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► **To cite this version:**

C. Hernandez-Aguilar, A. Dominguez-Pacheco, C. Valderrama-Bravo, A. Cruz-Orea, E. Martínez Ortiz, et al.. Photoacoustic Spectroscopy in the Characterization of Bread with Turmeric Addition. Food and Bioprocess Technology, 2020, 13 (12), pp.2104-2119. 10.1007/s11947-020-02546-2 . hal-03381815

**HAL Id: hal-03381815**

**<https://hal.science/hal-03381815>**

Submitted on 18 Oct 2021

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## Photoacoustic spectroscopy in the characterization of bread with turmeric addition

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### ABSTRACT

In this research, we to apply photoacoustic spectroscopy and evaluate bread added with turmeric (*Curcuma longa* Linnaeus) at different concentrations in relation to wheat flour weight (0, 1.25, 2.5, 5 and 10%). The amplitude of the photoacoustic signal was related to measures of antioxidant activity and evaluate the sanitary quality and also, the texture, color and sensory acceptability of the breads. It is shown that: *i*) The photoacoustic signal produced by bread significantly increases with the turmeric concentration, such that it raises more than 100% for a concentration of 10% at the wavelengths of 428 and 515nm. *ii*) The Bread antioxidant activity is directly proportional to the level of photoacoustic signal at 515 nm and its value increases from 73.21% to 91.73%, when the concentration increases from 0 to 10%. *iii*) The sanitary quality of bread added with 2.5 % of turmeric is relatively higher than the ones obtained for other lower concentrations. No fungal colonies were observed in bread mixed with 2.5%, 5% and 10%, after six days of storage. *iv*) The addition of turmeric powder for bread making can slow down their textural changes (hardness, elasticity, cohesiveness, resilience and chewiness) during storage. *v*) Bread at 2.5 and 5% of turmeric concentration showed the yellowest color, while the one with a 10% concentration exhibited the most reddish color. Finally, through Principal component analysis it was possible to demonstrate that a concentration of either 5 or 10% of turmeric added to wheat flour could be used to improve its sanitary quality and antioxidant activity.

**Key words:** bread, wheat flour, turmeric, photoacoustic spectroscopy, antioxidant activity.

### 1. INTRODUCTION

In recent years there has been a trend to consume foods that contain natural substances with antioxidant properties due to their nutritional potential and therapeutic effects. Bread, mainly wheat, is considered a source of energy and nutrition for the population (Mousavi Khaneghah *et al.*, 2018; Mousavi *et al.*, 2019), it also contains some antioxidant compounds (Zhou *et al.*, 2015), which they are important in the control of degenerative diseases. However, the antioxidant content in wheat bread is low; so, it is necessary to enrich it with natural raw material rich in phenolic antioxidants such as cereals and seeds, parts of green plants, fruits, vegetables or spices, (Dzikia *et al.*, 2004 ).

Antioxidants neutralize free radicals damaging the human body due to alter cell membranes, causing cell damage and inflammation, promoting abnormal cell growth including various kinds of cancer, and even causing cell death (Lee *et al.* 2004; Krishnaswamy *et al.*, 2013). Free radicals are generated naturally in the body by metabolic process and from exogenous factors like ultraviolet light, radiation, smoke, and air pollution (Kim *et al.* 2006; Krishnaswamy *et al.*, 2013). As these factors have been increasing in some urbanized cities, it is necessary to increase the consumption of antioxidants. Hence it is required to incorporate nutraceuticals food in the prevention of diseases one way to incorporate them is through basic consumer foods such as bread.

Various nutraceutical breads based on wheat mixed with powders of grape seeds, grapes, amaranth, quinoa, green tea, black tea, turmeric, etc. have been prepared (Peng *et al.*, 2010; Wang *et al.*, 2007; Mariotti *et al.*, 2009; Alvarez-Jubete *et al.*, 2009; Zhu *et al.*, 2016; Kiskini *et al.*, 2011). In general, breads have been proposed to fortify protein, iron, gluten-free, low carbohydrate and glycemic index, high in fiber and to increase their antioxidant activity, among others (Sabanis and Tzia, 2009, Demirkesen *et al.*, 2013; 2014; Zhu, 2017; Rinaldi *et al.*, 2015; Durazzo *et al.*, 2013; Zhang *et al.*, 2015; Hernandez-Aguilar *et al.*, 2019b; Dall'Asta *et al.*, 2013; Lee, 2013). **Likewise, HoltekjØlen *et al.* (2008) evaluated the antioxidant capacity by means of FRAP (ferric reducing / antioxidant power) expressed as Fe 3+, of wheat bread added with barley flour; while Lin *et al.* (2009) incorporated buckwheat (buckwheat) in wheat breads, measuring the antioxidant capacity through the formation of conjugated diene compounds, using linoleic acid as a substrate. In both studies, improvement in antioxidant capacity was reported.**

One of the most powerful antioxidants is turmeric (*Curcuma longa* L.). This is a rhizome member of the Zingiberaceae family, constituted by polyphenolic compounds (Rahaie *et al.*, 2014; Zorofchian *et al.*, 2014; Jeevarathinam and Pandiarajan, 2016) and it is one of the most studied species due to its active substances, such as curcuminoids among which are the curcumin and phenolic pigments (Rahaie *et al.*, 2014). *Curcuma longa* L. and curcumin provide health benefits, such as anti-protozoal, anti-tumour, anti-inflammatory, anti-venom activities, anti-carcinogenic and wound-healing effects among many others (Sun *et al.*, 2017; Hernández-Aguilar *et al.*, 2015a; Zorofchian *et al.*, 2014; Khalil *et al.*, 2012). This is the reason why incorporating it as an additive in basic foods would bring pro-health properties to the human body. The in-depth investigation of the characteristics of turmeric bread is thus desirable (Lim *et al.*, 2011).

In the Literature there are only some works, report the addition of turmeric in bakery products such as butter cake (Lean and Mohamed, 1999), cookies (Choi *et al.*, 2013; Ju and Hong, 2011), cakes (Park *et al.*, 2012), Yaksik (Min and Hwang, 2017), snack of rice (Lim and Han, 2016) and bread (Lim *et al.*, 2011; Jeon *et al.*, 2013; Jung *et al.*, 2014), although there are other reports using turmeric powder in food products such as Noodles (*Garakguksu*) (Song and Jung, 2009), jeung-pyun (Shin *et al.*, 2018), pyeonyuk (Bae *et al.*, 2018), sunsink (Wu *et al.*, 2016), etc. In the case of rice snack, the authors shown that the turmeric snacks lengthen the shelf life of the product and in the butter cake, the authors reported antimycotic effect of the turmeric. Therefore, the use of turmeric powder as an additive in wheat bread could be an alternative to improve the antioxidant properties of a product with nutraceutical value that benefits health.

However, one of the major challenges facing the food industry is the development of techniques to obtain healthier and safer food products (Velázquez, 2011; Nemes and Orsat, 2010; 2011), because the quality of food has an impact on the health of the population and their quality of life (Hernández-Aguilar *et al.*, 2019a) as well as in consumer acceptance. In this sense, there have been a number of comprehensive reviews on technologies for both process control in the food industry (Kondakci and Zhou, 2017; Neethirajan and Jayas, 2017) and food evaluation (Lembe *et al.* 2012; Qu *et al.*, 2015; Ravikanth *et al.*, 2017; Vadivambal and Jayas, 2011; Karoui and Blecker, 2011). Finding, there are several conventional methods for their evaluation, despite their effectiveness, some of them are laborious, destructive, expensive, time-consuming, require calibration and use of chemicals (Su *et al.*, 2017). In this sense, various authors have proposed other alternatives methods, e.g. analysis of spectral and thermal images, color measurement, as well as the use of spectroscopic techniques. Among the spectroscopic techniques that have been applied for food analysis, are Raman, Nuclear magnetic resonance, Fluorescence, Near infrared, visible / infrared and UV / VIS, etc. (Pojić and Mastilović, 2013; Karoui and Blecker, 2011; Pankaj *et al.*, 2018). These techniques have been successfully used in the food area. Using them for the determination of pesticide contamination, determination of fumonisins, microorganisms, level of deterioration, identification and differentiation of grains, analysis of phenolic compounds in food, etc. (Sowoidnich *et al.*, 2010; Lee and Herrman, 2016; Pojić and Mastilović, 2013; Foca *et al.*, 2011; Lu *et al.*, 2011).

