



Effect of Pink Rock Rose Extract with or Without Ascorbic Acid and Sodium Ascorbate for the Preservation of Ready-to-Eat Frankfurter Type Sausages

Ayça Gedikoğlu^{1,a,*}, Hale İnci Öztürk^{1,b}, Sencer Buzrul^{1,c}, Münevver Sökmen^{2,d}, Ezgi Aytac^{3,e}

¹Department of Food Engineering, Konya Food and Agriculture University, Konya, Türkiye

²Department of Bioengineering, Faculty, Konya Food and Agriculture University, Konya, Türkiye

³Department of Plant Production and Technologies, Konya Food and Agriculture University, Konya, Türkiye

*Corresponding author

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ABSTRACT

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This study aimed to determine the effect of pink rock rose extract (PRR) with or without ascorbic acid and sodium ascorbate for improving the shelf life of sausages. Analyzed parameters were DPPH radical scavenging capacity of PRR extract; total aerobic count, thiobarbituric acid reactive substance, heme iron, pH, water activity, proximate composition, and color values of MAP packaged sausages for 12 weeks at 4 °C. Treatments: (1) Control (0.02% ascorbic acid and 0.05% sodium ascorbate – AA-SA), (2) electrostatic spray application of PRR extract (2%) – ES-PRR, (3) 0.02% AA and, 0.05% PRR extract, (4) 0.05% SA and 0.02% PRR, (5) 0.07% PRR extract. PRR extract had the half maximal inhibitory concentration (IC₅₀) value of 13.04 ± 0.133 µg/mL. Sausages formulated with 0.07% PRR had the lowest microbial growth rate, followed by AA-PRR formulation. The AA-PRR treatment had the lowest TBARS values for most of the storage. This study reveals that PRR extract can be added as a natural antioxidant in sausages, and it could be used as a replacement or for the reduction of ascorbic acid and sodium ascorbate in sausage formulations.

^a ayca.gedikoglu@gidatarim.edu.tr <https://orcid.org/0000-0002-5105-141X>

^c sencer.buzrul@gidatarim.edu.tr <https://orcid.org/0000-0003-2272-3827>

^e ezgi.aytac@gidatarim.edu.tr <https://orcid.org/0000-0001-6447-5273>

^b inci.ozturk@gidatarim.edu.tr

^d munevver.sokmen@gidatarim.edu.tr

<https://orcid.org/0000-0001-8334-0403>

<https://orcid.org/0000-0002-7671-4160>



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Introduction

Meat is one of the nutritious foods to consume due to presence of essential amino acids, vitamins, and minerals as well as presence of mono and poly unsaturated fats (Pereira and Vicente, 2013). In addition to these important macro and micro nutrients, meat particularly beef have been studied for its bioactive components such as, coenzyme Q₁₀, carnosine, anserine, creatine, and taurine. It has been reported that these compounds can help with improvement in cardiac function, scavenging radical oxidative species, improving physical capacity and muscle strength, and therapeutic agent in chronic inflammatory disorders, respectively (Ribas-Agusti, et al., 2019). Despite of these health benefits, adverse effects of meat consumption especially processed meat in human health have been reported. These are cardio vascular disease, type 2 diabetes and colorectal cancer (Richi et al., 2015). To

diminish the possible health risks associated with the consumption of processed meat products, healthier alternatives have been developed to replace saturated fats (Nieto et al., 2017), increase fiber content (Gedikoğlu and Clarke, 2019; Madane et al., 2020), replace synthetic nitrite with plant extracts (Kim et al., 2017), reduce sodium content (Cheng et al., 2013), use essential oils and/or plant extracts as antimicrobial agents (Dos Santos et al., 2021; Gedikoglu, 2022), and use antioxidants obtained from natural sources in product formulations (Tamkute et al., 2021; Wang et al., 2019).

Antioxidants in particular gained major interest in recent years for their involvement in protecting cells against damaging effect of free radicals either by breaking down and removing free radicals via enzymatic actions or by interrupting free radical chain reactions through non-

enzymatic activities (Nimse and Pal, 2015). Ascorbic acid, vitamin E, and phenolic and flavonoid compounds found in fruits, vegetables, and plants are some of the non-enzymatic antioxidants (Nimse and Pal, 2015). Non-enzymatic antioxidants can work as chain breakers during lipid peroxidation reactions or by donating electrons to lipid radicals to terminate lipid peroxidation reactions (Felix et al., 2020). This is especially important for the meat industry since meat products are prone to oxidation due to presence of unsaturated fats. As a result of lipid oxidation, meat products can have deterioration in final quality such as color discoloration, development of rancid flavor, change in texture, loss of nutritive value, and decrease in shelf life (Akcan et al., 2017; Madane et al., 2020). With the continuous efforts, different plant extracts have been investigated for their antioxidant potential. Alirezalu et al. (2017) used green tea extract, stinging nettle extract, and/or olive leaves extract to improve the quality attributes of frankfurter type sausage. Furthermore, Lee et al. (2021) tested 49 different natural antioxidant extracts for the preservation of emulsion sausage and found successful results.

