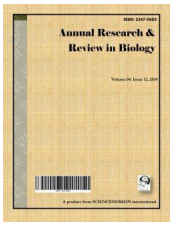

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Mycotoxin and Fungal Contamination of Fresh and Dried Tomato

Eman M. Hegazy[1*]

[1]Department of Food Toxicology and Contaminants, National Research Center, Dokki, Giza, Egypt.

Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

Background: Tomato is an important crop cultivated in Egypt. Huge amount of fresh tomatoes were lost and negatively affected human health because of fungal diseases. Drying process represent easy and applicable techniques to minimize tomatoes infection notably mycotoxigenic fungi. The economical problem of tomato comes from its price had 10-fold increase in the last decade. **Methodology:** The commercial ripe tomato samples (30) obtained from local super-market at Giza during October 2015 were used to evaluate the effect of drying with those from Egyptian agriculture centre, Giza, which considered as control. *A. flavus* and *Alt. alternata* have been isolated from sample, *Alt. alternat*a observed inside tomato using scanning electron microscope. Aflatoxins, ochratoxin A, and tenuazonic acid were determined. The infected and spoiled samples were discarded and the uniform with healthy were selected to study and were divided into 4 groups, three of them were cut into slices; the fourth group was converted to juice. The product of all groups were divided into 2 sets the first set was dried by solar energy, and the second treated by freeze-dryer. All sets sample packaged in plastic bags, stored at 25°C for one year, and inspected for fungal growth. **Results and Discussion:** The freeze-dried samples color appeared nearest to fresh tomato by highly coast. No fungal growth appeared through storage. The isolated fungi weren't able to produce either *aflatoxin or tenuazonic* acid. None of ochratoxin A or aflatoxins was detected in analyzed

**Corresponding author: E-mail: eman_hegazy@hotmail.com, emanhegazy2020@gmail.com;*

samples. Tenuazonic acid detected at low levels of concentration (0.680 ug/kg) in inside rotted ripped samples.
Conclusion: All tomato samples were free from either aflatoxins or ochratoxin *A. alternaria* fungi recorded as inside rotted. However the effectiveness and promising results of freeze-dried samples which near to fresh-like samples it's expensive cost considered as major problem.

Keywords: Tomato; Alternaria alternate; mycotoxins; solar drying; freeze drying; SEM.

1. INTRODUCTION

Tomatoes are exceptionally helpless to resist the parasitic infection in the field, amid transportation, preparing, and storage [1]. Tomato is a sensitive crop with delicate skin, exceedingly powerless to organisms' defilement through harmed skin or harmed tissues in the field, during transportation, processing, and through storage. In the United State of America, the dominant isolated fungi from tomatoes are *Alternaria*, *Aspergillus*, *Botrytis*, Cladosporium, *Fusarium*, *Mucar*, *Penicillium*, and *Rhizopus*.

Chain et al. [2], recorded that; The European Food Safety Authority proposed in 2021 that *Alternaria* toxin are the high worry for general wellbeing. Roughly thirty metabolites with lethality types of *Alternaria* species can produce mycotoxins of various substance classes, though the polyketidemycotoxins (Alternariol, altenuene, tenuazonic corrosive, tentoxin and altertoxins) [3]. A stem canker disease of fresh tomatoes (*Lycopersicon esculentum*), has been occurred in the field-resistant cultivars in greenhouse inoculation and caused by pathotype of *Alt. alternata*. Dark-brown-to-black cankers with concentric zonation occur on stems near the soil or in on tomato.

The great problem was related to the losses of tomatoes fruits either for its infection by diseases or through its contamination by fungi. The lost yield may affected to increase the gap between the production and the marketing, which related to food safety and food security. The presence of fungi on tomato crops not only considered as a type of outside infection decreases the amount of the production, but also it is a great source for mycotoxin contamination [4].

Mycotoxins are extremely harmful auxiliary metabolites delivered by a wide assortment of fungi. It is evaluated that it might influence as much as 25% of the world's nourishment edits every year. The most widely recognized mycotoxins found in sustenance's and nourishes are aflatoxins, ochratoxin, fumonisins, zearalenone and Ochratoxin A (OA) [5]. OA is a powerful mycotoxin with nephrotoxic, teratogenicity and cancer-causing properties in light of adequate proof of cancer-causing nature in exploratory creatures, it is viewed as a conceivable human cancer-causing agent 2B [6].

