

# Optical Determination of the Effects by Thermal Treatment (TT) in Honey of *Apis mellifera* Bees

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## Abstract

Spectroscopy in the UV-Vis and NIR ranges of light provides great convenience for the characterization and evaluation of the characteristics and optical modifications that occur during life, the processes for the preparation of some food in order to modify their characteristics or eliminate Micro-organisms that can affect their quality and shelf life, work is carried out at elevated temperatures, even above 100°C. The study has made it possible to identify changes in the Absorbance, Transmittance and Optical Intensity of *Apis mellifera* honey from four different botanical sources and States of the Mexican Republic: Citrus (*Citrus*) from the state of Veracruz, Mangle (*Rizophora mangle*) of the Pacific zone of the state of Sinaloa, Polyfloralis of the state of Morelos and Polyfloralis of the state of Tabasco.

## Keywords

Heat Treatment, Spectroscopy, Food Conservation, Honey

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## 1. Introduction

Honey is a product known worldwide and appreciated by all cultures since ancient times, but honey presents some inconvenience for its conservation depending on the moisture content, the climate, the storage place and the production practices [1], extraction, packaging, storage and care by the final consumer. Such situations have led people in charge of these activities to develop assurance methods for the good conservation of the product, even in situations of bad ha-

bits which have place during the process of obtaining, until its final consumption in the various applications and uses of the product in medicine, cosmetology, nutrition, food, wine production [2], beers, and food preservation.

With a global production of 183 million tons during 2015 and 179 million tons in 2016 [3] and a growing demand from the consumer market, it is interesting to ensure the conservation of honey for as long as possible. In order to achieve this objective, thermal treatment is used more frequently every day, since this method is easy to apply and ensures the reduction or elimination of micro-organisms [4] that can significantly affect the shelf life and therefore its quality for consumption [5].

Warming in honey produces an accelerated aging effect, which increases hydroxymethylfurfural and decreases Diastase, substances that international standards cite as an index of freshness [6].

In order to detect the effects caused by the heat treatment, we have used, in this work, the search for modifications that honey could suffer due to the effect of said method. Currently, there are new methods that can be agile, non-invasive [7], and reliable to identify if there is any effect on some types of food and the use of spectroscopy is a booming tool to identify the characteristics and changes in food [6] and thus ensure the quality of products.

## 2. Materials and Methods

### **Sample selection:**

Producers have been used to obtain the samples (some are honey exporters) who apply in their processes the specifications recommended by the Manual of Good Livestock Practices in Honey Production [1].

### **Sample Preparation:**

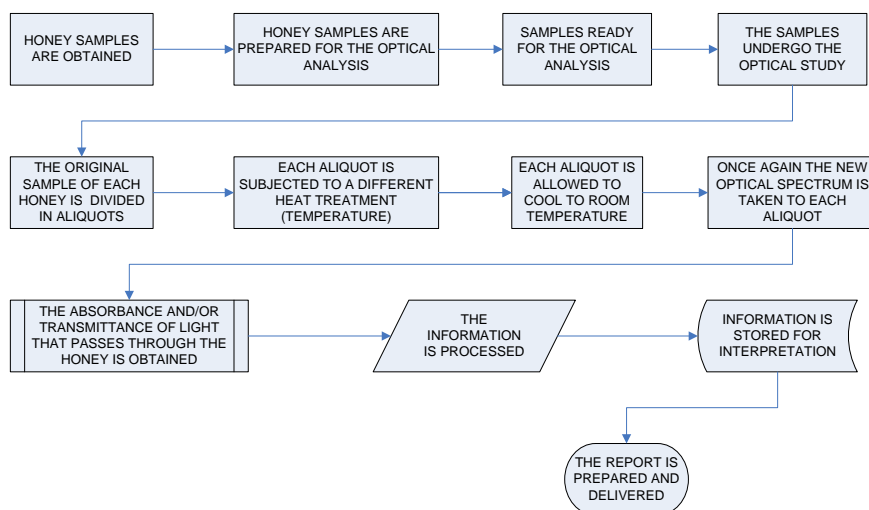
Once the honey is obtained, it is prepared for optical analysis, according to the following flow **Figure 1**.

