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DETERMINATION OF SODIUM NITRITE IN SOME LOCALLY MANUFACTURED MEAT PRADUCTS

(With 3 Tables and One Figure)

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تقدير نيتريت الصوديوم فى بعض منتجات اللحوم المصنعة محليا

إبراهيم على القويعى ، فايزة عبد العزيز التداوى

تعتبر اللحوم المحفوظة بمادة نيتريت الصوديوم من أكثر منتجات اللحوم انتشارا لدى الغالبية العظمى من المستهلكين خاصة الاطفال وذلك يعزى الى لونها الجذاب والنكهة والمذاق اللذيذ. وبالرغم من ذلك هناك جدال حول ما قد تسببه من مخاطر صحية اذا تعدت نسبتها الحدود المسموح بها طبقا للمواصفات القياسية المصرية والعالمية. وقد أجريت هذة الدراسة لتقديرمستوى نيتريت الصوديوم المتبقى فى بعض منتجات اللحوم المحفوظة به والأكثر إستهلاكا وأظهرت النتائج أن مستوى نيتريت الصوديوم فى البسطرمة واللانشون البقرى والسجق الاسكندرانى كان كالاتى ٦٧.٨١٥± ١٢.٦٩ و٩٧.٢٥٥±١٢.٦٦ و ٤٨.٢٠٧ ±٨.٩١ جزء من المليون على التوالى. وبعد المعاملة الحرارية لهذة المنتجات بطريقة القلى قد تبين انخفاض مستوى نيتريت الصوديوم بها إنخفاضا معنويا ليصبح ٥٠.٤٦٧± ١٠.٦٥ و٥٨.٧٧٣ ± ٧.٥٧١ و٣٨.٢١٨± ٦.٨٢٣ جزء من المليون على التوالى. وقد سجلت النتائج أن ٣٥٪ و٥٠ ٪ و ١٥ ٪ من عينات البسطرمة واللانشون البقرى والسجق الاسكندرانى على الترتيب جاوزت الحد المسموح به (١٠٠ جزئ من المليون) تبعا للمواصفة القياسية المصرية الخاصة بنيتريت الصوديوم فى الاغذية وقد انخفضت هذة النسب انخفاضا معنويا بعد معاملتها حراريا بطريقة القلى لتصبح ١٥٪ و١٠٪ و١٠٪على التوالى.

SUMMARY

Cured meat constitute the large proportion of the processed meats favoured by the great majority of the consumers especially the children due to its attractive colour, flavour, palatable and moreover its delicious taste. Despite of benefits and multi-functional properties of sodium nitrite,it has been often a source of doubt due to its health hazards. This study was conducted to investigate the residual level of nitrite in the

most popular cured meat products retailed in food outlets. The results revealed that the residual nitrite levels in prifried pastirma, beef luncheon and Alexandria semi-dry sausage were 67.815 ±12.69, 97.255 ±12.66 and 48.207 ± 8.91ppm, respectively. After frying of these products, the estimated values of nitrite were significantly decreased and the means ± S.E.values were recorded as 50.467 ±10.65, 58.773±7.571 and 38.218± 6.823 ppm, respectively. 35% , 50% and 15% of retailed pastirma, beef luncheon and semi-dry sausage were exceeded the Egyptian maximum permissible limits (100ppm) but after frying these percentages were decreased significantly to 15%, 10% and 10%, respectively.

Key words: *Meat products, sausage, luncheon, pastirma, sod.nitrite.*

INTRODUCTION

Meat is the main source of animal protein essential for human nutrition.Curing of meats as a method of preservation would help in diminishing perishability and improves palatability and increases its shelf-life.

The usual processing of cured meat is to incorporate sodium chloride, sodium nitrite, a reducing agent (such as ascorbate) and other ingredients such as seasonings (Kramlich *et al.,* 1973). The result is a meat product with a characteristic pink colour and with a specific flavour. Because of the curing ingredients, the heat processing and the usual use of vacuum packaging, the product will maintain freshness and safety for several weeks with assuming a proper refrigeration chain.

