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ORIGINAL ARTICLE

Dehiscence and prolonged storage of 'Kerman' Pistachios: Effects on morphometry and nutraceutical value

Perla Judith Garcia-Moreno[1] · Laura Alejandra de la Rosa[1] · Jazmin Cristina Stevens-Barron[1] · Roberto Rodríguez-Ramirez[2] · Baltazar Corral-Diaz[1] · Emilio Alvarez-Parrilla[1] · Francisco Javier Olivas-Aguirre[3] · Abraham Wall-Medrano[1]

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Abstract 'Kerman' pistachios (KP; *Pistacia vera* L.) are an important crop for several countries but their commercial value is diminished by their shell dehiscence status and prolonged storage in popular marketplaces. The aim was to evaluate the independent/synergistic effect of prolonged storage (1–4 year) and dehiscence status (split/unsplit) on KP's morphometry and chemical composition. Whole nut's and kernel's length, width, thickness, surface area, and volume were more affected by dehiscence (split > unsplit; $p \leq 0.01$) than storage time; Kernel's mass, macronutrient composition and tocopherols (T)/tocotrienols (T3) were not much affected by dehiscence but time-trend correlations were observed with macronutrient composition (split/unsplit; $\rho = -0.57$–0.42) and T + T3 (unsplit; $\rho = 0.81$). Specific/total fatty acids were affected by a complex dehiscence × storage time interaction, and they linearly correlated with certain morphometric characteristics ($r \geq 0.6$). Shell dehiscence status more than prolonged storage substantially modifies KP's quality.

Keywords *Pistacia vera* l. · Tocopherol · Tocotrienol · Fatty acid · Dehiscence · Shelf life

Introduction

Pistachio (*Pistacia vera* L.) is an economically important crop for many countries including Mexico. The global production of pistachios reached 0.78 million tons (in-shell basis) and their consumption spiked to 0.67 million tons between 2016 and 2017 (U.S. Dept. of Agriculture; USDA, 2018); according to *Transparency Market Research*® the pistachio global market will witness a moderate expansion between 2017 and 2026 since worldwide sales will

✉ Laura Alejandra de la Rosa
ldelaros@uacj.mx

✉ Abraham Wall-Medrano
awall@uacj.mx

Perla Judith Garcia-Moreno
al129262@alumnos.uacj.mx

Jazmin Cristina Stevens-Barron
jasmin_stev@hotmail.com

Roberto Rodríguez-Ramirez
roberto.rodriguez@itson.edu.mx

Baltazar Corral-Diaz
bcorral@uacj.mx

Emilio Alvarez-Parrilla
ealvarez@uacj.mx

Francisco Javier Olivas-Aguirre
javier_olivas22_09@hotmail.com

[1] Departamento de Ciencias Químico-Biológicas/Ciencias de La Salud, Instituto de Ciencias Biomédicas, Universidad Autónoma de Ciudad Juárez, Anillo Envolvente del PRONAF y Estocolmo s/n, 32310 Ciudad Juárez, Chihuahua, Mexico

[2] Departamento de Biotecnología Y Ciencias Alimentarias, Instituto Tecnológico de Sonora, 5 de Febrero 818 sur, Col. Centro, Ciudad Obregón, Son., Mexico

[3] Departamento de Ciencias de La Salud, Universidad de Sonora (Campus Cajeme), Blvd. Bordo Nuevo s/n, Antiguo Ejido Providencia, 85010 Ciudad Obregón, Son., Mexico

generate US$ 5000 MN revenues by 2026. Iran is the leading producer of pistachio in the world (261,000 tons per year; Fattahifar et al. 2018) followed by USA and Turkey but Mexico is considered an emerging producer (54 tons from a 131 Ha cultivated area). Since pistachios are rich dietary fiber, macro/micronutrients and a wide range of hydrophilic and lipophilic phytochemicals with health-promoting bioactivities (e.g. anti-inflammatory and antioxidant) against cardiovascular disease, diabetes mellitus and cancer (Stevens-Barron et al. 2019; Paterniti et al. 2017; Dreher 2012) in such a way that their market share within the functional food segment is important (Vergari et al. 2010).

Pistachio is a member of the cashew family; it is a monosperm fruit (drupe; Fig. 1) constituted of an outer yellowish hull (epicarp, Fig. 1a), a hard white shell (endocarp, Fig. 1b) and an inner edible seed (kernel, Fig. 1c) that reduces its size and change its color when dried (Fig. 1d); during ripening, the hull separates from the shell and the latter is forced to split as a result of kernel's enlargement (longitudinal dehiscence) and degree of cytodifferentiation (apical dehiscence), giving early-split (Fig. 1e), grown-split (Fig. 1f) and unsplit (Fig. 1g) nuts (shell + kernel) with different physical properties (Polito and Pinney 1999; Kashaninejad et al. 2006); shell splitting depends on many factors such as irrigation scheduling, use of fertilizers, harvest timing, parental genotypes and certain environmental conditions (Rabadan et al. 2018; Aliakbarkhani et al. 2017) and postharvest procedures including storing under proper oxygen level, light, and temperature should be controlled to achieve a maximum yield of good-quality open-shelled pistachios (Ghasemi-Varnamkhasti 2015; Tavakolipour 2015; Kader et al. 1982). However, nearly 1–5% of all freshly harvested pistachios remain unsplit (indehiscent) and additional post-production steps (e.g. re-sorting) or special equipment (e.g. compressive/striking machines) are often needed to split them out, often resulting in cracked nuts with reduced marketability (Maghsoudi et al. 2012).

The nutritional and functional value of pistachios (Terzo et al. 2017; Dreher 2012) is greatly influenced by drying techniques, storage conditions and other post-harvest procedures (Ling et al. 2016; Tavakolipour 2015). Extreme temperatures, packing materials, and improper storage accelerate deteriorative reactions, mainly in the lipophilic components of pistachio kernels (Ling et al. 2016; Tavakolipour et al. 2010), although they seem to be quite resistant to dehydration (Bahramabadi et al. 2018). However, scientific studies on the effect of prolonged storage and endocarp (shell) dehiscence in kernel's nutritional/functional profile are very scarce (Rabadán et al. 2018; Kader et al. 1982); here, we analyze the morphometric and chemical changes of 'Kerman' pistachios as affected by prolonged storage (1–4 years) and/or endocarp dehiscence (split/unsplit). To our knowledge, this is the first report exploring the individual or synergistic effect of these two factors on pistachios' quality.

