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Technological and sensory feasibility of incorporating oregano essential oil-loaded nanoemulsions as antioxidants in chicken pâté

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Abstract

Nanoemulsions encapsulating oregano essential oil (OEO) were obtained using phase inversion temperature (PIT), and their antioxidant action was evaluated *in vitro* and after incorporating spreadable chicken pâté. Nanoemulsions containing 3.25 g of OEO/100 g of presented droplet sizes around 25 nm were highly stable for 90 days of storage under refrigeration. The minimum inhibitory concentration values (1.30 and 1.73 mg OEO/mL for *Staphylococcus aureus* and *Escherichia coli*, respectively) and minimum bacterial concentration (3.67 and 3.46 mg OEO/mL for *S. aureus* and *E. coli*, respectively) indicated a high antibacterial potential of nanoemulsions *in vitro*. Regarding *in vitro* antioxidant activity, 2,2-diphenyl-1-picrylhydrazyl reduction capacity (63.4%) and total phenolics (11 mg equivalent gallic acid/mL nanoemulsion) remained stable following 13 weeks of storage. The evaluation of lipid oxidation in chicken pâté revealed a higher antioxidant action of nanoemulsions than for the nonemulsified OEO and synthetic antioxidant butylated hydroxytoluene. Sensory evaluation data indicated no significant changes in color, odor, and overall acceptability of chicken pâtés incorporated with OEO-loaded nanoemulsions after 90 days of storage. These results revealed that OEO-loaded nanoemulsions obtained using the PIT method are promising in terms of replacing common antioxidants used in this meat product.

Keywords: nanoencapsulation; phase inversion temperature; meat product; lipid oxidation.

Practical application: Preservation of chicken pâté against oxidation without using synthetic antioxidants.

1 INTRODUCTION

Oxidative reactions in meat products can cause undesirable physicochemical changes, such as flavor deterioration and changes in the texture, color, and even nutritional values, influencing their quality and shelf life (Pateiro et al., 2018; Rodríguez-Carpena et al., 2011). However, regular consumption of synthetic antioxidants generally incorporated in meat products can cause hazardous effects on consumers' health, such as toxicological and carcinogenic effects (Kumar et al., 2015). Essential oils may be considered an alternative to reduce or even replace the synthetic antioxidants in these products as they exhibit remarkable antioxidant activity (Cunha et al., 2018). The antioxidant action of essential oils is associated with several mechanisms of action: (i) prevention of chain initiation, (ii) free-radical scavengers, (iii) reducing agents, (iv) termination of peroxides, (v) prevention of continued hydrogen abstraction (as quenchers of singlet oxygen formation), and (vi) binding of transition metal ion catalysts (Tongnuanchan & Benjakul, 2014). Regarding oregano essential oil (OEO), several studies have evaluated its antioxidant activity in meat products (Al-Hijazeen et al., 2016; Boskovic et al., 2019; Hernández-Hernández et al., 2017; Mahgoub et al., 2020; Ozaki et al., 2021). Such a strong antioxidant action is attributed to the high concentration of phenolic compounds, such as carvacrol and thymol (Pateiro et al., 2018).

However, the low water solubility, powerful aroma and bitter flavor, high volatility, and hydrophobicity of OEO are drawbacks that make their direct incorporation into foods (Artiga-Artigas et al., 2017; Donsì & Ferrari, 2016). The encapsulation in nanoemulsions can reduce their deleterious sensory impacts and increase their physicochemical stability in foods (Asensio et al., 2020; de Carli et al., 2018; Kaur & Kaur, 2021; Moraes-Lovison et al., 2017). Low-energy methods are classified as spontaneous emulsification and phase inversion methods (McClements & Rao, 2011). The latter includes the phase inversion temperature (PIT) method (Solans & Solè, 2012), which was used in this study. The PIT method depends on the change in the solubility of nonionic polyethoxylated (POE) surfactants with temperature (Solans et al., 2005). As the temperature increases, the POE heads are dehydrated, and when the phase inversion temperature (T_{PIT}) is reached, an oil–water–surfactant system changes from an oil/water emulsion to a water/oil emulsion. A nanoemulsion

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may be produced if the system above PIT is rapidly cooled to a temperature below PIT under continuous stirring. Fast cooling makes the surfactant present in the oil phase migrate to the water phase as the POE headgroups become increasingly hydrated again. Such a migration leads to an increase in the interfacial area, turbulence at the oil/water interface, and, finally, the formation of nanodroplets (Anton & Vandamme, 2009). PIT methods have already been used to produce nanoemulsions encapsulating OEO by Moraes-Lovison et al. (2017) with Cremophor RH 40 and Span 80 as surfactants, and the nanoemulsions presented enough antibacterial action to be considered as good systems for incorporation in food products to cause microbial deterioration.

