

Impact of the Application of Red Beetroot and Radish Powders as Natural Nitrite Substitutes on the Quality of the Oriental Sausage

¹A. Ghazy, ²M.A. Kassem Gehan and ²Mohamed M.T. Emara

¹Follow-up and Performance Development Department,
Veterinary Service Department of Armed Forces, Egypt

²Department of Food Hygiene and Control,
Faculty of Veterinary Medicine, Cairo University, Giza, Egypt

Abstract: To explore the efficacy of red beet and radish powder substitution to sodium nitrite in oriental sausage, four groups of sausage batter were processed. The first group (N) was formulated with 100ppm of Sodium nitrite, while the 2nd (BR) was produced with 0.065% beetroot powder, the 3rd (R) was prepared with 0.065% radish powder and finally, 4th group (NBR) was prepared with 0.065% beetroot powder + 100ppm Sodium nitrite. All sausage groups were examined for the changes in the sensory attributes, physicochemical criteria, nitrite residue and bacterial load immediately after production and every month for three months during frozen storage at -18°C. The achieved result revealed that the addition of both red beetroot and radish powder significantly improved the sensorial and instrumental color indexes through the whole storage time. Residual nitrite in NBR was significantly lower than the control group at zero time and 1st month of frozen storage, while at end of the storage period N group showed lower nitrite residue than NBR group. Sodium nitrite and beetroot powder treated group revealed good antimicrobial activity than that of radish powder alone. Beetroot and radish powder considered a good and stable colorant with more investigation is required to explore their role as nitrite substitution in oriental sausage.

Key words: Natural nitrite substitutes • Sausage • Red beet root powder • Radish powder • Quality attributes

INTRODUCTION

With the modern lifestyle and extension of working hours, the consumption of meals outside the home became essential. Cured meat products are highly preferable by a wide sector of Egyptian consumers, mainly children, teenagers and, youth, where it is consumed as fast food. Therefore, the production of high-quality and healthy cured meat products became crucial to consumers' demand.

Nitrite salts are conventionally applied as curing compounds in meat products many years ago, for their ability to produce cured pink color, improve flavor, delay rancidity, in addition to its antimicrobial effect, especially against *Clostridium botulinum*. Although legislations recently have been settled to reduce the permissible limit of nitrite added to meat products, WHO [1] reported that consumption of cured meat products processed with salt

and nitrite to enhance flavor and improve preservation is carcinogenic, this may be due to the formation of N-nitroso-compounds and polycyclic aromatic hydrocarbons, which classified as a potentially carcinogenic chemical. In turn, consumers became worried and looking forward to prohibiting synthetic nitrite in meat processing [2]. As a result, meat processors strive in searching for natural nitrite replacers to enhance both the color and stability of meat products.

Control of residual nitrite in the meat product is a critical issue, where nitrite residue level in Egyptian meat products ranging between 10.45- 251.6 ppm while for oriental sausage it ranged from 10.45 to 117 ppm with a mean value of 36.47 [3]. The use of alternative natural nitrate sources in meat products could fulfill both consumers' health demand and processing meat industry profit [4-6]. Red beet, celery, spinach, radish and lettuce are among vegetables naturally contain high nitrate level

[7, 8], where red beets and organic red radish contain up to 3, 288 and 4, 875.8 ppm of nitrate respectively, while their content of nitrite is much lower (2.4 – 14.2 ppm). Moreover, phenolic compounds and betalains content of red beet marks it as natural colorants [9-11]. Studies on the application of natural nitrate sources like red beet and radish in food processing are still in its beginning and scarce data are available [12, 13]. Therefore, the present study aimed to explore the efficacy of red beet and radish powders as natural alternative to nitrite in oriental sausage processing.

MATERIALS AND METHODS

To explore the efficacy of red beetroot and radish powder substitution to nitrite salt in oriental sausage production, a triplicate trials experiment (independent three replicates at different times) was performed and the quality parameters of fresh sausage were screened throughout three months of frozen storage.

Materials

Preparation of Red Beet Root and Radish Powders: Fresh red beet (*Beta vulgaris*) and radish (*Raphanus sativus*) were obtained from a local market in Giza, Egypt. The samples were washed, peeled and diced into small pieces, then dried at 60°C in a tray dryer. The dried vegetables products were ground by a miller (IKAM20, IKA, Staufen, Germany) to a powdered form, then complete dryness in a rotatory evaporator (EYELA N-1000, Rikakikai, Japan). Nitrate and nitrite content in beetroot and radish powders were analyzed by HPLC technique (table A) as recommended by Cheng and Tsang [14] at Central Laboratory of Residue Analysis of Pesticides And Heavy metals in food - Ministry of Agriculture – Cairo Egypt.

