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# Spectroscopic Analyses of Black Seed to Study the Temperature and Humidity effect

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***Abstract:****This study was conducted to find out the effect of physical factors such as temperature and humidity on the black seed, based on the narrations of folk medicine, which recommend chewing the black seed and moistening it with saliva in order to benefit from its nutritional properties as mentioned in the Prophet's medicine in what al-Tirmidhi narrated in his Sunnah on the authority of the Prophet - may God bless him and grant him peace. He said: "You have this black seed; it has a cure for all diseases except the poison." [1,3,14] The samples were scientifically made from the black seed to be exposed to varying degrees of humidity and temperatures, and to track the nutritional changes that occur in it. IR spectroscopy are a type of spectroscopy that is classified under absorption spectroscopy. It has been used as a useful method for confirming evidence of a particular structural composition. For a compound, the presence of a selected absorption rarely gives definitive proof of the details of a particular composition, but of course it can help to suggest one of several possibilities.*

*Then, the use of Fourier Transition Infrared Spectroscopy (FTIR). FT-IR spectrum analysis was also carried out and the results confirmed the presence of functional groups such as amines, alkanes,acids, esters, alkyl and alkenes. Thus, the result of our study offers a platform of using Nigella sativa seeds as herbal alternatives[2]. Several emission spectra were obtained from 3 different values of the absorption process in deffirent time, after which the emission sites of the compounds were determined using the method of infrared spectroscopy (FTIR). The resultsconducted using the database of National Institute Standard and Technology (NIST)[3] ,fluker company data base. and showed the compounds of black seed, some of which were affected by the humidity factor, and the clarity value was high, It was also found that the temperature has an effect on the formation of the black seed.*

***Keywords****: Nigella sativa,black seed,Black Cumin, FTIR.*

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## I. Introduction:

Nigella sativa(N. sativa) , black seed, and black cumin are all names for what is known scientifically as nigella sativa. Nigella sativa is cultivated in different parts of the world and grown especially in the countries of the eastern Mediterranean[1].Nigella sativa is an annual flowering plant, which belongs to the Ranunculaceae family [1, 2]. The plant produces small black seeds that are flat, trigonal and angular in appearance, about 2 to 3.5 mm long and 1 to 2 mm wide [4]. In addition, these dark gray or black seeds have a similar appearance to sesame seeds [5] The seeds, due to their aromatic nature, are used as a spice in cooking. It is also used as a carminative and diuretic by Eastern people (Hedrick, 1972). The active ingredients in N. sativa have beneficial effects against many diseases, including cancer. For example, this is effective in reducing the risk of developing atherosclerosis by lowering low-density lipoprotein cholesterol in the blood and elevation of high-density lipoprotein cholesterol in the blood[6,7] (Dahri et al., 2005; Nader et al., 2010); The main components of N. sativa. The seeds have been reported in previous studies. Papian et al. (1978) studied the fatty acid and amino acid combinations of N. sativa. Imported from the Middle East, it reported that the seeds showed a combination of 21% protein, 35-5% fat, 5-5% moisture and 3-7% ash, and the rest were total carbohydrates[1,3.11]. Compounds with antimicrobial activities are found in N. sativa volatile oil. Seed by Egyptian workers (E1-Alyf et al., 1975)[7,8]. Literature data on the chemical composition of N. sativa. The seeds are very limited. The purpose of this investigation was to determine the approximate composition and some of the exact components of N. sativa. Seeds. There is not much research on the effect of physical factors on black seed and its components in this area. There are very few review articles in this area. We searched scientific databases such as Pubmed, Web of Science,database of National Institute Standard and Technology (NIST)and A Google.

## II. Materials and Methods

**SamplesPreparation:**

Samples were purchased from N. sativa seeds. From the local marketm,the 2019 crop. Different humidity values were specified. By soaking in water of different humidity and temperatures with a device (BENETECH GM 1363B - Moisture and Temperature Meter) the digestion and milling and emulsifying process was carried out, and samples were prepared by Fourier spectroscopy method and grinding of the black seed after being exposed to the temperature and humidity required to reduce the size of the particles to less than 5 mm , Which is the diameter. Otherwise, the larger particles will scatter the infrared beam and cause a fundamental gradient of the spectrum[9].

To prepare the samples, 150 grams were taken and they were soaked in water to obtain different degrees of humidity (50 grame for the one sample 26.2 % ,30% ,41.5% for the period of time 5,10,15 minute), then they were milled and squeezed to obtain their nutritional juice in the form of an emulsified substance, and then the material was transferred to an oven with a temperature of 100 degrees to see the effect of temperature And that in different times of 5 , 10 and 15 degrees Celsius. Grind and grind into a fine powder until crystallized It can no longer be seen and becomes somewhat "doughy" and adheres to mortar(mixture approximates the consistency of a toothpaste). The mixture is then transferred to the mull plates & the plates are squeezed together to adjust the thickness of the sample between IR transmitting windows. This is then mounted in a path of IR beam and the spectrum is run. Results were recorded after 5 , 10 and 15 minutes with different humidity values (26.2%, 30%, 41.5%).

**Table (1)** Sample results at different humidity, temperatures and times

| Sample | Humidity | Temperature | Time | Search Best Hit Description | Search Best Hit |
|---|---|---|---|---|---|
| S1 | 26.2% | 100°C | 5min | methyl linoleate natural | F62290 |
| | | | 10min | methyl linoleate natural | F62290 |
| | | | 15min | methyl linoleate natural | F62290 |
| S2 | 30% | 100°C | 5min | methyl linoleate natural | F62290 |
| | | | 10min | methyl linoleate natural | F62290 |
| | | | 15min | methyl linoleate natural | F62290 |
| S3 | 41.5% | 100°C | 5min | methyl linoleate natural | F62290 |
| | | | 10min | methyl linoleate natural | F62290 |
| | | | 15min | methyl linoleate natural | F62290 |

**The Experimental Setup:**

At first, visible and ultraviolet rays were used to determine the expected values of sample components according to previous studies using the LAMBDA 365 UV / Vis spectrometer. Wavelength: 572 nm; Measurement Mode: Absorbance; Cell 10 mm from PerkinElmer.

From the results obtained from the above device, the device used the FT-NIR spectrometer for research use only (Spectrum Two N)Part Number (L160000A) . From the same company, with specifications value Dimensions 450 × 300 × 210 mm (W × D × H ),Weight Approximately 13 kg ,Power input 100–230 V, 50/60 Hz, Max 65 VA ,Laser Class 1 ,Detector $LiTaO_3$ ,Operating temperature range 5 °C to 45 °C , Storage temperature range −20 °C to 60 °C ,Maximum relative humidity 80% (non-condensing) with $CaF_2$ windows,optical system collect data over a total range of 8,300 to 350 $cm^{-1}$. The instrument is connected to a PC, via a wireless network using the optional wireless router. [4].

FTIR spectroscopy is a major step forward over the traditional dispersive infrared approach for a number of reasons, including the fact that the entire FTIR spectrum is collected in a fraction of a second and the spectra are summed to signal light.FTIR-spectroscopy (FTIR) is a methodical analogue that measures the size of the molecular structure of the molecule. FTIR spectroscopy is a case in point that can be used as an infrared and modular energy module. Infrared light is absorbed at certain frequencies associated with the vibration-binding energies of the functional groups in the molecule. A characteristic pattern of the bands, the molecule vibration spectrum, is formed. The location and intensity of these spectral bands give the impression of a molecular structure, making FTIR spectroscopy a highly adaptable and useful technique[3].

The Fourier Spectrometer simultaneously collects spectral high-resolution data over a wide spectral range. This provides a major advantage over a spectrophotometer, which measures intensity over a narrow range of wavelengths simultaneously[10,12].

The samples were prepared with the known scientific image in such cases by taking three samples and exposing them to different humidity and temperature and taking the measurement results every five minutes up to 15 minutes according to table (1).

**The Experimental Procedure**

A pressurized pellet technique was used for sample preparation in which a small amount of the ground black seed sample was closely mixed.Grind and grind into a fine powder until crystallized It can no longer be seen and becomes somewhat "doughy" and adheres to mortar (mixture approximates the consistency of a toothpaste).

This finely ground mixture was then pressed under very high pressure into a vacuum mold or small press to form small pellets (about 1-2 mm in diameter and 1 cm in diameter). This results in granules that are transparent to the infrared radiation and operate in this manner[8].

