# **Antioxidant Effect of Poleo and Oregano Essential** Oil on Roasted Sunflower Seeds

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The objective was to evaluate the stability of sensory and chemical parameters in roasted sunflower seeds supplemented with oregano and poleo essential oils; and the consumer acceptability of this product. Four samples were prepared: plain roasted sunflower seeds (Control = RS-C), and sunflower seeds added with oregano (RS-O) or poleo (RS-P) essential oils or BHT (RS-BHT). Consumer acceptance was determined on fresh samples. The overall acceptance averages were 6.13 for RS-C, 5.62 for RS-P, and 5.50 for RS-O (9-point hedonic scale). The addition of BHT showed greater protection against the oxidation process in the roasted sunflower seeds. Oregano essential oil exhibited a greater antioxidant effect during storage than poleo essential oil. Both essential oils (oregano and poleo) provided protection to the product, inhibiting the formation of undesirable flavors (oxidized and cardboard). The antioxidant activity that presents essential oils of oregano and poleo could be used to preserve roasted sunflower seeds.

Keywords: antioxidant, essential oils, oregano, poleo, sunflower

Practical Application: The addition of natural additives instead of synthetic ones covers the present trend in food technology. The food product shelf-life could be prolonged by the addition of natural preservatives as essential oils. This research showed that the addition of oregano and poleo essential oils preserved the intensity ratings of positive sensory attributes and quality parameter in roasted sunflower seeds during storage. The addition of these essential oils should be considered for the food industry as a natural source of antioxidant additives for preserving quality properties in this food product and, also, in food with high fat content.

#### Introduction

The lipid and protein contents of sunflower seeds are from 45% to 50% and 33% to 35%, respectively. The sunflower oil is rich in polyunsaturated fatty acids and this makes it susceptible to lipid oxidation. The chemical composition of sunflower oil shows 18.7% oleic acid and 67.5% linoleic acids as the major components in its oil (Belitz and others 2009). Because of this chemical composition, sunflower seeds are a source of oils and proteins (Brătfâlean and others 2008) and are extensively used for manufacturing protein meal used for feed and edible oils. From the human health point of view, linoleic acid (18:2) is essential to mammals and shows a great hypocholesterolemic action (Kris-Etherton and others 2001), decreasing the cardiovascular disease risk. Omega-6 and omega-3 fatty acids, medium chain triglycerides, conjugated linoleic acids, and phytosterols are functional lipids and have many beneficial effects on obesity, bone health, treating and managing depression, blood pressure, cardiovascular health, and other issues related to human health (Alabdulkarim and others 2012).

Oxidation reaction in lipids produces deterioration in edible oils and fat. Oxidation reactions produce considerable losses in qual-

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ity, sensory acceptance, and nutritional value, limiting lipidic food shelf-life (Asensio and others 2011, 2012). This degradation process involves the oxidation of unsaturated fatty acids or their derivatives by a free radical mechanism (Saad and others 2007). Peroxides and hydroperoxides are the main primary products formed in the initial stages of lipids oxidation reactions. Products of secondary lipid oxidation as ketones, alkenes, alkanes, and aldehydes are produced in the final period. These types of molecules are responsible for rancid odor (Pereira de Abreu and others 2011). The susceptibility of fatty acids to oxidation is thought to be directly dependent 61 on their degree of unsaturation (Nepote and others 2009; Riveros and others 2009).

Interest in naturally occurring antioxidants for food preservation is emerging due to their technological relevance and positive impact on consumer health. Multiple researches in the field of new antioxidant agents are being conducted in order to replace synthetic antioxidants by natural ones (Asensio and others 2011, 2012; Quiroga and others 2011, 2013). Due to their antioxidant effect, health properties, and high consumer acceptance, essential oils are gaining attention in the food industry as preservation agents (Irshaid and others 2012).

Oregano has a characteristic and attractive flavor used for flavoring foods. Mexico, Israel, Greece, Albania, Turkey, and Morocco have the main areas of cultivation of this herb (Koksal and others 2010). Origanum vulgare (L.) is the species of oregano cultivated in Argentina (Dambolena and others 2010). Oregano essential oil and leaves are consumed not only for their flavor profile but also for their positive properties on human health. These properties can be associated to the soluble phenolic fractions and essential oil antioxidant activity (Ruberto and others 2002).

Table 1-Definitions of sensory attributes, standard references, and warm-up intensity ratings used in descriptive analysis of roasted sunflower seeds with the addition of oregano and poleo essential oils.