Table A: Nitrate and Nitrite content in red beet and radish powder

Vegetable name	BR (beetroot)	R (red radish)
Nitrate mg/kg	3312	7100
Nitrite mg/kg	>50	>50

Meat and Non-Meat Ingredients: Twenty-five Kilograms of imported frozen beef chucks (MINISTÉRIO DA AGRICULTURA, Brazil) were obtained from the Giza market in, Egypt at their first third of storage shelf life and stored frozen at -18°C till processing. Five kilograms of beef fat tissue was obtained from a local butcher (Giza, Egypt) within 24h after animal slaughtering, where it washed and stored frozen at -18°C. Sodium nitrite, sodium tri-polyphosphate and, spice mix were gained from LobaChemie, Mumbai, India. While sodium chloride was

acquired from a local market, Giza, Egypt. Medium size mutton hank (salt dried intestine of sheep) was obtained from a local market in Giza Egypt.

Oriental Sausage Formulation and Processing: Beef sausage was prepared following the Good manufacturing practices using 65% lean beef and 15% added fat. Just before processing, the beef and fat were minced using 5-mm grinding plate (Seydelmann NW 114 E; Stuttgart, Deutschland, Germany). The minced beef and fat were mixed with the other ingredients using a paddle mixer (K150 BP, Butcher's' Line, Italy) for 5 minutes, using the formula given in Table (B).

Table B: The formulation of sausages treated with nitrite salt and vegetable powders

Ingredients (%)	Treatments			
	N	BR	R	NBR
Beef	65	65	65	65
Fat tissues	15	15	15	15
Ice Water	10	10	10	10
Bread crumps	10	10	10	10
Salt	1.6	1.6	1.6	1.6
SpicesMixture	QS	QS	QS	QS
Sodium polyphosphate	0.5	0.5	0.5	0.5
SodiumNitriteppm	100	-----	-----	100
Red Beetroot BR	-----	0.065	-----	0.065
Radish R	-----	-----	0.065	-----

N: sample with 100 sodium nitrite; BR: sample with 0.065% red beetroot powder; R: sample with 0.065% Radish powder; and NBR: sample with 0.065% red beetroot powder & 100ppm Sod. nitrite.

QS: Quantum sufficient

The produced sausage mix was divided into 4 groups. The first group (N) was produced with 100ppm sodium nitrite, while sodium nitrite was substituted in 2nd group (BR) and 3rd group (R) with 0.065% experimentally produced red beet root and radish vegetable powder respectively. The 4th group (NBR) was produced with 100ppm of sodium nitrite and 0.065% red beetroot powder. The Sausage mix was stuffed into medium size mutton using sausage and packed in white foam plates 500 grams each and aerobically stored frozen (-18°C). The produced sausage was investigated for their sensory attributes, residual nitrite, instrumental color, pH and microbial load at the day of production (zero time) then every month for 3 months during frozen storage at -18°C as follow:

Sensory Evaluation: Sensory analysis was conducted by 15 staff members (23-55 years) from the Food Hygiene Department at the Faculty of Veterinary medicine - Cairo University. The panelists had a good experience with the

sensory examination of fresh sausage. Sensory examination was performed by determining the color, odor and texture for raw samples as well as color, taste, flavor and juiciness panel scores for cooked samples using five points hedonic scale (1=extremely dislike and 5=extremely like). Cooked samples were prepared by boiling about 50 gm of each sausage group for 10 minutes and then pan-fried in oil at 180°C for 5 minutes. Samples were coded, serviced in white disposable dishes and examined immediately after cooking at room temperature at daylight with water was available.

Residual Nitrite Analysis: To determine residual nitrite, sausage samples were treated by Sulphanilamide solution and N-1 naphthyl ethylenediamine dehydrochloride (NED) to develop color, then the absorbance of the developed color was measured by spectrophotometer at 540 nm. The level of residual nitrite was determined by standard curve comparison as recommended by AOAC [15].

Instrumental Color Measurement: Instrumental measurements of color were done by Chromameter (Konica Minolta, model CR 410, Japan), white plate and light trap (supplied by the manufacturer) was used for the calibration of chromameter. The color was expressed using the CIE L*, a* and b* color system [16]. Three spectral readings were recorded for each sample. Lightness (L*) (dark (0) to light (100)), the redness (a*) values ((+) reddish to (-) greenish). The yellowness (b*) values ((+) yellowish to (-) bluish) were estimated.

Measurement of pH Value: Homogenate of five grams sample and 20 ml distilled water was prepared. The pH was measured using pH meter (Lovibond Senso Direct) coupled with probe type electrode (Senso Direct Type 330), Three readings were recorded and their mean was calculated [15].

