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MASTER THESIS

Microbiological Quality Assessment of Beef Meat in Laghouat Region

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تقييم الجودة الميكروبيولوجية للحم البقر في منطقة الأغواط

من تقديم: - محمد بن مويزة

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Dedication

In the Name of ALLAH, Most Gracious, Most Merciful All the Prays is due to ALLAH alone, the Sustainer of all the worlds

To my dear parents, it is by Allah's grace and then your support that I have reached where I am today. Your constant prayers and immense sacrifices have always been the light that guides my path. I ask Allah to reward you on my behalf with the best of rewards, to bless you with long lives, and to grant you happiness in this world and the hereafter.

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Dedication

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With deepest gratitude and endless love.

Ibrahim Derbal

Abstract

Theme: Microbiological Quality Assessment of Beef Meat in Laghouat Region

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Abstract

The beef meat market in Algeria has seen a significant increase recently. However, the increased importation of beef and otherwise materials and equipment as well as consumer awareness in Algeria may lead to challenges related to microbial contamination due to the increased handling of meat which can lead to health risks for consumers. The objective of this study has enabled us to provide a quantified microbiological quality assessment of beef meat in laghaout region of the levels of contamination by spoilage indicator germs (total aerobic mesophilic flora: FAMT), faecal contamination (thermotolerant coliforms) and *Staphylococcus aureus* contamination.the Methodology is to carry out microbiological analyses of 15 beef meat samples taken from 5 selected butchers' shops on a 3 days period, in the regain of laghouat. During our study, we noted that that the number of butcheries with a number of microorganisms statistically exceeding ($p<0.05$) the acceptance limit set by the standard is One for AFMT, three for *staphylococci*, and for thermotolerant coliforms all butcheries It did not exceed the acceptance limit and the other butcheries, the enumeration of flora sought indicated statistically lower loads ($p<0.05$) compared to the microbiological criteria set by regulation, poor handling during meat preparation operations leads to very high levels of contamination. These contaminations can cause various serious health diseases and rapid meat spoilage, thus limiting its shelf life. A complementary study was conducted to obtain indices of consumer preferences for beef products. A questionnaire was developed for this purpose. The questionnaire included 17 questions to gather information on demographic and social variables also consumer behavior, perceptions of beef, and beef consumption preferences. The results show that the local consumer is somewhat familiar with these products and their behavior reflects the consumption habits of the region. This necessitates a thorough study in the future concerning this topic and the significance of recommending Configuring workers responsible for meat handling in good hygiene practice. in perspective, it would be useful to control the microbiological quality of meats at various levels from slaughterhouses to butchers to better understand and identify the origins of contamination and microbiological contamination issues.

Keywords: Beef meat; Butcher shop; *Staphylococcus aureus;* thermo-tolerant coliforms; Total Mesophilic Aerobic Flora; Questionnaire;Laghouat.

