

EFFECT OF NITRATE LEVEL ON THE CHEMICAL AND
MICROBIOLOGICAL PROPERTIES OF SAUSAGE DURING ITS STORAGE

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A B S T R A C T

In an attempt to reduce the amount of residual sodium nitrate in sausage 3 portions from minced meat were treated with 10, 20 and 30mg NaNO₃/100gm. The fourth portion was left without nitrate to serve as control. No nitrate was added. After stuffing in mutton casings sausages were stored at 4°C for one month. Samples were withdrawn after 1, 10, 20 and 30 days to be analyzed for moisture content, nitrate content, total volatile nitrogen, ammonia, TBA, total microbial count (TC) and organoleptic properties (color, aroma and taste).

After 1 day storage the nitrate content was 8.2, 43.0, 87.1 and 127.3 mg/100 gm sausage for samples prepared with 0, 10, 20 and 30mg nitrate respectively. It seemed possible that added NaCl contained some nitrate as impurity. Only the sample prepared with 30mg nitrate had a very slightly higher residual nitrate than that permitted by the Egyptian Food Regulations (<125). After 10-30 days storage all samples either fresh or cooked had lower amounts of nitrate than that permitted owing to the progressive reduction of nitrate upon storage.

At any given period of storage the higher the level of added nitrate and residual nitrate the less the microbial load, total volatile nitrogen, ammonia contents and TBA were found.

The red color intensity was increased by increasing the nitrate added. Similar trend was noticed in taste and aroma. Nevertheless, no considerable differences were found in colour, aroma and taste scores among samples prepared with 20 and 30 mg nitrate.

Accordingly, a concentration of 20 mg nitrate/100 gm sausage could be recommended for production of sausage with adequate organoleptic properties, high storage stability and a minimum residual from nitrate.

I N T R O D U C T I O N

Sodium nitrate is widely used in meat curing to enhance its color and flavor; beside its antibacterial affect.

On the other hand, nitrite could react with secondary and tertiary amines to form nitrosamines which were found to be toxic (Sugimura *et al.*, 1981). Moreover, nitrate is not considered so toxic, because it acts as a reservoir for nitrite via conversion reactions by reducing bacteria (Jensen and Hess, 1940 and Sokolov 1965). So, this work was conducted to determine the proper dose of nitrate which leads to a minimum residual of nitrite in sausage. Also, the keeping quality of such sausage was studied.

MATERIAL AND METHODS

Sausage samples containing 0, 10, 20 and 30 mg nitrate/100 gm were prepared. Sausage ingredients were (minced lean meat 64.66%, cattle fat tissues 20.05%, water (as ice flakes) 10.03%, salt (NaCl) 3.0%, black pepper 0.50%, red pepper 0.25%, Cubeb 0.25%, parsley 1.0% and ascorbic acid 0.25%). Sausage in mutton casings was stored at 4 C. Samples were analyzed after 1, 10, 20 and 30 days. Spoilage was detected by the development of off odors. Boiled sausages for 15 minutes in water were also examined.

The moisture content was determined according to the A.O A.C. (1970). Nitrite content was estimated according to the method suggested by Grau and Mirna (1957). Total volatile nitrogen (TVN) and thiobarbituric acid (TBA) value were measured according to Pearson (1970). Ammonia was estimated using the method described by Winton and Winton (1958). Total bacterial count was carried out by the plate count method reported by Frazier and Foster (1950). The red color intensity was colorimetrically measured by the method of Husaini *et al.* (1950). Sensory evaluation of cooked and uncooked sausage was applied according to the method described by Sorour (1978).

RESULTS AND DISCUSSIONS

- a. Effect of nitrate level, cooking and storage of sausage on its moisture content. As shown in Table (1) moisture content was reduced as a result of cooking process. Generally, the decrease in moisture was continuing during storage especially in the first period. These findings were in agreement with those reported by Ameen (1968) and Abd El-Salam (1978). Cooking and storage were found to decrease the water holding capacity (WHC); consequently decreasing the moisture content.
- B. Effect of nitrate level, cooking and storage on the residual nitrite in sausage. Results in Table (2) indicated that there was a residual of (8.2% WW and 20% DW) nitrite in the uncooked control samples after 1 day storage which pointed out that salts and spices added may be responsible for the nitrite residual appeared. Further more, the higher the added

Table (1) Effect of nitrate level, cooking and storage of sausage on its moisture content.

