Original

Lipid and protein oxidation in the internal part of Italian type salami containing basil essential oil (*Ocimum basilicum* L.)

Oxidação dos lipídios e das proteínas na parte interna do salame tipo italiano contendo óleo essencial de manjericão (Ocimum basilicum L.)

Alexandre José CICHOSKI[1]*, Rogério Luis CANSIAN[2], Débora de OLIVEIRA[2], Iloir GAIO[2], Adriana Galon SAGGIRATO[2]

Abstract

Different concentrations of basil essential oil (*Ocimum basilicum* L.) (0.19; 0.38; 0.75; 1.87; 3.75 and 6.00 mg.g^{-1}) were evaluated in relation to their antioxidant activity using the DPPH• radical methodology. From the IC_{50} obtained data, the concentrations of 0.19; 0.38; 0.75; 1.87; 3.75; 6.00 and 12.00 mg.mL^{-1} were applied directly to the product and these were sensorially evaluated by the test of control difference. The concentrations related to the highest acceptability (0.19; 0.38 and 0.75 mg.g^{-1}) were tested for antioxidant activity in the internal part of Italian type salami - during the processing and after 30 days of storage, in terms of lipid and protein oxidation. The oxidation of lipids was determined using the method of TBARS. The method of carbonyl compounds was employed for proteins oxidation. Five different formulations of salami were elaborated: blank (without the use of antioxidant); control (using sodium eritorbate as antioxidant); and adding 0.19; 0.38 and 0.75 mg.g^{-1} of basil essential oil. The product was kept between 25 °C and 18 °C and UR between 95% and 70%, for 28 days. Analyses were carried out on the processing day and after 2, 7, 14, 21 and 28 days, and also following 30 days of storage. The basil essential oil in vitro presented an antioxidant activity of IC_{50} 12 mg.mL^{-1}. In the internal part of the Italian type salami the commercial antioxidant (control) and the formulation containing 0.75 mg.g^{-1} of basil essential oil presented antioxidant activity in relation to the lipids, but not to the proteins - during processing and storage.

Keywords: *TBARS; Carbonyl; salami; Ocimum basilicum L.; natural antioxidant.*

Resumo

Diferentes concentrações de óleo essencial de manjericão (*Ocimum basilicum* L.) (2,5; 3,75; 5,0; 7,5; 10; 12,5; 15; 17,5; e 20 mg.mL^{-1}) foram avaliadas com relação à sua atividade antioxidante, utilizando a metodologia do radical DPPH•. A partir dos dados de IC_{50} obtidos, as concentrações de 0,19; 0,38; 0,75; 1,87; 3,75; 6,00; e 12,00 mg.mL^{-1} foram aplicadas diretamente no produto e foram avaliados sensorialmente pelo teste de diferença do controle. As concentrações com maior aceitabilidade sensorial (0,19; 0,38; e 0,75 mg.g^{-1}) foram testadas com relação à atividade antioxidante na parte interna de salame tipo italiano, durante a etapa de processamento e após 30 dias de armazenamento, em termos da oxidação de lipídios e proteínas. Para a oxidação dos lipídios utilizou-se o método de TBARS, enquanto que para a oxidação das proteínas no salame, empregou-se o método dos compostos carbonil. Foram elaboradas 5 formulações de salames: branco (sem nenhum tipo de antioxidante), controle (com eritorbato de sódio, antioxidante comercial) e com 0,19; 0,38 e 0,75 mg.g^{-1} de óleo essencial de manjericão, que permaneceram 28 dias, em temperaturas entre 25 °C e 18 °C e UR entre 95% e 70% (etapa de processamento). As análises foram realizadas: no dia do processamento e decorridos 2, 7, 14, 21 e 28 dias deste, assim como com 30 dias de armazenamento. O óleo de manjericão "in vitro" apresentou IC_{50} 12 mg.mL^{-1} de atividade antioxidante e, na parte interna do salame, o antioxidante comercial (controle) e o óleo de manjericão na concentração de 0,75 mg.g^{-1} apresentaram atividade antioxidante frente aos lipídios, mas não frente às proteínas, durante o processamento e armazenamento.

