Hovenia dulcis T. extract and G*lycyrrhiza glabra* as natural antioxidants in Bologna-type mortadela

Darlene Cavalheiro[1]*, Gisiéli Carla Morandin[2], Sabrina Vicentini Schaefer[3], Adrieli Maiandra Piccinin do Amaral[4], Luana Bettanin [5], Georgia Ane Raquel Sehn [6]

[1] darlene.cavalheiro@udesc.br, [2] gisieli_@hotmail.com, [3] sabrina.schaefer95@gmail.com, [4] adrielirosapiccinin@gmail.com, [5] luana.bettanin@udesc.br, [6] georgia.sehn@udesc.br. Departamento de Engenharia de Alimentos e Engenharia Química, Universidade do Estado de Santa Catarina (UDESC), Brazil
* corresponding author

Abstract: Pseudofruits of *Hovenia dulcis* T. present sweet-tasting edible and containing many nutrients, as well have a significant amount of phenolic compounds and could be a source of natural antioxidant. In this regard, this study aimed to evaluate the addition of *H. dulcis* T. extract and licorice root extract (licrezz™) in the formulation of Bologna-type mortadella and to investigate inhibition of the oxidative effect of lipids during cold storage, in order to assess its antioxidants properties. The mortadella samples were prepared, in duplicate, with sodium erythorbate (control), licrezz™ (commercial extract from *Glycyrrhiza glabra*), and H. dulcis extract, all of them with 0,1% amount, and were evaluated by proximate composition and microbiological characteristics, texture profile, lipid oxidation, presence of nitrite, instrumental color, pH and water activity. Mortadella with H. dulcis extract did not present a significant difference to the control in proximate composition, microbiological characteristics, or texture profile. However, they presented inferior results, but of the same order regarding the Thiobarbituric Acid Reactive Substances (TBARS) assay during the 28-day refrigerated storage. The control and *H. dulcis* extract samples presented at final storage increased approximately nine times of initial TBARS value while licrezz™ was twenty-six times. On the other hand, when compared to licrezz™, *H. dulcis* extract presented a similar behavior regarding the residual nitrite content. In this sense, *H. dulcis* pseudo-fruits extracts showed potential use as natural antioxidants to emulsified meat products, both used alone and in a mixture with *Glycyrrhiza glabra*.
Keywords: antioxidant; emulsified meat product; Japanese grape; lipid oxidation.

Extrato de Hovenia dulcis T. e Glycyrrhiza glabra como antioxidantes naturais para mortadela tipo Bologna

Resumo*: Os pseudofrutos da Hovenia dulcis T. apresentam um sabor adocicado, são comestíveis e contêm muitos nutrientes, além de possuírem quantidade significativa de compostos fenólicos e poderem ser fontes naturais de antioxidantes. Dessa forma, este estudo teve como objetivo avaliar a adição de extrato de H. dulcis T. e extrato de raiz de alcaçuz (licrezz™) na formulação de mortadela Bolonha e investigar a inibição do efeito da oxidação lipídica durante o armazenamento refrigerado, a fim de avaliar suas propriedades antioxidantes. As amostras de mortadela foram preparadas em duplicata com eritorbato de sódio (controle), licrezz™ (extrato comercial de Glycyrrhiza glabra) e extrato de H. Dulcis, todas com 0,1% das espécies citadas. As mortadelas foram avaliadas quanto à composição centesimal e características microbiológicas, perfil de textura, oxidação lipídica, presença de nitrito, cor instrumental, pH e atividade de água. A mortadela preparada com extrato de H. dulcis não apresentou diferença significativa em relação ao controle, no teste de composição centesimal, nas características microbiológicas e no perfil de textura. Porém apresentaram resultados inferiores, mas da mesma ordem, em relação ao ensaio de Substâncias Reativas ao Ácido Tiobarbitúrico (TBARS), durante o armazenamento refrigerado de 28 dias. As amostras preparadas com o controle e com extrato de H. Dulcis apresentaram um incremento de 9 vezes nos valores de TBARS, enquanto o licrezz™ apresentou valores vinte e seis vezes maior. Por outro lado, quando comparado ao licrezz™, o extrato de H. dulcis apresentou comportamento semelhante quanto ao teor de nitrito residual. Nesse sentido, extratos de pseudofrutos de H. dulcis mostraram potencial*

Revista Principia - Early View

utilização como antioxidante natural para produtos cárneos emulsionados, em ambos os casos, na formulação pura ou na mistura com Glycyrrhiza glabra.
Palavras-Chave: antioxidante; produtos cárneos emulsionados; oxidação lipídica; uva Japão.

1 Introduction

Mortadella are extremely susceptible to lipid oxidation as they have up to 30% fat. In addition, high temperatures of their thermal treatment enhance oxidation. Then, the quality of meat products is directly linked with the influence of lipid oxidation (Biasi *et al*. 2023; Domínguez *et al*., 2019; Santos *et al*., 2023).

Lipid oxidation is a process that includes multiple mechanisms with very complex reactions and interactions between substrates and catalysts. Intrinsic (meat composition) and extrinsic factors (processing and storage conditions) can promote or inhibit oxidative reactions. Meat and meat products are very complex matrices with a composition that makes them susceptible to oxidation processes. The oxidative processes on lipids, proteins, pigments, and vitamins are frequent and interrelated, negatively affecting the quality of the meat, including color and texture changes, rancidity development, nutrient losses, and the formation of toxic compounds (Domínguez *et al*., 2019). Then, inhibition of lipid oxidation is a major concern regarding the storage of meat products.

