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Hovenia dulcis T. extract and *Glycyrrhiza glabra* as natural antioxidants in Bologna-type mortadella

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ABSTRACT: Pseudo-fruits of *Hovenia dulcis* T. are sweet-tasting, edible, and contain numerous nutrients and a significant amount of phenolic compounds, which could serve as a source of natural antioxidants. In this context, 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 the inhibition of lipid oxidation during cold storage, to assess their antioxidant properties. Mortadella samples were prepared in duplicate with sodium erythorbate (control), licrezz™ (commercial extract from *Glycyrrhiza glabra*), and *H. dulcis* extract, at a concentration of 0,1%. The samples were evaluated for proximate composition, microbiological characteristics, texture profile, lipid oxidation, nitrite content, instrumental color, pH, and water activity. Mortadella with *H. dulcis* extract did not show significant differences from the control in terms of proximate composition, microbiological characteristics, or texture profile. However, they exhibited slightly lower, but comparable results in the Thiobarbituric Acid Reactive Substances (TBARS) assay during the 28 days of refrigerated storage. The control and *H. dulcis* extract samples showed approximately a ninefold increase in TBARS values by the end of storage, while licrezz™ showed a twenty-sixfold increase. On the other hand, H. dulcis extract exhibited similar behavior to licrezzTM regarding residual nitrite content. Thus, *H. dulcis* pseudo-fruits extract shows potential for use as a natural antioxidant in emulsified meat products, alone or in combination with *Glycyrrhiza glabra*.

Keywords: antioxidant; Bologna-type mortadella; emulsified meat product; lipid oxidation.

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

RESUMO: *Os pseudofrutos de Hovenia dulcis T. são doces, comestíveis e contêm vários nutrientes e uma quantidade significativa de compostos fenólicos, que*

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podem servir como fonte de antioxidantes naturais. Nesse contexto, 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 tipo Bologna e investigar a inibição da oxidação lipídica durante o armazenamento a frio, para avaliar suas propriedades antioxidantes. Amostras de mortadela foram preparadas em duplicata com eritorbato de sódio (controle), licrezz™ (extrato comercial de Glycyrrhiza glabra) e extrato de H. dulcis, todas na concentração de 0,1%. As amostras foram avaliadas quanto à composição centesimal, características microbiológicas, perfil de textura, oxidação lipídica, teor de nitrito, cor instrumental, pH e atividade de água. A mortadela com extrato de H. dulcis não apresentou diferenças significativas em relação ao controle em termos de composição centesimal, características microbiológicas ou perfil de textura. No entanto, ela exibiu resultados ligeiramente mais baixos, mas comparáveis, no ensaio de Substâncias Reativas ao Ácido Tiobarbitúrico (TBARS) durante 28 dias de armazenamento refrigerado. As amostras de controle e extrato de H. dulcis mostraram um aumento de aproximadamente nove vezes nos valores de TBARS ao final do armazenamento, enquanto o licrezz™ mostrou um aumento de vinte e seis vezes. Por outro lado, o extrato de H. dulcis exibiu comportamento semelhante ao licrezz™ em relação ao conteúdo residual de nitrito. Assim, o extrato de pseudofruto de H. dulcis mostra potencial para uso como um antioxidante natural em produtos de carne emulsionados, sozinho ou em combinação com Glycyrrhiza glabra.

Palavras-chave: *antioxidante; mortadela tipo Bologna; oxidação lipídica; produtos de carne emulsionados.*

1 Introduction

Mortadella is extremely susceptible to lipid oxidation due to its fat content of up to 30%. Additionally, the high temperatures involved in its thermal treatment further promote oxidation. Therefore, the quality of meat products is directly linked to the influence of lipid oxidation (Biasi *et al*., 2023; Domínguez *et al*., 2019; Santos *et al*., 2023).

