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Induced changes of phenolic compounds in turmeric bread by UV-C radiation

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ABSTRACT

Phenolic compounds of breads added with turmeric at different concentrations (A: 0, B: 1.25, C: 2.5, D: 5 and E:10%) and radiated by UV-C (I. 0, II. 15, III. 30 and IV. 60 s), have been evaluated by HPLC (High-performance liquid chromatography). It is shown that: (i) UV-C radiation modifies the content of phenolic compounds as a function of the percentage of addition of turmeric and the exposure time. There were significant differences (ρ ≤ 0.05) in the concentration of phenolic acids of the turmeric bread (TB): 0 s (sinapic, chlorogenic, protocatechuic), 15 s (chlorogenic, ferulic, protocatechuic, p-hydroxybenzoic, gallic), 30 s (chlorogenic and gallic) and 60 s (chlorogenic). (ii) In TB without radiation appeared, the sinapic, beta resorcylic, syringic and ferulic acids. In the radiation of bread at 15 s, the phenolic acids chlorogenic, ferulic, protocatechuic, p-hydroxybenzoic, gallic, had the highest concentration in the breads added with turmeric at 10% (0.02 μg mL−1), 10% (0.38 μg mL−1), 1.25, 2.5, 5% (0.39 μg mL−1), 10% (1.06 μg mL−1) and 0% (1.10 μg mL−1). (iii) There was a degradation of phenolic acids due to UV-C radiation at 30 and 60 s. At 15 s radiation, sinapic, beta resorcylic, syringic and ferulic acids were not detected in turmeric breads from breads added with turmeric at (1.25, 1.25, 0 and 0%). In radiation at 60 s, beta resorcylic, syringic and ferulic acids were not detected in any bread added with turmeric. In addition, measurements of proximate chemistry, color, sensory analysis, and number of fungal colonies were performed. It is important to mention that the sanitary quality is improved by both UV-C radiation and turmeric. However, the highest results in sanitary quality improvement were due to turmeric.

Key words: Bread, secondary metabolites, UV-C radiation, turmeric.
Food security is becoming more and more relevant every day as it has a direct impact on the quality of life of the population. According to FAO (1996), it implies, among other things, having enough safe and nutritious food to lead an active and healthy life. Thousands of people fall ill due to the consumption of contaminated food (Delorme et al., 2020; Gunter et al., 2018). In this way, various environmentally friendly methods have been used for disinfection processes, among them UV-C radiation is found (Otto et al., 2011; Shah et al., 2016; Zhao et al., 2019), which has been considered as one of the emerging trends in food processing (Jermann et al., 2015).

UV-C method has been applied in agriculture and food (Wang et al., 2017; Guerrero-Beltran and Barbosa-C, 2004; Melini and Melini, Condón-Abanto et al., 2016), due to its germicidal effect at wavelengths in the 200-280 nm wavelength range (Alothman et al., 2009; Zhao et al., 2019). Evidence of its potential use to reduce or eliminate bacteria, fungi, viruses, and protozoa (Hirneisen et al., 2010; Yusaf et al., 2014; Islam et al., 2016; Delorme et al., 2020), has been reported by various authors. The main applications have been in water, agricultural seed and grain, seedlings, plants, post-harvest products and various solid and liquid food products such as milk, cereals, tuna fillets, bread, etc. (Frohnmeyer and Staiger, 2003; Vicente et al., 2005; Ouhibi et al., 2014; De Araujo et al., 2019; Urban et al., 2016; Ferreira et al., 2021; Fan et al., 2021).

Bread is one of the main foods in the human diet worldwide (Melikoglu and Webb, 2013; Ravimannan et al., 2016; Melini and Melini, 2018; Pinilla et al., 2019; Agunbiade et al., 2020), whose tendency is to make them fortified and without preservatives or chemical additives (Axel et al., 2017; Pinilla et al., 2019; Parenti et al., 2020; Taglieri et al., 2020; Klopsch et al., 2018). In this way, when preservatives are not used, a microbial problem occurs due to fungi; the main cause of bread deterioration since it can reduce its useful life generating economic losses and consumer dissatisfaction (Unachukwu and Nwakanma, 2015; Ravimannan et al., 2016). The problem increases in high temperature areas (García et al., 2019), being the most common to develop the Mucor, Rhizopus, Aspergillus, Penicillium y Fusarium sp (Ravimannan et al., 2016; Ogodo et al., 2017; Fraberger et al., 2020; Hernández et al., 2020). Some of these fungi have been associated with problems of hepatotoxicity, nephrotoxicity, and hyperestrogenism since they are producers of mycotoxins (Thanushree et al., 2019; Bezerra et al., 2014; Torrijos et al., 2021). Mycotoxins are
highly toxic metabolites and some fungi that produce them are contained in bread (El-Banna and Scott, 1983), occurring even before fungi are visible (Axel et al., 2017).

Some authors have reported the contamination of bread after baking, due to the deposit of fungal spores in the environment (Knight and Menlove, 1961; Axel et al., 2017; Weidenbörner et al., 2000). Although there are other studies that indicate the existence of the fungus in the raw material used, in the flour and in the wheat used in its preparation (Weidenbörner et al., 2000; Nasir et al., 2003; Al-Defiery and Merjan, 2015).