Synthetic antioxidants play a key role in the prevention of oxidative reactions, delaying quality loss and extending the life of meat products (Kumar *et al*., 2015). However, the toxicological effects associated with the consumption of synthetic antioxidants have not been explained, and conflicting results have been reported in animal studies (Yehye *et al*., 2015). In addition, the possible carcinogenic activity of synthetic antioxidants mainly used in the meat industry indirectly contributes to the increasing search for knowledge about natural antioxidants that also attract the attention of consumers and researchers (Chandra *et al*., 2014). Consumers have presented a growing interest in foods processed with "natural ingredients or raw materials" (Baldin *et al*., 2016).

Natural antioxidants, particularly those obtained from unexplored sources, are attracting more attention due to their health benefits and potential applications in a range of categories, such as food, pharmaceutical, and chemical industries, besides reducing environmental impact and economic issues related to agribusiness (Kumar *et al*., 2015). Possible sources of natural antioxidants include extracts from plants, such as *Hovenia dulcis* T. (Rhamnaceae), which produces sweet-tasting edible pseudo fruits containing many nutrients (Carvalho, 1994; Maieves *et al.*, 2015). Studies indicate pseudo fruits have an inhibition effect on lipid oxidation due to their high content of phenolic compounds (Sehn *et al*., 2021; Xiong *et al*., 2012). When harvested in different maturation periods and evaluated for antioxidant potential, pseudofruits showed a high antioxidant potential *in vitro* testing, especially in an early stage of maturation, presented higher levels of ascorbic, citric, and tartaric acids (Maieves *et al.*, 2015).

Another extract plant is the licrezz™ from licorice (*Glycyrrhiza glabra*), an herb that has been used for centuries as a food flavoring ingredient and medicinal additive to treat main diseases in humans. Licorice contains a variety of bioactive molecules, such as different phenolic compounds (Li *et al*., 2017) and saponin glycyrrhizin, which present biological and pharmaceutical properties, including immunoregulatory, anti-inflammatory, anti-viral, anticarcinogenic activities (Quintana, *et al*., 2019), antifungal properties (Fatima *et al*., 2009), as well as antioxidant and antimicrobial activities (Thakur *et al*., 2016).

Additionally, licorice root has some important phenolic compounds, which have highly antioxidant activity. Some of these compounds are glabridin, hispaglabridin (A and B), 4'-o-methylglabridine, isoprenylchalcone, isoliquiritigenin, and formononetin (Martins, *et al*., 2015; Li *et al*., 2013). Furthermore, Kong *et al*. (2010) reported that licorice root extract, and many other herb/spice extracts, showed high effectiveness in inhibiting lipid oxidation in fresh meat during cold storage.

Therefore, this study aimed to evaluate the addition of *Hovenia dulcis* T. extract and licorice root extract (licrezz™) in the formulation of Bologna-type mortadella and to investigate inhibition of

Revista Pubvet - Early view

the oxidative effect of lipids during cold storage compared with sodium erythorbate, commercially used as an antioxidant agent.

In this way, the rest of this paper is divided into two sections. Section 2 presents the experimental procedures used in the development of this project, and Section 3 contains a discussion of the results obtained and correlations with other works already published in the literature. Finally, the Section 4 has the main conclusions of this research.

2 Material and methods

Hovenia dulcis T. extract was prepared using the methodology described by Larrauri, Rupérez and Saura-Calixto (1997), with some modifications. The extraction used water as a solvent, with a ratio of 1.5:10 (m/v). The mixture was stirred at 40 rpm in a shaker (Lucadema, Luca-223, Brazil) for 60 minutes at 30 °C. Then it was filtered using Whatmann quantitative filter paper nº 40 (GE Healthcare) and the filtrate was stored in a container, wrapped in aluminum foil, and frozen in an ultra-freezer at –18 °C until application.

Antioxidants sodium erythorbate, licrezz™ (extracted from licorice root), and the other ingredients: sodium caseinate, curing salt, salt, sodium polyphosphate, mortadella condiments, and cochineal carmine dye were provided by ICL Brazil. The raw materials: pork, beef, pork fat, cassava starch, and ice were bought at local stores.

Mortadella was prepared in duplicate batches for each treatment, the only difference was the antioxidant: 0.1% sodium erythorbate (control); 0.1% licrezz™, and 0.1% *Hovenia dulcis* extract. The formulation consisted of pork (52%), beef (21%), pork back fat (17%), ice (4%), sodium caseinate (1.5%), curing salt (nitrite, nitrate, and salt) (0.13%), salt (1.0%), sodium polyphosphate (tetrasodium pyrophosphate, sodium acid pyrophosphate and potassium metaphosphate) (0.3%), cassava starch (2%), cochineal carmine dye (0.02%) and mortadella spices (salt, red pepper, black pepper, natural flavor of spices, natural smoked flavor) (1%).

Mortadella paste was obtained by homogenization of all ingredients in a cutter (Frigomaq, Brazil) until they were fully mixed, at temperature control up to 10 °C. Then it was embedded in plastic casings of 6 cm diameter and around 100 g. All mortadella was cooked in a water bath for one hour and 45 minutes under a heat ramp: 30 minutes at 55 ºC, 30 minutes at 65 ºC, 30 minutes at 75 ºC and 15 minutes at 85 ºC, and ensuring the final core temperature of 72 °C. Finally, all mortadella were immersed in a cold-water bath (0 °C) and stored at a controlled temperature of 4±1 °C in a BOD chamber (Lucadema, Luca-161/01) until the moment of analysis, in triplicate.