Attributes <sup>a</sup>	Definition <sup>b</sup>	References	References intensity <sup>c</sup>	Warm-up intensity <sup>c,d</sup>
Appearance				
Brown color	The intensity or the strength of brown color from light to dark brown.	Cardboard (lightness value, $L = 47 \pm 1.0$ ) <sup>d</sup>	65	52
Roughness	The appearance associated with uneven surface.	Corn flakes <sup>e</sup>	85	14
Glossiness	The appearance associated with the amount of light reflected by the product surface.	Peanuts coated with chocolate <sup>f</sup>	58	21
Aromatics				
Oregano essential oil	The aromatic associated with essential oil of oregano.	Sunflower oil with oreganog	35	0
Poleo essential oil	The aromatic associated with essential oil of poleo.	Sunflower oil with poleog	28	0
Roasted sunflower flavor	The aromatic associated with medium roasted sunflower.	Dry roasted sunflowerh	57	57
Oxidized	The aromatic associated with rancid fats and oils.	Rancid sunfloweri	30-110	7
Cardboard	The aromatic associated with wet cardboard.	Moist cardboard <sup>j</sup>	70	20
Tastes				
Saltiness	Taste on the tongue associated with sucrose solutions.	2.0% sucrose solution	20	8
		5.0% sucrose solution	50	
		10.0% sucrose solution	100	
Sweetness	Taste on the tongue associated with sodium	0.2% NaCl solution	25	8
	chloride solutions.	0.35% NaCl solution	50	
		0.5% NaCl solution	85	
Sourness	Taste on the tongue associated with acid	0.05% citric acid solution	20	7
	agents such as citric acid solutions.	0.08% citric acid solution	50	
		0.15% citric acid solution	100	
Bitterness	Taste on the tongue associated with bitter	0.05% caffeine solution	20	19
	solutions such as caffeine.	0.08% caffeine solution	50	
		0.15% caffeine solution	100	
Feeling factor				
Astringency Texture	The shrinking or puckering of the tongue surface.	Tea infusion <sup>k</sup>	80	35
Hardness	Force needed to compress a food between molar teeth.	Almonds <sup>1</sup>	80	19
Crunchiness	Force needed and amount of sound generated from chewing a sample with molar teeth.	Corn flakes <sup>e</sup>	95	23

<sup>&</sup>lt;sup>a</sup>Attributes listed in order as perceived by panelists.

Lippia turbinata GRISEB, whose common name is "poleo," is a native South American shrub that grows in north-eastern region of Argentina, which is used in traditional medicine to relieve gastrointestinal disorders and in the industry as a bitter-flavor agent (Ricciardi and others 2005). In a previous work, the chemical composition, free antilipase activities, and radical scavenging of oregano and poleo essential oils were studied (Quiroga and others 2013). In that research, it was observed that their essential oils show interesting properties as potential natural agents for preserving the quality of fats and oils in food products.

The research objective was to evaluate (1) the stability of sensory and chemical parameters in roasted sunflower seeds supplemented with oregano and poleo essential oils; and (2) the consumer acceptability of this product.

#### Materials and Methods

#### Materials

Sound and mature 2010 harvest sunflower seeds were provided by Argensun S.A. (Buenos Aires, Argentina). The essential oils were obtained from Lippia turbinata GRISEB harvested in Altas Cumbres, Cordoba, Argentina and from Origanum vulgare L. harvested in San Carlos farm, Mendoza, Argentina. Plant materials were collected at the full flowering stage in November 2010. The plants (2-y-old) were cut 5 cm above the soil including leaves, flowers, and stems. Hydro-distillation in a Clevenger-type apparatus was used for extracting the essential oils (Quiroga and others 2013).

# Sample preparation and treatments

Sunflower seeds were roasted at 150 °C in an oven (Memert, model 600, Schwabach, Germany) for 30 min. Commercial sunflower oil (Natura, Aceitera General Dehesa, General Cabrera, Argentina) was used at 2% (w/w) for glossing the product and as a vehicle to include the essential oil. The glossing procedure was made using a stainless steel coating pan that was kept rotating for 5 min until the sunflower oil covered evenly the roasted sunflower seed. Four samples were prepared (1) roasted sunflower seeds without additives (Control sample, RS-C), roasted sunflower seeds with BHT (RS-BHT), and roasted sunflower seeds with the

<sup>&</sup>lt;sup>b</sup>The attributes definitions were based on a lexicon for roasted peanuts (Johnsen and others 1988) and adapted for roasted sunflower seeds.

<sup>&</sup>lt;sup>c</sup>Intensity ratings are based on 150-mm unstructured line scales

<sup>&</sup>lt;sup>d</sup>Piece of cardboard with color lightness value,  $L=50\pm1$  measured in colorimeter Hunter Lab.

<sup>&</sup>lt;sup>e</sup>Corn flakes, Granix, Buenos Aires, Buenos Aires Province, Argentina.

<sup>&</sup>lt;sup>f</sup>Peanuts coated with chocolate, ARCOR, Colonia Caroya, Córdoba Province, Argentina.

gRefined sunflower oil (Natura, AGD, General Cabrera, Cordoba Provincia, Argentina) with 0.1% oregano or poleo essential oils.

<sup>&</sup>lt;sup>h</sup>Dry roasted sunflower seeds (Argensun S.A. Buenos Aires, Buenos Aires Province, Argentina).

Dry roasted sunflower seeds (Argensun S.A. Buenos Aires, Buenos Aires Province, Argentina) stored at 40 °C during 7 and 15 d with intensity ratings of 30 and 80 in a 150-mm line scale, respectively.

Moist cardboard: 1 mL distilled water absorbed by 0.5-g cardboard.