As indicated in the flow chart above, the original sample (ONE LITER OF HONEY) is divided into aliquots (4 samples) and each one is subjected to different temperature ranges to carry out the intentional heat treatment (heating), then left cooling by the normalized method (natural convection), that is to say, without any procedure to accelerate the cooling. Once the samples (aliquots) are at room temperature, the optical analysis of each one is carried out.

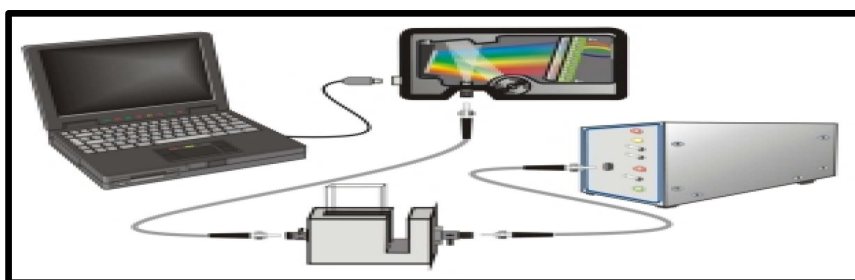
### **Equipment:**

The equipment used to obtain the characteristic and particular optical signals for each honey is an arrangement of source, spectrometers, fiber optic conductors, cell holder, quartz cell and computer equipment, whose arrangement is shown in **Figure 2**.

The wavelengths included in the UV-Vis and NIR [8] zones were those selected for the execution of optical analysis that could show the effects that the Thermal Treatment technique causes on honey, when it is used for tasks to de-crystallize and/or eliminate microorganisms that induce fermentation [9], significantly reducing the shelf life, mainly in honeys that have been harvested



**Figure 1.** Flow chart of the process for taking the optical spectrum to honey samples in this work.



**Figure 2.** Arrangement for obtaining the optical signals of the spectra generated by the passage of light through honey. (Image is taken from the Spectra Suite installation and operation manual, p. 133, document number: 000-20000-300-02-2011307).

before their complete maturation.

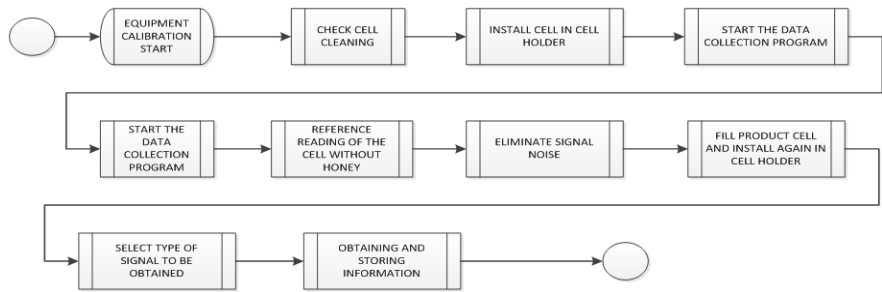
**Figure 3** shown below describes the preparation process of the equipment that allows obtaining the information for the construction of the spectral graphs of the analyzed honey samples.

### 3. Results and Discussion

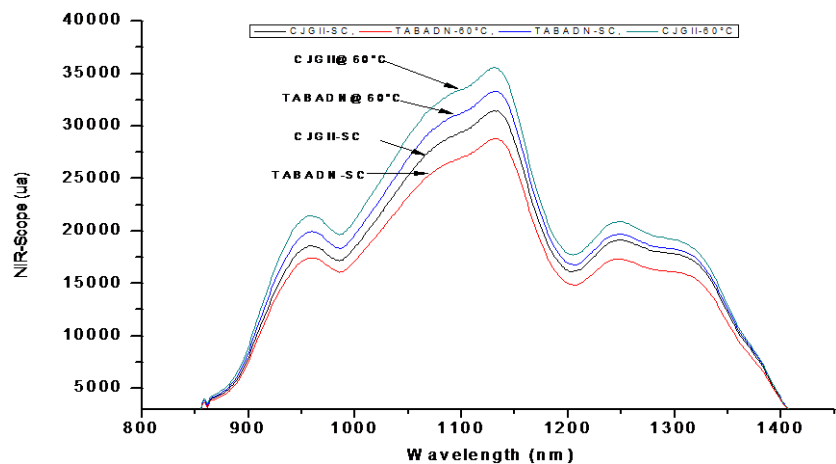
**Figure 4** obtained and set forth below represents the profiles (spectra) obtained by the incidence of light at different wavelengths that have been passed through the honey samples. In each of the images of the spectra obtained, the honey sample is included if heat treatment vs. the sample treated with heat at different temperatures (**Table 1** & **Table 2**).

