

MICROBIOLOGICAL QUALITY AND ELEMENTAL ANALYSIS OF SOME READY-TO-EAT MEAT PRODUCTS

Darwish, Soumia M.I.¹; Naema M. H. Yousef² and M. A. Ismail²

¹ Food Science and Technology Department, Fac. of Agric., Assiut Univ., Egypt

² Botany Department, Fac. of Science, Assiut Univ., Egypt

ABSTRACT

The ready-to-eat meat sandwiches of Meat dinner (shawerma), Sausages, Fried liver pieces and Minced meat with onion are of the most popular food in Assiut city, Egypt. These kinds of food may be contaminated by bacteria, fungi and heavy metals which cause many bad effects on the consumer health. In this study, different samples of these meat products were purchased from different restaurants. The concentration of heavy metals (Cu, Zn, Pb, Ni and Cd) in these samples were determined and compared with the permissible limits in Egypt and those recommended internationally. Elemental Nickel and Cadmium were not detected in any of the four meat products investigated. Copper ranged from 1.28-1.64 mg kg⁻¹ in meat dinner, sausages and minced meat with onion which is within the permissible limit internationally and in Egypt. However in fried liver, the level of copper was higher (46.91 mg kg⁻¹) than that of the permissible limit. Zinc concentration ranged from 13.13 - 33.42 mg kg⁻¹ in the four meat products investigated and this concentration was within the permissible limit recommended internationally. Unfortunately lead concentration ranged between (1.04 - 1.24 mg kg⁻¹) which is much higher than the recommended level in Egypt (0.5 mg kg⁻¹) and internationally (0.05 mg kg⁻¹). Species of *Klebsiella*, *Staphylococcus*, *Salmonella*, *Proteus* and *Escherichia* were isolated from the four meat products investigated. Of fungi species of *Aspergillus*, *Penicillium* and white-coloured yeasts were recorded from all products. Other fungi were reported only from sausage (*Acremonium roseum* and *Cladosporium cladosporioides*) or from fried liver pieces (*Stachybotrys chartarum*). The public health implications of these findings are discussed.

INTRODUCTION

Because of the changing life style in Egypt, people are consuming a variety of fast ready-to-eat (RTE) meals. Ready-to-eat traditional meat sandwiches such as meat dinner (shawerma), sausages, fried liver pieces and minced meat have become more popular in Middle East countries. They are considered the most common RTE sandwiches sold by street vendors and fast food restaurants (Ayaz *et al.* 1985; Williamson *et al.* 2005). Ingredients for RTE meat sandwiches may include raw products such as lettuce, onions, parsley, green pepper, spices, sliced tomatoes and tahina sauce (sesame paste).

Heavy metals are potential environmental contaminants with the capability of causing human health problems if present to excess in the food we eat. Potential sources of heavy metals in field include sewage sludge, industrial effluents, unsafe or excess application of pesticides, fungicides and fertilizers (Krishna Murti 1989).

They are given special attention throughout the world due to their toxic effects even at very low concentrations (Das 1990; Gilbert 1984; Khan *et al.* 1996). Several cases of human disease, disorders, malfunction and malformation of organs due to metal toxicity have been reported (Jarup 2003). The trace metal contents of individual foods depend upon those introduced in the growing, transport, processing and fortification of food. Other technological processes used to bring the food to the consumer can significantly increase the total trace metal contents of the food (Cabrera *et al.* 2003). This has led researchers all over the world to study the pollution with heavy metals in foods to avoid their harmful effects (Kennish 1992; Oehme 1989; Zakrzewski 1991) and to determine their permissibility for human consumption. Essential heavy metals are absolutely required by an organism to grow and complete its life cycle, but become toxic when its concentration levels exceed those required for correct nutritional response by factors varying between 40 and 200 folds (Venugopal *et al.* 1975). Meanwhile, some other metals such as Pb, Hg and Cd are toxic at quite low concentrations (Ogino and Yang 1978; Ogino and Yang 1980).