Mortadella proximate composition was determined in triplicate on the first day of product conservation. Ashes were obtained from incineration in a muffle furnace at 550 ºC by method nº 923.02, (AOAC, 2016). Protein determination was performed by the micro-Kjeldahl method nº 960.52, which assessed the total organic nitrogen content (AOAC, 2016). Factor 6.25 was used to convert the result into crude protein. The parameter of ether extract or total lipids was determined by the Soxhlet extraction method nº 920.39 (AOAC, 2016). The moisture content was determined by the drying oven method (105±5 °C), according to AOAC methodology nº 925.45 (2016), until constant mass. Texture profile analysis was performed according to Pires *et al.* (2017) on day 1 of storage using texture analyzer TAXT2i (Stable Micro Systems, United Kingdom). Samples of around 2 cm thick and 4 cm diameter were compressed axially in two consecutive cycles of 50% compression using a 50.8 mm diameter acrylic probe at a constant speed of 2 $mm.s^{-1}$. The following parameters were evaluated: hardness, cohesiveness, adhesiveness, springiness, and chewiness. Six repetitions were analyzed for each formulation.

The microbiological evaluation of mortadella was performed according to the Manual of Official Methods for Analysis of Animal Foods (Brazil, 2022) on day 1 of storage, evaluating the presence of *Salmonela spp*., sulfite-reducing Clostridium count, total and thermotolerant coliform, and *Staphylococcus aureus*. Moreover, some other analyses were performed during cold storage. Mortadella was evaluated on days 1, 7, 14, 21, and 28, in triplicate, for pH analysis, applying the official AOAC methodology nº 943.02 (2016) and using a pH meter (mPA210). However, it is important to highlight that due to the lack of time to carry out the analyses, it was possible to monitor

Revista Principia - Early View

the pH variation for up to 28 days, even though evidenced that the mortadella's shelf life is around 90 days. Water activity was measured using a Pre-Water Activity Analyzer, Decagon, USA, according to the manufacturer's instructions.

The instrumental color was determined by colorimetry (EZ 0374 4500L, Hunter Lab MiniScan, Brazil), operating in the CIE system (L*, a*, b*, where L* represents luminosity and a* and b* are the chromaticity coordinates). The color variation (ΔE) was calculated by variation lightness (L*), chromaticity a* (green to red), and chromaticity b* (blue and yellow) of the control formulation regarding the formulations with licrezz™ and *Hovenia dulcis* extract, according to Ripoll *et al*. (2013). A stock solution of 1.0×10^{-8} $mL.L^{-1}$ of distilled water was prepared for the construction of the standard curve of 1,1,3,3-tetraethoxypropane, and 1 mL to 5 mL of 1,1,3,3-tetraethoxypropane stock solution was added to test tubes containing 5 mL of 0.02 M thiobarbituric acid (TBA) solution. The tubes were submitted to a water bath at 97 °C for 20 minutes, observing their pink color development during cooling until room temperature with the aid of a chilled water bath. The absorbance of each solution was measured at 538 nm in a spectrophotometer (FEMTO, Cirrus 80SA), and a blank solution was prepared by adding distilled water to replace the stock solution. For sample analysis, about 5 g of the sample was extracted with 10% trichloroacetic acid solution. The mixture was centrifuged, and 5 mL of the supernatant was added to a test tube containing 5 mL of 0.02 M TBA. The same steps were performed as described for the construction of the standard curve. Malonaldehyde concentration in mg per kg of the sample was determined by the equation obtained with the standard curve of 1,1,3,3-tetraethoxypropane.

Nitrite analysis was performed using the residual meat nitrite methodology described by AOAC (2016), for which 10 g of the sample was ground. Then, the deproteinization steps of the samples were performed using a 5% (w/v) borax solution, heated in a water bath at 80 °C for 15 min, after the addition of 15% (w/v) potassium ferrocyanide and 30% (w/v) zinc acetate at room temperature. The solution was filtered using filter paper, and 0.5% (w/v) sulfanylamide and 0.5% (w/v) alpha-(naphthyl)ethylenediamine were added. Nitrite was determined in a spectrophotometer (FEMTO, Cirrus 80SA) at 540 nm after 15 minutes of reaction. To determine the standard curve, dilutions of a standard solution containing 1 $\mu g.mL^{-1}$ sodium nitrite were used.

For the statistical treatment, an analysis of variance (ANOVA) with a mixed model was performed for all variables considered in the study. These parameters were set as dependent variables, the formulation was included in the model as fixed effects, while the different batches and replicates were considered random effects. The comparisons of treatments were performed by Tukey's test ($p < 0.05$), using the Statistica 14 Trial software Statsoft. The values were expressed as mean values and standard deviation.

3 Results and discussions

In this section, the results obtained for proximate composition, texture profile, microbiological and physicochemical analyses will be presented, analyzed, discussed, and compared with works already published in the literature and, thus, to demonstrate the effectiveness of *Hovenia dulcis* T. as a natural antioxidant.

3.1 Proximate composition

The results of proximate composition showed no significant statistical difference ($p < 0.05$) between the control sample and samples with different antioxidant substances to moisture, protein, and lipids (Table 1). The differences in ash content were possibly due to inherent differences in the raw material. The values are by the recommendations of the Brazilian legislation (Brazil, 2000), which establishes the following limits: max. 70% moisture, max. 30% lipid, and min. 12% protein. Additionally, some works published also reported values like those of this work. This proves that formulations are suitable for obtaining excellent quality mortadella (Pires *et al*., 2017; Alves *et al*., 2016).

