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The use of beetroot extract and extract powder in sausages as natural food colorant

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Abstract: Four colorants (control, carmine, beetroot extract and beetroot extract powder) and two methods (fermentation and heat treatment) were used in the production of sausages. The betalain content, total phenolic substance content and ORAC and TEAC values of concentrated beetroot extract were 562.08 mg/L, 27.72 mg GAE/ mL, 33.96 μmol TE/mL and 35.70 mmol TE/L, respectively. The moisture content, pH value, lightness (L^*), yellowness (b*) and odor values of heat-processed sausages were higher than those of fermented sausages. 2-thiobarbituric acid reactive substances (TBARS) values were lower in sausages with beetroot extract (20.51 µmol·MDA/kg) and powder (19.03 µmol MDA/kg) than for control and carmine treatments. The use of beetroot extract and powder positively affected the sensory appearance, color, flavor and overall acceptance of sausages. Thus, beetroot extract and powder could be used as alternatives to carmine in sausage production.

Keywords: beetroot; betalain; carmine; colorant; sausage.

1 Introduction

Beetroot (*Beta vulgaris* L. ssp. *vulgaris*) is a two-year herbaceous plant from the Amaranthaceae family, a good antioxidant source for phenolic compounds and betalains [1, 2]. Betalain is a water-soluble nitrogenous pigment, and its basic structure consists of betalamic acid [3]. Betalamic acid, which forms the structure of the betalains, evolves into betacyanins (red) and betaxanthin (yellow) as a result of biosynthesis with different molecules [4, 5]. Betacyanins are betanidine glycosides; betaxanthins are condensation

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products of betalamic acid and amino acids or amines, respectively. Approximately 60 betacyanins and 33 betaxanthins have been found in nature [4, 6].

According to Food Additives Regulation (EC) No 1333/ 2008 of the Council of Europe, beet juice is not considered as a food additive, whereas betalains extracted from beetroot have been accepted as an additive and are labeled as E-162. E-162 gives a pink color when applied to food [2]. It is commercially used in the production of wine, jams, marmalade and strawberry-flavored dairy products such as yogurt and ice cream. Betalains are not toxic when compared to synthetic colorants and do not cause allergic reactions [7]. While acid-resistant anthocyanins are mostly used as natural pigments, the color of betalains is relatively stable over a wide pH range (3–7), and they are a suitable colorant for low-acid foods [8]. Their coloring power is very high because betalains have a higher molar extinction coefficient than synthetic colorants [7]. On the other hand, betalains have been proven to be beneficial to human health due to their potential to possess anti-inflammatory activity, inhibit lipid oxidation and peroxidation, increase resistance to the oxidation of low-density lipoproteins and provide chemo-preventive effects [9].

Sodium nitrate and nitrite are used in meat products for various purposes such as providing an antimicrobial effect, typical taste and aroma, and desired curing color, and preventing autooxidation [10]. However, nitrate and nitrite cause the formation of carcinogenic nitroso compounds such as N-nitrosodimethylamine in cured meat products [11]. It is also stated that nitrate is a potentially toxic food additive. Because of these, the use of synthetic additives has been reduced because of their toxicity, which in turn has gradually increased the demand for natural colorants such as annatto, curcumin, betalains, cochineal carmine, tomato, pepper and *Monascus* fungi, which provide a stable red color [12–14].

Carmine colorant is a colorful red extract obtained from female cochineal insects living on *Opuntia* cacti in tropical regions such as South Africa, Mexico and Arizona [15, 16]. The color of heat-resistant carmine is very similar to the color of cured meat products [17]. In the European Union, the use of carmine is restricted due to its allergen effect, and the amount permitted for use as a food additive in meat products is between 100 and 250 ppm [14]. Consumer concerns about the use of carmine in food

products have increased over the past years, due to the fact that this colorant is obtained by crushing insects [18]. Therefore, carmine is not preferred by most manufacturers, and the search for alternative food colorants is ongoing. In this study, the effect of different colorants (control, beetroot extract, beetroot powder and carmine) and treatments in sausage production (fermentation and heatprocessing) on lipid oxidation, color stability and sensory characteristics of sausage was investigated. For this purpose, the functional properties of beetroot extract were also analyzed.

2 Materials and methods

Beetroot used in the production of colorant, and lean-boneless beef, beef fat and spices for use in the production of sausages were obtained from well-known grocery store in Antalya.