Materials and methods

Chemicals

Pure (≥ 93%) standards [tocopherols (T), tocotrienols (T3), fatty acid methyl esters (FAME)] and ACS-grade salts and acids were obtained from Sigma-Aldrich-Fluka (St. Louis, MO, USA) or Cayman chemicals (Ann Arbor, MI, USA) while HPLC- or analytical-grade solvents were obtained from JT-Baker (Avantar Performance Materials S.A. de C.V., Mexico).

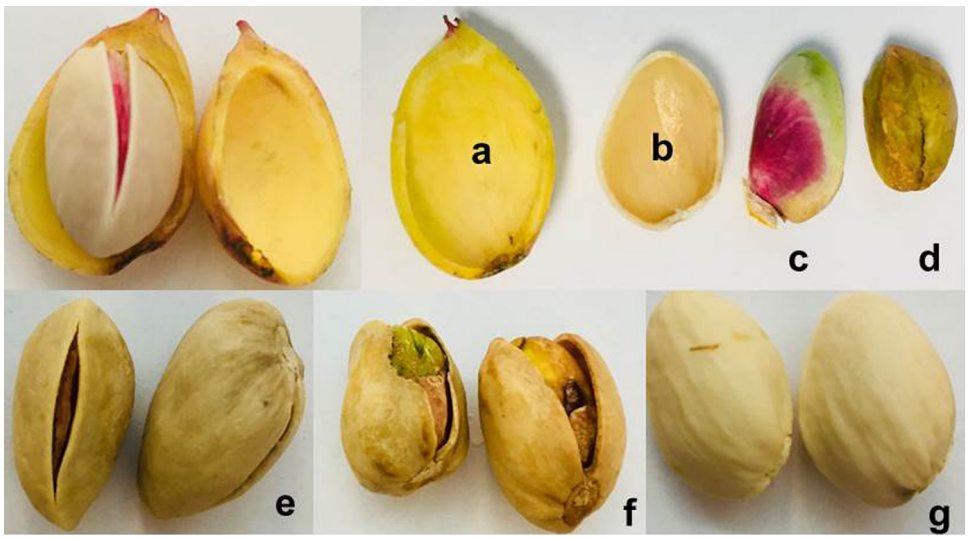

Fig. 1 'Kerman' pistachios. Hull (**a**), shell (**b**), un-dried (**c**) and dried (**d**) kernels, early-split (**e**), grown-split (**f**) and unsplit (**g**) pistachio nuts (shell + kernel)

Pistachio samples

Ripe 'Kerman" pistachio drupes (3–5% moisture; Fig. 1) were harvested at the University ranch property of the Autonomous University of Ciudad Juarez located in Praxedis G. Guerrero, Chihuahua, Mexico (31°21′10″N, 105°59′52″W; 1100 m altitude), during their harvest season (late August–September) from 2013 to 2016. Every year, mixed drupe lots were manually cleaned for foreign matters (dirt/dust), immature or damaged fruits, and harmful insects. Hulls were manually removed to separate complete endocarps (shell + kernel or "whole nut") from broken ones; whole nuts (1–2 kg) were distributed uniformly over a thin (2–5 mm) paper layer and left in the shade for 7 days at an average temperature of 33 ± 3 °C and average relative humidity of 41%. Dried in-shell nuts were then packed in conventional polyethylene bags and immediately stored at room temperature (~ 20 °C) and controlled humidity (15–20%) until august 2017, in order to obtain samples with one (2016) to four (2013) years of storage.

Triplicate samples from each lot were screened for grown-split (95–96%) early-split (3–4%) and unsplit (~ 1–2%) kernels. After morphometric analyses, kernels coming from split (grown) and unsplit whole nuts were finely ground in a coffee grinder (Black and Decker Canada Inc., Brockville, ON, Canada), sifted using a 1-mm mesh sieve and kept in vacuum-sealed plastic containers at − 20 °C until used for chemical analyses.

Morphometric analysis

The length (L; mm), width (W, mm), thickness (T, mm) and weight (g) of 50–70 in-shell nuts (shell + kernel) and kernels alone per lot (storage time) and dehiscence status (split, unsplit) were measured with a digital Vernier (0.001 mm) and an electronic scale (± 0.01 g), following the recommendations of Kashaninejad et al. (2006). Assuming that W = T for ripe 'Kerman' pistachios with kernel's moisture ≤ 8% (Hsu et al. 1991), the total 'ellipse' surface area (A, mm^2) and 'ellipsoid' volume (V, mm^3) were calculated with Eqs. 1 and 2.

$$A\left(cm^2\right) = [\pi \cdot (1/2L) \cdot (1/2W)]/10 \quad (1)$$

$$V\left(cm^3\right) = [(4\pi/3) \cdot (1/2L) \cdot (1/2W) \cdot (1/2T)]/10 \quad (2)$$

Chemical analysis

Macronutrient analyses (g 100 g^{-1}; cv ≤ 10%) were performed in kernel samples by triplicate, using the Association of Official Analytical Chemists methods (AOAC 2002): Moisture (925.10), protein (920.10), ash (942.05), lipid (920.39) and total carbohydrates (by difference).

Oil extraction

Oil was extracted from pistachio samples at room temperature to minimize loss of tocols. Ten grams of shelled pistachios were homogenized (Ultra-Turrax T 25 basic; IKA®WERKE, Germany) with 100 mL of hexane (1:10 w/v) for 3 min (Stevens-Barron et al. 2019), sonicated (3510-model ultrasonic bath; Branson, Wethersfield, CT, USA) three times for 15 min and the mixture was filtered through a Whatman #4 filter and the solid re-extracted two more times. Filtrates recovered from all three extractions were combined and hexane removed by rotary evaporation at 40 °C. Water residues were removed from the oil by filtering through a layer of Na_2SO_4 anhydride. Oil samples were weighed, transferred to amber bottles, sealed with nitrogen and stored at − 80 °C until use.

