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Effect of vegetable powders as nitrite sources on the quality characteristics of cooked sausages

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Due to the potential health risk associated with nitrites, nitrite alternatives from vegetable sources in meat products have been investigated. Therefore, in this study cooked sausages, manufactured with 0.438 % celery powder, 0.425 % parsley powder, 0.29 % red beet powder and 0.404 % spinach powder were produced. These sausage samples were compared to a traditionally nitrite-cured control. The inclusion of red beet powder increased a* value of samples and resulted in the protection of the desired red color during storage. There were significant differences among samples treated with vegetable powders and the sausage with sodium nitrite (control) at the end of storage period. The lactic acid bacteria count was higher in samples treated with vegetable powders. After 4 weeks of storage, no significant difference in TBARS value was observed between the sausages with sodium nitrite (control) and the sausages with red beet and spinach powder.

Keywords: Nitrite, Red Beet, Celery, Spinach, Parsley, Cooked Sausage, *Staphylococcus xylosum*

INTRODUCTION

For centuries nitrate and nitrite have been used extensively in preserving meat products. Nitrate and, more specifically, nitrite, create the distinctive cured meat characteristics (Zhang et al., 2014). A primary function of nitrite is the production of the characteristic pink color of cured meats, which is desired by the consumer and is usually indicative of quality of cooked products, nitrite also inhibits lipid oxidation and contributes to desirable meat product flavor (Sebranek and Bacus, 2007). In addition the most important role of nitrite is its antimicrobial activity, specifically against controlling the growth of *Clostridium botulinum* (Terns et al., 2011).

Despite all of its desired properties, the safety of nitrite to human health has been questioned. Nitrite can cause the formation of carcinogenic N-nitrosamines in cured products due to its reaction with secondary amines and amino acids in muscle proteins. Residual nitrite in cured meats may form

N-nitrosamines in the gastrointestinal tract Thus; the meat industry continues to search for alternative methods to produce nitrite-free meats that maintain the color characteristics of nitrite cured meat products (Riel et al., 2017).

Regarding the potential health concerns of nitrite, consumers prefer natural additives instead of synthetic additives in meat products due to the health risks involved. Therefore, the replacement of chemical additive nitrite with natural additives have increased in recent years (Riel et al., 2017). Some Vegetables with high content of nitrate such as carrot, celery, spinach, red beet and parsley in the form of powder or extracts, qualify as nitrate sources (Riel et al, 2017; Ko et al., 2017).

Celery (*Apium graveolens* var. *graveolens*) products like juice concentrate and powder are the main widely used additives as nitrate sources in studies related to cured meat products (Sindelar et al., 2007).

Parsley (*Petroselinum crispum*) extract

powder is often used in spice mixtures. Due to its high nitrate content it is an innovative alternative for the direct addition of nitrite in the production of meat products (Riel et al., 2017).

Spinach (*Spinacia oleracea L*) has often been seen as a major vegetable source for nitrate in the human diet. According to Walker (1990), nitrate level in spinach is as high as 2,470 ppm.

Red Beet (*Beta Vulgaris*), is regarded as a rich source of nitrate and it contains bioactive phytochemicals, including phenolic compounds which functions as antioxidant and natural colorant components in meat products (Sucu & Turp, 2018).

Nitrate in these vegetables must be reduced to nitrite by microorganisms such as *Staphylococcus xylosum* and/or *Staphylococcus carnosus* (Sebranek and Bacus, 2007).

The objective of this study was to evaluate the possibility of replacing sodium nitrite with vegetable powders (celery, spinach, parsley and red beet), as a nitrite alternatives in the production of cooked sausage by examining the changes in some quality characteristics during 28 days of storage period at 4 °C.

MATERIALS AND METHODS

Production of vegetable powders

Celery, parsley, spinach and red beet were obtained from a local market in Giza, Egypt. After washing, the vegetables dried in a tray dryer at 60 °C. The dried vegetables were then ground using miller to obtain vegetable powders.

Production of cooked beef sausage

Fresh beef (semitendinosus muscle) cuts and beef fat were obtained from a local market in Giza. Meat was trimmed of fat and connective tissues. Meat and fat were then ground through a 3 mm diameter plate in a mincer, and then mixed with the other ingredients together. During manufacture, a commercial nitrate-reducing starter culture consisting of *Staphylococcus xylosum* (10^6 cfu/g) was added to the batter.