Weidenbörner et al. (2000) mainly identified in whole wheat and white flour fungi of the genus Aspergillus and to a lesser amount Penicillium and Rizopus. Bosly (2014) identified the species of fungi of the genus Aspergillus in stored flour, reporting species flavus, niveus, terreus and niger at a rate of 44.5%, 37.8%, 10.9% and 6.7%, respectively. Aziz et al. (1997) conducted studies of fungi in wheat grain, flour, and bread, coinciding with the types of fungi that were found. In this way, methods that favor its elimination or reduction are required. In this way, UV-C has shown to be useful to extend the shelf life of food products (Frohmeyer and Staiger, 2003). In this sense, UV-C light has been applied to the raw material or even to the final product (Ataila et al., 2004; Campagna et al., 2020; Kumar et al., 2020; Demir and Elgün, 2013, 2014).

UV-C can be considered an emerging environmentally friendly non-thermal technology for decontamination (Zhang et al., 2011), although it is necessary to generate knowledge related to its effects on nutritional elements such as secondary metabolites (bioactive elements). Different authors have reported that these secondary metabolites are modified according to UV-C radiation parameters and characteristics of the biological sample (Islam et al. 2016; Gopisetty et al., 2018). Faced with a trend to fortify foods, it is important to know the range of UV-C light exposure, where it does not alter the nutritional characteristics of fortified foods and not like fortified as the wheat and turmeric nutraceutical bread. In this way the aim of this work is to evaluate phenolic acids of wheat bread additionated by turmeric radiated with UV-C.

Phenolic acid compounds occurring naturally in the plant kingdom (Khan et al., 2015; Shahidi and Ambigaipalan, 2015), are known because to these have been attributed antioxidant, antimutagenic, anticancer, anti-inflammatory, antimicrobial properties among other biological properties (Xu and Chang, 2009). In such a way that it is important to know its behavior in wheat bread added with turmeric after UV-C radiation, due to that has been reported to improve the shelf life of different
foods, but it also induces the biosynthesis of phenolic compounds (González Aguilar, 2007; Liu et al., 2018).

2. Materials and methods

2.1 Elaboration of bread

Nutritional supplement of turmeric powder-TP (with certification kosher and nutritional information: proteins = 10g, fats = 3 g, carbohydrates 67 g, sugars = 3 g and dietary fiber = 23 g, sodium = 27g and energy content of 335 Kcal for each 100 g) was acquired in the city of Mexico. Bread dough was elaborated at different concentrations of TP (A: 0, B: 1.25, C: 2.5, D: 5 and E: 10%) in proportion to weight of the wheat flour (600 g). Bread formulations according to Hernandez et al. (2020) were elaborated with basic ingredients of yeast (11 g), egg (48-50g), olive oil (70 g), salt (1.25 g), sugar (4g) and warm water (300 ml). The formulations expressed by percentage are possible observe in Table (1).

Table 1. Turmeric bread formulations

| Treatments Different % of TP | bread digital image | Wheat Flour (%) | Olive oil (%) | Egg (%) | Salt (%) | Sugar (%) | Yeast (%) | Turmeric powder (%) |
|----------------------------|---------------------|----------------|--------------|--------|---------|-----------|-----------|---------------------|
| A). CB (0 g)               |                     | 81.72          | 9.53         | 6.54   | 0.17    | 0.54      | 1.5       | 0                   |
| B). TB-.25% (7.5 g)        |                     | 80.7           | 9.53         | 6.54   | 0.17    | 0.54      | 1.5       | 1.02                |
| C). TB-2.5% (15 g)         |                     | 79.68          | 9.53         | 6.54   | 0.17    | 0.54      | 1.5       | 2.04                |
| D). TB-5.0% (30 g)         |                     | 77.63          | 9.53         | 6.54   | 0.17    | 0.54      | 1.5       | 4.09                |
| E). TB-10 (60 g)           |                     | 73.55          | 9.53         | 6.54   | 0.17    | 0.54      | 1.5       | 8.17                |

TP: Turmeric powder; TB: Turmeric bread

Turmeric powder was incorporated in A: 600, B: 592.5, C: 585, D: 570 and E: 540 g of wheat flour weight.

The ingredients were incorporated into the container of the bread mixer (OSTER - Perform inox 600 W), starting with the solid ingredients and then the liquid ingredients, adding the sporulated yeast in 150 ml of warm water. The mixer is turned on by placing the spiral hook and it begins to beat. Add the rest of the warm water and continue beating to complete the 5 min. The resulting ball of dough is kneaded with your hands for another 10 min. Then, it is placed in a first rest in a previously greased
container and covered with a cotton cloth. Subsequently, it is kneaded again for another five minutes and placed in a box bread mold (previously greased and sprinkled with wheat flour). Arranging in such a way that the entire bread is covered and letting it rest for 25 minutes (second rest). The same procedure was applied for all the breads made. Finally, they were baked in an electric oven (OSTER-42 liters) at a temperature of 180 ° C, for 50 min. Once baked, they were placed on bread racks and allowed to cool under room temperature conditions for 24 h. Subsequently, the pieces of bread were cut into slices (1.5 cm thick) using an electric knife (Hamilton beach type EK08, 121 V, 11 Hz). The final bread samples show white and yellow in different shades as seen in Table 1.

