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LONG TERM FREEZING STORAGE STUDY TOWARD SAFETY, NUTRITIVE VALUE AND CHEMICAL CHANGES OF PISTACHIO NUTS

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ABSTRACT

Consumption of nut kernels has shown an upward trend due to people's increasing tendency to eat healthy snacks. Pistachio (*Pistacia vera L.*) is one of the most famous nut trees in the world. The aim of the study is to asses' nutritive value, fatty acid, chemical and sensory parameters, total aflatoxins & aflatoxin B_1, and microbiological counts changes after long term storage in freezer (-18±1 °C). Twenty samples of pistachio (raw, unpacked) were collected from the local markets in Egypt. They were subjected to chemical, aflatoxins and microbiological analyses. It was found that pistachio nuts become rancid and not safe to be consumed. It was found that fatty acids profile changed after prolonged storage, where saturated fatty acids increased from 5.77% to 13.57% (+2.35 times), and the unsaturated fatty acids decreased from 47.61% to 41.98% (-11.82%). The most decrease was noticed in unsaturated linoleic acid (-57.84 relative % and -50.20 fats %). Also peroxide value increased. The pistachios nuts after two years of storage at freezing condition were shrinking (moisture content decreased from 5.18% to 4.5%, i.e. -13.13% decreases) resulting in raising the level of relative humidity around the nuts which leads to activation of AFs production. The microbial counts were sharply reduced after long term freezing storage application at refrigerator freezer for 2 years at (-18±1 °C). *Staphylococcus aureus* was not found in all the pistachio samples. This study has confirmed that prolonged storage render pistachio nuts are a suitable target for deterioration and aflatoxins accumulation, in spite of the reduction of microbial counts was considerable. So prolonged storage must be avoided to reduce deterioration and the threat to human health can be minimized.

Keywords: Pistachio nuts, Freezing storage, Rancidity, Aflatoxins, AF B_1, Microbiological contamination.

INTRODUCTION

Pistachio (*Pistacia vera L.*) is one of the most famous and popular nut trees in the world. It is mainly cultivated in dry hot areas as in Iran, Turkey, USA, and Mediterranean countries (Kashaninejad *et al.,* 2003 & 2006; and USDA, NRCS. 2007). Pistachios have laxative effect, helping the body detoxification. Pistachio is also used to treat edema, anemia, and malnutrition. Pistachio can also prevent cell aging, helps lower cholesterol, and relieve acute stress reactions (Gebauer *et al.,* 2008). Pistachio nuts have been clinically evaluated for use in hypercholesterolemia (Alma *et al.,* 2004; and Tsokou *et al.,* 2007).

Pistachio seeds are very appreciable owing to their high nutritional value. They contain unsaturated fatty acids and sterols (Food composition tables for Egypt 2006; and Arena *et al.,* 2007). They also contain about 20.71% proteins and high amounts of carbohydrates and minerals (Kuçukoner & Yurt 2003; and Food composition tables for Egypt 2006). The pistachio is a well-

used nut as an ingredient of many foods, owing to its flavour, taste and deep green colour. Unfortunately this delicious popular nut is most of the time consumed after being stored and not fresh, this leads to the series problems due to spoilage or being rancid due to the high fat content (Gentile *et al.,* 2007).

Food quality control is necessary to ensure that food supplies are safe, of good quality and available in adequate amounts in time, at affordable prices to ensure an acceptable nutritional and health status for all population groups. Food quality control includes all activities carried out to ensure the quality and safety of the food at all stages of the food supply chain from primary production or purchase, through processing and storage, to distribution and consumption (IFST, 1993).

