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Polyphenolic Herbal Extract of *Cistus incanus* as Natural Preservatives for Sausages Enriched with Natural Colors

Marta Sośnicka [1,*], Agnieszka Nowak [1], Agata Czyżowska [1], Ilona Gałązka-Czarnecka [2] and Aleksandra Czerbniak [1]

[1] Institute of Fermentation Technology and Microbiology, Lodz University of Technology, Wolczanska 171/173, 90-530 Lodz, Poland; agnieszka.nowak@p.lodz.pl (A.N.); agata.czyzowska@p.lodz.pl (A.C.); 212004@edu.p.lodz.pl (A.C.)

[2] Institute of Food Technology and Analysis, Lodz University of Technology, Stefanowskiego 4/10, 90-924 Lodz, Poland; ilona.galazka-czarnecka@p.lodz.pl

* Correspondence: marta.sosnicka@dokt.p.lodz.pl

Abstract: This study evaluates the effects of polyphenolic extract of *Cistus incanus*, lycopene dye from tomatoes, and betanin dye from red beet on selected parameters of model meat products with reduced nitrate contents. The polyphenolic composition and activity of the *C. incanus* extract was analyzed, revealing the presence of elagotannins, flavanols, and glycosylated flavanols. We studied the effects of the extract and dyes as well as of mixtures of the extract and dyes on the growth of bacteria characteristic of the meat environment: *E. coli*, *S. enterica*, *P. fragi*, *L. monocytogenes*, *B. thermosphacta*, and *L. sakei*. We studied the effects of the extract and dyes on the lipid oxidation, color, and microbiological quality of pork sausages with reduced nitrate content over 28 days of storage. During storage, the amounts of malon dialdehyde reduced, which indicates that the extract and dyes exhibited antioxidant activity and slowed lipid oxidation in the sausages. An increase in red color was also observed in the sausages with natural additives, despite their decreased nitrate content. It was found that the *C. incanus* extract combined with coloring agents positively influenced the selected parameters of the analyzed pork sausages.

Keywords: *Cistus incanus*; lycopene; beetroot; polyphenols; natural preservatives; meat

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1. Introduction

Curing processes used for the production of sausages in the meat industry involve the addition of salt, nitrites, nitrate salts, and spices to preserve and enhance the flavor, texture, and color of the final products [1]. Nitrates are artificial compounds added to processed meat products to inhibit the growth of pathogenic bacteria such as *Listeria monocytogenes* and *Clostridium botulinum*. Nitrates also prevent spoilage by retarding lipid oxidation and impart characteristic flavor and pink-red coloration to cured meats [2]. Despite their many benefits, nitrates may form carcinogenic N- nitroso compounds (NOCs), in particular nitrosamines, which increase the risk of esophageal, gastric, and bladder cancers [3]. In recent years, there has been growing consumer concern over the health risks of nitrates [1,4]. There has also been increasing consumer interest in healthy meat products enriched with natural ingredients [5,6]. Possible alternatives to nitrates include plant extracts and essential oils that contain bioactive compounds -polyphenols, flavonoids, and terpenoids characterized by strong antioxidant and antimicrobial activity [4,7,8]. Research using plant and vegetable extracts has been ongoing for several years [9,10]. It has been shown that natural extracts can be used as additives in the meat industry [4,7].

Color is one of the most important quality characteristics in the food industry. It signals that the correct technological processes have been implemented [11]. Meat coloration is also a sensory indicator that provides consumers with information about quality and freshness [12]. If the color is not acceptable by the consumer, then other characteristics such

as consistency or taste lose interest [13]. Color is also an indicator of various properties of products, including nutritional value and food safety [14]. There has been considerable research conducted to optimize the addition of nitrates to cured products and replace them with other natural compounds [11].

Many natural additives can be used by the meat industry that have health-promoting properties [15]. Tabaka and co-authors prepared model meat products using sweet red peppers, cayenne pepper, rosehips, acai berries, cranberries and grape seeds as additives, to reduce the content of sodium nitrate III. The use of dyes imparted an attractive color despite the reduction in nitrate III. Products containing acai berry and grape seed pigments had the most stable color [16]. Dias and co-authors obtained cooked ham with a pink color using natural extracts from red radish, hibiscus, and red beetroot. Both the red beet extract and the hibiscus extract provided the desired color, but a higher quantity of the hibiscus dye was required [17]. Other researchers have tested the effect of adding lycopene from tomatoes to ground meat. The ground meat showed increased stability during storage. Good color characteristics were obtained, and the additive enhanced the meat product with documented health-promoting properties [18].

The aim of this study was to determine the effects of aqueous extract of *Cistus incanus* separately and in combination with natural lycopene dye from tomatoes and betanin dye from red beet. *Cistus incanus* is a Mediterranean plant with pink or white flowers depending on the species, which is found in North African and Southern European countries and is able to grow in difficult climates and soil conditions. Folk medicine has used *C. incanus* in the form of infusions to treat many diseases. *Cistus incanus* extracts are characterized by antimicrobial, antiviral, antifungal, and antibacterial activities [19–21]. Aqueous extracts of *C. incanus* actively inhibit the growth of human epithelial prostate cells [22]. We investigated the effects of the extract and dyes on the oxidative stability of fat, color, as well as organoleptic and microbiological characteristics of sausages with limited amounts of nitrate III. According to the best of our knowledge, no studies have been conducted on the effect of *C. incanus* extract on nitrate-reduced meat products. The effect of *Cistus incanus* extract, dyes, extract and dyes together on bacterial growth parameters has not been researched yet.

2. Materials and Methods

The research materials consisted of aqueous plant extract of *C. incanus* prepared using commercially available dried plants from (Batom, Poland), powdered betanin dye from beetroot (Trzy ziarna, Poland), and lycopene dye from tomatoes (Chinese Herbs Health wish, China).

2.1. Preparation of C. incanus Plant Extract

Dried *C. incanus* (2.0 g) was weighed and extracted with 50 mL of distilled water for 1 h at room temperature. The sample was then centrifuged for 15 min at 4 °C at 7000 rpm. The supernatant was decanted and the microwave sterilized.

2.2. Preparation of Dyes

Lyophilized dye (1.0 g) was weighed and suspended using 100 mL of distilled water with a few drops of Tween 20 for 1 h at room temperature. The dye was subjected to microwave sterilization.

2.3. Determination of Total Phenolics in C. incanus Using Folin-Ciocalteu Reagent

The total polyphenol content in the *C. incanus* plant extract was determined by the Folin-Ciocalteu method. First, to 1 mL of the *C. incanus* extract was added 0.2 mL of Folin-Ciocalteu reagent, Next, 1 mL of 20% calcium carbonate and 2 mL of distilled water were added. The samples were incubated for 60 min in a dark place at room temperature. The absorbance was further measured at λ = 756 nm against the reagent sample. The

polyphenol content was determined as the amount of gallic acid equivalents (mg GAE/L of extract).

2.4. Antioxidant Activity (DPPH and ABTS)

The antioxidant activity of the extract and dyes was determined using the DPPH radical method [23] and ABTS [23]. In the DPPH method, 1.95 mL of the reagent solution (2,2-diphenyl-1-picrylhydrazyl) and 50 µL extract solution were used. The sample was incubated for 30 min at room temperature, after which time the absorbance was measured at λ = 515 nm against the reagent sample. Antioxidant activity according to the DPPH method was defined as the amount of trolox equivalents (mg trolox/100 mL). Antioxidant activity was also determined according to the ABTS using ABTS reagent (2,2'-azino-bis(3-ethylbenzothiazoline-6 sulfonic acid)) by adding 30 µL of extract solution to 3 mL ABTS solution [23]. The solution was then incubated for 15 min in a dark place at room temperature and the absorbance of the samples was measured at λ = 734 nm in distilled water. Antioxidant activity according to the ABTS method was defined as the amount of trolox equivalents (mg trolox/100 mL).