In this first analysis, the changes that occur in the profiles of the optical spectra influenced by the Heat Treatment to honey can be seen.

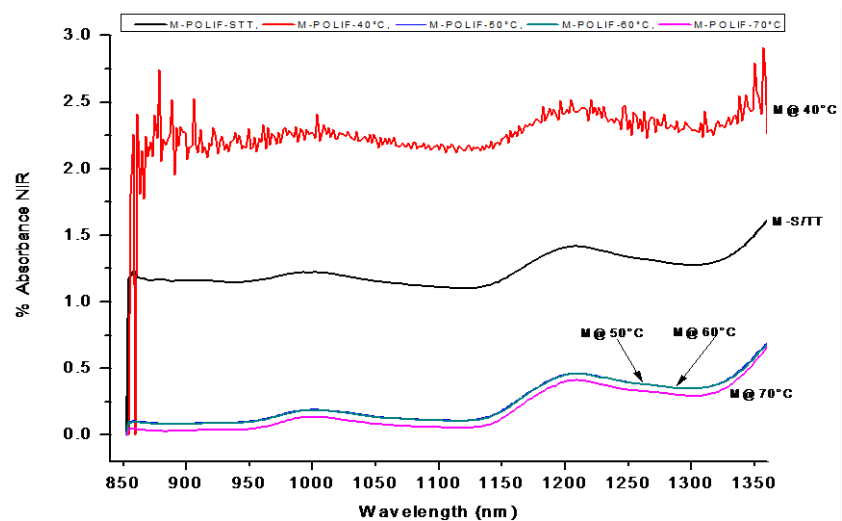
The results obtained in the first optical analysis that highlights the effect of TT on honey from *Apis mellifera* Bees, led to optical analysis of the modification of the profiles of the Electromagnetic Spectrum of honey at different temperatures (**Figure 5**).



**Figure 3.** Flow chart of the process to calibrate the equipment used to detect the modification in the profiles (spectra) of European *Apis mellifera* honey bees undergoing Thermal Treatment (TT).



**Figure 4.** Profiles of the spectra of two honey samples and tables with data of the intensity spectra for the two honey samples that come from different regions of the Mexican Republic, the TABADN sample comes from the state of Tabasco and the CJGII samples which comes from the state of Morelos.



**Figure 5.** Modification of the Absorbance of the optical spectrum of honey without heat treatment (STT) vs. the same honey after heat treatment (TT), at different Temperature Ranges, the sample corresponds to a Polyfloralis honey from the Chalcatzingo region, Morelos.

**Table 1.** Data of the effect of TT on the percentage of Absorbance of the honey sample (TABDN) at different wavelengths (nm).

HONEY SAMPLE	Wavelength (nm)	TEMPERATURES FOR HEAT TREATMENT IN APIS MELLIFERA HONEY BEES	
		STT	60°C
		% Absorbance NIR	
TABADN	961.040	17,377.180	19,878.820
TABADN	1130.980	28,806.640	33,337.000
TABADN	1208.060	14,874.000	16,821.000
TABADN	1247.410	17,336.73	19,698.73

**Table 2.** Data on the effect of TT on the percentage of Absorbance of the honey sample (CJGII) at different wavelengths (nm).