The mixture is then transferred to the mull plates & the plates are squeezed together to adjust the thickness of the sample between IR transmitting windows. This is then mounted in a path of IR beam and the spectrum is run .Then the mixture was inserted between the two screws and the upper screw A was joined until the powder was pressed onto a thin disc, after pressing the sample screws A & A1 were removed and a steel cylinder with granules inside was placed in the path of the infrared beam and the granule .Then I repeated the above steps to other samples of different time to show what happen in the sample [9,11].

## III. Results and Discussion

Tables (2-7) show the results of different values of the effect of temperature and humidity on samples using FTIR spectroscopy. Figures (1-24) list the results of the search for the compounds included in the composition of the samples after the influence of temperature and humidity on them.

**Results of black seed samples after the influence of different values of temperature and humidity:**

**Table (2)** The analyzed data of sample S1, at 26.2% and 100º C

| Sample | Humidity | Temperature | Time | Search Best Hit Description | Search Best Hit |
|---|---|---|---|---|---|
| S1 | 26.2% | 100 °C | 5min | methyl linoleate natural | F62290 |
| | | | 10min | methyl linoleate natural | F62290 |
| | | | 15min | methyl linoleate natural | F62290 |

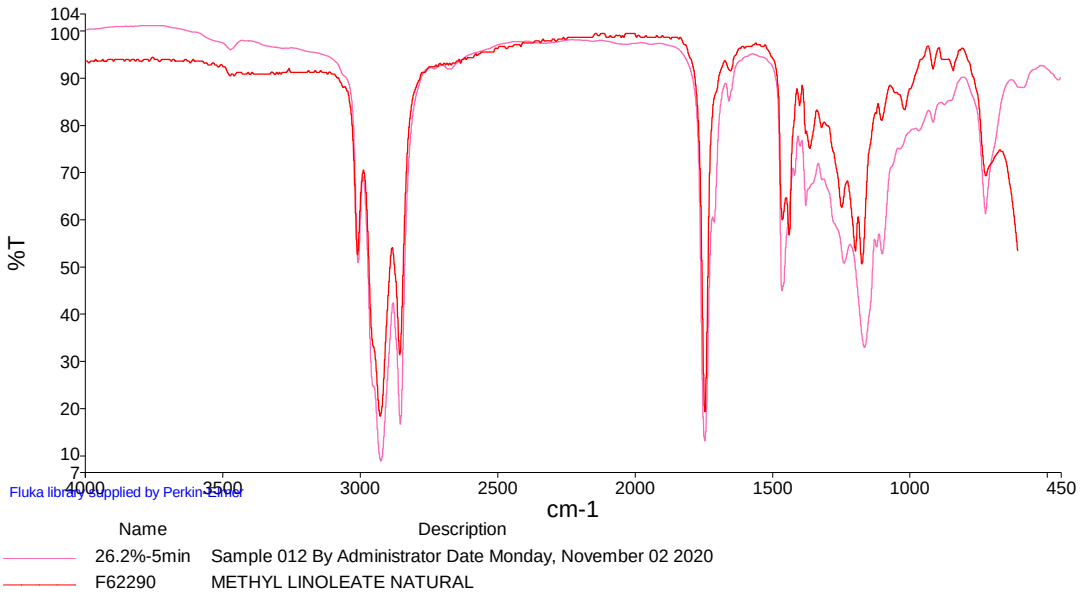


**Figure 1**. FTIR spectrum of sample (S1) at 26.2% -5min compare with Fluka library.

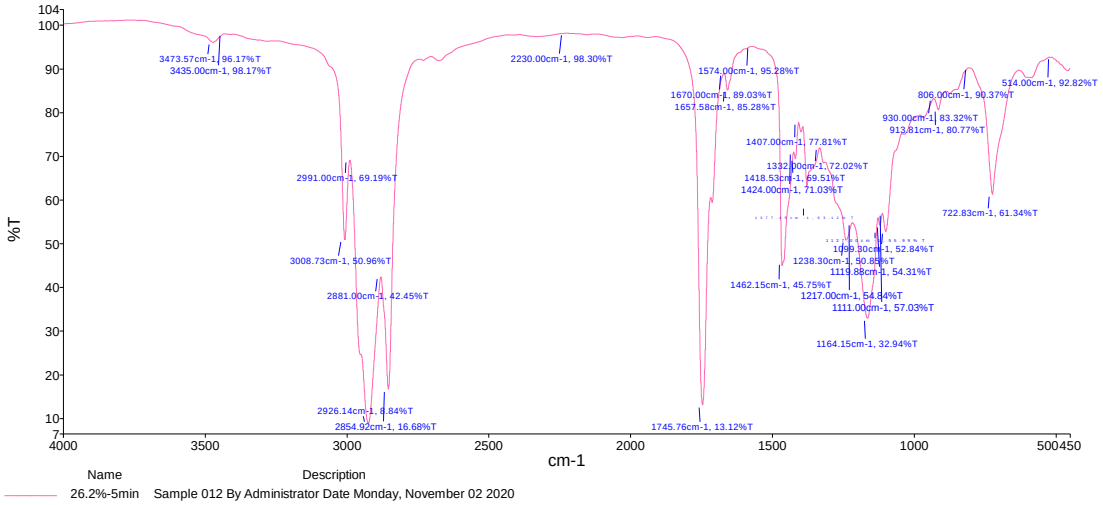