2.5. LC-MS Analysis of Polyphenols

The samples were filtered through a 0.45 µm membrane into vials. They were analyzed using an HPLC coupled to an LTQ Velos MS mass spectrometer. Chromatographic separation was conducted using a column operating at 45 °C. The mobile phase consisted of solvent A (1 mL formic acid and 1 mL deionized water) and solvent B (95% acetonitrile). A Hypersil Gold 150 × 2. 1 column was used, particle size 1.9 µm. Elution conditions: 96% to 85% A for 8 min, 85% to 82% A for 12 min, 82% to 60% A for 40 min, 60% to 50% A for 4 min, 60% to 50% A for 3 min, 50% to 96% A for 2 min, further washing and rebalancing of the column. The mass spectra were recorded over 60 min. The flow rate was 220 µL/min, and the injection volume was 10 µL. Electrospray ionization mass spectrometry was conducted using an LTQ Velos mass spectrometer equipped with an ESI interface and controlled by Excalibur software. The spray voltage was 4 kV, the shielding gas flow rate was 25. The auxiliary gas flow rate was 10, the desolvation temperature was 28 °C, and the source temperature was 35 °C. Mass spectra were acquired in the negative mode over the range m/z 120–1000. The I spray voltage was 4 kV. The sheath gas flow rate was 25 and the aux gas flow rate 10. The desolvation temperature was 280 °C, and the source temperature 350 °C. Peak identification was performed by comparing the retention time and mass spectra to standards and spectra from the literature.

2.6. Evaluation of the Activity of Extracts and Dyes against Selected Bacterial Strains

The three Gram positive bacteria *Listeria monocytogenes* (ATCC 13992), *Brochotrix thermosphacta* (MMAP4 HQ8909431*), and *Latilactobacillus sakei* (ATCC 15521), as well as the three Gram negative strains *Escherichia coli* (ATCC 10536), *Pseudomonas fragi* (ATCC 4973), and *Salmonella enterica* (MCH1 MG911721*), were used in the study. The strains *S. enterica* and *B. thermosphacta* were from natural environments. The 16s RNA sequence numbers in GenBank are given in parentheses above. The selected bacterial strains were activated and incubated to obtain 10^7 cfu/mL. The samples were then diluted in saline solution to obtain concentrations of 10^4 cfu/mL. A TSB culture medium was prepared in conical flasks, to which the extract (3, 6, and 12 mL), dyes (10 mL), or mixtures of the extract (3 or 6 of or 12 mL) and dyes (10 mL) were added, with inoculum to a concentration of 10^4 cfu/mL. The reference was without extract or dyes. Subsequently, deep seeding was performed for 0, 4, 6, 24, 30, 48 h or, for certain strains, 72 h. The cultures were incubated at 30 °C. The samples were diluted for seeding, and then the plates were flooded with PCA culture medium and incubated for 24 h at 30 °C. The bacterial colonies were counted. The Excel add-on DmFit 3.5 was used and the growth parameters were calculated on the basis of the number of bacterial cells, by substituting under the Gompertz equation

$L(t) = A + C \exp\{-\exp[-B \times (t - M)]\}$: the maximum specific growth rate μmax, the final multiplication of yEND, and tlag time lag.

2.7. Preparation of Model Sausages

White sausages were prepared under laboratory conditions from pork meat, curing salt, salt, water, spices, and extract of *C. incanus* (6 mL/100 g of meat) with either lycopene or betanin dyes (in the amount of 0.2 g of dye/100 g of meat). Pork of different classes (I—lean, up to 15% fat; and II—medium fat, 16–20%) was cut into cubes (2 ± 1 cm). Spices, extracts, salt, and curing salt were added, depending on the type of sausage (Table 1). A curing time of 24 h was used, after which the meat was ground using a ZELMER meat grinder, stuffed into natural casings, and steamed until the temperature in the thermal center reached 74 °C. After cooling, the sausages were vacuum-packed and stored in a refrigerator for 28 days at 2 ± 1 °C. Four variants of sausages were prepared, differing in additives: CS—control sausage, containing pork meat and salt; NS—sausage containing pork meat, water, and a mixture containing salt and nitrates; CLS—sausage made of pork meat with 50% less nitrate content, with the addition of *C. incanus* herb extract and enriched with lycopene dye; CBS—sausage made of pork meat with 50% less nitrate content, with the addition of *C. incanus* herb extract and beetroot coloring.

Table 1. Content of additives in the sausage variants.

Ingredients		CS	NS	CLS	CBS
Water	mL/ 100 g	20	20	14	14
Cistus incanus Extract		-	-	6	6
Salt	g/ 100 g	1.8	-	0.9	0.9
Curing salt		-	1.8	0.9	0.9
Pepper		0.2	0.4	0.4	0.4
Granulated garlic		0.4	0.4	0.4	0.4
Beetroot powder		-	-	-	0.2
Lycopene powder		-	-	0.2	-

CS, sausages with salt; NS, sausages with curing salt (content nitrates); CLS, sausages with *C. incanus* extract and lycopene; CBS, sausages with *C. incanus* extract and betanin.

2.7.1. TBARS Index

Samples of the four types of sausages were analyzed after 0, 7, 14, 21, and 28 days of storage. The stability of lipids was evaluated by determining the TBARS index, according to a modified method [24]. The sausages were ground in a ZELMER 686 mincer with a mesh of 8 mm holes. Next, 5.0 g of each sample was weighed and transferred to a distilled flask, to which 75 mL distilled water and 7 mL of 3N hydrochloric acid were added. Steam distillation was performed to obtain 20 mL of distillate. Next, 3 mL of TBA solution was added and the samples were heated in a water bath under reflux coolers for 30 min. The solutions were cooled, and the absorbance was measured at λ = 530 nm. The concentration of malondialdehyde was calculated from the prepared standard curve.

2.7.2. Color Determination

The color of the sausage samples was measured after 0, 7, 14, 21, 28 days of storage using the CIELa* b* method, where the L parameter measures the brightness on a range from 0 to 100, positive values for a* indicate red color, negative values for a* show green color, positive values for b* show yellow color, and negative values for b* indicate blue color. Spectrophotometric measurements were performed using a Konica Minolta Chroma Meter CR-400 (aperture 8 mm) colorimeter (Japan). The ΔE indices was calculated from the measurements, according to the formula:

$$\Delta E = \sqrt{(\Delta a)^2 + (\Delta b)^2 + (\Delta L)^2}$$

2.7.3. Microbiological Analysis

Microbiological analysis of the sausages was performed after 0, 3, 5, 7, 14, 21, 28 days of storage. The tests were conducted in accordance with ISO 6887-2-2017 [25]. The total numbers of psychrotrophs and mesophiles were determined after incubation at 6 °C for 10 days and at 30 °C for 72 h by counting bacterial colonies on agar plates (PCA, Merck). Enterobacteriaceae were also incubated at 30 °C for 24 h on plates with growth medium (VRBG, Merck). Lactic acid bacteria were incubated at 30 °C for 72 h on plates with MRS medium (Merck).

2.7.4. Sensory Analysis

Sensory analysis of the sausage variants was conducted by a selected panel of 10 people. The panel rated the taste, odor, color, and appearance of the meat products on a hedonic 5-point scale, where: 5 = extremely desirable, 4 = desirable, 3 = neither desirable nor undesirable, 2 = undesirable, 1 = extremely undesirable. Sensory analysis of the sausages was performed at time 0, then after 5, 14, 21, and 28 days. The taste of the meat products was evaluated at time 0 and 5 days after production.

2.7.5. Statistical Analysis

All assay variants were performed in triplicate. Excel 2010 was used to calculate the means and standard deviations of the samples. Analysis of variance (one-way ANOVA) was performed using STATISTICA 13.1. software. Tukey's honestly significant differences (HSD) post hoc test was used to determine differences between variables ($p < 0.05$).

3. Results and Discussion

3.1. Antioxidant Activity C. incanus Extract and Dyes

All the plant materials showed free radical scavenging ability (Table 2). The *C. incanus* extract, the beetroot extract, and the *C. incanus* extract in combination with beetroot and lycopene, had higher antioxidant activity than the lycopene extract, which showed the lowest activity. Higher values for free radical scavenging capacity were observed using the ABTS method than with DPPH. These differences were due to the properties of the reagents. ABTS interacts in both hydrophilic and lipophilic systems, whereas DPPH interacts in hydrophobic systems [26]. Previous studies have reported similar results to those produced in our study. Sayah [27] also observed strong antioxidant activity by *Cistus* extract. As in our study, Koubaier et al. [28] observed high antioxidant activity by beetroot extract. Many studies have shown that lycopene is a potent antioxidant and neutralizes free radicals. Its activity is increased by vitamins C and E as well as by β carotene [29–31].

Table 2. Antioxidant activity measured by DPPH and ABTS methods (mean ± SD, n = 3).

