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Antioxidant effects of *Satureja montana* L. essential oil on TBARS and color of mortadella-type sausages formulated with different levels of sodium nitrite

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ABSTRACT

This study aimed to evaluate the effect of adding winter savory (*Satureja montana* L.) essential oil (EO) at concentrations of 7.80, 15.60 and 31.25 μl/g on color and lipid oxidation (TBARS) in mortadella-type sausages formulated with different sodium nitrite ($NaNO_2$) levels (0, 100 and 200 mg/kg) and stored at 25 °C for 30 days. The EO was extracted by hydrodistillation and analyzed by gas chromatography–mass spectrometry (GC–MS). Twenty-six chemical compounds were identified; the most prominent of which were thymol (28.99 g/100 g), *p*-cymene (12.00 g/100 g), linalool (11.00 g/100 g) and carvacrol (10.71 g/100 g). Among the nitrite levels tested, a concentration of 100 mg/kg of sodium nitrite appeared to be sufficient for the formation of the characteristic red color. The use of EO at concentrations exceeding 15.60 μl/g adversely affected the color of the product by reducing redness (a^*) ($p \leq 0.05$) and increasing yellowness (b^* h^*). The EO antioxidant activity was confirmed by β-carotene bleaching method and DPPH assay. Reduced values of thiobarbituric acid reactive substances (TBARS) ($p \leq 0.05$) were observed in mortadellas formulated with the lowest concentrations of EO without added nitrite. This significant effect on lipid oxidation was also observed in samples containing EO and reduced amounts of sodium nitrite. The results suggest possible benefits from the combined use of EOs and minimal amounts of sodium nitrite in cured meat products.

1. Introduction

Meat products are widely consumed foodstuffs. In addition to appreciable sensory aspects, processed meat products are relatively inexpensive compared with traditional fresh meat cuts. Mortadella is a cured, emulsified and stuffed meat product that provides inexpensive access to animal proteins, making the minimal recommended protein intake possible (Feiner, 2006). Cured meat products contain nitrite, which is a key ingredient in the curing process. Nitrite performs the following functions: First, it contributes to the development of the typical cured meat flavor and prevents lipid oxidation, inhibiting the development of rancid off-flavors; second, it reacts with myoglobin to produce nitrosylhemochrome, which gives the cured meat its characteristic pink color; third, it inhibits spoilage and pathogenic bacteria, most importantly *Clostridium* sp. (Cammack et al., 1999; Marco, Navarro, & Flores, 2006). However, a high nitrite intake presents human health risks, including possible allergenic effects, vasodilator effects and metamyoglobin production *in vivo* (Cammack et al., 1999). In addition, nitrous acid (from the nitrite oxide hydration produced by sodium nitrite ($NaNO_2$) reduction) may react with secondary amines and amino acids naturally present in muscle foods and meat products to form *N*-nitroso compounds, especially nitrosamines. These are chemical substances with strong toxic, mutagenic, neurotoxic, nephrotoxic and carcinogenic effects (Karl-Otto, 2008; Rywotycki, 2002). These potential risks make the reduction or elimination of nitrite in foods desirable. According to Brazilian legislation for additives and preservatives in meat products, the maximal concentration of sodium or potassium nitrite, with or without nitrate, should not exceed 150 mg/kg or 0.015% in the final product (Brazil, 2009).

Reducing nitrite in meat emulsions, however, can lead to fat auto-oxidation, a major deteriorative reaction that results in off flavors and color alteration. In a complex sequence of chemical changes, this process promotes the formation of compounds that

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react easily with oxygen; the production of these highly reactive compounds can be delayed by adding antioxidants. However, when lipid oxidation occurs, hemepigments (myoglobin and hemoglobin) also oxidize in a coupled lipid-pigment reaction, which results in a color change (Hernández-Hernández, Ponce-Alquicira, Jaramillo-Flores, & Guerrero Legarreta, 2009). Various synthetic antioxidants, such as BHA, BHT, TBHQ, are used in the food industry to inhibit lipid oxidation. However, their use has been restricted because of possible health risks and toxicity.

Consumers increasingly demand natural products as alternative preservatives in foods because the safety of synthetic additives has been questioned in the last few years. Alternative preservation techniques using naturally derived ingredients are being investigated for their application in food products. Due to negative consumer perceptions of artificial preservatives, attention is shifting toward alternatives that consumers perceive as natural, including essential oils (EOs) and essences of plant extracts. In particular, plant EOs are attracting interest as potential preservatives because they are generally recognized as safe (GRAS) and have a wide acceptance from consumers (Burt, 2004; Gutierrez, Barry-Ryan, & Bourke, 2009; Smith-Palmer, Stewart, & Fyfe, 1998). The use of natural additives has attracted attention, and some authors report that natural compounds have antioxidant capabilities similar to or better than synthetic preservatives.

EOs are volatile, natural and complex compounds that are characterized by a strong odor. They are formed by aromatic plants as secondary metabolites. In addition to their use as flavoring agents in foods, EOs exhibit antibacterial, antifungal and antioxidant properties (Bakkali, Averbeck, Averbeck, & Idaomar, 2008). *Satureja montana* L., commonly known as winter savory or mountain savory, belongs to the *Lamiaceae* family, *Nepetoideae* subfamily and *Mentheae* tribe. It is a perennial semi-shrub (20–30 cm in height) that inhabits arid, sunny and rocky regions. *S. montana* L. is native to the Mediterranean and is found throughout Europe, Russia and Turkey. It is a strongly aromatic herb that has been used for centuries as a spice for food and teas; it is used in Mediterranean cooking, mainly as a seasoning for meats and fish as well as in flavoring agents for soups, sausages, canned meats and spicy sauces (Bezbradica, Tomovic, Vukasinovic, & Siler-Marinkovic, 2005; Ćetković, Mandić, Čanadanović-Brunet, Djilas, & Tumbas, 2007; Mastelić & Jerković, 2003; Silva et al., 2009; Slavkovska, Jancic, Bojovic, Milosavljevic, & Djokovic, 2001). *S. montana* L. has biological properties related to the presence of its major EO chemical compounds, thymol and carvacrol (Mirjana & Nada, 2004; Radonic & Milos, 2003).

This study aimed to evaluate the effect of winter savory (*S. montana* L.) essential oil (7.80, 15.60 and 31.25 μl/g) on color and lipid oxidation as measured by thiobarbituric acid reactive substances (TBARS) in mortadella-type sausages formulated with different levels of sodium nitrite (0, 100 and 200 mg/kg) and stored at 25 °C for 30 days. Using the results observed for the evaluated parameters, we aimed to determine the feasibility of reducing the amount of nitrite used in product formulation by adding savory essential oil.