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

Synthetic antioxidants play a critical role in preventing oxidative reactions, delaying quality deterioration, and extending the shelf life of meat products (Kumar *et al*., 2015). However, the toxicological effects associated with the consumption of synthetic antioxidants have not been fully explained, and conflicting results have been reported in animal studies (Yehye *et al*., 2015). Additionally, the potential carcinogenic activity of synthetic antioxidants commonly used in the meat industry has indirectly contributed to the growing interest in natural antioxidants, which are increasingly attracting the attention

of both consumers and researchers (Chandra *et al*., 2014). Consumers have increasingly preferred foods processed with "natural ingredients or raw materials" (Baldin *et al*., 2016).

Natural antioxidants, particularly those obtained from unexplored sources, are garnering increased attention due to their health benefits and potential applications across various sectors, including the food, pharmaceutical, and chemical industries, while also addressing environmental and economic concerns related to agribusiness (Kumar *et al*., 2015). Potential sources of natural antioxidants include extracts from plants, such as *Hovenia dulcis* T. (Rhamnaceae), which produces sweet-tasting edible pseudo-fruits rich in nutrients (Carvalho, 1994; Maieves *et al*., 2015). Studies indicate pseudo-fruits have an inhibitory effect on lipid oxidation due to their high content of phenolic compounds (Sehn *et al*., 2021; Xiong *et al*., 2012). When harvested at different maturation stages and evaluated for antioxidant potential, pseudo-fruits demonstrated high antioxidant potential *in vitro*, particularly in the early stage of maturation, where higher levels of ascorbic, citric, and tartaric acids were observed (Maieves *et al*., 2015).

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

Furthermore, licorice root contains important phenolic compounds with high antioxidant activity. Some of these compounds include glabridin, hispaglabridin (A and B), 4'-O-methylglabridine, isoprenylchalcone, isoliquiritigenin, and formononetin (Li *et al*., 2013; Martins *et al*., 2015). Additionally, Kong, Zhang and Xiong (2010) reported that licorice root extract, along with many other herb and spice extracts, was highly effective 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 the inhibition of lipid oxidation during cold storage compared to sodium erythorbate, which is commercially used as an antioxidant agent.

The remainder of this paper is structured as follows: Section 2 outlines the experimental procedures used in the development of this project, Section 3 discusses the results obtained and their correlations with other published works, and Section 4 presents the main conclusions of this research.

2 Material and methods

The *Hovenia dulcis* T. extract was prepared following the methodology described by Larrauri, Rupérez, and Saura-Calixto (1997), with modifications. Water was used as the solvent, in 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. It was then 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.

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

Mortadella was prepared in duplicate batches for each treatment, with the only difference being 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, and natural smoked flavor) (1%).

Mortadella paste was obtained by homogenization of all ingredients in a cutter (Frigomaq, Brazil) until fully mixed, maintaining the temperature below 10 °C. The mixture was then stuffed into plastic casings of 6 cm in diameter, weighing approximately 100 g. All mortadella batches were cooked in a water bath for 1 hour and 45 minutes, following a heat ramp of 30 minutes at 55 ºC, 30 minutes at 65 ºC, 30 minutes at 75 ºC, and 15 minutes at 85 ºC, ensuring the final core temperature of 72 °C. Finally, the mortadella samples 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 analysis, was conducted in triplicate.

The proximate composition of the mortadella was determined in triplicate on the first day of product conservation. Ash content was obtained via incineration in a muffle furnace at 550 ºC according to method nº 923.02, (AOAC, 2016). Protein content was determined using the micro-Kjeldahl method nº 960.52, assessing total organic nitrogen, and a factor of 6.25 was used to convert the result into crude protein. The ether extract or total lipid content was determined using the Soxhlet extraction method nº 920.39 (AOAC, 2016). Moisture content was measured using the drying oven method (105 ± 5 °C) by AOAC methodology nº 925.45 (AOAC, 2016) until a constant mass was achieved.

Texture profile analysis was conducted on day 1 of storage, following the method of Pires *et al*. (2017), using a TAXT2i texture analyzer (Stable Micro Systems, United Kingdom). Samples approximately 2 cm thick and 4 cm in diameter were axially compressed in two consecutive cycles of 50% compression, using a 50.8 mm acrylic probe at a constant speed of 2 $mm.s^{-1}$. The following parameters were evaluated: hardness, cohesiveness, adhesiveness, springiness, and chewiness, with six repetitions analyzed for each formulation.