الموضوع: التقييم الميكروبيولوجي لجودة لحم البقر في منطقة الأغواط

الاسم واللقب: بن مويزة محمد، إبراهيم دربال

بإشراف: د. لعيد جخدم

الملخص

شهد سوق لحوم البقر في الجزائر زيادة كبيرة مؤخراً. مؤخرًا. ومع ذلك، فإن زيادة استيراد اللحوم البقرية والمواد والمعدات وكذلك وعي المستهلكين في الجزائر قد يؤدي إلى تحديات تتعلق بالتلوث الميكروبي بسبب زيادة التعامل مع اللحوم، مما يمكن أن يؤدي إلى مخاطر صحية للمستهلكين. الهدف من هذه الدراسة إلى تقديم تقييم كمي للجودة الميكروبيولوجية للحوم البقرية في منطقة لقاوة من حيث مستويات التلوث بواسطة الجراثيم المؤشرة على Mesophilic Aerobic Total Flora (FAMT) والتلوث البرازي (thermo-tolerant coliforms)، وتلوث بكتيريا (*Staphylococcus aureus*). وكانت الطريقة المتبعة هي إجراء تحاليل ميكروبيولوجية على 15 عينة من لحم البقر المأخوذة من 5 محلات جزارة مختارة على مدى 3 أيام في منطقة لقاوة. خلال دراستنا، لاحظنا أن عدد المحلات الجزارة التي تجاوزت إحصائياً ($p<0.05$) الحد الأقصى المقبول وفقاً للمعيار هو واحدة لـFAMT ، ثلاث *Staphylococcus aureus*، وبالنسبة thermo-tolerant coliform جميع المحلات الجزارة لم تتجاوز الحد الأقصى المقبول، وفي المحلات الجزارة الأخرى، أظهر تعداد الفلورا المطلوبة أقل بشكل إحصائي ($p<0.05$) مقارنة بالمعايير الميكروبيولوجية المحددة بالتشريعات. سوء التعامل أثناء عمليات تحضير اللحوم يؤدي إلى مستويات عالية جدًا من التلوث. يمكن أن تسبب هذه الملوثات أمراضًا صحية خطيرة وتفسد اللحوم بسرعة، مما يحد من مدة صلاحيتها.أجريت دراسة مكملة للحصول على مؤشرات تفضيلات المستهلكين لمنتجات اللحوم البقرية. تم تطوير استبيان لهذا الغرض. تضمن الاستبيان 17 سؤالًا لجمع معلومات حول المتغيرات الديموغرافية والاجتماعية، وكذلك سلوك المستهلكين، تصوراتهم عن اللحوم البقرية وتفضيلاتهم في استهلاكها. أظهرت النتائج أن المستهلك المحلي مألوف بعض الشيء مع هذه المنتجات وسلوكه يعكس عادات الاستهلاك في المنطقة. هذا يستدعي دراسة معمقة في المستقبل حول هذا الموضوع وأهمية توصية بتكوين العاملين المسؤولين عن التعامل مع اللحوم في سلامة الغذاء. ومن المفيد مراقبة الجودة الميكروبيولوجية للحوم على مختلف المستويات من المسالخ إلى الجزارين لفهم وتحديد مصادر التلوث ومشاكل التلوث الميكروبيولوجي بشكل أفضل.

الكلمات المفتاحية: لحم البقر؛ محل الجزارة؛ *Staphylococcus aureus*؛ thermo-tolerant coliforms؛ Mesophilic Aerobic Total Flora؛استبيان؛ الأغواط.

Thème : Évaluation de la qualité microbiologique de la viande bovine dans la région de Laghouat

Nom et prénom : Ben Mouiza Mohammed ; Ibrahim Derbal

Encadreur: Dr. Djokhdem Laid

Résumé

Le marché de la viande bovine en Algérie a connu une augmentation significative récemment. Cependant, l'importation accrue de viande bovine, de matériaux et d'équipements ainsi que la sensibilisation des consommateurs en Algérie peuvent entraîner des défis liés à la contamination microbienne en raison de la manipulation accrue de la viande, ce qui peut entraîner des risques pour la santé des consommateurs. L'objectif de cette étude nous a permis de fournir une évaluation quantifiée de la qualité microbiologique de la viande bovine dans la région de Laghaout, en ce qui concerne les niveaux de contamination par des germes indicateurs d'altération (flore aérobie mésophile totale : FAMT), de contamination fécale (coliformes thermotolérants) et de contamination par *Staphylococcus aureus*. La méthodologie consiste à réaliser des analyses microbiologiques sur 15 échantillons de viande bovine prélevés dans 5 boucheries sélectionnées sur une période de 3 jours, dans la région de Laghouat. Au cours de notre étude, nous avons constaté que le nombre de boucheries présentant un nombre de micro-organismes dépassant statistiquement ($p<0,05$) la limite d'acceptation fixée par la norme,un pour la FAMT, trois pour les staphylocoques, et pour les coliformes thermotolérants toutes les boucheries n'ont pas dépassé la limite d'acceptation. Dans les autres boucheries, le dénombrement de la flore recherchée a indiqué des charges statistiquement inférieures ($p<0,05$) par rapport aux critères microbiologiques fixés par la réglementation. Une étude complémentaire a été menée pour obtenir des indices des préférences des consommateurs pour les produits de viande bovine. Une mauvaise manipulation lors des opérations de préparation de la viande entraîne des niveaux de contamination très élevés. Ces contaminations peuvent causer diverses maladies graves pour la santé et une détérioration rapide de la viande, limitant ainsi sa durée de conservation. Un questionnaire a été élaboré à cet effet. Le questionnaire comprenait 17 questions pour recueillir des informations sur les variables démographiques et sociales, ainsi que sur le comportement des consommateurs, les perceptions de la viande bovine et les préférences de consommation de la viande bovine. Les résultats montrent que le consommateur local est familier avec ces produits et que son comportement reflète les habitudes de consommation de la région. Cela nécessite une étude approfondie à l'avenir concernant ce sujet et l'importance de recommander la formation des travailleurs responsables de la manipulation de la viande en matière de bonne pratique d'hygiène. Dans cette perspective, il serait utile de contrôler la qualité microbiologique des viandes à différents niveaux, des abattoirs aux boucheries, afin de mieux comprendre et identifier les origines de la contamination et les problèmes de contamination microbiologique.

