# THE EFFECT OF MICROWAVE TREATMENT ON SUNFLOWER SEEDS AND PASILLA CHILI PEPPERS WHEN USED AGAINST *Aspergillus flavus*

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*Abstract*

Molds of the genus *Aspergillus* contaminate cereal, legume, and oilseed crops, both in the field and during storage when environmental conditions permit, causing losses and risk of mycotoxins. The present study aimed to evaluate the effect of microwave treatments on sunflower seeds and pasilla peppers against Aspergillus flavus. Microwave treatments were applied until the target temperature of 60°C was reached, followed by cooling with air. Effectivity of 100% was reached, reducing the *A. flavus* population by 4 log cycles. Meanwhile, in sunflower seeds, population was reduced by 1 log cycle. Microwave treatment did not affect the moisture content of Pasilla pepper, and had no effect on the moisture content, water activity and color of sunflower seeds ($p>0.05$).

## Introduction

Post-harvest losses are currently a problem for global food security. Twenty-five percent of agricultural crops can be affected by the presence of mycotoxins, impacting product availability and human health (WHO, 2023). *Aspergillus* molds grow in soil, cereals, and decaying vegetation. *A. flavus* and *A. parasiticus* produce dangerous mycotoxins that can contaminate various crops. The most vulnerable to these species are cereals, oilseeds, spices, nuts, and chili peppers (WHO, 2023).

Microwave heating treatments have been used and researched as non-chemical alternatives to control post-harvest pests in dry products such as cereals and legumes (Yadav et al., 2014). Microwaves interact with the water molecules present in food; the energy from the microwaves is mainly absorbed by the water in the sample, causing the water molecules to vibrate and agitate rapidly. This vibration generates internal friction, converting electromagnetic energy into heat, which efficiently and rapidly increases the temperature of the food. This process kills microorganisms: the high heat that is quickly reached is lethal to bacteria, viruses, and other microorganisms, achieving their inactivation or destruction (Feng et al., 2002).

Sunflower seeds belong to the Asteraceae family and the species Helianthus annuus L. They are found in the center of the flower, with a dark shell and white stripes. This seed is native to America, in Mexico, where it is grown together with corn, beans, and squash within the milpa system. They are also rich sources of essential minerals such as manganese, calcium, copper, phosphorus, and selenium, which contribute to bone health and other vital bodily functions. They contain linoleic acid, a healthy fat that promotes cardiovascular health, unlike the less beneficial fats found in meat and fried foods. Furthermore, their high fiber content makes them a nutritious and complete snack (Espinoza, 2018).

The pasilla chili pepper, also known as “Chile Negro”, is a type of chili pepper obtained by dehydrating the chilaca chili pepper; the word “Pasilla” comes from its wrinkled appearance, which is similar to that of a raisin. The pasilla chili pepper is a variety of the *Capsicum annum L.* species. It is less spicy than other chili peppers, although it has a mild and pleasant spicy touch. Its pungency level is between 1,000 and 2,500 on the Scoville scale. This type of chili pepper is more notable for its slightly fruity, earthy flavor with a smoky touch than for its spiciness (Tko, 2024).

This chili pepper is a long, wavy fruit with a pointed or flat tip (NMX-FF-107/1-SCFI-2006).

The objective of this project is to analyze the effectiveness of microwave heat treatment on sunflower seeds and pasilla chili peppers for the inactivation of Aspergillus flavus mold and the change in the physicochemical properties of the samples analyzed before and after being subjected to the designed treatment.

**Materials and Method**

Physical-chemical analysis

Pasilla chili peppers and sunflower seeds were purchased at the Abastos de Irapuato market in Guanajuato. Physical-chemical tests (moisture content, color, and water activity) were performed before and after treatment.

Moisture content was determined in accordance with NOM-116-SSA1-1994 by placing 1 g of sample in duplicate in an oven for 24 hours at 105°C in boxes at constant weight. After this time, the dry sample was weighed.

Color was measured in triplicate with a colorimeter (Color Flex EZ, CFE204483 HunterLab, USA) using the CIELab scale to evaluate the color of the pasilla chili and sunflower seed samples.

The Water activity ($a_w$) was determined by placing 0.25 g of sample in an electric hygrometer (Aqua Lab, CX2, Decagon Devices, WA, USA), previously calibrated with activated carbon.

Inoculation of *Aspergillus flavus* mold

*A. flavus* mold was seeded on solid medium (Potato Dextrose Agar) and incubated for 5 days in an incubator at room temperature (25°C). Spores were washed with sterile water and 10 g of sunflower seeds and 2 g of pasilla chili peppers were inoculated. The samples were previously cleaned with cotton and alcohol. The samples were incubated for 3 days in an incubator at a temperature of 25°C to ensure the growth and fixation of the mold.

