GUAVA AND BEETROOT RESIDUES EXTRACTS AS NATURAL ANTIOXIDANTS IN COOKED CHICKEN MEAT STORED IN AEROBIC AND VACUUM PACKAGING

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Abstract **- The aim of this study was to evaluate the effect of beetroot stems extract (BE) and guava pomace extract (seeds and peels) (GE) on lipid oxidation, instrumental colour (CIELAB) and pH of cooked processed chicken meat stored in vacuum and aerobic packages at 4±1°C for 14 days. BE and GE were effective to retard the lipid oxidation compared to the control in aerobic packages, and proved to be as effective as BHT in both aerobic and vacuum packages. No changes in pH, L* and b* values were found between samples in both packages. Nonetheless, in aerobic packages the addition of both natural extracts reduced the intensity of the red color of samples and it was observed a decrease in a* values during the storage time.**

Key Words - agro-industrial residues, natural antioxidants, lipid oxidation

I. INTRODUCTION

Agro-industrial activities generate millions of tons of residues all over the world and since most of them have no specific destination, they may be inappropriately disposed and cause damage to the environment. However, many of them are rich in bioactive compounds, including antioxidants, substances capable of preventing oxidative damage caused by free radicals [1].

During beetroot processing, a great amount of residues is generated, such as stems, leaves and peels. This vegetable is the main source of betalaines, natural water-soluble pigments, that are important antioxidant substances in human diet [2]. In addition to betalains, both the roots and the peels of beetroot contain phenolic compounds, such as ferulic acid, phenolic amides, and flavonoids [3].

The residues generated by guava processing industry are composed of seeds and residual pulp. Red guava presents high levels of antioxidant compounds, such as vitamin C [4], the carotenoids licopen and β-caroten [5], and phenolic compounds, such as myricetin and apigenin [6] The skin of this fruit has a considerable amount of ascorbic acid [7] and the seeds contain phenolic compounds and flavonoids [8].

Concerns about synthetic antioxidants safety and toxicity, together with the consumer demand for natural and healthy products, have aroused particular interest in the use of natural antioxidants in the food industry, principally those found in plants [9] and agro-industrial by-products [10].

As chicken meat is relatively rich in unsaturated fatty acids [11], it is more vulnerable to lipid oxidation, which affects the physicochemical parameters flavor, odor, color, and texture, and it is also responsible for the degradation of liposoluble vitamins and essential fatty acids [12]. Because of this, the use of antioxidants in this kind of products is extremely important in order to prevent or delay the onset of lipid oxidation, avoiding the loss of product quality.

The present study aimed evaluate the effect of beetroot stem (BE) and guava pomace (GE) extracts on lipid oxidation, instrumental color and pH of cooked processed chicken meat stored in vacuum and aerobic packages at 4±1°C for 14 days.

II. MATERIALS AND METHODS

A. A. Preparation of grape extracts: Beetroot stems and guava pomace (peels and seeds) were frozen, lyophilized and grounded in an analytical mill. The extracts of both residues were prepared in triplicate, using 2 g of lyophilized residue and 20 mL of ethanol/water (80/20 v/v). Extraction was carried out in ultrasound equipment at room temperature, for 15 min. After that, the extracts were centrifuged at 5000 x g for 15 min and a part of the supernatant, was concentrated in rotary evaporator under vacuum, at 37°C, up to complete solvent removal. The extracts were then redissolved with water to a final volume of 50 mL.

B. Preparation of chicken samples: Boneless and skinless chicken thighs and drumsticks were ground

(0.8 cm plate) in a grinder and divided into 4 treatments: 1) beetroot stems extract (BE) (concentration of 60 mg total phenolic compounds (TPC)/kg of meat) (concentration determined in a preliminary experiment); 2) guava pomace extract (GE) (concentration of 60 mg TPC/kg meat) (concentration determined in a preliminary experiment); 3) BHT (0.01% according to Decree No 1004 of the Secretariat of Health Surveillance, Brazil) dissolved in 5 ml of soybean oil without antioxidant; 4) control, without antioxidant. In all treatments, sodium chloride (1.5%) was added. After the addition of ingredients, the treatments were homogenized in a cutter, 25g portions were shaped into meatballs and cooked on a hot plate for 3 min to an internal temperature of 76°C. Part of the samples were packaged in PVC film (aerobic packaging) and the other part were vacuum packaged in vacuum bags with ethylene-vinyl acetate (EVA) copolymer multilayer structure with an oxygen permeability rate of < 25 m^3/m^2 day at 1 atm/23°C/0% relative humidity (RH) and a water vapor permeability rate of <10 g H_2O/m^2 day at 1 atm/38°C/90% RH (Cryovac, São Paulo, SP, Brazil). All the samples were stored under refrigeration (4±1°C) for 14 days.