Bacteriological Examination: Enumeration of mesophilic and psychrotrophic bacteria was performed as recommended by APHA [17]. Ten grams from each sample were homogenized in 90 ml of 1/4 Ringer's solution (Oxoid BR 52) for one minute using a stomacher (Lab blender 400, Seward lab. Model No. AB 6021). Then, ten-fold decimal dilutions were prepared using the same diluents. One hundred µl from each dilution of the previously prepared sample homogenate was aseptically spread over the dry surface of a double set of Aerobic plate count agar medium (Oxoid CM 463) for 48 hours for mesophilic bacteria count and 7°C for 7 days for psychrotrophic bacteria count. The average count of the duplicate plates was enumerated and the mesophilic and psychrotrophic bacterial count CFU/g were calculated.

Statistical Analysis: All data were recorded as means and standard errors. One-way ANOVA test was used for the analysis of variance between the different treatments and storage period using SPSS program (V17.0) and a probability level of $P < 0.05$ is applied.

RESULTS AND DISCUSSION

Sensory attributes of oriental sausage processed by nitrite and natural vegetable powders during 3 months of frozen storage

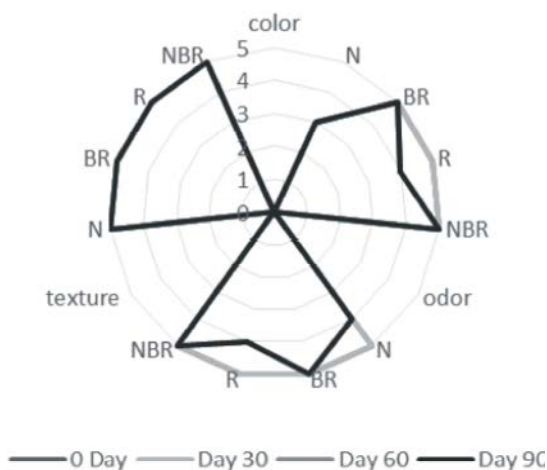


Fig. 1: Raw

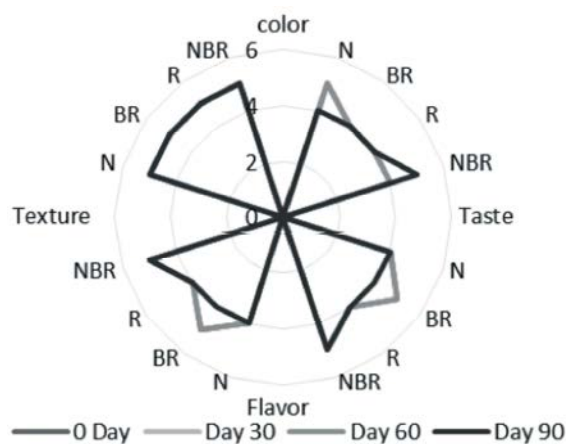


Fig. 2: Cooked

Table 1: Mean value of Instrumental color analysis of oriental sausage processed by synthetic nitrite and natural vegetable powders during

Parameters	Storage period	N	BR	R	NBR
L* (lightness)	0	46.23±6.30 ^{Bb}	47.32±4.11 ^{Aa}	46.87±6.19 ^{Bb}	42.96±5.12 ^{Cc}
	30	46.30±6.30 ^{Bb}	47.12±4.12 ^{Aa}	46.82±6.19 ^{Bb}	42.76±5.12 ^{Cc}
	60	47.36±3.36 ^{Aa}	47.60±6.14 ^{Aa}	49.50±5.14 ^{Aa}	47.99±4.19 ^{Bb}
	90	47.46±4.66 ^{Aa}	46.40±4.17 ^{Bc}	49.68±6.19 ^{Aa}	49.09±4.19 ^{Aa}
a* (redness)	0	9.96±6.33 ^{Ad}	18.59±3.93 ^{Ab}	16.8 6±5.1 ^{Ac}	22.06±4.12 ^{Aa}
	30	9.86±6.36 ^{Ad}	18.39±3.91 ^{Ab}	16.5 6±5.17 ^{Ac}	20.06±4.15 ^{Aa}
	60	9.17±5.17 ^{Ad}	15.43±4.13 ^{Ba}	14.60±6.18 ^{Bb}	13.10±3.19 ^{Bc}
	90	7.58±5.19 ^{Bd}	9.50±1.54 ^{Cb}	8.08±1.18 ^{Cc}	10.12±2.14 ^{Ca}
b* (yellowness)	0	10.13±4.19 ^{Ac}	9.86±1.88 ^{Ad}	11.27±1.10 ^{Ab}	13.2±2.17 ^{Aa}
	30	10.15±5.19 ^{Ac}	9.80±1.88 ^{Ad}	11.27±1.17 ^{Ab}	12.84±2.19 ^{Aa}
	60	9.04±4.19 ^{Bb}	8.68±1.16 ^{Bc}	10.20±1.10 ^{Ba}	6.99±1.14 ^{Cd}
	90	7.84±4.17 ^{Cb}	6.01±1.01 ^{Cd}	8.90±1.19 ^{Ca}	7.15±1.17 ^{Bc}

Values are shown as mean ± standard error

Means within the same row of different small litters are significantly different at ($P < 0.05$).