In the case of UV / VIS spectroscopy have been used for determination of phenolic content and antioxidant activity. Muhammad *et al.* (2019) used UV – visible spectrophotometry to measure the absorbance at 760 nm to estimate the total phenolic content of the cocoa powder, the colloidal cinnamon nanoparticles and the chocolate beverage, applying the Folin – Ciocalteu method. Regarding antioxidant activity, various methods have been reported, among them, DPPH scavenging assay that is the widely used one (De and Nariya, 2013; Yamaguchi *et al.*, 1998; Krishnaswamy, 2013). This method has been applied to determine the antioxidant activity of Grape (*Vitis vinifera*), lemon (*Citrus limon*), pomegranate (*Punica granatum*), orange (*Citrus sinensis*), hemp (*C. sativa*) seed, seaweeds, Golden oyster mushroom (*Pleurotus cornucopiae*), samples of extrudates and raw flours for corn and yams, among much other food (Paari *et al.*, 2012; Krishnaswamy *et al.*, 2013; Teh *et al.*, 2014; Devi *et al.*, 2008; Jo *et al.*, 2013). The measure of antioxidant activity has been obtained from obtaining optical absorption measurements of the extracts of samples obtained around 520 nm (517 and 515 nm). Although has also been obtained at optical absorption measurement of 330 nm (De and Nariya, 2013). Chemical and reagents are required to carry out these measurements (Sellami *et al.*, 2012); methods that could avoid the use of these have been reported in the literature, such as is the case of Photoacoustic spectroscopy (PAS) technique.

Photoacoustic spectroscopy (PAS) is considered as a “green” technology (He *et al.*, 2007) and could hence be a convenient option to be incorporated in the food industry for diverse specific applications (Hernández-Aguilar *et al.*, 2019a). PAS technique allows probing the quality of food by means of its color (Bicanic *et al.*, 2006), which is closely related to the quality and flavor of food (Kaur and Gupta, 2012). This was demonstrated by various authors through the optical characterizing of food products, such as marine algae, coffee, milk, cheese, yogurt, tortillas, maize, ketchup, pastas, chocolates, vegetables, etc. (Rosencwaig and Hall, 1975; Reis *et al.*, 1986; Martel *et al.*, 1987; Bicanic *et al.*, 2011; Dóka *et al.*, 2010; 2013; Hernández-Aguilar *et al.*, 2011 ; 2012). Likewise, few researches of PAS applied to the study of bread products have been found. Hernández-Aguilar *et al.* (2019b)

investigated the relation between optical absorption spectra obtained by PAS and secondary metabolites on bread of wheat flour added with lentil sprouts. Taking into account that the world population is becoming more aware of what it consumes for disease prevention and improved quality of life, the interest for nutraceutical food has been significantly increasing due to its direct relationship with human health (Šporin *et al.*, 2017; Serpa-Guerra *et al.*, 2019).

In our study we thought it could be interesting to characterize by photoacoustic spectroscopy (non-conventional method), wheat bread added to different concentrations of turmeric, relating the level of the photoacoustic signal at the wavelength of 515 nm with the value of the antioxidant capacity of the bread measured at the same wavelength by a conventional method (DPPH Method). Complementing the study, other properties of the turmeric bread were evaluated as texture, color, sensory acceptance, and sanitary quality. There are no reports in the literature to apply PAS and evaluate bread added with curcuma and related to measures of antioxidant activity and evaluate the sanitary quality of the product as it is done in the present study.

## **2. MATERIALS AND METHODS**

### **2.1 Elaboration of bread**

Turmeric powder (TP) with certification kosher as natural nutritional supplement was acquired in a local supermarket of the city of Mexico. Dough was prepared at concentrations of TP of 0, 1.25, 2.5, 5 and 10% in relation to wheat flour weight (600 g). Five types of bread formulations were elaborated with common ingredients of egg (average weight 48g), salt (1.25 g), sugar (4g), olive oil (70 g), and yeast (11 g). Turmeric powder was incorporated 0, 7.5, 15, 30 and 60 g, in 600, 592.5, 585, 570 and 540 g wheat flour, respectively. The concentrations of Turmeric used in this research were chosen according to that reported by other authors in the literature (Jung *et al.*, 2014; Jeon *et al.*, 2010) and also considering not to exceed 10g of it to avoid toxic effects of consumption or adverse effects by oral ingestion that have been reported by other authors (Vincent, 2011). The final formulations five expressed by percentage are showed in Table (1). The ingredients were mixed for 5 min, in a blender with a spiral hook. After its fermentation in 300 g warm water (30 °C), the yeast was incorporated into the mixture, which was then kneaded for 10 min and placed in a mold covered with a moistened cotton fabric during 30 min. Next, the dough was again kneaded for 10 min and put in a greased mold for bread, where it increased its volume during 25 min. Afterwards, each type of the doughs was placed in different molds and simultaneously baked in a pre-heated electric oven (Black+Decker) at 180° C, for 50 min. After baking, the bread was allowed to cool down to room temperature during 24 h and subsequently cut it into slices (1.5 cm thick) through an electric knife (Hamilton beach type EK08, 121 V, 11 Hz).

### **2.2 Photoacoustic Spectroscopy experimental setup**

The experimental setup of PAS consists of a Xenon lamp used as the light excitation source, a monochromator applied to obtain a monochromatic light beam, and a mechanical chopper employed to modulate the light beam at the fixed frequency  $f = 17$  Hz ( $f$  at which the electret microphone used has the best response), as shown in Fig. 1. The modulated beam was focused onto an optical fiber to guide it to the photoacoustic (PA) cell, whose signal was then detected by an electret microphone (photoacoustic sensor) connected to the cell through a fine channel (1 mm diameter). The PA signal was amplified by means of a lock-in amplifier (EG&G, Mod. 5210) before being recorded

in a personal computer, as a function of beam wavelength. Experiments were performed for wavelengths between 300 nm to 700 nm (Range where it is possible to observe the absorbent bands of the compounds contained in the bread) and by placing the samples (0, 1.25, 2.5, 5 and 10% of turmeric concentration) into the PA cell with a diameter of 6 mm. The samples of each type of bread were cut into circular slides with a diameter of 5 mm, such that they do not obstruct the microphone sensor. The operating principle is based on the photoacoustic effect discovered by A. G. Bell in 1880 and modeled by Rosencwaig and Gersho (1970) (Hernández.Aguilar *et al.*, 2019a). Which is based on the absorption capacity of each material when a modulated beam of light falls on it. The light absorbed by the sample is transformed into a modulated heat emitted by it within a hermetically sealed FA cell. As air is present inside the FA cell, an adiabatic process occurs that causes pressure changes inside the FA cell. The pressure changes are captured by an electret microphone and this is what generates a photoacoustic signal value.

### **2.3 Antioxidant activity (DPPH method)**

#### **2.3.1 Chemicals**

The 2,2-diphenyl-1-picrylhydrazil (DPPH) and 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma Aldrich (St. Louis, MO, USA). All others chemicals of analytical grade were purchased from Merck Company (Darmstadt, Germany).

