A Study of Some Microbial and Chemical Contaminants in Packaged Mortadella Available in the Local Markets

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KEYWORDS

Beef Mortadella roll, Microbial Contaminants, Chemical Contaminants, Aerobic Bacteria.

ABSTRACT

Meat is one of the important sources of protein, as it is one of the basic nutrients that the body needs, and a lack of protein in the body can lead to malnutrition. Despite the importance of meat, it is also necessary to conduct studies to identify its contents of pollutants, whether microbial or chemical, to ensure safety In general, foodborne diseases are a major health problem facing not only the patient at risk, but also physicians, epidemiologists, food inspectors and laboratory microbiologists. The researchers studied the quality of imported beef mortadella rolls by collecting ten samples from local markets in The city of Baghdad and analyzed it microbiologically, and found that the levels of the total number of aerobic bacteria for samples of imported beef mortadella roll ranged between (1× 102-2.5× 106) CFU/g, while the level of the total number of coliform bacteria in the study samples was (1×101- 5× 104) CFU/g ,the levels of numbers of Staphylococcus spp. bacteria ranged from (1× 101 - 2.4× 103) CFU/g,some study samples varied in the total count of yeasts and molds (1× 102- 2.5× 103) CFU/g ,with significant differences P≤0.05, and all study samples were empty from Salmonella spp. and Escherichia coli O104:H4, the results of this study showed the presence of metals Lead, Pb, and Cadmium, Cd, in all tested samples, at varying concentrations. The highest concentration of Lead was 0.0624 mg/kg and the lowest was 0.0371 mg/kg with significant differences P≤0.05, while the lowest concentration of Cadmium was 0.0102 mg/kg and the highest concentration of the same element was 0.0189 mg/kg.

1. Introduction

Meat is considered one of the necessary and most consumed foods by humans in all countries of the world. It is one of the most important sources of protein of high vital value that comes from the high quality and quantity of protein present in it, along with fats and mineral elements, especially phosphorus, potassium, iron and a large group of vitamins, especially vitamin B group, although there are many other animal products that can replace red meat in human nutrition such as milk and its products, eggs, fish and poultry meat, red meat is the important source of nutrition in many countries of the world (Domínguez et al.,2020, Eze et al., 2017) Red meat contains the elements necessary for the growth of most microbes, such as moisture, pH, vitamins and salts.

The internal tissues of a healthy animal often do not contain any microbes or contain limited numbers of them, while the skin and its viscera contain huge numbers of microorganisms that help in the occurrence of internal tissue contamination (Domínguez et al.,2020) Mortadella is a food product prepared from meat (red meat or poultry meat) or a mixture of them with permitted additives after it has been cooked through heat treatments (to a sufficient degree to ensure its consistency, safety and suitability for human consumption under normal conditions of transportation and storage),it may be smoked. Mortadella was located in the city of Bologna in Italy, mortadella is very popular in the world, because of its wonderful taste, high nutritional value, it is found in the market in the form of cylindrical rolls cut or sliced and vacuum-packed, and it is used to prepare low-cost sandwiches (Mohamadi et al., 2023, Martins et al., 2019).

Heavy elements are an important element in the food process, and they are necessary, but in a small percentage determined by the competent authorities, and the deficiency of these elements leads to a weakness in the vital biological process and when their concentration exceeds the permissible limits, they become toxic and have health effects on humans (Rahman et al., 2012, Al-Musawi et al., 2016) .

Heavy metals are known as minerals with a high specific weight of more than 5 g/cm3 (Al-Musawi, 2019) in addition to being minerals that do not have a beneficial effect on the body, if their concentrations exceed the permissible limits and their presence in the body leads to many biological

changes, is also one of the most important problems Environmental and one of the main pollutants of food and this problem has become a major international concern due to food safety issues and health risks caused by heavy metals, due to their bio accumulative and non-degradable properties, these metals are found everywhere in the environment, especially due to their continuous release from natural and human sources origin, such as mining, industrial wastewater treatment, agricultural activities, fuel transportation, coal, oil and forest combustion and then it is transmitted from the environment to living organisms through food exposure, so it poses a serious threat to human, animal and plant health, because it contains a lot of heavy elements, which are some are in concentrations that may exceed the allowable limit, these persistent environmental pollutants have been linked to serious health outcomes ranging from cancer and immunotoxicity to death (Al-Soufi et al.,2015, Bahareh et al.,2022).

