



ISSN 2357-0725

<https://jsasj.journals.ekb.eg>

JSAS 2022; 7(1): 51-59

Received: 13-04-2022

Accepted: 29-05-2022

Mohamed A Sorour
Abul-Hamd E Mehanni
El- Sayed A Mahmoud
Rasha M El-Hawashy

Food Science and Nutrition Department
Faculty of Agriculture
Sohag University
Sohag
Egypt
82524

Corresponding author:**Rasha M El-Hawashy**rashamagdy@agr.sohag.edu.eg

Chemical Preservatives and Microbiological Contamination of Some Locally Processed Meat Products

Mohamed A Sorour, Abul-Hamd E Mehanni, El- Sayed A Mahmoud and Rasha M El-Hawashy

Abstract

The purpose of this study was to assess the levels of nitrate and nitrite in various available meat products (luncheon, frankfurter, hot dog, corned beef, smoked beef, salami, and pastrami) obtained from local markets in Sohag Governorate, Egypt, as well as their relationship to the levels of N-nitrosamines in those products. A total of 18 random samples of the 7 meat products were collected from Sohag Governorate's supermarkets. The result indicated that the average nitrate levels (ppm) in the tested meat products ranged from 4.54 to 163.63ppm, while the average of nitrite levels (ppm) in the all tested meat products ranged from 63.37 to 125.06 ppm. N-nitrosamine content (ppm) in the same products were ranged from 0.41 to 3.73 ppm, but it was not detected in salami. Total bacterial count in all tested meat products ranged from 6.066 to 8.232 Log cfu/g. The *E. coli* count in all tested meat products ranged from 2.617 to 3.642 Log cfu/g, and it was not detected in smoked beef. *Staph. aureus* was found in all of the tested meat products, with count ranging from 4.405 to 4.886 Log cfu/g. *Clostridium botulinum* and *Salmonella* were not detected in all tested meat samples.

Keywords:

Nitrate, nitrite, N-nitrosamine, meat products, and microbial contamination.

INTRODUCTION

Food preservatives are required in meat products because of the risk resulted from contamination and deterioration. Nitrite and nitrate are the most widely utilized preservatives in meat processing (Farag and Abd-el-fatah, 2011). Nitrite (E249, E250) and nitrate (E251, E252) are frequently used in meat preservation and are permitted food additives in the European Union (EU Regulation No. 1129/2011/EC 2011) (Merino *et al.*, 2016). The sodium or potassium salts of these compounds are added to meat products to improve the colour and taste, and it has antibacterial qualities since it creates nitrous acid, nitrous oxide, or nitric oxide (Sindelar *et al.*, 2011). Except for somewhat higher levels in some traditional cured foods, the maximum amount of nitrite approved for use as a food additive in cured beef is presently 150 mg /kg (represented as NaNO₂) (Merino *et al.*, 2016). By this amount, nitrite (and its predecessor, nitrate) improves the appearance, flavour, safety and quality of cured meats, the distinct reddish-pink coloring, and improving meat safety by reducing the growth of microbes, particularly *Clostridium botulinum* (Bedale *et al.*, 2016). To achieve the antimicrobial activity, a mixture of 75 and 500 ppm or 100 and 250 ppm of nitrite and ascorbate from natural sources should be used by the weight of the finished product (FSIS, 2018). Finally, by suppressing lipid peroxidation, nitrite protects and may even improve the flavor by reducing rancidity (Richards, 2013). Because of its beneficial characteristics, nitrite has long been a popular curing ingredient for meat products (Sindelar and Milkowski, 2012). Since the early 1960s, nitrates and nitrites have been considered an environmental issue (Ogunmodede, 2014). Nitrate is a harmless substance in and of itself. Toxicity is typically caused by the conversion of nitrate to the more hazardous nitrite. Despite the advantages of nitrite application in the meat industry (pinkish-red color, and antioxidant and antimicrobial properties), it is harmful to human health (Bedale *et al.*, 2016). Myoglobinemia can be caused by nitrite, which can lead to tissue hypoxia and mortality. Nitrite can also generate N-nitroso compounds when it reacts with secondary N-alkylamides (carcinogenic) (Ez-Eldain-Afaf and El-Nemr, 2016). Recent

