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# STUDY ON THE SHELF LIFE OF VACUUM-PACKED MATURED BEEF

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***Abstract***

*The research conducted on the quality and safety characteristics of matured beef, packaged in polyethylene foil, aimed to optimize the shelf life of this food product. The investigations were carried out on samples taken from the local producer and the organoleptic properties (external appearance, cross-sectional appearance, consistency, odor and color) which present appreciable characteristics, and the physicochemical indices (pH value, protein, fat content, peroxidase and peroxidase reaction, etc. were evaluated) demonstrated the requirements in parametric terms. Following microbiological investigations, no pathogenic microorganisms were detected such as: Salmonella spp., E. coli beta-glucuronidase positive, UFC/g, Listeria monocytogenes, etc., which would affect the health of consumers. Heavy metal residues (Cd, Pb) do not exceed the maximum permitted limits for this product, which proves the conformity and safety of matured beef during the shelf life.*

***Key words****: heavy metal residues, mature beef, shelf life.*

## INTRODUCTION

Whether animal, plant or mixed, food is essential for humans. This is because it provides energy and the nutrients needed to maintain health. The human body remains healthier when the diet is balanced, diversified and correctly composed in terms of calories and respecting the optimal ratio of main substances (carbohydrates, proteins, lipids, dietary fibers), (Hodoşan et al., 2023). Food is the most important element, which affects the body, and the maintenance of human health is closely linked to the "health of food".

The main purpose of food safety is to ensure the protection of human health at a high level, and the main objective is to represent the interests of consumers with regard to food products. The EU food safety policy is based on the "farm to fork" concept, which is a holistic approach that vertically integrates every stage of the product approval process, from feed production, plant health, animal welfare, primary production and secondary processing, packaging, storage, retail, delivery for import and export (Georgescu et al., 2000; Diaconescu et al., 2007).

Meat is consumed worldwide due to its high nutrient content, aroma and taste appreciated by consumers. Various health deficiencies (protein malnutrition, iron deficiency, anemia, vitamin deficiencies) can be prevented or corrected by consuming meat and other animal products (Ianiţchi et al., 2024). Due to these special properties, meat consumption has increased significantly, especially in developing countries, and is expected to double by 2050.

Beef, in particular, is appreciated for ensuring a healthy and balanced diet, due to its important content of macro and micro nutrients, proteins, proteolytic enzymes, etc. However, beef requires a longer time for culinary preparation, compared to other types of meat, meanwhile prolonged heat treatment can have negative effects on the chemical composition of the product.

According to studies on meat consumption - the quality of red meat consumption depends on aging (maturation) and this has been included in various guidelines for food quality (Tudor et al., 2009; Pop et al., 2009).

To improve the properties of meat, especially taste and aroma, juiciness and tenderness, meat can be subjected to the maturation process (Oprică, 2011; EFSA, 2022a; Jeremiah, 2001; Air Products SAS, 2016). Another form of improving the tenderness and juiciness of meat is tumbling or massaging, which also ensures a

homogeneous dispersion of salting and flavoring components (Pătraşcu et al., 2011).

Matured beef is a product appreciated by consumers in several countries, in recent years it has also been of interest to the Republic of Moldova.

The main benefits of meat maturation are:

• reducing meat hardness, improving consistency;
• increasing protein solubility, hydration level;
• increasing protein digestibility due to the destruction of the actomyosin complex;
• improving the process of collagen digestibility;
• forming the taste and aroma of meat due to enzymatic transformations of proteins and other enzymes;
• dry maturation has a positive effect in reducing the microbiological load on meat over longer periods, due to the combined effect of cooling and drying.

During maturation, the formation and accumulation of products of enzymatic decomposition of proteins and peptides (glutamic acid, sulfur-containing amino acids), nucleotides (inosine, hypoxanthine, etc.), carbohydrates (glucose, fructose, pyruvic and lactic acids), lipids (low molecular weight fatty acids), as well as creatine, creatinine and other nitrogenous extractive substances, precursors of the taste and aroma of meat, occurs.