Reproductive disorders and potential neurotoxicity in humans due to its accumulation in tissues (Badr & El-Habit, 2018) And since processed foods are widely consumed, as a result of changes in the lifestyle and diet of the population, this has led to an increase in the demand for processed foods, therefore the consumer expects that the products of these foods will be safe, but they can be considered a source of danger to his health and safety, especially if they are not manufactured and handled in accordance with the requirements. Due to the lack of local studies (Fardet &Rock, 2020).

Aim of this study to investigate the presence of bacterial and chemical contaminants in the imported beef mortadella roll products that are available in the local markets.

2. Methodology

Collection of sample

In September to October 2021, ten Samples of imported beef mortadella rolls were obtained from different origins and available in the local markets of Baghdad city and with three replicates for each sample, they were transferred to a small icebox prepared for this purpose, then transferred to the laboratory for analysis. Samples were prepared according to the method described in International Commissions on Microbiological Specification of Food (ICMSF-1978) (Mohamadi *et al.*, 2023 Martins *et al.*, 2019) as follows:

A) 25 g of the study samples were weighed separately under sterile conditions to the cover of the Stomacher mixer, after adding an appropriate amount of the liquid medium, 1% sterile peptone water, placed in glass containers at a rate of 1%. 225 mL Then the sample was mixed at 2000 rpm for 2 minutes, after that the mixture was added to the rest of the contents of the sterile vial containing the liquid medium to obtain a 10^{-1} dilution, then a series of dilutions up to 10^{-5} were performed, and the following tests were performed.

B) Total Viable Bacterial Count

The pour plate method was used in the process of counting aerobic bacteria counting by transferring 1 ml of pre-prepared dilutions to petri dishes and adding nutrient agar after cooling it to 45°C under sterile conditions, stirring the dishes carefully and leaving them to solidify. The dishes were incubated aerobic at a temperature of 30°C for 72 hours, after the end of the incubation period, the cfu/g was calculated.

C) Detection of coliforms

Dilutions were transferred from previously prepared using a sterile carrier loop to Petri dishes pre-cast with sterile crystal violet neutral red bile lactose agar (VRBL) medium and spread by streaking on this medium, and the dishes were incubated aerobically at a temperature of 37°C for 48 hours.

D) Enumeration of *Staphylococus* spp.

This test was carried out with the aim of counting *Staphylococus* spp., by transferring 0.1 ml of a series of pre-prepared dilutions prepared in (A) to Petri dishes prepared in advance with Baird-Parker Agar (BPA) and spread using a sterile L-Shape glass rod. The dishes were incubated in an

air at a temperature of 37°C for 48 hours, Then the colonies were counted on the basis of coagulase positive Staphylococci test.

E) Enumeration of Yeast and Moulds

0.1 ml of the series of dilutions prepared in (A) was transferred to Petri dishes prepared with Rose Bengal Agar + Cholamphenicol + Dichloran (DRBC Agar) medium and spread using a sterile L-Shape glass rod. The plates were incubated at a temperature of 25°C for 5 days, the colonies were counted on the basis of cfu /g.

F) Detection of *Salmonella* spp.

Put 25g of each sample in sterile conditions in the envelope of the Stomacher mixer, after adding an appropriate amount of liquid medium, sterilized peptone water, placed in glass vials at a rate of 225 ml, then mix the sample at a speed of 2000 rpm for two minutes, then add the mixture to the rest of the contents of the sterile vial. The container on the liquid medium to obtain a dilution of 10^{-1}. The bottles were incubated at 37°C for 24 hours. 1 ml of peptone broth was transferred to 10 ml of Selenite cystine broth and Tetrathionate broth medium, then incubated at 37°C for 24 hours, then transferred using a sterile carrier loop to Petri dishes pre-molded with sterile Salmonella-Shigella Agar medium and spread by streaking on this medium. The selection medium was used to isolate *Salmonella* bacteria and it was incubated for 24 hours at a temperature of 37°C. The result is positive if the colonies appear black surrounded by a transparent halo.

