Original Paper

SCIENTIAFUNGORUM

DOI: 10.33885/sf.2023.54.1428
((2023) Vol. 54: e1428

Mycobiota of dried fruits from different regions of Mexico

Micobiota de frutos secos provenientes de diferentes regiones de México

David González-Albarrán, Nelly Ramírez-Corona, Aurelio López-Malo, Enrique Palou

Departamento de Ingeniería Química, Alimentos y Ambiental, Universidad de las Américas Puebla. San Andrés Cholula, Puebla 72810, México.

RESUMEN

Antecedentes: Las nueces y frutos secos son productos muy rentables, altamente vulnerables a contaminarse por mohos como *Penicillium* spp., *Aspergillus* spp. y *Rhizopus* spp. En México, la contaminación microbiana de las nueces y frutos secos ha sido poco estudiada, en particular del producto para su consumo a granel, que además tiene una regulación laxa.

Objetivo: Estudiar el estado de contaminación fúngica de cacahuates, nueces pecanas, semillas (pepitas) de calabaza y nueces de Castilla de diferentes regiones del país.

Métodos: Se midió el contenido de humedad y actividad de agua de 111 muestras de cacahuates (36), nueces pecanas (30), nueces de Castilla (12) y semillas de calabaza (33). Se aislaron e identificaron distintas especies de mohos presentes en las muestras.

Resultados y conclusiones: Sólo algunas muestras, principalmente de pepita de calabaza, contenían mohos y levaduras por encima de lo permitido por la normativa mexicana. Sin embargo, muchas muestras presentaron especies toxígenas (*Aspergillus flavus, Penicillium* spp. y *Fusarium* spp.) y otros mohos. Aunque los niveles de contaminación no fueron alarmantes, se debe de monitorear y reglamentar la venta de frutos secos a granel, ya que existe un riesgo potencial para el consumidor si las condiciones de almacenamiento no son las adecuadas.

Palabras clave: *Arachis hypogaea, Carya illinoinensis,* contaminación por mohos, *Cucurbita argyrosperma, Juglans regia*

ABSTRACT

Background: Tree nuts and dried fruits are very profitable products, highly vulnerable to contamination and subsequent deterioration by molds such as *Penicillium* spp., *Aspergillus* spp. and *Rhizopus* spp. In Mexico, microbial contamination of nuts and dried fruits, particularly of the product sold for bulk consumption, which also has a lax regulation.

Objective: To study the fungal contamination status of peanuts, pecans, pumpkin seeds, and walnuts from different regions of the country.

Methods: The moisture content and water activity of 111 samples of peanuts (36), pecans (30), walnuts (12) and pumpkin seeds (33) were measured. Additionally, the different species of molds present in the samples were isolated and identified.

Results and conclusions: Only some samples, mainly pumpkin seed, were found with mold and yeast contents above what is allowed by Mexican regulations. However, many samples were contaminated with toxigenic mold species, such as *Aspergillus flavus, Penicillium* spp. and *Fusarium* spp., as well as other deteriorating molds. Although contamination levels do not seem to be cause for alarm, the sale of nuts in bulk should be monitored and regulated, as there is a potential risk to the consumer if storage conditions are not adequate.

Keywords: *Arachis hypogaea, Carya illinoinensis, Cucurbita argyrosperma*, fungal contamination, *Juglans regia*

ARTICLE HISTORY

Received 13 May 2022 Accepted 15 September 2023
On line 28 November 2023

CORRESPONDING AUTHOR

✉ Enrique Palou, e-mail: enrique.palou@udlap.mx
Orcid: 0000-0003-1431-7490

INTRODUCTION

Tree nuts and dried fruits are usually considered stable against microbial spoilage, mostly due to their low water activity. Tree nuts and peanuts are usually protected from spoilage microorganisms by their thick shells (Pitt and Hocking 2009). However, many times peanuts, nuts, and edible seeds are dehulled before being sold to consumers, which creates avenues for microbial contamination, growth, and spoilage. The nature of the edible part of seeds, nuts and other dried fruits makes it impractical to decontaminate them by traditional means, such as washing or steam-based thermal treatments. Of particular concern is the contamination with toxigenic mold species, such as *Aspergillus flavus, Aspergillus parasiticus*, and *Fusarium* and *Penicillium* species.

Peanuts are an important food crop all around the world, valued for their edible seed as well as a source of edible oils. As a crop that grows underground, peanuts are highly susceptible to microbial contamination. Mycobiota of peanuts mostly consists of *Aspergillus* and *Penicillium* species, including *A. flavus* and *A. parasiticus*, both known to produce highly toxic aflatoxins (Khandaker *et al*. 2019a). Spoilage of peanuts is often caused by mold due to their low water activity and is often caused by improper storage when water activity is allowed to rise above 0.6.

Tree nuts are similar to peanuts in composition and spoilage characteristics: they have a high oil content and low water activity. Contamination of tree nuts with mold occurs mostly when they are handled improperly after being dehulled, since their thick shells are usually able to protect them from microbial contamination. After being dehulled, tree nuts are susceptible to spoilage by molds when their water activity is allowed to increase above the threshold at which molds are capable of growth. Tree nuts are important food crops all around the world, and their nutritional and flavor attributes make them of economic importance to the countries in which they are produced.

The seeds from different species of squash or gourds are consumed either on their own or as an ingredient on dishes all around the world. The species *Cucurbita pepo* and *Cucurbita argyrosperma* are of particular importance for their production of edible seeds (Sánchez-de la Vega *et al*. 2018). Amongst edible seeds, squash seeds are unique in that they have a relatively high moisture content, which makes them more susceptible to microbial spoilage (Chayjan *et al*. 2013). Despite this, little information exists regarding the mycobiota of edible squash seeds.

In Mexico, dry and intermediate moisture foods, such as grains, seeds, and nuts are of great cultural and economic importance. Mexico is one of the main worldwide producers of nuts, which are grown in most regions of the country, and are also important and commonly used ingredients in traditional Mexican cuisine.

In this context, the objective of this research was to carry out an investigation of the status of contamination of selected edible nuts and seeds sold in local markets throughout the country.

MATERIALS AND METHODS

Survey and sampling of select dried fruits

Peanut (*Arachis hypogaea*), pecan (*Carya illinoinensis*), walnut (*Juglans regia*), and squash seed (*Cucurbita argyrosperma*) samples were purchased in municipal produce markets of cities and towns across different regions of Mexico, where dried fruits can be purchased loose. The different regions were defined as Northwest, Northeast, West, Center, and Southeast based on climate and land use as reported by the National Institute of Statistics and Geography (INEGI), summarized in Table 1. Samples consisted of at least 250 g of the selected dried fruit dehulled, unsalted, and unroasted. A total of 111 samples (36 peanut samples, 30 pecan samples, 12 walnut samples, and 33 squash seed samples) were obtained and transferred to the Laboratory of Food Microbiology of the Universidad de las Americas Puebla for further analysis. Details about the samples can also be seen in Table 1.

Physicochemical characteristics of dried fruits

For the physicochemical analysis of dried fruits, approximately 10 g of each sample were manually ground using a ceramic mortar and pestle. Ground samples were immediately transferred to sealable plastic cuvettes for water activity analysis to prevent as much exposure to the environment as possible. Both moisture content and water activity determinations were carried out within a day of the sample having been received.