The extraction of compounds in the bread samples was carried out based on the methodology proposed by Ranjbar *et al.* (2019) and Ragaee *et al.* (2011). The dry flour sample was mixed for 5 minutes with a methanol-acetone-water solution (7:7:6; v/v/v) to obtain a concentration extract 33g/L. Then the solution was centrifuged for 5000 rpm for 10 min. The supernatant was removed, and the pH adjusted with 4N HCl. Subsequently, the antioxidant capacity was determined by the DPPH method, following the methodology of Yu *et al.* (2013). A solution of DPPH methanol (4  $\mu\text{mol} / \text{L}$ ) was mixed with 0.1 mL of the extract. To evaluate the antioxidative activity of specific compounds, the latter can react with a stable radical, 2,2-Diphenyl-picrylhydrazyl (DPPH) in a methanol solution. The reduction of DPPH is followed by monitoring the decrease in its absorbance at a characteristic wavelength during the reaction; in its radical form, DPPH absorbs at 515 nm (Brand *et al.*, 1995). Then, Absorbance was measured at 515 nm by UV-vis spectrophotometer (VE-5100UV) and the results were reported as  $\mu\text{mol}$  equivalent of Trolox per gram of dry sample.

### **2.4 Sanitary quality**

Sanitary quality test adapted according to Neergaard (1979) and Pérez *et al.* (2015) was established under a randomized complete block design with four repetitions for each bread sample. We randomly selected 4 slices of each bread type (0, 1.25, 2.5, 5 and 10% of turmeric concentration) and subsequently place them in a hermetically sealed container of polystyrene properly sterilized. The incubated samples were then placed in a closed room at an average temperature of 25 °C. The observation and quantification of the existing fungal colonies were performed at 3 and 6 days of incubation. Incubation Elapsed, observations were performed by a stereoscopic microscope to record the development of fungal colonies in the breads. Their gender was determined, according to the type of mycelium and spores, and proceeded to quantify the numbers of colonies in the breads contaminated containing each type of fungus. The gender of fungus was identified according to taxonomic keys (Ravimannan *et al.*, 2016).

## 2.5 Texture Profile Analysis (TPA)

The texture of the bread samples (turmeric and control) was evaluated by using a Texture Analyzer Brookfield Model CT3 25 K USA with a load cell of 25 kg. From each slice the bread samples were cut the crust and the crumb of the central part was used, to which cubes of 3 x 3 x 1.5 cm were marked to obtain regular geometric bodies and, 5 repetitions of each type of bread were cut with the electric knife (Hamilton beach type EK08, 121 V, 11 Hz). The samples were packed in polyethylene bags, placed separately to avoid damage to the texture of the bread, and were allowed to stabilize 10 minutes before measurement. The analysis was performed for two 20% compression cycles by means of a TA General Probe Kit with a TA25/1000 with a cylinder of diameter and length of 50.8 and 20 mm, respectively: operating at a speed of 1.7 mm/s. The obtained parameters were the hardness, elasticity, resilience, cohesiveness, and chewiness. The TPA analysis with four replicas at  $24 \pm 1^\circ\text{C}$  was performed 18 and 90 hours after the bread elaboration.

## 2.6 Color analysis

The color of the bread samples was determined on a Minolta CR 300 colorimeter. The color parameters corresponding to the uniform color space CIELAB (a, b y L\*) were directly obtained by the equipment. L\* indicates brightness (100 = White y 0 = Black), “a”, indicates greenish-reddish [negative (green) to red (positive)] and “b”, indicates blueish-yellowish [negative (blue) to yellow (positive)]. The equipment was calibrated by using a reference white L\*= 96.65, a= -0.03 and b= 1.86.

## 2.7 Sensory acceptability

The sensory acceptability test was carried by using of nine points hedonic scales method (where 1 = Dislike Extremely, 2 = Dislike Very Much, 3 = Dislike Moderately, 4 = Dislike Slightly, 5 = Neither Like nor Dislike, 6 = Like Slightly, 7 = Like Moderately, 8 = Like Very Much and 9 = Like Extremely) according to Laureati *et al.* (2012). In this study, were selected consumers (15) of bread, between 20 and 50 years old (11 woman and 4 men), reside in the city and the state of Mexico.

Each consumer was given an evaluation instrument (questionnaire) and received a set of five complete pieces of bread with thickness of 1.5 cm, placed inside a plastic bag (low-density polyethylene) hermetically sealed. The bread was made seven hours before being placed in the bags, from each mass according to the elaboration process, 23 pieces were obtained. In addition to the questionnaire, water to rinse the mouth and coffee grains to neutralize odors was given to each participant. The questionnaire applied was divided in three sections: 1) consumer general data, 2) Bread score on 10 selected attributes: color, porosity, sponginess, hardness, cohesiveness, aroma, chewiness, flavor and healthy preferences and attributes in general, and 3) direct questions related to their purchase decision on the proposed breads and their healthy preferences, as well as their health status of them and / or relatives mainly (Hernández-Aguilar *et al.*, 2019b).

## 2.8 Statistical analysis and Principal Component Analysis (PCA)

### 2.8.1 Variance analysis

Bread texture and color measurements were carried out under an experimental design with three repetitions of each type of bread (0, 1.25, 2.5, 5, and 10%). The mean difference between breads was determined using analysis of

variance (ANOVA), followed by a Tukey test at 95% confidence level using Minitab® version 17 statistical software (Minitab Inc., State College PA, USA).

### 2.8.2 PCA

The principal component analysis was applied to the experimental data obtained for the variables evaluated in wheat bread elaborated with (a) 0, (b) 1.25%, (c) 2.5%, (d) 5% and (e) 10% of turmeric concentration. The analysis was performed using the R Project software version 0.10-47, with R Commander and factoMiner. The data matrix used was formed from the measurements of the photoacoustic signals (515 and 475 nm), absorbance (A515 nm), fungal colonies (on days 3 and 6), color parameters ((a, b and L \*), antioxidant activity (DPPH) and texture variables (hardness, elasticity, resilience, cohesiveness, and chewiness). Finally, the linear equation of the correlation and the intensity of linear correlation ( $r$ ) between the variables: photoacoustic signal (normalized) at 515nm and DPPH was determined (Table 4). The  $r$  was quantified through Pearson's linear correlation coefficient. It was calculated from the quotient of the covariance between the standard deviations of the data obtained from the variables (Vila *et al.*, 2004). The normalized signal was obtained by the quotient of the amplitudes of the photoacoustic signals at each wavelength with respect to the initial value of the signal at the beginning of the photoacoustic spectrum.

### 2.8.3 Statistical analysis of photoacoustic spectra using kernel estimator

Statistical analysis of the data was performed using the probability density function (PDF), which specifies how the probabilities are distributed over the values adopted by the random variable [Soong, 2004]. To calculate the PDF, among other non-parametric methods are used: kernel estimators. They are used to estimate a continuous and uniform distribution from a finite set of observed points (Rojas *et al.*, 2016).

The kernel density estimate method is based on each data point  $X_1, X_2, \dots, X_n$  of a sample of size  $n$  from a random variable with a unknown probability density function density  $f_X(x)$ , is replaced by a specified function  $K(\cdot)$  called the kernel, centered at each data point  $X_i$  and with a scaling parameter  $h$  called smoothing constant. The kernel functions are added together and the resulting function is scaled to have a smooth curve called the density estimate (Rojas *et al.*, 2016). One of the most used kernels is the Gaussian kernel. The equations for the calculation of Density estimate of  $f_X(x)$  and Gaussian kernel are possible to observe in Table 2 and the data processing was carried out using MatLab R2020a software.