<sup>&</sup>lt;sup>k</sup>Tea infusion: 4 tea bags (La Virginia, Córdoba, Cordoba Province, Argentina) soaked in 1 L of distilled water at 80 °C during 10 min.

<sup>&</sup>lt;sup>1</sup>Almonds, Grandiet, Córdoba, Cordoba Province, Argentina.

Table 2-Means and standard deviation (n = 100) of the consumer tests for color, odor, flavor, texture, and overall acceptances measured in a hedonic scale of 9 points of roasted sunflower seeds (RS-C), roasted sunflower seeds with BHT (RS-BHT), and roasted sunflower seeds with the addition of oregano (RS-O) and poleo (RS-P) essential oils.

Consumer acceptance	RS-C*	RS-BHT*	RS-P*	RS-O*
Color	5.75 ± 0.12a	5.88 ± 0.14a	5.78 ± 0.11a	5.67 ± 0.11a
Odor	$6.10 \pm 0.12a$	$6.16 \pm 0.10a$	$5.27 \pm 0.13b$	$5.32 \pm 0.15b$
Flavor	$6.29 \pm 0.13a$	$6.33 \pm 0.14a$	$5.41 \pm 0.17b$	$5.07 \pm 0.19b$
Texture	$6.37 \pm 0.13a$	$6.35 \pm 0.18a$	$6.02 \pm 0.13a$	$6.05 \pm 0.13a$
Overall	$6.13 \pm 0.09a$	$6.18 \pm 0.11a$	$5.62 \pm 0.10$ b	$5.50 \pm 0.11b$

<sup>\*</sup>Means  $\pm$  standard error followed by different letters mean significant differences among samples (ANOVA and DGC test,  $\alpha = 0.05$ ).

addition of oregano (RS-O) and poleo (RS-P) essential oils. The Storage and sampling inclusion of BHT and essential oils in a proportion of 0.02 g/ after the roasting process of the seeds.

The samples packaged into 27 × 28 cm plastic bags (Ziploc; 100 g final product were made using the refined sunflower oil SC Johnson & Son, Buenos Aires, Argentina) were kept for 35 d at room temperature (23 °C) in darkness and 60% to 70%

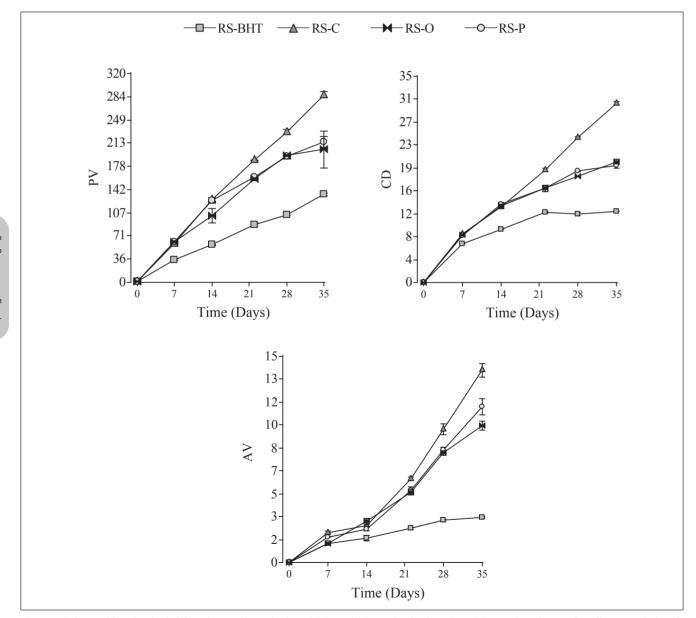


Figure 1-(A) Peroxide value (PV), (B) conjugate dienes (CD), and (C) p-anisidine value (AV) evaluated in samples of roasted sunflower seeds (RS-C), roasted sunflower seeds with BHT (RS-BHT), and roasted sunflower seeds with the addition of oregano (RS-O) and poleo (RS-P) essential oils during storage.

Table 3-Mean and standard deviation (n = 3) of sensory attributes in fresh samples evaluated at day "zero" of storage. Samples: roasted sunflower seeds (RS-C), roasted sunflower seeds with BHT (RS-BHT), and roasted sunflower seeds with the addition of oregano (RS-O) and poleo (RS-P) essential oils.