HONEY SAMPLE	Wavelength (nm)	TEMPERATURES FOR HEAT TREATMENT IN APIS MELLIFERA HONEY BEES	
		STT	60°C
		% Absorbance NIR	
CJGII	900.000	18,528.730	21,389.090
CJGII	1130.980	31,474.550	35,568.000
CJGII	1208.060	16,179.730	17,760.640
CJGII	1247.410	19,150.45	20,893.73

The effect of TT at 40°C, produces an increase in Absorbance that subsequently decreases below the transmittance of the STT sample when the TT is performed at a higher temperature (Table 3).

Below (Figure 6) an approach of Figure 5 is shown, shown above. The approach is carried out on the “y” axis from 0% to 0.2% Absorbance, in the near infrared (NIR) zone.

The approach in Figure 6 shows the best separation zone between the Absorbance profiles of heat-treated honey at 50°C and 60°C where the zone has been limited from 850 nm to 1200 nm.

The sample with TT at 40°C and STT are excluded by the approach.

The samples M at 40°C and M-STT were excluded from the image by the approach.

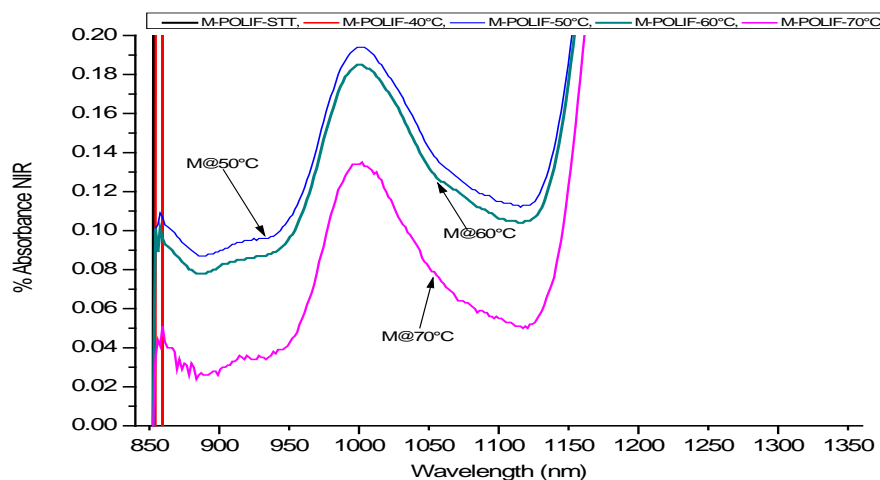
The following spectra (profiles) show the behavior caused by the heat treatment effect in a sample of Citrus honey from Martínez de la Torre Veracruz region (Figure 7 & Table 4).

Next, the NIR Absorbance spectrum for Citrus honey on a reduced scale (zoom) is shown, with the intention of facilitating the observation of the effects of the heat treatment effect (Figure 8).

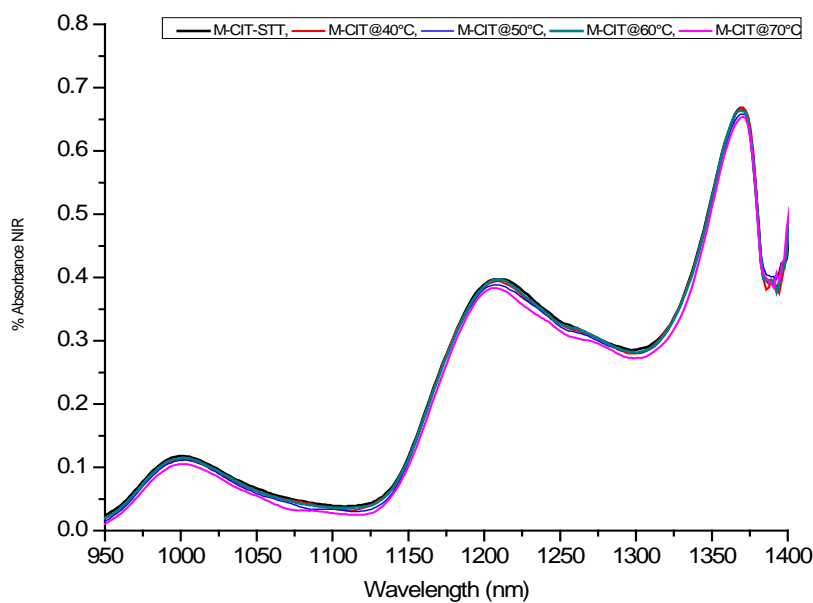
The next honey, from Mangle, analyzed by optical means in this work, comes from a region near the Mexican Pacific coast in the state of Sinaloa (Figure 9 & Table 5).