The microbiological evaluation of the mortadella was performed on day 1 of storage, following the Manual of Official Methods for Analysis of Animal Foods (Brazil, 2022), assessing the presence of *Salmonella spp*., sulfite-reducing Clostridium counts, total and thermotolerant coliforms, and *Staphylococcus aureus*. Additional analyses were conducted during cold storage, with triplicate evaluations on days 1, 7, 14, 21, and 28. The pH was measured using AOAC methodology nº 943.02 (AOAC, 2016), employing a pH meter (mPA210). However, due to time constraints, pH monitoring was limited to 28 days, even though the shelf life of mortadella is approximately 90 days. Water activity was measured using a Pre-Water Activity Analyzer (Decagon, USA) by the manufacturer's instructions.

Instrumental color was determined by colorimetry (EZ 0374 4500L, Hunter Lab MiniScan, Brazil), operating in the CIE system (L*, a*, b*, where L* represents lightness and a* and b* represent chromaticity coordinates). The color variation (ΔE) was calculated by comparing the lightness (L*), chromaticity a* (green to red), and chromaticity b* (blue and yellow) of the control formulation with those containing licrezz™ and *Hovenia dulcis* extract, as described by 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, with 1 mL to 5 mL of stock solution added to test tubes containing 5 mL of 0.02 M thiobarbituric acid (TBA) solution. The tubes were placed in a water bath at 97 °C for 20 minutes, and pink color development was observed as they cooled to room temperature, aided by a chilled water bath. The absorbance of each solution was measured at 538 nm using a spectrophotometer (FEMTO, Cirrus 80SA), and a blank solution was prepared by replacing the stock solution with distilled water. For sample analysis, approximately 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 followed as described for the standard curve construction. The malonaldehyde concentration, expressed as mg per kg of sample, was determined using the equation obtained from the standard curve of 1,1,3,3-tetraethoxypropane.

Nitrite analysis was conducted using the residual meat nitrite methodology described by AOAC (2016). A 10 g was ground, and deproteinization steps were performed using a 5% (w/v) borax solution, followed by heating in a water bath at 80 °C for 15 minutes after the addition of 15% (w/v) potassium ferrocyanide and 30% (w/v) zinc acetate at room temperature. The solution was filtered, and 0.5% (w/v) sulfanilamide and 0.5% (w/v) alpha-(naphthyl)ethylenediamine were added. Nitrite content was determined using a spectrophotometer (FEMTO, Cirrus 80SA) at 540 nm after 15 minutes of reaction. The standard curve was constructed using dilutions of a standard solution containing 1 µg.mL^{-1} of sodium nitrite.

For statistical analysis, variance analysis (ANOVA) was performed using a mixed model for all variables considered. These parameters were treated as dependent variables, with the formulation included in the model as fixed effects, while different batches and replicates were considered random effects. Treatment comparisons were performed using Tukey's test ($p < 0.05$), employing Statistical 14 Trial software (Statsoft). Values are 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 previously published works, demonstrating the effectiveness of *Hovenia dulcis* T. as a natural antioxidant.

3.1 Proximate composition

The results of proximate composition showed no statistically significant difference ($p < 0.05$) between the control sample and samples with different antioxidant substances in terms of moisture, protein, and lipid content (Table 1). The differences in ash content were likely due to inherent variations in the raw material. The values comply with the recommendations of Brazilian legislation (Brazil, 2000), which sets the following limits: max. 70% moisture, max. 30% lipid, and min. 12% protein. Additionally, other published studies reported similar values to those found in this work, confirming that the formulations are suitable for producing high-quality mortadella (Alves *et al*., 2016; Pires *et al*., 2017).

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

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 lowercase letters on the same line indicate statistically significant differences ($p < 0.05$). ns: not statistically significant ($p > 0.05$).

