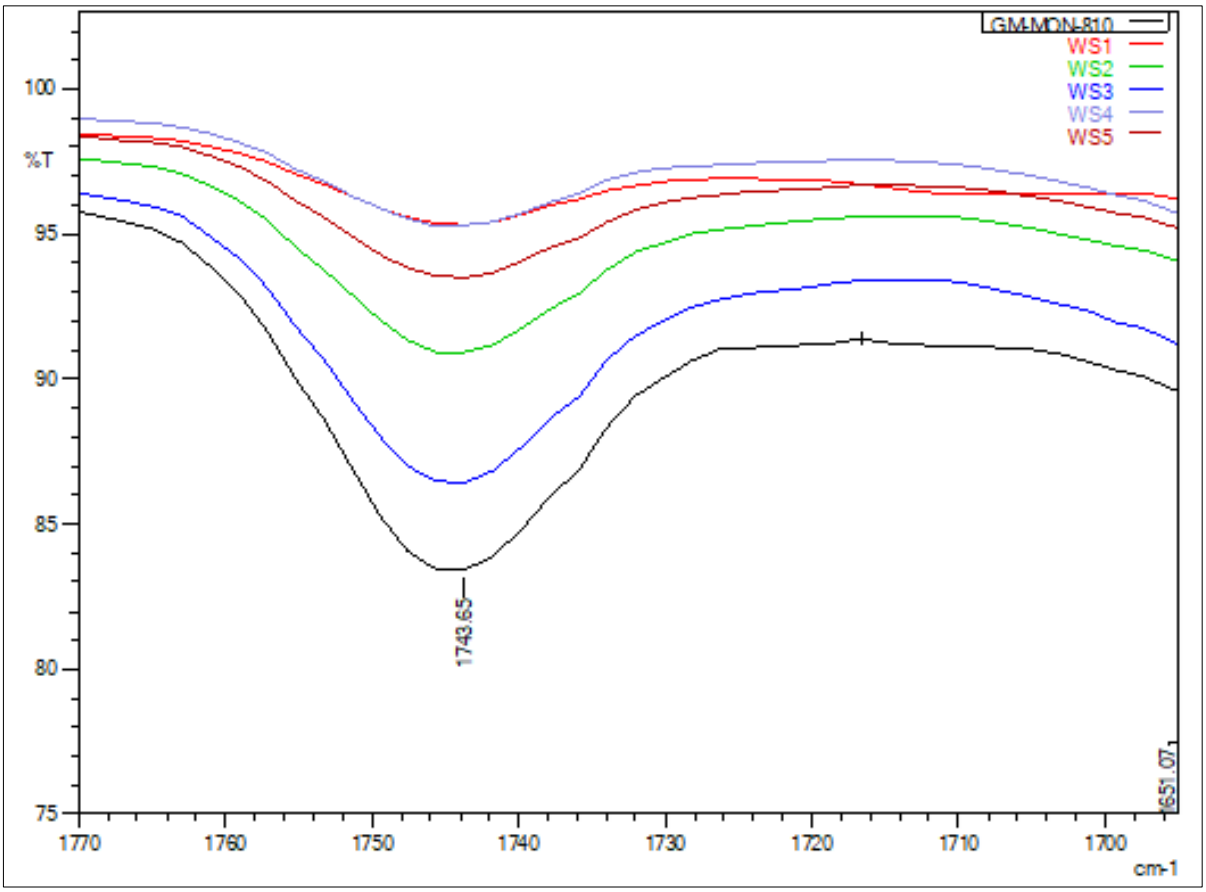


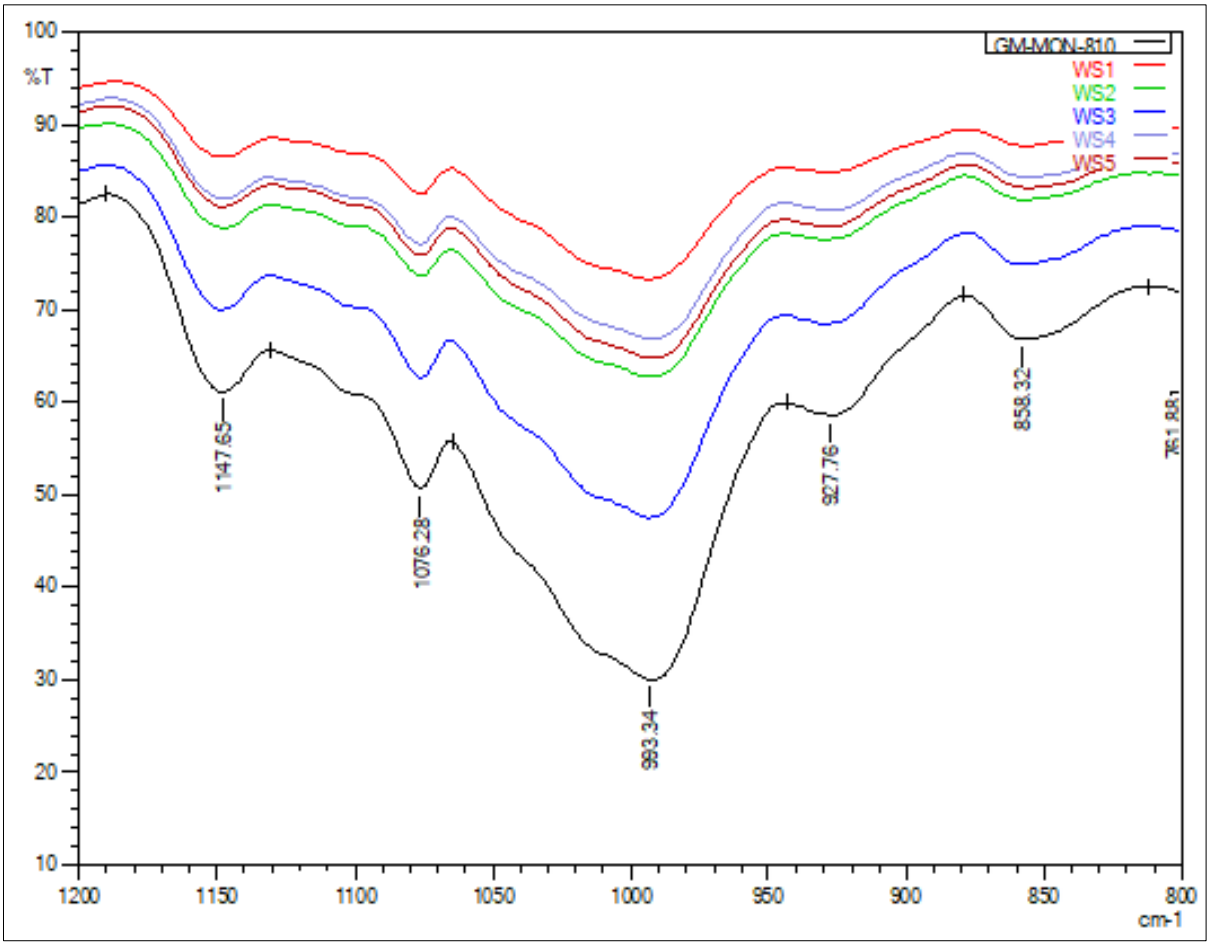
Fig 3: FTIR spectra of maize grains and Standard GM maize



