ANTIOXIDANT ACTIVITY OF *ILEX PARAGUARIENSIS* IN GROUNDED CHICKEN MEAT SUBMITTED TO THERMAL TREATMENT

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Introduction

Lipid oxidation is an important factor for meat and meat product quality since it can cause deterioration by changing the sensorial and nutritional value of meat products (TOKUSOGLU e BASMACIOGLU, 2004). Another factor to be considered is that the consumption of products that have suffered oxidative alterations cause countless health problems, being toxic to the cells, the liver, the kidneys, the cardiovascular system and also causing cancer and arteriosclerosis (FERRARI, 1999).

The use of synthetic antioxidants or controlling lipid oxidation can preserve meat quality. Studies have shown that synthetic antioxidants such as butylated hydroxy anisole (BHA) and butylated hydroxytoluene (BHT) have been implicated into toxic effects. Thus these findings together with consumer interests in natural food additives have reinforced the need for effective antioxidants from natural sources (MELO & GUERRA, 2002).

"Mate" is a plant that is cultivated in Argentina, Uruguay, Paraguay and Brazil. The aerial parts of this plant are used to prepare a drink called "mate" (FILIP et al., 2000). Experiments have investigated that diseases caused by oxidative reactions in biological systems can be decelerated by the ingestion of natural antioxidants found in diets, especially phenolic compounds. In this sense, there are reports of antioxidant effects of *llex paraguariensis* (GUGLIUCCI & STAHL, 1995; SCHINELLA et al. 2000).

The results found in some studies have shown that "mate" extracts present antioxidant properties in boneless poultry meat (MILANI et al. 2001; MILANI et al. 2002) and some meat products (TERRA et al. 2002; FURTADO et al. 2004).

The NaCl is added in foods for a variety of purposes, including flavor and inhibition of microorganisms. However, it has been shown that NaCl accelerates lipid oxidation in a variety of meats, such as beef, pork, chicken and fish (CHEN et al. 1984, SAKAI et al., 2004).

Objectives

The objective of this study was to investigate the antioxidant activity of hydro-ethanollic extract of “mate” in grounded chicken meat submitted to thermal treatment as well as to test its antioxidant activity in presence of salt.

Methodology

Hydro-ethanollic “mate” extract: The hydro-ethanollic “mate” extract was donated by GERMINAL (Company of SP-International Speciality Products Group).

Chicken meat samples: Thigh and leg boneless, skinless chicken meat were used by grinding in a 5 mm disc. Four equal portions were separated to correspond to four treatments as: control (without salt or antioxidant), treatment 1 (addition of 0,5% of hydro-ethanollic “mate” (*Ilex paraguariensis*) extract, treatment 2 (addition of 2% of sodium chloride) and treatment 3 (addition of 2% of sodium chloride plus 0.5% of hydro-ethanollic “mate” extract). All portions, after homogenization, were molded as hamburger patties and thermically treated at 75 C/20minutes. Later, the samples were packaged in plastic bags and stored at 4C for 10 days.

Microbiological analysis: mesophyllic microorganism counts were done using plate count agar (PCA) and incubated at 36C/48 hours (BRASIL, 2003).

Thiobarbituric acid-reactive substances (TBARS) assay: The general extension of lipidic oxidation was determined by the TBARS content (RAHARJO et al., 1992) and expressed as mg of malonaldehyde per kg of sample.

pH assay: To the pH determination, 10 grams of sample were homogenized with 100 mL of distilled water (TERRA e BRUM, 1998).

Sensorial analysis: Hedonic scale with five points was used to evaluate the cooked samples in a conventional oven (ANSALDUA-MORALES, 1994).

Statistical analysis: The mean values were subjected to analysis of variance (ANOVA) and Tukey´s test was applied when appropriated at statistical significance of 5% level (COSTA NETO, 1977).

Results & Discussion

Figure 1 shows the variation of the TBARS values during storage period of the grounded chicken meat samples submitted to 75°C/20 minutes. The addition of 2% of salt accelerated the lipid oxidation of the samples showing TBARS values significantly higher than the control samples during the analyzed period. At the end of this period, it was observed that the addition of 2% of NaCl presented TBA value higher than 3.0 while the control samples obtained approximately a value of 0.8 mg of malonaldehyde/kg sample.