Five different formulations of produced sausages of 3 kg each were prepared. The formulations of produced sausages have been given in Table (1). Sausage batter was stuffed into natural casing using a sausage filling machine. Sausage was heated with steam at 80 °C for an hour with the aim of achieving a core temperature of 72 °C. The sausage was then chilled to room temperature,

packed and kept in refrigerated conditions (4°C) during analysis (28 days).

Proximate composition analysis

Chemical composition of the sausage samples including crude fat, moisture, ash and crude protein contents were determined according to the methods described by AOAC (2005).

pH determination

The pH value of the samples was determined using a pH-meter (Hanna Instruments, HI9219; Georgantelis et al., 2007). For each formulation, the pH was measured in duplicate.

Residual nitrite analysis

Residual nitrite in sausage samples was analyzed according to the method described by AOAC (2005).

Color measurement

Color measurements were conducted using a Konica Minolta Chroma-meter (model CR-410; Konica Minolta Sensing, Inc., Osaka, Japan) based on the CIE L*a*b* color space. CIE (Commission International de L'Eclairage) lightness "L*", redness "a*", and yellowness "b*" values were determined from four random different surfaces of the samples. The instrument was set for illuminant D-65 and 10° observer angle, and standardized using a white standard plate.

Thiobarbituric acid value (TBARS)

Lipid oxidation was evaluated on the basis of changes in Thiobarbituric acid-reactive substances (TBARS) during the chilled storage. The resulting color was measured at 538nm in a UV/VIS Spectrophotometer (Perkin-Elmer Lambda 15, Boston). The results were expressed as mg malonaldehyde/kg of sample (MD/ kg). TBARS determinations were performed in triplicate. The procedure for measurement of TBARS was based on methods used by Delgado-Pando et al., (2011).

Lactic acid bacteria

For each sample, 10 g (in replicate) was taken and placed in a sterile plastic bag (Sterilin, Stone, Staffordshire, UK) with 90 ml of peptone water (0.1%) for 1 min in a stomacher blender (Colworth 400, Seward, London, UK), appropriate decimal dilutions were pour-plated on De Man, Rogosa, Sharpe Agar (MRS agar, Merck, Germany)

Table (1): The formulation of sausages treated with vegetable powders

Ingredients	Treatments				
	C	CP	PP	RP	SP
Beef Meat (%)	70	70	70	70	70
Fat tissues (%)	14	14	14	14	14
Ice Water (%)	7.038	6.602	6.615	6.750	6.636
Starch (%)	3	3	3	3	3
Salt (%)	2	2	2	2	2
Onion (%)	1.2	1.2	1.2	1.2	1.2
Garlic (%)	1	1	1	1	1
Dried Skim Milk (%)	0.4	0.4	0.4	0.4	0.4
Glucose (%)	0.1	0.1	0.1	0.1	0.1
Spices Mixture (%)	1.2	1.2	1.2	1.2	1.2
Cherry Powder (%)	0.050	0.050	0.050	0.050	0.050
Starter Culture (%)	-	0.01	0.01	0.01	0.01
Sodium Nitrite (%)	0.012	-	-	-	-
Natural Nitrate (%)	-	0.438	0.425	0.290	0.404

C= control; CP= celery powder; PP= parsley powder; RP= red beet powder; SP= spinach powder

Plates for lactic acid bacteria (LAB) were incubated at 30 °C for 72 h (Sucu & Turp, 2018). The results were expressed as logarithms of colony forming units per gram (Log cfu/g).

Statistical analysis

A one-way analysis of variance (ANOVA) with two factors (treatments and storage period) was applied for each parameter using the SPSS program (V17.0). Means and standard errors were calculated and a probability level of $P < 0.05$ was used in testing the statistical significance of all experimental data.

RESULTS

Proximate composition

The results for the proximate composition of moisture, crude fat, crude protein and ash are presented in Table (2). There were a significant differences ($p < 0.05$) in the proximate composition of the samples treated with vegetable powders and the control (nitrite added). The moisture of the sausages increased with the addition of vegetable powders. In contrast, crude fat of the control sample was higher than that of samples treated with vegetable powders. The ash contents showed the same trend as that for moisture. There was a significant difference between the samples in protein content.

pH determination

pH associated with the chemical and microbiological reactions occurring food deterioration. The changes in the pH values of cooked sausages treated with vegetable powders

during refrigerated storage at 4 °C for 4 weeks are shown in figure (1). At zero time of refrigerated storage, the pH values of all treatments were ranged between 6.09 and 6.22 with significant differences between treatments. In general, the pH values gradually decreased with refrigerated storage time. At the end of storage, the pH values of all treatments were ranged between 5.14 and 5.46. The decreasing of pH values could be associated with the potential survival and slow growth of lactic acid-producing bacteria during and after product manufacture may also explain a decrease in pH values over time. Sindelar et al, (2007) noted that, the pH values of cured meat decreased after adding vegetable powder.