## 3 RESULTS AND DISCUSSION

### 3.1 Photoacoustic Spectroscopy and antioxidant activity

Figures 2a and 2b show the photoacoustic spectra and their densities for the wheat bread elaborated with 0, 1.25, 2.5, 5 and 10% of TP. Note that the amplitude of the photoacoustic signal (PA-s) is quite sensitive to the TP concentration and the wavelength ( $\lambda$ ). For the absorption band (250-300 nm), the photoacoustic spectra of different samples exhibit similar behaviors and values. This maximum absorption band could be related to the presence of flavonoids (Amarowicz *et al.* 2003) and proteins contained in wheat (Martin *et al.*, 2004). Note also that a second band of maximum absorption only appears for turmeric breads (*i.e.*, in pure wheat bread it does not occur) and its bandwidth increases with the TP concentration. The photoacoustic signal of the widest bandwidth (350-550 nm) for bread with 10% TP, tends to increase for 350-450 nm and decrease within 450-550 nm. This increase is maximum at the wavelength of 428 nm, which is a point of interest, since it corresponds to the maximum absorption peak of

curcuminoids (Hegge *et al.*, 2009), such as curcumin, the polyphenol responsible of the yellow color of turmeric (Akram *et al.*, 2010). It was found that in this absorbent point ( $\lambda = 428$  nm) there are significant statistical differences when comparing the PA- signal obtained in amplitude of the different breads elaborated in this investigation (Table 2, column 3). It is possible observe that the value of the photoacoustic signal increases with the increase of turmeric powder to the bread. This means that the photoacoustic signal is a function of the added turmeric for making bread. For this latter wavelength, the amplitude of the PA-signal increases from 0.05 mV (control- bread made without turmeric) to 0.181 mV (bread added with 10% TP), which represents an increase of more than 100%. The spectrum of bread without turmeric does not show the absorption band corresponding to the characteristic yellow pigment of turmeric bread. On the other hand, the PA-signal amplitude also increases (from 0.02 to 0.08 mV) with the TP concentration at 515 nm, which is the wavelength where the antioxidant activity is measured (Ranjbar *et al.*, 2019). Although this latter increase is smaller than the one at 428 nm, the increase of the PA-signal amplitude thus indicates the improvement of antioxidant activity, as shown in Fig. 3a.

Table 3 shows significant statistical differences in antioxidant activity between breads with and without TP (0, 1.25, 2.5, 5 and 10%). The different letters in the columns indicates that the values are statistically different ( $\rho \leq 0.05$ ). The lowest value of antioxidant activity (DPPH ( $\mu\text{mol}$  equivalent of Trolox/g) was found in the control bread (20.24) and the highest one in the turmeric bread at 10% (35.25), which represents an increase by 74%.

The results (Table 3, column 5) indicate that there is a significant difference between antioxidant potential and turmeric powder concentrations added to wheat flour in the bread making process, which is consistent with the data reported by Sikkhamondhol *et al.* (2009) and Lim *et al.* (2011), when determining that the total level of antioxidant with DPPH is higher with the addition of turmeric powder in bread. In the food industry, antioxidants are used as bio-ingredients to prevent oxidative deterioration of fats and oils (Nanditha and Prabhasankar, 2008). Recent research on species has suggested that its active components as antioxidants can be used as natural bio-ingredients (Gottardi *et al.* 2016) in bakery products. Curcumin which is the main active component of turmeric is one of the most widely used species as natural antioxidants and is used as a medicinal herb for its medicinal properties, as an antifungal, antitumor and anti-inflammatory (Tuba *et al.*, 2008).

The probability distribution function estimated by the Gaussian kernel of the photoacoustic spectra shows multimodal behaviors, *i.e.*, the data obtained from the photoacoustic signals come from different data populations, related to the different components of bread (Fig 2.b). It is possible observe that wheat bread B<sub>0</sub> (0% TP) exhibits a range with maximum values between 0-0.05 mV, it is found the maximum value around of 0.03 mV of the amplitude of the photoacoustic signal (photoacoustic signal data), which indicates that in this region the values occur with a relatively frequency high. In the range of values of the amplitude of the photoacoustic signal (0-0.05), note that in the pan B<sub>0</sub> they are within the wavelength range of 400-800 nm (Fig. 2a). The highest values of the photoacoustic spectrum (0.05-0.25 mV) (Fig-2a), have a lower frequency of occurrence of the data (Fig. 2b) and are in the wavelength range of 250 - 400 nm (Fig. 2a). Similarly, for bread B<sub>1</sub> (1.25% TP), it is possible to observe that the maximum occurs for 0.03 mV, although this value occurs with less frequency than bread B<sub>0</sub> (without TP), it would correspond to the range of 525-800 nm (Fig. 2a). However, in this density function (B<sub>1</sub> - 1.25% TP) a second region appears (0.10-0.15 mV) with a maximum value close to 0.12 mV, when observing Fig. 2a, it corresponds to the wavelength range of 325-475 nm. In the curve corresponding to the density function of turmeric bread B<sub>2</sub> (2.5%), the first maximum coincides with that found in bread B<sub>1</sub> (1.25%) with a value of the photoacoustic signal of

0.03mV and have a similar frequency of occurrence. The second region formed (0.106-0.156 mV) presents a maximum peak around 0.143 mV, these values located in Fig. 2a within the wavelength range of 325-475 nm. The increase in the value of the maximum point of the amplitude of the photoacoustic signal (photoacoustic signal data) in this region of the kernel for B<sub>2</sub>, is related to an increase in yellow pigment, with respect to bread B<sub>1</sub> (1.25%). For both regions of curve de kernel de bread B<sub>1</sub> and B<sub>2</sub> the wavelength range of 325-475 nm is associated (Fig. 2a). According to the literature, curcuminoids are found in this wavelength range, as has been shown by several authors such as Silva-Buzanello *et al.* (2015) who obtained the UV-VIS spectrum of pure curcumin, where an absorption band was found in the range of 300-500 nm. Finally, the first and second maxima of the density functions of turmeric breads at 5 and 10% of TP occur at 0.05 and 0.14 mV, respectively. In general, the density function for the spectrum of bread B<sub>0</sub> (without turmeric) exhibits two populations, one with high and the other with low occurrence. For breads (B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub> and B<sub>4</sub>), in the range of values of approximately 0.10-0.17 mV (Fig. 2b), they belong to the wavelength range of 300-525 nm, which is associated to the presence of turmeric in the bread, since in wheat bread (without turmeric) this absorption band is not present. Photoacoustic spectra of turmeric powder from various sources have been found with an absorbent band around the range of 300-550 nm (Hernández-Aguilar *et al.*, 2015b; Alvarado *et al.*, 2018). Therefore, the kernel curves of bread with and without turmeric showed different pattern of behavior.

### 3.2 Sanitary quality

Figure 3b shows that when the amount of turmeric increases, the sanitary quality of bread is improved; such that there are no fungal colonies developed for breads with turmeric concentration of 2.5, 5, and 10%. The fungi found in the bread slices were: *Penicillium* sp. (growing colonies in green color with dense conidia), *Rhizopus* sp. (white cottony mycelia, with black dots) and *Aspergillus* sp. (yellow or yellowish green colonies), being the most abundant the fungus *Penicillium*.

This fact is consistent with the antifungal and fungistatic properties of turmeric due to the curcuminoids (Apisariyakul *et al.*, 1995, Abbasi and Shah, 2015). These curcuminoids, including curcumin (González-Albadalejo *et al.*, 2015), possess various biological activities, including the suppression of the proliferation of a wide variety of tumor cells from the skin, lung, colon, stomach and breast (Khalil *et al.*, 2012); as well as antidiabetic and antimicrobial properties making it one of the most studied natural remedies with high medicinal value (Kunnumakkara *et al.*, 2017). So, the addition of turmeric to staple food as bread makes it a functional product with pro-health extensive properties.

### 3.3 Texture Profile Analysis

Table 4 shows the results of the texture profile analysis, indicating significant difference ( $\rho \leq 0.05$ ) between the evaluated variables (hardness, elasticity, cohesiveness, resilience and chewiness) of the different types of bread on days 0 and 3 of evaluation. Hardness results, corresponding to the maximum force of the first product compression cycle, indicate higher hardness in bread with 10% turmeric powder, with an increase of 31% from day 0 and day 3. This increase is even higher (48%) with respect to the control bread (no turmeric content). The hardness of the turmeric bread at 5% increased from day 0 to day 3 by 15%, while no differences were observed for turmeric breads with lower concentrations (1.25% and 2.5%). The bread elasticity on day 0 representing the material ability to recover its form after being deformed, took its highest ( $6.01 \pm 0.28$ ) and lowest ( $4.4 \pm 0.35$ ) values for bread with

2.5% and 0% of turmeric, respectively. The resilience, a textural parameter used to describe the elastic properties of samples (Peng *et al.*, 2017), did not exhibit significant differences ( $p > 0.05$ ) between days 0 and 3 for turmeric bread at 10%.