Nuts, in general and pistachio nuts, in specific, are very sensitive to *Aspergillus flavus* and *Aspergillus parasiticus*, weak opportunistic plant pathogenic fungi (Mojtahedi *et al*., 1979 and Sinha and Bhatnagar, 1998]). Mycotoxins are naturally-occurring substances produced by fungi growing on food and animal feed. Aflatoxins are the most toxic group of mycotoxins, and they are produced by two species of the *Aspergillus*, a fungus which is especially found in areas with hot and humid climates. The climatic conditions of the Mediterranean countries favours mould infestation (Bacha *et al.,* 1988). Both *Aspergillus flavus* and *Aspergillus parasiticus* have a wide host ranges and by producing aflatoxins (AFs) cause to contaminate agriculture crops, feed and food products (Shephard, 2005). AFs, especially AFB_1, result in carcinogenic, teratogenic, hepatotoxic and immunosuppressive effects on human and animals (Guengerich *et al.*, 1996) and (Hussein and Brasel, 2001). Exposure to aflatoxin contamination through food storage should be kept as low as possible since aflatoxins are known to be genotoxic and carcinogenic (Molyneux *et al*., 2007; and Ehrlich *et al.,* 2007). Because of the carcinogenic nature of aflatoxin, national organizations as well as buyers commonly limit the acceptable level of this toxin; thus, the guideline level set in the United States by the Food and Drug Administration (FDA) for human consumption is 20 ng /g (nut plus shell basis) (European Commission 2006), while European consuming countries commonly set levels at 1-2 ng /g (B_1) and 4-5 ng /g (total). For export, as well as human health reasons, it is desirable to have the level in a lot as low as possible. Pistachio nuts are among the commodities with the highest risk of AFs contamination (Pittet, 1998), (Cheraghali and Yazdanpanah, 2010) and (Food and Agriculture Organization of the United Nations (FAO), 2001). AFs contamination has been reported in pistachio nut (Mojtahedi *et al*., 1979) especially during harvest/postharvest period and storage stage. In a survey in Iran, 7.5% of the analyzed pistachio nuts contained total aflatoxins (AFT) higher than maximum tolerated level (15 ng/g) (Cheraghali *et al*., 2007). Aflatoxin level in raw pistachios was several times higher than that in finished pistachios. Several studies have shown the natural occurrence of AFB_1 in pistachio nuts in many countries with high levels (Ghali and Belouaer*,* 2009). Such contamination constitutes a threat to public health.

The main risks are contamination by micro-organisms, toxins, chemical contaminants, excess of moisture content, and degradation of the nutritional

value. Deterioration due to repeated packaging and sealing materials due to mould growth often results in spoilage, damp and mouldy nut which is difficult to handle. The significance of microorganisms in stored food commodities depends on a number of conditions, including type and number of microorganism present, treatments to which it has been exposed, the processing or storage treatment, in addition to the handling (McCluggage, 2010).

Occasionally, it is possible to find low levels of micro-organisms in healthy mature nuts taken directly from the tree. However, this is rare; the majority of intact nuts on the tree are sterile. An intact shell is an effective barrier to microbial invasion, and the presence of a hull surrounding the shell further reduces the possibility of microbial contamination of the nutmeat (Roberts and Greenwood, 2003).

The Codex International Code of Hygienic Practice for tree nuts states that these products should be free from pathogenic microorganisms (CAC, 1972). Although nut-associated outbreaks of infection are relatively uncommon, recent outbreaks of salmonellosis associated with the consumption of peanuts, peanut products, and almonds have raised awareness of nuts as a potential vehicle for foodborne illness (Centers for Disease Control and Prevention (CDC), 2004) . Some microorganisms cannot multiply on nuts, but can survive on and in these products for more than one year and has been isolated from nut kernels, such as pistachios ([(CDC), 2009] and [Little *et al*., 2009]).

In a study of the effect of freezing on microbial growth and survival of microorganisms at low temperature by (Chattopadhyay, 2004).He reported that the low temperature preservation of foods is based on the principle that the activities of food-borne microorganisms can be slowed down at temperatures around freezing point and stopped at temperatures below freezing point. As well yeasts and moulds are more likely to grow at temperatures below 0°C compared to bacteria. Although there was a significant reduction in viable numbers of Salmonella over the 270 days of storage at −25.5°C with most species, in no case did all cells die. Although some microorganisms are killed by freezing, approximately 50% may survive depending on the type of organism, the rate of freezing and the composition of substrate being frozen. Bacterial spores are unaffected by freezing and in general Gram-positive rods and cocci are more resistant than Gram-negative bacteria. The viability of organisms is enhanced as the freezing rate is increased. This increase in survival may be due to the diminishing time that the susceptible organism is in contact with harmful high solute concentrations in the unfrozen water.

There is little published data on the microbial contamination of edible nut kernels and the effect of freezing storage on it.