epidemiological studies have found a link between colorectal cancer, stomach cancer, pancreatic cancer, cardiovascular disease, and other causes of death and a high intake of processed meat preserved with nitrate and nitrite, in addition to the presence of Nitrosamines, which increased the risk of, and other causes of death (Larsson and wolk, 2012; Rohrmann *et al.*, 2013). The allowable levels of nitrite or nitrate in the United States are determined by the individual items being processed (Redondo-Solano *et al.*, 2013). Maximum ingoing sodium nitrate and sodium nitrite concentrations for immersion cured products are 700 ppm and 200 ppm, respectively; maximum ingoing sodium nitrate and sodium nitrite concentrations for massaged or pumped cured products are 700 ppm and 200 ppm; and maximum ingoing sodium nitrate and sodium nitrite concentrations for comminuted cured products are 1718 ppm and 156 ppm, respectively (USDA, 1995). The U.S. Environmental Protection Agency (EPA) limits the quantity of sodium nitrate in finished meat products to 500 ppm and the amount of sodium nitrite in finished meat products to 200 ppm (Colditz, 2015).

The objective of this study was to assess the nitrate, nitrite, and N-nitrosamine level (ppm) in the most common meat products available in Sohag markets like (luncheon, frankfurter, hot dog, corned beef, smoked beef, salami, and pastrami), and comparing with their allowed limits from world health organization, as well as bacterial evaluation.

MATERIALS AND METHODS

A total of 18 random samples of the 7 meat products including (luncheon, frankfurter, hot dog, corned beef, smoked beef, salami, and pastrami) were collected from various supermarkets in the governorate of Sohag for determination of nitrate, nitrite, and N-nitrosamine. The collected samples were immediately transported to the laboratory for testing the acceptability of the meat products in accordance with FAO/WHO (1991) specifications.

Chemicals

Sodium tetraboratedecahydrate (Na₂B₄O₇.10H₂O) 5%, Carrez reagent I (Potassium hexacyanoferrate II trihydrate) [K₄Fe(CN)₆.3H₂O] 10.6% and

Carrez reagent II (Zinc acetate solution) [$\text{Zn}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$] 22%, solutions were made with double-distilled deionized water.

Reagents

Color development reagents (Griess reagents) included:

Griess I: 0.25g of sulfanilamide was dissolved in 25 ml acetic acid conc. and diluted with 180 ml warm distilled water (50°C).

Griess II: 0.20g N-naphthyl-(1) ethyldiamine. 2HCl was dissolved in 25 ml acetic acid conc. and diluted with 180 ml distilled water with adding 90 ml ammonia solution 10%.

Sample preparation

According to FAO/WHO (1991), the preparation of the samples were done as follow:

In a 100 ml beaker, about 5 g of the well homogenized specimen was taken. 5mL borax solution (5% borax) was applied and thoroughly mixed with the sample, followed by 100 mL hot distilled water (70-80°C). This mixture was transferred to a 200 mL volumetric flask and put in a water bath at 100°C for 15 minutes, shaking occasionally. Then 2 ml of Carrez I and 2 ml of Carrez II were added and stirred together. Allow standing for 20 minutes after diluting to volume, then filtered through a whatman filter paper No. 41.

Determination of nitrite

According to FAO/WHO (1991), the determination of nitrite content in the samples were done as follow:

5ml filtrate, pipetted in a test tube, was mixed with 5 ml combined reagent, and allowed to stand for 20 minutes until color developed. A portion of the solution was then transferred to a spectrophotometer cuvette and absorbance was measured at 538 nm against a blank. The amount of nitrite in the sample was determined by comparing it to a standard curve.

