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*Research Article*

# The Antioxidant Capacity of Rosemary and Green Tea Extracts to Replace the Carcinogenic Antioxidant (BHA) in Chicken Burgers

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The present study aimed to evaluate the effect of natural extracts (rosemary and green tea extracts) in frozen storage of chicken burgers. Chicken burger treatments were prepared as follows: control (CON), 20 mg BHA/kg (BHA20), 10 mg green tea extract/kg (GT10), 38 mg green tea extract/kg (GT38), 18.6 mg rosemary extract/kg (RO18), and 480 mg rosemary extract/kg (RO480). Analysis of physicochemical parameters, color, TBAR index, and sensory acceptance were performed at 0, 30, 60, and 120 days of storage at −18°C in burgers packaged in LDPE plastic bags. The addition of natural antioxidants did not affect ($p > 0.05$) the color and physicochemical parameters of the chicken burgers. After 120 days at −18°C, the RO480 sample showed a TBAR index similar ($p > 0.05$) to BHA20 (0.423 and 0.369 mg, resp.). Sensory acceptance did not differ ($p > 0.05$) among the treatments throughout the storage period ($p > 0.05$).

## 1. Introduction

Synthetic food additives, such as butyl hydroxyl anisole (BHA), are normally used by the food industry in order to control lipid oxidation, although these compounds are considered toxic to human health [1–3]. At the present time, much investigative research is being carried out to replace carcinogenic antioxidants, mainly in processed meat. A recent study suggested that processed meats could be carcinogenic [4] or that chemical contaminants could be added to meat products [5]. Kumar et al. [6] confirmed this in the review "Toxicological and Carcinogenic Effects of Synthetic Antioxidants." For this reason, natural extracts with antioxidant potential can be a valuable alternative to synthetic compounds.

Herbs and spices such as rosemary and green tea are effective protectors against oxidation due to their antioxidant capacity [7]. The ability to inhibit oxidation is associated with the chemical structure of phenolic compounds that are similar to chemical antioxidants. Efficiency of natural extracts in food systems depends on factors such as the chemical reactivity of their constituents, extraction procedure, and interaction with food components [8]. The main studies about plant extracts are related to quantification of antioxidant compounds in order to identify the potential in antioxidant mechanisms [7]. In general, plants, herbs,

Table 1: Antioxidant capacity values according to the different methodologies.

| Sample | Folin-Ciocalteu (mg GAE/g) | FRAP ($\mu$mol Trolox/g) | $EC_{50}$ (mg/L) |
|---|---|---|---|
| BHA | 1476.67 ± 33.00[a] | 3327.32 ± 202.15[a] | 24.13 ± 0.018[a] |
| Rosemary extract (4.4% phenolics) | 114.50 ± 0.24[b] | 140.88 ± 4.08[b] | 22.46 ± 0.025[a] |
| Pure rosemary extract (supposed value) | 2602.27 | 3201.82 | 0.98 |
| Green tea extract (20% diterpenes) | 1497.97 ± 19.88[a] | 1757.96 ± 47.14[c] | 11.70 ± 0.017[b] |
| Pure green tea extract (supposed value) | 7489.85 | 8789.80 | 2.34 |

Results are expressed as mean value ± standard deviation ($n = 3$). Different letters indicate significant difference (estimated by ANOVA analysis and Tukey's test, $p \leq 0.05$).

and fruits are known to contain a wide variety of phytochemicals, such as polyphenols, carotenoids, flavonoids, and catechins [9]. These products could be natural antioxidants because the compounds could scavenge free radicals and provide oxidative stability to many food items including high fat meat products [10]. The mechanism involved in the antioxidant activity of either natural or synthetic antioxidants is dependent on molecular structure. Additional galloyl, catechoyl, or hydroxyl groups in phenolic compounds have been associated with an increase in antioxidant activity [11].

There are now several methods of quantifying the antioxidant activity of natural extracts but none of them are considered official because the matrix of each method presents different reaction system and complexity; for example, the total polyphenol method is based on the Folin-Ciocalteu reagent, and the FRAP assay is based on the reduction of ferric ions under acidic conditions. These studies have evaluated only the antioxidant activity without concern as to whether it represents the same performance in the food matrix, for example, meat products. Meat is susceptible to oxidation; in particular, chicken meat is more susceptible than other meats due to having more unsaturation lipids in its lipid structure [12–14].