MATERIALS AND METHODS

Twenty samples of raw pistachio nuts were collected from the local markets in Cairo, Egypt. Part of the samples was stored at refrigerator freezer for 2 years at (-18±1 °C).

Chemical analysis:

The samples (before and after storage) were subjected to determine fibers, total fat, ash, moisture, protein and carbohydrate using the methods described by (A.O.A.C 2009). Minerals and vitamins (A,C and E were also determined according to (AOAC, 2009).

Peroxide Value (PV):

The PV values were determined by the iodometric titration method as described by the British Standard Institute (BS,1987).

Fatty acid Profile:

a) Lipid extraction was conducted using method of AOAC 2000. Separation of fatty acids (saponification, preparation of diazomethane, then methylation) was carried out using method of Vogel 1975.

b) Identification and determination of fatty acids was conducted using gas liquid chromatography (GLC, GC trace GC ULTRA) according to Farag *et al.,* 1986. The gas chromatographic analysis was performed on a GC trace GC ULTRA equipped with an FID fitted with a column (30m) packed with 70% cyanopropyl polysilphenylene siloxane. The carrier gas was N_2 with a flow rate of 1.5 ml/min. The column was run isothermally at 195 ˚C and the injector and detector were at 220 ˚C. The fatty acids were identified by the retention time by comparing with standards. Peak area was measured by using a computing integrator (PU 4810, Philips).

Microbiological Examination:

The collected samples were conducted to microbial detection and count experiments which achieved in triplicates. At each sampling time, the aerobic bacterial plate counts (CFU/g) at 35 °C, as well detection of coliforms (MPN/g), *Escherichia coli* (MPN/g), *Bacillus cereus* (CFU/g), and *Staphylococcus aureus* (CFU/g) , mold and yeast counts (CFU/g) at 28 °C were determined. The microbiological procedures were those recommended and described by the international commission on microbiological specification for foods (ICMSF 2002).

Aflatoxins content:

Sample preparation procedure was performed according to the instruction of the test kit (Rida Aflatoxin column Art No: R 5001/5002, R-Biopharma, Darmstadt, Germany (Anonymous, 2005). Test procedure of total aflatoxins were according to Rida screen Aflatoxin total (Art No: 4701) test kit (Anonymous 2002). Test procedure of AFB_1, according to Rida screen Aflatoxin B_1 30/15 (Art No: 1211) test kit (Anonymous, 2004). Measurement of the absorbance was performed with an Elisa-Reader (Stat fax 3200-Awareness Technology) at a wavelength of 450 nm. Evaluation method: Evaluation of the method used was performed according to the Rida Soft windows program distributed by R-Biopharma. The recovery rate was determined by experimentally spiking blank pistachio nuts with 2.5 µg/ kg of AFB_1, and AFT. Triplicate of the concentration were analysed for Both AFB_1, and AFT. The recovery rate was 83% with a relative standard deviation (RSD) for the whole method 3.6% of the detection limit of the AFT test and AFB_1 test in the Elisa procedure was 1ppb (µg /kg) (Hilal and Enver, 2006).

Sensory Evaluation:

Initially and after 24 month of storage, the stored pistachio nuts were given to a panel of twenty persons to evaluate on a 10 point scale, with 10 for excellent and one for highly disliked.

Statistical analysis:

The data were analyzed using the SPSS (1995) software, version 11.0.

RESULTS

Tables (1 a & b) reveal nutritive value, mineral and vitamin content of pistachio nuts. The kernels are a rich source of oil (53.67%). It contains around 20.71% protein, 16.87% carbohydrate and 5.18% moisture. Pistachio nut also contains high amounts of K and P, and various amounts of Ca, Mg and Fe. The caloric value of the pistachio nuts kernel was 633.35-645.54 Kcal/100gm of the edible parts. Table (I) reveal that moisture of pistachio nuts (either fresh or stored) did not exceed 6%.

Table (1-a): Chemical composition of Pistachio nuts (g/100g).

	Protein	Ash	Fibres	Moisture	Carbohydrate	Total Fat	Calories (Kcal/ 100g)
Fresh (before storage)	20.71	2.14	1.43	5.18	16.87	53.67	633.35
After storage	20.99	2.42	1.62	4.5	14.53	55.94	645.54

Table (1-b): Minerals and Vitamins content of fresh Pistachio nuts.