In relation to the cohesiveness, which is the limit at which a material can deform during the second compression cycle, it took its highest values for the control and 2.5% turmeric breads, on day 0. This represents 20% more cohesiveness than the one for bread with 10% TP, which exhibited the lowest cohesiveness ( $0.38 \pm 0.01$ ). The chewiness that is the energy required to chew bread produced at different turmeric concentrations, showed no significant difference between days 0 and 3 for turmeric breads at 2.5% and 10%. For breads with 1.25% and 5% of turmeric concentration, the chewiness decreased on day 3, while that of control bread increased by 70% from day 0 to 3. This is consistent with the relatively high increase of its hardness. He and Hosney (1990) reported that the firmness of the bread increases with the loss of moisture from the crust. So, the addition of TP for bread making could slow their changes during storage. Bread making is a complex process that involves various unit operations such as: mixing, fermentation and baking, in which there is water evaporation, volume increase, starch gelatinization, protein denaturation and crust formation (Manu and Rao, 2008). On the other hand, turmeric is a yellow phenolic pigment and numerous studies have reported its pharmacological activity; including, effective antioxidant activity that can eliminate superoxide, hydrogen peroxide and nitric oxide radicals (Akter *et al.*, 2019; 2018., Ho *et al.*, 2011). Phenolic antioxidants can form complexes with proteins by means of hydrogen links between the hydroxyl groups of the phenols and the carbonyl group of the peptide residue of proteins (Almajano *et al.*, 2007). Therefore, among the components of the dough such as wheat proteins, fiber, fat, sugars, and phenolic antioxidants; the addition of turmeric alters the properties of the dough and the texture attributes of bread, as shown in Table 2.

### 3.4 Color analysis

The results of the color analysis of the bread samples at the different evaluation days are shown in Table 4, which indicates significant differences ( $p \leq 0.05$ ) between the different types of bread and evaluation days (0 and 3). The highest value of "b" color is for bread with 2.5% turmeric (51.72); while the yellowest color ( $b = 49.9$ ) of bread at 5% did not show significant differences ( $p > 0.05$ ). This fact indicates that the bread with 2.5% concentration of turmeric exhibits a high sanitary quality, since no colonies of fungi were observed after six days of storage. In this time, the bread with 10% concentration of turmeric underwent a decrease of its "b" value to  $b=41.33$  and a significant increase of its "a" value to  $a=5.78$ , which indicates the bread tendency to a redder color, because of the relatively high "a" value of the turmeric powder. On the other hand, the value of  $L^*$  decreased as the turmeric concentration increased, such that the bread samples on day 0 ( $L^*=75.85, 70.09, 71.06, 63.58, 56.71$ ), were clearer than on day 3 ( $L^*=63.95, 63.32, 60.76, 51.33, 46.48$ ), for the different concentrations of turmeric (0, 1.25, 2.5, 5 and 10%). In this regard, Ho *et al.* (2011) reported that the color change of bread is related to the turmeric pigments and phenolic compounds, which have oxidation reactions. So, when changing bread color, it would be necessary to evaluate the consumer preferences. So, despite of its enhanced sanitary quality, the elaboration of functional bread with 10% of turmeric concentration would perhaps no longer be attractive for the consumer. This is the reason why we recommend the production of bread with 2.5% of turmeric concentration.

### 3.5 Sensory acceptability

The results of the ANOVA for the 10 variables evaluated in the bread acceptability test, significant statistical differences ( $P < 0.05$ ) were obtained in seven of them: color, porosity, sponginess, aroma, flavor, healthy option, and general attributes (Table 5). It can be seen, in the color attribute, bread added to the 1.25% concentration of turmeric powder ( $B_2$ ) was preferred, followed by bread  $B_3$  (2.5% TP), and  $B_4$  (5% TP). The least accepted bread in the color attribute was  $B_5$  (10% concentration of turmeric powder).

Sensory acceptability for the attributes porosity, sponginess, aroma, and flavor attributes was evaluated in a higher score for wheat bread without curcuma powder ( $B_1$ , 0% of TP), with a score of 7.63, 7.0 and 6.45 and 5.90, for each one of these attributes, respectively. It should be noted that as the addition in the concentration of turmeric powder, increased the preference of these attributes in the bread decreased. Bread with a 10% concentration of turmeric ( $B_5$ ) being the least accepted bread with a score of 6.36, 5.18, 4.72 and 4.09, for each of the attributes mentioned. It is important to note that the attribute most affected in acceptance due to the addition of turmeric was the taste of the bread, with a decrease in acceptance of around 30% when compared to control bread (0% addition of turmeric). In the evaluation of bread as a healthy option, consumer acceptance increased as the concentration of turmeric powder added to bread increased.

Choosing the bread with the best healthy characteristics, the one with the highest concentration of turmeric ( $B_5$ ). Increasing its acceptance by more than 100% with respect to control wheat bread (0% of TP). It should be noted that all consumers participating in the evaluation or family members are diabetic and / or hypertensive. In this way, they are interested in the consumption of healthy products, such as bread added with turmeric. In relation to the attributes in general, the most accepted breads were  $B_3$  (2.5% of TP) and  $B_2$  (1.25). It was found that the least accepted breads by consumers are  $B_1$  (0%) and  $B_5$  (10% of TP). It is important to note that the most influential attributes in consumer preferences are that the bread is a healthy option, followed by taste, smell and color. The evaluated population mentions their interest in health care in these times of pandemic, being a definitive aspect for the acquisition of a new type of bread; is that can be healthy. Another important aspect to clarify is that consumers have purchasing power and, at least one member of the family has an education at the University level.

Despite the various benefits of turmeric, it is well known that curcumin, i.e. the active element of curcuma, has poor absorption and rapid metabolism (Stohs et al., 2020). To overcome this, there is evidence from numerous studies that to increase bioavailability, formulations should be prepared, for example, with pepper or phospholipids (Kesarwani and Gupta, 2013; Patil et al. 2013). Thus, in this present investigation Olive oil is used in bread making to improve the bioavailability of Turmeric by adding it to wheat flour in the bread making process.

In the maximum concentration of turmeric (10%), 60 g of Turmeric powder is added to 540 g of wheat flour. Twenty three slices of bread are generated from the dough that is formed. A maximum common person could eat three slices on average, which would imply consuming less than 10 g of turmeric allowed consumption and in this way there would be no risk of toxicity.

### 3.6 Principal component analysis

Figure 4 shows the plot of the PCA, it is possible to observe that 4 groups of data were formed, where only group 4 showed two types of bread with similarities, which corresponds to the types of wheat bread elaborated with 5 and 10% of turmeric concentration (TC). Note that the similarities of these two types of bread (5 and 10% of TC), are

due to a similar behavior of the variables: PA428nm, PA515nm, chewiness, DPPH, hardness and health options (A). The other three clusters formed (1-bread (0% of TC), 2-bread (1.25%) and 3-bread (2.5%)) had no similarities in behavior of variables. It is relevant to highlight that, according to the PCA (quadrant 3), turmeric-free bread (0%) is characterized by the existence of fungal colonies for the days evaluated (3 and 6). Conversely, for the breads of cluster four (5 and 10% of TC), showed opposite behavior (There were no fungal colonies).

Furthermore, it is possible to observe in the circle of correlations (Figure 4) that the variables DPPH and amplitude of the photoacoustic signal (at 515 nm) are highly correlated, since they form an angle of less than 90 ° between them. On the other hand, they have a negative correlation with the resilience variables and the color component L\*. They also have a negative correlation with the variables that represent the number of fungal colonies (fungi day 1 and 2).

