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Combined Effects of Parsnip Fermented Juice and Hawthorn Extract Regarding Pork Mince Stability: Physico-Chemical and Microbiological Aspects

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Abstract: Parsnip fermented juice (PFJ) and hawthorn extract (HE) were identified as natural nitrite and antioxidant sources for pork mince. This study aimed to determine the effects of varying levels of HE added to a constant concentration of PFJ on lipids stability, heme pigment conversion degree, residual nitrite content, and spoilage bacteria growth, during refrigeration, compared with the combined effect of synthetic nitrite and sodium ascorbate (SA). Pork mince was formulated in six different ways with sterile distilled water (NC), 100 ppm synthetic nitrite and 50 ppm SA (PC), PFJ in the concentration of 100 ppm NO_2^- (T1), constant level of PFJ (100 ppm NO_2^-), and increased level of HE, 50, 25 and 10 ppm GAE (T2, T3 and T4). During the experiment, pH increased for all the treatments, but the addition of PFJ alone or in combination with HE, it was maintained below the NC pH value. The lowest TBARS values and the highest PUFA concentrations were found in the T3, T4, and PC treatments. Of all the samples, the lowest residual nitrite values were found for T2. The highest NO-heme values were found for T2 and PC. After 9 days of storage, TVC results were higher than 5.69 logs CFU/g for all treatments. Overall, the obtained results showed that the combination of HE and PFJ could be a promising natural preservative for minced meat that could replace synthetic preservatives.

Keywords: parsnip fermented juice; hawthorn extract; natural nitrite; natural antioxidant; lipids stability; spoilage bacteria; heme pigment conversion degree; bioactive compounds

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1. Introduction

Oxidative processes and meat spoilage bacteria are the most important factors in decreasing the shelf life of meat and meat products. Meat oxidation starts during slaughtering and continues in post-slaughtering conditions, handling, processing, and storage [1,2]. Meat is susceptible to spoilage bacteria due to its favourable growth conditions, high water content, nitrogen-containing molecules, carbohydrates, lipids, lactic acid, vitamins, and minerals. These degradative processes result in the development of off-odours, off-flavour, off-taste, loss of colour, loss in nutritional value, slim formation, and toxic compounds generation, making the meat undesirable for human consumption [3–5]. In the meat industry, synthetic antioxidants and nitrates and nitrites inhibit oxidative processes, bacterial growth, and input attractive colour. Lipid oxidation may be slowed down by synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butylhydroquinone (TBHQ), propyl gallate (PG), and sodium ascorbate and ascorbate.

To inhibit the grow spoilage bacteria and improve meat colour, synthetic additives, such as potassium/sodium nitrite and potassium/sodium nitrate are used. According to commission regulation (EU) No 1129/2011, the amount of nitrite permitted for use as a food additive in cured meat is currently 150 mg kg^{-1}. Many studies have suggested the toxicity and carcinogenicity of synthetic antioxidants with phenolic structures [6–8] and synthetic nitrites and nitrates [9,10] and for this reason, the researchers interested in replacing these synthetic substances with natural ones has increased in recent decades [11–16]. Many research studies have demonstrated the antioxidant and antimicrobial activity of plant extracts rich in phenolic compounds [14]. Vegetables are important sources of natural nitrates, but nitrate concentrations vary widely among plants, plant parts, and growing conditions. During the fermentation process, nitrates from plant juices can be converted into nitrites and, after concentration, concentrated fermented vegetable plant juices or fermented plant powders rich in natural nitrites result. The high nitrate content in the fermented vegetable juice/powder led researchers to study it as a nitrite natural source for the meat industry [17]. In recent years, consumer preferences have shifted towards requiring health-friendly, high-quality, nutrient-rich natural products. Parsnip fermented juice appears to be highly compatible with processed meat products because it has a high content of nitrates, very little vegetable pigment, and a mild, pleasant flavour profile. On the other hand, extracts of hawthorn berries are sources of natural antioxidants with previously proven preservative effects for meat products [5]. In fact, the present research highlights the preservative activity of the active compounds in hawthorn and nitrite-rich fermented juice. This study emphasizes the effects of varying levels of hawthorn berries ethanolic extract (HE) added to a constant concentration of parsnip fermented juice (PFJ) on lipids stability, heme pigment conversion degree, residual nitrite content, and spoilage bacteria growth in pork mince during refrigeration, compared to the combined effect of synthetic nitrite and sodium ascorbate. This study aimed to evaluate the combined effects of parsnip fermented juice and hawthorn phenolics on lipids and colour stability, and on spoilage bacteria growth in pork mince.

2. Materials and Methods

2.1. Parsnip Fermented Juice (PFJ) Obtaining

Parsnip roots were bought from a local market near Bucharest. The parsnip roots were cut into small pieces (1 × 1 cm) and then placed in a homogenizer with sterile distilled water in a ratio of 1:2 (*w*/*v*), and left to rest for 3 h at 4 °C. After homogenization, filtration and sterilization *Staphylococcus xylosus* (ATCC 29971) were added at 10^8 cfu/mL in a shaker incubator at 37 °C for 36 h [18]. Next, the mixture was filtered through Whatman No. 1 filter paper and evaporated using a rotary evaporator (Heidolph Laborota 4000). The concentrated fermented parsnip juice had a pH of 5.31 and 6237.5 ppm nitrite content. The nitrite concentration was assayed by the AOAC method [19]. The concentrated juice was kept in the refrigerator until used.

2.2. Hawthorn Extract (HE) Obtaining

The dried hawthorn fruits were collected from the forests near Câmpulung Muscel, Argeș county. After grinding, using a kitchen milling machine, the obtained powder was mixed with 60% ethanol (*v*/*v*), in an extraction rapport of 1:10 (*w*/*v*). Then was vigorously mixed and left to stand for 5 h. After that, the extraction was continued in a water bath (GFL 1092) at 60 °C for 3 h. The mixture was filtered through Whatman No. 1 filter paper and concentrated with a rotary evaporator (Heidolph Laborota 4000) to a temperature lower than 80 °C. The total phenolic content was assayed using Folin Ciocalteu reagent [20] and was expressed as mg gallic acid equivalent/mL (mg GAE/mL).

