

Article

Effect of Addition of Grape Pomace Extract (Pinot Noir Variety) on the Physical-Chemical Properties and Safety of Frankfurter Sausages

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Abstract

This study was designed to demonstrate the effect of Pinot Noir grape pomace extract (GPE) on the quality and shelf-life of frankfurter sausages during 35 days of storage at 4 °C. Due the high polyphenolic compounds of GPE, there is an increasing interest in potentially replacing the artificial additives with natural alternatives generated from waste. The GPE was prepared using ultrasound-assisted extraction (UAE). Following ultrasound-assisted extraction, the extracts were filtered through filter paper to remove solid residues. The meat batter was divided into four groups: control (NFC) and three experimental batches with 1%, 2%, and 3% Pinot Noir grape pomace extract (FE1, FE2, FE3). Results showed that increasing GPE significantly raised water and protein levels while reducing fat content and water activity ($p < 0.05$). Except for a decrease in the b^* color value, GPE did not notably affect pH, collagen, or L^* and a^* color parameters. Although the chemical parameters changed, the collagen content remained unchanged, indicating that GPE does not significantly affect connective tissues. The best effect was shown in the sample FE3, which had the highest GPE addition and exhibited the highest protein content (16.46%) and moisture content (64.99%), as well as the lowest water activity (0.93). Microbiologically, GPE reduced aerobic mesophilic bacteria, with all samples remaining safe for up to 28 days. These findings confirm GPE as a viable ingredient for producing safe, high-quality frankfurter sausages.

Keywords: grape pomace extract; frankfurter sausages; plant extracts; polyphenolic compounds; physicochemical properties; additives

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1. Introduction

The aim of this research was to investigate the potential of grape pomace extract from Pinot Noir as a natural preservative and its antimicrobial effect on meat products. The study was conducted by adding different weight fractions ($w = 0\text{--}3\%$) of Pinot Noir grape pomace extract (GPE) and observing the effect on physicochemical properties and health safety of frankfurter sausages during storage for 35 days at 4 °C. Frankfurter sausages are categorized as cooked sausages and they are typically produced from beef and/or pork (30–40% of meat), along with connective and fatty tissues, and can contain up to 60% water and up to 30% fat. Furthermore, they are pasteurized meat products that are typically

encased and may undergo smoking as part of their processing [1]. Due to their rich nutritional and biological value, meat and meat products are considered an important element of a balanced diet. Besides being a source of high-quality protein, meat also contains essential micronutrients such as iron, zinc, magnesium and B vitamins [2]. The presence of easily digestible, high-quality nutrients, moisture, and unsaturated fatty acids makes meat perfect food for spoilage by microbial growth, lipid peroxidation, and enzymatic autolysis [3]. Using food additives (e.g., preservatives, antioxidants) is one of the main methods for preserving meat and meat product quality (edible, sensory, and technological) during processing and storage. Conversely, they show negative health implications, so scientists are focusing on finding alternatives for these compounds [4]. However, food safety has become more important than before and it is closely linked to sustainable waste management of by-products to improve the nutritional and functional value of food. Plant by-products are typically discarded despite being rich in valuable bioactive compounds such as phenolics, flavonoids, and polysaccharides, which makes them natural substitutes for additives and nitrites in meat processing [2]. To prevent spoilage of meat products, various preservation methods are used, the most well-known of which are salting and brining. During brining, besides NaCl, ingredients such as nitrates, nitrites, carbohydrates (most commonly glucose), phosphates, ascorbates, spices, and other permitted additives are incorporated into meat products [3]. Moreover, there is a growing interest in natural additives to improve their nutritional and functional properties. In addition, a study was conducted with a by-product of winemaking, a grape pomace, which is recognized for its high content of polyphenolic compounds.

