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Application and evaluation of propolis, the natural antioxidant in Italian-type salami

Aplicação e avaliação de própolis, o antioxidante natural, em salame tipo Italiano

Cléria Andréia Kunrath[1], Daniele Cristina Savoldi[1], João Paulo Fernando Mileski[2], Cláudio Roberto Novello[3], Alexandre da Trindade Alfaro[4], João Francisco Marchi[4], Ivane Benedetti Tonial[3]*

[1] *Universidade Tecnológica Federal do Paraná (UTFPR), Coordination of Food Technology, Francisco Beltrão/PR - Brazil*

[2] *Universidade Tecnológica Federal do Paraná (UTFPR), Food Engineering Department, Graduate Program in Food Technology (PPGTAL), Francisco Beltrão/PR - Brazil*

[3] *Universidade Tecnológica Federal do Paraná (UTFPR), Department of Chemistry and Biology, Francisco Beltrão/PR - Brazil*

[4] *Universidade Tecnológica Federal do Paraná (UTFPR), Food Engineering Department, Francisco Beltrão/PR - Brazil*

***Corresponding Author**

Ivane Benedetti Tonial, Universidade Tecnológica Federal do Paraná (UTFPR), Department of Chemistry and Biology, Linha Santa Bárbara, s/n, CEP: 85601-970, Francisco Beltrão/PR - Brazil, e-mail: ivane@utfpr.edu.br

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Summary

This study aimed to characterize propolis with respect to its antioxidant activity and apply it to the elaboration of Italian-type salami. A propolis sample was collected and subjected to chemical and physicochemical characterization and its antioxidant capacity determined. Four salami formulations were developed: F1 (no antioxidants); F2 (addition of 0.01% BHT); F3 (addition of 0.01% propolis) and F4 (addition of 0.05% propolis). The salamis were evaluated with respect to their physicochemical properties and lipid oxidation. The characterization of the propolis showed a high level of waxes and low levels of phenolic compounds and flavonoids, although in sufficient quantity to prove their antioxidant activity. The Italian-type salamis showed moisture, protein and lipid contents which conformed to the limits preconized by Brazilian legislation. The F4 formulation (0.05% propolis) showed a better result when compared to the formulations F3 (0.01% propolis) and F1 (no antioxidant). However, formulation F2 (0.01% BHT) showed the lowest value of lipid oxidation. The results showed that propolis inhibits oxidative action and can be added to meat products as a natural antioxidant.

Keywords: *Meat products; Shelf life; Lipid oxidation.*

Resumo

O presente estudo teve por finalidade caracterizar a própolis quanto à sua atividade antioxidante e aplicá-la na elaboração de salame tipo italiano. Amostra de própolis foi coletada e submetida à caracterização físico-química, química e capacidade antioxidante. Quatro formulações de salame foram elaboradas: F1 (sem antioxidante), F2 (adição BHT 0,01%), F3 (adição de própolis 0,01%) e F4 (adição de própolis 0,05%). Os salames foram avaliados quanto às propriedades físico-químicas e oxidação lipídica. A caracterização da própolis mostrou alto teor de ceras e baixo teor de compostos fenólicos e flavonoides, porém em quantidades suficientes para comprovar sua atividade antioxidante. Os salames tipo italiano apresentaram teor de umidade, proteínas e lipídeos conforme limites preconizados pela legislação brasileira. A formulação F4 (0,05% de própolis) apresentou melhor resultado quando comparado às formulações F3 (0,01% de própolis) e F1 (sem antioxidante). No entanto, a F2 (0,01% de BHT) foi a formulação que apresentou menor valor de oxidação lipídica. Os resultados demonstraram que a própolis inibe a ação oxidativa, podendo ser adicionada em produtos cárneos como antioxidante natural.

Palavras-chave: *Produtos cárneos; Prazo de validade; Oxidação lipídica.*

1 Introduction

Salami is a raw, cured, fermented, matured and dried food that may or may not have been smoked. It is an industrialized meat product obtained from pork or pork and/or beef, with the addition of fat, ingredients, and built-in natural and/or artificial wrappings (BACKES, 2011).