In this study, OEO was encapsulated in nanoemulsions obtained by the PIT method using Cremophor RH 40 and Brij 30 as surfactants. These nanoemulsions were evaluated regarding their physicochemical stability during storage and *in vitro* antioxidant activities. These nanoemulsions were incorporated into chicken pâtés, and their antioxidant effects were also evaluated in the meat product, along with the effect of this incorporation on the sensory quality of the product.

2 MATERIALS AND METHODS

2.1 Chemicals

Nanoemulsions were obtained using oregano (*Origanum vulgare*) essential oil (Ferquima, Cotia, SP, Brazil), sunflower oil (Cargill, Mairinque, SP, Brazil), 40PEG hydroxylated castor oil (Cremophor RH 40, BASF, Ludwigshafen, Germany), and polyoxyethylene(4)lauryl ether (Brij 30, Sigma-Aldrich, St. Louis, MO, USA). Lean chicken meat, subcutaneous chicken fat, chicken liver, refined salt, margarine, maltodextrin (MOR-REX1920, Ingredion, Mogi-Guaçu, SP, Brazil), dehydrated onion, dehydrated garlic, xanthan gum (Grindsted 80, Du Pont, Cotia, SP, Brazil), butylated hydroxytoluene (BHT), sodium nitrite, and lactic acid were used to produce the chicken pâtés.

2.2 Production of nanoemulsions encapsulating OEO by phase inversion temperature method

Nanoemulsions were produced by the PIT method (Moraes-Lovison et al., 2017). A surfactant–oil–water system was prepared with the following composition: deionized water (80.5 g/100 g nanoemulsion), Cremophor RH 40 (9.75 g/100 g nanoemulsion), Brij 30 (3.25 g/100 g nanoemulsion), OEO (3.25 g/100 g nanoemulsion), and sunflower oil (3.25 g/100 g nanoemulsion). The mixture was heated to 20°C above T_{PIT}, stirred magnetically at 1,350 rpm, and then cooled to 20°C (cooling rate: 10°C/min) in a jacketed beaker (while being stirred magnetically at 585 rpm). The samples underwent two cycles of heating and cooling. Subsequently, the nanoemulsions were stored under refrigeration (7°C–10°C). The value of T_{PIT} was determined via a conductivity meter (Inolab 740, Tetracon cell 325, WTW, Weilheim, Germany), using conductivity as a function of temperature, and calculated from the average temperature between the onset of conductivity decrease and the minimum temperature reached by the system after phase inversion (Bovi et al., 2017).

2.3 Determination of average particle size and polydispersity

The average hydrodynamic diameter and polydispersity of the nanoemulsions were determined using light scattering quasi-elastic ZetaPlus equipment (Brookhaven Instruments Company, Holtsville, NY, USA) at 25°C and a He–Ne laser at a wavelength of 627 nm with an incidence angle of 90°. To prevent multiple light scattering, samples were diluted with ultrapure water before measurements were taken.

2.4 Quantification of volatile compounds using gas chromatography

Anhydrous ethanol was added (1/10, v/v) to destabilize and quantify the volatile compounds (carvacrol, thymol, and γ-terpinene) encapsulated in the nanoemulsions. An HP-5971 mass selective detector in electron impact ionization mode (70 eV) was used with GC (HP 5890 Series II, Palo Alto, CA, USA) that had a capillary column HP-5 (25 m × 0.2 mm × 0.33 μm), a split/splitless injector, and He as the carrier gas (1.0 mL/min). The injection temperature was set to 220°C. In the column, the temperature was initially set to 60°C and raised to 240°C at a rate of 3°C/min. When the temperature reached 240°C, the sample remained in the column for 7 min (Sartoratto et al., 2004). Analyses were performed in triplicate, and an analytical curve of the carvacrol, thymol, and γ-terpinene compounds was prepared with concentrations of 40–400 μg/mL.