Due to the formation of a mold crust on the surface of the meat, during the maturation process, the meat is protected from the effects of pathogenic microflora, softens and acquires a pleasant sweetish (corn) taste. Before sale, cooking, the crust is, of course, removed.

Thus, in the meat maturation process, a significant improvement in organoleptic and technological characteristics, nutritional value compared to refrigerated, fresh meat is observed (UKHSA, 2024; Farber et al., 2006; Jeremiah, 2001; EFSA, 2022b).

## MATERIALS AND METHODS

Laboratory investigations were carried out to determine the quality (physico-chemical), safety, microbiological and sensory evaluation indices during the storage of 21 days matured beef (at temperature 0-2°C) in order to establish the required shelf life, packaged in polyethylene foil, in pieces with a net mass of 0.2-0.4 kg.

The analyses were carried out during September-October 2023. The evaluation of the quality and safety parameters of matured meat, packaged in polyethylene foil, produced for public consumption was carried out by sampling three batches of meat with different production dates. The meat was subjected to the dry maturation process, half-carcasses, quarters and pieces on the bone in unpackaged form, in refrigerated warehouses, on shelves (with controlled temperature and humidity) at a temperature of 0-2°C and a relative air humidity of 75-80% for 21 days, with the provision of a constant air flow with an indicator of 0.5 to 2 m/s. At the end of the maturation period (after 21 days), the cut meat, sliced into smaller pieces (0.2-0.4 kg), is vacuum-packed for transmission to the retail chain, where it continues to be stored under similar conditions at a temperature of 0-2°C and a relative air humidity of 75-80%.

Laboratory investigations were carried out in the Republican Center for Veterinary Diagnostics, in the Food Products Testing Laboratory. Laboratory evaluations included organoleptic, physicochemical, microbiological and safety indices, which were compared with the requirements of the standards in force for this category of products.

The frequency of research for "Mature beef" prepared according to the manufacturer's technological instructions was established as follows: initially (background), after 5 days, 10 and 13 days of storage. Investigations for each stage and date of production were carried out on intact, previously unopened samples.

All investigations were carried out in an accredited laboratory using classical reference methods. Heavy metals were determined by atomic absorption spectrometry with a graphite furnace using the standardized method EN 14083 and EN 13806.

The determination of quality indices was made using classical physicochemical methods: protein determination using the ISO 937 method, fat content using the standardized method ISO 1443.

The microbiological indicators were determined using classical reference methods according to EN and ISO standards: Colony count at 30°C, ufc/g – ISO 4833; *E. coli* beta-glucuronidase positive, ufc/cm$^2$ – ISO 16649-2; *Enterobacteriacae*, log ufc/cm$^2$ – ISO 21528-2;

*Salmonella* spp. – ISO 6549; *Listeria monocytogenes* in 25.0 g – ISO 11290.
The obtained results were compared with the requirements of the regulations in force for this product category; Government Decision no. 696/2010 for the approval of the Requirements regarding the production, import and placing on the market of raw meat; Government Decision no. 221/2009 – Rules regarding microbiological criteria for food products; Government Decision no. 520/2010 – Sanitary Regulation regarding contaminants in food products.