Chromatographic analysis of fatty acids

Fatty acids from pistachio edible oils (g 100 g^{-1}) were analyzed by gas chromatography. FAME were obtained according to Isbell et al. (2008) as follows: 0.25 mL of KOH (0.5 M) in methanol was added to 1 g of oil and incubated at 60 °C for 1 h; then, 0.25 mL of H_2SO_4 (1 M in methanol) was added and incubated for another 15 min at 60 °C; finally, 0.25 mL of a saturated saline solution (NaCl) + 1 mL of hexane were added, allowing to stand for two-phase separation (FAME were recovered in upper layer). Quantification was performed as described by Núñez-Gastélum et al. (2018) with certain modifications. The equipment consisted of a gas chromatograph 3800 (Varian Inc., Palo Alto, CA) with a flame ionization detector, a capillary column CP7485 88 (25 mm, 0.32 mm i.d. and thickness). Running conditions were: Injection volume (1 µL), gas carrier (helium, 0.6 mL min^{-1}), constant detector temperature (250 °C), and column temperature (50 °C for 1 min, 220 °C at a rate of 4 °C min^{-1} for 1 min, and 240 °C, maintained for 5 min). Quantification of FAME was achieved by comparing the area under the curve (AUC) of each peak with those of pure standards and expressed as g 100^{-1} per 100 g^{-1} oil; lastly, the following ratios were calculated: monounsaturated/saturated (MUFA/SFA), polyunsaturated/saturated (PUFA/SFA), MUFA/PUFA, linoleic/palmitic (c18:2/C16:0; oil stability index) fatty acid ratios (Wall-Medrano et al. 2017).

Chromatographic analysis of tocols

The content of tocols [tocopherols (T) + tocotrienols (T3)] in cold hexane-extracted oils (mg 100 g^{-1}oil) was

analyzed by high-performance liquid chromatography (HPLC) system (Perkin Elmer model 200 series HPLC equipment) with a quaternary pump, auto-sampler and fluorescence detector (p/n N292-2006). The isocratic normal phase was 0.9% isopropanol in *n*-hexane, normal phase column (SupercosilL, 250 × 4.6 mm, 5 µ, Phenomenex Inc., Torrence CA) and wavelengths were 285 (excitation) and 325 (emission) nm (Chun et al. 2006). The flow rate was 1 mL/min. Sixty mg of oil in 1 mL of *n*-hexane was filtered through syringe membrane filters (Nylon, 0.45 µM) and placed in HPLC vials. Isoforms of T and T3 peaks were identified by retention times of individual standards [αT, αT3, γT, γT3, δT] and a tocotrienol-enriched extract from palm oil (Tocomin SupraBio ®) that contains 17% T3 (8% γ-T3, 5% α-T3, 3% δ-T3, 1% β-T3) was used to provide reference retention from βT3 and δT3. The results were expressed in mg/100 g^{-1}oil; βT3 and δT3 were estimated by linear regression equations from built for γT3 and δT, respectively.

Statistical analysis

All variables were expressed as the mean ± standard deviation (SD) from at least three replicates per evaluated parameter. All variables were checked for normality (± 1.96) and homogeneity of variance to avoid biased analysis (Boukid et al. 2019). One-way ANOVA followed by Tukey's HSD post hoc test was used to evaluate pairwise differences (95% confidence limits) between storage time (1–4 years) and endocarp dehiscence status (split vs. unsplit). Spearman's rank correlation coefficients (ρ) were used to establish all possible monotonic relationships between response variables (morphometric, macronutrient composition, fatty acids and tocols) and storage time (ordinal variable), both in split and unsplit nuts while Pearson's product-moment correlation (r; + 1 to − 1) was used to establish the strength of linear association between two continuous variables.

To delve into which variables (including storage time) were responsible for the associated variance in total lipids, MUFA and T + T3 concentrations by shell dehiscence status, step-wise (logistic and linear) regression analysis was used after testing variables for multicollinearity. The best prediction models were selected based on the lowest number of independent variables at which a maximum coefficient of determination (R^2) and the lowest root-mean-square error (RMSE) was achieved. All statistics were performed with the NCSS® (version 6.0; NCSS LLC, Kaysville, UT, USA) or Minitab® (version 16.0; Minitab Inc., State College, PA) statistical software.

Results and discussion

In-shell pistachios are normally consumed as raw, salted/seasoned or toasted nuts while cracked kernels are used as ingredients in fermented meats, ice creams, bakery/confectionery products, sauces and puddings (Tavakolipour et al. 2010). However, pre- and post-harvest procedures have a huge impact on the final sensorial traits and phytochemical profile of edible nuts (Christopoulos and Tsantili 2012) including pistachios (Rabadán et al. 2018; Ghasemi-Varnamkhasti 2015; Tavakolipour 2015) while shell (endocarp) dehiscence and log-term storage are important factors for their marketability.

Morphometry

As compared to other varieties, 'Kerman' pistachios are preferred by consumers, producers, and industrial processors, due to their excellent quality and sensorial characteristics (Rabadan et al. 2018; Polito and Pinney 1999; Hsu et al. 1991), their size is above average and their kernels are easily removed from shells (Fig. 1). However, they are often bulk-stored in woven baskets or wood/metal containers in local farms and popular marketplaces or silo-stored at agro-industrial facilities (Beck et al. 2017), kept in the shadow for very long periods. Whether these common practices affect the quality and nutritional/phytochemical profile this "green nut" is practically unknown (Kader et al. 1982).