Table 1. Characteristics of the five regions under analysis (INEGI 2020) and number of samples obtained by region of each studied dried fruit

No.	Region and conforming states	Description of region	Dried fruit	No. of samples
1	Northwest (Baja California, Baja California Sur, Chihuahua, Sinaloa and Sonora)	Predominantly desert or semiarid climate (Köppen BSh/BWh). Region is not densely populated and is predominantly rural. Largest population center in the region is the city of Tijuana, B.C.	Peanut Pecan Walnut Squash seed	3 6 3 9
2	Northeast (Coahuila, Durango, Nuevo León, San Luis Potosí and Tamaulipas)	Predominantly semiarid climate (Köppen BSh/BSk). Region is mostly scarcely populated and rural with some densely populated urban and suburban areas. Largest population center in the region is the city of Monterrey, N. L.	Peanut Pecan Walnut Squash seed	6 6 0 6
3	West (Aguascalientes, Colima, Guanajuato, Jalisco, Michoacán, Nayarit, Querétaro and Zacatecas)	Varied climate with predominantly semiarid areas (Köppen BSh/BSk) with important presence of tropical savannah areas (Köppen Aw). Region is mostly densely populated and highly urbanized. Largest population center is the city of Guadalajara, Jal.	Peanut Pecan Walnut Squash seed	3 3 3 3
4	Center (Mexico City, State of Mexico, Guerrero, Hidalgo, Morelos, Puebla and Tlaxcala)	Predominantly temperate oceanic climate (Köppen Cfb). Region is densely populated and extremely urbanized, as it contains the Mexico City megalopolis urban complex. Largest population center is Mexico City.	Peanut Pecan Walnut Squash seed	18 12 6 12
5	Southeast (Campeche, Chiapas, Oaxaca, Quintana Roo, Tabasco, Veracruz and Yucatan)	Predominantly tropical climate (Köppen Aw). Region is mostly sparsely populated and rural with some urban and suburban areas. Largest population center is the city of Mérida, Yuc.	Peanut Pecan Walnut Squash seed	6 3 0 3

Moisture content determination

For moisture content determination, 2 g of ground samples were precisely weighed on constant-weight aluminum trays with a bed of silica sand using an analytical scale. The initial weight was recorded, and the samples were transferred to a 130 ° C oven. Samples were dried until constant weight was achieved (~24 h.). Samples were then removed from the oven and allowed to cool at ambient temperature (~25 °C) in a desiccator with silica gel until temperature equilibrium was achieved (15-20 min). Afterwards, dry weight was measured using an analytical scale and moisture content was calculated as the ratio of the difference between sample weight and dry weight to sample weight.

Water activity determination

For water activity (a_w) determination, enough ground sample was placed in sealable plastic cuvettes to cover the bottom of the container. Water activity was then measured in a 4TEV dew-point water activity meter (AquaLab, USA) at 25 °C. Assays were carried out in triplicates.

Yeast and mold count

Total yeast and mold counts were determined on dried fruit samples. 10 g of each sample were weighed and placed in sterile sampling bags. Samples were then diluted with 90 mL of 0.1 % peptone (Becton-Dickinson, Mexico) solution and homogenized using a Stomacher ® laboratory blender (Seward, United Kingdom.). Serial dilutions of the sample were prepared using the same 0.1 % peptone solution and plated in potato dextrose agar (PDA) (Becton-Dickinson, Mexico) plates acidified with 1.6 mL/100 mL of a 10 % tartaric acid solution to a final pH of 3.5. Colonies in inoculated plates were counted after being incubated at 25 °C over a period of 5 days. For samples in which *Rhizopus* species were abundant, Dicloran Chloramphenicol Rose Bengal agar (DRBC) (Becton-Dickinson, USA) was used instead of PDA to limit colony growth and allow for a more accurate quantification of yeasts and molds. Assays were carried out in triplicates.

Fungal isolation and identification

Individual mold colonies were taken from counting plates and inoculated in PDA plates, without tartaric acid, for isolation. Plates were incubated at 25 °C for 7 days, or 3 days when a *Rhizopus* colony was apparent, and inspected afterwards for contamination. Resampling and inoculation of the mold colonies was repeatedly carried out until only a single mold species was apparent in the agar plate. Afterwards, molds were point-inoculated in malt extract agar (MEA) (Becton-Dickinson, Mexico), Czapek Yeast Extract agar (CYA) (Becton-Dickinson, Mexico), Czapek Dox agar (CZD) (Becton-Dickinson, Mexico), and G25N agar, the latter of which was prepared according to the formula given by Pitt and Hocking (2009). For identification, molds were incubated at 5, 25 and 36 °C for 7 days, or 3 days when a *Rhizopus* spp. colony was readily apparent.

After incubation of the fungal isolates, macro and microscopic colony morphology was observed and identification was carried out following dichotomous keys as outlined by Pitt and Hocking (2009) and Samson *et al*. (1995).

For microscopic observation and measurement, a sample was taken from the outer edges of the colony using a sterile dissection needle, cutting a small (16 mm^2, approx.) and shallow (< 1 mm deep) square of media along with the desired colony portion. The colony sample was then placed in a microscope slide and a drop of aniline blue solution (0.1 % aniline blue; Química Meyer, Mexico) in 85 % lactic acid (Química Meyer, Mexico) was added, as mounting fluid. Afterwards, a drop of 70 % ethanol was added. A slide cover was then placed on top of the sample, and the sample was carefully heated with a Meker-Fisher burner to melt the culture medium. The sample was then observed under 100x magnification in an optical microscope (American Optical, USA.) equipped with an Axiocam ERc 5S camera (Zeiss Microscopy, Germany.) and associated software for microphotography and measurements.

Identification of *Penicillium* subgenus *Penicillium* species

For identification of *Penicillium* subgenus *Penicillium* species, an additional culture was prepared using

creatine sucrose neutral agar (CSN), as described by Pitt and Hocking (2009). The medium adds an additional criterion for discrimination of otherwise similar species of *Penicillium* by producing an acid (yellow), alkaline (violet), or neutral (grey) reaction based on whether and how the mold metabolizes sucrose and/or creatine. CSN reaction, along with culture morphology, is part of the criteria used in the dichotomous keys presented by Pitt and Hocking (2009).

Incidence of individual genera or species of mold

Incidence of individual genera or species of mold was calculated as the fraction of samples contaminated with a particular species or genus of mold, using the following equation:

$$I_s = \frac{n_c}{n_t} \times 100$$

Where I_s is the incidence (%) of a genus or species of mold in a subset of samples; n_c is the number of samples from which the genus or species in question was isolated at least once within a subset of samples, and n_t is the total number of samples in that subset. Samples were categorized by region and seed type and incidence was expressed thusly. A sample was considered contaminated when the genus or species in question was isolated and identified from the sample.

Data analysis
Analysis of variance (ANOVA)

Statistical data analysis was carried out using Minitab 19 (Minitab LLC, USA). An analysis of variance (ANOVA) was carried out, preceded by an equal variance test and a normality test. Equal variance testing was deemed necessary due to the different sample sizes among dried fruit samples and was tested by the Levene method. Normality was tested by the Ryan-Joiner method. Grouping information was then calculated by the Games-Howell method. Finally, correlation between variables was calculated by the spearman correlation method. The level of significance was set at 0.05 for all statistical analyses.

Principal component analysis (PCA)

To illustrate the relationships between variables, a principal component analysis (PCA) was carried out between the system variables of a_w, % H, yeast and mold count, and number of different identified species. Grouping was decided based on categorical variables (dried fruit type and region). The cophenetic correlation coefficient (CCC) was computed to measure the accuracy of the PCA, where a value closer to 1.0 corresponds to a more faithful preservation of correlations between variables. For these statistical analyses, XLSTAT 2019 addon for Microsoft Excel was used (Addinsoft LLC, Paris, France).