2.1 Preparation of beetroot extract

The extraction of beetroot was performed according to the method of Wiley and Lee, with modifications [19]. Once beetroots were washed, their shells were peeled off and their size was reduced. In order to increase mass transfer to solvent, the beetroots were chopped into cubes $(0.5 \times 0.5 \times 0$

The beetroot extract was concentrated in a rotary evaporator (Laborota 4000, Heidolph Instruments GmbH & Co. KG, Schwabach, Germany) in a 200-mm Hg vacuum and a temperature of 50 °C. Some of the concentrated extracts were adjusted to 25°Brix for analysis, and the remainder were dried by the freeze-drying technique (Operon fdu & fdb type, Gyeonggi-do, South Korea).

2.2 Freeze drying of beetroot extract

The concentrated extract was spread in a thin layer over trays and frozen at $-80\,^{\circ}\text{C}$ for 24 h. After the freezing process, the trays were put into a freeze-dryer (Operon FDU&FDB type, Gyeonggi-do, South Korea). Freeze drying was started at $-40\,^{\circ}\text{C}$ (shelf temperature) at an absolute chamber pressure of 5333 Pa (40 mm Hg), and the process temperature reached room temperature at the end of the freeze-drying process in 48 h. The powder obtained was stored in a hermetically sealed glass jar until it was analyzed.

2.3 Production of sausages

Boneless beef (Simmental breed) cuts and beef fat were purchased from a local manufacturer (Veli Cengiz Meat Products Company, Antalya). For sausage production, the beef and beef fat were minced

together in a mincing machine. Then, other additives (salt, seasoning mix and sodium tripolyphosphate) were added, and the mixture was homogenized for about 5 min. The batter was divided into four groups for different colorant sources (control, carmine, beetroot extract concentrate and beetroot extract powder). The amount of colorant to be used in the sausage production was determined according to its coloring power and the literature [12, 13]. Carmine and beetroot extract powder were diluted in the water used in the formulation and mixed into the batter. The formulations for all sausage groups are given in Table 1. The mixtures were stuffed into artificial sausage casings with 28 mm diameter, and these casings were knotted at 15-cm intervals. Each colorant group included two different treatments (fermentation and heat-processing). Some of the sausages were ripened in a climate room for 15 days at 10 \pm 1 °C and 78% relative humidity (RH) for fermentation treatment. The rest of the sausages were cooked until the core temperature reached 68 °C and were stored in refrigerator conditions until analyzed. A total of 8 kg was used for one replication, and two replicates were carried out for analyses.

2.4 Total betalain analysis

The betaxanthin and betacyanin content of concentrated beetroot extract was determined spectrophotometrically at 480 and 538 nm, respectively, according to the method of Stintzing et al. [7] Total betalains were the sum of both betacyanins and betaxanthins. The betalain content of the extract was calculated from the following equation.

$$Betalain content \left(\frac{mg}{L}\right) = \left[\frac{A \times DF \times MW \times 1000}{e \times l}\right]$$

where *A* is absorbance, *DF* is the dilution factor, and *l* is the optical path length of the tube (1 cm). The molecular weight (MW) and molar extinction coefficient (e) of betacyanin and betaxanthin are given as MW = 550 g/mol; e = 60.000 L/mol·cm and MW = 308 g/mol; e = 48.000 L/mol cm, respectively.

Table 1: The formulations of sausages.

Ingredients				Quantity (%)
	Control sausage	Sausage with carmine	Sausage with beetroot extract	Sausage with beetroot extract powder
Beef	70	69.90	68	68
Beef fat	25	25	25	25
Water	3	3	0	3
Salt	0.70	0.70	0.70	0.70
Seasoning mix	1	1	1	1
Sodium tripolyphosphate	0.30	0.30	0.30	0.30
Carmine	0	0.10	0	0
Beetroot extract	0	0	5	0
Beetroot extract powder	0	0	0	2
Total	100	100	100	100

2.5 Total phenolic substance analysis

The analysis method used is based on the reduction of Folin-Ciocalteu reagent by the total soluble phenolic substances present in beetroot extract, and the conversion of the phenolic compounds to the oxidized form. Extract was diluted 1,000 times with distilled water. For the analysis, 5 mL 0.2 N Folin-Ciocalteu reagent was added to 1 mL extract and incubated for 2 min. A 4-mL sodium carbonate solution (7.5% w/v) was added, and the reaction was completed in 15 min at 50 °C in a water bath. Absorbance was measured spectrophotometrically at 760 nm. The standard calibration curve was prepared using gallic acid. The results were given as mg gallic acid equivalent (GAE)/L extract [20].

