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Wine industry residues extracts as natural antioxidants in raw and cooked chicken meat during frozen storage

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ABSTRACT

The effect of Isabel (IGE) and Niagara (NGE) grape seed and peel extracts on lipid oxidation, instrumental colour, pH and sensory properties of raw and cooked processed chicken meat stored at −18 °C for nine months was evaluated. The pH of raw and cooked samples was not affected by the addition of grape extracts. IGE and NGE were effective in inhibiting the lipid oxidation of raw and cooked chicken meat, with results comparable to synthetic antioxidants. The extracts caused alterations in colour, as evidenced by the instrumental (darkening and lower intensity of red and yellow colour) and sensory results of cooked samples. In the sensory evaluation of odour and flavour, IGE produced satisfactory results, which did not differ from synthetic antioxidants. These findings suggest that the IGE and NGE are effective in retarding lipid oxidation of raw and cooked chicken meat during frozen storage.

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1. Introduction

Changes in eating habits arising from the development of society in recent decades have led people to search for affordable and healthier foods with satisfactory taste and pleasant appearance. Thus, the food industry continually seeks to adapt and develop new formulations designed to increase shelf life and to improve quality and food safety.

Chicken meat, especially its industrial products, presents serious problems of processing and storage. Unsaturated lipids, fine grinding, incorporation of air, haem pigments, metal contact and high temperature during processing contribute to lipid oxidation (Field, 1988). After microbial deterioration, lipid oxidation is the main process that results in loss of quality (Gray, Gomaa, & Buckley, 1996). Lipid oxidation generates undesirable products from the sensory point of view, making the food unfit for consumption. In addition, it causes the degradation of fat soluble vitamins and essential fatty acids, and it interferes with the integrity and safety of foods through the formation of potentially toxic compounds (Silva, Borges, & Ferreira, 1999), such as malonaldehyde (MDA).

In an attempt to control this process, food industries use synthetic additives with antioxidant properties. However, due to reports of possible toxic effects from synthetic antioxidants and to increasingly demanding consumer preferences for natural products and health benefits, the interest for alternative methods to retard lipid oxidation in foods, such as the use of natural antioxidants, has increased. These methods include spice extracts (El-Alim, Lugasi, Hóvári, & Dworchák, 1999), fruit juice (Naveena, Sen, Vaithiyanathan, Babji, & Kondaiah, 2008), tea extracts (Rababah, Hettiarachchy, & Horax, 2004), seed extracts (Brannan & Mah, 2007) and others.

Residues from the wine industry account for approximately 30% of the total volume of grapes used for wine production. These by-products, such as seeds and peels, are rich in phenolic compounds, which are responsible for their high antioxidant activity (Guendez, Kallithraka, Makris, & Kefalas, 2005). Flavonols are the most abundant phenolic compounds in grape peels, while grape seeds are rich in flavan-3-ol (Cheynier & Rigaud, 1986; Souquet, Cheynier, & Moutounet, 2000). According to Shirahigue et al. (2010), the grape residue extracts (mixture of seeds and peels) from the Isabel and Niagara varieties showed considerable amounts of total phenolic compounds, containing the flavonoids catechin and epicatechin as major compounds. In addition, the extracts from both varieties had high antioxidant activity in vitro as determined by methods measuring of DPPH free radical scavenging and the inhibition of lipid peroxidation. The possibility of using this residue as a natural antioxidant in the food industry, allowing not only reduction in environmental impact but also higher food utilisation rate, has attracted considerable attention.

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Thus, studies have been conducted to evaluate the potential use of grape residues as natural antioxidants in poultry meat (Lau & King, 2003; Rababah, Ereifej, Mahasneh, & Rababah, 2006; Brannan & Mah, 2007; Brannan, 2009; Shirahigue et al., 2010).

The objective of this study was to evaluate the effects of Isabel (IGE) and Niágara (NGE) (*Vitis Labrusca* L.) grape seed and peel extracts on lipid oxidation, instrumental colour, pH and sensory properties of raw and cooked chicken meat that was vacuum-packed and stored at −18 °C for nine months.

2. Materials and methods

2.1. Materials

The raw material consisted of pressed grape residue (mixture of seeds and peels) derived from Niagara and Isabel (*Vitis labrusca*) wine production provided from a local winery. Chicken meat, (drumsticks and thighs), was obtained from a local slaughterhouse. The following chemicals were used in this study: Butylated hydroxytoluene (BHT), ethanol and sodium carbonate (Na_2CO_3) (Synth, Diadema, São Paulo, Brazil); the commercial mixture of sodium erythorbate, citric acid and sugar (SE) (Ibracor − 501, Ibrac, Rio Claro, São Paulo, Brazil); 1,1,3,3-tetraethoxypropane (TEP; approximately 97%) and 2-Thiobarbituric acid (TBA; minimum 98%) (Sigma-Aldrich, St. Louis, Missouri, USA); Propyl-3,4,5-trihydroxy-benzoate (PB) (Merck, Hohenbrunn, Baviera, Germany); Trichloroacetic acid (TCA), Ethylenedinitrilotetraacetic acid, disodium salt dihydrate (Titriplex III) (Merck, Darmstadt, Hessen, Germany), Gallic acid (Vetec, Rio de Janeiro, Rio de Janeiro, Brazil), Folin-Ciocalteau (Dinâmica, Diadema, São Paulo, Brazil). Packages with an ethylene vinyl acetate (EVA) multilayer structure with an oxygen permeability rate of $<25\ m^3/m^2$ day at 1 atm/23 °C/0% relative humidity (RH) and with a water vapour permeability rate of $<10\ g\ H_2O/m^2$ day at 1 atm/38 °C/90% RH (Cryovac, Sao Paulo, São Paulo, Brazil) and were used for characterising vacuum packaging.