	DPPH	ABTS
	mg Trolox/100 mL	
C	24.7 ± 0.6 ^{a}	33.2 ± 0.4 ^{a}
L	18.5 ± 0.3 ^{c}	21.4 ± 0.2 ^{c}
B	25.6 ± 0.5 ^{a}	31.3 ± 0.3 ^{b}
C L	23.8 ± 0.5 ^{b}	33.3 ± 0.5 ^{a}
C B	24.3 ± 0.1 ^{a,b}	32.4 ± 0.5 ^{a}

C, *Cistus incanus* extract; L, lycopene; B, betanin; C L, *Cistus incanus* extract with lycopene; C B, *Cistus incanus* with betanin; ^{a}, ^{b}, ^{c}, significant differences between types of extracts ($p < 0.05$).

3.2. Polyphenolic Extract Composition

The total polyphenolic content in the extract of *C. incanus* was 1642.98 mg GAE/L of extract. Table 3 presents the qualitative composition of the aqueous extract of *C. incanus*. Forty-three compounds were identified. The largest groups in order of concentration were flavonols (39.5%) > tannins (13.95%) > benzoic acid derivatives (13.95%) > procyanidins (11.63%) > hydroxycinnamic acid derivatives, phenolic acids (4.65%), flavan-3-ols and other compounds. The *C. incanus* extract contained quinic and succinic phenolic acids. The following tannins were identified: punicalin isomer, cornusin B, HHDP (hexahydroxydiphenyl-glucose isomer), galloyl- HHDP-glucoside, punicalagin isomer, and punicalagin-gallate isomer. The flavonoids included myricetin derivatives, kaempferol derivatives, and quercetin. The extract contained the following procyanidins: gallocatechin, gallic acid, gallocatechin trimer, gallocatechin-(4α-8)-catechin, epicatechin-3-gallate, catechin and epicatechin.

Table 3. Polyphenols in aqueous extract of C. incanus identified using the MS method.

Retention Time [min]	λmax	*m/z*	ms^2	Compound
2.20	345; 378	781	601; 301	Punicalin isomer
2.21	255; 360	1085	783; 451; 425; 301	Cornusin B
2.29	218; 245	195	138	Caffeine
2.37	250; 277	191	173;171	Quinic acid
2.96	256; 280	331	481; 673; 779; 963	Galloyl glucose
2.96	279	913	305	Gallocatechin trimer
3.00	238; 376	481	301;275; 257;229	Hexahydroxydiphenyl-glucose isomer (HHDP)
3.02	260	117	73	Succinic acid
3.49	265	331	225; 481; 897	Galloyl glucose
3.88	270	343	191; 169; 125	3-*O*-galloquinic acid
3.93	213; 261	169	125	Gallic acid
4.01	275	609	591; 483; 441; 423; 305	Gallocatechin-(4α-8)-gallocatechin
4.34	280	483	169	Digalloyl glucose
4.50	260; 379	781	601; 301	Punicalin isomer
5.20	255; 275	913	305	Gallocatechin trimer
5.21	260; 285	609	423; 305; 483; 591	Gallocatechin-(4α-8)-gallocatechin
5.30	265	331	225; 481; 897	Galloyl glucose
5.47	256; 288	913	305	Gallocatechin trimer
5.84	259; 288	593	575; 467; 425; 407; 289	Gallocatechin-(4α-8)-catechin
6.09	278	483	169	Digalloyl glucose
6.25	240; 267	153	109	Protocatechuic acid
6.38	260; 273; 299	305	287; 261;221; 219; 179	Gallocatechin
6.91	260; 278	609	423; 305; 483; 591	Gallocatechin-(4α-8)-gallocatechin
7.04	305; 345	593	447;285	Kaempferol-3-rutinoside
7.10	310	355	195	Cis or trans ferulic acid hexoside
8.27	238	633	301;	Galloyl-HHDP-glucoside
9.01	320; 365	1083	781; 601; 301	Punicalagin isomer
9.01	255; 370	1085	783; 451; 425; 301	Cornusin B
9.45	376	1251	1207; 1082; 781;601; 301	Punicalagin-gallate isomer
9.53	278	289	271; 245; 205; 179	Catechin
9.79	249;333	351		Ni
9.90	320; 365	1083	781; 601; 301	Punicalagin isomer
9.90	255; 370	1085	783; 451; 425; 301	Cornusin B
9.90	278	633	301	Galloyl-HHDP-glucoside
10.10	265; 289	305	287; 261;221; 219; 179	Gallocatechin
10.37	238	481	301;275; 257;229	Hexahydroxydiphenyl-glucose isomer(HHDP)
10.57	267; 286	179	135	Caffeic acid
11.07	256; 375	461	415	Diosmetin 8-C-glucoside
11.22	258; 376	1251	1207; 1082; 781;601; 301	Punicalagin-gallate isomer
11.24	260; 350	625	316	Myricetin derivative
11.29	238; 377	481	301;275; 257;229	Hexahydroxydiphenyl-glucose isomer(HHDP)

Table 3. *Cont.*

Retention Time [min]	λmax	*m/z*	ms^2	Compound
11.70	260; 349	449	447	Myricetin-pentoside
12.01	256; 350	433	301; 271	Myricetin derivative
12.05	276; 289	441	169	Epicatechin-3-gallate
13.29	285	163	119	*p*-Coumaric acid
13.44	355	475	285	Kaempferoldimethyletherhexoside
13.75	280	483	169	Digalloyl glucose
13.89	345	625	316	Myricetin derivative
14.11	351	479	317; 289; 271; 179, 151	Myricetin-galactoside
14.18	266; 278	137	93	Salicylic acid
15.88	260; 351	449	447	Myricetin-*O*-xyloside
16.17	279	913	305	Gallocatechin trimer
16.21	260; 351	479	317; 289; 271; 179, 151	Myricetin-3-*O*-galactoside
16.21	260; 355	463	317; 289; 271; 179; 151	Myricetin-rhamnoside
16.31	255; 345	609	463	Quercetin rhamno glucoside
16.71	260; 355	625	317; 271; 179;1 51	Myricetin-rutinoside
18.21	270	289	245; 205; 271	Epicatechin
18.42	255; 345	447	301; 271; 255; 179	Quercetin-3-*O*-rhamnoside
18.58	258; 350	433	301	Quercetin derivative
18.75	260; 345	593	285	Keampferol- rhamno-glucoside
19.62	267; 350	447	285	Kaempferol-3-*O*-glucoside
19.66	281; 296	483	169	Digalloyl glucose
23.85	355	625	607; 479; 317; 179	Myricetin rhamno-glucoside
24.70	253; 284	301	151	Quercetin

HHDP, hexahydroxydiphenyl-glucose isomer, Ni, not identified.

Barrajón-Catalán et al. [32] and Riehle et al. [33] identified flavan-3-ols in *C. incanus* extracts, and found various flavanols, especially the compounds myricetin, quercetin, and kaempferol. Wittpahl et al. [19] also identified tannins: punicalin isomers, punicalagin isomers, and cornusin B.

3.3. Antibacterial Activity of C. incanus Extract and Dyes

The culture method was used to study the impact of the *C. incanus* extract and dyes on the activity of selected Gram negative and Gram positive bacteria. *Cistus incanus* was applied in concentrations of 0.5%, 1.0%, and 2.0% with 1% of both lycopene and betanin dyes, or with 1% of either dye. The numbers of microorganisms were determined by the average colony count method on three plates after a specific seeding time. Based on the results, the growth parameters were calculated using the Gompertz function. After fitting the results, a correlation coefficient of between 0.962 and 0. 999 was obtained. We calculated the following parameters: proper growth rate (μmax), duration of lag-phase (tLag), final number of cells (yEnd). The dye extracts had different effects on the selected bacteria. The results are presented in Tables 4 and 5. It was observed that as the concentration of *C. incanus* extract increased, the multiplication rate of the tested bacteria decreased. The application of *C. incanus* extract in concentrations of 0.5%, 1.0%, and 2.0% decreased the growth rate to a statistically significant degree. The use of the extract resulted in a lower growth rate and lag time of the bacterial phase compared to the control samples. The *C. incanus* extract interacted against the Gram positive bacteria better than against the Gram negative bacteria.

Table 4. Effects of *C. incanus* extract and dyes on the maximum specific growth rate (μmax) of bacteria (mean ± SD, n = 3).

