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SPINACH POWDER ADDITION TO SUCUK FOR ALTERNATIVE TO NITRITE ADDITION

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Abstract: *The effect of spinach powder addition on lipid oxidation, color and sensorial characteristics of traditional fermented meat product (Sucuk) was studied. Sucuk was manufactured with three treatments (control, 1% spinach powder added, 3% spinach powder added) and two replicates. Sucuks fermented by spontaneous fermentation. The pH of the spinach powder added samples were significantly ($p<0.01$) lower than the control group. L*and a* values of the control group was significantly ($p<0.01$) higher than treament groups but b* values of 3% spinach powder added group was significantly ($p<0.01$) higher than the others. TBA values of the treatment groups were significantly ($p<0.051$) lower than the control group during the beginning of the fermentation period. On the third day, TBA value of the control group was low, on the 7th and 14th days TBA values of the treatment groups were found to be significantly lower. There was no significant difference was found between the sucuk samples at the end of the storage. When the sensory properties were analyzed, the color, taste, texture and general acceptability values of spinach powder added sucuk samples scores were found to be significantly ($p<0.01$) lower than the control group.*

Keywords: *Color, Nitrite, Spinach powder, Sucuk, TBA.*

1. Introduction

Sucuk is a traditional dry-fermented sausage which consumed commonly in Turkey. Sausage produced by beef and/or water buffalo and sheep meat, sheep tail fat, curing ingredients (nitrite and/or nitrate), salt, sucrose, and various spices. After filling the sucuk dough into the natural air-dried bovine small intestines or small casings with same characteristics, the fermentation and drying process steps are occurred under climatic or natural conditions [8]. Fermentation occurs by two different way; spontaneous fermentation or commercial starter culture addition [5]. In recent years, consumers have been preferred the

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traditional products without the additives which have harmful adverse effects. Consumers avoid the consumption of synthetic additives used processed meat products such as sucuk. The use of vegetable-based nitrate containing additives in the curing of meat products is insufficient to achieve the desired benefits at sausage characteristics. When naturally cured products compared to the products which nitrite is added, requires more research for preventing or developing color, texture, lipid oxidation inhibition and antimicrobial effect [10]. Chang et al. [3] said that the green vegetables are rich in nitrate/nitrite in human nutrition. Nitrate levels are significantly higher in plants such as spinach [17]. Nitrite forms carcinogenic, teratogenic and mutagenic N-nitroso compounds by reacting with secondary and tertiary amines [18].

The aim of the research is to determine some quality characteristics of sucuk which have been produced by spinach powder addition.

2. Materials and Methods

2.1. Material

Beef and fat purchased from a local butcher. The beef and fat stored at 4ºC. Natural casings and other spices etc. purchased from a local market in Afyonkarahisar.

2.2. Sucuk Preparation

The cattle of 2.5, 3-year-old Montafon race meat was purchased from a local butcher in Afyonkarahisar. The sucuk dough formulation given in Table 1. Meat and fat divided into 6 equal pieces in duplicate as control (C), 1% spinach powder added samples (S1) and 3% spinach powder added samples (S2).

2.3. Proximate Analysis

Moisture were determined according to AOAC (1990) [1] method, and pH was measured by Metler Toledo model pH-meter. The lactic acid content was determined with [1] and the results obtained were expressed as %w/w lactic acid. LabTouch-aw (Novasina AG, Switzerland) water activity meter was used to determine a_w of sucuk samples [13].

Sucuk formulation Table 1

Ingredients [g]	**C**	**S1**	**S2**
Meat+Fat	1000	1000	1000
Salt	18	18	18
Garlic	15	15	15
Red pepper	4.5	4.5	4.5
Red pepper (hot)	7.5	7.5	7.5
Black pepper	7.5	7.5	7.5
Cumin	7.5	7.5	7.5
Allspice	2.2	2.2	2.2
$NaNO_2$ (ppm)	80	-	-
Spinach powder	-	10	30

2.4.Thiobarbituric Acid (TBA) Determination

Lipid oxidation was determined by the method of [16]. The amount of malondialdehyde (MDA) calculated by multiplying the absorbance values by the factor 7.8.