Minerals (mg/dl)									Vitamins (mg/dl)		
Mg	**Ca**	**Fe**	**Na**	**K**	**Cu**	**P**	**Zn**	**Mn**	**C**	**A**	**E**
129.00	107.14	3.93	270	1039.29	1.43	460	2.4	1	5.71	42.14	26.07

Table (2) shows sensory characteristic of stored pistachio nuts at (-18±1 °C for 2 years where overall acceptability did not exceed 53.6%.

Table (2): Sensory evaluation of stored pistachio nuts at -18±1 °C for 2 years.

No. of samples		Appearance	Colour	Texture	Odour	Taste	% Over all acceptability
	Score	10	10	10	10	10	
20		5.1	6.8	5.4	5.0	4.55	53.6

Pistachio nuts are highly nutritious foods, its fat content as high as 53.67%. Table (4) reveals that the mean fatty acid composition of the pistachio nut is 60.51% oleic acid, 27.69% linoleic acid, 10% palmitic acid. It also reveals that unsaturated fatty acid of the nut is 88.71%.

Table (3) and Figure (1) show the peroxide value of pistachio nuts stored for 0, 6, 12, 18, and 24 months at (-18±1 ˚C (Each point represents the mean of three determinations). The PV increases as times increase. In our study the PV at 0 time of purchasing nuts from the local markets is somewhat high. The increase in PV after 6 months are not so high compared with the increase in PV after 0,6,12,18 and 24 months,

Table (3): Peroxide value of pistachio nuts before and after storage at -18±1 °C for 2 years.

Peroxide Value (meq O_2/ kg)				
Before	**After**			
Zero	**6 months**	**12 months**	**18 months**	**24 months**
15	20	35	60	80

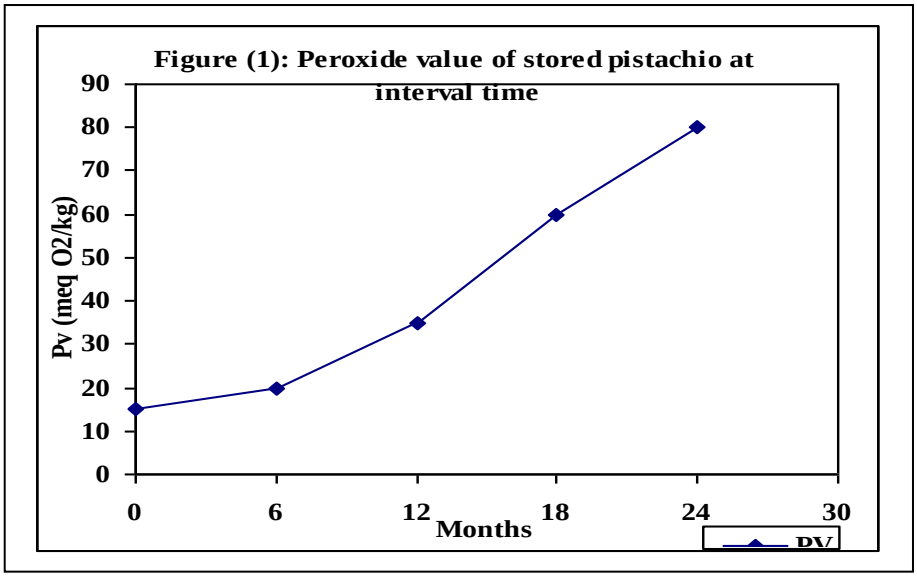

Table (4) show the change in fatty acid composition after storage where saturated fatty acid increase from 5.77% to 13.57% (+3.52 times), and the unsaturated fatty acids decreases from 47.61 to 41.98 (-11.81%). The most decrease was noticed in unsaturated linoleic acid (-57.84%).

Results of Table (5) showed that the incidence of AFB_1 and AFT in unpacked raw pistachio nuts at zero time of purchase was 50% and 60% respectively. In this study 10 out of 20 samples and 13 out of 20 samples found to be contaminated with AFB_1 and AFT respectively. Table (5) showed that the concentration of AFB_1 and AFT was found to be ranging from ND to 2 µg/kg and from ND to 4 µg/kg respectively for all tested samples. However storage in freezing condition for two years leads to increasing the contamination in all tested samples which clearly recorded 3.1 µg/kg and 5 µg/kg for AFB_1 and AFT respectively and that all samples became positive. Data of Table (5) revealed that raw pistachio kernel samples collected from local markets in Cairo has high level of AFB_1 and AFT which indicate high contamination (European Commission, 2006).

