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Fungal Incidence, Aflatoxin B_1, Tocopherols and Fatty Acids Dynamics in Ground and Tree Nuts during Storage at Two Moisture Levels

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ABSTRACT

Any damage in the shell or kernel in field, during transportation, processing, and handling or storage enhances the prospect for fungal invasion in nuts. Processing in any way lowers microbial load of food but nuts are directly consumed so the microbiological characteristics of nuts are of special attention to food manufacturers. The presence of mycoflora also intimidates about the possible incidence of mycotoxins. The objective following the present study was to investigate the effect of different moisture levels on the multiplication of mycoflora, detection of aflatoxin B_1 (AFB_1), tocopherols and fatty acid profiles in tree and ground nuts during storage. Nuts; walnut, almond, pistachio and peanut, randomly selected from the retail market of Faisalabad, Pakistan were found infected with *Aspergillus parasiticus, A. flavus* and *Penicillium*. The moisture content of the nuts was increased to 10±3% and 16±3% to facilitate mould growth. In nuts after a storage period of 12 weeks, the fungi were found growing in all nuts along with considerable aflatoxin B_1 production. Peanut samples maintained at 16±3% moisture level showed highest level of fungal contamination as well as that of AFB1 (158.67 μg kg^{-1}). The tocopherol contents of ground and tree nuts were significantly affected during storage period of 12 weeks. Storage period brought about an increase in free fatty acids in all the analyzed nuts. © 2012 Friends Science Publishers

Key Words: Aflatoxins; Tree nuts; Ground nut; Aspergillus; Tocopherol; Storage period

INTRODUCTION

Tree nuts as walnuts (*Juglans regia*), almonds (*Prunus duclus*) and pistachios (*Pistachio vera*) and peanuts (*Arachis hypogea*) as ground nuts, are inspired worldwide for their nutritional, sensory and health promoting attributes. Exceptional nutrient profile of the nuts constructively affects lipids and lipoproteins (Griel & Kris-Etherton, 2006). The fat contents of nuts are generally in the range of 50-75%. Tree nut oils generally contain high percentage of monounsaturated fatty acid predominantly oleic acid and low percentage of polyunsaturated fatty acids. In addition, small percentage of saturated fatty acids is also reported. Ground nut oil contains high percentage of mono and polyunsaturated fatty acids and low percentage of saturated fatty acids (Ozcan & Seven, 2003; Miraliakbari & Shahidi, 2008).

Fungal invasion of food and feed has resulted in a foremost socio-economic crisis through out the world. Array of microorganisms infect nuts and cause spoilage leading to production of toxic metabolites (Khan et al., 2010) e.g., biogenic amines are produced by lactic acid bacteria and mycotoxins by the fungi (Molyneux *et al.*, 2007). Several factors contribute to such infection. Optimum conditions of temperature and moisture favor the growth of mycoflora. Wind and insects carry the spores to the foliage and developing buds of nuts with the opportunity of producing aflatoxins. Aflatoxins are produced by more than one fungal species. Aflatoxin B_1 (AFB_1) and B_2 (AFB_2) are produced by *Aspergillus flavus* while *A. parasiticus* produces aflatoxin G_1 (AFG_1) and G_2 (AFG_2) along with AFB_1 and AFB_2 (Chareonpornsook & Kavisarasai, 2006). Total permissible aflatoxins content in nuts is 20 ng g^{-1} (20 ppb) in the United States (Food & Drug Administration, 1996) but a threshold limit of 4 ng g^{-1} (4ppb) is permissible in the European Community (Commission of the European Community, 1998).

Aflatoxin B_1 (AFB_1) is produced both under moderate and under subtropic and tropic climatic conditions. Because of its hazardous nature, different physical and physico-chemical techniques have been developed to detoxify and to degrade AFB_1 (Hormisch *et al.,* 2004). AFB_1 can be metabolized by constitutive cellular enzymes and this property is thought to be related to its noxious and carcinogenic effects. The metabolism of AFB_1 results in oxidative derivatives including hydroxylated species such as aflatoxin M_1 (AFM_1) and aflatoxin Q_i (AFQ_i) in addition to covalent macromolecular adducts with DNA and proteins. Biological punishment of oxidative metabolism of AFB_1 extends from cellular alteration to cell death (Groopman *et*

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al., 1985).

Storage time and conditions prevailing during storage have a significant bearing on the quality of nuts. In view of this, the effect of two different moisture levels is studied on the fungal load, AFB_1 detection, tocopherols and free fatty acids during storage in nuts.