Fermented meat products such as salami are susceptible to the process of lipid oxidation, which is considered to be one of the main degradation reactions that can occur during the processing, distribution and storage of foods, giving them unpleasant colours, flavours and odours, and making them inadequate for consumption (LUZIA; JORGE, 2009).

The food industry employs synthetic antioxidants to inhibit lipid oxidation, such as BHA (butyl-hydroxyl-anisole), BHT (butyl-hydroxyl-toluene), TBHQ (*tert*-butylhydroquinone) and PG (propyl gallate), which have been accused of causing heart diseases and of being carcinogenic, and hence Brazil has established limits for the usage of these kinds of compound in foods (TAKAMOTO et al., 2009). With the intent of preserving consumer health, the food industry has been investing in the search for natural compounds with antioxidant properties to replace the synthetic ones (ATUNGULU et al., 2007) and many products have appeared on this promising scenario, amongst which propolis.

Propolis is a product created by bees (*Apis mellifera* L.) from a resinous, gummy and balsamic material collected from buds, flowers and plant exudates. The material receives salivary secretions and wax, to be used in the protection of the hive against animals and intruder microorganisms (BRASIL, 2001; SILVA, 2014).

Propolis can be acquired from a wide range of plants, and is characterized by an extremely complicated chemical composition. Currently between 300 and 400 chemical constituents are known to be part of this composition, including flavonoids, terpenoids, aromatic acids, steroids and proteins, amongst other classes of organic compounds (DIAS et al., 2012).

However, the composition may vary both qualitatively and quantitatively according to the vegetable source, geographical location, botanical classification and phenology of the plant visited by the bees, and also the period the resin was collected (KUMAZAWA et al., 2004).

The biological activity is normally dependent on the number of polyphenolic compounds present, especially flavonoids, and these compounds are indicated as being those responsible for the great variety of therapeutic properties, such as anti-inflammatory, anti-microbial and anti-viral activities, and also the antioxidant characteristic (SILVA et al., 2013).

According to Valente et al. (2010) the phenolic compounds and their derivatives act as excellent antioxidants, playing the role of an efficient free radical scavenger, metal ion chelator, and free radical inhibitor and eliminator (RUSSO et al., 2004), hence preventing lipid oxidation. Thus propolis could be a source of natural antioxidant compounds with great application in the food industry.

This work aimed to add propolis extract to Italian-type salami, evaluating its potentiality as a natural antioxidant and verifying its influence on the physicochemical characteristics of the product.

2 Material and methods

2.1 Acquiring and preparing the propolis

In natura propolis was acquired from honey producers collected on a single occasion in August, 2014 from the municipality of Francisco Beltrão, Paraná state, Brazil (Geographical coordinates: 26° 4' 42" and South, 53° 3' 11" West). The impurities were manually removed and the propolis kept under refrigeration at a temperature of 8 °C and relative humidity of 60%.

A previously powdered sample (1.00 kg) was extracted by turbolysis with 70% ethanol/water (m/m) in the proportion of 10% (m/m) of propolis/solvent (BRUSCHI, 2006). A quantity of 125.4 g of carboxymethyl cellulose and 65.7 g of sodium alginate, previously dissolved in ultrapure water, were then added to the extract obtained (8.9 kg). The mixture was concentrated in a rotary evaporator under reduced pressure and dried by nebulization in a Spray Dryer, obtaining 420.0 g of dried sample. All reagents used were of analytical grade.

2.2 Propolis characterization

The sample of propolis was evaluated in different forms: *in natura;* alcoholic extract and dried extract. The ash and moisture contents were evaluated according to the *Association of Official Analytical Chemists* (CUNNIFF, 1995). The wax content was determined employing the method described by Woisky and Salatino (1998) and Salatino et al. (2005) and expressed in mass percentage (AL-MAMARY et al. 2002).

The total flavonoids were evaluated using the method described by Bruschi (2006) and the polyphenols were quantified using the Folin-Ciocalteau method according to Obanda et al. (1997). The antioxidant activity was evaluated according to Rufino et al. (2006) and Rufino et al. (2007).

