1. Introduction

Nowadays, substances are characterized and quantified using different techniques [1]. Some methods are time consuming, expensive, and destructive. These techniques are based on establishing a relationship with chemical properties, such as the presence and amount of a specific substance, and the response to a specific stimulation such as light [2]. When synthetic or biological materials interact with radiation, they either absorb or scatter the radiation. This physical phenomenon is univocal, that is, when a material absorbs some of the radiation that interacts with it, the rest of the radiation is transmitted or scattered [3]. Experimental analysis of materials in the visible wavelength range is simplified by many optical components and detectors optimized for use in this range. Moreover, portable or handheld visible wavelength spectrophotometers enable measurements in the field [4]. To reach the goal of this research is used diffuse reflectance spectrometer, to easily transfer the measurement design directly to chilies.

Analysis of a material using visible radiation allows the estimation of its optical properties, such as the extinction coefficient, which is related to the absorption of light. This optical property can be used in specific ways, such as determining the amount of material desired [1]. Capsaicin is an active compound in “capsicum fruits” [5]. Capsaicin in chilies (capsicum) causes the pungent feeling in the mouth. Moreover, although its properties make it widely used in medicine and foods [6], capsaicin is not well understood. Commercially, it provides flavor to foods and is used in pepper spray. Recent work has shown that topical or systemic use of capsaicin has medicinal value, but sometimes with potential side effects [7].

While the exact molecular mechanisms of capsaicin are still being studied, research has shown that capsaicin activates the metabolism, triggering euphoria and nasal decongestion. Topical capsaicin has been used for skin diseases like psoriasis [8]. Thus, study natural sources of capsaicin are of major interest to help in the development of tools, that will enable rapid quantification of capsaicin in the laboratory, which could then be used to make synthetic capsaicin. Obtaining natural capsaicin can be a challenge because of the current analytical methods that require some homogenization or mixing of samples. Moreover, the amount of capsaicin in a pepper depends on its variety and cultivation conditions.

Researchers have used high-performance liquid chromatography (HPLC) and ultraviolet (UV) spectroscopy for quantifying capsaicin. González et al. [9] compared the predicted capsaicin concentrations in
the capsicum variety “Chilepín”, they used HPLC and compared versus UV spectroscopy, they reported that both HPLC and UV are suitable for quantifying capsaicin extract. Capsaicin extract has been studied using different methodologies and spectral bandwidths [10], Koleva L., et al, used two methods in the UV range based on the Beer-Lambert law to quantify capsaicin extracted from capsicum. They built a baseline using the data from five experiments and showed how the extinction coefficient was important in building the baseline to determine the capsaicin concentration. Among the different methodologies used to study capsaicin, spectroscopy is one of the most used. Spectroscopic methodologies are based on light interacting with turbid media. In theory, spectroscopy establishes how a medium composed of small particles absorbs or scatters light. The analytical problem can be solved by predicting the optical properties used to build an analytical model to quantify the concentration-measuring absorbance curves [11].

Changyeun et al. [12] developed a method based on absorption spectroscopy to quantify capsaicin for alimentary use and found the relationship between the shape of powdered capsaicin particles and their absorption curve. Previous studies compared different absorption spectra of powdered capsaicin samples using least-squares data analysis for near-infrared spectroscopy. Changyeun Mo et al [12], showed that the particle size affected the spectral absorption. The fitted data using least-squares methodologies by Beer-Lambert law to analyze experimental values is the most common used. Gonzalez et al. [9] used a similar methodology to determine the concentrations of capsaicin from different kinds of chilies.

In the present work, an oleoresin based on capsaicin was analyzed in the visible range and its extinction coefficient was calculated. The Beer-Lambert law was used to build a linear model and predict the concentration of capsaicin, and statistical procedures were used to predict extinction coefficients associated with optical parameters. One goal of this work was to calculate the extinction coefficient in the visible wavelength range using a simple linear data fit. Another goal was to build an analytical method using diffuse reflectance visible spectroscopy and standard reference capsaicin to understand experimental factors that have the potential to affect the performance of the method.

We hypothesized that capsaicin could be quantified using visible spectroscopy with backscattered light. Experimental analysis of materials using the visible wavelength range has been simplified by the many optical components and detectors optimized for the visible wavelength [1]. Moreover, portable or handheld visible spectrophotometers enable measurements in the field [4]. The use of a diffuse reflectance spectrophotometer facilitated the transfer of the method to direct chili measurements.