2.2 Treatment of UV-C Radiation

The bread was treated by UV-C radiator system (UV-C/RS-Homemade-Esime, Zacatenco) – (Figure 1). A system of 4 lamps (UV-C, 254 nm) accommodated on the top and bottom of a cylindrical stainless-steel base. The lamps when turned on emit light towards a bases with grids, on which the slices of bread were placed. The slices of bread received radiation from the upper and lower sides of the slices. Several radiation times were applied to the bread: 0, 15, 30, and 60 s, programmed through a timer. The light intensity (700 μw/cm²) was measured by the UV-C/254 measuring equipment.

2.3 Analysis of phenolic acids by HPLC

Extracts were obtained with 50 mg of dry and pulverized material in 1 mL of methanol of HPLC grade (Sigma-Aldrich number 36860) at 80%, incubated for 20 min in an ultrasound bath (BRANSON at Smithkline company 50/60 Hz, model B-220, USA) (Meneses Reyes et al., 2008). The crude extracts were centrifuged at 731 g (Centrifuge 5804 eppendorf, model 5804) for 10 min (Irakli and Ekateriniadou, 2018). Supernatants were filtered with 25 mm diameter acrodiscs with
nylon membrane and 0.45 mm pore size (Titan). These extracts were injected immediately for the analysis by HPLC of phenolic acids. The samples were analyzed in a Hewlett Packard® chromatograph mod. 1100 provided with diode array detector and an Agilent Technologies automatic injector mod. 1200. The column was a Hypersil ODS HP column of 125 mm length and 4 mm internal diameter, 5 μm particle size was used. The mobile phase was distilled water adjusted to pH 2.5 with trifluoroacetic acid (A) and acetonitrile (B) (Bilia et al., 2001). The analysis was by gradient: T1 0.10 min (85% A) (15% B); T2 20 min (65% A) (35% B) and T3 25 min (65% A), (35% B), \( \lambda = 254, 280, 330 \) and 365 nm, Column temperature 30 ° C and flow of 1 mL min \(^{-1}\). Calibration curves were performed for standards of phenolic acids: sinapinic, \( \beta \)-resorcylic, syringic, chlorogenic, ferulic, protocatechuic, p-hydroxybenzoic and gallic (Sigma-Aldrich®)

The interpolations of all the extracts were calculated with ChemStation software © Agilent Technologies, Inc. 2004.

2.4 Statistical Analysis and Principal Component Analysis

**Variance Analysis**

The acid phenolic amounts of each treatment (Table 1) were compared by an analysis of variance (P\( \leq \) 0.05) (ANOVA) followed by Tukey test (Figure 1). The compilation of the data and all test calculations were performed using SAS software (SAS Institute, 2002).

**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 and radiated by UV-C (I. 0, II. 15, III. 30 and IV. 60 s). The analysis was performed using the R Project software version 0.10-47, with R Commander and factoMiner and Fitopac program (2.1). The data matrix used was formed from the measurements of the phenolic compounds (sinapic, beta resorcylic, syringic, chlorogenic, ferulic, protocatechuic, p-hydroxybenzoic and gallic) obtained.

3. Results and Discussion

Table 1 presents the analysis of variance of different phenolic acids (sinapic, beta resorcylic, syringic, chlorogenic, ferulic, protocatechuic, p-hydroxybenzoic and gallic) identified in the box bread made with different percentages of turmeric exposed to different radiation times UV-C. This is possible to observe, for each type of radiation, the set of values corresponding to each type of bread added with turmeric (A, B, C, D and E) and each type of phenolic acid found. The first set of results corresponds to the bread without UV-C radiation (I. 0 s). It can be observed that there were statistically significant
differences (P ≤ 0.05) in the concentration of phenolic acids (sinapic, chlorogenic and protocatechuic) of the bread. Among these, the phenolic acid most abundant was protocatechuic (0.42 μg / mL), followed by Sinapic acid (0.132 μg / mL) in turmeric bread at 5 and 1.25% TP (D, E). For the type of phenolic compound sinapic, it was found that 0 to 0.132 μg / mL increased between bread without turmeric (A) and bread with turmeric at 1.25% (B) and for protocatechuic acid it increased to approximately 9% when comparing bread without turmeric (A), with the added bread that most increased its content (D). However, chlorogenic acid decreased its content in turmeric added breads (B, C, D, E) by up to 61.5% with respect to turmeric-free bread.