Therefore, the aim of this study was to evaluate potential replacements of BHA (synthetic antioxidant) in frozen chicken burgers. For this purpose, the antioxidant potential of all the antioxidants tested (natural and synthetic) was determined and the concentration of natural antioxidants was also defined from these results. The color and physical-chemical and lipid stability of the chicken burgers were also evaluated.

## 2. Material and Methods

*2.1. Chemicals and Raw Material.* The synthetic and natural antioxidants employed in the evaluation were obtained from Dupont™ Danisco, Brazil [BHA pure synthetic antioxidant, rosemary extract (*Rosmarinus officinalis* L.) containing 4.4% phenolic diterpenes and green tea extract (*Camellia sinensis*) containing 20% of catechins]. Chicken meat and spices were purchased from the local market.

*2.2. Estimation of Total Polyphenol Content.* The activity of the antioxidants was assessed by analysis of reducing power measured with the Folin-Ciocalteu reagent described by Singleton and Rossi [15] and Georgé et al. [16] and evaluated at 760 nm against a blank in absence of extract in a spectrophotometer SP-22 (Biospectro, Brazil). The values were expressed as milligram of gallic acid equivalent per gram of antioxidant (mg GAE/g).

*2.3. Ferric Reducing Antioxidant Power (FRAP) Assay.* The FRAP method was based on the reduction of the ferric ion ($Fe^{3+}$) to ferrous ion ($Fe^{2+}$) under acidic conditions [17]. It was quantified at 593 nm after 30 min. and expressed as $\mu$mol Trolox equivalent per gram of antioxidant (TE/g).

*2.4. Determination of Antioxidant Capacity: Free Radical Scavenging Using the DPPH Radical.* The free radical scavenging capacity of antioxidants was measured using stable $DPPH^{\bullet}$ as previously described [18]. The absorbance (Abs) was measured at 515 nm until the end point time, approximately 3 hours, determined in studies to prove the sample's stability [19]. The percentage of DPPH inhibition was calculated according to the formula: DPPH inhibition (%) = [(Abs control − Abs sample)/Abs control] × 100 and results were expressed in $EC_{50}$ (mg/L of antioxidant).

*2.5. Manufacture of Chicken Patties and Sampling Procedures.* All batches of chicken burgers were prepared using the same formulation: 75% of chicken breast, 20% of chicken skin (both minced in a 3 mm plate using a meat mincer), 1.23% condiments (salt and white pepper), and water and antioxidant according to each treatment. The samples were packaged in low density polyethylene (LDPE) plastic bags and stored at −18 ± 1°C for 4 months.

Four different dosages of the natural antioxidants were determined according to the results of antioxidant capacity analyses (FRAP and DPPH) (Table 1) based on the maximum permitted level of BHA according to Brazilian legislation (100 mg/kg) regarding fat content in the meat product, therefore, 20 mg/kg BHA [20] (taking 20% chicken skin into account). Dosages of natural extracts were calculated according to the following example: in the analysis of green tea extract by the FRAP method, the result was 1757.96 $\mu$mol TE/g, whereas the BHA was 3327.32 $\mu$mol Trolox/g. The following inverse rule of three was then considered: (1757.96/3327.32) = (100%/$x$), $x$ = 189.27%. From this, it was determined that to obtain the same capacity of the antioxidant BHA, the green tea extract should be dosed *at* 89.27% more than the synthetic. That is, 89.27% × 0.002 (BHA concentration) = 0.0038% (38 mg/kg). The concentration of green tea extract was also determined considering the DPPH radical assay following a similar calculation

applied to the FRAP assay which resulted in 10 mg/kg. Rosemary extract concentrations based on the FRAP and DPPH radical assays were also calculated and indicated 480 and 18.6 mg/kg, respectively. However, once the dosages prescribed by the Folin-Ciocalteu method showed intermediate values between the FRAP and DPPH, they were disregarded. After determination of the antioxidant dosages, the following six treatments were assigned: control (without antioxidant), BHA20 (20 mg BHA/kg), GT38 (38 mg green tea extract/kg), GT10 (10 mg green tea extract/kg), RO480 (480 mg rosemary extract/kg), and RO18 (18.6 mg rosemary/kg).

*2.6. Proximate Composition, pH, and Color of Burgers.* The proximate composition of uncooked burgers was analyzed as follows: the moisture content was determined by drying in an oven at a temperature of 105°C for about 24 hours until constant weight was obtained (950.46 [21]). The amount of ash and mineral content was determined with the residue obtained in moisture and placed in an oven at 550°C for approximately 96 hours at constant weight (AOAC 920 153 [21]). The protein determination was performed according to the Kjeldahl method, according to AOAC 981.10 [21]. Lipid content was determined according to Bligh and Dyer [22].