Microwave treatment and cooling.

Weighed 490 g of sunflower seeds + 10 g of inoculated sunflower seeds and 98 g of pasilla chili peppers + 2 g of inoculated pasilla chili peppers. The inoculated samples were wrapped in sterile gauze for identification and recovery after treatment.

The heat treatment was carried out using a domestic microwave oven (LG, Model MS-0745VS, Mexico), and its output power was determined using the IEC 60705 method (George, 2008) using a power of 392 W for sunflower seeds and 520 W for pasilla chili peppers. Worked with 500 g of sunflower seeds and 100 g of chili peppers; initially, the temperature was measured in five different areas of the sample. Subsequently, they were subjected to microwave treatment for 40 s in the case of pasilla chili peppers and 120 s for sunflower seeds, generating movement every 20 s to homogenize the sample, until the target temperature of 60°C was reached. Following heating, cooling was applied by distributing the sample in a container with holes and blowing air using an electric fan placed 1 m away for 270 seconds for the sunflower seeds and 120 seconds for the pasilla chili peppers. The temperature profiles are shown in Figures 1 and 2. The treatments were performed in duplicate.

Figure 1. Heating-Cooling Curve in sunflower seed treatment at 50% power (392 W, respectively) with MW.

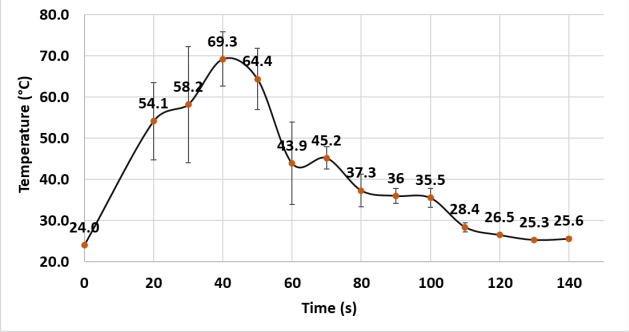
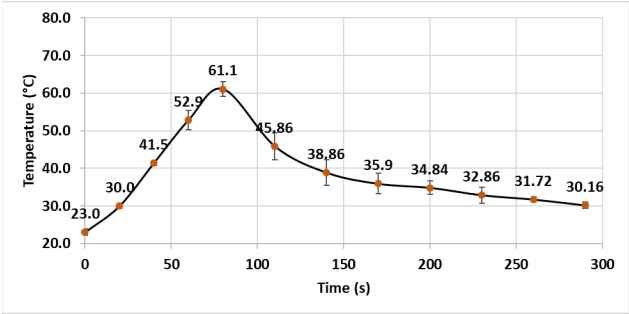



Figure 2. Heating-Cooling Curve in pasilla chili pepper treatment at 100% power (874 W, respectively) with MW.




Microbial count

In previously sterilized test tubes containing 9 mL of water, 1 g of each of the following samples was placed: control sample, sample identified as Bulk 1, and sample identified as Bulk 2. The three tubes were shaken and, using a micropipette, 1000 µL was extracted from each one and transferred to new tubes, thus obtaining a dilution of $1\times10^{-1}$.

The procedure was then repeated: 1000 µL was taken from the tubes with a dilution of $1\times10^{-1}$ and transferred to other tubes, achieving a dilution of $1\times10^{-2}$. After seeding, the samples were left to dry in the aseptic area and then incubated for 5 days at 25°C, keeping the boxes upside down.

A count of Colony Forming Units (CFU) was performed in accordance with NOM-210-SSA1-2014 for the control samples and the treated samples.

Statistical Analysis

An analysis of variance (ANOVA) was performed, applying a Tukey test with a 95% confidence level using the Statgraphics program, considering that there are no significant differences in the treatments if the value of ($p > 0.05$), while if the value ($p < 0.05$) indicates that there are significant differences in the treatments, this is to evaluate the effects of heating and cooling treatment on the physicochemical properties of sunflower seeds and dried pasilla chili peppers before and after applying microwave heating.

**Results and discussion**

Analysis of physicochemical properties

According to Table 1, water activity shows a significant decrease after microwave treatment in pasilla chili peppers ($p<0.05$). As for moisture content, there was no change when comparing the treated batches and the control ($p>0.05$), mainly due to variation between samples. This phenomenon has been documented in previous studies: Feng et al. (2002) explain that, during microwave drying, the mobility of water within plant tissues can vary, causing internal redistributions that alter measurements of total moisture content. This redistribution can cause an apparent increase in moisture content, without necessarily representing a real variation in the moisture present in the sample. According to Mexican Standard NMX-FF-107/1-SCFI-2006, the maximum permitted moisture content for pasilla chili peppers is 13.5%. Both the control sample and the treated sample comply with this value.