Table (4): Fatty acid composition of pistachio nuts before and after storage at -18±1 °C for 2 years.

Fatty Acids	No of Fatty Acids	Relative %			Fats (g/100g)		
		Before	After	% change	Before	After	% change
Myristic	C14:0	0.75	2.65	253.33	0.40	1.48	270.00
Palmitic	C16:0	10	20.43	104.30	5.37	11.43	112.85
Palmitoleic	C16:1	0.51	3.03	494.12	0.27	1.69	525.93
Stearic	C18:0		1.18			0.66	
Oleic (Omega 9)	C18:1	60.51	58.79	-2.84	32.48	32.89	1.26
Linoleic (Omega 6)	C18:2	27.69	13.22	-52.26	14.86	7.40	-50.20
		99.46	99.3		53.38	55.55	
Sat./ Unsat.					5.77/47.61	13.57/41.98	
					12.12	32.33	

Table (5): Aflatoxins content of pistachio nuts, before and after freezing storage at -18±1 °C for 2 years.

Treatment	µg /kg	AFT	AFB_1
Fresh (before storage)	Mean	3	1.2
	Range	ND-4	ND-2
After storage	Mean	5	3.1
	Range	4-7	2.5-4

ND: not detected

In respect of the microbiological profile of pistachio nuts; the incidence, ranges and means of aerobic bacteria count, coliforms count, *E.coli, Bacillus cereus, Staphylococcus aureus,* and mold & yeast count for pistachio samples are shown in Table (6). *Staphylococcus aureus* was not found in all the pistachio samples. However, all pistachio samples were positive for prevalence of aerobic bacteria, coliforms, *E.coli, Bacillus cereus,* and mold & yeast. The mean count of: aerobic bacteria was $1.4x\ 10^4$ CFU/g, coliforms was $1.2x10^1$ MPN/g, *E.coli* was 3 MPN/g, *Bacillus cereus* was $1.1x\ 10^2$ CFU/g and mold & yeast was $2.1x\ 10^3$ CFU/g. On the other hand, the same samples but after long term freezing storage application, revealed overall means of 3.5×10^3 CFU/g, 4 MPN/g, < 3 MPN/g, 7×10^1 CFU/g and 5.5×10^2 CFU/g for aerobic bacteria, coliforms, *E. coli*, *Bacillus cereus* and mold & yeast respectively. Table (6) shows that the microbial counts were sharply reduced after long term freezing storage application at refrigerator freezer for 2 years at (-18±1 °C).

Table (6): Microbiological profile of pistachio nuts, before and after freezing storage at -18±1 °C for 2 years.

Microbiological Examination		Microbial Counts (CFU or MPN/g)	
		Before Storage	After Storage
Aerobic bacterial count	Range	<1000-2.8x10^5 *	<1000-3.2x10^4 *
	Mean	14000 •	3500 •
Coliforms count	Range	<3-140 *	<3-64 *
	Mean	12 •	4 •
E. coli	Range	<3-21*	<3-11 *
	Mean	3 •	<3 •
Bacillus cereus	Range	<100-7.5x10^2 *	<100-1.5x10^2 *
	Mean	110 •	70 •
Staphylococcus aureus	Range	-ve	-ve
Molds and Yeasts count	Mean	100-2.1x10^4 *	<100-2.9x10^3 *
	Range	2100 •	550 •

*** Counts range, ● Counts mean**

DISCUSSION

The caloric value of the pistachio nuts kernel was 633.35-645.54 Kcal/100 gm of the edible parts which is in agreement with Breuer 1993 and Food composition tables for Egypt 2006. Pistachio nuts dried to 4-6% moisture are very stable and could be held for up to 12 months at temperatures as high as 20 °C without significant losses in its quality attributes (Hsu *et al.,* 1991).

