Research Article

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Antioxidant effects of the monoterpenes carvacrol, thymol and sabinene hydrate on chemical and sensory stability of roasted sunflower seeds

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Abstract

BACKGROUND: Oxidation products and rancid flavors decrease the sensory quality of food products, making them unacceptable to consumers. Synthetic antioxidants are used in many foods to prevent rancidity, though their safety is questioned. Monoterpenes are obtained from essential oils and many of them have shown antioxidant activity. The objective of this study was to evaluate the stability of sensory and chemical parameters in roasted sunflower seeds supplemented with carvacrol, thymol and sabinene hydrate monoterpenes.

RESULTS: Five samples were prepared: control roasted sunflower seeds (RS-C) and sunflower seeds treated with carvacrol (RS-Car), sabinene hydrate (RS-S), thymol (RS-T) and butylated hydroxytoluene (RS-BHT). The three monoterpenes (carvacrol, sabinene hydrate and thymol) provided protection to this food product, inhibiting the formation of oxidative deterioration compounds such as peroxides and hexanal and undesirable off-flavors such as oxidized and cardboard flavors. Sabinene hydrate had greater effect preventing peroxide formation during storage than the other monoterpenes.

CONCLUSION: Carvacrol, sabinene hydrate and thymol could be used as an alternative to synthetic antioxidants for preserving the quality of roasted sunflower seeds.

Keywords: sunflower; monoterpenes; antioxidant; lipids

INTRODUCTION

Sunflower seeds are a food with high nutritional value owing to their lipid composition, being rich in polyunsaturated fatty acids such as linoleic acid (18:2), and are primarily used in the production of edible oil and animal feed.[1] The susceptibility of fatty acids to oxidation is directly dependent on the degree of unsaturation,[2,3] and their fatty acid composition makes sunflower seeds susceptible to deterioration. The low-molecular-weight compounds that are responsible for off-flavors in oils are produced during the oxidation process and make the oil unacceptable to consumers as well as for use as an industrial or food ingredient. Oxidative stability is an important indicator determining the quality and shelf life of oils and lipid-rich foods,[4] since oxidation reactions are responsible for a considerable loss of quality, sensory acceptance and nutritional value of foods.[5–7]

Sensory descriptive analysis methods involve the detection and description of qualitative and quantitative sensory attributes of food products evaluated by trained panelists. These sensory tests are useful to obtain details of appearance, aroma, flavor and texture, providing a complex sensory description of foods.[8] Sensory attributes described by trained panelists can also be related to instrumental, chemical or physical properties.[9–11] In addition, changes in the intensity of specific attributes can be measured over time in order to follow up deterioration reactions that alter the products and make them unacceptable to consumers. Attributes such as oxidized and cardboard flavors are commonly evaluated in oils and lipid-rich foods. These attributes are related to lipid oxidation and increase during storage.[2,3,5,7,12–19]

Antioxidants are compounds that prevent lipid oxidation reactions.[20] The addition of antioxidants to lipid-rich foods is a technically simple way to reduce lipid oxidation, and numerous antioxidant compounds are commercially available for use in foods. However, natural antioxidants (from edible aromatic plants) are more accepted by consumers because they are perceived as safe and for their functional and sensory properties.[6,13]

In previous works the antioxidant effects of different oregano essential oils were studied.[21–23] Oregano species such as *Origanum vulgare* ssp. *virens* (Hoffm. et Link) letswaart and *O. vulgare* L.

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ssp. *vulgare* with higher contents of thymol, sabinene hydrate and carvacrol exhibited higher antioxidant activity. Sabinene hydrate in both species was the major component in the oregano essential oil, ranging between 27 and 38.2%.[23,24] These three monoterpenes could be responsible for the antioxidant activity that is observed in oregano essential oil. Thymol, carvacrol and sabinene hydrate have not been extensively studied as antioxidants in food products. The aim of the present study was to evaluate the antioxidant effects of carvacrol, sabinene hydrate and thymol monoterpenes on the chemical and sensory stability of roasted sunflower seeds.

EXPERIMENTAL

Materials

Sound and mature sunflower seeds (2010 harvest) were provided by Argensun SA (Buenos Aires, Argentina).

The monoterpenes carvacrol, thymol and sabinene hydrate were purchased from Sigma-Aldrich (Buenos Aires, Argentina).

Sample preparation and treatments

Sunflower seeds were roasted at 150 °C in an oven (Model 600, Memmert, Schwabach, Germany) for 30 min. Refined sunflower oil (Natura, Aceitera General Dehesa, General Cabrera, Argentina) was added at 20 g kg^{-1} to the roasted seeds as a vehicle for the essential oil. The sunflower seeds and sunflower oil were placed in a stainless steel coating pan that was kept rotating for 5 min until the oil was distributed evenly on the kernels. Five samples were prepared: roasted sunflower seeds without additives (control sample, RS-C), roasted sunflower seeds with BHT (RS-BHT) and roasted sunflower seeds with carvacrol (RS-Car), sabinene hydrate (RS-S) and thymol (RS-T). BHT and monoterpenes were added in a proportion of 0.2 g kg^{-1} final product using the added sunflower oil as a vehicle after the roasting process of the seeds. The Argentinean Food Code (CAA, 1996) allows a maximum of 0.2 g kg^{-1} BHT and other synthetic antioxidants in vegetable oils. Considering this limit, it was decided to use the maximum allowed (0.2 g kg^{-1}) for BHT and the same concentration for the studied monoterpenes to make the results more comparable.