2.2. Preparation of grape extracts

Residues (mixture of seeds and peels) were dried in an oven with forced air circulation (Nova Ética 400/D, Vargem Grande Paulista, Brazil) at 40 °C for 24 h and ground in an analytical mill (Quimis Q298A21, Diadema, São Paulo, Brazil) to a grain diameter of less than 0.5 mm. Twenty grams of dried and ground residue were macerated with 100 ml of ethanol 80% (v/v) under constant mechanical agitation on a rotary shaker (Nova Ética 304D, Vargem Grande Paulista, São Paulo, Brazil) at room temperature and protected from light for 48 h. The extract was filtered (12.5 mm qualitative filter paper), and the filtrate obtained was concentrated in a vacuum rotary evaporator (Tecnal TE-210, Piracicaba, São Paulo, Brazil) at 65 °C until the solvent was evaporated. The residues were dissolved in water to a final volume of 50 ml. These grape seed and peel extracts, NGE and IGE, were stored in amber glass bottles and kept under refrigeration (6 ± 2 °C).

2.3. Measurement of total phenolic content

The total phenolic content (PC) was analysed each time the chicken samples were processed to determine the volume of grape residue extract that needed to be added in the meat (the volume was calculated to obtain a concentration of 60 mg PC/kg of meat). The analysis was performed in triplicate. Total phenolic content of IGE and NGE was determined colorimetrically through the method proposed by Singleton, Orthofer, and Lamuela-Raventos (1999). The extract solutions (0.1 mL) were mixed with 5 mL of distilled water and 0.5 mL of Folin-Ciocalteau reagent. After 3 min of reaction, 1.5 mL of Na_2CO_3 (20%) and 2.9 mL of distilled water were added. The absorbance was measured in a spectrophotometer (Shimadzu UV-1240, Chiyoda-ku, Tokyo, Japan) at 765 nm after 2 h of incubation in the dark at room temperature. PC were expressed in gallic acid equivalents (GAE) per 100 g (dry weight) of grape residue.

2.4. Preparation of chicken meat samples

In a preliminary experiment, using seeds and peels of grapes from the same batch as those used in this study, IGE and NGE were more effective at preventing lipid oxidation of processed chicken drumsticks and thighs when added at a concentration of 60 mg of total phenolic compounds (PC)/kg of meat (Shirahigue et al., 2010). This concentration was used for this study.

Boneless and skinless chicken thighs (8 kg) and drumsticks (8 kg), obtained from birds slaughtered at approximately 42 days of age, were ground (0.8 cm plate) in a grinder (Hobart 4B22-2, Troy, Ohio, USA) and divided into 5 treatments as follows: 1) IGE (concentration of 60 mg PC/kg of meat); 2) NGE (concentration of 60 mg PC/kg meat); 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) SE (0.37%, which is the concentration usually employed in industry) dispersed in salt; and 5) control without addition of antioxidants. In all treatments, sodium chloride (1.5%) was added. Immediately after the addition of ingredients, the treatments were homogenised in a cutter (Hobart 84142, Troy, Ohio, USA). From the homogenised meat mixture, 25 g portions were shaped in the form of meatballs. Some of the samples were cooked in a hot plate (Edanca, São Bernardo do Campo, São Paulo, Brazil) until the internal temperature reached 72 °C for 5 min. Raw and cooked samples were vacuum packaged separately (Sealer Selovac 300B, São Paulo, São Paulo, Brazil) in vacuum bags with ethylene-vinyl acetate copolymer multilayer structure (Cryovac, São Paulo, São Paulo, Brazil), with 6 samples from the same treatment/bag and stored under freezing temperature (−18 °C) for nine months. The procedure was performed in triplicate.

2.5. Analysis of samples

2.5.1. Thiobarbituric acid reactive substances (TBARS)

The TBARS values were determined in duplicate using an extraction method described by Vyncke (1970), (1975) and Sorensen and Jorgensen (1996) with modifications. For extraction, 5 g of meat was homogenised in an Ultra Turrax (Ika T18 basic, Wilmington, North Carolina, USA) at 10,000 rpm for 30 s with 15 ml of a solution (7.5% TCA, 0.1% PB and 0.1% EDTA). After filtration with qualitative filter paper (12.5 mm), 5 ml of the filtrate was mixed with 5 ml of an aqueous solution (0.02 M TBA) in capped test tubes. The samples were incubated in a water bath at 100 °C for 40 min and then cooled in cold water. The absorbance was measured at 532 nm and 600 nm by a spectrophotometer (Shimadzu, UV–Vis mini 1240, Chiyoda-ku, Tokyo, Japan) against a blank containing 5 ml of the same TCA, PB and EDTA solution and 5 ml of TBA solution. The difference ($A_{532\ nm}$–$A_{600\ nm}$) was used as absorbance values corrected for turbidity. The results were calculated from the standard curve of TEP and expressed as mg of MDA per kg of meat. The TBARS value determination was performed after processing and after three, six and nine months of freezing.