Palavras-chave: *TBARS; Carbonil; salame; Ocimum basilicum L.; antioxidante natural.*

1 Introduction

Several varieties of condiment plants are used in meat products with the purpose of enhancing flavor and appearance, but whether these plants exercise antioxidant and/or antimicrobial activity is not taken into consideration (SHARMA et al., 1981). From these plants, through hydrodistillation, for example, one can extract essential oils from leaves, flowers and seeds. These oils are characterized as colorless or slightly yellowish, and have an intense aroma (OUSSALAH et al., 2007).

Fresh or dry basil leaves (*Ocimum basillicum* L.) are commercially used for flavoring and seasoning; they contain phenolic compounds such as cinamic, caffeic, synaptic and ferulic acids that have comproved antioxidant activity (GRAYER et al., 1996; LOUGHRIN; KASPERBAUER, 2001).

Basil essential oil extracted from its leaves (fresh or dry) and flowers has been used as flavoring agent by the pharmaceutical

Received 30/06/2009
Accepted 30/11/2009 (004279)

[1] *Departamento de Tecnologia e Ciencia dos Alimentos, Universidade Federal de Santa Maria – UFSM, Centro de Ciencias Rurais, Campus Universitário, Av. Roraima, 1000, CEP 97105-900, Camobi, Cidade universitária, Santa Maria, RS, Brasil, E-mail: ajcichoski@hotmail.com*
[2] *Departamento de Engenharia de Alimentos, Universidade Regional Integrada – URI, Campus de Erechim, Av. Sete de Setembro, 1621, CEP 99700-000, Erechim, RS, Brasil*
**Corresponding author*

industry (JAVANMARDI et al., 2002) due to its antimicrobial and antioxidant properties. In view of those properties, basil oil can also be used in food, since the substitution of synthetic antioxidants by natural ones is becoming relevant in the modern concept of food industry (BOZIN et al., 2006).

Gas chromatography (GC) is broadly employed in the evaluation of chemical compounds of essential oils and is considered as an efficient separation method of the different complex structures present in oils (RADULESCU; CHILIMENT; OPREA et al., 2004; BOZIN et al., 2006).

The literature points out that toxicity caused by basil essential oil was observed in rats at doses of 1500 $mg.kg^{-1}$ of body weight and higher, when the administration occurred daily for 14 days. In this case, the eugenol present in the oil can cause dhermatitys and other undesirable effects. The mean lethal dose (LD_{50}) of basil essential oil in rats was 3250 $mg.kg^{-1}$ of body weight and the secure dose in humans is 0.7 $mg.kg^{-1}$/day (FANDOHAN et al., 2008).

Several methods can be used to determine the antioxidant activities of extracts and essential oils in vitro. One of the most frequently used methods consists of evaluating the scavenging activity of the 2.2-diphenyl-1-picryl-hydrazil free radical (DPPH•) (MIRANDA; FRAGA, 2006). *Hippomarathrum microcarpum, Rosmarinus officinalis, Artemisia fragrans* and *Artemisia austriaca* essential oils show efficient concentrations (EC) or inhibitory concentrations (IC_{50}) of 10.69 $mg.mL^{-1}$, 20 $mg.mL^{-1}$, 7.86 $mg.mL^{-1}$ and 8.06 $mg.mL^{-1}$, respectively (WANG et al., 2007), through the DPPH• radical method.

Lipid oxidation is the main deterioration factor in meat products during processing and mainly during storage, contributing to the development of undesirable flavors, rancid odor, color change and formation of toxic compounds such as 4-hidroxinonenal. Researchers have been focusing on malonaldehyde and other products formed during lipid oxidation due to a probable carcinogenic action (ZANARDI et al., 2004).