## RESULTS AND DISCUSSIONS

The evaluation of the quality and safety of mature beef for 21 physical days packaged in polyethylene foil in order to ensure the stability of the shelf life requires the study of quality indices, namely: safety and microbiological.
Following the laboratory tests carried out, it was established that matured beef for 21 days met the requirements of the normative documents in force, Government Decision No. 696/2010 - Requirements for the production, import and introduction on the market of meat - raw material, and from a nutritional point of view is a rich source of protein (21-22%), and moderate source of fat (4.7%).
Laboratory investigations for the assessment of organoleptic indices, presented that pieces of beef have appearance of marbled meat (with the presence of strips/threads of fat between the muscle fibers). The surface of the meat is clean, without foreign impurities and visible blood stains, with a glossy surface, of a bright, natural color from red to burgundy, without stains, the threads of fat are white. In the package without leaked liquid: in the section - the samples of matured beef for 21 days presented well-developed muscles, the meat slightly moist, shiny, with thin strips of intermuscular fat. The meat is red-burgundy in color, uniform over the entire surface of the muscle, without gray spots. The threads of fat are white. The elastic consistency of the meat, firm, the dimple from pressing with a finger quickly disappears. When opening the package with meat and cutting the meat in section, a pleasant smell was felt, characteristic of fresh meat, without foreign odor.
Thus, beef matured for 21 days, packaged in polyethylene foil and stored at a temperature 0-4°C retained its sensory characteristics (appearance, appearance in section, consistency, odor) for 13 days, without changing the organoleptic characteristics and correspond to the normative documents GD no. 696/2010 and the Technological Instruction.
Along with the organoleptic indices, the physicochemical quality indices of beef matured for 21 days, packaged in polyethylene foil were also assessed (Table 1).

Table 1. Physico-chemical indices of matured beef

| Indices examined | Regulatory requirements | Results achieved | | | DN to test method |
|---|---|---|---|---|---|
| | | Batch I (n=3) X ± Sx | Batch II (n=3) X ± Sx | Batch III (n=3) X ± Sx | |
| Protein mass fraction, %, min. | De facto | 22.295 ± 0.465 | 21.288±0.071 | 21.125±0.275 | ISO 937 |
| Fat mass fraction, %, max. | De facto | 2.300±0.050 | 4.750±0.076 | 4.775±0.247 | ISO 1443 |
| pH value, un. | De facto | 5.750±0.050 | 5.780±0.082 | 5.775±0.076 | ISO 2917 |

Following the analysis of the meat, proteins values of 21.120- 22.29% were recorded, which are specific and ISO 937 compliant.
The protein value of the meat is also influenced by the way it is prepared (the highest value is recorded in boiling or frying, when the internal temperature of the meat does not exceed 65-70$^0$C). The fat content depends largely on the degree of fat cover and the amount of adipose tissue. The fat content in beef varies (1-3.5%), which is why beef is considered dietetic.
After the research carried out on the matured beef samples, it can be said that the batches of meat examined had fat content values ranging from 2.1% to 5.1%. The results obtained meet the requirements in force and comply with ISO1443.
It should be noted that meat quality depends on the initial microbiological contamination as well as the pH value (when the meat has a pH above 6.2, its shelf life is reduced).

Acidity is one of the main factors that inhibit the development of bacterial microflora, thereby preventing spoilage.

The concentration of hydrogen ions in meat depends on the glycogen content in the muscles at the time of slaughter. In living muscle, the pH ranges between 7.0 and 7.5, and after the animals are slaughtered, under normal conditions, the pH value of the meat decreases relatively quickly due to the process of glycolysis, which leads to the formation of lactic acid. As a result, 12 to 14 hours after slaughter, the pH reaches its final value of 5.4-5.6. After that, the pH value stabilizes.

Therefore, pH values between 5.6 and 6.0 indicate an environment in which microbial growth is reduced or slowed down, and this favors the stability of the sensory attributes of the meat. At the same time, the variation in pH influences the technological properties of meat (water retention capacity, hydration capacity), which improve with increasing pH (Ianiţchi et al., 2019).

In order to monitor possible lipid oxidation processes, which may occur as a result of biochemical processes, tests were carried out to determine the peroxide value and the acidity value in the fat extracted from beef matured for 21 days. Thus, in Figure 1, the results are shown regarding the acidity values in beef matured for 21 days and stored at t =0-4°C.