Attributes	RS-BHT	RS-C	RS-P	RS-O
Color	54.04 ± 1.08	54.13 ± 0.54	54.17 ± 0.64	$53.79 \pm 0.79$
Roughness	$14.00 \pm 0.33$	$13.84 \pm 0.51$	$13.92 \pm 0.80$	$14.33 \pm 0.52$
Glossy	$23.29 \pm 0.61$	$22.09 \pm 1.14$	$22.67 \pm 1.61$	$21.92 \pm 1.00$
Oregano essential oil	$0.00 \pm 0.00 a$	$0.00 \pm 0.00 a$	$0.00 \pm 0.00 a$	$24.07 \pm 0.32 \mathrm{b}$
Poleo essential oil	$0.00 \pm 0.00 a$	$0.00 \pm 0.00 a$	$19.13 \pm 1.50 \mathrm{b}$	$0.00 \pm 0.00 a$
Roasted sunflower	$53.84 \pm 3.13 a$	$55.46 \pm 0.81 a$	$51.50 \pm 1.11 \mathrm{b}$	$48.54 \pm 2.35 \mathrm{b}$
Oxidized	$6.54 \pm 0.26$	$6.71 \pm 0.71$	$6.13 \pm 0.63$	$5.46 \pm 0.38$
Cardboard	$16.71 \pm 0.69$	$16.88 \pm 0.63$	$17.42 \pm 0.69$	$15.13 \pm 1.60$
Salty	$7.84 \pm 0.19$	$7.42 \pm 0.07$	$7.75 \pm 0.33$	$7.63 \pm 0.63$
Sweetness	$8.51 \pm 0.38$	$8.13 \pm 0.13$	$7.79 \pm 0.64$	$8.25 \pm 0.76$
Sour	$6.96 \pm 0.52$	$7.08 \pm 0.38$	$7.17 \pm 0.31$	$7.75 \pm 0.22$
Bitterness	$17.50 \pm 0.33 \mathrm{b}$	$17.59 \pm 0.61 \mathrm{b}$	$19.29 \pm 0.36 a$	$18.96 \pm 0.69 a$
Astringent	$32.09 \pm 0.64$	$32.79 \pm 0.92$	$33.67 \pm 0.69$	$33.09 \pm 1.30$
Hardness	$18.67 \pm 0.50$	$19.34 \pm 0.71$	$19.00 \pm 0.45$	$18.88 \pm 1.19$
Crunchiness	$21.71 \pm 0.88$	$22.5 \pm 0.21$	$23.21 \pm 0.88$	$22.58 \pm 0.14$

<sup>\*</sup>Means  $\pm$  standard deviation followed by different letters mean significant differences among samples (ANOVA and DGC test.  $\alpha=0.05$ ).

relative humidity. Chemical and sensory variables were evaluated on samples on day "zero" and on samples removed every 7 d from storage.

# Chemical analysis

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 peroxide value (AOAC 1980), p-anisidine value (AV) (IUPAC 1987), and conjugated dienes (COI 2001).

# Sensory analysis

Consumer acceptability. Fresh samples (RS-C, RS-BHT, RS-O, and RS-P) at storage day 0 were served to consumer panelists in order to determine acceptability. Consumer panelists (n = 100) were recruited from Cordoba (Argentina) using the following criteria: people aged between 18 and 65 y, nonsmokers, without food allergies, and consumers of roasted sunflower and/or sunflower products at least once a week. The samples (3 g) were served in plastic cups with lids coded with 3-digit random numbers together with a water cup and paper ballots. Panelist evaluated the samples in random order during the test day. The panelists had to consume the whole sample and also had to rinse their mouths with water between them. Color, odor, flavor, and texture acceptances from roasted sunflower seeds were evaluated in a 9-point hedonic scale ranging from 1 = dislike extremely to  $9 = like \ extremely$  (Nepote and others 2009).

Descriptive analysis. Samples of roasted sunflower seeds (RS-C, RS-BHT, RS-O, and RS-P) were removed for descriptive analysis. A trained panel conformed for total 10 panelists (8 female and 2 male) were prepared for sample evaluation. The panelists had a long experience (6 y) evaluating similar food products. The panelist criteria selection were: people with natural dentition, without food allergies, nonsmokers, with age between 18 and 64 y, available for all sessions, interested in participating, and able to verbally communicate the observations regarding the product. All panelists had to pass a screening test before being selected (Grosso and Resurreccion 2002).

Eight training sessions that lasted 2 h were performed with the panelist. A hybrid method was used for descriptive analysis consisting of the SpectrumTM (Sensory Spectrum, Inc., Chatham, N.J., U.S.A.) and the Quantitative Descriptive Analysis (Tragon

Corp., Redwood City, Calif., U.S.A.) (Grosso and Resurreccion 2002; Meilgaard and others 2010). A list of definitions and warmup and reference intensity ratings (Table 1) were posted in the booths during the training and evaluation sections. For sample evaluation, an unstructured line scale of 150 mm was used (Johnsen and others 1988; Grosso and Resurreccion 2002).

Partitioned booths under fluorescent light at room temperature were used for sample evaluation. The samples (10 g) were served in plastic cups with lids. Each sample was identified with a code of 3-digit consisting in random numbers. In each evaluation session, 6 samples and the warm-up sample were evaluated per day. A randomized complete block design were used for sample testing (Grosso and Resurreccion 2002).