The amounts of antioxidants added to the mortadella did not influence the proximate composition of the samples. Furthermore, the data obtained complied with current legislation and were consistent with results from 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 ($p < 0.05$) among the samples for any of the parameters evaluated. The addition of natural antioxidants, *H. dulcis* extract, and licrezz™ did not alter the texture profile of Bologna-type mortadella. These results may be attributed to the low amount of antioxidants added, which did not negatively affect the texture properties of the samples. Sucu and Turp (2018) investigated the use of beetroot powder in sausages as a nitrite replacement agent and observed that the addition of 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.
Source: research data

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: not statistically significant ($p > 0.05$).

Other studies have also reported no significant alteration in the texture profile of mortadella after adding different substances. Doménech-Asensi *et al*. (2013) used tomato paste in mortadella. They obtained similar results for the texture profile, though their findings indicated lower values for hardness (64.46 N), cohesiveness (0.53), and higher values for elasticity/springness (0.07 m), possibly due to the specific formulation of Bologna-type mortadella, which typically includes a higher content of meat proteins.

Barbieri *et al*. (2013) also evaluated the texture profile of different commercial Bologna-type mortadellas and found lower hardness values (14.84 N) compared to those in this study. Notably, mortadella is commonly consumed in slices, so higher hardness and elasticity may enhance the slicing ability of meat products and increase consumer acceptance (Delahunty *et al*., 1997).

3.3 Microbiological characterization

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, from the Technical Regulation for Mortadella Identity and Quality (Brazil, 2000), which requires the absence of *Salmonella* in 25 g of the sample, a maximum of 5.0×10^3 CFU.g^{-1} for *Staphylococcus aureus*, and a maximum of 1.0×10^3 CFU.g^{-1} for *Clostridium perfringens*. The different antioxidants used in the mortadella did not affect the microbiological characteristics during storage.

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

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	$<1.0 \times 10^1$	$<1.0 \times 10^1$	$<1.0 \times 10^1$

The results for total and thermotolerant coliforms are consistent with the findings of Baldin *et al*. (2016), who evaluated the application of jabuticaba extract in Bologna-type mortadella and reported 3.0×10^0 CFU.g^{-1}, which is expected for this product, as it undergoes thermal treatment during cooking.

3.4 Results of analyses performed during cold storage of mortadella

During the storage period, the pH ranged from 5.7 to 6.5, with all samples showing significant differences ($p < 0.05$) throughout the storage period (Figure 1). On day 7, a significant increase in pH ($p < 0.05$) was observed in the control, licrezz™, and *Hovenia dulcis* T. extract samples compared to day 1. Days 1 and 14 showed significant differences ($p < 0.05$) between the licrezz™ samples and the others. On day 1, the licrezz™ samples had a lower pH and, while on day 14, they exhibited a higher pH compared to the control and *H. dulcis* extract samples. The mortadella containing *H. dulcis* extract did not differ from the control sample at any of the evaluated periods. On the other days, the samples did not show significant differences ($p < 0.05$), indicating that the addition of different antioxidant compounds did not affect the pH behavior after day 21 of storage.

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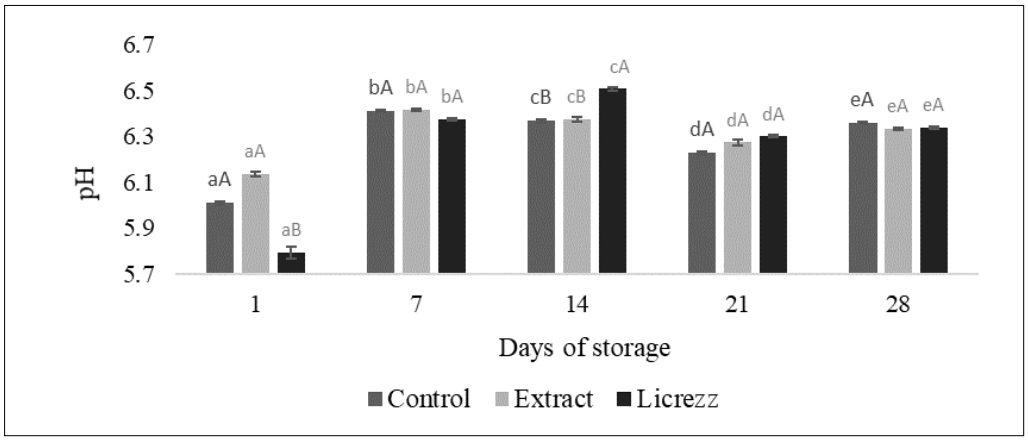
Figure 1 ▶
pH results during cold storage of Bologna-type mortadella samples prepared with different antioxidants: sodium erythorbate (control), licrezz™, and *Hovenia dulcis* T. extract.
Source: research data

