ANTIOXIDANT ACTIVITIES OF LYOPHILIZED ROSEMARY AND SAGE EXTRACTS AND ITS EFFECT TO PREVENT LIPID OXIDATION IN POULTRY PÁTÊ

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RESUMO – O objetivo deste estudo foi avaliar a atividade antioxidante (AA) de extratos liofilizados de Rosemary (ELR) e Sálvia (ELS) e seus efeitos na estabilidade oxidativa de patês de frango. Para isso, quatro formulações foram preparadas: um tratamento contendo ELR, ELS, eritorbato de sódio e um tratamento sem a adição de antioxidantes. O ELR e o ELS foram caracterizados quanto ao teor de compostos fenólicos totais (TPC) e AA por métodos espectrofotométricos. Os patês de frango armazenados à 4°C foram avaliados quanto a oxidação lipídica. Altas concentrações de TPC foram detectados nos ELR e ELS (46.48 e 41.61 mg GAE/g: ácido gálico equivalente, respectivamente). AA do ELR e ELS foram 4745.72 e 2462.82 µmol Trolox/g, respectivamente. Em relação a oxidação lipídica, a mesma aumentou de 1.35 para 2.87 mg malonaldeideo/kg de patê de frango. Este estudo sugere que os ELR e ELS podem ser usados como antioxidante natural em produtos contendo frango.

ABSTRACT – This study aimed to evaluate the antioxidant activities (AA) of lyophilized rosemary extract (LRE) and lyophilized sage extract (LSE) and their effects on the oxidative stability of poultry pâtê. For this purpose, four poultry pâtê formulations with LRE, LSE, sodium erythorbate, and a control (without antioxidants) were produced. The LRE and LSE were characterized according to total phenolic compounds (TPC), and AA by several methods. The poultry pâtês stored at 4°C were evaluated by the lipid oxidation. High concentrations of TPC were detected in LRE and LSE (46.48 and 41.61 mg GAE/g: Gallic Acid Equivalent respectively). The AA of LRE and LSE by free radical-scavenging were 4745.72 and 2462.82 µmol Trolox/g, respectively. Regarding lipid oxidation, it increased, on average, from 1.35 mg to 2.87 mg malondialdehyde/kg of poultry pâtê. This study suggests that LRE and LSE may be used as a natural antioxidant in poultry products.

PALAVRAS-CHAVE: *Rosmarinus officinalis; Salvia officinalis;* antioxidante natural; compostos fenólicos totais; TBARS.

KEYWORDS: *Rosmarinus officinalis; Salvia officinalis*; natural antioxidant; total phenolic compounds; TBARS.

1. INTROCUCTION.

Rosemary (*Rosmarinus officinalis*) and sage (*Salvia officinalis)* are plant of the Lamiaceae family, and both are native to the Mediterranean, however grows in many parts of the world and have adapted easily in Brazil. Several researchers reported that the rosemary and sage demonstrate excellent antioxidant potential (Pereira et al., 2017; Bianchin et al., 2017). The antioxidant activity of rosemary can be attributed to the presence of phenolic

compounds, mainly rosmarinic acid, carnosol, rosmanol, isorosmanol, rosmadiol, and carnosic acid (Ibañez et al., 2003). While rosmarinic acid and derivatives of caffeic acid are mainly responsible for the effect of the antioxidant activity of sage extracts. Rosemary and sage essential oil has been used in several types of food, such as mayonnaise, meat products, pie dough, and salad dressing as a natural antioxidant. In fact, the rosemary essential oil and lyophilized rosemary extract exhibited an excellent inhibitory effect on lipid oxidation of pork sausage with high acceptance rate (Bianchin et al., 2017). In another study, Pereira et al. (2017) reported that the rosemary extract had strong anti-oxidative effects in chicken burgers at 21 days of storage at refrigerated temperature. In addition, according to Estevéz et al. (2007), a mix of sage and rosemary essential oils inhibited oxidative deterioration of liver pâtés to a higher extent than BHT did.

The consumption of chicken meat has increased in recent years, and there is a tendency to substitute the use of red meat for white meat, due to the low cost of chicken meat when compared to that of other animal proteins. However, chicken meat is rich in iron and have several proteins indispensable to humans. Additionally, chicken meat is also a source of energy and other nutrients such as B vitamins (niacin and riboflavin), minerals and lipids. In 2019, a total of 5.81 billion chicken heads were slaughtered in the Brazilian market. The states of the Southern Region together slaughtered 60.6% of the total national slaughter, more than double the Region's share Southeast (19.9%), Midwest (13.9%), Northeast (3.8%) and North (1.6%). The state of Paraná in 2019 led the national ranking of chicken slaughter, with +94.52 million heads slaughtered with the participation of > 32.5 % followed by Santa Catarina, and Rio Grande do Sul (IBGE, 2019).