# Experimental design

The storage study had an experimental design of 4 treatments × 6 storage periods × 3 repetitions. The treatments were roasted sunflower control (RS-C), roasted sunflower with BHT (RS-BHT), and roasted sunflower with the addition of oregano (RS-O) and poleo (RS-P) essential oils. The storage periods were days 0, 7, 14, 21, 28, and 35. Three different lots of sunflower seeds were used considering each lot as a repetition. Samples for storage day, repetition, and treatment were packaged in individual bags. Statistical analysis

The InfoStat software, version 2012p (Facultad de Ciencias Agropecuarias, Univ. Nacional de Cordoba) was used for data analyses. To find significant differences among means, analysis of variance ( $\alpha = 0.05$ ) and Di Rienzo, Guzmán, and Casanoves (DGC) test were used. Regression analyses were made to figure out the effect of storage time on the different treatments. Linear model was used:  $y = \beta_0 + \beta_1 x$ , where "y" = chemical and sensory variables; "x" = time. Correlation analyses (Pearson's coefficient) were made to obtain associations between dependant variables from the storage study. Principal component analysis (PCA) was made on the correlation matrix of normalized data constituted by the results of chemical and sensory analyses of the storage with the purpose to explore associations between variables (Johnson and Wichern 2007).

#### Results and Discussion

In a previous study, the chemical compositions of oregano and poleo essential oils were determined (Quiroga and others 2013).

The main compounds in essential oil of *Origanum vulgare* reported in that study were 32.1%  $\gamma$ -terpinene, 15.1%  $\alpha$ -terpinene, 8.0% p-cymene, 8.0% thymol, and 7.1%  $\beta$ -phellandrene. On the other hand, the major components found in poleo essential oil were  $\alpha$ -limonene (76.8%) and 1,8-cineole (5.0%). In that study, it was also reported that both essential oils present antioxidant activity, measured as rancimat and IC50 of antilipase activity and radical-scavenging activity (DPPH). Poleo essential oil showed lower phenolic content, IC50-DPPH, and antilipase activity than oregano essential oil. Similar antioxidant indexes measured by rancimat were observed in both essential oils. Finally in that study, it was reported a positive relation among phenolic content, radical-scavenging, and antilipase activities.

# Consumer acceptance test of roasted sunflower seeds

A consumer analysis was made in order to evaluate how affects the addition of poleo and oregano essential oils on roasted sunflower seed acceptability. For color, odor, flavor, texture, and overall acceptances, all roasted sunflower samples showed means of

consumer responses between 5 (I neither like nor dislike) and 6.5 (I like slightly) on a 9-point hedonic scale (Table 2). The samples did not have significant differences in color and texture acceptances. Odor and flavor acceptances were higher in RS-C and RS-BHT than in the samples with essential oils (RS-O and RS-P) added. Overall acceptances were higher for RS-C (6.13) and RS-BHT (6.18) than for RS-O (5.50) and RS-P (5.62). RS-O and RS-P did not exhibit significant differences in any consumer acceptance attributes. Therefore, the addition of these essential oils affected consumer acceptance in the attributes flavor and odor but the presence of BHT did not affect any consumer acceptance attributes in the roasted sunflower product studied. Food sample may be considered unacceptable to the consumer when the acceptability has a value less than 5 on a 9-point hedonic scale. For acceptability values higher than 5 mean positive acceptance for a determined food product. Values higher than 5 (neither like nor dislike) were observed in all consumer acceptance attributes and in all samples; therefore, all samples with or without essential oil addition were positively accepted by consumers.

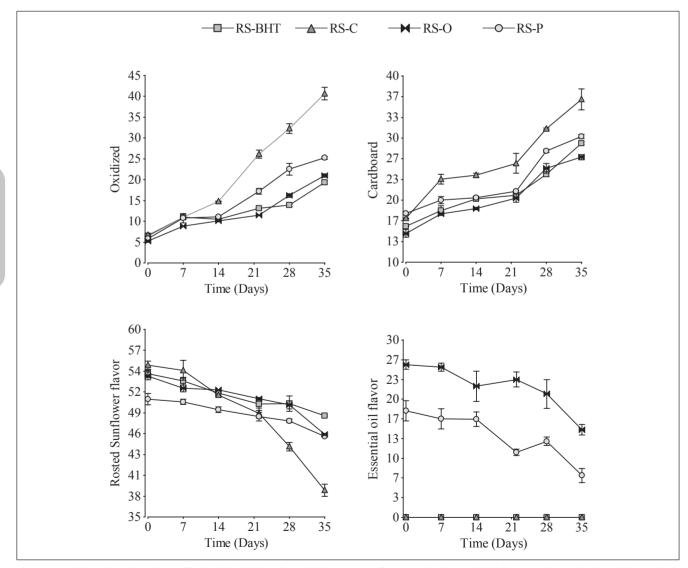


Figure 2—Attribute intensity ratings of (A) oxidized, (B) cardboard, (C) roasted sunflower, and (D) essential oil flavors evaluated by descriptive analysis in samples of roasted sunflower seeds (RS-C), roasted sunflower seeds with BHT (RS-BHT), and roasted sunflower seeds with the addition of oregano (RS-O) and poleo (RS-P) essential oils during storage.

Table 4-Regression equations and  $R^2$  for dependent variables as peroxide value, conjugated dienes, p-anisidine value, and cardboard, roasted sunflower, oxidized, and oregano/poleo essential oil flavors of roasted sunflower seeds (RS-C), roasted sunflower seeds with BHT (RS-BHT), and roasted sunflower seeds with the addition of oregano (RS-O) and poleo (RS-P) essential oils.