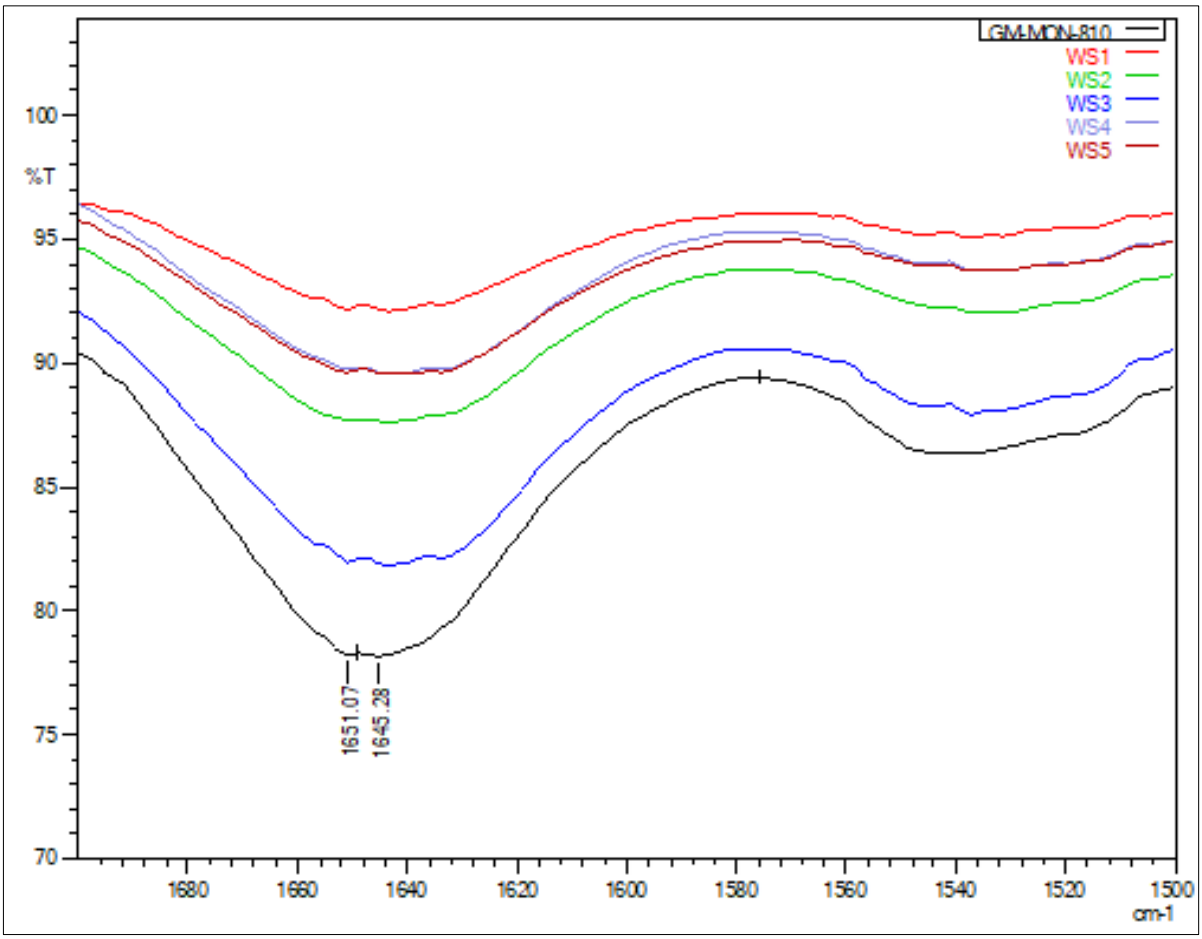
a)


GM-MON-810
WS1
WS2
WS3
WS4
WS5
%T
100
95
90
85
80
75
1743.65
651.07
1770
1760
1750
1740
1730
1720
1710
1700
cm-1

b)


GM-MON-810
WS1
WS2
WS3
WS4
WS5
%T
100
90
80
70
60
50
40
30
20
10
1147.65
1076.28
993.34
927.76
858.32
761.88
1200
1150
1100
1050
1000
950
900
850
800
cm-1

c)



d)

Fig 4: FTIR absorption spectra of maize grains representing different regions. a) Fat region 1 (2800-2980 cm^{-1}) b) Fat region 2 (1700-1770 cm^{-1} c) Carbohydrate region (1200-800 cm^{-1}) d) Protein region (1650-1500 cm^{-1})

4. Conclusion

Although there are some compositional variations between the GM maize standard and the non-GM samples, they are not likely to be biologically substantial because they are well within the range of values expected in the literature range (Ridley *et al*. 2002). Based on compositional variations, it is difficult to compare GM and non-GM maize. Hence FTIR served as an alternate way to compare functional groups of conventional maize and GM maize and distinguish them based on the area percentage under the peaks of the spectrum. FTIR can thus be a new approach for detection which should be confirmed with the standard method of GM maize detection like PCR for further conclusions.

5. Acknowledgement

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