2.6 Determination of antioxidant activity by the ORAC method

The antioxidant activity of extract was determined in a fluorescence spectrophotometer (Cary Eclipse, Agilent Technologies, Inc., Santa Clara, CA, USA). Sample dilutions were prepared with phosphate buffer (PBS) pH solution (75 mM, pH 7.4). Samples (150 µL) were left for incubation in darkness for 30 min at 37 °C after 2738 µL fluorescein solution (0.6136 μM) and 37 μL PBS were added. Then, 75 μL 2,2'-azobis-2-methyl-propanimidamide dihydrochloride (AAPH) solution (0.32 µM) was added to set off the reaction. Readings were performed at excitation-emission wavelengths of 490-512 nm. Results were expressed in µmol Trolox equivalent (TE)/mL [21].

2.7 Determination of antioxidant activity by the TEAC method

2,2'-Azinobis(3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt radical cation (ABTS⁺) was prepared by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulfate (K₂S₂O₈). ABTS⁺ radical cation gives a maximum absorbance at a wavelength of 734 nm. ABTS+ radical cation was diluted with PBS in order that its absorbance was 0.700 ± 0.02 at 734 nm. Then 100 μL of sample or Trolox solution prepared at different concentrations was added to 2 mL ABTS+ radical, and the reaction was completed in 6 min at 30 °C. The reaction of antioxidants with the radical was measured by the reduction in absorbance at 734 nm. The TEAC value of extract was calculated from the Trolox standard curve and expressed as mmol·TE/L extract [22].

2.8 Color analysis

About 20 mL of concentrated extract was placed into a standard glass container, and color values (L^* , a^* and b^*) were measured using a CR-400 chromameter (Konica Minolta, Japan). According to the CIE Lab color system, the L^* , a^* and b^* values represent black-white, red-green and yellow-blue color values, respectively. The device was calibrated with its own calibration plate before analysis [9].

2.9 Transmittance analysis

The clarity of the obtained extract was determined spectrophotometrically by measurement of transmittance (%T) at 620 nm.

2.10 Physicochemical properties of sausages

The moisture content of the samples was determined gravimetrically by drying (Memmert UNB 500, Schwabach, Germany) the samples at 105 °C. The pH values of the samples were determined using a pH meter (Hanna HI 2210, Woonsocket, RI, USA) [23]. The 2-thiobarbituric acid reactive substances (TBARS) were determined spectrophotometrically according to the method of Lemon [24] and expressed as umolmalondialdehyde (MDA)/kg. The color values (L^* , a^* , b^*) of sausage slices were measured at eight different points on the sample surface using a CR-400 Chromameter (Konica Minolta, Japan).

2.11 Sensory analysis of sausages

For the sensory evaluation, sausage slices were heated in a microwave oven for about 2 min until an internal temperature of 72 °C was reached, then immediately presented to panelists in random order. The appearance, color, odor, flavor, texture and overall acceptability specifications were evaluated by a 10-person educated panelist group, consisting of undergraduate and graduate students of the Food Engineering Department at Akdeniz University (Turkey) using a ninepoint hedonic scale (1: dislike extremely, 9: like extremely). In addition, the panelist group assessed all the aspects at the same time.

2.12 Statistical analysis

Four colorants (control, carmine, beetroot extract and beetroot extract powder) and two methods (fermentation and heat treatment) were used in the production of sausages. The sausages that included no colorant were used as a control. Sausage production was done in two replicates, and analyses of the samples were held in parallel. Variance analysis (ANOVA) was done for the data, and a Duncan multiple comparison test was applied to the important factors. All statistical calculations were done using SAS Statistics Software (v.7.00, SAS Institute Inc., Cary, NC, USA), and the values were given as mean ± standard error. Further, Pearson correlation analysis was done by using Excel program (Microsoft Office 365, WA, USA) to find out the correlation between TBARS, color values and sensory scores.

