Effect of natural antioxidant combinations on lipid oxidation in cooked chicken meat during refrigerated storage
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The effect of combinations of sage, oregano and honey on lipid oxidation in cooked chicken meat during refrigeration at 4 °C for 96 h was determined. Chicken samples (thigh and breast) were then separated into five groups: control; butylated hydroxytoluene; oregano + sage; oregano + sage + 5%honey and oregano + sage + 10%honey. Quantitative measurements of thiobarbituric acid reactive substances, conjugated dienes, hexanal, fatty acids, cholesterol and cholesterol oxides were used as indicators of lipid oxidation. Acceptability and preference were also evaluated. The effectiveness of the natural antioxidants for reducing the velocity of lipid oxidation in cooked chicken thigh and breast was demonstrated after 48 and 96 h of refrigeration at 4 °C. The treatments that presented the lowest hexanal values after 96 h of refrigeration were oregano + sage + 5%honey and oregano + sage + 10%honey. Only traces of free cholesterol oxides were found (25-OH, 7-k, 7α-OH and 7β-OH). The natural antioxidants protected cooked chicken meat from oxidation processes and resulted in great acceptability.

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1. Introduction

Lipid oxidation is one of the most important parameters that influence the quality and acceptance of meat and chicken. The products of lipid oxidation are responsible for unacceptable off-flavours and off-odours in cooked meats and limit the shelf life of such meats. In contrast to raw meats, in which lipid oxidation occurs over days or weeks, these reactions proceed rapidly in cooked meats, such that oxidized flavours are detectable within hours of cooking (Kingston, Monahan, Buckley, & Lynch, 1998).

Thermal processes can promote lipid oxidation by disrupting cell membranes and releasing pro-oxidants, thereby inducing “warm-over flavour” (WOF), which rapidly develops in cooked meat products during refrigerated storage and subsequent reheating (Sato & Hegarty, 1971). Precooked meats are likely to oxidize and produce secondary oxidative compounds such as hexanal, pentanal, 2,4-decadienal, 2,3-octanediene, and 2-octenal (Trout & Dale, 1990). Hexanal is the most important WOF compound (Beltran, Pla, Yuste, & Mor-Mur, 2003). It is formed during the oxidation of linoleic acid via 13-hydroperoxide, and is easily detected because it has a low odor threshold (Goodridge, Beaudry, Festka, & Smith, 2003).

In order to investigate “WOF” development in meat, other chemical methods are used, including measuring changes in conjugated dienes and malonaldehyde. The latter, is a product of autoxidation of polyunsaturated fatty acids in muscle tissues (Pegg & Shahidi, 2007).

Lipid oxidation in meat during cooking may affect cholesterol, through leading to formation of cholesterol oxides (COPs) in cooked meat. This is related to the temperature and duration of the heat treatment (Larkeson, Dutta, & Hansson, 2000).

Use of either synthetic or natural antioxidants is one of the major strategies for preventing lipid oxidation. Current recommendations restrict synthetic food additives, which encourages their replacement by naturally occurring ingredients with similar functions (Sasse, Colindres, & Brewer, 2009).

Apart from traditional antioxidants, a variety of compounds with oxidation inhibition activity could be proposed for use in food. The desire for new sources of safe and inexpensive antioxidants of natural origin has resulted in considerable interest in herbs and spices as sources of natural antioxidants (Yanishlieva, Marinova, & Pokorny, 2006). Plants from the Lamiaceae family contain the substances for which the plant is used in the pharmaceutical, food or fragrance industries. Essential oils represent a small fraction of the plant composition; the main compounds are terpens and sesquiterpenes, and several oxygenated derivatives compounds (alcohols, aldehydes, ketones, acids, phenols, ethers, esters, etc.) all of them responsible for the characteristic plant odour and flavour (Yanishlieva et al., 2006). These compounds include natural flavourings such as sage, oregano, rosemary and others (Mariutti, Orlien, Bragagnolo, & Skibsted, 2008).
Studies have shown wide effective in spices and honey to retard lipid oxidation in meat products (Antony, Rieck, & Dawson, 2000; Johnston, Sepe, Miano, Brannan, & Alderton, 2005; Juntachote, Berghofer, Siebenhandl, & Bauer, 2006, 2007a, 2007b; Lee et al., 2010; Mariutti et al., 2008; McKibben & Engeseth, 2002; Sasse et al., 2009; Trout & Dale, 1990).

Consequently, the aim of this study was to investigate the potential role of natural antioxidant combinations on lipid oxidation in cooked chicken meat during refrigerated storage.