2.5. Color Values

CIE color values (L*, a*, b*) of the sucuk samples were determined by X-Rite (Ci6X) colorimeter. The color was measured at the cross-sectional surface of sucuk samples during the ripening.

2.6. Nitrite Analysis

Residual nitrite was analyzed according to the method described by [1]. A standard curve (r>0.99) used for the determining of the residual nitrite content of sucuk.

2.7. Sensorial Analysis

Sucuk samples were served to 12 trained assessors contained 6 of the male and 6 of the female, from Afyonkarahisar Afyon Health School Nutrition and Dietetics students. Their ages were between 21-25 and trained about the production of sucuk and the sensorial characteristics of sucuk before panel Sucuk sampleswas grilled and evaluated for the taste, odor, texture and overall acceptance parameters at 14^{th} day. Sensorial characteristics were scored from 1 to 9 (1-3: bad, 4-5: not bad, 6-7: good, 8-9: excellent).

2.8. Statistical Analysis

The analyze results were subjected to statistical analysis using SPSS 17. Analysis of variance (ANOVA) was used to evaluate the effect of ripening time (days) and treatment (spinach powder addition) on the analyzes. Values were further analyzed, using Duncan's Multiple Range Test when the effect of the ripening time and treatment were found significant.

3. Results and Discussion

Table 2 shows the pH, lactic acid content, moisture and a_w of sucuk samples.

Table 2

pH, lactic acid moisture content and water activity of sucuk samples

Treatment	pH	Lactic acid [%]	Moisture [%]	a_w
Control	5,47 ± 0,74^{a}	0,01 ± 0,005^{b}	38,26 ± 12,87	0,85 ± 0,11
S1	5,38 ± 0,71^{b}	0,02 ± 0,005^{a}	37,21 ± 12,97	0,86 ± 0,10
S2	5,33 ± 0,74^{c}	0,01 ± 0,004^{b}	36,53 ± 12,44	0,85 ± 0,11
Sig	**	**	ns	ns
Days				
0	6,04 ± 0,04^{a}	0,01 ± 0,001^{b}	51,94 ± 1,35^{a}	0,93 ± 0,004^{a}
1	6,06 ± 0,10^{a}	0,01 ± 0,004^{b}	49,65 ± 2,51^{a}	0,93 ± 0,002^{a}
3	4,09 ± 0,05^{b}	0,01 ± 0,002^{b}	42,16 ± 3,75^{b}	0,92 ± 0,004^{a}
7	5,12 ± 0,08^{c}	0,02 ± 0,004^{a}	34,67 ± 1,86^{c}	0,86 ± 0,030^{b}
14	5,17 ± 0,06^{d}	0,02 ± 0,002^{a}	28,15 ± 1,12^{d}	0,82 ± 0,002^{c}
28	5,88 ± 0,32^{e}	0,02 ± 0,002^{a}	17,46 ± 1,40^{e}	0,65 ± 0,016^{d}
Sig	**	**	**	**
Treatment*Days				
Sig	**	ns	ns	**

The spinach powder addition to samples and the storage time were significantly (p<0.01) affected the pH values of the sucuk samples. The lowest pH value was found in S2 samples in the treatment groups. This may be the usage of the high ratio of spinach powder (Table 1). Sucu & Turp [15] found that the beetroot powder addition to the sucuks didn't affect the pH values moisture contents. Also, Djeri et al.

[4] reported that there was no significant difference were found between the control and treatment groups of celery juice added turkey Bologna samples. Riel et al. [12] showed that the pH of the parsley extract addition with different concentrations on mortadella-type sausages significantly lowers the pH of the samples. It has been reported by Hwang et al. [6] , added the nitrite extracts to sausage samples significantly affects the pH values. Also, Kim et al., [9] demonstrated that the positive and negative control groups of samples have higher pH values, than the fermented spinach extracts added raw cured pork loins. Our results are similar because the pH of the control group (5.47 ± 0.74) is higher than the treatment groups. Pexara et al., [11] reported that the fermentable carbohydrates decreases the pH of the meat. In Table 2, it was observed that S1 group had more lactic acid ratio but ph value was among other samples. Lactic acid bacteria produce acids like lactic acid, acetic acid, formic acid, and the formation of acid levels depends on the genus, species and growth conditions [2]. The pH values of samples can be affected by the development of bacteria.

