RESEARCH ARTICLE

Peanut Storage

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Improving Quality Preservation of Raw Peanuts Stored under Different Conditions During a Long-Term Storage

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This research evaluated the chemical, microbiological, and sensory quality preservation of raw peanuts packed in high barrier ethylene vinyl alcohol bags (EVOH) under vacuum and in polypropylene (PP) bags, during 720 days of storage at 10° (T10) and 25 °C (T25). Peroxides (PV) and conjugated dienes (CD) remained almost stable in EVOH at 10 °C (EVOH-T10). The free fatty acid increase was greater in peanuts packaged in EVOH at 25 °C than at 10 °C. EVOH-T10 presented the lowest saturated/unsaturated and oleic/linoleic ratios, and the highest iodine values. The lowest γ-tocopherol decrease was for EVOH-T10. Peanuts stored in PP bags at 25 °C (PP-T25) showed the highest alkanes and the lowest decane,5,6-bis(2,2-dimethylpropylidene)-,(E,Z)- content at the end of storage. PP-T25 had the highest rate of roasted peanutty flavor decrease. Less than 10 CFU g^{-1} molds, yeasts, and aerobic mesophilic bacteria are detected for peanut samples, irrespective of packaging and storage temperature. Storage at 10 °C is associated with lesser quality deterioration than storage at 25 °C. All studied quality parameters are better preserved in raw peanuts packaged in EVOH except for free fatty acids.

***Practical Applications*: Raw peanuts are used as primary ingredients which can be converted into value-added products like peanut butter, peanut flour, peanut oil, snacks, and other by-products. Nevertheless, preserving raw peanut quality has become a critical issue for peanut industry owing to peanut susceptibility to deterioration process. This research presents results about the chemical and sensory quality parameter changes of raw peanuts stored under different packaging materials and temperatures during a long-term storage to figure out which are better conditions to preserve raw peanuts as a high-quality product.**

1. Introduction

With unique nutty flavors and pleasant aromas, peanuts (*Arachis hypogaea* L.) are greatly appreciated by consumers worldwide. Furthermore, raw peanuts are an excellent source of polyunsaturated fatty acids, proteins, antioxidants, minerals, vitamins and other micronutrients, such as magnesium.[1] Raw peanuts can be consumed directly or used as a primary ingredient that can be converted into value-added products such as peanut butter, peanut flour, peanut oil, and snack products. Thus, they occupy a unique position among oilseeds. However, high levels of unsaturated fatty acids make peanuts susceptible to lipid oxidation during storage, which can lead to an important quality loss, with deleterious effects and toxic metabolite occurrence.[2]

The shelf life of stored peanuts is impacted by external factors including the type of packaging, temperature, oxygen availability, exposure to light, and relative humidity. Suitable packaging material selection and storage temperature control are key to preserving the nutritional and oxidative stability of peanuts.[3] The principal function of food packaging is protection and preservation from external contamination including environmental factors such as oxygen and water permeation, and spurious aromas and flavors. The main packaging approaches currently include polymeric film materials and modified atmospheres.[4]

Polypropylene (PP) films dominate the largest part of the flexible packaging market. However, ethylene vinyl alcohol (EVOH) copolymer has many desirable properties as an oxygen, water and undesirable aroma barrier, making it particularly appropriate for packaging oilseeds. When this type of packaging is combined with a form of modified atmosphere (e.g., vacuum), oilseeds are less susceptible to lipid oxidation and growth of spoilage microorganisms.[5]

The world peanut trade involves extreme transportation conditions and long-term storage at low or ambient temperatures. Therefore, it is very important to know the best packaging and storage conditions to maintain the safety and quality of this raw material throughout the entire marketing chain.[6] Many researchers

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have reported the shelf life of roasted peanuts and other seeds stored under various conditions.[7,8] However, few studies have investigated the quality of raw peanuts stored under diverse environments.[9] Moreover, there is a lack of information about the chemical and sensory behavior of raw peanuts throughout long term storage under various temperature conditions.

In this context, the present research aimed to evaluate the raw peanuts preservation (chemical, microbiological, and sensory quality) by combination of certain storage temperatures (cold storage and room temperature) and packaging materials (normal and high barrier) during a long-term storage (around 2 years).

2. Materials and Methods

2.1. Materials

Raw peanut seeds type Runner (cv. Granoleico), size 38/42 kernels per ounce (2013 crop), were obtained from the Lorenzati, Ruetsch & Cia company (Ticino, Prov. Córdoba, Argentina).

2.2. Storage Conditions and Sampling

Peanut samples (2 kg) were placed in bags (25 cm × 35 cm × 3.6 cm = 3150 cm^3) of two different packaging materials: a) polypropylene (PP) ventilated pouches (Córdoba Envases, Córdoba, Argentina) having 75 μm total thickness with holes all over the material surface contributing to a free oxygen transmission and b) high barrier plastic pouches made of ethylene vinyl alcohol (EVOH) having 175 μm total thickness with an oxygen transmission rate of 1–5 $cm^3/m^2/bar/24$ h (DISE S.A., Cordoba, Argentina) packaged under vacuum condition (−760 mmHg) using an industrial packaging machine.[10]

The packaged peanuts were stored in the dark at two temperature conditions: a) room temperature (25 ± 2 °C, 60–80% relative humidity), and b) cold storage (10 ± 2 °C, 60–80% relative humidity). Temperature and relative humidity were monitored using a digital temperature and moisture meter (CASIO ID-16, Tokyo, Japan). The experiment was run in three repetitions for a period of 720 days, from February 2014 to February 2016. Three packages from each storage temperature (10 and 25 °C) were withdrawn from each sampling time since day 0 and every 60 days to perform chemical, microbiological and sensory analysis.

2.3. Moisture Content

Moisture content (MC) was determined according to the AOAC method number 925.40.[11]

2.4. Chemical Analysis

Peanuts were pressed using a 20-ton hydraulic press (HE-DU, Hermes I. Dupraz S.R.L., Córdoba, Argentina) to express the oil. The following parameters were used to determine the degree of deterioration: peroxide value (PV), conjugated dienes (CD), free fatty acids (FFA), changes of tocopherol and fatty acid compositions, and volatile analysis.