RESULTS AND DISCUSSION

Physicochemical analysis of dried fruits

Both water activity and moisture content of dried fruit samples was analyzed. Equal variance tests showed significantly different variance ($p<0.05$) for several groups of samples. Variance was found to be significantly different for water activity, moisture content, and yeast and mold counts for all sample groupings except for walnuts, where no significant difference was found for variances among regions. For this reason, ANOVAs were run without assuming equal variances among samples and the Games-Howell post-ANOVA pairwise comparison method was used. The results of these analysis (Figures 1 and 2) show most dried fruits exhibited water activity levels below 0.6 and moisture content below 6 %. Only squash seeds from the South-East region showed moisture content higher than 6 %, while pecans from the same region showed water activity close to 0.6 (0.597±0.004). Peanuts had moisture levels ranging from 4.17±0.02 % to 2.40±0.80 %, which is considerably lower than the 8.79±0.07 % moisture content found by Ghorbani and Hosseini (2017), but closer to the moisture content reported by Liu *et al*. (2018) of 3.65±0.05 to 4.05±0.09. On the other hand, water activity levels of peanuts are within the range considered by Ghorbani and Hosseini (2017) as ideal quality for peanut kernels (from 0.33 to 0.43 at 25 °C) with only peanuts from the north-east and north-west regions showing water activity values outside that range.

Peanut samples from different regions showed significant differences ($p<0.05$) in water activity and moisture content. Peanuts from the Northeast and Northwest regions had significantly higher water activity than peanuts from the Western region. Peanuts from the Northwest also had significantly higher water activity than peanuts from the West and Center regions. This probably indicates a difference in composition in the peanuts, as water activity is not only affected by moisture content, but also by content of low-molecular-weight soluble solids, as well as other components. It is known that moisture content and water activity of peanuts can also vary depending on the state of maturity of the seed at the time of harvest (Dorner 2008). It is unlikely that the water activity of the peanuts was influenced by storage conditions or local climate, as peanuts from the arid Northwest region had significantly higher water activity that peanuts from other regions, as well as no significant difference with peanuts from the more tropical Southeast region.

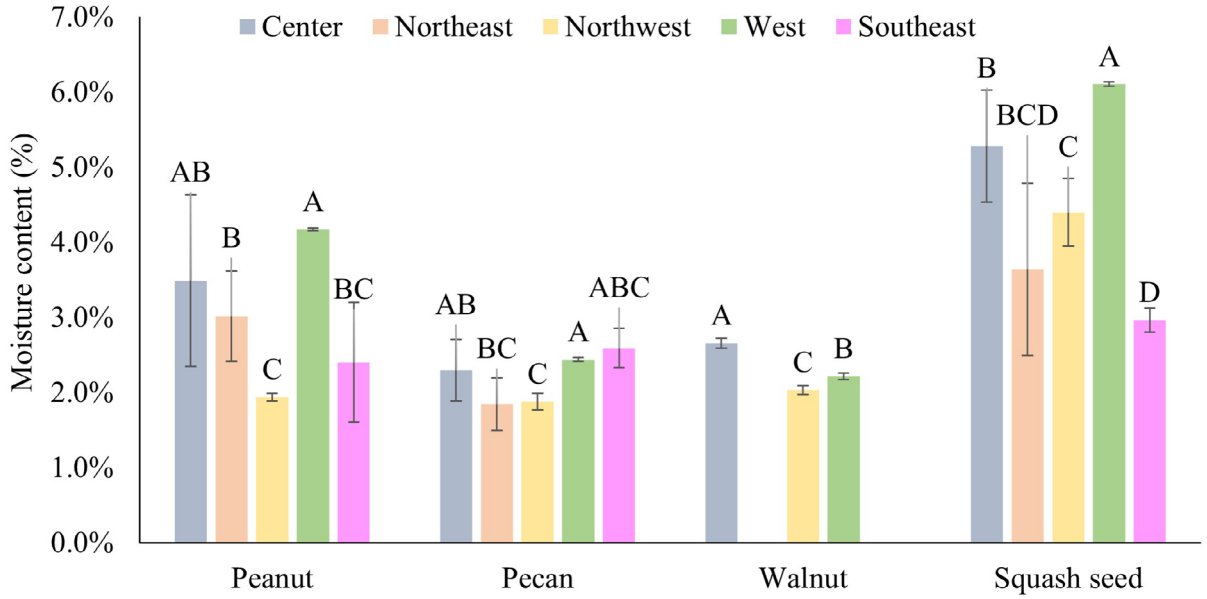

Figure 1. Moisture content of studied dried fruits from selected regions of Mexico. Different letters indicate significant ($p<0.05$) differences amongst dried fruits from different regions.

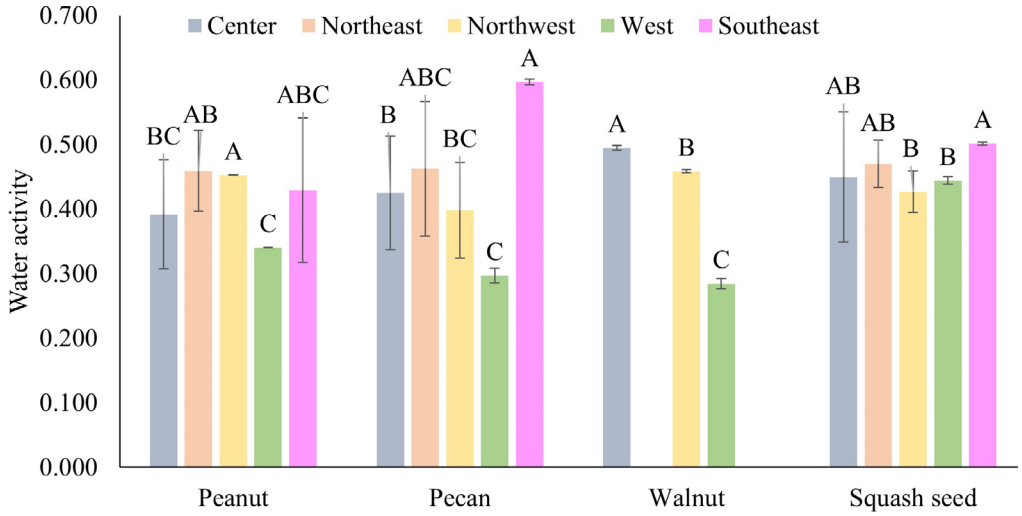

Figure 2. Water activity of studied dried fruits from selected regions of Mexico. Different letters indicate significant ($p<0.05$) differences amongst dried fruits from different regions.

Pecan samples also showed significant differences in both water activity and moisture content, with nuts from the Southeast region having a significantly higher water activity ($p<0.05$) than samples from all other regions, except from those from the Northeast region. Nuts from the Southeast region were also close to the threshold for growth and development of mold spores ($a_w > 0.6$) which would make them vulnerable to spoilage and/or aflatoxin generation. Water activity levels were considerably lower than those reported by Valle-Garcia *et al.* (2019), who found all pecan samples tested above the 0.6 threshold and up to 0.9. Moisture content levels appeared lower than those reported by Reis Ribeiro *et al.* (2020), who found moisture levels ranging from 4.56 % to 5.50 %. In the case of pecans, regional climate appeared to have an influence on their water activity, as only nuts from the wetter Southeast region had water activity close to the mold growth threshold.

Walnuts from different regions showed significant differences ($p<0.05$) in both water activity and moisture content, with walnuts from the West region showing a significantly lower value than from the other two studied regions. Walnuts from this region also show a much smaller water activity value than what was reported by Schmidt and Fontana (2019) of 0.57-0.58. Walnuts from the other regions presented values closer to what was reported, yet still considerably smaller. Moisture content of all samples is within the range reported by Peng *et al.* (2021), who found moisture content for walnuts ranged between 1.20 to 9.92 %. The water activity values found in this study for walnuts show a remarkable resistance to ambient conditions, as it has been reported that walnuts are highly hygroscopic and will readily absorb moisture at relative humidity levels as low as 1.5 % (Boaghi *et al.* 2019).