2.5 Determination of the in vitro *antioxidant activity of OEO-loaded nanoemulsions*

The *in vitro* antioxidant activity of OEO-NEs was evaluated by measuring the percentage inhibition of 2,2-diphenyl-1-picrylhydrazyl (DPPH•) and by quantifying the total phenolic content. The percentage inhibition of DPPH• was determined by adding 20 μL of OEO-NEs to 1,980 μL of an ethanolic solution of DPPH• (0.5 mM). This mixture was vortexed and kept in the dark at 25°C for 30 s. After 1 h, the absorbance of the mixture was measured at 517 nm in a spectrophotometer (DR-2800, Hach, Loveland, CO, EUA). Anhydrous ethanol was used as the blank.

The total phenolic compounds were determined by spectrophotometry using the Folin–Ciocalteu reagent according to Singleton et al. (1999), with modifications. Initially, OEO-NEs were diluted in deionized water, and 250 μL of these samples was added to 2 mL of deionized water along with 250 μL of Folin–Ciocalteu reagent. Then, the mixture was vortexed, and after 3 min, 250 μL of a saturated sodium carbonate solution was added. After stirring, the samples were heated at 37°C for 30 min, and the absorbance was measured at 760 nm (Libra S22, Biochrom, Cambridge, UK). The total phenolic content was calculated using a standard gallic acid curve, with concentrations ranging from 0 to 0.05 mg/mL. The results were expressed as mg equivalent of gallic acid/mL of sample.

2.6 Production of chicken pâtés

Chicken pâtés were produced according to de Carli et al (2018). Four treatments were made: T1—control (no antioxidants), T2—chicken pâté with OEO-NEs (6 g dispersion/100 g

pâté—the total amount of OEO: 0.195 g/100 g pâté), and T3—chicken pâté with BHT (100 mg/kg pâté) and sodium nitrite (150 mg/kg pâté) (maximum permitted concentrations of these additives by the Brazilian legislation).

2.7 Instrumental colorimetry of chicken pâtés

Instrumental color in the CIELAB space (L* for lightness, a* for redness, and b* for yellowness) was measured by using a MiniScan XE portable colorimeter (HunterLab, Reston, VA, USA), with a D65 illuminant, an observation angle of 10°, and a cell opening of 30 mm. The chroma (C*) and hue angles (H*) were calculated from the a * and b * values according to Equations 1 and 2:

$$C^* = (a^* + b^*)^{0.5} \quad (1)$$

$$H^* = \text{arctg}\,(b^*/a^*) \times 57.29 \quad (2)$$

The total color differences (ΔE) in pâtés between weeks 1 and 16 of storage were calculated as follows (Equation 3):

$$\Delta E = [(L_{16}-L_1)^2 + (a_{16}-a_1)^2 + (b_{16}-b_1)^2]^{1/2} \quad (3)$$

2.8 Determination of lipid and protein oxidation in chicken pâtés

Primary lipid oxidation in chicken pâtés, expressed in meq O_2/kg pâté, was evaluated by the peroxide value index method. The TBARS (2-thiobarbituric acid reactive substances) method was used to quantify secondary lipid oxidation (values were expressed in milligrams MDA/kg pâté) according to Pateiro et al. (2014). According to Oliver et al. (1987), the levels of oxidatively modified proteins were determined, and carbonyl compounds were expressed as nM/mg protein.

2.9 Sensory evaluation

This research was submitted to and approved by the Ethics Research Committee from the School of Animal Science and Food Engineering (FZEA), Universidade de São Paulo (USP, Brazil) (code CAAE 59015916.5.0000.5422), and the 100 non-trained participants signed a Term of Consent. The pâté was spread on a small toast before being served to the panelists. The sensory test was effective and was used to evaluate consumer acceptance. The panelists were asked to rate their liking or disliking of the chicken pâtés based on attributes such as odor, color, and overall acceptance using a 9-point hedonic scale for fresh and 90-day stored samples of pâtés. Quantification of total viable bacteria and enterobacteria was performed before sensory evaluation. These analyses were performed one day after pâté production, based on Silva et al. (2010), in triplicate.

2.10 Statistical analyses

All experiments were performed in triplicate. The software SAS (Statistical Analysis System, version 9.2 SAS Institute Inc., Cary, NC, USA) was used to analyze variance, and Tukey's test was chosen to determine significant differences at a 5% significance level.