Treat- ments Time of storage in days	0mgN		10 mgN		20mg N		30 mg N	
	Control							
	BC	AC	BC	AC	BC	AC	BC	AC
1	58.99	50.52	59.03	49.97	58.53	49.89	58.66	49.75
10	57.82	49.95	57.18	49.85	57.91	49.94	57.40	50.12
20	57.75	48.87	57.30	48.43	57.68	49.75	57.22	48.51
30	57.57	47.42	56.85	47.72	57.05	47.88	56.90	47.22

BC = Before cooking

AC = After cooking

N = Nitrate

nitrate, the higher the residual nitrite formed by bacterial reduction of nitrate. Generally, the residual nitrite in the different treatments was in the permitted limits of the Egyptian food standard organization. When samples were cooked; a decrease in the residual nitrite content occurred which may be due to the effect of leaching in the boiling water or to the weakened structure of sausage by storage which helped in reducing the residual nitrite amount. Upon prolonged storage from 10-30 days; samples of both cooked and uncooked sausage containing 30 mg nitrate showed a lower residual nitrite than the permitted dose; which indicates that the rate of nitrite formation during storage was less than its decomposition by the organisms activity.

Total count of bacteria :

C. Effect of nitrate level, cooking and storage on the total bacterial count in sausage. Results in Table (3) showed that after one day storage of sausage the total count was very low. Although, differences between treatments were not marked. It could be noticed that the higher the amount of nitrate, the less the total count was found. The effect of nitrate on the production of toxin by *Cl. botulinum* was slight, but storage at lower temperature (4°C) was found to be more effective to inhibit toxin production. On the other hand, the cooking reduced the total count (Sokolov, 1965), but this thermal reduction was less marked by increasing the storage period. Generally, the increase in total count after the storage period (30 days) was in the permitted limits of the Egyptian food standard for sausages (10 X 10⁶ / gm.). Prolongation of storage period led to the development of off odour.

Table (2) Effect of nitrate level, cooking and storage on the residual nitrite content in sausage.

Nitrate dose																	
Storage in days	Treat-ments	0mg				10mg				20mg				30mg			
		BC		AC		BC		AC		BC		AC		BC		AC	
		WW	DW	WW	DW	WW	DW	WW	DW	WW	DW	WW	DW	WW	DW	WW	DW
1		8.20	20	8.41	17	43.02	105	43.53	87	87.09	120	90.20	180	127.33	308	143.21	285
10		6.75	16	6.61	13	33.83	79	32.60	65	67.34	160	62.07	124	83.07	195	84.80	170
20		5.92	14	5.62	11	25.62	60	25.27	49	55.02	130	50.75	100	73.15	171	72.60	141
30		4.24	10	4.21	8	20.71	48	20.39	39	49.82	116	49.51	95	69.82	162	69.67	132

WW = Wet weight basis DW = Dry weight basis

Table (3) Effect of nitrate level cooking and storage on the total bacterial count in sausage (count $\times 10^6$ / gm).

Storage in days	Treatments		0mg		10mg		20mg		30mg	
			BC	AC	BC	AC	BC	AC	BC	AC
1			0.007	0.005	0.006	0.003	0.005	0.002	0.004	0.001
10			4.33	3.30	4.17	2.19	3.12	1.19	3.03	1.00
20			7.20	5.40	7.01	4.30	6.36	3.07	4.70	1.52
30S			16.96	14.50	14.58	11.30	10.54	8.17	10.00	5.20

S = Spoilage.

and increased to total count more than the permitted limits.

4. Total volatile nitrogen (T.V.N.) and ammonia: from Table (4) it could be observed that T.V.N. as well as ammonia contents were affected by nitrate level.

Table (4) Effect of nitrate level, cooking and storage on the total volatile nitrogen and ammonia.

Treat- ment	0 mg Nitrate (control)				10 mg Nitrate				20 mg Nitrate				30 mg Nitrate			
	Before cooking		after cooking		before cooking		after cooking		before cooking		after cooking		before cooking		after cooking	
Time of storage in days	T.V.N.	NH ₃	T.V.N.	NH ₃	T.V.N.	NH ₃	T.V.N.	NH ₃	T.V.N.	NH ₃	T.V.N.	NH ₃	T.V.N.	NH ₃	T.V.N.	NH ₃
1	19.28	13.15	15.52	10.07	16.48	9.34	12.28	8.12	13.25	7.05	10.15	6.17	10.26	5.11	7.42	4.05
10	26.71	20.09	19.33	17.23	21.34	14.28	16.86	14.24	18.72	11.40	12.05	11.38	15.58	9.32	9.53	8.09
20	39.33	37.33	29.12	28.30	34.17	32.95	26.01	23.22	29.15	29.04	19.95	19.73	24.51	23.58	16.25	14.23
30	49.58	40.38	39.50	31.45	44.22	35.95	34.33	27.83	30.75	30.75	31.16	24.33	35.05	25.37	28.81	18.81

5. Thiobarbituric acid value (TBA) : Results in Table (5) showed that oxidation of lipids was less marked by increasing the nitrate level. Although, nitrate, and nitrosmyoglobin accelerated the lipid oxidation (Pavolovski and Palmin, 1963); the decreased amount recorded by increasing nitrate level may be attributed to the decrease occurred in the microbial load. Moreover, cooking resulted in a decrease in TBA value; which may be due to the leaching of some malonaldehyde from sausage, whereas storage increased such component.