Revista Pluricia - Early View

Table 1 – Proximate composition of Bologna-type mortadella prepared with different antioxidants: sodium erythorbate (control), licrezz™, and *Hovenia dulcis* T. extract

Analysis (%)	Control	Extract	Licrezz™
Moisture	61.05 ± 0.14^{ns}	61.15 ± 0.16^{ns}	60.76 ± 0.20^{ns}
Protein	15.44 ± 0.41^{ns}	15.57 ± 0.20^{ns}	15.45 ± 0.21^{ns}
Lipids	11.59 ± 1.11^{ns}	11. 64±0.57^{ns}	12,99 ± 0.89^{ns}
Ash	1.94 ± 0.20^{b}	2.43±0.17^{a}	2.31 ± 0.10^{a}

Mean ± standard deviation (n = 3). Different small letters, on the same line, indicate statistically significant differences (p < 0.05). ns: statistically not significant differences (p < 0.05).
Source: research data

The amounts of antioxidants added to mortadella did not influence the proximate composition of the samples. Also, the data obtained were following the current legislation and were similar to the results of other studies analyzing Bologna-type mortadella.

3.2 Texture profile analysis

Table 2 presents the results of the texture profile analysis of mortadella with different antioxidants compared to the control sample, showing no significant difference (p < 0.05) between the values of different samples in all parameters evaluated. The addition of natural antioxidants, *H. dulcis* extract, and licrezz™ did not change the texture profile of Bologna-type mortadella. These results may be related to the low amount of antioxidants added, not presenting negative effects on the texture properties of the samples. Sucu and Turp (2018) investigated the use of beetroot powder in sausage as a nitrite replacement agent and observed that adding small proportions of natural ingredients did not significantly affect such characteristics.

Table 2 –. Texture profile analysis of Bologna-type mortadella samples prepared with different antioxidants: sodium erythorbate (control), licrezz™, and *Hovenia dulcis* T. extract

Parameter	Control	Extract	Licrezz™
Hardness (N)	232.4 ± 30.8^{ns}	220.7 ± 8.0^{ns}	205.5 ± 24.7^{ns}
Cohesiveness	1.1 ± 0.1^{ns}	1.0 ± 0.1^{ns}	1.2 ± 0.2^{ns}
Adhesiveness (mJ)	0.2 ± 0.1^{ns}	0.3 ± 0.0^{ns}	0.2 ± 0.1^{ns}
Springiness (mm)	10.9 ± 0.3^{ns}	10.9 ± 0.1^{ns}	11.3 ± 0.1^{ns}
Chewiness (mJ)	2723.8 ± 266.4^{ns}	2424.7 ± 199.8^{ns}	2851.4 ± 437.7^{ns}

Mean±standard deviation (n = 4). ns - statistically not significant differences (p < 0.05).
Source: research data

Other studies reported the texture profile of mortadella without significant alteration after adding different substances, such as Doménech-Asensi *et al.* (2013), who used tomato paste in mortadella. These authors obtained similar results for texture profile, but lower values than those found in this study for hardness (64.46 N), cohesiveness (0.53), and higher values for elasticity/springness (0.07 m), possibly due to the typical formulation of Bologna-type mortadella, which has higher addition of meat proteins.

Also, the study conducted by Barbieri *et al.* (2013), who evaluated the texture profile of different commercial Bologna-type mortadella of different brands, presented lower results for hardness (14.84 N) when compared to those found in this study. Of note, mortadella is commonly consumed in slices, so higher hardness and elasticity results can positively affect the slicing ability of meat products and may increase product acceptance (Delahunty *et al.*, 1997).

3.3 Microbiological characterization

Revista Principia - Early View

Table 3 shows the results of microbiological analyses. All samples complied with the legal standard described in Normative Instruction nº 4 of March 31, 2000, of the Technical Regulation for Mortadella Identity and Quality (Brazil, 2000), that is, *Salmonella* absence in 25 g of the sample, max. 5.0×10^3 CFU.g^{-1} for *Staphylococus aureus*, and max. 1.0×10^3 CFU.g^{-1} for *Clostridium perfringens*. Different antioxidants applied in mortadella did not affect the microbiological characteristics during sample storage.

Table 3 – Microbiological characterization of Bologna-type mortadella prepared with different antioxidants: sodium erythorbate (control), licrezz™ and *Hovenia dulcis* T. extract

Analysis (CFU.g^{-1})	**Control**	**Extract**	**Licrezz™**
Staphylococus aureus	$<1.0 \times 10^2$	$<1.0 \times 10^2$	$<1.0 \times 10^2$
Salmonella spp.	Absence	Absence	Absence
Total coliform count	$<3.0 \times 10^0$	$<3.0 \times 10^0$	$<3.0 \times 10^0$
Thermotolerant coliform count	$<3.0 \times 10^0$	$<3.0 \times 10^0$	$<3.0 \times 10^0$
Clostridium sulfite reducer	$<1.0 \times 10^1$	$<1.0 \times 10^1$	$<1.0 \times 10^1$

