Characterization of Natural Hepatoprotectors and Added Foods by Photoacoustic Spectroscopy and Colorimetry

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Research Article

Keywords: Hepatoprotectors, photoacoustic spectroscopy, “Tortilla, Raphanus sativus

Posted Date: July 5th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1794731/v1

Abstract

The current pandemic events make it necessary to develop foods with a higher content of nutrients that have a better impact on the health of the population. The improvement of daily food mixtures by adding nutraceutical materials with bioactive substances has a favorable effect on food products and consumers. In the present study, photoacoustic spectroscopy and colorimetry were used to characterize hepatoprotective plants and tortillas added with black radish (2 and 8%) and black radish and turmeric (3%). The main findings were: 1) Of the evaluated hepatoprotectants *Silybum marianum, Peumus boldus* and *Raphanus sativus*; the black radish peel is the one that presents the highest level of photoacoustic signal at the wavelength (250 -900 nm). 2) The "tortillas" characterized by PAS and colorimetry, presented a positive correlation between the photoacoustic signal at the wavelength of 450 nm. and the color variables a* and b*. Likewise, there is a correlation between the color parameter L* and the amplitude of the photoacoustic signal at the wavelength of 350 nm. 3). The dehydration process modifies the color of the tortilla, being the variable L*, the most affected and to a lesser degree a* and b*. The PAS technique and colometry permit the study of naturals hepatoprotectors, and blends of cornmeal added with black radish.

Introduction

Currently there is a great food and nutrition problem that is increasing, due to the poor quality of nutrients contained in them on the one hand and on the other hand the excess of carbohydrates which is one of the factors that triggers the problem of fatty liver which is simply the initial stage of more serious diseases such as cirrhosis and cancer [1–8]. Thus, the relationship between diseases and diet is increasingly confirmed [9–10]. The WHO reports that 8.2 million deaths worldwide are caused by these dietary problems. Thus, food characterization is becoming more and more necessary. Currently, one of the non-destructive and environmentally friendly alternatives for food characterization are spectroscopic and color measurements. These can be used for characterization without prior sample preparation. FT techniques are developed based on the photoacoustic phenomenon discovered by A. Bell in 1880 [11], and later mathematically modeled by Rosencwaig, A. Gersho in 1976 [12]. The photoacoustic effect is based on the response of materials when a modulated light source is incident on them and produces a modulated heating generated by the sample inside a hermetically sealed cell manifesting adiabatic changes due to the expansion and contraction of the gas contained in the photoacoustic cell. These pressure variations can be censored by means of an electret microphone that generates a response in power difference (voltage) and by means of an electronic device it is digitized obtaining its amplitude and phase components. With the use of FT techniques have been characterized from metals, liquids, semiconductors [13], to homogeneous and non-homogeneous biological materials [14–17]. In the area of food and agriculture, grains, seeds, and plants have been characterized [18–22]. As well as specific foods such as tomato, margarines, flours, cocoa, soups, milk, etc. [23–29]. On the other hand, characterization can be performed by using colorimetric techniques to obtain the optical differences of materials in the variables (CIEL*a*b*) that can be used for food analysis [30]. In this research, the characterization of different parts of hepatoprotective plants such as black radish (*Raphanus sativus* var. Niger), boldo leaves (*Peumus boldus*) and milk thistle (*Silybum marianum*), which contain a large amount of substances that have beneficial effects on liver diseases [30–31], as well as tortillas made with a mixture of corn flour and black radish added with curcuma (*Curcuma longa* L.), of the hepatoprotective plants and of the "tortillas", their photoacoustic spectra were obtained, as well as their color parameters and their interaction to later obtain their main parameters. Corn is known to be made up of carbohydrates [32], which when added with nutraceutical elements or fiber have been shown to modify the glycemic impact on those who consume them. In addition, using bioactive compounds that have been reported as hepatoprotectors, could benefit human health, using an option such as black radish and turmeric to add to the corn tortilla.

Materials And Methods

Biological material

Three hepatoprotective biological materials were used: *Silybum marianum* (plant: exterior and interior of the flower and leaves), *Peumus boldus* (plant: leaves), *Raphanus sativus* L. var. Niger (vegetable: shell: exterior and interior part) acquired in a supply market in Mexico City. From the black radish fruit peel (RN), flour (radish powder) was obtained and used to prepare a food product: Tortilla with black radish, black radish, and turmeric. Thus, turmeric powder was used as an ingredient with bioactive elements, with Kosher certification and nutritional information (protein content: 10 g, carbohydrates: 67 g, sugar: 3 g, dietary fiber: 23 g, sodium: 0 g and energy content: 335 Kcal, per 100 g).

Food formulation: "Tortilla"

The formulations for the preparation of the tortillas can be observed in Table 2. It is possible to appreciate the ingredients used to make the dough in percentages (pepper, salt, corn flour-commercial, black radish powder (obtained from the interior-HIRN and exterior (pel)-RCN), turmeric (3%) and *Psyllium* (5%).

Corn tortillas were added with RCN at 2 and 8% without and with turmeric at 3% concentration in relation to the amount of commercial corn flour used (100 g).

Case I. Corn tortilla (control (TN2) - without addition of RCN) and with two different types of black radish flour percentage (2% (TC1) and 8% (TC4)).

Case II. Corn tortilla (control (TN2) - without addition of RCN)) and added only with 3% turmeric (TNC) and added with both, black radish, and turmeric (2%RCN and 3% turmeric (TCC1) and 8% RCN and 3% turmeric (TCC4).

Black radish powder (RCN)

The black radish, purchased fresh from supermarkets, was sliced, and dehydrated at 50 °C (using a Hamilton beach dehydrator) for 10 h. The radish was then crushed for 10 min in a food processor (Hamilton beach 70725) to obtain the radish powder. It was then ground for 10 min in a food processor to obtain horseradish powder. This RCN powder was stored in a glass container for later use with the dough. The total processed material was 1 kg to obtain 350 g of black radish flour.