Means within the same column of different capital litters are significantly different at ($P < 0.05$).

N: sample with 100 ppm sodium nitrite; BR: sample with red beetroot powder (0.065%); R: sample with Radish powder (0.065%); and NBR: sample with 100ppm sodium nitrite and red beet root powder (0.065%)

Table 2: Mean values pH value of oriental sausage processed by nitrite and natural vegetable powders during 3 months of frozen storage

Storage period in days	Treatment			
	N	BR	R	NBR
0	6.36±1.36 ^{Aa}	6.34±1.35 ^{Aa}	6.28±1.35 ^{Ab}	6.29±1.37 ^{Ab}
30	6.00±1.16 ^{Ba}	5.95±1.19 ^{Ba}	6.06±1.31 ^{Ba}	6.01±1.25 ^{Ba}
60	6.17±1.17 ^{Aa}	6.2±2.17 ^{Aa}	6.2±1.36 ^{Aa}	6.1±1.37 ^{Ba}
90	6.37±1.13 ^{Aa}	6.4±2.14 ^{Aa}	6.3±1.17 ^{Aa}	6.2±1.39 ^{Aa}

Values are shown as mean ± standard error

Means within the same row of different small litters are significantly different at ($P < 0.05$).

Means within the same column of different capital litters are significantly different at ($P < 0.05$).

N: sample with 100 ppm sodium nitrite; BR: sample with red beetroot powder (0.065%); R: sample with Radish powder (0.065%); and NBR: sample with 100ppm sodium nitrite and red beet root powder (0.065%)

Table 3: Residual nitrite in oriental sausage processed by nitrite and natural vegetable powders during 3 months of frozen

Parameters	Storage days	Treatments			
		N	BR	R	NBR
Nitrite residues (ppm)	0	101±3.25 ^{Ab}	0 ^{Ac}	0 ^{Ac}	78.3±6.24 ^{Aa}
	30	100±3.25 ^{Ab}	0 ^{Ac}	0 ^{Ac}	78±6.25 ^{Aa}
	60	30.82±4.25 ^{Bb}	0 ^{Ac}	0 ^{Ac}	33.46±3.67 ^{Ba}
	90	29.81±5.33 ^{Bb}	0 ^{Ac}	0 ^{Ac}	30.1±5.25 ^{Ca}

Values are shown as mean ± standard error

Means within the same row of different small litters are significantly different at ($P < 0.05$).

Means within the same column of different capital litters are significantly different at ($P < 0.05$).

N: sample with 100 ppm sodium nitrite; BR: sample with red beetroot powder (0.065%); R: sample with Radish powder (0.065%); and NBR: sample with 100ppm sodium nitrite and red beet root powder (0.065%)

Table 4: Mean value of aerobic plate count log₁₀CFU/g of oriental sausage processed by nitrite and natural vegetable powders during 3 months of frozen storage

Storage period in days	Treatments			
	N	BR	R	NBR
0	4.01±1.04 ^{Bc}	4.74±1.14 ^{Ab}	5.52±2.02 ^{Aa}	4.20±0.24 ^{Ac}
30	2.30±1.13 ^{Cc}	2.47±1.14 ^{Cc}	4.45±1.04 ^{Ba}	3.25±1.09 ^{Bb}
60	2.47±1.14 ^{Cc}	2.81±1.61 ^{Cb}	5.30±1.08 ^{Aa}	2.30±1.04 ^{Dd}
90	4.70±1.13 ^{Ab}	2.32±1.06 ^{Bd}	5.61±1.17 ^{Aa}	2.60±1.14 ^{Cc}

Values are shown as mean ± standard error

Means within the same row of different small litters are significantly different at ($P < 0.05$).

Means within the same column of different capital litters are significantly different at ($P < 0.05$).

N: sample with 100 ppm sodium nitrite; BR: sample with red beetroot powder (0.065%); R: sample with Radish powder (0.065%); and NBR: sample with 100ppm sodium nitrite and red beet root powder (0.065%)

Table 5: Mean value of Psychrotrophic bacterial counts log10CFU/g of oriental sausage processed by nitrite and natural vegetable powders during 3 months of frozen storage

Storage period in days	Treatments			
	N	BR	R	NBR
0	3.65±1.06 ^{Aa}	3.39±1.11 ^{Ab}	3.30±1.06 ^{Bb}	3.17±0.24 ^{Bc}
30	2.30±1.11 ^{Ba}	2.17±1.10 ^{Cc}	3.14±1.00 ^{Cb}	1.54±1.02 ^{Dd}
60	2.19±0.22 ^{Cb}	3.14±0.5 ^{Ba}	3.14±1.00 ^{Ca}	2.37±1.02 ^{Cb}
90	2.39±1.13 ^{Bb}	3.11±1.06 ^{Bc}	3.61±1.17 ^{Aa}	3.60±1.14 ^{Aa}

Values are shown as mean ± standard error

Means within the same row of different small litters are significantly different at ($P < 0.05$).