2.5.2. pH

The pH was determined directly on samples using a potentiometer (Oakton pH 300 series 35618, Vernon Hills, Illinois, USA) with automatic temperature compensation and a glass penetration electrode (Digimed, Presidente Prudente, São Paulo, Brazil). For the analysis, the samples were thawed for 12 h at 6 ± 2 °C. The analysis was performed on five processed samples of each treatment after three, six and nine months of freezing using two readings in each sample.

2.5.3. Instrumental colour

The instrumental colour was determined using a colorimeter (Konica Minolta, Chroma Meter, CR-400, Mahwah, New Jersey, USA) using the following parameters: L* (lightness), a* (redness) and b* (yellowness) (CIELAB). The parameters were calibrated in a standard white porcelain with $Y = 93.7$, $x = 0.3160$ and $y = 0.3323$ and with a measurement area of 8 mm in diameter, an observation angle of 10° and an illuminant D65 with the specular component included. For the analysis, the samples were thawed for 12 hours at 6 ± 2 °C. To determine the colour, a surface layer was removed from both sides of the product. The analysis was performed on five samples from each treatment after three, six and nine months of freezing with two readings in each sample.

2.5.4. Sensory evaluation

For the sensory evaluation, a group of 12 panelists was trained. Two training sessions (2 h each) were performed. At these sessions, the description of the colour, odour and flavour attributes of the processed chicken meat were studied. After the description of the attributes, reference standards (Table 1) were presented to the panelists to delimit the ends of the scale in the evaluation of the samples. The objective of this training was to evaluate the possible alterations caused by the addition of the extracts and by the lipid oxidation on the colour, flavour and odour of the product. During the experiment, the panelists were trained to recall the reference standards that were used.

The trained panel evaluated the products after processing and after three, six and nine months of freezing. At each storage time, the panelists evaluated the product in two replications. Five samples per session were presented to the panelists that were coded with random numbers of three digits. For the analysis of flavour and odour alterations, the samples were cut into cubes of uniform size and heated in a microwave (10 s). Regarding the colour alteration, whole samples were presented with only the surface layer removed of the product for better visualisation of the inner colour. The panelists evaluated the samples for colour, flavour and odour alterations using a 10 point unstructured scale ranging from absent (0) to intense (10).

2.6. Statistical analysis

A 5 × 4 full factorial experiment was conducted, considering as factors the treatments (control, BHT, IGE, NGE and SE) and the storage times (0, 3, 6 and 9 months). The experiment was performed in triplicate. An analysis of variance -ANOVA ($p \leq 0.05$) was carried out to analyse the experimental results considering in the statistical model the effects of treatments, storage time and interaction of treatment x storage time. The comparisons of treatments averages and storage time averages were performed using the Tukey HSD test ($p \leq 0.05$).

Table 1
Attributes, standard references and rating used in the sensory evaluation of cooked chicken meat with different antioxidant treatments after processing and after three, six and nine months of frozen storage (−18 °C).

Attribute	Reference	Rating
Odour alteration	Unoxidised soybean oil	0
	Cooked fresh chicken meat, without concentrated grape juice	0
	Oxidised soybean oil	10
	Cooked fresh chicken meat, with concentrated grape juice	10
Colour alteration	Cooked fresh chicken meat, without dye	0
	Cooked fresh chicken meat, with dark red dye	10
	Cooked fresh chicken meat, with green dye	10
Flavour alteration	Unoxidised soybean oil	0
	Cooked fresh chicken meat, without concentrated grape juice	0
	Oxidised soybean oil	10
	Cooked fresh chicken meat, with concentrated grape juice	10

3. Results and discussion

3.1. Total phenolic content

The Isabel grape residue extract had a significantly higher content of total phenolic compounds compared to the Niagara grape residue extract (Table 2). Similar results were found by Soares, Welter, Kuskoski, Gonzaga, and Fett (2008), who observed values of 854.03 mg GAE/100 g (dry weight) in the Isabel grape peel extract and 1014.04 mg GAE/100 g (dry weight) in the Niagara grape peel extract.

The content of phenolic compounds obtained from the grape residues of the Isabel and Niagara varieties was higher compared to other fruits residues. Oliveira et al. (2009), who used extraction with methanol, found values of 681 mg GAE/100 g (dry weight) in an acerola pulp and peel extract, 275 mg GAE/100 g (dry weight) in a pineapple seed, pulp and peel extract and 103 mg GAE/100 g (dry weight) in a passion fruit seed and pulp extract. Soares, Welter, Gonzaga, et al. (2008), who derived extracts from apple pulp with acetone at concentrations of 75% and 100%, found values of 467.24 mg GAE/100 g (dry weight) and 522.74 mg GAE/100 g (dry weight), respectively.

3.2. Thiobarbituric acid reactive substances (TBARS)

The treatments had a significant effect on the lipid oxidation of cooked samples ($p \leq 0.05$), and no significant effect was observed for the storage period and interaction ($p > 0.05$).

The control treatment had significantly higher TBARS values ($p \leq 0.05$) when compared to other treatments with antioxidants (Table 3). According to Al-Kahtani et al. (1996), meat products can be considered well preserved in regards to oxidative changes, when they had less than 3 mg MDA/kg sample. Thus, in the cooked product, only the control treatment showed values higher than 3 mg MDA/kg meat, indicating that the samples had advanced oxidation and were unsuitable for consumption. In relation to natural antioxidants, the concentration that was used was sufficient for maintaining the oxidative stability of the chicken product for nine months of frozen storage.