Through our investigations, we found that *Cistus creticus* also known as pink rock rose (PRR) can be one of these plants, owing to its rich phenolic and flavonoid content (Lukas et al., 2021; Papaefthimiou et al., 2014) and high antioxidant capacity (Lahcen et al., 2020; Matlok et al., 2020). Even though cistus has been used for pharmacological purposes for ages, it has not been investigated fully for its use as a natural antioxidant food additive (Nicoletti et al., 2015). In the food industry, synthetic antioxidants such as butylated hydroxytoluene, and natural antioxidants such as ascorbic acid and/or tocopherol are commonly used in the sausage formulations. It has been established that synthetic antioxidants are very effective at low concentrations. However, they can have a negative effect on health. For this reason, in recent years, considerable attention is given to natural antioxidants (Wongnen et al., 2022). It is important to determine the effectiveness of plant extracts in comparison to these commonly used additives in product formulations. Natural antioxidants should be as effective at same concentrations as these additives. Only few studies investigated the antioxidant potential of different plant extracts against these antioxidant additives (Haak et al., 2009; Hwang et al., 2017; Lorenzo et al., 2013). Furthermore, antioxidant additives are mostly added into the formulation of the products; however, these ingredients can be applied in dipping, spraying or in an edible coating as well. Recently, electrostatic spraying is gaining major interest in the food industry due to ease of application, providing uniform coverage, and producing less waste. There are only few studies available that used electrostatic spray technology for antimicrobial (Stella et al., 2017) or antioxidant (Nam et al., 2011) application.

The aim of study was to determine the antioxidant potential of pink rock rose (PRR) extract in comparison to ascorbic acid and sodium ascorbate in ready to eat frankfurter type sausages. Also, for the first time, the effect of electrostatic spray application of an antioxidant, PRR extract, on the sausage surface was tested.

Materials and Methods

Extraction Process

Cistus creticus L. plant materials were obtained from Mediterranean region of Türkiye. Plants were stored at 25°C in a dark room with open shelves providing even air flow. Plants were dried until reached to constant weight. All plants consisted of wood/stalks, bark, and leaves were milled using a mechanical mill to a particle size of 3-5 mm. A 100 g of ground PRR was placed in a bottle and 1 L of methanol was added and the bottle was shaken for 24 h at 400 rpm (Toros, Türkiye). After that plant material was filtered using a Whatman® no. 1 filter paper and a vacuum pump (Rocker 400, Taiwan). The PRR extract was concentrated at 50°C by rotary evaporation. Later, the extract was frozen at – 80°C and freeze dried.

Antioxidant Assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging capacity was measured according to Cuendet et al. (1997). In this spectrophotometric method, after obtaining absorbance values, the inhibition (%) of the DPPH radical was calculated based on this formula:

$$I(\%) = \left[\frac{(A_{\text{blank}} - A_{\text{sample}})}{A_{\text{blank}}} \right] \times 100 \quad (1)$$

Then, the extract concentrations were plotted against the inhibitions (%). Using the equation obtained from the plot, the extract concentration providing 50% inhibition (IC₅₀) was determined. Tests were conducted in triplicate, and ascorbic acid was used as a positive control.

Sausage Production

Sausage treatments were produced in three replications. Approximately 10 kg batches were prepared for each treatment. Formulation of the control sausage treatment was; 50.85% beef trim, 22.5% ice, 15% fat emulsion, 6.45% functional mix, 2.5% liquid smoke, 1.1% nitrite salt, 1.05% spice mix, 0.3% mix of sodium lactate and sodium diacetate, 0.15% powder smoke, 0.04% natural colorant, 0.05% sodium ascorbate, and 0.02% ascorbic acid. The other treatments were prepared replacing either ascorbic acid and/or sodium ascorbate with the PRR extract, keeping the rest of the formulation intact. There were five treatment groups, including a (1) control group with 0.02% ascorbic acid and 0.05% sodium ascorbate as antioxidants (AA-SA); (2) 2% PRR extract electrostatically applied to the control sausage surfaces (ES-PRR), (3) 0.02% ascorbic acid and 0.05% PRR extract (AA-PRR), (4) 0.05% sodium ascorbate and %0.02 PRR extract (SA-PRR); and (5) 0.07% PRR extract (PRR). Figure 1. displays the flow diagram of sausage production. For the ES-PRR treatment, the PRR extract (2%) dissolved in water (w/v), then, it was placed into the Nalgene bottle with liquid hose. The extract was applied to sausage surface using electrostatic spraying system SC-EB (GA, USA) with the MaxCharge™ nozzle, including 30-micron flow disk. Sausages were allowed to dry for 10 min, while the operation room temperature was less than 15°C. Figure 2. shows the 2% PRR extract electrostatic spray application. Later, all the treatments were modified atmosphere packaged using a gas mixture of 20-25% CO₂ and 75-80% N₂, and labeled for the treatment and the storage.