MATERIALS AND METHODS

Chemicals: All the reagents used were of HPLC-grade. Acetonitrile and methanol (LABSCAN, ANALYTICAL SCIENCES), acetic acid (Riedel- deHaën), aflatoxin standards were purchased from sigma chemical company, USA, potato dextrose agar and sabrod liquid (Oxoid Ltd. Basingstoke, Hampshire, England).

Samples: Aflatoxin free samples of walnut, almond, pistachio and peanut with initial moisture content of 0.38%, 0.68%, 0.54% and 0.71%, respectively were randomly collected from local market of Faisalabad. Moisture content was determined by drying replicate portions of 5-10 g of ground and tree nuts at 106 °C nuts for 24h and then subsequently up to constant weight and loss in weight was expressed as percentage calculated on wet weight basis (USDA, 1978).

Storage conditions for mold growth: The conditions for storage of nuts were adjusted according to (Albores *et al.,* 2005) with little modifications. The moisture content of the samples was adjusted to 10±3 and 16±3% and stored in wooden containers. The containers were placed at a store room with proper ventilation at a temperature of 25-30 °C for a period of 12 weeks. After 12^{th} week of storage, the nuts were placed under a 1000 mg ethylene oxide gas environment for 3 h to hinder multiplication of microorganisms.

Enumeration of fungal load: The ground and tree nuts were analyzed for the fungal count following the method of Arici *et al.* (2007). All the reagent solutions used for fungal load were autoclaved before use and all the glass ware after proper washing was dried in oven at 180°C for 3 h. One gram of each finally ground nuts was transferred into 10 mL of autoclaved (120°C for 15 min) 2% peptone water in a conical flask. Wash water was collected for further analysis after vigorously shaking for 5 min., and filtering through Whatman filter paper No.1. After extracting microorganisms from samples with 2% peptone water, these were plated on pre sterilized Petri plates in hot air oven at 180 °C for 3 h containing nutrient agar potato dextrose to obtain fungal biomass and pure cultures were obtained using streaking method. Fungal species were identified by microscopy in the department of Microbiology, University of Agriculture, Faisalabad, Pakistan. Pure cultures isolated from the 12 weeks stored nuts were diluted up to 10^1 spores mL^{-1} and absorbance was taken at 620 nm at u-quart (Bio Tech., USA.) Average standard curve was drawn for pure isolates plotting spores mL^{-1} versus optical density. The fungal count of the stored nuts was calculated using spread plate method and direct observation method. The data presented is the average count in three Petri dishes for each sample.

Extraction and purification of aflatoxins: Aflatoxin B_1 extraction in nut samples was made according to the procedure reported by Fu *et al.* (2008) with little modifications. Samples of ground and tree nuts were randomly selected from the lot during 12^{th} week of storage. Samples were ground in a laboratory mill (culatti, JANKE & KUNKEL, GmbH) and weighed 25 g in Erlenmeyer. Aflatoxins were extracted using 80 mL of a mixture of ACN: H_2O (84:16) by shaking for 30 min. The extract was filtered through Whatman (Maidatone, UK) filter paper (NO. 3). From the filtrate 9 mL was taken in a glass Vail, acidified with 70 µL acetic acid and vortex. The acidified mixture was then passed through a mycosep # 226 Aflazon + column (Romer labs) with a flow rate of 2mL min^{-1}. The pure aflatoxin solution (2 mL) was then dried through stream of N_2 and the residue was dissolved in 2mL of mobile phase.

HPLC conditions for aflatoxins analysis: All analysis of aflatoxins were performed on HPLC apparatus Prominance™, Shimadzu® (Shimadzu, Japan) equipped with Mediterranae C-18 ® 5 µm25 cmx0.46 Serial No. N45074 (Teknokroma, Spain) fitted with CTO-20A® (Shimadzu, Japan) column oven and (LC-20AT® (Shimadzu, Japan) pump. For the determination of aflatoxins in nuts, isochratic mobile phase consisting of methanol: acetonitrile: water (22.5:22.5:55) was used with a flow rate of 1 mL/min with an injection volume of 20 µL. The eluate was detected using fluorescence detector RF-10AXL ® (Shimadzu, Japan) set at emission 440 nm and excitation was at 360 nm.