Proteins are more susceptible to oxidation when there is a decrease in the quantity of antioxidant agents, with the development of oxidized lipids and in the presence of metallic ions and oxidizing enzymes (LIU; XIONG, 1996; PARK et al., 2006). Oxidation modifies the structures of proteins, causing the destruction of amino acids, lowering solubility and causing loss of enzymatic activity. The amino acids that are more susceptible to oxidation are cysteine, methionine, lysine, arginine, histidine, triptofan, tyrosine, valine, serine, leucine, phenylalanine, proline and glutamic acid (XIONG, 2000), and the products formed belong to the carbonyl group, which can be quantified, thus indicating the oxidation level (LEVINE et al., 1990).

Several studies on antioxidant activity of essential oils have been published recently. Nevertheless, there is still a gap in the literature concerning its practical utilization, including basil essential oil in salami and its antioxidant activity on lipids and proteins, and also the sensorial acceptance of the final product. As a matter of fact, Koutsoumanis et al. (1998) stated that it is necessary to know which concentrations of essential oil are necessary to achieve a balance between efficiency as an antimicrobial and/or antioxidant and sensorial acceptability.

Taking into account the statements presented above, the purpose of this study was to evaluate the antioxidant activity of basil essential oil in vitro and in the internal part of Italian type salami by monitoring the oxidation of lipids and proteins during the processing stage (0 to 28 days) and after 30 days of storage at 22 °C, using the oil concentrations previously determined as sensorially acceptable.

2 Material and methods

2.1 Material

One hundred grams of basil leaf (*Ocimum basilicum*) were dried at 25 (±2 °C) until constant weight, they were then submitted to hydrodistillation (Clevenger) with 300 mL of distilled water at 100 °C for 90 minutes, cooling down the condensate at 4 °C. The yield was calculated in the device itself, with measurements taken at each 5 minutes. The residual water present in the oil was initially separated by density difference, and later, by filtration with sodium sulfate anhydrous. The oil was placed in an amber glass recipient and stored at -18 °C until the moment of use.

2.2 Chemical compounds of basil oil

The chemical compounds of basil oil were determined through Gas Chromatography and Mass Spectrometry (GC/MS) in Shimadzu device (Model QP 5050A) with the use of an oil sample dissolved in dichloromethane (Merck) at 5,000 ppm concentration. It was used a capillary column DB-5 (Dimethyl polysiloxane) 30 m × 0.25 mm; helium gas as carrier (1 mL/minute) at 1.0 Kv; Split Mode (1:20), and injector and interface at 280 °C. The initial temperature was programmed as from 50 °C (4 minutes); 2.5 °C/minute from 50 °C to 180 °C and 5 °C/minute from 180 °C to 280 °C. The solvent cut time was 4 minutes and the total time of analysis was 76 minutes. The mass spectra was compared to standards from the Willey library, considering a minimal similarity of 90% and also by visual comparison of the mass spectra to substances found in the literature. The compounds were listed based on percent of normalized area and retention time.

2.3 Antioxidant activity in vitro by free radical test 2.2–diphenyl–1 picryl-hydrazil (DPPH•) methodology

The antioxidant activity in vitro of essential oil was evaluated through the DPPH• method. The methodology was based in the measurement of absorption extinction of the 2.2-diphenyl-1-picryl-hydrazil free radical (DPPH•), related to solutions containing different concentrations of basil oil (2.5, 3.75, 5.0, 7.5, 10, 12.5, 15, 17.5 and 20 $mg.mL^{-1}$) in ethanol. The scavenging percentage of the DPPH• radical was calculated in terms of percentage of antioxidant activity (AA%), using the following Equation 1:

$$AA\% = 100 - \{[(Abs._{sample} - Abs._{white}) \times 100]/Abs._{control}\} \quad (1)$$

A spectrophotometer was used (Agilent Technologies, model 8453E) and the readings were carried out at 515 nm (MIRANDA; FRAGA, 2006). The concentration of essential oil was calculated through regression analysis, in order to scavenge 50% of the DPPH• radical, which corresponded to the efficient concentration (EC) or inhibitory concentration (IC_{50}).