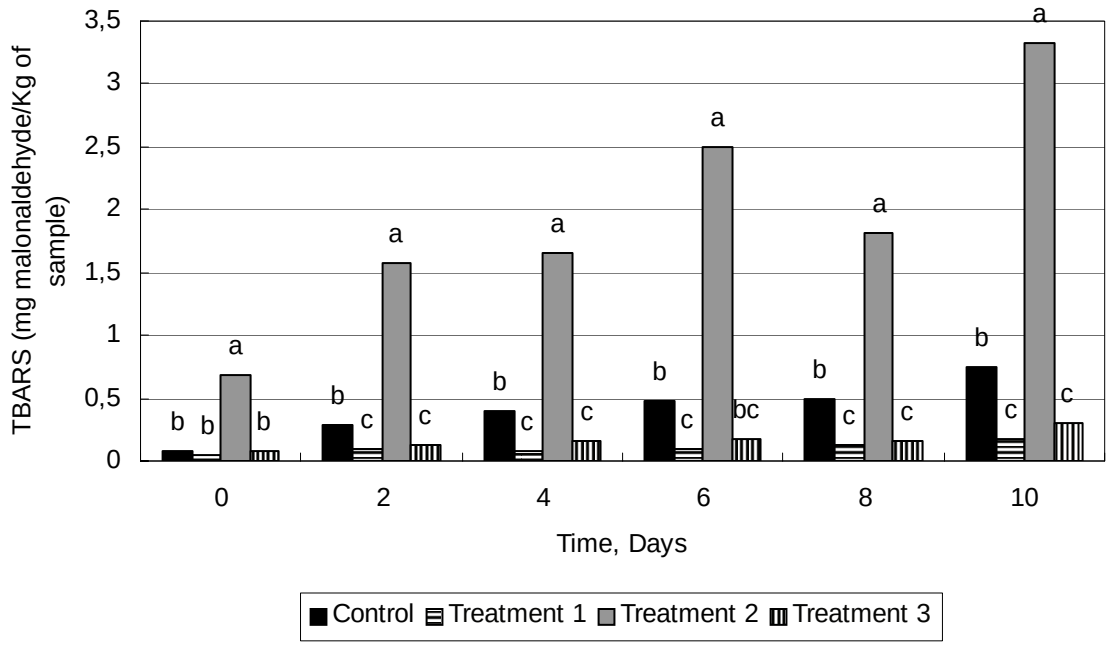

FIGURE 1: Antioxidant activity of grounded and cooked chicken meat control treated with hydro-ethanollic extracts, 2% of salt or a combination of both during days of storage at 4°C.

The pro-oxidant activity of salt is well known (CHEN et al. 1984; SAKAI et al., 2004). According to BELTRAN et al. (2004) sodium chloride is an important meat pro-oxidant for several reasons such as: ionic iron release, damaging of the cell membranes and decrease in reduction of antioxidant enzymes activity. Also cooking has been found to be a pro-oxidant treatment and if NaCl is present it releases the ionic iron from the heme pigment facilitating lipid oxidation (HALLIWELL e GUTTERIDGE, 1984).

AGUIRREZÁBAL et al. (2000) investigated that salt modified lipid peroxydation in sausage, especially in the absence of paprika. It was also noted that the values of reactive substances with the tiobarbituric acid (TBA) did not increase during the ripening of the sausage samples that contained paprika.

CHEN et al (1984) studied the effects of salt alone and with a few antioxidants on raw and cooked beef. These researchers also observed that NaCl accelerates lipid oxidation. In using both Tenox 4-coated salt and the mixture of BHT and the BHA with salt completely inhibited lipid oxidation in cooked meat during cooking and storage, although the tocopherol-coated salt did not control lipid oxidation during the storage after cooking.

BELTRAN et al. (2004) used 5% of sodium chloride and rosemary and acerola extracts to minimize rancidity in pressurized and cooked chicken slurries. Rosemary extracts showed as an antioxidant for pressure-treated samples, although had little effect on cooked ones while the acerola extract had none antioxidant effect.