	Escherichia coli	*Pseudomonas fragi*	*Salmonella enterica*	*Brochothrix thermosphacta*	*Latilactobacillus sakei*	*Listeria monocytogenes*
	μmax [h^{-1}]					
Control	0.844 ± 0.021 $^{a\,A}$	0.438 ± 0.010 $^{a\,E}$	0.630 ± 0.035 $^{a\,C}$	0.542 ± 0.013 $^{a\,D}$	0.547 ± 0.007 $^{a\,D}$	0.819 ± 0.014 $^{a\,A}$
C 0.5	0.726 ± 0.032 $^{b\,B}$	0.407 ± 0.005 $^{b\,E}$	0.593 ± 0.018 $^{a\,D}$	0.412 ± 0.005 $^{e\,E}$	0.267 ± 0.014 $^{e\,F}$	0.844 ± 0.025 $^{a\,A}$
C 1.0	0.621 ± 0.035 $^{c\,B}$	0.405 ± 0.011 $^{b\,D}$	0.483 ± 0.011 $^{c\,D}$	0.312 ± 0.015 $^{g\,E}$	0.263 ± 0.005 $^{e\,F}$	0.721 ± 0.008 $^{b\,A}$
C 2.0	0.432 ± 0.024 $^{e\,B}$	0.395 ± 0.013 $^{b\,C}$	0.469 ± 0.023 $^{c\,B}$	0.292 ± 0.010 $^{g\,D}$	0.260 ± 0.022 $^{e\,D}$	0.582 ± 0.007 $^{c\,A}$
L	0.451± 0.005 $^{e\,B}$	0.410 ± 0.021 $^{a,b\,B}$	0.444 ± 0.007 $^{c,d\,B}$	0.293 ± 0.027 $^{g\,C}$	0.500 ± 0.021 $^{b\,A}$	0.429 ± 0.014 $^{e\,B}$
B	0.560 ± 0.017 $^{d\,A}$	0.351 ± 0.015 $^{c\,C}$	0.432 ± 0.021 $^{c,d\,B}$	0.409 ± 0.017 $^{e\,B}$	0.512 ± 0.004 $^{b\,A}$	0.567 ± 0.017 $^{c\,A}$
C 0.5 L	0.825 ± 0.034 $^{a\,A}$	0.455 ± 0.021 $^{a\,E}$	0.613 ± 0.017 $^{a\,C}$	0.520 ± 0.009 $^{a\,D}$	0.277 ± 0.013 $^{e\,F}$	0.493 ± 0.013 $^{d\,E}$
C 1.0 L	0.810 ± 0.021 $^{a\,A}$	0.445 ± 0.003 $^{a\,E}$	0.583 ± 0.015 $^{a,b\,D}$	0.454 ± 0.010 $^{d\,E}$	0.289 ± 0.008 $^{e\,G}$	0.391 ± 0.025 $^{e,f\,F}$
C 2.0 L	0.803 ± 0.019 $^{a\,A}$	0.426 ± 0.010 $^{a,b\,E}$	0.573 ± 0.021 $^{a,b\,D}$	0.381 ± 0.017 $^{e,f\,F}$	0.269 ± 0.019 $^{e\,G}$	0.413 ± 0.011 $^{e,f\,E}$
C 0.5 B	0.795 ± 0.024 $^{a\,A}$	0.435 ± 0.015 $^{a\,D}$	0.591 ± 0.009 $^{a\,C}$	0.534 ± 0.015 $^{a\,C}$	0.340 ± 0.011 $^{c\,E}$	0.456 ± 0.025 $^{d,e\,D}$
C 1.0 B	0.780 ± 0.021 $^{a,b\,A}$	0.345 ± 0.025 $^{c\,E}$	0.564 ± 0.028 $^{a,b\,C}$	0.505 ± 0.006 $^{b\,C}$	0.315 ± 0.007 $^{d\,E}$	0.434 ± 0.014 $^{e\,D}$
C 2.0 B	0.720 ± 0.015 $^{b\,A}$	0.266 ± 0.004 $^{d\,E}$	0.500 ± 0.010 $^{c\,C}$	0.495 ± 0.013 $^{b,c\,D}$	0.253 ± 0.023 $^{e\,E}$	0.437 ± 0.008 $^{e\,D}$

C 0.5, *Cistus incanus* extract 0.5%; C 1.0, *Cistus incanus* extract 1.0%; C 2.0%, *Cistus incanus* extract 2.0%; L, lycopene; B, betanin; C 0.5 L, *Cistus incanus* extract 0.5% with lycopene; C 1.0 L, *Cistus incanus* extract 1.0% with lycopene; C 2.0 L, *Cistus incanus* extract 2.0% with lycopene; C 0.5 B, *Cistus incanus* extract 0.5% with betanin; C 1.0 B, *Cistus incanus extract* 1.0% with betanin; C 2.0 B, *Cistus incanus* extract 2.0% with betanin; $^{a–g}$, significant differences between variants against the same bacterial strain ($p < 0.05$). $^{A–G}$, significant differences between types of bacterial strain for the same variant ($p < 0.05$). The colors indicate values from the highest (red) through medium (yellow) to the lowest (green).

The application of 0.5% *C. incanus* extract contributed to a 51.2% reduction in the growth rate of L. sakei. However, against Gram negative *E. coli* bacteria, 2.0% *C. incanus* extract slowed the growth by 48.8% compared to the control. The weakest effect was observed in the case of *P. fragi*. Both lycopene and beetroot pigments slowed the growth rate of Gram positive bacteria, with the best results against *B. thermosphacta* (45.9% reduction in growth rate compared to the control). Lycopene was stronger against Gram negative bacteria, reducing the growth rate of *E. coli* by 46.6%. No synergistic effect between the *C. incanus* extract and the lycopene and betanin dyes was observed at the tested concentrations. No statistically significant changes were observed for Gram negative *E. coli* and *S. enterica*. *Cistus incanus* extract at concentrations of 1.0 and 2.0 in combination with betanin slowed the specific growth rate of *P. fragi* the most. The extract of *C. incanus* with both lycopene and betanin slowed the growth rate of the Gram positive bacteria L. sakei and *L. monocytogenes* best. Against *B. thermosphacta*, 2.0% extract of *C. incanus* in combination with lycopene was the most effective.

Another measured parameter was the duration of the lag phase. As the concentration of *C. incanus* extract was increased, the duration of lag increased compared to the control sample. For Gram negative bacteria, lag was best extended by *C. incanus* at a concentration of 2.0% against *P. fragi*, and for Gram positive bacteria by *B. thermosphacta* and *L. sakei*. Lycopene and beetroot dye extracts prolonged lag phase duration the most, by 202.7% and 205.3%, respectively, against *L. sakei*. The dyes also increased the lag time of *L. monocytogenes* by 70%. Against Gram negative bacteria, the dyes had the strongest effect on the lag phase of S. enterica, extending its duration by 136.3% in the case of lycopene and by 128.0% for beet. When *C. incanus* extract was used at three concentrations together with dyes, 1.0% and 2.0% *C. incanus* with lycopene and 2.0% *C. incanus* with beetroot had the best effects against *B. thermosphacta* and *P. fragi*, respectively. The differences in the final bacterial proliferation (yEnd) values were not statistically significant.

According to the literature, *C. incanus* extracts inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* bacteria [19,34]. Rajnbar [35] studied the antimicrobial activity of lycopene oleoresin against the pathogens *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus ureuse*, *Salmonella typhimurium*, *Listeria monocytogenes*, *Bacillus cereus*, and *Bacillus licheniformis*. It was found that tomato peel oleoresin containing 2% lycopene can inhibit and restrain both Gram negative the Gram positive bacteria. There are no data in the literature on the effects of *C. incanus*, lycopene and beetroot extracts on microbial growth parameters.

Table 5. Effects of *C. incanus* extract and dyes on lag time of bacteria (mean ± SD, n = 3).