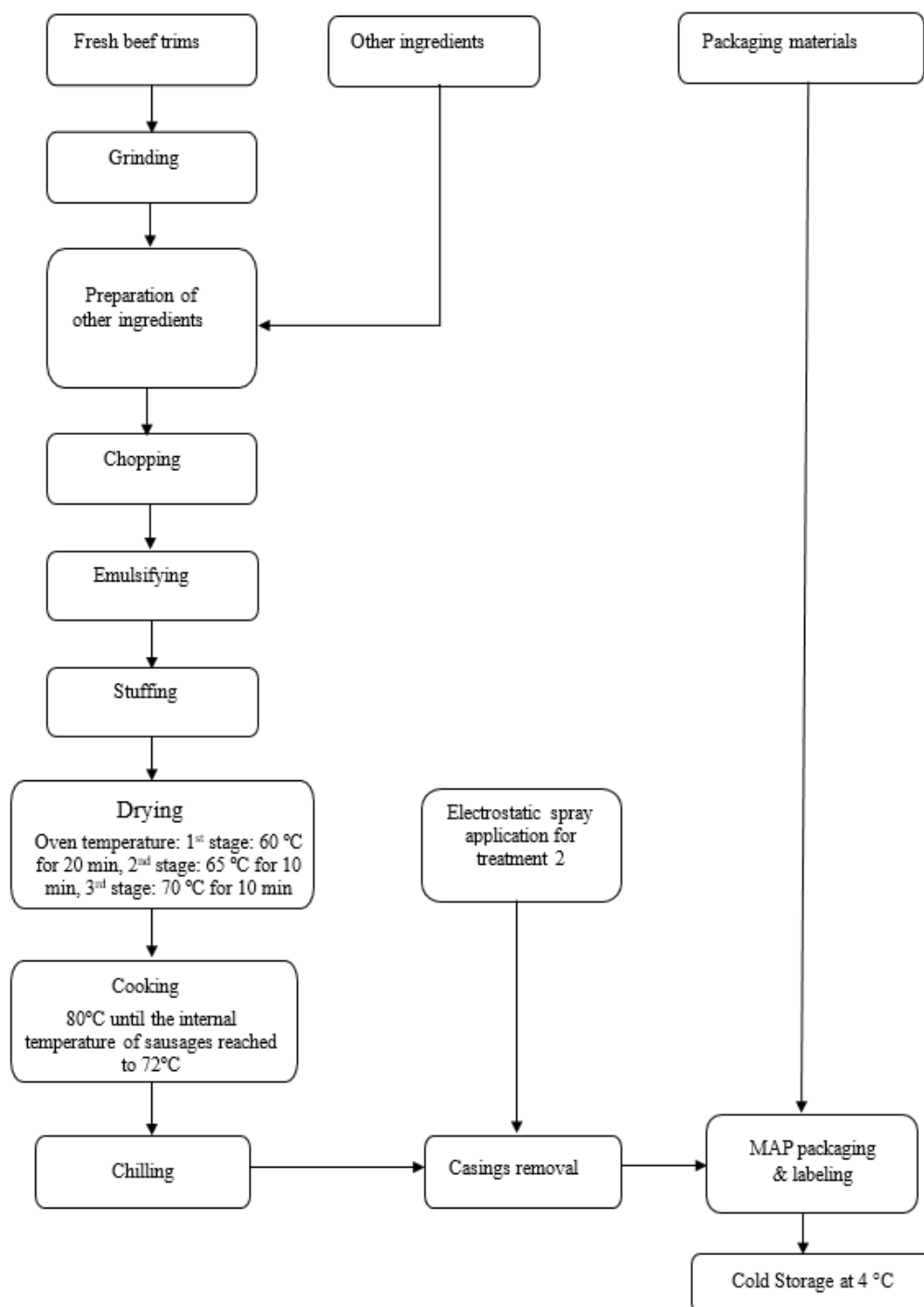


Figure 1. Sausage production flow chart.