Morphometric changes of 'Kerman' whole nuts (in-shell kernels) during storage for both shell dehiscent (split) or not (unsplit) samples are reported in Table 1. One-way ANOVA analysis revealed that morphological changes in whole nuts were more evident by dehiscence status (unsplit > split) than by storage time (no clear time-trend). Splitted nuts were 2.8, 0.7 and 0.8 shorter (mm) and 0.4, 0.3 and 0.3 wider (mm) than unsplit ones ($p \leq 0.001$) in samples with 1–3 years of storage and the same phenomenon occurred with their length-to-width ratio [1.46 (split) vs. 1.52 (unsplit); data not shown], surface area and volume in the first 2 years of storage; these results are in agreement with all morphometric parameters reported by Hsu et al. (1991) and Kashaninejad et al. (2006).

Also, kernel's length (3–4 year), width (1–3 year), area and volume (2–4 year) but not its mass were more affected by dehiscent status (split > unsplit) than by storage time (no clear time-trend) and kernel's length-to-width ratio [1.65 (split) vs. 1.69 (unsplit); data not shown] was lesser to that reported by Boukid et al. (2019) for pistachio kernels from Iran and USA (∼ 1.76). Many factors could be explaining these physical differences including different agronomic practices, environmental and geographical

Table 1 Morphometric and macronutrient composition of 'Kerman' pistachios

Storage (Y)	Length (mm)	Width (mm)	Area (cm^2)	Volume (cm^3)	Mass (g)	Moisture (g 100 g^{-1})	Protein (g 100 g^{-1})	Lipids (g 100 g^{-1})	Ash (g 100 g^{-1})	Carbs (g 100 g^{-1})
Shell—Split										
1	18.2 (0.8)^{b}	12.9 (0.7)^{a}	18.4 (1.5)^{ab}	159 (21)^{ab}	0.6 (0.1)^{a}	–	–	–	–	–
2	18.0 (0.9)^{b}	12.3 (0.5)^{c}	17.4 (1.3)^{b}	142 (21)^{b}	0.5 (0.1)^{b}	–	–	–	–	–
3	19.5 (0.8)^{a}	12.9 (0.6)^{a}	19.8 (1.5)^{a}	171 (20)^{a}	0.6 (0.1)^{a}	–	–	–	–	–
4	18.0 (0.8)^{b}	12.5 (0.6)^{b}	17.6 (1.2)^{b}	147 (17)^{b}	0.6 (0.1)^{a}	–	–	–	–	–
Shell—Unsplit										
1	21.0 (1.2)^{a}*	13.3 (0.6)^{a}*	22.0 (1.8)^{a}*	195 (24)^{a}*	0.8 (0.1)^{a}	–	–	–	–	–
2	18.7 (0.9)^{c}*	12.6 (0.6)^{b}*	18.5 (1.4)^{b}*	156 (19)^{b}*	0.6 (0.1)^{c}	–	–	–	–	–
3	20.3 (0.8)^{b}*	13.2 (0.6)^{a}*	21.1 (1.2)^{a}	185 (18)^{a}*	0.7 (0.1)^{b}	–	–	–	–	–
Kernel- Split										
4	18.1 (0.8)^{c}	12.3 (0.6)^{c}	17.4 (1.3)^{c}	143 (17)^{c}	0.5 (0.1)^{c}	–	–	–	–	–
1	14.4 (1.5)^{b}	9.4 (1.3)*	11.0 (1.4)^{b}	71 (14)	0.6 (0.1)^{b}	3.3 (0.3)	20.2 (0.8)^{a}	45.2 (1.0)^{a}*	2.9 ± 0.1^{a}	28.6 (1.6)^{a}
2	15.6 (0.8)^{a}	9.2 (0.5)*	11.4 (1.0)^{a}*	70 (10)	0.6 (0.1)^{b}	3.6 (0.2)	19.9 (0.8)^{a}	48.8 (2.3)^{b}	2.9 ± 0.0^{a}	23.3 (1.9)^{b}
3	16.0 (1.0)^{a}*	9.6 (0.8)*	11.8 (1.2)^{a}*	75 (12)*	0.7 (0.1)^{a}	3.2 (0.3)	19.7 (1.4)^{ab}	49.7 (0.8)^{b}	2.9 ± 0.2^{ab}	24.5 (1.9)^{b}
4	15.8 (0.6)^{a}*	9.2 (0.6)^{b}	11.5 (1.0)^{a}*	71 (11)*	0.6 (0.1)^{b}	3.6 (0.1)	18.8 (0.6)^{b}	49.8 (0.3)^{b}	3.1 ± 0.1^{b}	24.7 (0.5)^{b}
Kernel- Unsplit										
1	15.2 (2.3)^{a}	8.8 (1.3)^{ab}	11.5 (1.3)^{a}*	71 (14)^{a}	0.7 (0.1)^{a}	4.5 (0.4)^{b}*	20.4 (0.8)^{ab}	42.9 (0.6)^{a}	3.1 (0.1)	29.1 (0.4)^{a}
2	15.3 (1.3)^{ab}	8.4 (1.3)^{b}	10.5 (1.3)^{a}	61 (9)^{ab}	0.6 (0.1)^{b}	3.7 (0.3)^{a}	21.5 (0.8)^{a}*	46.6 (2.0)^{b}	3.0 (0.1)	24.8 (1.0)^{b}
3	14.1 (2.2)^{b}	8.9 (2.0)^{ab}	9.1 (2.1)^{b}	52 (17)^{b}	0.7 (0.1)^{a}	3.8 (0.4)^{a}	21.3 (0.6)^{a}	48.8 (2.0)^{b}	2.9 (0.2)	23.0 (2.9)^{b}
4	14.8 (0.8)^{ab}	9.2 (0.8)^{a}	10.8 (0.9)^{a}	67 (10)^{a}	0.6 (0.1)^{b}	3.8 (0.2)^{a}	20.5 (0.5)^{b}*	47.8 (0.5)^{b}	3.1 (0.1)	24.8 (0.8)^{b}

values are expressed as mean (SD)

Different superscript letters for a same parameter, same dehiscence status (split or unsplit) and same type of sample (shell + kernel or kernel alone) means statistical differences ($p \leq 0.01$) by storage time while an asterisk (in the higher value) means statistical differences ($p \leq 0.001$) for a same parameter and type of sample between split (dehiscent) and unsplit endocarps

Mass (g) = shell (g) + kernel (g). Not analyzed (–)

conditions or parental genotypes (Rabadan et al. 2018; Aliakbarkhani et al. 2017). According to Polito and Pinney (1999), the width of dried 'Kerman' shells is 12.3 mm (unsplit) and 11.7 mm (split) while that of its kernel remain the same (9.7 mm), as happened in our study for split nuts.