		Linear regression equation*		
Dependent variable	Samples	$oldsymbol{eta}_0$	$oldsymbol{eta}_1$	$R^2$
PV	RS-BHT	$5.0665 \pm 1.8142$	$3.6810 \pm 0.1347 c$	0.9931
	RS-C	$3.2286 \pm 2.2271$	$8.0547 \pm 0.3062$ a	0.9929
	RS-P	$18.7738 \pm 0.7153$	$6.0881 \pm 0.3050 \mathrm{b}$	0.9604
	RS-O	$15.1162 \pm 12.5272$	$5.9564 \pm 1.0160 \mathrm{b}$	0.9168
CD	RS-BHT	$2.8689 \pm 0.0759$	$0.3220 \pm 0.0060 c$	0.8057
	RS-C	$0.8965 \pm 0.0896$	$0.8374 \pm 0.0117$ a	0.9918
	RS-P	$3.0891 \pm 0.1833$	$0.5600 \pm 0.0173 \mathrm{b}$	0.8993
	RS-O	$2.9891 \pm 0.1721$	$0.5629 \pm 0.0113 \mathrm{b}$	0.9083
AV	RS-BHT	$0.4064 \pm 0.1112$	$0.0902 \pm 0.0060 \mathrm{d}$	0.9289
	RS-C	$-1.0802 \pm 0.2045$	$0.3882 \pm 0.0223$ a	0.9326
	RS-P	$-0.8075 \pm 0.1193$	$0.3291 \pm 0.0246 \mathrm{b}$	0.9400
	RS-O	$-0.5594 \pm 0.0780$	$0.2903 \pm 0.0102 \mathrm{c}$	0.9758
Essential Oil flavor	RS-P	$18.9124 \pm 2.6192$	$-0.2939 \pm 0.0794$ a	0.6970
	RS-O	$26.9001 \pm 0.6317$	$-0.2723 \pm 0.0490$ a	0.5670
Roasted Sunflower flavor	RS-BHT	$54.0250 \pm 0.9975$	$-0.1577 \pm 0.0141$ a	0.7961
	RS-C	$57.1146 \pm 0.5925$	$-0.4702 \pm 0.0330 \mathrm{b}$	0.9036
	RS-P	$51.1108 \pm 0.7529$	$-0.1353 \pm 0.0189$ a	0.8268
	RS-O	$54.1214 \pm 0.5407$	$-0.1898 \pm 0.0157$ a	0.8297
Cardboard	RS-BHT	$15.3511 \pm 0.6050$	$0.3430 \pm 0.0209 \mathrm{b}$	0.9113
	RS-C	$17.6309 \pm 0.4035$	$0.4938 \pm 0.0285 a$	0.8879
	RS-P	$17.5564 \pm 0.4194$	$0.3360 \pm 0.0129 \mathrm{b}$	0.8491
	RS-O	$15.5446 \pm 0.5891$	$0.3255 \pm 0.0584 \mathrm{b}$	0.8340
Oxidized	RS-BHT	$6.9680 \pm 0.1650$	$0.3062 \pm 0.0120 \mathrm{d}$	0.8770
	RS-C	$4.2890 \pm 1.1092$	$0.9973 \pm 0.0417 a$	0.9664
	RS-P	$5.5117 \pm 0.7576$	$0.5615 \pm 0.0211 \mathrm{b}$	0.9403
	RS-O	$4.8309 \pm 0.2683$	$0.4134 \pm 0.0273 c$	0.9292

\*Regression equations:  $Y = \beta_0 + \beta_1 X$ , where Y = dependent variable and X = independent variable (time). ANOVA and DGC multiple range test: The slope  $(\beta_1)$  of each variable followed with different letters in the column are significantly different  $(\alpha = 0.05)$ .

Other research has reported the flavor and odor acceptances of sausage prepared with the essential oil of Cinnamonum zeylanicum as a natural antioxidant and antibacterial (Moarefian and others 2013). Those authors prepared mixtures of essential oil and nitrites in different ratios. Then, they made a sensory evaluation analysis using a 5-point scale. Those authors reported that C20 and C40 mixture samples have higher scores for flavor and odor acceptances. The C40 samples showed higher score than the control samples. The C20 sample showed no significant difference from the control sample. The flavor scores of samples C20 and C40 did not present significant differences between them but these scores were higher than those of the control sample. According to those authors, the Cinnamonum zeylanicum essential oil addition in sausages improves their consumer acceptance.

Chemical and sensory analysis in roasted sunflower seeds during storage

Chemical analysis. Peroxide value (PV), conjugated dienes (CD), and *p*-anisidine value (AV) are lipid oxidation indicators. Changes in these lipid oxidation indicators of the roasted sunflower seeds during storage are shown in Figure 1. Significant differences were observed between samples in these variables during storage. RS-C and RS-BHT had the highest and lowest PV, CD, and AV values, respectively. Samples with essential oils (RS-O and RS-P) had intermediate PV, CD, and AV values, without significant differences between them in PV or CD. RS-P had higher AV values than RS-O after 28 storage days.