**Figure 2**. FTIR spectrum of sample (S1) at 26.2% -5min with peaks by Fluka library.

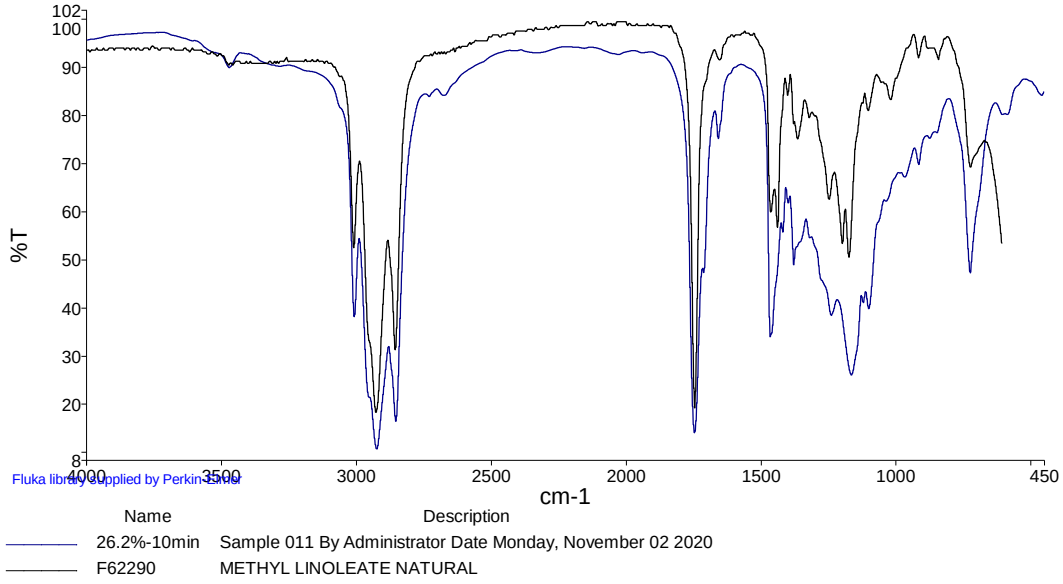


**Figure 3.** FTIR spectrum of sample (S1) at 26.2% -10min compare with Fluka library.

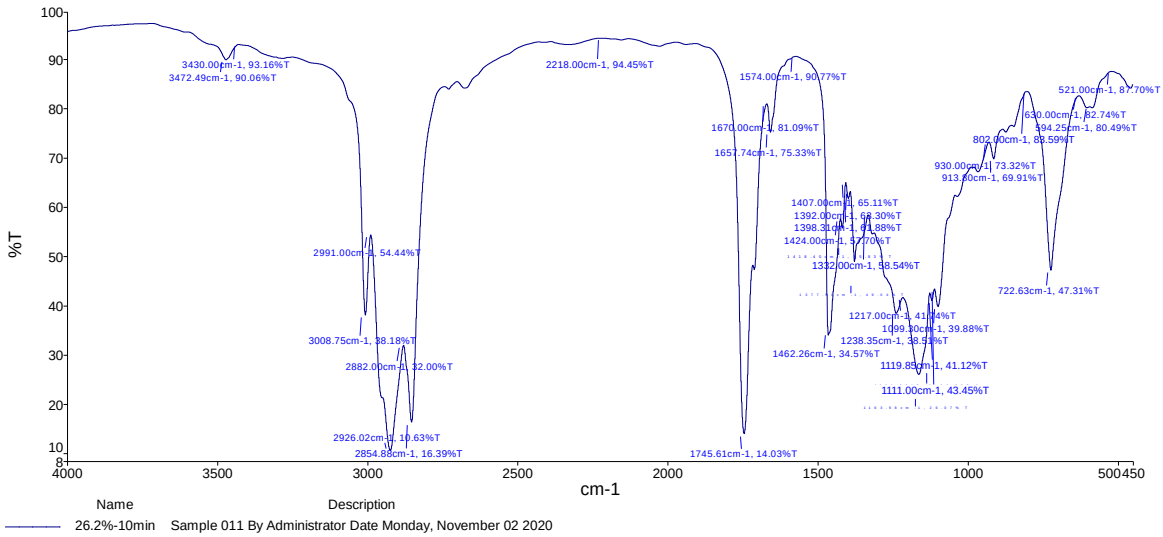


**Figure 4.** FTIR spectrum of sample (S1) at 26.2% -10 minwith peaks by Fluka library.

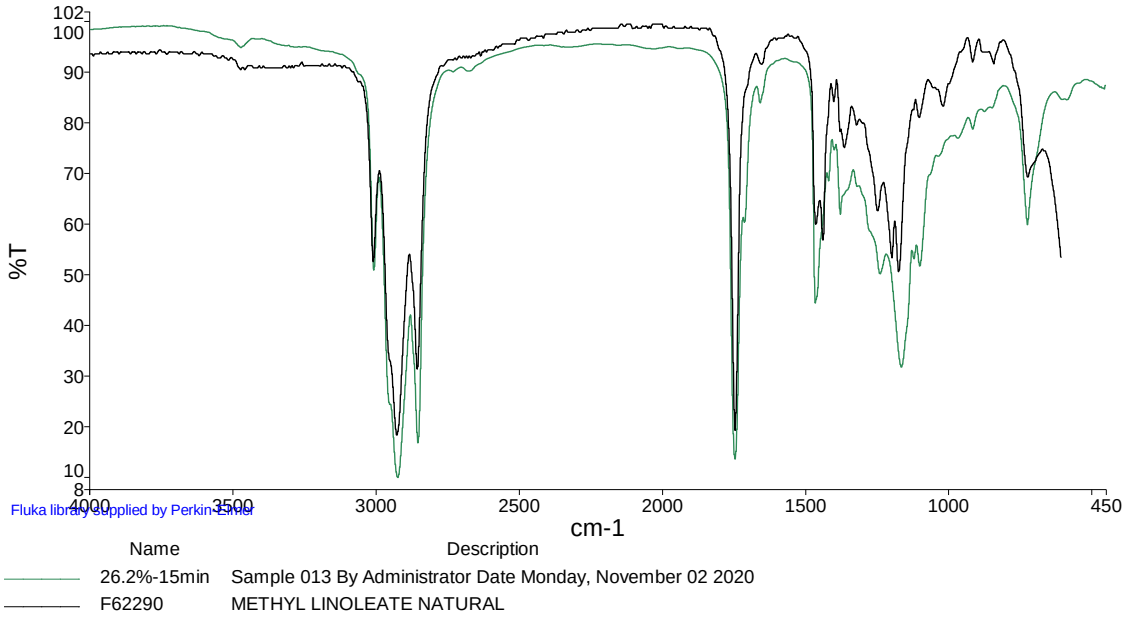


**Figure 5.** FTIR spectrum of sample (S1) at 26.2% -15min compare with Fluka library.

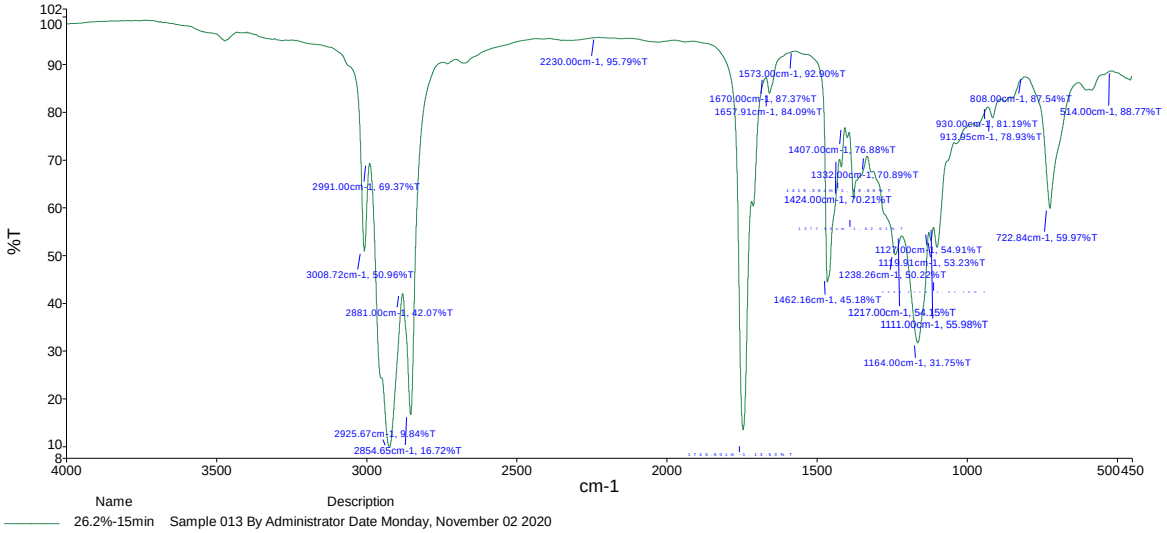


**Figure 6**. FTIR spectrum of sample (S1) at 26.2% -15 min with peaks by Fluka library.

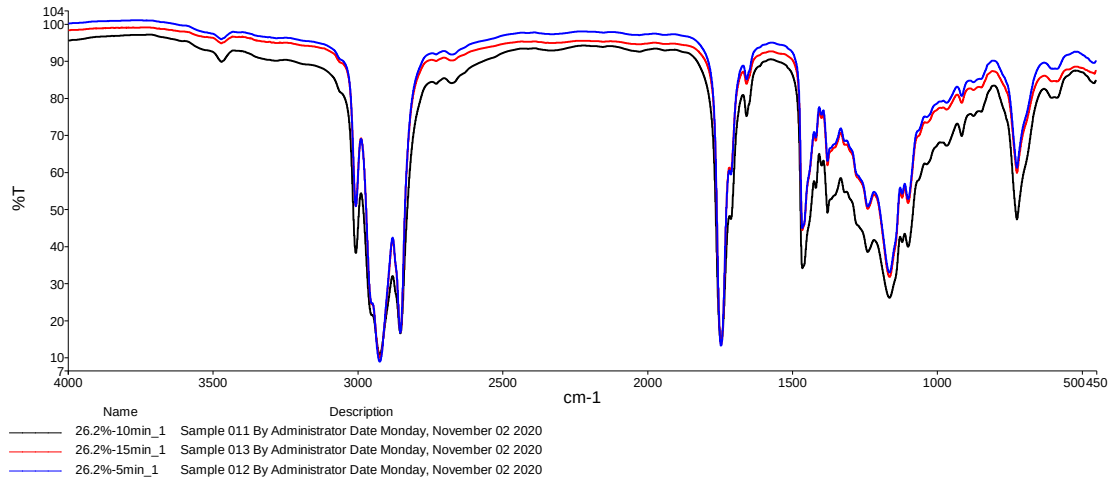


**Figure 7.** FTIR spectrum of sampled (S1) comparein different time.

**Table (3)** List of Searched Library References of sample S1, at 26% and different time.

| | Search Reference Spectrum Description | Search Score in 5min | Search Score in 10 min | Search Score in 15 min |
|---|---|---|---|---|
| 1 | CIS-ANDROSTERONE | 0.629926 | 0.608116 | 0.628518 |
| 2 | (+)-CAMPHOR-10-SULFONYL CHLORIDE | 0.685122 | 0.641309 | 0.689136 |
| 3 | BIS(2-ETHYLHEXYL) SEBACATE | 0.749377 | 0.675344 | 0.748268 |
| 4 | DIMETHYL AZELATE 90-95% | 0.794502 | 0.744857 | 0.792787 |
| 5 | ETHYL LINOLEATE | 0.826481 | 0.787519 | 0.825535 |
| 6 | ETHYL MYRISTATE | 0.83096 | 0.738249 | 0.828048 |
| 7 | ETHYL PALMITATE | 0.869538 | 0.775036 | 0.866414 |
| 8 | BUTYL STEARATE | 0.877618 | 0.783842 | 0.875753 |
| 9 | METHYL ELAIDATE GC REFERENCE | 0.901784 | 0.833905 | 0.900066 |
| 10 | METHYL LINOLEATE NATURAL | 0.937858 | 0.906069 | 0.93753 |