Assessment of the quality and safety of foods requires microbiological analysis. Growth of bacteria and or fungi can result in organoleptic changes in food including off-colours and off-odours rendering it unacceptable to the consumer. The presence of pathogenic bacteria such as *Salmonella*, *Campylobacter*, *Listeria Monocytogenes*, *E.coli* as well as toxigenic fungi in foods possess a poisoning threat.

The main objective of this study was to assess the presence of heavy metals (Cu, Zn, Pb, Ni, and Cd) in meat dinner (shawerma), sausages, fried liver pieces and cooked minced meat purchased from different restaurants in Assiut city, Egypt, to compare the maximum acceptable standards for human health. In addition, Microbiological (including bacterial and fungal) analysis were also assessed.

MATERIALS AND METHODS

Collection of meat product samples.

A variety of foods including 24 samples meat dinner (shawerma), sausages, fried liver pieces and minced meat with onion were included in the study. These samples were purchased from 6 different restaurants in Assiut city, Egypt (4 meat product samples each). The samples were transferred to the laboratory in an ice box, and then kept frozen until heavy metal and microbiological analyses.

Determination of heavy metals

Digestion of samples.

Five grams from each sample was digested by using a mixture of nitric and perchloric acids (Khan *et al.* 1995).

Estimation of heavy metals.

Copper (Cu), zinc (Zn), lead (Pb), nickel (Ni), and cadmium (Cd) were analyzed using atomic absorption spectrophotometer (model GBC 906 AA).

The data were statistically analyzed using the Microsoft Excel 7.0 program and were presented as mean, minimum, maximum and standard error (S.E.) following Gomez & Gomez (1984).

Bacteriological examination

The foods were initially checked for their background flora on nutrient agar. Five grams of each meat sample were homogenized in motor in a one to ten ratio of sterilized distilled water. A 100 µl of the homogenate was plated on McConkey's agar medium (MERK, Germany). Three replicate plates for each sample were incubated at 37 °C for 48 hrs, after which the developing bacterial colonies were counted. Preliminary identification based on their colony morphology, Gram reaction, shape; spore and capsule formation were performed. Bacterial strains were plated on media specific for some enteric species (Endo agar and *Salmonella* and *Shigella* agar media). Bergey's Manual of systematic Bacteriology (Sneath 1986) was used for identification. KBOOL HILMVIC™ Biochemical test kit was also used for confirming the identification of enteric species.

Mycological examination

Appropriate dilutions of the samples were prepared, and then plated on dichloran rose Bengal agar medium of Pitt (1981). Three replicate plates for each sample were used. After incubation for 7 – 10 days at 25°C; the developing colonies were counted, isolated and identified. Identification was based on the macro- and microscopic features using the keys presented by (Pitt and Hocking 1997). It is worth to mention that some fungal species had been grown on McConkey's agar (the medium used for isolation of enteric bacteria and these were taken into consideration in the results.

RESULTS AND DISCUSSION

Heavy metal analysis of meat products

The levels of Cu, Zn and Pb in the four meat products (Meat dinner, Sausages, Fried liver pieces and Minced meat with onion) were presented in table (1). Unfortunately lead concentration ranged between 1.04-1.24 mg kg⁻¹ which is much higher than the recommended level in Egypt (0.5 mg kg⁻¹, E. O. S. Q. C ,1993) and internationally (0.05 mg kg⁻¹, FAO/WHO, 1984). Elemental Nickel and Cadmium were not detected in any of the four meat products investigated. Copper ranged in the current results from 1.28 to 46.91 mg kg⁻¹ for meat dinner (shawarma), sausages and minced meat with onion that is within the permissible limit in Egypt and recommended internationally. However in fried liver, copper limit was higher 46.91 mg kg⁻¹ than that of the permissible limit of the Egyptian Organization for Standardization and Quality Control (E.O.S.Q.C 1993); Codex Alimentarius Commission (CAC 1993). Zinc concentration ranged from 13.13-33.42 mg kg⁻¹ in the four meat products investigated and this concentration was within the permissible limit recommended internationally by USDA (2003).

