ANTIOXIDANT AND ANTIMICROBIAL POTENTIAL OF BLACKTHORN AND RED CHERRY EXTRACTS IN NATURAL SAUSAGE CASINGS

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ABSTRACT

This study explores the use of natural plant extracts as a sustainable method to enhance the quality and safety of long-life sausages by incorporating them into natural edible casings. Ethanol (E) and aqueous (A) extracts of blackthorn (BT) and red cherry (RC), as well as their ethanol and water-based solutions, were prepared and analysed for their antioxidant and antimicrobial properties. The analyses included the quantification of total phenols, non-flavonoids, flavonoids, flavonols, and anthocyanins, along with antioxidant activity assessments using FRAP, DPPH, and ABTS assays. Antimicrobial efficacy was tested against Gram-positive and Gram-negative bacteria (*Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, and *Salmonella enterica*) and mold *Penicillium expansum* via agar dilution methods to determine MIC and MBC/MFC.

Results showed that ethanol extracts had higher antioxidant activity than aqueous ones ($p<0.05$), with the ethanol extract of blackthorn (EBT) containing the highest levels of phenolics (54.11 mg GAE/g d.e.) and exhibiting the strongest antioxidant, antimicrobial, and antifungal activities. Casings treated with EBTE (ethanol BT extract dissolved in ethanol) demonstrated significant antibacterial properties, particularly against Gram-negative bacteria. This study confirms that plant-extract-enriched natural casings can protect against oxidation and microbial spoilage, thereby enhancing the overall quality and safety of dry-cured sausages.

Keywords: plant extracts, edible casings, antioxidants, antimicrobial activity.

INTRODUCTION

The food industry has recently been searching for natural, safe, and economically profitable antioxidants and antimicrobial agents to replace existing synthetic additives. Plant extracts can be very beneficial in the food industry due to their potential as antimicrobial agents and antioxidants, as they are recognized as safe (GRAS, Generally Recognized as Safe) and do not have negative effects on human health. Their influence and

application in various foods, especially in meat and meat products (pork, beef, lamb), are being extensively studied [1], [2], [3].

Their effects should not adversely impact sensory properties (e.g., color, smell, or aroma), must be effective at low concentrations, be easy to use, remain stable during processing and storage, and, of course, should also be economically viable [3]. Plant extracts are primarily used to prevent the oxidation of fats and proteins, inhibit the growth and development of bacteria, yeasts, and molds, or prevent product spoilage. The antimicrobial and antioxidant activities of these substances are primarily attributed to their high content of phenolic compounds [4], which can extend shelf life, enhance oxidation resistance, slow the growth of microorganisms and molds, and thereby positively affect the properties of the product [5].

Wild forest fruits, mainly berries, represent one of the most popular and widespread groups of edible wild plants. Among the phytochemicals present in berries, polyphenolic compounds hold the most significant position, displaying strong antioxidant and notable antimicrobial activities [6]. Blackthorn (*Prunus spinosa*), as a rich source of phenols including phenolic acids, anthocyanins, and flavanols, is recognized in the literature as a source of antioxidants. Moreover, blackthorn demonstrates selective inhibition of the growth of certain potentially pathogenic bacterial strains. It is regarded as having significant biological importance as an antioxidant and microbiological agent and can find various applications in the food and pharmaceutical industries [7], [8], [9], [10]. Wild red cherry (*Prunus avium*) fruits are particularly rich in polyphenols (especially flavonoids, anthocyanins, and hydroxycinnamic acids) [11], and contain significant amount of anthocyanins that affect the overall antioxidant activity [12], [13], [14], [15].

Recent findings suggest that cherry extracts are effective against the growth of microorganisms, including both Gram-positive and Gram-negative bacteria, along with the understanding that cherries possess not only antioxidant properties but also antimicrobial properties [16]. Wild red cherries are considered a promising functional food for human health [13].

By incorporating plant extracts or certain natural biopreservatives, films with antimicrobial and antioxidant properties can be created [17], [18]. The term "active" packaging refers to a material intended to release active components into food or absorb them from food with the goal of enhancing food sustainability while maintaining or improving packaging conditions. The use of edible coatings and films to preserve food quality has increased recently [2], [19]. The protective properties can be improved by adding antimicrobial agents or antioxidants. Edible films and coatings offer an opportunity to improve food quality, extend its shelf life, enhance safety, and increase functionality. They can be utilized as individual packaging materials, coatings for food, or carriers of active ingredients [20].