The pâté is defined by Brazilian legislation as a pasty product, obtained from meat and meat products and edible offal and added with food ingredients. The paste must be subjected to an appropriate thermal process and can be cooked, pasteurized or sterilized (Brasil, 2000). In these products, the lipid oxidation is one of the main reactions that can occur and can affect the nutritional quality of the food products. These oxidative processes in meat products are influenced by polyunsaturated fatty acids in the presence of prooxidating agents such as iron, oxygen, salt and even mechanical processes. However, its effects can be reducing or inhibited by the use of antioxidants in meat products (Carpes et al., 2020). In fact, the use of synthetic antioxidants to extend the shelf life of meat and meat products is common in the food technology. However, the synthetic antioxidants used in the meat industry, such as butylatedhydroxyanisole (BHA), butylatedhydroxytoluene (BHT), tert-butylhydroquinone (TBHQ) and propyl gallate (PG) has been restricted by many countries due to possible risks to consumer health (Rocha et al., 2007). The toxicological effects of these chemical additives is growing the interest in studies of natural substances with antioxidant activity in meat (Ibrahim et al., 2010).

The main mechanism of action of phenolic compounds from plants is the inactivation of lipid free radicals. However, reduction of the production of reactive species ao oxigênio (ROS) and, consequently, interrupting the propagation phase of lipid autoxidation can also occur. Thus, this work aimed to determine the antioxidant properties, total phenolic compounds of lyophilized rosemary extract (LRE) and lyophilized sage extract (LSE), and the effects of their adding in poutry pâtês, to avoid the lipid oxidation during refrigerated storage at 4 °C.

2. MATERIAL AND METHODS

2.1 Material and preparation of the extracts

Rosemary (*Rosmarinus officinalis*) and sage *(Salvia officinalis)* samples were purchased at a fair of farmers in Pato Branco, Paraná, Brazil. Rosemary and sage leaves were dried in an oven with forced air circulation (Nova Ética 400/D, São Paulo, Brazil) at 42 ºC for 42 h and ground in an analytical mill (less than 0.5 mm).

Samples containing 10 g of sample (dry basis) were subjected to the extraction process with 100 mL of ethanol solution (800 mL/L) in a water-bath at 70 ºC for 30 min at a stirring rate of 150 rpm. The extract was filtered through qualitative filter paper, and the supernatants were evaporated in a rotary evaporator (vacuum pressure of 600 mm Hg and 40 °C) until completely dry and lyophilized (Liotop L101, São Carlos, Brazil). The extracts were named as lyophilized rosemary extract (LRE) and lyophilized sage extract (LSE)

2.2 Total phenolic compounds and total flavonoids content

The total phenolic content (TPC) was performed using Folin-Ciocalteu method described by Singleton et al. (1999) using gallic acid as the standard. After two hours in darkness, the absorbance of the extract was measured at 764 nm in spectrophotometer (UV-VIS Bel Photonics 2000 Piracicaba, Brazil). The results were expressed as mg GAE/g of sample (GAE: gallic acid equivalent). For the total flavonoid content (TFC) was quantified by the colorimetric method with aluminum chloride, following methodology used by Carpes et al. (2008). After 40 min at room temperature, the absorbance was measured at 415 nm in a spectrophotometer. A quercetin standard curve was obtained, and the results were expressed as Quercetin equivalent (mg QE/g). All the assays were carried out in triplicate.

2.3 DPPH (2,2-diphenyl-1-picryl-hydrazyl) radical scavenging

DPPH (2,2-diphenyl-1-picryl-hydrazyl) radical scavenging according to the methodology described by Carpes et al. (2008). The mixture was incubated at room temperature in the dark for 45 min and the absorbance was read using a spectrophotometer (Bel Photonics 2000, Piracicaba, Brazil) at 517 nm. The result was expressed in µmol Trolox/g. Additionally, the EC_{50} (concentration required to obtain a 50% antioxidant effect) values were calculated by means of a linear regression between the concentration in µg/mL (axis of the abscissas) and the mean percentage of antioxidant activity (ordinal axis).