Sucu & Turp (2018) reported that, Vegetable-based additives such as leek, lyophilized celery product, parsley extract powder and fermented spinach extract led to a decrease in pH of cured meat products.

Kim et al., (2017) reported that, addition of different concentrations of spinach extract affected the pH values of meat products. The pH values of the meat product can be affected by some factors such as product characteristics, production process, type and amount of additives.

Residual nitrite contents

Addition of a nitrate reducing starter culture (*staphylococcus xylosus*), nitrate was reduced to nitrite and resulted in some typical properties of cured sausage in samples treated with vegetable powders, although no chemical curing salt was added to these samples.

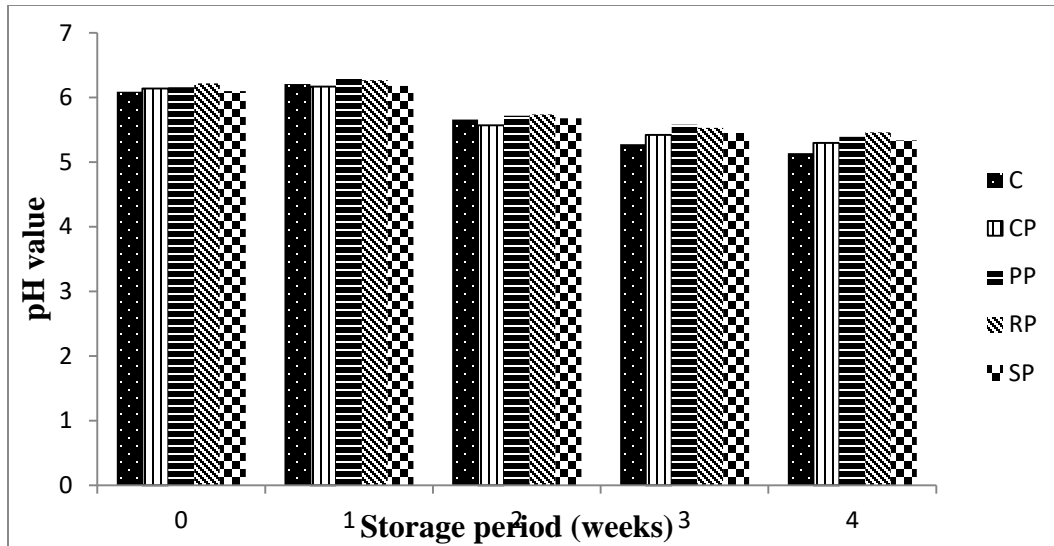


Figure (1): pH values of cooked sausages formulated with vegetable powders during refrigerated storage

C= control; CP=celery powder; PP=Parsley Powder; RP=red beet powder; SP=spinach powder

Table (2): proximate composition of the cooked sausages formulated with vegetable powders

Treatments	Moisture (%)	Fat (%)	Protein (%)	Ash (%)
C	62.03 ±0.14 ^c	19.84 ±0.53 ^a	12.25±0.15 ^c	2.80±0.13 ^c
CP	64.00±0.05 ^{ab}	17.30±0.12 ^b	12.51±0.33 ^a	3.30±0.17 ^a
PP	64.46 ±0.09 ^a	17.00±0.25 ^c	12.45±0.16 ^b	3.23±0.05 ^a
RP	63.65 ±0.08 ^b	17.10±0.45 ^{bc}	12.48±0.19 ^b	3.02±0.07 ^{ab}
SP	63.80±0.05 ^{ab}	17.15±0.61 ^{bc}	12.32±0.16 ^b	3.15±0.18 ^b

C= control; CP=celery powder; PP=Parsley Powder; RP=red beet powder; SP=spinach powder
All values are the mean ± SD (n=3).

Different small letters indicate significant among means in the same column (p< 0.05).