It is important to highlight that the sensory acceptability of the breads is better in the breads without turmeric and with turmeric at 1.25% (characteristics with less added amount of turmeric), which was found with the lower antioxidant activity and sanitary quality of the bread. The consumer evaluation in the sensory test, in the attribute of bread as a healthy option, coincides with the characteristics of a higher concentration of added turmeric and therefore, higher values in antioxidant activity, levels of photoacoustic signals in the wavelengths of 428 and 515 nm (PA428nm and PA515nm), etc. Finally, the PA signal (normalized) at 515 nm was also related with its DPPH by linear equation of the correlation and the intensity of linear correlation ( $r$ ). Obtaining the equation:  $y = ax + b$ ; where  $a = 17.683168$ ,  $b = 14.01203$ ; and  $R^2 = 0.86841$  (see Figure 5).

In Fig. 5 In Fig. 4a, it is possible to observe that when the antioxidant activity increases, the photoacoustic signal is increased. In this way, there is a relationship between these measures. What could lay the foundation for future research, where a correlation between the photoacoustic signal and antioxidant activity is established, with a possible opportunity to apply to have a quantitative measure of that antioxidant activity.

, it is possible to observe that when the antioxidant activity increases, the photoacoustic signal is increased. In this way, there is a relationship between these measures. What could lay the foundation for future research, where a correlation between the photoacoustic signal and antioxidant activity is established, with a possible opportunity to apply to have a quantitative measure of that antioxidant activity.

The main idea lies in relating the photoacoustic signal of five types of bread (averaged from 4 repetitions) with the value of the antioxidant activity, obtained using a conventional technique. Then, we were interested in knowing the correlation between the photoacoustic signal at 515 nm, and the antioxidant activity obtained at that same wavelength. Photoacoustic spectroscopy is not often used, especially for testing bread products. There are no reports in the literature to apply photoacoustic spectroscopy and evaluate bread added with curcuma and related to measures of antioxidant activity. Through these results, it could open the possibility that future work will be carried out considering the development of devices under the photoacoustic principle, using a near light source or at 515 nm that can measure the antioxidant activity of bakery products. Future studies should focus on two perspectives 1) Assemble portable equipment that uses a laser light source or LED diodes, at a wavelength of 515 nm or close. 2) Carry out a study using a larger quantity of samples to specify, depending on the photoacoustic signal obtained, which value of antioxidant activity corresponds to. In this way, demonstrate the effectiveness of photoacoustic

spectroscopy to determine antioxidant activity in turmeric bread. So, it is possible said that future work could be directed to employ portable photoacoustic instrumentation to determine antioxidant activity

#### 4. CONCLUSIONS

In the present research project, it has been found:

- (i) Existing changes in characteristics of hardness, elasticity, cohesiveness, resilience and chewiness, color, DPPH, PA signals (515 nm and 428 nm) and sanitary quality could be due to the existing compounds in the absorption band (325-500 nm) that presented differences in the photoacoustic spectra obtained from the bread without turmeric (control) and the breads at different concentrations of turmeric (1.25, 2.5, 5 and 10%). The addition of turmeric powder for bread making can slow down their textural changes (hardness, elasticity, cohesiveness, resilience and chewiness). On the other hand, it increased the antioxidant properties beneficial to health and to the sanitary quality of bread, but consumer acceptability (flavor, color and aroma) decreases with increasing curcuma concentration.
- (ii) The photoacoustic signal amplitude produced by bread significantly increases with the turmeric concentration, such that it raises more than 100% for a concentration of 10% at the wavelengths of 428 and 515 nm. There was a positive correlation between the amplitude level of the photoacoustic signal obtained by photoacoustic spectroscopy (unconventional method) and the antioxidant activity of bread obtained by conventional method (DPPH).
- (iii) The Bread antioxidant activity is directly proportional to turmeric powder concentration and the amplitude of the photoacoustic signal at 515 nm, being the maximum increase (74%) at the concentration of 10%. At a 10% concentration of turmeric, three slices of bread contain approximately 8g of turmeric.
- (iv) The probability distribution function estimated by the Gaussian kernel of the photoacoustic spectra shows multimodal behaviors and we have observed that bread with different concentrations of turmeric showed different pattern of behavior.
- (v) The sanitary quality increase with the turmeric concentration. No fungal colonies were observed in bread mixed with 2.5%, 5% and 10%, after six days of storage, with possible fungistatic effect.
- (vi) Bread at 2.5 and 5% of turmeric concentration showed the yellowest color. Additionally, through Principal component analysis it was possible to demonstrate the strong positive correlation between DPPH and the PA signal (515 nm). As well as a negative correlation of both variables with respect to the number of fungal colonies in bread. It is shown that bread to 5 and 10% of TC have a similar behavior in relation to these variables. In this way, a concentration of either 5 or 10% of turmeric added to wheat flour could be used to improve its sanitary quality and antioxidant activity.

**5. ACKNOWLEDGEMENTS:** The authors would like to thank the “Instituto Politécnico Nacional”, through the CONACYT, COFAA, EDI and projects SIP scholarships. The support Research and Postgraduate Secretariat (Project SIP 20196252 and SIP 20201809), and the Photothermal Techniques Laboratory of Physics Department,

CINVESTAV-IPN for the support to develop the experiments of the present study. We also thank Ing. Esther Ayala for her technical support at the Physics Department, CINVESTAV-IPN.

*Complying with ethical standards.*

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethical approval:

The manuscript has not been sent to more than one journal to be considered.

The work submitted is original and has not been published elsewhere in any form or language (partial or complete).

This study has not been divided into several parts and has not been sent to several magazines or to a magazine over time.

The results will be presented clearly, honestly and without fabrication, falsification or inappropriate manipulation of data (including image-based manipulation).

It complies with the specific rules of the discipline to acquire, select and process data.

No data, texts or theories of others are presented as if they were the author's own. And the appropriate awards must be given to other works

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## Table captions

**Table 1.** Turmeric bread formulations

**Table 2.** Equations used to calculate density estimate, linear correlation, and correlation coefficient

**Table 3.** Photoacoustic signal levels at 428 and 515 nm, as well as antioxidant activity and effects on the sanitary quality of bread

**Table 4.** Texture profile and color analysis of bread samples with different concentrations of turmeric.

**Table 5.** Mean consumer scores for sensory acceptability of 5 breads (0, 1.25, 2.5, 5 and 10% of turmeric powder).

Table 1.

Ingredients	CB (%)	TB-1.25 (%)	TB-2.5 (%)	TB-5.0 (%)	TB-10 (%)
Wheat flour	81.72	80.7	79.68	77.63	73.55
Olive oil	9.53	9.53	9.53	9.53	9.53
Egg	6.54	6.54	6.54	6.54	6.54

Salt	0.17	0.17	0.17	0.17	0.17
Sugar	0.54	0.54	0.54	0.54	0.54
Yeast	1.5	1.5	1.5	1.5	1.5
Turmeric powder	0	1.02	2.04	4.09	8.17

CB: Control bread (without addition of turmeric), TB: Turmeric bread

Table 2

Description	Equation
Density estimate	$\hat{f}_h(x) = \frac{1}{nh} \sum_{i=1}^n K\left(\frac{x-X_i}{h}\right)$
Gaussian kernel $K(z)$	$K(z) = \frac{1}{\sqrt{2\pi}} \exp\left(-\frac{z^2}{2}\right)$
Linear equation of correlation	$y = ax + b$ $a = \frac{n \sum xy - \sum x \sum y}{n \sum x^2 - (\sum x)^2}$ $b = \frac{\sum y - a \sum x}{n}$
Pearson's linear correlation coefficient (r)	$-1 \leq r = \frac{Cov(X, Y)}{S_x S_y} = \frac{\sum_{t=1}^n (X_t - X) * (Y_t - Y)}{\sqrt{\sum_{t=1}^n (X_t - X)^2} * \sqrt{\sum_{t=1}^n (Y_t - Y)^2}} \leq +1$

Table 3.