2. Materials and methods

2.1. Essential oil (EO)

2.1.1. Plant material and EO extraction

Dried aerial parts of winter savory spice (*S. montana* L.) originating from Albania (a mountainous country in southeastern Europe on the Balkan peninsula, 41° 21′ N and 19° 59′ W, with a Mediterranean climate) were acquired from a spice store (Mr. Josef Herbs and Spices) at the local market in São Paulo (SP, Brazil). The EO was extracted by hydrodistillation, using a modified Clevenger apparatus. Dry plant material was added to water in a 6 l volumetric distillation flask. The flask was coupled to the modified Clevenger apparatus, and the extraction was performed for 3 h at 100 ± 5 °C. The obtained hydrolate (water/oil fraction) was centrifuged at 322 *g* for 10 min at 25 °C. The EO was collected with a Pasteur pipette, and the water traces were removed with anhydrous sodium sulfate. The oil was refrigerated at 5 ± 2 °C in glass flasks wrapped in aluminum foil (Oliveira, Brugnera, Cardoso, Alves, & Piccoli, 2010).

2.1.2. Determination of moisture content and yield

Aerial parts of the winter savory (5 g) were added to 80 ml of cyclohexane in a 250 ml volumetric distillation flask. The flask was coupled to a condenser with a graduated volumetric collector and heated at 100 ± 5 °C for 2 h. After distillation, the volume of water in the collector was measured and expressed as the moisture content per 100 g sample. To calculate the yield, 350 g of dry spice was extracted by hydrodistillation, and the resulting EO was quantified. Along with the moisture content measurement, the EO yield for dried plants was obtained (g/100 g) as the moisture-free basis (MFB) (Pimentel et al., 2006).

2.1.3. Identification and quantification of chemical constituents

The EO chemical components were identified by gas chromatography with mass spectrometry (GC–MS). A Shimadzu gas chromatograph (model GC 17A) equipped with a mass selective detector (Model QP 5000) was operated under the following conditions: fused silica capillary column (30 m × 0.25 mm) coated with a DB-5 MS stationary phase; ion source temperature of 220 °C; column temperature programmed at an initial temperature of 40 °C and increased by 3 °C/min up to 240 °C; helium carrier gas (1 ml/min); initial column pressure of 100.2 kPa; split ratio of 1:10 and volume injected of 1 μl (1% solution in dichloromethane). The following conditions were used for the mass spectrometer (MS): impact energy of 70 eV; decomposition velocity of 1000, decomposition interval of 0.50 and fragments of 45 Da and 450 Da decomposed. A mixture of linear hydrocarbons (C_9H_{20}; $C_{10}H_{22}$; $C_{11}H_{24}$;…$C_{24}H_{50}$; $C_{25}H_{52}$; $C_{26}H_{54}$) was injected under identical conditions. The mass spectra obtained were compared to those of the database (Wiley 229), and the Kovats retention index (KI) calculated for each peak was compared to the values described by Adams (2007).

The quantification of EO constituents was conducted with a Shimadzu gas chromatograph (Model GC 17A) equipped with a flame ionization detector (FID) under the following conditions: DB5 capillary column; column temperature programmed at an initial temperature of 40 °C and a final temperature of 240 °C; injector temperature of 220 °C; detector temperature of 240 °C; nitrogen carrier gas (2.2 ml/min); split ratio of 1:10; volume injected of 1 μl (1% solution in dichloromethane) and column pressure of 115 kPa. The quantification of each constituent was obtained by means of area normalization (%).

2.2. Mortadella-type sausages

2.2.1. Essential oil concentrations and sodium nitrite levels

Batches of mortadella-type sausages were formulated with different concentrations of sodium nitrite (0, 100 and 200 mg/kg) and winter savory EO at concentrations of 7.80, 15.60 and 31.25 μl/g. The EO concentrations were determined according to the results obtained from the microbiological assays in another step of study (Oliveira et al., 2011); the sodium nitrite concentrations were determined according to Brazilian legislation limits for additives and preservatives in meat products (Brazil, 2009). The different

treatments evaluated (Essential oil × Sodium nitrite) were based on Minimum inhibitory concentration (MIC concentrations) and the possible combined effects of EO and minimized amounts of sodium nitrite.

2.2.2. Sausage formulation and manufacturing

Batches of mortadella-type sausages were formulated with different concentrations of $NaNO_2$ (0, 100 and 200 mg/kg) and EO from winter savory (0.00, 7.80, 15.60 and 31.25 μl/g). Refrigerated, vacuum packaged lean beef and frozen pork backfat were obtained within 48 h of slaughtering from a local meat packer. Each batch was prepared using a typical Brazilian formula as follows: ground meat (58 g/100 g), pork backfat (14 g/100 g), NaCl (1.9 g/100 g), ice water (20 g/100 g), cassava starch (5 g/100 g), polyphosphate Fosmax (0.3 g/100 g, New Max Industrial, Brazil), ascorbic acid (0.05 g/100 g), spice mix for Mortadella 913 (0.5 g/100 g, New Max Industrial, Brazil) and $NaNO_2$ (0 mg/kg, 100 mg/kg and 200 mg/kg: Vetec, Brazil). The sausages samples were packed with a weight of 200 ± 5 g, and showed a pH = (6.29 ± 0.11) and water activity (Decagon-Aqualab) Aw = (0.941 ± 0.008). The mortadella-type sausages were made in a pilot plant in the Products of Animal Origin Laboratory at the Federal University of Lavras (Brazil).

Lean beef, salt, phosphate and $NaNO_2$ were placed in a cutter (Sire, Filizola S.A., Brazil) and mixed for approximately 1 min. Fifty percent of the ice and spices were then added and mixed at a high speed. After complete homogenization, the speed of the cutter was reduced. Ground pork backfat was then added and mixed until the temperature of the mixture reached 10 °C. The remaining 50% of the ice, cassava starch, ascorbic acid and EO were added and mixed until the temperature of the mixture reached 13 °C. The total emulsification time was approximately 10 min, and the processing room temperature was approximately 20 °C. The batters were stuffed into nylon bags (Unipac Darlon, Brazil, 50 μm thickness) and were cooked by immersion in water using the following program: 55 °C for 30 min, 65 °C for 30 min, 75 °C for 30 min, and 85 °C until the temperature of the product reached 73 °C (measured by a thermometer inserted into the center of the packed sausage batter). The cooked sausage was cooled in a water bath for 10 min and stored in a controlled chamber (Thermostat cabinets *LS* Logen Scientific) at 25 °C before analysis at 1, 10, 20 and 30 days.

2.3. Analyses of objective color

Color measurements were taken with a colorimeter (Chroma Meters CR-300, Konica Minolta Sensing Inc.) established at a 10° angle for the observer and illuminated at D_{65} to calculate color indices in the CIELAB system, following the recommendations of Ramos and Gomide (2007). The color parameters lightness (L^*), redness (a^*) and yellowness (b^*) were obtained from an average of six readings taken at different points in slices approximately 40 mm wide. The a^* and b^* coordinates were transformed to polar coordinates: (h^*) hue = $\tan^{-1}(b^*/a^*)$ and (C^*) chroma = $(a^{*2} + b^{*2})^{1/2}$.