2.3. Experimental Setup and Preparation of Meat Samples

Fresh pork leg was purchased from a local butcher. After washing with distilled sterile water, all subcutaneous and intramuscular fat and visible connective tissue were removed

with a knife. The meat was manually chopped into cubes of approximately 2 cm^3 and then minced in a grinder equipped with a 10 mm plate, followed by grinding through a 3.5 mm plate. The meat was divided into 6 portions of one kilogram. The samples were treated with sodium nitrite (SN) and sodium ascorbate (SA), parsnip fermented juice (PFJ), and hawthorn extract (HE) at different concentrations, as shown in Table 1. Sodium ascorbate and sodium nitrite were analytical reagents and were obtained from Sigma-Aldrich, St Louis, MO, USA.

Table 1. Pork mince treatments with sodium nitrite (SN), sodium ascorbate (SA), parsnip fermented juice (PFJ), and hawthorn extract (HE).

Additives / Treatments	SN (ppm NO_2^-)	SA (ppm)	PFJ (ppm NO_2^-)	HE (ppm GAE)
NC	0	0	0	0
PC	100	50	0	0
T1	0	0	100	0
T2	0	0	100	50
T3	0	0	100	25
T4	0	0	100	10

Abbreviation: SN, sodium nitrite; SA, sodium ascorbate; PFJ, parsnip fermented juice; HE, hawthorn extract; NC, negative control; PC, positive control; GAE, gallic acid equivalent; ppm, parts per million and it expresses milligrams per kg (mg/kg); T1, treatment 1; T2, treatment 2; T3, treatment 3; T4, treatment 4.

For the pork-minced formulation, the ingredients (SN, SA, PFJ, HE) were dissolved in cold (4 °C) sterilized distilled water in a 100 mL final volume. For the negative control, 100 mL of sterilized distilled water was used. All meat samples were homogenized using a food processor (Moulinex DP 700), packed in plastic film, and stored at 4 °C for 9 days.

2.4. Physicochemical Analysis

pH Value

The pH value of all samples was measured using a pH meter (Hanna Instruments, Cluj Napoca, Romania) by direct measurement with a glass electrode calibrated with the phosphate buffers 4.0 and 7.0 at room temperature (21 °C). Ten grams of sample was homogenized with distilled water in a ratio of 1:100 (w/v) for 30 min. After filtration, the pH of the filtrate was measured [21].

2.5. Chemical Analysis

2.5.1. Chromatographic Profile of Fatty Acids

Lipid Extraction and Fatty Acid Methyl Esters

The fatty acids profile of lipids from minced meat was determined as fatty acids methyl esters (FAME). Lipid extractions were made according to [22] method and FAME was prepared by transmethylation using 2 M KOH in methanol and normal heptane according to the method described by [23].

Gas-chromatographic Analysis of Fatty Acid Methyl Esters

Fatty acid methyl esters (FAME) were quantified by gas chromatography (GC) using a Perkin-Elmer-Clarus 500 system with a flame ionisation detector (FID), capillary column injection system and a silica capillary column SGE (BPX70, 60 m; 0.25 mm inner diameter, 0.25 µm film, Agilent). Gas-chromatographic conditions were according to the procedure described by [5]. Each fatty acid was expressed as g/100 g fatty acid methyl esters (FAME).

2.5.2. Thiobarbituric Acid Reactive Substances (TBARS) Value

Lipid oxidation in the minced pork was monitored by measuring thiobarbituric acid reactive substances (TBARS) every 3 days during refrigeration storage. TBARS value was assayed by the method described by [24]. Briefly, 0.5 g minced meat was treated with 2.5 mL thiobarbituric acid solution. After homogenisation, the tube with the mixture was

immersed in a boiling water bath for 10 min. After cooling under running tap water, sonication for 30 min, and centrifugation, the absorbance of the supernatant was read at 532 nm. A standard curve was prepared using 1,1,3,3-tetramethoxypropane and the concentration ranging from 0 to 10 ppm. TBARS value was expressed as mg of malondialdehyde equivalents/kg of the sample (mg MDA/kg).

2.5.3. Nitrosyl Hemochrome, Total Pigment Content, and the Heme Pigment Conversion Degree

Nitrosyl hemochrome and total pigments were measured after extraction with acetone and acidified acetone [25]. For quantification of the nitrosyl hemochrome, a 10 g sample was mixed with 43 mL of acetone-water solution (acetone: water, 40.5:2.5, *v*/*v*) in reduced light. After 5 min, the mixture was filtered through Whatman No. 1 paper, and the filtrate absorbance was measured at 540 nm using acetone water solution (80% acetone, 20% water) for blank. NO-heme pigment concentration was calculated using formula (1).

$$\text{NO-heme concentration (ppm hematin acid)} = \text{sample A540} \times 290 \quad (1)$$

For total pigment measurement, a 10 g sample (65% water) was mixed with 40.4 mL acetone, 1.6 mL water and 1 mL of concentrated HCl. The mixture was stored in dark at the room temperature, stirring from time to time. After 1 h, the homogenates were filtrated and the absorbance of the filtrate was read at 640 nm against the same solvent mixture used for the homogenate preparation. The optical density read at 640 nm was multiplied by 680, according to formula (2), to express the total pigment concentration.

$$\text{Total pigment concentration (ppm hematin acid)} = \text{A640} \times 680 \quad (2)$$

The heme pigment conversion degree was calculated using formula (3).

$$\%\text{Conversion} = (\text{NO-Heme/Total-Heme}) \times 100 \quad (3)$$

2.5.4. Residual Nitrite Level

Residual nitrite levels in the minced samples were assayed using the method recommended by [26]. For nitrite extraction, 5 g of sample was mixed completely with 40 mL hot water and quantitative transferred to a 500 mL volumetric flask. After the addition of about 300 mL, the volumetric flask was immersed in a shaking water bath for 2 h at 80 °C. Then, the mixture was cooled at room temperature, diluted to volume with water, mixed and filtrated. The colourimetric method using Griess diazotization was performed. Residual nitrite level was determined by comparison with the prepared standard curve and was expressed as ppm NO_2^- (μg NO_2^-/g sample).