The mean values of lead (Pb) in ready- to- eat (RTE) meat sandwiches ranged from 1.04 ± 0.42 to 1.24 ± 0.38 mg kg⁻¹ are much higher than those reported earlier in meat products in Zagazig (Fatin 1998), (Morshdy *et al.* 2000) and in Assiut (Sharkawy and Amal 2003). However higher levels (4.806 ± 0.519 mg kg⁻¹) in liver sandwiches were reported by (El-Shorbagy 2004) than those reported in the current study (the highest level being 1.24 ± 0.38 mg kg⁻¹). These variations in lead concentration in different meat products may be attributed to the nature and age of animals; dietary habits; transportation condition, environmental pollution with heavy metals, and traffic density (Hafez 1995; Leita *et al.* 1991; Sabir *et al.* 2003). Table (1) indicates that the lead had no significant differences meat products ($P < 0.05$; $P < 0.01$).

The data showed higher level of Pb in RTE meat sandwiches. This may be attributed to their making and selling then outside the restaurants that make them liable for Pb pollution by motor vehicle exhaust especially at areas of high traffic density like squares and near stations. (Hu 2002; Morshdy *et al.* 2000). Sandwich fixings such as onions, pickles, tomatoes, and lettuce may also be a source of Pb contamination (Ward and Savage 1994). Also, addition of spices such as pepper, mustard and other common spices have been reported to contain significant quantities of some heavy and trace metals (Gupta *et al.* 2003). In addition, equipments and utensils used for cooking may be the source of Pb in this meal (Benneth 1981) mentioned that Pb in take accurse from the consumption of food stored in lead lined containers. Also, Pb can pathway to the food supply through leach from older plumbing into drinking water or water used for food manufacturing or processing (Health 1998).

Lead is recognized as toxic substance which is accumulated inside the body due to its low rate of elimination. Thus the damage of the central nervous system is a marked and common feature particularly in children due to low lead tolerance (Johansen *et al.* 2004; lidsky and Schneider 2003).

The levels of copper (Cu) in all RTE meat sandwiches were generally below the permissible levels except in fried liver pieces 46.91 ± 13.85 mg kg⁻¹. Similer results have been reported earlier (El-Seady 2001; Essa *et al.* 2004). However, this level in liver was higher than that obtained by Ibrahim, *et al.* (2001) Fatin (2005).

These results may be attributed to the widely use of Cu in cooking utensils and water distribution systems, as well as fertilizers, bactericides, fungicides, algicides and antifouling paints. It is also used as an animal feed additives and growth promoters, as well as for disease control in livestock and poultry (WHO 1998).

The actual concentration of copper in food from various countries varies widely depending upon the food product, the growing conditions (soil, water, use of copper containg fungicides and fertilizers) and type of processing used; in particular, pH levels and the use of copper vessels (Müller *et al.* 1996).

Copper is known to be essential at low concentrations but it is toxic at high levels. Accordingly, ingestion of an excessive dose of Cu may lead to sever nausea, bloody diarrhea, hypetension and jaundice. Moreover, chronic

Cu poisoning may result in what is known "Wilson's disease" which manifested by destruction of nerve cells, liver cirrhosis, ascitis, oedema and hepatic failure (Gossel and Bricker 1990). Statistically, there were highly significant ($P < 0.01$) differences in Cu contents among the studied meat products.

Bacteria reported from different meat products

The mean values of total aerobic plate (CFU) counts of meat dinner, sausages, liver pieces and cooked minced meat were 7.7×10^3 , 3.6×10^2 , 4.9×10^3 and 1.2×10^3 CFU / g respectively (Table 2).

Table 2. Bacterial species found in different meat products.

Enteric bacteria	Meat Dinner		Sausages		Fried liver pieces		Minced meat with onion		All meat products	
	CFU	NCI	CFU	NCI	CFU	NCI	CFU	NCI	CFU	NCI
<i>Escherichia coli</i>	266	1	—	—	—	—	—	—	266	1
<i>Klebsiella pneumoniae</i>	2433	2	200	2	1766	2	366	3	4765	9
<i>Proteus penneri</i>	—	—	166	1	—	—	—	—	166	1
<i>p. vulgaris</i>	2166	1	—	—	—	—	—	—	2166	1
<i>Salmonella enteritidis</i>	833	1	—	—	—	—	—	—	833	1
<i>S. typhi</i>	1832	2	—	—	1133	1	532	1	3497	4
<i>Staphylococcus aureus</i>	366	1	100	1	2000	1	333	1	2799	4
Total CFC/ g meat	7696	4	366	3	4899	3	1231	4	14192	14

CFU: Colony forming units / g meat.