2.3 Elaboration of Italian-type salami

The experiment was organized using a completely randomized design (CRD) with four treatments and three repetitions. The materials used for the elaboration of the salami were: meat, pork fat (saturated) and starter culture (TEXEL®AS-308). Table 1 show all the ingredients used in the elaboration of the Italian-type salami. Seven kilograms (7 kg mass) of each formulation were prepared, giving a total of twenty-eight kilograms (28 kg mass) of Italian-type salami, corresponding to 30 pieces of 200 g for each of the four formulations.

Table 1. Ingredients used in each formulation of Italian-Type Salami.

Ingredients	F1	F2	F3	F4
Pork (%)	86.86	86.85	86.85	86.87
Fat (%)	10.00	10.00	10.00	10.00
Salt – sodium chloride (%)	2.00	2.00	2.00	2.00
Curing salt-IBRAC (Hungarian powder) (%)	0.25	0.25	0.25	0.25
Fixative– IBRAC (erythorbate) (%)	0.25	0.25	0.25	0.25
White pepper (%)	0.05	0.05	0.05	0.05
Garlic powder (%)	0.07	0.07	0.07	0.07
Nutmeg (%)	0.02	0.02	0.02	0.02
Flavour enhancer- IBRAC (%)	0.10	0.10	0.10	0.10
Sugar (%)	0.30	0.30	0.30	0.30
Starter culture- DANISCO (%)	0.025	0.025	0.025	0.025
Propolis extract powder (%)	-	-	0.01	0.05
Synthetic antioxidant BHT (%)	-	0.01	-	-

For the preparation of the Italian-type salami, the pork meats (palette and ham) and the pork fat were first selected. The meats (temperature 4 °C) were weighed and ground using an 8 mm disc, and the fat cut into cubes (temperature < 0 °C). The amount of meat and fat required for each formulation was then weighed and homogenized for the addition of the other ingredients. The fixative (erythorbate) was dissolved in water and added to the mass.

The fermentation phase was carried out by adding the starter culture (TEXEL®AS-308) containing the following bacteria: *Lactobacillus sakei, Staphylococcus carnosus* and *Staphylococcus sylosus* plus dextrose as the vehicle.

After adding all the ingredients, the mass was homogenizing for 5 minutes. To obtain the salamis, the mass was embedded in artificial collagen to approximately 25 cm in a manual stuffer. The salamis were then placed in an industrial refrigerator at 7 °C and maintained there for 12 hours for partial dehydration of the surface. This process is used to improve the smoking process. The salami was cold-smoked with natural smoke, a natural humidity of 80% and a temperature of 30 °C for 2 hours and 30 minutes in order to produce the colour and dry the casing.

After smoking, the pieces were maintained in the chamber at a temperature of 26 °C, and the temperature progressively reduced (1 °C by day) until it reached 18 °C. During the first seven days of fermentation the relative humidity was maintained between 70 and 85%, but as from the 8th day onwards it remained constant at 70% with a temperature of 18 °C and air velocity of 0.2 m/s (Table 2). From this stage on the fermentation continued to occur, but the dehydration phase of the product was accentuated, completing the maturation by the 35th day.

2.4 Physicochemical analyses of the salami

The physicochemical analyses were carried out with three repetitions (duplicates) for each of the four formulations. The pH and acidity were determined according to the methodologies of the Adolfo Lutz Institute (IAL, 2008) after 01, 06, 08, 13, 15, 22 and 28 days, and the water activity – Aw (Aqualab lite) 7, 14, 16 and 28 days after elaboration of the salamis. The other analyses were only carried out 28 days after production of the salamis.

Table 2. Temperature, relative humidity and air velocity parameters during the fermentation and drying of the salamis.

Parameters	Values (variation)
Temperature (°C)	18-26
Relative humidity (%)	70-85
Air velocity ($m.s^{-1}$)	0.2-1.0

The moisture, ash and lipid contents were determined using the Adolfo Lutz Institute methods (IAL, 2008), the protein content was determined using the method described by Tedesco et al. (1995) and the percentage of carbohydrates was calculated by difference (BRASIL, 2003).

Lipid oxidation was determined using the methodology described by Tarladgis et al. (1964) and modified by Crackel et al. (1988), and the results expressed in mg of TBARs per kilogram of sample.