Value of pH was determined by pHmetro Hanna and meat color measurements were made using a colorimeter (mod. MiniScan XE, Hunterlab brand) in which the equipment was calibrated with a standard white and another black pattern in the CIE system. Evaluating measures absolute coordinates of brightness ($L^*$), red color ($a^*$), and yellow ($b^*$).

*2.7. Evaluation of Oxidative Stability and Sensory Analysis.* Stability of the burgers during the frozen storage (−18 ± 1°C) was monitored at zero, 30, 60, 90, and 120 days, respectively, applying the thiobarbituric acid-reactive substances (TBARS) method and sensory evaluation. TBAR assay was performed as described by Vyncke [23]. Absorbance was read at 538 nm and values are expressed as mg of TBARS per kg of meat (TBAR index). For sensory evaluation, the burgers were cooked on an electric griddle (Croydon, GRSE 20665-6, Brazil) at 165°C for 4 minutes on each side, until internal center temperature reached 90°C, as measured by a thermocouple. Samples were evaluated by 60 regular chicken burger consumers, for "general acceptability" using a nine-point hedonic scale, varying from "1 = dislike extremely" to "9 = like extremely," according to Meilgaard et al. [24].

*2.8. Statistical Analysis.* Experimental data were analyzed using repeated measures ANOVA ($p \leq 0.05$), considering the repeated measures factor and the five levels of storage time. The comparisons of treatment averages and storage time averages were performed using the Tukey HSD test ($p \leq 0.05$). Two replicates were performed for each treatment at each storage time. All statistical analyses were performed using the Statistica™ software (Statsoft Inc., Tulsa, OK).

## 3. Results

*3.1. Proximate Composition, pH, and Color.* In all treatments, moisture, fat, and protein content met the standards set by current Brazilian legislation for this type of meat product [25] which should not exceed 23% of lipids and contain at least 15% protein. The average of results was moisture 68.99 ± 0.66%, protein 18.53 ± 0.20%, fat 9.07 ± 0.31%, and ash 2.21 ± 0.07%.

The pH values did not differ ($p > 0.05$) among all samples and were close to 6.00, indicating that the pH samples were not influenced by the addition of different antioxidants. Similar values were found by Trindade et al. [26] in MSM (mechanically separated meat) chicken with antioxidants during frozen storage.

ANOVA results for the $L^*$, $a^*$, and $b^*$ parameters did not differ significantly, neither between treatments nor over time ($p > 0.05$). The average $L^*$ values were between 45.91 and 51.14 and the $b^*$ values between 4.88 and 7.00. The $L^*$ values observed in the present study were in accordance with the results obtained by Yogesh and Ali [10]. These authors studied the effect of *Thuja occidentalis* and *Prunus persica* natural antioxidants in ground chicken meat and found $L^*$ values around 48.2. Concerning $b^*$, the researchers obtained a different value around 20.0, which could be because of the type and quality of the raw meat material and the country of production. For the $a^*$ parameter, the values are close to zero, indicating that chicken burgers present a less intense red color compared with other meats, which would be expected.

*3.2. Antioxidant Capacity.* There was no significant difference in antioxidant capacity between green tea and BHA evaluated by the Folin-Ciocalteu method (Table 1). However, rosemary presented a lower value ($p \leq 0.05$) compared with other antioxidants. A possible explanation for this behavior is the quantity of phenols contained in each extract and their different antioxidant mechanisms. Erkan et al. [27] analyzed rosemary extract and found that it contained 6% carnosic and 8% rosmarinic acids. They affirmed that there are different phenolic diterpenes in rosemary extracts and that these components could explain the antioxidant activity of natural extracts.

Values from the FRAP assay indicated that the three antioxidants have different antioxidant capacities ($p \leq 0.05$), with higher values for BHA and then the other two natural extracts, with rosemary the lowest. High value of antioxidant capacity of BHA was also reported by Hossain et al. [28].

DPPH$^\bullet$ values of rosemary and BHA (expressed as EC50 on Table 1) were superior to green tea ($p \leq 0.05$) which therefore showed the lowest antioxidant capacity, for the DPPH$^\bullet$ radical assay is based on the decrease of DPPH$^\bullet$ radical absorbance. A possible explanation for this is that, in this present study, 3 hours was necessary to reach an absorbance plateau for extracts exposed to light and heat in a bath at 25°C, which may have affected the result since some extracts are more sensitive to these conditions than others [29].