Tortilla preparation

The ingredients were placed in a container (99.4 g of commercial corn flour, 120 ml of warm water (36°C), 0.5 g of salt, and 0.1 g of pepper), adding for each type of tortilla the respective concentrations of black radish and turmeric. The ingredients were mixed, kneading the dough formed. It was left to rest for 1 h (in a container covered with a moist cloth), and then the dough was divided into 25 g portions. These portions were placed in a manual machine used for flattening the tortillas. Finally, the tortillas were passed to the cooking process in a black+decker flat grill at 180°C, cooking for 40 seconds on the first side, 45 s on the second side, and 30 s, and on the third side until the tortillas puffed. For tortilla characterization by photoacoustic spectroscopy, the tortillas were dehydrated for 12 h at 40°C in a Hamilton Beach model 32100 dehydrator. These samples were crushed (by means of a microprocessor) for 5 min until powder was obtained. The samples were placed in test tubes covered with aluminum for analysis.

Table 1

Tortilla Formulation

Type of Tortilla	Pepper (%)	salt (%)	Corn flour (%)	HIRN (%)	HRCN (%)	Turmeric (%)	*Psyllium (%)*
TI1	0.1	0.5	97.4	2	0	0	5
TI4	0.1	0.5	91.4	8	0	0	5
TC1	0.1	0.5	97.4	0	2	0	5
TC4	0.1	0.5	91.4	0	8	0	5
TCC1	0.1	0.5	94.4	0	2	3	5
TCC4	0.1	0.5	88.4	0	8	3	5
TN2	0.1	0.5	99.4	0	0	0	5
TNC	0.1	0.5	96.4	0	0	3	5

HIRN: flour of Interior Black raddish, HRCN: Flour of peel Black Raddish

Experimental Setup for PAS

Experimental setup PAS (Fig.1) has as excitation source a 700W xenon lamp (Oriel). The light is focused to a monochromator with which a 250 to 900 nm scan is made, emitting a monochromatic light beam at the output. This light passes through a mechanical chopper to modulate the beam at f=17 hz, which is transmitted by an optical fiber to impinge on the PA cell containing the sample in its interior. The photoacoustic cell is hermetically sealed with two quartz crystals with silicone grease. The modulated light is absorbed by the sample and transformed into modulated heat which causes a variation in the temperature on the sample surface, causing the air layer surrounding the sample to heat up in a modulated manner. These periodic temperature changes cause fluctuations in the surrounding air pressure, which produce sound waves that are detected by an electret microphone that is used as a sensor. This is connected to a lock-in amplifier where the amplitude and phase components of the photoacoustic signal are obtained and sent to a computer where it is stored. The amplitude signal was recorded in the range of 250-900 nm for the hepato-protectants and the added tortillas.

Color

The color of tortillas was determined in two conditions: dehydrated and nondehydrated. Using pre-calibrated manual colorimeter (FRU WR-10QC, Shenzhen Wave Optoelectronics Technology Ltd, China). The color parameters corresponding to the CIELAB uniform color space (L*, a* and b*) were obtained directly from the instrument. L* indicates lightness (100=White and 0=Black), "a" indicates greenish-reddish [negative (-a) (green) to red (+a) (positive)] and "b" indicates bluish-yellowish [negative (-b) (blue) to yellow (+b) (positive)]. The equipment was calibrated using a reference blank L*= 96.65, a= -0.03 and b= 1.86.

Additional color parameters were determined as the whiteness index (WI), hue (h_{ab}) y chroma (C_{ab}) [29, 33-34]. Finally, color differences between tortillas were determined in CIELAB space, according to the equations presented in Table 2.

Table 2

Equations used to determine color variables.

Description	Equation
hue	$h_{ab} = \ arctan\left(b^*/_{a^*}\right) C_{ab} = [\ (a^*\ )^2 +\ (b^*\ )^2]^{1/2}$
Chroma	$C_{ab} = \dfrac{[\ (a^*\ )^2 +\ (b^*\ )^2]^{1/2}}{arctan\left(b^*/_{a^*}\right)}$
Whiteness index	$WI = \ 100\ -\ [(100 - L^*\ )^2 + (a^*\ )^2 +\ (b^*\ )^2]^{1/2}$
Differences in color	$\Delta E = \ [(\Delta L^*\ )^2 + (\Delta a^*\ )^2 +\ (\Delta b^*\ )^2]^{1/2}$

Tortilla Sanitary Test.

The sanitary quality test was established to evaluate the number of fungal colonies growing on the tortillas. It was established under a randomized complete block experimental design with three replicates for each type of tortilla prepared with the different formulations. The tortillas were selected and placed in polystyrene bags previously sterilized with alcohol. The samples were incubated in a closed space with a maximum temperature of 28 ± 1 °C and a minimum of 15 ± 1 °C. The observation and quantification of the existing fungal colonies was performed at 3 and 9 days of incubation. The observations were made according to Hernández *et al*. [35]. The fungal genus was identified according to the taxonomic keys [36]. Finally, the fungal colony count was performed, and the data were recorded.

Análisis estadístico

Analysis of variance

For the analysis of variance of the variables evaluated, an analysis of variance (ANOVA) was used by means of Statgrafic and MiniTab software. Additionally, the least significant difference (LSD) test was performed at the 5% probability level to compare the variables for each type of tortilla.

Principal component analysis.

PCA was used to correlate the color variables evaluated and the amplitude values of the photoacoustic signal at wavelengths 350, 450, 600 and 650, as well as to classify the tortillas evaluated. The analysis was performed using R Project software version 0.10-47, with R Commander and the program factoMiner and Fitopac (2.1).

Results And Discussion

Photoacoustic spectra

Hepatoprotective plants

Figure 2 shows the spectra obtained from *Silybum marianum* (plant: exterior and interior of the flower and leaves), *Peumus boldus* (plant: leaves), *Raphanus sativus* L. var. Niger (vegetable: shell: exterior and interior part). The behavior pattern of the spectrum of the radish peel (black color) presents a maximum amplitude value of the photoacoustic signal (0.145 mV) at the wavelength of 250 nm. Subsequently, it presents the minimum value of the spectrum at 300 nm (0.075 mV), with a tendency to increase the level of the photoacoustic signal, until the wavelength of 350 nm, from which it tends to have a small decrease, for example at 900 nm, a decreasing value of the photoacoustic signal of 0.09 mV is presented. However, the spectrum of the inner part of the radish (white color), presents the spectrum with the lowest amplitude values of the photoacoustic signal. It is observed that from the wavelength value of 300 nm, the amplitude value of the photoacoustic signal is between 0.005-0.015 mV.