3 Results and discussion

3.1 Chemical properties of beetroot extract

The betacyanin and betaxanthin content in beetroot extract were 300.82 and 261.27 mg/L, respectively, with a collective betalain pigment content of 562.08 mg/L. However, the betacyanin and betaxanthin content obtained in this study was lower than that found by Latorre et al. [25] for red beetroot tissue (400–800 mg/L), and by Bazaria and Kumar [26] for beetroot juice concentrate (1523.89 mg/L). Our findings are in agreement with previous studies demonstrating that beetroot treated with thermal processes such as blanching, boiling, drying and roasting [2, 9, 27] loses betalains depending on the process time and

temperature. Bazaria and Kumar [9] reported that changes in betalain (betacyanin and betaxanthin) in beetroot juice concentrate were affected significantly by concentration temperature and time. In addition, the betalain content has been reported to vary among different raw materials, mainly due to differences in the part of the root, cultivar and climatic and agricultural conditions [8].

The color properties (L^*, a^*, b^*) and transmittance values) were evaluated in 100-fold diluted samples since the color of the concentrated extract was too dark. The diluted samples had positive a^* and b^* values, mentioned as "redness" and "yellowness" in the color scale (CIE), respectively. The color of the extract might be affected by the concentration temperature and hence the dominant color in beetroot extract was red-brown. Herbach et al. [28] reported that the color of fresh beet juice was the typical red-purple, but heating for 8 h induced an unexpected shift to a yellow-orange solution. Betacyanin (red-violet) pigment is heat-sensitive, and betacyanin pigment degradation has been observed at higher temperatures (60 °C) which were accompanied by a vacuum [9]. It was determined that diluted samples showed very good transmittance (95.64%), and this trend of transmittance value might be explained by the presence of less unfilterable particulate matter which is the cause of turbidity.

The total phenolic substance content in beetroot extract was 27.72 mg · GAE/mL. Similar to that result, total phenolic content was between 34.21 and 112.49 mg · GAE/ mL in concentrated beetroot juice samples [9]. Beetroot juice exhibits particularly high antioxidant activity because it is rich in polyphenols [29]. 4-Hydroxy benzoic acid has been determined as the major constituent among the phenolic substances from beetroot extracts, followed by cinnamic, vanillic, chlorogenic, trans-ferulic and caffeic acids [1]. It has been reported that the total polyphenol values of heat-treated beetroot cultivars are higher than those of raw ones, showing the sensitivity of these compounds towards high temperature [30]. This may arise from the release of phenolic compounds because of thermal treatment, since these compounds are linked to cell wall components in the fresh material. Jiratanan and Liu [27] also reported that the total phenolic substance content of beet increased with an increase in process time (from 0 to 45 min) and temperature (from 105 to 125 °C).

Two methods (ORAC and TEAC) were used in our study to determine the antioxidant activity of beetroot extract (Table 2). The ORAC and TEAC values of beetroot extract were 33.96 µmol TE/mL and 35.70 mmol TE/L, respectively. In some literature, it is reported that antioxidant activity is between 40 and 50 µmol vitamin C equivalent/g beet in

Table 2: Properties of the beetroot extract.

Parameter	Content
Betalain (mg/L)	562.08 ± 39.36
Betacyanin (mg/L)	300.82 ± 21.28
Betaxanthin (mg/L)	261.27 ± 18.07
Transmittance (%T)	95.64 ± 0.12
L*	18.92 ± 0.07
a*	2.52 ± 0.07
b*	2.91 ± 0.08
Total phenolic substance (mg · GAE/mL)	27.72 ± 0.11
ORAC (µmol·TE/mL)	33.96 ± 1.38
TEAC (mmol·TE/L)	35.70 ± 2.29

thermally processed beet [27], between 47.37 and 78.90% (scavenging activity) in vacuum-concentrated beetroot juice [9], and between 5 and 25% in microwave-treated red beet [2]. The antioxidant activity of 13 red beetroot varieties was determined by TEAC and DPPH methods as between the ranges of 35.50-49.71 and 24.06-32.87 µmol·Trolox/g dm of beetroot, respectively [8]. The processed beetroot samples in this study had higher antioxidant activity compared to raw or fresh ones because the antioxidant activity depends on the presence of betalains and polyphenols which could have been increased during the treatments.

3.2 Physicochemical properties of sausages

The moisture content and pH value of heat-processed sausages were significantly (p < 0.01) higher than those of fermented sausages (Table 3). These moisture and pH results might be attributed to the greater degree of water loss and lactic acid formation in sausages during fermentation. Since lactic acid bacteria and other acid-producing

Table 3: Moisture, pH and TBARS values of sausages.