There was no significant difference ($p > 0.05$) between treatments with synthetic antioxidants (BHT and SE) and treatments with natural extracts (IGE and NGE), demonstrating the efficiency of grape residue extracts as antioxidants in chicken meat. These results agree with those observed by Rababah et al. (2006) and Shirahigue et al. (2010), who found a reduction in TBARS in chicken meat with grape seed extract during refrigerated storage. The mechanism of the protective effect on lipid oxidation may be due to the fact that the grape seed extract obtained from wine and juice processing is rich in proanthocyanidins, which has multiple mechanisms for its antioxidant activity and the abilities to sequestrate radicals, chelate metals and synergise with other antioxidants (Lu & Foo, 1999).

Table 2
Averages values (± standard deviations) of total phenolic content (PC) in IGE and NGE expressed in terms of gallic acid equivalents (GAE).

Samples	PC (mg GAE/100g dry weight)
IGE	784.25 ± 175.86^{b}
NGE	941.66 ± 126.59^{a}

Averages followed by different letters in the same column differ significantly ($p \leq 0.05$) by the Tukey HSD test.
IGE: Isabel grape seed and peel extract; NGE: Niagara grape seed and peel extract.

A significant effect ($p \leq 0.05$) of treatments and storage time was observed in raw samples, however there was no interaction effect (Table 3). The BHT treatment was the only treatment that significantly differed ($p \leq 0.05$) from the control with the lowest TBARS values. IGE and NGE treatments had no significant difference ($p > 0.05$) when compared to the control or treatments with synthetic antioxidants (Table 3), suggesting that there was little difference in the lipid oxidation among raw products with grape extracts, synthetic antioxidants and the control. Lack of cooking, vacuum packaging and frozen storage are factors that provide additional protection for a product against the development of lipid oxidation. For that reason, the TBARS values of the raw samples (Table 3) showed averages ranging from 0.13 to 0.86 with values much lower than those found in the cooked product. Thus, due to the low rates of lipid oxidation that were observed, the effect of the antioxidants in raw samples was not substantial. These results indicate that IGE and NGE are comparable to commercial antioxidants and that their action is more effective in cooked samples, in which oxidation is induced by cooking.

For the storage period, the TBARS values increased over time with an average of 0.13 mg MDA/kg meat at the beginning of the experiment and an average of 0.74 mg MDA/kg meat at the end of the experiment (nine months). The development of oxidative rancidity occurs even during the storage of frozen chicken meat because while the rate of the deteriorative reactions (microbiological and enzymatic) can be inhibited by low temperatures, lipid oxidation still occurs normally, although at low rates (Grau, Guardiola, Boatella, & Codony, 2000). Similar results were reported by Brannan (2008), that observed an increase in the TBARS values of chicken meat during refrigerated storage.

In contrast to this study in which no significant difference ($p > 0.05$) between the control and the treatments (IGE and NGE) was observed, the study of Brannan (2008), with chicken meat, found significant reductions in TBARS values in treatments with the addition of grape extract when compared to control. The differences between this study and the study of Brannan (2008) may be due to the quality of the grape residue extract used, since this study used extract obtained in the laboratory, without purification steps and the study of Brannan (2008) used commercial grape seed extract. Moreover factors such as maturity, variety, cultivation practices, geographic origin, growth stage, harvest conditions and storage process will influence the total content of phenolic compounds (Kim, Jeong, & Lee, 2003).

3.3. pH

There were no significant differences ($p > 0.05$) in the pH values between treatments in both raw and cooked samples, indicating that the pH of the extracts did not affect the pH of the chicken product. No effect was observed from the interaction ($p > 0.05$). The pH values were approximately 6.5 for cooked samples and 6.32 for raw samples (data not shown). These results were similar to previous research with cooked and refrigerated ground chicken meat (Brannan, 2009) and cooked and refrigerated chicken breast (Rababah et al., 2006), which also demonstrated no change in pH values of samples to which grape seed extract was added. These previous reports were in agreement with the findings of this research.

However, there were significant changes ($p \leq 0.05$) in pH values during the storage period of the samples. The pH values after processing and after three, six and nine months of frozen storage for cooked products were 6.47, 6.59, 6.48 and 6.44, respectively, and the pH values for raw product were 6.19, 6.51, 6.31 and 6.29, respectively. Although pH has a significant effect during storage time, differences of 0.15 (cooked samples) and 0.32 (raw samples) were marginal and not significant on a practical level.

3.4. Instrumental colour

The results of the three colour parameters (L^*, a^* and b^*) for the cooked samples showed no significant effect ($p > 0.05$) during the storage time. However, there was a significant effect ($p \leq 0.05$) due to the treatments, which indicates that the addition of the grape residue extracts promoted colour alterations in the chicken product (Table 4). The treatments IGE, NGE and SE generated a significant reduction in the L^* value compared to the BHT treatment, which had the clearest samples. The reduction in L^* values of the treatments IGE and NGE may have been caused by addition of the extracts, especially IGE, which presented a dark red colour. The darkening of samples with the addition of grape extract was also reported in precooked chicken meat (Brannan, 2009).