2.4 Salami manufacturing procedure

The salami used in this study consisted of 55.34% pork meat, 30% cow meat, ground into 8 mm pieces; 10% bacon fat cubes; starters *Pediococcus pentosaceus* and *Staphylococcus xylosus* (Lyocarni RHM 33, Sacco Brazil); 2% NaCl; 0.3% $NaNO_2$ (Kraki); 0.25% sodium eritorbate (Kraki); 1% condiment for Italian type salami (Kraki); 0.06% and 0.05% ground pepper (white and black, respectively); and 1% of sucrose. Calculations were based on the amount of meat used in the salami production. Sucrose was used as the means to insert basil oil in the meat mixture and the other ingredients were added in the traditional way and mixed for 5 minutes. The meat mixture remained at 20 °C for 3 hours; it was then submitted to stuffing in collagen casing caliber 50 mm (Kraki), previously immersed in water containing 8% NaCl at 30 °C. The salami treatments had an average weight of 650 g. The product was placed in a chamber for 28 days. The temperature and relative humidity (RH) were slowly decreased, according to the following conditions: 25 °C and RH 95% (1st day), 24 °C and RH 92% (2nd day), 23 °C and RH 89% (3rd day), 22 °C and RH 86% (4th day), 21 °C and RH 83% (5th day) , 20 °C and RH 80% (6th day), 19 °C and RH 80% (7th day), and at 18 °C and RH 75% 8th to 28th day).

Five treatments of salami were determined: control (with sodium eritorbate commercially available as antioxidant), blank (without antioxidant) and three treatments with basil oil at concentrations of 0.75, 0.38 and 0.19 $mg.g^{-1}$. To define the concentrations, the treatment containing salami IC_{50} in vitro (12 $mg.g^{-1}$) was determined first, and the other concentrations were determined in sequence (6, 3.75, 1.87, 0.75, 0.38 and 0.19 $mg.g^{-1}$, which corresponded to half, up to 1/64 of IC_{50}). These concentrations were sensorial evaluated by 40 panelists, along with the control and blank formulations, according to the methodology described by Faria and Yotsuyanagi (2002). The difference of control test, constituted by a structured scale of 10 points (0 – with no difference from the control and/or blank and 9 – extremely different from the control and/or blank), had the purpose to determine which concentrations would be sensorially accepted.

In order to monitor the antioxidant activity in the different salami formulations, the analyses were performed as day zero and after 2, 7, 14, 21 and 28 days of processing, as well as after the salami was vacuum packed and stored for 30 days at 22 °C, protected from the incidence of light.

2.5 Lipid oxidation

Lipid oxidation was measured with tiobarbituric acid reactive substances (TBARS) (RAHARJO; SOFOS; SCHMIDT et al., 1992), modified by Wang et al. (2002) by monitoring the interference of sugar in the reaction. Results were expressed as mg malonaldehyde (MDA) per kg of salami. This analysis is an estimation of lipid oxidation, since it determines the substances reactive to tiobarbituric acid. Results were expressed in $mg.MDA.kg^{-1}$ of product.

2.6 Protein oxidation

Protein oxidation was measured through estimation of carbonyl groups formed during the experiment (LEVINE et al., 1990) with slight modifications. The concentration of protein was calculated at 280 nm in the chloride acid (HCl) control using bovine serum albumin (BSA) in 6 m guanidine as standard. Carbonyl concentration in the treated sample was measured with 2,4-dinitrophenylhydrazine (DNPH) incorporated to the basis of absorption of 21.0 $nm^{-1}.cm^{-1}$ at 370 nm of protein hydrazones. Results were expressed as nanomoles of DNPH per milligram of protein.

2.7 Statistical analysis

The analysis was performed in threefold and results were statistically evaluated through the variance analysis (ANOVA) and Tukey's test, to significance level of $p < 0.05$ with the use of software Statistic version 5.0 (Statsoft Inc. USA).

3 Results and discussion

3.1 Extraction and chemical compounds of basil essential oil

The amount of oil obtained after 60 minutes of extraction presented an average yield of 1.2% ± 0.14.