Squash seeds showed significant differences ($p<0.05$) in water activity only between the Southeast region and the West and Norwest regions. On the other hand, most regions showed a significant difference in moisture content, with seeds from the West region having the highest moisture content, at 6.10±0.03 %. Ardabili *et al.* (2011) reported a moisture content for *Cucurbita pepo* seeds of 5.20±0.28 %, which is similar to some of the values found in this study. *Cucurbita argyrosperma* seeds remain poorly studied as a food source. To the authors' knowledge, no study has reported their moisture content or water activity levels before, though they can be assumed to be similar to those of the seeds of other species in the *Cucurbita genus.*

Water activity value of samples was comfortably below the optimum water activity levels for aflatoxin production of 0.96, and below minimum water activity for aflatoxin production of 0.85 (Liu *et al.* 2017). This alone is not enough to rule out the possibility of aflatoxin contamination, as cross-contamination is still a possibility. However, it is an indication that storage conditions were adequate to prevent the development of mold spores and the endogenous production of aflatoxins.

Microbiological analysis of dried fruits

Total yeast and mold count

Several dried fruit samples exceeded the permitted number of total molds and yeasts as outlined in Mexican official norm NOM-247-SSA1-2008, which lists a maximum of 300 UFC/g of yeasts and molds in edible seeds and their derivatives. There was great variability in the yeast and mold counts of individual dried fruit samples. Figure 3 shows the distribution of yeast and mold counts in dried fruit samples. It is notable that no peanut or walnut samples were above the maximum allowable limit; however, one pecan sample from the center region and three squash seed samples (one from center, one from west, and one from northeast) were, with a squash seed sample having the highest registered yeast and mold count of all samples at 4200 CFU/g. In some sample groupings, significant correlation ($p<0.05$) between either water activity or moisture content and yeast and mold count was found. Table 2 shows the correlation coefficients and p values for the correlation between yeast and mold counts and both moisture content and water activity. In the case of peanuts, yeast and mold count was found to be significantly positively correlated to moisture content, whereas correlation was not significant to water activity. The opposite was true for pecan, where a significant positive correlation was found between yeast and mold count and water activity, but not moisture content. In the case of walnut, a significant negative correlation was found between water activity and yeast and mold count, while no significant

correlation between either parameter was found in the case of squash seed. In general, a positive correlation is expected between water activity and/or moisture content and yeast and mold counts; however, this might not always be the case since fungal development is limited at lower water activity and/or moisture content values. Lack of correlation between a_w/moisture content and yeast and mold counts would appear to indicate no endogenous mold growth was possible in dried fruit samples, and thus that the yeasts and molds detected and isolated come primarily from exogenous contamination.

Despite conditions and practices that could be perceived as "unsanitary" in Mexican plantations, produce markets, and distribution centers (García-Gómez *et al.* 2002, Cedillo Camarena 2013), seeds and nuts are generally in good microbiological condition, in respect to yeast and mold content, in accordance with national regulations. The low water activity and moisture content values found also indicate that the seeds were properly stored prior to sale, as even in regions with a tropical climate (center and southeast), seeds did not show a_w values that could be considered risky for mold development and all samples were below 7 % moisture content, which is considered optimal for long-term storage of dried fruits (Norlia *et al.* 2019).

Table 2. Correlation coefficients (r) and p values for yeast and mold counts versus water activity (aw) and moisture content (% H) for studied dried fruits

	a_w		%H	
Dried fruit	r	p	r	p
Peanut	0.128	0.478	0.620	0.000
Pecan	0.546	0.003	0.403	0.037
Walnut	-0.975	0.000	-0.527	0.145
Squash seed	-0.975	0.986	-0.070	0.713

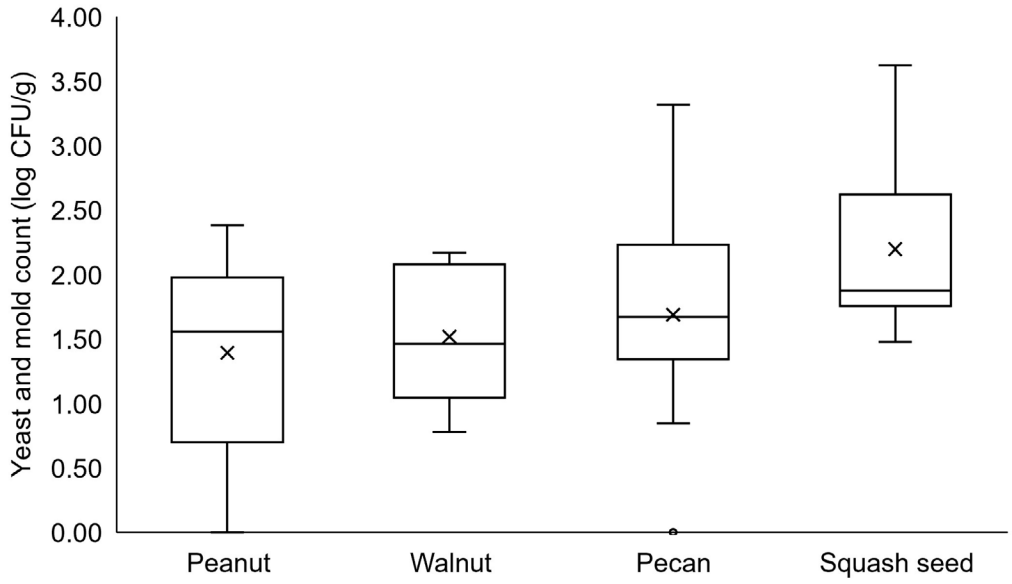

Figure 3. Box plot of the distribution of yeast and mold content in the four studied dried fruits.

Fungal isolation and identification

From the many different genera and species of mold isolated and identified from dried fruit samples (Table 3), several spoilage and toxigenic molds stand out. *Aspergillus* species (including *A. flavus*) were found throughout the different samples. *Penicillium* and *Rhizopus* species were also ubiquitous. These three genera are important since many of the species contained in them are either responsible for spoilage of seeds and grains or capable of producing toxic metabolites. The presence of *A. flavus, Fusarium* spp., and *Penicillium* spp. also stand out as known producers of mycotoxins. *Alternaria* spp. was also found on some samples, as were other molds associated with field contamination, such as *Phoma* spp. and *Cladosporium* spp. that otherwise are not known to deteriorate dried fruits or produce mycotoxins.

Table 3. Species of molds identified in peanut, pecan, walnut, and squash seed

Peanut	Pecan	Walnut	Squash seed
Alternaria alternata	*Alternaria* spp.	*Aspergillus niger*	*Aspergillus candidus*
Aspergillus candidus	*Aspergillus flavus*	*A. ustus*	*A. flavus*
A. flavus	*A. niger*	*Paecilomyces variotii*	*A. niger*
A. niger	*Cladosporium* spp.	*Penicillium rugulosum*	*A. niveus*
A. terreus	*Eurotium amstelodami*	*Rhizopus* spp.	*Eurotium* spp.
A. ustus	*Fusarium* spp.		*Fusarium* spp.
A. versicolor	*Mucor hiemalis*		*Penicillium expansum*
Cladosporium cladosporioides	*Penicillium expansum*		*Penicillium* spp.
Epicoccum spp.	*P. funiculosum*		*Phoma* spp.
Neosatorya fisheri	*P. nalgiovense*		*Rhizopus oryzae*
Penicillium citrinum	*Rhizopus* spp.		
P. echinulatum	Yeast		
P. glabrum			
P. nalgiovense			
Penicillium spp.			
Phoma spp.			
Ulocladium chartarum			
Yeast			

Incidence of different genera and species of mold

A high incidence of relevant toxigenic and spoilage mold was found across all four tested dried fruits. *A. flavus* incidence was highest in squash seed, where around 80 % of the seeds tested showed contamination with this mold (Figure 4). In contrast, only 30 % of peanuts tested showed contamination with *A. flavus*. Peanut samples, in turn, showed a high proportion of contamination with *A. niger*, a mold not usually associated with mycotoxin production, but capable of spoiling dried fruits. Pecan nuts showed a similar incidence of contamination with *A. flavus* and *A. niger*. These two species of mold are known to be antagonistic to each other (Aziz and Shahin 1997, Fendiyanto and Satrio 2020, Griffin *et al.* 2001) and are therefore seldom found together in a sample. In this case, only six out of the 30 samples tested contained both mold species at the same time, while only five contained neither. Walnuts showed a similar trend, with a high incidence of *A. niger*, but no incidence of *A. flavus*.