Present-day consumers have an intense interest in health, as related to food consumption patterns and especially to the intake of meat. Two components of cured meats have come under withering attack, fat and sodium nitrite. Composition of cured meats, especially emulsion products, has changed greatly in the past decades. The changes have been driven by consumer demand for low-fat products. While the allowable fat content is basically ≤30%, they have been altered so that products containing 1 to 5% are now commonly available. The distress about fat has been its possible association with diseases of the heart and circulatory system and also with a tendancy to some forms of cancer (Cassens, 1997).

Sodium chloride levels added in meat preservation may vary between 3 and 6% although this is not high enough to exert a complete bacteriostatic action. Therefore, other preservation techniques such as refrigeration, dehydration, acidification, cooking and smoking are required. On the other hand, salt may cause undesirable effects in that it may accelerate oxidation of meat pigments and fats, resulting in brown off colour and rancid taste. Nitrite plays an important role in the prevention of these changes (Muller, 1991; Flores and Toldrá, 1993). Most commercially cured meats are not heated sufficiently to kill the heat resistant bacterial spores such as those of *Clostridum botulinum*, a food poisoning microorganism, but they have had an excellent safety record. The suggestion of the microbiological safety of such heat processed meat products may be due to the "Perigo factor" an inhibitor of bacteria, that is formed when nitrite is heated (Perigo *et al.,* 1967) and by the reaction of nitrite with sulfhydryls and iron (Moran *et al.,* 1975). Nitrate may be added as sodium or potassium salts which is transformed to nitrite by the bacteria, naturally present in foods or added as a starter culture, which have nitrate reductase activity. The nervousness about nitrite has centered on the potential link to cancer. Because nitrite can exert acute toxic effects and contribute to the total body burden of N-nitroso compounds, it has been recommended that exposure to these agents should be reduced. The use of nitrate salts in curing should be eliminated, with the exception of dry-cured products, due to the great uncertainty of convertion of nitrate to nitrite (Cassens, 1995). The absorbed nitrite in the blood may oxidized haemoglobin to methaemoglobin, thereby reducing or destroying its oxygen carring ability "methaemoglobinemia" (Cassens, 1997). Two subpopulations are known to be more susceptable to methaemoglobinemia, young infants have a high gastric pH (pH>4) and thus any ingested nitrite is less rapidly degraded in the stomach and also have lower concentrations of oxidation agents as ascorbic acid in their stomachs to inhibit the reduction of nitrates to nitrites. Individuals suffering from anemia are the second subpopulation that may be more susceptible to methaemoglobinemia because of their lower baseline oxygen-carrying capacity (McKnight *et al.,* 1999). Thus, this study was conducted to monitor the residual nitrite concentrations in the most popular meat products, pastirma, beef luncheon and Alexiandria semi-dry sausage, in retailed and fried states, retailed in El-Bohiera governorate to ensure their quality and safety.

MATERIALS and METHODS

1. Samples collection:

A total of 60 randomly collected meat products samples (20 each of pastirma, Beef luncheon and Alexandria semi-dry sausage) were purchased from Damanhour supermarkets during the summer of 2010. Each sample of 250g weight was placed separately into polyethylene plastic bag, identified and then delivered as soon as possible to the laboratory in an ice box.

2. Chemical examination:

2.1. Preparation of the samples:

Samples of pastirma and beef luncheon were sliced into thick slices and the semi-dry sausages into several unites. Then, the weight of each sample was halved into 2 portions. The first half was analysed directly and the second portion was fried in a preheated uncovered frying pan using corn oil at 176ºC until an internal temperature of 76ºC was reached as monitored by hand thermometer (about 2 minutes).