The extracts of both grape varieties caused a significant reduction ($p \leq 0.05$) in the redness of cooked chicken meat compared to other treatments (Table 4). Treatment with SE had the highest a^* value and gave greater stability to the samples with regard to red discolouration. Significant changes in a^* values were also observed in chicken meat

Table 3
Averages values (± standard deviations) of TBARS in cooked and raw chicken meat with different antioxidant treatments after processing and after three, six and nine months of frozen storage (−18 °C).

Treatment	TBARS (mg malonaldehyde/kg meat)				Average
	Storage time (months)				
	0	3	6	9	
Cooked chicken meat					
C	4.75 ± 2.37	7.24 ± 1.19	7.83 ± 0.81	7.71 ± 1.68	6.88 ± 1.90 ^{a}
BHT	0.84 ± 1.37	0.90 ± 0.24	0.86 ± 0.97	0.91 ± 0.73	0.88 ± 0.94 ^{b}
IGE	1.66 ± 1.72	2.04 ± 1.15	1.86 ± 0.92	2.24 ± 1.38	1.95 ± 1.15 ^{b}
NGE	1.35 ± 1.48	1.79 ± 1.34	1.50 ± 1.36	2.12 ± 1.53	1.69 ± 1.26 ^{b}
SE	1.42 ± 2.19	0.83 ± 1.26	0.94 ± 1.02	1.88 ± 2.38	1.27 ± 1.60 ^{b}
Average	2.00 ± 2.14 ^{A}	2.54 ± 2.68 ^{A}	2.60 ± 2.87 ^{A}	2.97 ± 2.86 ^{A}	
Raw chicken meat					
C	0.24 ± 0.13	0.42 ± 0.06	1.48 ± 1.51	1.29 ± 0.91	0.86 ± 0.88 ^{a}
BHT	0.10 ± 0.06	0.08 ± 0.01	0.21 ± 0.04	0.13 ± 0.01	0.13 ± 0.06 ^{b}
IGE	0.12 ± 0.02	0.25 ± 0.04	0.58 ± 0.17	0.81 ± 0.40	0.43 ± 0.33 ^{ab}
NGE	0.10 ± 0.01	0.29 ± 0.04	0.75 ± 0.55	0.81 ± 0.30	0.49 ± 0.40 ^{ab}
SE	0.10 ± 0.06	0.19 ± 0.02	0.54 ± 0.12	0.70 ± 0.13	0.38 ± 0.27 ^{ab}
Average	0.13 ± 0.08 ^{C}	0.24 ± 0.12 ^{BC}	0.71 ± 0.70 ^{AB}	0.74 ± 0.52 ^{AB}	

For each treatment, averages followed by different lowercase letters in the same column differ significantly ($p \leq 0.05$) by the Tukey HSD test.
For each storage time, averages followed by different capital letters in the same row differ significantly ($p \leq 0.05$) by the Tukey HSD test.
C: control, BHT: Butylated hydroxytoluene; IGE: Isabel grape seed and peel extract; NGE: Niagara grape seed and peel extract; SE: sodium erythorbate, citric acid and sugar.

Table 4
Average values (± standard deviations) of instrumental colour (L*, a* and b*) in cooked chicken meat with different antioxidant treatments after processing and after three, six and nine months of frozen storage (−18 °C).

Treatment	Storage time (months)				Average
	0	3	6	9	
L (lightness)*					
C	68.99 ± 0.50	69.32 ± 0.81	67.76 ± 0.80	68.26 ± 0.51	68.58 ± 0.86 ac
BHT	69.86 ± 0.69	69.64 ± 0.63	68.72 ± 0.71	69.81 ± 1.16	69.51 ± 0.85 a
IGE	66.53 ± 0.51	66.31 ± 1.72	66.47 ± 1.44	66.93 ± 1.74	66.56 ± 1.25 b
NGE	67.28 ± 1.49	67.09 ± 0.64	67.27 ± 0.74	67.19 ± 1.51	67.21 ± 1.00 bc
SE	68.27 ± 1.89	67.62 ± 0.80	68.09 ± 1.53	67.97 ± 2.60	67.99 ± 1.57 bc
Average	68.19 ± 1.57 A	68.00 ± 1.58 A	67.66 ± 1.22 A	68.03 ± 1.75 A	
a (redness)*					
C	5.04 ± 0.10	5.09 ± 0.25	5.07 ± 0.24	5.18 ± 0.21	5.10 ± 0.19 b
BHT	5.04 ± 0.24	5.11 ± 0.18	5.20 ± 0.26	5.20 ± 0.42	5.14 ± 0.25 b
IGE	4.30 ± 0.15	4.31 ± 0.36	4.30 ± 0.02	4.65 ± 0.22	4.39 ± 0.25 d
NGE	4.63 ± 0.18	4.71 ± 0.32	4.71 ± 0.23	4.80 ± 0.26	4.71 ± 0.23 c
SE	5.56 ± 0.16	5.49 ± 0.04	5.69 ± 0.08	5.75 ± 0.26	5.62 ± 0.17 a
Average	4.91 ± 0.46 A	4.94 ± 0.47 A	4.99 ± 0.51 A	5.12 ± 0.46 A	
b (yellowness)*					
C	16.21 ± 1.74	15.41 ± 0.58	16.13 ± 0.71	15.11 ± 0.50	15.72 ± 0.99 a
BHT	15.84 ± 1.13	16.04 ± 0.47	15.95 ± 0.67	15.15 ± 0.79	15.75 ± 0.78 a
IGE	13.74 ± 0.80	13.49 ± 0.94	13.33 ± 1.01	13.34 ± 0.93	13.48 ± 0.81 b
NGE	14.16 ± 1.33	14.18 ± 0.57	14.23 ± 1.00	14.11 ± 1.23	14.17 ± 0.92 b
SE	15.97 ± 1.24	15.98 ± 1.71	16.26 ± 0.84	15.52 ± 1.32	15.93 ± 1.15 a
Average	15.18 ± 1.52 A	15.02 ± 1.33 A	15.18 ± 1.42 A	14.65 ± 1.19 A	