Figure 2. Electrostatic spray application of pink rock rose extract to sausages.

The total mesophilic counts were completed at weeks 0, 2, 4, 6, and 8, and the rest of the analysis were carried out at weeks, 2, 4, 6, 8, 10 and 12. All the analysis were carried out in three independent replications.

Thiobarbituric Acid Reactive Substances (TBARS) Assay

The method of Jridi et al. (2018) was used to determine the lipid oxidation in sausage samples. First, the sample (10g) was mixed with 20 mL of 10% trichloroacetic acid, centrifuged, and filtered. An aliquot of filtrate was incubated with the same amount of 20 mM thiobarbituric acid at 97°C in a water bath for 30 min. Using an ice bath, samples were cooled to room temperature, then the absorbance values were obtained at 532 nm. The TBARS values were expressed as mg malonaldehyde/kg of sample.

Heme Iron Content

Heme iron content was determined by mixing sausage samples with acidified acetone (90% acetone, 8% distilled water and 2% HCl) and incubating in the dark at room temperature for 1 h. Later, the absorbance values were obtained at 640 nm. Using the following formula,

$$\text{Heme iron} = A_{640} \times 680 \times 0.0882 \quad (2)$$

Heme iron content was calculated and expressed as $\mu\text{g/g}$ of meat (Clark et al., 1997).

Water Activity, pH and Proximate Composition

The water activity of grounded sausage samples was determined as described in our previous study (Gedikoglu, 2022) using a AQUALAB 4TE water activity meter. For the determination of pH values, the slurry of sausage samples (1:9, w/v) were prepared by homogenizing sausage samples in distilled water, then the pH of samples was measured using a calibrated pH meter. Near-infrared spectrometer was used to determine the protein, collagen, moisture, fat, salt, and ash content of the sausage treatments (Gedikoğlu, 2022).

Hunter Color Measurements

CIE color values [Lightness (L^*), redness (a^*), and yellowness (b^*)] were determined using a CR-410 colorimeter (Minolta Chroma Meter, Osaka, Japan) with a D65 illuminant and 10° observer. The instrument was calibrated using white and black standard plates. Measurements were taken in triplicate within each replication.

Microbiological Analysis

The total aerobic mesophilic bacteria count (TAMB) of treatments was determined by the pour plate method at weeks 0, 2, 4, 6, and 8. After incubation of plates at 37°C for 48 h, TAMB was expressed as logarithms of colony forming units per gram of meat (\log_{10} CFU/g) (Gedikoglu, 2022).

Statistical Analysis

Three independent experimental replications were conducted. A two-way analysis of variance (two-way ANOVA) in Stata IC 14 (Stata Corp., College Station, Texas, USA) was used to analyze the data for the sausage

treatments. The significant differences between the treatments and storage periods were determined by Tukey multiple comparison test ($P \leq 0.05$).

Results and Discussion

Antioxidant Activity

Results of the DPPH radical scavenging activity showed that pink rock rose methanolic extract had a high antioxidant capacity, and IC_{50} value of the extract was $13.04 \pm 0.133 \mu\text{g/mL}$. In addition, pure compound such as ascorbic acid had an IC_{50} value of $5.59 \pm 0.01 \mu\text{g/mL}$. In comparison to our results, Kilic et al. (2019) reported lower DPPH radical scavenging activity for *C. creticus* L. ethanol, dichloromethane and n-hexane extracts with 165.10, 189.71, and 397.29 $\mu\text{g/mL}$, respectively. While, Nicoletti et al. (2015) reported much higher radical scavenging activity for *Cistus monspeliensis* methanol extract with 3 $\mu\text{g/mL}$. Similarly, Sayah, Marmouzi, Mrabti, Cherrah, and Faouzi (2017) reported high radical scavenging activity for *Cistus salviifolius* (3.28 $\mu\text{g/mL}$) and *Cistus monspeliensis* (3.30 $\mu\text{g/mL}$). It has been shown that several factors affect the antioxidant capacity of a plant extract including location, climatic conditions, soil, time of cultivation, extraction methodology, and extracted parts (Papaefthimiou et al., 2014). Furthermore, the composition and the concentration of phenolic and flavonoid compounds can cause difference in the antioxidant capacity of pink rock rose extracts (Lukas et al., 2021; Matlok et al., 2020; Sayah et al., 2017). In this study, methanol extract of pink rock rose had high antioxidant capacity due to its high phenolic or flavonoid content.