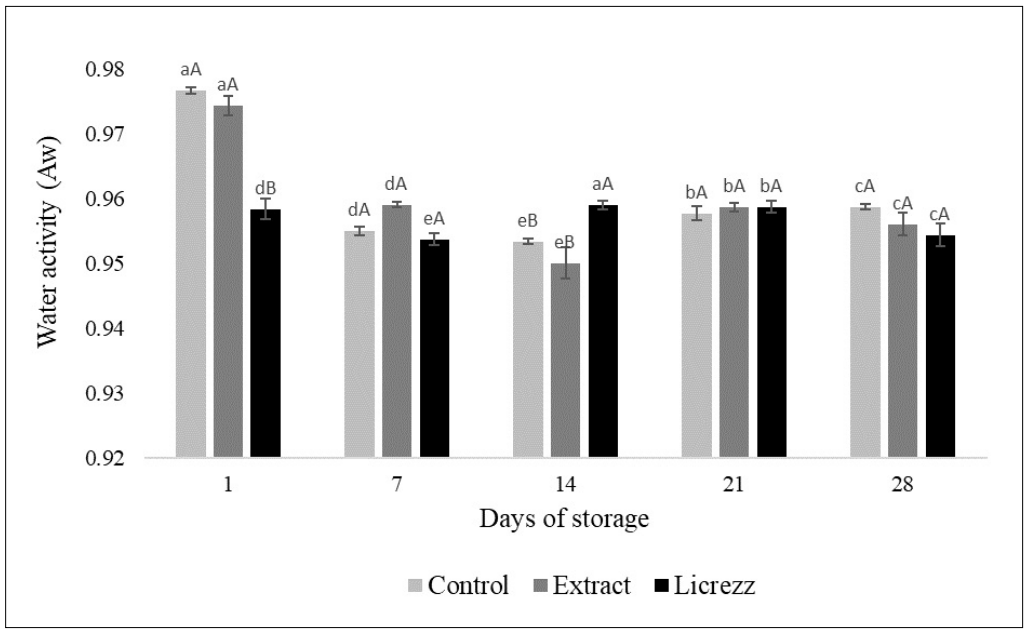


Different lowercase letters indicate statistically significant differences ($p < 0.05$) for the same sample across different storage periods; different uppercase letters indicate statistically significant differences ($p < 0.05$) between samples within the same storage period.

According to Mendonça *et al*. (2013), the increase in pH may be due to the formation of non-protein compounds and ammonium ions with buffering action from proteins, promoting pH elevation during mortadella storage. The higher pH observed in the licrezz™ sample may be related to the acidity of the licorice root extract. Similar results were reported by Pires *et al*. (2017), who evaluated sodium reduction in Bologna-type mortadella, and by Almeida *et al*. (2015), who assessed the effects of lipid oxidation when jabuticaba extract was added to mortadella. These studies also observed an initial pH increase followed by stabilization, indicating this is typical behavior of Bologna-type mortadella, likely due to the meat's buffering capacity.

Water activity (Aw) 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 ($p < 0.05$); while in the other periods, the treatments were similar to the control 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.
Source: research data



Different lowercase letters indicate statistically significant differences ($p < 0.05$) for the same sample across different storage periods; different uppercase letters indicate statistically significant differences ($p < 0.05$) between samples within the same storage period.

A significant difference ($p < 0.05$) in the Aw profile was observed for all samples during storage. On the first day, all samples showed a reduction in water activity, followed by an increase in the control and extract samples. In contrast, the licrezz™ sample remained stable by the end of storage. The initial variation in water activity may be due to the stabilization of the emulsified meat batter, as the emulsion is stabilized after cooking and cooling, when fat globules and other components become immobilized due to protein gelation, resulting in the final product characteristics (Ignácio, 2011).