2.5.5. Volatile Basic Nitrogen (VBN) Value

To determine the extent of protein deterioration during refrigeration storage, volatile basic nitrogen value was performed by the method of [27]. Briefly, 5 g of sample were mixed with 30 mL of 5% (*w*/*v*) trichloroacetic acid (TCA). The homogenate was made up to 50 mL of final volume with 5% (*w*/*v*) TCA and filtered using the Whatman filter paper No. 1. One mL of filtrate and 1 mL of borate buffer were placed in the outer and inner Conway dish, respectively. After 100 min incubation at 37 °C, the inner solution was titrated with 0.01 N HCl and the titration volume was recorded. The results were expressed as mg%.

2.5.6. Microbiological Analysis

Total viable count (TVC) was determined in plate count agar by the pour-plate method [28]. A total of 25 g of sample was aseptically weighed and homogenized with sterile 0.1% peptone water in the ratio 1:9 (*w*/*v*) for 1 min using a stomacher (400 circulators Seward Ltd.UK) at a speed of 6000 rpm. The homogenized sample was serially diluted (1:10) in sterile 0.1% peptone water. One mL sample of serial dilutions was plated into plate

count agar and then incubated at 35–37 °C for 48 h. Microbiological data were expressed as the logarithm of the number of colony-forming units (logCFU/g). All counts were performed in triplicate.

2.6. Statistical Analysis

The effect of treatments on the fatty acids profile of minced pork was performed using one-way ANOVA (XLStat, Addinsoft, New York, NY, USA). The effect of treatments and storage time on pH value, TBARS, NO-heme, total heme, heme pigment conversion degree, residual nitrite level, volatile basic nitrogen, and the total viable count was performed using two-way ANOVA (XLStat, Addinsoft, New York, NY, USA). The Tuckey test was used to predict differences among the criteria; the effects were considered significant if $p < 0.05$. The statistical model included the fixed effects of treatments (NC, PC, T1, T2, T3, T4) and storage time (0, 3, 6, 9 days) and their interactions. For correlations between parameters, Pearson's correlation was used.

3. Results and Discussions

3.1. Physico-Chemical Analysis

pH Value

Several studies demonstrated that increased refrigeration storage increased raw meat's pH [29–31]. It was suggested that increases in pH during refrigeration are due to the volatile basic compounds, such as ammonia, methylamine, dimethylamine and trimethylamine, and microbial catabolites, which result in meat spoilage [4,32,33]. The pH variations for meat samples according to the storage time are shown in Table 2.

Table 2. Changes in pH values of pork mince depending on treatment and storage time.

Days	Treatments						Treatments		Main Effects Time		*p* Values	
	NC	PC	T1	T2	T3	T4	NC	5.788 [a]	0	5.460 [c]	Treatments	<0.0001
0	5.52 [a]	5.40 [b]	5.43 [ab]	5.45 [ab]	5.47 [ab]	5.49 [ab]	PC	5.495 [d]	3	5.492 [c]	Time	<0.0001
3	5.59 [a]	5.43 [b]	5.45 [b]	5.47 [b]	5.49 [b]	5.52 [ab]	T1	5.628 [b]	6	5.625 [b]	Treatments × time	<0.0001
6	5.81 [a]	5.47 [c]	5.7 [b]	5.51 [c]	5.55 [c]	5.71 [b]	T2	5.533 [d]	9	5.867 [a]		
9	6.23 [a]	5.68 [d]	5.93 [b]	5.7 [d]	5.81 [c]	5.85 [bc]	T3	5.580 [c]				
							T4	5.643 [b]				

[a–d] Means in a row without a common letter significantly differ. NC—negative control; PC—positive control; T1 –treatment with PFJ; T2—treatment with PFJ + HE, 50 ppm GAE; T3—treatment with PFJ + HE, 25 ppm GAE; T4– treatment with PFJ +HE, 10 ppm GAE.

On day 0, a slight difference was found between the positive control pH values and others, due to the residual acidity of hawthorn phenolics and PFJ. After 3 days of refrigeration, the pH increased for all samples, but the highest increase was found in the NC. The increase in the pH during storage was lower in the first 3 days and higher in the last 6 days. On the 6th day, T2 and T3 pH values were similar to PC. After 9 days of storage, the pH value of T1 was significantly lower than the pH value found in NC ($p < 0.05$). T2 pH value was significantly lower than the pH values found in T3 and T4 ($p < 0.05$). HE addition in pork mince inhibited pH values increasing in a relation depending on HE levels; thus, after 9 days of refrigeration storage, the pH values decreased in order T4, T3, and T2. By comparison of pH values for samples treated with PFJ + HE, it can be seen that samples treated with 25 and 10 ppm GAE (T3 and T4) resulted in a greater increase in pH ($p < 0.05$); formulation with 50 ppm GAE (T2) resulted in an increase in pH values similar to PC. The slight increase in the pH in the meat samples treated with the PFJ and PFJ + HE treatments (T1, T2, T3 and T4) is attributed to the inhibitory effect of antimicrobial compounds found both in HE and PFJ on the growth and proliferation of spoilage microorganisms that metabolize basic nitrogen compounds, such as amino acids, L-carnitine, lecithin, and choline [30,31].

3.2. Chemical Analysis

pH Value

Chromatographic Profile of Fatty Acids

Lipid deterioration is the major cause of the loss of bioavailability and sensory quality of the meat and is due to fatty acids oxidation and, to a lesser extent of fatty acids catabolism by spoilage bacteria. Lipid oxidation in muscle systems is initiated at the membrane level in the intracellular phospholipid fractions, with high polyunsaturated fatty acids (PUFA) content [34]. In minced meat, PUFA oxidation is the result of the interaction with endogenous prooxidants, such as metalloproteins [35], and with exogenous prooxidants, such as iron from grinding machines and singlet oxygen. The effects of PFJ and PFJ + HE, in concentrations 50, 25, and 10 mg GAE/kg, on the fatty acids profile, after 9 days of refrigeration are shown in Table 3.