Tocopherol and Free fatty acids analysis: Tocopherol analysis was carried out according to the method suggested by Swiglo and Sikorska (2004). The oil (0.5 g) extracted from ground and tree nut samples at different time intervals was dissolved in 1 mL of 2-propanol, vortexed for 5 min and were directly injected into high performance liquid chromatograph. Mobile phase used was acetonitrile and methanol in the ratio of 1:1. Fluorescence detector was used with an excitation wavelength at 295 nm and emission at 325 nm.

Fatty acid methyl esters of stored ground and tree nuts were prepared by the method followed by Sheshata *et al.* (1970). To 0.2 mL of extracted oil methanolic sulphuric acid (4:1) was added. After heating on water bath at 80 °C for 2 hours, 2 mL distilled water was added. The contents were extracted with 2mL petroleum ether thrice. The samples were concentrated to semi-liquid for GC analysis using Gas chromatograph (Perkin Elmer-3920) attached with C-R4A chromatopac.

Statistical analysis: Experimental data including fungal count, aflatoxin B_1 detection, tocopherols and free fatty acids was subjected to analysis of variance. Means, standard deviations and ANOVA were estimated using Microsoft Excel 2003. Means were separated using t-test.

RESULTS AND DISCUSSION

Mycoflora multiplication in tree and ground nuts during storage: The mycoflora were detected in all samples of tree and ground nuts immediately after collection from market. The results concerning the monitoring of mycoflora, which were analysed in ground and tree nuts samples during a storage period of 12 weeks, are shown in Fig. 1. The identification of fungal spores by microscopy showed the presence of fungi belonging to *A. flavus, A. parasiticus* and *Penicillium*. In peanut and pistachio samples, *A. flavus* and *A. parasiticus* were detected, whereas in almond and walnut samples *Penicillium* along with *A. flavus* and *A. parasiticus* were detected. Our finding was found to be in agreement with (Aziz & Moussa, 2002; Nakai *et al.*, 2008). The fungal count increased significantly at high moisture level (16±3%) in comparison to that at low moisture level (10±3%) in all samples of ground and tree nuts. The peanut kernels had higher incidence of *Aspergillus* and *Penicillum* due to the better adaptation of these fungi to this substrate throughout storage (Fig. 1).

In case of walnuts, the fungal count reached 5.7 log 10 during 12th week of storage at low moisture level and 6 log 10 at high moisture level. This finding was in consistency with Kakashita *et al.* (1994) and Aquino *et al.* (2007).The incidence of toxigenic mould represents a potential risk of mycotoxins contamination. Statistical analysis of variance of the present results indicated a positive correlation ($P \leq 0.05$) between storage period, moisture content and multiplication of mycoflora in nuts (Fig. 1).

The rate of growth of toxin producing fungi and aflatoxin production depends generally on the prevailing physical, biological, biochemical and environmental conditions. However, in the present work all physical and environmental conditions were fixed. Hence, difference in fungal growth may be related to biochemical conditions within each commodity. The growth of *Aspergillus* has also been observed at 10% moisture level but substantially lower than that at 20% moisture levels (Ghosh *et al.*, 1996).

Detection of aflatoxin B_1 in ground and tree nuts at different moisture levels during storage: Samples of ground and tree nuts with moisture level adjusted at 10±3% and 16±3% were evaluated for aflatoxin B_1 production by the storage fungi during 1st, 2nd, 4th and 12th week of storage. During 1st week of storage, AFB_1 was not detected in any nut sample at both moisture levels. At the end of 12th week of storage all of the nuts were infected with AFB_1. The levels of AFB_1 in ground and tree nuts at different moisture levels during storage are shown in Fig. 2. The level of AFB_1 was found to be very high in all nut samples at both moisture levels in comparison to control nuts. This finding was in consistence with Kaaya *et al.* (2006) and Chun *et al.* (2007).

The maximum AFB_1 found in peanuts stood at 158.67 µg kg^{-1} at 16±3% and 46.77 µg kg^{-1} at 10±3% moisture level. This observation is also supported by the fungal count

Fig. 1: Multiplication of mycoflora in ground and tree nuts during storage at different moisture levels

*Commodities marked with star * are adjusted at high moisture level (16±3%) and without star * are at low moisture level (10±3%) * cfus g^{-1} is colony forming units per gram * Y- error bars are representing standard deviations

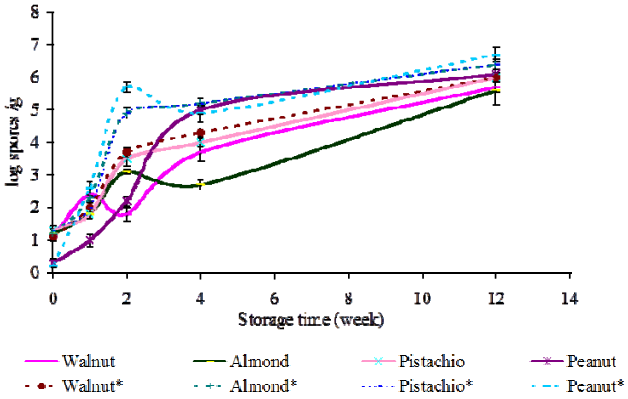


Fig. 2: Detection of AFB_1 in ground and tree nuts during storage adjusted at different moisture levels.

