

RESEARCH ARTICLE

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The differences in the microbial numbers of the raw minced meat preparation during the shelf life of the productERMELINDA NEXHIPI¹, ELVIRA BELI²¹The Department of Microbiology, the Institution Food Safety and Veterinary “, of Tirana²The Faculty of Agriculture and Environment, the University of Agriculture of Kames, of Tirana* corresponding author e-mail: emanexhipi@yahoo.com**Abstract:**

In this study we have examined the raw minced meat preparation, during the shelf life, in aim to see the differences in the microbial number during, the declared on the label, shelf life. The *APC* and *Escherichia coli* were enumerated in 130 samples of raw minced meat preparation, by the respective Standard ISO methods. The first enumeration was checked on the date of production. The samples were stored in 2-4°C according to the producers recommendation, and reanalyzed on the 7th day, on the 14th day and 2 days before the expired date. At the first examination for *E.coli* 32 out of 130 samples resulted without the presence; 36 samples resulted with account from 1.6×10^2 cfu/g to 3.2×10^2 cfu/g, 26 samples resulted with 3.5×10^2 cfu/g to 4.9×10^3 cfu/g, 36 samples resulted with a count from 1.3×10^4 - 2.1×10^5 cfu/g. At the last examination made two days before the shelf life, 52 samples resulted with a count from 5.4×10^3 - 3.3×10^5 cfu/g for *E.Coli*. Also it was determined the *APC* on both temperatures where we took this results: in the produced day, 25 samples resulted with a count from 1.2×10^1 - 1.4×10^2 cfu/g, 56 samples resulted with 1.8×10^3 - 5.1×10^5 cfu/g, 49 samples resulted with 5.1×10^5 - 2.1×10^6 cfu/g. At the last examination two days before the shelf life, 56 samples varied from 5.2×10^6 - 6.1×10^8 cfu/g for *APC* where the highest indicators, were belonged to meatballs stored in 4°C. The analyses of the results obtained in last enumeration indicated a very increased number of microbial flora. This means that microbiological quality, and safety of the product isn't stable, during the shelf life and the processing establishment of Tirana needs to be improve the production technology and determinate the expiry date based on the study and research.

Key words: *APC*, *E. coli*, during shelf life, raw minced meat preparation, Tirana establishments.

1. Introduction

Food borne infections and illnesses are a major international health problem with consequent economic reduction [16]. It is the major cause of illness and death worldwide, and recognizing this, the World Health Organization (WHO) developed its Global Strategy for Food Safety. In the developing world, foodborne infection leads to the death of many children and the resulting diarrhoeal disease can have long-term effects on children's growth as well as their physical and cognitive development [16]. In the industrialized world, foodborne infection causes considerable illness, heavily affecting health care systems [1]. According to [4], foodborne diseases are diseases resulting from ingestion of bacteria, toxins and/or cells produced by microorganisms present in foods. The intensity of the signs and symptoms may vary with the amount of contaminated food ingested and susceptibility of the individual to the toxin [4]. Food security is therefore a complex issue, where animal proteins such as meats are generally regarded as high risk commodity in respect of pathogen contents, natural toxins and other possible contaminants and adulterants [22]. Nowadays, the

changes in individual's consumption habits and advances in food technology have caused an increase in the demand for different style convenience and semi-processed convenience foods. Meatball is one of the most preferred foods due to the ease of preparation [2]. Meatball is prepared by mince which is a quite suitable environment for microbial growth. The quality of mince and other additives used in preparation of meatballs determines the quality of meat products like meatballs [3]. The shelf-life of meatballs depends on the microbiological quality of meat and other ingredients, especially spices used in its manufacturing, the hygienic precautions taken during production and finally type of packaging and storage conditions [9]. The safety of the product can easily be limited by microbial pathogenic bacteria such as *Cl.perfringens*, *Escherichia coli*, *Listeria monocytogenes* and *Salmonella spp.* [21, 7, 3, 17]. Non-hygienic prior / during and post processing, including primary and secondary contamination may shorten the shelf-life of meatballs [15, 20]. In this study we shall examine the raw minced meat preparation, during the shelf life, in aim to see the differences in the microbial number during, the declared on the label, shelf life.

2. Material and Methods

2.1 Sample's collection

130 samples of prepared minced meat were collected in a casual way from processing establishments of Tirana.