2. Materials and methods

2.1. Preparation of capsaicin samples

Samples were prepared using a capsaicin-based oleoresin made for alimentary use (Reproquín, Ecatepec de Morelos, Mexico), and diluted in pure ethanol (Sigma Aldrich, St. Louis, MO, USA). Liquids samples were analyzed to ensure a homogeneous medium and avoid noise due the particle shape Figure 1. Three groups of samples with low capsaicin concentration were prepared to calculate the extinction coefficient and multiplicative values for the Kubelka-Munk “reemission-function” [13]. Each group of 10 samples were prepared starting at a capsaicin concentration of 0.1% [g/mL × 100%] up to 1% [g/mL × 100%] in 0.1% increments. Samples were prepared at low concentrations to be compatible with the linear range of the detector and to mimic capsaicin concentrations found in natural samples [14].

An error analysis was done for sample preparation despite the error associated with experimental devices they are significantly low, at around ± 0.0005 %.

2.2. Experimental setup for absorbance and diffuse reflectance measures

Quartz cuvettes (Starlab Cells, Atascadero, CA, USA) were used [1-cm path length (l)] to measure the absorption spectra of the liquid samples. A spectrometer USB 400 (Ocean Optics, Largo, FL, USA) with a TCD134AD detector (Toshiba Tokyo Japan), with a wavelength range from 350 to 1100 nm was used. Each cuvette was placed on a cuvette holder. The setup, shown in Figure 2, was manipulated in absorbance mode using special software (Spectrasuite, Ocean Optics). After calibrating the system, all samples were measured 10 times to have 300 measurements (30 per concentration sample).

For diffuse reflectance measurements, the light source and the spectrometer were connected via a bifurcated optical fiber (Ocean Optics, model No. R600-7-UV-125F). The fiber containing six individual fibers were connected to the light source and one central fiber to detect the backscattered light. For this experimental setup Spectrasuite software in reflectance mode was used to take the measures (Figure 3).

Figure 1. Capsaicin samples.

Figure 2. Experimental setup used to measure capsaicin for absorbance spectra.

Figure 3. Experimental setup to measure light absorption by capsaicin samples. The light that passes through the liquid samples in the cuvettes originates from a light source with a bandwidth of 300-1100 nm. The light source is connected to an attenuator.
A custom probe holder was built in-house to ensure consistent contact of the probe with the sample cuvette. The test probe, cuvette, and Spectralon®, (Spectralon® is a fluoropolymer with high scattering coefficient, normally used as standard for diffuse reflectance measures) (Lab sphere, North Sutton, NH, USA) were placed in a simple layered configuration, parallel and in contact, as shown in Figure 4.

This setup was designed in this manner to minimize the glide between the boundary surfaces, moreover, the transmitted light is completely backscattered by Spectralon into the cuvette. The effectively increment light going the sample cuvette. The system was calibrated by blank measure.

Finally, a few samples of “Jalapeño” Chilies was measured direct in the placenta, to compare the spectra from capsaicin oleoresin. Experimental Results.

2.3. Extinction coefficient calculations: absorption experiment results

Figure 5 shows the absorption spectra in the bandwidth from 500 nm to 700 nm. The absorption spectra started at 500 nm, then all of them showed a reduction until reach a minimum around 640 nm. Moreover, after this last wavelength mentioned, the spectra have another maximum peak at 663 nm. These peaks are related to the extinction coefficient of capsaicin present in chilies.

2.4. Absorption data fit

Based on Lambert-Beer Law, the data were fitted to get the molar extinction coefficient for bandwidth reported, a lineal model was used to fit the data. The results are in Figure 6.

According to the Lambert-Beer law, the absorbance is proportional to extinction coefficient and the concentration. In addition, the extinction coefficient is calculated in the wavelength range of 500–700 nm because it is different for each wavelength.

Figure 6 shows that the extinction coefficient has a maximum at 507 nm and decays up to 640 nm, whereon another peak appears at 663.1 nm.

In addition, the results for the correlation coefficients indicate the similarity between the experimental data and the linear model at the corresponding wavelength, for our work the correlation coefficient are close to 1 for all spectral range computed.

3. Diffuse reflectance results and data analysis

3.1. Experimental results for diffuse reflectance spectroscopy

Figure 7 shows the diffuse reflectance signals as a function of wavelength. The curves are the mean of 30 measurements per concentration. The reflectance values of all the concentrations curves in the interval from 500nm to 560 nm starts around 0. As the concentration increases from 0.1% to 0.5% in the 500-560-nm wavelength range, the reflectance signals have greater intensity than those for concentrations from 0.6% to 1%. On the other hand, all curves rise in the interval from 560 to 640 nm. Then the curves decrease to a minimum at 663.1 nm and all the
reflectance signals increase to a maximum at around 700 nm. Furthermore, in the interval from 640 to 680 nm, the minimum concentration of 0.1% has a stronger signal than that for the concentration of 1%. The absorption and diffuse reflectance curves have a similar behavior, meaning that the absorption is strong when the backscattered light is weak and vice versa.