2. Materials and methods

2.1. Reagents and chemicals

Butylated hydroxytoluene (BHT), 2-thiobarbituric acid (TBA), 1,1,3,3-tetraethoxypropane (TEP) and hexanal were obtained from Sigma–Aldrich (USA). Silicone anti-foaming agent was obtained from Merck (Germany). SPME carboxen/polydimethylsiloxane (PDMS/DVB) fibres were obtained from Supelco (Bellefonte, PA, USA). Cholesterol and COPs (7α-OH, 7β-OH, 7-k and 25-OH) were purchased from Steraloids (Wilton, NH, USA). HPLC-grade solvents (2-propanol and hexane) were purchased from Carlo Erba (São Paulo, Brazil). H2O2 was purchased from Synth (São Paulo, Brazil). All other chemicals used were of analytical grade.

2.2. Sample preparation

Commercially samples of dried oregano leaves (Origanum vulgare) and dried sage leaves (Salvia officinalis) were obtained from Fuchs Gewürze (São Paulo, Brazil).

Commercially available honey (orange source) was obtained from a local market (São Paulo, Brazil).

Fresh chicken thigh and breast (around 30 kg of each) were obtained from a local market (São Paulo, Brazil) and transported to the laboratory under chilled condition in ice boxes.

Skin, bone and visible fat accompanying each type of meat were removed. The chicken samples (about 6 kg of each meat per group) of both types were then divided into five groups. The samples were subjected to the following treatments: group 1 – control; group 2 – BHT (100 ppm); group 3 – oregano + sage (0.2% w/w of oregano and 0.2% w/w of sage); group 4 – oregano + sage + 5% honey (0.2% w/w of oregano, 0.2% w/w of sage and 5% w/w of honey) and group 5 – oregano + sage + 10% honey (0.2% w/w of oregano, 0.2% w/w of sage and 10% w/w of honey). Commercial sodium chloride (2% w/w based on finished product weight) was added to all samples. Immediately after these dried herbs cutted into small pieces, BHT and honey had been added, each group was thoroughly marinated in a vacuum tumbler (model MGH-20, Vakona Quality) for 30 min at 4 °C.

After marination, the chicken samples were packed in polyester film (Assa Pratsy)® and then heated in a conventional oven at 200 °C for 1 h (until reaching an internal temperature of 80 °C). The temperature was monitored using a thermometer (TDPK digital thermometer; Novus, Brazil). After cooling at room temperature, the samples were minced in food processors (Wallita Master, Brazil), divided into portions (200 g) and wrapped in oxygen-permeable cling film. The samples were stored in a refrigerator (4 °C) for 96 h.

2.3. Methods

The cooked chicken samples were analyzed immediately after cooking and after 48 and 96 h of refrigerated storage. Three replicates were made for each treatment.

2.3.1. Chemical analysis, pH and thiobarbituric acid-reactive substances (TBARS)

The moisture content of the meat was determined as described (AOAC, 1995). The total lipids of chicken muscle were extracted in accordance with the method described by Folch, Lees, and Stanley (1957).

The pH value of chicken meat was determined using 5 g samples, homogenized with 5 mL of Milli-Q water using an Ultra Turrax T-18 homogenizer (16,000 rpm) (IKA, Brazil). The pH was measured using a digital pH-meter (Gehaka PG 1800, Brazil) with direct insertion of the probe electrode after calibration.

Thiobarbituric acid reactive substances (TBARS) were measured by following the distillation method described by Tarladgis, Watts, Younathan, & Dugan, 1960). In brief, 10 g of sample were homogenized and transferred to a flat-bottomed flask. One drop of silicone anti-foaming agent was added, plus 2.5 mL of HCl (4 N) and 97.5 mL of distilled water. This sample was then distilled and the first 50 mL of distillate was collected. Next, 5 mL of the distillate was added to 5 mL of 0.02 M thiobarbituric acid and were heated in a boiling water bath (100 °C) for 35 min for colour development. The samples were immediately cooled on ice and the absorbance was measured at 532 nm on a spectrophotometer (Shimadzu UV-1650, Tokyo, Japan). The TBARS value was calculated from a standard curve of malondialdehyde (MDA), which was freshly prepared by acidification of 1,1,3,3-tetraethoxypropane (TEP). The results were reported as mg of MDA per kilogram of sample.

2.3.2. Hexanal content

Minced samples (5 g) and 25 mL of distilled water were thoroughly mixed using a T-18 homogenizer (16,000 rpm) (IKA, Brazil). Aliquots (3 mL) of the homogenates were dispensed in a vial, which was then closed using a polytetrafluoroethylene (PTFE) silicone septum (Branton, Cronin, Monahan, & Durcan, 2000). An SPME fibre (75 µm carboxen–polydimethylsiloxane coating) was inserted through the septum and exposed to the headspace of the vial. Extraction was carried out at 40 °C for 20 min with stirring in a water bath (Büchi HB-140, Büchi Laboratory Equipment, Switzerland). After extraction, the SPME fibre was immediately desorbed in the chromatograph injector, using a splitless mode at 260 °C.