C. Analysis of samples:

Thiobarbituric acid reactive substances (TBARS): TBARS values were determined in duplicate using an extraction method described, with modifications [13-14].

Instrumental colour: The instrumental colour was determined in triplicate with a colorimeter using CIELAB system.

pH: The pH was determined in triplicate, using a potentiometer with automatic temperature compensation and a glass penetration electrode.

The determinations was performed after 1, 7 and 14 days of refrigerated storage.

D. Statistical analysis: This experiment was designed as randomised complete blocks with a 4 x 3 complete factorial arrangement of treatment factors and storage times. The experiment was performed in duplicate and the results were submitted for analysis of variance (ANOVA). The comparison of treatment means was performed using the Tukey test ($p < 0.05$).

III. RESULTS AND DISCUSSION

A. Thiobarbituric acid reactive substances (TBARS)

Vacuum packaging

On the first day of storage, the treatments with BE and GE showed significantly ($p < 0.05$) reduced TBARS values compared to the control, indicating a fast action of these natural extracts to prevent lipid oxidation (Table 1). At the end of the storage period (14 days), although no significant ($p>0.05$) difference was detected between the natural antioxidants and the control, the treatments with BE and GE reduced TBARS values by 46.4% and 51.55%, respectively, compared to the control. During the storage period, the treatments with GE and BE did not present significant ($p > 0.05$) difference in relation to the treatment with BHT, which suggests that both extracts at the concentration of 60 mg PC/kg chicken meat showed antioxidant efficiency comparable with that of BHT.

Table 1. Average values (± standard deviations) of TBARS values in cooked chicken meat with different antioxidant treatments and stored at 4°C up to 14 days

Treatment	TBARS (mg malonaldehyde/kg meat) Storage time (days)		
	1	7	14
Vacuum Packaging			
C	1.47 ± 0.28^{aA}	1.46 ± 0.19^{aA}	1.94 ± 0.40^{aA}
BHT	0.58 ± 0.18^{bA}	0.63 ± 0.23^{bA}	0.64 ± 0.09^{bA}
BE	0.61 ± 0.05^{bA}	0.76 ± 0.27^{abA}	1.04 ± 0.29^{abA}
GE	0.49 ± 0.09^{bB}	0.56 ± 0.01^{bB}	0.94 ± 0.01^{abA}
Aerobic Packaging			
C	4.77 ± 1.14^{aB}	11.19 ± 1.2^{aA}	15.34 ± 1.25^{aA}
BHT	1.85 ± 0.78^{bC}	6.05 ± 0.11^{bB}	9.62 ± 0.11^{bcA}
BE	2.11 ± 0.31^{abC}	7.06 ± 0.22^{abB}	10.58 ± 0.37^{bA}
GE	1.16 ± 0.02^{bB}	4.46 ± 2.07^{bAB}	7.44 ± 0.50^{cA}

Different lowercase letters between treatments and different capital letters between days indicate significant difference at the 5% level by the *Tukey* test.

C-control; BHT-butylated hydroxytoluene; BE-beetroot stems extract at the concentration of 60 mg PC/kg chicken meat; GE guava pomace extract at the concentration of 60 mg PC/kg chicken meat.

The effect of grape seed extract and green tea extract on vacuum packaged cooked chicken meat stored at 5°C was evaluated by other authors [15]. They found TBARS values ranging from 24.5 to 32.0 mg MDA/kg chicken meat for the treatment with grape seed extract and from 25.0 to 38.0 mg MDA/kg chicken meat for the treatment with green tea extract. Compared with other natural extracts used in vacuum packaged chicken meat, the TBARS values found in our study can be considered low.

Regarding storage time, no significant ($p>0.05$) increase in TBARS values was observed for the control, BHT, and BE treatments. Other authors [16-17] affirmed that removing the oxygen from the sealed cooked meat package is a good option to

control lipid oxidation, and that both vacuum and modified atmosphere packaging are suitable for this purpose.

Aerobic packaging

During storage, GE and BE treatments did not differ significantly ($p > 0.05$) from BHT treatment, with proven effectiveness, evidencing the antioxidant action of the natural extracts tested (Table 1). After 14 days under refrigeration, GE was significantly ($p < 0.05$) superior to BE. The presence of bioactive compounds in the GE, such as quercetin, gallic acid, and epicatechin (determined in a preliminary experiment), may have inhibited lipid oxidation of processed chicken meat, similar to BHT action, for samples stored in vacuum and aerobic packages.