Total Aerobic Mesophilic Bacteria Count

The changes in the total aerobic mesophilic bacteria count during storage are displayed in Figure 3. According to ANOVA results both the treatment and the storage had a significant ($P \leq 0.05$) effect on the TAMB count. There was no significant difference ($P > 0.05$) between the treatments during week 0 and week 2. Starting from week 4, PRR treatment had a significantly ($P \leq 0.05$) lower TAMB than the control treatment (AA-SA). Also, the PRR treatment had the lowest microbial growth throughout the storage period. At the final week of storage, the PRR treatment had 1.6 \log_{10} CFU/g less TAMB than that of AA-SA treatment. This indicates that PRR extract showed antimicrobial property against TAMB. In our preliminary study (data not shown), we observed that PRR extract was rich with quercetin and gallic acid. Since, antimicrobial and antioxidant activities of these phenolic compounds have been reported in different studies (Fernandes and Salgado, 2016; Franco et al., 2018), antimicrobial properties of PRR extract could be due to presence of these phenolic compounds.

Changes in Physicochemical Characteristics

Lipid oxidation of sausage samples were determined according to TBARS values. The results of the TBARS analysis are illustrated in Figure 4. According to ANOVA results both the treatment and the storage had a significant effect ($P \leq 0.05$) on the TBARS values of sausages. For all the treatments, the final or 12th week of storage, had a significantly higher TBARS values than rest of the weeks.

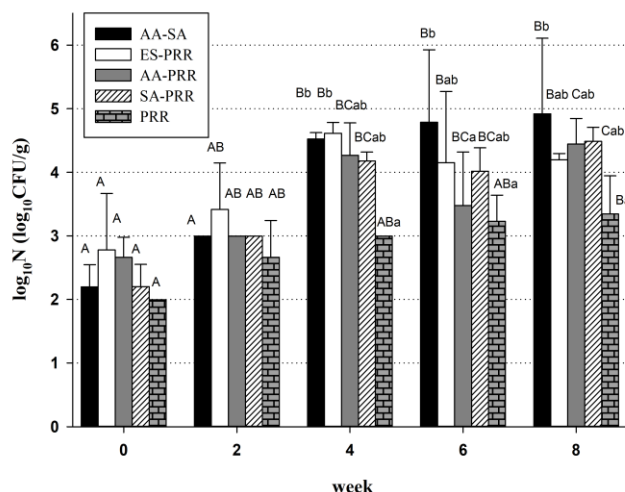


Figure 3. Total aerobic mesophilic bacteria count of sausages at 4 °C for different storage times.

AA-SA: control cocktail sausages formulated with 0.02% ascorbic acid and 0.05% sodium ascorbate, ES-PRR: control cocktail sausages electrostatically sprayed with 2% pink rock rose extract, AA-PRR: cocktail sausages formulated with 0.02% ascorbic acid and 0.05% pink rock rose extract, SA-PRR: cocktail sausages formulated with 0.05% sodium ascorbate and 0.02% pink rock rose extract, PRR: cocktail sausages formulated with 0.07% pink rock rose extract. Error bars represent standard deviation of three replicates. a, b: indicate significant difference between treatments within the same week by the Tukey test ($P \leq 0.05$). A, B, C: indicate significant difference within the treatment at different weeks by the Tukey test ($P \leq 0.05$).

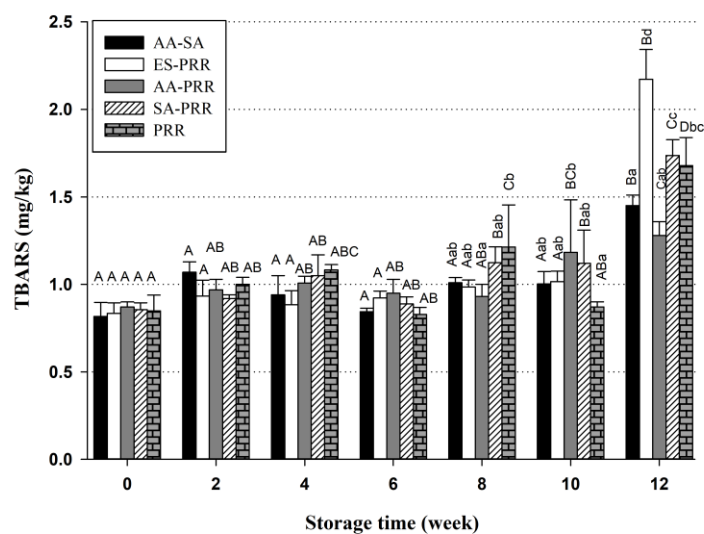


Figure 4. Change in lipid oxidation (TBARS) values of sausages at 4 °C for different storage times.