**Table (4)** The analyzed data of sample S2, at 30% and 100 º C

| Sample | Humidity | Temperature | Time | Search Best Hit Description | Search Best Hit |
|---|---|---|---|---|---|
| S2 | 30% | 100 °C | 5min | methyl linoleate natural | F62290 |
| | | | 10min | methyl linoleate natural | F62290 |
| | | | 15min | methyl linoleate natural | F62290 |

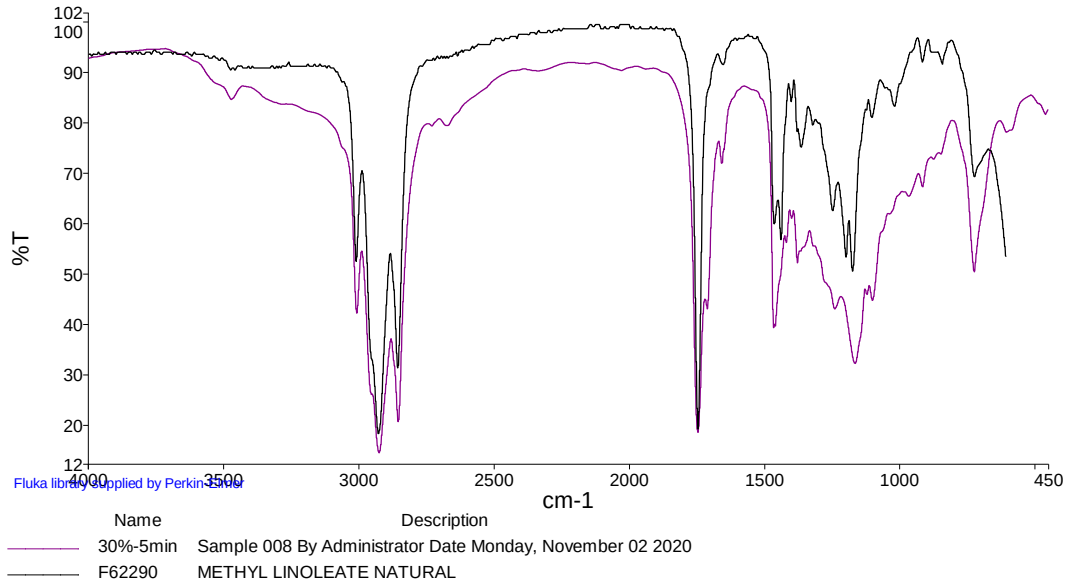


**Figure 8**. FTIR spectrum of sample (S2) at 30% -5min compare with Fluka library.

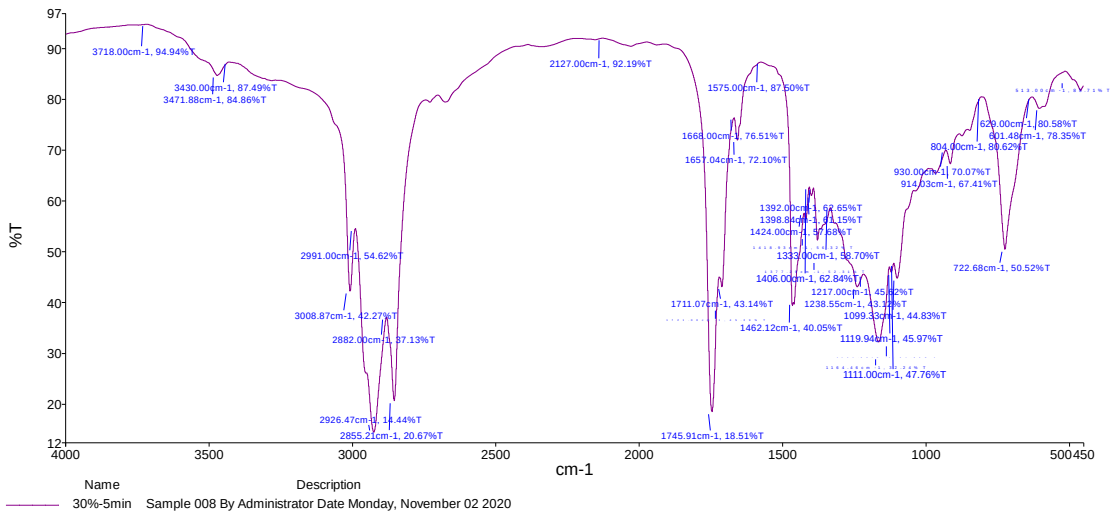


**Figure 9**. FTIR spectrum of sample (S2) at 30% -5 min compare with peaks by Fluka library.

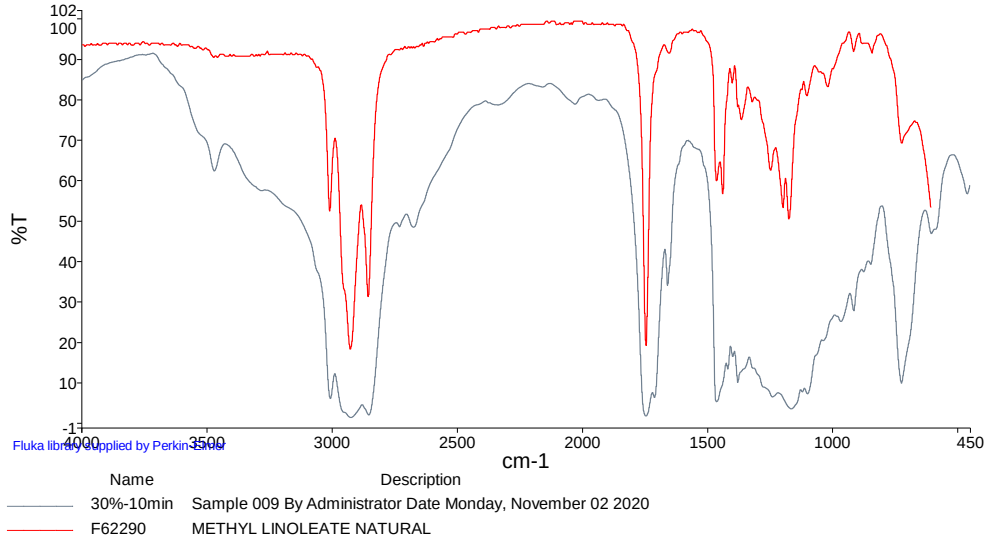


**Figure 10.** FTIR spectrum of sample (S2) at 30% -10min compare with Fluka library.

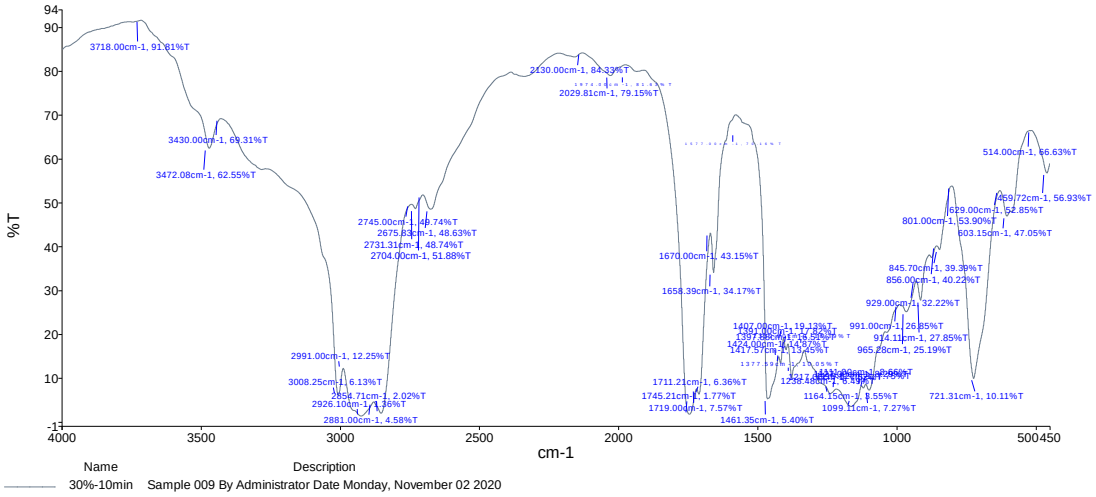


**Figure11.** FTIR spectrum of sample (S2) at 30% -10 min compare with peaks by Fluka library.

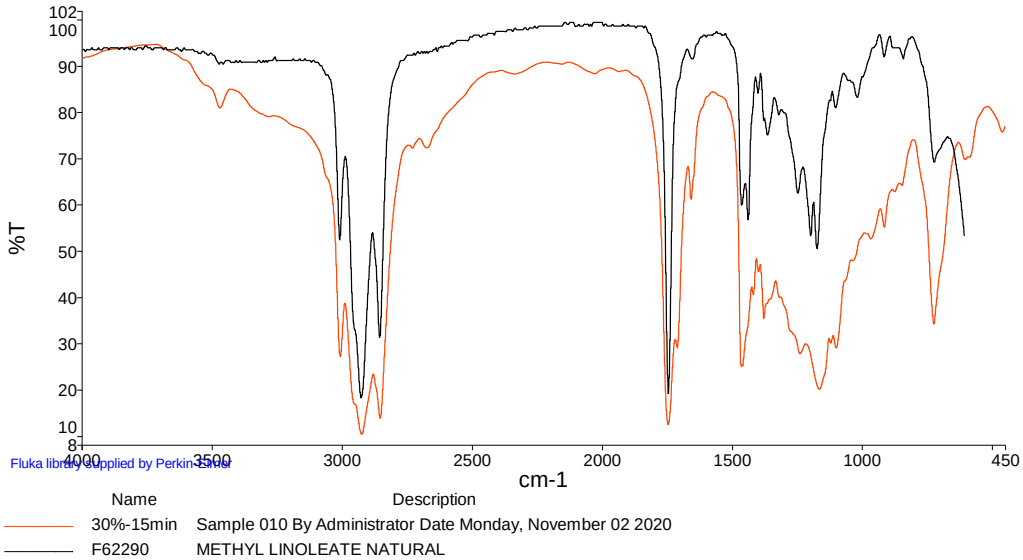


**Figure 12.** FTIR spectrum of sample (S2) at 30% -15min compare with Fluka library.

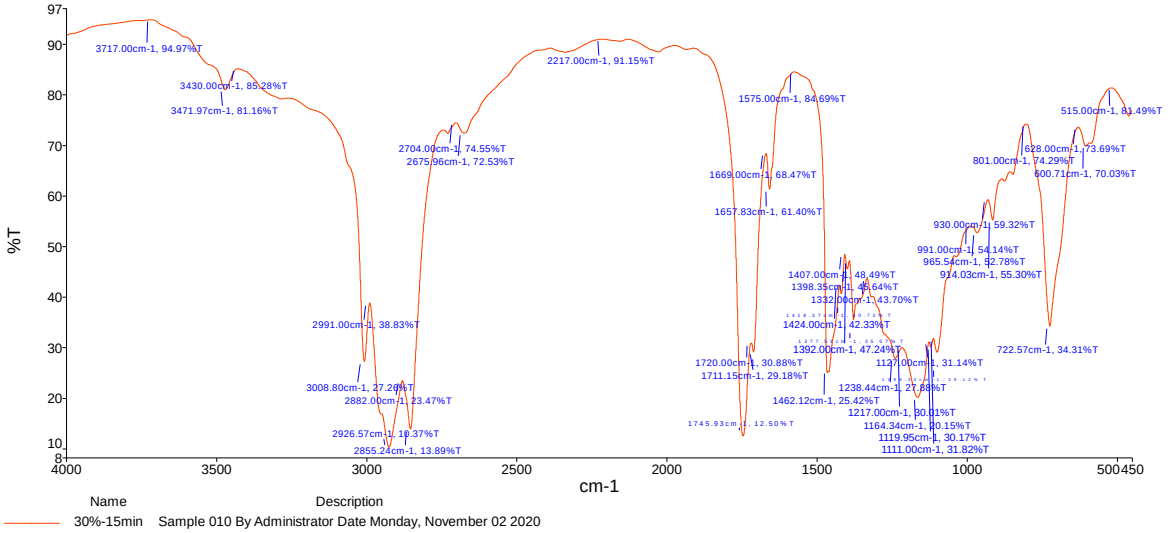


**Figure13.** FTIR spectrum of sample (S2) at 30% -15 min compare with peaks by Fluka library.

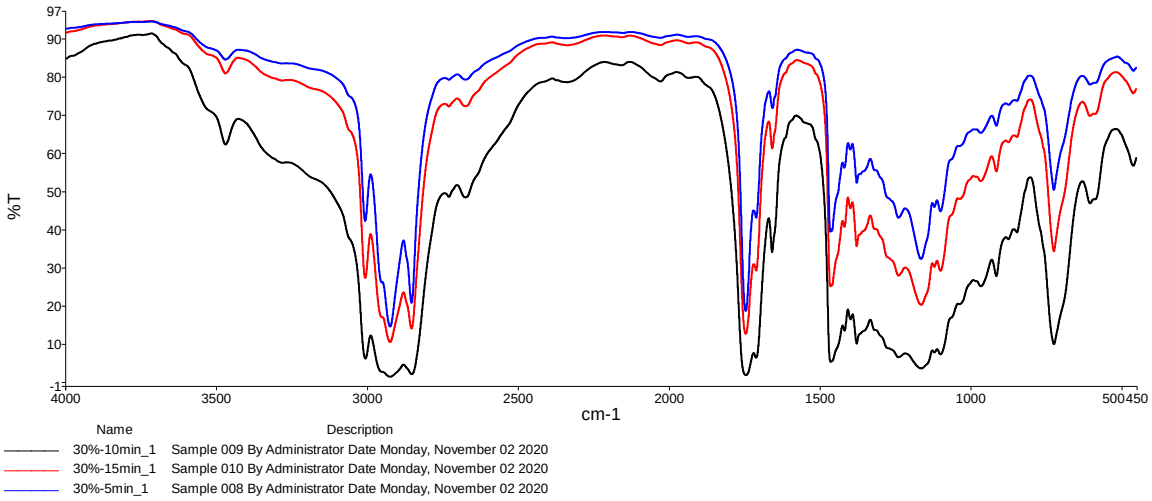