3 RESULTS AND DISCUSSION

3.1 Production and physicochemical stability of OEO-loaded nanoemulsions

The T_{PIT} of OEO-NEs, containing 13% (w/w) surfactants (Cremophor RH40 and Brij 30), was here determined to be 52°C. In a previous study (Moraes-Lovison et al., 2017), lower values for this temperature (44–46°C) were obtained. In that case, the surfactants were Cremophor RH40 and Span 80. In this study, Brij 30 was used, whose structures certainly influenced the value of T_{PIT}. Span 80 has 20 POE groups, while Brij 30 has only 4. Such a fact makes the latter more difficult to dehydrate and hydrate, resulting in a higher T_{PIT} value. Furthermore, the value of T_{PIT} increased as the value of the total surfactant concentration decreased. This helped to explain why the value obtained in this study was higher than the T_{PIT} obtained by Moraes-Lovison et al. (2017). The average droplet size was very small (25.5 ± 0.21 nm), and the droplets were highly stable during the 90 days of storage (Table 1). Moreover, Table 1 shows the extremely low values of PDI as well as their high stability over 90 days of storage under refrigeration (0.05 ± 0.01).

Regarding the capacity of OEO-NEs to preserve the primary components of the OEO, there was a significant reduction ($p < 0.05$) in their concentrations during storage: 46.4% for carvacrol, 10.4% for thymol, and 93.0% for γ-terpinene. Such a decrease in these three components of our OEO is shown in Figure 1. The reason for these high losses may be associated with the migration rate of these volatile compounds from the oil core of the droplets to the aqueous phase, followed by their volatilization.

3.2 In vitro *antioxidant activity of OEO-loaded nanoemulsions*

Regarding the quantification of phenolic compounds during the storage of the OEO-NE (Figure 2), there was no reduction in total phenolic values after 24 weeks.

Table 1. Hydrodynamic diameters and polydispersity indexes of the OEO-NE over the storage.

	Storage (day)							
	1	**11**	**21**	**27**	**60**	**75**	**90**	**Sign.**
Hydrodynamic diameter (nm)	25.5 ± 0.21	24.8 ± 1.56	24.6 ± 1.34	23.1 ± 0.57	23.9 ± 0.28	24.3 ± 0.57	23.8 ± 0.85	n.s.
Polydispersity index	0.05 ± 0.01	0.08 ± 0.06	0.04 ± 0.05	0.05 ± 0.001	0.07 ± 0.02	0.04 ± 0.04	0.04 ± 0.03	n.s.

n.s.: There were not any statistically significant differences among the values in the same row according to Tukey's test ($p < 0.05$).

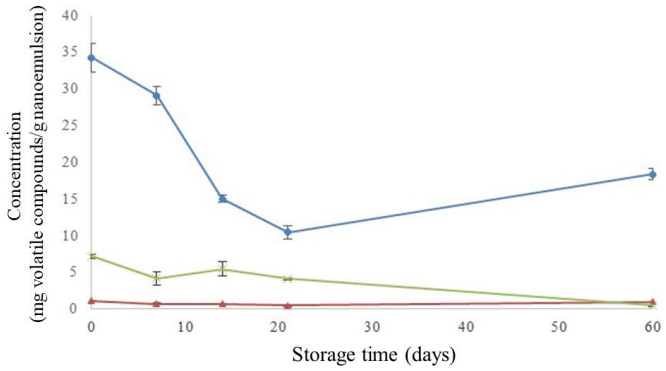

Figure 1. Temporal profiles of the concentration for (•) carvacrol, (▪) γ-terpinene, and (▲) thymol during 60 days of refrigerated storage of OEO-NEs.

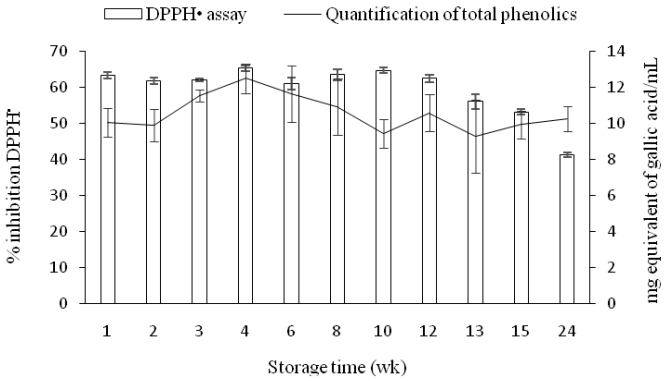

Figure 2. *In vitro* antioxidant activity of nanoemulsions encapsulating oregano essential oil over the storage period, expressed in % inhibition DPPH• and quantification of total phenolic content.