Experimental design

An experimental design of 5 treatments × 6 storage periods × 3 repetitions was used in the storage study. The treatments were roasted sunflower as control sample (RS-C) and roasted sunflower with the addition of BHT (RS-BHT), carvacrol (RS-Car), sabinene hydrate (RS-S) and thymol (RS-T). Three different lots of each product were prepared separately and each lot was considered a repetition. Samples of roasted sunflower seeds (100 g) were packaged in 27 cm × 28 cm plastic bags (Ziploc, SC Johnson & Son, Buenos Aires, Argentina) in an aerobic atmosphere and stored at room temperature (23 °C) in darkness at 60–70% relative humidity for 35 days. Samples were removed from storage every 7 days for the evaluation of chemical and sensory indicators of product quality. Samples were also evaluated on day 0.

Lipid content

Lipids were extracted from samples with hexane using a Soxhlet apparatus. The lipid content was determined by weight difference.

Fatty acid methyl esters (FAMEs)

FAMEs were prepared from the extracted lipids by transmethylation using a 30 g L^{-1} solution of sulfuric acid in methanol.[25] The FAMEs were analyzed in a Perkin Elmer Clarus 600 gas chromatograph (GC) coupled with a mass detector (Perkin Elmer, Shelton, CT, USA). An EconoWax capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness) (Perkin Elmer, Shelton, CT, USA) was used. The column temperature was increased from 100 to 200 °C at 10 °C min^{-1} and from 200 to 250 °C at 5 °C min^{-1}. The carrier gas was helium at a flow rate of 0.9 mL min^{-1}. The separated FAMEs were identified by comparing their retention times with those of authentic samples purchased from Sigma Chemical Co. (St Louis, MO, USA). Quantitative fatty acid analysis was performed using heptadecanoic acid methyl ester (Sigma Chemical Co.) as internal standard.

Chemical analysis

Peroxide (PV) and p-anisidine (AV) values

Roasted sunflower samples were cold pressed in a 20 ton press (HE-DU/Hermes I, Dupraz SRL, Cordoba, Argentina). The extracted oils were used for measuring PV[26] and AV.[27]

Volatile analysis (VA)

Volatile compounds of sunflower samples were extracted by headspace solid phase microextraction (HS-SPME) and analyzed by gas chromatography/mass spectrometry (GC/MS).[28] The SPME fiber was divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) 50/30 µm, StableFlex, 1 cm long (Supelco, Bellefonte, PA, USA). Extraction conditions were selected according to a method previously optimized in our laboratory. Roasted sunflower seeds (2 g) were placed in a vial at 70 °C for 10 min. The needle of the SPME device was inserted into the vial through a septum and the fiber was exposed to the vial headspace for 30 min. The fiber was then transferred to the injection port of the GC and desorbed for 5 min in splitless mode.[28] Analysis of volatile compounds was performed using a Perkin Elmer Clarus 600 GC coupled with a mass detector (Perkin Elmer). A DB-5 column (30 m × 0.25 mm i.d., 0.25 µm film thickness; Supelco) was used. The column temperature was programmed from 50 °C (10 min hold) to 280 °C (5 min hold) at a rate of 4 °C min^{-1}. The injector was held at 250 °C. Helium was used as carrier gas at a flow rate of 0.9 mL min^{-1}. Ionization was performed by electron impact at 70 eV.

Identification of volatile compounds in sunflower seed samples was performed in full scan mode (*m/z* 40–550) via a combination of the NIST mass spectral library and gas chromatographic retention times of standard compounds. When standards were not available, volatile compounds were tentatively identified using GC/MS spectra only. Chromatographic responses of detected volatile compounds (peak area electronic accounts) were monitored for comparison of each compound between samples.[28]

Hexanal, since it can easily be determined by headspace analysis, is used as an indicator for the characterization of off-flavors resulting from lipid peroxidation.[29] For this reason, hexanal was the aldehyde selected for the evaluation of oxidative stability. Hexanal is a volatile compound and its content is directly related to the development of oxidative off-flavor. It has a low odor threshold and is considered an indicator of oil quality.[30] The peak area (electronic accounts) for hexanal obtained by HS-SPME-GC/MS analysis of roasted sunflower samples can be directly related to the content of this compound in the samples. This peak area was used for

comparative purposes to evaluate the changes in hexanal content in samples during storage.

The monoterpenes (carvacrol, sabinene hydrate and thymol) and BHT added to the roasted sunflower seeds were also detected and followed by VA during storage.

Sensory descriptive analysis

Samples of roasted sunflower seeds (RS-C, RS-BHT, RS-C, RS-S and RS-T) taken from storage were used for the evaluation of sensory descriptive attributes by ten (eight female and two male) trained panelists. All panelists had at least 6 years of experience in evaluating similar products (sunflower and peanut products) and were selected according to the following criteria: people between 18 and 64 years old without food allergies, with natural dentition, non-smokers, available for all sessions, interested in participating and able to verbally communicate their observations regarding the product. Panelists were selected by a screening test. Selected panelists showed a perfect score in a taste sensitivity test and the ability to identify five of seven commonly found food flavors.[8]

Training and calibration of panelists were conducted in eight sessions of 2 h. Panelist training and sample evaluation were based on a hybrid descriptive analysis method formed by the Quantitative Descriptive Analysis (Tragon Corp., Redwood City, CA, USA) and Spectrum Descriptive Analysis (Sensory Spectrum, Inc., Chatham, NJ, USA) methods.[12] Samples were evaluated using a 150 mm unstructured line scale. A list of attribute definitions and a sheet with warm-up and reference intensity ratings (Table 1) were developed during the training sessions. Standard references were employed as a reference point of intensity rating for a determined sensory attribute used during training and evaluation sessions. In addition, roasted sunflower seeds (medium roast, 150 °C for 30 min) were used as a warm-up sample that served as reference for all attributes.[12,31]

All samples were evaluated in partitioned booths under fluorescent light at room temperature. Samples (10 g) were placed in plastic cups with lids and coded with three-digit random numbers. Panelists evaluated 12 samples plus the warm-up sample per day. Before beginning the evaluation of samples, the panelists retested all references and the warm-up sample. The final lists of warm-up and reference intensity ratings and definitions were posted in the booths for all test sessions. Samples were tested using a randomized complete block design. The reported results were mean values obtained from all intensity measurements performed by the panelists for every attribute of each sample.