The results align with typical values for mortadella, which range from 0.950 to 0.960 according to Yunes *et al*. (2013). Furthermore, these authors replaced pork fat with vegetable oils in mortadella and reported Aw values between 0.954 to 0.970, which are similar to the findings of this study. Rodrigues (2016) used ora-pro-nobis in pork mortadella and found water activity values ranging from 0.960 to 0.980 during storage.

Table 4 presents the mean values for luminosity (L*), showing a significant reduction ($p < 0.05$) in luminosity across all treatments from day 1 to day 7, indicating that all samples became darker during this period. After day 7, no significant difference was observed during storage. This change may be related to fat oxidation over time. Luminosity in food is influenced by pigment concentration and type, as well as the nitrite content in the 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.
Source: research data

		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

Different lowercase letters indicate statistically significant differences ($p < 0.05$) for the same sample across different storage periods; different uppercase letters indicate statistically significant differences ($p < 0.05$) between samples within the same storage period.

Additionally, the samples containing *Hovenia dulcis* T. extract and licrezz™ did not show significant differences ($p < 0.05$) in luminosity compared to the control sample, except on day 7 of storage, when the samples with extract and licrezz™ presented better results than the control.

The* parameter, which indicates the red color of the samples, exhibited a significant difference between treatments ($p < 0.05$) on all analysis days, suggesting that the addition of *H. dulcis* extract and licrezz™ negatively influenced the typical red color of the samples compared to the control, which showed higher a* values, especially from day 14 onward.

Regarding the storage period, a significant difference ($p < 0.05$) was observed in the control sample on day 7, with a reduction in red color intensity. However, no significant difference was noted during the rest of the storage period compared to day 1. For the samples containing licrezz™ and extract, the behavior differed from that of the control, showing a significant difference ($p < 0.05$) between the evaluated days, indicating that the color was not stable throughout storage.

A more intense red color was observed in the samples containing *Hovenia dulcis* extract compared to licrezz™, which showed lower values, particularly in the initial days of storage. This difference may be related to the fact that licrezz™ does not dissolve easily in the formulation.

One factor that may explain the different results in the instrumental color parameters is the addition of nitrite, as higher amounts corresponded to higher a* values and lower b* values (Baldin *et al*., 2016). Although the amount of nitrite added was the same for all samples in this study, the conversion of nitrate to nitrite may have varied (Table 4), as residual nitrite in the samples containing licrezz™ and extract differed significantly on certain storage days but was higher in the extract samples compared to the control. Over the entire storage period, residual nitrite was lower in the control samples, suggesting that the amount of nitrite did not impact the a* parameter differences.

According to Farhi and Al-Sawalha (2023), the addition of antioxidants significantly affects the characteristic color of mortadella. To achieve a pink-red color, nitrate (NO_3) must first be converted to nitrite (NO_2), and then to nitric oxide (NO), which reacts with iron from the heme pigment, producing nitrosohemochrome pigment (in cooked products). These pigments provide a stable pink-red color typical of cured products, with the intensity (a*) influenced by factors such as the presence of certain microorganisms, reducing agents (e.g., natural antioxidants and vitamins), enzymes, pH, redox potential, and free iron.

For the b* parameter, where higher b*+ values indicate a tendency towards yellow, the samples generally exhibited low values during storage. Significant differences ($p < 0.05$) were observed only on the final day of storage for the control and extract samples. Additionally, the licrezz™ samples varied during storage, potentially due to the difficulty in dissolving the extract. No significant difference ($p < 0.05$) was observed between the control and extract samples. However, the licrezz™ samples differed significantly ($p < 0.05$) from the control on days 7 and 21.

Studies conducted by Cáceres, García, and Selgas (2008) reported similar results for a* (13.15), b* (10.57), and L* (48.28) in Bologna-type mortadella. Pereira *et al*. (2011) found higher L* values (60.12) after adding mango seed extract to Bologna-type mortadella. These variations are likely due to differences in mortadella formulations.