Therefore, OEO-NEs were effective at protecting the phenolic compounds in OEO during this period. The DPPH• inhibition capacity was 63.4% in the first week of storage (Figure 2), and it remained constant for up to 13 weeks of storage. Furthermore, it is important to emphasize that the high antioxidant activity of the nanoemulsions is primarily related to OEO as the percentage reduction in DPPH• for the OEO-NE was significantly higher than the nanoemulsion value without the OEO (only 1.97%). However, in the 24th week of storage, the reduction in DPPH• was 41.2%; such a decrease in the antioxidant activity may be associated with the loss of carvacrol and thymol that was previously reported in Section 3.1.

3.3 Physicochemical characterization of chicken pâtés

First, it is important to justify the absence of a pâté incorporated with nonencapsulated OEO in this part of the study. Due to the remarkable sensory changes the free OEO produced in the chicken pâté, it became unacceptable to be included in the sensory analysis step. Therefore, as it would be unrealistic in terms of sensory acceptance to compare such an intolerable product with the other treatments, the authors decided not to include nonencapsulated OEO in the chicken pâté treatments submitted to the physicochemical characterization. However, a comparison with a commercial antioxidant (BHT) was performed.

Regarding colorimetric parameters, in the first week of storage, the values of L* and b* did not vary significantly ($p < 0.05$) with any treatment. Regarding the parameter a*, there was a significant increase in treatment T3 compared to the other treatments due to the addition of sodium nitrite, which preserved the red coloration of the meat products following heat treatment.

Table 2 shows that chroma (C*) did not vary significantly among the treatments during storage. Regarding the hue angle (H*), the significant increase observed with all treatments at the end of the storage period (16 weeks) indicated discoloration of the pâtés. This color loss could be associated with protein oxidation (Figure 3) observed in all treatments of chicken pâtés during storage (Estévez & Cava, 2004, 2006). In the case of total color differences between weeks 1 and 16 (ΔE_{1-16}), treatment T3 containing BHT showed higher values than the control treatment without antioxidants (T1). However, such an increase was not significant. Moreover, the same behavior was reported in a study conducted by Estévez et al. (2006) on pork liver pâté, which presented a higher value of ΔE between days 0 and 90 for treatments with BHT and essential oils of sage and rosemary when compared to a control without antioxidants. The same authors associated color change with chemical changes or alterations in the composition of pork liver pâté, which may have occurred during storage and were not necessarily directly related to oxidative processes.

3.4 Antioxidant activity (protein and lipid) of OEO-loaded nanoemulsions in chicken pâté

Protein oxidation is associated with the development of oxidative reactions that degrade essential amino acids and the subsequent formation of crosslinks (protein carbonyls) (Stadtman & Levine, 2003). In the 1st week, the carbonyl content varied between 0.87 and 1.54 nM carbonyl/mg protein (as shown in Figure 3), and after 16 weeks, there was a significant increase in the carbonyl contents of all chicken pâtés. Lorenzo et al. (2014) associated protein oxidation in pâté over time with the temperatures applied in heat treatment during pâté production, as well as with meat grinding during homogenization. Heating degrades myoglobin, releasing iron and increasing its pro-oxidant potential in cooked meats. On the other hand, the rupture of tissues during product homogenization leads to the release of pro-oxidants naturally present in the animal muscle, increasing the amount of oxygen in the system and facilitating the oxidation of the meat product. Significant inhibition of protein carbonyl formation due to the addition of phenolic antioxidants (such as essential oils) has not been observed (Estévez et al., 2007; Jongberg et al., 2013).

Regarding lipid oxidation, the peroxide index was 0.88–1.05 meq O_2/kg of sample on the first week of storage (Figure 4). For all pâtés, there was an increase in the peroxide index up to week 5 and a significant reduction in these values (Figure 4). Such behavior was associated with the auto-oxidation of lipids in the meat product. In its first stages, the hydroperoxides' formation

Table 2. Values of instrumental color (L*, a*, and b*), chroma (C*), hue angles (H*), and the total color differences (ΔE) during 16 weeks of chicken pâté refrigerated storage.