Table (3): Changes in residual nitrite content (mg/kg) of cooked sausages formulated with vegetable powders during refrigerated storage

Treatments	Storage period (weeks)				
	0	1	2	3	4
C	95.50 ±0.17 ^a	73.53 ±0.24 ^a	65.71±0.21 ^a	50.93±0.18 ^a	42.45±0.35 ^a
CP	52.41±0.21 ^c	47.33 ±0.34 ^c	42.15±0.32 ^c	36.67 0.14 ^c	32.75 0.26 ^c
PP	55.34 ±0.48 ^{bc}	50.63 ±0.43 ^{bc}	45.49 ±0.11 ^{bc}	39.03 ±0.33 ^{bc}	35.15±0.24 ^b
RP	61.48 ±0.33 ^b	55.41 ±0.18 ^b	50.60 ±0.36 ^b	44.30 ±0.1 ^{fab}	40.51 ±0.15 ^a
SP	57.80 ±0.37 ^{bc}	54.18 ±0.22 ^b	47.90 ±0.43 ^b	42.12±0.25 ^b	37.45±0.41 ^{ab}

C= control; CP=celery powder; PP=Parsley Powder; RP=red beet powder; SP=spinach powder.
All values are the mean ± SD (n=3).

Different small letters indicate significant among means in the same column (p< 0.05).

Table (4): Changes in lightness (L) values of cooked sausages formulated with vegetable powders during refrigerated storage

Treatments	Storage period (weeks)				
	0	1	2	3	4
C	63.05 ±1.2 ^a	64.63±0.8 ^a	65.58±0.6 ^a	64.91±0.7 ^a	64.25±1.2 ^b
CP	61.95 ±0.6 ^a	62.10±0.5 ^a	63.03±0.9 ^{ab}	64.93±1.2 ^a	65.45±0.7 ^a
PP	61.23 ±0.07 ^a	62.61 ±1.3 ^a	63.93±1.2 ^{ab}	64.17±1.2 ^a	65.59±0.5 ^a
RP	57.45±1.7 ^b	59.29±1.3 ^b	59.80±1.2 ^c	60.18±0.6 ^b	60.85±1.5 ^c
SP	60.43±1.4 ^{ab}	61.12±0.8 ^{ab}	62.60±1.6 ^b	63.38±0.5 ^{ab}	64.69±0.8 ^{ab}

C= control; CP=celery powder; PP=Parsley Powder; RP=red beet powder; SP=spinach powder

All values are the mean \pm SD (n=3).

Different small letters indicate significant among means in the same column ($p < 0.05$).

Table (5): Changes in redness (a) values of cooked sausages formulated with vegetable powders during refrigerated storage

Treatments	Storage period (weeks)				
	0	1	2	3	4
C	13.15 \pm 0.4 ^b	11.81 \pm 0.3 ^b	10.74 \pm 0.5 ^{bc}	9.33 \pm 0.3 ^{bc}	8.65 \pm 0.4 ^c
CP	10.76 \pm 0.6 ^c	10.45 \pm 0.3 ^c	10.17 \pm 0.4 ^c	8.70 \pm 0.4 ^c	8.38 \pm 0.3 ^c
PP	12.75 \pm 0.4 ^{bc}	11.30 \pm 0.6 ^{bc}	11.06 \pm 0.3 ^b	10.15 \pm 0.6 ^b	9.65 \pm 0.2 ^b
RP	21.00 \pm 0.3 ^a	18.93 \pm 0.2 ^a	18.71 \pm 0.3 ^a	18.49 \pm 0.4 ^a	17.80 \pm 0.3 ^a
SP	13.54 \pm 0.5 ^b	11.95 \pm 0.2 ^b	11.50 \pm 0.6 ^b	10.70 \pm 0.5 ^b	9.68 \pm 0.4 ^b

C= control; CP=celery powder; PP=Parsley Powder; RP=red beet powder; SP=spinach powder.

All values are the mean \pm SD (n=3).

Different small letters indicate significant among means in the same column ($p < 0.05$).