2.4.1. Peroxide Value (PV)

PV was evaluated following AOAC method number 965.33, using 5 g oil from each sample and expressed as milliequivalents of active oxygen per kilogram of oil (meq O_2/kg).[11]

2.4.2. Conjugated Dienes (CD)

CD were determined following the COI method T 20/Doc n° 19/Rev 1, in a UV-V diode array spectrophotometer Spectrum SP-2100 UV (Zhejiang, China) at 232 nm. The results were reported as the extinction coefficient E (1%, 1 cm).[12]

2.4.3. Free Fatty Acids (FFA)

FFA were evaluated by the AOAC method number 940.28 and results expressing as g oleic acid/100 g peanut oil.[11]

2.4.4. Tocopherol Composition

The tocopherol composition of peanut oil (α, β, γ, and δ) was measured by high pressure liquid chromatography according to the method of Silva et al.,[13] using a HPLC chromatograph (Agilent 1100, Agilent Technologies, Palo Alto, CA, USA) equipped with a normal phase HPLC column, Zorbax RX-SIL column (5 μm particle size, 4.6 × 250 mm, Agilent Technologies). The mobile phase was a mixture of isopropanol and n-hexane (0.5:99.5 v/v) and tocopherols were detected at 298 nm. Identification and quantification of each tocopherol was made by standards purchased from Sigma–Aldrich (St Louis, MO, USA).

2.4.5. Fatty Acid Composition

Crude Fatty acid methyl esters were analyzed by gas-liquid chromatography on a Perkin Elmer Clarus 600 (Waltham, Massachusetts, USA) equipped with a flame ionization detector (FID). A SAC™-5 capillary column, 30 m length, 0.25 mm internal diameter and 0.25 μm film thickness (C#24156, Supelco) was used. The chromatographic analysis was performed in accordance to Martín et al.[10]

2.4.6. Volatile Analysis (VA)

The volatile compounds were extracted using a solid phase microextraction (HS-SPME) fiber and analyzed by gas chromatography/mass spectrometry (GC/MS) using a chromatograph Perkin Elmer Clarus 600 (Waltham) according to Quiroga et al.[14] A SPME fiber (Supelco, Bellefonte, PA, USA) coated with divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) was used. Raw peanut samples (2 g) were

ground and placed in a 10 mL glass flask at 70 °C for 20 min. The SPME fiber was placed into the flask for 10 min. Subsequently, fiber was injected into a GC-MS, and volatiles were thermally desorbed into an ELITE 5MS (30 m × 0.25 mm i.d., 0.25 μm film thickness; Perkin Elmer) column.[10] Peak identifications were based on the comparison of their mass spectra with the NIST mass spectral library and, additionally, in some cases, by comparison of retention times with those of standard compounds: propanal, hexanal, heptanal, and nonanal (Sigma–Aldrich).

2.5. Microbiological Count

The microbiological analysis was performed on 10 g of each milled peanut sample. Enumeration of total aerobic microorganisms and total yeasts and molds was carried out according to Asensio et al.[15] and ISO method 7954,[16] respectively. All counts were expressed as colony forming units per gram of sample (CFU/g).

2.6. Descriptive Sensory Analysis

A "hybrid" method developed by Meilgaard et al.[17] was used for descriptive analysis. The trained panel (seven female and two male) was selected, trained, and calibrated according to Grosso and Resurreccion[18] and all of them had at least 6 years of experience in sensory descriptive analysis. A 150 mm unstructured linear scale was employed for samples evaluation. Samples were roasted at 155 °C for 20 min in an air circulation oven (Garmont, Alta Gracia, Argentina) and blanched (removing skins) before descriptive analysis.[18] During the roasting process, non-enzymatic reactions occur allowing to develop the typical roasted peanut flavor, color, and texture. Therefore, this is the way they are commonly consumed.[19]

The descriptive analysis used for sample evaluation included the following attributes: brown color, roughness, glossiness, roasted peanut, oxidized, cardboard, sweetness, saltiness, sourness, bitterness, astringent, hardness, and crunchiness. All samples were evaluated in partitioned booths under fluorescent light at room temperature. A completely randomized block design was used for testing samples. The data were registered on paper ballots.

2.7. Statistical Analysis

Experimental results were the averages of three repetitions. The experiment was conducted in a factorial (2 levels for packaging materials, 2 levels for temperature conditions, and 13 storage times) and completely randomized design via a three-way analysis of variance (ANOVA) to assess the effect of packaging material, temperature condition, storage time and their interaction (packaging*temperature*time). The Fisher's least significant difference (LSD) was used as a post hoc test for comparison of means among results. Significance was accepted at 0.05 probability level or less. Correlations were determined by the Pearson coefficient, which denotes the strength of the linear association between two dependent variables. Linear regression equations were obtained for the regression analyses. Exploration of associations between treatments, chemical, and sensory variables was made by Principal Component Analysis (PCA). All statistical data were computed using INFOSTAT software Version 2016 (Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Córdoba, Argentina).

3. Results and Discussion

3.1. Chemical Analysis from Stored Raw Peanuts

There was a significant increase ($p < 0.0001$) in MC between storage days 0 and 720 (**Figure 1**a). The differences between storage at 25 °C and storage at 10 °C had a greater impact on MC compared to that observed for the different assessed packaging materials. These trends were also observed by Shishir et al.[20] Samples stored at 25 °C suffered a greater MC increase during storage than samples stored at 10 °C. This property is pivotal due to the negative influence of moisture increases leading to flavor deterioration in peanut seeds and in microorganism loads.[21]

The PV and CD were evaluated as primary lipid oxidation indicators. Significant effects of packaging, temperature, and storage time were observed on PV and CD, among peanut treatments. These oxidative markers increased with storage time for both packaging materials and temperature conditions. Similar trends were documented in previous studies.[22,23] The PV increase for EVOH bags was significantly lower than that of PP bags at both temperatures. The highest and lowest PV at the end of the storage period were recorded for raw peanuts packed in PP ventilated bags at ambient temperature (5.3 meq O_2/kg) and for raw peanuts packed in EVOH under vacuum at 10 °C (1.9 meq O_2/kg), respectively (Figure 1b).

At both storage temperatures, the EVOH pouches under vacuum preserved raw peanuts with a PV under the limit of 2.0 meq O_2/kg oil, considered an indicator of a fresh nut product.[24] As shown in Figure 1c, similar trends were observed for CD. The presented results indicated that the combination of a packaging with low oxygen permeability under vacuum, along with lower storage temperature, is a better alternative to preserve the oxidative stability of raw peanuts.