6. Color intensity : The color intensity increased by increasing the nitrate level (Table 6) Nitrate via its conversion to nitrite and afterwards to nitric oxide resulted in the formation of nitrosmyoglobin and nitroshaemochromogen which acquire a desirable red color in both cooked and uncooked samples. Color was considerably improved by increasing the nitrate dose. Samples containing 30 mg nitrate showed a better color than those containing 10 mg. nitrate. On the other hand, cooking and storage decreased slightly the color intensity due to pigment leaching during cooking or to oxidation process especially by action of microorganisms (Pavlovski and Palania 1963 and Abd El- Salam 1978).

7. Sensory evaluation : a Color : - Samples containing higher dose of nitrate scored higher degree than those contained lower one (Table 7). Increasing the storage period reduced these scores. Samples containing 20 and 30 mg nitrate showed similar scores. Cooked samples did show sig-

nificant differences than uncooked which indicated that cooking process did not affect the color appeal till 20 days storage especially in high nitrate doses. i.e. 20 and 30 mg.

Table (5) : Effect of nitrate level, cooking and storage on the thiobarbituric acid value of sausage (mg malonaldehyde/kg).

Treat- ment	0 mg N.		10 mg N.		20 mg N.		30 mg N.	
	Before Cooking	After cooking	Before cooking	After cooking	Before cooking	After cooking	Before cooking	After cooking
Time of storage in days								
1	1.56	0.65	1.48	0.25	1.37	0.25	1.25	0.25
10	4.85	0.86	4.45	0.75	4.03	0.64	3.72	0.56
20	7.74	1.31	6.95	1.18	6.33	1.01	5.82	0.87
30 S	10.85	1.85	9.75	1.65	9.13	1.46	8.87	1.33

Table (6) Effect of nitrate level, cooking and storage on the color intensity of sausages (absorbance at 542 μm).

Treat- ment	0 mg(control)		10 mg N.		20 mg N.		30 mg N.	
	Before cooking	after cooking	before cooking	after cooking	before cooking	after cooking	before cooking	after cooking
Time of st- orage days								
1	0.95	0.25	0.95	0.25	0.97	0.30	1.00	0.40
10	0.85	0.20	0.87	0.25	0.90	0.30	0.95	0.35
20	0.70	0.15	0.72	0.25	0.77	0.30	0.90	0.30
30 S	0.55	0.16	0.55	0.20	0.65	0.25	0.75	0.30

b- Odor : Table (8) showed that the odor was clearly pronounced by increasing the nitrate doses. Cooked samples scored higher than the uncooked ones containing 20 or 30 mg. Nitrate showed similar scores which assumed that 20 mg. of nitrate could be considered enough for enhancing and improving the odor.

c- Taste : Results obtained showed that cooked sausage samples containing 20 and 30 mg nitrate scored a very full taste; while the control sample or that containing 10 mg nitrate scored

a full taste.

Table (7) : Effect of nitrate level, cooking and storage on average color scores of sausages.

Time of storage in days	0 gm N(control)		10 gm N.		20 gm N.		30 gm N.	
	Before cooking	after cooking	Before cooking	After cooking	Before cooking	After cooking	Before cooking	After cooking
1	6	6	8	8	9	9	9	9
10	5	4	7	7	8	8	9	9
20	5	4	6	5	8	8	8	8
30	4	3	4	3	6	5	7	5

From the previous results it could be concluded that addition of 20 mg. of nitrate was quite satisfactory for production to enhance the color, odor, and taste; beside the residual nitrite which was in the level allowed by the food standard. If salt and spices are free of nitrite, 30 mg of nitrate will be recommended.

Table (8) : Effect of nitrate level cooking and storage on the average aroma scores of sausages

Time of storage in days	0 gm N.(controls)		10 gm		20 mg		30 mg	
	Before cooking	after cooking	before cooking	after cooking	before cooking	after cooking	before cooking	after cooking
1	7	8	8	9	8	9	8	9
10	6	7	8	9	8	9	8	9
20	5	6	7	8	8	9	8	9
30 S	1	1	1	2	2	3	2	3

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