In general, there were no apparent time-trend changes in kernel' mass (g) (Table 1). Regression analysis confirmed that kernel's mass, surface area and volume have no monotonic correlation with storage time (Table 2), even though the statistical correlation power (ρ) is particularly stronger in unsplit than split nuts and the fact that all morphometric parameters correlated with each other in split and unsplit nuts ($r \geq 0.69$, $p \leq 0.03$; data not shown). It is necessary to remember that longitudinal shell splitting (Fig. 1) is the most common dehiscence form but also the most important for consumers and is the result of a plethora of biochemical and physiological factors originated in kernel's embryo producers (Bahramabadi et al. 2018; Polito and Pinney 1999).

Chemical composition

According to Table 1, the average macronutrient composition of 'Kerman' pistachio kernels was as follows: water (~ 4%), protein (~ 20%), fat (~ 47%), carbohydrates (~ 25%) and ash (~ 3%), regardless storage time and shell dehiscence status. One-way ANOVA comparison of kernel's from split vs. unsplit samples showed statistical differences for certain parameters at a certain storage time: moisture (1st year), fat (1st year) and protein (2nd and 4th years). It is well known that water activity (A_w) influences certain quality parameters of edible nuts such as mold and aflatoxin contamination (high A_w), shrivel (low A_w), color and rancidity (Tavakolipour 2015; Barden and Decker 2016; Ghirardello et al., 2013). The structural rearrangement of the kernel (split and unsplit) as resulting from mild dehydration in the first year seems to be responsible for these changes in macronutrient composition (Bahramabadi et al. 2018). Best storage conditions for Iranian kernels ranges between 65 and 70% of relative humidity and during processing should be at 55–65% and 6% kernel's moisture, in order to preserve the chemical stability of its volatile compounds (Beck et al. 2017), as seems to be the case in our study.

Statistically significant ($p \leq 0.05$) time-trend relationships with storage time were evidenced by Spearman's monotonic correlation (ρ; Table 2) for the following kernel's parameters by shell dehiscent status [Split: protein

Table 2 Bivariate and multivariate regression analyses

Dependent	Independent	r_s-S	r_s-US	Dependent	Independent	r_s-S	r_s-US
Bivariate (Spearman rank correlation, SRC)							
Mass	Storage	0.11	− 0.22	Total lipids	Storage	0.58*	0.66*
Area	Storage	0.02	0.17	Tocopherols	Storage	0.56*	0.81*
Volume	Storage	0.02	0.17	Tocotrienols	Storage	− 0.10	− 0.11
Moisture	Storage	0.26	− 0.54*	Total tocols	Storage	0.53*	0.81*
Protein	Storage	− 0.48*	− 0.05	SFA	Storage	0.29	− 0.29
Carbohydrates	Storage	− 0.41*	− 0.57*	MUFA	Storage	− 0.24	0.04
Ash	Storage	0.42*	0.03	PUFA	Storage	0.39	0.00

Dependent	Independent	Shell	β	R^2	p	RMSE
Multivariate (Stepwise linear regression, SLR)						
Total lipids	Carbohydrates	Split	− 0.95	0.90	< 0.001	0.52
Total tocols	PUFA	Split	0.83	0.68	0.003	3.60
MUFA	Area (M1)	Split	1.24	0.71	0.002	2.50
	PUFA (M2)	Split	− 0.61	0.17^{Δ}	0.040	
Total lipids	Carbohydrates	Unsplit	− 0.97	0.93	0.001	0.80
Total tocols	Total lipids	Unsplit	0.87	0.76	0.001	2.54
MUFA	Area (M1)	Unsplit	0.66	0.43	0.001	2.10
	Moisture (M2)	Unsplit	− 0.59	0.34^{Δ}	0.002	

Chemical composition data from kerman pistachio kernels

Statistically significant (p-trend ≤ 0.04)

SLR model of one (M1) and two (M2) factors, Monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids, Spearman's coefficient (rs) for split (rs-S) and unsplit (rs-US) samples, determination coefficient (R^2), incremental value (Δ from M1), root mean square error (RMSE)

(− 0.48), ash (0.42); unsplit: moisture (− 0.54); both: carbohydrates (split − 0.41, unsplit − 0.57) and lipids (split 0.58, unsplit 0.66)]. According to Kader et al. (1982) 'Kerman' pistachios dried to 4–6% are very stable and can be held for up to 12 months at a temperature as high as 20 °C without losses in quality attributes, as long as they are kept at a relative humidity that is in equilibrium with their moisture content. Also, Ghasemi-Varnamkhasti (2015) stated that storing four Iranian pistachio varieties ('Kale-Ghouchi', 'Akbari', 'Ohadi' and 'Momtaz') at low temperatures improves their chemical stability up to 3 months. Hsu et al. (1991) reported that in 'Kerman' pistachios kept at different moisture levels from 7.6 to 37.5%, their length, width and thickness increased with increasing moisture content as represented by third-degree regression equations ($R^2 \geq 0.98$); the authors also reported almost perfect linear relationships between moisture content and bulk density, specific gravity, surface area and several thermal properties of pistachios ($R^2 \geq 0.92$) and that shell splitting increases as moisture content decreases. Based on these facts it is clear that maximum drying is achieved during the prolonged storage of pistachios, a fact that improves its quality and nutritional profile (Kader et al. 1982).

Fatty acids

Pistachios are considered nutrient-dense snacks with health-promoting properties (Stevens-Barron et al. 2019; Paterniti et al. 2017; Dreher 2012). However, the market share of pistachios not only relies on its sensorial and nutritional characteristics but also in its incremental demand by health-conscious consumers who seeks for functional foods for preventing many chronic diseases such as type 2 diabetes, endothelial diseases and cancer (Vergari et al. 2010). Particularly, pistachio's (MUFA + PUFA)/SFA ratio (and possibly other lipophilic phytochemicals) promote heart-healthy blood lipids (Terzo et al. 2019; Dreher 2012).