2.4 Ferric reducing antioxidant power (FRAP)

The FRAP was determined as described in Carpes et al. (2020). The reaction mixture was incubated for 30 min in water bath at 37ºC, and the absorbance was measured at 595 nm. Aqueous solutions of ferrous sulfate were used for calibration, and the results were expressed as µmol of Fe^{2+}/g.

2.5 Scavenging activity of $ABTS^{\bullet+}$

The ABTS (2,2'-Azino-bis (3-ethylbenzthiazoline-6-sulphonic acid)) method was performed as described by Carpes et al. (2020) in the LRE and LSE. The stock solutions included 7.4 mM $ABTS^{\bullet+}$ and 2.6 mM potassium persulfate. The solution was diluted by mixing 1 mL $ABTS^{\bullet+}$ solution with 60 mL ethanol to an absorbance of 0.70 ± 0.02 units at 734 nm on a spectrophotometer (Femto UV 2000, Brazil). Antioxidant activity was expressed in µmol/g of TEAC (Trolox-equivalent antioxidant capacity).

2.6 Coupled oxidation of β-carotene and linoleic acid assay

The measure of antioxidant activity was determined by the coupled oxidation of β-carotene and linoleic acid according to Carpes et al. (2008). Emulsion oxidation was spectrometrically monitored (Bel Photonics 2000, Piracicaba, Brazil) by measuring its absorbance at 470 nm, at time zero (t = 0) and subsequently after every 20 min, until the characteristic color of β-carotene disappeared in the control reaction (t = 100 min). The antioxidant activity was determined as percent inhibition relative to control sample.

2.7 Poultry pâté elaboration and oxidative stability of pâtés

The LRE and LSE were applied in poultry pâté to evaluate the effect at inhibition of lipid oxidation of the product, based on the preliminary studies with chicken pâtes (Carpes et al., 2020), chicken burguer (Pereira et al., 2017) and pork sausage (Bianchin et al., 2017). The pâté formulation was prepared with drumstick and leg (330 g/Kg), breast (80 g/Kg), chicken fat (246 g/Kg). The cuts of the thigh, drumstick, breast and fat were ground twice through a 5 mm plate at -2 ºC before the application of the other ingredients. 70% of this portion was cooked slowly at 60 ± 3 ºC, in order to soften the connective tissue. The raw and cooked pasta were mixed and then the remaining ingredients were added: ice water (274 g/Kg), salt (13 g/Kg), a mix of dehydrated garlic, onion, chives, parsley and pepper (4 g/Kg), isolated soy protein (6.0 g/Kg), cassava starch (40 g/Kg), sodium polyphosphate (5 g/Kg), and carmine (0.73 g/Kg). This basic formulation was divided into treatments. T1: was added 2 g/Kg of lyophilized rosemary extract (LRE); T2: was added 2 g/Kg of lyophilized sage extract (LSE); T3: was added

sodium erythorbate (SE) (0.2 g/kg), and the T4: no additional ingredients were included. Separately, the pâté lots were stored in sterile glasses, autoclaved and submitted to a baking process in water bath at 80°C for 30 min. After baking, the pâtés were cooled with ice and stored at 4 °C in a refrigerator during 28 days to evaluate the oxidative stability of the pâtés.

The oxidative stability of poultry pâtês was estimated by using 2-thiobarbituric acid reactive substances (TBARS) according to Carpes et al. (2020). Tetramethoxypropane (TMP) was used as standard reference and substances reacting with thiobarbituric acid were measured spectrophotometrically at 532 nm at times 0, 7, 14, 21, and 28 days of the storage. The results were expressed as mg of MDA/kg of sample (MDA: malondialdehyde). The lipid oxidation was assessed in triplicate.

2.8 Statistical analysis

Analysis of variance (ANOVA) was performed to analyze the data, and the means were compared by Tukey's test for TBARS values, using the Statistica 8.0 software (StatSoft, USA). The results were considered statistically significant when $p<0.05$. All tests were performed in triplicate.