Means within the same column of different capital litters are significantly different at ($P < 0.05$).

N: sample with 100 ppm sodium nitrite; BR: sample with red beetroot powder (0.065%); R: sample with Radish powder (0.065%); and NBR: sample with 100ppm sodium nitrite and red beet root powder (0.065%)

DISCUSSION

Sensory attributes of both raw and cooked oriental sausage produced by sodium nitrite and dry vegetable powdered of beetroot and radish during 3 months of frozen storage is presented in Fig. 1 & 2 respectively. The addition of beetroot only or with sodium nitrite combination and radish powder significantly improve the color scores ($P < 0.05$) of raw oriental sausage formulations at zero time and continuous throughout the three months of frozen storage period than the sausage produced by sodium nitrite alone. On the other hand, no significant difference ($P < 0.05$) could be established in odor and texture scores of raw sausage as a result of the addition of nitrite, beetroot, radish and beetroot and nitrite mix to sausage formulation at zero time and during whole storage time at -18°C for three months. The results reported are in harmony with Jin *et al.* [18], Sucu and Turp [19]. The cooking of oriental sausage samples significantly improved color in N group over the other groups at zero time and through 60 days of frozen storage. This is explained by the degradation effect of heat treatment on the colorant pigment in beetroot and radish (betalaine and anthocyanins) with the enhancement effect of heat treatment in fixation of cured color in sausage processed with sodium nitrite.

The flavor scores were significantly improved ($P < 0.05$) by the addition of beetroot powder and the combination of sodium nitrite and beetroot powder to sausage than that produce with nitrite only at zero time and through the whole storage time. In the contrary, the usage of radish powder negatively affected flavor scores due to the pungent odor trans-4-methylthio-3-butenyl-isothiocyanate present in radish root [20]. No significant differences could be detected in juiciness scores among different groups as a result of vegetable powder or

sodium nitrite. Similar results are reported [21]. Nonetheless [22] found that the addition of 2-3% leave and root radish powder in pork patties improved juiciness due to its high fibers.

The means value of instrumental color indexes (CIE L^* , a^* and b^*) of oriental sausage produced by sodium nitrite, beetroot and radish powder during 3 months of frozen storage are presented in Table 1. The lightness L^* were greatly affected by the added materials, where the lightness of NBR group was significantly ($P < 0.05$) improved (reduce) than N, R and BR groups at zero time and 30 days of frozen storage. Nonetheless, a gradual increase in lightness value among all groups was proved during 60 and 90 days of frozen storage with no significant difference among all groups at the end of storage time.

Incorporation of natural vegetable powder with and without sodium nitrite improved redness a^* significantly ($P < 0.05$) at zero time and though the whole storage period. Where the highest redness value (20.06 ± 4.15) was reported in NBR group at zero time and constitute over double the value in sausages produced by sodium nitrite only (9.96 ± 6.33). Also, the use of vegetable powders alone improved redness in produced sausages. This could be explained by the high betalain content in beetroot powder and which is considered a natural colorant compound and consisted of yellow beta-xanthins and red-violet betacyanins [23, 24]. Radish powder considers a potential natural colorant because of its content of anthocyanins pigment with good stability and gorgeous red hue [25].

The data of instrumental color was well correlated with sensory scores of color (Table 1). Significant reduction ($P < 0.05$) in redness values in all groups was observed during 2nd and 3rd month of frozen storage, with NBR group had the highest redness value followed by BR

and R groups, while N group showed the lowest redness value. The reduction of redness in sausages produced with vegetable powders may be referring to pigment degradation as mention by Fernandez-Gines *et al.* [26] who showed that lipid oxidation could play a role in the reduction of redness. The obtained results were in harmony with that recorded by Jin *et al.* [18], Sucu and Turp [19] who reported an increase in a^* values of sucuk (Turkish fermented beef sausage) during 84 days of cold storage.

Significant higher yellowness b^* values ($P<0.05$) were reported for NBR and R groups than N and BR groups at zero time and 1st month of frozen storage, nonetheless, yellowness values were reduced with the extension of the storage period in all groups to be the lowest in BR group followed by NBR, N and the highest in R group. The obtained results were in harmony with that recorded by Sucu and Turp [19] who found that, red beet powder in Turkish fermented beef as it significantly reduced yellowness (b^*) values on day 0 and at the end of the storage period.