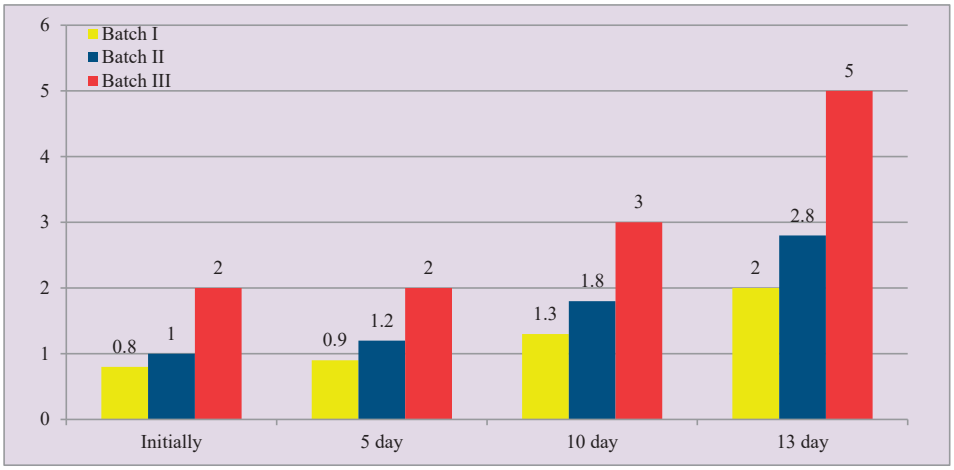


Figure 1. Variation in acidity index of matured beef

In accordance with the obtained results, the acidity index evaluated in the 21 day matured beef samples taken into study for all three batches showed values ranging from 0.8 to 5.0 on the 13th day. These values show that the acidity index on the 10th day was 3 mg KOH/g, which confirms that the beef is considered fresh, because, according to GOST 33818, beef is considered fresh if the acidity is up to 4 mg KOH/g. Next, in Figure 2, the results of laboratory tests on peroxide values in matured beef for 21 days and stored at temperature 0-4°C are presented.

Therefore, laboratory investigations carried out on possible oxidation processes (hydrolytic rancidity, aldehyde rancidity) on the 21 day matured beef samples (Figure 2) demonstrated that samples corresponds in terms of quality, because they were not affected the aroma, texture, color, nutritional value, and neither acidity index and peroxide, according to the GOST 34118 standards.

An indication of meat quality preservation is the peroxidase reaction. The essence of the reaction is that the peroxidase decomposes hydrogen peroxide, and the resulting atomic oxygen rapidly oxidizes benzidine to paraquinodiimide, which with benzidine residues forms a blue-green compound that turns brown in a few seconds, a characteristic reaction for fresh meat. When the initial compound turns blue-green and very slowly turns brown, the reaction is partially negative and the meat is suspect regarding freshness. In case of negative reaction, the

compound immediately turns brown, a process characteristic of spoiled meat. For beef matured for 21 days, peroxidase tests were performed for all three batches of products at each stage: initially, after 5, 10 and 13 days of storage (Table 2).