Linalool (71.01% of area with retention time of 14.175 minutes) was the major component found in the basil oil, but other compounds were also identified, such as 1,8 cineol (8.27% of area with retention time of 9.325 minutes), aromadendrene (6,73% of area with retention time of 40.325 minutes) and trans-caryophyllene (4,84% of area with retention time of 30.333 minutes). Similar results were found by Lee et al. (2005) and Bozin et al. (2006), who also obtained basil oil through hydrodistillation, identifying the same compounds.

Tepe et al. (2007) identified the essential oil of *Nepeta flavida* with 1.8-cineol and linalool as the main compounds, but attributed the high antioxidant activity of the oil to the 1.8-cineol conpound, and less to linalool. The basil oil used in our work, as presented above, is constituted mainly by 1.8-cineol and linalool, indicating its potential use in antioxidant activity. The extraction process used in this work favored the production of a high amount of linalool and a small amount of of 1.8 cineol. These compounds present high affinity to lipids (SIMIONATTO, 2004). As salami presents a fat content of 10-12%, a good solubility was verified between the product and the essential oil, favoring the antioxidant action. Therefore, eugenol, also present in the basil essential oil composition can act synergistically with other compounds, contributing to the antioxidant activity of this natural product (LEE et al., 2005; POLITEO; JUKIC; MILOS et al., 2007).

3.2 Antioxidant activity in vitro

With the values obtained from the determination of antioxidant activities of basil oil in vitro with concentration between 2.5 and 20 $mg.mL^{-1}$, a calibration curve was

determined, obtaining the inhibitory concentration value (IC_{50}) 12 mg.mL^{-1}. This concentration value was higher than the one found for *Hippomarathrum microcarpum*, *Artemisia fragrans* and *Artemisia austriaca* (IC_{50} de 10.69 mg.mL^{-1}, 7.86 mg.mL^{-1} and 8.06 mg.mL^{-1}, respectively), but lower than that found for *Rosmarinus officinalis* with value of 20 mg.mL^{-1} (WANG et al., 2007). The IC_{50} of basil essential oil (12 mg.mL^{-1}) can be considered similar to those found by *Hippomarathrum microcarpum*, *Artemisia fragrans* and *Artemisia austriaca*. The supra-mentioned plants are considered as good antioxidant fonts by the specialized literature. Based on this information, basil essential oil can be a good alternative to food industries because of its antioxidant properties.

The use of essential oils as antioxidant substances in food products is still limited because of the alteration they promote in flavor, making it necessary to determine the concentrations of oils that guarantee the product acceptability (KOUTSOUMANIS et al., 1998).

To achieve this information, sensory analyses were carried out in this study to evaluate the influence of essential oil concentration in product flavor. For this, different concentrations of oil were added to salamis. The higher the punctuation attributed by the panelists, higher the difference related to the Standard, where score 0 refers to no difference, score 2 is slightly different, 4 is moderately different, 6 highly different and 9 extremely different.

The formulation tested in this work presented the following punctuation: blank (0.94^{c}), 0.19 mg.g^{-1} (1.63^{bc}), 0.38 mg.g^{-1} (2.31^{b}), 0.75 mg.g^{-1} (2.77^{b}), 1.87 mg.g^{-1} (6.37^{a}), 3.75 mg.g^{-1} (7.09^{a}), 6 mg.g^{-1} (8.28^{a}) and 12 mg.g^{-1} (8.92^{a}). Mean of punctuation followed by equal letters do not differ at a confidence level of 95% by Tukey's test. The formulation containing up to 0.75 mg.g^{-1} of essential oil could be classified as slightly different from the standard, accepted by the panelists.

3.3 Lipid oxidation

The TBARS index for the internal part of the salami determined with different basil oil concentrations, with and without commercially available antioxidant, is presented in Figure 1.

The salami containing 0.75 mg.g^{-1} of basil oil, from processing days 2 to 28 showed TBARS values varying from 0.093 to 0.268 mg.MDA.kg^{-1}, consisting on the lowest values among the five treatments. The White lot contained the higher values, varying from 0.252 to 0.375 mg.MDA.kg^{-1} and differed significantly from the lowest values found on all other days of analysis. The TBARS values from the control treatment, and treatment with 0.75 mg.g^{-1} of basil oil, only differed significantly on processing days 2 and 14. Regarding the other concentrations of basil oil (0.38 and 0.19 mg.g^{-1}), the TBARS values significantly differed from the ones found at concentration 0.75 mg.g^{-1}, during the whole processing stage (Figure 1).