Reduction of nitrate to nitrite

According to Cortesi *et al.*, (2015), reduction of nitrate to nitrite was done as follow:

Weigh 600 mg Zn powder into 50 mL volumetric flasks for each sample and scatter powder over the bottom of the flask. To make a homogeneous mixture, carefully apply 4 mL of 10% (w/v)

cadmium sulfate ($3\text{CdSO}_4 \cdot \text{H}_2\text{O}$) solution to zinc powder in the flask. Allow the freshly shaped spongy metallic cadmium to rest for 10 minutes before moving it. 2 ml NH_4OH at a concentration of 25% and 10 ml sample solution were added. To loosen spongy cadmium, shake the flask for exactly 1 minute, then set it aside for 10 minutes. Filter after diluting with H_2O to volume. Pour the contents of the volumetric flasks into a waste bottle after use and remove any residues in the volumetric flasks with concentrated HCl in another waste bottle. Fill a second bottle with waste from the color reaction. Decide on proper waste bottle disposal.

Determination of nitrate

According to FAO/WHO (1991), the determination of nitrate content in the samples were done as follow:

5ml filtrate, pipetted in a test tube, was mixed with 5 ml combined reagent, and allowed to stand for 20 minutes until color developed. A portion of the solution was then transferred to a spectrophotometer cuvette and absorbance was measured at 538 nm against a blank. The amount of nitrite and nitrate in the sample was determined by comparing them to a standard curve.

Determination of total N-nitrosamine

Total N-nitrosamine was determined according to Hassan and Ali, (2010).

To a 5 ml sample solution pipetted in a test tube, 0.1 ml Fe^{3+} was added, mixed well, and exposed to UV light for 30 minutes, during which the nitrosamine is photochemically cleaved to yield an amine and nitrite ion, then 5 ml of the combined reagent was added, mixed, and allowed to stand for 20 minutes until the color developed, then a portion of the solution was transferred to a photometer cuvette. The amount of nitrite in the sample was determined by comparing it to a standard curve.

Microbiological methods

Sample preparation

10 g of each sample is combined with 90 ml sterile distilled water and homogenized thoroughly under sterile conditions to produce a 1/10 dilution. Several different types of bacteria were counted using serial dilutions (Mahmoud, 2013). The

outcomes are stated as a logarithm of colony forming units (log cfu) per gram of sample.

Total bacterial count

According to A.P.H.A (1976) and Difco-Manual (1984), the total bacterial count was calculated using a plate count technique on a nutrient agar medium. The plates were incubated for 48 hours at 37 °C.

***Clostridium botulinum* count**

The count of *C. botulinum* was calculated using a blood agar medium, as defined by Difco-Manual (1984). The plates were incubated for 24 to 48 hours at 37 °C under anaerobic conditions.

***Staphylococcus aureus* count**

The presence of *Staph. aureus* was determined using mannitol salt agar medium, as defined by Difco-Manual (1984). The plates were incubated for 24 to 48 hours at 35 °C.

Coliform bacteria count

A.P.H.A (1976) and Difco-Manual (1984) identified a method for determining coliform group bacteria using MacConeky agar medium. The plates were incubated for 24 hours at 37 °C.

Detection of *Salmonella*

The presence or absence of *Salmonella* was determined using Salmonella Shigella agar medium, as defined by FAO (1979). Plates were incubated for 24 hours at 35 °C. *Salmonella* emerged as black colonies with metallic sheets on some of them.

Statistical analysis

The measured data were summarized statistically as means and standard deviations (SD), as well as one sample t-test was used to comparing the measured values of various products with its standard values using Proc MEAN procedure (SAS ver. 9.2, SAS Institute 2008). All measured data were subjected to analysis of variances (ANOVA) by Proc GLM procedure (SAS ver. 9.2, SAS Institute 2008), as well as the least significant differences (LSD) test among the means was used at 5% level of significance according to Gomez and Gomez (1984).