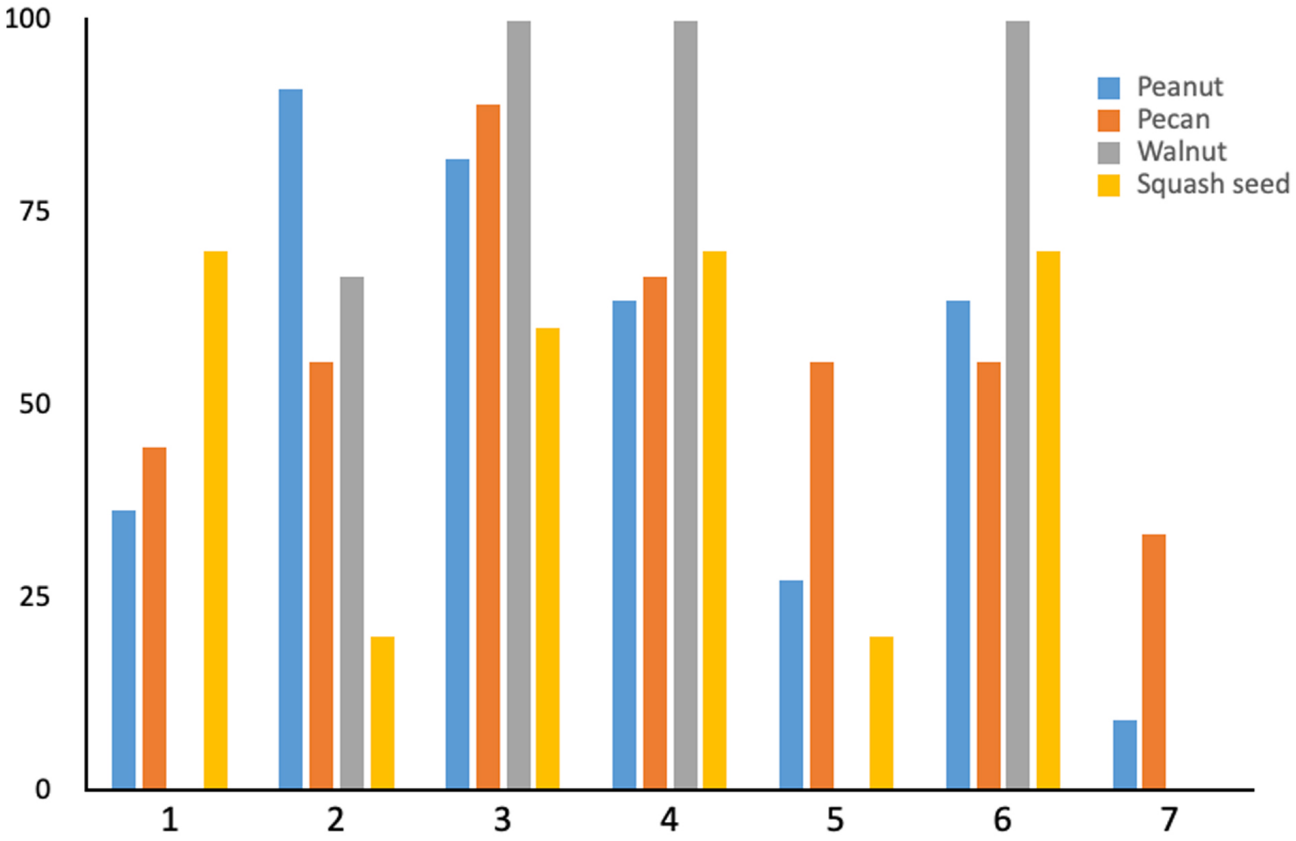

Figure 4. Incidence of contamination with some important pathogenic and deteriorative molds in select dried fruits. 1: *Aspergillus flavus*. 2: *Aspergillus niger*. 3: *Penicillium* spp. 4: *Rhizopus* spp. 5: *Fusarium* spp. 6: *Aspergillus* spp. 7: *Cladosporium* spp.

Incidence of species of *Penicillium* was above 50 % across all four kinds of dried fruits. *Penicillium* are a ubiquitous genus of molds, and so are expected to appear in high rates in dry foods. Some *Penicillium* species can produce mycotoxins such as ochratoxin, and therefore their presence is cause for concern (Pitt and Hocking 2009).

Incidence of *Rhizopus* species, mostly represented by *R. oryzae*, was also above 50 % in all four of the tested dried fruits. *R. oryzae* is a common contaminant of grains in tropical locations and capable of spoiling improperly stored dried foods. It is, however, not known to produce toxins (Pitt and Hocking 2009).

Mold species found in peanuts were similar to those reported by other authors: *Aspergillus flavus* has been reported in roasted peanuts from South Africa (Adetunji *et al.* 2020) and Bangladesh (Khandaker *et al.* 2019a, 2019b), and in raw peanuts from Benin

(Adjou et al. 2012), Pakistan (Abbas *et al.* 2019), and Turkey (Gürses 2006). These same authors have also variously reported presence of *A. niger, Fusarium* spp., *Penicillium* spp., *Alternaria alternata, Rhizopus* spp., *Cladosporium* spp., *Mucor* spp., and other mold genera and species. In general, mycobiota of peanuts appears varied, though the most prevalent molds are from the *Aspergillus genus*, with a high incidence of *A. flavus*, and *A. niger.*

The mold species found in pecans were like those reported by Valle-Garcia *et al.* (2019) in pecans from Rio Grande do Sul State in Brazil, although the frequency at which the different species were found seems to differ. Valle-García *et al.* (2019) reported complete absence of *A. flavus* in the analyzed samples, which was not the case in the samples analyzed in this study. They also reported a predominance of *Aspergillus* species, which was also the case in the samples analyzed on this study. However, in these samples, *Penicillium* species were found at a higher frequency than in Valle-García *et al.* (2019) report. Finally, they also reported an incidence of *Cladosporium* spp. of 30 %, which was like the rate at which the genus was isolated from pecan samples in this study. *Cladosporium* is a known pathogen of pecan nuts that has been reported previously in Mexican pecan (Walker *et al.* 2016).

On the other hand, mold species found in walnut samples are different than those reported in other studies, where *Aspergillus species,* such as *A. flavus, A. terreus, A. niger, A. fumigatus* and *A. versicolor* have been shown to predominate (Abbas *et al.* 2019), although *Penicillium* species have also been reported in walnut previously (Gürses 2006). Contamination with *A. flavus* has been demonstrated in Mexican walnuts before, although aflatoxin content was reportedly low (Adaya-González *et al.* 2015).