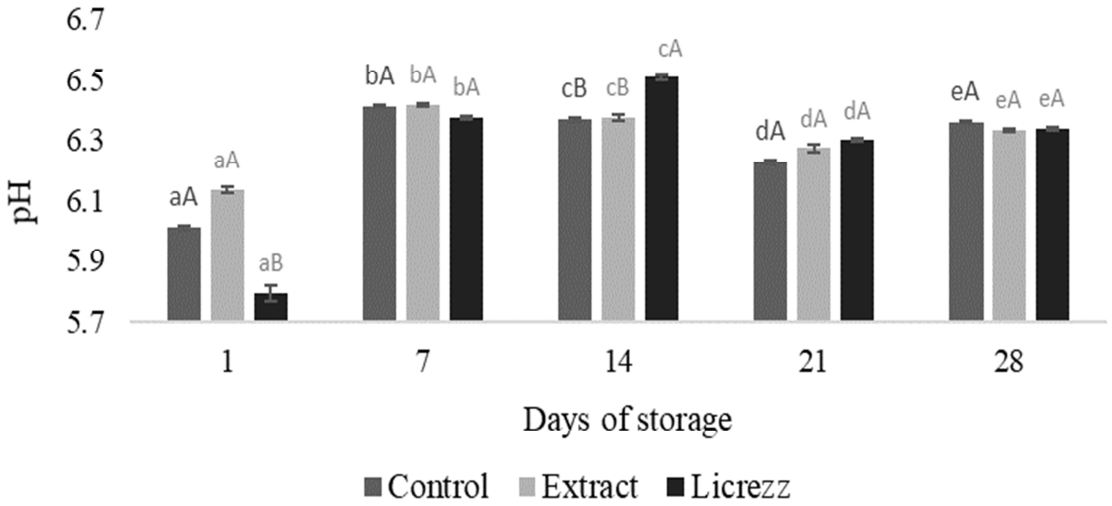
Source: research data

The results for total and thermotolerant coliform agree with the studies conducted by Baldin *et al.* (2016), who evaluated the application of jabuticaba extract to Bologna-type mortadella and found 3.0×10^0 CFU.g^{-1}, which is expected for this product, as it is subjected to a thermal treatment in the cooking stage.

3.4 Results of analyses performed during cold storage of mortadella

During sample storage, pH ranged from 5.7 to 6.5, with all samples presenting significant differences ($p < 0.05$) during the storage period (Figure 1). At day 7 of storage, a significant increase in pH ($p < 0.05$) was observed in control, licrezz™, and *Hovenia dulcis* T. extract samples when compared to day 1. Day 1 and day 14 of storage showed a significant difference ($p < 0.05$) between the licrezz™ samples and the others. On day 1, the samples with licrezz™ showed lower pH and at day 14, a higher pH than the control and *H. dulcis* extract samples. Mortadella containing *H. dulcis* extract did not differ from the control sample in all periods evaluated. On the other days, the samples did not differ significantly from each other ($p < 0.05$), indicating the addition of different antioxidant compounds did not change the pH behavior after day 21 of storage.

Figure 1 – pH results during cold storage of Bologna-type mortadella samples prepared with different antioxidants: sodium erythorbate (control), licrezz™, and *Hovenia dulcis* T. extract



Different small letters indicate statistically significant differences ($p < 0.05$) for the same sample in different storage periods; different capital letters indicate statistically significant differences ($p < 0.05$) between samples in the same storage period.

Principia - Early View

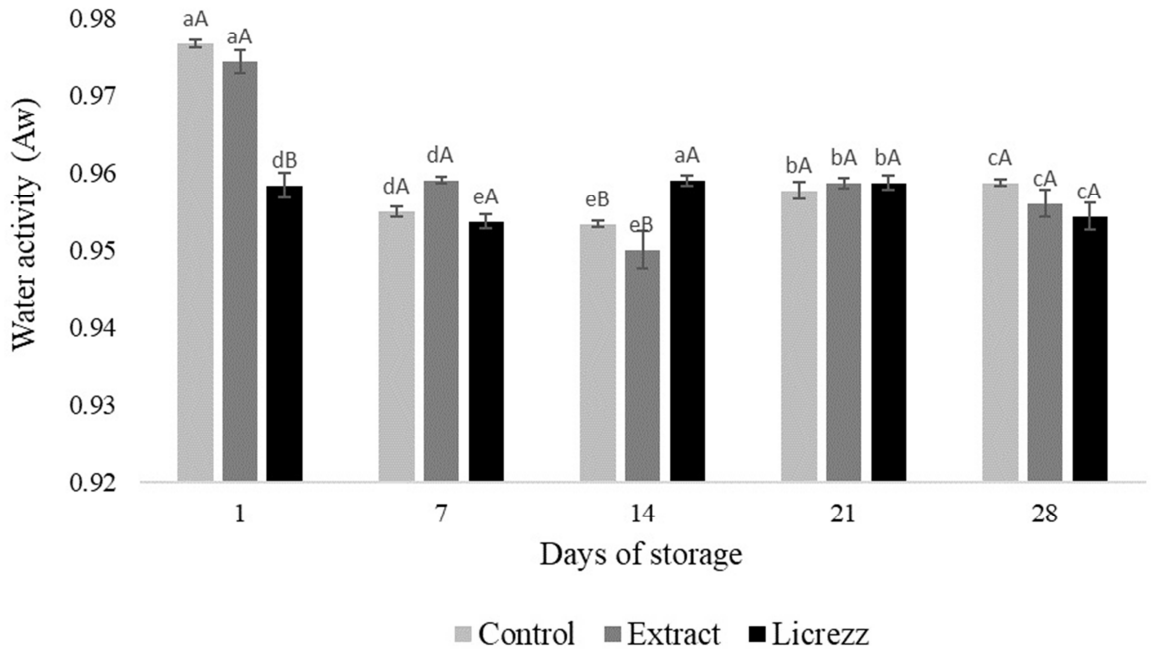
Source: research data

According to Mendonça *et al.* (2013), increased pH may be due to the formation of non-protein compounds and ammonium ions with protein buffer actions, promoting pH elevation during mortadella storage. Increased pH is more evident in the sample with licrezz™, possibly due to the acidity of the extract origin (licorice root).

Similar results were observed by Pires *et al.* (2017), who evaluated sodium reduction in Bologna-type mortadella, and by Almeida *et al.* (2015), who evaluated the effect on lipid oxidation of jabuticaba extract added to Bologna-type mortadella. These authors reported increased pH at the beginning of the mortadella storage, with subsequent stabilization, indicating a characteristic behavior of Bologna-type mortadella, possibly due to the meat buffering capacity.

Water activity (Aw) results during the 28-day storage period ranged from 0.976 to 0.945 (Figure 2). A significant difference was observed between the samples on day 1 and day 14 ($p < 0.05$); in the other periods, the treatments were similar to the control sample and did not differ significantly.