FFA indicate the degree of triacylglycerol deterioration that occurs by hydrolysis, fermentation and oxidation, promoted by temperature and presence of water.[25] The initial value (day 0) for FFA was lower with respect to the maximum acceptable limit of 1.0 g oleic acid/100 g peanut oil (CODEX STAN PEANUTS 200–1995). This low initial value for FFA indicates that this study started using fresh peanut samples. FFA increased with storage time ($p < 0.0001$), in concurrence with Domingues de Oliveira et al.[26] for palm seeds stored under various conditions. Likewise, significant differences were observed for packaging materials ($p < 0.0001$) and temperature conditions ($p < 0.0001$). The packaging*temperature*time interaction also presented significant differences ($p < 0.001$) demonstrating that raw peanuts stored in EVOH packaging under vacuum at 25 °C at the end of storage time (day 720) showed the highest FFA content (Figure 1d). In our experiments, irrespective of the type of packaging, the FFA increase was more pronounced at

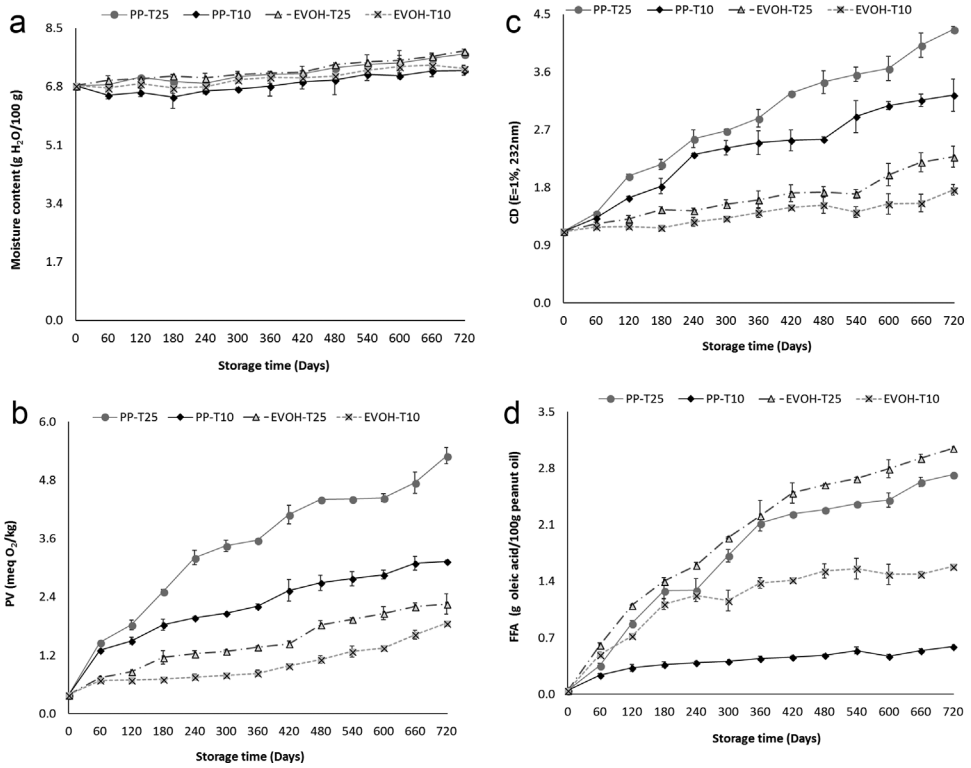

Figure 1. a) Moisture content (MC), b) peroxide value (PV), c) conjugated dienes (CD), and d) free fatty acids (FFA) in raw peanuts packaged in polypropylene ventilated pouches (PP) and high barrier plastic pouches (EVOH) under vacuum, during 720 days of storage at 10 and 25 °C ($p < 0.05$, $n = 3$). Treatments: polypropylene bags at 25 °C (PP-T25), polypropylene bags at 10 °C (PP-T10), ethylene vinyl alcohol bags under vacuum at 25 °C (EVOH-T25) and ethylene vinyl alcohol bags under vacuum at 10 °C (EVOH-T10).

25 °C than at 10 °C, whereby the rates were almost stable; but, the samples packed in EVOH stored at this temperature exhibited greater values at the end of storage than the samples packed in PP. This implied that storage temperature had a greater impact on glyceride lipolytic decomposition than the packaging materials. These results evidence that EVOH material did not have a protective effect on this quality parameter. Similar trends were observed in previous studies, which indicated that high temperatures produced a high fat content deterioration in various oilseeds.[27]

3.2. Changes in Fatty Acid Composition

Analysis of the fatty acid composition is a useful tool for detecting classes of lipids that are involved in the oxidative changes.[28] The saturated/unsaturated fatty acid ratio (S/U), oleic/linoleic ratio (O/L), and iodine value (IV) of raw peanuts as a function of packaging material, and storage time at 10 and 25 °C are given in **Figure 2**. The O/L ratio is a quality parameter used for the determination of breeding lines, with normal peanuts having an O/L ratio of 1.5–2.0 and high-oleic varieties showing O/L values greater than 9.0.[29] In this work, the assayed samples included high-oleic cultivars (O/L > 9.0).

Although no significant differences were observed for the packaging*temperature*time interaction, the S/U and O/L ratios increased significantly with storage time ($p < 0.001$). Conversely, IV decreased during storage ($p < 0.0001$). Such tendencies were also observed by Bhatti et al.[30] Furthermore, the S/U, O/L, and IV were significantly ($p < 0.001$) affected by the storage temperature (10 and 25 °C) ($p < 0.0001$) and packaging material (EVOH and PP) ($p < 0.0001$). The combination of EVOH packaging and storage at 10 °C, resulted in samples with the lowest S/U and O/L, and the highest IV averages. Conversely, S/U, O/L, and IV exhibited the highest values in PP packaged samples stored at 25 °C. In contrast, Mourad et al.[7] concluded that there was no alteration in the lipid profile of sunflower seeds stored in two different packaging materials under two storage conditions. In the current research, storage time had a deleterious effect on the lipid profile, while high barrier packaging materials and lower temperatures better preserved the fatty acid composition.