2.2. Quantitative determination of nitrite levels: by Griess-llosvay reaction (AOAC, 1990).

2.2.1: Reagents:

a- Modified Griess-llosvay reagent:weigh 0.5g sulphanilic acid and 0.1g N-1-naphthyl ethylenediamine dihydrochloride and dissolved in 300 ml 15%v/v acetic acid. Mix with a stirrer to dissolve. Store in a brown-coloured glass bottle.

b- Saturated mercuric chloride: weigh 6.9g $HgCl_2$ in a 250 ml beaker, dissolve in100ml distilled water, then mixing for 1h. Add more $HgCl_2$ if necessary to ensure saturation.

c- Standardised sodium nitrite stock solution:weigh 0.5g dried $NaNO_2$ and dissolve in1000ml distilled water, mix. (1ml=500µg $NaNO_2$).

d- Standardised sodium nitrite working solution: Transfer 10ml of stock $NaNO_2$ solution with a volumetric pipette to a 1000ml volumetric flask. Dissolve and make up to volume with distilled water and mix. (1ml=5µg $NaNO_2$).

2.2.2. Procedure:

a- Prepartion of standard curve:

I. Prepare working standards from the dilute standard solution of $NaNO_2$ following the table below to seven 50ml volumetric flasks, using volumetric pipettes to transfer aliquots.

Flask	Volume dilute standard (ml)	Final concentration(µg $NaNO_2$/ml
1	0	0
2	2	2
3	3	0.30
4	4	0.40
5	5	0.50
6	10	1.00
7	20	2.00

II. Fill each flask to 3/4 full with distilled water and mix. Then add 2ml Griess-llosvay reagent to each flask. Dilute to volume with distilled water and mix.

III. Allow the standards to stand 1h to allow maximum colour development. Measure and record the absorbance on a spectrophotometer at 540nm against the reagent blank.

b. Sample preparation and analysis:

I. Weigh 10.00g of meat product into a 150ml beaker. Add approximately 50ml D.W. Heat on a hot plate, boil for1-2 min., stirring with a glass rod to break up the sample.

II. Quantitatively transfer sample and dilute to a 250ml vol. flask. Add 5ml $HgCl_2$ to clarify the extract. Mix. and filter through nitrite free filter paper, the filtrate must be free from turbidity.

III. With a vol.pipette transfer a 25ml aliquot of the filtrate to a 50ml vol. flask. Fill the vol. flask to 3/4 full with D.W. and mix. Add 2ml Griess-llosvay reagent. Dilute to volume with D.W. and mix.

IV. Allow the sample to stand 1h to allow maximum colour development. Measure and record the absorbance on a spectrophotometer at 540nm against a reagent blank.

C. Calculation:

I. Calculate the slope of the standard curve:

m=[(A2/0.20)+(A3/0.30)+(A4/0.40)+(A5/0.50)+(A6)+(A7/2.0)]/6

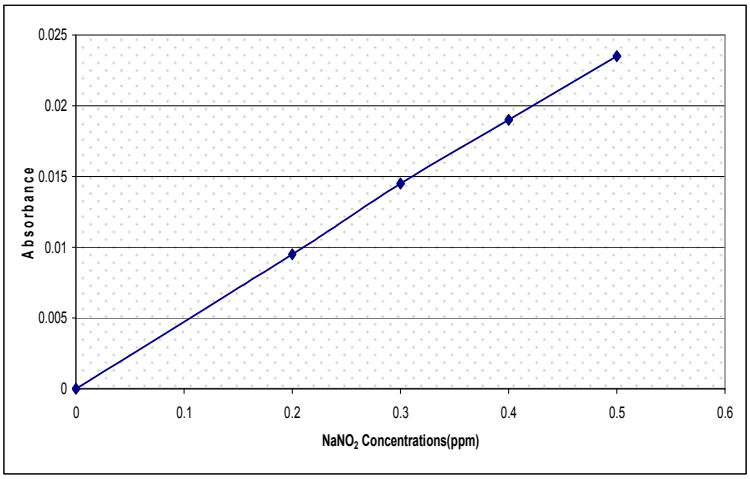
where: A2,A3,A4,A5,A6,A7= the absorbance of the working standards at 540 nm.