2.2 Samples' preparation

2.2.1 *Escherichia coli*

The "Sample preparation" was referred to ISO 6887-1 [10], where 10 g of sample were weight and homogenized with 90 ml of BPW. Using the pipettes, will transfer them in Petri sterile plates (1 ml of primary and serial dilutions). In every Petri sterile plate we put 15 ml of TBX media, warmed before in 44⁰ C – 47⁰ C. After that we mixed inoculate to the media as soon as it possible and we let it to be hardened in a cold horizontal surface. Then we turn overthrown the plates and we put them in a thermostat, at 44⁰ C for 18-24 hours. The total incubation time should not be more than 24 hours.

2.2.2 Aerobic Plate Count

The "Sample preparation" was referred to ISO 6887- 2 [13], where 10 g of sample were weight and homogenized with 90 ml of BPW. Using the pipettes, will transfer them in Petri sterile plates (1 ml of primary and serial dilutions). In every Petri sterile plate we put 15 ml of PCA media, warmed before in 44⁰ C – 47⁰ C. After that we mixed inoculate to the media as soon as it possible and we let it to be hardened in a cold horizontal surface. Then we turn overthrown the plates and we put them in a thermostat at 30⁰ C for 72 hours.

2.3 Enumeration

2.3.1 *Escherichia coli* enumeration.

The enumeration technique of colonies in plate in 44⁰C by using 5-bromo-4-chloro-3-indolyl β -glucuronide and results expressed it was perform according to the standard method – ISO/TS 16649-2 [11], the horizontal method for enumeration of *Escherichia coli* β -glucuronidase-positive.

2.3.2 Aerobic Plate Count enumeration.

We used the regulated ISO 4833:2004 [12] the horizontal method for the enumeration of microorganisms -Colony-count technique at 30⁰C and expressed the result.

3. Results and Discussions

On the processing establishments of Tirana, in a casual way, were taken 130 products based on minced-meat. The aim of the study was the analysis of the change of the microbial count for *Escherichia Coli* and APC, according to the declared data on labels from the producer. According to the label's data, 65 samples were preserved on 2⁰C and 65 other samples on 4⁰C. The samples had long utilization for 2 months. The analyses were made in Institute of Food Safety and Veterinary (ISUV), Tirana. The first analysis was made on the production date of product. Then it was worked with an interval time for a week. The last analysis was made 2 days before the shelf life of the product. From samples' analyzes us took these results:

I- The results for the change of the microbial count of E.coli of preserved products on 2-4⁰ C.

Table 1. The microbial count of preserved products on 2⁰C (*E.coli*)

Days	0 cfu/g	10 ² cfu/g	10 ³ cfu/g	10 ⁴ cfu/g	10 ⁵ cfu/g
Production Day	19 ¹	18	10	18	0
7 th Day	19	12	14	19	1
14 th Day	19	8	18	13	7
2 days before the shelf life	19	5	17	7	17

¹-Samples' number

Table 2. The microbial count (*E. coli*) for preserved products on 4⁰C

Days	0 cfu/g	10 ² cfu/g	10 ³ cfu/g	10 ⁴ cfu/g	10 ⁵ cfu/g
Production Day	13	18	16	15	3
7 th Day	13	12	14	21	5
14 th Day	13	6	8	26	12
2 days before the shelf life	13	2	12	10	28

Table 3. The microbial count preserved on 2°C

Days	10^1-10^2 cfu/g	10^3-10^4 cfu/g	10^5-10^6 cfu/g	over 10^6 cfu/g
Production Day	15	21	12	17
7 th Day	12	18	16	19
14 th Day	10	15	19	21
2 days before the shelf life	5	9	27	24

The initial count was 19 samples without a count for *E.coli*, 46 samples were varied with a count from 10^2-10^4 cfu/g. During the examination of the later days it was seen a gradual microbial count increase, where 17 samples were resulted with a count of 10^5 cfu/g.

At the first examination for *E.coli* 32 out of 130 samples resulted without the presence; 36 samples resulted with a count from 1.6×10^2 cfu/g to 3.2×10^2 cfu/g, 26 samples resulted with 3.5×10^2 cfu/g to 4.9×10^3 cfu/g, 36 samples resulted with a count from 1.3×10^4 - 2.1×10^5 cfu/g. At the last examination made two days before the shelf life, 52 samples resulted with a count from 5.4×10^3 - 3.3×10^5 cfu/g for *E.Coli*. The presence of *E. coli* in raw minced meat preparation is considered an indication of direct or indirect fecal contamination. Indirect contamination can occur through sewage and polluted water. Direct fecal contamination occurs during the processing of raw foods of animal origin and because of poor personal hygiene of food handlers.