Aflatoxins (B_1, B_2, G_1, and G_2) are occurring worldwide in a large variety foods and feeds. Also, they are thermo stable compound and can cause carcinogenic, teratogenic and mutagenic damages for both human and animals [7,8]. The first aim was to study the occurrence of toxigenic fungal species and mycotoxins in fresh tomatoes. The second one was to prepare dried tomatoes using solar energy or freeze-dried, thus to compare between the two application effect either on quality or on safety of the product, also the product of dried tomato was stored for one year to evaluate the impact of the two drying application on dried tomato.

2. MATERIALS AND METHODS

2.1 Sampling

A total of 30 samples of fresh ripe tomato (1kg for each) were purchased from supermarkets at Giza, Egypt in October 2015, and from Agriculture research center (as a standard samples used in drying treatment).

2.2 Isolation and Identification of Fungi

The isolation of fungi from studied samples were performed according to the method described by Wilson et al. [9]. The dilution plate with PDA (Potato Dextrose Agar medium). The identification of fungi based on morphologically macro and microscopic characteristics was determined as described by Summerell et al. [10].

2.3 Determination of Mycotoxins Production by Isolated Fungal

Determination of aflatoxins production by *A. flavus*, as well as ochratoxin A by *A. ochracus*

Photo 1. Ripe rotted tomato (outside inside)

in liquid media of yeast extract sucrose, the method was done using HPLC techniques as illustrated by Josep Rubert et al. [11], the cleanup and preparation described by Mariana et al. [12]. Production tenuazonic acid by *Alt. alteranta* was done by cultured on rice media [13].

2.4 Drying Fresh Tomato

Fresh tomato obtained from Agriculture Research Center, Giza, Egypt. The dried samples were chosen as free from any contamination of fungi. The fresh tomato were washed in tap water and divided into four groups, three groups were cut to slices, half slices and square, the fourth group was made juice. The four groups above were divided into two groups the first one were dried by solar energy, the temperature inside solar dryer was 70°C for 4 days. The second one were dried by freeze dryer (LABCONCO- Free zone 12 Liter Console Freeze Dry System with Coppering Tray Dryer, catalog Number 7759030-plug type: Schuko, Style: Console and Pressure a/MP. The temperature inside it was 50°C for 48 hours).

2.5 Applications for Tomato Dried

The tomato samples for inspection was differ from the samples for drying. All dried tomato (solar dried as well as freeze dried) were dehydrated through soaked in distilled water for 5 to 10 minutes depending on the shape of the tomato as the usual application with tomato was made. Samples were dried well using sterile soft tissues after soaking, then it was ready for the drying treatment (solar dried or freeze dried).

2.6 Storage of Dried Tomato

All dried tomato were packaged in plastic bags and were stored at 25°C, observation for fungal growth all over one year was carried out at zero time, 6 and 12 months.

2.7 Determination of Moisture Content

Moisture content was determined by the oven at 105°C for 24 hr for both fresh ripe tomato (none and inside rotted) as well as dried tomato by (solar and freeze dried) according to AOAC [14].

2.8 Scanning Electron Microscope (SEM)

Ripped inside rotted tomato fruit were fixed in 2.5% glutaraldehyde at 4°C according to Harley and Fergusen [15]. Examined on SEM, National Research Center, Quanta FEG250 (Photo 1 above).

2.9 Statistical Analysis

All experiments were carried out in triplicate and the results are expressed as mean ± SD. Duncan's multiple range tests were used to compare the difference among mean values of beverage's properties at the level of 0.05 and SAS software (version 9.1; statistical analysis system institute Inc., Cary, NC, USA) was used for analysis [16].

3. RESULTS AND DISCUSSION

3.1 Isolation and Identification of Fungal

Data presented in Tables (1 and 2) revealed the isolation and identification of fungal. Data presented revealed that; the total fungal count in fresh ripe none rotted tomato was higher than the rotted tomato. Also, *A. flavus* and *A. niger* were found in both kind of tomato, but *Alternaria alternata* was only found in rotted tomato which recorded colony forming unit at 1.00 CFU×10^{-1}/g (Table 2). This result agreed with Lee et al. (2015), who reported that, black molds of the genus *Alternaria* contamination in different food and feed (Cereals, tomato, figs, wine, sunflower seed, olives, citrus fruit, pecans, and apples). *Aspergillus niger* was the common fungi of none rotted tomato and was found in both Fresh ripe

tomato. Abdel-Mallek et al. [17] studied the fungi which associated to tomato fruits in Egypt, he found that; *Aspergillus niger* was observed to be the most predominant contagious among 39 fungi species detected on the assortment having 116 sound tomato organic products gathered from market sectors in Asyut, Egypt, in 1994.