Figure 2 – Water activity results during cold storage of Bologna-type mortadella samples prepared with different antioxidants: sodium erythorbate (control), licrezz™, and *Hovenia dulcis* T. extract



Different small letters indicate statistically significant differences ($p < 0.05$) for the same sample in different storage periods; different capital letters indicate statistically significant differences ($p < 0.05$) between samples in the same storage period.
Source: research data

When the Aw profile was observed during storage, a significant difference ($p < 0.05$) was observed for all samples. During the first day of storage, all samples presented reduced water activity, and later an increase was observed in the control and extract samples, unlike the licrezz™ sample, which remained stable at the end of storage.

The initial variation in water activity of the samples may be due to the stabilization of the emulsified meat batter; the emulsion is stabilized after cooking and cooling when fat globules and other components are immobilized due to protein gelation, so the end product characteristics are achieved (Ignácio, 2011).

The results are in agreement with typical values for mortadella, from 0.950 to 0.960 according to Yunes *et al.* (2013). In addition, these authors replaced pork fat with vegetable oils in mortadella and found Aw values from 0.954 to 0.970, similar to this study. Rodrigues (2016) used ora-pro-nobis in a pork mortadella and found water activity results from 0.960 to 0.980 along the product storage period.

Table 4 presents the mean values for luminosity (L*), showing a significant reduction ($p < 0.05$) of luminosity in all treatments from day 1 to day 7. It indicates all samples were darker during this period. After day 7 no significant difference was observed during storage. This fact may be related to fat oxidation during the storage period. Luminosity in foods is affected by the concentration and type of pigments, which may also be influenced by the content of nitrite in samples (Yunes *et al.*, 2013).

Table 4 – Results of instrumental color (L*, a*, b*) during cold storage of Bologna-type mortadella samples prepared with different antioxidants: sodium erythorbate (control), licrezz™ and *Hovenia dulcis* T. extract

		Storage days				
Parameter	**Sample**	**1**	**7**	**14**	**21**	**28**
L*	**Control**	48.60 ± 0.21 ^{aA}	42.16 ± 0.31 ^{bB}	43.61 ± 0.31 ^{bA}	42.07 ± 0.09 ^{bA}	41.72 ± 0.30 ^{bA}
	Extract	48.96 ± 0.81 ^{aA}	44.03 ± 1.22 ^{bA}	43.29 ± 0.96 ^{bA}	43.30 ± 1.10 ^{bA}	41.78 ± 1.27 ^{cA}
	Licrezz	49.32 ± 0.55 ^{aA}	43.47 ± 0.11 ^{bA}	44.14 ± 0.46 ^{bA}	41.34 ± 0.85 ^{bA}	41.82 ± 0.08 ^{bA}
a*	**Control**	10.90 ± 0.15 ^{aA}	9.96 ± 0.18 ^{bA}	11.34 ± 0.31 ^{aA}	10.26 ± 0.21 ^{bA}	11.40 ± 0.49 ^{aA}
	Extract	10.60 ± 0.17 ^{aA}	9.15 ± 0.26 ^{bA}	9.63 ± 0.28 ^{bB}	8.51 ± 0.26 ^{bB}	8.86 ± 0.08 ^{bC}
	Licrezz	9.12 ± 0.19 ^{bB}	7.87 ± 0.29 ^{cB}	9.56 ± 0.09 ^{aB}	8.94 ± 0.22 ^{bB}	10.01 ± 0.18 ^{aB}
b*	**Control**	12.84 ± 0.63 ^{aA}	11.60 ± 0.02 ^{bA}	11.87 ± 0.18 ^{aA}	12.14 ± 0.24 ^{aA}	11.53 ± 0.21 ^{bA}
	Extract	12.81 ± 0.64 ^{aA}	11.57 ± 0.19 ^{aA}	11.65 ± 0.30 ^{aA}	12.06 ± 0.30 ^{aA}	11.50 ± 0.28 ^{bA}
	Licrezz	11.76 ± 0.26 ^{Aa}	10.47 ± 0.39 ^{bB}	11.51 ± 0.21 ^{aA}	10.01 ± 0.18 ^{bB}	11.64 ± 0.19 ^{aA}
ΔE	**Extract**	0.69	2.71	1.61	3.06	2.63
	Licrezz	3.58	4.53	2.67	2.72	1.38

On each line, different small letters indicate statistically significant differences ($p < 0.05$) for the same sample in different storage periods; on each column, different capital letters indicate statistically significant differences ($p < 0.05$) between samples in the same storage period.
Source: research data

Also, the samples containing *Hovenia dulcis* T. extract and licrezz™ did not differ significantly ($p < 0.05$) from the control sample in luminosity, except on day 7 of storage, when the samples containing extract and licrezz™ presented better results than the control sample.

Parameter a*, which promotes a red color of the samples, showed a significant difference between treatments ($p < 0.05$) on all days of analysis, suggesting that adding *H. dulcis* extract and licrezz™ had a negative influence on the typical red color of samples when compared to the control sample, which showed higher values of a*, especially starting at day 14.

When the storage period was evaluated, at day 7 of storage a significant difference ($p < 0.05$) was observed in the control sample, which presented reduced intensity of red color, but in the rest of the period, no significant difference was reported when compared to day 1 of storage. However, when the storage period of licrezz™ and extract samples was evaluated, the behavior was different from the control sample, with a significant difference ($p < 0.05$) between the evaluated days, suggesting that color did not remain stable during the storage period.

A more intense red color was observed in samples containing *Hovenia dulcis* extract when compared to licrezz™, which presented lower values, especially on the first days of storage. This

Revista Principia - Early View

difference between the samples may be associated with the fact that licrezz™ is not easily dissolved in the formulation.