Statistical analysis

Data were analysed using InfoStat Version 2012p software.[32] Analysis of variance (ANOVA, $\alpha = 0.05$) and the Di Rienzo, Guzmán and Casanoves (DGC) test were performed to find significant differences among means. Linear regression equations were used to determine the effect of the independent variable (storage time). The regression analysis was performed by adjusting the linear model $y = \beta_0 + \beta_1 x$, where y is the dependent variable (chemical indicators and sensory attributes), β_0 is a constant that is equal to the value of y when $x = 0$, β_1 is the coefficient of x, and x is the independent variable (time). Correlation analyses were performed using Pearson's coefficient in order to obtain associations between the chemical and sensory variables from the storage study. Principal component analysis (PCA)[33] was applied to the correlation matrix of the standardized (normalized) data formed by results of chemical variables (PV, conjugated dienes and PV) and sensory variables (oxidized, cardboard, roasted sunflower and essential oil flavors). The purpose of the PCA was to explore associations between the chemical and sensory variables of roasted sunflower samples.

RESULTS AND DISCUSSION

Chemical analyses

The lipid content and fatty acid composition did not show significant differences between samples. The average fat content among samples was 469.94 ± 10.16 g kg^{-1}. The fatty acid composition of the samples (expressed as g kg^{-1} seeds) was 20.44 ± 0.69 g kg^{-1} palmitic acid, 14.160 ± 0.20 g kg^{-1} stearic acid, 160.94 ± 1.44 g kg^{-1} oleic acid, 266.72 ± 2.13 g kg^{-1} linoleic acid, 0.80 ± 0.07 g kg^{-1} linolenic acid, 1.03 ± 0.09 g kg^{-1} arachidic acid, 2.91 ± 0.24 g kg^{-1} behenic acid and 0.99 ± 0.018 g kg^{-1} lignoceric acid.

Changes in the lipid oxidation indicators PV and AV of roasted sunflower seeds during the storage period are shown in Fig. 1. PV and AV increased in most samples during storage, with significant differences between samples ($\alpha = 0.05$). After 21 days of storage, control roasted sunflower seeds (RS-C) and roasted sunflower seeds with BHT (RS-BHT) had the highest and lowest PVs (Fig. 1(a)) and AVs (Fig. 1(b)) respectively. Samples treated with monoterpenes, i.e. roasted sunflower seeds with carvacrol (RS-Car), sabinene hydrate (RS-S) and thymol (RS-T), had intermediate PVs and AVs.

During storage, RS-Car and RS-T samples showed no significant differences in PV. From day 21, RS-S (59.09 meq O_2 kg^{-1}) had the

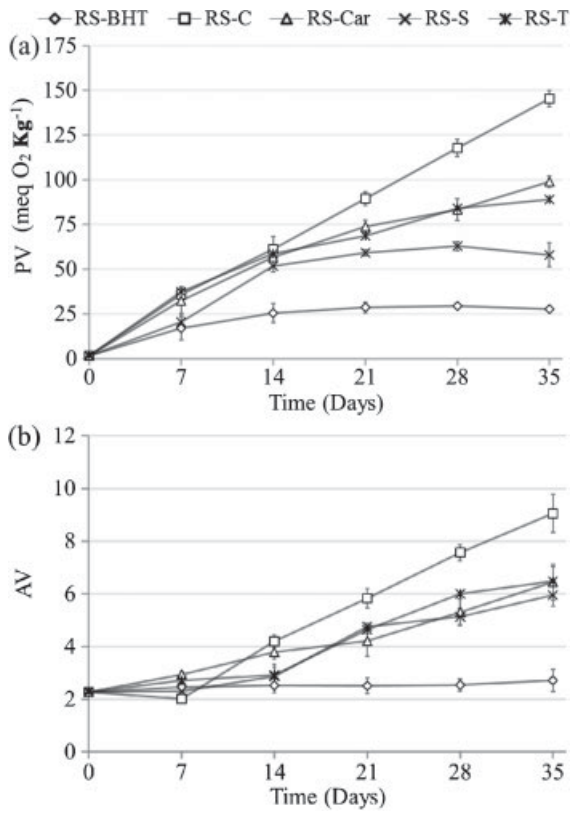

Figure 1. (a) Peroxide value (PV) and (b) *p*-anisidine value (AV) evaluated in samples of roasted sunflower control (RS-C), with BHT (RS-BHT) and with monoterpenes carvacrol (RS-Car), sabinene hydrate (RS-S) and thymol (RS-T) during storage.

lowest PV in comparison with the other samples treated with monoterpenes (RS-T and RS-Car, 68.73 and 73.93 meq O_2 kg^{-1} respectively). On day 35 (the final day of storage) the RS-BHT sample had the lowest PV (27.74 meq O_2 kg^{-1}) and the RS-C sample had the highest PV (145.35 meq O_2 kg^{-1}), while RS-S had a lower PV (58.05 meq O_2 kg^{-1}) than both RS-Car (98.95 meq O_2 kg^{-1}) and RS-T (89.12 meq O_2 kg^{-1}), which did not differ significantly. AVs in samples containing monoterpenes (RS-T, RS-Car and RS-S) did not differ significantly during storage (Fig. 1(b)). On day 35, AVs were 9.05, 2.71, 6.45, 5.95 and 6.48 for RS-C, RS-BHT, RS-Car, RS-S and RS-T respectively.