Beta resorcylic (1.25, 2.5, 5, and 10%), syringic (5 and 10%) and ferulic acids (1.25, 2.5, 5, and 10%), were identified in turmeric breads at respective percentages of addition of turmeric, i.e. wheat bread (without turmeric) does not possess these phenolic compounds. The level of this phenolic acids tends to present a similar content level between the different breads with the different concentrations of turmeric since no significant statistical differences (P ≤ 0.05) were found between them. Although these acids tended to decrease in turmeric bread E (10% of TP), with respect to the other breads added with turmeric. Among these compounds, syringic acid was the most abundant (1.20 μg / mL).

The p-hydroxybenzoic and Gallic acids did not present statistically significant differences between the curcuma breads (0, 1.25, 2.5, 5 and 10%). These were present in the bread without turmeric and those added with turmeric. Although the p-hydroxybenzoic acid tended to increase in the bread with the increase in the percentage of addition of turmeric and the gallic acid was identified in the lowest levels of content in the breads C (2.5%) and E (10%).

In the set of data presented in Table 1, corresponding to the case of bread radiated with UV-C during 15 s (I), significant statistical differences (P ≤ 0.05) were found in phenolic acids of the chlorogenic, ferulic, protocatechuic, p-hydroxybenzoic and gallic types. Among these, the most abundant acids were protocatechuic (0.394 and 0.393 μg / mL) acid in the samples of bread to B (1.25%) and C (2.5%) and gallic (1.1 and 0.8 μg / mL) in the samples of breads, A (0%) and D (5%). It is important to note that this last acid (gallic) was not identified in the bread samples E (10%). However, p-hydroxybenzoic (1.06 μg / mL), chlorogenic (0.02 μg / mL) and ferulic (0.38 μg / mL) acids had the highest content in these breads (10%). It should be noted that chlorogenic and ferulic acids were not identified in the bread samples without turmeric (A) at this radiation level (15 s). Although in the case of chlorogenic acid in bread A (0 % of TP) without radiation (I. 0 s), these acids were present (0.024 μg / mL).
On the other hand, beta resorcylic acid was present in the samples of bread added with turmeric and radiated at 15 s, with a similar statistical behavior, although the breads A (0%) and E (10%) had the highest (0.228 μg / mL) and the lowest (0.088 μg / mL), content of this type of phenolic acid.

Regarding the sinapic and syringic acids, these were not identified in wheat bread without turmeric and radiated during 15 s. They were identified in the breads B (1.25%) and C (2.5%), respectively. It should be noted that syringic acid was present in the bread radiated at 15 s, at a lower level of addition of turmeric to the bread (2.5%), with respect to the bread without radiation (1.0 s) that was identified from the bread added with turmeric at 5% (D).

It is also important to emphasize that although there were no statistically significant differences between the breads evaluated with this radiation time (15 s), in these types of acids (sinapic and syringic). Their contents tended to decrease as the addition of turmeric increases, obtaining the lowest contents of these acids for the breads added to 10% (0.05 and 0.59 μg / mL, respectively). Among these compounds, syringic acid was the most abundant (0.59 - 1.19 μg / mL).

The concentration of phenolic acids identified in bread is decreased by increasing UV-C radiation by 30 s (6: sinapic, Beta resorcylic, chlorogenic, protocatechuc, p-hydroxybenzoic and gallic) and 60 s (5: sinapic, chlorogenic, protocatechuc, p -hydroxybenzoic and gallic). Due to UV-C radiation was found an apparent degradation of phenolic acids.

Note in Table 1, that for 30 s (case III), in gallic and chlorogenic phenolic acids, statistically significant differences were found when comparing the different breads (A, B, C, D and E). Gallic acid was the most abundant at this level of radiation (30 s) of added turmeric breads (0, 1.25, 2.5, 5, and 10%). The breads at 10% of TP had the highest contents of gallic acid (1.7 μg / mL) and chlorogenic (0.031 μg / mL). The protocatechuic and p-hydroxybenzoic acids did not present statistically significant differences (P ≤ 0.05), however, the highest values of these acids were found also in the bread E samples (0.381 and 0.137, respectively).