		Storage time (weeks)						
	T	**1**	**3**	**5**	**7**	**13**	**16**	
L*	T1	54.07 ± 1.28^{a}	53.15 ± 3.40^{a}	58.14 ± 1.19$^{a.1}$	57.66 ± 0.88$^{a.1}$	53.98 ± 0.59^{a} 53.99	53.12 ± 3.48^{a}	*
	T2	53.26 ± 0.22^{b}	53.15 ± 0.49^{b}	55.43 ± 1.23$^{a.b.2}$	56.83 ± 0.27$^{a.1.2}$	53.26 ± 2.66^{b}	54.76 ± 0.72$^{a.b}$	*
	T3	53.42 ± 0.14^{b}	51.94 ± 1.31^{b}	55.44 ± 0.36$^{a.2}$	56.19 ± 0.23$^{c.2}$	52.81 ± 0.67^{b}	52.91 ± 0.56^{b}	*
	Sign.	n.s	n.s	*	*	n.s	n.s	
a*	T1	1.18 ± 0.30$^{b.2}$	1.68 ± 0.23$^{a.b.2}$	1.67 ± 0.14$^{a.b.2}$	1.65 ± 0.18$^{a.b.2}$	1.22 ± 0.25$^{b.1.2}$	-0.05 ± 0.11^{c}	*
	T2	0.87 ± 0.07$^{b.c.2}$	0.70 ± 0.24$^{c.3}$	1.24 ± 0.18$^{a.b.3}$	1.21 ± 0.10$^{a.b.2}$	1.10 ± 0.18$^{b.2}$	-0.12 ± 0.03^{d}	*
	T3	4.07 ± 0.41$^{a.1}$	3.52 ± 0.56$^{a.1}$	3.63 ± 0.11$^{a.1}$	2.66 ± 0.24$^{b.1}$	1.62 ± 0.07$^{b.1}$	0.17 ± 0.12^{c}	*
	Sign.	*	*	*	*	*	n.s	
b*	T1	14.84 ± 0.72$^{a.b}$	15.8 ± 0.44^{a}	14.86 ± 0.07$^{a.b}$	16.13 ± 0.67^{a}	14.69 ± 0.82$^{a.b}$	13.64 ± 0.80$^{b.c}$	*
	T2	14.89 ± 0.11^{a}	15.81 ± 0.95^{a}	15.04 ± 0.14^{a}	15.68 ± 0.47^{a}	14.42 ± 0.71^{a}	14.37 ± 0.71^{a}	*
	T3	15.34 ± 0.44^{a}	15.32 ± 0.69^{a}	15.07 ± 0.48^{a}	15.59 ± 0.31^{a}	15.68 ± 0.35^{a}	15.10 ± 0.27^{a}	*
	Sign.	n.s	n.s	n.s	n.s	n.s	n.s	
C*	T1	14.89 ± 0.72$^{a.b}$	15.47 ± 0.45^{a}	14.96 ± 0.07$^{a.b}$	16.21 ± 0.65^{a}	14.74 ± 0.83$^{a.b}$	13.64 ± 0.80$^{b.c}$	
	T2	14.91 ± 0.11^{a}	15.82 ± 0.95^{a}	15.09 ± 0.15^{a}	15.73 ± 0.48^{a}	14.47 ± 0.72^{a}	14.37 ± 0.71$^{a.b}$	
	T3	15.87 ± 0.49^{a}	15.73 ± 0.56^{a}	15.50 ± 0.45^{a}	15.82 ± 0.27^{a}	15.76 ± 0.36^{a}	15.10 ± 0.27^{a}	
	Sign.	n.s	n.s	n.s	n.s	n.s	n.s	
H*	T1	85.41 ± 1.12$^{b.1}$	83.74 ± 0.77$^{b.c.2}$	83.53 ± 0.55$^{b.c.2}$	84.10 ± 0.78$^{b.1}$	85.22 ± 0.67$^{b.1.2}$	89.54 ± 0.17^{a}	
	T2	86.62 ± 0.26$^{b.c.1}$	87.41 ± 0.85$^{b.1}$	85.23 ± 0.66$^{d.1}$	83.55 ± 0.24$^{c.d.1}$	85.59 ± 0.52$^{c.d.1}$	89.47 ± 0.13^{a}	
	T3	75.09 ± 1.28$^{d.2}$	76.94 ± 2.50$^{c.d.3}$	76.41 ± 0.73$^{d.3}$	80.25 ± 1.05$^{b.c.2}$	84.04 ± 0.13$^{b.2}$	89.30 ± 0.45^{a}	
	Sign.	*	*	*	*	*	n.s	
ΔE	T1						2.59 ± 0.43	
	T2						2.48 ± 0.58	
	T3						3.67 ± 0.56	
	Sign.						n.s	