3.3. Changes in Tocopherol Composition

Tocopherols are natural liposoluble metabolites capable of acting as antioxidants by interrupting the initiation or propagation step of lipid oxidation reactions due to quenching of free radicals, thus, improving the oxidative stability of edible oils.[30] The α- and γ-tocopherols were present in higher concentrations than β- and δ-tocopherols. Comparable results were reported by Seppanen et al.[31] In the present study, in general, α-tocopherol did not undergo oxidation over the period studied in samples

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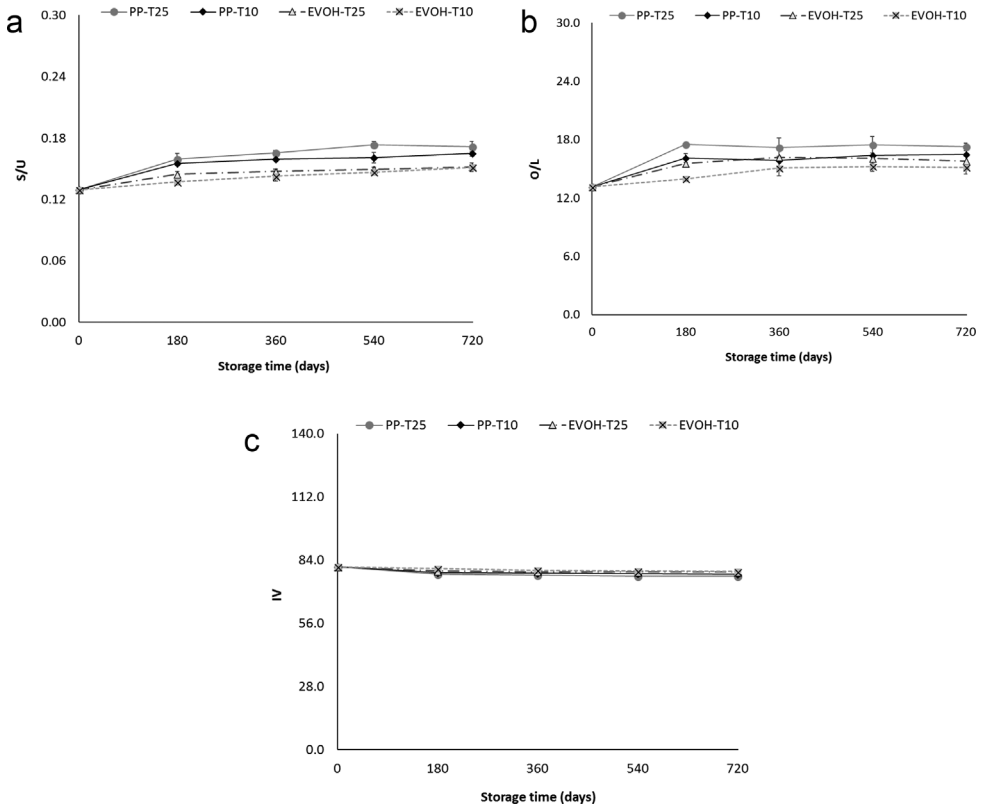

Figure 2. a) Saturated/unsaturated ratio (S/U), b) oleic/linoleic ratio (O/L), and c) iodine value (IV) in raw peanuts packaged in polypropylene ventilated pouches (PP) and high barrier plastic pouches (EVOH) under vacuum, during 720 days of storage at 10 and 25 °C ($p < 0.05$, $n = 3$). Treatments: polypropylene bags at 25 °C (PP-T25), polypropylene bags at 10 °C (PP-T10), ethylene vinyl alcohol bags under vacuum at 25 °C (EVOH-T25), and ethylene vinyl alcohol bags under vacuum at 10 °C (EVOH-T10).

packaged in EVOH. Samples packaged in PP bags at 10 and 25 °C exhibited a significant decrease ($p < 0.002$) between days 0 and 180 (**Table 1**). Wilkin et al.[32] also revealed α-tocopherol as the most stable tocopherol for high-oleic peanuts stored under various conditions. According to our results, α-tocopherol probably played an important role in hindering the autocatalytic lipid peroxidation processes, particularly for peanuts stored in high barrier packaging materials and lower temperatures.

γ-tocopherol presented the greatest deterioration during storage for both treatments and temperatures (Table 1). The decrease in γ-tocopherol began after day 540 for samples packaged in EVOH-T10. Chun et al.[33] also demonstrated the effectiveness of vacuum packaging in controlling the quality of tocopherols.

β-tocopherol decreased significantly between day 0 and day 180, remaining constant from then on for both packaging materials and temperature conditions while δ-tocopherol did not display changes during storage.

According to Subramaniam,[34] one of the most important trends linked to the stability and shelf life of food, is the growing area of plant extracts with natural antioxidant properties. Hence, it is crucial to preserve tocopherols in raw peanuts as a source of natural antioxidants. In this sense, high barrier packaging materials and lower temperatures help to preserve these kinds of molecules.

3.4. Volatile Headspace Profile

Gas chromatographic techniques are commonly used to identify and quantify oxidative degradation markers such as aldehydes, ketones, alcohols, and hydrocarbons.[35] Volatiles generated from roasted peanuts and other oilseeds have been extensively discussed in the literature.[36] Conversely, there are few data associated with volatile compounds in raw peanuts.[37] Consequently, it was difficult to compare results found in the present research with previous studies. Overall, nine peaks were identified by the GC-MS analysis of raw peanut samples. These peaks corresponded to the classes of alkanes (undecane, dodecane, tridecane, tetradecane, pentadecane, hexadecane, octadecane, and nonadecane) and a complex compound (decane,5,6-bis(2,2-dimethylpropylidene)-,(E,Z)-). It is well known that hydrocarbons and, in particular, alkanes, are derived from the thermal degradation of long-chain fatty acids by autoxidation or homolysis.[38] Furthermore, an earlier study demonstrated that the homologous series of hydrocarbons reported in the present research are deviations from normal volatile profiles and appear in samples stored under non-ideal conditions, indicating future off-flavor problems.[39] The homologous series was not found at day 0 of storage. However, these hydrocarbons appeared since day 180 and increased with storage time for all peanut samples. Moreover, there were significant

Table 1. Tocopherol content (means ± standard deviations) of raw peanuts packaged in polypropylene ventilated pouches (PP) and high barrier plastic pouches (EVOH) under vacuum, during 720 days of storage at 10 and 25 °C.