II. Calculate $NaNO_2$ concentration in examined samples:

$$\textbf{µg } NaNO_2 \textbf{ µ/g} = \frac{(A_s)\,(1/m)\,(50)\,(250)}{(\text{Volume aliquot})\,(\text{Sample weight})}$$

Where: A_s is sample absorbance ; **m** is the slope of the standard curve (assume the slope is linear); **50** is the volume of the volumetric flask; **250** is the volume of meat product sample extract; **volume aliquot** is the volume of filtrate.

Standard calabiration curve:



RESULTS

Table 1: Statistical analytical results of residual nitrite levels (expressed as ppm of sodium nitrite) in examined meat products `(n = 20 of each).

Product Type	Before Heat Treatment					After Heat Treatment				
	Detected Samples		Min.	Max.	Mean ± S.E.	Detected Samples		Min.	Max.	Mean ±S.E.
	No	%				NO	%			
Pastirma	20	100	5.257	171.379	67.815 ±12.69	20	100	1.051	166.121	50.467 ±10.65
Beef Luncheon	20	100	31.542	200.818	97.255 ±12.66	20	100	14.72	150.35	58.773 ±7.571
Semi-dry Sausage	20	100	6.308	139.836	48.207 ±8.91	20	100	6.308	103.037	38.218 ± 6.823

Min.= Minimum Max.= Maximum S.E.= Standard Error of Mean

Table 2: Frequency distributions of nitrite levels in examined meat prod**ucts*.**

Levels ranges	Pastirma				Beef Luncheon				Semi-dry Sausage			
	Before H.T.		After H.T.		Before H.T.		After H.T.		Before H.T.		After H.T.	
	No	%	No	%	No	%	NO	%	No	%		%
ND	0	0	0	0	0	0	0	0	0	0	0	0
1-25	7	35	7	35	0	0	2	10	5	25	7	35
>25-50	4	20	7	35	7	35	7	35	10	50	9	45
>50-75	1	5	1	5	3	15	6	30	0	0	1	5
>75-100	1	5	2	10	0	0	3	15	2	10	1	5
>100-125	4	20	1	5	3	15	1	5	1	5	2	10
>125-150	1	5	0	0	4	20	0	0	2	10	0	0
>150-175	2	10	2	10	0	0	1	5	0	0	0	0
>175-200	0	0	0	0	2	10	0	0	0	0	0	0
> 200-225	0	0	0	0	1	5	0	0	0	0	0	0

H.T.= Heat treatment **P.L.=** Permissible limit **No** = Number **ND**=Not detected

*** P.L.=** permissible limit according to **Egyptian Standard Specification (E.S.S.) No. 3597 / 2005** for sodium nitrite used in foods products=100ppm.

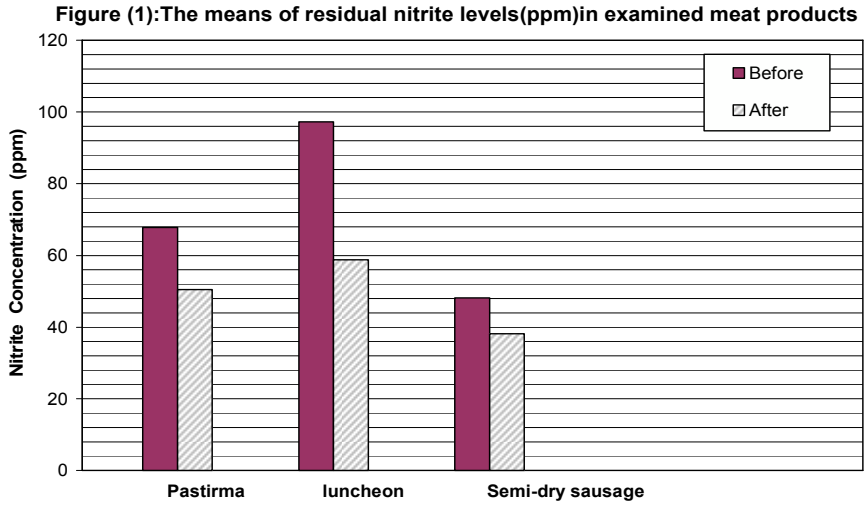


Table 3: Statistical analytical results by using paired t-test.