RESULTS AND DISCUSSION

The numerous preservatives applied to meat products during processing are important for extending shelf life and improving food safety. Consumers have always been concerned about their health and safety. As a result, determining their identity is critical for both statutory purposes and consumer health (Frag and Abd-el-fatah, 2011).

Nitrate, Nitrite, and N-nitrosamine content in different meat products

According to the findings of this study, all products sold in the markets contain measurable amounts of nitrate, nitrite, and N-nitrosamine. The average nitrite levels (ppm) in the tested meat products were ranged from 79.67 to 107.22 ppm for luncheon, from 104.25 to 147.31 ppm for Hot dog, from 71.07 to 104.04 for smoked beef, from 78.85 to 79.72 ppm for corned beef, from 76.16 to 89.30 for frankfurter, from 77.08 to 90.06 for salami and from 63.37 to 71.42 ppm for Pastrami. These results were in line with (Abdel-Moemin, 2016; Benli, 2017; Gürbüz and güngör, 2020; Abd-Elghany *et al.*, 2020) they determined the residual nitrite in fermented sausages and it was between (58.65- 216.63 ppm). The nitrite amounts found were significantly varied among the various examined types of beef products. The highest content of residual nitrite was found in hot dog (mean concentrations equal to 125.06 ppm) and the lowest content of residual nitrite was found in pastrami (mean concentrations equal to 67.30 ppm). This variation between samples may be due to the diversity in raw materials, formulation, processing, and time after manufacture (Sullivan, 2011).

The levels of nitrate detected in the various studied types of beef products were significantly different. The average of nitrate levels (ppm) in the tested meat products were ranged from 14.43 to 39.22 ppm in luncheon, from 4.11 to 9.52 ppm for Hot dog, from 36.97 to 37.11 for smoked beef, from 27.26 to 35.09 ppm for corned beef, from 21.91 to 23.18 for frankfurter, from 22.72 to 47.81 for salami and from 154.30 to 172.96 ppm for Pastrami. These results are in the line with (Benli, 2017; Gürbüz and güngör, 2020) who determined the residual nitrate in fermented sausages and it was between 4.30- 161.08 ppm. The highest level

of nitrate found in pastrami (mean concentrations equal to 163.63 ppm) and the lowest content of nitrate found in hot dog (mean concentrations equal to 4.54 ppm). The presence of nitrate could be due to the use of nitrate in the manufacturing process and the introduction of products in a short period without adequate ripening. Furthermore, the presence of nitrate may be attributed to the oxidation of nitrite to nitrate, as well as nitrate found in water and spices used during manufacturing (Honikel, 2008; Gürbüz and güngör, 2020).

The number of N-nitrosamine compounds in different meat products was significantly different. The average of N-nitrosamine levels (ppm) in the tested meat products were ranged from 0.041 to 0.083 ppm for luncheon, from 2.48 to 3.72 for smoked beef, from 3.31 to 4.14 for Pastrami, around 1.24 ppm for corned beef, around 1.24 for frankfurter, around 1.66 ppm for Hot dog, but it was not detected in salami samples as described in

Figure (2). This result was similar to (Hsu, 2009; Moradi *et al.*, 2021). The highest concentrations of N-nitrosamine compounds were found in smoked beef (mean concentrations equal to 2.07 ppm) and the lowest concentration of N-nitrosamine compounds were found in salami (mean concentrations equal to 0.00 ppm). The different concentrations of N-nitrosamine compounds could be influenced by elements such as the type of processing used, including curing time, smoking duration, and smoking temperature (Herrmann, 2014). This also may be due to the quantity of residual nitrite in meat products, especially if some heating is used during processing, as well as other processing factors such as pH and the presence of any chemical that could function as a catalyst or inhibitor such as reducing substances like ascorbic acid, all influence the existence of N-nitrosamines in meat products (Biswas and mandal, 2019).