Color values of spinach powder added sucuk samples are shown in Table 3. The effect of spinach powder addition on L*, a* and b* values of the groups was found significantly ($p<0.01$) different. The lightness of the control group is higher than the other treatment groups. The highest redness was found in the control group and the lowest was at S2 samples. The lightness of samples significantly ($p<0.01$) decrased by the fermentation and ripening time. This can be explained with the pigments of spinach powder adversely affect the a* value of the sample.

Table 3

Color parameters of spinach powder added sucuk samples

Treatment	**L***	**a***	**b***
Control	47,92 ± 4,73^{a}	11,29 ± 2,83^{c}	17,73 ± 2,00^{b}
S1	42,94 ± 3,68^{b}	9,59 ± 2,64^{b}	18,32 ± 2,61^{b}
S2	44,50 ± 3,02^{b}	7,20 ± 1,45^{a}	19,43 ± 1,82^{a}
Sig	**	**	**
Days			
0	49,18 ± 4,05^{a}	8,13 ± 1,40^{c}	20,55 ± 1,66^{a}
1	45,05 ± 2,65^{b}	11,65 ± 2,95^{a}	20,82 ± 1,59^{a}
3	44,96 ± 1,82^{b}	11,11 ± 2,46^{a}	17,44 ± 1,06^{b}
7	43,29 ± 2,32^{b}	10,34 ± 2,04^{ab}	17,71 ± 2,12^{b}
14	42,96 ± 2,40^{b}	9,00 ± 2,37^{bc}	17,45 ± 0,81^{b}
28	45,29 ± 7,69^{a}	5,93 ± 1,79^{d}	16,99 ± 2,31^{b}
Sig	**	**	**
Treatment*Days			
Sig	ns	ns	*

$^{a-c}$: Mean values within the same column followed by the different lowercase letters show significant difference ($P< 0.05$) by Duncan test. Results were given Mean±SD.

The a* values of samples increases by the fermentation period of the beginning and reaches the highest value at 3rd day. Then decreases by the time. This can be explained by the microorganisms which reduce nitrate to nitrite during fermentation and NO-myoglobin formation may occur. Kim et al. [9] found that the raw curing of meats with fermented spinach extract decreases the L* and b* values by the increasing the extract addition. They also concluded that the a* value of meat can be improved by increasing the level of fermented spinach extract so the brine contains more nitrite. Thus NO-myoglobin formation improved. Hwang et al. [6] reported the pork batters formulated with fermented lettuce extract shows lower redness compared with other samples. Djeri and Williams [4] showed that the celery and cherry juice addition to bologna results darker samples also higher a* and b* values were seen in the same sample.

Lipid oxidation causes the rancidity and also affects the sensorial and nutritive value of the food [7]. According to Table 4 TBA values of groups increases by the time. At the beginning of the fermentation treatment groups TBA values significantly ($P< 0.05$) lower than the control group (Day 0, Day1). There was no significant difference was found between the groups at the end of the storage period. Sindelar et al. [14] concluded that there was no significant difference was found between sodium nitrite or celery juice powder added groups TBA values. Hwang et al. [6] reported fermented lettuce and spinach extract added pork sausages have lower TBA values than the other groups. Sucu and Turp [15] concluded that the TBA values of samples don't differ significantly at the end of storage at beetroot added fermented sausages. But they found that during storage (28th and 56th days) the TBA values of treatment groups was higher than control group.