Based on these results, it was observed that BHT and essential oil addition provides protection against the oxidation of roasted sunflower seeds. Previous works have reported that oregano essential oil has an antioxidant effect on foods such as olive oil (Asensio and

others 2011, 2012), peanut products (Olmedo and others 2012a), and cream cheese (Olmedo and others 2013).

**Descriptive analysis.** The attribute intensity ratings of fresh roasted sunflower seeds (storage day "zero") evaluated by descriptive analysis are shown in Table 3. The essential oil and roasted sunflower flavors and bitterness intensity ratings showed significant differences between samples. The essential oil addition to the roasted sunflower seeds affected the intensity ratings of roasted sunflower flavor and bitterness in RS-O and RS-P, showing lower intensity ratings for roasted sunflower flavor and higher bitterness with respect to RS-C and RS-BHT. Significant differences between samples were not detected in the other sensory attributes. Olmedo and others (2012a) found that the oregano essential oil addition in roasted peanuts increased the intensity of bitterness and decreased the intensity rating of roasted peanut flavor.

Changes in the intensity ratings for oxidized, cardboard, roasted sunflower, and essential oil flavors were detected in the roasted sunflower treatments during storage (Figure 2). During storage, all roasted sunflower seed samples exhibited an increase in intensity ratings for oxidized (Figure 2A) and cardboard (Figure 2B) flavors. RS-C showed the highest values of these attributes. Significant differences in those flavors between samples were detected after 14 and 7 storage days, respectively. The results evidence that the essential oil addition protected the product, inhibiting the formation of undesirable flavors that come from secondary lipid oxidation process. Previous works have also reported increases in oxidized and cardboard flavors in peanut products (Riveros and others 2010; Olmedo and others 2012b).

Roasted sunflower flavor is a positive characteristic for this product. The intensity ratings for roasted sunflower flavor decreased in all samples during storage (Figure 2C). RS-C showed the highest values at zero storage time. From storage day 21 and on, RS-C the other samples. The lowest slope of oxidized flavor was obexhibited the lowest intensity for this attribute. Lower decrease in the roasted sunflower flavor intensity was detected in samples added with BHT and poleo and oregano essential oils. These results indicate that BHT and essential oils preserved the product against deterioration reactions. The loss of roasted flavor intensity is associated to oxidation process in the kernels and also, this flavor could be masked by rancid and off-flavors (Nepote and others 2006). A decrease of roasted flavor during storage is considered nonpositive effect affecting the product quality at a sensory level (Riveros and others 2010; Olmedo and others 2012b).

The intensity rating for essential oil flavor also decreased in the RS-O and RS-P samples during storage (Figure 2D). RS-O showed higher intensity ratings for this attribute during storage than RS-P. Other authors have also reported that the oregano, laurel, and rosemary essential oil flavors decreases in roasted peanuts during storage (Olmedo and others 2012a).

## Regression analysis

The linear regression equations calculated from the chemical analysis data observed during storage showed high  $R^2$  (higher than 0.81), which means that the chemical data were well adjusted to the storage time (Table 4). The slopes  $(\beta_1)$  of the linear regression are an indicator of the deterioration tendency of each treatment. Significant differences were found between the slopes for PV, CD, and AV. RS-BHT showed the greatest chemical stability (lower PV, CD, and AV slopes), while RS-C exhibited the highest slopes. RS-O and RS-P had PV, CD, and AV slopes with values between those of RS-C and RS-BHT.

The linear regression slopes for oxidized and cardboard flavors were higher for RS-C (0.9973 and 0.4938, respectively) than for

served in RS-BHT (0.3062), followed by RS-O (0.4134) and RS-P (0.5615), with significant differences between them. RS-BHT (0.3430), RS-P. (0.3360), and RS-O (0.3255) did not differ in their cardboard flavor slopes. Rancid odor and flavor are caused by volatile compounds that are produced in advanced stage of the lipid oxidation process. These off-flavors are related to the oxidized and cardboard flavor sensory attributes (Grosso and Resurreccion 2002; Frankel 2005; Nepote and others 2009). The results indicate that the addition of essential oils afforded protection to the product, inhibiting the formation of undesirable flavors that come from secondary lipid oxidation processes. Previous studies also reported increases in the intensity of these oxidized and cardboard flavors during storage in high lipid content food products such as peanut products (Nepote and others 2006; Riveros and others 2010; Olmedo and others 2012b). In addition, food products like salami (Larrauri and others 2013) and olive oil (Asensio and others 2011) have also shown higher intensity ratings of oxidized flavor with the storage time.