*Commodities marked with star are maintained at 16±3% moisture level while those not marked are at 10±3% moisture level; * n.d. is not detected; * Y-error bars are standard deviations

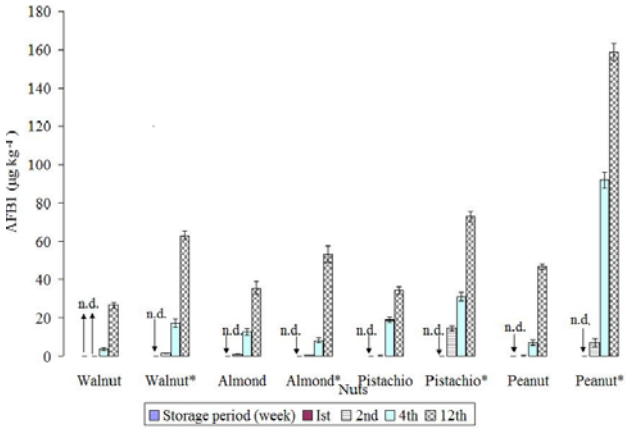
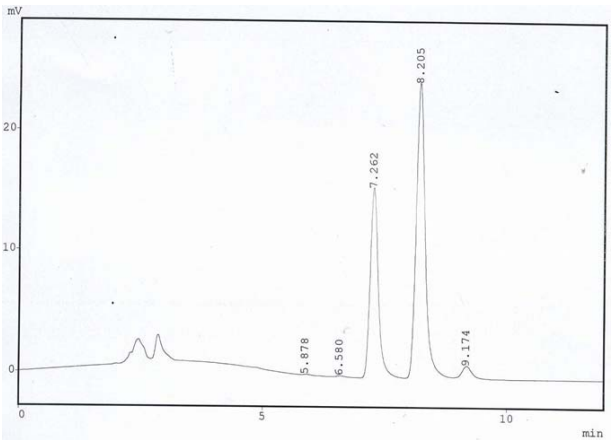


Fig. 3: Standard chromatogram of tocopherols analyzed using HPLC-FLD. 1. δ-tocopherol, 2. γ-tocopherol, 3. α-tocopherol



of peanuts in comparison to that of tree nuts. Previous studies support that peanut is one of the most vulnerable foodstuffs to be contaminated by toxigenic fungi producing aflatoxins (Escobar & Reueiro, 2002; Chun *et al.,* 2007). At low moisture level, tree nuts (almond & pistachio) showed almost same level of AFB_1 (34.21 & 35.52 μg kg^{-1}, respectively), however, at high moisture level, the difference between AFB_1 levels was quite large.

Walnut showed 26.61 μg kg^{-1} of AFB_1 at low moisture, and this was the minimum level of AFB_1, while at high moisture level the level of AFB_1 was 2nd last in ground and tree nuts. This finding is in agreement with Hassan *et al*. (2010).

Tocopherols content in tree and ground nuts: The tocopherols content of tree and ground nuts was estimated by high performance liquid chromatography at different time intervals during storage. Table I represents the data obtained from triplicate analyses of lipid samples of walnuts during storage at different moisture levels. In walnut lipid sample taken during first week of storage, γ tocopherol was found to be the predominant isomer of vitamin E followed by δ and α- tocopherols. It is in agreement with the tocopherol contents reported in thirteen different varieties of walnuts reported by Savage *et al.* (1999). In our analyzed nuts, α- tocopherol is predominant followed by γ and δ-tocopherol which is in accordance with tocopherol content in almond varieties reported by Ortiz *et al.* (2008) and in different tree nuts reported by Miraliakbari and Shahidi (2008). The results are also in agreement with Miraliakbari and Shahidi (2008) in the absence of β-tocopherol in almond. In our analyzed samples of almond and pistachio, δ-tocopherol was detected in a considerable amount but was below detection limits in the samples analyzed by these authors.