As shown in Figure 2, in all the spectra there is an absorption band 300-400 nm, corresponding to the highest amplitude values of the spectra. It is worth mentioning that this absorbing band is related to the presence of flavonoid pigments [37]. All the spectra of the hepatoprotective samples from 300 nm tend to increase and then decrease from 370 nm. Except for the spectral curve of boldo leaves (green color), which is characterized by a third spectral band, in the wavelength range of 650-700 nm, with a maximum peak value at the wavelength of 680 nm, As shown in Figure 2, in all the spectra there is an absorption band 300-400 nm, corresponding to the highest amplitude values of the spectra. All the spectra of the hepatoprotective samples from 300 nm tend to increase and then decrease from 370 nm. Except for the spectral curve of the boldo leaves (green color), which is characterized by a third spectral band in the wavelength range of 650-700 nm, with a maximum peak value at the wavelength of 680 nm, corresponding to the absorbing point of the wavelength of chlorophyll *a* [38]. The characteristic photoacoustic patterns show significant differences between the spectra of each type of hepatoprotective element, mainly in the 300-400 nm band, followed by the 600-700 nm band, where the differences between the spectra of the samples are smaller. In this way, the black radish peel is the one that presents the best performance, linked to a higher content of pigments and consequently a higher quantity of bioactive elements, which are generally therapeutic. Black radish (*Raphanus sativus* var. Niger) has been used since ancient times for the treatment of different diseases such as liver disorders [39]. Some recent studies indicate that it has hepatoprotective effects on CCL4-induced lesions [40]. It has also been found to have effects on high-fat diets by improving scavenging activity, as well as protecting the mucous membrane against lipid peroxidation [10]. Different chemical compounds have been reported in this plant, which are gallic acid, pyrogallic acid, chlorogenic acid, l-tyrosine, pyrocatechol, caffeic acid, p-coumaric acid, 3,4-dihydroxybenzoic acid and floroglucinol [39]. In this way, the black radish husk powder was used in the preparation of the tortilla. Then, the changes observed in tortillas made with black radish and radish and turmeric are analyzed.

Figure 3 represents the curves of the characteristic photoacoustic signals of black radish peel, reference tortilla (control-not added) and added with black radish at 2 and 8% (Fig. 3a) and added with 3% turmeric and added with black radish (2 and 8%) and turmeric (3%) (Fig. 3b).

Figure 3a shows that the photoacoustic signal spectra of TN2, TC1 and TC4 are below the amplitude level of the PA signal of the RCN. When comparing the TN2, TC1 and TC4 tortillas, in the wavelength range of 450-900 nm, the photoacoustic signal with the highest amplitude corresponded to the TC4 "tortilla". The maximum changes with respect to the TN2 tortilla in the 500-700 nm range. This range of high values is related to a major capacity of antioxidant activity, due to the compounds present in this range.

On the other hand, in Figure 3b, it is possible to observe that just like the tortillas added with radish, those added with turmeric and turmeric and radish are below the amplitude level of the PA signal of the RCN. The TN2 (control-not added) and TNC "tortillas" (added only with turmeric) had the lowest amplitude level compared to the "tortillas" added with radish and turmeric (TCC1 and TCC4). The latter presented a higher maximum spectral band (300-500 nm) with higher PA signal values (0.7-0.95 mV) compared to the tortilla prepared without radish and with turmeric (0.2-0.6 mV). This means that the "tortilla" added with turmeric (3%) and radish (8%) had the best nutraceutical properties.

Comparing Figures 3a and 3b, it can be seen how the combined effects of turmeric and radish have a synergistic effect that allows to potentiate their nutraceutical properties, since the photoacoustic signal level is elevated to a major extent than when a "tortilla" is added with either radish or curcuma. But when both are combined (TCC1 and TCC4), the effects are enhanced, as evidenced by the higher spectral bandwidth and higher photoacoustic signal values.

It is important to highlight the reddish color and not the yellowish color of the "tortilla" added with turmeric powder. This may be due to the reaction to the nixtamalized corn flour used to make the "tortilla", which gives it a certain alkalinity, reflected in the "tortilla" with a color change.

Color

Table 3 shows the means of the Cielab values (L*, a* and b*) in the non-dried and dehydrated "tortillas", as well as the chroma, hue and whiteness index. It is possible to observe significant differences ($p \leq 0.05$) between all the "tortillas" and in all the evaluated variables. The brightness of the "tortillas" added with black radish peel (TC, TCC) presents a decrease when compared with the normal tortillas (without radish addition), this is because the addition of black radish peel gives a grayish color to the "tortilla", so that when the whiteness of an object decreases, lower values of the color parameter L* are obtained.

Regarding the "tortillas" added with turmeric (TNC, TCC) they present the same behavior of "tortillas" added with black radish, *i.e.*, they present a decrease L* with respect to the "tortillas" (Control-not dehydrated). It is observed that all the dehydrated tortillas lost luminosity with respect to the values of the "tortillas" not dehydrated. For the color a* variable, the "tortillas" added with turmeric (TNC, TCC) presented an increase in values with respect to the tortillas (TN), while the tortillas added with black radish peel (TC1 and TC4) showed a decrease in value (2.43, 2.31, respectively). In this dimension of color, a*, decreases in the values of the dehydrated tortillas were also observed with respect to the non-dried tortillas, in a percentage between 28-33% of the values. In relation to the color parameter b*, it can be observed that the values of the tortillas added with turmeric (TNC) or black radish and turmeric present the highest values when compared to the tortillas TN2 (Control), TI1, TI4 ("tortillas" added with the powder obtained from the inside of the radish) and TC1 and TC4 (tortillas added with black radish peel). Likewise, it can be observed that the dehydrated tortillas present a decrease in the values with respect to the non-dehydrated tortillas (decreasing in a range between 0.5 and 15 %). In the color parameter whiteness index (WI), a decrease is observed due to the presence of black radish hull flour (TC) and turmeric (TNC, TCC) with respect to the TN2 "tortilla" (Control), with values of TNC = 26.08, TC4 = 46.89, TCC1 = 29.86 and TN2 = 56.44. In the chroma, an increase is observed in the tortillas added with turmeric (TCC, TNC) and a decrease in the values of the tortillas added only with black horseradish peel (TC). It is important to mention that both the whiteness and chroma index values of the dehydrated tortillas showed decreases with respect to the non-dehydrated "tortillas". Finally, the values of the hue color variable showed the least changes, either when comparing the values between tortillas or when comparing the values between dehydrated and non-dehydrated tortillas (Fig. 4).