			Tocopherol content [mg/100 g peanut oil]				
			Storage time [days]				
Packaging	Temperature	Tocopherols	0[b)]	180	360	540	720
	10	α-T	25.1 ± 0.64c	23.1 ± 0.08ab	23.1 ± 0.53ab	23.4 ± 0.08b	23.4 ± 0.71b
		β-T	0.8 ± 0.07b	0.5 ± 0.01a	0.6 ± 0.02a	0.6 ± 0.02a	0.6 ± 0.10a
		γ-T	21.5 ± 0.12gh	19.7 ± 0.35e	19.5 ± 0.22de	19.1 ± 0.19cde	18.2 ± 0.23ab
		δ-T	0.5 ± 0.01abc	0.6 ± 0.04de	0.6 ± 0.03e	0.5 ± 0.04abc	0.5 ± 0.01bcd
PP[a)]							
	25	α-T	25.1 ± 0.64c	22.7 ± 0.63ab	22.5 ± 0.05ab	23.0 ± 0.16ab	22.2 ± 0.71a
		β-T	0.8 ± 0.07b	0.6 ± 0.01a	0.6 ± 0.01a	0.6 ± 0.02a	0.6 ± 0.03a
		γ-T	21.5 ± 0.12gh	19.3 ± 0.19cde	18.8 ± 0.24bcd	18.6 ± 0.51abc	18.0 ± 0.41a
		δ-T	0.5 ± 0.01abc	0.5 ± 0.03a	0.5 ± 0.03cde	0.5 ± 0.03abcd	0.5 ± 0.01ab
	10	α-T	25.1 ± 0.64c	25.0 ± 1.00c	25.4 ± 0.19c	25.3 ± 0.31c	25.6 ± 0.54c
		β-T	0.7 ± 0.07b	0.6 ± 0.05a	0.5 ± 0.04a	0.6 ± 0.04a	0.6 ± 0.01a
		γ-T	21.5 ± 0.12gh	21.6 ± 0.39h	21.6 ± 0.40gh	21.4 ± 0.10fgh	19.5 ± 0.51de
		δ-T	0.5 ± 0.01abc	0.5 ± 0.01abcd	0.5 ± 0.01abcd	0.5 ± 0.01abcd	0.5 ± 0.01abcd
EVOH[a)]							
	25	α-T	25.1 ± 0.64c	24. ± 0.33c	24.7 ± 0.47c	25.0 ± 0.72c	25.6 ± 0.31c
		β-T	0.8 ± 0.07b	0.6 ± 0.02a	0.6 ± 0.03a	0.6 ± 0.04a	0.6 ± 0.02a
		γ-T	21.5 ± 0.12gh	21.3 ± 0.40fgh	20.9 ± 0.51fg	20.8 ± 0.47f	19.1 ± 0.26cde
		δ-T	0.5 ± 0.01abc	0.5 ± 0.01abcd	0.5 ± 0.01abcd	0.5 ± 0.01abcd	0.5 ± 0.01abcd

[a)] Means ± standard deviations for each tocopherol isoform followed by different letters within and between rows are statistically different ($\alpha = 0.05$) for the packaging*temperature*time interaction ($n = 3$, LSD Fisher).
[b)] Before storage.

differences ($p < 0.001$) for the interaction packaging*temperature*time. To better visualize the data, the total content of these alicyclic hydrocarbons in each sample is presented in **Figure 3**a. PP-T25 showed the highest increase in the total alkane content, while EVOH-T10 undergone the lowest increase during the 720 days of storage. The total accumulated alkane content increase visualized at the end of storage, was in accordance with previous reports, which demonstrated that high volatile concentrations are good indicators of reduced peanut quality. For instance, previous researchers stated that volatile organic compounds in roasted peanuts increased with increasing storage temperature.[38]

The decane,5,6-bis(2,2-dimethylpropylidene)-,(E,Z)- content decreased with storage time for all peanut packaging materials and temperature conditions (Figure 3b) but the lowest rate of decrease was observed for samples packaged in EVOH combined with storage under vacuum at 10 °C. Previously, Moniruzzaman et al.[40] established this compound as the most important active component in legumes with antimicrobial effect. According to the current results, decane,5,6-bis(2,2-dimethylpropylidene)-,(E,Z)- is considered a fresh product marker, which was affected by the type of packaging, temperature and long-term storage, undergoing degradation during the evaluated period.[10]

In summary, the low oxygen permeability of the high barrier packaging and low storage temperature (10 versus 25 °C), produced the lowest alkane content and the highest preservation of fresh quality.

3.5. Microbiological Counts

There are no specific limits for total mesophilic microorganisms or yeast and mold counts for raw peanuts, however, they must be free from microorganisms or substances derived from microorganisms, in amounts which may represent a health hazard (CODEX STAN PEANUTS 200–1995). The roasting process contributes to eliminate microorganism spoilage due to the reduction in seed moisture content. Hence, it is critical that the peanut industry ensures the safety of the final product.[41] In this study, the MC of all peanut samples reached values under 10%, which is considered the highest strict limit up to no tolerance.[42] However, microbiological studies were performed to detect general contamination. Over the 720 days of storage, less than 10 CFU g^{-1} molds, yeasts, and aerobic mesophilic bacteria were detected for the peanuts, irrespective of packaging and storage temperature, which was correlated to the low MC found in the samples. Pothakos et al.[43] demonstrated that the packaging of food products combined with storage at low temperature under vacuum, extends the shelf life for long-term storage. The temperature conditions and packaging materials used in the present research avoided microbial contamination of the