2.5 Statistical Analysis

The results were analysed using the analysis of variance ($p<0.05$) and Tukey's means test with the software STATISTICA 7.0 (STATSOFT, 2005).

3 Results and discussion

3.1 Composition and antioxidant activity of the propolis

Table 3 shows the results obtained in the evaluation of the physicochemical composition and antioxidant activity of the propolis.

The propolis was evaluated in the *in natura* and dry extract forms. The average values obtained for the moisture and ash contents, for the *in natura* and dry extract samples

Table 3. Physicochemical composition and antioxidant activity of the propolis in the *in natura,* alcoholic extract and dry extract forms.

Chemical Variables	Propolis		
	In natura	Alcoholic extract	Dry extract
Moisture	7.46±0.06[a]	N.A	3.30±0.04[b]
Ash	2.04±0.02[b]	N.A	3.12±0.05[a]
Waxes	34.07±0.71	N.A	N.A
Total flavonoids (g.100g^{-1})	0.26±0.01[c]	0.03±0.0006[a]	0.40±0.01[b]
Total polyphenols (%)	4.17±0.07[c]	0.31±0.006[a]	5.13±0.04[b]
Antioxidant Activity			
A.A (FRAP)- µM iron sulphate per g of propolis	352.04±2.20[c]	50.98±0.379[a]	543.40±3,73[b]
A.A (DPPH) (EC50) – g of propolis/g of DPPH	7.42±0.06[a]	89.94±0.62[c]	5.86±0.05[b]

N.A: Not applicable. Averages with the same letters in the same line do not show significant differences (p>0.05) in the Turkey's means test.

were below the maximum limits established by the Brazilian legislation (8.00% for moisture and 5.00% for ash) (BRASIL, 2001). According to Siqueira et al. (2014) propolis samples with high moisture contents are less stable and can make their maintenance difficult during storage, while the ash level reflects the mineralization of the sample as well as the presence of impurities, due to the production process (wood and bee remains and dirt) resulting in an increase in the ash level (D'ETTORRE et al., 2006).

However the wax content for the *in natura* sample was above 25%, the maximum limit established by the legislation. This expressive value found for the waxes in the samples analysed could, according to Lopes (2014), be related to and depend on the botanical origin of the sample and on the collection process used by the apiarist. Similar values to those found in the present study for the moisture (3.30% - dry extract), ash (2.04% - *in natura;* 3.12% - dry extract) and wax (34.07%) contents, were also found by Lopes (2014) in propolis samples, with values of: 3.86% for moisture; 38.00% for ash and 4.00% for waxes. The antioxidant activity of propolis is characterized by the presence of polyphenols and, normally, of flavonoids (MARQUELE et al., 2005), with the concentration of polyphenols generally being directly related to its antioxidant property. However, Brazilian propolis may show a great concentration of prenylated phenylpropanoids (SALATINO et al., 2005), over the majority presence of flavonoids. In this study, the total flavonoid content was higher in the nebulized dry extract (0.40%) than in the *in natura* sample and hydro-alcoholic extract. In relation to the total polyphenolic compound contents, the same values were obtained in this study as observed by Vieira (2012), varying between 9.56% and 16.24% in propolis extract samples.

The behaviour observed for the total flavonoid and polyphenol contents was reflected in the evaluation of the antioxidant capacity as measured in terms of µM of iron sulphate per g of propolis (FRAP), which is based on the capacity of the phenols to reduce Fe^{3+} to Fe^{2+} (STRATIL et al., 2006; HUKKANEN et al., 2006). This was evidence that the dry extract had a greater antioxidant capacity than the *in natura* propolis and alcoholic propolis extract.