For ΔE (Table 4), according to Francis and Clydesdale (1975), values close to zero indicate samples similar to the control, while values of two or more suggest perceptible differences between two treatments. Therefore, the treatment using extract and licrezz™ resulted in a noticeable color change compared to the control. The addition of licrezz™ had a greater influence on the color difference, likely because licrezz™ is not easily dissolved in the mortadella paste.

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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.
Source: research data

Lipid oxidation occurs during the processing and storage of meat products. While it cannot be entirely prevented, it can be delayed with the addition of antioxidant substances that provide stability and mitigate the negative effects of oxidation on sensory attributes (Doménech-Asensi *et al*., 2013). Lipid oxidation levels in mortadella prepared with different antioxidants ranged from 0.25 to 2.05 mg malonaldehyde/kg of the sample (Table 5).

		Storage days				
Analyze	**Sample**	**1**	**7**	**14**	**21**	**28**
TBARS	**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±0.0^{3e}A	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	**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}
	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}

Different lowercase letters on each row indicate statistically significant differences ($p < 0.05$); different uppercase letters in each column indicate statistically significant differences ($p < 0.05$). TBARS expressed in mg malonaldehyd kg sample^{-1}; Nitrite expressed in ppm $NaNO_2$.

Trindade *et al*. (2008) indicated that rancid products 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. Consequently, only the licrezz™–containing samples on day 28 would likely exhibit a rancid smell.

The TBARS values obtained from the different treatments differed significantly ($p < 0.05$), indicating variation between the treatments and the control throughout storage (Table 5). For all treatments, TBARS values increased significantly ($p < 0.05$) over the storage period. At the end of storage, the control and *H. dulcis* extract samples showed an approximate nine-fold increase in TBARS values compared to the initial value, while licrezz™ samples showed a twenty-six-fold increase, indicating that the *H. dulcis* extract behaved similarly to the control.

TBARS values were also significantly different between treatments ($p < 0.05$) on day 1 of storage, with higher values of *H. dulcis* extract and the control samples on days 1, 7, 14, and 21. After 28 days of cold storage, the control and *H.dulcis* extract samples exhibited the lowest values and stabilization, while the extract and licrezz™ samples were less effective than the antioxidant agent added to the control. Sodium erythorbate (control) is a concentrated antioxidant powder, likely offering stronger lipid oxidation control. Trindade *et al*. (2008) found that sodium erythorbate effectively controls lipid oxidation when added in amounts exceeding 100 ppm.

Doménech-Asensi *et al*. (2013) obtained similar results when applying tomato paste to mortadella, with increased tomato paste concentrations leading to reduced lipid

oxidation compared to the sodium erythorbate control. However, when added in the same proportion, oxidation was not reduced. This suggests that increasing the amount of *H. dulcis* extract and licrezz™ added to mortadella, which in this study matched the erythorbate concentration (0.1%), may improve results.

Almeida *et al*. (2015) found no significant difference ($p < 0.05$) during cold storage between control samples containing 0% antioxidant and the sample containing 0.25% jabuticaba extract in Bologna-type mortadella. Similarly, Nissen *et al*. (2004) successfully inhibited lipid oxidation in processed meat products using natural plant antioxidants, including *Prunus domestica* extract. Rodrigues (2016) reported malonaldehyde levels of 0.395 to 0.500 mg/kg in mortadella containing 2% ora-pro-nobis as an antioxidant agent, replacing sodium erythorbate.

The effect of plant antioxidants on lipid oxidation-reduction is attributed to their phenolic nature, as phenolic compounds act as radical scavengers and metal chelators, interrupting both the initiation and propagation of the oxidative process. The antioxidant activity of phenolic compounds is linked to the hydroxyl group attached to the aromatic ring, which donates electrons via hydrogen atoms to neutralize free radicals. This mechanism prevents the formation of more active oxidative compounds, such as malonaldehyde (Krishnan *et al*., 2014).

Nitrite reactions impact cured meat color, microbial inhibition, antioxidant effects, and taste (Sucu; Turp, 2018). However, because nitrite is potentially toxic (Kurćubić *et al*., 2014), its residual levels in final products must be controlled. In Brazil, the maximum residual nitrite content allowed in cured products is 150 ppm (Brazil, 2000). However, nitrite reduction is limited by the need to prevent the growth of *C. botulinum* (O'Sullivan; Kerry, 2012).