As with many aspects of *Cucurbita argyrosperma*, the mycobiota of the seeds remains poorly studied. Phytopathogenic molds such as *Phytophthora capsici*, *Rhizoctonia solani*, and *Sclerotium rolfsii* have been reported to cause fruit rot (Díaz-Nájera *et al.* 2015, 2018); however, no studies have been made over the mycological ecology of the seeds of this gourd. It can be assumed that mycobiota of *C. argyrosperma* seeds is similar to the mycobiota of seeds of other cucurbits, such as that of *C. pepo.* In that regard, Rahim *et al.* (2013) have reported the presence of at least 100 different species of mold in the seeds of *C. pepo*, among which are those found in the present study. As was the case with the other dried fruits analyzed, the genus *Aspergillus* appeared with a high incidence, and the degree of contamination with *Aspergillus flavus* is of particular concern, being present in over 70 % of the samples tested, and on a higher degree than on peanuts and pecans.

Despite high incidence of contamination with molds, most of the samples tested were within the allowable limits for yeast and mold counts. This would seem to indicate that Mexican dried fruits are safe for consumers despite contamination with molds. However, care must be taken when transporting and storing dried fruits, since mold spores can develop and increase in number in the right conditions. Data is pertaining mostly to dried fruits from the center region of Mexico, which encompasses the most highly urbanized states in the country and contains the Mexico City megalopolis. It is known that rural areas are more vulnerable to contamination with toxigenic molds than urban areas, and the results of this study reflect this (Zuki-Orozco *et al.* 2018).

Regional variation in dried fruit mycobiota

Variation was observed in mycobiota of dried fruits across different regions, as is shown in Figure 5. It is notable that *A. flavus, A. niger,* and *Fusarium* spp. were much more prevalent in samples from the Northeast and Southeast regions, whereas other species of mold showed a more evenly distributed incidence across all regions. Regional variations in mycobiota have been reported in other countries and have been shown to be related to regional climate; in particular, to relative humidity and temperature (Fatahinia *et al.* 2018, Jayashree and Wesely 2019, Temperini *et al.* 2019, Aveling *et al.* 2020). Locations and seasons with high relative humidity show increased fungal load and diversity when compared to drier environments (Fatahihia *et al.* 2018). This influence can also be observed in the present study: the incidence of mold species in the Northwest region, the most arid of the five regions under study, consistently shows lower incidence of all mold species, except for species of *Aspergillus* other than *A. flavus* and *A. niger.*

Principal component analysis

Figure 6 shows the scatter plot of the main three principal components: F_1, F_2 and F_3. The obtained cophenetic correlation coefficient was 0.98, indicating that the three main principal components represent an accurate reduction in the number of variables for this analysis. Together, F_1, F_2 and F_3 account for 84.4 % of variability, with each individual component accounting for 38.6, 28.6, and 17.2 % of variability, respectively.

From the results of the PCA, it is clear that seed type is a stronger predictor for characteristics than region. This is to be expected, as there is bound to be more similarity between the same dried fruits across different regions, as opposed to different dried fruits from the same region. Squash seeds and pecan nuts show distinct clustering in separate areas of the [F_1, F_2, F_3] space, along the F_2 axis and the [F_1, F_2] plane. This is due to the correlation of F_1 and F_2 with yeast and mold content (Table 4), where squash seeds have the highest and most widely distributed values of all tested dried fruits.

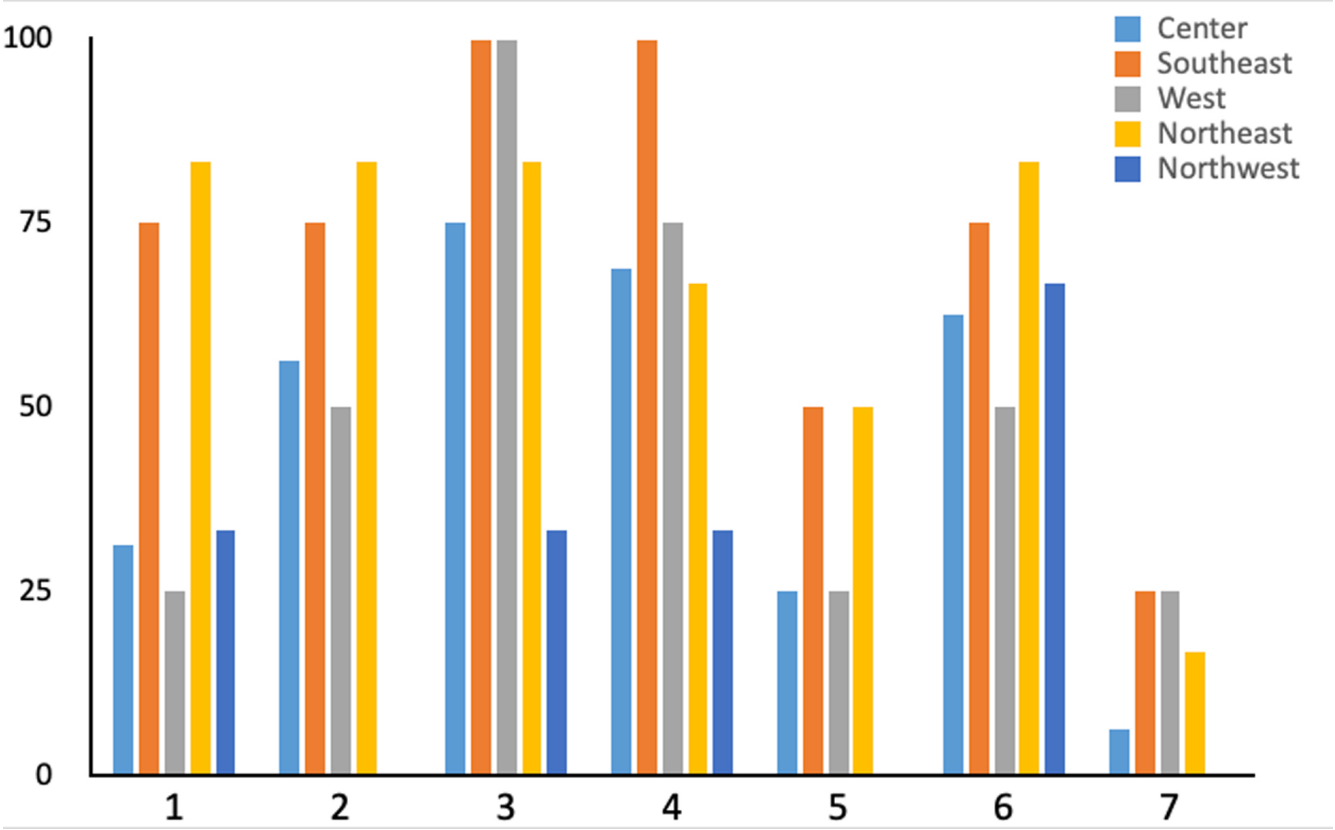

Figure 5. Incidence of contamination with some important pathogenic and deteriorative molds in studied dried fruits from selected regions of Mexico. 1: *Aspergillus flavus*. 2: *Aspergillus niger*. 3: *Penicillium* spp. 4: *Rhizopus* spp. 5: *Fusarium* spp. 6: *Aspergillus* spp. 7: *Cladosporium* spp.