***Table 1.*** *Analysis of the physicochemical properties of Pasilla chili peppers before and after microwave treatment.*

| Treatment | $a_w$ | Moisture Content (%) | Color CIELab | | |
|---|---|---|---|---|---|
| | | | L | a | b |
| Control | 0.548 ±0.00[a] | 11.54 ±0.52 [a] | 14.99 ± 0.86 [a] | 1.33 ± 0.06 [a] | 1.19 ± 0.21 [a] |
| Treatment MW #1 | 0.535 ±0.00[bc] | 12.21 ±1.07 [a] | 19.24 ± 1.69[b] | 4.27 ± 1.88 [b] | 5.90 ± 3.30 [b] |
| Treatment MW #2 | 0.525 ±0.01[c] | 13.30 ±3.54 [a] | 20.23 ±1.57 [b] | 4.80 ± 1.26 [b] | 5.23 ±1.41[b] |

Equal letters indicate that there is no significant difference between the treatments evaluated.

The color of the pasilla chili pepper was affected by microwave treatment ($p<0.05$). There is an increase in lightness (L), giving it a lighter appearance. Parameter a (red-green) reflects an intensification of the red tone, and parameter b (yellow-blue) shows a greater yellow tone.

***Table 2.*** *Analysis of physicochemical properties of Sunflower seeds before and after microwave treatment.*

| Treatment | $a_w$ | Moisture content (%) | Color CIELab | | |
|---|---|---|---|---|---|
| | | | L | a | b |
| Control | 0.616 ±0.00[a] | 7.94 ±0.52 [a] | 36.33 ± 1.98 [a] | 3.04 ± 0.06 [a] | 9.57 ± 0.49 [a] |
| MW Treatment Lote #1 | 0.616 ±0.01[b] | 7.54 ±1.45 [a] | 33.30 ± 1.15 [a] | 2.69 ± 1.88 [b] | 8.17 ± 0.60 [b] |

Equal letters indicate that there is no significant difference between the treatments evaluated.

For sunflower seeds, water activity was not affected by microwave treatment, maintaining the same value (0.616), as was moisture content ($p>0.05$), with values ranging from 7.5 to 7.9%. The color of the sunflower seed shell showed significant changes after microwave treatment ($p<0.05$).

Microbiological inactivation

In pasilla chili peppers, an average of $4x10^4$ CFU was counted in the inoculated sample without treatment (Figure 3) and zero growth in the inoculated sample that received microwave treatment (Figure 4), thus achieving a reduction of 4 logarithmic cycles after microwave treatment under the indicated conditions.


***Figure 3.*** Sample of pasilla chili inoculated with *Aspergillus flavus,* untreated.

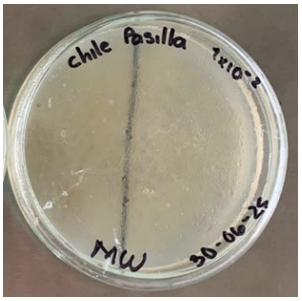



***Figure 4.*** Sample of pasilla chili peppers inoculated with *Aspergillus flavus* after microwave treatment.

In sunflower seeds, an average of $5x10^3$ CFU was counted in the inoculated sample (Figure 5) and $4.2 x10^2$ CFU in the inoculated sample that received microwave treatment (Figure 6), meaning that only 1 logarithmic cycle was reduced after the microwave treatment was applied.


***Figure 5.*** Sample of sunflower seed inoculated with *Aspergillus flavus*, untreated.

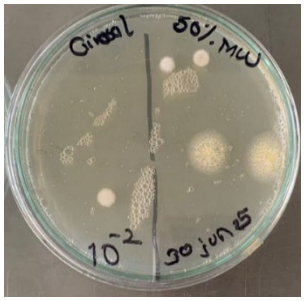



***Figure 6.*** Sample of sunflower seed inoculated with *Aspergillus flavus* after microwave treatment.

**Conclusions**

Microwave treatment at 520 W in pasilla chili peppers inoculated with Aspergillus flavus was effective in reducing

the mold population by 4 logarithmic cycles. The treatment did not affect the moisture content, which complies with Standard NMX-FF-107/1-SCFI-2006. In the case of sunflower seeds with shells, microwave treatment at 392 W achieved a reduction of one logarithmic cycle in the A. flavus population, and there were no significant changes in water activity and moisture content, which complies with Mexican Standard NOM-247-SSA1-2008. However, a decrease in the b parameter (chromaticity on the yellow-blue axis) of color on the CIELAB scale was observed. The proposed microwave treatment was effective for pasilla chili peppers, and more time or temperature will be required for the treatment of sunflower seeds to further reduce the population of this mold.

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