The analyses were conducted using a GC–MS Focus GC coupled to an ion trap mass-spectrometer selective detector (Polaris Q, Thermo Electron Corporation, San Jose, CA, USA). Hexanal separation was performed on a DB-5MS fused silica capillary column of dimensions 30 m × 0.25 mm × 1.0 µm film thickness (Thermo Finnigan, San Jose, CA, USA), operating in a helium flow of 2.0 mL/min. The oven program was 60 °C initially for 1 min, and then 5 °C/min up to 160 °C. The transfer line temperature was maintained at 200 °C. The ion trap mass spectrometer was operated in the electron impact mode (70 eV) and the ion source temperature was 200 °C. Selected ion monitoring (SIM) was used as the data acquisition mode, and data were collected and integrated by means of the Xcalibur software (Thermo Electron Corporation).

2.3.3. Analysis of conjugated dienes (CDs)

Approximately 70 mg of lipid were weighed into a 100 mL volumetric flask and filled up to volume with isooctane. The flask was thoroughly shaken to obtain homogeneous distribution. The absorbance was measured using a spectrophotometer (Shimadzu UV-1650, Tokyo, Japan) at 232 nm against a blank of isooctane. CDs were calculated in accordance with the Ti 1a-64 methods (AOCS, 2003), using the formula: CD = 0.84 [(A/λc) – K0].

A is the absorbance at 232 nm, b is the cuvette length (cm), c is the sample concentration in isooctane (g/L), and K0 represents the absorptivity by acid or ester groups (0.07 for esters, 0.03 for acids).
2.3.4. Fatty acid composition

Lipids were extracted as described by Folch et al. (1957). Fatty acid methyl esters (FAMEs) were prepared by means of saponification and methylation in the presence of BF₃ in methanol (Metcalfe, Schmitz, & Pelka, 1966). Fatty acid analysis was performed in a Shimadzu GC 2010 gas chromatograph equipped with a FID detector, under the following conditions: split injection (1:30 injection volume 1 μl); capillary column SP 2560 (Supelco) (100 m × 0.25 μm × 0.2 μm film); injection port and detector temperatures of 250 °C and 260 °C respectively; initial oven temperature of 140 °C, increasing at 4 °C/min to 240 °C, which was maintained for 15 min; and carrier gas of hydrogen at 1.5 mL/min flow rate. Identification of the fatty acids was achieved by comparing their retention times with pure standards (FAME 37, code 47885, Sigma Chemical Co.). Fatty acid composition was expressed as the percentage of total FAMEs.

2.3.5. Free cholesterol and free cholesterol oxide contents (7α-OH, 7β-OH, 7-k and 25-OH)

Free cholesterol and four free COPs were determined in accordance with the methodology described by (Casllany & Ayaz, 1976). Each sample (50 mL of total lipids) was dried under vacuum at 25 °C on a rotary evaporator (Rotavapor® R-111, Buchi Laboratory Equipment, Switzerland). After that, the residue was dissolved in 3 mL of hexane/isopropanol (97:3 v/v) and passed through a syringe-tight membrane filter (Millex 0.22 μm pore size; Millipore Co., Ireland).

Free cholesterol and COPs were analyzed simultaneously by means of HPLC (Thermo Separation Products, USA) equipped with a UV/VIS detector (UV-2000, SpectraSystem UV/VIS detectors), an automatic injector (AS-3000, Spectra Series autosamplers) and a quaternary pump (P-4000, Spectra Systems gradient pumps).

The analytical column was a CN-bonded stainless steel CPS-type column (Thermo Hypersil, UK) of dimensions 25 cm × 0.4 cm (spherical particles of 5 μm). The mobile phase was hexane/isopropanol (97:3 v/v) at a flow rate of 1 mL/min, and the sample injection volume was 20 μL. Wavelength used for the cholesterol and cholesterol oxides (7α-OH, 7β-OH and 25-OH) was 206 nm, except for 7-k, which was taken at 233 nm (Vicente & Torres, 2007).

2.3.6. Sensory evaluation

Fifty panelists (42 women and 8 men), recruited from among the staff and graduate students of the School of Public Health, University of São Paulo, tested the cooked chicken meat, without any knowledge as to the formulation of the products. Pieces of cooked chicken thigh and breast meat of approximately 2.5 cm in length were presented to each panelist in random order. Each panelist received three different uniform samples: oregano + sage, oregano + sage + 5% honey and oregano + sage + 10% honey. The samples were coded with three-digits. The thigh and breast samples were served immediately after cooking. The testing was done in individual booths and under fluorescent light. Acceptance was scored on a structured hedonic scale: 1 – dislike extremely; 2 – dislike very much; 3 – dislike moderately; 4 – dislike slightly; 5 – neither like nor dislike; 6 – liked slightly; 7 – liked moderately; 8 – liked very much; and 9 – like extremely. The panelists were asked to assign an order to the samples, in accordance with taste preferences (Meilgaard, Carr, & Civille, 1999). The panelists were also invited to provide their own comments about the samples.