Table 1. Isolated fungi from fresh ripe tomato none rotted at zero time

Isolated fungal	Fungal count CFU×10/g			
	10^{-1}	10^{-2}	10^{-3}	10^{-4}
Penicillium spp	1.00	ND	ND	ND
Aspergillus flavus	1.00	ND	2.48	ND
Aspergillus niger	ND	ND	2.48	ND

ND: Not detected

Table 2. Isolated fungi from fresh ripe tomato rotted at zero time

Isolated fungal	Fungal count CFU×10/g			
	10^{-1}	10^{-2}	10^{-3}	10^{-4}
Alternaria alternata	1.00	ND	ND	ND
Mucor	ND	1.00	ND	ND
Aspergillus niger	ND	ND	ND	2.60

ND: Not detected

Logrieco et al. [18] study the epidemiology of toxigenic fungi and its mycotoxin on some Mediterranean crops. The main *Alternaria* mycotoxins had belonged to 3 structural orders: the derivatives of tetramic acid, tenuazonic acid; the di-benzopyrone derivatives (alternariol, alternariol mono-methyl ether, and altenuene); and the perylene derivatives (altertoxins). In the same study he recorded that, Toxigenic strains of *A. alternata* and linked tenuazonic acid and alternariols were generally set up as a tomato black fungi, as it was found on olive, citrus, cereals, and black fungi for several vegetables.

3.2 Mycotoxins Produced by Isolated Fungi

The nearness of a toxigenic fungi in a food stuff is not by any means the only variable in charge of the poison creation since contagious development and aflatoxin generation are the outcome of connections among the growth, the host, and nature. Different variables like the degree of the contamination and conditions for poison generation, for example, temperature, water content, medium structure and the non-appearance of opposing microorganisms are likewise imperative perspectives that prompt perceptible measures of poison despite the fact that the exact elements that starts poison arrangement has not been surely know yet [19].

All strains of *Alt. alternata* isolated from rotted tomato relieved that, no ability to produce any amount of tenuazonic acid at rice culture these results agreement with Morris et al. [20] who showed that from 62 strains of *A. alternata* isolated from Californian tomatoes fruits they found most-isolated strains were none-pathogenic. Also, Graf [21] found 63 species of *A. alternata* isolated from tomato, none of them was pathogenic. On the other hand, Ackermann et al. [22] found that; despite the low pathogenic activity of *Alternaria*, tomatoes and tomato products are very frequently contaminated with Alternaria toxins such as tenuazonic acid (TeA), Alternariol (AOH) or alternariol mono methyl ether (AHE), sometimes even in very high levels.

3.3 Mycotoxins Detection in Samples

No Ochratoxin A and aflatoxins (B_1, B_2, G_1, and G_2) were detected in any of 30 samples analyzed samples of both fresh ripe tomato (none rotted and inside rotted). These results were in agreement with Gelosa [23], who found no aflatoxins were distinguished in any of the 64 investigations, tests of the tomato items, considering the recognition furthest reaches of the connected strategy. Otherwise in a Survey for market infection and aflatoxin contents in Nigerian tomato samples [24], the Rotten tomato fruits were heavily contaminated with fungi, Eight molds were associated to rotten tomato fruits. *A. flavus* as well *A. niger* were the dominant fungi. Aflatoxins were detected in tomato diseases sampled and marketed rotten tomato as their tested on thin layer chromatography. The results of Mariutti and Soares [1] illustrated that, on a Survey of aflatoxins in more than sixty samples of tomato products, *A. flavus* and *A. parasiticus* strains which able to producing aflatoxins were presented, while all samples under the study were free from aflatoxins according to their detection method.