Table 3

omparison of color parameters of the different "tortilla" formulations.

Type of tortilla	Non-dehydrated						Dehydrated “tortilla”					
	L*	a*	b*	WI	Chroma	Hue	L*	a*	b*	WI	Chroma	Hue
TN2	61.08	4.95	18.93	56.44	19.56	1.315	59.22	3.53	16.23	55.96	16.61	1.35
TNC	43.78	26.05	40.29	26.08	47.98	0.996	42.97	17.61	40.15	28.06	43.84	1.15
TI1	70.75	2.88	16.68	66.21	16.93	1.39	60.48	3.27	15.29	57.49	15.64	1.36
TI4	58.07	4.59	15.82	54.95	16.48	1.28	46.31	4.55	13.03	44.56	13.81	1.23
TC1	60.15	2.43	10.85	58.62	11.12	1.35	48.07	2.72	10.78	46.89	11.12	1.32
TC4	47.53	2.31	7.843	46.89	8.17	1.28	38.70	2.39	8.06	38.12	8.418	1.28
TCC1	41.40	15.28	35.38	29.86	38.54	1.16	38.32	11.27	33.42	28.94	35.28	1.24
TCC4	40.05	5.60	28.72	33.29	29.26	1.37	35.82	5.41	27.94	29.79	28.46	1.38
R^2	.155	.135	.0978	1.00	1.00	1.00	.237	.166	.089	1.00	1.00	1.00
CV	21.47	105.16	49.82	32.04	57.59	10.66	20.14	77.07	52.51	26.97	56.96	5.87
Media	55.04	7.343	21.23	48.86	22.85	1.29	46.65	6.242	20.08	41.89	21.10	1.28
α	0.00	0.00	0.00	*	*	*	0.00	0.00	0.00	*	*	*

L*: brightness, a*: green-reddish dimension, b*: blue-yellowish dimension, WI: whiteness index, TN2: “tortilla” not added (Control), TNC: “tortilla” added with turmeric, TI1 and TI4: “tortilla” added with 2 and 8% of radish (interior), TC1 and TC4: “tortilla” added with 2 and 8% of black radish, TC1 and TC4: “tortilla” added with 2 and 8% black radish powder, TCC1 and TCC4: “tortilla” added with 2 and 8% black radish powder and turmeric (3%), R2: coefficient of determination, CV: coefficient of variance, α: significance.

Table 4

Differences in color of dehydrated and non-dried black radish tortillas.

Type of condition and tortilla		$\Delta E_{Factor-TN2}$	$\Delta E_{Factor-TNC}$	$\Delta E_{Factor-TI1}$	$\Delta E_{Factor-TI4}$	$\Delta E_{Factor-TC1}$	$\Delta E_{Factor-TC4}$	$\Delta E_{Factor-TCC1}$	$\Delta E_{Factor-TCC4}$
Not- dehydrated	TN2	0	43.84204	2.11199	14.7899	14.072	26.8247	38.2779	34.5842
	TNC	43.842	0	42.685	35.545	41.137	40.3802	12.0767	23.7937
	TI1	2.11198	42.68498	0	12.8239	12.111	24.8603	36.9469	33.0943
	TI4	14.7899	35.54504	12.824	0	5.8016	13.4237	27.8337	22.1874
	TC1	14.0715	41.13699	12.1118	5.80162	0	12.9754	33.4371	27.0768
	TC4	26.8247	40.38015	24.8603	13.4237	12.975	0	31.0509	22.4191
	TCC1	38.2779	12.07667	36.9469	27.8337	33.437	31.0509	0	11.8278
	TCC4	34.5843	23.79377	33.0944	22.1874	27.077	22.4191	11.8278	0
dehydrated	TN2	0	32.16092	1.59191	13.3374	12.429	22.1106	28.1489	26.2274
	TNC	32.1609	0	33.6147	30.2809	33.318	35.7627	10.3426	18.6785
	TI1	1.59191	33.6147	0	14.4049	13.209	22.9621	29.7291	27.7904
	TI4	13.3374	30.28086	14.4049	0	3.3961	9.33911	22.9107	18.2455
	TC1	12.4292	33.3175	13.2095	3.39609	0	9.76517	26.095	21.2502
	TC4	22.1106	35.76273	22.9621	9.33911	9.7652	0	26.871	20.3044
	TCC1	28.1489	10.34265	29.7291	22.9107	26.095	26.871	0	8.4066
	TCC4	26.2274	18.67851	27.7904	18.2456	21.25	20.3044	8.4066	0

ΔE: Differences in color, TN2: “tortilla” not added (Control), TNC: “tortilla” added with turmeric, TI1 and TI4: “tortilla” added with 2 and 8% of radish (interior), TC1 and TC4: “tortilla” added with 2 and 8% of black radish, TC1 and TC4: “tortilla” added with 2 and 8% black radish powder, TCC1 and TCC4

In Fig. 4 it is possible to observe the evaluated color variables for non-dehydrated (L*, a*, b*, WI, Chroma, Hue) and dehydrated (L*', a*', b*', WI, Chroma, Hue) tortillas (Fig. 4a) and the amplitude values found by photoacoustic spectroscopy at wavelengths 350, 450, 600 and 650 nm (Fig. 4b). Based on the heat map represented with colors ranging from blue to red, it is noted which variables show more changes with respect to others. In Fig. 4a, the variables with the most homogeneous behavior are a*, hue and a*'and hue*, followed by b*, b*'. In the case of the behavior of the photoacoustic signal amplitude, the ones that showed the least change were the signal amplitude at the wavelength of 600 and 650 nm. This means that the addition of radish and turmeric, do not modify the response at these wavelength absorbing points, so they are not associated with chlorophyll compounds for example, which is located at that wavelength level, in the case of 650 nm, corresponds to chlorophyll b [38].