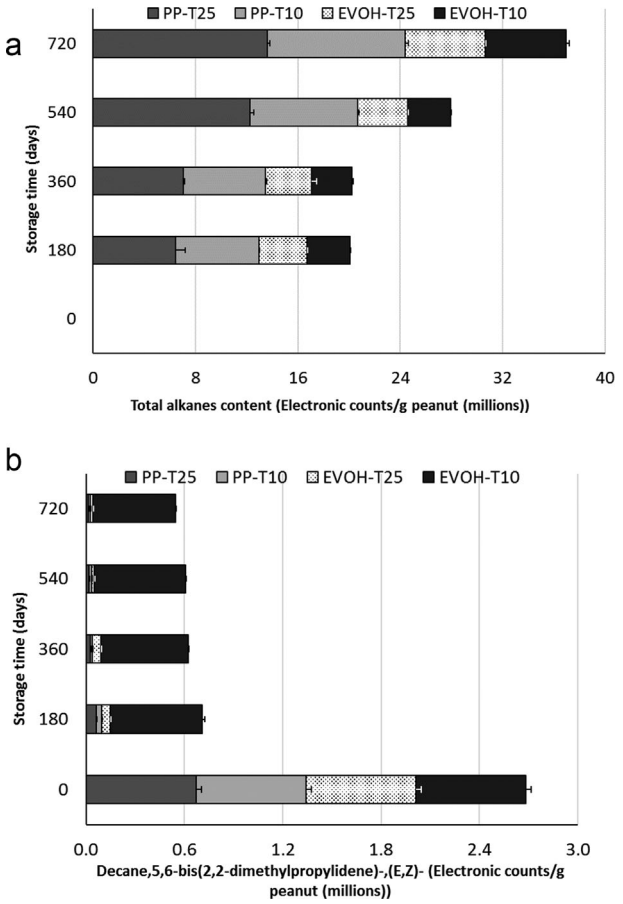

Figure 3. a) Total alkanes content and b) decane,5,6-bis(2,2-dimethylpropylidene)-,(E,Z), in raw peanuts packaged in polypropylene ventilated pouches (PP) and high barrier plastic pouches (EVOH) under vacuum, during 720 days of storage at 10 and 25 °C ($p < 0.05$, $n = 3$). Treatments: polypropylene bags at 25 °C (PP-T25), polypropylene bags at 10 °C (PP-T10), ethylene vinyl alcohol bags under vacuum at 25 °C (EVOH-T25) and ethylene vinyl alcohol bags under vacuum at 10 °C (EVOH-T10).

samples. Lianou et al.[44] indicated that key factors influencing microorganism proliferation include applied storage conditions, mainly packaging and temperature conditions.

3.6. Descriptive Sensory Analysis

The panelists could distinguish significant differences only in the roasted peanut flavor. For the other attributes, no significant differences were observed. Storage time ($p < 0.001$), temperature ($p < 0.001$), and type of packaging ($p < 0.001$) significantly affected the roasted peanut intensity ratings (**Figure 4**). All the samples showed a progressive decrease in the roasted peanut attribute throughout storage. In other studies,[45,46] decreases in roasted peanutty for other peanut products during storage were also observed. The interaction between packaging material, temperature condition and storage time, also had a significant influence ($p < 0.0351$) on the roasted peanut flavor. The highest decrease was observed in raw peanuts packed in PP foils and stored at 25 °C (PP-T25), with significant differences observed

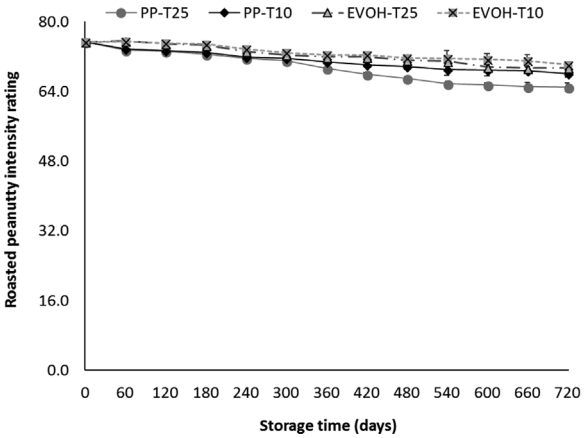

Figure 4. Roasted peanutty intensity ratings in raw peanuts packaged in polypropylene ventilated pouches (PP) and high barrier plastic pouches (EVOH) under vacuum, during 720 days of storage at 10 and 25 °C ($p < 0.05$, $n = 3$). Treatments: polypropylene bags at 25 °C (PP-T25), polypropylene bags at 10 °C (PP-T10), ethylene vinyl alcohol bags under vacuum at 25 °C (EVOH-T25) and ethylene vinyl alcohol bags under vacuum at 10 °C (EVOH-T10).

after 300 days of storage. At the end of the storage period (day 720), raw peanuts packed in EVOH foils under vacuum at 10 °C exhibited the highest intensity rating (70.2) for this flavor.

Davis and Dean[25] highlighted that studies utilizing diverse peanut genotypes, demonstrate that the total sugar content of the seed is a good predictor of the roasted peanut flavor. Changes in sugar content of raw peanuts during storage would affect the final quality of the roasted peanut flavor. The sugar concentration effect could have affected on peanut sensory quality, but sugar content was not analyzed in this experiment.

Several studies have demonstrated the association between off-notes (oxidized and cardboard) and volatile lipid oxidation products, like nonanal, octanal and hexanal, derived from the deterioration of unsaturated fatty acids.[14] In the current study, no volatile compounds directly associated with lipid peroxidation were detected.

3.7. Correlation and Regression Analysis

The variables of interest in this study were the chemical oxidative markers (PV and CD), FFA, MC, roasted peanutty flavor, S/U ratio, O/L ratio, IV, γ-tocopherol, decane,5,6-bis(2,2-dimethylpropylidene)-,(E,Z)-, and total alkane content. Pearson correlation coefficients higher than 0.65 were observed between PV, CD, FFA, MC, O/L ratio, S/U ratio, and total alkane content for each peanut treatment. These positive correlations between these variables were due to the fact that all of them increased with storage time for all samples. Negative correlation coefficients higher than −0.65 were observed between roasted peanutty flavor, IV, γ-tocopherol, decane,5,6-bis(2,2-dimethylpropylidene)-,(E,Z)-, and the variables mentioned above for both samples. These results were due to the fact that roasted peanutty flavor, IV, γ-tocopherol and decane,5,6-bis(2,2-dimethylpropylidene)-,(E,Z)- decreased while PV, CD, FFA, MC, S/U ratio, O/L ratio, and total alkane content increased with storage. In

previous works, relations between chemical and sensory variables were reported for others peanut products.[45,47]

Regression coefficients for chemical and sensory variables (dependent variables) and storage time (independent variable) for each packaging and temperature are shown in **Table 2.** The R^2 values were higher than 0.45 for all variables in all treatments. The regression equations obtained could be used to predict the effects of storage time on these peanut samples packaged in EVOH and PP at 10 and 25 °C. Considering that the maximum tolerance level in many markets for PV in raw peanuts is

Table 2. Regression coefficients and adjusted R^2 for the dependent variables: peroxide value (PV), conjugated dienes (CD), free fatty acids (FFA), moisture content (MC), roasted peanutty flavor (RP), saturated/unsaturated ratio (S/U), oleic/linoleic ratio (O/L), iodine value (IV), γ-tocopherol, decane, 5,6-bis(2,2-dimethylpropylidene)-,(E,Z)- and total alkane content in raw peanuts packaged in polypropylene ventilated pouches (PP) and high barrier plastic pouches (EVOH) under vacuum, during 720 days of storage at 10 and 25 °C.