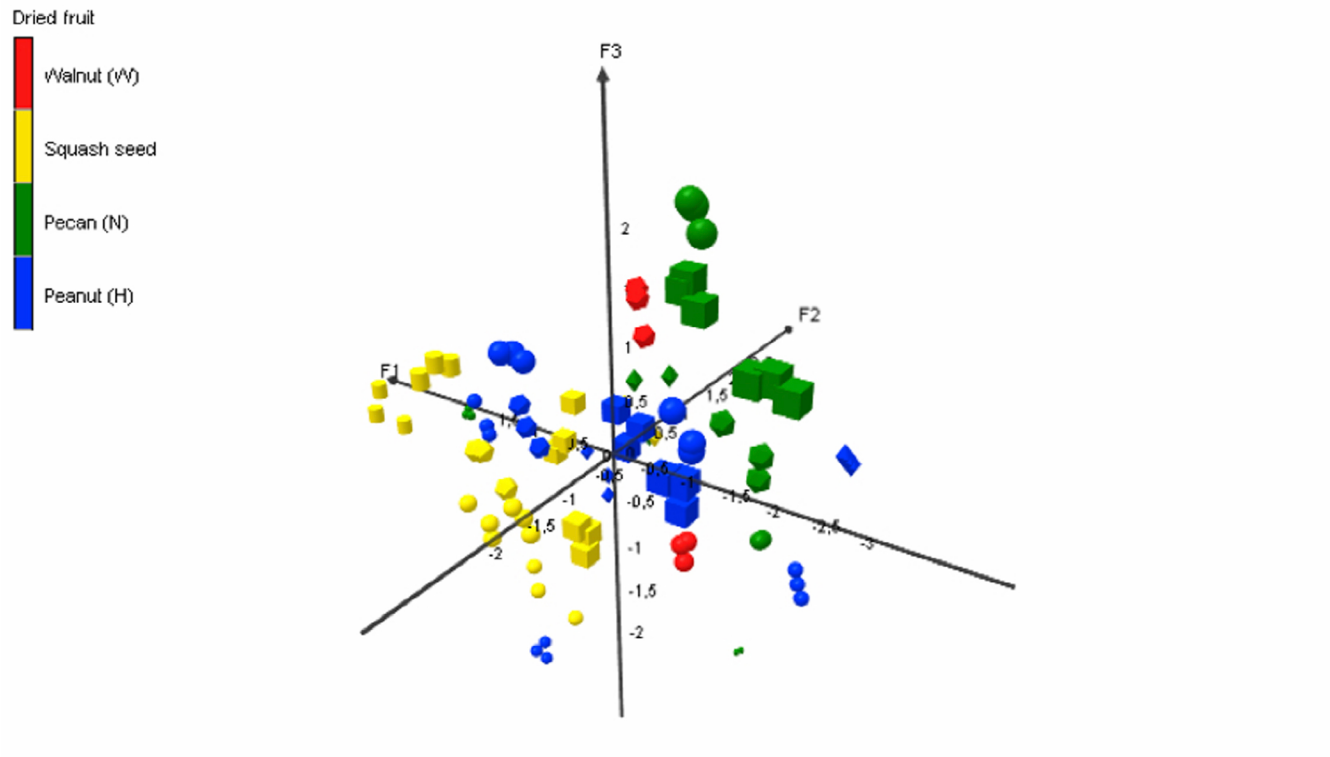

Figure 6. Scatter plot for observations on principal component axes F_1, F_2, and F_3. Shapes indicate region as follows: center (sphere), northeast (cube), northwest (cylinder), southeast (octahedron), and west (icosahedron).

Table 4. Correlations between variables and principal components

	F_1*	F_2*	F_3*
Water activity (a_w)	0.703	0.375	-0.436
Moisture content (% H)	0.693	-0.427	-0.269
Yeast and mold content (CFU/g)	0.750	-0.073	0.643
Number of identified species	0.096	0.902	0.106

* Sign indicates direct (+) or indirect (-) correlation between the variables and the principal components.

CONCLUSIONS

High incidences of contamination with spoilage and toxigenic molds were found in Mexican dried fruits. Despite this, only one pecan sample showed water activity above the threshold for mold growth and only one other pecan sample showed yeast and mold counts over the allowed limit of 300 UFC/g. Squash seeds, on the other hand, showed multiple samples that exceeded the allowable limit for yeast and mold count, while also having a high incidence of contamination with *Aspergillus flavus*. This indicates that dried fruits sold in open-air markets throughout the country are generally safe to consume but must be properly stored to avoid increasing mold counts to unacceptable levels. This study's data pertained mostly to samples collected on urbanized areas which are known to be less prone to contamination with molds. This situation was reflected in the results of all but the squash seeds, which showed worrying levels of contamination. Although it is important to note that this was an initial exploratory study; thus, it would be necessary to expand the number of samples for further investigations. In relation to the mycotoxigenic fungi found, we were not able to carry out analyses of mycotoxins associated with them; therefore, we recommend performing these explorations in future studies.

ACKNOWLEDGEMENTS

David González Albarrán appreciates the financial support provided by the National Council of Humanities, Sciences and Technologies (CONAHCYT) and the University of the Americas Puebla (UDLAP) for his doctoral studies.

REFERENCES

Abbas M, Naz SA, Shafique M, Jabeen N, Abbas S. 2019. Fungal contamination in fried fruits and nuts: a possible source of mycosis and mycotoxicosis. Pakistan Journal of Botany 51, 1523-1529. https://doi.org/10.30848/PJB2019-4(31)

Adetunji MC, Ezeokoli OT, Ngoma L, Mwanza M. 2020. Phylogenetic diversity and prevalence of mycoflora in ready-to-eat supermarket and roadside-vended peanuts. Mycologia 113, 1-11. https://doi.org/10.1080/00275514.2020.1804235

Adjou ES, Dahouenon-Ahoussi E, Soumanou MM. 2012. Investigations on the mycoflora and processing effects on the nutritional quality of peanut (*Arachis hypogea* L. var. TS 32-1). Journal of Microbiology Biotechnology and Food Science 2(3), 1025-1039.

Adaya-González J, Carvajal-Moreno M, Rojo-Callejas F, Ruiz-Velazco S. 2015. Aflatoxins in walnut (*Juglans regia* L.), pecan (*Carya illinoinensis* (Wangenh.) K. Koch) and cashew (*Anacardium occidentale* L.) nuts of Mexico. Pharmaceutica Analytica Acta 6, 338. https://doi.org/10.4172/2153-2435.1000338

Ardabili AG, Farhoosh R, Khodaparast MHH. 2011. Chemical composition and physicochemical properties of pumpkin seeds (*Cucurbita pepo* Subsp. *pepo* var. styriaka) grown in Iran. Journal of Agricultural Science and Technology 13, 1053-1063.

Aveling TAS, de Ridder K, Olivier NA, Berger DK. 2020. Seasonal variation in mycoflora associated with asymptomatic maize grain from smallholder farms in two provinces of South Africa. Journal of Agriculture and Rural Development in the Tropics and Subtropics 121, 265-275. https://doi.org/10.17170/kobra-202011262275

Aziz NH, Shahin AAM. 1997. Influence of other fungi on aflatoxin production by *Aspergillus flavus* in maize kernels. Journal of Food Safety 17, 113-123. https://doi.org/10.1111/j.1745-4565.1997.tb00180.x

Boaghi E, Reşitca V, Ciumac J. 2019. Water activity influence on walnuts (*Juglans regia* L.) microbiological and oxidative stability. International Journal of Food Science and Nutrition Dietary 8(2), 401-404. https://doi.org/10.19070/2326-3350-1900071

Cedillo Camarena MC. 2013. Iniciativa orientada a locatarios para mejorar la seguridad sanitaria del mercado municipal de Villa Guerrero, Estado de México. Tesis de maestría. Instituto Nacional de Salud Pública, Cuernavaca.