The quality of many foods decreases during storage. Consumer is generally concerned with the length of time of food can be stored in home before it can no longer be used (this is known as shelf life). Here the consumer can not judge it easily (safety, chemical, physical and microbial changes). Mainly he can feel taste, aroma, and colour i.e. sensory evaluation (Pegg 1999). There are several approaches to determine shelf life as Accelerated Shelf Life Testing (ASLT), Sensory evaluation, Instrumental Measurements, Chemical, Physical property and Microbial tests (Pegg 1999 and IFST,1993). According to Sedaghat (2010), maximum shelf life was 284 days for samples stored at < 2%O_2, and 5 °C; minimum shelf life was 127 days for samples stored at < 21%O_2, and 45 °C. Here in this study we perform sensory evaluation. The overall acceptability determined by the sensory evaluation scale (53.6) is not in favor for storing and using pistachio nuts after long time (2 years) storage.

Pistachio nuts are highly nutritious foods, its fat content as high as 53.67%. The fatty acid composition of the pistachio nut agrees to somewhat with (Gamlyi and Hayoglu 2007). The unsaturated fatty acid content of the nut is 88.71%. This high unsaturated fatty acid content makes it more susceptible to autoxidation, and rancidity.

Rancidity, which is the development of undesired flavor which makes food unacceptable for eating is generally a major problem in nuts during storage. Deterioration in the quality of pistachio nuts during storage is also

attributable to lipid oxidation. When oxygen in the air reacts with unsaturated fats through process called autoxidation, various break down products are formed which can cause the unacceptable taste and flavors. Factors influencing lipid peroxidation include nature and position of the free fatty acids, oxygen concentration, temperature, water content, physical condition, exposure to light or certain minerals, pro-oxidants and antioxidants, the total number of unsaturated linkages in the sample and the degree of unsaturation. The initial oxidation of fat is usually slow and at a relatively uniform rate. This is known as induction period. At the end of the induction period, the oxidation process accelerates very rapidly, and here the fat begins to smell or taste rancid (Gamlyi and Hayoglu 2007).

The peroxide value (PV) increases as times increase. In our study the PV at 0 time of purchasing nuts from the local markets is somewhat high, and this may be due to that the purchased pistachio were unpacked exposed to air, temperature, and %RH of the temperature which facilitate the activity of probable available lipoxygenase and lipase enzymes (Bonvehi & Coll 1993; and Lopez *et al.,* 1997 a, b).

Oxidative deterioration has often been quantified in terms of the peroxide value or the content of volatile secondary lipid oxidation products, such as hexanal (hexanaldehdye or caproic aldehdye, or capronaldehyde). Hexanal is a good indicator of the degree of oxidation and has along with other volatiles been applied to follow the quality deterioration (Jensen and Risbo 2007). The common polyunsaturated fatty acid, linoleic acid, is very susceptible to oxidation and during this process, hexanal is produced (Jensen and Risbo 2007). It is formed during the oxidation of linoleic and/or linolenic acid via the 9- or 13-hydroperoxide; these molecules are very unstable and are rapidly converted to an array of other compounds such as short-chain aldehydes. Hexanal is the principle material responsible of the unpleasant “grassy-beany; off-flavour” (Piccirillo *et al.,* 2005).

After long freezing storage of nuts, the saturated fatty acids increased from 5.77% to 13.57% (+2.35 times), and the unsaturated fatty acids decreased from 47.61% to 41.98% (-11.82%). The most decrease was noticed in unsaturated linoleic acid (52.26% as relative and 50.2 % as fats g/100 g) In this study we tried to find a relation between peroxide formation and fatty acid change, we noticed the increase of saturated and the decrease of saturated fatty acids. The peroxide value increases rapidly during the propagation stage of the lipid oxidation free radical reaction (Rudolph *et al.,* 1992 and Maskan & Karata 1998 & 1999;).

Storage in freezing condition for two years leads to increasing the contamination in all tested samples which clearly recorded 3.1 µg/kg and 5 µg/kg for AFB_1 and AFT respectively and that all samples became positive which agree with Punam and Shukla, 2007, Agustin 2009, and Juan *et al.,* 2008).

Data of Table (5) revealed that raw pistachio kernel samples collected from local markets in Cairo has high level of AFB_1 and AFT which indicate high contamination according to EU Standards not according to ES. Aflatoxin standard guide lines sets by European union (EU) established 2 µg/kg for AFB_1 and 4 µg/kg for AFT in nuts (Richard 2007, and Juan *et al.,*

2008) and the Egyptian standards (ES) for maximum levels of aflatoxins in food (ES: ES-1875/1/2007) sets 5 µg/kg for AFB_1 and 10 µg/kg for AFT in nuts.