Polyphenols, such as anthocyanins, tannins and flavonols, and essential oils (mainly composed of terpenes) are the main bioactive components found in plants that can be used by the meat industry. These compounds can be obtained from different parts of plants such as seeds, leaves and fruits [5]. These molecules exert antioxidant and antimicrobial effects; hence, they are widely utilized as potential natural preservatives by partial or fully replacing the synthetic preservatives [6]. Some of the most used synthetic preservatives are sodium erythorbate, sodium ascorbate, propyl gallate, butylated hydroxyanisole (BHA), tert-butylhydroquinone (TBHQ), and butylated hydroxytoluene (BHT), as well as nitrites and nitrates. Some of these compounds have been described in the literature to be associated with potential toxic and carcinogenic properties [7]. Although phenolic compounds contribute to more than 69% of the total antioxidant capacity, some studies have shown that effectiveness depends not only on the overall capacity of polyphenols but also on their specific type. For example, green tea, fruit mix, olive and grape, and olive extracts exhibited high inhibition of N-nitrosation at pH 5 (41–49%), while being slightly less active at pH 2.5 (21–44%) [8]. Furthermore, grape fruit extract behaves differently from grape seed extract, even though both contain high levels of phenolic compounds, which confirms that composition is more important than quantity alone. Also, certain plant extracts may exert different effects during cured meat processing (pH ~5) and throughout gastric digestion (pH ~2–3). Plant extracts could be used in cured and smoked meat products as potential alternatives to nitrites/nitrates by reducing residual nitrite levels, N-nitrosamine formation, and exhibiting antimicrobial effect [6]. Some studies have investigated the incorporation of grape pomace into meat products through different technological approaches such as the addition of GP powder and extract, as well as marination in solutions containing grape pomace compounds, reporting both beneficial and potential negative effects on product quality [9]. Overall, these studies have proved that the addition of GP should be carefully optimized to balance technological functionality with sensory quality [9].

1.1. Composition and Nutritional Value of Grape Pomace

The chemical composition of grape pomace, shown in Table 1, depends on the vinification process, pressing intensity and grape variety. Grape pomace is the main solid by-product formed throughout the pressing and fermentation phases of wine production [10]. Grape pomace consists of seeds, skin, and stems, where the seeds are highly rich in nutrients such as polyphenolic compounds (60–70%), fibers, proteins, oils, whereas the remaining fraction consists of minerals, sugars, and non-phenolic antioxidants such as β -carotene. The skin, however, contains the highest proportion of dry matter (up to 65%). Grape seeds can be utilized for the production of extracts and oils due to their antimicrobial properties and are widely used as a supplement or additive in food industries. The high concentration of polyphenols in grape pomace has a positive effect on the stability of the final product as well as the organoleptic properties [10,11]. Beyond polyphenols, GP also contains organic acids, dietary fibers, and minor bio-actives such as tocopherols, which may act synergistically to modulate microbial growth [12]. In general, the amount of phenolic compounds in red grape pomace is higher than that of white grape pomace, which is also affected by the different grape varieties [10].

Table 1. Chemical composition of grape pomace [10,13–17].

Component	Content (% DM)
Sugars	2.7–49.1
Tannins	12.1–22.3
Lipids	8.2–13.5
Total dietary fiber	46.2–74.5
Cellulose	9.2–14.5
Hemicellulose	4.0–10.3
Lignin	28.7–42.2
Pectin	3.7–6.2
Ash	4.7–9.5
Total carbon	44.3–52.9
Total polyphenols	4.8–6.7
Total nitrogen	1.2–4.5
Proteins	7.0–23.5

Besides their high nutritional value, they also have anti-inflammatory, antioxidant, antimicrobial, antiviral, and antimutagenic activities. However, no standardized method for polyphenol isolation has yet been established due to their structural diversity and variability in extraction conditions [18].

1.2. Technological Procedures for GPE Extraction and Stabilization

To isolate polyphenols, an extraction must be performed. Extraction separates a compound from a solid or liquid mixture using a suitable solvent in which the compound is more soluble than the other components. The process can be classified as liquid–liquid or solid–liquid, depending on the physical state of the mixture’s phases [19]. Different solvents and methods can be utilized to prepare the plant extracts, and the properties of an extract will depend on the method and solvent used for extraction [19]. Due to their phenolic structure, polyphenols tend to demonstrate greater hydrophilicity than lipophilicity. Therefore, polyphenols can be extracted using solvents such as methanol, ethanol, acetonitrile and acetone, as well as their mixtures with water [20]. Since the use of organic solvents has an undesirable environmental impact, researchers are focused on developing green and sustainable methods [20]. There are multiple extraction methods that can be used, such as high-pressure hydrostatic extraction (HHP), ultrasound-assisted extraction

(UAE), microwave-assisted extraction (MAE), and supercritical fluid extraction (SFE). The ultrasonic energy treatment provided high polyphenol yield due to better extraction by enhancing mass transfer and enabling the solvent penetration into the cell. Furthermore, it minimizes the risk of polyphenol degradation and fastens the extraction [21]. The selection of solvent should be based on its viscosity to enhance mass transfer efficiency [20].