	Escherichia coli	*Pseudomonas fragi*	*Salmonella enterica*	*Brochothrix thermosphacta*	*Latilactobacillus sakei*	*Listeria monocytogenes*
	tLag [h^{-1}]					
Control	2.59 ± 0.05 $^{c\,C}$	4.67 ± 0.31 $^{d\,A}$	1.82 ± 0.13 $^{d\,D}$	3.17 ± 0.22 $^{g\,B}$	3.01 ± 0.07 $^{g\,B}$	3.38 ± 0.25 $^{f\,B}$
C 0.5	2.52 ± 0.07 $^{c\,D}$	5.33 ± 0.15 $^{c\,A}$	1.79 ± 0.15 $^{d\,E}$	4.51 ± 0.11 $^{f\,B}$	3.37 ± 0.15 $^{f\,C}$	4.29 ± 0.09 $^{c\,B}$
C 1.0	3.09 ± 0.09 $^{b\,C}$	5.50 ± 0.22 $^{c\,A}$	2.73 ± 0.11 $^{b\,D}$	5.15 ± 0.07 $^{e\,A}$	5.38 ± 0.11 $^{d\,A}$	4.39 ± 0.05 $^{c\,B}$
C 2.0	3.68 ± 0.15 $^{a\,E}$	6.05 ± 0.15 $^{b\,B}$	2.80 ± 0.16 $^{b\,F}$	7.18 ± 0.13 $^{c\,A}$	6.87 ± 0.25 $^{b\,B}$	4.94 ± 0.12 $^{b\,D}$
L	3.53 ± 0.03 $^{a,b\,F}$	4.74 ± 0.20 $^{d\,E}$	4.30 ± 0.13 $^{a\,E}$	3.82 ± 0.08 $^{g\,F}$	9.11 ± 0.15 $^{a\,A}$	5.73 ± 0.03 $^{a\,D}$
B	3.42 ± 0.09 $^{a,b\,F}$	4.65 ± 0.15 $^{d\,E}$	4.15 ± 0.08 $^{a\,E}$	5.65 ± 0.15 $^{d\,D}$	9.19 ± 0.09 $^{a\,A}$	5.65 ± 0.07 $^{a\,D}$
C 0.5 L	2.84 ± 0.16 $^{b\,D}$	4.85 ± 0.08 $^{d\,B}$	1.92 ± 0.20 $^{d\,E}$	3.47 ± 0.11 $^{g\,C}$	5.82 ± 0.11 $^{c\,A}$	3.76 ± 0.08 $^{e\,C}$
C 1.0 L	3.15 ± 0.11 $^{b\,E}$	6.23 ± 0.05 $^{b\,B}$	2.48 ± 0.11 $^{c\,F}$	7.45 ± 0.07 $^{b\,A}$	6.02 ± 0.09 $^{c\,B}$	3.80 ± 0.12 $^{e\,E}$
C 2.0 L	3.57 ± 0.15 $^{a\,E}$	6.78 ± 0.31 $^{a\,B}$	2.81 ± 0.09 $^{b\,F}$	7.47 ± 0.11 $^{b\,A}$	6.06 ± 0.09 $^{c\,B}$	4.26 ± 0.13 $^{c\,D}$
C 0.5 B	2.95 ± 0.07 $^{b\,C}$	4.70 ± 0.16 $^{d\,A}$	1.85 ± 0.08 $^{d\,D}$	3.49 ± 0.05 $^{g\,B}$	3.50 ± 0.03 $^{f\,B}$	3.28 ± 0.15 $^{f\,B}$
C 1.0 B	3.45 ± 0.09 $^{a,b\,C}$	4.78 ± 0.13 $^{d\,B}$	2.65 ± 0.04 $^{b\,D}$	5.71 ± 0.11 $^{d\,A}$	4.59 ± 0.21 $^{e\,B}$	3.88 ± 0.13 $^{e\,C}$
C 2.0 B	3.73 ± 0.10 $^{a\,F}$	5.28 ± 0.07 $^{c\,D}$	2.60 ± 0.09 $^{b,c\,G}$	8.11 ± 0.21 $^{a\,A}$	4.75 ± 0.05 $^{e\,E}$	4.05 ± 0.01 $^{d\,E}$

C 0.5, *Cistus incanus* extract 0.5%; C 1.0, *Cistus incanus* extract 1.0%; C 2.0%, *Cistus incanus* extract 2.0%; L, lycopene; B, betanin; C 0.5 L, *Cistus incanus* extract 0.5% with lycopene; C 1.0 L, *Cistus incanus* extract 1.0% with lycopene; C 2.0 L, *Cistus incanus* extract 2.0% with lycopene; C 0.5 B, *Cistus incanus* extract 0.5% with betanin; C 1.0 B, *Cistus incanus* extract 1.0% with betanin; C 2.0 B, *Cistus incanus* extract 2.0% with betanin; $^{a–g}$, significant differences between types of extracts against the same bacterial strain ($p < 0.05$). $^{A–G}$, significantly differences between types of bacterial strain the same extract ($p < 0.05$). The colors indicate values from the highest (red) through medium (yellow) to the lowest (green).

The antimicrobial activity is not due to the total amount of polyphenols but to the chemical composition of the extracts [36]. Numerous flavonoids: flavan—3—ols, flavonols and tannins were identified in the extract of *Cistus incanus* we studied. According to literature data, flavonoids are characterized by strong antimicrobial properties. Quercetin, myricetin, kaempferol and their derivatives, as well as flavones, flavanones, flavan—3—ols and chalcones found in plants inhibit bacterial growth [37]. In contrast, tannins have weak antimicrobial activity against Gram negative bacteria [38]. The punicalagin isomers found in our extract are known for their antioxidant, antibacterial and antiviral properties. The available studies checked the activity of punicalagin against *S. aureus* by agar diffusion method. Based on the results, punicalagin showed good activity against *Staphylococcus aureus*. Moreover, it had a significant inhibitory effect on *S. aureus* biofilm formation [39]. In a study by Kuchta et al., who also tested the aqueous extract of *Cistus incanus* for antimicrobial activity, it was observed that the aqueous extract of *C. incanus* showed no activity against Gram negative bacteria [40]. The previous studies also confirm the good activity of aqueous extracts of *Cistus incanus* against Gram positive and to a lesser extent against Gram negative [19,41]. Our study is in agreement with literature data which indicate that Gram positive bacteria are more sensitive to polyphenols than Gram negative bacteria [42]. Although the walls of Gram negative bacteria are covered with a lipophilic outer membrane that provides protection, and this contributes to a lower permeability of hydrophilic substances, the presence of polyphenols can disrupt the outer membrane of Gram negative bacteria, thereby increasing the permeability of the membrane [43,44]. To the best of our knowledge, the effect of *C. incanus* extract on bacterial growth parameters, proper growth rate and lag phase duration, has not been studied yet. Thus, further studies are required to investigate the mechanism of action of *Cistus incanus* extract and the bioactive compounds present in it.

3.4. Effect of Polyphenolic Extract and Dyes on Lipid Oxidation in Pork Sausages

Figure 1 shows the effects of *C. incanus* extract with lycopene and betanin dyes on lipid oxidation during cold storage of pork sausages. The content of malondialdehyde, which is one of the lipid oxidation products, varied during storage. The highest content was observed in the control sausage with salt (CS) and was 3.35 mg/100 g meat. However, in the other meat products the aldehyde content was several times lower. After the first week of storage, an increase in aldehyde content was observed in all the analyzed sausages. The highest increase was in sausages with salt and was 3.36 mg/100 g of meat. The lowest increase was observed in the case of sausages with *C. incanus* and lycopene.

After two weeks, the malondialdehyde content of the salted sausages (CS) increased by 17.31% compared to the baseline sample. In the remaining samples, a slight increase was observed compared to samples after 7 days of storage, with the lowest increases for sausages with *C. incanus* extract and lycopene (CLS) and with *C. incanus* and betanin (CBS). This indicates that extracts in combination with dyes slow lipid oxidation processes. After three weeks of storage, the highest increase in malondialdehyde content (eight times higher) was recorded for sausages with nitrates (PS). Conversely, a significant increase was observed in the malondialdehyde content of sausages with *C. incanus* and betanin (as high as six times higher than in the initial samples). After four weeks of storage, the highest malondialdehyde concentration at 4.81 mg/100 g of meat was obtained for sausages with salt (CS). On the basis of these results, it can be concluded that the use of *C. incanus* extract in combination with beetroot and tomato peel colorant and a reduced amount of nitrates slows the process of lipid oxidation in stored pork sausages.

Zhang et al. [45] evaluated the effects of clove extract on the quality and fat oxidation of sausages. As in our study, they observed slowing of lipid oxidation processes and inhibited deterioration. Efenberger-Szmechtyk et al. [36] observed that the addition of leaf extracts of *A. melanocarpa, C. superba, and C. mas* to pork sausages stored in MAP inhibited lipid oxidation. The *C. superba* extract had the greatest effect on the MDA concentration in the sausages.