Peanut, a ground nut, showed a distinctive tocopherol contents than all the tree nuts. The variation in tocopherol content in peanut during storage at two moisture levels is shown in Table IV. In peanut, γ and α tocopherols were present in excessively large amount than δ tocopherol. A number of studies have reported the similar vitamin E content in ground nut with little or no variation in the concentrations of vitamin E homologues (Maguire *et al.,* 2004; Kornsteiner *et al.,* 2006; Ryan *et al.,* 2006).

During storage period of 12 weeks, a decrease in tocopherol content was observed in all nut samples at both moisture levels. There was a significant change ($P < 0.05$) in individual and total tocopherol content of all nuts at both moisture levels. This finding is in consistence with Shin and Godber (1996) who reported that high destruction of vitamin E vitamers may be related to the amount of free fatty acids hydrolyzed by lipolytic enzymes in food or feed commodity. The loss of tocopherol content in nuts during storage may be attributed to the development of mycoflora on nuts during storage and loss of antioxidant activity as a natural phenomenon. The maximum loss in tocopherol content was observed in almond and pistachio adjusted at high moisture level.

Changes in free fatty acids profile of ground and tree nuts during storage: Free fatty acids composition of the lipid component of ground and tree nuts was estimated qualitatively and quantitatively by GC/FID during 1st, 2nd, 4th and 12th weeks of storage. The analysis was performed in triplicate, the means of triplicate analysis and their standard deviations are shown in Tables V, VI, VII and VIII for walnut, almond, pistachio and peanut, respectively.

Nuts are rich sources of oils. The lipid contents of walnut, almond, pistachio and peanut were estimated to be 70.17, 52.86, 54.28 and 52.65% (W/W), respectively. The tree and ground nuts exhibited more than 50% (W/W) lipid fraction. Several authors report similar findings (Grosso *et al.,* 2000; Satil *et al.,* 2003; Cherif *et al.,* 2004; Miralikbari & Shahidi, 2007; Sanchez-Bel *et al.,* 2008). The profile of lipid fraction extracted from nuts show a little variation according to the climatic conditions of their habitat.

The saturated fatty acids in tree and ground nuts are palmitic acid ($C_{16:00}$) and stearic acid ($C_{18:00}$). The unsaturated fatty acids are oleic acid ($C_{18:1}$), linoleic acid ($C_{18:2}$) and linolenic acid ($C_{18:3}$). Only the lipid content of peanut was solidified at room temperature (25±3°C), whereas the tree nut lipids were in liquid phase. The percentage of stearic acid in saturated fatty acids was very low. It is a well known fact that the oils of plant origin contain very small stearic acid fraction. The fatty acid profiles of ground and tree nuts revealed in our study were in synchronization with a number of already published repots on oils extracted from different commodities (Amara *et al.,* 2003; Cherif *et al.,* 2004; Venkatachalam & Sathe, 2006; Kornsteiner *et al.,* 2006). Walnut contains low concentration of monounsaturated fatty acids in comparison to the rest of the tree nuts and ground nut analyzed in our study that has also been reported previously (Corinna *et al.,* 2003).

The storage period of 12 weeks selectively affected the free fatty acids in the nuts. The concentrations of palmitic and stearic acids increased throughout during storage period in all the nuts analyzed. The increase in saturated fatty acids was statistically significant in all the nuts as shown in Tables V-VIII for walnut, almond, pistachio and peanut, respectively. The concentration of monounsaturated fatty acid showed a little decrease during 2nd and 4th weeks of storage. However, during 12th week of storage, there was a considerable increase in oleic acid concentration. The concentration of polyunsaturated fatty acids decreased throughout the storage period in all the nuts analyzed. This leads to a corresponding decrease in ∑UFA/∑SFA (oxidation index) throughout storage period for walnut (Table V), almond (Table VI), pistachio (Table VII) and peanut (Table VIII). The highest reduction in oxidation index was observed in peanut adjusted at high moisture level. Decrease in polyunsaturated fatty acids and increase in saturated fatty acids was more pronounced for nuts adjusted at high moisture level as clear

Table I: Vitamin E (alpha, sigma & gamma tocopherols) levels during storage in walnut at different moisture levels