The fatty acid profile (g 100 g^{-1} oil) of 'Kerman' pistachio kernels by storage time and endocarp dehiscence status is shown in Table 3. Oleic (C18:1n9*c*) and linoleic (C18:2n6*c*) acids represented 76–85%, followed by palmitic acid (C16:0; ∼ 13%), which coincides with the profile reported for several pistachios grown in USA (California), Iran, USA, Turkey, Syria, Kyrgyzstan and Italy (Boukid et al. 2019; Kader et al. 1982) and 'Kerman' pistachios from Spain (Rabadán et al. 2018). However, the high linoleic content has a negative impact on the chemical stability of edible nut oils (Ghirardello et al. 2013) including that of 'Kerman' pistachios which is characterized by a high production of dienes (K_{232}, 1.5) and conjugated trienes (K_{270}, 0.1) and a low oxidative stability (25–31 h; rancimat technique) as compared to other varieties (Rabadán et al. 2018). Storage temperatures should be selected upon the expected shelf life since the lower temperature and the lower peroxidation rate (Tavakolipour 2015).

One-way ANOVA showed that both storage time and shell dehiscence status had significant ($p \leq 0.05$) effects in all fatty acids (Table 3), besides the obvious differences in concentration for α-linolenic (C18:3n3), arachidic (C20:0) and behenic (C22) acids. In fact, a complex interaction between storage time and dehiscence status affected the specific amount of certain fatty acids. For example, capric (C10:0; split) and palmitoleic (C16:1; unsplit) acids were affected in a time-dependent manner ($p \leq 0.04$) but not by dehiscence status while the opposite happened for oleic acid (C18:1n9*c*; $p \leq 0.04$); statistical differences by shell dehiscence status were more evident in total SFA (split > unsplit) and PUFA (unsplit > split) but not for MUFA concentration. While the amount of fatty acid subgroups (SFA/MUFA/PUFA) did not showed any monotonic relationship with storage time ($\rho = -0.29$ to 0.39; Table 2) or total lipids ($r = -0.59$ to 0.15, $p > 0.05$; data not shown) although they did with kernel's mass ($r \geq 0.60$), surface area ($r \geq 0.62$), volume ($r \geq 0.69$) in split pistachios ($p \leq 0.03$; data not shown).

Ghirardello et al. (2013) showed that lipid content and fatty acid subgroups in hazelnuts are very stable for 1 year when stored at room temperature and 70% or relative humidity. Since the fatty acid profile of pistachio oil is also affected by genotype and seasonality (Rabadán et al. 2018), the underlying explanation to our results seems to go beyond the studied factors, possibly as a result of the interaction of fatty acids with other phytochemicals including tocols (Yalcin and Schreiner 2018; Ojeda-Amador et al. 2018) and phenolic compounds (Tsantili et al. 2011; Christopoulos and Tsantili 2012). Also, SFA, MUFA, and PUFA content when expressed as ratios did not provide additional evidence as to a time-trend effect in both split and unsplit pistachios. These fatty acid ratios in split pistachios with one year of storage are quite similar to those from Iran and Kyrgyzstan while unsplit ones are more similar to those from Turkey (Boukid et al. 2019). Moreover, the oil stability index (c18:2/C16:0; ∼ 2.3) is similar to that reported by Rabadán et al. (2018) for 'Avidon' and 'Kerman' pistachios which implicates a better stability than grapeseed (∼ 9) and corn (∼ 4) oils (Wall-Medrano et al. 2017).

Tocols

From a nutraceutical standpoint, 'Kerman' pistachios have almost the same oil and sterol content yet are richer in linoleic acid and γ-tocopherol when compared to other

Table 3 Fatty acid composition of 'Kerman' pistachio kernel oil

Fatty acid (g 100 g^{-1})	Storage (y)			
	1	2	3	4
Split				
C10:0	2.3 (0.2)^{c}	2.8 (0.2)^{bc}	3.2 (0.3)^{b}	5.9 (0.5)^{a}*
C16:0	13.2 (1.1)^{ab}	11.8 (0.8)^{b}	14.7 (1.0)^{a}	12.2 (1.0)^{b}
C16:1	1.2 (0.1)	1.4 (0.1)	1.2 (0.1)	1.4 (0.1)
C18:0	1.6 (0.1)^{ab}	1.4 (0.1)^{b}	1.8 (0.1)^{a}	nd
C18:1n9*c*	57.4 (4.6)^{ab}*	59.6 (4.8)^{a}	54.5 (4.4)^{b}*	55.2 (5.0)^{b}
C18:2n6*c*	23.8 (1.7)^{a}	20.9 (1.7)^{b}	24.2 (1.9)^{a}	24.8 (1.7)^{a}
C18:3n3	0.4 (0.0)	nd	0.4 (0.0)*	0.4 (0.0)
C20:0	0.2 (0.0)^{b}	0.7 (0.1)^{a}*	nd	nd
Σ SFA	17.3 (1.4)^{c}*	17.2 (1.4)^{c}*	19.7 (1.6)^{a}	18.1 (1.5)^{b}*
Σ MUFA	58.5 (4.7)*	61.2 (4.9)	55.7 (4.5)*	56.6 (5.1)
Σ PUFA	24.2 (1.9)^{b}	21.6 (1.7)^{c}	24.6 (2.0)^{b}	25.3 (2.0)^{a}
Unsplit				
C10:0	2.1 (0.2)^{b}	2.7 (0.2)^{b}	4.2 (0.3)^{a}*	nd
C16:0	12.4 (1.0)^{ab}	11.6 (0.9)^{b}	15.0 (1.2)^{a}	12.1 (1.1)^{b}
C16:1	1.0 (0.1)^{b}	1.3 (0.1)^{a}	1.3 (0.1)^{a}	1.4 (0.1)^{a}
C18:0	1.4 (0.1)^{a}	1.1 (0.1)^{b}	1.6 (0.1)^{a}	1.0 (0.1)^{b}
C18:1n9*c*	53.6 (4.3)^{b}	60.2 (4.8)^{a}	50.5 (4.6)^{b}	57.3 (4.0)^{ab}
C18:2n6*c*	28.4 (2.0)^{a}*	22.8 (1.6)^{c}*	25.3 (2.0)^{b}	27.9 (2.0)^{a}*
C18:3n3	0.5 (0.0)^{a}	0.4 (0.0)^{ab}*	nd	0.4 (0.0)^{ab}
C20:0	0.3 (0.0)^{b}	nd	0.5 (0.0)^{a}*	nd
C22:0	0.4 (0.0)^{b}*	nd	1.2 (0.1)^{a}*	nd
Σ SFA	16.5 (1.3)^{b}	15.3 (1.2)^{c}	22.4 (2.0)^{a}*	13.0 (1.2)^{d}
Σ MUFA	54.6 (4.4)^{bc}	61.6 (4.9)^{a}	52.4 (4.2)^{bc}	58.6 (4.7)^{ab}
Σ PUFA	28.9 (2.3)^{a}*	23.1 (1.9)^{b}*	25.3 (2.0)^{ab}	28.3 (2.6)^{a}*