**Table 3.** The table, generated from data in **Figure 5**, shows the percentages of absorbance at different temperatures and wavelengths (nm) influenced by the TT to the Polyf-loralis honey sample (M-POLIF).

HONEY SAMPLE	Wavelength (nm)	TEMPERATURES FOR HEAT TREATMENT IN APIS MELLIFERA HONEY BEES				
		STT	40°C	50°C	60°C	70°C
% Absorbance NIR						
M-POLIF	900.85	1.157	2.067	0.090	0.082	0.029
M-POLIF	1000.57	1.221	2.267	0.194	0.185	0.134
M-POLIF	1208.06	1.417	2.425	0.467	0.459	0.410
M-POLIF	1355.07	1.563	2.410	0.647	0.638	0.603



**Figure 6.** Approach by scale reduction to facilitate the observation of the samples M at 50°C and M at 60°C.



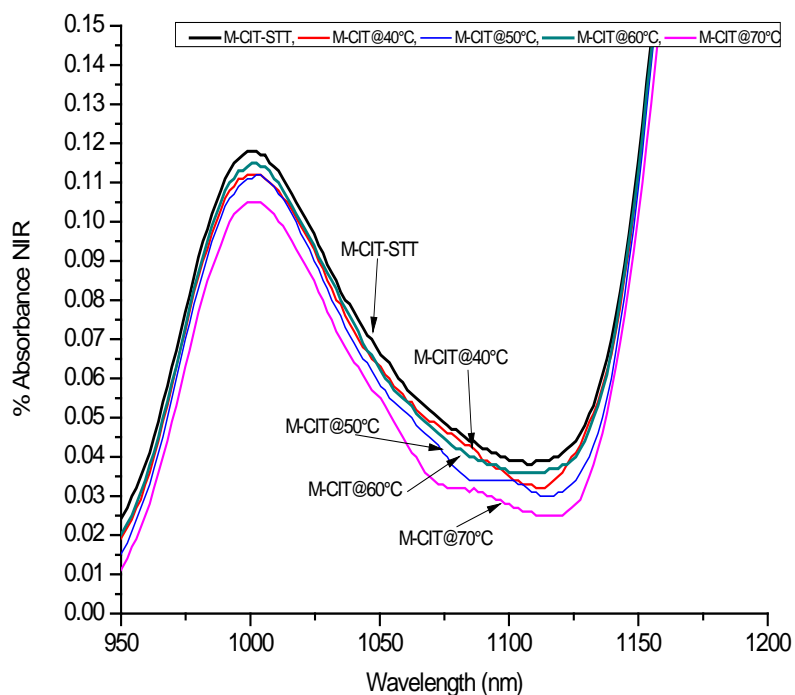
**Figure 7.** NIR absorbance spectrum for the citrus honey sample (M-CIT), without heat treatment (STT) vs. the optical effect after heat treatment (TT).