		Sample[c]					
		PP			EVOH		
		Regression[a]					
Dependent variable	Temperature [°C]	β_0	β_1[b]	R^2	β_0	β_1[b]	R^2
PV							
	10	0.3843	0.0045c	0.7351	0.3843	0.0017a	0.9113
	25	0.3843	0.0076d	0.8231	0.3843	0.0028b	0.9490
CD							
	10	1.1092	0.0033c	0.9238	1.1092	0.0008a	0.9090
	25	1.1092	0.0046d	0.9594	1.1092	0.0015b	0.9442
FFA							
	10	0.0469	0.0009a	0.4539	0.0469	0.0027b	0.4583
	25	0.0469	0.0043c	0.8647	0.0469	0.0049d	0.8482
MC							
	10	6.8304	0.0004a	0.6613	6.8304	0.0007a	0.7914
	25	6.8304	0.0011b	0.9000	6.8304	0.0012b	0.9457
RP							
	10	75.3700	−0.0112b	0.9316	75.3700	−0.0069b	0.9509
	25	75.3700	−0.0160a	0.9725	75.3700	−0.0087b	0.9582
S/U							
	10	0.1294	0.00006b	0.5683	0.1294	0.00003a	0.9727
	25	0.1294	0.00008c	0.6078	0.1294	0.00004a	0.6379
O/L							
	10	13.1400	0.0059a	0.6715	13.1400	0.0035a	0.7671
	25	13.1400	0.0078a	0.5176	13.1400	0.0052a	0.6594
IV							
	10	80.9945	−0.0057ab	0.4836	80.9945	−0.0033b	0.9139
	25	80.9945	−0.0071a	0.4734	80.9945	−0.0041b	0.4792
γ-tocopherol							
	10	21.5400	−0.0049ab	0.8061	21.5400	−0.0016c	0.4639
	25	21.5400	−0.0056a	0.6771	21.5400	−0.0026bc	0.7639
decane, 5,6-bis(2,2-dimethylpropylidene)-,(E,Z)-							
	10	670283	−1215a	0.5100	670283	−260b	0.5586
	25	670283	−1200a	0.5617	670283	−1189a	0.5608
Total alkane content							
	10	0	16212b	0.7786	0	8327a	0.7600
	25	0	20708c	0.9112	0	8861a	0.7167

[a] Regression equation: $Y = \beta_0 + \beta_1 x$, where y is the dependent variable (PV, CD, FFA, MC, RP, S/U, O/L, IV, γ-tocopherol, decane, 5,6-bis(2,2-dimethylpropylidene)-,(E,Z)-, and total alkane content), β_0 is a constant that it is equal the value of y when the value of $x = 0$, β_1 is the coefficient of x; x is an independent variable (days of storage), and R^2 is the adjusted determination coefficient.
[b] ANOVA and LSD Fisher test: The slope (β_1) of each variable and sample followed by the same letters are not significantly different at $\alpha = 0.05$.
[c] PP, polypropylene ventilated pouches; EVOH, high barrier plastic pouches.

2 meqO_2/kg oil and using the prediction equation, PVs higher than the mentioned limit were reached after 950, 577, 359, and 213 days in peanut samples packaged in EVOH-T10, EVOH-T25, PP-T10 and PP-T25, respectively. These results indicate that the maximum shelf life for raw peanuts was found at 10 °C storage temperature in EVOH packaging. In addition, the differences in stability between samples could be analyzed through the oxidation tendencies. In Table 2, significant differences between the slopes (β1) from regression analysis were detected among samples for dependent variables PV, CD, FFA, roasted peanutty flavor, S/U ratio, IV, γ-tocopherol, decane,5,6-bis(2,2-dimethylpropylidene)-,(E,Z)-, and total alkane content. Higher slopes (β1) in PV, CD, FFA, S/U ratio, and total alkane content indicate higher tendency to oxidation. β1 values of PV, CD, S/U ratio and total alkane content in PP-T25 were higher than the other treatments. In the case of FFA, higher slope was obtained for EVOH-T25 than for the remaining peanut samples which indicate that higher temperatures produce higher triglyceride deterioration and EVOH material did not have a protective effect on this quality parameter as explained earlier. On the other hand, lower negative slopes (β_1) for roasted peanutty flavor, γ-tocopherol, IV and decane,5,6-bis(2,2-dimethylpropylidene)-,(E,Z) observed in EVOH-T10 than in the remaining treatments indicated lower tendency to product deterioration in the former. Previous authors examined the influence of temperature, type of atmosphere and physical shape on the oxidative stability of almonds kernels during storage. They concluded that modified atmosphere packaging under vacuum along with refrigeration temperatures were the most effective method for protect almonds against oxidation.[24] According to the conclusions obtained by those researches and the results of this study, high barrier plastic materials (EVOH) under vacuum along with 10 °C storage temperature would provide peanut kernels with a higher protection against lipid deterioration than polypropylene ventilated materials (PP) and 25 °C. In addition, raw peanuts packaged in EVOH pouches and stored at refrigeration temperatures (10 °C) would have longer shelf life than raw peanuts packaged in PP pouches at ambient temperature.

3.8. Principal Component Analysis (PCA)

PCA was performed to create an overall impression of the most effective combination of packaging material and temperature condition that best preserves the oxidative and sensory stability of raw peanuts exposed to long-term storage. The data matrix of variables analyzed included MC; PV; CD; FFA; tocopherols (α, β, γ, and δ); fatty acids (16:0, 18:1, 18:2, 20:0, 20:1, 22:0, 22:1, and 24:0); S/U; O/L; IV; volatile compounds [decane,5,6-bis(2,2-dimethylpropylidene)-,(E,Z)-, undecane, dodecane, tridecane, tetradecane, pentadecane, hexadecane, octadecane, and nonadecane]; and roasted peanutty flavor.