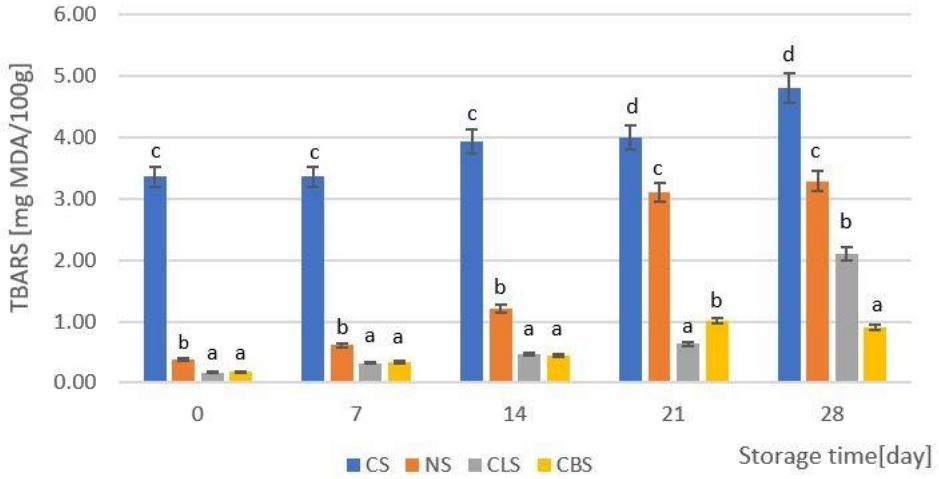

Figure 1. Effects of *C. incanus* extract with lycopene and betanin on lipid oxidation in vacuum packed pork sausages stored at 2 ± 1 °C. The results are expressed as mean ± SD, n = 3. CS, sausages with salt; NS, sausages with curing salt; CLS, sausages with *C. incanus* extract and lycopene; CBS, sausages with *C. incanus* extract and betanin; a–d, statistically differences between the sausage variants ($p < 0.05$).

3.5. Effect of Polyphenolic Extract and Dyes on Sausage Color

Color and color stability are important quality parameters for meat and meat products [46]. Table 6 shows the values for the parameters of brightness L*, redness a*, yellowness b*, and color stability ΔE in pork sausages with and without the *C. incanus* extract and natural dyes. The sausages were initially characterized by brightness ranging from 60.01 to 64.82. The darkest sausage was the one with *C. incanus* extract and beetroot. The brightest was the sausage with salt. Similar brightness levels were recorded for all the sausages with the extract and dyes and reduced nitrate content. After one week of storage, the sausage with *C. incanus* extract and betanin (CBS) was still the darkest, while the sausage with NS nitrate was the lightest. After two weeks, the sausages with salt (CS) and nitrates (NS) were darker, as were the sausages with natural additives. However, after 3 weeks of storage, the sausages with salt and the sausages with nitrates darkened, while sausages with the extract and dyes became lighter. After four weeks of storage, the sausages with salt (CS) and curing salt (NS) were darker and the other sausages were lighter.

The highest proportion of red color (parameter a*) was measured for sausages with a lower content of nitrates, with *C. incanus* extract and enriched with betanin (CBS). The share of red color was the lowest in the sausages with salt (CS) but was 50% higher in the sausages with nitrates compared to the sausages without added nitrates. This confirms that curing salt fulfils its function and provides meat products with their characteristic pink color. At the same time, the addition of the extract and natural colorants increased the proportion of red color in the sausages. After the first week of storage, the greatest decrease in the content of red color of as much as 5% was recorded for sausages with nitrates (NS). A reduction in parameter a* was also observed in the sausages with *C. incanus* and betanin (CBS). After 7 days, the sausage with *C. incanus* and betanin extract (CBS) was still characterized by the highest proportion of red color. This trend continued throughout the 28-day storage period. After 14 days, the proportion of red color in the sausages with salt (CS) decreased by 9% in comparison to the color immediately after manufacture. The proportion of red increased in the remaining sausages. The greatest increase was observed for sausages enriched with lycopene dye (CLS). In the next stage of storage, the CBS sausages were characterized by the highest share of red color, which indicates that the addition of the *C. incanus* extract and enrichment with betanin increased and stabilized the share of red color in the sausages. The lowest proportions of red color after 3 and 4 weeks were in the CS and NS sausages.

In our previous study, which analyzed the effects of *C. incanus* extract on selected parameters of meat products, the plant extract positively increased the proportion of red color in sausages with reduced nitrates [47]. The b* parameter, indicating the proportion of yellow color, was initially highest in the sausages with CS salt and was maintained during storage. The lowest b* value was detected in sausages with nitrates (NS) and in sausages with C. incanus and beetroot (CBS). After 7 days of storage, an increase in the proportion of yellow color was observed in all the sausages. During storage, the b* value increased in all the analyzed sausages. The samples containing C. incanus extract and enriched with lycopene (CLS) and betanin (CBS) had a greater color difference ΔE compared to the salt and nitrate samples. A similar result was obtained by Nowak et al. [48] using cherry and black currant leaf extracts as additives in sausages. Noticeable changes in color $\Delta E > 2$ were noticed for only one type of CBS sausage.

3.6. Effect of Polyphenolic Extract and Dyes on Microbial Stability of Sausages

The results of microbiological test on the vacuum-packed pork sausages made during the 28-day storage period are shown in Table 7. At the end of the storage time, the amounts of mesophilic bacteria, psychrotrophs, lactic acid bacteria, and *Enterobacteriaceae* in the pork sausages increased by statistically significant amounts. After production, the pork sausages containing the plant extract and colorants showed small amounts of the tested bacterial groups and there were no bacteria from the *Enterobacteriaceae* family. The smallest amount of mesophilic bacteria was recorded in the sausages containing lycopene and extract of *Cistus incanus*. During storage, the *C. incanus* extract and beetroot increased the bacterial count in the sausages. The greatest increase in the amount of psychrotroph bacteria was noticed after day 7 of storage. On days 7 and 14 of storage, the smallest number of *Enterobacteriaceae* was recorded for sausages with *C. incanus* extract with lycopene. In terms of the amount of psychrotrophs, the smallest significantly statistical differences ($p < 0.05$) were recorded on day 14 of storage. Further research is needed to test whether higher concentrations of the plant extract and dyes could inhibit the growth of the studied groups of bacteria. The reduced amount of nitrates in the sausages with *C. incanus* extract and dyes did not impair the microbiological quality of the meat products.

Table 6. Effects of *C. incanus* extract with lycopene and betanin on color parameters (L*, a*, b*) of pork sausages stored in vacuum packaging at 2 ± 1 °C (mean ± SD, n = 3).

Lightness L*					
Day	0	7	14	21	28
CS	64.82 ± 0.25 $^{c\,A}$	64.22 ± 0.09 $^{c\,A}$	64.67 ± 0.30 $^{c\,A}$	66.04 ± 0.42 $^{c\,B}$	65.21 ± 0.28 $^{d\,A,B}$
NS	64.71 ± 0.07 $^{c\,A}$	64.77 ± 0.23 $^{c\,A}$	65.22 ± 0.19 $^{c\,B}$	65.46 ± 0.21 $^{c\,B}$	65.29 ± 0.37 $^{d\,B}$
CLS	61.41 ± 0.16 $^{b\,A}$	62.60 ± 0.29 $^{b\,B}$	63.07 ± 0.26 $^{b\,B}$	62.75 ± 0.36 $^{b\,B}$	62.94 ± 0.15 $^{b\,B}$
CBS	60.01 ± 0.28 $^{a\,A}$	60.61 ± 0.15 $^{a\,A}$	61.30 ± 0.24 $^{a\,B}$	60.30 ± 0.45 $^{a\,A}$	60.67 ± 0.21 $^{a\,A}$
Redness a*					
Day	0	7	14	21	28
CS	4.44 ± 0.03 $^{a\,C}$	4.53 ± 0.16 $^{a\,C}$	5.04 ± 0.39 $^{a\,C}$	3.64 ± 0.24 $^{a\,B}$	3.13 ± 0.21 $^{a\,A}$
NS	8.91 ± 0.07 $^{c\,B,C}$	8.47 ± 0.45 $^{c\,B}$	9.48 ± 0.22 $^{b\,C}$	9.58 ± 0.10 $^{b\,C}$	7.86 ± 0.18 $^{b\,A}$
CLS	9.38 ± 0.11 $^{c\,A}$	9.61 ± 0.20 $^{c\,A}$	10.43 ± 0.14 $^{c\,B}$	10.39 ± 0.17 $^{c\,B}$	9.29 ± 0.23 $^{c\,A}$
CBS	11.65 ± 0.09 $^{d\,C}$	10.41 ± 0.28 $^{d\,A}$	11.44 ± 0.03 $^{c\,B}$	11.96 ± 0.16 $^{d\,C}$	10.31 ± 0.03 $^{d\,A}$
Yellowness b*					
Day	0	7	14	21	28
CS	15.36 ± 0.29 $^{c\,A}$	15.77 ± 0.11 $^{c\,A}$	15.74 ± 0.18 $^{d\,A}$	15.77 ± 0.03 $^{b\,A}$	16.23 ± 0.08 $^{d\,B}$
NS	12.74 ± 0.08 $^{a\,A}$	13.65 ± 0.16 $^{a\,B}$	12.79 ± 0.33 $^{a\,A}$	13.34± 0.17 $^{a\,B}$	13.53 ± 0.16 $^{a\,B}$
CLS	14.69 ± 0.35 $^{b\,A}$	15.88 ± 0.27 $^{c\,B}$	15.03 ± 0.19 $^{d\,A}$	15.27 ± 0.37 $^{b\,A}$	15.38 ± 0.22 $^{c\,A,B}$
CBS	12.92 ± 0.16 $^{a\,A}$	14.46 ± 0.14 $^{b\,B}$	14.02 ± 0.30 $^{c\,A,B}$	13.46 ± 0.42 $^{a\,A}$	14.00 ± 0.26 $^{a\,A,B}$
Δ E					
Day	0	7	14	21	28
CS	-	0.73 ± 0.05 $^{a\,A}$	0.67 ± 0.18 $^{a\,A}$	1.96 ± 0.12 $^{c\,C}$	1.07 ± 0.10 $^{a\,B}$
NS	-	1.01 ± 0.11 $^{a\,B}$	1.40 ± 0.04 $^{b\,C}$	0.61 ± 0.15 $^{a\,A}$	1.74 ± 0.04 $^{b\,D}$
CLS	-	1.70 ± 0.03 $^{b\,C}$	1.27 ± 0.09 $^{b\,B}$	0.40 ± 0.03 $^{a\,A}$	1.11 ± 0.09 $^{a\,B}$
CBS	-	2.07 ± 0.06 $^{c\,C}$	1.31 ± 0.20 $^{b\,A}$	1.26 ± 0.08 $^{b\,A}$	1.78 ± 0.03 $^{b\,B}$