The mean pH values of oriental sausage processed by sodium nitrite and natural vegetable powders during 3 months of frozen storage are shown in table 2. The mean pH values ranged from 6.28 in R group to 6.36 in N group at zero time with significantly higher values in N and BR group than R and NBR group. However, a significant reduction in pH value in all groups was noticed after 30 days of frozen storage, with no significant differences among all groups and the lowest reduction was noticed in BR group. Nearly similar results were recorded by Ahn *et al.* [22] and attributed this to the enhancing growth of lactic acid bacteria and increase lactic acid fermentation as a result of the addition of vegetables as radish and beetroot [27]. Continuous increase in pH value was observed at the end of the 2nd and 3rd month of frozen storage with the highest pH value in R and the lowest value in NBR. It is worth mentioning that pH value of all examined groups during the storage period was within the acceptable limit stated by ESS [28].

Means value of residual nitrite in oriental sausage processed by synthetic nitrite and natural vegetable powder during 3 months of frozen storage is recorded in table 3. Residual nitrite is detected in N and NBR groups, while for BR and R groups no residual nitrite could be identified. Although vegetable powders of beetroot and radish are used as a natural replacer for synthetic nitrite [18, 21, 22, 26], residual nitrite could be only detected in

samples that contain synthetic nitrite (N and NBR group). This may be referred to as the production of nitrite in a group containing vegetable powder is consumed in a chemical reaction with meat and an external source of the fermentative bacterial source is required to produce detectable quantities of nitrite. Residual nitrite ranged from 101 ± 3.25 to 78.3 ± 6.24 ppm at zero time for N and NBR groups respectively, with a non-significant decrease ($P<0.5$) after 30 days of frozen storage in the same groups. Nevertheless, a significant reduction ($P<0.5$) of residual nitrite is noticed during frozen storage to be 30.82 ± 4.25 , 33.46 ± 3.67 and 29.81 ± 5.33 , 30.1 ± 5.25 ppm after 60 and 90 days of frozen storage in N and NBR groups respectively. The obtained results were in harmony with that recorded by Sullivan [29], who stated that the amount of residual nitrite ranged from 40 to 100 ppm at the end of storage time in ham. In general, residual nitrite and nitrate detected in meat products are lower than the initial added amount as a result of the reaction of nitrate and nitrite with the meat component. This process depends on the type of meat product, processing condition and muscle composition [30-32]. It is worth mentioning that residual nitrite in NBR group was significantly higher ($P<0.05$) than in group N, where sodium nitrite is only used, although its value was significantly lower than N group at zero time and this indicated that beetroot powder added more nitrite continuously. Myers *et al.* [33] emphasized these results and stated that residual nitrite in meat products treated with synthetic sodium nitrite declined faster than those treated with natural nitrite. Residual nitrite in all examined samples at the production time and during the frozen storage period was within the permissible limit stated by ESS [28] that stated 100 ppm as the acceptable level in oriental sausage.

The means value of aerobic plate count (APC) and psychrotrophic bacterial count \log_{10} CFU/g of oriental sausage processed by nitrite and natural vegetable powder during 3 months of frozen storage are shown in Tables 4 and 5, respectively. The sausage produced with sodium nitrite (N) showed a significant reduction ($P<0.05$) in APC at zero time, in comparison with NBR, BR, R groups, this result emphasized the antimicrobial effect of synthetic nitrite [34]. While sausage produced using radish powder revealed the highest APC at zero time and continues to be the highest count till the end of storage time. Significant reduction ($P<0.05$) in APC was observed after 30 days of storage in N, BR, R and NBR as a result of the exhausting freezing effect on bacteria. The fluctuation

in APC among the different groups was noticed at 60 and 90 days of frozen storage, with significantly lower ($P<0.05$) APC in BR and NBR at the end of the frozen storage period in comparison to N and R groups. Sebranek *et al.* [35] nearly recorded similar counts and stated that a mix of vegetable powder and sodium nitrite has a good antibacterial effect. Lower results for pork patties treated by radish leave and root powder are recorded [22].

Concerning psychrotrophic bacterial count, a significant reduction ($P<0.05$) counts in NBR group was observed than all other groups. The obtained results showed that, storage at -18°C for 30 days proved a significant decrease ($P<0.05$) in psychrotrophic bacterial count in all examined groups with NBR group as the lowest psychrotrophic bacterial count. At the end of the frozen storage period, an increase in psychrotrophic bacterial count is recorded.