The first two principal components (PC1 and PC2) were sufficient to explain 93.8% of the data variability, as shown in **Figure 5**. High positive PC1 scores (right-hand side of the biplot) were obtained for the variables IV, unsaturated fatty acids (18:1, 18:2 and 20:1) most of the tocopherols (α-, γ-, and δ-tocopherols),

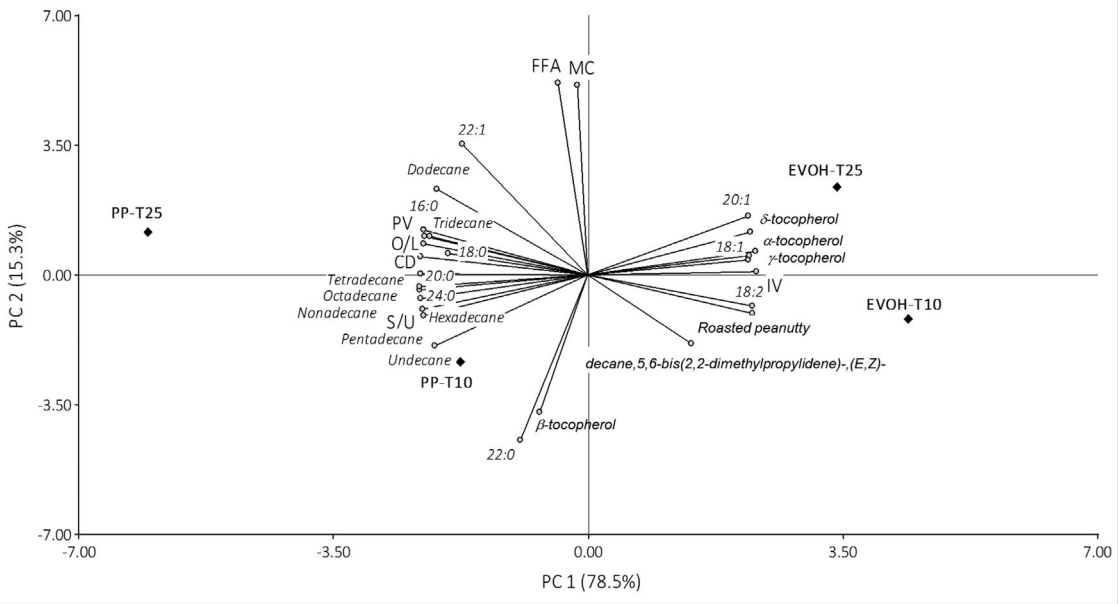

Figure 5. PCA loading scores for raw peanuts packaged in high barrier plastic pouches (EVOH) under vacuum and in polypropylene ventilated pouches (PP) during 720 storage days at 10 and 25 °C ($n = 3$). Variables: peroxide value (PV); conjugated dienes (CD); free fatty acids (FFA); moisture content (MC); fatty acids (16:0, 18:1, 18:2, 20:0, 20:1, 22:0, 22:1, and 24:0); saturated/unsaturated ratio (S/U); oleic/linoleic ratio (O/L); iodine value (IV); tocopherols (α, β, γ, and δ); roasted peanutty flavour; and volatile compounds (decane,5,6-bis(2,2-dimethylpropylidene)-,(E,Z), undecane, dodecane, tridecane, tetradecane, pentadecane, hexadecane, octadecane, and nonadecane). Treatments: polypropylene bags at 25 °C (PP-T25), polypropylene bags at 10 °C (PP-T10), ethylene vinyl alcohol bags under vacuum at 25 °C (EVOH-T25) and ethylene vinyl alcohol bags under vacuum at 10 °C (EVOH-T10).

decane,5,6-bis(2,2-dimethylpropylidene)-,(E,Z)-, and roasted peanutty flavor. Positive correlation coefficients, higher than 0.65, were found between most of the above-mentioned variables, for each peanut sample. Conversely, the same variables were inversely associated with chemical oxidative indicators (PV and CD), saturated fatty acids (16:0, 18:0, 20:0, 22:0, and 24:0), S/U, O/L, and alkanes, which were placed on the left-hand side of the biplot. FFA and MC presented positive association between them. However, poor associations were observed between the above-mentioned variables and the remaining variables assessed in the current study. Ajith et al.[27] also found a direct association between FFA and MC variables, indicating that a high MC increases lipase activity, facilitating oil degradation, and occurrence of FFA in raw cashew nuts.

PCA resulted in efficient clustering the data obtained by GC/MS analysis (volatiles and fatty acids). It was noted that volatiles considered potential markers of oxidative problems (alkanes), had high negative values and were negatively associated with decane,5,6-bis(2,2-dimethylpropylidene)-,(E,Z)-, which was a fresh peanut indicator. In the present research, the unsaturated fatty acids, with high, positive PC1 values (right-hand side of the biplot), were inversely associated with the saturated fatty acids (negative PC1 values). This association indicates that the unsaturated fatty acids were deteriorated in greater proportion in raw peanuts packaged in normal atmosphere (PP material) increasing the relative percentage of saturated fatty acids.

Figure 5 illustrates that peanut samples packed in PP-T25 were located closest to the higher results for the variables associated with lipid deterioration (negatives values in PC1), followed by PP-T10. Conversely, peanut samples packed in EVOH-T10 were located more to the right-hand side of the biplot (positives PC1 values), forming an angle near 180° with PP-T25, thereby, indicates a negative correlation between PP-T25 and EVOH-T10. These associations were confirmed by Pearson coefficients.

In the current research, the PCA results indicated that high barrier plastic bags combined with storage under vacuum at refrigerated conditions (10 °C), provided raw peanuts with a more effective barrier against oxidative and sensory deterioration during long-term storage. Previous researches also demonstrated the effectiveness of high barrier packaging and low temperature on products shelf life.[48] However, there is a quality parameter, free fatty acids that increased in a greater proportion when the raw peanuts were packed in high barrier plastic bags.

4. Conclusions

Refrigeration temperatures, along with high barrier plastic packaging under vacuum, effectively contribute to delay oxidation and sensory deterioration of raw peanuts in comparison to storage at room temperature (25 °C) under normal atmosphere conditions. Only free fatty acids (FFA), which are considered a quality parameter, were not better preserved in peanuts packed in high barrier plastic bags under vacuum. Nevertheless, the remaining results of chemical and sensory analysis, demonstrate that raw peanuts subjected to long storage periods, show enhanced preservation of their nutritional properties (unsaturated fatty acids and tocopherols) and roasted peanut flavor when they are packed under these conditions.

Abbreviations

CD, conjugated dienes; EVOH, ethylene vinyl alcohol bags; FFA, free fatty acids; IV iodine value; O/L oleic to linoleic ratio; MC, moisture content; PP, polypropylene ventilated bags; PV, peroxide value; RP, roasted peanutty flavor; S/U, saturated to unsaturated fatty acid ratio.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

oxidation, packaging, peanuts, quality, storage

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