On the other hand, amid in the vicinity of the period 1979 and 1982, the Public Health Laboratory in Milan, Italy, dissected 40 specimens of tomato glue and 40 examples of canned tomatoes and found none aflatoxins (discovery restrict: 5– 10 µg/kg). Besides, these

same specimens were observed to be free from patulin and ochratoxin. In another review likewise completed in Italy, no aflatoxins were identified in 70 tomato items industrially accessible: 40 of juice, 20 of glues, and 10 of glues (recognition limit: 1 µg/kg) [25,26]. Then again, on an overview of aflatoxin defilement in the vicinity of 2001 and 2002 in Nigeria, spoiled tomatoes from 5 neighborhood markets were certain (discovery restraint not announced) for aflatoxin sullying even after autoclave treatment at 121°C for 15 min. Aflatoxin, an *Aspergillus* poison, was distinguished in spoiled tomatoes popularized in the Nigeria nation [24]. Approximately 30 metabolites with possible toxicity are known from various species of *Alt.* such as *Alternaria* (AOH), Alternariol ether (AME), altenuene (ALT), tenuazonic acid (TeA), and Tentoxin (KN) [3].

Our results had detected Tenuazonic acid at low levels at concentration 0.680 µg/kg in ripe inside rotted tomato only from Faisal city. In these accept, an alt. toxin (Tenuazonic acid) was found in Canada and U.S.A. in National Tomato Products at low levels [27,28]. Tomatoes fruit can be contaminated by *Alt. alternata*, which is the main reason to produce the Alternaria toxins such as altermariol, alternarial mono methyl ether ortenuazonic acid.

3.4 Scanning Electron Microscopy (SEM)

The structural characteristics were determined by scanning electron microscope (SEM), the differences in spore morphology of *Alternaria alternata* from ripe and inside rotted tomato (Photos 2: C, E, F, I and K) and *A. alternata* with the tissue of tomato were appeared in (Photos 2: A, B, D, and H). The SEM observation cleared that ripe and rotten inside tomato had the phyto-pathogenic fungus *Alternaria alternata* (Photo 2 {A to K}), which found naturally infected on several tomatoes. These results were agreement with Lee et al. [3] who reported that tomatoes are very susceptible to fungal growth due to soft tissue, and Alt. is a dominant fungus cause its spoilage. Dominant tomato diseases mainly caused by fungi [29].

3.5 Storage of Dried Tomato

All dried tomato (solar dried and freeze dried) were packaged in plastic page and storage at room temperature (25°C) observed daily for any fungal growth for one year (Photo 3).

So, Janaína-Muniz [29] revealed that, lessening definitely the nature of the organic product, impacting its chemical and physical qualities, other than appeasing pollution by growths and microorganisms. Also, several origins and are caused by fungi, bacteria, viruses, nematodes, and phyto-plasmas, but diseases caused by fungi are more abundant. Janaína Muniz reported that, reducing drastically the quality of the fruit, influencing its chemical and physical characteristics, besides propitiating contamination by fungi and bacteria.

Although all storage tomato hadn't any treatment before tomato hadn't any contamination with fungal through six and twelve months. Freeze dried tomato showed good appearance like the fresh tomato for the first six months (Photo 3) but this method was a very expensive one to dried tomato. Solar dried tomato had good color but through the end of storage the color changed to the red bleak.

3.6 Determination of Moisture Content

The moisture content of the dried tomato was presented in Table (3). Results appeared that; the moisture content of dried tomato with solar energy has the highest value of moisture content than the dried tomato with freeze dryer except for the powder tomato the value of moisture content was nearest which recorded 20,22 and 19.7% of

Table 3. The moisture content of fresh and dried tomato

Drying methods	Moisture content (%)			
	Dried tomato			
	Slices	Half-Slices	Square	Powder
Solar energy	18.00±0.33	17.82±0.51	18.33±0.22	20.22±0.23
Freeze dryer	4.02±0.28	5.95±0.59	6.12±0.45	19.70±0.57
	Fresh ripe tomato			
None rotated	80.05±0.18 to 82.36±0.51			
Rotated	90.00 ±0.25 to 91.56±0.61			