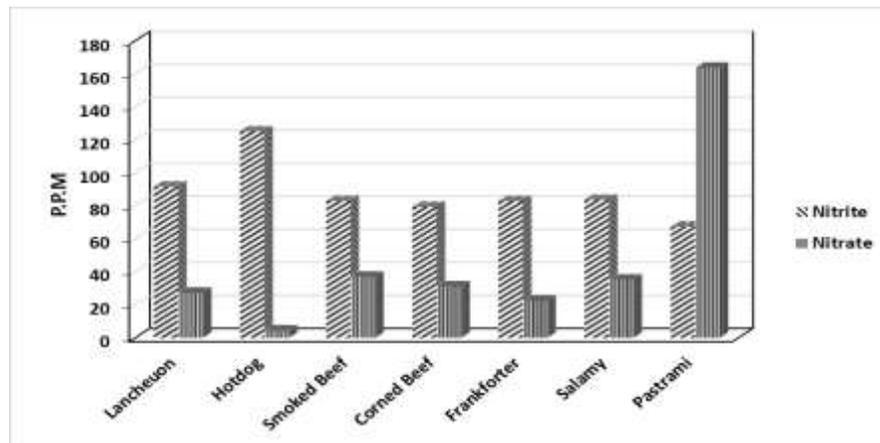


Figure (1) Nitrate, and Nitrite content in different meat products.

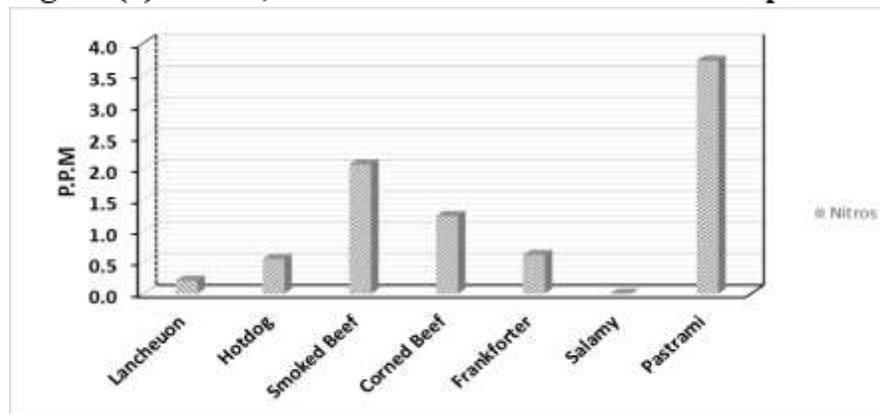


Figure (2) N-nitrosamine content in different meat products.

Microbiological quality of different meat products

There are significant differences in total bacterial count among all purchased meat products (Figure 3). The highest total bacterial count was found in corned beef (8.232 Log cfu/g) and the lowest total bacterial count was found in pastrami (6.941 Log cfu/g). The total bacterial count ranged from 7.142 to 7.264 Log cfu/g for luncheon, from 7.083 to 7.137 Log cfu/g for hot dog, from 6.041 to 6.099 Log cfu/g for smoked beef, from 8.183 to 8.281 Log cfu/g for corned beef, from 6.280 to 7.159 Log cfu/g for frankfurter, from 7.295 to 7.312 Log cfu/g for salami and from 6.893 to 6.988 Log cfu/g for pastrami. The Egyptian organization for standardization and quality control (E.O.S., 2010) indicated that the total bacterial count in different meat products must not exceed 6.699 Log cfu/g. From the collected data, all the meat products exceeded the permissible limits and this difference in total bacterial count among different meat products may be due to poor meat handling and factory environmental conditions (Soepranianondo and wardhana, 2019). Other variables contributing to the increased bacterium load, according to Haileselassie *et al.*, (2013) were the factory workers' low standard sanitary operational practices. Also maybe due to other parameters, such as salt concentration, heat treatment, various curing agents, and pH value, influence nitrite's bactericidal activity (Govari and pexara, 2015; Gassara *et al.*, 2016).