The aim of this work was to investigate the antioxidant and antimicrobial effects of blackthorn and red cherry extracts, as well as their influence on the properties of natural casing.

MATERIAL AND METHODS OF WORK

Extract preparation. Wild forest fruits (blackthorn - BT, and red cherry - RC) were purchased from the local market. First, fruits were washed, and the petioles and the seeds were removed. For extract, 40 g of fruits were homogenized with 160 mL of extragens (80% (v/v) ethanol – E, and distillated water - A), first on a Polytron PT 3100 homogenizer, for 10 minutes at 8000 rpm, then in an ultrasonic bath for 30 minutes, and 30 minutes on a magnetic stirrer. The mixture was filtered, and the obtained filtrate was evaporated to a dry residue, first on a vacuum evaporator and then in a dry sterilizer at 50˚C. The dry extracts were stored in a dark until use.

Dry extracts analysis. The total phenolic content (TPC) was determined using the modified method of Folin-Ciocalteu [21]. The content of non-flavonoids (TN) was determined according to the formaldehyde method [22]. The content of flavonoids (TF) was calculated from the difference between the content of total phenols and non-flavonoids. The content of flavonols (F) was determined according to the method of

Kumaran and Karunakaran [23]. For the determination of anthocyanins (monomeric anthocyanins - MA, total anthocyanins – TA, degradation index (ID) values) the pH differential and "single" pH methods were used [24]. The testing of antioxidant activity using the Ferric reducing/Antioxidant power (FRAP) assay was carried out by Benzie and Strain [25]; the 2,20-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) assay using the modified method of Re et al. [26] and the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) assay using modified method of Liyana-Pathirana and Shahidi [27]. All the chemicals and reagents used were of analytical grade.

Extract solutions. Extracts were dissolved in 80% ethanol (v/v) (E) and in distilled water (W) to obtain solutions for further analysis (EBTE, EBTW, ABTE, ABTW, ERCE, ERCW, ARCE, ARCW)[1].

Extract solutions analysis. To determine the antibacterial activity of extracts against selected G+ (*Staphylococcus aureus* and *Bacillus cereus*) and G- (*Escherichia coli* and *Salmonella enterica*) bacteria (minimum inhibitory - MIC, and minimum bactericidal - MBC concentrations), the agar dilution method was used [28], [29], [30]. To test the inhibition of the growth of the mycelium of the mold *Penicillium expansum*, the dilution method in agar was used [30] and the diameter of the mold growth was monitored (5 days).

Casing treatment. Based on the results of the antimicrobial tests, concentrations for casing treatment were determined for further analysis (EBTE, ABTE - 22.5g/L, ERCE, ERCW, ARCE - 30 g/L, EBTW - 45 g/L, ABTW - 90 g/L and ARCW - 120 g/L). Natural salted beef casings are soaked in water and washed of salt. For further analyses, the intestines were cut into pieces (5 cm long for antioxidant tests, 3.5x2.4 cm long for antimicrobial tests, dried and sterilized with a UV lamp for 30 minutes), submerged in extracts and kept for 24 hours on a shaker (SHO-2D, Witeg, Germany) at 120 rpm. After 24 hours of shaking, the casings were removed from the extracts, and dried at room temperature under sterile conditions.

[1]etanolic-E/aqueous-A blackthorn BT/red cherry RC ekstract disolved in ethanol-E/water-W

Casings analysis. The antioxidant activity of the casings was evaluated by TPC analysis, and FRAP, ABTS, and DPPH assays. To test the antimicrobial activity of the casing with the extract, the agar diffusion method was used [31].

Statistical analysis of the obtained results was performed using Microsoft Excel 2013 software package and the IBM SPSS Statistics 22.0 (Armonk, NY, United States). Results were presented as mean values of individual measurements ± standard deviations. The significance of differences between arithmetic means was determined and expressed with 95% probability (Tukey's test).

RESULTS AND DISCUSSION

Table 1 presents the average values of the phenolic compounds in extracts, confirming statistically significant differences between the samples for all evaluated parameters ($p<0.05$), except TNF. The content of total phenols ranged from 14.83 to 54.11 mg GAE/g dry extract (ABT and EBT, respectively). Higher TPC content was obtained in ethanol extracts of all tested plants, as a result of better solubility of polyphenolic components in diluted ethanol compared to water, due to selective extraction of certain phenolic compounds [32]. Some authors report TPC values in blackthorn ranging from 8 to 20 mg GAE/g fresh fruit [33] [34].