The capacity of propolis to capture free radicals was also evaluated by analysing the solutions of the extracts with DPPH. The results showed that the hydro-alcoholic extract presented a minor capacity to capture free electrons as compared to the *in natura* propolis and the dry extract, since the latter had a higher solvent concentration (approximately 90%). According to Alves et al. (2007), the higher the consumption of DPPH by the sample, the greater is its antioxidant activity. The method was previously validated using the positive standard Trolox, which possesses a high capacity to capture free electrons. The EC_{50} was achieved with 0.36 g of Trolox/g of DPPH, that is, 0.36 g of Trolox was required to inhibit 50% of 1.00 g of DPPH. The lowest value for EC_{50} amongst the propolis samples was obtained by the nebulized dry extract (5.86±0.05 g/g DPPH), showing the high antioxidant capacity of this extract when compared to Trolox.

3.2 Proximate composition of Italian-type salami with added propolis extract

Table 4 shows the average results obtained together with their respective standard deviations for six replicates of the parameters that make up the proximate composition of the Italian-type salami samples evaluated 28 days after their production.

The physicochemical parameters evaluated in the Italian-type salami samples showed no significant difference amongst the four formulations 28 days after production for the moisture, ash and lipid contents, only differing for the protein content, where the F2 formulation had an average of 28.86%, different from the others.

According to Normative Instruction Number 22 of the Ministry of Agriculture, Livestock and Supply (MAPA) (BRASIL, 2000), the physicochemical characteristics of Italian-type salami should be as follows: moisture content (≤ 35%), lipid content (≤ 32%), and protein content (≥ 25%) according to the values preconized for this type of product.

Table 4. Physicochemical parameters of the different formulations 28 days after production.

Formulation	Moisture (%)	Ash (%)	Lipid (%)	Protein (%)
F1	28.38±1.39[a]	11.88±0.87[a]	23.99±0.70[a]	32.78±1.17[b]
F2	31.15±2.82[a]	13.20±1.15[a]	24.32±1.98[a]	28.86±0.62[a]
F3	30.21±0.64[a]	11.49±0.53[a]	25.44±0.38[a]	31.70±0.90[b]
F4	29.06±0.64[a]	11.77±1,32[a]	23.72±0.44[a]	32.65±0.38[b]

Averages with the same letters in the same column do not show significant difference (p>0.05) according to the Turkey's means test. F1: control; F2: 0.01% BHT; F3: 0.01% propolis; F4: 0.05% propolis.

The moisture content was not significantly different between the four salami formulations developed and evaluated in this study. The moisture content of salami is due to the aging process (drying) of the product, which must be controlled to avoid the formation of a dry crust on its surface which would maintain the moisture within the product, causing storage problems (GARCIA et al., 2000).

The ash content represents the total mineral content of a food, being composed of K, Na, Ca, Mg (larger amounts), Al, Fe, Cu, Mn, Zn (smaller amounts), and Ar, I and F (trace amounts) (CECCHI, 2003). The ash contents found in the present study were higher (11.49 to 13.20%) than the values found by Dalla Santa (2008) in salami samples from different manufacturers produced by spontaneous fermentation (3.76% to 8.84%), and by Fieira et al. (2015), who evaluated different formulations of Italian-type salami in order to evaluate the interference of different salts on the starter culture (6.35% to 7.51%). The high ash content found in the present study may be related to the greater moisture loss (level of dryness) and addition of salt (sodium chloride - 2.00%) (DALLA SANTA, 2008).

In meat products, the protein content has various roles which determine the yield, quality, structure and sensory attributes (OLIVO; SHIMOKOMAKI, 2006). In this study, the protein content ranged from 28.86% to 32.78%, with a significant difference (p<0.05) between formulations. These values are within the range of those determined by Dalla Santa (2008) (11.32% - 41.27%), and lower than the values reported by Marangoni (2007) (42.5% - 43.8%) at the end of the 35 days of maturation of salami. The protein content is directly related to the amount of lean meat added to the product, and in the present study its values are within the quality parameters required by the legislation (BRASIL, 2000).

Lipid is considered to be an important constituent of this meat product, since it give the products juiciness, flavour and aroma (OLIVO; SHIMOKOMAKI, 2006). The formulations showed a level of fat ranging from 23.72% to 25.44%. These values are intermediate when compared to those found by Dalla Santa (2008), who found values ranging from 7.44% to 48.83%, and lower than those found by Zanardi et al. (2002) (31.91%) in Milano-type salami. The amount of lipids found in the present study can be attributed to the amount of fat used in the manufacture of the products, and the values found conformed to the values recommended in the Brazilian legislation (BRASIL, 2000).