Results in figure (3) revealed that a significant difference in *Escherichia coli* count among the different meat products. The hot dog had a significantly higher mean *E. coli* count (3.642 Log cfu/g) than the other products. On the other hand, corned beef had a quite lower mean *E. coli* count (3.161 Log cfu/g) than other products.

However, the *E. coli* count ranged from 3.096 to 3.261 Log cfu/g for luncheon, from 3.492 to 3.842 Log cfu/g for hot dog, from 3.085 to 3.236 Log cfu/g for corned beef, from 3.612 to 3.628 Log cfu/g for frankfurter, from 3.567 to 3.646 Log cfu/g for salami, from 2.541 to 2.693 Log cfu/g for pastrami and it was not detected in smoked beef. As *E. coli* is a microbiological indication of fecal contamination and the probability of enteric pathogens being present. However, contamination can occur at any point in the food supply chain,

including retail (e.g., during meat slicing, grinding, or packing) and household (wrong food handling habits) (Toldra, 2017).

Data in the same figure also revealed a significant difference in *Staphylococcus aureus* count among the purchased meat products. Pastrami had a substantially higher mean *Staph. aureus* count than the other products (4.886 Log cfu/g). In comparison to the other products, Hot dog had a much lower mean *Staph. aureus* count (4.405 Log cfu/g). Data also revealed that, *Staph. aureus* was found in all of the products, with count ranging from 4.690 to 4.812 Log cfu/g for luncheon, from 4.331 to 4.452 Log cfu/g for hot dog, from 4.301 to 4.489 Log cfu/g for smoked beef, from 4.661 to 4.810 Log cfu/g for corned beef, from 4.628 to 4.751 Log cfu/g for frankfurter, from 4.389 to 4.452 Log cfu/g for salami and from 4.852 to 4.920 Log cfu/g for pastrami. This result was in line with (Naas *et al.*, 2019). The variance in *Staph. aureus* counts in meat, chicken meat, fish, and their products may be due to a variety of factors including mishandling, freezing, and food additives (Naas *et al.*, 2019).

Clostridium botulinum and *Salmonella* were not detected in all samples. This result was in the line with those reported by (Khaleghi *et al.*, 2016). This result was also in agreement with the limits of the Egyptian organization for standardization and quality control (E.O.S., 2010) which indicated that all meat products must be free of *Salmonella* and *Clostridium botulinum*.

CONCLUSION

In conclusion, a total of 18 random samples of the 7 meat products including luncheon, frankfurter, hot dog, corned beef, smoked beef, salami, and pastrami were collected from Sohag Governorate's supermarkets. The samples were prepared to assess the nitrate, nitrite, and N-nitrosamine concentrations (ppm), as well as bacterial counts evaluation. The levels of nitrate, nitrite and N-nitrosamine were within the permissible limits stated by the Egyptian organization for standardization and quality control, while total bacterial count exceeded the permissible limits of the Egyptian organization for standardization and quality control.