% concentration of turmeric at wheat bread	Digital image of the bread	PA- s 515 nm	PA -s 428 nm	DPPH ( $\mu$ mol equivalent of Trolox/g)	Digital image of bread on the sixth day	No. of Colonies 3 d	No. of colonies 6 d
(a) 0.00% (White)		0.02c	0.05d	20.24a		2	16
(b) 1.25%		0.05b	0.132c	20.37 a		1	1
(c) 2.50%		0.06b	0.161b	27.66 b		0	0
(d) 5.00%		0.082a	0.163b	29.39b		0	0
(e) 10.00 %		0.083a	0.181a	35.25c		0	0

PA- s: Photoacoustic signal; d: day

Table 4.

Treatment	CB		TB 1.25%		TB 2.5%		TB 5%		TB 10%	
	Day 0	Day 3	Day 0	Day 3	Day 0	Day 3	Day 0	Day 3	Day 0	Day 3
Hardness	14.13 <sup>f</sup> ± 1.47	20.93 <sup>cd</sup> ± 0.59	16.42 <sup>ef</sup> ± 0.47	16.58 <sup>ef</sup> ± 0.62	17.33 <sup>ef</sup> ± 2.20	15.2 <sup>f</sup> ± 0.53	18.89 <sup>de</sup> ± 0.64	21.81 <sup>c</sup> ± 0.31	24.98 <sup>b</sup> ± 1.20	32.67 <sup>a</sup> ± 1.44
Elasticity (mm)	4.4 <sup>d</sup> ± 0.35	5.49 <sup>abc</sup> ± 0.02	4.81 <sup>bcd</sup> ± 0.37	4.63 <sup>cd</sup> ± 0.01	6.01 <sup>a</sup> ± 0.28	5.45 <sup>abc</sup> ± 0.26	5.78 <sup>ab</sup> ± 0.41	4.29 <sup>cd</sup> ± 0.24	4.92 <sup>abcd</sup> ± 0.08	4.42 <sup>d</sup> ± 0.25
Cohesiviness	0.5 <sup>bc</sup> ± 0.02	0.51 <sup>ab</sup> ± 0.02	0.44 <sup>de</sup> ± 0.01	0.55 <sup>a</sup> ± 0.01	5.45 <sup>abc</sup> ± 0.26	0.49 <sup>bcd</sup> ± 0.02	0.46 <sup>cd</sup> ± 0.01	0.48 <sup>bcd</sup> ± 0.01	0.41 <sup>ef</sup> ± .01	0.38 <sup>f</sup> ± 0.01
Resilience	0.25 <sup>b</sup> ± 0.02	0.23 <sup>bcd</sup> ± 0.008	0.2 <sup>de</sup> ± 0.01	0.3 <sup>a</sup> ± 0.04	0.22 <sup>cde</sup> ± 0.02	0.17 <sup>f</sup> ± 0.01	0.19 <sup>e</sup> ± 0.01	0.24 <sup>bc</sup> ± 0.008	0.16 <sup>f</sup> ± 0.01	0.16 <sup>f</sup> ± 0.004
Chewiness (mJ)	33.3 <sup>c</sup> ± 2.86	56.63 <sup>a</sup> ± 2.07	33.13 <sup>c</sup> ± 0.75	38.27 <sup>c</sup> ± 1.25	52.23 <sup>ab</sup> ± 3.01	33.87 <sup>c</sup> ± 2.36	47.37 <sup>b</sup> ± 3.56	37.6 <sup>c</sup> ± 1.78	57.23 <sup>a</sup> ± 2.51	54.83 <sup>a</sup> ± 2.07
a	-0.85 <sup>d</sup> ± 0.05	-1.23 <sup>d</sup> ± 0.12	-6.18 <sup>i</sup> ± 0.09	-5.58 <sup>h</sup> ± 0.07	-4.11 <sup>g</sup> ± 0.05	-1.62 <sup>f</sup> ± 0.14	1.25 <sup>d</sup> ± 0.01	4.29 <sup>e</sup> ± 0.23	5.78 <sup>b</sup> ± 0.05	8.3 <sup>a</sup> ± 0.22
b	17.09 <sup>f</sup> ± 0.34	18.10 <sup>f</sup> ± 0.94	47.42 <sup>bc</sup> ± 0.38	46.71 <sup>c</sup> ± 1.46	51.72 <sup>a</sup> ± 0.06	50.54 <sup>a</sup> ± 0.71	49.89 <sup>ab</sup> ± 0.07	45.59 <sup>c</sup> ± 1.49	41.33 <sup>d</sup> ± 0.07	37.7 <sup>e</sup> ± 1.90
L*	75.85 <sup>a</sup> ± 0.50	63.95 <sup>c</sup> ± 1.46	70.09 <sup>b</sup> ± 0.49	63.32 <sup>cd</sup> ± 2.14	71.06 <sup>b</sup> ± 0.11	60.76 <sup>d</sup> ± 0.43	63.58 <sup>c</sup> ± 0.03	51.33 <sup>f</sup> ± 0.90	56.71 <sup>e</sup> ± 0.11	46.48 <sup>g</sup> ± 0.72

Mean ± standard deviation values followed of different letter in each row and column between days 1 and 3, are significantly different ( $p < 0.05$ ). L\* indicates brightness (100 = White y 0 = Black), “a”, indicates greenish-reddish [negative (green) to red (positive)] and “b”, indicates blueish-yellowish [negative (blue) to yellow (positive)]. **CB: Control bread (without addition of turmeric), TB: Turmeric bread**

Table 5

	Color	Porosity	Sponginess	Hardness	Cohesiveness	Aroma	Chewiness	Flavor	Healthy option	General attributes
0% (B1)	7.0a	7.63a	7.0a	6.90a	6.27b	6.45a	6.81b	5.90ba	4.18b	5.54c
1.25% (B2)	7.9a	7.54a	6.18ba	5.72a	6.54b	6.18ba	6.81b	6.54a	4.18b	6.72ba
2.5 % (B3)	7.63a	7.27a	6.00bc	5.90a	6.63ba	5.72bc	7.09ba	5.27bc	5.27b	6.90a
5 % (B4)	7.18a	7.45a	5.90bc	6.45a	6.90ba	5.27dc	7.09ba	4.63dc	8.09a	6.81a
10% (B5)	5.45b	6.36b	5.18c	6.81a	6.63 <sup>a</sup>	4.72d	7.45a	4.09d	8.54a	5.81bc
<b>LSD</b>										
(0.05%)	0.77	0.82	0.99	1.2	0.5	0.63	0.5	0.69	1.22	0.93
<b>Media</b>	7.03	7.25	6.05	5.67	6.6	5.6	7.05	5.29	6.05	6.36
<b>Significance</b>	0.0001**	0.022*	0.01*	0.6ns	0.17ns	0.0001**	0.20ns	0.0001**	0.0001**	0.01*
<b>C.V.</b>	12.84	13.20	19.07	13.07	8.87	13.07	9.86	15.33	23.39	17.13
<b>R<sup>2</sup></b>	0.62	0.49	0.46	0.81	0.85	0.81	0.85	0.80	0.76	0.43

## Figure captions

**Figure 1.** Scheme of PAS experimental setup used in our experiments.

**Figure 2.** Characterization of turmeric bread with different TP concentrations (0, 1.25, 2.5, 5 and 10%):  
a) Spectra of the photoacoustic signals and their b) density functions.

**Figure 3.** Photoacoustic signal as a function of the concentration of turmeric bread (0, 1.25, 2.5, 5 and 10%)  
and a) DPPH and b) Number of fungal colonies formed on bread after six days.

**Figure 4.** Principal component analysis (Dim 1 (68.57%) x Dim 2 (20.70%)) of the variables evaluated in the bread samples at 0, 1.25, 2.5, 5 and 10%.

**Figure 5.** Correlation between the normalized PA signal of the sample with its DPPH ( $\mu\text{mol}$  equivalent of Trolox /g).