	Moisture (%)	рН	TBARS (µmol MDA/kg)
Treatment	:		
Fermentation	$22.88^{b} \pm 0.96$	$5.86^{b} \pm 0.13$	$23.91^a \pm 0.69$
Heat processing	$30.15^a \pm 1.09$	$6.49^{a} \pm 0.08$	$17.64^{b} \pm 0.55$
Colorant			
Control	$26.27^a \pm 3.16$	$6.40^{b} \pm 0.17$	$21.98^a \pm 2.08$
Carmine	$28.61^a\pm2.86$	$6.51^a \pm 0.13$	$21.58^a \pm 1.28$
Beetroot extract	$24.41^a \pm 1.79$	$5.95^{c} \pm 0.19$	$20.51^{ab} \pm 2.53$
Beetroot extract powder	26.77 ^a ± 1.79	$5.85^{d} \pm 0.24$	19.03 ^b ± 1.63

a,b,c,d Means with different online letters within the column indicating significant difference (p < 0.05).

bacteria produce lactic acid and other organic acids during this period, the pH value of meat products decreases [31]. TBARS values were higher (p < 0.01) for fermented sausages compared to heat-processed sausages. This might be due to the lipolytic activity of bacteria in the sausage and hence the formation of free fatty acids during fermentation because they are the precursors of lipid oxidation. Similarly, the TBARS value was found to be 18.48 μ mol MDA/kg in fermented sausage at the end of a ripening period [32]. Some researchers have also stated that TBARS value increases gradually during the fermentation period of sausages [31, 33, 34].

The moisture content of the sausages was not significantly (p > 0.05) affected, while the TBARS value was significantly (p < 0.05) affected by the addition of colorants. The low TBARS values of sausages with beetroot extract and powder might be due to the antioxidant activity of beetroot due to betalains and phenolic compounds. Additionally, the TBARS values of sausages with beetroot powder were higher than those of sausages with beetroot extract, which indicates that the beetroot extract also maintains its antioxidant activity in powder form.

Baldin et al. [35] reported that the moisture and TBARS values of sausages showed non-significant differences among control, carmine and microencapsulated jabuticaba extract treatments. In addition, it was reported that the addition of *Hibiscus sabdariffa* was less effective (p < 0.05) in reducing the TBARS value in Turkish dryfermented sausages than other antioxidants because of its low phenolic content [33].

A significantly (p < 0.01) lower pH value characterized the sausages with beetroot extract and powder. This result may be explained by the low pH value of beetroot extract and powder due to the citric acid used in the extraction, besides other components of beetroot. Latoch and Stasiak [36] reported that $Urtica\ dioica$ water extract contains phenolic compounds, which contributes to lower pH values in cooked pork sausages. Baldin et al. [13] also reported that the addition of 2 and 4% microencapsulated jabuticaba extract caused a reduction in the pH value of pork sausages during storage.

The L^* and b^* values of heat-processed sausages were significantly higher than those of fermented sausage, while a^* values were higher (p < 0.05) for fermented sausage (Table 4). These results indicate that the fermented sausages were darker than the heat-processed sausages. While heat treatment provided color stability, fermentation treatment led to a darker color because of Maillard browning reaction and drying in the sausage. Karabacak and Bozkurt [33] reported that the lightness and yellowness

Table 4: L^* , a^* ve b^* values of sausages.

L*	a*	b*
$46.56^{b} \pm 1.65$	$27.02^a \pm 2.86$	$17.94^{b} \pm 0.84$
$47.62^a \pm 1.68$	$26.29^{b} \pm 2.60$	$18.58^a \pm 0.28$
$52.57^a \pm 0.43$	$20.92^{c} \pm 0.84$	$20.03^a \pm 0.95$
$40.37^{c} \pm 0.21$	$38.88^a \pm 0.47$	$16.94^{d} \pm 0.35$
$48.14^{b} \pm 0.50$	$23.10^{b} \pm 0.30$	$18.39^{b} \pm 0.50$
$47.27^{b} \pm 0.52$	23.72 ^b ± 0.97	$17.68^{c} \pm 0.89$
	$46.56^{b} \pm 1.65$ $47.62^{a} \pm 1.68$ $52.57^{a} \pm 0.43$ $40.37^{c} \pm 0.21$ $48.14^{b} \pm 0.50$	$46.56^{b} \pm 1.65 \qquad 27.02^{a} \pm 2.86$ $47.62^{a} \pm 1.68 \qquad 26.29^{b} \pm 2.60$ $52.57^{a} \pm 0.43 \qquad 20.92^{c} \pm 0.84$ $40.37^{c} \pm 0.21 \qquad 38.88^{a} \pm 0.47$ $48.14^{b} \pm 0.50 \qquad 23.10^{b} \pm 0.30$

 $^{^{}a,b,c,d}$ Means with different online letters within the column indicating significant difference (p < 0.05).

values of fermented sausage decreased during ripening due to browning.