Prior to the incorporation of by-products such as grape pomace, stabilization is essential to ensure an extended shelf life and maintain its functional properties [22]. High hydrostatic pressure (HHP) represents a non-thermal processing technology used for microbial inactivation, effectively preserving phenolic compounds. However, it does not inhibit polyphenol oxidase activity which will contribute to the loss of polyphenol contents during storage [22]. Furthermore, the combination of thermal blanching and HHP leads to the stabilization of polyphenolic compounds and inactivation of polyphenol oxidase [22].

2. Materials and Methods

This study examined the effect of Pinot Noir grape pomace extract (1%, 2%, and 3%) on the physicochemical properties and microbiological safety of frankfurter sausages during 35 days of storage at 4 °C, analyzing basic chemical composition, pH, water activity (a_w), color parameters, and the presence of pathogenic and spoilage microorganisms. The basic composition of frankfurter sausages included veal meat (42%) and veal fat (11%), pork meat (10%) and pork fat (14%), water, table salt (2.2%), and Pinot Noir grape pomace extract in concentrations ranging from 0 to 3%. The prepared meat batter was divided into equal batches, after which GPE was incorporated. The batch formulated without the addition of the grape pomace extract served as the control sample. The mixtures were thoroughly homogenized to ensure uniform distribution of the bioactive compounds. Following homogenization, the meat batter was stuffed into natural casings (sheep thin casings, diameter 22/24 mm) and subjected to thermal processing in a water bath at 80 °C for 20 min until a stable core temperature was achieved. Immediately following the thermal treatment, the sausages were rapidly chilled and stored at a constant temperature of 4 °C. The technological production process was considered complete upon the conclusion of the chilling stage. A similar technological production process was followed according to the method described by Eyiler and Oztan [23]. Pinot Noir grape pomace extract was prepared in the laboratory of the Faculty of Food Technology Osijek. Grape pomace of Pinot Noir cultivar was obtained from the Belje winery in Kneževi Vinogradi, Croatia. Firstly, the grape pomace was cleaned to remove impurities and the grape skins were separated from the seeds, as the skins were further milled using an SM-4500C sample mill (MRC Sample Mill, Brannenburg, Germany) for 1 min at 4000 rpm. Grinding is an important sample preparation step because reducing particle size increases the specific surface area of the material, therefore improving the contact between the sample and the solvent enhancing the efficiency of bioactive compound extraction [24]. For the extraction procedure, 1.5 g of the ground sample was weighed into test tubes, and 30 mL of the 48% aqueous ethanol solution (MilliporeSigma, Burlington, MA, USA; Gram Mol d.o.o., Zagreb, Croatia) was used as extraction solvent. Furthermore, the samples were homogenized and subjected to ultrasound-assisted extraction using a Hielscher UP400St ultrasonic probe (Hielscher Ultrasonics GmbH, Teltow, Germany). Following ultrasound-assisted extraction, the extracts were filtered through filter paper to remove solid residues. A second filtration step was also performed using 0.45 µm PTFE membrane filters to eliminate any remaining suspended particles. The total phenolic content in the extract was determined spectrophotometrically using the Folin–Ciocalteu method (Folin–Ciocalteu reagent, Sigma-Aldrich, Steiheim, Germany) (ISO 14502-1:2005) [25]. The highest total phenolic content, 60.26 mg GAE/L, was obtained when 48% (v/v) aqueous ethanol was used as the extraction solvent at 100% amplitude and 100% pulse. Furthermore, HPLC (Agilent 1260

Infinity 0 II, Agilent Technologies, Santa Clara, CA, USA) characterization analysis of GPE, performed in our laboratory, indicated the presence of several phenolic compounds, such as syringic acid, p-coumaric acid, ellagic acid, myricetin and procyanidin B2, with caftaric acid being among the most abundant. These bioactive compounds are widely reported for their antioxidant and antimicrobial activity [10,11].

The prepared meat batter was divided into four batches: a control sample and three experimental groups with the addition of grape pomace extract (Pinot Noir) at mass fractions of 1, 2, and 3% (Table 2). The homogenized mixture was stuffed into natural casings (sheep thin intestines) and thermally processed at 80 °C for 20 min (Figure S1). The frankfurter sausages were rapidly cooled to 4 °C to complete the technological production process.

Table 2. Sample designations, abbreviations, and descriptions.