In this experiment, the hydro-ethanollic “mate” extract showed antioxidant activity since the samples showed TBA values significantly lower than the control after 2 days of

storage (Figure 1), maintaining TBA values under 0.5 until the end of the analyzed period. In the presence of NaCl the hydro-ethanollic extract of “mate” (treatment 3) maintained its antioxidant activity, obtaining TBA values very close to the ones obtained in the samples treated with only the hydro-ethanollic extract of “mate” (Figure 1). Thus it was observed that even after the samples have received thermal treatment and in the presence of sodium chloride the hydro-ethanollic “mate” extract preserved its antioxidant activity showing its great antioxidant potential.

Others authors also observed the effect of different “mate” extracts. GUCLIUCCI and STAHL (1995) observed that aqueous and alcoholic “mate” extracts showed antioxidant capacity *in vivo*, protecting low density lipoprotein against oxidation. SCHINELLA et al. (2000) confirmed the antioxidant properties of “mate” extracts as inhibitor of lipid peroxydation when rat liver microssomes were used.

MILANI et al. (2002) studied the antioxidant effect of hydro-ethanollic and methyllic extracts of apple peel, artichoke leaves and methyllic “mate” extracts in mechanically deboned chicken meat (MDCM) maintained under refrigeration and freezing. It was observed that the methyllic extract of “mate” presented higher antioxidant effect when compared to the other extracts tested. After a month of storage the samples under refrigeration presented a 7.95 TBA level while the sample treated with methyllic extract showed a 1.68 value.

Antioxidant activity of hydro-ethanollic of “mate” extract is most likely related to the presence of phenolic compounds. FILIP et al. (2000) tested the presence of antioxidants in “mate” extracts of six natural and two commercial species. All the species presented higher antioxidant activity. TERRA et al. (2002) added BHA and the hydro-ethanollic “mate” extract to 0.5% and 1% in Italian type sausage to inhibited rancidity. It was observed that in the treatments with the addition of “mate” extract there was a protection against lipid oxidation comparable to the BHA. The treatments with 0.5% “mate” extract and BHA obtained the best sensorial results when compared to the treatments with the 1% “mate” extracts and control. The analysts considered the typical aroma of “mate” and rated the 1% “mate” extract samples as the worst result.

Table 1 shows that the hydro-ethanollic 0.5% “mate” extract did not alter significantly the sensorial characteristics in the analyzed samples. The NaCl treated samples showed higher values to flavor, color, odor, texture and for the acceptability.

Salt addition not only adds a salty taste in the final product but also favors the solubilization of muscle proteins that in turn allows gel formation and development of an optimum texture (ANDRÉS, 2004), which could explain the higher acceptability of the salt-treated samples comparing to the others.

The pH affects chemical and biochemical reactions and microorganisms development. The sample pH varied from 6.4 to 7.0 (Table 2). The samples began with pH levels superior to 6.4, which most likely favored microbial development. According to PRICE and SCHWEIGERT (1971) meats that have pH 6.5 are modified bacteriologically faster than meats with pH 5.3.

TABLE 1 - Sensorial analysis of grounded and cooked chicken meat control and treated samples with hydro-ethanollic extracts, 2% of salt or a combination of both during 10 days of storage at 4°C

Treatments	Storage Period (days)		
	0	**5**	**10**
COLOR			
Control	6.72[b]	8.15[a]	6.47[a]
Treatment 1	6.77[b]	7.45[a]	4.77[a]
Treatment 2	8.52[a]	8.05[a]	7.28[a]
Treatment 3	7.92[ab]	4.84[b]	6.21[a]
ODOR			
Control	7.65[a]	5.22[a]	4.38[a]
Treatment 1	7.58[a]	4.31[a]	3.88[a]
Treatment 2	8.75[a]	6.07[a]	5.43[a]
Treatment 3	8.41[a]	6.56[a]	4.82[a]
TASTE			
Control	6.89[b]	nd	nd
Treatment 1	5.88[b]	nd	nd
Treatment 2	8.78[a]	nd	nd
Treatment 3	8.15[ab]	nd	nd
TEXTURE			
Control	6.80[b]	nd	nd
Treatment 1	6.99[b]	nd	nd
Treatment 2	8.80[a]	nd	nd
Treatment 3	8.50[ab]	nd	nd
ACCEPTABILITY			
Control	6.15[b]	nd	nd
Treatment 1	6.18[b]	nd	nd
Treatment 2	8.18[a]	nd	nd
Tretment 3	7.84[ab]	nd	nd

Mean score in the same column which are not followed by the same lower case letter are significantly different ($p<0,05$).