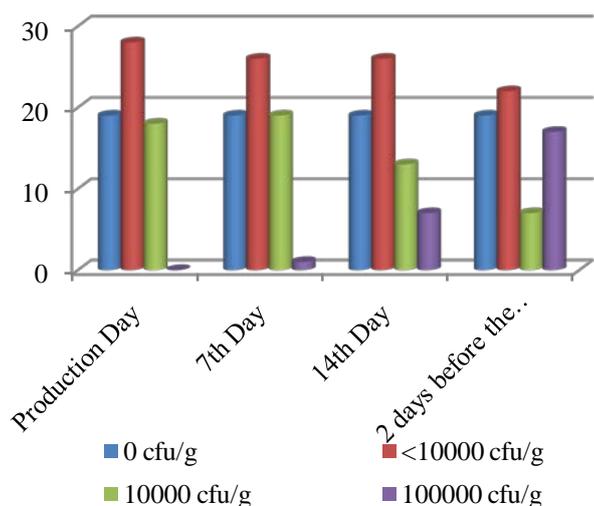


Figure 1 Graphical presentation of the microbial count increase of *E. coli*.

As it's seen from the graphical presentation of the samples' number with a high presence of *E. coli*, it had been raised from the first analysis up to the last analysis with a count of 10^3 cfu/g. 26% of samples had a count from 5.4×10^3 to 3×10^5 cfu/g.

The preserved samples on 4°C showed us a big increase of *E. coli*. From 27% of samples with account of 10^2 cfu / g, resulted on the first analysis, 43% resulted with an increase of the count to 10^5 cfu / g. Davidson [5] reported *E. coli* at the level of 4.8×10^3 cfu / g. Tekinsen [19] examined 20 samples and found that *E. coli*, were counted at the average levels of 6.7×10^3 cfu / g. Hampikyan [8] determined coliform group microorganisms in 8 (40 %) of the 20 meatball samples ranging from 10^1 to 10^4 cfu / g. They found that 3 (15 %) samples contained *E.coli* at levels changing between 10^1-10^3 cfu/g. Soyutemiz [18] determined an average number of coliform bacteria between 10^4 and 10^5 cfu/g. *E. coli* was found in 33.3 % of these meatball samples. In their study, Yildiz [21] found the number of coliform bacteria as 5.2×10^3 cfu / g. *E.coli* was determined in 32 % (24/75) of the samples.

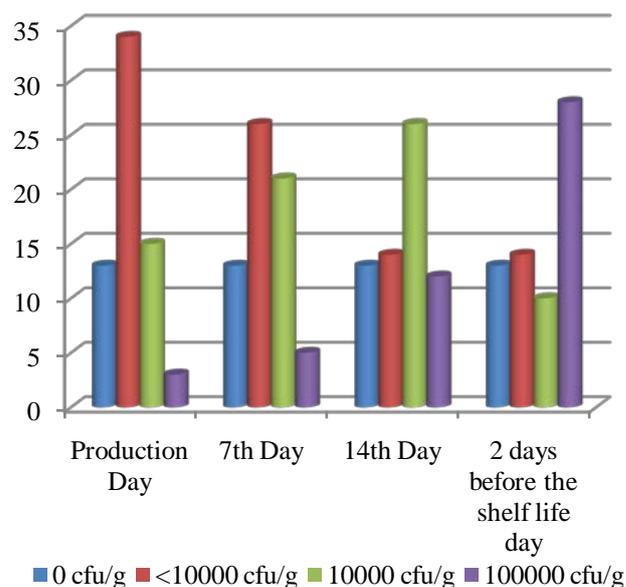


Figure 2 Graphical presentation of the microbial count increase of *E. coli*.

II-The results for the microbial count change of APC for the preserved product on 2-4oC

From the performed analyzes made on the production day, 5 samples resulted with an APC count of 10^1-10^2 cfu/g, 21 samples with count of 10^3-10^4 cfu/g, 12 samples with a count 10^5-10^6 cfu/g, 17 samples with a count over 10^6 cfu/g.