It has been reported that TBARS value higher than 1.2 mg MDA/kg has been considered as rancid for sausage products (Ham et al., 2016). At the final week of storage, all the treatments passed the threshold of lipid oxidation. In addition, until the week 8 there was no significant difference ($P > 0.05$) among the treatments. At weeks 8 and 10, only statistically significant difference ($P \leq 0.05$) was observed between the treatment AA-PRR and PRR. The last week of storage, AA-PRR treatment had significantly lower ($P \leq 0.05$) TBARS values in comparison to all the treatments. In addition, ES-PRR treatment had the highest TBARS value at week 12, and it had significantly higher ($P \leq 0.05$) TBARS value than other treatments. While the electrostatic spraying provided even coverage and less use

of material, application of the PRR extract to the surface of the sausage had no effect on delaying the lipid oxidation. This was most probably due to limited interaction between the antioxidant and lipids on the product surface. Contrary to our findings, Nam et al. (2011) reported that electrostatic spraying of sesamol as an antioxidant to the surface of ground beef was very effective to control rancidity. Furthermore, AA-SA treatment, which is generally used in the regular sausage formulation, was statistically same ($P > 0.05$) with the other treatments during shelf life, which revealed that PRR extract might be used in sausage formulations either by itself or in combination with ascorbic acid or sodium ascorbate as an antioxidant to control lipid oxidation.

Heme iron is one of the contributors of lipid oxidation in meat products due to the oxidizing potential of iron. When iron is released from porphyrin ring, ferrous iron induces oxidation of lipids and formation of secondary oxidation products such as malondialdehyde. It has been also reported that processing techniques such as frying, grilling or cooking, and storage conditions could affect the release of iron from porphyrin ring and lead to heme iron associated oxidation (Macho-Gonzalez et al., 2020). Results of the heme iron changes of sausage treatments during 12 weeks of cold storage are shown in Figure 5. Treatment had no significant effect ($P>0.05$) on the heme iron values, on the other hand, heme iron values were significantly ($P\leq 0.05$) affected by the storage. There was also treatment \times storage interaction effect. The heme iron content of the ES-PRR, the SA-PRR and the PRR treatments wasn't significantly ($P>0.05$) affected by the storage period. On the contrary, the AA-SA and the AA-PRR treatments heme iron content was influenced by the storage ($P\leq 0.05$). The heme iron content decreased until the 8th week, which is expected due to oxidation, however, the major increase in heme iron content was not expected.

Particularly, heme iron content of AA-SA treatment increased by the 8th week and the heme iron value of AA-SA treatment was significantly ($P\leq 0.05$) higher than AA-PRR, SA-PRR and PRR treatments. This increase could be also attributed to experimental error since the uncertainty (standard deviation) was high (Figure 5). In addition, the heme iron content was differed significantly ($P\leq 0.05$) between the treatments at week 8, 10, and 12. The decrease in heme iron content was also reported in other studies. Wang et al. (2018) noted that the heme iron content in rabbit meat decreased during storage.

The changes in the pH, water activity (a_w) and color characteristics of sausages during shelf life are all displayed in Table 1. The results indicated that both the treatment and the storage had no significant effect ($P>0.05$) on the pH values. This shows that addition of PRR extract did not cause any negative changes in pH of sausage samples. On the contrary, a_w of sausage treatments was significantly ($P\leq 0.05$) affected by both the treatment and storage. Although, this difference was statistically significant ($P\leq 0.05$), a_w values of treatments were between 0.960 and 0.989, and could be considered normal for this product.