Following the mechanism of action, the basil essential oil is classified as a primary antioxidant, since it acts removing or inactivating the free radicals produced during the oxidation reaction, through the donation of hydrogen atoms, interrupting the reaction in chain. On the other hand, the sodium eritorbate is classified as a secondary antioxidant, acting through different mechanisms of oxygen removal (DO PRADO, 2008). The amount of basil essential oil added to the product (0.75 mg.g^{-1}) was lower than the eritorbate (2.5 mg.g^{-1}) and the values of TBARS (Figure 1) in the salami produced using essential oil were lower than that using sodium eritorbate as antioxidant (control). This result demonstrated that the basil essential oil was efficient against lipid oxidation, showing the possibility of using this natural antioxidant in food products formulation.

Torres et al. (1994) reports that the perception of rancidity in cooked meat takes place when the TABRS values are between 0.6 and 2.0 mg.MDA.kg^{-1} sample. The TBARS values of the salami with 0.75 mg.g^{-1} of oil were lower than the one with 0.6 mg.MDA.kg^{-1}, consisting on a difference when compared to the 0.332 mg.MDA.kg^{-1} (0.6 mg-0.268 mg) of processing day 28, followed by the salami which contained the commercially available antioxidant (control), where there was a difference of 0.313 mg.MDA.kg^{-1} (0.6 mg-0.287 mg). Those values demonstrate that basil oil at 0.75 mg.g^{-1} exercised antioxidant activity during the processing stage.

After 30 days of storage, the salami from the control treatment and from the treatment containing 0.75 mg.g^{-1} of oil (Figure 1) had the lowest TBARS values, with no significant difference between each other ($p < 0.05$), but differing significantly from the remaining treatments of salami (White, 0.19 and 0.38 mg.g^{-1}).

During the storage period, the TBARS values decreased in all salami treatments, result also observed by Nassu et al. (2003) and Sammet et al. (2006), due to the reactions that occur between malonaldehyde (MDA) and proteins. Proteins more susceptible to this reaction contain in their structures the aminoacids cistein, metionine, lisin, triptofan, arginine, proline and histidine and, as a consequence, there are modifications in their structures and isoeletric points. The compounds produced

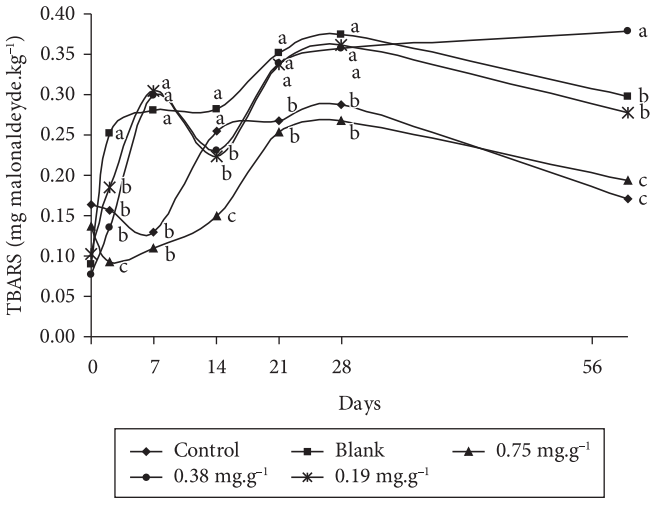

Figure 1. TBARS values (mg.MDA.kg^{-1} sample) in the internal part of the salami with different basil oil concentrations, with and without commercialized antioxidant, for 28 days of processing and after 30 days of storage at 22 °C.

are Schiff bases and other carbonyl compounds (GARDNER, 1979; LIU; XIONG, 2000; CHOPIN; KONE; SEROT et al., 2007).