Table 4 shows the differences in color (ΔE) of the “tortillas” in the applied treatments (not dehydrated and dehydrated). The factors considered for the tortilla comparison are TN2, TNC, TI1, TI4, TC1, TC4, TCC1, TCC4. It is observed that there are significant differences according to the standards established by ISO 12647-2, which establishes that color differences are visible to the human eye as from ΔE> 5. It is observed that there are only two values which do not have significance since they are less than 5 (as the norm says), being these the values between the non-dehydrated and dehydrated “tortillas”, and the added with 2% of radish powder (inner zone of the radish - white zone), this means that there is no perceptible difference for the human eye between these tortillas. With respect to the other tortillas, it is possible to observe that they presented differences perceptible to the human eye when compared with each other.

Sanitary Test.

According to the results, there were statistical differences ($p < 0.05$) in the number of fungal colonies found among the different "tortillas" prepared. Figure 5 shows the behavior of the appearance of fungal colonies on days 3 and 7 of the samples of control tortillas, tortillas added with radish and tortillas added with radish and turmeric.

It is observed that in the control "tortillas" and "tortillas" added with black radish, the fungi appeared on the third day. The number of fungi increased in relation to the addition of black radish to the "tortilla". It is important to point out that the "tortilla" added with turmeric and black radish and turmeric did not present any fungal colony during the test period. Other authors adding turmeric (3%) to wheat bread added with corn, found that turmeric has a fungistatic effect [29]. In this investigation a fungal effect is found, since in the test period there were no fungi in any of the tortillas added with turmeric.

In this way, it is possible to mention the advantages of incorporating turmeric to the tortillas added with black radish. This not only potentiates its nutraceutical properties, but also improves its sanitary quality, thus increasing the probable shelf life of a product of this type.

Análisis de componentes principales (ACP)

Figure 6 shows the plot of the principal component analysis of the variables (Non-dehydrated tortilla, L*, a*, b*; dehydrated tortilla L*', a*', b*' and the amplitude levels of the photoacoustic signal at wavelengths (l) of 350, 450, 600 and 650 nm. In the Figure, it is observed that there are 4 clusters (I. TNC; II. TCC1 and TCC4, III. TN2 and TI1, TI4 and IV. TC1 and TC4). It is noted that there is a correlation between the color variables a* and b* with the amplitude values of the photoacoustic signal at the wavelength of 450 nm.

In relation to the brightness color variable L*, it is more correlated with the photoacoustic signal at the wavelength of 350 nm. Thus, it can be said that the levels of the photoacoustic signal at a given wavelength are related to the color parameters of the tortilla. Another interesting fact that can be observed by means of this principal component graph is that the vectors of the color variables a* and b* of the non-dehydrated and dehydrated tortilla, form a smaller angle (2°) of separation with respect to the angle formed by the vectors of the color parameter L* (15°), between the tortillas of both conditions. This means that there are more color changes in the L* component with respect to the color dimensions a* and b* during the tortilla dehydration process.

The present study allows us to know the spectroscopic and color characteristics of black radish. Which has been reported to have a therapeutic effect. But it is important to combine other bioactive compounds, since it could give a potentialized result. In this research it was observed that the levels of the photoacoustic signal, related to the bioactive components of black radish or curcuma, increase when there is fusion of both in the preparation of the tortilla,

In addition, both compounds have been reported as possible hepatoprotectors. This is of interest in populations that base their diet on high carbohydrate content, a factor that, among others, triggers the problem of fatty liver in the population [32].

It is observed that the black radish peel tortilla added with turmeric is a food with better characteristics in antioxidant capacity and carotenoid content [41]. This could be useful for the consumption of the population as well as for the shelf life of the food product.

Conclusions

Conventional photoacoustic spectroscopy shows characteristic patterns of tortillas added with black radish and black radish and turmeric, which confirms that the effects of combining both are enhanced, since the maximum spectral bandwidth and the level of the photoacoustic signal increase.

There is a correlation between the values of the photoacoustic signal at 450 nm and the color parameters a* and b*. On the other hand, the color variable L* is associated with the photoacoustic signal at the wavelength of 350 nm.

The dehydration process modifies the color of the tortilla, being the variables with less changes a* and b*. The color parameter L* was the one that changed the most.

Among the hepatoprotectants from *Silybum marianum*, *Peumus boldus* and *Raphanus sativus*. The black radish peel is the one that presents the highest level of photoacoustic signal in the evaluated wavelength (250–900 nm), so it could be a suitable nutraceutical element to be added to the tortilla.

Declarations

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

The authors are thankful for the funding provided by the National Polytechnic Institute (SIP 20221589, SIP 20211699 EDI, COFFA), and the CONACYT.