Table 5 presents the results of residual nitrite in Bologna-type mortadella samples prepared with different antioxidants. The average residual nitrite values for all samples showed significant differences ($p < 0.05$), decreasing throughout storage, indicating that nitrite was converted into other compounds. These results comply with regulatory limits, remaining below 150 ppm (Brazil, 2000).

These findings align with those of Li *et al*. (2013), who reported that residual nitrite levels in meat products can decrease significantly immediately after production, as nitrite reacts with myoglobin, sulfhydryl groups, lipids, and proteins, converting through oxidation into nitric acid (HNO_3) or gaseous forms (N_2O and N_2).

When comparing the mean values between treatments, the samples containing *Hovenia dulcis* extract and licrezz™ exhibited higher residual nitrite levels than the control sample throughout the cold storage period. The samples containing plant extracts differed significantly ($p < 0.05$) from the control sample. However, the samples containing *H. dulcis* extract and licrezz™ only showed significant differences ($p < 0.05$) from each other only after 21 days of storage. The variation on the final day may be attributed to the difficulty of homogenizing the compound in the paste, as licrezz™ is not easily dissolved in water.

Bologna-type mortadella containing sodium erythorbate (control) exhibited lower residual nitrite content than the samples containing natural antioxidants, with statistically significant differences ($p < 0.05$) observed during each storage period. These findings align with those of Li *et al*. (2013), who reported the presence of sodium erythorbate promotes nitrite reduction, converting nitrite oxide more rapidly than plant polyphenols.

According to Li *et al*. (2013), reductions in residual nitrite levels are likely to occur due to polyphenols and flavonoids, which react with various bio compounds present in

plant extracts. Hwang *et al*. (2014) observed similar behavior in emulsified pork sausage, where the application of 0.2% ethanolic extract of Artemisia reduced residual nitrite levels during the storage period. In a study by Kurćubić *et al*. (2014), 0.1% to 0.2% ethanolic extract of *Kitaibelia vitifolia* was applied to sausage as an antioxidant agent, and the samples containing the extract demonstrated superior residual nitrite levels during storage.

Therefore, all the evidence and works presented in the literature support the excellent results reported in this study and highlight the use of *H. dulcis* extract as an effective natural antioxidant alternative.

4 Conclusion

The addition of plant extracts from *Hovenia dulcis* T. pseudo-fruits and licorice root (licrezz™) did not affect the microbiological composition or the texture profile of Bologna-type mortadella. However, changes in instrumental color-parameters L* and a* were observed, possibly due to the difficult dissolution of licrezz™ and the limited impact of plant extracts on color development.

During cold storage, no significant changes were observed in the pH and water activity of the samples. However, mortadella containing natural antioxidants exhibited variations in lipid oxidation, suggesting that these agents did not achieve the same level of effectiveness as the antioxidant added to the control formulation. Similarly, in the analysis of residual nitrite content, natural antioxidants were able to reduce the amount after 28 days, but with lower efficiency than the control formulation, which contained a commercial antioxidant.

Nonetheless, *H. dulcis* extract demonstrated potential as a natural antioxidant, and further studies are needed to explore its concentration, of the effect of adding larger amounts, as well as sensory evaluations of the mortadella. Additionally, based on the promising results of this study, there is potential to expand the application of *Hovenia dulcis* T powder in the preparation of sausages or hamburgers, given its suitability for similar meat bases.

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Declaration of Competing Interest

The authors declare no potential conflicts of interest for the research, authorship, and/or publication of this article.

Contributions to the article

CAVALHEIRO, D.; SEHN, G. A. R.: study/research conception or design; data analysis and/or interpretation; final review with critical and intellectual contributions to the manuscript. **MORANDIN, G. C.:** study/research conception or design; data analysis and/or interpretation. **SCHAEFER, S. V.; AMARAL, A. M. P.:** data analysis and/or interpretation. **BETTANIN, L.;** final review with critical and intellectual contributions to the manuscript. All authors participated in the writing, discussion, reading, and approval of the final version of the article.

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