2.4. In vitro antioxidant activity assay

2.4.1. Determination of antioxidant activity with the β-carotene bleaching method

Antioxidant activity of the *S. montana* L. essential oil was determined using β-carotene bleaching test (Lopes-Lutz, Alviano, Alviano, & Kolodziejczyk, 2008). As a reference the antioxidant activity of the Timol (essential oil major compound) was assessed. Approximately 10 mg of β-carotene (Sigma–Aldrich) was dissolved in 10 ml chloroform. The carotene–chloroform solution, 0.2 ml, was pipetted into a boiling flask containing 20 mg linoleic acid (Sigma–Aldrich) and 200 mg Tween 40 (Sigma–Aldrich). Chloroform was removed using a rotary evaporator (RE-52AA) at 40 °C for 5 min, and to the residue, 50 ml of distilled water was added, slowly with vigorous agitation, to form an emulsion. The emulsion (5 ml) was added to a tube containing 0.2 ml of the samples solution and the absorbance was immediately measured at 470 nm against a blank, consisting of an emulsion without β-carotene. The tubes were placed in a water bath at 50 °C and the oxidation of the emulsion was monitored spectrophotometrically (UV–visible spectrophotometer, Shimadzu UV 1601PC) by measuring absorbance at 470 nm over a 60 min period. The concentrations of essential oil evaluated were: 0, 25, 50, 100, 150, 200 and 250 μg/mL. The antioxidant activity was expressed as inhibition percentage with reference to the control after a 60 min incubation using the following equation: %AA = 100 [1 – (Ai – At)/Ac – Act)], where % AA = antioxidant activity; Ai = sample absorbance at time 0; At = sample absorbance at 60 min; Ac = absorbance of control at time 0; Act = absorbance of control at 60 min.

2.4.2. Determination of antioxidant activity by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method

The hydrogen atom or electron donation ability of the savory essential oil and the timol pure compound (reference) were measured from the bleaching of purple-colored ethanol solution of DPPH. This spectrophotometric assay uses the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a reagent (Amarowicz, Pegg, Rahimi-Moghaddam, Barl, & Weil, 2004). An aliquot of the sample (100 μl) was mixed with 1.4 ml of ethanol and then added to 1 ml of 0.004% DPPH (Sigma–Aldrich) in ethanol. The mixture was shaken vigorously and then immediately placed in a UV–vis spectrophotometer (UV – 1601PC Shimadzu) to monitor the decrease in absorbance at 517 nm. Monitoring was continued for 60 min until the reaction reached a plateau. The radical-scavenging activities of samples, expressed as percentage inhibition of DPPH, were calculated according to the formula: Antioxidant activity %AA = 100 – [(As × 100)/Ac] where As and Ac are the absorbance values of the sample and of the control checked after 60 min, respectively.

2.5. Lipid oxidation

The effect of *S. montana* L. EO on lipid oxidation in the sausages was evaluated using a spectrophotometer (Cary, *Varian*) and the 2-thiobarbituric acid (TBA) extraction method described by Raharjo, Sofos, and Schmidt (1992). Ten-gram portions of sausages were combined with 40 ml of 5% trichloroacetic acid (TCA) and 1 ml of 0.15% antioxidant BHT (Sigma–Aldrich) and homogenized for 5 min. The homogenate was filtered through Whatman No. 1 filter paper, and 2 ml of filtrate was combined with 2 ml of 0.08 mol/l TBA reagent and heated in boiling water (100 ± 5 °C) for 5 min. The absorbance of the resulting solution was measured at 531 nm, and the TBARS values were expressed as mg of malondialdehyde (MDA) per kg sample, calculated using 1,1,3,3-tetraethoxypropane (TEP) as the standard.

2.6. Experimental design and statistical analysis

Treatments were arranged in split-plot factorial designs, with EO concentrations (0.00, 7.80, 15.60 and 31.25 μl/g) and nitrite levels (0, 100 and 200 mg/kg) as plots and times of storage (1, 10, 20 and 30 days) as subplots. The whole experiment was conducted in three independent batches, and the collected data were subjected to analysis of variance (ANOVA) to verify the interactions between the effects. The differences among the treatments at each day of storage were also determined by ANOVA, and the means were compared with a Scott–Knott test, adopting a 5% significance level.

The statistical analyses, plots and regression plots were performed using Statistical R® software (2010).

3. Results and discussion

3.1. Essential oil

Winter savory (*S. montana* L.) EO was subjected to a detailed GC–MS analysis to determine its chemical composition. As shown in Table 1, 26 compounds were identified, representing 99.48% of the total EO. The average extraction yield of the *S. montana* EO was 4.7 ml/kg of dried aerial parts in an MFB. The major compound groups were monoterpene hydrocarbons and phenolic compounds. Thymol (28.99 g/100 g), *p*-cymene (12.00 g/100 g), linalool (11.00 g/100 g) and carvacrol (10.71 g/100 g) were the major chemical constituents. The extraction yield value of *S. montana* EO was similar to that found by Ćavar, Maksimović, Šolic, Mujkić, and Bešta (2008); however, the yield found in our study was lower than the yield reported by the following groups: Bezbradica et al. (2005); Mastelić and Jerković (2003) and Radonic and Milos (2003). The phytochemical profile of the winter savory EO in this study was in agreement with the results of several authors who have also evaluated this vegetal species (Mastelić & Jerković, 2003; Radonic & Milos, 2003; Silva et al., 2009; Skočibušić & Bezić, 2003). In contrast, the savory EO evaluated by Ćavar et al. (2008) was characterized by a high content of alcohols, such as geraniol and terpinen-4-ol. The final composition of EO is genetically influenced, with additional influence from the following: each organ and its stage of development; the climatic conditions of the plant collection site; the degree of terrain hydration; macronutrient and micronutrient levels; and the plant material's drying conditions (Bakkali et al., 2008; Burt, 2004). Slavkovska et al. (2001) and Mirjana and Nada (2004) reported that the chemical profile of *S. montana* EO varied according to factors such as the plants' stage of development and geographic location.

Table 1
Chemical constituents of *Satureja montana* L. essential oil identified by GC–MS and their contents.