Mots-clés : Viande bovine Boucherie ; *Staphylococcus aureus* ; Coliformes thermotolérants ; Flore mésophile aérobie totale ; Questionnaire ; Laghouat.

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FNLS	Faculty of Natural and Life Sciences.
CFU/g	Colony-Forming Units per gram.
PCA	Plate Count Agar.
VRBL	Violet Red Bile Lactose.
BP	Baird-Parker.
TMAF	Total mesophilic aerobic flora.
TTC	Thermos-Tolerant Coliforms.
NaCl	Sodium Chloride.
pH	Potential Hydrogen.
ATP	Adenosine Tri Phosphate.
Spss	Statistical Package for Social Sciences.
HACCP	Hazard analysis and critical control points

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INTRODUCTION

Introduction

Beef is considered an important meat in the Algerian diet, representing a significant proportion of meat production at 34.5%, with an average consumption of around 10.5 kg per person annually (Sadoud, 2011; Chikhi and Bencharif, 2016), Despite domestic production, imports of red beef were high in Algeria, reaching $122 million during the first ten months of 2020 (Nora et al., 2022).

Beef and its derivative products hold a special place in our diet due to their nutritional value. The high-water content and high biological value proteins make meat an essential food for a balanced diet. However, these factors also make meat a favorable environment for microbial proliferation (Clinquart et al., 1999).

Meat, due to its nutritional characteristics, serves as a conducive habitat for a wide range of microbial contaminations. The sanitary state of meat is contingent upon factors such as the upbringing conditions of animals, their transportation pre-slaughter, and the likelihood of contamination during the slaughtering process. The slaughtering facility stands out as a pivotal juncture for ensuring meat hygiene, with the act of slaughter being identified as the primary stage where contamination risks are most pronounced. Studies indicate that a significant proportion, ranging from 80 to 90%, of the microbial flora present in meat and eventually consumed by individuals can be traced back to contamination incidents at the slaughterhouse (Djenidi, 2016). Noteworthy among the microorganisms involved are spoilage bacteria, which contribute to meat spoilage, and pathogenic bacteria, known to be causative agents of foodborne diseases. The occurrence of such microbial contamination is frequently associated with deficiencies in hygiene practices (Durand et al., 2006; Cartier and Moëvi, 2007).

In Algerian farms, beef meat showed a contamination rate of 64% in 2020, with the lowest contamination rate observed in beef slaughterhouses at 33.84% (Khireddine et al., 2021).

The microorganisms present in meat can also cause organoleptic changes and alter market qualities, or pose a danger to public health due to their pathogenic power for humans.Thus, contamination can be caused by individuals (germs on the skin, hands, intestines, throat, or cuts), soil, dust, wastewater, surface water, manure, and already spoiled foods (Hammoudi and Riad, 2013).