CS, sausages with salt; NS, sausages with curing salt; CLS, sausages with *Cistus incanus* extract and lycopene; CBS, sausages with *Cistus incanus* extract and betanin; $^{a–d}$, significant differences between the sausage variants (in columns) ($p < 0.05$); $^{A–D}$, significant differences during storage (in rows) ($p < 0.05$).

Table 7. Effect of *C. incanus* extract with lycopene and betanin on the microbiological quality of pork sausages (n = 3).

TMC [cfu/g]							
day	0	3	5	7	14	21	28
CS	2.43 ± 0.05 $^{b\,A}$	3.85 ± 0.12 $^{B\,b}$	4.64 ± 0.13 $^{C\,b}$	6.72 ± 0.06 $^{D\,a}$	6.94 ± 0.04 $^{D\,a}$	7.91 ± 0.14 $^{E\,b}$	7.70 ± 0.10 ^{E}
NS	2.38 ± 0.08 $^{b\,A}$	2.48 ± 0.03 $^{a\,A}$	3.90 ± 0.04 $^{a\,B}$	6.81 ± 0.10 $^{a\,E}$	6.72 ± 0.07 $^{a\,E}$	6.95 ± 0.03 $^{a\,E}$	6.59 ± 0.05 $^{a\,E}$
CLS	1.78 ± 0.11 $^{a\,A}$	2.97 ± 0.15 $^{a\,B}$	4.40 ± 0.17 $^{b\,D}$	6.15 ± 0.13 $^{a\,F}$	6.80 ± 0.10 $^{a\,F}$	7.53 ± 0.05 $^{b\,G}$	7.34 ± 0.07 $^{b\,G}$
CBS	2.30 ± 0.05 $^{b\,A}$	3.30 ± 0.07 $^{b\,B}$	5.12 ± 0.02 $^{c\,D}$	6.93 ± 0.11 $^{a\,E}$	6.54 ± 0.08 $^{a\,E}$	7.40 ± 0.02 $^{b\,F}$	7.56 ± 0.17 $^{b\,F}$
LAB [cfu/g]							
day	0	3	5	7	14	21	28
CS	2.28 ± 0.09 $^{b\,A}$	3.01 ± 0.05 $^{b\,B}$	4.63 ± 0.09 $^{b\,C}$	6.76 ± 0.14 $^{a\,E}$	7.21 ± 0.12 $^{b\,F}$	8.15 ± 0.06 $^{c\,G}$	8.48 ± 0.04 $^{c\,G}$
NS	2.51 ± 0.03 $^{b\,A}$	2.11 ± 0.18 $^{a\,A}$	3.70 ± 0.15 $^{a\,B}$	6.72 ± 0.03 $^{a\,E}$	6.77 ± 0.07 $^{a\,E}$	7.90 ± 0.02 $^{b\,F}$	7.34 ± 0.09 $^{b\,F}$
CLS	1.90 ± 0.14 $^{a\,A}$	2.65 ± 0.07 $^{a\,B}$	4.15 ± 0.04 $^{b\,D}$	6.23 ± 0.10 $^{a\,F}$	7.12 ± 0.15 $^{b\,G}$	6.57 ± 0.13 $^{a\,F}$	7.02 ± 0.11 $^{b\,G}$
CBS	1.95 ± 0.11 $^{a\,A}$	2.93 ± 0.14 $^{b\,B}$	5.19 ± 0.08 $^{c\,D}$	6.89 ± 0.06 $^{a\,E}$	7.19 ± 0.08 $^{b\,F}$	6.67 ± 0.05 $^{a\,E}$	6.86 ± 0.16 $^{a\,E}$
TPC [cfu/g]							
day	0	3	5	7	14	21	28
CS	2.26 ± 0.16 $^{b\,A}$	3.30 ± 0.11 $^{b\,B}$	3.85 ± 0.07 $^{a\,B}$	6.91 ± 0.14 $^{a\,E}$	7.45 ± 0.08 $^{b\,F}$	7.42 ± 0.11 $^{b\,F}$	7.57 ± 0.07 $^{b\,F}$
NS	2.00 ± 0.04 $^{b\,A}$	2.42 ± 0.02 $^{a\,A}$	3.00 ± 0.05 $^{a\,B}$	6.83 ± 0.18 $^{a\,D}$	6.93 ± 0.06 $^{a\,D}$	6.78 ± 0.03 $^{a\,D}$	6.28 ± 0.17 $^{a\,D}$
CLS	1.48 ± 0.09 $^{a\,A}$	2.40 ± 0.16 $^{a\,B}$	3.30 ± 0.12 $^{a\,C}$	6.26 ± 0.03 $^{a\,E}$	7.06 ± 0.10 $^{a\,F}$	7.41 ±0.14 $^{b\,F}$	7.21 ± 0.02 $^{b\,F}$
CBS	1.30 ± 0.05 $^{a\,A}$	3.13 ± 0.13 $^{b\,C}$	3.70 ± 0.06 $^{a\,D}$	6.90 ± 0.09 $^{a\,E}$	7.48 ± 0.04 $^{b\,F}$	7.37 ± 0.12 $^{b\,F}$	7.60 ± 0.10 $^{b\,F}$
Enterobacteriaceae [cfu/g]							
day	0	3	5	7	14	21	28
CS	<1	1.70 ± 0.05 $^{a\,A}$	2.32 ± 0.16 $^{a\,B}$	4.38 ± 0.04 $^{b\,D}$	5.95 ± 0.07 $^{c\,E}$	5.58 ± 0.03 $^{b\,E}$	5.85 ± 0.17 $^{b\,E}$
NS	<1	1.30 ± 0.09 $^{a\,A}$	2.04 ± 0.02 $^{a\,B}$	4.20 ± 0.11$^{b\,D}$	4.23 ± 0.14 $^{b\,D}$	4.28 ± 0.05 $^{a\,D}$	4.81 ± 0.02 $^{a\,D}$
CLS	<1	2.00 ± 0.14 $^{a,b\,A}$	2.46 ± 0.07 $^{a\,A}$	3.48 ± 0.15 $^{a\,B}$	3.00 ± 0.03 $^{a\,B}$	4.72 ± 0.08 $^{a\,C}$	4.69 ± 0.14 $^{a\,C}$
CBS	<1	2.79 ± 0.06 $^{b\,A}$	2.85 ± 0.08 $^{a\,A}$	3.85 ± 0.09 $^{a\,B}$	4.28 ± 0.12 $^{b\,C}$	4.72 ± 0.04 $^{a\,C}$	5.16 ± 0.10 $^{b\,D}$

CS, sausages with salt; NS, sausages with curing salt; CLS, sausages with *Cistus incanus* extract and lycopene; CBS, sausages with *Cistus incanus* extract and betanin; $^{a–c}$, significantly differences between the sausage variants (in columns); $^{A–F}$, significant differences during storage (in rows) ($p < 0.05$). The colors indicate the values from the highest (red) through medium (yellow) to the lowest (green).