3.2. Theoretical background: Kubelka-Munk reemission function

According to the Kubelka-Munk theory, a turbid media can be characterized by optical properties using diffuse reflectance spectroscopy. The optical properties absorption and scattering are used to quantify the concentration of a specific substance [11]. The Kubelka-Munk theory allows associating a turbid media with the concentration by optical coefficients, as absorption and scattering. Incident light on a sample is scattered unless the material is homogeneous. This light traveling in the turbid media undergoes absorption and scattering, losing intensity but at the same time gaining intensity due to the backscattered light. The reflectance from an turbid media is given by Eq. (1) [15]:

$$f(R) = \frac{(1 - R)^2}{2R} = \frac{k'}{s}$$  \hspace{1cm} (1)

where $R$ is the reflectance light in the ratio of incident light to backscattered light, $k'$ is the absorption coefficient, and $s$ is the scattering coefficient. In this second-degree equation, the minimum of parabola is expressed as $s = 2[k' - (k'/s)]$ and $R = 1 - \left(\frac{s - k'}{s}\right)^2$.

The solution to the reemission function is as follows:

$$R = 1 + \k \pm \sqrt{2k' + \left(\frac{k'}{s}\right)^2}$$  \hspace{1cm} (2)

If absorption by the medium is low, the quadratic term in Eq. (2) is insignificant. According to the Mie theory, absorption and scattering are strongly related because if one increases, then the other decreases. Thus, for this model, $k' < s$ and $s \neq 0$ [16]. This is similar to the Lambert-Beer law, so $k'$ can be proportional to the concentration ($k' = \varepsilon c$) and still depends on the scattering coefficient [19]. In addition, in solving Eq. (2), the negative root is chosen because the value of $R$ can be only between 0 and 1. Using this information and algebra, Eq. (2) can be rewritten as

$$R = 1 - \left(\frac{2\varepsilon c}{s}\right)^{1/2} + \frac{\varepsilon}{s}c$$  \hspace{1cm} (3)

where $\varepsilon$ is the extinction coefficient, $s$ is the scattering coefficient and $c$ the independent variable. The ratio $\varepsilon/s$ can be defined as a multiplicative constant as follows:

$$a_1 = -\left(\frac{2\varepsilon}{s}\right)^{1/2}$$

$$a_2 = \frac{\varepsilon}{s}$$

and $a_0 = 1$. So, Eq. (3) is rewritten as follows.

$$R(c) = a_0 - a_1c^{1/2} + a_2c$$  \hspace{1cm} (4)

To fit the experimental data to Eq. (4), we used the least-squares method for the nonlinear expression to calculate the coefficients ($a_0$, $a_1$, $a_2$) and the correlation coefficient [17]. The calculations were performed using Matlab 2015 code, which can fit the necessary parameters to build the mathematical expression needed to calculate the concentration for each wavelength. To fit the experimental data for each wavelength, the code uses the diffuse reflectance versus the concentration to fit the nonlinear model [18].

3.3. Data-fitting results: diffuse reflectance

The coefficients computed for the diffuse reflectance experiments are plotted in Figure 8. These data were used to generate the corresponding mathematical expression for each wavelength. The expression is used in the reemission function to calculate the concentration.

Figure 8 shows that in the interval from 500 to 560 nm, coefficient $a_0$ is initially around 0 and then increases to approximately 1 at 560 nm and remains close to 1 up to 700 nm. All $a_1$ values are negative, which agrees with the sign from the mathematical model. Finally, all $a_2$ values are positive from 500 to 670 nm, then become negative up to 700 nm due the capsaicin in this part of the spectrum has a high reflectance value.

The accuracy of these diffuse reflectance measurements was shown via statistical data fitting using the correlation coefficient [19]; the results are shown in Figure 9. The correlation coefficient increases from under 0.55 up to 0.95 for the wavelengths from 500 to 560 nm. Above 560 nm, the correlation coefficient is over 0.95 and increases to close to 1 up to the end on the spectral wavelength range. The correlation coefficients for the interval from 500 to 560 nm are low because capsaicin has high absorption in this part of the spectrum.

3.4. Concentration

To determine the concentration, the reemission function is formulated for each wavelength using equation (10) and the computed coefficients $a_0$, $a_1$, and $a_2$. The diffuse reflectance experimental data from the spectrometer for each wavelength is used to solve the quadratic

Figure 7. Diffuse reflectance versus wavelength. The curves show the relationship between the signal and the concentration. For low-concentration samples, the signal is strong, and as the concentration increases, the curves lose intensity.