In relation to sinapic and beta resorcylic acids, they were present only in the breads to which no turmeric was added (A: 0%). Apparently, the degradation of these acids is intensified with the addition of turmeric (1.25, 2.5, 5, and 10%). On the contrary, chlorogenic acid was only present in the bread added with turmeric. Its concentration increasing as the addition of turmeric in the bread increased, with the highest concentration in the bread having 10% addition of turmeric.
Finally, at the radiation time of 60 s (IV) of the turmeric added breads (0, 1.25, 2.5, 5 and 10%), there were no statistically significant differences in the different types of acids identified (sinapic, chlorogenic, protocatechuic, p-hydroxybenzoic and gallic). Being gallic and protocatechuic acids, the most abundant, presenting the highest values both, in bread D (5%), with values of 1.2 and 0.378 μg/mL. Followed by p-hydroxybenzoic acid with values that ranged between 0.111 and 0.118 μg/mL. Regarding chlorogenic acid, it was identified from the 1.25% turmeric breads (i.e., breads without turmeric was not identified), with the highest level of acid content being the D bread (5%). In relation to synapic acid, it was not found in curcuma breads (A, B, D and E). It was only found in samples of bread C (2.5%) with a value of 0.06 μg/mL. It should be noted that syringic and ferulic acid occurred only in the radiation of 0 and 15 s, i.e., the breads radiated at 60 s, they were not found.
| Time (seconds)* | Treatment | Sinapic acid | Beta resorcylic acid | Syringic acid | Chlorogenic acid | Ferulic acid | Protocatechuic acid | p-hydroxybenzoic acid | Gallic acid |
|----------------|-----------|--------------|----------------------|--------------|-----------------|-------------|---------------------|----------------------|-------------|
| A. 0% (control) | NP        | NP           | NP                   | 0.02486638 a | NP              | NP          | 0.3824985 a         | 0.112323 a          | 1.21359 a   |
| B. 1.25%       | 0.13232025 a | 0.20401625 a | NP                   | NP           | 0.00976228 b    | 0.06055525 a | NP                  | 0.3960535 a        | 0.11211525 a | 0.74181 a |
| I. 0           | 0.1182655 b | 0.20153575 a | NP                   | NP           | 0.00970065 b    | 0.05125125 a | NP                  | 0.3933325 a         | 0.1112895 a | 1.0228625 a |
| C. 2.5%        | 0.11964525 b| 0.22864325 a | 1.205635 a           | 0.01331943 b | 0.05235175 a    | 0.4149025 b  | NP                  | 0.12641625 a        | 1.5305175 a | 0.938615 a |
| D. 5%          | 0.12056775 a| 0.19829977 a | 1.19136 a            | 0.0117385 b  | 0.03524255 a    | 0.38399225 a | NP                  | 0.12272925 a        | 0.938615 a |
| E. 10%         | 0.0059     | 0.035        | 0.0127               | 0.0054       | 0.0039          | 0.0287      | NP                  | 0.0121             | 0.7736      |
| DMS            | 0.999558   | 0.994616     | 0.99986              | 0.972003     | 0.999037        | 0.840574    | NP                  | 0.733081           | 0.422809    |
| R²             | 0.09816    | 0.166499     | 0.479399             | 0.479399     | 0.04348         | 0.394156    | NP                  | 0.116975           | 1.089479    |
| Means          | 0.228811 a | NP           | 0.21231              | 1.5066       | 0.0203          | 0.017       | NP                  | 0.0378933 a         | 0.11426875 a | 1.102185 a |
| B. 1.25%       | 0.120778 a | 0.1986425 a  | NP                   | NP           | 0.00929915 b    | 0.05324175 b| NP                  | 0.394325 a         | 0.1112965 a | 0.4951965 a |
| C. 2.5%        | 0.129145 a | 0.207212 a   | 1.188565 a           | 0.00952553 b | 0.05834425 b    | 0.393645 a  | NP                  | 0.11263075 b        | 0.56748475 b | 0.81492 b   |
| D. 5%          | 0.124358 a | 0.19864975 a | 1.192567 a           | 0.0105215 a  | 0.05573925 b    | 0.393186 a  | NP                  | 0.1168985 b         | 0.81492 b   |
| E. 10%         | 0.05968 a  | 0.0887945 a  | 0.593845 a           | 0.0233517 a  | 0.3829525 a     | 0.1138825 a | NP                  | 1.0613075 a         | NP          |
| DMS            | 0.1533     | 0.2312       | 1.5066               | 0.0203       | 0.017           | 0.0386      | NP                  | 0.0379 a           | 0.5153      |
| R²             | 0.775723   | 0.591808     | 0.800773             | 0.812835     | 0.99527         | 0.996214    | NP                  | 0.999689           | 0.942042    |
| Means          | 0.086792   | 0.184422     | 0.594996             | 0.01054      | 0.110056        | 0.334231    | NP                  | 0.30328            | 0.595957    |
| III. 30        | 0.058667 a | 0.2110205 a  | NP                   | NP           | NP              | 0.377098 a  | NP                  | 0.11371125 a        | 0.8831375 b |
| B. 1.25%       | NP         | NP           | 0.01507665 a         | NP           | 0.3777675 a     | 0.05557925 a| NP                  | 0.6064975 b         | 0.9483975 b |
| C. 2.5%        | NP         | NP           | 0.0162039 b          | NP           | 0.38025025 a    | 0.11022775 b| NP                  | 0.7657575 b         | 0.942042 b   |
| D. 5%          | NP         | NP           | 0.01374298 b         | NP           | 0.37661575 a    | 0.110545 a  | NP                  | 0.610925 b          | 0.942042 b   |
| E. 10%         | NP         | NP           | 0.03127028 a         | NP           | 0.38152575 a    | 0.13761575 a| NP                  | 1.70368 a           | 0.942042 b   |
| DMS            | 0.1488     | 0.007        | NP                   | 0.0141       | NP              | 0.1030      | NP                  | 0.1411             | 0.8778      |
| R²             | 0.444444   | 0.997978    | NP                   | 0.94091      | NP              | 0.523634    | NP                  | 0.540438           | 0.87426     |
| Means          | 0.011733   | 0.042204     | NP                   | 0.015259     | NP              | 0.378651    | NP                  | 0.105536           | 0.914364    |
| IV. 60         | 0.012103   | 0.0        | 0                    | 0.0134       | 0               | 0.377775    | NP                  | 0.114169           | 0.907834    |