Values are expressed as mean (SD)

Different superscript letters within a same line (parameter) means statistical differences ($p \leq 0.04$) while an asterisk (in the higher value) means statistical differences ($p \leq 0.04$) for a same parameter between split (dehiscent) and unsplit endocarps

Saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids. Not detected (nd)

varieties (Ojeda-Amador et al. 2018) and dry roasted/salted pistachios have more γ-tocopherol but less α-tocopherol than dry roasted/salted almonds (Dreher 2012). The 'Kerman' pistachios studied here were also good sources of γ-T (4.9–18.1 mg 100 g^{-1} oil) and δ-T3 (1.2–1.6 mg 100 g^{-1} oil), regardless shell dehiscence status and storage time (Table 4). The natural distribution and amount of tocols in edible nuts greatly depends on pre- and post-harvest procedures and storage conditions, but pistachios always rank within the highest as tocol sources (~ 116.4 mg/kg of fresh kernels; Stevens-Barron et al. 2019); it is noteworthy that tocopherols (T) play important roles as radical scavengers and COX-2 inhibitors while tocotrienols (T3) are more effective anticancer agents (Dreher 2012).

Also, the content of γ-T and total-T increased but δ-T decreased overtime in unsplit but not split pistachios (Table 4) while a time-trend monotonic relationship was found for T + T3 in split (ρ = 0.53) but mostly unsplit (ρ = 0.81) pistachios (Table 2). To our knowledge, this is the first report observing this particular issue, suggesting that the metabolic activity of kernel's embryo extends throughout shelf life when it is not exposed to adverse environmental conditions. Pearson's product-moment correlation analysis (data not shown) revealed that total tocol (T + T3) content directly correlated with total lipids ($r = 0.87$) in unsplit samples and PUFA ($r = 0.83$), SFA ($r = 0.64$) and protein ($r = -0.63$) content in split samples; total-T content linearly correlate with total lipid content ($r = 0.82$; unsplit kernels) and PUFA ($r = 0.80$; split kernels) and protein ($r = -0.66$; split kernels) contents while total tocotrienols (T3) correlated linearly with total mass ($r = 0.61$; split kernels), SFA ($r = 0.74$; split kernels), protein ($r = 0.77$; unsplit kernels), SFA ($r = 0.77$; unsplit kernels) and ash ($r = -0.82$; unsplit kernels).

Table 4 Tocol content in 'Kerman' pistachio kernels

Tocol (mg kg^{-1})	Storage (y)			
	1	2	3	4
Split				
αT	1.8 (0.1)	1.9 (0.1)	2.0 (0.4)	2.0 (0.1)
βT	1.5 (0.0)	1.8 (0.2)	1.5 (0.2)	1.7 (0.1)
γT	13.2 (2.4)*	13.4 (1.1)	13.2 (3.6)	18.1 (1.3)
δT	1.5 (0.0)	1.5 (0.0)	1.4 (0.1)	1.5 (0.1)
αT3	nd	0.5 (0.0)	nd	nd
βT3	nd	nd	nd	nd
γT3	0.3 (0.0)^{b}	0.2 (0.0)^{b}	1.5 (0.5)^{a}	0.5 (0.0)^{b}
δT3	1.4 (0.1)	1.5 (0.0)	1.4 (0.1)	1.5 (0.1)
ΣT	17.9 (2.3)^{ab}*	11.8 (7.6)^{b}	18.1 (4.3)^{ab}	23.3 (1.6)^{a}
ΣT3	1.7 (0.2)^{b}	2.2 (0.1)^{ab}	2.9 (0.7)^{a}	1.8 (0.3)^{b}
ΣT + T3	20.1 (2.2)^{ab}	13.5 (7.6)^{b}	21.2 (5.2)^{ab}	25.2 (1.9)^{a}
Unsplit				
αT	2.0 (0.1)^{a}	1.9 (0.0)^{ab}	1.6 (0.2)^{b}	1.7 (0.2)^{ab}
βT	1.8 (0.3)	1.8 (0.3)	1.3 (0.2)	1.3 (0.2)
γT	4.9 (1.8)^{b}	10.5 (1.4)^{a}	10.5 (0.7)a	13.8 (2.1)^{a}
δT	1.6 (0.1)^{b}	1.6 (0.1)^{b}	1.2 (0.2)^{a}	1.3 (0.1)^{a}
αT3	nd	0.6 (0.0)	0.4 (0.1)	nd
βT3	nd	nd	nd	nd
γT3	0.3 (0.1)^{b}	0.1 (0.0)^{c}	1.7 (0.1)^{a}	0.3 (0.1)^{b}
δT3	1.4 (0.1)	1.5 (0.0)	1.2 (0.2)	1.3 (0.1)
Σ T	9.1 (3.0)^{c}	15.7 (1.6)^{ab}	14.5 (1.3)^{b}	18.1 (0.8)^{a}
Σ T3	2.0 (0.3)^{b}	1.9 (0.4)^{b}	3.3 (0.3)^{a}	1.7 (0.3)^{b}
Σ T + T3	11.2 (2.9)^{b}	17.6 (2.0)^{a}	17.8 (1.6)^{a}	19.8 (0.7)^{a}