Table 1. Physicochemical properties of frankfurter type sausages

Attribute	Treatment	Storage Time (Weeks)							SEM
		0	2	4	6	8	10	12	
pH	AA-SA	6.04	6.05	6.04	6.03	6.01	6.02	6.05	0.031
	ES-PRR	6.03	6.08	6.05	6.06	5.98	5.95	5.79	
	AA-PRR	6.03	6.11	6.11	6.13	6.10	5.79	6.11	
	SA-PRR	6.03	6.10	6.04	6.09	6.04	6.01	5.89	
	PRR	5.97	6.05	6.03	6.08	6.12	6.10	6.04	
	SEM	0.027							
a_w	AA-SA	0.977B	0.972AB	0.963ABab	0.964AB	0.972ABab	0.960Aa	0.970AB	0.0016
	ES-PRR	0.971	0.971	0.979cd	0.974	0.975ab	0.968ab	0.968	
	AA-PRR	0.971A	0.969A	0.989Bd	0.973A	0.980Ab	0.976Abc	0.971A	
	SA-PRR	0.977B	0.962A	0.959Aa	0.970AB	0.967ABa	0.970ABabc	0.969AB	
	PRR	0.974AB	0.961A	0.973ABbc	0.971AB	0.968Aab	0.982Bc	0.967A	
	SEM	0.002							
L^*	AA-SA	51.74	51.58	51.78	52.41	58.21	51.52	52.27	0.34
	ES-PRR	52.07	52.25	51.45	52.34	57.76	52.21	53.35	
	AA-PRR	51.05	51.67	50.83	51.75	57.22	50.85	52.87	
	SA-PRR	51.66	51.91	51.39	52.41	57.62	53.56	54.05	
	PRR	52.76	52.41	51.37	53.37	59.59	52.82	53.62	
	Sample Mean	51.86AB	51.96AB	51.37A	52.45AB	58.08C	52.19AB	53.24B	
a^*	SEM	0.55							
	AA-SA	26.43Ab	29.65Bc	27.69ABb	27.63ABb	26.05Ab	25.53Ac	25.57Ad	0.47
	ES-PRR	26.07ABb	29.08Cbc	27.86BCb	27.85BCb	26.21ABb	24.96Abc	24.39Acd	
	AA-PRR	22.58Aba	25.87Ca	24.96BCa	24.93BCa	24.15BCab	22.43ABa	20.72Aa	
	SA-PRR	24.74ABb	27.16Cab	25.84BCab	25.59ABa	23.95ABa	23.09Aab	23.21Abc	
	PRR	22.33ABa	25.41Ca	24.37ABa	24.99BCa	22.08ABa	21.52Aa	21.32Aab	
SEM	0.38								
b^*	AA-SA	9.15	10.43	9.69	10.19	9.95	8.94	8.51	0.133
	ES-PRR	9.42	10.48	9.81	10.26	10.07	9.34	9.16	
	AA-PRR	9.77	10.11	9.63	10.26	9.63	9.19	9.02	
	SA-PRR	9.21	10.09	9.57	9.94	9.60	9.02	9.15	
	PRR	9.51	10.52	9.68	10.12	9.53	8.52	8.94	
	Sample Mean	9.41B	10.32D	9.68B	10.16CD	9.76BC	9.01A	8.96A	
SEM	0.096								

a, b, c, d: Different letters in the same column are significantly different by the Tukey test ($P\leq 0.05$). A, B, C, D: Different letters in the same row are significantly different by the Tukey test ($P\leq 0.05$). Treatment and week with no letters indicate no significant different by the Tukey test ($P>0.05$). SEM: Standard error of mean. AA-SA: Control sausages formulated with 0.02% ascorbic acid and 0.05% sodium ascorbate, ES-PRR: Control sausages electrostatically sprayed with 2% pink rock rose extract, AA-PRR: Sausages formulated with 0.02% ascorbic acid and 0.05% pink rock rose extract, SA-PRR: Sausages formulated with 0.05% sodium ascorbate and 0.02% pink rock rose extract, PRR: Sausages formulated with 0.07% pink rock rose extract.

Table 2. Proximate composition of frankfurter type sausages

Treatments	Parameters					
	Protein (%)	Collagen (%)	Moisture (%)	Fat (%)	Salt (%)	Ash (%)
AA-SA	9.49 ^a	2.05	61.97	17.11	2.42	2.69
ES-PRR	9.70 ^{ab}	1.98	63.14	15.88	2.49	3.43
AA-PRR	10.43 ^{ab}	2.29	64.41	13.96	2.51	3.34
SA-PRR	10.69 ^b	2.14	63.22	14.76	2.61	3.52
PRR	10.25 ^{ab}	2.18	62.90	14.80	2.59	3.80
SEM	0.25	0.11	0.79	0.82	0.11	0.39

a, b: Different letters in the same column are significantly different by the Tukey test ($P \leq 0.05$). Treatment and week with no letters indicate no significant difference by the Tukey test ($P > 0.05$). SEM: Standard error of mean. AA-SA: Control sausages formulated with 0.02% ascorbic acid and 0.05% sodium ascorbate, ES-PRR: Control sausages electrostatically sprayed with 2% pink rock rose extract, AA-PRR: Sausages formulated with 0.02% ascorbic acid and 0.05% pink rock rose extract, SA-PRR: Sausages formulated with 0.05% sodium ascorbate and 0.02% pink rock rose extract, PRR: Sausages formulated with 0.07% pink rock rose extract.