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Figures

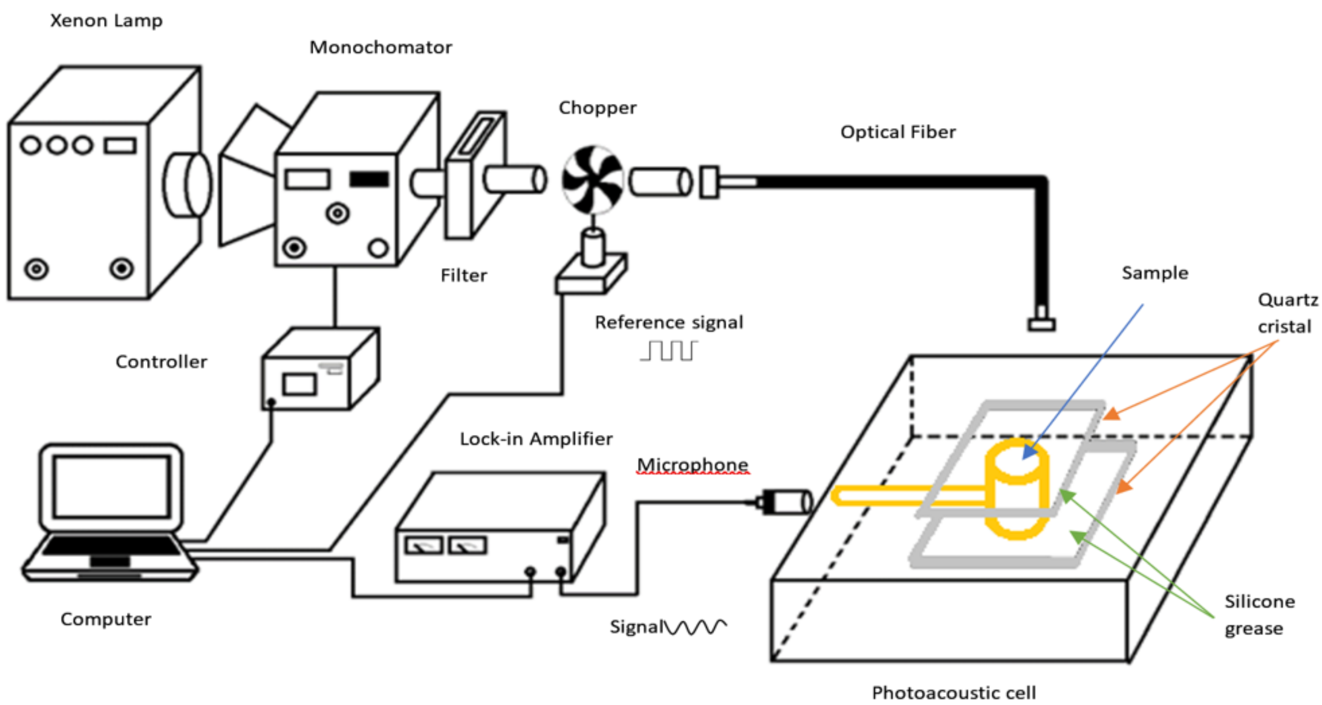

Figure 1

Experimental setup for Photoacoustic Spectroscopy (PAS), adapted from [26].

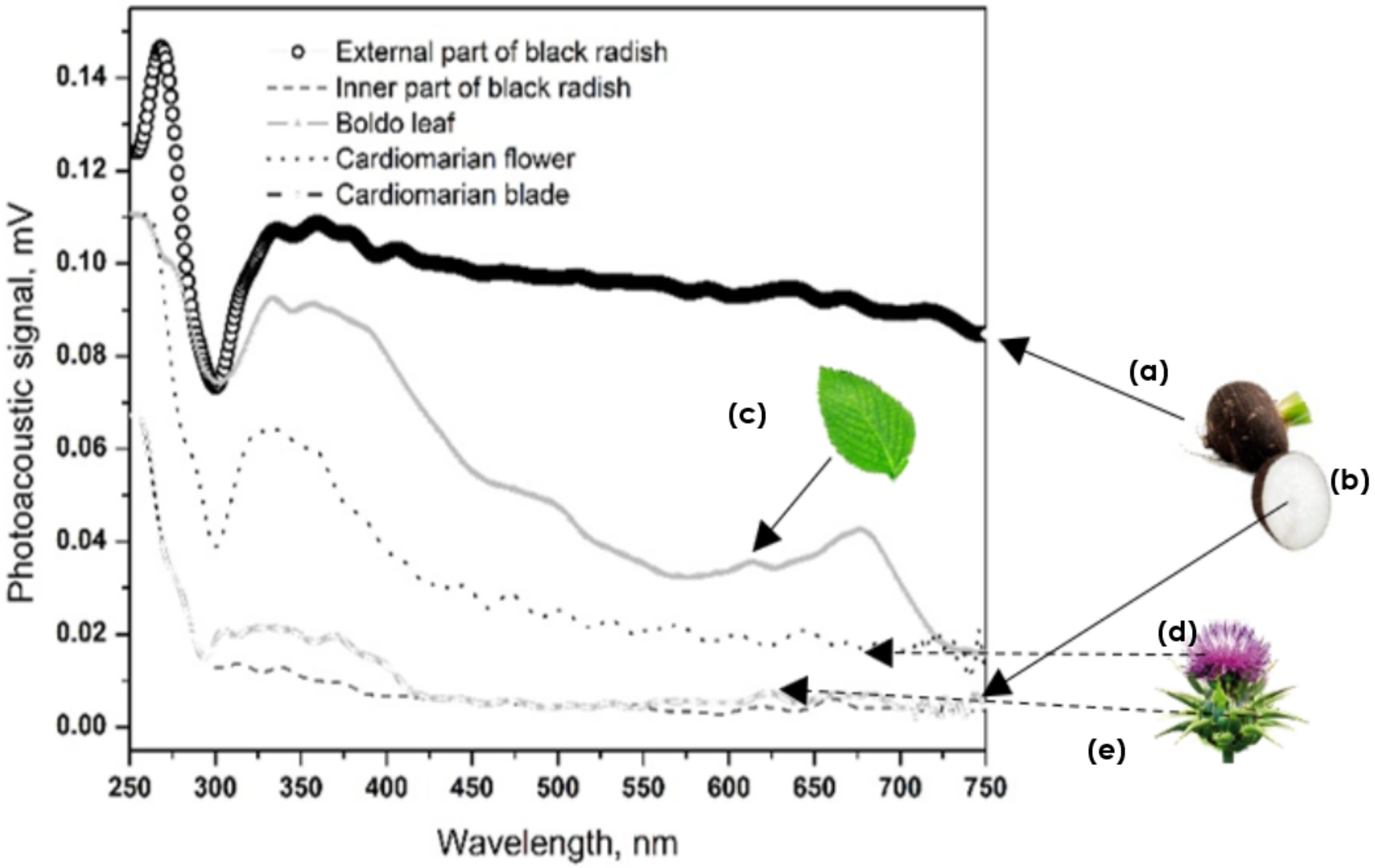

Figure 2

Photoacoustic absorption spectra a) black radish (husk-outer), b) black radish inner, c) boldo leaf, d) milk thistle-flower, e) milk thistle -leaf.