Similar $EC_{50}$ values of BHA were observed by Bubonja-Sonje et al. [30], and Duarte-Almeida et al. [31] obtained 28.20 mg/L and 25.00 mg/L, respectively.

In fact, the antioxidant activity of different extracts is directly related to the concentration of active components, which, in this present study, was 4% in the rosemary extract and 20% in the green tea extract. Thus, if the antioxidant

Table 2: Results of sensory acceptance of chicken burgers during storage period.

| Treatments | Storage time (days) | | | | |
|---|---|---|---|---|---|
| | 0 | 30 | 60 | 90 | 120 |
| Control | 7.0 ± 1.47 | 6.6 ± 1.55 | 7.0 ± 1.38 | 7.0 ± 1.52 | 6.8 ± 1.36 |
| BHA20 | 7.2 ± 1.43 | 6.8 ± 1.47 | 6.8 ± 1.51 | 6.6 ± 1.43 | 6.8 ± 1.50 |
| GT38 | 7.0 ± 1.55 | 6.9 ± 1.53 | 7.1 ± 1.22 | 6.7 ± 1.19 | 6.7 ± 1.48 |
| GT10 | 7.2 ± 1.25 | 6.7 ± 1.40 | 6.8 ± 1.21 | 6.7 ± 1.32 | 6.5 ± 1.61 |
| RO480 | 6.7 ± 1.34 | 6.6 ± 1.64 | 7.2 ± 1.18 | 6.7 ± 1.47 | 6.9 ± 1.14 |
| RO18 | 7.1 ± 1.34 | 7.0 ± 1.39 | 6.8 ± 1.35 | 6.8 ± 1.32 | 6.9 ± 1.34 |

Results are expressed as mean value ± standard deviation. Averages showed no difference ($p \geq 0.05$) by ANOVA test.

potential of both extracts is evaluated and compared with the pure synthetic antioxidant (100%), we can assume that the natural extracts have a higher antioxidant activity than the synthetic, as shown in Table 1 (supposed values for pure extracts). According to Wojdyło et al. [32], the antioxidant potential of phenolics depends on a number of factors, such as their skeleton structure and pattern of functional groups on this skeleton. To extract the total phenolics of natural substrates is critical due to phenolic derivatives, because polyphenols constitute one of the most numerous groups of phenolic structures and the chemical diversity of antioxidants makes it difficult to extract [33].

From this point of view, Soobrattee et al. [33] affirmed that it is critical to evaluate antioxidant activity only *in vitro*. They confirmed that it is essential to evaluate the behavior of antioxidants at different points. In this present study the antioxidants were evaluated in terms of potential interacting with a specific target molecule (diluted in an aqueous compound) and applied directly in food structure (burger application). The phenolic compounds may interact with free radicals to delay lipid oxidation which are generated in the initiation phase, propagation phase, or during the breakdown of the hydroperoxides [6, 33].

*3.3. Oxidative Stability during Frozen Storage.* Regarding lipid oxidation determined by the TBARS method, it was observed that there were significant effects of treatment and storage time, showing differences between treatments during storage time ($p \leq 0.05$).

Initial TBAR values (zero time) showed no differences ($p \geq 0.05$) between any of the samples (Figure 1). Samples BHT20 and RO480 were more stable in terms of lipid oxidation level over time. In spite of that control, RO18, GT10, and GT38 showed increased lipid oxidation level during storage time, insofar as the control test showed an increase of 65% in the TBAR index at 120 days. However, the burgers applied with a higher dosage of green tea extract also presented a difference from the control ($p \leq 0.05$) after 120 days of storage, showing that the higher experimental dosages were effective in order to control the lipid oxidation of samples. These results show the greater effectiveness of rosemary in relation to green tea, since the rosemary extract showed the same efficacy as BHA treatment.

In this present study the TBAR index showed values lower than 1.0, which is extremely important, because high levels of TBAR are toxic, carcinogenic, and mutagenic [34].

*3.4. Sensory Stability.* Regarding sensory evaluation, the ANOVA results for sensory data indicated that the effect of treatments and storage time were not significant ($p > 0.05$), showing that the addition of natural extracts (rosemary and green tea) at any tested concentration did not affect consumers' sensory acceptance of the chicken burgers (Table 2). O'Neill et al. [35] argue that the rancid flavors in meat are initially detected by assessors in amounts from 0.5 to 2.0 on the TBAR index, which could be a possible explanation for good acceptability in this present study.