For each treatment, averages followed by different lowercase letters in the same column differ significantly ($p \leq 0.05$) by the Tukey HSD test.
For each storage time, averages followed by different capital letters in the same row differ significantly ($p \leq 0.05$) by the Tukey HSD test.
C: control, BHT: Butylated hydroxytoluene; IGE: Isabel grape seed and peel extract; NGE: Niagara grape seed and peel extract; SE: sodium erythorbate, citric acid and sugar.

(Brannan, 2009), however, this author reported an increase in a* values of samples with grape seed extract, which was different from what we observed. This variation in results may be due to different colourations of the grape extracts used, which may have interfered with the meat colour in different ways.

The b* value was also affected by the addition of the extracts, which significantly decreased ($p \leq 0.05$) the yellowness of the cooked samples. Similar results were observed in cooked ground chicken (Brannan, 2009).

In raw samples, no significant change was observed ($p > 0.05$) in the L*, a* and b* colour parameters in all of the treatments (Table 5) and no effect was observed from the interaction. Rojas and Brewer (2008) observed that the instrumental colour of raw and frozen pork samples with natural antioxidants, including grape seed extract, was

Table 5
Average values (± standard deviations) of instrumental colour (L*, a* and b*) in raw chicken meat with different antioxidant treatments after processing and after three, six and nine months of frozen storage (−18 °C).

Treatment	Storage time (months)				Average
	0	3	6	9	
L (lightness)*					
C	57.45 ± 1.51	59.24 ± 1.15	57.88 ± 2.84	58.66 ± 0.93	58.31 ± 1.53 a
BHT	58.79 ± 0.59	60.33 ± 0.25	61.06 ± 4.00	60.60 ± 2.93	60.20 ± 2.10 a
IGE	53.45 ± 5.82	55.60 ± 3.26	54.36 ± 6.75	55.38 ± 6.38	54.70 ± 4.42 a
NGE	56.11 ± 4.71	55.31 ± 4.19	54.58 ± 6.84	56.53 ± 6.07	55.63 ± 4.27 a
SE	55.86 ± 4.09	56.15 ± 3.43	56.10 ± 7.74	57.49 ± 9.08	56.40 ± 4.99 a
Average	56.33 ± 3.45 A	57.33 ± 3.05 A	56.80 ± 5.14 A	57.73 ± 4.73 A	
a (redness)*					
C	9.70 ± 0.94	9.51 ± 2.23	8.35 ± 0.21	9.74 ± 0.47	9.33 ± 1.12 a
BHT	10.47 ± 0.30	8.86 ± 1.05	7.34 ± 1.04	7.93 ± 0.44	8.65 ± 1.40 a
IGE	9.53 ± 1.26	9.17 ± 1.00	8.19 ± 1.61	8.93 ± 0.11	8.96 ± 1.01 a
NGE	8.76 ± 0.36	9.23 ± 0.34	8.22 ± 0.76	9.44 ± 0.57	8.91 ± 0.65 a
SE	10.10 ± 0.94	9.64 ± 0.04	9.11 ± 0.82	10.10 ± 1.41	9.74 ± 0.83 a
Average	9.71 ± 0.88 A	9.28 ± 0.94 AB	8.24 ± 0.95 BC	9.23 ± 0.97 AB	
b (yellowness)*					
C	15.59 ± 1.43	14.47 ± 0.89	14.22 ± 2.18	13.13 ± 0.01	14.35 ± 1.40 a
BHT	15.39 ± 0.92	15.22 ± 0.25	16.40 ± 2.33	16.46 ± 1.32	15.87 ± 1.23 a
IGE	12.81 ± 3.30	12.18 ± 2.51	11.97 ± 4.07	12.02 ± 2.47	12.25 ± 2.41 a
NGE	13.84 ± 3.46	12.81 ± 3.08	12.69 ± 4.00	13.36 ± 2.74	13.18 ± 2.58 a
SE	14.49 ± 3.18	13.40 ± 2.79	12.95 ± 5.34	13.90 ± 3.49	13.69 ± 2.96 a
Average	14.42 ± 2.27 A	13.62 ± 2.02 A	13.65 ± 3.26 A	13.77 ± 2.34 A	

For each treatment, averages followed by different lowercase letters in the same column differ significantly ($p \leq 0.05$) by the Tukey HSD test.
For each storage time, averages followed by different capital letters in the same row differ significantly ($p \leq 0.05$) by the Tukey HSD test.
C: control, BHT: Butylated hydroxytoluene; IGE: Isabel grape seed and peel extract; NGE: Niagara grape seed and peel extract; SE: sodium erythorbate, citric acid and sugar.

similar to the colour of the control samples. Furthermore, Sasse, Colindres, and Brewer (2009) reported that the addition of grape seed extract did not change the a* and b* values of raw pork.