Abbreviation	Sample Description
NFC	Nitrite-free control
FE1	Frankfurter + 1% Pinot Noir grape pomace extract
FE2	Frankfurter + 2% Pinot Noir grape pomace extract
FE3	Frankfurter + 3% Pinot Noir grape pomace extract

Furthermore, the proximate composition (moisture, fat, protein, and collagen) of the control and grape pomace extract-enriched frankfurter sausages was determined using a FoodScan™ Meat Analyzer (Foss, Hilleroed, Denmark). The analysis relied on Near-Infrared (NIR) transmission spectroscopy (850–1050 nm) utilizing an Artificial Neural Network (ANN) calibration following ISO 12099:2017 [26] and was validated according to AOAC Official Method 2007.04 [27]. This correlative technique allowed for the precise prediction of constituent concentrations in all samples. Subsequently, water activity (a_w) of the control and grape pomace extract-enriched frankfurter sausages was determined using a HygroLab 3 (Rotronic, Bassersdorf, Switzerland) water activity meter in accordance with ISO 18787:2017 [28]. Homogenized samples were placed in dedicated measurement chambers, and readings were taken using a calibrated probe until equilibrium was reached. Additionally, the pH values of the control and grape pomace extract (GPE)-enriched frankfurter sausages were determined using a WTW pH meter (Xylem Analytics, Weilheim, Germany) equipped with a penetration electrode in accordance with ISO 2917:1999 [29]. Measurements were performed by inserting the electrode directly into the homogenized samples, with readings recorded once the values reached stability.

Following this, color parameters of the control and grape pomace extract (GPE)-enriched frankfurter sausages were determined using a MiniScan XE Plus (HunterLab, Reston, VA, USA). Measurements were performed directly on homogenized samples, with results expressed according to the CIE $L^*a^*b^*$ color space following ISO/CIE 11664-4:201 [30].

Finally, microbiological analyses were conducted according to relevant ISO standards (Table 3) to determine the counts of *Enterobacteriaceae* (HRN EN ISO 21528-2:2017) [31], sulfite-reducing clostridia (HRN ISO 15213-1:2023) [32], coagulase-positive staphylococci (*Staphylococcus aureus*; HRN EN ISO 6888-1:2021) [33], and aerobic mesophilic bacteria (HRN EN ISO 4833-1: 2013) [34]. Furthermore, samples were screened for the presence of pathogens, specifically *Salmonella* spp. (HRN EN ISO 6579-1:2017) [35] and *Listeria monocytogenes* (HRN EN ISO 11290-1:2017) [36], in compliance with standardized food safety protocols.

All measurement were performed in triplicate. For analysis of variance, and then for Fischer's LSD test of least significant difference ($p < 0.05$), the statistical program Statistica 13.0 (TIBCO Software Inc., San Ramon, CA, USA) was used.

Table 3. Valid ISO standards and microorganisms.

Microorganisms	Standard Designation
<i>Enterobacteriaceae</i>	HRN EN ISO 21528-2:2017
<i>Salmonella</i> spp.	HRN EN ISO 6579-1:2017 HRN EN ISO 6579-1:2017/A1:2020
<i>Listeria monocytogenes</i>	HRN EN ISO 11290-1:2017
CPS/ <i>Staphylococcus aureus</i>	HRN EN ISO 6888-1:2021
Sulfite-reducing clostridia	HRN ISO 15213-1:2023
Aerobic mesophilic bacteria	HRN EN ISO 4833-1:2013 HRN EN ISO 4833-1:2013/A1:2022