For TPC in wild red cherry, the type of solvent had little influence. Serra et al. [13] consider that the phenolic composition of cherries includes flavonoids (anthocyanins, flavan-3-ols, and flavonols), hydroxycinnamic and hydroxybenzoic acids, with total TPC content ranging from 440 to 1300 mg/100 g dry matter.

Different concentrations of TNF (total non-flavonoids) and TF (total flavonoids) can be explained by the significant influence of various factors (location and cultivation technique, genotype, plant maturity, environmental conditions, climate, temperature, light) on the content of these substances in the plant [35].

Blackthorn extracts had higher flavonol content than red cherry (Table 1). Ruiz-Rodríguez et al. [7] report that flavonol content varies depending on the harvest year,

and values ranged from 0.9 to 2.3 mg Rutin/g fresh blackthorn. The obtained flavonol content in wild red cherry compared to literature data is somewhat higher [11], [13].

Table 1. Content of total phenols (TPC), non-flavonoids (TNF), flavonoids (TF), and flavanols (F) in extracts

		TPC	TNF	TF	F
		(mg GAE/g d.e)		(mg QE/g d.e)	
BT	E	54.11^{a} ±5.14	17.28^{a} ±6.05	33.21^{a} ±5.43	10.37^{a} ±0.38
	A	14.83^{b} ±0.38	14.08^{a} ±0.17	0.75^{b} ±0.26	7.00^{b} ±0.97
RC	E	38.34^{c} ±1.43	20.91^{a} ±3.22	17.43^{c} ±1.15	1.45^{c} ±0.51
	A	31.87^{c} ±1.55	18.74^{a} ±1.80	13.13^{bc} ±1.02	1.47^{c} ±0.56

$^{a–c}$ – different letters within the same column indicate statistically significant difference at $p < 0.05$ by Tukey's test

Table 2 presents the anthocyanin content, indicating that ethanol extracts exhibited significantly higher values ($p<0.05$) compared to aqueous extracts, values for TA ranged from 3.65-37.48 mg/g d.e. (ABT and EBT, respectively). Pinacho et al. [36] state that anthocyanin content depends on the polarity of the solvent used and report values for blackthorn of 165–180 mg malvidin glucoside equivalent/g. Similar values were reported by Ruiz-Rodríguez et al. [7].

Table 2. Content of monomeric (MA) and total (TA) anthocyanins in extracts (mg cyanidin-3-glucoside/g dry extract) and degradation index (ID) values

		MA	TA	ID
		(mg/g d.e)		
BT	E	8.33^{a} ±0.02	37.48^{a} ±0.59	4.50^{a} ±0.08
	A	2.45^{b} ±0.66	3.65^{b} ±0.54	1.67^{b} ±0.67
RC	E	6.43^{c} ±0.93	12.96^{c} ±1.00	2.04^{b} ±0.14
	A	1.53^{b} ±0.35	5.57^{b} ±0.45	3.75^{a} ±0.54

$^{a–c}$ – different letters within the same column indicate statistically significant difference at $p < 0.05$ by Tukey's test

Total anthocyanin content in cherries, depending on the harvest location, ranged from 0.6 to 6.8 mg/g [37]. Various studies report differing anthocyanin contents, likely due to geographic differences and post-harvest factors such as storage conditions and extraction methods, with values ranging from 24-225 mg/100g [38]. Leichtweis et al. [39] suggest that ultrasound extraction and diluted ethanol can extract larger amounts of anthocyanins. Pliszka et al. [40] attribute the variations in degradation index (ID) values to differences between fruit species and extraction methods, both of which have a significant impact on anthocyanin stability.

Table 3 presents the results of the ABTS, DPPH, and FRAP assays, used to evaluate the antioxidant capacity of blackthorn and red cherry extracts. The strongest antioxidative activity in all tests was observed in the ethanol extract of blackthorn, while the aqueous extract of blackthorn showed relatively weak antioxidant activity, likely due to insufficient dissolution of polyphenolic compounds in the aqueous medium. The lowest activity was observed in aqueous extracts of red cherry (431.29 mg/L, 2588.14 mg/L and 58.97 µmol Fe+2/g d.e., for ABTS, DPPH and FRAP, respectively).