**Figure 14.** FTIR spectrum of sampled (S2) compare in different time .

**Table (5)** List of Searched Library References of sample S2, at 30% and different time.

| | **Search Reference Spectrum Description** | Search Score in 5min | Search Score in 10 min | Search Score in 15 min |
|---|---|---|---|---|
| 1 | BIS(1-BUTYPHENTYL) ADIPATE | 0.640684-2 CIS-ANDROSTERONE | 0.67447 -2 | 0.560167 |
| 2 | (+)-CAMPHOR-10-SULFONYL CHLORIDE | 0.625926 -1 | 0.721536 -7 METHYL N-VALERATE | 0.564359 |
| 3 | BIS(2-ETHYLHEXYL) SEBACATE | 0.661766 - 3 | 0.716513 -5 | 0.575813 |
| 4 | ETHYL MYRISTATE | 0.738957 - 5 | 0.679279 - 4 | 0.614808 |
| 5 | ETHYL PALMITATE | 0.80428 - 7 | 0.668349 -1 | 0.640955 |
| 6 | DIMETHYL AZELATE 90-95% | 0.73261 - 4 | 0.733814 - 8 | 0.648429 |
| 7 | BUTYL STEARATE | 0.809522 - 8 | 0.675055 -3 | 0.650169 |
| 8 | ETHYL LINOLEATE | 0.772048 -6 | 0.797126 - 9 | 0.718366 |
| 9 | METHYL ELAIDATE GC REFERENCE | 0.838258 -9 | 0.716571 - 6 | 0.719914 |
| 10 | METHYL LINOLEATE NATURAL | 0.91403 -10 | 0.845012 - 10 | 0.816062 |

**Table (6)** The analyzed data of sample S3, at 41.5% and 100 º C

| Sample | Humidity | Temperature | Time | Search Best Hit Description | Search Best Hit |
|---|---|---|---|---|---|
| S3 | 41.5% | 100 °C | 5min | methyl linoleate natural | F62290 |
| | | | 10min | methyl linoleate natural | F62290 |
| | | | 15min | methyl linoleate natural | F62290 |