3. Results and Discussion

3.1. Influence of GPE Addition on the Physicochemical Properties of Frankfurter Sausages

Results showed that the addition of grape pomace extract (GPE) at levels of 1–3% significantly influenced the physicochemical and microbiological properties of frankfurter sausages during refrigerated storage at 4 °C (Tables 4–6). The incorporation of GPE resulted in a statistically significant and linear increase in moisture and protein content ($p < 0.05$), while concurrently leading to a reduction in fat content and water activity (a_w). The decrease in water activity despite an increase in moisture content can be explained by stronger interactions between water molecules and biopolymers such as proteins and phenolic compounds, particularly polyphenols, which are rich in hydroxyl groups capable of forming hydrogen bonds with water. In systems with higher polyphenol content, this effect is further enhanced due to increased binding sites for water molecules, leading to a greater proportion of immobilized water. Since water activity represents the fraction of free, thermodynamically available water, its value decreases when total moisture content increases, as more water becomes associated with solutes and is no longer available in its free form [37]. Sample FE3 exhibited the maximum protein mass fraction (16.46%) following a 35-day storage period at 4 °C. In addition, sample NFC exhibited the maximum fat mass fraction (19.77%) in frankfurter sausages after 35 days of storage at 4 °C. The highest water content (64.99%) was observed in sample FE3 on the first day of storage. The results indicate that the water content in frankfurter sausages shows a statistically significant increase ($p < 0.05$) across all storage intervals at 4 °C as the mass fraction of GPE increases. These changes occurred without affecting the collagen content, whereas the highest collagen content (2.66%) in veal frankfurter sausages was recorded in the NFC sample after 35 days of storage. The pH value of frankfurter sausages samples, after 28 and 35 days of storage at 4 °C, ranged between 5.17 and 5.99. The lowest a_w (0.93) was measured in sample FE3 on the first day of storage at 4 °C. Although increasing the proportion of GPE resulted in lower a_w values than those of the nitrite-free control sample, a slight increase in a_w was observed during 35 days of storage. This increase may be attributed to the potential absorption of moisture from the surrounding environment as well as the structural changes within the meat matrix, resulting in water redistribution. In addition, interactions between polyphenols from grape pomace and meat proteins may modify protein structure and water-binding capacity, therefore promoting the release of previously immobilized water [38–40]. Generally, the addition of GPE significantly ($p < 0.05$) reduced all storage time intervals at 4 °C (Table S1).

Table 4. Proximate composition, water activity (a_w), and pH value of veal frankfurters formulated with grape pomace extract (GPE; w = 0–3%) during 35-day refrigerated storage at 4 °C.

	Protein Content (%)	Fat Content (%)	Moisture Content (%)	Collagen Content (%)	pH	a_w
Day 1						
NFC	14.64 c	18.58 a	63.77 b	1.47 a	5.77 a	0.96 a
FE1	14.58 c	17.76 b	64.19 ab	1.44 a	5.76 a	0.94 b
FE2	14.95 b	17.22 c	64.08 ab	1.44 a	5.77 a	0.94 b
FE3	15.33 a	16.40 d	64.99 a	1.50 a	5.76 a	0.93 c
Day 14						
NFC	15.71 c	19.47 a	61.72 b	2.15 a	5.96 a	0.96 a
FE1	15.89 b	18.59 b	62.94 a	2.15 a	5.99 a	0.96 a
FE2	15.93 b	18.55 c	63.00 a	2.25 a	5.98 a	0.95 b
FE3	16.19 a	18.56 c	62.98 a	2.18 a	5.97 a	0.94 b
Day 28						
NFC	15.72 c	19.77 a	61.88 c	2.33 a	5.31 b	0.96 a
FE1	15.91 b	19.12 b	62.28 b	2.41 a	5.57 a	0.95 ab
FE2	16.23 a	18.76 c	63.00 a	2.34 a	5.58 a	0.95 bc
FE3	16.29 a	18.49 d	62.97 a	2.43 a	5.55 a	0.95 c
Day 35						
NFC	16.03 b	19.78 a	61.98 c	2.66 a	5.17 b	0.96 a
FE1	16.00 b	19.11 b	62.79 b	2.47 a	5.32 a	0.96 a
FE2	16.00 b	18.79 c	63.08 a	2.34 a	5.34 a	0.95 b
FE3	16.46 a	18.35 d	63.03 a	2.26 a	5.36 a	0.95 b

Results are expressed as the mean of three replicates; values in the same column followed by the same letter (a–d) are statistically significant ($p < 0.05$).

Table 5. Instrumental color parameters of Frankfurter sausages formulated with GPE (w = 0–3%) during 35-day storage at 4 °C.

	L*	a*	b*
Day 1			
NFC	63.63 a	18.13 ab	17.90 a
FE1	62.82 a	18.62 a	17.46 a
FE2	63.50 a	18.69 a	16.32 b
FE3	62.59 a	18.63 ba	16.51 b
Day 14			
NFC	71.18 a	10.61 a	15.24 a
FE1	71.53 a	10.26 a	14.72 b
FE2	68.94 a	10.44 a	15.14 ab
FE3	71.97 a	10.38 a	14.72 b
Day 28			
NFC	72.65 a	10.30 b	14.96 a
FE1	72.50 a	10.46 ab	14.90 a
FE2	72.62 a	10.50 ab	14.23 b
FE3	70.70 a	10.62 a	14.29 b
Day 35			
NFC	70.56 ab	10.21 a	14.33 a
FE1	70.66 ab	10.30 a	14.06 ab
FE2	70.41 ab	10.16 a	13.00 ab
FE3	71.42 a	10.58 a	13.60 b

Results are expressed as the mean of three determinations; values within the same column followed by the same letter (a,b) are statistically significant ($p < 0.05$).