Table (6): Changes in yellowness (b) values of cooked sausages formulated with vegetable powders during refrigerated storage

Treatments	Storage period (weeks)				
	0	1	2	3	4
C	12.91 \pm 0.8 ^b	11.52 \pm 0.1 ^{ab}	10.75 \pm 0.4 ^b	10.40 \pm 0.5 ^b	9.38 \pm 0.7 ^b
CP	16.05 \pm 0.8 ^a	14.78 \pm 0.3 ^a	14.08 \pm 0.6 ^a	13.28 \pm 0.7 ^a	12.13 \pm 0.4 ^a
PP	14.48 \pm 0.3 ^{ab}	13.35 \pm 0.5 ^a	12.98 \pm 0.8 ^{ab}	12.40 \pm 0.6 ^a	11.83 \pm 0.3 ^{ab}
RP	9.12 \pm 0.6 ^c	10.95 \pm 0.5 ^b	11.40 \pm 0.4 ^b	12.18 \pm 0.4 ^a	12.75 \pm 0.6 ^a
SP	10.21 \pm 0.4 ^{bc}	9.66 \pm 0.8 ^b	9.26 \pm 0.4 ^b	8.93 \pm 0.3 ^b	8.18 \pm 0.6 ^b

C= control; CP=celery powder; PP=Parsley Powder; RP=red beet powder; SP=spinach powder.

All values are the mean \pm SD (n=3).

Different small letters indicate significant among means in the same column ($p < 0.05$).

Residual nitrite contents (The nitrite content after the production process) of the cooked sausages treated with vegetable powders during storage period are shown in Table (3). The amount of nitrite formed during production depends on the quantities of ingoing nitrate. However, when using vegetable extract the amount of nitrite formed during production is unknown (Sindelar, 2014). The residual nitrite contents decreased over time in all samples. Although a greater nitrite reduction was observed in the control sample (55.5 % reduction) than in celery powder (37.5 %), parsley powder (36.48 %), spinach powder (35.20 %) and red beet powder (34.10%) after 4 weeks storage. After 4 weeks of storage, the control sample (sausage with sodium nitrite) had significantly more residual nitrite than the sausages treated with vegetable powders. In addition there were significant differences ($p < 0.05$) among samples treated with vegetable powders and the sausage with sodium nitrite (control) throughout 4 weeks of storage. Myers et al., (2013) reported that, gradual decline of residual nitrite throughout the shelf life of meat products. The decrease in the nitrite content during processing was reported to be due to the conversion of nitrite into nitric oxide and nitrous

oxide as well as nitrite oxidation to nitrate over time.

Color

The effect of adding vegetable powders instead of sodium nitrite on the color of produced sausages during refrigerated storage can be seen in Table (4). Lightness (L) values of all samples increased significantly during storage ($p < 0.05$). The color of sausages treated with sodium nitrite (control) was lightest on day 0, while the color of the cooked sausages treated with vegetable powders was darker. There are some studies which put forth that a decrease was detected in L* values of sausages as a result of using cabbage and radish as natural curing sources (Ko et al, 2017) and low fat frankfurter by the use of red beet extract (Hwang et al, 2017).

Overall, a* values in all samples decreased during storage (Table 5). The a* values of the sausages treated with red beet was higher as compared to the control and sausages with CP, PP and SP on the final day of storage. These results are in agreement with Sucu & Turp (2018), they found that, the addition of red beet powder caused an increase in a* values of Turkish fermented beef sausage (sucuk) in both the 0th and 84th days. These results indicate that the red

beet is very effective in increasing the redness and maintaining the desired red color of sausages. Similarly, it has been pointed out in studies with emulsified pork sausage (Jin et al, 2014) and emulsified beef sausage (Turp et al, 2016) that redness increased with an increased amount of red beet addition which was attributed to the betalain content.

Results in Table (6) show that, the yellowness b^* values of parsley powder and celery powder treatments were significantly more ($p < 0.05$) yellow than the control sample (nitrite added), red beet powder and spinach powder samples. These results are in agreement with Riel et al, (2017) they found that, the ingoing parsley extract as a nitrite source into mortadella sausages led to the rise in b^* values of the sausages. Likewise, the addition of celery powder to ham resulted in greater yellowness in comparison with the conventional product (Horsch et al, 2014).

Sucu & Turp (2018) reported that, use of red beet powder in Turkish fermented beef sausage as nitrite alternative caused a significant decrease in the yellowness (b^*) values on day 0 and at the end of storage period.

Kim et al (2017) found that, the yellowness b^* values of cooked cured meat were decreased with increasing levels of fermented spinach extract.