Campos et al. (2007) evaluated the antioxidant activity of the extract of mate tea in Italian type salami, following a formulation similar to that used in this work. After 30 days of processing, the TBARS values were lower than 0.200 mg.MDA.kg^{-1}, differing from the use of sodium eritorbate. After 28 days of storage, TBARS values of 0.266 mg.MDA.kg^{-1} were obtained using 0.75 mg.g^{-1} of essential oil, not differing significantly from that using sodium eritorbate (Figure 1). Basil essential oil presents terpenic non-polar compounds. The mate tea leaf extract, on the other hand, presents polar and amphiphilic phenolic compounds, combining easily with proteins and fats, presenting higher antioxidant activity (SIMIONATTO, 2004; MENDES, 2007).

3.4 Protein oxidation

The concentration of carbonyl compounds (nmol carbonyl.mg^{-1} of protein) in the internal part of the salami determined with different concentrations of basil oil and with and without commercially available antioxidant is shown in Figure 2.

The meat mass used for salami production referred to control, blank, and with 0.75, 0.38 and 0.19 mg.g^{-1} of basil essential oil, presented values of 18.61, 12.61, 18.40, 13.42 and 13.10 nmol of carbonyl mg^{-1} of protein, respectively (Figure 2). The carbonyl values of the control and the formulation using 0.75 mg.g^{-1} of oil did not differ significantly ($p < 0,05$), but both presented statistical difference to other formulations.

The meat used in the composition of the salami was ground and later received the additives (NaCl and $NaNO_2$), forming the meat mixture, which remained for 3 hours on a tray in contact with oxygen at 20 °C. As it was ground, the contact surface increased, and the presence of free Fe^{+2} ions - associated

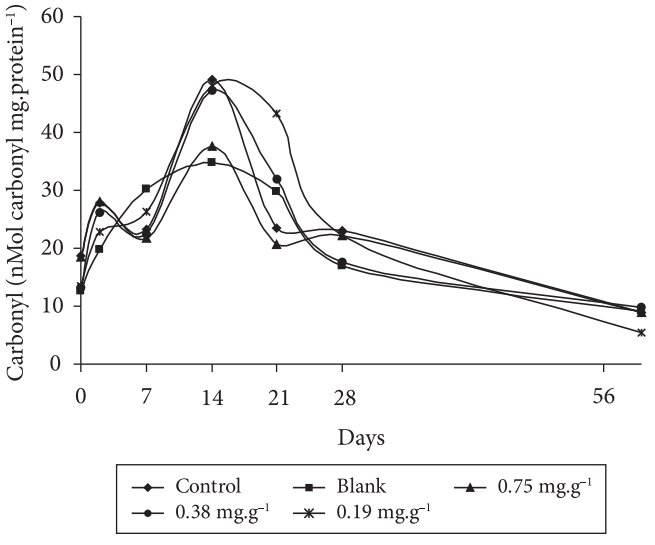

Figure 2. Values of carbonyl (nmol Carbonyl mg^{-1} of protein) in the internal part of the salami with different concentrations of basil oil, and with and without commercial antioxidant, during 28 days of processing and after 30 days of storage at 22 °C.

to the formation of Metmyoglobin, initially promoted by the action of the $NaNO_2$ - accelerated the oxidation (VARNAM; SUTHERLAND, 1998; ESTÉVEZ; CAVA, 2006). When Metmyoglobin was formed, there was also the formation of Hydrogen peroxyde (H_2O_2), Singlet oxygen (O_2•), and Ferrimyoglobine radical (Fe^{+4}), resulting from the reaction between the Metmyoglobin and H_2O_2. Because it is very unstable, it resulted in the formation of the Peroxyl radical (ROO•) (BATIFOULIER et al., 2002). All those compounds associated to the concentration of the NaCl (2%), and the time and temperature at which the meat mixture remained (3 hours at 20 °C), promoted the oxidation of the lipids and proteins, consisting on the possible factors that influenced the initial values of protein oxidation (carbonyl mmol.mg^{-1} of protein) found in the meat mixture soon after being stuffed (day zero, Figure 2). These reactions occurred in a pronounced way where sodium eritorbate (control) and 0.75 mg.g^{-1} of essential oil was used, demonstrating that both substances favored the protein oxidation at the first day of analysis (Figure 2).