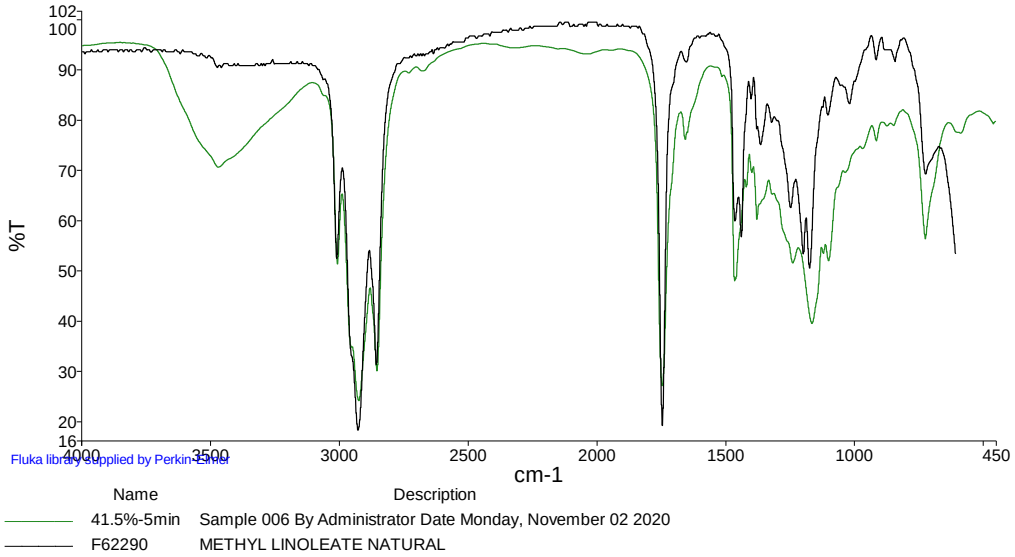


**Figure 15.** FTIR spectrum of sample (S3) at 41.5% -5min compare with Fluka library.

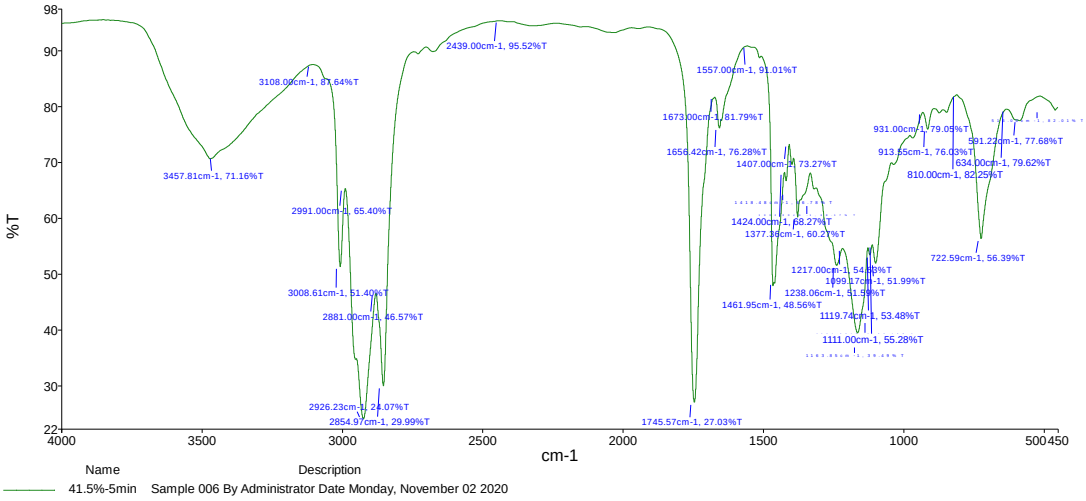


**Figure 16.** FTIR spectrum of sample (S3) at 41.5% -5min with peaks by Fluka library.

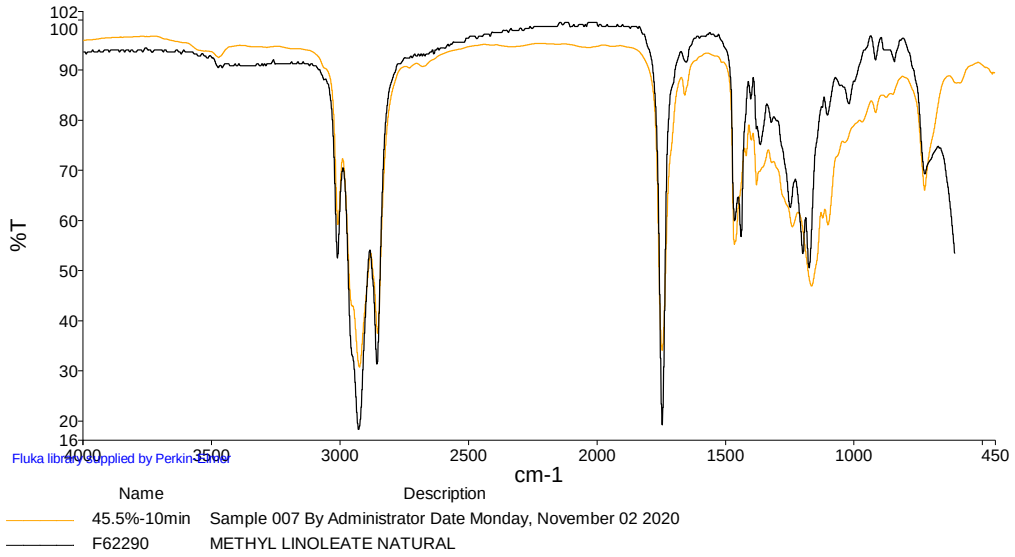


**Figure 17.** FTIR spectrum of sample (S3) at 41.5% -10min compare with Fluka library.

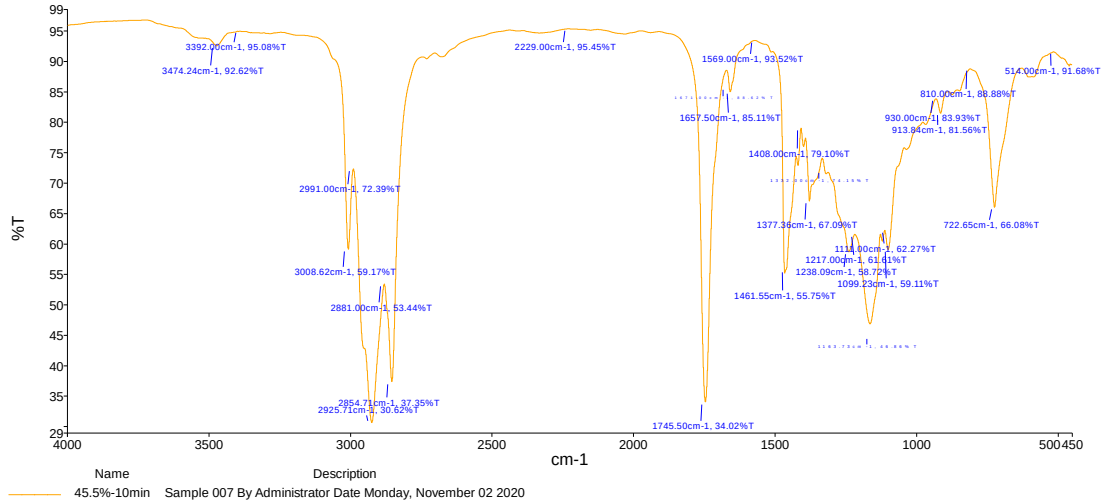


**Figure 18.** FTIR spectrum of sample (S3) at 41.5% -10 min with peaks by Fluka library.

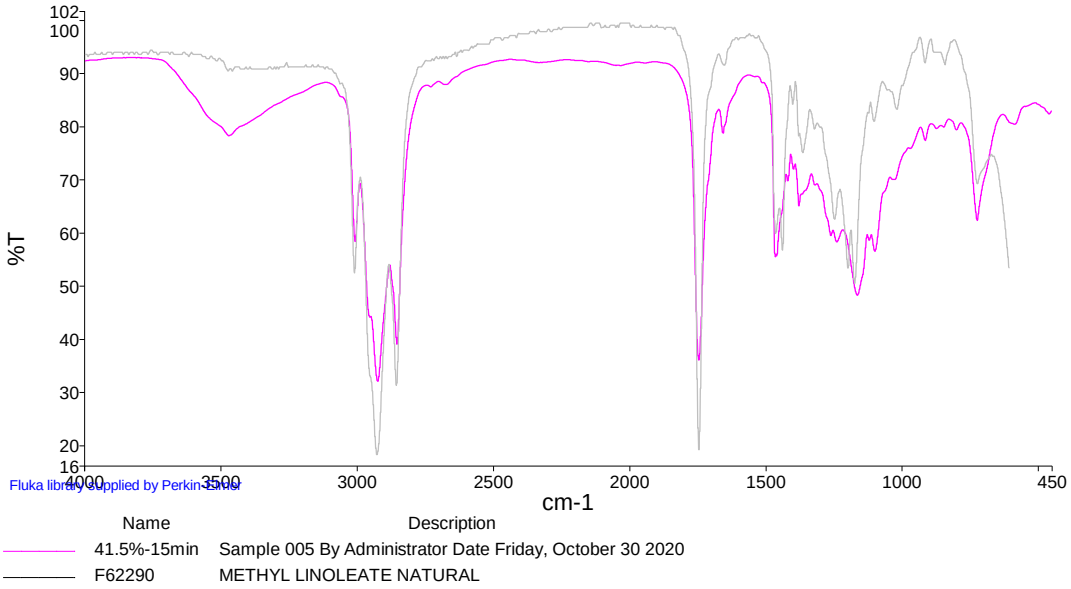


**Figure 19.** FTIR spectrum of sample (S3) at 41.5% -15min compare with Fluka library.

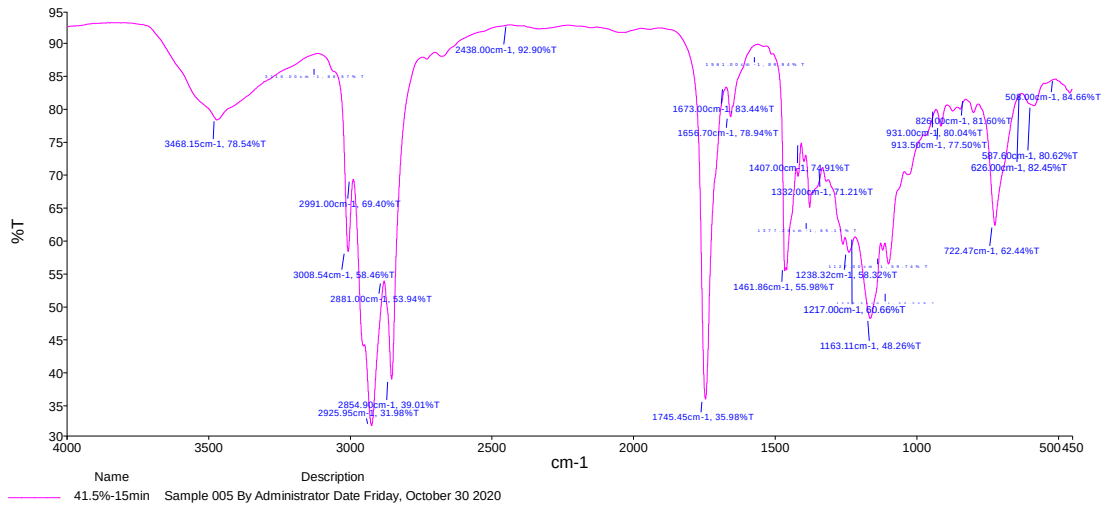


**Figure 20.** FTIR spectrum of sample (S3) at 41.5% -15min with peaks by Fluka library.

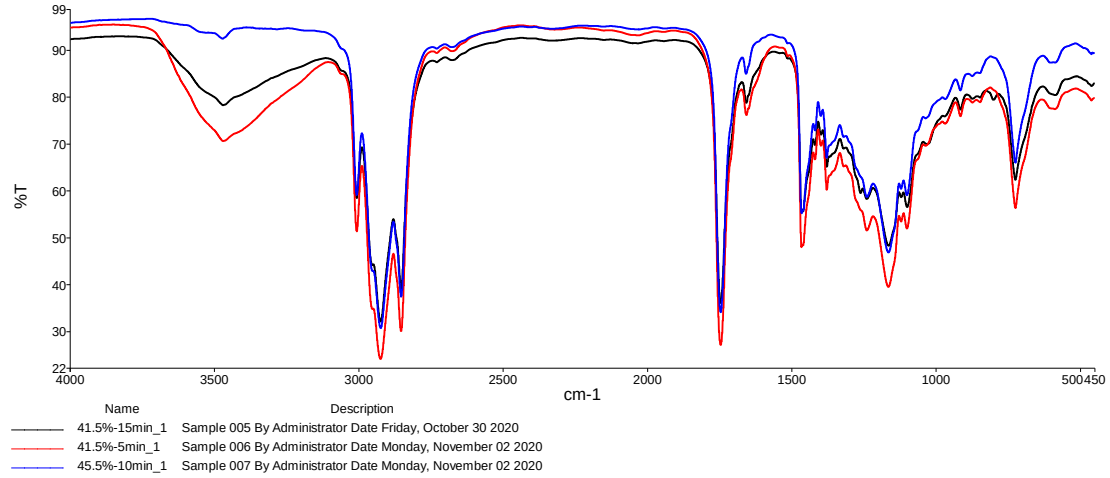