T = treatments: (T1) control (no additives); (T2) nanoemulsion (6 % w/w, nanoemulsion concentration was selected based on the MIC results), and (T3) synthetic antioxidants BHT (100 mg/kg) and sodium nitrite (150 mg/kg). Means with a different letter (^{a,b,c,d}) within a line are significantly different ($p < 0.05$). Means with a different number (^{1,2,3}) within a column are significantly different ($p < 0.05$). Sign.: Significance: n.s.: not significant ($p \geq 0.05$), significant different: *($p < 0.05$).

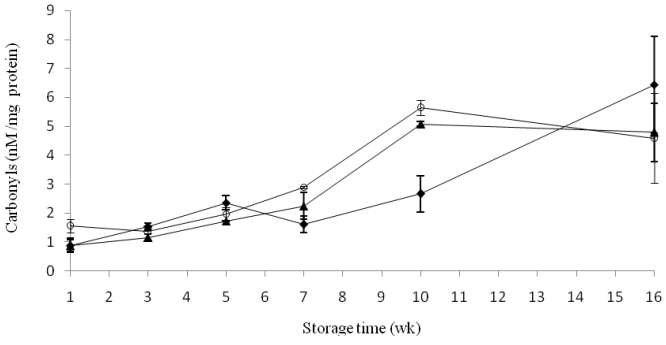

Figure 3. Protein carbonyl in chicken pâté during storage: (◆) T1, control (no additives); (▲) T2, nanoemulsion (6 % w/w); and (○) T3, BHT (100 mg/kg) and sodium nitrite (150 mg/kg).

rate was higher than their decomposition rate. The reverse occurred in the later phase, wherein the hydroperoxides decomposed faster than when they were formed (Akarpat et al., 2008).

Despite the reduction of peroxide index values in the 10th week, there was no significant increase ($p < 0.05$) in TBARS values (indicative of secondary oxidation) (Figure 4). Therefore, it can be suggested that hydroperoxides may have already been degraded

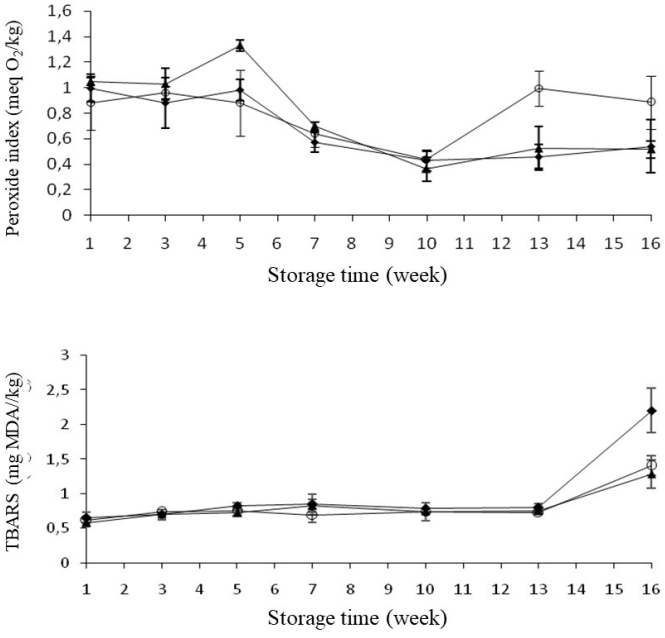

Figure 4. Values of peroxide index and TBARS during 16 weeks of refrigerated storage: (◆) T1, control (no additives); (▲) T2, nanoemulsion (6 % w/w); and (○) T3, BHT (100 mg/kg) and sodium nitrite (150 mg/kg).

in compounds not detected in TBARS analysis, such as ketones, alcohols, aldehydes (except malonaldehyde), hydrocarbons, and volatile organic acids or epoxy (Shahidi & Zhong, 2010).