Values are expressed as mean (SD)

Different superscript letters within a same line (parameter) means statistical differences ($p \leq 0.04$) while an asterisk (located in the higher value) means statistical differences ($p \leq 0.04$) for the pairwise comparison between split (dehiscent) and unsplit endocarps; not detected (nd). Tocopherol (T), tocotrienol (T3) isoforms (α, β, γ, δ)

Many factors can be acting synergistically to explain tocol fluctuation during storage including water loses, kernel's microstructure rearrangement, chemical interaction with other lipophilic compounds (e.g. fatty acids) or antioxidant phytochemicals (e.g. phenolic compounds) and kernel's embryo metabolic activity (Bahramabadi et al. 2018; Polito and Pinney 1999). On the later, it is known that α-T, β-T and α-T3 concentrations (the natural pathway of tocol accumulation) have a very strong positive correlation with total lipids ($R^2 \geq 0.76$, $p < 0.0001$) in almonds during ripening (Zhu et al. 2017) an so, the observed relationship between total tocol (T + T3) content and total lipids ($r = 0.87$) and the fact that γ-T increase while δ-T decreases over time, could be also related to kernel's metabolic activity during storage (Bahramabadi et al. 2018; Polito and Pinney 1999). Another possible explanation relies on isoform fluctuations as resulting from antioxidant defense: α-T, produced from γ-T, plays important roles on maintaining the redox status and chloroplast function in plants exposed to abiotic stress while T + T3 scavenge lipid peroxy radicals to avoid membrane damage, resulting in tocopheroxyl and tocotrienoxyl radicals which are recycled back to T and T3 by a concerted action with other antioxidants (Fritsche et al. 2017; Munné-Bosch and Alegre 2002).

Chemometrics

In food science, chemometric tools are more often used to stablish statistical relationships between chemical composition parameters and influencing factors; studies on the influence of harvesting time, parental genotypes, short-term storage or geographic location on the chemical composition of edible pistachios and oils (Rabadan et al. 2018; Beck et al. 2017), sensory traits (Ghasemi-Varnamkhasti 2015), nutrient profiles (Aliakbarkhani et al.

2017) or physical properties (Boukid et al. 2019) of pistachios, have been documented. In this study, Spearman-rank correlations between certain physicochemical characteristics (morphometric and chemical composition) and storage time/dehiscence status (Table 2) prompt us to conclude the following (data not shown): (A) There is a complex interaction between dehiscence status and storage time affecting the pistachio kernel's morphometry and fatty acid content, (B) Kerman pistachio's macronutrient composition was not much affected by both factors and, (C) its total and specific tocol composition is more influenced by other lipophilic compounds than by dehiscence status or storage time.

Furthermore, multivariate stepwise linear regression (SLR) helped to identify certain predicting compositional factors associated to changes in major lipophilic components in Kerman pistachio kernels (Table 2):

- *First*, since the content of lipids + carbohydrates represented ~ 70% of the macronutrient composition of 'Kerman' pistachios (Table 1), it was not surprising to find out that ≥ 90% of total lipid's associated variance was inversely explained by their total carbohydrate content ($p \leq 0.001$; RMSE ≤ 0.80), while other macronutrient variables did not.
- *Second*, 88% ($p \leq 0.04$, RMSE = 2.50) of the associated variance to MUFA content was explained by changes in surface area ($R^2 = 0.71$) and PUFA content ($R^2 = 0.17$) in split nuts while 77% of that of unsplit nuts ($p \leq 0.002$, RMSE = 2.10) was explained by surface area ($R^2 = 0.43$) and moisture content ($R^2 = 0.34$). It is well known that heat transfer in split nuts is higher than in unsplit ones, triggering deteriorative reactions (e.g. lipid peroxidation) and possibly adaptive metabolic transformations between fatty acid subclasses, although total fatty acids also increase during kernel's drying (Tavakolipour et al. 2010).
- *Third*, the associated variance to T + T3 content was explained by PUFA content ($R^2 = 0.68$, $p = 0.003$, RMSE = 3.60) or total lipids ($R^2 = 0.76$, $p = 0.001$, RMSE = 2.54) in split and unsplit nuts respectively. As previously mentioned, this linear relationship may be related to an active unsplit kernel that conveniently turns on/off tocol anabolic pathways upon kernel's defense against abiotic stress in a more controlled environment than that of the split nuts (Fritsche et al. 2017; Munné-Bosch and Alegre 2002).

Many factors may be involved in the transient fluctuations of specific tocols and fatty acid subgroups including oxygenation exposure, cross-talk between chemical species, kernel's metabolic activity, and many others; also, water may help to reduce lipid peroxidation by building hydrogen bonds with lipid hydroperoxides, increasing the oxidative stability of lipophilic phytochemicals in low-moisture foods (Barden and Decker, 2016).

Conclusion

In this study, we demonstrated that the nutritional and phytochemical profile of 'Kerman' pistachios shell is more affected by shell dehiscence than prolonged storage (up to 4 years) when packed in conventional polyethylene bags and immediately stored at room temperature (~ 20 °C) and controlled humidity (15–20%). Prolonged stored unsplit 'kerman' pistachios may have a market opportunity in the functional food-nutraceutical segment, as evidenced in this study on their preserved fatty acid and tocol profile.

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Author contributions PJG-M and JCS-B carried out most of the experiments, analyzed and interpreted the original data and contributed in drafting the manuscript; LA-DLR and AW-M designed the study and significantly contributed with data analysis as well as drafting and revising the manuscript; RR-R contributed with data acquisition and interpretation for fatty acid composition of the samples; BC-D, E. EA-P, and FJO-A, significantly contributed with statistical analyses revising for important intellectual content the final version of the manuscript. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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