One factor that may be associated with the different results obtained for instrumental color parameters is the addition of nitrite, as higher additions meant higher a* values and lower b* values (Baldin *et al*., 2016). In this study, the amount of nitrite added was the same for all samples, but the conversion of nitrate to nitrite was possibly different for the samples (Table 4) as residual nitrite in the samples with licrezz™ and extract was significantly different on some storage days but higher for the extract, and when compared to the control sample. During the whole storage period, residual nitrite was lower than in the other samples, indicating that the amount of nitrite present in the samples did not impact differences in parameter a*.

According to Farhi and Al-Sawalha (2023) the addition of antioxidants significantly affects the characteristic color of mortadella, and to obtain a pink-red color, first, nitrate (NO_3) must be converted to nitrite (NO_2) and then to nitric oxide (NO), which reacts with iron from the heme pigment molecule, generating the nitrosohemochrome pigment (in cooked products). These pigments ensure a stable compelling pink-red color, typical of cured products. Its intensity (a*) depends on some conditions, including the presence and action of certain microorganisms, reducing agents in the formulation such as natural antioxidants and vitamins, enzymes, pH of the medium, potential redox reduction, and presence of free iron.

For parameter b*, considering that b*+ indicates a tendency to yellow, in general, the samples presented low values during storage. The results differed significantly ($p < 0.05$) only on the last day of storage for control and extract samples. Also, the samples with licrezz™ differed along the storage period possibly because the extract is not easily dissolved. No significant difference was observed between the control and extract samples ($p < 0.05$); however, the samples with licrezz™ differed statistically ($p < 0.05$) from the control samples on days 7 and 21.

Studies conducted by Cáceres, García and Selgas (2008) presented similar results for parameters a* (13.15) and b* (10.57) and L* (48.28) for Bologna-type mortadella. Pereira *et al*. (2011) found higher results for L* (60.12) after adding mango seed extract to Bologna-type mortadella. This variation in the parameters is mainly due to different formulations to produce mortadella.

For ΔE (Table 4), according to Francis and Clydesdale (1975), values close to zero indicate samples that are similar to control, and values of two or more indicate differences between two treatments that can be perceptible to the human eye. Then, the treatment using extract and licrezz™ resulted in a visible color change when compared to the control sample. However, the addition of licrezz™ had a more significant influence on the color difference from the control sample, possibly since licrezz™ is not easily dissolved in the mortadella paste.

Lipid oxidation occurs during the processing and storage of meat products; it cannot be fully inhibited, but it can be delayed with the addition of antioxidant substances that provide stability and reduce the negative impact of oxidation on the sensory attributes of products (Doménech-Asensi *et al*., 2013). The results of lipid oxidation in mortadella prepared with different antioxidants ranged from 0.25 to 2.05 mg malonaldehyde/kg of the sample (Table 5).

Table 5 – Results of lipid oxidation (TBARS) and residual nitrite during cold storage of Bologna-type mortadella samples prepared with different antioxidants: sodium erythorbate (control), licrezz™, and *Hovenia dulcis* T. extract

		Storage days				
Analyze	**Sample**	**1**	**7**	**14**	**21**	**28**
TBARS[α]	**Control**	0.09±0.01^{cB}	0.51±0.04^{bA}	0.54±0.01^{bB}	0.89±0.04 ^{aB}	0.90±0.03^{aC}
	Extract	0.19±0.03^{eA}	0.31±0.02^{dB}	0.66±0.03^{cA}	1.27±0.04^{bA}	1.76±0.03^{aB}
	Licrezz	0.08±0.01^{eB}	0.19±0.01^{dC}	0.45±0.04 ^{cC}	0.55±0.01^{bC}	2.12±0.05^{aA}
Nitrite[β]	**Control**	101.00±0.01^{aB}	37.00±0.01^{bB}	24.00±0.01 ^{Cb}	23.00±0.01^{cB}	4.00±0.01^{dC}
	Extract	176.00±0.10^{aA}	66.00±0.08^{bA}	66.00±0.01^{bA}	26.00±0.01^{cA}	16.00±0.01^{dA}

Revista Principia - Early View

Licrezz	175.00±0.01^{aA}	63.00<0.01^{cA}	68.00 <0.01^{Ba}	26.00<0.01^{dA}	11.00<0.01^{eB}

On each line, different small letters indicate statistically significant differences ($p < 0.05$); on each column, different capital letters indicate statistically significant differences ($p < 0.05$). MDA - malonaldehyde. $^{\alpha}$ (mg MDA kg sample^{-1}); $^{\beta}$ (ppm $NaNO_2$)
Source: research data

According to Trindade *et al.* (2008), a rancid product can be detected by trained and untrained tasters in the range of 0.5-1.0 and 0.6-2.0 mg malonaldehyde/kg of sample, respectively. Then, only samples containing licrezz™ at day 28 would likely present a rancid smell.

The results of lipid oxidation (TBARS) obtained from the different treatments were significantly different ($p < 0.05$), indicating a difference between the treatments and the control over the storage period (Table 5). For all treatments, a significant increase ($p < 0.05$) of TBARS values was observed during the storage period. The control and *H. dulcis* extract samples presented at final storage an increase approximated nine times of initial TBARS value while licrezz™ was twenty-six times, indicating that *H. dulcis* extract showed similar behavior to control.

In addition, TBARS values were significantly different between treatments ($p < 0.05$) already at day 1 of storage, higher for samples with *H. dulcis*, and control at days 1, 7, 14, and 21. After a 28-day cold storage period, the control and *H.dulcis* samples presented the lowest value and stabilization, followed by the samples with extract and licrezz™, suggesting these agents did not achieve the same efficiency as the antioxidant agent added to the control formulation in the evaluated period. Sodium erythorbate (control) is a concentrate powder antioxidant, possibly with a stronger action of lipid oxidation control. Studies conducted by Trindade *et al.* (2008) report that sodium erythorbate has a positive effect on lipid oxidation control when added in amounts exceeding 100 ppm.