Control:did not contain any salt or antioxidant, treatment 1: addition of 0,5% of hydro-ethanollic extract of “mate”; treatment 2: addition of 2% of sodium chloride and treatment 3: addition of 2% of sodium chloride and 0,5% of hydro-ethanollic extract of “mate”.

Figure 2 shows the mesophyllic aerobic microorganism counts of samples during the storage period at 4ºC. It can be seen that the control samples and the ones that received “mate” extracts treatment obtained a very close microbial development, increasing in both samples during the storage period. The observed results agree with antioxidant and antimicrobial effects of methanollic and ethanollic green tea, black tea and “mate” extracts in MDCM studies of MILANI et al. (2001).

TABLE 2. pH values of grounded and cooked chicken meat control and treated samples with hydro-ethanollic extracts, 2% of salt or a combination of both during 10 days of storage at 4°C

Treatments	Storage Period (days)					
	0	2	4	6	8	10
pH						
Control	6.6[a]	6.6[a]	6.7[a]	6.7[a]	6.7[a]	6.8[b]
Treatment 1	6.6[a]	6.5[a]	6.7[a]	6.6[a]	6.6[b]	6.6[d]
Treatment 2	6.4[b]	6.5[a]	6.6[b]	6.6[a]	6.6[b]	7.0[a]
Treatment 3	6.5[ab]	6.5[a]	6.6[b]	6.6[a]	6.6[b]	6.8[b]

Means scores in the same column which are not followed by the same lower case letter are significantly different (P<0.05).

Control:did not contain any salt or antioxidant, treatment 1: addition of 0,5% of hydro-ethanollic extract of “mate”; treatment 2: addition of 2% of sodium chloride and treatment 3: addition of 2% of sodium chloride and 0,5% of hydro-ethanollic extract of “mate”.

FURTADO et al. (2004) while studying antimicrobial activity of Achyrocline satureioides extract in sausage also utilized hydro-ethanollic extract of “mate”. It was observed that this extract did not present antimicrobial activity on the total aerobic microorganisms.

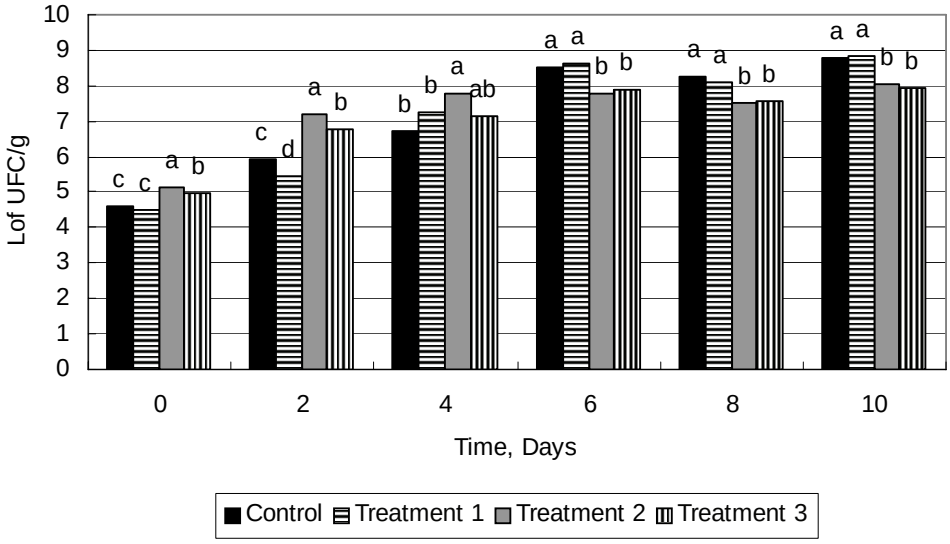

Figure 2. Mesophyllic aerobic microorganisms of grounded and cooked chicken meat control and treated samples with hydro-ethanollic extracts, 2% of salt or a combination of both during 10 days of storage at 4°c.

Conclusions

The hydro-ethanollic “mate” extract presented antioxidant activity in grounded chicken meat submitted to cooking at 75C/20 minutes and in the presence of salt. The 0.5% of hydro-ethanollic “mate” extract did not show antimicrobial activity and did not affect sensorial characteristics of the samples.

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