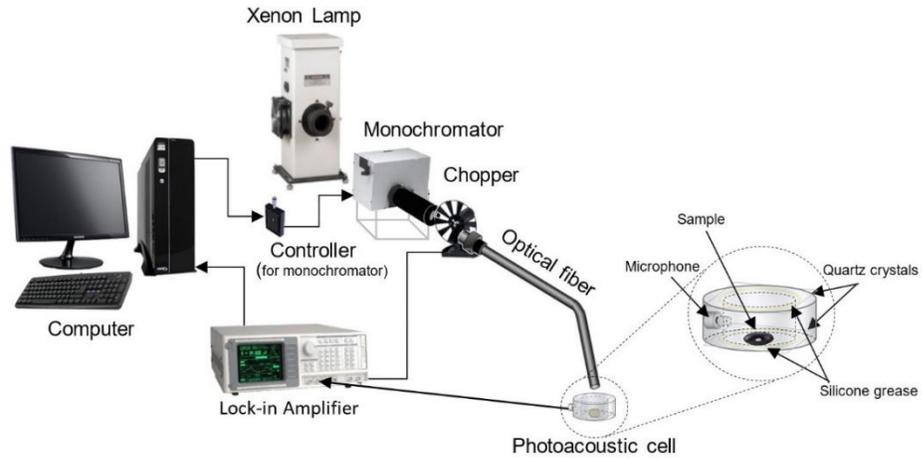


Figure 1.

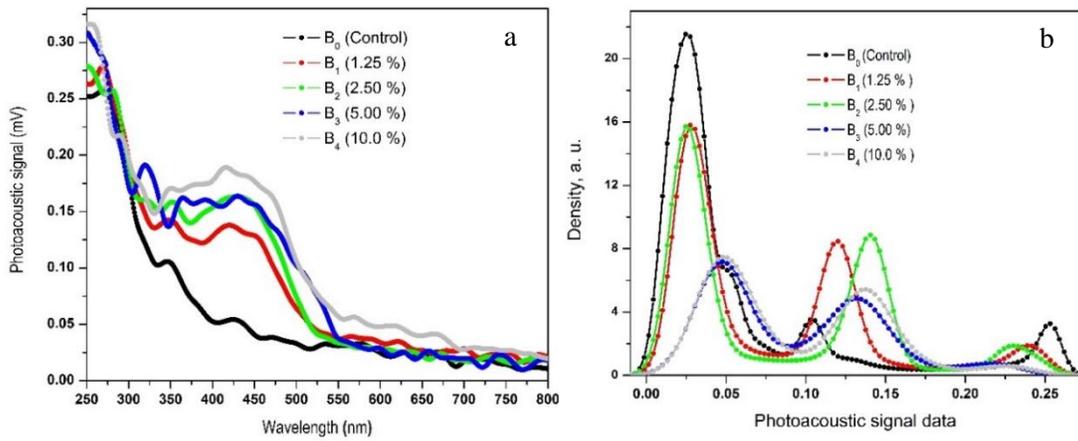


Figure 2.

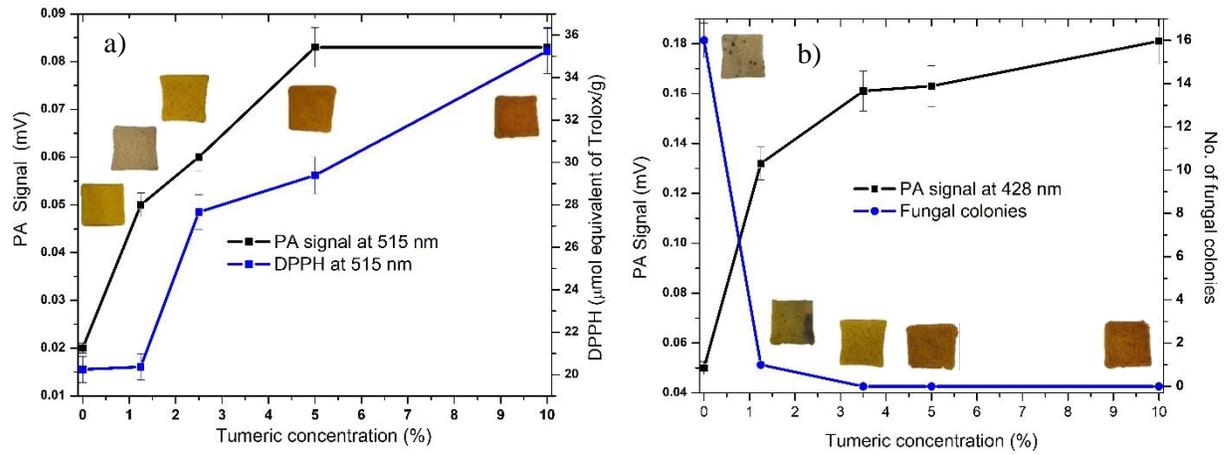


Figure 3.

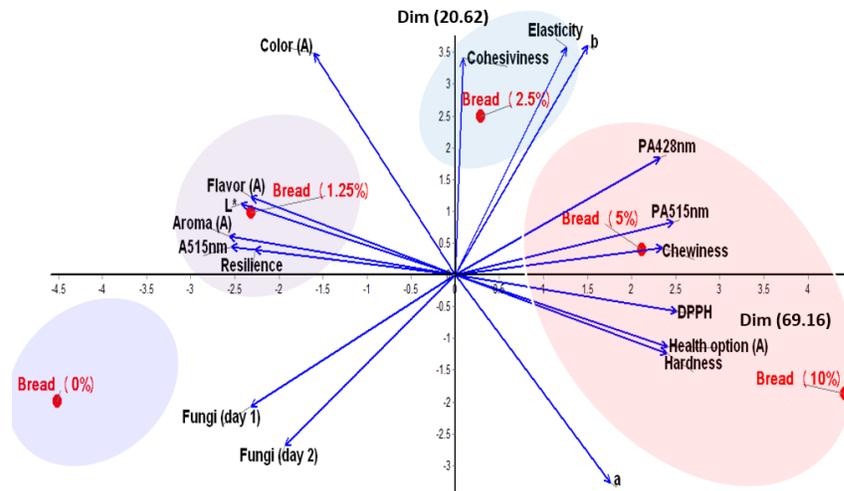


Figure 4.

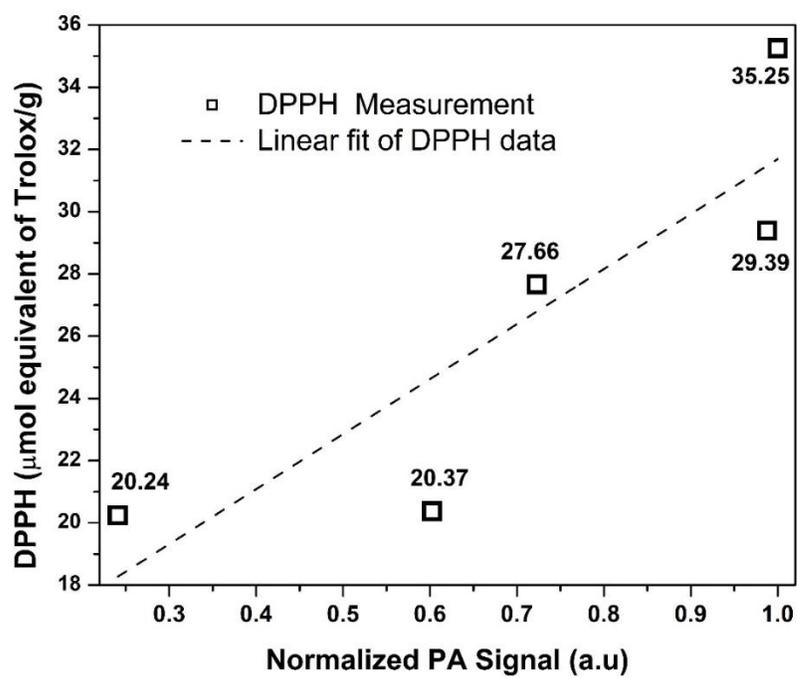


Figure 5.