The main volatile compounds detected by GC/MS analysis were hexanal, nonanal and 2-heptenal. In particular, hexanal was the component that showed the highest significant changes observed

Table 1. Definitions of sensory attributes, standard references and warm-up intensity ratings used in descriptive analysis of roasted sunflower seeds with addition of monoterpenes carvacrol, sabinene hydrate and thymol

Attribute[a]	Definition[b]	Reference	Reference intensity[c]	Warm-up intensity[c]
Appearance				
Brown color	The intensity or the strength of brown color from light to dark brown	Cardboard (lightness value $L = 47 \pm 1.0$)[d]	65	52
Roughness	The appearance associated with uneven surface	Corn flakes[e]	85	14
Glossiness	The appearance associated with the amount of light reflected by the product surface	Peanuts coated with chocolate[f]	58	21
Aromatics				
Roasted sunflower flavor	The aromatic associated with medium roasted sunflower	Dry roasted sunflower[g]	44	57
Oxidized	The aromatic associated with rancid fats and oils	Rancid sunflower seeds[h]	100	7
Cardboard	The aromatic associated with wet cardboard	Moist cardboard[i]	30	20
Carvacrol flavor	The aromatic associated with monoterpene carvacrol	Roasted sunflower seeds with carvacrol (0.2 g kg^{-1})	35	0
Sabinene hydrate flavor	The aromatic associated with monoterpene sabinene hydrate	Roasted sunflower seeds with sabinene hydrate (0.2 g kg^{-1})	27	0
Thymol flavor	The aromatic associated with monoterpene thymol	Roasted sunflower seeds with thymol (0.2 g kg^{-1})	21	0
Taste				
Bitterness	Taste on the tongue associated with bitter solutions such as caffeine	0.5 g kg^{-1} caffeine solution	20	19
		0.8 g kg^{-1} caffeine solution	50	
		1.5 g kg^{-1} caffeine solution	100	
Sourness	Taste on the tongue associated with acid agents such as citric acid solutions	0.5 g kg^{-1} citric acid solution	20	7
		0.8 g kg^{-1} citric acid solution	50	
		1.5 g kg^{-1} citric acid solution	100	
Feeling factor				
Astringency	The shrinking or puckering of the tongue surface	Tea infusion[j]	34	35
Texture				
Crunchiness	Force needed and amount of sound generated from chewing a sample with molar teeth	Corn flakes[e]	110	23
Hardness	Force needed to compress a food between molar teeth	Almonds[k]	70	19

[a] Attributes are listed in order as perceived by panelists.
[b] Attribute definitions were based on a lexicon for roasted peanuts[31] and adapted for roasted sunflower seeds.
[c] Intensity ratings are based on 150 mm unstructured line scales. Warm-up sample: roasted sunflower seeds (medium roast) that were roasted at 150 °C for 30 min.
[d] Piece of cardboard with color lightness value $L = 50 \pm 1$ measured in Hunter Lab colorimeter.
[e] Corn flakes: Granix, Buenos Aires, Argentina.
[f] Peanuts coated with chocolate: ARCOR, Colonia Caroya, Argentina.
[g] Dry roasted sunflower seeds: Argensun SA, Buenos Aires, Argentina.
[h] Dry roasted sunflower seeds (Argensun SA) stored at 40 °C for 7 and 15 days until developing rancid flavor with intensity ratings of 30 and 80 on a 150 mm line scale respectively.
[i] Moist cardboard: 1 mL of distilled water absorbed by 0.5 g of cardboard.
[j] Tea infusion: four tea bags (La Virginia, Córdoba, Argentina) soaked in 1 L of distilled water at 80 °C for 10 min.
[k] Almonds: Grandiet, Córdoba, Argentina.

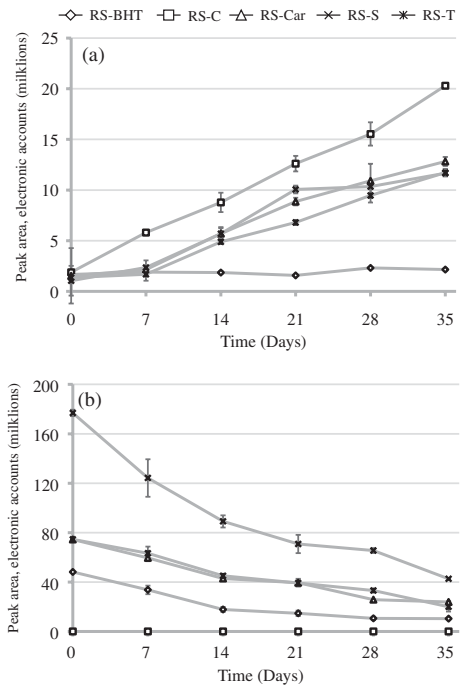

Figure 2. Volatile analysis: (a) hexanal content and (b) antioxidants (BHT, carvacrol, sabinene hydrate and thymol) evaluated in samples of roasted sunflower control (RS-C), with BHT (RS-BHT) and with monoterpenes carvacrol (RS-Car), sabinene hydrate (RS-S) and thymol (RS-T) during storage.

in the samples during storage. In addition, the formation of this aldehyde is related to the oxidation process of linoleic acid, which is the major fatty acid in sunflower oil.[4]

Linoleic acid is the major fatty acid in roasted sunflower seeds. It is sensitive to autoxidation and is a precursor of hexanal that is predominant in the volatile fraction.[29] Hexanal content is directly related to the development of oxidative off-flavors in lipid-rich foods.[30] In general, the hexanal content (peak area) in roasted sunflower samples increased significantly during storage ($\alpha = 0.05$) with the exception of RS-BHT (Fig. 2(a)), which remained constant. After 14 days of storage, RS-C and RS-BHT exhibited the highest and lowest hexanal contents, 8.78 and 1.87 million (electronic accounts) respectively, while samples treated with monoterpenes had intermediate hexanal contents (RS-Car, RS-S and RS-T, 5.70, 5.70 and 4.88 million respectively). Initially, all treatments showed similar hexanal peak area (electronic accounts) values between 1.05 and 1.89 million, which were significantly different after 7 days of storage. By day 35, samples had the following peak areas: 20.30, 2.16, 12.82, 11.66 and 11.72 million (electronic accounts) for RS-C, RS-BHT, RS-Car, RS-S and RS-T respectively. The concentrations of monoterpenes (carvacrol, thymol and sabinene hydrate) decreased faster than the concentration of BHT (Fig. 2(b)). This higher decrease in monoterpenes could have been responsible for lower antioxidant activity.