CONCLUSION

From the results achieved in the present study, it could be concluded that the use of beetroot and radish powders in oriental sausage manufacture significantly improve color characteristics through three months of frozen storage. Beetroot powder incorporated sausage showed a high flavor score and antibacterial effect. Also, at zero time, the mix of beetroot powder and synthetic nitrite used in oriental sausage processing indicated improvement in sensory attributes (color and flavor), with lower nitrite residue and good antibacterial activity. Although the addition of radish powder to sausage formula enhanced color score and redness of the product but negatively affected flavor score, with low antibacterial activity in comparison to beetroot and sodium nitrite treatment. Beetroot and radish powders revealed good coloring matter in oriental sausage with antibacterial activity and flavor enhancer. Further studies are required to investigate the role of natural nitrate in production of cured meat products.

REFERENCES

1. WHO's IARC, 2015. World Health Organization's International Agency for Research on Cancer: Ingested Nitrate and Nitrite and Cyanobacterial Peptide Toxins. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Volume 94 IARC ISBN-13 (Print Book).
2. Jeong, H.J., H.C. Lee and K.B. Chin, 2010. Effect of red beet on quality and color stability of low-fat sausages during refrigerated storage. *Korean J. Food Sci. Anim. Resour.*, 30: 1014-1023.
3. Zahran, Dalia and Kassem, Gehan, 2011. Residual Nitrite in Some Egyptian Meat Products and the Reduction Effect of Electron Beam Irradiation. *Advance Journal of Food Science and Technology*, 3: 376-380.
4. Lorenzo, J.M., J. Sineiro, I.R. Amado and D. Franco, 2014. Influence of natural extracts on the shelf life of modified atmosphere -packaged pork patties. *Meat Science*, 96(1): 526-534. <https://doi.org/10.1016/j.meatsci.2013.08.007>.
5. Bedale, W., J. Sindelar and A.L. Milkowski, 2016. Dietary nitrate and nitrite: benefits, risks and evolving perceptions. *Meat Sci.*, 120: 85-92. <https://doi.org/10.1016/j.meatsci.2016.03.009>.
6. Choi, Y.S., T.J. Jeong, K.E. Hwang, D.H. Song, Y.K. Han, H.W. Kim, Y.B. Kim and C.J. Kim, 2016. Combined effect of *Laminaria japonica* and transglutaminase on physicochemical and sensory characteristics of semi-dried chicken sausages. *Poultry Sci.*, 95: 1943-1949. doi: 10.3382/ps/pew093.
7. Chang, A.C., T.Y. Yang and G.L. Riskowski, 2013. Ascorbic acid, nitrate and nitrite concentration relationship to the 24 hour light/dark cycle for spinach grown in different conditions. *Food Chemistry*, 138(1): 382-388. <http://doi.org/10.1016/j.foodchem.2012.10.036>.
8. Choi, J.H., J.W. Jung, H.S. Ko, S.H. Kwon and J.S. Park, 2015. Process for the preparation of fermented broth using fruits and vegetables and process for the preparation of meat products without synthetic sodium nitrite using the same. *Korea Patent* 10-1526694.
9. Matilla, P. and J. Hellstrom, 2007. Phenolic acids in potatoes, vegetables and some of their products. *Journal of Food Composition and Analysis*, 20: 152-160. 10.1016/j.jfca.2006.05.007.
10. Ravichandran, K., A.R. Ahmed, D. Knorr and I. Smetanska, 2012. The effect of different processing methods on phenolic acid content and antioxidant activity of red beet. *Food Res. Int.*, 48: 16-20.
11. Ravichandran, K., N.M.M.T. Saw, A.A. Mohdaly and A.M. Gabr, 2013. Impact of processing of red beet on betalain content and antioxidant activity. *Food Research International*, 50(2): 670-675.