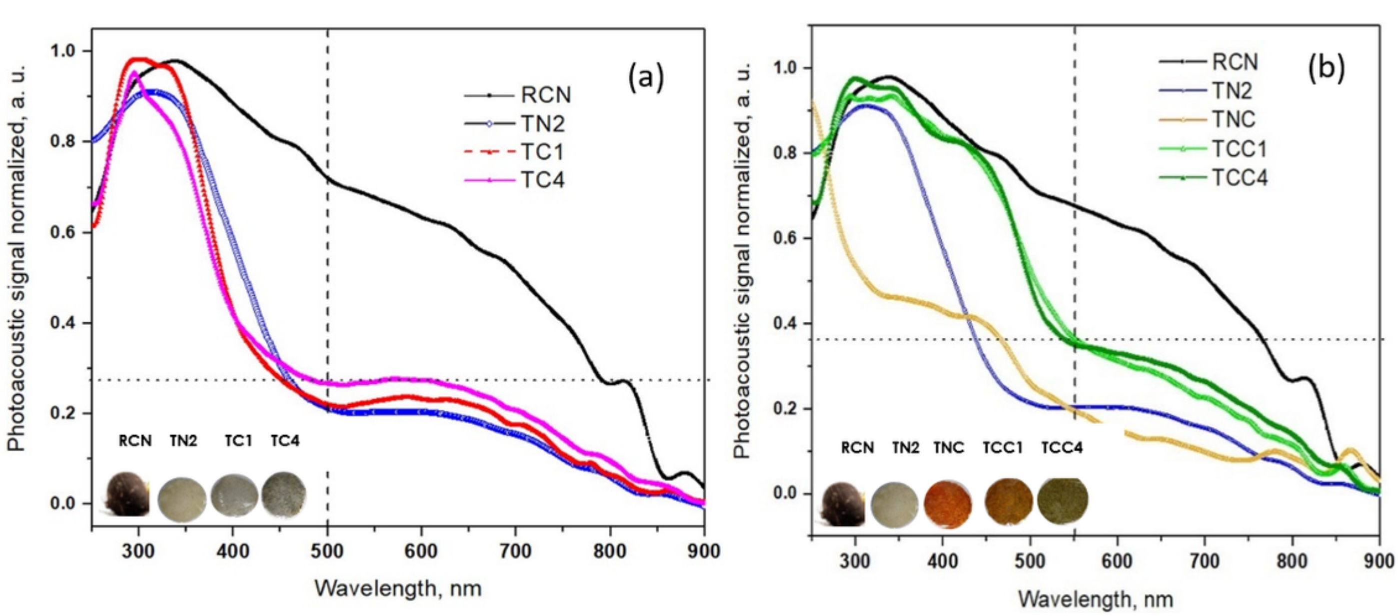

Figure 3

Photoacoustic absorption spectra of black radish powder and "tortillas" a) control and added with black radish, b) control and added with black radish and turmeric.