Rt	Compound	(%)	IRRexp	IRRlit
6.580	α-Thujene	0.23	924	930
6.801	α-Pinene	1.37	932	939
7.314	Canphene	0.55	948	954
8.297	1-Octen-3-ol	1.32	979	979
8.607	Myrcene	0.68	989	990
9.549	α-Terpinene	1.13	1016	1017
9.826	*p*-Cymene	12.00	1024	1024
9.985	Limonene	0.62	1028	1029
10.094	1,8-Cineole	1.26	1031	1031
11.030	γ-Terpineno	2.91	1057	1059
11.480	*cis*-Sabinene	1.34	1069	1070
12.586	Linalool	11.00	1100	1096
12.815	*cis*-Thujene	1.62	1106	1102
14.307	Camphor	1.59	1146	1146
15.241	Borneol	3.43	1171	1169
15.558	Terpinen-4-ol	3.96	1180	1177
16.100	α-Terpineol	1.33	1194	1188
17.344	Ether methyl thymol	0.45	1229	1235
17.672	Ether methyl carvacrol	2.16	1238	1244
19.356	Isobornyl acetate	0.35	1284	1285
19.576	Thymol	28.99	1290	1290
19.845	Carvacrol	10.71	1298	1299
24.007	(E)-Caryophyllene	4.54	1418	1419
26.502	NI*	0.52	1494	-
26.909	β-Bisabolene	1.86	1507	1505
29.071	Spathulenol	1.00	1576	1578
29.236	Caryophyllene oxide	3.08	1581	1583
Total identified		99.48%		
Moisture (dry spice)		9.9561% (±1.9586)		
Yield (MFB**)		0.4721% (±0.0006)		

Rt = retention time (min). IRRexp – experimental index. IRRlit – literature index. NI* not identified compound. MFB** Moisture Free Basis.

3.2. Antioxidant activity

3.2.1. Lipid oxidation

The interaction between the effects (essential oil concentration × nitrite levels × storage time) was significant ($p \leq 0.05$) for TBARS values. Fig. 2 shows the results for the TBARS values during storage, according to the EO concentration and sodium nitrite levels used. The control samples, which were produced without sodium nitrite or EO, differed significantly ($p \leq 0.05$) in their lipid oxidation behavior; they suffered a more rapid and intense oxidation than those with added EO. After 20 days of storage, sausages formulated with 7.80 μl/g EO showed lower TBARS values ($p \leq 0.05$) among the treatments formulated without sodium nitrite. These results demonstrate the potential antioxidant effect of this EO. The antioxidant activity of savory EO can be credited to the presence of its major phenolic compounds, particularly thymol and carvacrol, and their recognized impact on lipid oxidation (Table 1). The antioxidant activity of phenolic compounds is related to the hydroxyl groups linked to the aromatic ring, which are capable of donating hydrogen atoms with electrons and stabilizing free radicals (Baydar, Sağdiç, Gülcan, & Karadoğan, 2004; Dorman, Peltoketo, Hiltunen, & Tikkanen, 2003; Yanishlieva, Marinova, & Pokorny, 2006). This prevents further degradation to more active oxidizing forms, such as malonaldehyde. Fig. 1 shows the *in vitro* antioxidant results for evaluated essential oil. Radonic and Milos (2003), using the same methodology as this study (TBARS), and Ćavar et al.

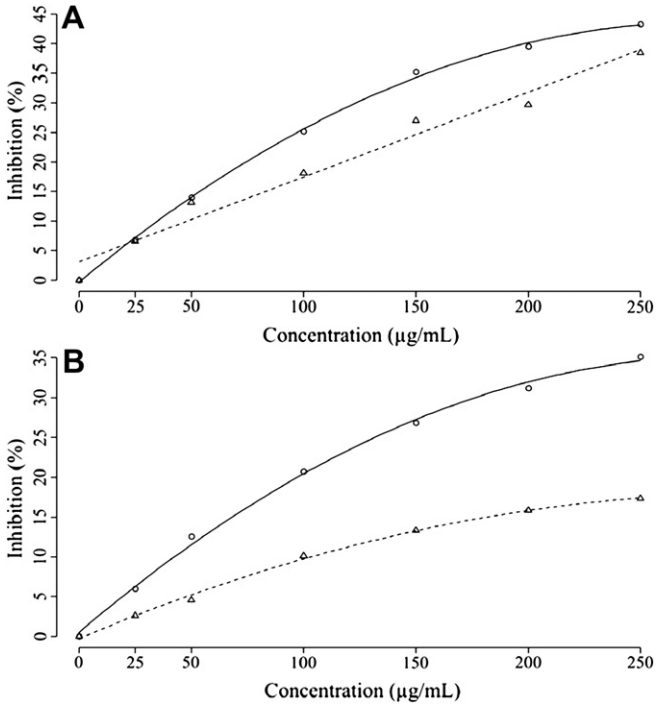

Fig. 1. (A) Antioxidant activity, as inhibition ratio, of total *Satureja montana* L. essential oil and Timol (majority compound and reference) determined by β-carotene bleaching method; (——○——) Timol $f(x) = -3.159e^{-01} + 3.155e^{-01}x - 5.667e^{-04}x^2$ ($R^2 = 0.9823$); (– –△– –) Essential oil $f(x) = 3.108342 + 0.143438x$ ($R^2 = 0.9385$). (B) Antioxidant activity assessed by 1,1-diphenyl-2 picrylhydrazyl (DPPH) assay of total savory essential oil (– –△– –) $f(x) = -2.863e^{-01} + 1.199e^{-01}x - 1.961e^{-04}x^2$ ($R^2 = 0.9718$) and Timol (——○——) $f(x) = 4.954e^{-01} + 2.416e^{-01}x - 4.203e^{-04}x^2$ ($R^2 = 0.9958$).