From processing days 2 to 14, the values of carbonyl increased in the five treatments of salami. The higher values occurred on day 14, and the control treatment had a value of 49.13 nmol, the White one of 34.68 nmol, and the ones containing basil oil 0.75, 0.38 and 0.19 mg.g^{-1} had values of 37.58, 47.08 and 48.02 nmol carbonyl mg^{-1} of protein, respectively (Figure 2). The value of carbonyl from the control treatment differed significantly ($p < 0.05$) from the value of the White treatment and from the one with 0.75 mg.g^{-1} oil. The same occurred between the blank and the treatment that used 0.75 mg.g^{-1} of essential oil. The increase in values of carbonyl would be related to the proteolysis promoted by the endogenous enzymes of the meat, due to the decrease of pH to values considered ideal for the action of those enzymes (pH 4.5 to 5.5). That is promoted by the lactic bacteria (*Pediococcus pentosaceus*) added to the meat mixture (CASABURI et al., 2008). This factor associated to the temperatures applied in the processing chamber (25 °C to 18 °C), assisted the action of endogenous enzymes in the salami, with the release of higher quantities of tyrosine, proline, histidine, methionine, triptofane, and lysine (CASABURI et al., 2008) which are more susceptible to oxidation (XIONG, 2000). Another factor that could also have contributed to the increase of carbonyl values was that the internal part of the salami in that period presented brownish color, caracteristic of Metmyoglobin pigment, and according to Shahidi (1992), whenever Metmyoglobin is present, there is also non-heme Fe^{+3}, with oxidizing action.

On processing day 21, the salami from the control treatment had a carbonyl value of 23.37 nmol, the White lot 29.71 nmol, whereas the ones with basil oil 0.75, 0.38 and 0.19 mg.g^{-1}, had values of 20.68; 31.87 and 43.24 nmol of carbonyl.mg^{-1} of protein (Figure 2). From that day until the end of storage (60 days, Figure 2), the carbonyl values decreased, reaching values of 8.98 nmol in the control treatment, 9.18 nmol in the White lot, 9.02 nmol in the treatment with 0.75 mg.g^{-1} of oil, 9.70 nmol in the treatment with 0.38 mg.g^{-1} and of 5,42 nmol in the treatment with 0.19 mg.g^{-1} of basil oil. Maillard condensation would be one of the possible causes that promoted a reduction at the carbonyl values (VARNAM; SUTHERLAND, 1998), taking

place from day 21 till the end of the storage period (Figure 2). This is due to the fact that, in Maillard condensation, the reaction of the carbonyl compounds (formed through protein oxidation) takes place with the NH_2 group, released when the proteolysis occurred (between days 2 and 14), promoted by endogenous enzymes. Another factor is that, the high index of protein oxidation in the first 14 days of processing may have promoted an alteration in the structure of the endogenous protease enzymes (XIONG, 2000), which assisted in the reduction of the a_W value (from 0.86 to 0.82), and an increase in the concentration of salt reduced the enzymatic activity, and consequently, of the proteolysis. Batifoulier et al. (2002) observed a reduction of carbonyl values in the meat of turkeys that received a diet with vitamin E supplement, reaching a value of 8 nmol carbonyl.mg^{-1} of protein. A similar fact occurred with Mercier, Gatellier and Renerre et al. (2004), who also observed a reduction in the carbonyl values along the time, reaching values of 6 nmol carbonyl mg^{-1} of protein in the meat of *Charolis* cattle, which received vitamin E supplement in their diet.

4 Conclusions

Basil oil in vitro exercises antioxidant activity in consideration to free the 2.2–diphenyl–1 picryl-hydrazil free radical (DPPH•).

Basil oil in the quantity of 0.75 mg.g^{-1} showed good antioxidant activity regarding oxidation of lipids during the processing stage.

The different concentrations of basil oil and commercially available antioxidant did not show antioxidant activity regarding protein oxidation during the processing stage.

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