Moisture contents (%)	Tocopherol (mg kg^{-1})			
	α-tocopherol	δ-tocopherol	γ-tocopherol	Total
10±3	8.71±0.61	25.61 ± 0.62	266.28 ± 0.93	300.61 ± 1.7
	5.21±0.29	22.57 ± 0.33	245.07 ± 0.44	272.86 ± 1.9
	3.03±0.08	17.32 ± 0.33	224.44 ± 0.38	244.78 ± 2.4
	2.95±0.05	6.52 ± 0.05	214.81 ± 0.31	224.29 ± 0.88
16±3	8.71±0.61	25.62 ± 0.62	266.28 ± 0.93	300.62 ± 1.7
	4.29±0.34	9.96 ± 0.40	225.25 ± 0.65	239.51 ± 1.67
	2.61±0.02	5.82 ± 0.02	212.70 ± 0.29	221.14 ± 1.41
	1.56±0.01	1.96 ± 0.04	205.26 ± 0.22	208.79 ± 1.40

Table II: Vitamin E (alpha, sigma & gamma tocopherols) levels during storage in almond at different moisture levels

Moisture contents (%)	Storage period (week)	Tocopherol(mg kg^{-1})			
		α-tocopherol	δ-tocopherol	γ-tocopherol	Total
10±3	Ist	279.11 ± 0.91	15.61 ± 0.46	43.91±0.55	338.63±2.25
	2nd	238.71 ± 1.45	9.89 ± 0.11	32.01±0.65	280.61±2.11
	4th	194.16 ± 1.61	4.73 ± 0.26	27.58±1.10	226.47±1.92
	12th	161.98 ± 1.15	1.77 ± 0.45	25.72±0.71	189.47±1.53
16±3	Ist	279.11 ± 1.24	15.61 ± 0.77	43.91±0.51	338.62±2.49
	2nd	205.08 ± 2.16	6.63 ± 0.62	28.01±0.62	239.73±2.45
	4th	176.45 ± 1.98	3.78 ± 0.81	25.64±0.91	205.87±2.03
	12th	134.45 ± 2.01	1.01 ± 0.01	21.79±0.95	157.25±2.61

Table III: Vitamin E (alpha, sigma & gamma tocopherols) levels during storage in pistachio at different moisture levels

Moisture contents (%)	Storage period (week)	Tocopherol (mg kg^{-1})			
		α-tocopherol	δ-tocopherol	γ-tocopherol	Total
10±3	Ist	276.26 ± 1.75	8.86 ± 0.22	18.62 ± 1.53	303.75 ± 2.21
	2nd	225.31 ± 1.41	7.90 ± 0.45	13.50 ± 1.09	246.71 ± 2.42
	4th	197.37 ± 1.16	6.88 ± 0.21	9.85 ± 0.99	214.09 ± 1.81
	12th	175.37 ± 1.89	1.95 ± 0.10	8.85 ± 0.45	186.17 ± 2.15
16±3	Ist	276.27 ± 2.11	8.86 ± 0.18	18.63 ± 0.47	303.74 ± 1.96
	2nd	168.38 ± 2.75	6.75 ± 0.39	10.24 ± 0.61	185.01 ± 1.85
	4th	153.08 ± 1.79	4.80 ± 0.41	6.33 ± 0.55	164.21 ± 2.02
	12th	149.39 ± 1.42	1.57 ± 0.80	5.79 ± 0.42	156.75 ± 2.21

Table IV: Vitamin E (alpha, sigma & gamma tocopherols) levels during storage in peanut at different moisture levels

Moisture contents (%)	Storage period (week)	Tocopherol (mg kg^{-1})			
		α-tocopherol	δ-tocopherol	γ-tocopherol	Total
10±3	Ist	119.16 ± 1.11	8.80 ± 0.83	135.07 ± 1.73	263.04 ± 2.19
	2nd	107.61 ± 1.10	7.31 ± 0.44	142.26 ± 1.23	257.18 ± 2.08
	4th	98.20 ± 2.01	5.15 ± 0.51	116 ± 1.47	219.35 ± 2.45
	12th	76.46 ± 1.45	3.67 ± 0.22	102.17 ± 1.35	182.31± 1.11
16±3	Ist	119.17 ± 1.66	8.80 ± 0.29	135.07 ± 2.11	263.04 ± 1.41
	2nd	101.17 ± 1.15	6.47 ± 0.18	128.12 ± 2.58	236.00 ± 2.15
	4th	84.56 ± 0.91	4.02 ± 0.25	109.42 ± 2.09	198.01 ± 2.11
	12th	69.04 ± 1.29	1.88 ± 0.29	92.02 ± 1.88	162.93± 1.83