Similar results were found by Doménech-Asensi *et al.* (2013) by applying tomato paste to mortadella, demonstrating that an increased concentration of tomato paste leads to reduced lipid oxidation when compared to the control sample containing sodium erythorbate, but when added in the same proportion, oxidation was not reduced. Then, this suggests increasing the amount of *H. dulcis* extract and licrezz™ added to mortadella, which in this study was the same as erythorbate (0.1%).

Almeida *et al.* (2015), when applying jabuticaba extract to Bologna-type mortadella at various concentrations, observed that the control samples containing 0% antioxidant and the sample containing 0.25% jabuticaba extract showed no significant difference ($p < 0.05$) along the cold storage period.

Nissen *et al.* (2004) also reported successful inhibition of lipid oxidation in processed meat products using natural plant antioxidants, including *Prunus domestica* extract. Rodrigues (2016) found 0.395 to 0.500 mg malonaldehyde/kg of sample in mortadella with the addition of 2% ora-pro-nobis as an antioxidant agent replacing sodium erythorbate.

The effect of plant antioxidants on reduced lipid oxidation can be attributed to their phenolic character, as phenolic compounds act as radical sequestrants and sometimes as metal chelators, acting both in the beginning and propagation of the oxidative process. The antioxidant activity of phenolic compounds is associated with the hydroxyl group connected with the aromatic ring, which can donate electrons with hydrogen atoms and neutralize free radicals. This mechanism blocks the degeneration of more active oxidizing forms such as malonaldehyde (Krishnan *et al.*, 2014).

Nitrite reactions result in changes in cured meat color, microbial inhibition, antioxidant effects, and taste (Sucu; Turp, 2018). However, as it is a potentially toxic compound Kurćubić *et al.* (2014), it should be controlled in end products. In Brazil, the maximum residual amount of nitrite, expressed as sodium nitrite, allowed in cured products is 150 ppm (Brazil, 2000). However, its reduction is limited due to risks related to the development of *C. botulinum* (O'Sullivan; Kerry, 2012).

Table 5 shows the results of residual nitrite in Bologna-type mortadella prepared with different antioxidants. The mean values of all samples showed significant differences ($p < 0.05$), decreasing over the storage period, showing that nitrite was converted into other compounds. These results comply with the regulatory requirements for product characteristics, below 150 ppm (Brazil, 2000).

The results agree with those reported by Li *et al.* (2013), for which residual nitrite levels can be significantly reduced in meat products immediately after the production process, because when nitrite

Revista Principia - Early View

is added to the paste, it reacts with myoglobin, sulfhydryl groups, lipids, and proteins, transforming due to oxidation into nitric acid (HNO_3) or gaseous forms (N_2O and N_2).

When comparing the mean values between the treatments, the samples containing *Hovenia dulcis* extract and licrezz™ presented higher residual nitrite than the control sample during the whole period of cold storage, and the samples containing plant extracts differed significantly ($p < 0.05$) from the control sample. The samples containing *H. dulcis* extract and licrezz™ differed significantly ($p < 0.05$) from each other only after 21 days of storage. Last-day variation may be due to the difficult homogenization of the compound in the paste, as licrezz™ is not easily dissolved in water.

Bologna-type mortadella containing sodium erythorbate (control) presented lower residual nitrite content than samples containing natural antioxidants, differing statistically ($p < 0.05$) in each of the storage periods investigated. These data agree with those of Li *et al*. (2013), who reported the presence of sodium erythorbate produces nitrite, reducing nitrite oxide faster than plant polyphenols.

Also, according to Li *et al*. (2013), reductions in nitrite residual levels are likely to occur due to polyphenols and flavonoids, which react with the various biocompounds present in plant extracts. Hwang *et al*. (2014) also observed the same behavior in emulsified pork sausage, in which the application of 0.2% ethanolic extract of Artemisia reduced the residual nitrite contents during the storage period. In a study conducted by Kurćubić *et al*. (2014), 0.1% to 0.2% ethanolic extract of *Kitaibelia vitifolia* was applied to sausage as an antioxidant agent, and samples containing the extract showed superior residual nitrite results during storage.

Therefore, all the evidence and works published in the literature, previously presented, only contribute to the proof of the excellent results reported in this work, as well as showing that the use of *H. dulcis* extract is an alternative natural antioxidant.

4. Conclusion

The addition of plant extracts from *Hovenia dulcis* T. pseudofruits and licorice root (licrezz™) did not affect the composition microbiological characteristics and texture profile of Bologna-type mortadella. Regarding the instrumental color, changes in parameters L* and a* were observed, possibly due to the difficult dissolution of licrezz™ and poor action of plant extracts in color development.

During the cold storage of mortadella, in general, no significant change was observed in the pH and water activity of the samples. However, mortadella containing natural antioxidants showed alterations in lipid oxidation, suggesting these agents did not achieve the same efficiency as the antioxidant agent added to the control formulation. Likewise observed for the analysis of residual nitrite content, where natural antioxidants managed to reduce the amount found after 28 days, but with a lower effectiveness than the control formulation, with a commercial antioxidant.

However, *H. dulcis* extract showed potential use as a natural antioxidant, and further studies on its concentration and the addition of larger amounts are required, as well as sensory evaluation of mortadella. Furthermore, based on the promising results obtained in this work, there is the possibility of expanding the application of *Hovenia dulcis* T powder in the preparation of sausages or hamburgers, due to its use of similar meat bases.

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Declaration of competing interest

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