Table 3. Antioxidant activity of extracts

		ABTS	DPPH	FRAP
		Ic50% (mg/L)		(µmol Fe^{+2}/g d.e.)
BT	E	30.45[a] ±1.33	142.85[a] ±7.69	512.22[a] ±9.80
	A	276.48[b] ±6.67	1571.63[b] ±22.93	107.51[b] ±10.29
RC	E	187.26[c] ±2.93	950.32[c] ±25.73	216.59[c] ±5.32
	A	431.29[d] ±14.97	2588.14[d] ±157.03	58.97[d] ±13.07

[a–d] – different letters within the same column indicate statistically significant difference at $p < 0.05$ by Tukey's test

Several studies have reported the antioxidant properties of blackthorn and wild cherry extracts, with notable differences depending on the extraction method and solvent used. Values of Ic50% for ethanol extracts of blackthorn reported by Veličković et al. [41] for DPPH were around 258 µg/mL, for ABTS about 184 µg/mL, while FRAP values were 0.10 µmol Fe^{2+}/g dry matter. The same authors report values for aqueous extracts of blackthorn about 490 µg/mL (DPPH), 217 µg/mL (ABTS), and 0.01 µmol Fe^{2+}/g dry matter (FRAP). Using aqueous ethanol and methanol solutions, and water on dry blackthorn, Tahirović et al. [9] report DPPH values of 140.80 µmol TE/g, ABTS 223.98 µmol TE/g, and FRAP 249.13 µmol TE/g. According to the same authors, 50% ethanol proved the best extraction solvent, while aqueous solutions showed the weakest activity.

Many authors believe that the content of polyphenolic compounds (phenols, anthocyanins) contributes to the overall antioxidant activity of wild red cherries [12], [42], while Serra et al. [13] report that in methanolic cherry extracts the most active substances with the best antioxidant properties are flavonoids (including catechin, epicatechin, quercetin, and anthocyanins) and derivatives of quinic acid (chlorogenic acid, neochlorogenic acid).

Analysis of cherries from Spain showed DPPH values of 242 µmol TE/100 g fresh fruit, ABTS 640 µmol TE/100 g fresh fruit, and FRAP 763 µM Fe^{2+}/100 g fresh fruit [43].

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Different ripening stages also influence antioxidant activity, with ABTS values ranging from 317.92 mg TE/100 g fresh fruit in the early ripening stage to 439.10 mg TE/100 g fresh fruit in the final ripening stage [42]. Analysis of antioxidant activity in dark and light cherries from Turkey by Hayaloglu and Demir [14] indicate that darker cherries have stronger activity, which correlates with the anthocyanin content in the samples. The same authors report ABTS values of 4.5–6.15 mg TE/g fresh fruit, DPPH of 4.5–6.02 mg TE/g fresh fruit, and FRAP of 0.44–1.61 mg TE/g fresh fruit. Research has shown that bioactive compounds vary depending on the degree of ripeness and that with increased fruit color intensity, antioxidant activity increases [44].

As shown in Table 4, the minimum inhibitory concentration (MIC) of analyzed blackthorn extracts ranged from 7.5-30 g/L, and ethanol extract solutions (EBTE and EBTW) showed much better activity. MBC values for blackthorn extracts ranged from 15 to >120 g/L, with ABTW being the weakest. Red cherry extracts showed very weak antibacterial activity against selected G (+) bacteria. MIC values ranged from 30-120 g/L and 30-60 g/L (*S. aureus* and *B. cereus*, respectively). MBC values ranged from >30 to >120 g/L for *S. aureus* and 30-120 g/L for *B. cereus*. Considering the extract type and solvent, the best effect against selected bacteria was by ethanol extracts dissolved in ethanol, while aqueous extracts dissolved in water showed the weakest effect.