Table V: Changes in free fatty acids profile in walnut during storage adjusted at different moisture levels

Moisture contents (%) →	10±3				16±3			
Storage period (week) →	1st	2nd	4th	12th	1st	2nd	4th	12th
Fatty acids (g/100 g) ↓								
16:00	8.39 ± 0.21	9.32 ± 0.15	9.68 ± 0.04	9.85 ± 0.65	8.39 ± 0.32	9.74 ± 0.01	10.09 ± 0.19	10.31 ± 0.71
18:00	4.26 ± 0.01	4.46 ± 0.21	5.18 ± 0.24	5.59 ± 0.19	4.27 ± 0.08	4.82 ± 0.26	5.48 ± 0.71	6.18 ± 0.19
18:01	27.31 ± 0.78	26.97 ± 1.67	27.59 ± 0.46	27.97 ± 0.11	27.32 ± 0.78	25.36 ± 0.38	26.51 ± 0.28	28.70 ± 1.7
18:02	47.52 ± 0.51	44.76 ± 0.20	41.42 ± 0.2	38.91 ± 0.92	47.52 ± 1.04	44.23 ± 1.21	40.16 ± 0.94	38.49 ± 0.43
18:03	12.48 ± 0.11	12.28 ± 0.12	11.07 ± 0.88	9.62 ± 0.83	12.49 ± 0.07	11.14 ± 0.39	10.36 ± 0.78	9.18 ± 0.46
∑SFA	12.66 ± 0.25	13.79 ± 0.48	14.87 ± 0.44	15.45 ± 0.29	12.67 ± 0.12	14.56 ± 0.12	15.57 ± 0.61	16.49 ± 0.29
∑UFA	87.33 ± 1.2	84.03 ± 1.05	80.08 ± 1.41	76.50 ± 1.08	87.33 ± 1.18	80.73 ± 1.00	77.04 ± 0.54	76.37 ± 0.78
∑UFA/ ∑SFA	6.8	6.1	5.4	4.9	6.8	5.5	4.9	4.6

*Mean ± standard deviation (n = 3)

Table VI: Changes in free fatty acids profile in almond during storage adjusted at different moisture levels

Moisture contents (%) →	10±3				16±3			
Storage period (week) →	1st	2nd	4th	12th	1st	2nd	4th	12th
Fatty acids (g/100 g) ↓								
16:00	8.74 ± 0.44	9.36 ± 0.26	9.85 ± 0.03	10.05 ± 0.1	8.74 ± 0.06	9.74 ± 0.04	10.03 ± 0.14	10.22 ± 0.29
18:00	1.89 ± 0.03	1.92 ± 0.02	2.07 ± 0.03	2.31 ± 0.01	1.89 ± 0.02	1.94 ± 0.06	2.32 ± 0.04	2.51 ± 0.03
18:01	74.10 ± 0.55	72.78 ± 0.52	72.59 ± 0.40	74.42 ± 0.15	74.10 ± 0.11	73.12 ± 0.32	74.62 ± 0.24	75.01 ± 0.45
18:02	14.99 ± 0.12	14.59 ± 0.46	14.85 ± 0.88	13.62 ± 0.50	14.99 ± 0.03	14.12 ± 0.15	13.06 ± 0.87	12.78 ± 0.1
18:03	0.28 ± 0.02	0.27 ± 0.04	0.25 ± 0.02	0.22 ± 0.02	0.28 ± 0.01	0.27 ± 0.06	0.24 ± 0.04	0.22 ± 0.03
∑SFA	10.62 ± 0.07	11.28 ± 0.52	11.91 ± 0.04	12.36 ± 0.13	10.62 ± 0.93	11.68 ± 1.11	12.34 ± 0.48	12.73 ± 0.17
∑UFA	89.37 ± 1.02	87.64 ± 1.08	87.69 ± 0.78	88.27 ± 0.83	89.37 ± 0.48	87.51 ± 0.40	87.93 ± 0.01	88.01 ± 0.44
∑UFA/ ∑SFA	8.4	7.7	7.3	7.1	8.4	7.4	7.1	6.9

Table VII: In pistachio changes in free fatty acids profile during storage maintained at different moisture levels