The results from the hedonic scale were subjected to one-way analysis of variance (P < 0.05), frequency distribution analysis, and the Tukey test. The preference ranking (P < 0.01) was analyzed using the Friedman test (Resurrección, 1998). FIZZ (sensory analysis and consumer test management software version 2.00, Biosystems) was used for the statistical analysis.

2.3.7. Statistical analysis

The results from the experiments were used as variables and analyzed by means of one-way analysis of variance (ANOVA). The means for the different antioxidant combinations on lipid oxidation in samples were compared using the Tukey multiple comparisons test, and statistical significance was set at P < 0.05. The statistical assessment was carried out using the SPSS for Windows software system (version 10.0).

3. Results and discussion

3.1. Chemical analysis, pH and thiobarbituric acid-reactive substances (TBARs)

The moisture and total lipid content of the cooked chicken thigh and breast meat during refrigerated storage are presented in Table 1. The total lipids were calculated on a dry weight basis. The moisture levels ranged from 6.1 ± 0.9 to 24.4 ± 4.6 g/100 g in cooked thigh and 14.4 ± 0.4 – 63 ± 1.4 g/100 g (dry basis) for cooked breast, respectively. The increase in moisture content of the samples could be to the effect of salt in increasing the binding of water to the protein molecules. Oxidative damages to the sarcoplasm may also involve changes in moisture loss and texture (Ladikos & Lougovois, 1990). One possible explanation for the observed effect with the honey could be that the sugar-containing provided a hygroscopic effect, thereby reducing moisture loss from the inter- and intra-muscular fibres. In addition, the protection of muscle membrane from lipid oxidation by applying lipid-soluble antioxidants can also maintain membrane integrity of muscle fibres and reduce moisture loss (Mitsumoto, Arnold, Schaefer, & Cassens, 1995).

The lipid values ranged from 6.1 ± 0.9 to 24.4 ± 4.6 g/100 g (dry basis) in cooked thigh and 14.4 ± 0.4 – 63 ± 1.4 g/100 g (dry basis) for cooked breast, respectively. The lipid contents were significantly lower after 96 h of refrigeration in all samples. Thus, the important losses of total lipids contents in chicken samples have been considered to be related to the antioxidation of lipids. The results agree with those reported by (Igene, Pearson, Merkel, & Coleman, 1979) this authors showed that losses observed in total lipids during storage were primarily to changes in the triglycerides levels.

The pH values for cooked thigh during refrigeration ranged from 6.3 to 6.9, while in cooked breast, the measured values were 5.7 – 6.4 (data not shown). Chicken breast presented lower pH values than in chicken thigh (p < 0.05).

The results from the analysis on the oxidative degradation of lipids from cooked chicken thigh and breast during refrigeration, as measured by TBARs numbers, are shown in Fig. 1.

The effectiveness of the natural antioxidants for reducing the velocity of lipid oxidation in cooked chicken thigh and breast was demonstrated after 48 and 96 h of refrigeration at 4 °C. The analysis of variance on the TBARs data indicated that the TBARs values were significantly affected by natural antioxidants throughout refrigeration (p < 0.05).

The initial TBARs values (0 h) for all the samples containing antioxidants were significantly lower than those found for the control group (p < 0.05). After 96 h, the samples with added herbs and honey presented lower TBARs values than those of the control and BHT samples, thus suggesting that sage, oregano and honey presented a better antioxidant effect than shown by BHT. Oregano + sage + 5% honey was the most effective treatment for reducing lipid oxidation in cooked chicken breast after 96 h, these results are in agreement with the study of (McKibben & Engeseth, 2002). The treatments with oregano + sage, oregano + sage + 5% honey and oregano + sage + 10% honey in cooked chicken thigh after 96 h of refrigeration presented similar behaviour (p > 0.05). These results...
suggest that these antioxidants retarded lipid oxidation immediately after cooking and during storage under refrigeration. The results agree with those reported by Juntachote et al. (2006).

Lipid oxidation occurred more rapidly in cooked chicken breast than in cooked chicken thigh (Fig. 1). These results were also observed in the studies by Gong, Parker, and Richards (2010) in analyses on duck breast and thigh meat and by Alasnier, Meynier, Viau, and Gandemer (2000) in evaluations on lipid oxidation in chicken breast and thigh muscles. Part of the explanation for this probably involves the high pH of chicken thigh, and this may be the factor that slowed lipid oxidation during refrigerated storage, compared with chicken breast. Elevated pH decreases the ability of metals and heme proteins to oxidize lipids (Gong et al., 2010). In addition, Alasnier et al. (2000) suggested that even though thigh muscles contained more pro-oxidant agents such as iron and potential substrate, the antioxidant status of thigh muscles represented by vitamin E and the antioxidant system (such as glutathione and antioxidant enzymes) remained efficient during the first stage of storage, such that they slowed down the rate of lipid oxidation.