Table 3. Fatty acid profile of pork mince with different treatments after 9 days of refrigeration.

Fatty Acid [1] (g/100 g)	NC	PC	T1	T2	T3	T4	SEM	*p* Value
C8:0	0.18 ^{ab}	0.17 ^{abc}	0.19 ^{a}	0.16 ^{bc}	0.14 ^{c}	0.16 ^{abc}	0.006	0.0001
C10:0	0.18 ^{a}	0.17 ^{b}	0.18 ^{ab}	0.17 ^{b}	0.05 ^{c}	0.17 ^{b}	0.007	<0.0001
C12:0	0.10 ^{cd}	0.11 ^{d}	0.14 ^{a}	0.11 ^{d}	0.13 ^{bc}	0.14 ^{ab}	0.005	<0.0001
C14:0	1.72 ^{c}	0.75 ^{b}	1.68 ^{a}	1.66 ^{a}	1.68 ^{a}	1.67 ^{a}	0.014	<0.0001
C15:0	0.17 ^{a}	0.17 ^{a}	0.11 ^{b}	0.17 ^{a}	0.11 ^{b}	0.07 ^{c}	0.010	<0.0001
C15:1	0.07 ^{bc}	0.08 ^{ab}	0.07 ^{bc}	0.10 ^{a}	0.05 ^{c}	0.06 ^{bc}	0.008	0.0001
C16:0	23.94 ^{b}	23.35 ^{f}	24.35 ^{a}	23.44 ^{e}	23.66 ^{c}	23.58 ^{d}	0.021	<0.0001
C16:1	3.67 ^{a}	3.39 ^{c}	3.58 ^{b}	3.59 ^{b}	2.73 ^{e}	3.08 ^{d}	0.016	<0.0001
C17:0	0.18 ^{c}	0.19 ^{bc}	0.22 ^{b}	0.19 ^{bc}	0.26 ^{a}	0.20 ^{bc}	0.010	<0.0001
C17:1	0.20 ^{a}	0.19 ^{a}	0.19 ^{a}	0.18 ^{ab}	0.04 ^{c}	0.15 ^{b}	0.011	<0.0001
C18:0	10.61 ^{a}	10.13 ^{c}	10.40 ^{b}	10.20 ^{c}	10.42 ^{b}	10.32 ^{bc}	0.062	<0.0001
C18:1	44.67 ^{b}	44.6 ^{c}	44.79 ^{a}	44.75 ^{a}	44.58 ^{c}	44.45 ^{d}	0.021	<0.0001
C18:2n−6	12.21 ^{d}	13.80 ^{a}	12.24 ^{d}	12.99 ^{c}	13.52 ^{b}	13.52 ^{b}	0.044	<0.0001
C18:3n−6 (γ)	0.14 ^{b}	0.22 ^{a}	0.16 ^{b}	0.17 ^{b}	0.20 ^{a}	0.20 ^{a}	0.009	<0.0001
C18:3n−3(α)	0.54 ^{c}	0.71 ^{a}	0.55 ^{c}	0.55 ^{c}	0.65 ^{ab}	0.60 ^{bc}	0.021	<0.0001
C20:2n−6	0.64 ^{a}	0.56 ^{b}	0.43 ^{d}	0.49 ^{cd}	0.54 ^{bc}	0.46 ^{d}	0.082	<0.0001
C20:3n−6	0.45 ^{bc}	0.57 ^{a}	0.30 ^{d}	0.50 ^{b}	0.46 ^{bc}	0.43 ^{c}	0.017	<0.0001
C20:4n−6	0.43 ^{c}	0.72 ^{a}	0.39 ^{d}	0.52 ^{b}	0.7 ^{a}	0.69 ^{a}	0.014	<0.0001
Others fatty acids	0.08 ^{a}	0.07 ^{a}	0.03 ^{b}	0.06 ^{ab}	0.03 ^{b}	0.05 ^{ab}	0.008	0.001
∑FA	100	100	100	100	100	100	-	-
∑SFA	40.98 ^{d}	35.04 ^{e}	35.71 ^{a}	36.10 ^{c}	36.46 ^{b}	36.31 ^{bc}	0.037	<0.0001
∑MUFA	44.61 ^{a}	48.26 ^{b}	48.63 ^{a}	48.62 ^{a}	47.40 ^{d}	47.74 ^{c}	0.038	<0.0001
∑PUFA	14.41 ^{e}	16.67 ^{a}	14.30 ^{f}	15.22 ^{d}	16.12 ^{b}	15.90 ^{c}	0.060	<0.0001

[1] FAME—fatty acids methyl esters. FA—fatty acids; SFA—saturated fatty acids; MUFA—monounsaturated fatty acids; PUFA—polyunsaturated fatty acids. $^{a–f}$ Means in a row without a common superscript letter significantly differ ($p < 0.05$). NC—negative control; PC—positive control; T1—treatment with PFJ; T2—treatment with PFJ + HE, 50 ppm GAE; T3—treatment with PFJ + HE, 25 ppm GAE; T4—treatment with PFJ +HE, 10 ppm GAE.