Moisture contents (%) →	10±3				16±3			
Storage period (week) →	1st	2nd	4th	12th	1st	2nd	4th	12th
Fatty acids (g/100 g) ↓								
16:00	7.76 ± 0.71	8.31 ± 0.12	8.40 ± 0.18	9.00 ± 0.46	7.76 ± 0.17	8.57 ± 0.31	8.93 ± 0.04	9.66 ± 0.37
18:00	1.39 ± 0.13	1.25 ± 0.10	1.41 ± 0.24	1.56 ± 0.01	1.39 ± 0.27	1.59 ± 0.18	1.77 ± 0.04	2.13 ± 0.16
18:01	58.38 ± 0.06	54.29 ± 0.14	54.34 ± 0.38	59.18 ± 0.03	58.38 ± 0.55	52.30 ± 0.52	52.07 ± 0.41	56.18 ± 0.15
18:02	30.51 ± 0.87	30.57 ± 1.57	27.71 ± 0.17	27.65 ± 1.50	30.51 ± 0.06	29.44 ± 0.93	26.20 ± 0.29	24.46 ± 1.11
18:03	2.00 ± 0.10	n.d.	n.d.	n.d.	2.00 ± 0.10	n.d.	n.d.	n.d.
∑SFA	9.15 ± 0.03	9.57 ± 0.15	9.82 ± 0.26	10.57 ± 0.09	9.15 ± 0.41	10.16 ± 0.28	10.71 ± 0.26	11.79 ± 0.25
∑UFA	90.89 ± 1.21	84.86 ± 1.69	82.04 ± 0.88	86.83 ± 0.83	90.89 ± 1.10	81.74 ± 0.34	78.27 ± 0.31	80.64 ± 1.25
∑UFA/ ∑SFA	9.9	8.8	8.3	8.2	9.9	8.1	7.3	6.8

Table VIII: In peanut changes in free fatty acids profile during storage maintained at different moisture levels

Moisture contents (%) →	10±3				16±3			
Storage period (week) →	1st	2nd	4th	12th	1st	2nd	4th	12th
Fatty acids (g/100 g) ↓								
16:00	10.76 ± 0.39	11.01 ± 0.07	12.42 ± 0.22	14.90 ± 0.17	10.76 ± 0.44	12.15 ± 0.31	13.25 ± 0.24	15.31 ± 0.14
18:00	3.39 ± 0.71	3.90 ± 0.12	4.11 ± 0.19	4.65 ± 0.46	3.39 ± 0.31	4.27 ± 0.04	4.72 ± 0.10	5.14 ± 0.16
18:01	64.22 ± 0.51	62.62 ± 0.22	60.91 ± 0.43	64.42 ± 0.43	64.22 ± 0.54	62.03 ± 0.26	60.24 ± 0.46	64.60 ± 0.27
18:02	21.63 ± 0.38	18.25 ± 0.13	17.64 ± 0.38	16.55 ± 0.13	21.63 ± 0.96	17.82 ± 0.52	17.02 ± 0.04	16.19 ± 0.37
18:03	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
∑SFA	14.15 ± 0.87	14.91 ± 0.93	16.53 ± 0.17	19.55 ± 1.50	14.15 ± 0.06	16.41 ± 0.39	17.97 ± 1.11	20.45 ± 0.87
∑UFA	85.84 ± 0.32	80.87 ± 0.61	78.56 ± 0.92	80.96 ± 0.46	85.84 ± 0.56	79.84 ± 0.80	77.26 ± 0.67	80.79 ± 1.27
∑UFA/ ∑SFA	6.1	5.4	4.7	4.1	6.1	4.8	4.2	3.9

*Mean ± standard deviation (n = 3)

from the data. The behavior of the free fatty acids during storage in nuts, reported in our study, was found to be in harmony with Sanchez-Bel *et al.* (2005) Shin and Godber (1996) and Shin *et al.* (1997).

CONCLUSION

All samples of ground and tree nuts were found contaminated by *A. flavus, A. parasiticus* , and some with *Penicillium* as well, immediately after random collection from retail outlets. The fungal load increased significantly during storage and was more pronounced at high moisture levels. The level of AFB_1 was significantly affected during storage period. Ground and tree nuts exhibited almost similar tocopherol contents except walnut, which showed different tocopherol profile. The tocopherol contents of ground and tree nuts were substantially affected during storage period of 12 weeks. The decrease in tocopherol contents may be also be related to the fungal growth during storage. Tree and ground nuts showed free fatty acids profiles with major contribution by unsaturated and minor by saturated ones. Prolonged storage period was associated with an increase in free fatty acids in all the nuts under investigation. It is suggested that there must be permissible limits of shelf life according to the prevailing conditions of the storage site to meet the wholesomeness of unsealed stored commodities.

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