Table 4. Antibacterial activity of tested extracts against selected Gram-negative bacteria

	Staphylococcus aureus ATCC 25923		***Bacillus cereus WDCM 00151***	
g/L	MIC	MBC	MIC	MBC
EBTE	7.5	30	7.5	15
EBTW	30	>30	15	15
ABTW	30	>120	30	60
ABTE	15	30	15	15
ERCE	30	>30	30	30
ERCW	>30	>30	30	>30
ARCW	120	>120	60	120
ARCE	30	>30	30	>30
Ampicillin 10 mg (mm)	34.40±1.50		11.50±2.32	
Ciprofloxacin 5 mg (mm)	30.00±5.90		29.10±4.41	
Erytromycin 15 mg (mm)	28.20±3.66		25.60±2.94	
Gentamicin 10 mg (mm)	28.60±2.94		23.30±2.14	

MIC values for Gram-negative bacteria (Table 5) of blackthorn extracts ranged from 15-60 g/L, and MBC values ranged from 30-120 g/L. The aqueous extract of blackthorn dissolved in water showed the weakest activity. Red cherry extracts did not show significant antibacterial activity against selected G (-) bacteria (MIC 30-120 g/L, MBC 30->120 g/L). Ethanol extracts dissolved in ethanol also showed stronger activity against G (-) bacteria, while aqueous extracts dissolved [8] in water had the weakest effect. These results align with previous findings.

Veličković [32] and Radovanović et al. [33] established that ethanol extracts of blackthorn show significant activity against some bacteria (*S. abony, E. coli*, *P. aeruginosa*, and *S. aureus*). *B. subtilis* showed resistance to ethanol blackthorn extract [8]. Blackthorn extracts have significant biological importance both as antioxidants and antimicrobial agents, as confirmed by many authors [8], [33], [41]. Testing aqueous and ethanol cherry extracts on pure and clinical strains of *E. coli*, Rovčanin et al. [45] observed larger inhibition zones for the pure strain with ethanol extracts, as well as lower extract concentrations needed to inhibit this strain compared to clinical strains. Hanbali et al. [16] also note antimicrobial activity in wild red cherry extracts.

Table 5. Antibacterial activity of tested extracts against selected Gram-negative bacteria

	Escherichia coli WDCM 00013		***Salmonella enterica WDCM 00030***	
g/L	MIC	MBC	MIC	MBC
EBTE	15	>30	15	30
EBTW	>30	>30	>30	>30
ABTW	60	120	60	60
ABTE	15	30	15	30
ERCE	30	>30	30	30
ERCW	>30	>30	>30	>30
ARCW	120	>120	120	>120
ARCE	30	>30	30	>30
Ampicillin 10 mg (mm)	16.50±6.00		22.20±2.64	
Ciprofloxacin 5 mg (mm)	32.60±3.32		36.60±5.43	
Erytromycin 15 mg (mm)	9.00±1.26		8.20±1.17	
Gentamicin 10 mg (mm)	23.00±5.06		24.60±3.38	

Many authors have shown that wild fruit extracts exhibit antibacterial activity against most tested G (+) and G (-) bacteria [32], [33]. G (+) bacteria are generally considered more susceptible to the inhibitory effects of plant extracts, likely due to structural differences in their cell walls compared to G (-) bacteria [46].

Figure 1 shows the effect of solutions on *P. expansum*. Solutions ranging from 3.75-30 g/L were tested, but lower concentrations (3.75 and 7.5 g/L) showed no activity. Aqueous solutions of all tested extracts did not exhibit antifungal activity. The EBTE solution showed activity at concentrations of 15 and 30 g/L, with mycelial growth inhibition (MGI) of 31.4% and 63.7%, respectively. Veličković et al. [41] report that the aqueous blackthorn extract had slightly better antifungal properties than the ethanol extract, but its effect was significantly weaker than commercially used antimycotics. Gegiu et al. [47] state that blackthorn extracts have no antifungal activity. ERCE and ARCE solutions showed activity at a concentration of 30 g/L, while other tested concentrations showed no activity against the selected mold. The ERCE solution demonstrated inhibitory activity (MGI of 63.3%) at 30 g/L. The solution of EBTE, in concentration 15 g/L showed a steady increase but very weak activity. Results from Hanbali et al. [16] confirm a measurable effect of red cherry extracts on the tested molds. The ARCE solution showed significant inhibitory activity at 30 g/L (MGI of 54.3%). For red cherry extract solutions ERCW and ARCW, the diameter increased with concentration, making these extracts also suitable environments for the development of *P. expansum* mold.