NCI: Number of cases of isolation (out of 6 samples in each meat product).

Many food-borne bacteria including the non pathogenic spoilage types, as well as pathogens are capable of causing food-borne diseases (Bean et al. 1990; Cunningham and Cox 1987). *Salmonellosis* caused by *Salmonella* is the most common food-borne bacterial disease in the world. *Salmonella* as shown in table 2 was isolated from all studied meat products except sausages. *Salmonella* was identified into two species *S. typhi* and *S. enteritidis*. In agreement with our results, *Salmonella* was isolated also from raw meat products (Busani et al. 2005; DAoust 1989; Essa et al. 2004; Rose et al. 2002; Yasmine et al. 2005).

Klebsiella pneumoniae and *Staphylococcus aureus* were isolated from all types of meat products samples, but the number of colony forming units (CFU) of *K. pneumoniae* were much more than those of *S. aureus* (Table. 2). *Klebsiella pneumoniae* that cause pneumonia was isolated from all meat products under study with a high number of colony forming units (CFU). *K. pneumoniae* was also isolated from raw meat products (Essa et al. 2004).

Escherichia coli, *Proteus vulgaris* and *Salmonella enteritidis* were isolated only once from meat dinner sandwiches (Shawerma). While *Proteus penneri* was isolated only once from sausages. In agreement with our results,

E. coli and *P. vulgaris* were also isolated but from raw meat products (Hassouba *et al.* 2007; Phillips *et al.* 2001).

In this respect, some strains of *E. coli* can cause a distinctive and sometimes deadly disease. enteropathogenic *E. coli* is known as a causative agent for nursery school outbreaks in London (George 2004).

The enteric bacteria (represented by species of *Escherichia*, *Klebsiella*, *Proteus*, and *Salmonella*) as well as *Staphylococcus aureus* were isolated from four restaurants out of six studied (Table 2). One of the restaurants have sandwiches contaminated with only *Salmonella typhi* in all meat products except sausages.

Staphylococcus aureus which could be killed at high temperature is able to produce enterotoxin that is heat resistant and causes illness. *Staphylococcus aureus* was also isolated previously from raw meat products (Hassouba *et al.* 2007; Ploatijs *et al.* 2004).

Fungi reported from different meat products

Thirteen fungal species (related to five genera) in addition to some unidentified white coloured yeasts were recorded from the four meat products investigated on dichloran rose Bengal agar (DRBC) and Mc- Conkey agar media at 25 °C.