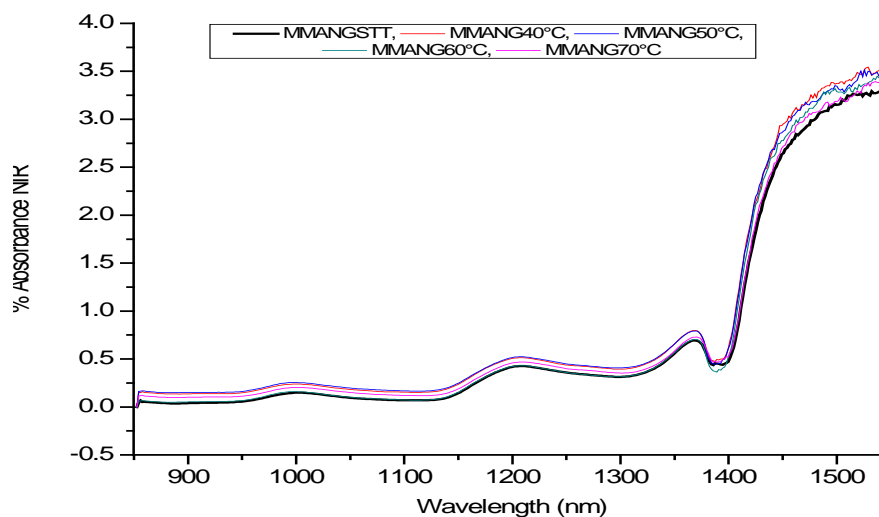
**Table 4.** The data correspond to the variations in the percentage of NIR Absorbance in a sample of Monofloralis Citrus honey at different wavelengths (nm) and temperatures.

HONEY SAMPLE	Wavelength (nm)	TEMPERATURES FOR HEAT TREATMENT IN APIS MELLIFERA HONEY BEES				
		STT	40°C	50°C	60°C	70°C
% Absorbance NIR						
M-CIT	955.89	0.032	0.027	0.024	0.028	0.019
M-CIT	1000.57	0.118	0.112	0.111	0.115	0.105
M-CIT	1110.41	0.039	0.032	0.031	0.036	0.025
M-CIT	1355.07	0.580	0.579	0.572	0.579	0.564

**Table 5.** Variation of the percentages of Absorbance (NIR) at different wavelengths (nm) and degrees of Celsius temperature caused by the effect of TT in Mangle honey (*Rhizophora mangle*).

HONEY SAMPLE	Wavelength (nm)	TEMPERATURES FOR HEAT TREATMENT IN APIS MELLIFERA HONEY BEES				
		STT	40°C	50°C	60°C	70°C
% Absorbance NIR						
M-MANG	900.85	0.040	0.137	0.151	0.053	0.101
M-MANG	1000	0.148	0.239	0.254	0.159	0.203
M-MANG	1200.34	0.426	0.513	0.523	0.430	0.468
M-MANG	1368.73	0.629	0.799	0.791	0.703	0.727

**Figure 8.** The graph shows the resistance and optical stability that Citrus honey presents against the effects of heat treatment compared to the Polyfloralis honey of Chalcatzingo, Morelos (see **Figure 4**), that is, the separation of the spectra with respect to the “Y” axis is less in this Monofloralis Citrus Honey (M-CIT) from Martínez de la Torre, Veracruz.



**Figure 9.** NIR Absorbance Spectra that shows the effects by heat treatment in Mangle Honey from the Pacific coast of the state of Sinaloa.

The effect that the heat treatment exerts on the optical characteristics of this honey sample is not easily appreciated. In order to have a clearer vision, the amplified image of the graph is presented below (**Figure 10**).

For additional information, the NIR Transmittance spectra obtained for the same mangrove honey sample from the Mexican Pacific coast in the state of Sinaloa are presented (**Figure 11**).

The Transmittance in the NIR zone of the EEM is useful, since the difference in the percentage (of Transmittance) can be appreciated for each temperature recorded in the different stages of the TT, as it is the case of this Mangle honey coming from the Pacific coast on the shores of the state of Sinaloa.

The Absorbance results obtained in the Visible range of the Electromagnetic Spectrum of Mangle honey are shown below (**Figure 12**).

The following image shows the effect of the heat treatment on the Transmittance optical spectrum for the same sample of M-MANG honey from the Pacific coast of the state of Sinaloa (**Figure 13**).