In all samples, the monounsaturated fatty acids (MUFA) showed the highest degree (44.61–48.63%), with the oleic acid (C18:1) being the most abundant fatty acid (44.58–44.79%), whereas polyunsaturated fatty acids (PUFA) showed the lowest percent (14.30–16.67%), with linoleic acid (C18:2n−6) the most abundant PUFA (12.21–13.80%). Saturated fatty acids (SFA) were found in the intermediate degree (35.04–40.98%), with palmitic (C16:0) and stearic (C18:0) acids the most abundant (23.35–24.35% and 10.13–10.61%, respectively). The results are in agreement with the results obtained by [36] that found a similar fatty acid profile in pork meat. Results show differences in fatty acid profile for all treatments, although some of them were statistically significant. For PFJ + HE treatments, (T2, T3 and T4), the total PUFA degree was significatively higher than the degree found in NC ($p < 0.05$), on the last day of determination. The highest total PUFA degree was found in PC

samples, treated with sodium nitrite and sodium ascorbate (PC). The total PUFA degree in sample T3, treated with PFJ + HE, 25 ppm GAE, follows an increasing path as PC ($p < 0.05$). Except for the formulation with PFJ + HE 50 ppm GAE/kg (T2), the other treatments with PFJ + HE (T3 and T4), significantly ($p < 0.05$) increased the stability of unsaturated fatty acids to oxidation, compared with samples formulated with PFJ alone (T1). The lowest degree for total PUFA was found for T1. Comparing the PUFA results obtained for T2, T3, and T4, a prooxidant effect on minced pork lipids by HE added at 50 ppm GAE/kg can be suggested. According to the results from the present study, the concentrations of 25 and 10 mg GAE/kg, seem to protect the essential fatty acids, linoleic (C18:2n−6), linolenic (C18:3n−3), and arachidonic (C20:4n−6) acids against oxidative damage, comparatively with T1. Some authors have shown that colonial-type salami treated with rosemary extract alone or in association with celery powder had a higher PUFA content compared with control samples. [37]. Some authors used Mediterranean berries' ethanolic extracts as antioxidants in pork burgers subject to cooking and chilled storage and found that the protective effect of ethanolic extracts on PUFA decreased in the following order: *Rosa canina*, *Rubus ulmifolius*, *Arbusto unedo*, and *Crataegus monogyna*. The highest level of linoleic acid they found in patties treated with the ethanolic extract obtained from *Arbusto unedo* berries [38].

3.3. Thiobarbituric Acid Reactive Substances (TBARS) Value

Generally, lipid oxidation affects bioavailability and sensory quality in minced meat. A good indicator of lipid oxidation level is the TBARS value. It was suggested that the TBARS value at 0.5 mg/kg was a threshold value for rancidity perception by consumers [39]. The results regarding the changes in TBARS values for the six evaluated treatments are presented in Table 4.

Table 4. Changes in thiobarbituric acid reactive substances values of minced pork depending on treatment and storage time.

Days	Treatments						Treatments		Main Effects Time		*p* Values	
	NC	PC	T1	T2	T3	T4	NC	0.845 [a]	0	0.123 [b]	Treatments	<0.0001
0	0.121 [a]	0.122 [a]	0.121 [a]	0.130 [a]	0.122 [a]	0.121 [a]	PC	0.198 [e]	3	0.328 [a]	Time	<0.0001
3	0.650 [a]	0.17 [d]	0.45 [b]	0.27 [c]	0.16 [d]	0.27 [c]	T1	0.523 [b]	6	0.632 [d]	Treatments × time	<0.0001
6	1.340 [a]	0.22 [e]	0.87 [b]	0.65 [c]	0.27 [e]	0.44 [d]	T2	0.373 [c]	9	0.557 [c]		
9	1.27 [a]	0.28 [e]	0.65 [b]	0.44 [c]	0.35 [d]	0.35 [d]	T3	0.226 [e]				
							T4	0.295 [d]				

[a–e] Means in a row without a common letter significantly differ. NC—negative control; PC—positive control; T1 –treatment with PFJ; T2—treatment with PFJ + HE, 50 ppm GAE; T3—treatment with PFJ + HE, 25 ppm GAE; T4– treatment with PFJ +HE, 10 ppm GAE.

The analysis of variance for the TBARS data indicates that the TBARS values for pork mince were influenced by the refrigeration period ($p < 0.001$) and treatments. Overall, TBARS values increased during refrigeration storage, but the lowest TBARS values were found in PC pork mince ($p \leq 0.05$). The sample treated with PFJ alone (T1) had lower TBARS values than NC, but higher than PC ($p \leq 0.05$). The addition of HE in the minced pork affects the TBARS values in a concentration-dependent manner, after 3 days of refrigeration. The best antioxidant activity was found in the sample treated with PFJ + HE in a concentration equal to 25 ppm GAE (T3), followed by the samples treated with PFJ + 10 ppm GAE HE (T4), on the 6th day of the determination. On the last day of the investigation (9th day), the samples treated with PFJ + 50 ppm HE (T2) showed the highest TBARS values, comparatively with T3 and T4. The result obtained showed that the capacity of PFJ + HE to inhibit lipid oxidation in minced pork depends on the amount of HE used. HE in concentrations of 50 ppm GAE/kg meat has prooxidant activity, but HE in a concentration of 25 and 10 mg GAE/kg influenced the antioxidant activity as the association of sodium nitrite with sodium ascorbate (PC).

Another study showed the TBARS values after the preparation of beef patties using resveratrol as an antioxidant in concentrations as 110 µmol/kg meat and 550 µmol/kg meat, during 9 days of refrigeration. The highest TBARS values were found in samples treated with 550 µmol/kg meat, comparatively with TBARS values found in samples treated with 110 µmol/kg meat, but both were lower than those found in control samples [40]. It was reported different TBARS values for cooked pork patties, prepared with ethanolic berry extracts, and stored by refrigeration than those found in control samples (1.071 mg MDA/kg) [38]. So, higher TBARS values were reported for *Crataegus monogyna* (0.181 mg MDA/kg) and *Rosa canina* (0.143 mg MDA/kg) treatment extracts, but lower TBARS values for *Rubus ulmifolius* (0.082 mg MDA/kg) and *Arbusto unedo* (0.113 mg MDA/kg) treatment extracts.