The percentages of carbohydrate, obtained from the difference between 100 and the sum of the protein, lipid, moisture and ash contents, presented by formulations F1, F2, F3 and F4 were 2.97%; 2.47%; 1.38% and 2.80%, respectively.

Dalla Santa (2008) observed, in a study with salami samples from different manufacturers, that several of the samples evaluated showed one or more characteristics in violation of the law. This variation in the composition of salami may be related to the raw materials (meat) used in their preparation, since these can modify the moisture, protein and fat contents. In the same group of meat or meat products, the protein levels are often constant; but the fat and moisture levels are correlated, causing a compensatory relationship amongst the components (OLIVO; SHIMOKOMAKI, 2006).

3.3 pH and acidity values

The results for pH and acidity throughout the 28 days after production can be seen in Figures 1 and 2.

The first stage after processing of the salamis was fermentation, during which a rapid fall in pH occurred up to the 6th day, which can be seen in Figure 1. According to Terra (2006), this reduction in pH caused by the lactic acid bacteria is important in the control of deteriorative and pathogenic bacteria and to maintain the quality of the product.

The pH values of the Italian-type salami developed and evaluated in this study changed during the period of maturation, showing a higher value at the start of manufacturing (5.57 to 5.62). The decrease in pH up to the 6th day of maturation was probably due to the presence of *Lactobacillus* in the starter culture added to the formulation. On the 15th day after production, the values dropped to (5.31 to 5.41) and stabilized at the end of the process at (pH 5.21 to 5.29).

The decline in pH observed up to the 6th day as well as the oscillations occurring during the maturation period are characteristic of fermented meat products, and present a number of advantages amongst which the inhibition of undesirable microorganisms and the formation

of compounds that contribute to the enhancement of the flavour and aroma, and stabilize the colour (MATOS et al., 2007)

Marangoni (2007) found pH values (5.10 to 5.29) close to those observed in the present study, when evaluating the pH of Italian-type salami with the addition of essential coriander oil on the 28th day of maturation. Dalla Santa (2008) found higher pH values, ranging from 4.35 to 6.92, in salami from different manufacturers produced by spontaneous fermentation.

During the maturation process of the salami, it was observed an increase in the acidity index and the subsequent drop in pH. The fact is due to the lactic acid produced by bacteria during the fermentation process. According to Mendes et al. (2014), Brazilian law does not set the pH as a quality parameter for salami, but values close to the isoelectric point of proteins is considered the recommended ideal, since it will contribute to the loss of moisture and water activity of the product.

3.4 Determination of the Aw (water activity) and moisture content

The water activity (Aw) of Italian-type salami, according to Normative Instruction Number 22/2000 (BRASIL, 2000), should present a maximum value of 0.90. The moisture content (%) and water activity values of the salamis are presented in Figures 3 and 4 respectively.

In this study, the predicted and evaluated samples showed Aw values ranging from 0.89 to 0.90 (7 days) and from 0.73 to 0.75 (28 days) after manufacture, a value below the maximum limit recommended by law and below the value mentioned by Terra (2006) (0.87). This fact may contribute to the microbiological safety of the product, since the water activity is considered one of the most important factors for the conservation of fermented raw products (MONFORT, 2002).

Higher values for Aw (0.842 to 0.803) were found by Campagnol (2007) in samples of salami formulated

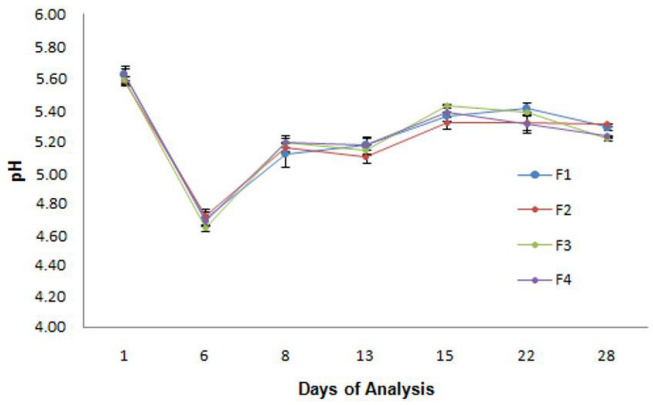

Figure 1. pH values throughout the 28 days after production for each formulation of Italian-type salami. F1: control; F2: 0.01% BHT; F3: 0.01% propolis; F4: 0.05% propolis.