**Figure 21.** FTIR spectrum of samples (S3) compare in different time .

**Table (7)** List of Searched Library References of sample S3, at 41.5% and different time.

| | **Search Reference Spectrum Description** | Search Score in 5min | Search Score in 10 min | Search Score in 15 min |
|---|---|---|---|---|
| 1 | CIS-ANDROSTERONE | 0.641572-2 (+)-CAMPHOR-10-SULFONYL CHLORIDE | 0.67447 -2 | 0.637778 |
| 2 | TRICHLOROACETIC ACID | 0.641543-1 | 0.641543 -1 METHYL N-VALERATE | 0.64012 |
| 3 | BIS(2-ETHYLHEXYL) SEBACATE | 0.679553- 3 | 0.716513 -5 | 0.670307 |
| 4 | ETHYL MYRISTATE | 0.743258- 5 | 0.679279 - 4 | 0.738888 |
| 5 | DIMETHYL AZELATE 90-95% | 0.739748- 4 | 0.668349 -1 | 0.739884 |
| 6 | ETHYL LINOLEATE | 0.796065- 6 | 0.733814 - 8 | 0.791 |
| 7 | ETHYL PALMITATE | 0.800857- 8 | 0.675055 -3 | 0.80035 |
| 8 | BUTYL STEARATE | 0.800521-7 | 0.797126 - 9 | 0.804161 |
| 9 | METHYL ELAIDATE GC REFERENCE | 0.845138-9 | 0.716571 - 6 | 0.838327 |
| 10 | METHYL LINOLEATE NATURAL | 0.92406-10 | 0.845012 - 10 | 0.92344 |