Regarding storage time, a significant effect was verified ($p \leq 0.05$) only in relation to the a* value of the raw samples. A slight reduction in the a* value was observed in the sixth month of freezing. Despite the significant effect, the variation in the a* value after the samples were processed (9.71) and after 6 months of frozen storage (8.24) was marginal. However, the reduction in the intensity of red colour during storage could be explained due to the interdependence between lipid oxidation and colour oxidation in meats (Lynch & Faustman, 2000). The pigment oxidation may catalyse lipid oxidation, and free radicals produced during oxidation may oxidise the iron atoms or denature the myoglobin molecules, negatively changing the colour of the products. Thus, because the TBARS values of raw samples in this study increased slightly throughout the storage time, this trend of decreasing a* values may be due to interference with the lipid oxidation in the myoglobin oxidation. Furthermore, the temperature (−18 °C) may have affected the a* value during the nine months of storage. According to Young and West (2001), the colour of meat stored under freezing temperatures becomes dark red (red-brownish) from the combination of low light reflection, surface dehydration and metmyoglobin formation.

Between the treatments, the BHT had a more evident reduction in the a* value over nine months of frozen storage. Similar results were reported by Decker and Crum (1991), who studied the effect of carnosine, sodium tripolyphosphate, α-tocopherol and BHT on lipid oxidation of raw and frozen pork. They observed that BHT and sodium tripolyphosphate were not effective in preventing colour alterations in pork. These results can be explained because, although lipid soluble free radical scavengers (Butylated hidroxytoluene) can inhibit myoglobin oxidation, their inability to inactivate water-soluble free radicals make them less effective than grape residue extracts at preventing colour changes.

Table 6
Average values (± standard deviations) of sensory scores in cooked chicken meat with different antioxidant treatments after processing and after three, six and nine months of frozen storage (−18 °C).

Treatment	Storage time (months)				Average
	0	3	6	9	
Colour alteration					
C	1.9 ± 1.19	2.2 ± 0.57	1.8 ± 0.86	2.1 ± 0.98	2.0 ± 0.81 [b]
BHT	1.8 ± 0.67	2.0 ± 0.75	2.0 ± 0.35	1.8 ± 0.91	1.9 ± 0.61 [b]
IGE	5.2 ± 2.25	5.3 ± 0.41	5.8 ± 0.71	5.2 ± 0.92	5.4 ± 1.13 [a]
NGE	5.1 ± 3.08	5.4 ± 1.49	5.2 ± 0.96	4.7 ± 0.47	5.1 ± 1.55 [a]
SE	3.0 ± 1.09	2.7 ± 0.96	3.3 ± 0.78	2.9 ± 0.86	3.0 ± 0.82 [b]
Average	3.4 ± 2.20 [A]	3.5 ± 1.74 [A]	3.6 ± 1.82 [A]	3.3 ± 1.59 [A]	
Flavour alteration					
C	4.0 ± 1.09	3.7 ± 0.80	3.7 ± 0.69	3.4 ± 0.62	3.7 ± 0.74 [ac]
BHT	2.4 ± 0.98	2.8 ± 0.38	2.4 ± 0.88	2.3 ± 0.36	2.5 ± 0.64 [b]
IGE	3.0 ± 0.61	3.2 ± 0.13	2.7 ± 0.59	3.0 ± 0.45	3.0 ± 0.46 [bc]
NGE	3.8 ± 1.14	4.2 ± 0.64	4.1 ± 0.42	4.1 ± 0.69	4.1 ± 0.67 [a]
SE	2.3 ± 0.93	2.3 ± 0.22	2.4 ± 0.51	2.5 ± 0.11	2.4 ± 0.47 [b]
Average	3.1 ± 1.10 [A]	3.3 ± 0.79 [A]	3.1 ± 0.91 [A]	3.1 ± 0.79 [A]	
Odour alteration					
C	3.1 ± 0.60	3.9 ± 0.59	3.5 ± 0.58	3.7 ± 0.64	3.5 ± 0.61 [a]
BHT	1.5 ± 0.24	2.3 ± 0.65	1.7 ± 0.58	2.2 ± 0.31	1.9 ± 0.52 [b]
IGE	2.2 ± 0.88	2.2 ± 0.76	2.5 ± 0.72	2.9 ± 0.19	2.5 ± 0.65 [b]
NGE	2.6 ± 1.31	4.0 ± 0.97	3.5 ± 0.43	4.0 ± 0.16	3.5 ± 0.94 [a]
SE	1.7 ± 0.59	2.2 ± 0.84	2.0 ± 0.28	2.4 ± 0.41	2.1 ± 0.55 [b]
Average	2.2 ± 0.90 [B]	2.9 ± 1.08 [A]	2.6 ± 0.90 [AB]	3.0 ± 0.81 [A]	

For each treatment, averages followed by different lowercase letters in the same column differ significantly ($p \leq 0.05$) by the Tukey HSD test.
For each storage time, averages followed by different capital letters in the same row differ significantly ($p \leq 0.05$) by the Tukey HSD test.
C: control, BHT: Butylated hydroxytoluene; IGE: Isabel grape seed and peel extract; NGE: Niagara grape seed and peel extract; SE: sodium erythorbate, citric acid and sugar.