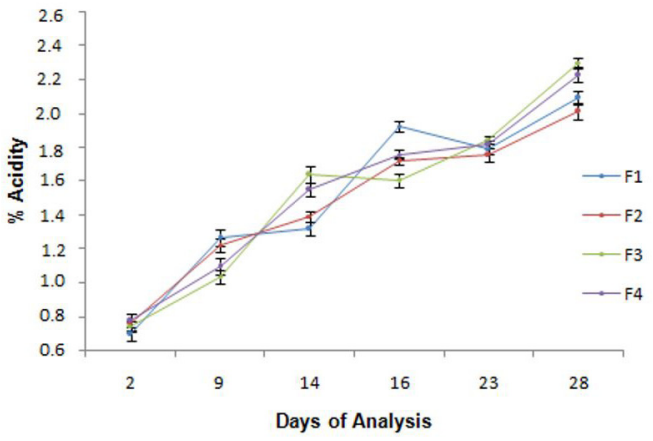

Figure 2. Acidity values throughout the 28 days after production for each formulation of Italian-type salami. F1: control; F2: 0.01% BHT; F3: 0.01% propolis; F4: 0.05% propolis.

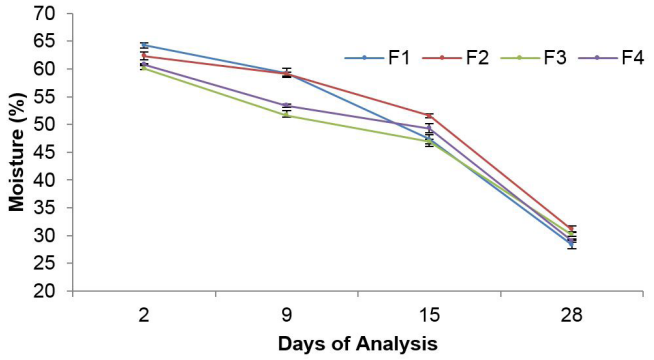

Figure 3. Values for moisture content throughout the 28 days after manufacture for each formulation of Italian-type salami. F1: control; F2: 0.01% BHT; F3: 0.01% propolis; F4: 0.05% propolis.

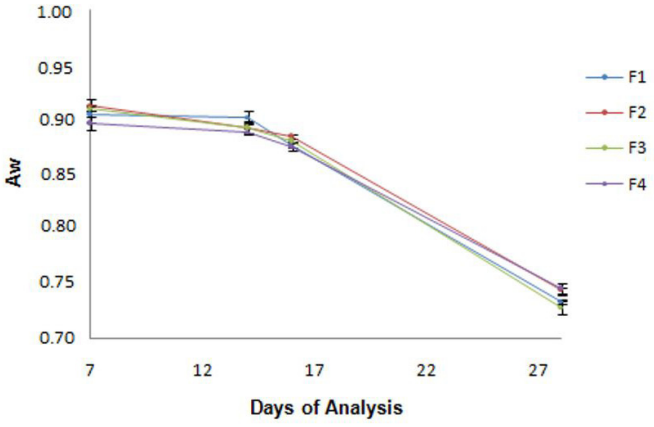

Figure 4. Values for water activity (Aw) throughout the 28 days after manufacture for each formulation of Italian-type salami. F1: control; F2: 0.01% BHT; F3: 0.01% propolis; F4: 0.05% propolis.

Table 5. Average TBARs values (mg of malonaldehyde/kg) in the samples of Italian-type salami according to the formulation and storage time (days).