Figure 8. Coefficients computed using least-squares fitting.
equation and recover the concentration. Figure 10 presents the results of this test for some wavelengths. Each mark on the y-axis corresponds to one recovered concentration, and each of the 10 lines represents one independent experiment. This methodology was applied to the entire wavelength range used in this work [20].

Finally, a few measures were carried out directly in chilies. The purpose was comparing the spectra between pure capsaicin and placenta chilies, where the concentration of capsaicin is high [21]. The results are in Figure 11.

4. Discussion

In this research, capsaicin was analyzed in the visible range from 500 to 700 nm, although normally it has been studied in the UV range from 250 to 300 nm [5]. The extinction coefficients of 2.537 and 0.288 at 507 and 663.1 nm, respectively, were reported from data analysis. In the maximum absorption (at 633.1 nm), the molar extinction coefficient reported gives new opportunities to make research for high concentrations of capsaicin in the part of the visible range. As the absorption of capsaicin has a linear behavior at each wavelength, these results could be used to build a baseline for applying the Lambert-Beer law using similar methods or improve new techniques in the visible region.

In the spectroscopic techniques used, the particle shape influences the measurements. Mo et al. showed how the particle size changes the absorption curve [21]. To avoid this extra variable, we measured liquid samples to calculate the extinction coefficient in the visible range. The advantages of this experimental setup used are; minimizes the glare between the boundary surfaces and the transmitted light is diffusely reflected from the Spectralon into the cuvette, thus effectively increasing the light going through the sample cuvette.

Particle shape restrict absorption methodologies that use diffuse illumination. Changyeun Mo et al [12] developed a method to quantify capsaicinoids from powdered samples. They reported the relationship between absorption spectra and particle shape and size. These properties could give noise signals due the scattering changes with the particle shape [22]. Due these behavior in this study, liquid samples were analyzed to reduce the effect of the shape of the particles and have a homogeneous medium. One of the objectives was reached, because we traduce diffuse reflectance signals of low concentrations of capsaicin in a useful information to build the methodology to identify it.

Different theories are used to describe the interaction of light with turbid media. The Kubelka-Munk reemission function is the most common optical model [23]. This model was used in this study to associate the experimental data from samples with different capsaicin concentrations with diffuse reflectance signals A square regression based on the reemission function approximating the experimental data to a polynomial expression was implemented to calculate the optical properties [18]. Statistical data-fitting analysis used to the experimental data using the Kubelka-Munk theoretical model, was applied to calculate the optical properties to build the diffuse reflectance equation for each wavelength.

In addition, the reflectance equation was used to compute the initial concentrations. These values calculated show that around these were 90% close to their experimental values.

To validate these percentage results, an error formula was used [23]. Diffuse reflectance spectroscopy is commonly used with powdered samples, and the most common wavelength ranges used are UV and near infrared (NIR). In this paper, we presented a new way to study capsaicin in another part of the electromagnetic spectrum. Moreover, the extinction coefficient at ~663 nm could provide a chance to improve a similar analysis for high-concentration samples. Although our research was on liquid samples, our results are quite like those reported by Won Young et al. for pure capsaicin [24].

Figure 11 shows the relation between direct measures in chiles and spectra from oleoresin. The graphics are quite similar around the reported maximum at 631nm, moreover, among 660nm and 680nm the graphics has a strong qualitative correlation.
5. Conclusions

The most significant extinction coefficients are $\varepsilon_1 = 2.573$ at 507 nm and $\varepsilon_2 = 0.288$ at 663.1 nm, where the peaks maximum of the capsaicin spectra were found. The reported data are accurate enough that they can be used to build a baseline for other applications. The advantage of having this optical property at different wavelengths in the visible range, is that it could be a useful tool for predicting concentration. The absorptions spectra were a significant information to relate the both optical phenomena scattering and absorption, proving that theoretical model are concordant with experimental data.

The behaviors of the signals from diffuse reflectance spectroscopy are closely related to the concentration. Moreover, the mathematical model of analysis allowed easily identify the optical coefficients of Kubelka-Munk reemission function. These coefficients used to report our quantification model. To conclude, our model was able to obtain the initial concentration of capsaicin of studied samples in part of the visible range, and in some samples with a low percentage error. This method was useful for low concentrations in the 560-700-nm range. In addition, the diffuse reflectance spectra in chilies and pure capsaicin are similar indicating that capsaicin is of easy to identify making direct measures in chilies. Diffuse reflectance signals allowed the identification of capsaicin and opened the possibility of this fast and easy to do method for classification and quantification of bioactive compounds.

Declarations

Author contribution statement

Manuel Abraham López Pacheco: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

José Javier Báez Rojas: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Jorge Castro-Ramos: Contributed reagents, materials, analysis tools or data; Wrote the paper.

José Fabian Villa Manríquez: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Karen Esmolde-White: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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