Table 6. Microbiological analysis results of veal hot dog samples with added GPE (w = 0–3%) during 35 days of storage at 4 °C.

Storage Time/Days	Sample	E (cfu/g)	AMB (cfu/g)	SRK (cfu/g)	CPS/SA (cfu/g)	SAL/25 g	LM/25 g
1	NFC	10	1.8×10^2	<10	<10	-	-
	FE1	1.2×10	2.1×10^2	<10	<10	-	-
	FE2	8.0×10	1.8×10^2	<10	<10	-	-
	FE3	7.0×10	1.5×10^2	<10	<10	-	-
14	NFC	1.4×10	2.1×10^3	<10	<10	-	-
	FE1	5.0×10	2.45×10^3	<10	<10	-	-
	FE2	2.0×10	1.89×10^3	<10	<10	-	-
	FE3	3.8×10	1.80×10^3	<10	<10	-	-
28	NFC	10^2	10^4	<10	<10	-	-
	FE1	7.1×10	2.6×10^3	<10	<10	-	-
	FE2	6.6×10	2.10×10^3	<10	<10	-	-
	FE3	5×10	1.95×10^3	<10	<10	-	-
35	NFC	4.5×10^2	7.0×10^4	<10	<10	-	-
	FE1	10^2	3.0×10^4	<10	<10	-	-
	FE2	9.0×10	2.0×10^4	<10	<10	-	-
	FE3	9.0×10	1.8×10^4	<10	<10	-	-

E—*Enterobacteriae*; AMB—aerobic mesophilic bacteria; SRK—sulfite-reducing clostridia; CPS—coagulase-positive staphylococci/*Staphylococcus aureus*; SAL—*Salmonella* spp.; LM—*Listeria monocytogenes*; —not detected.

3.2. Colorimetric Parameters

The addition of nitrites in meat production prevents sausage discoloration and is responsible for the characteristic pink color [41]. However, adding GPE from Pinot Noir affected color parameters, particularly b^* values, which were significantly reduced in treated samples. In the control sample (NFC) after 28 days of storage at 4 °C, results showed the highest L^* value (72.65) while the lowest (62.60) was recorded in frankfurter sausages with 3% GPE on day 1. Furthermore, results showed the highest a^* value (18.69) in samples with 2% GPE on day 1, and the lowest (10.16) after 35 days in the same formulation. The highest b^* value (17.90) occurred in the control sample (NFC) on the day 1, while the lowest (13.00) was observed in samples with 3% GPE after 35 days of storage. In general, the addition of GPE resulted in a significant decrease ($p < 0.05$) in b^* values whereas a^* and L^* values were not significantly affected ($p > 0.05$). Overall, a^* values decreased during storage across all samples, while no consistent effect of GPE on redness was observed, indicating a limited influence of GPE on this color parameter. This is consistent with previous reports describing a reduction in meat redness during storage due to myoglobin and lipid oxidation processes [42,43].

3.3. Microbiological Safety and Preservation Mechanisms

Pathogens such as *Escherichia coli*, *Salmonella enterica*, *Listeria monocytogenes*, and *Staphylococcus aureus* continue to pose significant challenges to food safety. These plant-derived compounds can reduce pathogenic loads, extend shelf life, and improve overall food safety [44].

The main mechanisms by which plant extracts display antibacterial activity are the disruption of cell membrane and cell wall integrity, the inhibition of enzymes and metabolic pathways, the interaction with nucleic acids and microbial proteins, the inhibition of efflux pumps and degrading enzymes (like β -lactamases), the induction of oxidative stress, and the blockage of quorum sensing and biofilm formation [44].

From a safety perspective, all GPE-enriched samples remained microbiologically safe throughout the storage period, with no detectable presence of coagulase-positive staphylococci (*Staphylococcus aureus*), sulfite-reducing clostridia, *Listeria monocytogenes*, or *Salmonella* spp. Furthermore, GPE exhibited notable antimicrobial activity, evidenced by a significant reduction in *Enterobacteriaceae* and aerobic mesophilic bacteria counts (cfu/g). These findings demonstrate that grape pomace extract serves as a viable functional ingredient that can enhance the nutritional profile and extend the microbial stability of frankfurter sausages as a natural preservative. Regarding microbial safety, control samples (NFC) exceeded recommended limits for *Enterobacteriaceae* (4.5×10^2 cfu/g) and aerobic mesophilic bacteria (AMB) (7×10^4 cfu/g) after 35 days of storage at 4 °C, rendering them microbiologically unacceptable. In contrast, the addition of GPE (0–3%) significantly reduced both *Enterobacteriaceae* and AMB counts across all storage intervals (14, 28, and 35 days), maintaining the samples within safe limits. This antimicrobial effect is attributed to the high content of bioactive compounds in GPE, particularly polyphenols and terpenes [45]. Similar reductions in AMB counts were reported by Riazi et al. [46] in beef sausages enriched with red grape pomace extract.