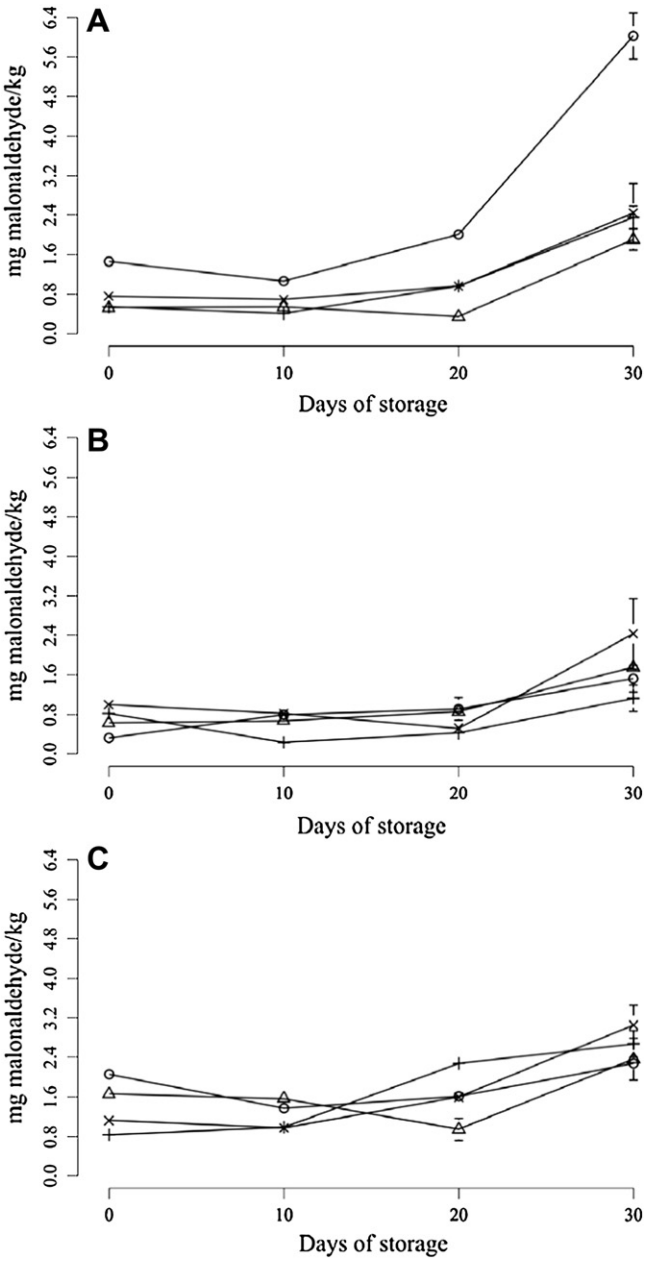

Fig. 2. Effect of different concentrations of *Satureja montana* L. essential oil and sodium nitrite ($NaNO_2$) levels on TBARS values (mg malonaldehyde/kg) in mortadella-type sausages stored at 25 °C for 30 days. (Average of three readings). A, B and C indicate 0, 100 and 200 mg/kg of $NaNO_2$, respectively. Essential oil concentration: 0.0 μl/g; 7.80 μl/g; 15.60 μl/g and 31.25 μl/g.

(2008), using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method, confirmed the antioxidant effect of winter savory EO *in vitro*. These authors also attributed the antioxidant activity of the EO to its thymol and carvacrol contents. Moreover, other components present in the *S. montana* L. EO evaluated in this study (Table 1) have antioxidant activity that has been reported in the literature. Ruberto and Baratta (2000) evaluated about 100 purified constituents of various essential oils and found pronounced antioxidant effects in the compounds α and γ-terpinene, myrcene, limonene, *p*-cymene and α-thujene; at high concentrations, their effects were comparable to those of phenolic compounds.

In the samples manufactured with sodium nitrite, however, the interaction between EO and nitrite should be considered. First, without added EO, TBARS values were significantly ($p \leq 0.05$) lower across all storage times in samples with nitrite added than without nitrite (control sample). The antioxidant effect of nitrite in cured meats is related to the formation of stable compounds with myoglobin, which make Fe unavailable to act as active catalyst of oxidation reactions (Karl-Otto, 2008). Al-Shuibi and Al-Abdullah (2002), in a study in which mortadella was produced with different levels of nitrite and stored for 14 weeks at 4 and 25 °C, also found lower TBARS values in samples with nitrite added. Moreover, these authors observed that 40 and 80 mg/kg nitrite, with TBARS values ranging from 0.53 to 0.59 mg MDA/kg, have a greater antioxidant effect than 120 mg/kg nitrite (TBARS value 0.65 mg MDA/kg). This result was also observed in this study because the antioxidant effect was more pronounced ($p \leq 0.05$) in sausages manufactured with 100 mg/kg of nitrite than with 200 mg/kg. According to Lücke (2000), the nitrite concentrations required for the antioxidant effect vary between 20 and 50 mg/kg, depending on the type of meat product.

In this study, all samples manufactured with nitrite and EO had TBARS values below 3.1 mg MDA/kg sample. Melton (1983) reported detectable oxidized flavor with TBARS values in the range of 0.3–1.0 for pork and beef, 1.0–2.0 for chicken and above 3.0 for turkey meat. However, these TBARS values should not be considered thresholds of rancid odors in meat because they were influenced by several factors. Spicy meat products seem to mask the effects of off flavors.

Although treatment with sodium nitrite and savory EO all significantly ($p \leq 0.05$) inhibited lipid oxidation, the antioxidant effect was only synergistic with the combination of 100 ppm nitrite and 15.60 μl/g EO. This combination showed lower ($p \leq 0.05$) TBARS values than other treatments after the 10th day of storage. Viuda-Martos, Ruiz-Navajas, Fernández-López, and Pérez-Álvarez (2010) evaluated the effect of rosemary and thyme EO (0.02 g/100 g) on TBARS values in mortadella (formulated with 150 mg/kg nitrite) stored for 24 days in packages with different atmospheres. The authors found lower lipid oxidation rates in samples with added EOs compared with controls.

The use of 100 mg/kg nitrite without savory EO had the same effect on lipid oxidation as use of more than 7.80 μl EO in samples without nitrite. However, the use of 200 mg/kg nitrite and savory EO resulted in no positive effect on lipid oxidation. Moreover, an antagonistic effect was observed in samples with 15.60 and 31.25 μl/g EO. This antagonistic effect suggests a possible interaction between nitrite and chemical compounds present in the fraction of *S. montana* EO. The phenolic compounds might interact with nitrite by linking portions of the aromatic ring, and the antagonism might impair the antioxidant effect of the EO and nitrite.

The use of natural additives has attracted attention, and some authors report that natural compounds have antioxidant effects similar to or better than those of synthetic preservatives. Sebranek, Sewalt, Robbins, and Houser (2005) compared the antioxidant activity of rosemary extracts with the synthetic antioxidants BHA/BHT in sausages, using the TBARS method. The authors found that the natural and synthetic products yielded similar results.

3.3. Color measurements

The interaction between the effects (essential oil concentration × nitrite levels × storage time) was significant ($p \leq 0.05$) for the color coordinates lightness (L^*), redness (a^*), yellowness (b^*), chroma (C^*) and hue angle (h^*).

Values of CIE L^* (lightness) for all treatments throughout the storage period are depicted in Fig. 3. Despite some differences during storage, in the samples with no nitrite, the addition of EO