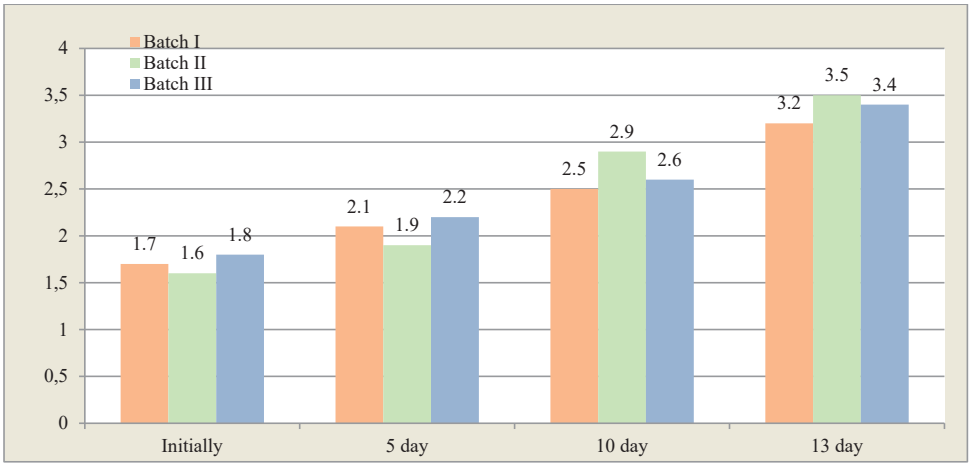


Figure 2. Variation of peroxide value in matured beef

Table 2. Evaluation of peroxidase and Kreiss reaction in matured beef

| Indices | Regulatory requirements | Results achieved | | | DN to test method |
|---|---|---|---|---|---|
| | | Batch I (n=3) X ± Sx | Batch II (n=3) X ± Sx | Batch III (n=3) X ± Sx | |
| Peroxidase reaction | Positive reaction | Positive reaction | Positive reaction | Positive reaction | GOST 7702.1 |
| Kreiss reaction ($H_2S$) | Negative reaction | Negative reaction | Negative reaction | Negative reaction | SR 9065-11 |

However, the peroxidase activity depends on the pH value:
- up to pH=6.0 the peroxidase reaction is positive;
- at pH=6.1-6.2 the peroxidase reaction is suspect;
- over pH 6.2 the peroxidase reaction is always negative.

Thus, at all stages of research the peroxidase reaction for the samples taken into study was positive – a characteristic reaction for fresh meat, or the pH for meat up to 10 and 13 days of storage was maintained within the limits of 5.5-5.6, a specific environment for the positive peroxidase reaction. Determining the stage of fat oxidation by the Kreis Reaction helps to identify the presence of epihydric aldehyde, which appears as a result, of the oxidative processes of linoleic acid in an advanced stage of fat oxidation. Also, a positive Kreis Reaction confirms an advanced stage of alteration in which significant organoleptic changes in taste, color and smell occur.

According to the obtained results, beef matured for 21 days corresponds in terms of quality, because the Kreis Reaction determined in the samples taken in the study was negative.

According to the initial research program, samples of matured beef were subjected to investigations to determine the safety indices: heavy metals (Pb, Cd) (Table 3).

Table 3. Safety indices for matured beef

| Indices | Normative Requirements | Results obtained | | | DN to test method |
|---|---|---|---|---|---|
| | | Batch I (n=3) X ± Sx | Batch II (n=3) X ± Sx | Batch III (n=3) X ± Sx | |
| Pb, mg/kg | max. 0.1 | 0.020±0.003 | 0.060 ± 0.005 | 0.050 ±0.003 | SM SR EN 14082:2006 |
| Cd, mg/kg | max. 0.05 | <0.005 | <0.005 | <0.005 | SM SR EN 14082:2006 |

The results presented in Table 3, referring to the safety of the 21 day matured beef, demonstrate that all the samples investigated, both for the content of Pb (lead), mg/kg, and for Cd (cadmium), mg/kg, presented values that do not exceed the maximum permissible limits, which confirms that the matured beef is safe and meets the requirements of the normative acts in force - Government Decree no. 520/2010 - Sanitary Regulation on contaminants in food products.

The intensity of microbiological processes depends on the composition and properties of the meat: the type of raw material, its water content, pH value, water activity, surface condition (presence of cuts, drying of the crust, etc.). The use of protein films with a protective role can be a solution for limiting microbiological contamination of meat (Cercel et al., 2017). The most important factor affecting the shelf life of raw meat is the initial microbial contamination. Contamination of meat carcasses and other slaughter products occurs both endogenously and exogenously. During the storage period, microbiological indices were examined, which could modify the microflora and product quality stroge – colony count at $30^0$C, *E. coli* beta-glucuronidase positive, *Enterobacteriaceae*, *Salmonella* spp. in 25.0 g, and *Listeria monoytogenes* in 25.0 g, and the results obtained on microbiological testing are presented in Table 4.