Species of *Aspergillus* and *Penicillium* were the most common on all meat products and were isolated on both types of media while the unidentified yeasts were isolated from all products but only on DRBC (Table 3). Species of *Aspergillus* and *penicillium* are of great importance in food spoilage and they produce the most important of known mycotoxins (Pitt and Hocking 1997). *A. flavus*, *A. niger*, *P. chrysogenum* and *P. pinophilum* were recorded from all product types on one or both isolation media used. *P. duclauxii* was recorded from meat dinner, sausages and fried liver pieces. These species were reported as *A. flavus* from different meat products (Aziz and Youssef 1991; El-kady *et al.* 1994; Ismail and Zaky 1999; Kaur *et al.* 1992), Smoked meats (Cvetnic and Pepeljnjak 1995), *A. niger* from meat products (Aziz and Youssef 1991; Dragoni *et al.* 1980b; Leistner and Ayres 1968) (Rojas *et al.* 1991; Takatori *et al.* 1975a), *P. chrysogenum* (Andersen *et al.* 1995; Hadlok *et al.* 1975; Leistner and Eckardt 1979; Takatori *et al.* 1975a), *P. pinophilum* (Nassar and Ismail 1994). *A. sydowii* and *A. terreus* were recorded only from sausages on MacConky, *A. ochraceus* from minced meat on MacConky and *A. fumigatus* on DRBC from fried liver pieces. *A. sydowii* (Wu *et al.* 1974), *A. terreus* (Hadlok *et al.* 1976; Nassar and Ismail 1994), *A. fumigatus* (Aziz and Youssef 1991; Hadlok *et al.* 1976; Harbans *et al.* 1992; Leistner and Ayres 1968; Nassar and Ismail 1994; Takatori *et al.* 1975b; Wu *et al.* 1974) are quite common on meat products in the tropics. *P. purpurogenum* was isolated only once from meat dinner on DRBC as it was reported from meat products (Leistner and Ayres 1968; Racovita *et al.* 1969). *Acremonium roseum* and *Cladosporium cladosporioides* were isolated from only sausages on MacConky agar, while *Stachybotrys chartorum* was isolated once from fried liver pieces on DRBC (Table 3). *C. cladosporioides* has been reported perilously from fresh and frozen meat (Aziz and Youssef 1991; Gill *et al.* 1981; Nassar and Ismail 1994; Ozari and Mansour 1988).

In conclusion: Nickel and Cadmium were not detected in any of the four products, and Zinc conc. in the four products was within the permissible limit recommended internationally. Lead concentration was much higher than the recommended level in Egypt and internationally. Out of the four meat products investigated, only fried liver had copper level higher than that of the permissible limit in Egypt. To obtain meals with a minimal heavy metal contamination, preparation of such foods should be inside the restaurants not in the streets, rearing animals near high traffic density roads should be prevented.

Some species of bacteria e.g. those of *Klebsiella*, *Staphylococcus*, *Salmonella*, *Proteus* and *Escherichia* and fungi e.g. those of *Aspergillus*, *Penicillium*, white colored yeasts and others were recorded from the four meat products. Such microbial incidence in these ready-to-eat meat products may be attributed to poor personal hygiene, house flies and cockroaches that found very close in the restaurants, utensils and working surfaces, additives such as tomatoes, lettuce, peanut paste and spices. In order to protect public health, it is essential to keep contaminants at levels which are toxicologically acceptable. This could be achieved by regular analysis of saved foods and their additives.

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الجودة الميكروبيولوجية وتحليل بعض العناصر لمنتجات اللحوم الجاهزة للأكل

سوميه محمد إبراهيم درويش^١، نعيمه محمد همام يوسف^٢ و ماضي أحمد اسماعيل^٢

١- قسم علوم وتكنولوجيا الأغذية - كلية الزراعة - جامعة أسيوط - مصر

٢- قسم النبات - كلية العلوم - جامعة أسيوط - مصر

أصبحت اليوم الأغذية السريعة أكثر انتشارا وشيوعا وعلى وجه الخصوص سندوتشات اللحوم الجاهزة مثل الشاورمة والسجق والحاوشى والكبد. وقد أثبتت الدراسة الحالية أن هذه الأغذية تحتوى على أعداد من البكتيريا والفطريات وكذلك بعض المعادن الثقيلة الضارة بصحة الإنسان. فقد تم جمع عينات من مطاعم مختلفة بمدينة أسيوط وتم تقدير تركيز المعادن الثقيلة (النحاس، الزنك، الرصاص، النيكل، الكاديوم) في هذه العينات ومقارنتها بالحدود المسموح بها في المواصفات القياسية المصرية والدولية. فوجد أن عنصر النيكل والكاديوم لم يكن موجودا في العينات المختبرة، بينما كان متوسط كمية عنصر النحاس (١,٢٨ - ١,٦٤ ملجم/كجم) في سندوتشات الشاورمة والسجق والحاوشى وهى في نطاق الحدود المسموح بها. وكان متوسط كمية عنصر الزنك في العينات المختبرة (١٣,١٣ - ٣٣,٤٢ ملجم / كجم) وهى أيضا في الحدود المسموح بها، بينما كان متوسط كمية عنصر النحاس (٤٦,٩١ ملجم/كجم) أعلى من الحدود المسموح بها. كما كانت كمية عنصر الرصاص تراوح ما بين (١,٠٤ - ١,٢٤ ملجم/كجم) وهى أعلى بكثير من المسموح به محليا ودوليا. وقد تم عزل أنواع بكتيريا *Klebsiella*, *Staphylococcus*, *Salmonella*, *Proteus* و *Escherichia* من العينات المختبرة كما تم عزل *white-* *Aspergillus*, *Penicillium* و *coloured yeasts*. أنواع أخرى من الفطريات تم عزلها فقط من السجق وهى *Acremonim* و *roseum* و *Cladosporium cladosporioides* و أنواع تم عزلها من سندوتشات الكبد فقط وهى *Stachybotrys chartarum*.. كما تم مناقشه الاشتراطات الصحيه لهذه النتائج.