TBARS values

Lipid oxidation is one of the main factors affecting the quality of characteristics of meat and meat products during storage period since it can result in the development of rancidity and have an impact on the product nutritive value, color and flavor (Kim et al., 2017). The results of TBARS values of the cooked sausages containing vegetable powders during refrigerated storage all shown in figure (2). TBARS values of all samples increased significantly with the storage period. The initial TBARS values for control and all treatments did not significantly ($p < 0.05$) differ. At the end of storage, the TBARS values of cooked sausage containing vegetable powders ranged between 0.31 and 0.35 mg MD/Kg sample and the sausage with sodium nitrite (Control) had the lowest TBARS value. After 4 weeks of storage, no significant difference in TBARS value was observed between the sausages with sodium nitrite (control) and the sausages with red beet and spinach powder. A TBARS value of 0.5 to 1.0 is considered to be the threshold for oxidized odor (Tarladgis et al, 1960). All of the TBARS values measured in this study were less than 0.5mg MD/Kg. Nitrite is an effective antioxidant in meat

products (Sindelar et al, 2007) and is known to possess antioxidant activity at concentrations as low as 40-50 ppm nitrite (Peg and Shahidi, 2008). Therefore, the initial amount of nitrite in the sausage with sod-nitrite (control) (95.5ppm) and the sausages containing vegetable powders (52.41-61.48) in the present study would be sufficient to prevent or reduce lipid oxidation.

Studies that used radish in sausage (Ko et al, 2017) and celery juice powder in turkey bologna (Djeri & Williams, 2014) have indicated that the use of vegetable resulted in no significant difference compared to conventional nitrite in terms of TBARS values. Kim et al, (2017) reported that, addition of fermented spinach extract into pork lion had a comparable protective effect against lipid oxidation with regard to that of nitrite added control. Hwang et al., (2017) concluded that TBARS values of frankfurters treated with fermented red beet extracts did not exceed 0.5mg MD/Kg by the end of the storage period. Sindelar et al, (2007), showed that no significant difference in lipid oxidation between control with nitrite and treatments with different levels of vegetable juice powder. Bergman et al., (2001), reported that aqueous spinach extract had considerable antioxidant capacity.

Lactic acid bacteria

Lactic acid bacteria are important since they limit the growth of some undesirable microorganisms, improve the physicochemical properties which contributing to the aroma and flavor of fermented meats (Leroy & Devugst, 2004). Figure (3) shows the lactic acid bacteria count in the samples treated with vegetable powders during refrigerated storage for 4 weeks. The initial counts of lactic acid bacteria (LAB) in all samples ranged between 1.59 and 2.91 log cfu/g. The counts of LAB in all samples increased significantly during the period of storage and at the end of the storage, LAB count of the control sample (nitrite added) reached 3.88 log cfu/g, meanwhile, the sausages treated with vegetable powders ranged between 4.13 and 4.73 log cfu/g. The lowest LAB count ($p < 0.05$) during storage period was observed in the control sample. Djeri & Williams (2014) reported that, LAB count in turkey bologna samples containing celery juice powder was higher than that of the control sample with nitrite. Fista et al., (2004) found that, no statistically significant difference in the LAB count was observed between the sausages containing nitrite and leek.