Different from the vacuum packaged samples, which presented oxidative stability during storage, samples stored in aerobic packages showed significant ($p < 0.05$) increase in TBARS values for all treatments during the storage period. The oxidation requires that an oxidizing agent has access to the substrate (lipid) and the most common oxidizing agent is the oxygen from the air [18]. Therefore, the increase in TBARS values for the samples packed in polystyrene trays covered with PVC film is certainly related to the oxygen permeability of this material, which permits the oxidation process to start and reach high speeds.

B. Instrumental colour

The instrumental color of chicken meat samples stored in vacuum and aerobic packages did not present significant ($p > 0.05$) effects of treatments, storage time, or interaction between treatment and storage time regarding L* (lightness) and b* (yellowness) values. For the a* value, a significant effect ($p<0.05$) of treatments and storage time was observed only for samples packaged aerobically (Table 2).

The lightness (L*) mean values of all treatments were 66.95 for aerobic packaging and 66.50 for vacuum packaging. Mean b* values were 13.02 and 12.53 for aerobic and vacuum packages, respectively (data not shown). It is known that color is one of the factors that determine food acceptance or rejection by consumers. In this sense, addition of WEB and WEG to cooked chicken meat produced good results in terms of L* and b* values, since they did not cause changes in these color parameters. Similar results were also observed in cooked chicken meat treated with grape seed extract [15].

Evaluating a*values (redness), samples stored in aerobic packages (Table 2) presented significant ($p<0.05$) difference between treatments only at day one of refrigerated storage, when samples treated with natural extracts showed lower intensity of red compared to the control. The natural pigments, such as lycopene in guava and betalains in beetroot, may have interfered in the color of the chicken meat product. Nevertheless, at the end of the storage period no significant ($p > 0.05$) difference was observed between treatments.

Table 2. Average values (± standard deviations) of a* value in cooked chicken meat with different antioxidant treatments and stored at 4°C up to 14 days in aerobic packaging

Treatment	a* value Storage time (days)		
	1	7	14
C	6.09 ± 0.11^{aA}	4.83 ± 1.84^{aA}	2.82 ± 1.27^{aA}
BHT	$5.72\pm0.12a^{bA}$	5.00 ± 0.34^{aAB}	4.20 ± 0.26^{aB}
EB	4.83 ± 0.41^{bA}	4.17 ± 0.20^{aA}	3.61 ± 0.78^{aA}
EG	4.79 ± 0.32^{bA}	4.31 ± 0.44^{aA}	3.93 ± 0.13^{aA}

Different lowercase letters between treatments and different capital letters between days indicate significant difference at the 5% level by the *Tukey* test.

C-control; BHT-butylated hydroxytoluene; BE-beetroot stems extract at the concentration of 60 mg PC/kg chicken meat; GE guava pomace extract at the concentration of 60 mg PC/kg chicken meat.

A significant ($p < 0.05$) effect of storage time was detected for the BHT samples stored in aerobic packages, with a decrease in a* values during the 14 days of refrigeration. The products of lipid oxidation can enter the cytoplasm to react with oxymyoglobin and accelerate metmyoglobin accumulation [19]. In our study, we found significant ($p<0.05$) increase in TBARS values during storage for the samples stored in aerobic packages. The products of lipid oxidation may have reacted with oxymyoglobin, since this package presents oxygen permeability, leading to the production of metmyoglobin, a brown pigment, and consequently to the decreased intensity of the red color of the chicken meat.

C. pH

There were no significant differences ($p>0.05$) in pH values between treatments in both aerobic and vacuum packages and no effect was observed from the interaction ($p>0.05$). We found pH values of 6.63 for both samples stored in aerobic and in vacuum packaging (data not shown). No change in pH values was observed by previous researches with cooked and refrigerated chicken breast [15].

During the storage period, pH values presented significant ($p<0.05$) changes. The pH values of the samples after 1, 7, and 14 days of refrigerated storage in aerobic packages were 6.72, 6.60, and 6.57, respectively (pH variation of 0.15 during storage), and in vacuum packages were 6.70, 6.59 and 6.60, respectively (pH variation of 0.11 during storage). A previous report also found significant pH effect during storage of a chicken product, but, according to these authors, pH variations of up to 0.15 do not present practical significance [20].

IV. CONCLUSION

BE and GE were effective to retard the lipid oxidation process compared to the control in aerobic packages, and proved to be as effective as BHT to maintain the oxidative stability of samples in both aerobic and vacuum packages. No changes in pH, L* and b* values were found between samples in both aerobic and vacuum packages. Nonetheless, the addition of both natural extracts reduced the intensity of the red color of samples.

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