In fact, the contamination of nuts can firstly occur in the field (WHO 1979; and Bennett & Klich 2003), which is chiefly related to the influence of certain meteorological factors as lengthy rains which leads to insufficient drying of the harvest and this may led to inducing the growth of *Aspergillus infestation* and production of mycotoxins. The extent of contamination will vary according to the toxigenic species that may infest one sample and not the other. In this study, we remarked that AFLs synthesis were not the same for all the samples. This might be due to the invasion of some samples of pistachio nuts by *A. flavus* rather than *A. parasiticus*. As Known *A flavus* has two types, toxigenic and non-toxigenic strains, which may influence the level of mycotoxin in some commodities since there is a correlation between *A flavus* and AFLs specially AFB_1 accumulation in pistachio nuts (Mojtahedi *et al.,* 1979 and Ghali and Bolouaer*,* 2009). Abarca *et al.,* 1988 reported that among 36 strains of *A. flavus*, only five were AF producers. The difference in AFLs detected or ND in analysed pistachio nut samples can be attributed to the susceptibility of some cultivars to the insect damage. The association of the aflatoxigenic species with suitable environmental factors contributed to the production of AFLs which vary from one cultivar to another. Such contamination levels emphasise the importance of controlling mycotoxins through harvesting and using post harvest machineries and packaging treatments. All these factors effect on aflatoxins contamination. Handling and transportation and even marketing for raw untreated unpacked unshelled pistachio nut may also affect in aflatoxins contamination.

In this study it was clearly observed that pistachios nuts after two years of storage at freezing condition were shrinking (moisture content decreased from 5.18% to 4.50%, i.e. -13.13% decreases) resulting in raising the level of relative humidity around the nuts which leads to activation of AFs production. However the most important condition influencing fungal development in stored-nut ecosystems are the humidity and storage temperature (Ellis *et al.,* 1994; Sinha 1995; and Saleemullah *et al.,* 2006). On the other hand, even the low temperature ((-18±1 °C) had controlled the levels of AFs, it exceed the maximum tolerable limits 2 µg/kg for AFB_1 and 4 µg/kg for AFT by EU regulation but still lower than the maximum permitted levels set by ES regulations. No samples exceed the level.

The bacterial incidence results in this study go in the same line of that reported in a study of (Farzaneh *et al.*, 2011) which has revealed the incidence of bacteria in pistachio nut samples. As well as the use of *E. coli* as a faecal indicator organism is based on the concept that its detection in food or water samples indirectly provides evidence that the sample has been contaminated with faecal material and that pathogenic organisms may also be present (Roberts and Greenwood, 2003). *E. coli* was present in 0.8% (23) of the 2886 kernel samples, of which one sample (0.03%) was of unsatisfactory microbiological quality due to an *E. coli* level of 150/g. The *E. coli* at unsatisfactory levels was present in a sample of pre-packed Brazils (0.2%) . *E. coli* was found at lower levels (range: 3.6–43/g) in additional

samples of pistachios (1.1%) (Little *et al*., 2010). The use of *E. coli* as an indicator organism in edible kernels and dried seeds has been recently challenged by a number of investigators ([Danyluk *et al*., 2007] , [Eglezos *et al*., 2008] and [Willis *et al*., 2009]), The results of this study show that the microbial counts were sharply reduced after long term freezing storage application at refrigerator freezer for 2 years at ((-18±1 °C). Generally, a comparison between the microbial counts before and after long term freezing storage application demonstrated the improvement of the safety of the pistachio samples in view of the fact that the microbial counts were found to reduce in the investigated samples.

Generally, the results in this study of the effect of long term freezing storage application on the microbial growth in pistachio samples go in the same lines of that mentioned by Chattopadhyay 2004, that the low temperature preservation of foods is based on the principle that the activities of food-borne microorganisms can be slowed down at temperatures around freezing point and stopped at temperatures below freezing point. The metabolic reactions of microorganisms are catalysed by enzymes and its rate is dependent on temperature. The rate of reaction is increased with increase in temperature. This reduction in the microbial counts in this study after long term freezing storage application, proves the successful application of long term freezing storage at refrigerator freezer for 2 years at ((-18±1±1 °C) in respect of microbiological profile of pistachio nuts regardless of the analysis results of the nutritive value, chemical changes and aflatoxins content.