G) Detection of *Escherichia coli* O104:H4

For the purpose of investigating the presence of *Escherichia coli* of the serotype H4: O104 and distinguishing it from the rest of the strains of *Escherichia col*i, the method adopted in (Al-Musawi *et al.,* 2018) was followed, and by following the same steps as the method of work mentioned in Paragraph (a). A glass of 225 ml, to which vancomycin was added at a rate of 0.8 mg/l, incubated at a temperature of 42 °C for a period of 6-24 hours. After the end of the incubation period, it was transferred by means of filling the sterile carrier lug and planted on the medium of cefixime tellurite sorbitol MacConkey agar and chromo agar by the planning method, then incubated at 42 °C for 24 hours. The colonies were elected for the purpose of conducting the necessary diagnostic tests, including the use of the Latex agglutination kit prepared by (*E. coli* O104:H4 latex agglutination kit /Product, Abraxis, Warminster, PA, USA) to ensure their return to this specifically isolation.

H) Determination of Lead and Cadmium

Wet Digestion Methods were used to prepare samples, weighing 10 g of them, each separately, using an acidic mixture consisting of nitric acid and perchloric acid, in a volume ratio of 4: 1 respectively, and placed in heat-resistant glass tubes at 190 °C , left for at least 3 hours. In order to form a transparent translucent solution as an indication of the completion of the digestion process, the samples were transferred to volumetric flasks with a capacity of 25 ml after filtering them with Watman filter paper No.1, In Lead and cadmium, in a flame atomic absorption device AAS7000 equipped from the Japanese company Shimadzu, and the measurement conditions were in the flame atomic absorption device using acetylene gas and air, the wavelength λ Max of the elements was Pb 217.0 nm, Cd 228.8 nm (Chouba *et al.,* 2007).

Statical analysis

The Statistical Analysis System- SAS (1998) program was used to detect the effect of difference factors in study parameters. Least significant difference –LSD test (Analysis of Variation-ANOVA) was used to significant compare between means in this study (Littell, 1988).

3. Result and Discussion

The levels of the total number of aerobic bacteria of imported beef mortadella roll samples ranged

between 1×10^2 - 2.5×10^6 CFU/g shows in (Table.1), with significant differences P≤0.05. The level of the total count of coliform bacteria ranged with a significant difference of P≤0.05 in this study from 1×10^1 - 5×10^4 CFU/g, as shown in (Table 1). with regard to *Staphylococcus* spp. Its numbers ranged from 1×10^1 - 2.8×10^3 CFU/g, with significant differences P≤0.05 (Table, 1), while the results of the study samples showed in (Table1) regarding the total count of yeasts and molds, the samples were empty. Others from the same table 1×10^2- 2.5×10^3 CFU/g. the results indicated that all samples were empty from *Salmonella* spp. and *Escherichia coli* O104:H4.

Table 1. Microbial Contaminants in Imported Beef Mortadella Roll (CFU/g)

Number of sample	**Total Viable Bacterial Count**	**Total Coliform**	***Staphylococus spp.***	**Yeast and moulds**	***Salmonella spp.***	***Escherichia coli* O104:H4**
1	2×10^5	2.3×10^4	2.4×10^3	1×10^2	Nill	Nill
2	1×10^5	1.6×10^4	1×10^3	2.5×10^3	Nill	Nill
3	1.9×10^4	3.4×10^4	1.8×10^3	3.1×10^2	Nill	Nill
4	6×10^3	1.2×10^2	1.5×10^2	N.D	Nill	Nill
5	1×10^2	1.1×10^1	1×10^1	N.D	Nill	Nill
6	2.5×10^6	5×10^4	2.8×10^3	1×10^2	Nill	Nill
7	3.6×10^3	1×10^1	2.1×10^1	N.D	Nill	Nill
8	8×10^5	2.3×10^3	3×10^2	1.2×10^2	Nill	Nill
9	1.9×10^5	5×10^4	2.1×10^3	3.4×10^2	Nill	Nill
10	1.2×10^4	2×10^2	2.5×10^1	N.D	Nill	Nill
LSD value	42.507 *	29.661 *	32.893 *	18.43 *	NS	NS
* (P≤0.05).						

Note : least significant difference at the level of P≤0.05, NS: no significant differences

Determination of Lead and Cadmium

The results of laboratory analysis to investigate the level of Lead and Cadmium metals for the study samples showed that the highest concentration of Lead was 0.0624 mg/kg and the lowest concentration was 0.0371 mg/kg of wet weight with significant differences P≤0.05, while the lowest concentration of Cadmium was 0.0102 mg/kg and the highest concentration for the same element 0.0189 mg/kg wet weight.