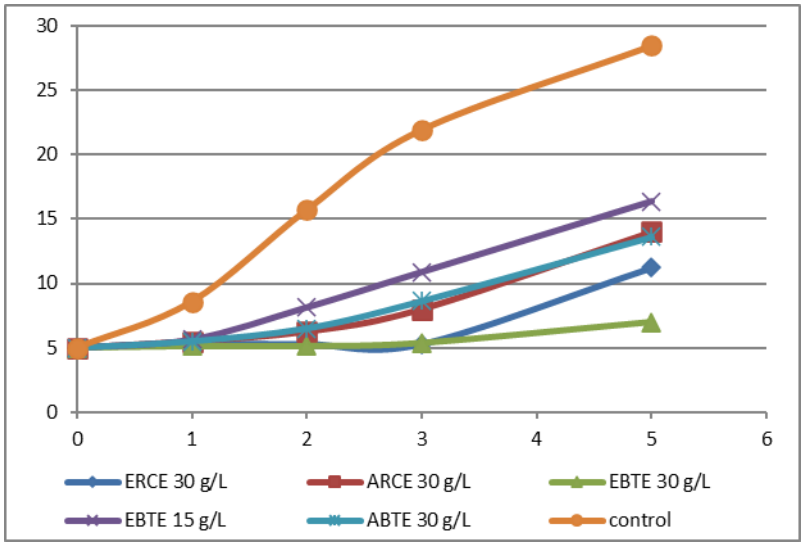

Figure 1. Antifungal activity of extracts against the mold *P. expansum*

The antimicrobial activity of the tested extracts is largely related to the quantity and structure of extracted compounds. The antimicrobial activity of plant extracts is often associated with polyphenolic compounds that also exhibit antioxidant effects. Numerous authors attribute strong antimicrobial properties to phenolic compounds, anthocyanins, iridoids, tannins, and other related substances[8], [10], [48].

Results of the total phenolic content and antioxidant activity (DPPH, ABTS and FRAP tests) for treated casings are presented in Table 6. After treatment of the casings with selected extract solutions, EBTE had the highest TPC (3.07 mg GAE/g), and ARCW the lowest (0.30 mg GAE/g). According to DPPH radical assay, ERCE had the best activity, and ABTE the weakest, while according to ABTS assay, ARCE showed the best activity (182.02 µg

GAE/g) and ABTE the weakest (53.95 µg GAE/g). FRAP test values ranged from 2.41-10.08 µmol Fe^{2+}/g (ABTE and ERCE, respectively). Although numerous studies confirm the positive effects of natural polyphenols obtained from various plant materials, limited information is available on the impact of edible films incorporating natural plant extracts [49]. Biologically derived active substances have strong antioxidant and antimicrobial effects, low toxicity, and are considered effective agents for active packaging used in food preservation and conservation [50].

Table 6. The total phenolic content and antioxidant activity (DPPH, ABTS and FRAP tests) of treated casings

	TPC	DPPH	ABTS	FRAP
	mg GAE/g	µgGAE/g		µmol Fe^{2+}/g
EBTE	3.07^{a} ± 0.32	128.95^{ab} ± 1.65	134.26^{ab} ±1.01	7.24^{ab} ± 0.46
EBTW	1.94^{b} ± 0.06	159.80^{a} ± 2.26	132.75^{ab} ± 2.11	6.14^{ac} ± 0.01
ABTW	2.07^{b} ± 0.05	84.99^{b} ± 1.07	105.81^{abc} ±2.02	6.15^{ac} ± 0.59
ABTE	1.02^{b} ± 0.21	28.24^{c} ± 1.89	53.95^{c} ± 2.08	2.41^{d} ± 0.44
ERCE	0.34^{d} ± 0.08	167.75^{a} ± 2.83	175.35^{b} ± 3.05	10.08^{b} ± 1.65
ERCW	1.88^{b} ± 0.18	133.51^{a} ± 2.44	129.56^{abc} ± 2.18	7.89^{ab} ± 0.94
ARCW	0.30^{d} ± 0.05	148.18^{a} ± 2.12	182.02^{b} ± 4.21	9.98^{b} ± 1.29
ARCE	2.35^{b} ± 0.13	34.76^{c} ± 1.15	66.52^{ac} ± 1.31	3.88^{cd} ± 1.04

$^{a–d}$ – different letters within the same column indicate statistically significant difference at $p < 0.05$ by Tukey's test