These results indicated that the addition of BHT and monoterpenes carvacrol, sabinene hydrate and thymol provided protection against lipid oxidation in roasted sunflower seeds. Previous studies report the antioxidant effects of oregano essential oil, rich in thymol and carvacrol, added to olive oil,[5,21] peanut products[14] and cheese.[2] Quiroga *et al.*[23] studied the antioxidant activity of essential oils from four different oregano species (Compacto, Cordobes, Mendocino and Criollo). They found that the essential oils from Compacto and Cordobes oregano species were rich in thymol and sabinene hydrate and both showed higher antioxidant activity than the other species.

Sensory descriptive analysis

The attribute intensity ratings of fresh roasted sunflower seeds (storage day 0) evaluated by descriptive analyses are shown in Table 2. Attributes with high intensity ratings (on a 0–150 scale) for all samples before storage were color (53.25–54.17) and roasted sunflower flavor (57.50–60.42), while negative attributes such as oxidized and cardboard flavors were low (<17). In general, fresh samples did not show significant differences in their intensity ratings for most of the evaluated attributes. The only exception was the monoterpene flavor attribute, where R-Car (33.50) had the highest monoterpene flavor intensity, followed by RS-S (25.13) and RS-T (17.25).

The roasting process is a heat treatment used to develop an attractive aroma and/or flavor for the consumer[34] and to decrease or eliminate microbes as required by food safety norms. In contrast, volatile compounds arising from lipid oxidation cause oxidized/rancid odors and flavors that are disliked by the consumer.

Table 2. Mean ± standard deviation ($n = 3$) of sensory attributes in fresh samples evaluated at day 0 of storage. Samples: control roasted sunflower seeds (RS-C), with BHT (RS-BHT) and with monoterpenes carvacrol (RS-Car), sabinene hydrate (RS-S) and thymol (RS-T)

Attribute	RS-BHT	RS-C	RS-Car	RS-S	RS-T
Color	53.25 ± 0.66	54.17 ± 0.58	53.75 ± 1.25	54.00 ± 0.50	53.42 ± 0.29
Roughness	14.25 ± 0.25	14.25 ± 0.25	14.25 ± .25	14.25 ± 0.25	14.00 ± 0.25
Glossiness	18.25 ± 0.50	18.08 ± 0.29	18.42 ± 0.52	18.25 ± 0.25	18.25 ± 0.50
Roasted sunflower	59.42 ± 0.80	60.42 ± 2.90	58.67 ± 1.89	57.5 ± 1.95	59.08 ± 0.52
Oxidized	4.00 ± 1.32	4.33 ± 1.61	4.00 ± 1.32	4.00 ± 1.32	4.00 ± 1.32
Cardboard	16.00 ± 0.25	15.75 ± 0.25	15.83 ± 0.29	16.33 ± 0.58	15.92 ± 0.38
Carvacrol flavor	ND	ND	33.50 ± 2.25	ND	ND
Sabinene hydrate flavor	ND	ND	ND	25.13 ± 1.88	ND
Thymol flavor	ND	ND	ND	ND	17.25 ± 1.39
Sourness	18.67 ± 0.38	17.67 ± 1.04	19.33 ± 1.89	19.42 ± 1.81	19.67 ± 1.59
Bitterness	0.13 ± 0.13	0.25 ± 0.25	0.13 ± 0.13	0.13 ± 0.13	0.13 ± 0.13
Astringency	30.50 ± 0.66	30.00 ± 1.50	31.17 ± 1.15	30.5 ± 0.66	30.67 ± 0.76
Crunchiness	20.75 ± 0.75	20.00 ± 1.32	21.25 ± 0.25	21.5 ± 0.25	20.67 ± 0.63
Hardness	21.58 ± 1.51	22.00 ± 0.50	21.92 ± 0.14	21.75 ± 0.25	21.08 ± 0.95

Means in a row followed by different letters are significantly different among samples (ANOVA and DGC test, $\alpha = 0.05$). ND, not detected.

These flavors are related to negative sensory attributes called oxidized and cardboard flavors.[31] In addition, positive attributes such as roasted flavor decrease while negative attributes increase during product storage.[10,12,35] Changes in the intensity ratings for oxidized, cardboard, roasted sunflower and monoterpene flavors in the samples during storage are presented in Fig. 3. All samples had increased intensity ratings for oxidized (Fig. 3(a)) and cardboard (Fig. 3(b)) flavors and decreased ratings for roasted sunflower (Fig. 3(c)) and monoterpene (Fig. 3(d)) flavors during storage.

RS-C showed the highest intensities for oxidized and cardboard flavors in comparison with other samples, with significant differences after 21 days of storage. At the end of the storage period (35 days), RS-C (52.00) had the highest intensity of oxidized flavor, which was significantly different from the other treatments. RS-Car (22.67) had the lowest oxidized intensity rating, not significantly different from that of RS-BHT (26.67). RS-T (31.33) and RS-S (34.00) had intermediate intensities for this attribute, with no significant differences between them (Fig. 3(a)). For cardboard flavor (Fig. 3(b)), on day 35, RS-C (40.00) showed the highest intensity rating and RS-Car (23.00) the lowest, followed by RS-T (26.00), while RS-S (34.00) and RS-BHT (32.00) had no significant differences between them. The results indicated that the addition of monoterpenes protected the roasted sunflower seeds, inhibiting the formation of undesirable flavors from secondary lipid oxidation.