*3.5. Comparison of TBAR x Antioxidant Capacity Evaluation.* Antioxidant extracts were applied in two different concentrations in the burgers, according to the antioxidant capacity analyses of FRAP and DPPH. The concentrations of natural extracts based on the Folin-Ciocalteu method results were not applied to the samples because the results obtained by this method showed intermediate values, between the two other methods, and the number of samples would be excessive to perform oxidation analyses. Calculations were carried out in order to determine whether natural extracts have the same antioxidant capacity as BHA, aiming at the same performance of the natural extracts in relation to BHA in the oxidative stability of chicken burgers. However, this behavior was not observed for all dosages applied. For this reason, it can be concluded that the method to determine the antioxidant capacity is a key factor in determining the dosage of natural antioxidants to replace synthetic antioxidants. Results obtained in this experiment demonstrated that dosages of natural extracts added to chicken burgers as determined by the FRAP method produced a TBAR index quite similar to products with BHA20 mg/Kg. This can be better observed in Figure 1 mainly for BHA20 and RO480 treatments, which presented a similar pattern throughout the frozen storage period. The three methods used in this study (Folin-Ciocalteu, FRAP, and DPPH) involve electron transfer reaction, which is a reaction involved in the impairment of oxidative reactions. It is worth noting that these methods have different arrays and can directly influence the result of the

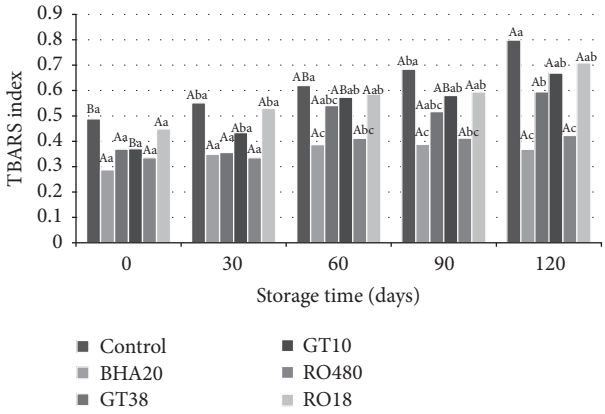


Figure 1: TBARS index mean values of chicken burgers treatments during storage period. Each point represents the mean value ± standard deviation ($n = 3$). For each treatment, averages followed by different capital letters differ significantly ($p \leq 0.05$) during storage time (see the same treatment during different storage times) and for each storage time, averages followed by different lowercase letters differ significantly per treatment (see the different treatments in the same storage time) ($p \leq 0.05$) by the Tukey HSD test.

analysis and, therefore, must be dosed in the correct quantity in the final product [36].

According to Huang et al. [7], there is great interest in research to define a convenient method to quantify antioxidant effectiveness. In fact, to measure antioxidant activity in model systems presents several problems to be extrapolated in food products, such as prooxidant effects, the mincing of meat and fat, the presence of salt, and long storage time, versus antioxidant effects, the presence of antioxidant compounds (phenolics), reduced temperature during storage, and polar paradox. The researchers suggested that a general protocol should test various oxidation conditions and compare antioxidants at the same molar concentrations as active components.

There seems to be no consensus of opinion, most probably due to the complexity of the composition of foods and different phenolic compounds. Studying this individually could be costly and inefficient. In fact, the area of antioxidant compounds in a food mixture is an extremely complex topic.

## 4. Conclusion

In conclusion, under the conditions evaluated in this study, commercial rosemary extract can replace the synthetic antioxidant BHA in the proportion of 20 mg/kg to rosemary at 480 mg/kg in chicken burgers, assuring its stability during the 4 months of frozen storage, providing a healthier and cleaner *label*, without changing the sensory acceptance of the product.

## Additional Points

*Practical Applications.* Concerns about the negative health effects of synthetic antioxidants, like BHA and BHT, widely used by the meat processing industry, have led to research in the food industry seeking alternatives. Natural extracts rich in phenolic compounds from sources already present in the diet are thought to have a central role in this trend. In our study, the effects of two promising natural extracts, rosemary and green tea natural extracts, were studied at two levels in chicken burgers frozen for 120 days. Results pointed to rosemary extract at 480 mg/kg as a commendable alternative for future industrial applications due to the similar protective effect against lipid oxidation compared to BHA (20 mg/kg).

## Competing Interests

The authors declare that they have no competing interests.

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