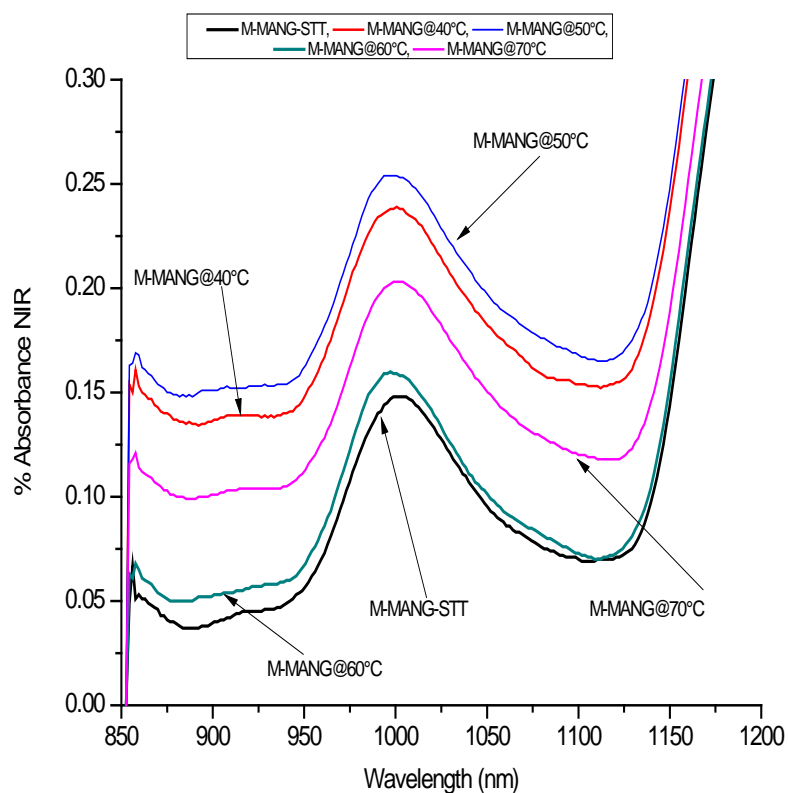
The above image shows that these wavelengths (Visible zone) of the EEM are not useful for detecting the effect of TT on Mangle honey at temperatures of 40°C, 50°C, 60°C and up to 70°C.

#### 4. Conclusions

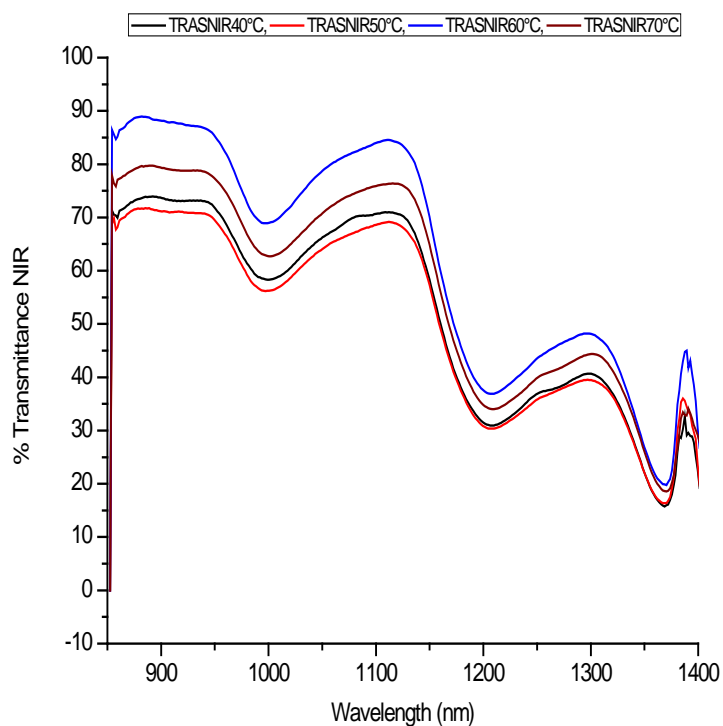
1) The Monofloralis nectar honeys analyzed in the present work were more optically stable against Thermal Treatment (TT) than Polyfloralis nectar honeys, as shown by the graphs of the NIR Absorbance optical spectra profiles.

2) The spectroscopic analysis in the Visible range of the EEM was not useful to identify the effect of the TT performed on the honey samples in the present work, but it can be evidenced in the NIR range, where the lines of the spectral profiles show Greater separation.