3.4. Nitrosyl Hemochrome, Total Pigment Content, and the Heme Pigment Conversion Degree

Nitrosyl hemochrome (NO-heme), the pink pigment of cured meat, is formed mainly from NO(II)Mb resulting in the reaction of myoglobin (Mb) with NO generated from nitrite conversion in the presence of ascorbate [41]. Some studies suggested that the addition of ascorbate in the muscle reduces met(III)Mb to deoxy(II)Mb and the simultaneously generated NO from nitrite binds to deoxy(II)Mb or can reduce nitrosylate met(III)Mb under anaerobic conditions, resulting in the formation of NO(II)Mb [41].

The studies regarding the effect of pH on the stability of nitrosyl hemochromogen suggest that NO-Heme is extremely unstable at weakly acid pH, and this explains why pH affects the apparent colour of cured meat products with storage time [42]. Table 5 showed the results obtained for nitrosyl hemochrome (NO-Heme) values of minced pork formulated with parsnip fermented juice (PFJ) alone, parsnip fermented juice + hawthorn extract (PFJ + HE) compared to untreated minced pork (NC), and synthetic added compounds (PC).

Table 5. Changes in NO-heme value, total heme value, and heme pigment conversion degree of minced pork depending on treatment and storage time.

Treatments	NO-Heme (ppm Hematin Acid)	Total Heme (ppm Hematin Acid)	Heme Pigment Conversion Degree (%)
0 days			
NC	0.00 ^{c}	121.23	0.00 ^{c}
PC	26.23 ^{a}	120.99	21.68 ^{a}
T1	20.50 ^{b}	121.45	16.87 ^{b}
T2	26.20 ^{a}	123.09	21.30 ^{a}
T3	26.00 ^{a}	121.40	21.41 ^{a}
T4	25.89 ^{a}	120.97	21.42 ^{a}
3 days			
NC	0.00 ^{d}	120.46	0.00 ^{c}
PC	44.60 ^{a}	120.90	36.68 ^{a}
T1	26.70 ^{c}	123.77	21.76 ^{b}
T2	30.50 ^{b}	123.09	23.99 ^{b}
T3	30.70 ^{b}	121.41	24.47 ^{b}
T4	27.98 ^{bc}	121.56	23.02 ^{b}
6 days			
NC	0.00 ^{e}	119.00 ^{b}	0.00 ^{e}
PC	55.60 ^{a}	120.35 ^{ab}	46.11 ^{a}
T1	32.5 ^{d}	123.56 ^{a}	26.31 ^{d}
T2	45.81 ^{b}	122.67 ^{ab}	37.34 ^{b}
T3	43.21 ^{bc}	120.50 ^{ab}	34.70 ^{bc}
T4	40.76 ^{c}	121.67 ^{ab}	33.50 ^{c}

Table 5. *Cont.*

Treatments	NO-Heme (ppm Hematin Acid)	Total Heme (ppm Hematin Acid)	Heme Pigment Conversion Degree (%)
9 days			
NC	0.00 ^{d}	118.00	0.00 ^{d}
PC	60.50 ^{ab}	119.30	50.71 ^{ab}
T1	38.45 ^{c}	120.15	32.00 ^{c}
T2	63.50 ^{a}	120.75	52.58 ^{a}
T3	43.21 ^{ab}	119.50	50.79 ^{ab}
T4	40.76 ^{b}	119.59	47.99 ^{b}
Main effects Treatments			
NC	0.000 ^{e}	119.672 ^{c}	0.000 ^{e}
PC	46.733 ^{a}	120.438 ^{bc}	38.794 ^{a}
T1	29.540 ^{d}	121.482 ^{ab}	24.235 ^{d}
T2	41.503 ^{b}	122.400 ^{a}	33.803 ^{b}
T3	40.153 ^{b}	120.703 ^{abc}	32.843 ^{bc}
T4	38.008 ^{c}	120.950 ^{abc}	31.482 ^{c}
Time			
0	20.803 ^{d}	121.522 ^{a}	17.113 ^{d}
3	26.747 ^{c}	121.364 ^{a}	21.653 ^{c}
6	36.315 ^{b}	121.327 ^{a}	29.660 ^{b}
9	46.758 ^{a}	119.550 ^{b}	39.012 ^{a}
p value			
Diet	<0.0001	0.001	<0.0001
Time	<0.0001	0.0001	<0.0001
Diet × time	<0.0001	0.769	<0.0001

$^{a–e}$ Means in a row without a common letter significantly differ. NC—negative control; PC—positive control; T1–treatment with PFJ; T2—treatment with PFJ + HE, 50 ppm GAE; T3—treatment with PFJ + HE, 25 ppm GAE; T4–treatment with PFJ +HE, 10 ppm GAE

The highest value for NO-Heme during 9 days of refrigeration was found in PC. In the sample formulated only with PFJ (T1), NO-Heme content was significantly lower, compared with PC and samples formulated with PFJ + HE (T2, T3 and T4) ($p < 0.05$). The formulation of the minced meat with PFJ and HE increased NO-Heme content, in a relation dependent on the HE level in samples (Table 5).

After 9 days of refrigeration storage, in the samples treated with PFJ + HE, the content of NO-Heme increased in the order: T2, T3, and T4. Obtained results showed that HE accelerates the heme pigments to react with NO in a concentration-dependent manner. The nitrosylation reaction stimulated by HE and SA may be due to the lowering of the pH values on day 0 and of the reaction between phenolics and nitrite [43]. It was reported that a pH decreasing only 0.2 unit, doubles the rate of colour formation through nitrite-myoglobin reaction [44]. It was suggested that the final colour properties of nitrite also increase with decreasing pH and this constitutes the basis for product formulation with acidulants to accelerate curing [44,45].

Table 5 presents the results regarding total heme pigment values obtained for all treatments during 9 days of refrigeration. In all samples, a slight decrease in total heme pigment was found after 9 days of storage. Significant differences ($p < 0.05$) in total heme pigment were found in the treated minced pork after 9 days of refrigeration storage compare with previous determinations (0, 3 and 6 days).