3.4. Discussion

The potential of grape pomace extract (GPE) as a functional ingredient in frankfurter sausages was evaluated, revealing that its incorporation leads to substantial improvements in the products' physicochemical profile, color stability and microbiological safety. Regarding the water relations within the food matrix, a specific thermodynamic behavior was observed. The decrease in water activity (a_w) despite an increase in moisture content can be explained by stronger interactions between water molecules and biopolymers such as proteins and phenolic compounds, particularly polyphenols, which are rich in hydroxyl groups capable of forming hydrogen bonds with water. In systems with higher polyphenol content, this effect is further enhanced due to increased binding sites for water molecules, leading to a greater proportion of immobilized water. Since water activity represents the fraction of free, thermodynamically available water, its value decreases when total moisture content increases, as more water becomes associated with solutes and is no longer available in its free form [37]. However, changes over time occurred throughout the refrigerated storage period. Although increasing the proportion of GPE resulted in lower a_w values than those of the nitrite-free control sample, a slight increase in a_w was observed during 35 days of storage. This increase may be attributed to the potential absorption of moisture from the surrounding environment as well as structural changes within the meat matrix, resulting in water redistribution. In addition, interactions between polyphenols from grape pomace and meat proteins may modify protein structure and water-binding capacity, therefore promoting the release of previously immobilized water [38–40]. Regarding color characteristics, adding GPE from Pinot Noir significantly affected yellowness (b^*), while redness (a^*) and lightness (L^*) of the sausages were not significantly altered. The significant decrease in b^* values in the GPE-treated samples can be attributed to the natural pigments present in the grape pomace, which can influence and modify color characteristics in meat products [47]. Overall, a^* values decreased during storage across all samples, while no consistent effect of GPE on redness was observed, indicating a limited influence of GPE on this color parameter. The reduction in a^* values during storage is attributed to changes in myoglobin redox chemistry, particularly the conversion of oxymyoglobin to metmyoglobin [43]. Furthermore, lipid oxidation in meat systems may indirectly contribute to color changes during storage [42].

4. Comparative Effects of Plant Extracts and Their Effects on Meat Product Quality

In the context of comparative phytochemical analysis, rosemary extract serves as a primary reference due to its robust profile of phenolic compounds. It possesses significant anti-inflammatory, anticancer, and antimicrobial properties, primarily mediated by rosmarinic acid, carnosol, and carnosic acid [48]. The study was conducted to evaluate different amounts of rosemary extract and nitrate which were incorporated into frankfurter sausages. Furthermore, the microbiological and physicochemical properties of the sausages during a six-day storage were investigated. Sausages were made from minced beef with added oil, ice, salt, spices, starch, rosemary extract, and nitrate. The mixture was cooked to 75 °C, rapidly cooled, and stored at 2–3 °C for further analysis and treatment. They were divided into two groups. One with plasma-activated-water (PAW) and one without PAW. The results demonstrated that the rosemary extract combined with PAW inhibited pathogenic microorganisms even at the lowest concentrations. This can be linked due to carnosic and rosmarinic acid. Furthermore, the addition of rosemary extract and PAW had no significant effect on the pH values or moisture content of the sausages during storage. Moreover, due to its antioxidant properties, rosemary extract can inhibit myoglobin and lipid oxidation [48].