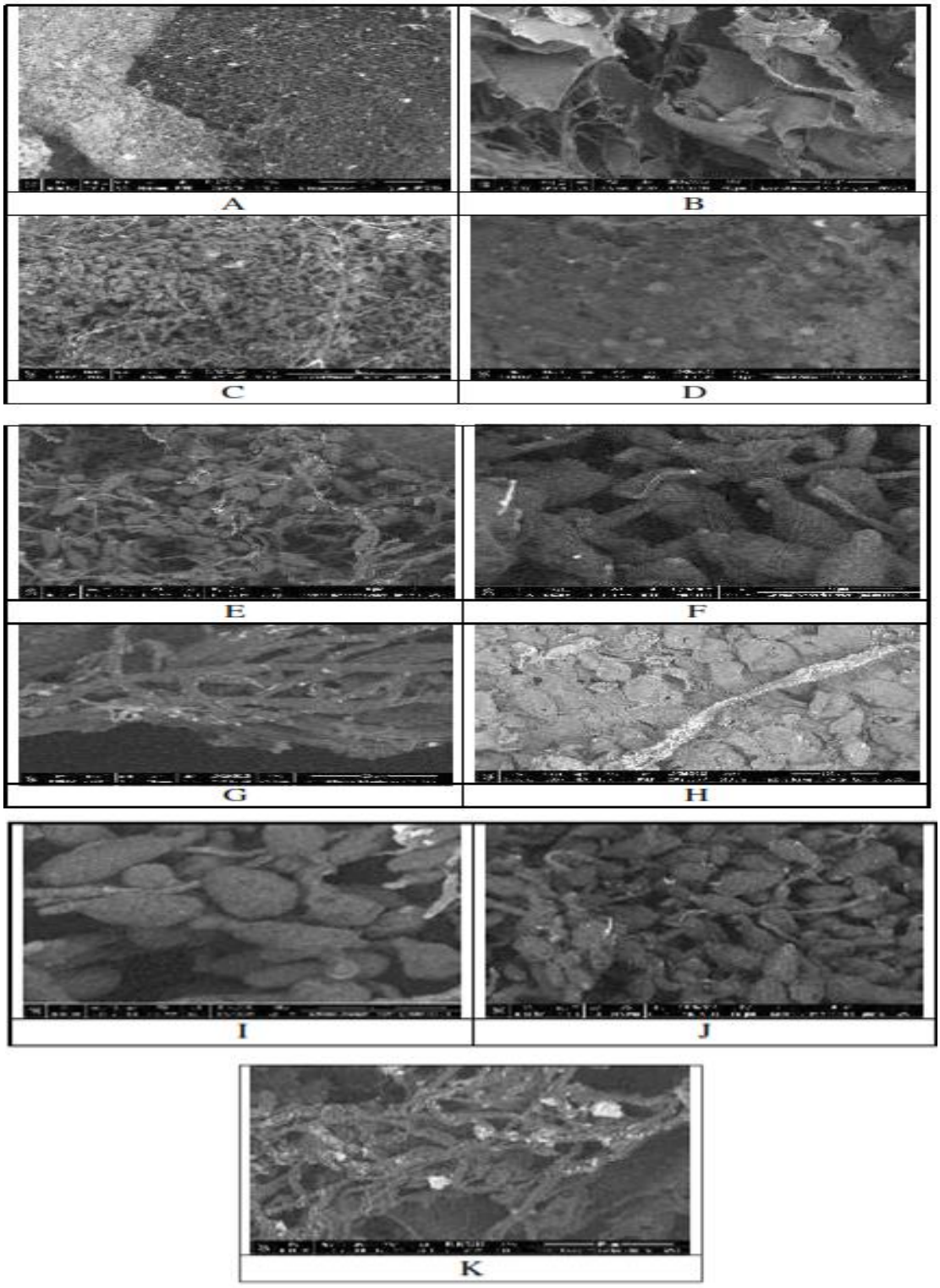

Photo 2. Scanning electron microscopy for ripe inside rotten tomato (A to K)

powder dried tomato with solar energy and freeze dryer, respectively. Moisture content is a very important factor fungal growth and mycotoxins content [30,31].

3.7 Dried Application of Tomatoes

All dried tomato (solar dried as well as freeze dried) are dehydrated through soaked in distilled water for 5 to 10 minutes depending on the shape of the tomato. Some application usually made by tomato was made like put in sandwiches cheese with tomato and made a pizza (slices or half slices), made red macaroni as well as the soup (powder), tomato jam (square). All these applications had a good test, color for the costume for both solar and freeze drying.