3.5. Sensory evaluation

According to the evaluation of colour alteration attributes, the treatments had a significant effect ($p \leq 0.05$). In contrast, the storage time and interaction had no significant effect ($p > 0.05$).

It was observed that the treatments with natural antioxidants had significantly higher ($p \leq 0.05$) colour alterations compared to control and synthetic antioxidant treatments (Table 6). In regards to colour, the results of the sensory evaluation corroborate with the results of the instrumental evaluation presented in Table 4, in which colour alterations were observed in cooked samples with grape seed and peel extract. This colour alteration of samples, as previously mentioned, may be due to the addition of the extracts, especially IGE, which has a dark colour. Significant colour alterations of samples with grape seed extract (meat darkening) were also observed by Brannan (2009) in precooked and refrigerated chicken meat and by Lau & King (2003) in turkey meat.

For data on flavour alteration attributes, there was a significant effect ($p \leq 0.05$) from the treatments, but no effect was observed from the storage time and interaction ($p > 0.05$) (Table 6). Treatment with the addition of NGE had the highest flavour alteration, which significant differed ($p \leq 0.05$) from the other treatments with antioxidants.

Between the natural antioxidants, IGE had better results that were more comparable to the synthetic antioxidants (BHT and sodium erythorbate). According to the sensory evaluation reports, some panelists noticed a grape/wine flavour in samples from the NGE treatment. Thus, it appears that the addition of NGE influenced the characteristic chicken meat flavour. In a preliminary sensory analysis with untrained panelists, Lau & King (2003) also found a light masking in the flavour of turkey meat with the addition of grape seed extract. The control treatment had a high score of flavour alteration and significantly differed ($p \leq 0.05$) from the synthetic antioxidant scores. This result may be indicative of alterations due to the lipid oxidation process, which may lead to the formation of undesirable flavours. However, the high value of flavour alteration at the beginning of the storage period (4.0) was probably caused by the warmed over flavour (WOF). The term WOF was first introduced by Tims and Watts (1958) to describe the rapid attack of rancidity in cooked meat during refrigerated storage, which was an undesirable sensory characteristic to the panelists (St. Angelo, Vercellotti, Dupuy, & Spanier, 1988). Unlike rancidity that develops slowly and becomes evident only after prolonged storage under freezing, the oxidised flavours were readily detectable after 48 h (Pearson, Gray, Wolzac, & Horenstein, 1983).

In regards to the attribute of odour alteration, the results demonstrated a significant effect ($p \leq 0.05$) from the treatments and storage time but no effect from the interaction ($p > 0.05$). NGE and control had significantly ($p \leq 0.05$) higher odour alterations than the other treatments (Table 6). These results were similar to the flavour data, and this alteration was probably due to the same causes. In samples from the NGE treatment, there may have been interference of the odour from the grape extract in the characteristic cooked chicken odour. In the sensory evaluation, a wine/grape odour was reported by the panelists. Wine odour in turkey meat samples with grape seed extract was also observed by Lau & King (2003), which supports the data from this study. In samples from the control treatment, the alteration was probably due to the occurrence of lipid oxidation with the formation of volatile compounds that are characteristic of oxidative rancidity, as was indicated by the high TBARS levels and the reports of bad/rancid odour by some panelists during the sensory evaluation.

The odour alteration of samples was lower in treatments with the addition of BHT, SE and IGE, with no significant differences ($p > 0.05$) among them. Thus, for this attribute, IGE had positive results.

The odour of processed chicken meat was affected by the frozen storage period ($p \leq 0.05$). There was a slight increasing trend in the odour alteration averages of samples, which started the storage period with an average of 2.2 and, after nine months of freezing temperatures, reached an average of 3.0. Considering that the panel evaluated the samples using a ten-point unstructured scale ranging from absent (0) to intense (10), an increase of 0.8 in odour alteration during the storage period of the samples could be considered marginal. However, this slight increase may be due to the development of lipid oxidation and consequent production of volatile compounds, which gave the product off-odours.

In the studies with pork (Sasse et al., 2009; Rojas & Brewer, 2007) and chicken meat (Brannan, 2009), it was found that control samples had greater rancid odour intensity, corroborating data from the present study, which presented the highest odour alteration score. According to Brannan (2009) and Rojas & Brewer (2007), this interference in both odour and flavour in the control treatment may be due to the development of uncharacteristic flavours, which were controlled in the treatments with the addition of grape seed extract. Furthermore, the grape seed extract reduced attributes associated with WOF, such as the musty and rancid odours, according to Brannan (2009).

4. Conclusions

Isabel and Niagara grape residue extracts were as effective as BHT and sodium erythorbate at preventing lipid oxidation in raw and cooked chicken meat. IGE and NGE did not alter the pH values of raw and cooked samples or the colour of raw samples, but they promoted alterations in the colour of the cooked product, which was evidenced by the results of the sensory and instrumental measures. In the sensory evaluation, only the NGE interfered with the natural chicken meat flavour and odour. The use of residues from the wine industry as natural antioxidants, combined with the use of vacuum packaging and storage under freezing temperatures, may be considered an effective method to retard lipid oxidation in both raw and cooked processed chicken meat.

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