Furthermore, during storage, there was a significant increase in TBARS with all treatments of chicken pâté, but in the case of treatment T1 (control), this value was significantly higher. The percent inhibition of lipid oxidation compared to the control, at the 16th week of storage, for treatments with nanoemulsions and BHT plus sodium nitrite was 41.9 and 36.2%, respectively. Similar results were obtained by Estévez et al. (2007), who evaluated the antioxidant activity of sage and rosemary essential oils vs BHT in pork liver pâté and obtained a percent inhibition of lipid oxidation for sage (48%) and rosemary (52%) superior to that of the synthetic antioxidant BHT (28%), proving the efficacy of essential oils against lipid oxidative reactions.

3.5 Sensory evaluation

Regarding sensory evaluation, the panelists were asked to grade the chicken pâtés in terms of odor, color, and overall appearance on the product's first and 90th day of storage. It is essential to emphasize that chicken pâtés with nonencapsulated OEO were not included in this analysis because the strong odor (it was described as even "nauseating" for some people) could influence the sensory analysis results. The data obtained are shown in Table 3.

According to Estévez et al. (2007) and Pateiro et al. (2014), the oxidation of lipids can generate residual compounds such as aldehydes and ketones, which cause nutritional and sensory deterioration of products, affecting the odor, color, and texture of meat products. The acceptance analysis for odor and color attributes, as well as the overall quality of the chicken pâté, submitted to the three treatments, was performed to verify if the panelists would detect sensory changes associated with the oxidation of the meat product on different days during the 90-day storage period.

Data indicated that, for the odor and color attributes and the overall quality of meat products, no significant difference ($p < 0.05$) was observed between days 1 and 90 for all treatments. As a result, sensory changes in the color and odor, which may have occurred because of the oxidation of meat products during storage, were not noticeable by the panelists in any treatment. Therefore, the discoloration of meat products observed via instrumental analyses of instrumental color during storage was not detected by the panelists in the sensory analysis. Moreover, the scores of odor and global quality obtained by T2 (chicken pâté incorporated with OEO-loaded nanoemulsions) were slightly higher than T1 (control) and T3 (the pâté formulated with BHT).

4 CONCLUSIONS

The PIT method produced OEO-loaded nanodispersions of a significantly reduced average diameter. Because of such extremely low average droplet sizes, the stability of nanoemulsions during the storage period was extremely high. Furthermore, the nanoemulsions were very effective in preserving the *in vitro* antioxidant activity of OEO. Moreover, this capacity was observed following their incorporation into chicken pâté and reflected in the sensory analysis results. Therefore, OEO nanoemulsions obtained using the PIT method in the conditions, described in this study, can be considered promising nanosystems for food preservation. However, this technological potential should still be complemented with data from deeper sensory evaluation approaches and nanotoxicological studies to ensure the safety of the developed ingredients for consumers.

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Table 3. Sensory evaluation data for the chicken pâtés on the first and 90th day of storage (odor, color, and overall acceptability).

Chicken pâté (treatment)	Storage (day)				
	1	30	60	90	Sign.
Odor					
T1	5.24 ± 1.8	5.45 ± 2.0	5.33 ± 2.1	5.25 ± 2.0	n.s.
T2	6.53 ± 1.9	6.13 ± 2.0	6.48 ± 1.8	6.39 ± 1.8	n.s.
T3	4.63 ± 1.9	4.61 ± 2.0	4.84 ± 2.0	5.25 ± 1.90	n.s.
Color					
T1	5.26 ± 1.7	5.30 ± 1.8	5.34 ± 1.7	5.02 ± 1.8	n.s.
T2	5.59 ± 1.6	5.77 ± 1.7	6.04 ± 1.6	5.60 ± 1.6	n.s.
T3	5.75 ± 2.1	5.59 ± 2.1	6.05 ± 2.1	6.03 ± 2.0	n.s.
Global quality					
T1	5.28 ± 1.6	5.53 ± 1.6	5.36 ± 1.6	5.21 ± 1.7	n.s.
T2	6.12 ± 1.6	6.13 ± 1.6	6.21 ± 1.4	6.07 ± 1.4	n.s.
T3	5.18 ± 1.9^{ab}	5.05 ± 1.9^{b}	5.48 ± 1.7^{ab}	5.74 ± 1.7^{a}	*

Means with a different superscript letter within a line are significantly different ($p < 0.05$); n.s.: there is no significant difference in any of the values in the same row.

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