Table 3. Fungal species found in different meat products on discoloring rose Bengal chloramphenicol agar (DRBC) and MacConky agar (Mac) at 25 °C.

Taxa	Meat Dinner				Sausages				Fried liver pieces				Minced meat with onion				All meat products			
	DRBC		Mac		DRBC		Mac		DRBC		Mac		DRBC		Mac		DRBC		Mac	
	CFU	NCI	CFU	NCI	CFU	NCI	CFU	NCI	CFU	NCI	CFU	NCI	CFU	NCI	CFU	NCI	CFU	NCI	CFU	NCI
<i>Acremonium roseolum</i>	—	—	—	—	—	—	66.7	2	—	—	—	—	—	—	—	—	—	—	66.7	2
<i>Aspergillus</i>	1133.3	2	33.3	1	1700.0	5	100.0	2	1233.3	5	333.3	2	1500.0	4	66.7	2	5566.6	16	533.3	7
<i>A. flavus</i>	966.7	2	—	—	1533.3	5	—	—	1200.0	5	33.3	1	1500.0	4	—	—	5200.0	16	33.3	1
<i>A. niger</i>	166.7	2	33.3	1	166.7	1	33.3	1	—	—	300.0	2	—	—	33.3	1	333.4	3	399.9	5
<i>A. sydowii</i>	—	—	—	—	—	—	33.3	1	—	—	—	—	—	—	—	—	—	—	33.3	1
<i>A. terreus</i>	—	—	—	—	—	—	33.3	1	—	—	—	—	—	—	—	—	—	—	33.3	1
<i>A. ochraceus</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	33.3	1	—	—	33.1	1
<i>A. fumigatus</i>	—	—	—	—	—	—	—	—	33.3	1	—	—	—	—	—	—	33.3	1	—	—
<i>Cladosporium</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>C. cladosporioides</i>	—	—	—	—	—	—	1100.0	5	—	—	—	—	—	—	—	—	—	—	1100.0	5
<i>Penicillium</i>	266.7	2	633.3	3	333.3	2	2066.7	3	100.0	3	366.7	1	366.7	2	266.7	1	1066.7	9	333.4	8
<i>P. duclauxii</i>	33.3	1	33.3	1	—	—	33.3	1	—	—	33.3	1	—	—	—	—	33.3	1	100.0	3
<i>P. chrysogenum</i>	—	—	600.0	3	—	—	2033.3	3	—	—	333.3	1	—	—	266.7	1	—	—	3233.3	8
<i>P. pinophilum</i>	200.0	2	—	—	333.3	2	—	—	100.0	3	—	—	366.7	2	—	—	1000.0	9	—	—
<i>P. purpurogenum</i>	33.3	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	33.3	1	—	—
<i>Stachybotrys chartarum</i>	—	—	—	—	—	—	—	—	33.3	1	—	—	—	—	—	—	33.3	1	—	—
Yeasts (white colour)	500.0	1	—	—	66.7	2	—	—	1366.7	5	—	—	33.3	1	—	—	1966.7	4	—	—
Total	1900.0	4	666.7	3	2100.0	5	3333.3	5	1366.7	5	700.0	2	1900.0	5	333.3	2	15266.6	19	888.6	12

Legnads as those below Table (2)