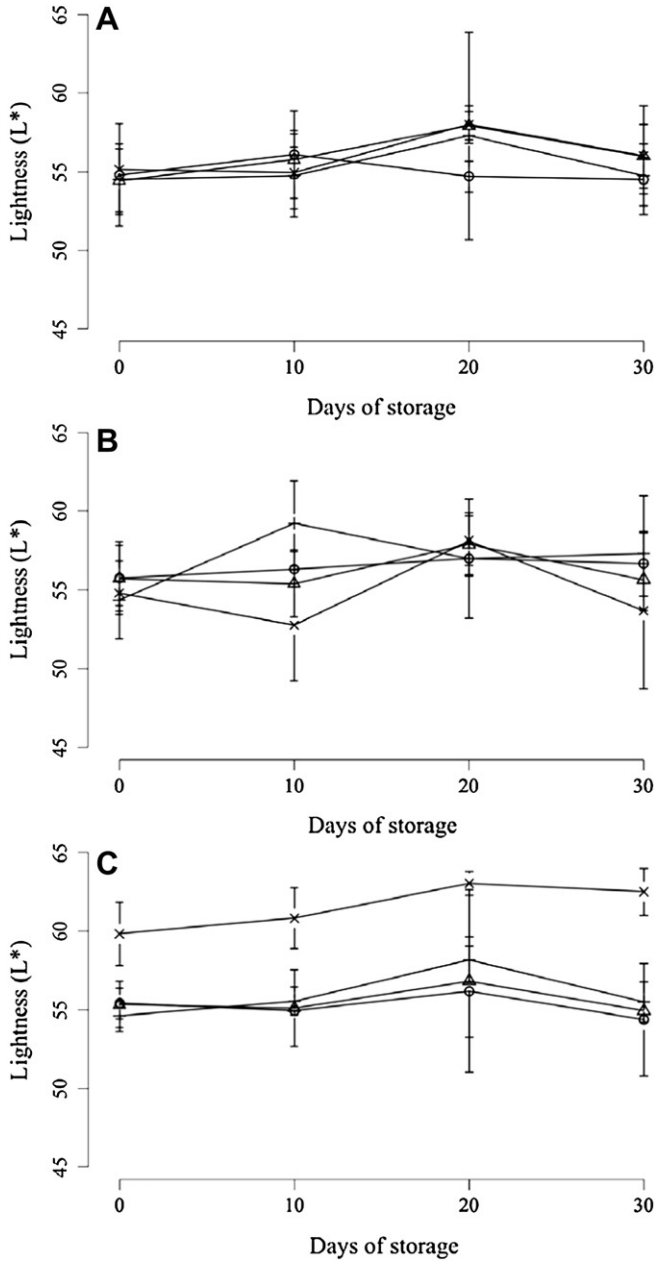

Fig. 3. Effect of different concentrations of *Satureja montana* L. essential oil and sodium nitrite ($NaNO_2$) levels on lightness (L^*) in mortadella-type sausages stored at 25 °C for 30 days. Average of six readings taken at different points in slices of approximately 40 mm wide. A, B and C indicate 0, 100 and 200 mg/kg of $NaNO_2$, respectively. Essential oil concentration: 0.0 μl/g; 7.80 μl/g; 15.60 μl/g and 31.25 μl/g.

had no effects ($p > 0.05$) in lightness of mortadella. However, in samples manufactured with nitrite, the addition of EO at 31.25 μl/g affected lightness, which was significantly different from other treatments ($p \leq 0.05$); this effect was most noticeable in samples with 200 mg/kg added. The effects of EO were dependent on the amount of nitrite added; the samples with 100 mg/kg nitrite were darkest (lower L^* values) at the end of storage, and those with 200 mg/kg nitrite had higher L^* values throughout the storage period. This result is not in accordance with Hernández-Hernández et al. (2009), who observed a higher and negative correlation between lightness and TBARS values in model raw pork batters manufactured without nitrite; as oxidation increased (as TBARS), lightness decreased (the samples became darker). In this study, no relationship was found between TBARS values and lightness, not even in the samples without nitrite and savory EO. These results suggest that the darkening or lightening of cured cooked meat is not only related to lipid oxidation (and TBARS values) but also depends on an interaction between nitrite and certain EO compounds.

All treatments with added nitrite had higher ($p \leq 0.05$) redness (a^*) than samples prepared without nitrite (Fig. 4), indicating a greater involvement of these additives in the red/pink product color. This finding was expected because nitrite plays a key role in

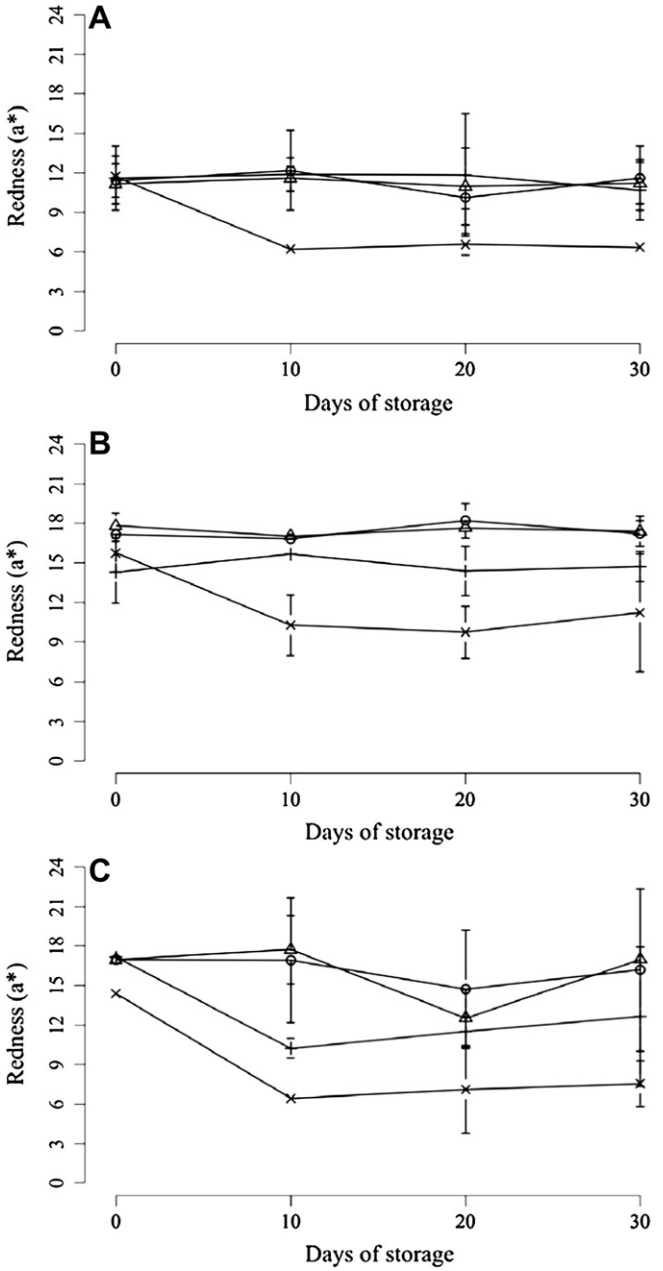

Fig. 4. Effect of different concentrations of *Satureja montana* L. essential oil and sodium nitrite ($NaNO_2$) levels on redness (a^*) in mortadella-type sausages stored at 25 °C for 30 days. Average of six readings taken at different points in slices of approximately 40 mm wide. A, B and C indicate 0, 100 and 200 mg/kg of $NaNO_2$, respectively. Essential oil concentration: 0.0 μl/g; 7.80 μl/g; 15.60 μl/g and 31.25 μl/g.

forming the characteristic color of cured meat products. Additionally, no significant differences ($p > 0.05$) were observed for redness (a^*) at the end of the first day of storage for treatments formulated with 100 and 200 mg/kg of nitrite and without oil. These results, along with the lack of differences ($p > 0.05$) in yellowness (b^*) between the samples manufactured with and without nitrite (Fig. 5), show that the lowest dose of nitrite (100 mg/kg) was sufficient for the formation of a pink color. In studies aiming to reduce the nitrite level used in the production of hot dogs, Jafari and Emam-Djomeh (2007) found that the color indices a^* and b^* were similar in samples fabricated with 50 and 120 mg/kg of nitrite; the authors reported that 50 mg/kg of nitrite appears to be sufficient to develop the color and flavor of the product, but higher concentrations are required for microbiological stability. Studies conducted by Al-Shuibi and Al-Abdullah (2002) evaluated the sensory aspects of color in mortadella produced with varying sodium nitrite levels replaced by sodium sorbate; the authors reported that panelists' comments on the color (range: 0–10) did not differ significantly between mortadellas produced with 120 and 40 mg/kg of nitrite.