Table 4

TBA values of spinach powder added sucuk samples

	Treatment		
Days	**Control**	**S1**	**S2**
0	0.203 ± 0.022^{eA}	0.070 ± 0.033^{dB}	0.055 ± 0.022^{cB}
1	0.339 ± 0.028^{dA}	0.051 ± 0.017^{dB}	0.035 ± 0.006^{cB}
3	0.425 ± 0.017^{cB}	0.542 ± 0.017^{bA}	0.581 ± 0.496^{bA}
7	0.644 ± 0.005^{bA}	0.591 ± 0.02^{bB}	0.517 ± 0.004^{bC}
14	0.577 ± 0.055^{bA}	0.41 ± 0.028^{cB}	0.558 ± 0.028^{bA}
28	1.069 ± 0.044^{a}	1.037 ± 0.011^{a}	1.115 ± 0.11^{a}

$^{A-C}$: Mean values within the same line followed by the different lowercase letters shows significant difference ($P< 0.05$) by Duncan test. $^{a-c}$: Mean values within the same column followed by the different lowercase letters shows significant difference ($P< 0.05$) by Duncan test. Results was given Mean±SD.

Color is an important criterion for purchase choice of consumers. When making sensory analyzes, the sucuks were quite dry (28.15 ± 1.12). Dry sausage samples which contain spinach powder make the color of the samples darker.

There was no significant difference was found in the manner of appearance and odor parameters. When analyzing the color, odor, texture and general acceptability of the sucuk samples, there were no significant differences was found between the spinach added samples but all scores were significantly lower than the control group. Sucu and Turp [15] reported that the beetroot powder addition to fermented sausages ensures some advantages. Sindelar et al. [14] reported the vegetable juice powder addition to ham didn't affect the color in treatment groups.

Aroma scores were found higher than some treatment groups but not higher than others. Riel et al. [12] concluded that there were no significant differences were found between the groups in the manner of redness of the products.

Table 5

Sensorial analysis of spinach powder added sucuk samples

Treatment	Color	Appearance	Odor	Taste	Texture	General Acceptability
Control	5,22 ± 1,44[a]	6,50 ± 1,38	7,11 ± 1,23	7,22 ± 0,94[a]	6,67 ± 1,24[a]	7,06 ± 1,26[a]
S1	3,89 ± 1,84[b]	5,72 ± 1,84	6,72 ± 1,49	5,39 ± 1,79[b]	4,78 ± 2,13[b]	5,56 ± 1,42[b]
S2	3,00 ± 1,78[b]	5,33 ± 1,41	5,94 ± 1,70	5,00 ± 1,49[b]	4,67 ± 1,57[b]	5,11 ± 1,41[b]
Sig	**	ns	ns	**	**	**

[a-c]: Mean values within the same column followed by the different lowercase letters shows significant difference by Duncan test. Results was given Mean±SD.

Table 6

Residual nitrite content of sucuk samples

	Residual Nitrite Content [mg/kg]
Control	0,09 ± 0,01[b]
S1	0,09 ± 0,00[b]
S2	0,15 ± 0,00[a]

[a-b]: Mean values within the same column followed by the different lowercase letters shows significant difference (P< 0.05) by Duncan test. Results was given Mean±SD.

As a result of the statistical analysis, there was no significant difference was found between the control group and the S1 spinach powder added group for nitrite, whereas the S2 group was significantly higher than the other groups ($p < 0.01$). The same nitrite formula was also made for spinach powder and the average nitrite value of spinach was found to be 0.54 ± 0.05.

4. Conclusions

There is a potential to use the nitrate containing vegetables to replacing of nitrate/nitrite in the meat products which provides the consumer choice. As a result of the research, it can be said that the spinach powder which is added to the sucuk can be used as a source of nitrite to inhibit the TBA formation but the color and sensorial characteristics adversely

affected. Further researches must be conducted on a transformation of spinach powder to obtain acceptable color formation in sucuk. Some research can be designed with the addition of starter cultures and spinach powder together which reduces nitrate to nitrite can be added to sucuk dough.