According to Ndiaye (2002), it can also occur through poorly washed instruments, domestic and companion animals, pests, or animals slaughtered in poor hygiene conditions.

Furthermore, meat preserved by techniques aimed at maintaining its microbiological quality by slowing down the rate of microbial proliferation and preserving its organoleptic and nutritional properties by eliminating intrinsic and extrinsic alteration mechanisms can be a site for microbial growth, leading to serious, and potentially fatal, health problems if strict hygiene standards during slaughter and carcass treatments to extend meat durability are not adhered to (Multon, 1984; Durand et al., 2006).

Therefore, foodborne infectious diseases are often linked to hygiene defects. The majority of these syndromes are related to the transmission of pathogens through foods from infected or carrier animals or meats contaminated by water and fecal matter (Goudiaby, 2005). In food microbiology, it is more important to determine the absence or presence of microorganisms and to be able to ascertain whether the product complies with human consumption standards (Lightfoot, 2002).

Given the importance of meat in our diet and its role as a conducive environment for the spread of various microorganisms, especially pathogenic species that cause diseases, we have decided to conduct a study on the bacteriological quality of beef sold in five butcher shops in the Wilaya of Laghouat. The aim is to verify if hygiene conditions are being followed to ensure whether this meat can be consumed or not, and how we can address contamination if it occurs. We have adopted the following structure, which consists of four chapter:

The first part is dedicated to a literature review comprising two chapters. The first chapter gathers all information related to the quality and consumption of meat, while the second chapter focuses on the bacteriology of this type of food.

In the third chapter, we present the methodology in tow part; the first adopted for conducting the experiment.

Specifically, for beef meat, the following will be counted:

- Total mesophilic aerobic flora (FMAT).
- Thermo-tolerant coliforms
- *Staphylococcus aureus*

In the second part we present the methodology of survey.

The fourth chapter discusses the results obtained of two parts, and we conclude our study with a summary, recommendations, and future outlooks.

CHAPTER I:

GENERALITIES OF BEEF MEAT

I.1 Definition

According to the World Organization for Animal Health (OIE), meat refers to all the edible parts of an animal, considering "animal" in this context as "any mammal or bird" (OIE, 2016). This vocabulary includes the flesh of mammals (such as sheep, cattle, goats, camels, etc.) and birds (such as chicken, turkey, guinea fowl, etc.).

The meat is the product of muscle transformation after the animal's death (Dennaï et al., 2001; Fosse et al., 2006). In general terms, meat is defined as any fresh or prepared flesh that humans use for consumption.

Meat results from the post-mortem evolution of skeletal (or striated) muscle tissue and adipose tissue. Thus, it is the product of muscle transformation after the animal's death (Salifou et all., 2013). Traditionally, it is considered the carrier of numerous foodborne illnesses in humans due to hygiene issues (Dennaï et all., 2001; Fosse et al., 2006).

I.2 Composition of Meats

The composition of muscle varies between animals and even within the same animal from one muscle to another (Coibion, 2008). However, the average composition of beef meat is reported in Table 1.

Table 1. Average biochemical composition of meat in cattle (Keeton and Eddy, 2004).

Components	Percentage
Water	74%
Proteins	19%
Lipids	5%
Carbohydrates	1%
Ashes	1%

I.3 Importance of Beef in a Balanced Diet

Meat provides us with several essential nutrients such as proteins, minerals (iron), and B-group vitamins. The quality of proteins provided by meat is so high that it covers human protein needs. These macromolecules perform numerous specific functions in the human body. Their essential role lies in the synthesis and renewal of the body's constitutive proteins (Jacotot and Parco, 1983).

Beef is indispensable in the diet of the entire family. It provides children and adolescents with the necessary proteins for the development of their skeleton and muscles. For adults, it provides the required amount of proteins per day (0.8 g/day per kg of body weight) (Collind, 1972).