Heme pigment conversion degrees for all minced pork treatments are presented in Table 5. After 9 days of refrigeration, the results showed that the minced pork with PFJ (T1) produced an increase in the heme pigment conversion % equal to 32.00%. When HE was

added to PFJ, even the lowest HE concentration, 10 ppm GAE (T4) produced an increase in heme pigment conversion degree (47.99%), compared to the formulated sample with PFJ alone. So, compared to PC, the samples formulated with PFJ + HE provided increases in the heme pigment conversion degree in T2 and T3 or slower in T4 (Table 5). Moreover, PFJ + HE and SN + SA have the same effect on the heme pigment conversion degree, meaning an increased percentage. Results found for pH values, NO—heme and heme pigment conversion degree show important aspects related to sample formulation. The results can be attributed to the reducing and acidic properties of SA or HE that favour met(III)Mb reduction, NO generation, and the reaction of heme pigments with NO also.

3.5. Residual Nitrite Level

Nitrite added in the meat reacts especially with heme proteins (myoglobin, haemoglobin), and less with nonheme proteins, lipids, and carbohydrates. Unreacted nitrite, named residual nitrite, can be depleted by the formation of nitrogen gasses [46]. Nitrite depletion in cured meat is influenced by pH, temperature, time, meat type, salt, and nitrite concentration. The residual nitrite level in meat treated with PFJ and PFJ + HE is shown in Table 6. For all samples, residual nitrite level decreased during refrigeration storage, but the most rapidly decreasing was found after 3 days of storage. Throughout the refrigeration, the lowest residual nitrite level was found in the negative control (NC), without adding any nitrite, and the highest level was found in pork mince treated with PFJ alone (T1). The addition of HE to the PFJ favours the decrease in the residual nitrite level. Therefore, the residual nitrite level in minced pork treated with PFJ + HE decreased with increasing levels of HE. In the pork mince treated with the PFJ + 50 ppm HE (T2), the residual nitrite level was significantly ($p < 0.05$) lower than those found in the positive control. Regarding the residual nitrite level in the minced pork treated with the PFJ + 50 ppm HE (T3), the rate of nitrite depletion with storage time was similar to the one found for the positive control. The residual nitrite depletion was a bit slower in the meat treated with PFJ + HE, at level 10 ppm GAE (T4), compared with the PC. The addition of HE showed a statistically significant effect in the nitrite depletion with storage time, and so, the decrease in the residual nitrite level ($p < 0.001$).

Table 6. Changes in residual nitrite level and volatile basic nitrogen of minced pork depending on treatment and storage time.

Treatments	Residual Nitrite Level (ppm)	Volatile Basic Nitrogen (VBN) mg%
0 days		
NC	0.00 ^{b}	12.89
PC	99.67 ^{a}	12.82
T1	99.33 ^{a}	12.89
T2	98.67 ^{a}	12.84
T3	100.00 ^{a}	12.81
T4	98.33 ^{a}	12.88
3 days		
NC	0.00 ^{e}	23.55 ^{a}
PC	20.30 ^{c}	16.84 ^{c}
T1	80.50 ^{a}	19.89 ^{b}
T2	13.50 ^{d}	17.87 ^{bc}
T3	25.60 ^{c}	18.02 ^{bc}
T4	33.50 ^{b}	20.10 ^{b}

Table 6. *Cont.*

Treatments	Residual Nitrite Level (ppm)	Volatile Basic Nitrogen (VBN) mg%
	6 days	
NC	0.00 ^{d}	30.45 ^{a}
PC	16.00 ^{c}	18.86 ^{c}
T1	75.00 ^{a}	23.78 ^{b}
T2	10.50 ^{c}	10.87 ^{bc}
T3	15.50 ^{c}	21.17 ^{bc}
T4	23.00 ^{b}	21.70 ^{bc}
	9 days	
NC	0.00 ^{d}	46.7 ^{a}
PC	10.00 ^{bc}	20.11 ^{d}
T1	69.00 ^{a}	33.70 ^{b}
T2	5.80 ^{c}	22.39 ^{cd}
T3	13.50 ^{b}	24.50 ^{c}
T4	14.00 ^{b}	31.80 ^{b}
	Main effects Treatment	
NC	0.000 ^{e}	28.398 ^{a}
PC	36.492 ^{c}	17.158 ^{d}
T1	80.958 ^{a}	22.565 ^{b}
T2	32.117 ^{d}	18.493 ^{c}
T3	38.650 ^{c}	19.125 ^{c}
T4	42.208 ^{b}	21.620 ^{b}
	Time	
0	82.667 ^{a}	12.855 ^{d}
3	28.900 ^{b}	19.378 ^{c}
6	23.333 ^{c}	22.805 ^{b}
9	18.717 ^{d}	29.867 ^{a}
	p value	
Diet	<0.0001	<0.0001
Time	<0.0001	<0.0001
Diet × time	<0.0001	<0.0001

$^{a–e}$ Means in a row without a common letter significantly differ. NC—negative control; PC—positive control; T1—treatment with PFJ; T2—treatment with PFJ + HE, 50 ppm GAE; T3—treatment with PFJ + HE, 25 ppm GAE; T4—treatment with PFJ +HE, 10 ppm GAE.

These results were in agreement with those found by other researchers [47,48], who reported continuous depletion of the residual nitrite level in cured meat during the storage time. A more pronounced decrease in residual nitrite level in minced pork formulated with SA and HE, compared to the sample formulated with PFJ alone may be the result of the pH decreasing and the interaction of nitrite with the SA and the HE. Some researchers reported a decrease in free nitrite in aqueous solutions of nitrite and sodium ascorbate [49]. Other results suggested that NO resulting in the reaction of free nitrite with SA is responsible for nitration reactions and residual nitrite depletion during meat curing [50]. A good correlation ($p < 0.001$) was found between pH value and residual nitrite depletion rate and between HE concentrations and residual nitrite depletion rate. Similar results were achieved in different research regarding cured meat products [51–54].

3.6. Volatile Basic Nitrogen (VBN) Value

Volatile basic nitrogen is a product of bacterial spoilage and endogenous enzyme action [55]. VBN is mainly composed of ammonia and primary, secondary, and tertiary

amines [56] resulting from amino acid degradation [57,58]. The changes in the VBN value of all samples with storage time are shown in Table 6.