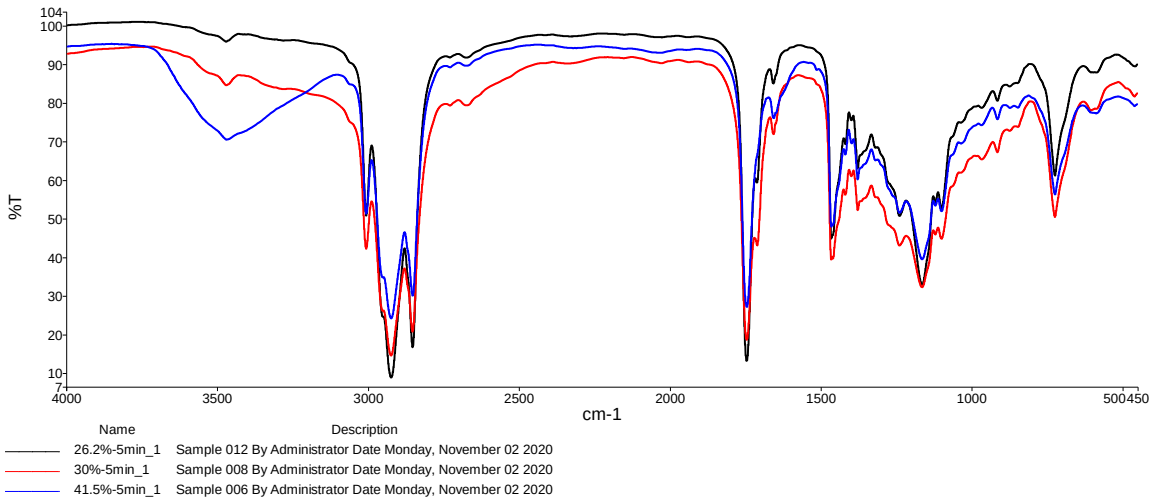


**Figure 22.** FTIR spectrum of samples compare with different humidity in 5 min.

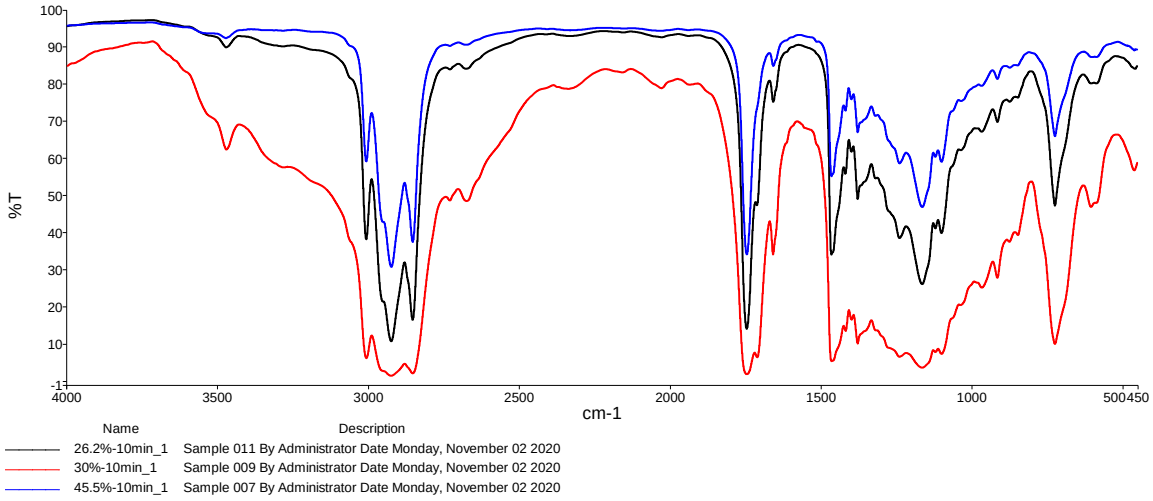


**Figure 23.** FTIR spectrum of samples compare with different humidity in 10 min.

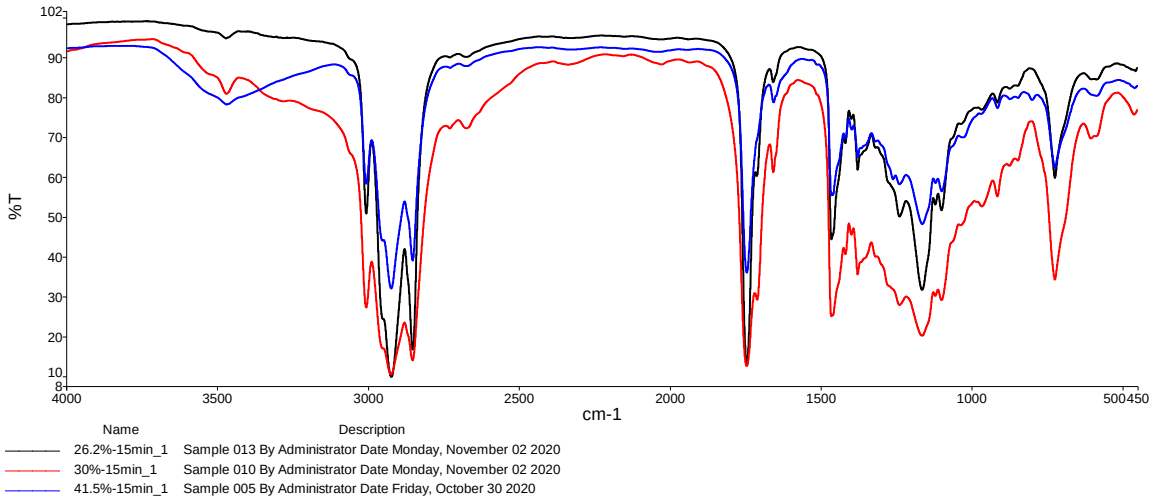


**Figure 24.** FTIR spectrum of samples compare with different humidity in 15 min.

the results of FTIR spectra have assigned the existence of a variety of sharp, strong, and weak peaks as well as crucial functional groups that correspond to C-H, -CH2, -CH3, C=O, C-O, N-H , -COOH , O-H and C=C, suggesting the presence of methyl linoleate natural and laurolactam. the major reference spectrum description search at compounds the bottom of the tables (see Figure 2- 4 - 6) after effect of humidity and tempretuer factor[11]. These functional groups with their rotation and molecular movements and despite being affected by the factors of moisture and heat confirms that the black seed as a natural product was not affected by its components and natural elements according to previous studies.To the best of our knowledge, a weak absorption peak at 2991 $cm^{-1}$ shown in the FTIR spectrum (see Figure 6-13-20) can be corresponded to the C-H stretching of the methyl group. Moreover, the two intense bands observed at 2926 $cm^{-1}$ and 3008 $cm^{-1}$ (see Figure 9,11,13) can be assigned to the C-H stretching of an aliphatic group, Mean while,in (Figure 4- 11- 18) another important strong band is observed at 1745 $cm^{-1}$ and 1657 $cm^{-1}$, which can be attributed to the C=O stretching of the forester and ketone groups, respectively.

In addition,(see Figure 16-18-20) a further remarkable absorption band was observed at 1500 $cm^{-1}$ belonging to the C=O stretching of because of the appearance of N-H in 3473 $cm^{-1}$ the resonance frequency effect of the carbonyl group . The two peaks at 1500 and 1700 $cm^{-1}$ can be related to N-H absorption scissoring and methyl rock in2960 $cm^{-1}$, respectively. In the end, a weak peak at 1332 $cm^{-1}$ owing to the C-N group and a band.

All these results are very similar to those in the literature [12]. These results refer to the fact that humidity and heat factor do not affect much in changing and determining the structure of organic black seeds. Therefore, the pharaohs used them in embalming materials. We also conclude that this material can preserve its properties under changing weather conditions.

* To anthor side compare the results of the experiment, it is necessary to note the changes that appeared at the same time and at the same degree of humidity, then move to compare between different times and with the same humidity, so through Figures ( 2,9,6 ) and( 4,18,13 )and( 16,18,20 ) through the tables( 3,5,7 ) which shows aclear comparison between the peaks at the same time (5,10,15 min) that change the result in tables (3,5,7)

For example, at the peak of 2991, we found that the absorption intensity was very close, with an average of (69.19, 54.62, 65.4) = 63.07 at the same time.

* We note the difference in the peaks at a rate of (0.1 to 1.5) with an average absorption of approximately 53.4

For example, at the peak of (3008.73 cm-1 ,3008.87 cm-1 ,3008.61cm-1) , we found that the absorption intensity was very close, with an average of (50.96, 42.27, 51.4) = 48.21

This is another indication that the black seed is not affected by humidity and heat.

## IV. Conclusions:

Through the tables (2-7) we obtained

*Among the basic compounds that were found when the temperature and humidity differed and were not affected by them is a compound (Natural methyl linoleate ) is a type of linoleic acid that helps in the fat burning process[6].

*Linoleic Acid Methyl Ester is classified under CAS No.112-63-0[11].

*Linoleic Acid Methyl Ester is also known as Methyl Linoleate, Linoleic Acid Methyl Ester, Methyl Ester of Linoleic Acid[9].

*Linoleic Acid Methyl Ester is colorless to pale yellow liquid which is soluble in alcohol and ether.Linoleic Acid Methyl Ester is a common methyl ester produced from soybean or canola oil and methanol[12].

* Through the observation results, we find that the black seed is rich in Linoleic Acid methyl ester$C_{19}H_{34}O_2$ , which applications in cosmetic, flavor and fragrances used as plasticizers, solubilizer, antistatic lubricant & rust inhibitors[8].

*Through the results of the observation and discussion, we found that the black seed was not significantly affected by the humidity and temperature factor, as it can thus be preserved in different storage conditions and makes it not affected by the climatic conditions.

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