Formulations	Days		
	01	15	35
F1 (Control formulation)	0.06±0.05^{aA}	0.49±0.01^{aB}	0.51±0.03^{aB}
F2 (0.01% BHT)	0.07±0.01^{aA}	0.17±0.01^{cB}	0.10±0.05^{bC}
F3 (0.01% propolis)	0.10±0.01^{bA}	0.22±0.01^{bB}	0.46±0.07^{aC}
F4 (0.05% propolis)	0.10±0.01^{bA}	0.18±0.01^{Cb}	0.22±0.04^{cB}

Averages with the same lower case letters in the same column do not show significant differences (p>0.05) according to Tukey's Means Test. Averages with the same capital letters in the same line do not show significant differences (p>0.05) according to Tukey's Means Test for those days.

with different starter cultures, and by Fieira et al. (2015) (0.894 - 0.899) in Italian-type salami samples prepared with different salts and starter cultures.

One possible explanation for the Aw values observed, both between the different formulations and the different studies may be related to the composition of the samples (higher or lower proportions of the different components) as well as the site of maturation of the salami (MARANGONI, 2007).

The low value for Aw on the 28th day after maturation may be related to the reduction in pH, since, due to the lower pH value being nearer the isoelectric point, with the increased acidity, the meat proteins lose part of their water retention capacity, causing dehydration of the product and a consequent loss of moisture (TERRA, 2006).

Like the Aw, the moisture content also declined during the maturation process, reaching the minimum value (approximately 30.00%) on the 28th day. Fieira et al. (2015) found values ranging from 31.11% to 37.71% for Italian-type salami after the maturation period, and Leite et al. (2014) found values ranging from 39.08% to 41.25%. High moisture levels facilitate the proliferation of microorganisms (TERRA, 2006), and low moisture contents together with the presence of lactic acid give the salami its characteristic flavour (GRIS; BORTOLUZZI, 2002). According to Martins (2006), the maximum moisture content in salami should be 40%.

3.5 Lipid oxidation in salami

The evaluation of lipid oxidation by determining the TBARs index, quantifies the malonaldehyde present in the sample, one of the main products in the decomposition of the peroxides formed during the oxidation process. The results obtained for TBARs, evaluated after 01, 15 and 35 days of storage are shown in Table 5.

The results for lipid oxidation showed a significant difference (p<0.05) between the formulations and also throughout the periods evaluated. Formulations F1, F3 and F4 showed an increase in the TBARs values throughout the maturation period of the salamis.

The variation in mg malonaldehyde/kg in sample F1 was from 0.06 (F1-1st day) to 0.51 (F1-35th day). No significant differences were found between the TBARs indexes in formulation F4 (0.05% dried propolis) after 15 days maturation in relation to formulation F2 (BHT – positive control) after the same period (15 days), indicating similarity in the protection granted by the propolis and BHT against oxidising agents.

It should be highlighted that the formulation with the addition of 0.05% propolis (F4) showed a significant reduction (p<0.05) in the oxidative activity in relation to the control sample (F1), showcasing the potential efficacy of propolis against oxidative damage.

On the other hand, the concentration of 0.01% propolis (F3) showed the same value statistically as the control formulation after 35 days of aging, which shows that at this concentration, the propolis extract does not contribute towards retarding the oxidative process.

Thus the data showed that the dried propolis extract had antioxidant activity comparable to that of BHT over certain periods of product maturation.

TBARs values close to those found in the present study (0.61 mg malonaldehyde/kg of sample) were observed by Marangoni (2007) in Italian-type salami with the addition of 0.01% of coriander, and values of approximately 0.47 mg malonaldehyde/kg measured in the mass and in the salamis were found on the first day of storage by Bernardi et al. (2013).

On the other hand, higher TBARs values (2.75 and 2.52 mg malonaldehyde/kg of the sample) were found by Macedo (2005) in Italian-type salami with the addition of *marcela do campo* (*Achyrocline satureioides*), a natural antioxidant, 28 days after processing.

4 Conclusion

The propolis used in this study showed a high level of waxes, low levels of phenolic compounds and flavonoids, and a proven antioxidant activity. The propolis extract was shown to be a potent natural antioxidant for application in meat products, where it showed great effectiveness in the control of the oxidative process in formulation F4. However, sensory evaluations are necessary in order to verify possible interferences by the propolis in the flavour of the product.

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