High concentrations of *S. montana* L. EO had a negative impact on color formation. In products manufactured without nitrite, the

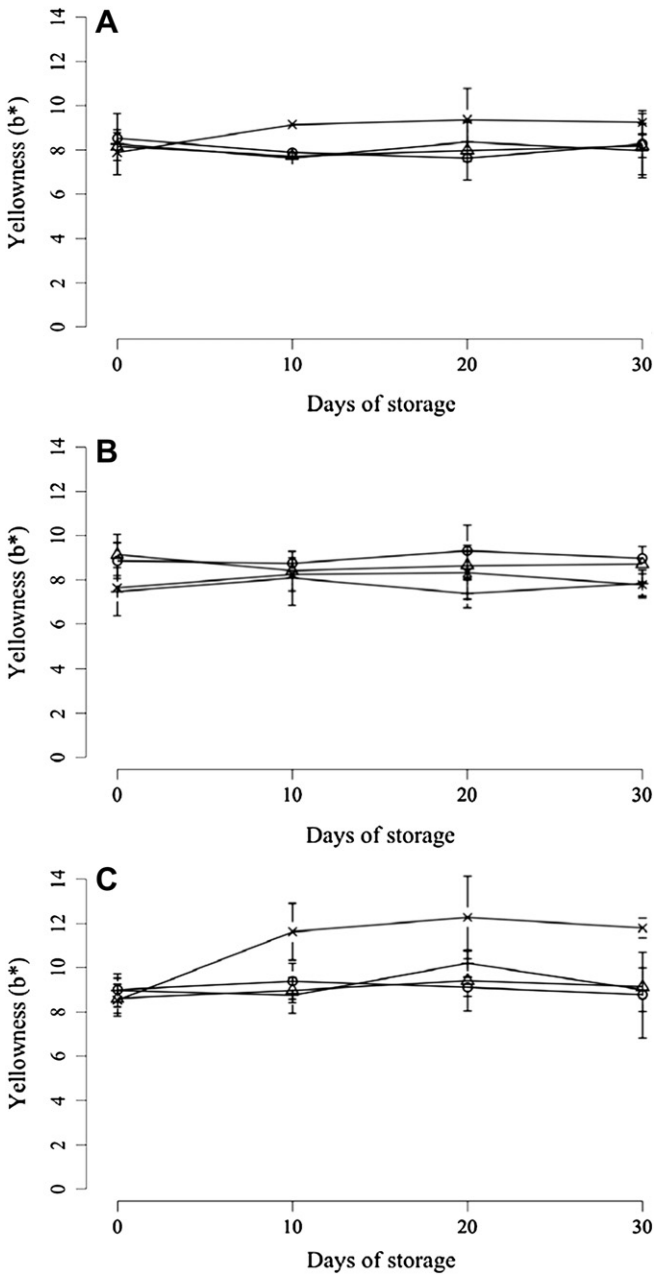

Fig. 5. Effect of different concentrations of *Satureja montana* L. essential oil and sodium nitrite ($NaNO_2$) levels on yellowness (b^*) in mortadella-type sausages stored at 25 °C for 30 days. Average of six readings taken at different points in slices of approximately 40 mm wide. A, B and C indicate 0, 100 and 200 mg/kg of $NaNO_2$, respectively. Essential oil concentration: 0.0 μl/g; 7.80 μl/g; 15.60 μl/g and 31.25 μl/g.

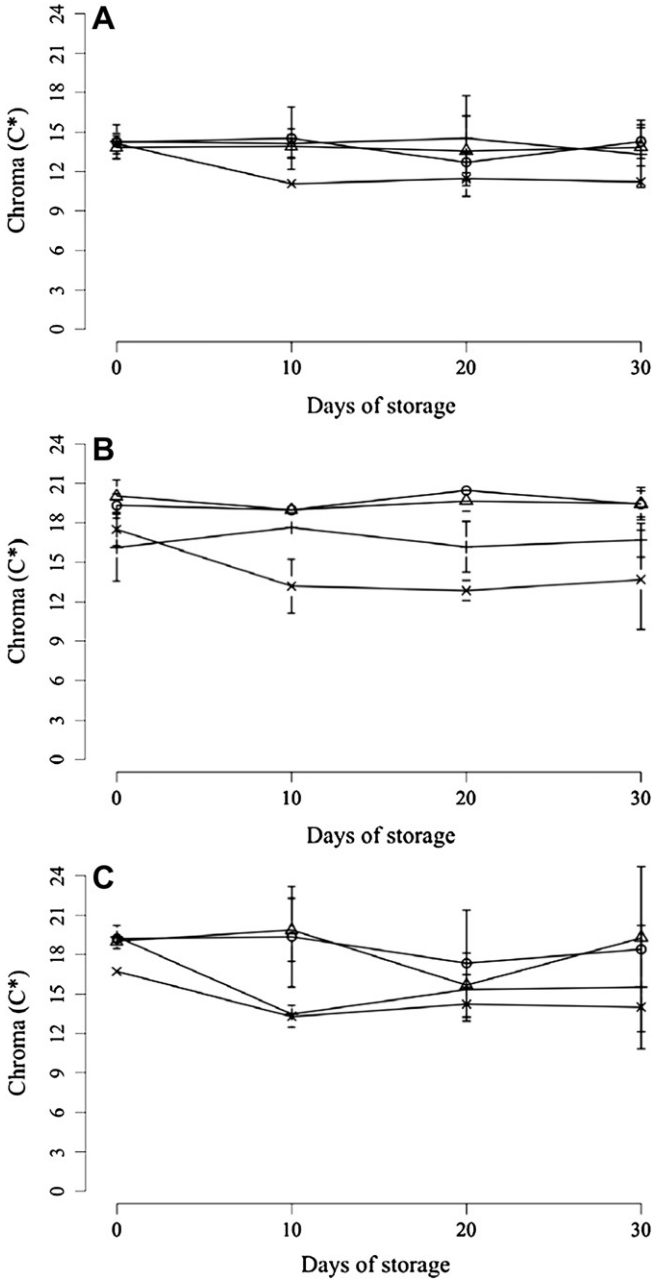

Fig. 6. Effect of different concentrations of *Satureja montana* L. essential oil and sodium nitrite ($NaNO_2$) levels on chroma (C^*) in mortadella-type sausages stored at 25 °C for 30 days. Average of six readings taken at different points in slices of approximately 40 mm wide. A, B and C indicate 0, 100 and 200 mg/kg of $NaNO_2$, respectively. Essential oil concentration: 0.0 μl/g; 7.80 μl/g; 15.60 μl/g and 31.25 μl/g.