Statistic parameters / Product	Calculated"t"value	df	Means differance	Significane "P value"
Pastirma	2.341	19	17.348	0.030
Beef luncheon	4.210	19	38.481	0.0005
Semi-dry sausage	2.330	19	9.988	0.031

Significance level at 5%

DISSCUSION

The function of nitrite in meat curing is four folds: To stabilize the colour of the lean tissues, to contribute to the characteristics flavour of cured meat, to inhibit growth of a number of anaerobic food poisoning and spoilage microorganisms and to retard development of rancidity (Pearson and Tauber, 1996). The most

important action of nitrite is the preventing of the growth of *Cl.botulinum* and its toxins. Moreover, Wirth (1991) realized that up till now no substance has been found and don't expect that any will be found that could take the place of nitrite with its varied actions in meat products. Nitrites have effects on microorganisms other than *Cl.botulinum*. They have proven to have inhibitory effects against *Cl.perfringens, E.coli O157:H7, Listeria monocytogens, Achromobacter*, *Enterobacter*, *Flavobacterium*, *Micrococcus* and *Pseudomonas* (Tarr, 1942; Gibson and Roberts, 1986; Pelroy *et al.,* 1994) certain strains of *Salmonella*, *Bacillus* and *Clostridum* are resistant (Rice and Pierson, 1982).

Pastirma:

The obtained results in Table 1 showed that the residual nitrite levels (ppm) in examined prifried samples of pastirma were ranged from 5.257 to171.379 with a mean ± S.E.value of 67.815 ±12.69 ppm; while after frying were ranged from 1.051 to166.121 with a mean ± S.E. value of 50.467 ± 10.65 ppm.

Higher results were obtained by Aiedia (1995) (126.7ppm) ; Tolba *et al.* (1995) (112.0 ±9.97); Hassan (1997) (124.73 ±1.519) and Farag and Abd El-Fatah (2011) (142.15 ± 9.13), but lower results were recorded by El-Khateib *et al.* (1987) as the residual nitrite levels in pastirma were low, on average 12 mg/kg. Kotzekidou (1990) Studied chemically the pastirma produced by five different plants in Greece. He found that the average of nitrite level was 100 ppm.

Table 2 showed that 65% and 85% of examined prifried and fried samples of examined pastirma not exceeded the maximum permissible limit of nitrite (100 ppm) according to *E.S.S., NO. 3597/2005* for sodium nitrite in foods products. Also, it was evident that the frying heat treatment could lead to a significant reduction of residual nitrite content of pastirma ($p<0.05$), (Table 3) *Egyptian standard specification No.3597/2005* stated 100 ppm as the maximum permissible limit for residual nitrite in cured meat products and didn't differentiate between cooked, raw, salted and dry meat products in their residual nitrite content.

About 55% of examined samples of retailed pastirma had lower nitrite concentrations than declared on the labels (50ppm), so the potential for botulinum toxin production can be exist for such samples especially this product was kept unrefrigerated, but it is recorded that the spores fail to germinate until the residual nitrite concentratin is reduced to below 4 ppm (Urbain, 1971). Current U.S.regulations allow the use of

nitrite and nitrate in meat products based upon the product category and method of curing (IFT, 1987). Immersion cured,massaged or pumped products such as hams or pastirma are limited to a maximum ingoing level of sodium or potassium nitrite and nitrate of 200 and 700 ppm, respectively, based on the raw product weight. If a combination of nitrite and nitrate are used, the combination must not result in more than 200ppm sodium nitrite in the finished product (USDA, 1995).