Chayjan RA, Salari K, Abedi Q, Sabziparvar AA. 2013. Modeling moisture diffusivity, activation energy and specific energy consumption of squash seeds in a semi fluidized and fluidized bed drying. Journal of Food Science and Technology 50, 667-677. https://doi.org/10.1007/s13197-011-0399-8

Díaz-Nájera JF, Alvarado-Gómez OG, Leyva-Mir SG, Ayvar-Serna S, Michel-Aceves AC, Vargas-Hernández M. 2015. Identification and control of fungi causing fruits rot in pipiana pumpkin (*Cucurbita argyrosperma* Huber). African Journal of Agricultural Research 10, 1150-1157. https://doi.org/10.5897/AJAR2015.9486

Díaz-Nájera JF, Sahagún-Castellanos J, Vargas-Hernández M, Ayvar-Serna S, Alvarado-Gómez OG, Villanueva-Verduzco C, Acosta-Ramos M. 2018. Diagnosis and integrated management of fruit rot in *Cucurbita argyrosperma*, caused by *Sclerotium rolfsii*. Plant Pathology 34, 171-181. https://doi.org/10.5423/PPJ.OA.08.2017.0185

Dorner JW. 2008. Relationship between kernel moisture content and water activity in different maturity stages of peanut. Peanut Science 35, 77-80. https://doi.org/10.3146/PS07-101.1

Fatahinia M, Zarei-Mahmoudabadi A, Shokri H, Ghaymi H. 2018. Monitoring of mycoflora in outdoor air of different localities of Ahvaz, Iran. Journal of Mycologie Medique 28, 87-93. https://doi.org/10.1016/j.mycmed.2017.12.002

Fendiyanto MH, Satrio RD. 2020. Identification, aflatoxin content, and antagonistic test of spoilage fungi in bread to *Aspergillus niger*. Bioteknologi 17(2), 60-66.

García-Gómez R, Chávez-Espinoza J, Mejía-Chávez A, Durán-de Bazúa C. 2002. Microbiological determinations of some vege-

tables from the Xochimilco zone in Mexico City, Mexico. Revista Latinoamericana de Microbiología 44, 24-30.

Ghorbani M, Hosseini H. 2017. Determination of water activity corresponding to the proper quality of peanut kernels. Journal of Food Processing and Preservation 41, 1-8. https://doi.org/10.1111/jfpp.13260

Griffin GJ, Smith EP, Robinson TJ. 2001. Population patterns of *Aspergillus flavus* group and *A. niger* group in field soils. Soil Biology and Biochemistry 33, 253-257. https://doi.org/10.1016/S0038-0717(00)00133-4

Gürses M. 2006. Mycoflora and aflatoxin content of hazelnuts, walnuts, peanuts, almonds and roasted chickpeas (LEBLEBI) sold in Turkey. International Journal of Food Properties 9, 395-399. https://doi.org/10.1080/10942910600596597

INEGI. 2020. Datos. Instituto Nacional de Estadística, Geografía e Informática. October 29. https://www.inegi.org.mx/datos/

Jayashree M, Wesely EG. 2019. The impact of weather on the fungal abundance and richness in sorghum grains in selected districts of Tamil Nadu, India. Infokara Research 8, 1985-1989.

Khandaker M, Ahmed R, Rahim M, Hassan T, Begum M. 2019a. Determination of mycoflora and mycotoxins in raw and roasted peanuts in Bangladesh. International Journal of Plant and Environment 5, 259-264. https://doi.org/10.18811/ijpen.v5i04.5

Khandaker MM, Rahman T, Rahim MM, Hassan MT, Begum M, Bhuiyan MAB. 2019b. Mycoflora and mycotoxin detection in roasted peanut kernels of Bangladesh. Bangladesh Phytopathological Society 35, 53-58.

Liu K, Liu Y, Chen F. 2018. Effect of gamma irradiation on the physicochemical properties and nutrient contents of peanut. LWT 96, 535-542. https://doi.org/10.1016/j.lwt.2018.06.009

Liu X, Guan X, Xing F, Lv C, Dai X, Liu Y. 2017. Effect of water activity and temperature on the growth of *Aspergillus flavus*, the expression of aflatoxin biosynthetic genes and aflatoxin production in shelled peanuts. Food Control 82, 325-332. https://doi.org/10.1016/j.foodcont.2017.07.012

Norlia M, Jinap S, Nor-Khaizura MAR, Radu S, Samsudin NIP, Azri FA. 2019. *Aspergillus* section flavi and aflatoxins: Occurrence, detection, and identification in raw peanuts and peanut-based products along the supply chain. Frontiers in Microbiology 10, 1-17. https://doi.org/10.3389/fmicb.2019.02602

Peng D, Liu Y, Yang J, Bi Y, Chen J. 2021. Nondestructive detection of moisture content in walnut kernel by near-infrared diffuse reflectance spectroscopy. Journal of Spectroscopy, article ID 9986940. https://doi.org/10.1155/2021/9986940

Pitt JI, Hocking AD. 2009. Fungi and food spoilage. Springer, New York.

Rahim S, Dawar S, Zaki J. 2013. Mycoflora associated with the seed samples of *Cucurbita pepo* collected from Pakistan. Pakistan Journal of Botany 45, 2173-2179.

Reis Ribeiro S, Klein B, Machado Ribeiro Q, Duarte dos Santos I, Gomes Genro AL, de Freitas Ferreira D, Janner Haman J, Smanioto Barin J, Chichoski AJ, Fronza D, Both V, Wagner R. 2020. Chemical composition and oxidative stability of eleven pecan cultivars produced in Brazil. Food Research International 136, 1-12. https://doi.org/10.1016/j.foodres.2020.109596

Samson RA, Hoekstra ES, Frisvad JC, Filtenberg O. 1995. Introduction to food-borne fungi, 4th ed. Centraalbureau voor Schimmelcultures, Wagening.

Sánchez-de la Vega G, Castellanos-Morales G, Gámez N, Hernández-Rosales HS, Vázquez-Lobo A, Aguirre-Planter E, Jaramillo-Correa P, Montes-Hernández S, Lira-Saade R, Eguiarte LE. 2018. Genetic resources in the "calabaza pipiana" squash (*Cucurbita agryrosperma*) in Mexico: Genetic diversity, genetic differentiation and distribution models. Frontiers in Plant Science 9, 400. https://doi.org/10.3389/fpls.2018.00400.

Schmidt SJ, Fontana AJ. 2019. Water activity values of select food ingredients and products. In: Barbosa-Cánovas GV, Fontana AJ, Schmidt SJ, Labuza TP (eds.) Water activity in foods: Fundamentals and applications, 2nd ed. Blackwell Publishing, Oxford. 407-420.

Temperini CV, Franchi ML, Benavides Rozo ME, Greco M, Prado AG, Pose GN. 2019. Diversity and abundance of airborne fungal spores in a rural cold dry desert environment in Argentinean Patagonia. Science of the Total Environment 665, 513-520. https://doi.org/10.1016/j.scitotenv.2019.02.115

Valle-Garcia M, Machado Moraes V, Olivier Bernardi A, Schneider Oliveira M, Mallmann CA, Boscardin J, Venturini Copetti M. 2019. Mycological quality of pecan nuts from Brazil: absence of aflatoxigenic fungi and aflatoxins. Ciência Rural 49, 1-8. https://doi.org/10.1590/0103-8478cr20190076

Walker C, Muniz MFB, Rolim JM, Martins RRO, Rosenthal VC, Maciel CG, Mezzomo R, Reiniger LRS. 2016. Morphological and molecular characterization of *Cladosporium cladosporoides* species complex causing pecan tree leaf spot. Genetics and Molecular Research 15, 1-11. https://doi.org/10.4238/gmr.15038714

Zuki-Orozco BA, Batres-Esquivel LE, Ortiz-Pérez MD, Juárez-Flores BI, Díaz-Barriga F. 2018. Aflatoxins contamination in maize products from rural communities in San Luis Potosi, Mexico. Annals of Global Health 84, 300-305. https://doi.org/10.29024/aogh.918