Roasted sunflower flavor is considered a positive sensory attribute related to a pleasant aroma or taste (nut-like) that is developed during the roasting process.[34] In all samples the intensity rating for roasted sunflower flavor decreased during storage (Fig. 3(c)). Initially, all treatments had similar intensities (~60) for this attribute (Table 2), but significant differences between samples were detected from day 21 onward. At the end of storage (35 days), RS-C (39.67) had the lowest value for this attribute compared with the samples protected with antioxidants (RS-BHT, RS-Car, RS-S and RS-T, 49.67, 50.00, 47.67 and 46.67 respectively). When the roasted flavor decreases during storage, it is considered a negative effect on the sensory quality of the product.[3,14]

The monoterpene flavor intensity rating also decreased in RS-Car, RS-S and RS-T samples during storage (Fig. 3(d)). RS-Car showed higher intensity ratings for this attribute than RS-S and RS-T during storage.

The descriptive sensory analyses of roasted sunflower seed samples indicated that the addition of monoterpenes protected the product, reducing the formation of undesirable flavors produced during lipid oxidation (oxidized and cardboard flavors) and preventing the loss of positive attributes such as roasted sunflower flavor. This protective effect was consistent with the results obtained for chemical indicators of oxidation (presented above). Other investigators have also reported an increase in the intensity of negative attributes (oxidized and cardboard tastes) and a decrease in positive attributes (roasted peanut and essential oil flavors) during storage in related products, indicating a deterioration in organoleptic properties.[3,6,14,15] Previous studies reported that the oregano essential oil flavor decreases in olive oil added with oregano essential oil, rich in thymol and carvacrol,[6] and the addition of other natural antioxidants such as essential oils and peanut skin extracts provides protection against lipid oxidation.[6,17,18]

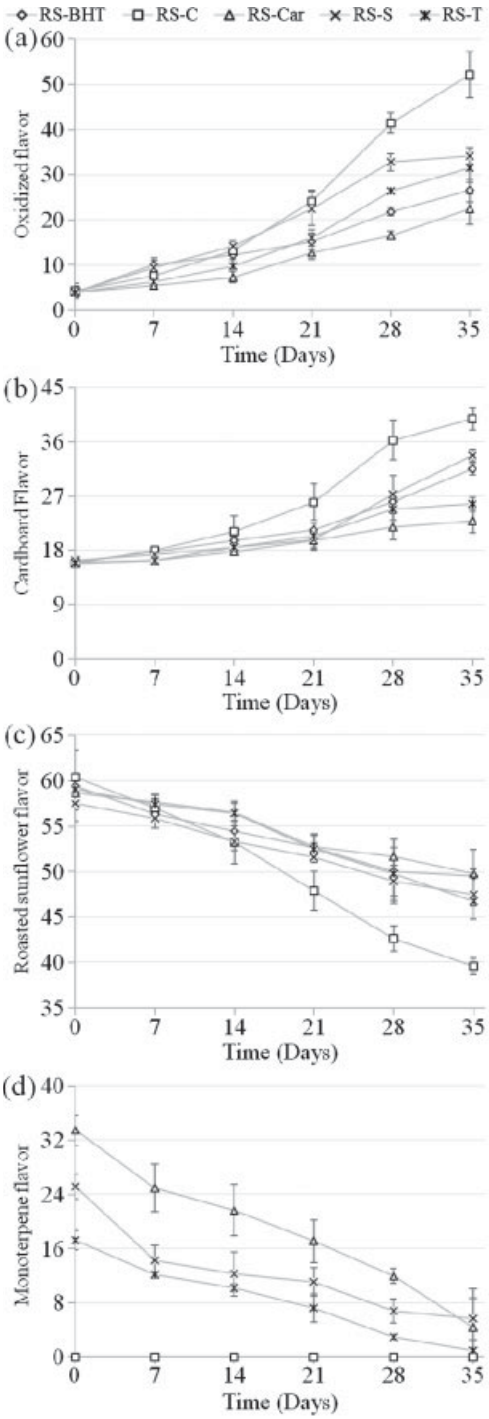

Figure 3. Attribute intensity ratings of (a) oxidized, (b) cardboard, (c) roasted sunflower and (d) monoterpene flavors evaluated in samples of roasted sunflower control (RS-C), with BHT (RS-BHT) and with monoterpenes carvacrol (RS-Car), sabinene hydrate (RS-S) and thymol (RS-T) during storage.

Regression analyses

Table 3 shows the linear regression equations obtained from the chemical and sensory analysis data during storage. In general, all regression models showed an R^2 higher than 0.65, indicating good adjustment between chemical data, sensory data and storage time, with the exception of the RS-BHT sample, which was in poor agreement with the models for the chemical variables AV and hexanal ($R^2 = 0.05$ and 0.35 respectively). The slopes (β_1) of the linear regressions are an indicator of the deterioration tendency of each treatment. Significant differences were found between the slopes for PV, AV, hexanal and antioxidants.