Beef luncheon:

Data presented in Table 1 showed that residual nitrite content of beef luncheon in prifried samples were ranged from 31.542 to 200.818 with a mean ± S.E.value of 97.255 ± 12.66 ppm, but after frying were ranged from 14.72 to150.35, with a mean ± S.E.value of 58.773 ± 7.571 ppm.

Aiedia (1995) stated that the residual nitrite content of beef luncheon was ranged from 75.0 to160.0 with a mean value of 118.9 ppm; also, Fath El-Bab and Sayed (2005) recorded that the nitrite concentration in beef luncheon was ranged from 110 to 128 with a mean ± S.E.value of 120.1±1.2 ppm. El-Bassuony *et al.* (2010) measured the mean value of nitrite content in luncheon at zero time as 100.5 ±4.46 mg/kg and this value was significantly decreased during the chilling storage time till it reached 40.8 ±1.78 mg/kg at the end of the chilling storage period (4 months).

The data presented in Table 2, showed that 50% & 90% of prifried and fried luncheon samples not exceeded the maximum permissible limit (100 ppm) and the incidence of detection of nitrite was 100%. The frying heat treatment had a highly significant reducing effect on nitrite levels in luncheon samples ($p<0.05$) (Table 3).

Hotchkis and Cassens (1987) reported that USDA allows 120ppm nitrite level with 550 ppm ascorbate are requested. The practice of curing with $NaNO_2$ and packaging in vacuum may provide protection against growth of *S.aureus*,both by extending duration of the adjustment phase of growth and by reducing the multiplication rate during the exponential phase of growth. The magnitude of inhibition is dependent on the interaction of $NaNO_2$ concentration, initial pH and O_2. *S.aureus* metabolizes nitrite when culture aerobically, but the nitrite content of anaerobic cultures remain essentially unchanged (Buchanan and Solberg, 1972). Comminuted products such as frankfurters, bologna and other cured sausages are limited to a maximum ingoing level of 156 ppm of sodium or potassium nitrite based on the raw weight of the meat block (USDA, 1995).

Alexandria semi-dry sausage:

It was evident from Table 1 that residual nitrite values in semi-dry sausages were ranged from 6.308 to 139.836, with a mean ± S.E. value of 48.207± 8.91 ppm in prifried samples, while were ranged from 6.308 to 103.037, with a mean ± S.E. value of 38.218 ±6.823 ppm in fried ones.

Higher results were recorded by Aiedia (1995), (131.6 ppm) who mentioned that 35%&50% of examined sausage samples collected from classes II&III meat processing plants, respectively, were showed relatively high nitrite contents. On the otherside, 20% &10% of sausage samples of classes II & III were showed relatively low nitrite contents than requested. Also, El-Bassuony *et al.* (2010) registrated higher residual nitrite level (94.8 ±4.17mg/kg) in the examined sausages at zero time and this value was significantly decreased during chilling storage till it reached 35.1± 2.06mg/kg at the end of chilling storage period (4 months).

Table 2 pointed to that only 15% of the examined prifried sausage samples exceeded the permissible limit (100ppm) of nitrite content and this percentage was regressed to 10% after frying treatment,and the statistical analysis was ensured that the frying heat treatment had a significant effect on nitrite content in semi-dry sausages ($p< 0.05$) (Table 3).

The second primary use for nitrite is to inhibit *C. botulinum* growth and toxin formation, although not necessarily spore germination. Its effectiveness is affected by temperature and salt concentration. Although 50 ppm (mg/kg) initial nitrite levels are sufficient for the meat color desired, up to 200 ppm, depending on the country, is used for antibotulinal activity. The result has been the almost total absence of botulism in cured meat. Its effect on non-spore-formers is distinctly genus dependent. As with the organic acids, lower pH noticeably improves the antimicrobial activity of nitrite (Labb´E and Nolan, 2009) .