3. RESULTS AND DISCUSSION

3.1 Total phenolic compounds, total flavonoids and antioxidant activity

The quantification of total phenolics and total flavonoids brings the perspective of how promising the sample's bioactive properties are, since they are related to antioxidant activity. In fact, phenolic compounds from fruits, vegetables and medicinal herbs are presumed to be a good source of antioxidants. The total phenolic compounds, total flavonoids and antioxidant activity present in the extracts of rosemary and sage are shown in Table 1. There was a statistical difference between the extracts for total phenolic compounds and total flavonoids ($p <0.05$). The rosemary extract have the highest content of phenolic compounds (46.48 mg EAG/ g of plant) and this result is in agreement with a previous study Bianchin et al. (2017) who found 45.67 mg EAG/g for lyophilized rosemary extract that was extracted with ethanol solution in a shaker at 40°C for 60 min. However, in this study, the sage extract showed superiority in the content of flavonoids (15.03 mg QE/ g of plant) in compared at lyophilized rosemary extract (11.89 mg QE/g). Our results showed that total phenolic content of lyophilized sage extract was lower than values reported by Kivrak et al. (2019), who found for total phenolic compounds 52.3 mg of pyrocatechol /g on methanolic extract of sage from Turkey. These differences between TPC and TFC amounts obtained in our study and that found in the literature can be attributed to the different methods of extraction, solvents and temperatures employed.

The antioxidant activity of these compounds may act at different stages in the oxidation process, lowering free radicals concentration, chelating ions and leading to non-radical compounds. The structure of the phenolic compound facilitate the electron donation of the hydroxyl portion to oxidizing radical species. Thus, the antioxidant activity of LRE and LSE was assessed by four distinct in-vitro methods, including: the free radical-scavenging ability by the use of ABTS, DPPH radical, ferric reducing antioxidant power (FRAP), and oxidation of β-carotene/linoleic acid. These spectrophotometric methods are complementary and have different mechanisms of action, which can depend on the reaction of an organic radical, a cation radical, or a complex with an antioxidant molecule capable of donating a hydrogen atom (Pisoschi and Negulescu, 2011). Besides, the absorbance can be measured to test the amount of iron reduced and can be correlated with the amount of antioxidants in several matrices. All this to be enabled to report complete information on the antioxidant capacity of these samples. Regarding the antioxidant activity by DPPH method expressed in EC_{50}, it's important to highlight that the EC_{50} value is inversely proportional to the antioxidant capacity of the compounds. Given all these considerations, the LRE and LSE showed antioxidant activities of 274.72 and 398.86 µg/mL, respectively. Based in our findings, the LRE showed antioxidant activities lower than reported by Bianchin et al. (2017), who found 127.33 µg/mL (EC_{50}) in extract of rosemary leaves. In addition Hamrouni-Sellami et al. (2013) found values of EC_{50} = 23.87 µg/mL for sage methanolic extract from Tunisia. Several studies evaluating the antioxidant activity of Salvia species can be found in the literature; although, each study presents a different methodology and expresses its results in many ways. Besides, many of these studies are with sage species originating from different regions where the soil and the climate interfere in the results.

Table 1: Total phenolic content, total flavonoid content and antioxidant activity measured by different methods of rosemary and sage extracts

Parameters	LRE	LSE
Total Phenolic Compound (mg GAE/g)	46.48 ± 0.08	41.61 ± 0.91
Total Flavonoid Content (mg QE/g)	11.89 ± 0.58	15.03 ± 0.44
EC_{50} (µg/mL)	254.72 ± 0.66	398.86 ± 0.10
AA %*	92.25± 0.46	79.81 ± 0.56
DPPH (µmol Trolox/g)	4745.72 ± 0.47	2462.82 ± 0.03
ABTS (µmol TEAC/g)	301.47 ± 0.76	182.89 ± 0.526
FRAP (µmol de Fe^{2+}/g)	180.09 ± 0.01	133.99 ± 0.68
β-carotene/linoleic (%)**	85.67 ± 0.66	89.29 ± 0.10

LRE: lyophilized rosemary extract; LSE: lyophilized sage extract; GAE: Gallic acid equivalent;
QE: Quercetin equivalent; EC50: Equivalent concentration; TEAC: Trolox-equivalent antioxidant capacity;
Values are presented as mean ± deviation (n = 3). *LRE and LSE at 0.33 mg/mL; ** LRE and LSE at 6.66 mg/mL.

The results obtained from DPPH expressed in Trolox, ABTS, FRAP and β-carotene/linoleic methods for LRE were 4745.72 µmol Trolox/g, 301.47 µmol TEAC/g, 180.09 µmol Fe^{2+}/g, and 85.67 %, respectively are shown in Table 1. With the exception of antioxidant activity by the β-carotene/linoleic acid model system, all these values of antioxidant activity for LRE were higher than those reported for sage.