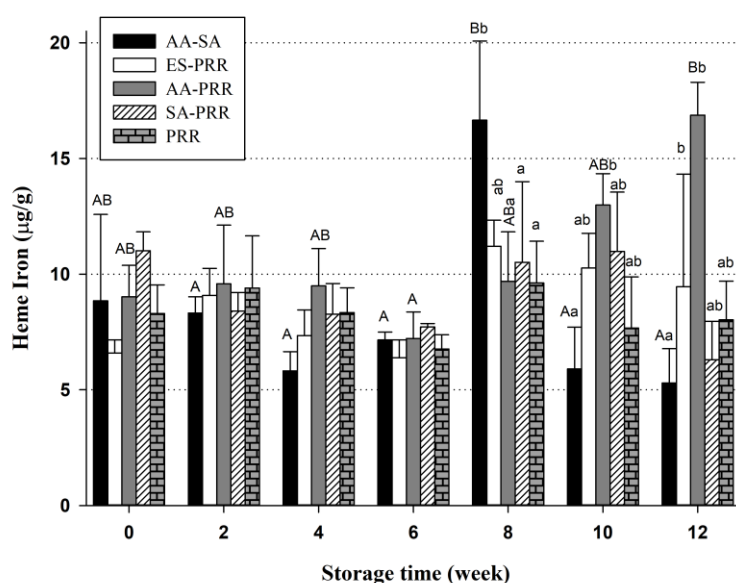


Figure 5. Change in heme iron values of sausages at 4°C for different storage times.

AA-SA: control cocktail sausages formulated with 0.02% ascorbic acid and 0.05% sodium ascorbate, ES-PRR: control cocktail sausages electrostatically sprayed with 2% pink rock rose extract, AA-PRR: cocktail sausages formulated with 0.02% ascorbic acid and 0.05% pink rock rose extract, SA-PRR: cocktail sausages formulated with 0.05% sodium ascorbate and 0.02% pink rock rose extract, PRR: cocktail sausages formulated with 0.07% pink rock rose extract. Error bars represent standard deviation of three replicates. a, b: indicate significant difference between treatments within the same week by the Tukey test ($P \leq 0.05$). A, B: indicate significant difference within the treatment at different weeks by the Tukey test ($P \leq 0.05$).

Lightness values were affected by the storage. For all the treatments L^* values increased over time. The lightness values were highest during week 8 and it was significantly ($P < 0.05$) different than rest of the weeks. Overall lightness values were increased during storage. Similarly, Lee et al. (2021) reported increase in lightness for emulsion sausage formulated with antioxidant during storage. Redness values were statistically significantly different ($P \leq 0.05$) for both the treatment and the storage. The addition of PRR extract to the sausage formulation caused decrease in redness values. The AA-SA treatment had the highest redness values and, it had significantly higher a^* values than AA-PRR and PRR treatments. With the addition of phenolic extract decrease in redness values were also reported in other studies (Cheng et al., 2013; Haak et al., 2009; Hwang et al., 2017). Yellowness values were not statistically different ($P > 0.05$) between treatments; however, the storage had a significant ($P \leq 0.05$) effect on

the yellowness values. The b^* value decreased over time across all the treatments. Seo et al. (2019) reported decrease in yellowness values for pork sausages during storage period. In this study, only the redness value affected by the addition of PRR extract, however, changes occurred during storage could be due to oxidation and microbial spoilage.

Proximate composition of the sausage treatments is shown in Table 2. Only, the protein content was statistically different ($P \leq 0.05$). SA-PRR treatment had the highest protein content with $10.69 \pm 0.28\%$, while AA-SA treatment had the lowest protein content with $9.49 \pm 0.27\%$. In contrast, the AA-SA treatment had the highest fat content. Ash content was also increased with PRR treatment. Similar patterns were observed by Nieto et al. (2017).

Conclusion

This study demonstrated the use of pink rock rose or *Cistus creticus* in sausages as an additive. It was found that pink rock rose had a high DPPH antioxidant capacity and showed antioxidant action in the frankfurter type sausages. Results of the lipid oxidation, heme iron content, and pH were similar for both control sausages and the PRR treatment. In addition, total aerobic mesophilic bacteria count was lowest for sausages formulated with the PRR extract. Overall, quality attributes were not affected by the addition of PRR extract, exception being decrease in redness value. Pink rock rose extract can be considered as a valuable natural antioxidant, and it could be used as a replacement for ascorbic acid and sodium ascorbate in sausage formulations.

Conflict of interest

There are no competing interests to declare.

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