Conclusion

This study has confirmed that prolonged storage render pistachio nuts is a suitable target for deterioration and aflatoxins accumulation, in spite of the reduction of microbial counts was considerable. So prolonged storage must be avoided to reduce deterioration and the threat to human health can be minimised (Rahmianna *et al.,* 2003, Raffi *et al.,* 2006, and Fatma *et al.,* 2010).

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دراسة تأثير تخزين الفستق بالتجميد لفترة طويلة من حيث السلامة والقيمة الغذائية والتغيرات الكيميائية

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مقدمة : يشير استهلاك حبوب المكسرات إلى الاتجاه الصاعد وذلك ناشئ عن النزعة المتزايدة لدى الأفراد لتناول وجبات خفيفة صحية، يعتبر الفستق واحداً من أكثر المكسرات شهرة وشيوعاً في العالم.
هدف البحث : تقييم التغيرات في القيمة الغذائية و الأحماض الدهنية و القياسات الكيميائية ، الحسية ومحتوى الفستق من السموم الفطرية (الأفلاتوكسينات الكلية ، الأفلاتوكسين ب١) والصورة الميكروبيولوجية بعد التخزين لفترة طويلة تحت ظروف التجميد (- ١٨ ± ١°م).
المواد المستخدمة وطرق العمل : تم جمع عشرون عينة من الفستق الخام والغير معبأ من أسواق مدينة القاهرة. تم إخضاع هذه العينات للتحاليل الكيميائية وتقدير المحتوى من السموم الفطرية بالإضافة إلى التحاليل الميكروبيولوجية.
النتائج والمناقشة : من نتائج تحليل العينات تحت الدراسة ، وجد أن عينات الفستق أصبحت زنخة الرائحة وكريهة المذاق وغير آمنة للاستهلاك الآدمي. كما لوحظ حدوث تغيرات في صورة الأحماض الدهنية بإطالة مدة التخزين ، حيث زادت الأحماض الدهنية المشبعة بما يقارب ضعفين ونصف قيمتها الأولية، بينما انخفضت الأحماض الدهنية الغير مشبعة بمقدار ١١,٨١ %. كما لوحظ أيضاً أن أكثر الأحماض الدهنية الغير مشبعة انخفاضاً كان الحامض (لينوليك) ، أيضا زادت قيمة البيروكسيد. دلت النتائج على أنه بعد التخزين تحت ظروف التجميد لمدة سنتين حدث تقلص في وزن حبوب الفستق (حيث انخفض محتوى الرطوبة بمقدار ١٣,١٣ %) أدى ذلك إلى زيادة مستوى الرطوبة النسبية حول حبوب الفستق مما أدى إلى تنشيط إنتاج الأفلاتوكسين، حيث أرتفع متوسط محتوى حبوب الفستق من الأفلاتوكسينات الكلية من ٣ إلى ٥ ميكروجرام/ كجم كذلك الأفلاتوكسين ب١ زاد من ١,٢ إلى ٣,١ ميكروجرام/ كجم. كذلك أشارت نتائج الدراسة إلى انخفاض الأعداد الميكروبية بحدة في عينات الفستق بعد معاملة التخزين لمدة سنتين تحت ظروف التجميد (- ١٨ °م). أثبتت التحاليل الميكروبيولوجية خلو جميع عينات الفستق من بكتيريا الإستافيلوكوكس أوريس.
الاستنتاج : تؤكد هذه الدراسة أن الفستق الذي تحولت فيه وتغيرت الأحماض الدهنية نتيجة لإطالة فترة التخزين ، يصبح هدف مناسب للفساد والتلف، على الرغم من انخفاض أعداد الميكروبات بعينات الفستق، لذلك يجب تجنب إطالة فترة التخزين لتقليل الفساد والتلف وتحقيق الحد الأدنى من المخاطر التي تهدد صحة الإنسان.
الكلمات الدالة : مكسرات الفستق ، التخزين بالتجميد ، التزنخ ، الأفلاتوكسينات ، أفلاتوكسين ب١ ، التلوث الميكروبي.

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