addition of 31.25 μl/g EO induced a reduction ($p \leq 0.05$) in a^* values and an increase in b^* values. When nitrite was used, the a^* value was significantly reduced in samples with EO concentrations greater than 15.60 μl/g, and even greater decreases were observed when 31.25 μl/g EO was added. The b^* value was increased only in samples containing 31.25 μl/g EO and 200 mg/kg nitrite. The decreased a^* (redness) values and increased b^* (yellowness) values, with or without L^* changes, are associated with the fading of the cured color (AMSA, 1991). The fading that resulted from adding high concentrations of EO can be explained by a possible interaction between nitrite and chemical components present in the aromatic fraction EO, making NO_2^- unavailable to combine with myoglobin to produce the characteristic red color. Moreover, this interaction and the high concentration of oil can lead to a prooxidant effect, separating nitric oxide from the cured pigment and subsequently oxidizing it to brown metmyoglobin, which is associated with a reduction in reddish color (fading). This finding is in agreement with Lindahl, Lundström, and Tornberg (2001), who found that the pigment content and the myoglobin form were the most important factors in the variation in a^* value. However, the myoglobin forms, and not the pigment content, were important for the b^* value.

The chroma (C^*) and the hue angle (h^*) are both based on the a^* and b^* values and, consequently, are influenced by both the pigment content and the myoglobin form. Compared with samples manufactured without nitrite and EO, all other treatments without oil had a lower hue angle (h^*) and higher chroma (C^*), indicating a more intense reddish color (Figs. 6 and 7).

Despite the significantly lower ($p \leq 0.05$) color intensity in samples with higher concentrations of savory EO, intensity also depended on the concentration of nitrite added and was more pronounced in the 100 mg/kg nitrite samples. In the samples without nitrite, the reduction was only significant with EO concentrations greater than l31.25 μl/g; in the samples with added nitrite, EO concentrations greater than 15.60 μl/g were sufficient to reduce chroma values. The inverse was observed for hue angle: EO additions greater than 31.25 μl/g induced a substantial increase in hue values in all samples, and in samples manufactured with low (100 mg/kg) or without nitrite, EO concentrations greater than 15.60 μl/g also increased hue values. These hue angle (h^*) increases suggest an increase in yellowness.

These changes (increased hue and reduced chroma) with the addition of high concentrations of savory EO, confirmed that a discoloration (fading) of the cured color of products occurred. This finding is in agreement with Sánchez-Escalante, Djenane, Torrescano, Beltrán, and Roncales (2003), who reported that myoglobin and oxymyoglobin oxidation to brown metmyoglobin was associated with a reduction in reddish color (higher hue values) and lower chroma.

Among the nitrite levels tested, the use of sodium nitrite at a concentration of 100 mg/kg appeared to be sufficient for the formation of the characteristic red color. Additionally, the use of savory EO at concentrations lower than 15.60 μl/g had no effects on the color of the products and produced a synergistic antioxidant effect when combined with nitrite. This result indicates that it is feasible to use this EO to reduce nitrite levels in mortadella.

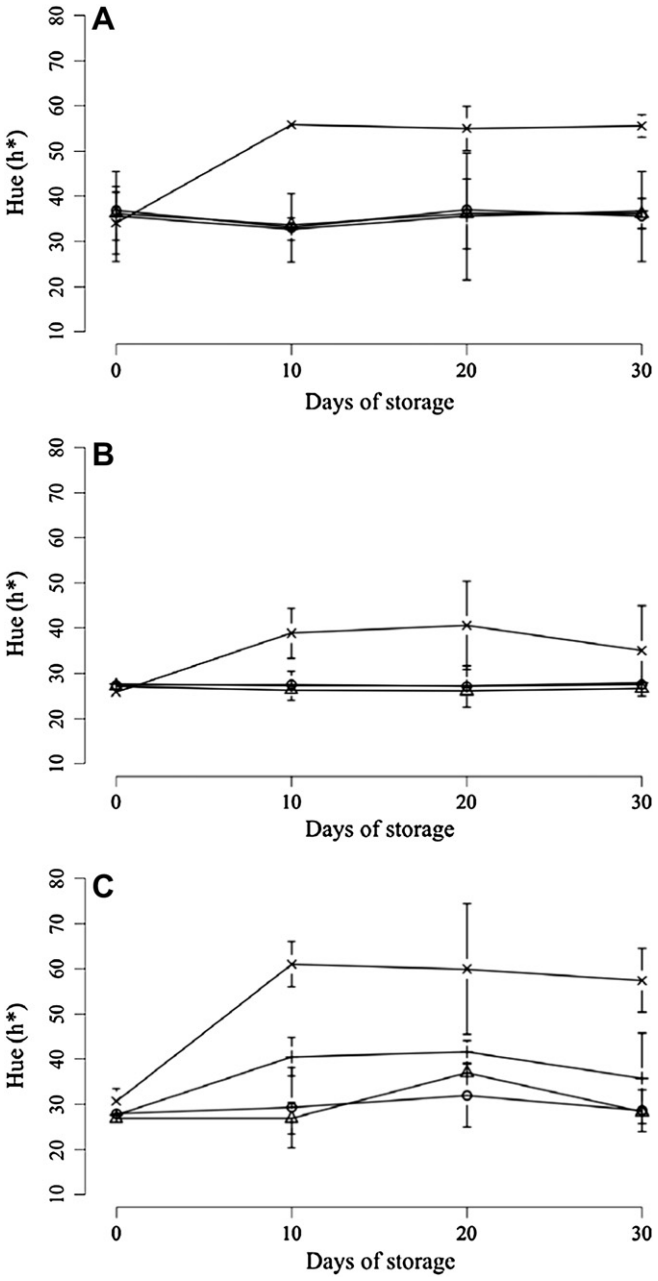

Fig. 7. Effect of different concentrations of *Satureja montana* L. essential oil and sodium nitrite ($NaNO_2$) levels on hue angle (h^*) in mortadella-type sausages stored at 25 °C for 30 days. Average of six readings taken at different points in slices of approximately 40 mm wide. A, B and C indicate 0, 100 and 200 mg/kg of $NaNO_2$, respectively. Essential oil concentration: 0.0 μl/g; 7.80 μl/g; 15.60 μl/g and 31.25 μl/g.

4. Conclusions

The use of savory EO in high concentrations with high levels of sodium nitrite can promote undesirable sensory changes by changing the characteristic color of the product. The antioxidant activity and effect of EO on lipid oxidation in mortadella was confirmed by reduced oxidative reactions. These results suggest a possible application of savory EO, combined with minimal doses of nitrite (100 mg/kg or lower), to meet the increased consumer demand for natural additives.

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