*C. Longa L.* 0.00234041 0.00357646

A. 0% (control) B. 1.25% C. 2.5% D. 5% E. 10%; * Different exposure time of UV-C radiation.

DMS is the least significant difference, and the same letter are statistically equal (Tukey, P ≤ 0.05).
Figure 2 shown that UV-C radiation modified the concentration of phenolic acids identified in the added bread according to the radiation time (0, 15, 30 and 60 s) and to the addition of turmeric in the bread (0, 1.25, 2.5, 5 and 10%). It is observed that with higher UV-C radiation, the concentration level of phenolic acids decreases, being at 0 and 15 s, eight types of phenolic acids identified and at 30 and 60 s, six and five were found. It is observed that phenolic acids protocatechuic, p-hydroxybenzoic and gallic, were less affected by UVC- radiation since they were maintained at all levels of applied radiation. Among these, the least affected were protocatechuic and p-hydroxybenzoic. The gallic acid concentration showed a tendency to decrease due to the increase in UV-C radiation for each type of turmeric bread (0, 1.25, 2.5, 5 and 10%). White bread (At zero 0 s radiation), the gallic acid content interval was between the values of 0.7-1.53 μg / mL and at 60 s radiation the content variation interval in the breads added with turmeric was between 0.6 - 1.2 μg / mL, having a decrease for these limit ranges of 11 and 21%, approximately by UV-C light.

Other authors have reported the stability of secondary metabolites as a function of UV-C light. Anthocyanin concentrations were modified in cranberry water depending on the dose of UV-C light applied (0, 15, 30, 60, 120 and 240 mJ cm$^{-2}$) with a tendency to decrease (10%) as the dose increased (240 mJ cm$^{-2}$) recommending the UV-C light treatment dose of 40 mJ cm$^{-2}$ (Gopisetty et al., 2018). On the other hand, it should be noted that studies related to the sensory preferences of consumers applying different doses UV-C (70, 140, and 210 mJ cm$^{-2}$) on white bread, consumers preferred bread with the dose of 70 mJ cm$^{-2}$ (Kawaguchi et al. 2019).

In the present investigation, the doses of UV-C radiation applied to bread were lower (0, 10.5, 21, 42 mJ cm$^{-2}$), where at the dose of around 40 mJ cm$^{-2}$, it inhibited some of the phenolic acids in the bread added with turmeric (sinapic, beta resorcylic, syringic, and ferulic). Thus, in our study, the dose of 42 mJ cm$^{-2}$ is not recommended in bread samples due to the instability that it could produce. The applied dose that implies intensity and time of exposure to radiation by UV-C light, influence the percentage of modification of secondary metabolites depending on the type of metabolite studied and the food sample used. In this case, there were phenolic acids that degrade more easily under the applied UV-C light and others were more resistant. Likewise, there are other types of secondary metabolites that are affected. In pomegranate juice exposed to UV-C, they modified secondary metabolites (anthocyanins) in the order of 8-16% (Pala et al., 2011). Even though some international organizations have authorized the use of UV radiation to control organisms (FDA, 2013), it is also important to consider the importance of applying the appropriate doses to food, which avoids degradation of nutrients, as antioxidants can be, as this research has shown.
It would be convenient not to exceed 21 mJcm\(^{-2}\) of UV-C radiation since with higher radiation the stability of phenolic acids decreases and then the addition of turmeric in the bread would decrease its benefits. Which would not be convenient to decrease since the concentration of phenolic compounds is linked to antioxidant activity and beneficial therapeutic effects to people. Therefore, the appropriate irradiation parameters must be reached where its nutritional characteristics are preserved: in this case they could be improved since it is another of the effects reported in the scientific literature.

In fresh fruits, in the postharvest stage, radiated by UV-C, the content of secondary metabolites such as resveratrol increased, the authors reported a change in resveratrol concentration from 1 mg to 2 or 3 mg due to UV-C radiation (Cantos et al., 2000). In this way, depending on the radiated food and the radiation parameters, oxidation processes or not of secondary metabolites have been found. Gurol and Vatistas (1987) reported degradation of phenolic compounds as a function of time of exposure to UV-C treatment. In the present investigation there were some phenolic acids that remained more stable under UV-C radiation, mainly protocatechuic, p-hydroxybenzoic and gallic acids. These phenolic acids existed in bread without and with turmeric, \textit{i.e.}, are phenolic acids from wheat flour and not due to the addition of turmeric.

Some authors have reported that the main phenolic compounds in grain are concentrated in the bran fraction (Gélinas, 2006; Adom and Liu, 2002), one of those reported in abundance being ferulic acid. These acids in this investigation were not identified in white bread. Being only identified in bread added with turmeric, although it is not very stable against UV-C radiation since, after 30 s, said phenolic compound disappears in the bread. Other authors have identified this ferulic acid in wheat germ (King et al., 1962), in our research in white bread said phenolic compound was not identified.