The roasting process is an important pretreatment commonly used before reaching the direct consumer because it helps to develop an attractive aroma/flavor called nut-like (Bozan and Temelli 2008) and to kill microbes for food safety. Also, oilseeds receive a heating process prior to processing that helps the oil extraction during processing. Chemical, physical, and nutritional characteristics change in seeds and extracted oils after having heat treatments (Veldsink and others 1999). Considering that roasted sunflower flavor is a positive attribute for the product, this attribute should keep high intensity during storage. In all samples, the intensity ratings of roasted sunflower flavor decreased during storage (Figure 2C). The linear slopes from Table 4 indicated RS-C

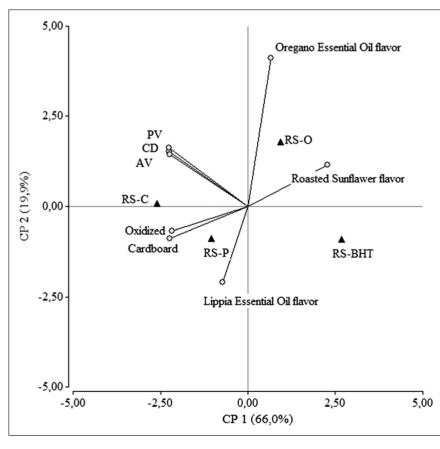


Figure 3-Biplot from the 1st and 2nd principal components of principal component analysis. Variables: chemical oxidation indicators like peroxide values, conjugated dienes, and p-anisidine values and sensory attributes like oxidized, cardboard, roasted sunflower, and essential oil flavors evaluated in roasted sunflower seeds (RS-C), roasted sunflower seeds with BHT (RS-BHT), and roasted sunflower seeds with the addition of oregano (RS-O) and poleo (RS-P) essential oils during storage.

(-0.4702) presented higher decrease of roasted sunflower flavor. RS-P slope (-0.1353) was lower in comparison with RS-BHT (-0.1577) and RS-O (-0.1898). The decrease of the roasted flavor intensity during storage were reported as a negative effect in food products like roasted peanuts (Nepote and others 2006; Olmedo and others 2012a).

The intensity ratings for essential oil flavor also decreased in RS-O and RS-P during storage (Figure 2D). For that reason, a negative slope was observed for both treatments. RS-O exhibited higher essential oil intensity than RS-P during the storage period, but without significant differences between the slopes in their linear regression equations (Table 4). Asensio and others (2012) found that the oregano flavor intensity decreased in virgin olive oil supplemented with oregano essential oil during storage. Other authors also reported that essential oil flavors like oregano, rosemary, laurel, aguaribay, and cedron essential oils loose intensity during the storage period when they are added in peanut products (Olmedo and others 2012a, 2012b).

# Principal component analysis

Principal component analysis is an unsupervised technique that reduces the dimensionality in a data set. Using PCA, the difference among objects can be demonstrated and related to which variables are principally involved. Multivariate analysis are every day more important for food characterization, classification, authenticity determination, and quality control (Arvanitoyannis and others 1999; Tzouros and Arvanitoyannis 2001). In Figure 3, it is presented as the biplot for the 1st and 2nd principal components of the PCA. The 1st 2 PCs of the PCA explain 85.9% variability in the samples during storage. This value was considered acceptable to make correlations between variables. The point dispersion in the graphic indicates high variability in the results among samples. PV, CD, and AV related to lipid oxidation in roasted sunflower seeds were positively associated with each other and with the negative sensory attributes from the descriptive analysis (oxidized and cardboard flavors), showing positive Pearson's correlation coefficients higher than 0.86. In addition, the latter 2 variables were negatively associated with the positive attribute roasted sunflower flavor (negative correlation coefficients between 0.83 and 0.89). The oxidized and cardboard flavors in a food product are related to lipid oxidation and rancidity. Therefore, these 2 sensory attribute are negative for roasted sunflower seeds. On the other hand, high intensity in roasted sunflower flavor is related to desirability in this product. RS-BHT and RS-O were associated with roasted sunflower flavor, which is a positive attribute; and RS-C was associated with PV, CD, and AV and oxidized and cardboard flavors, all of them related to lipid oxidation. In previous work, it was found that oregano essential oils with higher thymol contents (EO-Com and EO-Cor) in a canola oil storage study exhibited lower IC50 in a test of DPPH, meaning a greater ability to capture free radicals, and relatively lower PV and AV (Quiroga and others 2011). Carvacrol and thymol are phenolic structure that correspond to oxygenated monoterpenes with proved antioxidant properties (Ruberto and Baratta 2000). Similar correlations between sensory and chemical variables were also found in other researches that evaluated soybean and peanut products (Riveros and others 2010; Jáuregui and others 2012). Oregano essential oil included in olive oil showed also antioxidant activity delaying the increase of lipid oxidation indicators like CD and AV (Asensio and others 2011). Olmedo and others (2013) also reported antioxidant effect of oregano and rosemary essential oils when these essential oils were included in cream cheese. They found a positive correlation between chemical oxidation indicators and negative sensory attributes; samples

with essential oils added were negatively associated with lipid oxidation indicators and negative sensory attributes like rancid and fermented flavors.

#### Conclusions

According to the result observed in the present research, the addition of oregano and poleo essential oils retard the lipid oxidation process delaying the increase of chemical quality parameters (PV, CD, and AV) and the development of rancid flavors in roasted sunflower seeds. This food product added with these essential oils is accepted by consumers. The addition of these essential oils to this roasted sunflower seeds help to preserve the intensity of positive attributes in during storage. The use of natural additives instead of synthetic ones is convergent with the present trend in food technology; for this reason, poleo and oregano essential oils are aromatic plants that constitute as natural sources of antioxidant additives useful in the food industry for preserving quality properties in products with high lipid contents.

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