The color values of the sausages were significantly (p < 0.01) affected by colorant source (Table 4). The L^* and b^* values of the control were the highest, followed by the sausages with beetroot extract, beetroot powder and carmine, respectively. It has been pointed out that fermented red beet added to frankfurters results in lower lightness (L^*) and yellowness (b^*) values but higher redness (a^*) values [37]. Likewise, Sucu and Turp [38] reported that the addition of beetroot powder as a nitrate source into fermented sausages led to a decrease in the L^* and b^* values of sausages at the beginning of storage (p < 0.05). In another study, the addition of cochineal carmine slightly decreased L^* and b^* values in comparison to the control [35].

Regarding the redness of sausages, the addition of carmine colorant significantly (p < 0.01) increased the a^* values in comparison to those of other samples. A similar result was reported for mortadella sausage by Baldin et al. [35]. Moreover, the use of beetroot extract and powder caused an increase in the a^* values of sausages compared to the control. This result shows that beetroot extract and powder are effective in providing the desired red color in sausages due to their betalain and also nitrate content. Similar results have been reported by different authors for emulsified pork sausage [39], emulsified beef sausage [40] and Turkish fermented beef sausage (sucuk) [38]. Conversely, high water activity, high storage temperature, high luminosity and presence of oxygen and metal ions have been reported to have negative effects on the stability of betalains, natural colorant found in beetroot [2, 3]; therefore, beetroot extract and powder may not protect sausages from discoloration throughout the storage in spite of increasing redness.

Table 5: Sensory scores of sausages.

	Appearance	Color	Odor	Flavor	Texture	Overall
Treatment						
Fermentation	$5.71^a \pm 0.54$	$5.53^{a} \pm 0.57$	$4.78^{b} \pm 0.13$	$5.45^{a} \pm 0.36$	$5.54^{a} \pm 0.23$	$5.64^{a} \pm 0.33$
Heat processing	$5.75^{a} \pm 0.34$	$5.80^{a} \pm 0.47$	$5.55^{a} \pm 0.14$	$5.68^a \pm 0.23$	$5.83^{a} \pm 0.23$	$5.61^{a} \pm 0.31$
Colorant						
Control	$4.38^{c} \pm 0.17$	$4.08^{c} \pm 0.34$	$4.98^{a} \pm 0.31$	$5.03^{c} \pm 0.20$	$5.53^{a} \pm 0.38$	$4.48^{c} \pm 0.06$
Carmine	$4.83^{b} \pm 0.25$	$4.65^{b} \pm 0.29$	$5.08^a \pm 0.29$	$4.70^{c} \pm 0.25$	$5.65^{a} \pm 0.28$	$5.18^{b} \pm 0.14$
Beetroot extract	$6.73^{a} \pm 0.18$	$6.75^{a} \pm 0.16$	$5.28^{a} \pm 0.25$	$6.08^{b} \pm 0.17$	$5.90^a \pm 0.25$	$6.43^{a} \pm 0.02$
Beetroot extract powder	$7.00^a \pm 0.16$	$\textbf{7.18}^{a} \pm \textbf{0.10}$	$5.33^a \pm 0.32$	$6.45^a\pm0.13$	$5.65^{a} \pm 0.47$	$6.43^a\pm0.11$

 $^{^{}a,b,c}$ Means with different online letters within the column indicating significant difference (p < 0.05).