3.7. Effect of Polyphenolic Extract and Dyes on Sensory Evaluation of Sausages

At the time of production, the sausages with 1.8% curing salt (NS), as well as the sausages with *C. incanus* extract and lycopene (CLS), were rated by the panel as being desirable in terms of taste (Table 8, Figure 2). A slightly lower, but equally high score was given to the sausage enriched with *C. incanus* extract and beetroot. The sausage with 1.8% salt was rated lowest. After 5 days of storage, the panel rated the sausage with *C. incanus* extract and lycopene (CLS) the highest in terms of taste (desirable).

In terms of odor, at the time of production the sausages with 1.8% curing salt (NS), as well as the sausage with *C. incanus* extract and lycopene (CLS), were characterized by an exceptionally desirable aroma. Other sausage variants were considered desirable. After 5 days of storage, the product with 2% curing salt (NS) content was again evaluated most favorably. The sausage with added lycopene was rated the highest among all tested variants in terms of odor after the second week of storage. Three weeks after the sausages were made, the odors of all the meat products were judged to be undesirable. After the last week of storage, the odor was rated extremely undesirable. Throughout the storage period, the products with added *C. incanus* extract and lycopene (CLS) and 1.8% curing salt (NS) were characterized by the highest desirability in terms of color. The sausage with 1.8% salt (CS) was rated the lowest. At the time of manufacture, the color of the sausages with 1.8% curing salt content (NS) and *C. incanus* extract with lycopene (CLS) were rated extremely desirable, while products with *C. incanus* extract and beetroot (CBS) and 1.8% salt content (CS) were rated desirable. After four weeks of storage, the color of the sausage with 1.8% salt (CS) and the sausage enriched with *C. incanus* extract and beetroot (CBS) was considered extremely undesirable.

Table 8. Sensory analysis of the sausage variants during storage.

Storage Time [Day]	**0**	**5**	**14**	**21**	**28**	**0**	**5**	**14**	**21**	**28**
Sausage variant			**Taste**					**Odor**		
CS	4.5	3.4	n.t.	n.t.	n.t.	4.8	3.6	3.0	2.4	1.0
NS	5.0	3.5	n.t.	n.t.	n.t.	5.0	4.0	3.2	2.7	1.4
CLS	5.0	4.0	n.t.	n.t.	n.t.	5.0	3.8	3.4	2.5	1.0
CBS	4.8	3.6	n.t.	n.t.	n.t.	4.6	3.8	2.7	2.1	1.0
Sausage variant			**Color**					**Appearance**		
CS	4.5	4.0	3.5	2.2	1.0	4.6	4.0	3.3	2.2	1.0
NS	5.0	4.5	4.0	3.6	2.5	5.0	4.5	4.0	3.4	2.6
CLS	5.0	4.5	4.2	3.5	2.4	5.0	4.5	4.0	3.3	2.5
CBS	4.6	4.0	3.8	2.6	1.5	4.8	4.2	3.5	2.6	1.8

CS, control sausages with salt; NS, sausages with curing salt; CLS, sausages with *Cistus incanus* extract and lycopene; CBS, sausages with *Cistus incanus* extract and beetroot; n.t., not tested.

On average, the sausages received similar scores in terms of the assessment of color. After the storage period, the sausage enriched with *C. incanus* extract and lycopene (CLS) and the sausage with 1.8 % curing salt (NS) were rated the highest. The appearance of the sausage with the addition of *C. incanus* and beetroot (CBS) extract was rated slightly lower. The sausage with 1.8% salt (CS) content was the least desirable. Overall, in terms of flavor, odor, color, and appearance, the sausages with *C. incanus* extract and lycopene (CLS) and the sausages with 2% curing salt (NS) were rated the highest.

Swastike et al. [49] studied the effects of the addition of beetroot powder on the sensory characteristics of chicken sausages with tapioca flour as filler. Four ratios of beetroot powder and tapioca were used: 18:0, 17:1, 16:2, and 15:3 [%:%]. Organoleptic analysis of the chicken sausages was performed by a panel of 25 people. The panel evaluated the taste, color, texture, and tenderness of the sausages. The use of 2% beetroot powder had the most beneficial effect on the color, taste, and texture of the meat products. Aykin-Dicer et al. [50] showed that enrichment with beetroot extract and beetroot powder can have a beneficial effect on the sensory attributes (color, odor and taste) of sausages, concluding that natural

beetroot powder can be an alternative to carmine, a synthetic dye. Eyiler and Oztan [51] analyzed the effect of using tomato powder, a natural lycopene-rich colorant, on the taste, aroma, color, and overall desirability of sausage frankfurters. Organoleptic analysis of the products was conducted by a panel of 10 people. The addition of the natural colorant (lycopene) to the frankfurters had a positive impact on the organoleptic qualities of the product, which was characterized by high desirability in terms of taste, color, and odor.

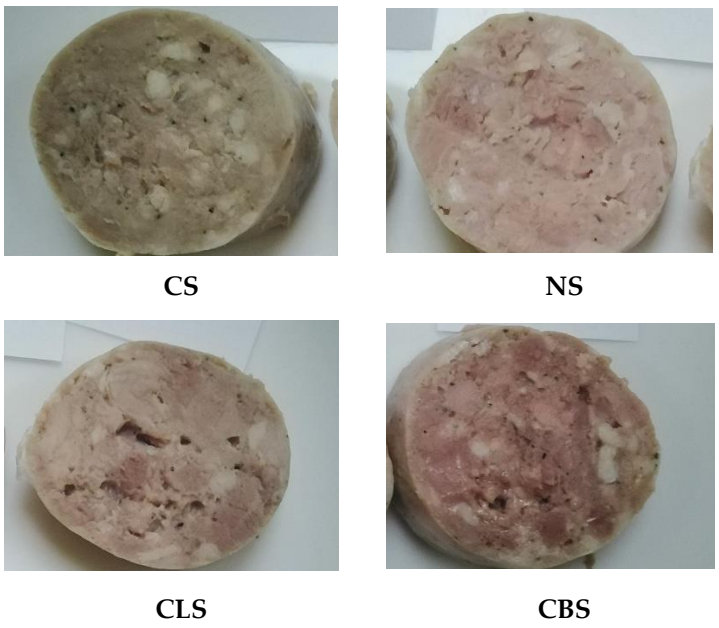

Figure 2. Sausages after 3 days of storage: CS, sausages with salt; NS, sausages with curing salt (containing nitrates); CLS, sausages with *Cistus incanus* extract and lycopene; CBS, sausages with *Cistus incanus* extract and betanin.

In our study, we confirmed high antioxidant activity of *Cistus incanus* extract resulting from the presence of many biologically active compounds. Many polyphenolic compounds were identified in which flavonoids were the largest group. When assessing the antimicrobial effect of *Cistus incanus* extract on selected bacteria, it was shown that increasing the concentration of the extracts decreased the growth rate of *E. coli*, *P. fragi*, *S. enterica*, *B. thermosphacta*, *L. sakei*, *L. monocytogenes* to an increasing extent. *C. incanus* extract also prolonged the duration of lag phase. The dyes lycopene and betanin affected the growth parameters of the bacteria tested. No synergistic effect of the extract with dyes was noted. The use of *C. incanus* extract and lycopene and betanin dyes effectively slowed down the lipid oxidation process in sausages. This is due to the presence of polyphenols in the plant extract. Therefore, *Cistus* extract in combination with dyes delays unfavorable changes in the product and allow to maintain the desired aroma and taste. *Cistus incanus* extract, lycopene and betanin significantly increase the proportion of red color, which is important information for consumers because the content of red color in meat products is a feature of their quality and freshness. From the microbiological point of view, the use of *C. incanus* extract and dyes and a 50% reduction in nitrate content did not deteriorate the quality of the sausages. In the sensory evaluation, the consumers positively assessed the sausages with the addition of *Cistus incanus* extract and natural dyes. However, they rated the sausage with *C. incanus* extract and lycopene the best.

4. Conclusions

In response to consumer demand for natural ingredients and the elimination of chemical additives, natural plant-based agents are being sought to replace commonly-used preservatives. Plants and especially plant extracts offer a promising alternative and exhibit a variety of properties including antimicrobial activity. In this study, *C. incanus* extract

in combination with the natural pigments lycopene (from tomatoes) and betanin (from beetroot) was found to exhibit antioxidant properties in vacuum-packed pork sausages, as well as giving a red color to the meat products. However, the additives did not show antimicrobial properties in the tested amounts. Further optimization of the dose is needed to achieve an antimicrobial effect.

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