With respect to the depletion or disappearance of nitrite from some samples, it is known that cooking reduced the amount of nitrite in the food product, depending on the heat treatment and the presence of reducing substances, so that immediately after heat processing 50% or less of the original nitrite will be found. This will diminish more rapidly and completely at increased storage temperature (Ingram and Simonsen, 1980). Nordin (1969) found the rate to be proportional to its concentration and to be exponentially related to both temperature and pH. The depletion rate doubled for every 12.2ºC increase in temperature

or 0.86 pH unit decrease and was not affected by heat denaturation of ham. These relationships did not apply at room temperature unless was the product was first heat treated, suggesting that viable organisms aided in its depletion.

In Chinese-style sausage, a non-fermented product containing natural flora, the pH decrease remarkably slow when manufactured by using traditional processing (Chen *et al.,* 1997). This indicated that bacteria did not degrade suger to lactic acid because of shorter fermention or non-fermentation. The maximum ingoing level of sodium nitrite permitted in Denmark is 60 ppm for most products with some specialty products allowed to have up to 150 ppm. From 1998 to 2006, the residual nitrite in Denmark sausages and salami-type products has varied between 6-20 ppm (Leth *et al.,* 2008) as a result of the ingoing levels allowed. In Australia, nitrites (sodium or potassium salts) are allowed at a maximum level of 125 ppm incured, dried and slow-dried cured meat and 50 ppm in sterile and canned meat. Nitrate may be incorporated at 500 ppm in slow-dried cured meats (Hsu *et al.,* 2009). Meat products surveyed in a Sydney market were found to have a nitrite content ranging from 3.7 to 86.7 ppm while nitrate levels ranged from 3.7 to 139.5 ppm. Gangolli *et al.* (1994) reported the nitrite and nitrate contents of bacon in the UK to be 24 and 43 mg, respectively, while ham levels were 26 and 22ppm, respectively. A multi-year survey of Canadian products indicated that the overall mean residual nitrite levels in cured meats had declined over the past 20-25 years averaging 28 ppm in1972, 44 ppm in 1983-1985, 31 ppm in 1993-1995, and 28 ppm in 1996 (Sen and Baddoo, 1997). Finnish cured meat products have been observed to range from 2.3-31.6 and 19-136 ppm for nitrite and nitrate contents, respectively (Penttila *et al.,* 1990).

Based on this brief overview of the nitrite in cured meat products from other countries and those values reported in Tables 1&2, comparable Egyptian products often contain nearly the same levels. Some factors contributing to lower residual nitrite levels could be the use of reductants such as sodium ascorbate (erythorbate), depletion of nitrite during refrigerated storage and the unstable nature of the nitrite (Anonymous, 1981). Interest in the use of sorbate as a preservative in cured meat products was proposed as an alternative to the use of nitrite, which might form nitrosamines in cured meats (Robach and Sofos, 1982; Sofos and Busta, 1983). Reduced nitrite levels (40-80ppm) resulted in decreased nitrosamine formation and appear to be adequate for the other functions of nitrite (e.g.,color, flavor, rancidity prevention,

etc.) while inclusion of sorbate in the formulation (0.2%) can be as effective as 120 ppm nitrite levels for control of *Cl.botulinum* (Sofos and Busta 1983). Another curing adjunct is phosphate, usually sodium pyrophosphate, tripolyphosphate and hexametaphosphate, they are added mainly to increase the water-holding capacity of muscle, thereby reducing shrinkage of finished products and also,as an effective antioxidant, can retard rancidity development and acting as a buffering agent to stabilize nitrite.

Finally, it is recommended that a periodic monitoring of nitrate, nitrite and nitrosamine in meat and meat products in meat plants before marketing and in the open markets, both for local and imported foods, should be permitted to the specialists, strict law enforcement should be undertaken by the governmental authorities, the primitive meat processing factories should be put under strict control of the specific food hygienic authorities and modern hurdle technology for meat and meat products preservation must be applied.

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