3.3 Sensory evaluation of sausages

The effects of different treatments and colorants on the appearance, color, odor, flavor, texture and overall acceptability scores of sausages are given in Table 5. The odor scores of heat-processed sausages were significantly (p < 0.01) higher than those of fermented sausages. This result shows that undesirable aroma compounds might form in meat products during fermentation. The appearance, color and flavor scores of sausages were significantly affected (p < 0.01) by colorant source. The scores of the sausages incorporating beetroot extract and powder were better than those of the other sausages. These results might be due to the fact that the beetroot provided a more natural color in the sausages and the carmine had a negative effect on the panelists with its dark pink color. In addition, the higher flavor score could be in fact related to the higher color score due to the influence of color on other sensory perception and liking. Similarly, the addition of (0.12, 0.24 and 0.35%) beetroot powder into cooked fermented sausages resulted in a statistically significant (p < 0.05) increase in inner color scores [38]. However, it was reported

that the color and aroma acceptance were significantly reduced by addition of microencapsulated jabuticaba extract in comparison to control and carmine-treated mortadella [35]. Because of that, the sensorial characteristics of meat products might be affected according to the specific characteristics of the plant additives used. The low score for flavor could be due to the presence of undesirable flavoring compounds which caused high TBARS values in control sausages and those with carmine.

The overall sensory quality of meat products depends on the formation of desired odor, color and flavor, and the addition of beetroot extract and powder increased the overall acceptability scores of sausages. These results suggest that the addition of beetroot extract and powder as a natural colorant to sausages enhances their sensory quality. These results are in accordance with a study indicating that the consumer acceptability score of fresh pork sausage with added red beetroot (1 mL/kg) was higher than those of control (no colorant) group sausages [12]. Moreover, some meat products such as low-salt frankfurters with fermented red beet extract (1, 3 and 5%) [37] and emulsified pork sausages with 1% red beet powder [39]

 Table 6: Pearson correlation matrix of sausage TBARS, color values and sensory scores.

Pearson corre	lation coeffic	ation coefficients (r)								
	TBARS	L*	a*	b*	Appearance	Color	Odor	Flavor	Texture	Overall
TBARS	1.000									
L*	-0.106	1.000								
a*	0.121	-0.933	1.000							
b*	-0.058	0.677	-0.622	1.000						
Appearance	-0.261	-0.016	-0.284	-0.340	1.000					
Color	-0.404	-0.012	-0.265	-0.355	0.955	1.000				
Odor	-0.706	0.032	-0.066	-0.029	0.306	0.346	1.000			
Flavor	0.027	-0.198	0.197	-0.258	0.098	0.016	0.609	1.000		
Texture	-0.297	-0.023	-0.010	-0.042	0.162	0.199	0.678	0.673	1.000	
Overall	-0.277	-0.152	-0.156	-0.367	0.948	0.959	0.291	0.070	0.258	1.000

Negative values indicate inverse correlation between the parameters, whereas positive values are an indicative of direct relationship between the parameters (p < 0.0001).

exhibited sensory characteristics similar to those of the control.

3.4 Correlation matrix studies between TBARS, color values and sensory scores

The Pearson correlation matrix to study the correlation behavior between various parameters for sausage is given in Table 6. The matrix indicated that there was generally negative relationship of TBARS, L^* , a^* and b^* values with appearance, color, odor, flavor, texture and overall acceptance scores of sausage samples. TBARS value had higher negative correlation (r = -0.706) with odor score than color, texture, overall acceptance and appearance scores. In addition, b^* value had a high positive correlation (r = 0.677) with L^* value and a high negative correlation (r = -0.622) with a^* value. Highest negative correlation (r = -0.933) amongst all the parameters was recorded for L^* and a^* values showing the inverse relation between these color parameters whereas, highest positive correlation (r = 0.959) was observed between color and overall acceptance scores indicating direct relationship between these sensorial parameters at the high significant level (p < 0.0001). Moreover, a direct positive correlation was observed among all sensorial parameters.

4 Conclusion

This study reveals that beetroot has significant potential for use as a natural colorant in fermented and heatprocessed sausages. The results confirmed that heat treatment contributes to an increase in moisture content, pH value, lightness (L^*), yellowness (b^*) and odor values of sausages, while it leads to lower TBARS and redness (a^*) values in comparison with fermentation. The use of beetroot extract and powder significantly prevented further development of lipid oxidation in sausages due to the antioxidant properties of beetroot, with a prevalence of betalains and phenolic compounds. Instrumental analysis of color showed that the addition of beetroot extract and powder to the sausages resulted in a reduction of L^* and b^* values, while a^* values were increased compared to the control. The use of beetroot extract and powder positively affected sensory appearance, color, flavor and overall acceptance of the sausages. In conclusion, beetroot extract and powder show the most potential as alternatives to carmine, not only maintaining color development but also inhibiting lipid oxidation of beef sausages.

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