3.2 Effect of LRE and LSE on lipid oxidation from poultry pâtês

The oxidative degradation of lipids is one of the main reactions that occur in food products and can be inhibited by the use of natural antioxidant in several kind of food products. Additionally, TBARS is generally used as an indicator of the degree of lipid oxidation that reflects the content of MDA formed during the oxidation of polyunsaturated fatty acids. The results obtained in the evaluation of lipid oxidation by TBARS in the four treatments are shown in Table 2.

Table 2. Average values of TBARS in poultry pâté with LRE and LSE during storage at 4 ºC.

Treatments	TBARS (mg of MDA/Kg of chicken pâté)				
	Storage times days				
	0	7	14	21	28
T1	0.98 ± 0.02^{Dd}	1.23 ± 0.04^{Cc}	1.87 ± 0.01^{Bb}	1.88 ± 0.02^{Bc}	2.23 ± 0.02^{Ac}
T2	1.18 ± 0.10^{Ec}	1.71 ± 0.04^{Db}	1.86 ± 0.01^{Cb}	2.37 ± 0.03^{Bb}	2.69 ± 0.02^{Ab}
T3	1.58 ± 0.04^{Eb}	1.73 ± 0.05^{Db}	2.00 ± 0.01^{Ca}	2.28 ± 0.03^{Bb}	2.60 ± 0.04^{Ab}
T4	1.66 ± 0.03^{Ea}	1.85 ± 0.02^{Da}	1.91 ± 0.03^{Ca}	2.87 ± 0.02^{Ba}	3.96 ± 0.30^{Aa}
Average	1.35 ± 0.05^{E}	1.63 ± 0.04^{D}	1.91 ± 0.02^{C}	2.35 ± 0.04^{B}	2.87 ± 0.06^{A}

Values are presented as mean ± deviation (n = 3). MDA: malondialdehyde. All measurement was carried out in triplicate.
T1: Treatment with LRE (added 0.2 g/Kg of lyophilized rosemary extract); T2: Treatment with LSE (added 2 g/Kg of lyophilized sage extract) T3: Treatment with sodium erythorbate (0.2 g/Kg); T4: Treatment control (no added antioxidant); Different lower-case letter in the same column indicate significant difference ($P < 0.05$) by Tukey's test. Different capital letters in the same row indicate significant difference ($P < 0.05$) by Tukey's test.

During the storage period of the pâtês, the TBARS values increased over time and ranged from 1.35 ± 0.05 at the beginning of the experiment at 2.87 ± 0.06 mg MDA/Kg pâté at the end of the experiment (28 days). Regarding to control treatment, the TBARS average values ranged of 1.66 at 3.96 mg MDA/Kg pâté and had significantly higher TBARS values ($p<0.05$) than other treatments. Besides, there wasn't a significant difference in the malonaldehyde values when compared to treatments with LSE and with sodium erythorbate (T2, T3) in processing day. Treatment of pâtês with LRE (T1) showed lower TBARS values during any day of storage than the LSE (T2), sodium erythorbate (T3) and control (T2) treated pâtês.

At the end of storage time (28 days), the poultry pâtês containing LRE (T1) and LSE (T2) inhibited respectively, 43.69% and 32.07% of lipid oxidation in comparison to the control pâtês (T4). In this point, the

treatment with LRE was better than treatment with synthetic antioxidant and the pâtês group with LSE wasn't significant difference ($p<0.05$) with the treatment with synthetic antioxidant (T3). These results indicate a strong anti-oxidative effect of lyophilized rosemary extract and sage extract in poultry pâtês, probably due to the gradual release of the bioactive compounds from these plants.

4. CONCLUSION

The present study represent a relevant contribution to the bioactivity knowledge of the rosemary and sage grown in Brazil, which are important sources of phenolic compounds with antioxidant activity. Therefore, these results suggested that these plants could be of great industrial importance and can support the development of natural additive with potential for application in the food technology. Besides, considering the interest in finding for natural antioxidants, it is possible to suggest that LRE and LSE have biological activity, mainly because of the presence of reducing compounds, free radical scavengers and hydrogen donors in the plant material. In fact, the LRE and LSE was more effective in the lipid oxidation inhibition in poultry pâtês than synthetic antioxidant, and these pâtês with rosemary and sage can be an alternative for consumer demand for healthy foods.

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