### Table 1

Moisture (g/100 g) and total lipid content (g/100 g) (dry basis) of cooked chicken thigh and breast meat.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Thigh</th>
<th>0 h</th>
<th>48 h</th>
<th>96 h</th>
<th>Breast</th>
<th>0 h</th>
<th>48 h</th>
<th>96 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (g/100 g)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Control</td>
<td>61.5 ± 0.8</td>
<td>62.0 ± 1.3</td>
<td>x</td>
<td>62.4 ± 0.2</td>
<td>x</td>
<td>65.2 ± 0.7</td>
<td>x</td>
<td>66.2 ± 1.5</td>
</tr>
<tr>
<td>BHT</td>
<td>60.5 ± 0.4</td>
<td>63.0 ± 0.5</td>
<td>x</td>
<td>64.8 ± 1.6</td>
<td>x</td>
<td>65.3 ± 0.6</td>
<td>x</td>
<td>65.4 ± 1.1</td>
</tr>
<tr>
<td>Oregano + sage</td>
<td>58.3 ± 1.7</td>
<td>59.3 ± 0.3</td>
<td>x</td>
<td>62.0 ± 0.1</td>
<td>x</td>
<td>66.3 ± 0.2</td>
<td>x</td>
<td>66.1 ± 0.9</td>
</tr>
<tr>
<td>Oregano + sage + 5% honey</td>
<td>58.5 ± 1.4</td>
<td>59.9 ± 1.1</td>
<td>x</td>
<td>63.5 ± 1.4</td>
<td>x</td>
<td>67.1 ± 1.0</td>
<td>x</td>
<td>65.3 ± 0.9</td>
</tr>
<tr>
<td>Oregano + sage + 10% honey</td>
<td>61.2 ± 0.3</td>
<td>52.4 ± 0.2</td>
<td>x</td>
<td>57.5 ± 1.2</td>
<td>x</td>
<td>67.0 ± 1.0</td>
<td>x</td>
<td>66.6 ± 1.1</td>
</tr>
<tr>
<td>Lipids (g/100 g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Control</td>
<td>14.8 ± 1.6</td>
<td>10.0 ± 0.6</td>
<td>x</td>
<td>8.45 ± 2.3</td>
<td>x</td>
<td>4.2 ± 0.8</td>
<td>x</td>
<td>6.3 ± 1.4</td>
</tr>
<tr>
<td>BHT</td>
<td>20.8 ± 1.1</td>
<td>10.6 ± 1.4</td>
<td>x</td>
<td>13.9 ± 2.2</td>
<td>x</td>
<td>6.0 ± 1.0</td>
<td>x</td>
<td>4.5 ± 0.6</td>
</tr>
<tr>
<td>Oregano + sage</td>
<td>9.2 ± 0.6</td>
<td>6.1 ± 0.9</td>
<td>x</td>
<td>7.0 ± 0.7</td>
<td>x</td>
<td>3.3 ± 0.4</td>
<td>x</td>
<td>1.2 ± 0.4</td>
</tr>
<tr>
<td>Oregano + sage + 5% honey</td>
<td>20.0 ± 2.8</td>
<td>14.9 ± 2.0</td>
<td>x</td>
<td>12.6 ± 0.6</td>
<td>x</td>
<td>5.9 ± 1.3</td>
<td>x</td>
<td>4.9 ± 0.4</td>
</tr>
<tr>
<td>Oregano + sage + 10% honey</td>
<td>24.4 ± 4.9</td>
<td>12.0 ± 1.3</td>
<td>x</td>
<td>15.6 ± 3.8</td>
<td>x</td>
<td>4.9 ± 2.3</td>
<td>x</td>
<td>2.2 ± 0.1</td>
</tr>
</tbody>
</table>

Results are expressed as means ± standard deviations of the three replicates. “Averages with different letters in the same row indicate significant differences (p < 0.05). “Averages with different letters in the same column indicate significant differences (p < 0.05).

**Fig. 1.** Progression of lipid oxidation during refrigeration storage of cooked chicken thigh and breast meat measured by TBARs values.
Analysis on our data showed that all of the three combinations of natural antioxidants tested would be beneficial for reducing the velocity of lipid oxidation in both chicken meats during storage. These findings are corroborated by other authors who have added honey and herbs and thereby inhibited the development of lipid oxidation in cooked meats during refrigeration time (Antony et al., 2000; McKibbon & Engeseth, 2002; Juntachote et al., 2007a).

3.2. Effect of antioxidant combinations on hexanal values of cooked chicken meat

The hexanal determination method was validated in accordance with (AOAC, 2002). Complete validation of the hexanal method was achieved through determining linearity ($R^2 = 0.990$), detection limits (0.268 µg/g), quantification limits (0.895 µg/g), repeatability (CV = 2.2%) and recovery (97.3%).