Table 3. Regression equations and R^2 for dependent variables peroxide value (PV), *p*-anisidine value (AV), hexanal content, antioxidants and oxidized, cardboard, roasted sunflower and monoterpene flavors of control roasted sunflower seeds (RS-C), with BHT (RS-BHT) and with monoterpenes carvacrol (RS-Car), sabinene hydrate (RS-S) and thymol (RS-T)

Dependent variable	Sample	β_0 [a]	β_1 [a, b]	R^2
PV	RS-BHT	9.46 ± 3.91	0.70 ± 0.09d	0.6567
	RS-C	4.40 ± 1.26	4.05 ± 0.15a	0.9931
	RS-Car	10.97 ± 4.46	2.68 ± 0.19b	0.9475
	RS-S	12.60 ± 2.41	1.70 ± 0.16c	0.7678
	RS-T	12.15 ± 9.21	2.48 ± 0.62b	0.8677
AV	RS-BHT	2.45 ± 0.46	0.01 ± 0.01 c	0.0469
	RS-C	1.43 ± 0.15	0.21 ± 0.01a	0.9472
	RS-Car	2.13 ± 0.14	0.12 ± 0.02b	0.9220
	RS-S	1.82 ± 0.13	0.12 ± 0.01b	0.9001
	RS-T	1.84 ± 0.05	0.13 ± 0.01b	0.9193
Hexanal content	RS-BHT	1.67 ± 0.22	0.01 ± 0.01c	0.3510
	RS-C	1.89 ± 0.27	0.51 ± 0.04a	0.9660
	RS-Car	0.86 ± 0.31	0.35 ± 0.02b	0.9434
	RS-S	1.05 ± 0.70	0.33 ± 0.02b	0.9324
	RS-T	0.48 ± 0.38	0.31 ± 0.02b	0.9659
Antioxidants (volatile analysis)	RS-BHT	41.23 ± 3.03	−1.07 ± 0.12a	0.8328
	RS-Car	69.91 ± 3.33	−1.46 ± 0.16b	0.9384
	RS-S	156.72 ± 7.53	−3.53 ± 0.28c	0.8925
	RS-T	72.25 ± 3.41	−1.50 ± 0.18b	0.9568
Oxidized flavor	RS-BHT	4.35 ± 1.18	0.62 ± 0.04c	0.9447
	RS-C	−1.03 ± 0.91	1.43 ± 0.06a	0.9286
	RS-Car	2.20 ± 1.65	0.54 ± 0.10c	0.9124
	RS-S	3.48 ± 0.39	0.93 ± 0.01b	0.9499
	RS-T	1.29 ± 0.83	0.82 ± 0.08b	0.9201
Cardboard flavor	RS-BHT	14.62 ± 0.05	0.43 ± 0.04b	0.9020
	RS-C	13.45 ± 0.40	0.73 ± 0.07a	0.9056
	RS-Car	15.27 ± 0.75	0.23 ± 0.07c	0.8352
	RS-S	13.73 ± 0.75	0.49 ± 0.07b	0.8079
	RS-T	14.78 ± 0.31	0.31 ± 0.03c	0.8654
Roasted sunflower flavor	RS-BHT	59.11 ± 0.85	−0.29 ± 0.03a	0.8039
	RS-C	61.25 ± 1.93	−0.63 ± 0.06b	0.9479
	RS-Car	59.64 ± 1.24	−0.28 ± 0.11a	0.8358
	RS-S	57.83 ± 1.75	−0.29 ± 0.10a	0.8708
	RS-T	60.30 ± 0.65	−0.37 ± 0.05a	0.8913
Monoterpene flavor	RS-Car	32.40 ± 3.29	−0.77 ± 0.10c	0.9065
	RS-S	21.13 ± 0.84	−0.49 ± 0.11b	0.7540
	RS-T	16.54 ± 0.73	−0.45 ± 0.02b	0.9425

[a] Regression equations: $Y = \beta_0 + \beta_1 X$, where Y = dependent variable and X = independent variable (time).
[b] ANOVA and DGC multiple range test: for each variable, slopes (β_1) followed by different letters are significantly different ($\alpha = 0.05$).

RS-BHT showed the greatest chemical stability (lowest PV, AV and hexanal content slopes), RS-C exhibited the highest slopes, while monoterpene-treated samples (RS-Car, RS-S and RS-T) had intermediate PV, AV and hexanal content slope values. RS-S samples had a lower PV slope than RS-Car and RS-T samples, but they did not differ in their AV and hexanal slopes.

Significant differences were also found between samples with respect to their sensory attributes. The linear regression slopes for RS-C were higher for oxidized and cardboard flavors and lower for roasted sunflower flavor than for other treatments. Lower slopes were found for RS-Car (0.5374) and RS-BHT (0.6149) for oxidized flavor and for RS-Car (0.2259) and RS-T (0.3143) for cardboard flavor, though the differences were not significant within the two pairs. Additionally, all samples treated with antioxidants (RS-Car, RS-T, RS-S and RS-BHT) had a lower tendency to lose the roasted sunflower flavor than the control sample (RS-C). The slopes indicated that the addition of monoterpenes protected the product and inhibited the formation of undesirable flavors. Similar results are reported for other food products treated with natural antioxidants.[3,6,14,17,19]

The intensity ratings for monoterpene flavor also decreased in RS-Car, RS-S and RS-T during storage (Fig. 3(d)), as indicated by the negative slopes (Table 3). RS-Car had a higher monoterpene flavor intensity rating than RS-S and RS-T at the beginning of the storage period but also had the highest decrease (higher negative slope, −0.7690) for this attribute compared with the other samples (RS-S, −0.4900; RS-T, −0.4530). These results are related to the slope decrease observed in the volatile analysis of monoterpenes and BHT (Table 3). The negative slopes for the amount of antioxidant in samples RS-Car (−1.4594), RS-S (−3.5341) and RS-T (−1.5041) were