Table 4. Dynamics of microbiological indices during shelf-life

| Sample Name/ Defrosting Date | Shelf Life, Days | Colony Count at 30°ufc/g | *E. coli* Beta-Glucuronidase Positive, ufc/cm$^2$ | *Enterobacteriaceae*, log ufc/cm$^2$ | *Salmonella* spp (detection) in 25.0 g | *Listeria monocytogenes* in 25.0 g |
|---|---|---|---|---|---|---|
| Test Method | | SM EN ISO 4833-1:2014 | SM SR ISO 16649-2:2011 | SM ISO 4831:2010 | SM EN ISO 6549-1:2017 | SM EN ISO 11290-1:2017 |
| Normative Requirements | | max. $1*10^6$ | max. 500 | Max 2,5 | Not Allowed | Not Allowed |
| Matured Beef for 21 Days - Lot I t = 2±0.6 °C (storage temperature) Packaged in PE film m.n= 0.2-0.4 kg | initially | $4.3*10^4$ | <10 | <1 | Not Detected | Not detected |
| | 5 | $2.7*10^5$ | <10 | 2.0 | - | Not detected |
| | 10 | $1.3*10^5$ | <10 | <1 | - | Not detected |
| | 13 | 7.4*105 | <10 | <1 | - | Not detected |
| Matured Beef for 21 Days - Lot II t = 2±0.6 °C (storage temperature) Packaged in PE film m.n= 0.2-0.4 kg | initially | $3.5*10^3$ | <10 | 1.7 | Not Detected | Not detected |
| | 5 | $1.5*10^3$ | <10 | <1 | - | Not detected |
| | 10 | 1.7*105 | <10 | <1 | - | Not detected |
| | 13 | $1.9*10^5$ | <10 | <1 | - | Not detected |
| Matured Beef for 21 Days -Lot III t = 2±0.6 °C (storage temperature) Packaged in PE film m.n= 0.2-0.4 kg | initially | $2.1*10^4$ | <10 | <1 | Not Detected | Not detected |
| | 5 | $2.5*10^5$ | <10 | <1 | - | Not detected |
| | 10 | $2.2*10^5$ | 40 | 1.5 | - | Not detected |
| | 13 | $6.5*10^5$ | 50 | 1.6 | - | Not detected |

The results presented regarding the microbiological indices did not show deviations from the admissible limits for the samples taken into study, and, the number of colonies at $30^0$C changed insignificantly, with a slight increase, for all three batches, but which fell within the admissible limits of the normative acts in force. However, the matured beef for 21 days is safe from a microbiological point of view even after 13 days of storage at t=0-$4^0$C.

## CONCLUSIONS

The organoleptic characteristics assessed in matured beef packaged in polyethylene foil and stored at a temperature of 0-4$^0$C preserved its maintained (appearance, cross-sectional appearance, smell, consistency) for 13 days without changing them. These are in accordance with Government Decree no. 696/ 2010.

The evaluated physicochemical indices (fat content, protein content and pH value) in matured beef presented concrete, compliant data that fall within the requirements of the regulations in force. Thus, the fat content in batch I is 2.300%, batch II is 4.750% and batch III is 4.775%. The protein content in batch I is 22.295%, batch II - 21.288%, batch III - 21.125%. The pH value of batch I is 5.750, batch II - 5.780%, batch III - 5.775%, results that fall within the limits of the requirements of the regulations in force for this product category.

The results on the safety indices (Pb and Cd), correspond to the requirements of the regulations in force, respectively Government Decision no. 520/2010.

Following the evaluation of the microbiological indices, it was established that the samples are safe and compliant even after 13 days of storage at t=0-4°C, with Government Decision 221/2009

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