The hexanal values of cooked chicken meat treated with natural antioxidants during refrigeration are shown in Table 2. The results from hexanal concentrations showed that all the samples presented increased hexanal levels at all durations of refrigeration. The treatments that presented the lowest hexanal values after 96 h of refrigeration were oregano + sage + 5% honey and oregano + sage + 10% honey in chicken meats. These results were in agreement with (Antony et al., 2000) who reported that hexanal was produced in the largest amounts in cooked meat samples and cooked samples stored for 48 h at 4°C. Similar observations have been also made by (Juntachote et al., 2007b) in cooked ground pork sausages with various added antioxidants. The results from our study indicated that honey, oregano and sage exhibited greater antioxidant efficacy than shown by BHT, regarding hexanal formation. The high capacity antioxidant of honey is related to the flavonoids, ascorbic acid, tocopherols, catalase, phenolic compounds and Maillard reaction products (Johnston et al., 2005; McKibbon & Engeseth, 2002). Sage and oregano exhibit antioxidants properties in different food systems (Mariuti, Nogueira, & Bragagnolo, 2011).

3.3. Changes in conjugated dienes during refrigeration of cooked chicken meat

The results from the analysis on conjugated dienes in cooked chicken meat are presented in Table 2. The evaluation on the results from conjugated diene analyses showed that all of the samples presented increases significantly ($p < 0.05$) with the exception of the control (breast) during 48 h of refrigeration and then decreased until the end of storage. After 96 h of refrigeration cooked chicken meat treated with BHT was the most resist to oxidation, as evidenced by the lowest value, followed by control, oregano + sage + 10% honey, oregano + sage + 5% honey and oregano + sage. The increase in conjugated diene formation was observed until the end of the period tested (96 h of refrigeration). This effect was enhanced by higher oxygen availability. According to the mechanism suggested by lipid oxidation the conjugated diene formation precedes the hexanal and TBARS formation during refrigeration of cooked chicken meat.

However, the results from the present study suggest that additions of oregano, sage, honey and BHT applied to cooked chicken meats were able to increase the resistance to oxidation, in comparison with the control group, as shown by the lower conjugated diene contents presented in antioxidant groups immediately after cooking. This is in agreement with previous studies, in which it was reported that the concentration of conjugated dienes significantly increased in cooked pork patties treated with antioxidants, over the refrigeration period (Juntachote et al., 2006; Lee et al., 2010).

3.4. Changes in fatty acid composition in processed chicken meat during refrigeration

The fatty acid composition and the effects of cooking on chicken meat, over the course of refrigeration, are presented in Tables 3 and 4.

Palmitic acid was the main saturated fatty acid (SFA) in the chicken thigh samples. The values of monounsaturated fatty acids (MUUFAs) were 2.6–11.1 (g/100 g) (dry basis), oleic acid the most predominant MUFA. The range of polyunsaturated fatty acids (PUFAs) was 1.50–4.9 (g/100 g) (dry basis). During refrigeration, the levels of PUFAs decreased in all treatments.

The total SFA in chicken breast meat ranged from 1.6 to 6.7 (g/100 g) (dry basis), of which palmitic acid accounted for the highest amount. The MUFA range was from 2.5 to 9.5 (g/100 g) (dry basis), and oleic acid was the most abundant MUFA. The levels of PUFA ranged from 1.7 to 6.0 (g/100 g) (dry basis), consisting mostly of linoleic acid. A significant decrease during refrigerated storage ($p < 0.05$) was observed in the PUFA profile for all samples.

The amount of MUFA and PUFA gradually decreased during refrigeration of cooked chicken meat as a likely consequence of the development of oxidative reactions. Results obtained in the present study suggest that the oxidative degradation of PUFA mainly happened after 96 h of refrigeration probably due to the gradual degradation of endogenous antioxidants and the release of iron from the heme molecule, indicating a close relationship between the lipid oxidation and the refrigeration period.