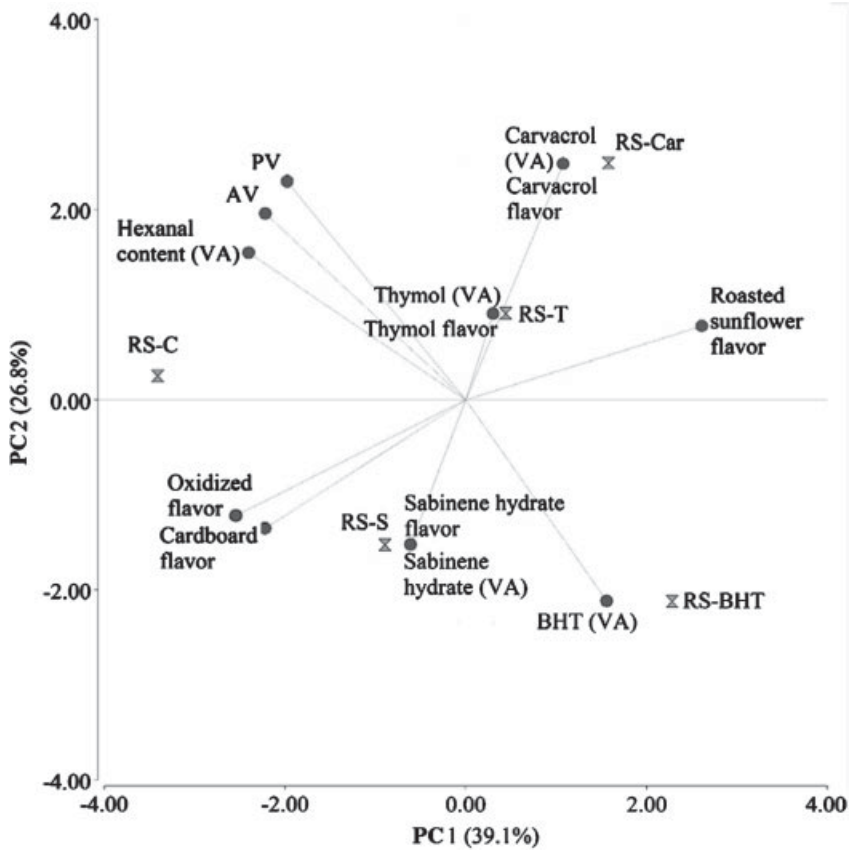

Figure 4. Biplot from first (PC 1) and second (PC 2) principal components of PCA. Variables: peroxide value (PV), *p*-anisidine value (AV), hexanal content by volatile analisis (VA), antioxidants (BHT, carvacrol, sabinene hydrate and thymol) by volatile analisis (VA) and oxidized, cardboard, roasted sunflower and monoterpene (carvacrol, sabinene hydrate and thymol) flavors evaluated in roasted sunflower control (RS-C), with BHT (RS-BHT) and with monoterpenes carvacrol (RS-Car), sabinene hydrate (RS-S) and thymol (RS-T) during storage.

higher than in RS-BHT (−1.0652). Therefore the monoterpenes exhibited higher loss by evaporation than BHT. This could explain the lowest activity of the monoterpenes during storage.

Principal component analysis

The biplot obtained from the first two principal components (PCs) of the PCA is presented in Fig. 4. The first two PCs explained 65.9% of variability in the samples during storage, which was considered acceptable for explaining correlations between variables. High variability among samples was indicated through the point dispersions. PV, AV and hexanal content (chemical lipid oxidation indicators) in stored roasted sunflower samples were positively associated and were also associated with the negative sensory attributes from descriptive analyses (oxidized and cardboard flavors), having a positive Pearson's correlation coefficient higher than 0.72. In addition, the oxidized and cardboard flavor sensory variables were negatively associated with the positive roasted sunflower flavor attribute (negative correlation coefficients −0.94 and −0.89 respectively), as were the chemical indicators PV, AV and hexanal content (−0.81, −0.93 and −0.94 respectively). RS-BHT and RS-Car samples were associated with roasted sunflower flavor, while RS-C was associated with lipid oxidation indicators (PV, AV and hexanal content) as well as oxidized and cardboard flavors.

A study of the antioxidant activity of different oregano essential oils in a canola oil storage study shows that thymol and sabinene hydrate contents in the essential oils were related to their antioxidant activity and ability to capture free radicals.[23] Carvacrol and thymol are oxygenated monoterpenes with phenolic structure and proven antioxidant properties.[36] Asensio *et al.*[5] researched the antioxidant effect of oregano essential oil, rich in thymol and carvacrol, in olive oil and found that lipid oxidation indicators (conjugated dienes and AV) were positively associated with samples lacking the essential oil. Similar correlations were found between chemical and sensory variables in stored soybean and peanut products.[3,16]

The mechanism of the oxidization of lipid systems leads to changes when an antioxidant molecule acts in the reaction medium. To explain the inhibitory action of antioxidants, there are two kinetic characteristics:[37] (i) affecting the induction period duration by interaction with peroxide radicals and (ii) changing the oxidation rate during the induction periods. In the case of thymol and carvacrol, which are isomeric molecules, they could take part in chain reaction initiation during the oxidation of sunflower oil triglycerides. In particular, carvacrol could be participating in a reaction of chain propagation. Thymol was a more active and effective antioxidant than carvacrol. With respect to sabinene hydrate, its mechanism of action as antioxidant in the oxidation process of sunflower oil is not clear.

CONCLUSIONS

The addition of monoterpenes (carvacrol, thymol and sabinene hydrate) improves the stability of roasted sunflower seeds,

preventing lipid oxidation and the development of rancid odors and tastes, which increases the shelf life of roasted sunflower seeds. The PCA suggested that the treatments with monoterpenes are associated with positive sensory attributes such as roasted sunflower flavor. Carvacrol and thymol show higher preserving action on sensory properties of this food product, while sabinene hydrate exhibits better behavior preventing peroxide formation. Considering the results of this research, the inclusion of monoterpenes (thymol, sabinene hydrate and carvacrol) in roasted sunflower seeds has an antioxidant effect. These compounds could possibly replace synthetic antioxidants such as BHT in other similar food products even though they had lower antioxidant activity.

The presence of the monoterpenes in the roasted sunflower seeds was detected by the sensory panel and will probably be perceived by consumers, affecting their acceptance. With the present results, it is not possible to know whether the effect of monoterpene addition will be positive or negative for the acceptability of the product until consumer tests have been performed.

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