Nazmi and Sarbon [51] state that total phenolic content (TPC) in protective coatings or composites (films) correlates with phenol content in the used extracts, and their study of C. asiatica extract showed a strong relationship between phenolic compounds and antioxidant activity. The same authors claim that the total phenol content in films increases with extract concentration, also supported by Shojaee-Aliabadi et al. [52]. In their work with gelatin-based films enriched with *C. asiatica* extract, Nazmi and Sarbon [51] report strong DPPH activity, attributing this to phenolic compounds including flavonoids, phenolic acids, and tannins. Pires et al. [53] during analysis of various essential oils added to films, observed the increase DPPH values, especially in films with coriander and citronella oils. Peighambardoust et al. [54] note that adding antioxidants to films significantly increases their DPPH values, although antioxidant activity and thermal stability of antioxidants differ, as confirmed by analysis of various composite films with synthetic antioxidants (BHT, BHA, sorbic acid). Many authors confirm that increasing antioxidant additives (essential oils) raises DPPH values [52] [55]. Teixeira et al. [56] argue that increased DPPH activity in fish protein films is due to the incorporation of essential oils, noting differences in antioxidant activity depending on the type of essential oil used, which is attributed to interactions between film components and essential oils and/or loss of volatile compounds during film drying.

Several authors agree that the addition of antioxidants (various essential oils or extracts) increases ABTS activity in films (chitosan, gelatin, fish proteins), mainly linked to the presence of phenols, flavonoids, and sulfur-containing compounds [57], [58].

FRAP values confirm that adding antioxidants enhances film activity, and it is considered that the amount of antioxidant added is proportional to the antioxidant activity of edible films [51]. Eça et al. [59] provide examples of significant increases in antioxidant activity of films (fish skin gelatin, chitosan, etc.) with antioxidant addition (various essential oils) as measured by DPPH and FRAP assays.

None of the casings with blackthorn extract solutions showed activity against G (+) *S. aureus* and B. *cereus*. Against G (-) bacteria, ABTW, ABTE, and EBTE exhibit contact inhibition (C.I.) on *S. enterica*, and only the casing treated with ABTW showed inhibition against *E. coli*. No casing showed any activity against *P. expansum* mold (n.a.). Although all casings used extract concentrations above the measured MIC values for certain extracts, it is assumed that the casing retained less than the required amount to act on the selected strains. No casing treated with red cherry extract solutions showed any activity against tested bacteria, or *P. expansum*.

The obtained results do not align with antioxidant activity measurements of the extracts, where casings treated with plant extracts show a positive influence on antioxidant properties. This indicates that the antimicrobial properties of plant extracts may be attributed to individual or synergistic effects of various factors, not only the phenolic content. A limitation of the agar diffusion method is the relatively long incubation time needed to detect inhibition zones, which can lead to loss of volatile or thermally unstable agents, likely causing the absence of antibacterial activity in tested samples. It is also impossible to quantify the amount of antimicrobial agent diffusing into the agar medium due to the gradient and matrix network of the agar used [30]. Pires et al. [53] report that varying antimicrobial activity was due to differences in active substances in agents (essential oils, plant extracts), interactions between agent compounds and films (soy, alginate-apple puree, alginate, and whey protein), and differences in agent quantity per film surface area. A possible explanation is that thinner film samples do not release sufficient amounts of the active agent to inhibit microbial growth [54], [56]. Shojaee-Aliabadi et al. [52] state that antibacterial activity is directly proportional to the agent concentration, consistent with our research and results.

CONCLUSIONS

Edible casings treated with various plant extracts showed significant differences in antioxidant performance, EBTE had the highest total phenolic content (TPC), while ERCE displayed the best antioxidant activity in DPPH and FRAP assays. However, antimicrobial effects were limited, only some casings inhibited specific Gram-negative bacteria, with no activity against Gram-positive strains or molds. These results suggest that antimicrobial activity depends on more than phenolic content alone, possibly involving complex synergistic interactions and the nature of the film matrix. The amount of extract retained and its interaction with the film material significantly influence antimicrobial efficacy. While natural plant extracts effectively boost antioxidant capacity, optimizing antimicrobial performance requires further research into extract concentration, film composition, and application methods.

Packaging infused with natural antioxidants and antimicrobials presents a promising approach to food preservation. By integrating these agents into edible films or coatings, oxygen transmission is reduced, and the structural and functional properties of the packaging are improved. Such active packaging systems not only help prevent oxidation but also enhance sensory quality and shelf life.

In the meat industry, this strategy represents a modern alternative to synthetic additives, providing an innovative and more natural method for extending the freshness of meat and its products.

DECLARATIONS OF INTEREST STATEMENT

The authors affirm that there are no conflicts of interest to declare in relation to the research presented in this paper.

LITERATURE

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