### Table 2

Hexanal content (µg/g – dry basis) and conjugated dienes (%) of cooked chicken thigh and breast meat during refrigeration.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Thigh</th>
<th></th>
<th></th>
<th>Breast</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0 h</td>
<td>48 h</td>
<td>96 h</td>
<td>0 h</td>
<td>48 h</td>
<td>96 h</td>
</tr>
<tr>
<td>Hexanal content (µg/g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.55 ± 0.0&lt;sup&gt;±&lt;/sup&gt;</td>
<td>4.20 ± 0.3&lt;sup&gt;±&lt;/sup&gt;</td>
<td>4.31 ± 0.1&lt;sup&gt;±&lt;/sup&gt;</td>
<td>7.20 ± 0.8&lt;sup&gt;±&lt;/sup&gt;</td>
<td>10.29 ± 0.7&lt;sup&gt;±&lt;/sup&gt;</td>
<td>10.36 ± 0.3&lt;sup&gt;±&lt;/sup&gt;</td>
</tr>
<tr>
<td>BHT</td>
<td>3.09 ± 0.2&lt;sup&gt;±&lt;/sup&gt;</td>
<td>4.09 ± 0.1&lt;sup&gt;±&lt;/sup&gt;</td>
<td>4.54 ± 0.2&lt;sup&gt;±&lt;/sup&gt;</td>
<td>6.05 ± 0.4&lt;sup&gt;±&lt;/sup&gt;</td>
<td>6.58 ± 0.3&lt;sup&gt;±&lt;/sup&gt;</td>
<td>8.29 ± 0.1&lt;sup&gt;±&lt;/sup&gt;</td>
</tr>
<tr>
<td>Oregano + sage</td>
<td>2.07 ± 0.0&lt;sup&gt;±&lt;/sup&gt;</td>
<td>2.21 ± 0.0&lt;sup&gt;±&lt;/sup&gt;</td>
<td>2.52 ± 0.0&lt;sup&gt;±&lt;/sup&gt;</td>
<td>4.53 ± 0.1&lt;sup&gt;±&lt;/sup&gt;</td>
<td>6.67 ± 0.5&lt;sup&gt;±&lt;/sup&gt;</td>
<td>7.26 ± 0.7&lt;sup&gt;±&lt;/sup&gt;</td>
</tr>
<tr>
<td>Oregano + sage + 5% honey</td>
<td>2.24 ± 0.0&lt;sup&gt;±&lt;/sup&gt;</td>
<td>2.29 ± 0.0&lt;sup&gt;±&lt;/sup&gt;</td>
<td>2.40 ± 0.0&lt;sup&gt;±&lt;/sup&gt;</td>
<td>8.48 ± 0.0&lt;sup&gt;±&lt;/sup&gt;</td>
<td>3.06 ± 1&lt;sup&gt;±&lt;/sup&gt;</td>
<td>4.02 ± 0.2&lt;sup&gt;±&lt;/sup&gt;</td>
</tr>
<tr>
<td>Oregano + sage + 10% honey</td>
<td>2.32 ± 0.0&lt;sup&gt;±&lt;/sup&gt;</td>
<td>1.88 ± 0.0&lt;sup&gt;±&lt;/sup&gt;</td>
<td>2.07 ± 0.0&lt;sup&gt;±&lt;/sup&gt;</td>
<td>7.90 ± 0.0&lt;sup&gt;±&lt;/sup&gt;</td>
<td>3.48 ± 0.4&lt;sup&gt;±&lt;/sup&gt;</td>
<td>4.96 ± 0.5&lt;sup&gt;±&lt;/sup&gt;</td>
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<table>
<thead>
<tr>
<th>Conjugated dienes (%)</th>
<th></th>
<th></th>
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<tr>
<td>Control</td>
<td>0.85 ± 0.0&lt;sup&gt;±&lt;/sup&gt;</td>
<td>1.60 ± 0.4&lt;sup&gt;±&lt;/sup&gt;</td>
<td>0.98 ± 0.2&lt;sup&gt;±&lt;/sup&gt;</td>
<td>2.59 ± 0.2&lt;sup&gt;±&lt;/sup&gt;</td>
<td>1.05 ± 0.2&lt;sup&gt;±&lt;/sup&gt;</td>
<td>2.03 ± 0.6&lt;sup&gt;±&lt;/sup&gt;</td>
</tr>
<tr>
<td>BHT</td>
<td>0.73 ± 0.0&lt;sup&gt;±&lt;/sup&gt;</td>
<td>1.32 ± 0.3&lt;sup&gt;±&lt;/sup&gt;</td>
<td>1.75 ± 0.4&lt;sup&gt;±&lt;/sup&gt;</td>
<td>2.07 ± 0.2&lt;sup&gt;±&lt;/sup&gt;</td>
<td>1.54 ± 0.2&lt;sup&gt;±&lt;/sup&gt;</td>
<td>2.35 ± 0.3&lt;sup&gt;±&lt;/sup&gt;</td>
</tr>
<tr>
<td>Oregano + sage</td>
<td>1.03 ± 0.1&lt;sup&gt;±&lt;/sup&gt;</td>
<td>0.34 ± 0.1&lt;sup&gt;±&lt;/sup&gt;</td>
<td>2.68 ± 0.4&lt;sup&gt;±&lt;/sup&gt;</td>
<td>1.64 ± 0.0&lt;sup&gt;±&lt;/sup&gt;</td>
<td>3.38 ± 1.3&lt;sup&gt;±&lt;/sup&gt;</td>
<td>3.28 ± 1.3&lt;sup&gt;±&lt;/sup&gt;</td>
</tr>
<tr>
<td>Oregano + sage + 5% honey</td>
<td>0.32 ± 0.2&lt;sup&gt;±&lt;/sup&gt;</td>
<td>0.34 ± 0.2&lt;sup&gt;±&lt;/sup&gt;</td>
<td>2.12 ± 0.3&lt;sup&gt;±&lt;/sup&gt;</td>
<td>0.80 ± 0.0&lt;sup&gt;±&lt;/sup&gt;</td>
<td>3.34 ± 0.5&lt;sup&gt;±&lt;/sup&gt;</td>
<td>3.38 ± 0.2&lt;sup&gt;±&lt;/sup&gt;</td>
</tr>
<tr>
<td>Oregano + sage + 10% honey</td>
<td>0.11 ± 0.0&lt;sup&gt;±&lt;/sup&gt;</td>
<td>1.05 ± 0.1&lt;sup&gt;±&lt;/sup&gt;</td>
<td>1.52 ± 0.5&lt;sup&gt;±&lt;/sup&gt;</td>
<td>0.32 ± 0.1&lt;sup&gt;±&lt;/sup&gt;</td>
<td>3.79 ± 0.3&lt;sup&gt;±&lt;/sup&gt;</td>
<td>3.24 ± 0.2&lt;sup&gt;±&lt;/sup&gt;</td>
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</tbody>
</table>