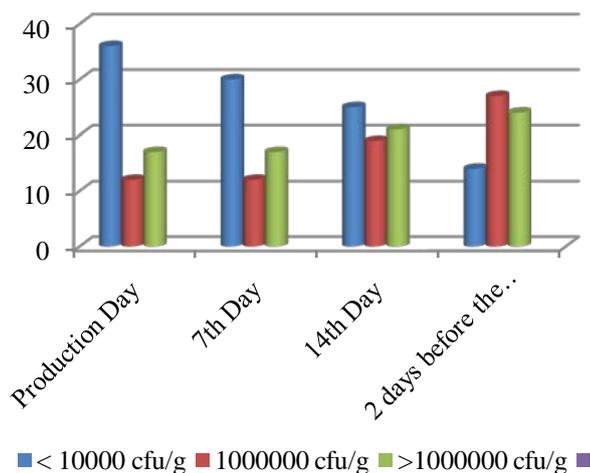


Figure 3 Graphical presentation of the microbial count increase of APC.

As it's seen from the graphic, the samples' number with a count over 10^5 - 10^6 cfu/g has been raised from 26% to 37% on the analysis performed 2 days before the shelf life.

Table 4. The microbial count preserved on 4°C (APC)

Days	10^1 - 10^2 cfu/ g	10^3 - 10^4 cfu/ g	10^5 - 10^6 cfu/ g	10^7 - 10^8 cfu/ g
Production Day	10	35	20	
7th Day	8	28	27	2
14th Day	6	25	25	9
2 days before the shelf life.	3	12	18	32

For samples preserved on 4°C, the microbial count starts changing from the 1st week of the preservation. From 20 samples with a count over 10^6 cfu/g had achieved on 27 samples. 3.1% of samples were varied with a count from 10^7 - 10^8 cfu/g. The performed analysis on 2 last days show us an increase of samples' number with a count over 10^7 - 10^8 cfu / g up to 32 samples, or 47.6%. Tekinsen [19] examined 20 samples and found that total *aerob* mesophiles were counted at the average levels of 8.4×10^7 cfu / g.

In the produced day, referred 130 samples, 25 samples resulted with a count from 1.2×10^1 - 1.4×10^2 cfu/g, 56 samples resulted with 1.8×10^3 - 5.1×10^5 cfu/g, 49 samples resulted with 5.1×10^5 - 2.1×10^6 cfu/g. At the last examination two days before the shelf life, 56 samples was varied from 5.2×10^6 - 6.1×10^8 cfu/g for APC where the highest indicators, were belonged to meatballs stored in 4°C. The aerobic plate count (APC) is important in food microbiology as an indicator of the

microbiological quality as well as a measure of sanitation used during handling of a food.

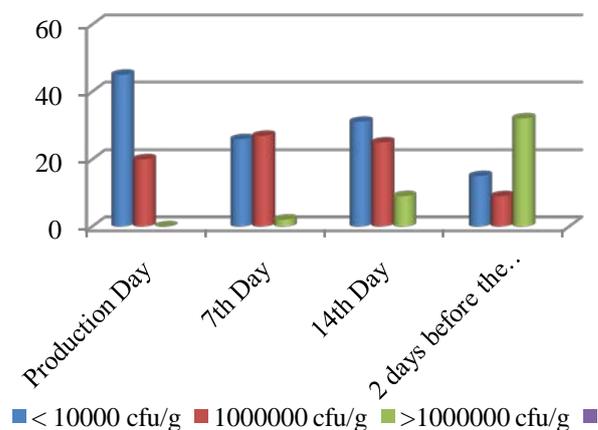


Figure 4 Graphical presentation of the microbial count increase of APC on 4°C

APC determines counts of the non-fastidious aerobic bacteria. In some foods, high APC may indicate poor quality. Higher bacterial numbers spoil the food faster and result in loss of quality. Food which appears normal may have high APC, indicating that the food is about to spoil. Standards for quality indicator tests for the various foods available are very limited. There are different acceptance levels of APC for different food products. For example, in UK, sliced cooked ham has a suggested level of $< 10^6$ cfu/g as a satisfactory level, 10^6 - $< 10^7$ cfu/g as an acceptable level and $\geq 10^7$ cfu/g as unsatisfactory level [6]. Kivanç and Kunduhoğlu [14] reported 4.35×10^7 cfu / g in Eskişehir and Yıldız [22] (5.6×10^5 cfu/g in İstanbul), Hampikyan [8] in İstanbul (1.6×10^2 - 3.8×10^5 cfu/g).

4. Conclusions

- The preserved temperature of raw minced meat preparation during the shelf life in 2-4 °C gave us an increase of microbial count and product decay.
- The determined date, placed on label for product utilization, doesn't fit with the microbiological quality of consumption according to the quality and safety way.
- The used hygiene on processing establishments and raw material and additional subjects, should be improved.

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