The absence of this phenolic compound and others; with a respective change in antioxidant capacity could be due to the thermal process carried out for the preparation of bread and the fermentation time, among other factors (Meral and Köse, 2019; Ibrahim, 2015). On the other hand, it should be noted that these wheat compounds are associated with genetics and growing environment in the pre-sowing, sowing and harvesting stages. These are a function of the genotype used and the associated diseases in its growth process (Abdel-Aal \textit{et al.}, 2001). As is known in the literature, secondary metabolites are modified by various stress conditions to which plants are exposed during their growth (Ouhibi \textit{et al.}, 2014).
So, the characteristics of the wheat used to produce flour, and its production process and later bread are relevant; due to these is the type and concentration level of secondary metabolites found in white bread; which is known for its low antioxidant level (Ibrahim, 2015).

In this way, in the literature and in some countries commercially, fortified breads are proposed or consumed to enrich nutrients and increase their antioxidant capacity. When chemicals are not added to preserve it, a serious problem is generated in this industry, due to the concentration of fungi that develop. In this way UV-C light has been proven as an alternative to reduce fungi in bread. Although it has not only been proposed to sterilize bread in problems in the industry, but also to keep product lines in good sanitary condition. Kawaguchi (2019) found that the most abundant and common fungus in bread is *penicillium* sp. In the application of this physical method, there is the problem that on the one hand the food product can be healthily benefited; but on the other hand, some nutritional components could be degraded. In our investigation, from the dose of 21 mJ cm$^{-2}$ syringic, and ferulic acids were degraded, which are important in human health.

In the case of ferulic acid, it has a wide range of therapeutic effects against various diseases such as cancer, diabetes, cardiovascular and neurodegenerative due to its strong antioxidant activity. It has also been reported to be an effective free radical scavenger and has been approved in certain countries as a food additive to prevent lipid peroxidation. Indeed, removes superoxide anion radical and inhibits lipid peroxidation (Srinivasan et al., 2007).

In the present investigation, the importance of having the appropriate radiation parameters is pointed out since it was demonstrated that UV-C radiation affects the stability of phenolic compounds in white bread and added with turmeric. Although there is also evidence of the positive effect of UV-C treatment in increasing the nutraceutical properties of food (Cisneros-Zevallos, 2003). In this research was pointed the need for found dose of radiation depending on the food, *i.e.*, find UV-C dose values that do not cause hormesis.

The need to find adequate doses of UV-C radiation to increase other types of micronutrients through UV-C has been reported. In bread (made with yeast), the phenomenon that occurs through UV-C radiation has been used to convert ergosterol to vitamin D$_2$ (Mau et al., 1998) to increase its level. The illumination time used for UV light (100-400 nm) can be from 0.1-60 s, or 2-30 seconds of treatment with UV light is preferable. With antimicrobial effects at the wavelength of 254 nm corresponding to UV-C light (EFSA, 2014: 2015). Vitamin D$_2$ in bread increases due to UV light
from 0.75 to 3 μg / 100 g in UV-treated bread, 1–5 g / 100 g of yeast in the dough. In this way, in the bakery industry this type of environmentally friendly and possibly beneficial applications is important, for the food production and storage process in the best possible conditions in the different quality, microbiological and nutritional attributes. Just as there is a tendency to increase some components of bread due to UV-C light, it can also decrease. At least in the case of the phenolic acids identified in this research. Similarly, with that reported with other authors in different types of food with doses around 200 mJ cm$^2$ (Xiang et al., 2020; Niu et al., 2021).

![Figure 2](image)

**Figure 2.** Concentration of phenolic acids in different percentages of *Curcuma Longa* L. added to box bread and exposed to different times of UV-C light (0, 15, 30 and 60 seconds)
1. Sinapic acid 2. Beta resorcylic acid 3. Syringic acid 4. Chlorogenic acid 5. Ferulic acid 6. Protocatechuic acid 7. p-hydroxybenzoic 8. Gallic acid.

A. 0% (control) B. 1.25% C. 2.50% D. 5% E. 10%

Figure 3. Concentration of phenolic acids in different percentages of Curcuma Longa L. added to box bread and exposed to different times of UV light (0, 15, 30 and 60 seconds).

This behavior results of phenolic acids are visualized in Figures 3 and 4. Figure 3, represents the behavior of each type of phenolic acid, for each type of bread at each level of UV-C radiation (0, 15, 30 and 60 s) applied. The horizontal axis represents the breads added with different
percentages of turmeric (A = 0, B = 1.25, C = 2.5, D = 5 and E = 10%). The left vertical axis marked from numbers 1 to 8 refer to the types of phenolic acids identified in the bread samples (1. Sinapic, 2. Beta resorcylic, 3. Syringic, 4. Chlorogenic, 5. Ferulic, 6. Protocatechuic, 7. p-hydroxybenzoic and 8. Gallic) in the loaves and on the right side is their content (μg / mL). Phenolic acids 6, 7 and 8 did not degrade due to UVC radiation.