12. Lee, J.H. and K.B. Jin, 2012. Evaluation of antioxidant activities of red beet extracts and physicochemical and microbial changes of ground pork patties containing red beet extracts during refrigerated storage. Korean J. Food Sci. Anim. Resour., 32: 497-503.
13. Martinez, L., I. Cilla, J.A. Beltran and P. Roncales, 2006. Combined effect of modified atmosphere packaging and addition of rosemary (*Rosmarinus officinalis*), ascorbic acid, red beet root (*Beta vulgaris*) and sodium lactate and their mixtures on the stability of fresh pork sausages. J. Agric. Food Chem., 54: 4674-4680.
14. Cheng, C.F. and C.W. Tsang, 1998. Simultaneous determination of nitrite, nitrate and ascorbic acid in canned vegetable juices by reverse-phase ion-interaction HPLC. Food Additives and Contaminants, 15(7): 753-758.
15. AOAC, 2005. Association of Official Analytical Chemist, Official Methods of Analysis, Method 935.14 and 992.24. 18th Edition, AOAC International, Suite 500, 481 North Frederick Avenue, Gaithersburg, Maryland 20877-2417, USA.
16. CIE (Commission Internationale de l'Eclairage), 1976. Official recommendations on uniform color spaces. Color difference equations and metric color terms, Suppl. No. 2. CIE Publication No. 15 Colorimetry. Paris.
17. APHA, 2001. American public health association. Compendium of Methods for the Microbial Examination of food. 3rd Ed. American public health association, Washington, Dc., USA.
18. Jin, S.K., J.S. Choi, S.S. Moon, J.Y. Jeong and G.D. Kim, 2014. The assessment of red beet as a natural colorant and evaluation of quality properties of emulsified pork sausage containing red beet powder during cold storage. Korean Journal for Food Science of Animal Resources, 34(4): 472-481.
19. Sucu, C. and G.Y. Turp, 2018. The investigation of the use of beetroot powder in Turkish fermented beef sausage (sucuk) as nitrite alternative. Meat Sci., 140: 158-166.
20. Gutiérrez, R. and R. Perez, 2004. *Raphanus sativus* (Radish): Their Chemistry and Biology. The Scientific World Journal, 4: 811-37. 10.1100/tsw.2004.131.
21. Choi, Y.S., T.K. Kim, K.H. Jeon, J.D. Park, H.W. Kim, K.E. Hwang and Y.B. Kim, 2017. Effects of pre-converted nitrite from red beet and ascorbic acid on quality characteristics in meat emulsions. Korean Journal for Food Science of Animal Resources, 37(2): 288-296.
22. Ahn, S.J., H.J. Kim, N. Lee and C.H. Lee, 2019. Characterization of pork patties containing dry radish (*Raphanus sativus*) leaf and roots. Asian-Australasian Journal of Animal Sciences, 32(3): 413-420. <https://doi.org/10.5713/ajas.18.0384>.
23. Georgiev, V.G., J. Weber, E.M. Kneschke, P. Nedyalkov Denev, T. Bley and A.I. Pavlov, 2010. Antioxidant activity and phenolic content of betalain extracts from intact plants and hairy root cultures of the red beetroot *Beta vulgaris* cv.detroit dark red. Plant Food Hum. Nutr., 65: 105-111.
24. Račkauskienė, I., A. Pukalskas, P.R. Venskutonis, A. Fiore, A.D. Troise and V. Fogliano, 2015. Effects of beetroot (*Beta vulgaris*) preparations on the Maillard reaction products in milk and meat-protein model systems. Food Research International, 70: 31-39.
25. Kucza, M., 1996. Analysis of flavor precursors in radish and radish color extracts: oregon state university. Master thesis, Oregon, USA.
26. Fernandez-Gines, J.M., J. Fernandez-Lopez, E. Sayas-Barbera, E. Sendra and J.A. Perez- Alvarez, 2003. Effect of storage conditions on quality characteristics of bologna sausages made with citrus fiber. J. Food Sci., 68: 710-715.
27. Park, K.S. and K.H. Kyung, 1992. Growth stimulation of lactic acid bacteria by a radish component. Korean J. Food Sci. Technol., 24: 528-34.
28. ESS 1972, 2005. Egyptian standard specification for frozen sausage. Egyptian Organization for Standardization and Quality Control.
29. Sullivan, G.A., A.L. Jackson-Davis, S.E. Niebuhr, Y. Xi, K.D. Schrader, J.G. Sebranek and J.S. Dickson, 2012. Inhibition of *Listeria monocytogenes* using natural antimicrobials in no-nitrate-or-nitrite-added ham. Journal of Food Protection, 75(6): 1071-1076.
30. Cassens, R.G., 1997. Residual nitrite in cured meat. Food Technology, 51: 53-55.
31. EFSA European Food Safety Authority, 2003. Opinion of the scientific panel on biological hazards on the request from the commission related to the effects of nitrites/nitrates on the microbiological safety of meat products. EFSA Journal, 14(1): 1-31.

32. Jiménez-Colmenero, F. and J.B. Solana, 2009. Additives: Preservatives. In L. M. L. Nollet, & F. Toldrá (Eds.). Handbook of processed meats and poultry analysis (pp: 91-108). Boca Raton, Florida: CRC Press.
33. Myers, K., J. Cannon, D. Montoya, J. Dickson, S. Lonergan and J. Sebranek, 2013. Effects of high hydrostatic pressure and varying concentrations of sodium nitrite from traditional and vegetable-based sources on the growth of *Listeria monocytogenes* on ready-to-eat (RTE) sliced ham. Meat Science, 94(1): 69-76.
34. Jackson, A.L., C. Kulchaiyawat, G.A. Sullivan, J.G. Sebranek and J.S. Dickson, 2011. Use of natural ingredients to control growth *Clostridium perfringens* in naturally cured frankfurters and hams. J. Food Protect., 74: 417-424.
35. Sebranek, J.G., A.L. Jackson-Davis, K.L. Myers and N.A. Lavieri, 2012. Beyond celery and starter culture: advances in natural/organic curing processes in the United States. Meat Sci., 92: 267-273.