Results are expressed as means ± standard deviations of the three replicates. *<sup>±</sup>Averages with different letters in the same row indicate significant differences ($p < 0.05$). **<sup>±</sup>Averages with different letters in the same column indicate significant differences ($p < 0.05$).
According to results, unsaturated lipids are more susceptible to lipid oxidation because hydrogen atoms can be more easily abstracted from polyunsaturated fats than saturated fats. This fact explains the higher values of hexanal, TBARs and conjugated dienes in chicken breast than in thigh meat.

3.5. Effect of antioxidant combinations on free cholesterol and cholesterol oxides values for cooked chicken meat during refrigeration.

Free cholesterol and cholesterol oxides extraction and quantification methods were validated to assure the reliability of the results. Quantification was performed by means of external calibration methods were validated to assure the reliability of the recoveries for cholesterol oxides values for cooked chicken meat. The quantification limits were 0.7 g/g for 7α-OH, 0.24 g/g for 25-OH, 0.53 g/g for 7β-OH and 0.35 g/g for cholesterol. Cholesterol presented recovery of 98.2%, whereas the cholesterol oxides presented recoveries from 94.1% to 99.3%.

Free cholesterol values between 208.1 ± 4.9 and 395.2 ± 22.5 mg/100 g (dry basis) were found for cooked chicken thigh, while in cooked chicken breast, values ranged from 221.5 ± 0.5 to 281.9 ± 1.5 mg/100 g (dry basis).

During the cooking and over the course of refrigerated storage, only traces of free cholesterol oxides were found (25-OH, 7-k, 7α-OH and 7β-OH), except for the BHT treatment on cooked thigh meat after 48 h, in which the presence of 7α-OH was observed (3.49 μg/g – dry basis). According to (Baggio & Bragagnolo, 2006; Vicente, Sampaio, Ferrari, & Torres, 2011) the use of antioxidants in the formulations and appropriated conditions of storage in processed meat products probably protected the cholesterol against oxidation.

3.6. Sensory acceptability and preference

The thigh and breast cooked chicken meats presented high sensory acceptability, as shown in Fig. 2. The panelists accepted (grades ≥ 6) all the samples of thigh meat, which reached 78% for oregano + sage, 92% for oregano + sage + 5% honey and 86% for oregano + sage + 10% honey. The samples with oregano + sage + 5% honey and oregano + sage + 10% honey were equally preferred by the panelists.

No significant difference (p > 0.05) in acceptability between oregano + sage + 5% honey (grades ≥ 6 = 90%) and oregano + sage + 10% honey (grades ≥ 6 = 94%) was observed in the breast meat. The results showed that the oregano + sage + 5% honey samples were significantly preferred (p < 0.05).

The samples were considered acceptable if 50% or more of the responses were in the range from 6 to 9 on the hedonic scale.

Flavour is an important meat quality attribute and is strongly associated with the perception of meat palatability, which is a...
determinant of whether a consumer will repurchase a specific type of meat or not (Goodson et al., 2002).

The sensory evaluation indicated that the use of sage, oregano and honey resulted in a pleasant flavour for cooked chicken meats.

4. Conclusion

In this study, the results confirmed the antioxidative effect of oregano, sage and honey when added prior to processing; these ingredients increased the shelf life of cooked chicken meat after 96 h of refrigeration at 4 °C. The addition of natural antioxidants on the breast and thigh cooked chicken improved the oxidative stability of lipids, reducing the degradation better than control samples used, and resulted in a product with a great acceptability.

Acknowledgements

We would like to address our thanks to the State of São Paulo Research Foundation (FAPESP) and Coordination Office for Academic Staff Improvement (CAPES) for their support and for granting scholarships to the authors, as well as to the Postgraduate in Applied Human Nutrition Program (PRONUT) of the University of São Paulo.

References