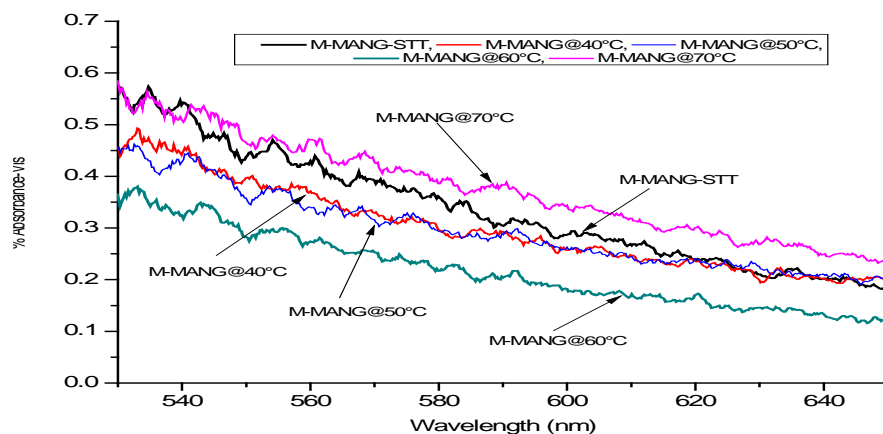




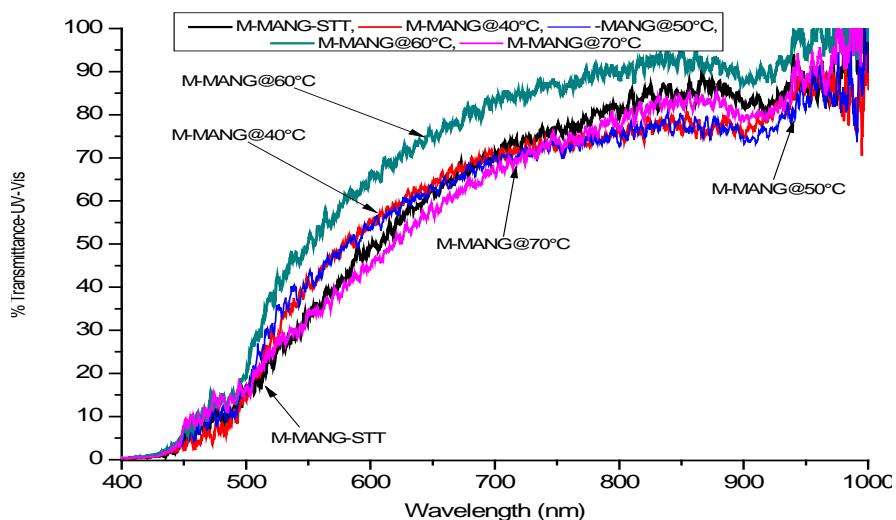
**Figure 10.** Amplification of **Figure 9**, to facilitate the observation of the effects on the NIR Absorbance of the thermal treatment in different temperature ranges on the Mangle honey sample from the Pacific zone of Sinaloa.



**Figure 11.** NIR transmittance for mangrove honey from the Pacific coast of the state of Sinaloa.



**Figure 12.** Absorbance in Wavelengths (nm), from 530 nm to 650 nm. The amount of noise generated by the signal does not allow a clear differentiation in the effect of the TT in the Visible range of the EEM for the sample of mangrove honey from the Pacific coast of the state of Sinaloa.



**Figure 13.** Vis Transmittance Spectra without Thermal treatment and with the effect of Thermal Treatment for the Mangle honey sample M-MANG of the Pacific coast of the state of Sinaloa.

3) It is possible to identify a different optical signal (modification in the optical properties of honey) due to the effect of TT in honey from *Apis mellifera* bees, as seen in the graphs of the profiles of the EEM shown in this work.

4) It is necessary to know the optical profile of the original honey before the TT to later identify the optical variations.

5) The result of the study of TT in honey bee evidences possible effects caused in the increase of Hydroxymethylfurfural and Diastase, both indicators of heating and/or freshness of honey.

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### Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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