References

1. AOAC., 1990. Association of Official Analytical Chemists, Arlington, VA..
2. Borch, E., Berg, H., Holst, O., 1991. Heterolactic fermentation by a homofermentative Lactobacillus sp. during glucose limitation in anaerobic continuous culture with complete cell recycle. In: Journal of Applied Microbiology, vol. 71(3), pp. 265-269.
3. Chang, A.C., Yang, T.Y., Riskowski, G.L., 2013. Ascorbic acid, nitrate, and nitrite concentration relationship to the 24 hour light/dark cycle for spinach grown in different conditions. In: Food Chemestry, vol. 138(1), pp. 382-388.
4. Djeri, N., Williams, S.K., 2014. Celery Juice Powder Used as Nitrite Substitute in Sliced Vacuum-Packaged Turkey Bologna Stored at 4C for 10 Weeks Under Retail Display Light. In: Journal of Food Quality, vol. 37(1), pp. 361-370.
5. Gençcelep, H., Kaban, G., Aksu, M.I. et al., 2008. Determination of biogenic amines in sucuk. In: Food Control, vol. 19, pp. 868-872.
6. Hwang, K., Kim, T., Kim, H. et al., 2018. Effect of natural pre-converted nitrite sources on color development in raw and cooked pork sausage. In: Asian-Australasian Journal of Animal Sciences, vol. 31(8), pp. 1358-1365.
7. Juntachote, T., Berghofer, E., Siebenhandl, S. et al., 2007. The effect of dried galangal powder and its ethanolic extracts on oxidative stability in cooked ground pork. In: LWT - Food Science and Technology, vol. 40(2), pp. 324-330.
8. Kaban, G., Kaya, M., 2009. Effects of *Lactobacillus plantarum* and *Staphylococcus xylosus* on the quality characteristics of dry fermented sausage 'sucuk'. In: Jornal of Food Science, vol. 74(1), pp. S58-S63.
9. Kim, T., Kim, Y., Jeon, K. et al., 2017. Effect of Fermented Spinach as Sources of Pre-Converted Nitrite on Color Development of Cured Pork Loin. In: Korean Journal for Food Science of Animal Resources, vol. 37, pp. 105-113.
10. Palamutoğlu, R., Sariçoban, C., 2012. Alternative Natural Curing Agents to Nitrate and Nitrite in Meat Products. In: Electronic Journal of Food Technology, vol. 3, pp. 46-58.
11. Pexara, E.S., Metaxopoulos, J., Drosinos, E.H., 2002. Evaluation of shelf life of cured, cooked, sliced turkey fillets and cooked pork sausages - 'Piroski' - Stored under vacuum and modified atmospheres at +4 and +10 °c. In: Meat Science, vol. 62, pp. 33-43.
12. Riel, G., Boulaaba, A., Popp, J. et al., 2017. Effects of parsley extract powder as an alternative for the direct addition of sodium nitrite in the production of mortadella-type sausages – Impact on microbiological, physicochemical and sensory aspects. In: Meat Science, vol. 131, pp. 166-175.
13. Rödel, W., Panert, H., Leistner, L., 1975. Verbessertes aw-Wert-Messer zur Bestimmung der Wasseraktivitat von Fleisch und Fleisch Waren. In: Fleischwirtschaft, vol. 4, pp. 557-558.

14. Sindelar, J.J., Cordray, J.C., Sebranek, J.G. et al., 2007. Effects of varying levels of vegetable juice powder and incubation time on color, residual nitrate and nitrite, pigment, pH, and trained sensory attributes of ready-to-eat uncured ham. In: Journal of Food Science, vol. 72, pp. 388-395.
15. Sucu, C., Turp, G.Y., 2018. The investigation of the use of beetroot powder in Turkish fermented beef sausage (sucuk) as nitrite alternative. In: Meat Science, vol. 140, pp. 158-166.
16. Tarladgis, B.G., Watts, B.M., Younathan, M.T. et al., 1960. A distillation method for the quantitative determination of malonaldehyde in rancid foods. In: Journal of the American Oil Chemists Society, vol. 37(1), pp. 44-48.
17. Walker, R., 1990. Nitrates, nitrites and N-nitrosocompounds: A review of the occurrence in food and diet the toxicological implications. In: Food Additives and Contaminants, vol. 7(6), pp. 717-768.
18. Zarringhalami, S., Sahari, M.A., Hamidi-Esfehani, Z., 2009. Partial replacement of nitrite by annatto as a colour additive in sausage. In: Meat Science, vol. 81(1), pp. 281-284.