The results show the progressively increasing VBN for all samples. The highest VBN values were found in NC and the lowest in the PC, throughout the refrigeration period. In pork mince formulated with PFJ alone, VBN values were lower than those found in NC but higher than those found in PC. Meat formulation with PFJ + HE decreased VBN values in total phenolics in HE concentration-dependent manner, the lowest values were found for PFJ + HE, in the concentration of 50 ppm GAE (T2) and the highest for PFJ +HE, 10 ppm GAE (T4). In the T2 samples, the geometry of the VBN values concentration dependence was similar to one obtained for PC, but the values were higher by an average of 11.14%. A previous study used fermented spinach for pork loin curing and reported similar results [15]. VBN values in samples treated with fermented spinach were higher than those found in the negative control and lower than those found in the positive control treated with synthetic nitrite.

3.7. Microbiological Analysis

The effect of PFJ and PFJ + HE on total viable count (TVC) is shown in Table 7. The initial number of bacteria in samples was between 2.60 log CFU/g and 2.77 log CFU/g which indicated the good quality of pork used in this study. For all samples, TVC increased with storage time and the value of NC and T1 increased faster than PC, T2, T3, and T4. From day 0 to day 3, there were strong significant differences between NC and T1 ($p < 0.05$). After 6 days of storage, significant ($p < 0.05$) differences between NC and T1, which indicated that PFJ inhibited the growth of TVC alone, were found. After 6 days of storage significant differences ($p < 0.05$) between T1 and samples T2 were found and these results indicated that HE added in PJF increased the antimicrobial activity of PFJ. A significant correlation was established between PC and samples T2, T3, and T4 ($p < 0.001$).

Table 7. Changes in total viable count of minced pork depending on treatment and storage time.

Days	Treatments						Treatments		Main Effects Time		*p* Values	
	NC	PC	T1	T2	T3	T4	NC	7.325 [a]	0	2.687 [d]	Treatments	<0.0001
0	2.77	2.67	2.67	2.75	2.6	2.66	PC	3.863 [d]	3	4.143 [c]	Time	<0.0001
3	5.2 [a]	2.87 [c]	4.66 [a]	3.34 [bc]	4.22 [ab]	4.57 [a]	T1	5.945 [b]	6	5.890 [b]	Treatments × time	<0.0001
6	8.22 [a]	4.11 [e]	6.89 [b]	4.56 [de]	5.55 [cd]	6.01 [bc]	T2	4.358 [d]	9	8.400 [a]		
9	13.11 [a]	5.8 [d]	9.56 [b]	6.78 [cd]	7.2 [c]	7.95 [c]	T3	4.893 [c]				
							T4	5.298 [c]				

[a–e] Means in a row without a common letter significantly differ. NC—negative control; PC—positive control; T1—treatment with PFJ; T2—treatment with PFJ + HE, 50 ppm GAE; T3—treatment with PFJ + HE, 25 ppm GAE; T4—treatment with PFJ +HE, 10 ppm GAE.

After 9 days of storage in all samples, TVC was higher than 5.69 logs CFU/g and so, not acceptable for safe consumption, but TVC values were lower for T1, T2, T3, and T4 than NC. Similar results were reported by the literature, showing that fermented spinach extract, celery juice powder, and cherry juice powder can inhibit the growth of microorganisms in different meat models [15,59]. Other researchers tested the antimicrobial activity of the ethanolic extracts obtained from rosemary and cloves and found important antimicrobial activity for extracts used alone or in combination [31].

3.8. Correlations between Analysed Parameters

The Pearson correlation coefficient (r) is the simplest way to measure a linear correlation. It takes values between −1 and 1 and indicates the relationship between two variables in strength and direction. When one variable changes, the other variable also changes. In this study, we analysed the effects of various concentrations of HE in combination with a constant PFJ concentration on the pH, fatty acids profile, TBARS value, NO-heme, total heme pigment, heme pigment conversion degree, residual nitrite, VBN, and TVC. pH value is a very important parameter for pork mince and was influenced by VBN value (r = 0.2488,

$p < 0.0001$) and TVC, in a positive and linear manner. NO-heme is a parameter related to meat colour stability. According to the results, a weak positive relationship was found between NO-heme and TBARS (r = 0.9349, $p < 0.0001$), but negative correlations were found between TBARS value and MUFA (r = −0.7275, $p < 0.0001$), and between TBARS value and PUFA (r = −0.8643, $p < 0.0001$). Colour stability is influenced by TBARS and could be related to fatty acids profile. A negative correlation was found between NO-heme and residual nitrite (r = −0.7786, $p < 0.0001$). The pH values determined in the present study established positive correlations with heme conversion degree, (r = 0.5848, $p < 0.0001$), and NO-heme (r = 0.5787, $p < 0.0001$) (data in Table S1).

4. Conclusions

The use of PFJ in combination with HE exerted an inhibitory effect on the growth of spoilage bacteria that metabolize nitrogen compounds and significantly increased the stability of unsaturated fatty acids to oxidation in minced pork. The addition of HE in the minced pork decreased the TBARS values compared to T1. At any time during the experiment, NO-heme increased in a concentration-dependent manner with HE. So, HE may influence the NO-heme concentration. The concentration of HE of 25 ppm together with PFJ was the most effective to delay the FA oxidation in pork mince, whereas the higher concentration of HE (50 ppm GAE) had decreased the total PUFA and increased TBARS, suggesting a prooxidant effect on the minced pork lipids. Colour and lipids stability may depend on the HE and PFJ combination. However, T1, T2, T3, and T4 showed lower TVC values than NC. Between them, T2 was the most effective against microbial growth. In conclusion, these combinations of natural sources of nitrites and antioxidants could be considered promising meat alternatives to synthetic nitrites and antioxidants.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture13020432/s1, Table S1: Pearson's correlation coefficients of analysed parameters.

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