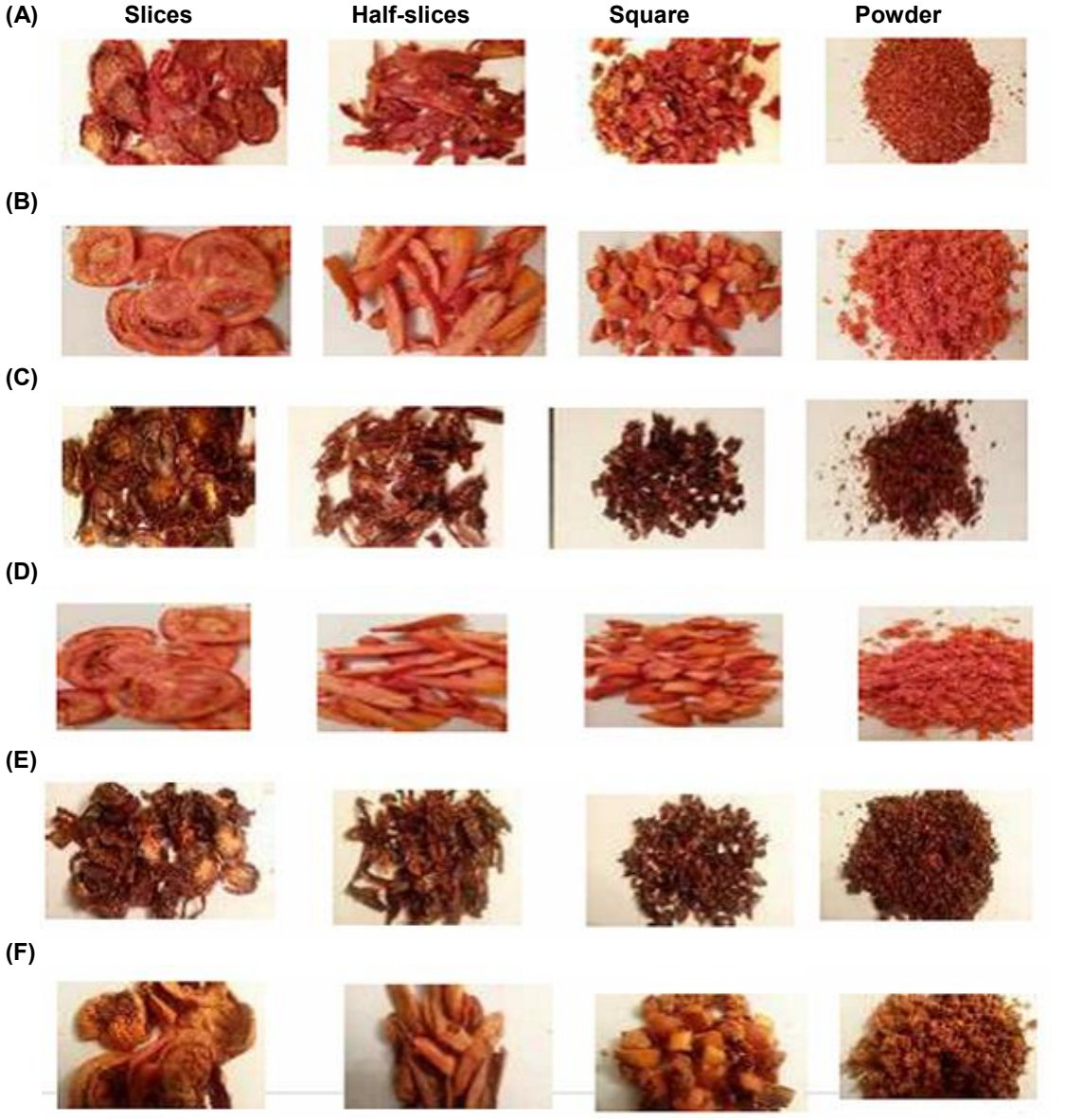

Photo 3. Dried tomato by (a) solar drying; (b) Freeze-drying; (c) solar drying after six months; (D) Freeze-drying after six months; (E) solar drying after 12 months, (F) Freeze-drying after 12 months

4. CONCLUSION

The dried method of tomato slices was done using two different methods, these methods were done by either solar energy or by freeze-dried. According to result; the application utilized solar energy was the best method for drying tomato. Dried tomatoes used freeze-dried gave an appearance lookalike the fresh but it was more expensive. The moisture content in tomato half slices was the lowest all over the four types of tomato pieces. Amongst the samples in this study, Aflatoxins and ochratoxin A were not detectable in the examined samples.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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