Table 2. Concentration of Lead and Cadmium in Imported Beef Mortadella Roll on Fresh Weight Basis (mg/kg)

Number of sample	Lead	Cadmium
1	0.0417	0.0139
2	0.0374	0.0103
3	0.0582	0.0126
4	0.0624	0.0189
5	0.0425	0.0123
6	0.0492	0.0156
7	0.0371	0.0149
8	0.0490	0.0130
9	0.0437	0.0102
10	0.0471	0.0117

LSD value	0.0177 *	0.0089 NS
* (P≤0.05).		

Note: least significant difference at the level of P≤0.05, NS: no significant differences

DISCUSSION

Mortadella is considered a desirable product, therefore its contamination is considered a potential source. For human infection, as the total bacterial count test is a means of detecting and inferring the extent of actual contamination and one of the indicators of the availability of health requirements during the manufacturing stages and the microbiological quality of the final products, as well as the fact that it includes a variety of microorganisms, as its height reflects the extent of The poor health conditions that the animal was exposed to before and after slaughter, on the contrary, when it is low, it is an indication of good conditions, this does not give an indication of the possibility of the presence of pathogenic microorganisms, which is a serious threat to the health of the consumer (Hiko *et al.*, 2015;Wardhana *et al.*, 2020), the total microbial count in our study can be considered acceptable It falls within the limits of confidence, which does not exceed 10^7cells/g, according to what was indicated by the Sudanese Standardization Metrology Organization (SSMO) (Mohammed *et al.*, 2015).

The cause of contamination with coliform bacteria in the samples is the lack of a healthy approach and lack of commitment to cleaning and disinfection of the tools used in manufacturing, as well as the low quality of the materials used in the manufacture of this product, With regard to Staphylococcus spp. it can be considered unclean manufacturing tools and workers lack of personal hygiene and their failure to follow sanitary methods during handling or adding parts From highly contaminated carcasses such as intestines to minced meat. which is the most important source of contamination of the product with pathogenic germs (Karisma *et al.*, 2021, Ágredo-Campos *et al.*,2023).

The reason for the contamination of some study samples with these fungi can be attributed to the low quality of the raw materials used, the failure to use good manufacturing practices during their preparation and preparation, which is reflected negatively on Product quality and safety (Mohammed *et al.*, 2015; Odeyemi *et al.*, 2020).

Determination of Lead and Cadmium

Contamination is a complex issue that depends on the quality of the environment in which the animal lives, the level of contamination in the feed, and the characteristics of potential contamination either during steps in manufacturing processes or during packaging and storage procedures. Moreover, adding spices to processed meats, while enhancing the flavor and texture of the food, may bring in more toxins (Badis *et al.*, 2014; Mohammed *et al.*, 2015; Al-Saleh *et al.*, 2017; Yakupa *et al.*, 2018; Barone *et al.*, 2021; Gade *et al.*, 2021) pointed out that there is a great concern about the extent of environmental pollution with chemical pollutants and their relationship to public health, as they considered pollution with heavy metals as one of the most dangerous problems in the world, and stressed the danger of eating food contaminated with the elements Lead Pb, mercury Hg and Cadmium Cd, which are considered environmental pollutants in Everywhere because it causes deep biochemical and neurological changes in the human body, causing severe health risks to the nervous system even at low concentrations, while (FAO/WHO, 2000; Li *et al.*, 2017) warned of the process of preparing and processing spices and additives that could make them a source of food poisoning, as lead could reach He pointed out that cadmium is one of the toxic metal elements when it is present in the systems of the animal body, and it is almost absent in the human body at birth, but it accumulates with age, and it was found that Cadmium can It affects the metabolism of Calcium and Phosphorus in the bones, especially for people who are exposed to it in a polluted environment, knowing that the permissible limit is 0.5 µ/gm (Yakupa *et al.*, 2018; Barone *et al.*, 2021; Olufemi *et al.*, 2022).

4. Conclusion and future scope

The results of microbial examinations of beef mortadella roll samples showed that there were different levels of the total number of aerobic bacteria, the total number of coliform bacteria, *Salmonella* spp. and *Escherichia coli* O104:H4 are not found in current samples. .

All samples gave been contain lead and cadmium.

While the recommendations included the following points

Introducing and improving food processing technology and controlling contamination that may occur during processing.

Urging researchers to increase and deepen their research in the field of food contamination with various pollutants to expand the detection of pollutants with different names such as heavy metals, pesticides, hormones, drug residues and antibiotics, which in their entirety are harmful to human health.

CONFIICT OF INTEREST

Authors are declaring no conflict of interest.

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