Figure 4. Principal component analysis of box bread added with turmeric (0, 1.25, 2.5, 5 and 10%) exposed to different times of UV light (0, 15, 30 and 60 seconds), according to the concentration of phenolic acids.

Figure 4 shown that three groups of breads were formed according to their content of the various secondary metabolites, group I (Quadrant I and II: 60 A, B, C, D, E; 30 B, C, D, E and 0 A), Group II (Quadrant III: 0 B, C, D, E; 15 A, B, C, D, E and 30 A) and Group 3 (Quadrant IV: 15 E). It can be observed that beta resorcylic, sinapic and syringic acid are correlated and show higher levels of these metabolites 15 A, 30 A, 0 C and 0 D (Figure 2). In contrast, panes 60 A, B, C, D, E and 30 A, B, C, D, E and 0 A, is related to lower levels of these phenolic acids. Furthermore, it can be observed that ferulic acid was more abundant in breads 0 B, E and 15 B, C, D, E; the highest value being bread 15 E. In contrast, bread 30 A, B, C, D, E and 60 A, B, C, D and E, which is situated at the other side of the plot, is related to lower levels, in this specific case there was no existence of ferulic acid.

The phenolic acid derivatives found are classified according to Shahidi and Ambigaipalan (2015) as hydroxybenzoic (beta resorcylic, syringic, protocatechuic, p-hydroxybenzoic, gallic) and hydroxycinnamic (sinapic, chlorogenic, ferulic). The concentration of phenolic acids in box bread
made with different percentages of curcuma Longa L are modified depending on the percentage of curcuma and the exposure times to UV-C radiation. Each phenolic compound responding differently.

Due the use of harmful chemicals in developing countries, it is important to continue exploring these possibilities offered by physical methods to improve the nutritional and sanitary quality of bread.

**CONCLUSIONS**

According to this research, phenolic acids were identified in white bread (chlorogenic, protocatechuic, p-hydroxybenzoic and gallic) and added with turmeric (sinapic, beta resorcylic, syringic, chlorogenic, ferulic, protocatechuic, p-hydroxybenzoic and gallic). The most abundant phenolic acids found were gallic and protocatechuic. Finding that there are phenolic acids more resistant to UV-C radiation (protocatechic, hydroxybenzoic and gallic) and others that are more affected (ferulic, syringic, and beta resorcylic) with increasing radiation.

The phenolic acids sinapic, beta resorcylic, syringic and ferulic (identified in turmeric bread) are the ones that existed in the least amount and at the same time are the ones that were sensitive to UV-C radiation, where beta resorcylic, syringic and ferulic disappeared for all the breads after 60 s of exposure to radiation applied to the bread.

UV-C radiation modified the concentration of phenolic acids identified in the added bread according to the radiation time (0, 15, 30 and 60 s) and to the addition of turmeric in the bread (0, 1.25, 2.5, 5 and 10%). It is observed that with higher UV-C radiation, the concentration level of phenolic acids decreases, being at 0 and 15 s, eight identified phenolic acids and at 30 and 60 s, six and five were found for 30 and 60 s.

White breads (0% of turmeric powder) were an apparent stimulation of phenolic acids due to UV-C radiation. Beta resorcylic was stimulated at 15 (0.22 μg / mL) and 30 s (0.21 μg / mL), after 60 s (0 μg / mL) it was not identified as well as at 0 s of radiation (0 μg / mL). Sinapic was stimulated at 60 s (0.586 μg / mL). In both phenolic acids, a state without presence was changed to presence.

The bread added with turmeric (1.25, 2.5, 5 and 10%), increases the presence of phenolic acids with respect to white bread (0% of turmeric), mainly sinapic, beta resorcylic, ferulic and syringic. Changing from a state without presence, in bread at 0% addition of turmeric powder (0 μg / mL), to presence with maximum values of 0.13, 0.22, 0.06 and 1.2 μg / mL. The phenolic acids sinapic, chlorogenic and protocatechuic had statistically significant differences when compared with the breads at different percentages of addition of turmeric (0, 1.25, 2.5, 5 and 10%).
Exposure of breads exposed to UV-C radiation duration of 15 s, produced significant statistical differences (P ≤ 0.05) in chlorogenic, ferulic, protocatechuic, p-hydroxybenzoic and gallic acids. The most abundant acids were protocatechuic (0.394 and 0.393 µg / mL) acid in the samples of bread to B (1.25%) and C (2.5%) and gallic (1.1 and 0.8 µg / mL) in the samples of breads, A (0%) and D (5%).

The UV-C radiation at 30 s of exposure of the breads produces statistically significant changes in the chlorogenic, and gallic phenolic compounds, having the maximum values of this type of acid in the breads at 10% addition of turmeric (reaching values of 0.03 and 1.7 µg / mL, respectively).

UV-C radiation during the 60 s exposure time did not lead to statistically significant changes in any of the constituent phenolic acids of bread at this radiation level (sinapic, chlorogenic, protocatechuic, p-hydroxybenzoic and gallic). Beta resorcylic, syringic and ferulic acid were not found in bread at this time of radiation exposure.

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