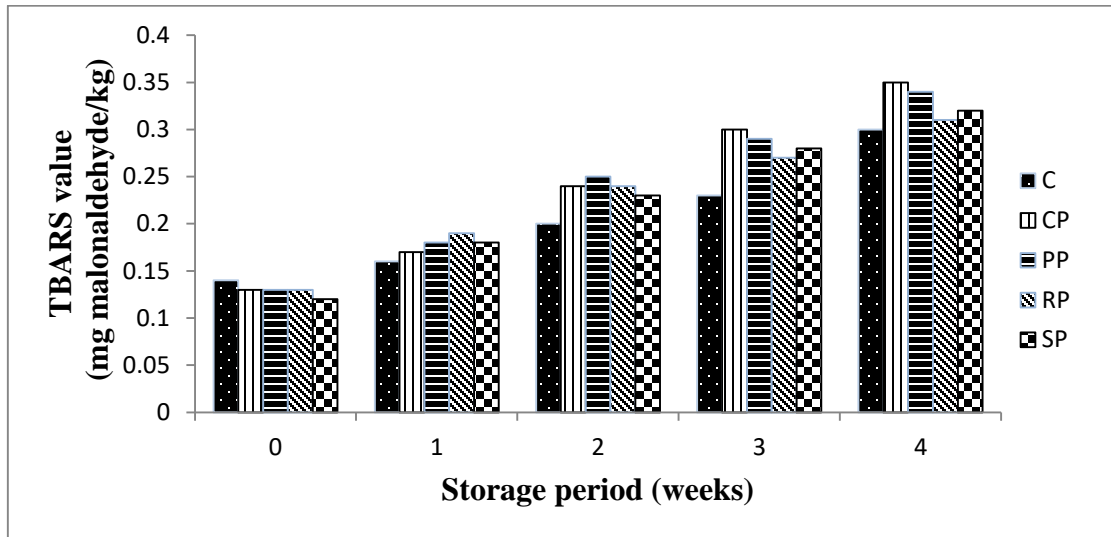


Figure (2): TBARS values of cooked sausages formulated with vegetable powders during refrigerated storage

C= control; CP=celery powder; PP=Parsley Powder; RP=red beet powder; SP=spinach powder

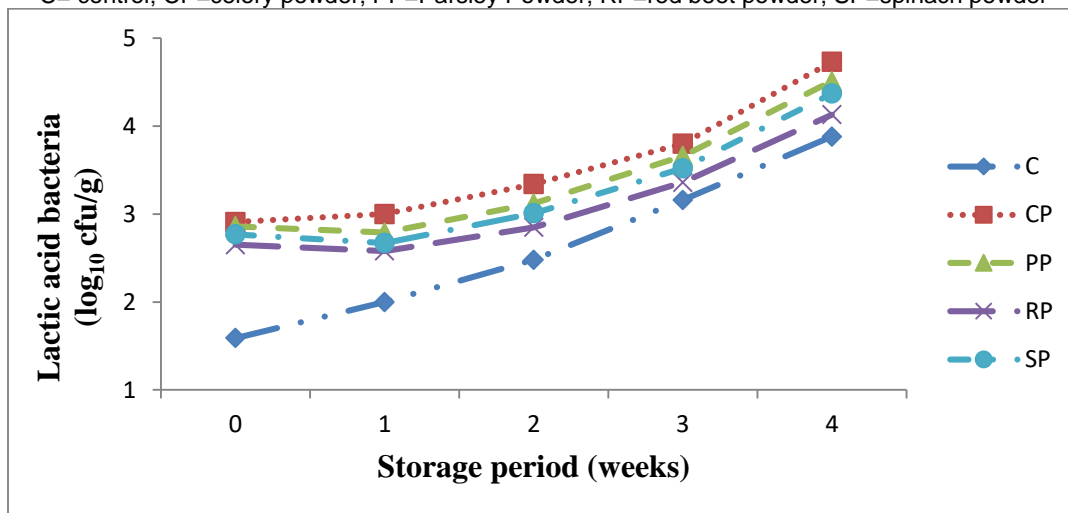


Figure (3): Lactic acid bacteria count of cooked sausages formulated with vegetable powders during refrigerated storage

C= control; CP=celery powder; PP=Parsley Powder; RP=red beet powder; SP=spinach powder

Tsoukalas et al., (2011) found that, sausages produced with 0.84% and 1.68% freeze-dried leek powder (FDLP) had the highest LAB count during storage period. Among the samples treated with vegetable powders, the LAB count in the present study was highest in the sausages containing celery powder.

CONCLUSION

This work showed that replacing nitrite by vegetable powders is an effective strategy to develop new meat products. At the same time, lower residual nitrite levels could be achieved with the use of vegetable powders and marketing of

these products may consequently reduce consumers' intake of nitrite. Redness value (a*) of the samples increased and was well protected during the storage when an increased amount of beetroot powder was used. No significant difference in TBARS value was observed between the sausages with sodium nitrite (control) and the sausages with red beet and spinach powder at the end of storage. After 4 weeks of storage, the control sample (sausage with sodium nitrite) had significantly more residual nitrite than the sausages treated with vegetable powders. The lowest LAB count (p<0.05) during storage period was observed in the control sample. The

traditional meat products with no/reduced synthetic nitrite will be a new opportunity for consumers.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest".

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AUTHOR CONTRIBUTIONS

Y. Riyad and M. Abdel-Aziz contributed in the research idea and designed the experiments and reviewed the manuscript, I. Ismail processed the experiments also wrote the manuscript. All authors read and approved the final version.

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