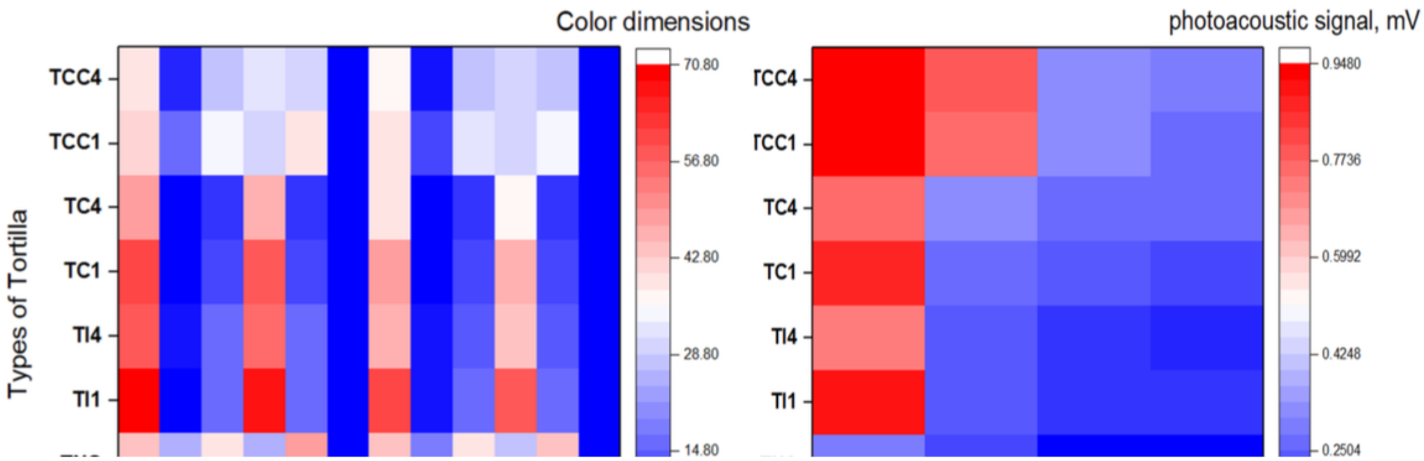

Figure 4

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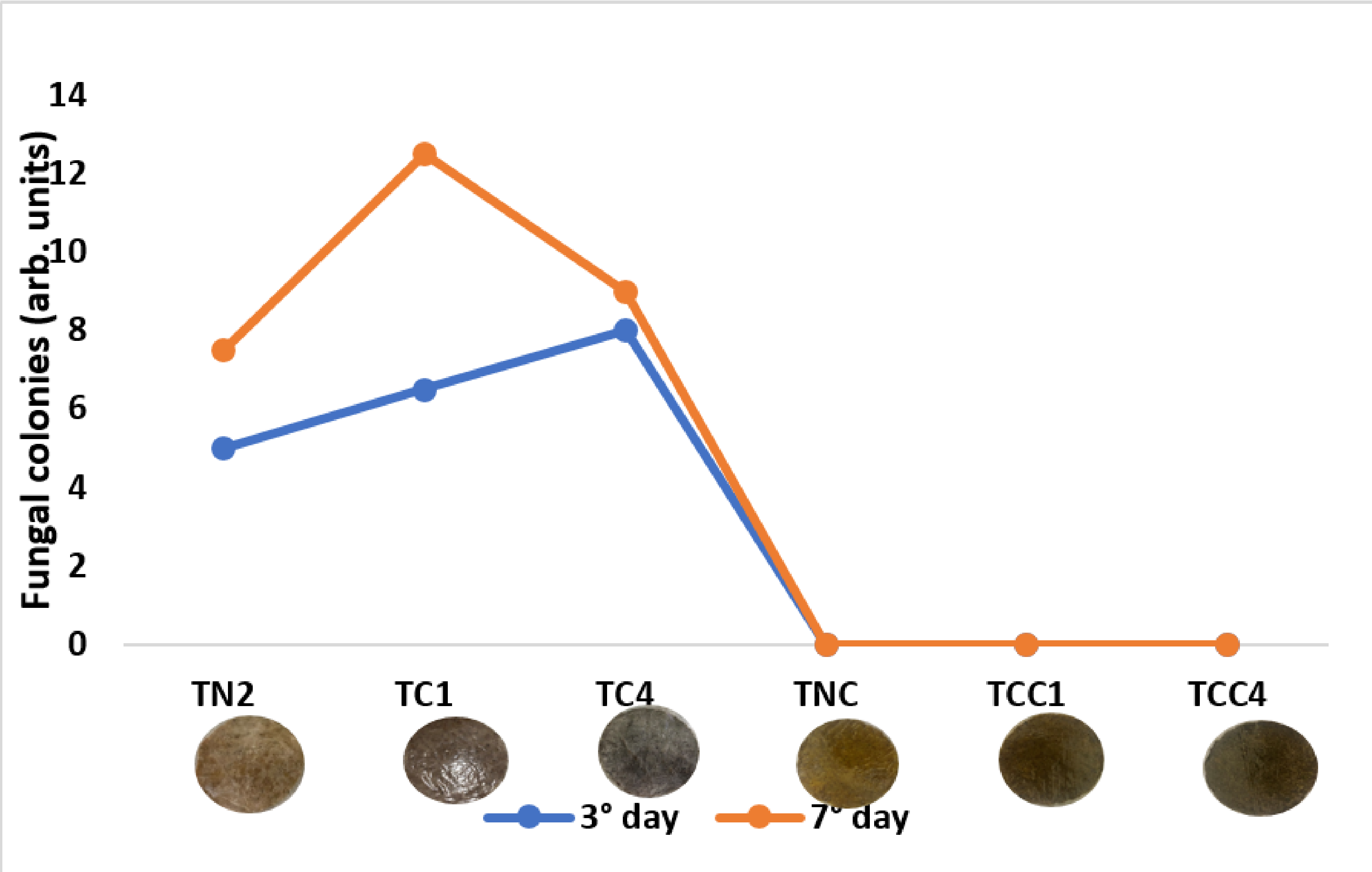

Figure 5

Fungal colonies in tortillas added with black radish flour (TC) and curcuma (TCC).

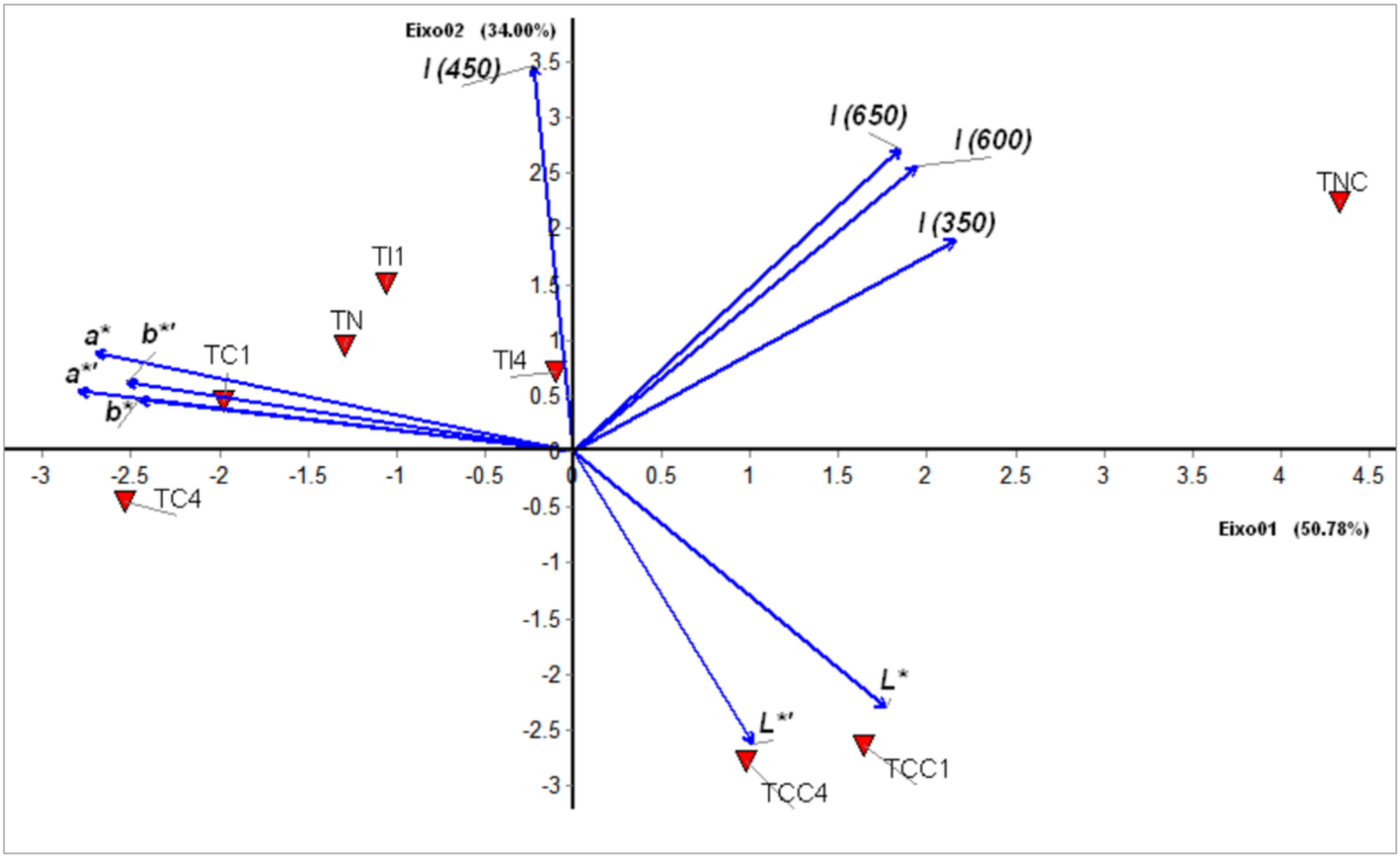

Figure 6

Principal component analysis of the variables (Non-dehydrated tortilla, L*, a*, b*; dehydrated tortilla L*', a*', b*' and the amplitude levels of the photoacoustic signal at wavelengths (l) of 350, 450, 600 and 650 nm.