Furthermore, the research was also conducted with the chokeberry extract, applied both individually and in combination with the vitamin C, to evaluate its effect on sensoric properties, firmness and color of frankfurter sausages during storage at 4 °C for 21 days. The results indicated that there were no negative changes on textural and color properties, but the sample with the highest concentration of chokeberry extract led to the best sensory evaluation score. Overall, the sample with combined chokeberry extract and vitamin C indicated the best antioxidant activity due to their synergistic capacity [49]. Another study has been conducted on mechanically deboned poultry meat to also evaluate the antioxidant potential of plant extracts such as grape, green tea, pomegranate, mate and rosemary. The concentrations of plant extracts were 0.125, 0.25, 0.50, and 1% during 2 °C storage. Grape extract had the best antioxidant capacity, whereas pomegranate extract demonstrated the lowest activity. This indicates that pomegranate extract had the highest lipid oxidation. Notably, all examined concentrations of plant extracts were equally effective in inhibiting lipid oxidation. The addition of the extracts led to a decrease in L* and b* values (lightness and yellowness), whereas grape extract specifically enhanced a* values (redness) during refrigerated storage [50]. Similar research was conducted to evaluate the effect of green tea extract during chilled storage (4 ± 1 °C). Meat samples were treated with green tea extract (700 ppm), green tea extract (1000 ppm), rosemary extract (0.2%), mixture of green tea extract (500 ppm) and rosemary extract (0.2%), and mixture of green tea extract (500 ppm) and rosemary extract (0.3%). The results showed that the untreated (control) sample remained acceptable until third day of storage, whereas the samples with green tea extract (1000 ppm) remained safe until the sixth day. Samples containing higher concentrations of GTE indicated the lowest microbial counts. Additionally, the rosemary extract has strong antibacterial activity, especially against Gram-positive bacteria. Notably, the pH of meat samples treated with higher green tea extract was slightly higher than control samples, likely due to the higher pH of green tea itself. However, the pH remained within acceptable limits [51].

When comparing these findings mentioning chokeberry and rosemary extract with our own results presented in Table 4, noticeable trends in the physicochemical properties can be observed. As mentioned before, significant increase in moisture content and water activity during refrigerated storage may be attributed to moisture migration from the environment, as well as dynamic water redistribution within the meat matrix [52]. Further-

more, possible interactions between muscle proteins and polyphenolic compounds originated from the plant extracts likely contributed to the enhanced water-binding capacity system [53]. The change in the meat matrix also explains the reduction in fat content, which occurred due to a formulation dilution effect where the plant extract substituted a portion of the lipid phase [54]. Consequently, this fat decrease, combined with the shifts in moisture, resulted in a relative increase in the total protein percentage.

5. Conclusions

This study demonstrates the strong potential of Pinot Noir grape pomace extract (GPE) as an effective, potential alternative to synthetic additives and nitrites in frankfurter sausage processing. The findings indicate that increasing the GPE mass fraction (1–3%) linearly induces higher protein and moisture content, while successfully reducing fat content and water activity (a_w). The optimum technological and nutritional profile was achieved in the FE3 formulation (3% GPE), which maintained the highest protein content (16.46%) after 35 days of refrigerated storage (4 °C) and the lowest initial water activity (0.93). The stability of collagen content confirms that the extract does not impair the connective tissue matrix, while the significant decrease in the parameter b^* from an initial 17.90 to a minimum of 13.00 highlights its potent capacity to suppress lipid peroxidation. From a microbiological standpoint, GPE incorporation guaranteed complete food safety up to day 28, successfully inhibiting pathogens such as *Listeria monocytogenes* and *Salmonella* spp. While the nitrite-free control (NFC) sample exceeded safe thresholds for *Enterobacteriaceae* (4.5×10^2 cfu/g) and aerobic mesophilic bacteria (7.0×10^4 cfu/g) by day 35, the extract-enriched formulations effectively maintained microbial counts within regulatory limits, owing to the synergistic antimicrobial effects of polyphenols and terpenes. In conclusion, valorizing this winemaking by-product as a functional ingredient offers an innovative framework for simultaneously enhancing the biosecurity, nutritional quality, and environmental sustainability of processed meat products.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/pr14142321/s1>, Figure S1: Veal frankfurters produced with 3% of Pinot Noir grape pomace extract; Table S1: Proximate composition of veal frankfurters formulated with grape pomace extract (GPE; w = 0–3% on dry matter basis) during 35-day refrigerated storage at 4 °C.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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Abbreviations

The following abbreviations are used in this manuscript:

GPE	Grape Pomace Extract
GTE	Green Tea Extract
PAW	Plasma Activated Water
NIR	Near-Infrared Transmission Spectroscopy
ANN	Artificial Neural Network Calibration
CIE	International Commission on Illumination
NFC	Nitrite-free Control
HHP	High Hydrostatic Pressure
TBARS	Thiobarbituric Acid Reactive Substances
PTFE	Polytetrafluoroethylene
UAE	Ultrasound-assisted extraction
MIC	Minimum inhibitory concentration

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