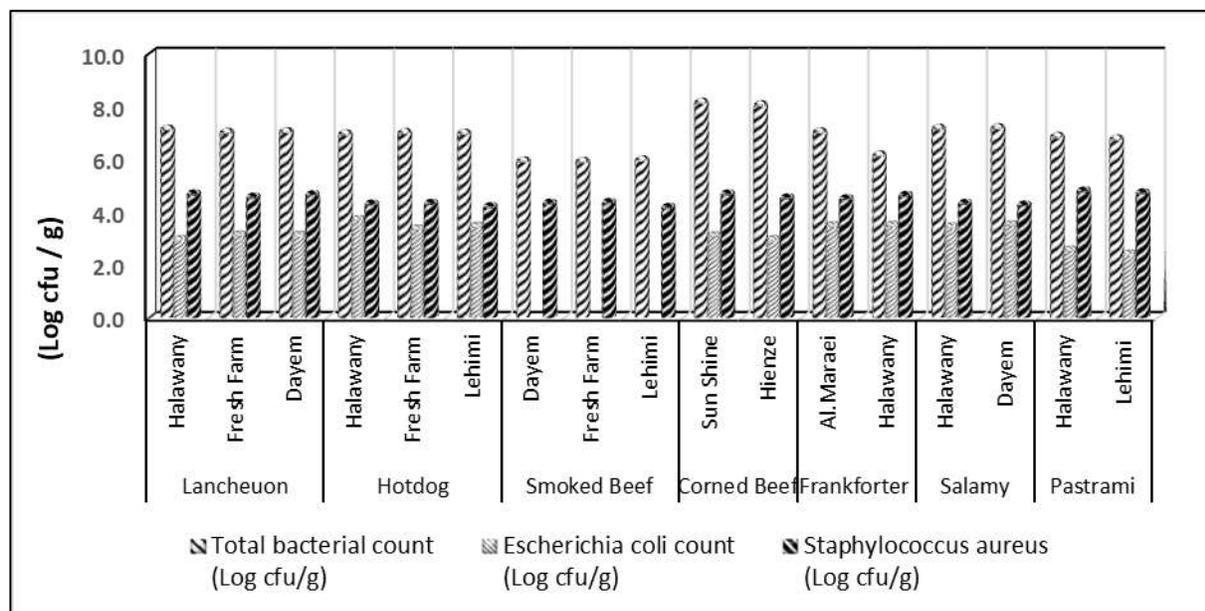


Figure (3) Microbiological quality of different meat products.

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المخلص العربي

المواد الحافظة الكيميائية والتلوث الميكروبيولوجي لبعض منتجات اللحوم المصنعة محلياً
 محمد عبد الحميد سرور، أبو الحمد مهني، السيد محمود، رشا مجدي الحواشي.
 قسم علوم الأغذية والتغذية، كلية الزراعة، جامعة سوهاج، سوهاج، مصر.

الغرض من هذه الدراسة هو تقييم مستويات النترات والنترت في منتجات اللحوم المختلفة (اللانسون - الفرافورتر - الهوت دوج - لحم البقر المقلب - لحم البقر المدخن - السلامي - البسطرمة) التي تم الحصول عليها من الأسواق المحلية في سوهاج ، وكذلك علاقتها مع مستويات مركبات النيتروزامين في تلك المنتجات. وقد تم جمع 18 عينة عشوائية من 7 منتجات لحوم محددة من محلات السوبر ماركت المختلفة في محافظة سوهاج، بالإضافة الى التقييم البكتريولوجي لها. أشارت النتائج إلى أن متوسط مستويات النترات (جزء في المليون) في منتجات اللحوم المختبرة تراوحت بين 4.54 إلى 163.63 جزء في المليون ، بينما تراوح متوسط مستويات النترت في جميع منتجات اللحوم تحت الدراسة من 63.37 إلى 125.06 جزء في المليون. وكان محتوى مركب النيتروزامين (جزء في المليون) في نفس المنتجات يتراوح من 0.41 إلى 3.73 جزء في المليون ، ولكن كان محتواه معدوماً في السلامي ، وقد تراوح العدد الإجمالي الكلي للبكتيريا في جميع منتجات اللحوم تحت الدراسة من 6.066 إلى 8.232 مستعمرة / جم. وكان عدد الإشريشيا كولاي *E. coli* في جميع منتجات اللحوم تحت الدراسة من 2.617 إلى 3.642 مستعمرة / جم ، ولم يتم اكتشافه في اللحم البقري المدخن. ومن ناحية أخرى تم العثور على البكتريا العنقودية *Staphylococcus aureus* في جميع منتجات اللحوم تحت الدراسة ، حيث تراوح العدد من 4.405 إلى 4.886 مستعمرة / جرام ، وكان محتوى جميع عينات اللحوم تحت الدراسة معدوماً من بكتريا السالمونيلا *Salmonella* وأبكتريا *Clostridium botulinum*.