Beef is rich in iron and proteins, making it particularly recommended for pregnant women. During pregnancy, the body's protein needs increase, while iron is necessary for the production of red blood cells, Beef should also not be absent from the daily diet of seniors. The proteins and iron it contain help them fight muscle atrophy and better resist infections (Roua, 1988).

I.4 Qualities of Meat

The concept of quality can be defined according to ISO 8402 as "the set of properties and characteristics of a service or product that give it the ability to satisfy expressed or implicit needs."

In other words, quality is about customer or user satisfaction and concerns all operators who expect satisfactions related to the profitability of their activity (Girard, 1990).

The notion of intrinsic quality of meats is a relative concept that depends on more or less objective elements: organoleptic, nutritional, hygienic, and sanitary quality (Frayasse and Darre, 1989).

I.4.1 Organoleptic Quality

The organoleptic quality of meat depends on numerous factors related not only to the animal and the rearing methods but also to the meat's cooking and processing (Renand et al., 1997; Dransfield, 2006). It encompasses color, flavor, juiciness, and tenderness (Monin, 1991).

I.4.1.1 Color

The primary characteristic noticed by consumers is the color of meat, which is closely linked to its freshness. Myoglobin, a chromoprotein, is the key pigment responsible for meat color. Upon exposure to air, myoglobin reacts with oxygen to create oxymyoglobin, a vibrant red color that signifies freshness and is preferred by consumers (Renerre, 1997; Coibion, 2008). Myoglobin serves as an oxygen-storing and -exchanging molecule and exists in three forms that determine meat color based on myoglobin's state (oxidized or reduced) and its concentration in the muscle (Renerre, 1997). These three forms of myoglobin—reduced (purplish-red), oxymyoglobin (bright red), and metmyoglobin (brown)—are depicted in Figure 01. The brown color of meat can lead to consumer aversion (Staron, 1982; Touraille, 1994).

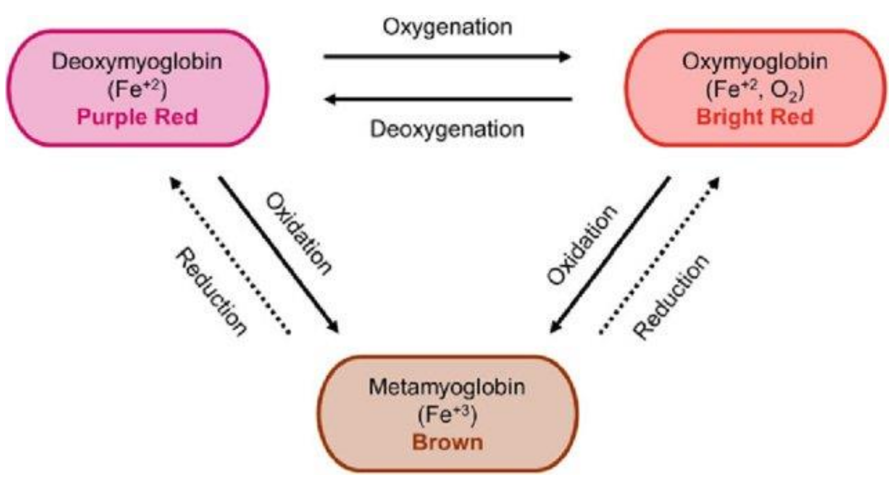

Figure 1. Meat color according to the oxidation state of myoglobin (Ribeiro,2022).

Additionally, the evolution of pH also affects meat color. However, a low pH causes meat to lose color, while a high pH results in darker meat coloration (Fraysse and Darre, 1989).

Other factors such as intramuscular fat content, dehydration, or the presence of a surface water film can also alter the perceived color (Moevi, 2006). Consequently, meat with a high bacterial load at the beginning of storage experiences quicker color deterioration due to the accelerated irreversible development of brown metmyoglobin, which is unattractive. Furthermore, various bacterial species *(Chromobacterium, Flavobacterium, Serratia, Bacillus, Micrococars, Sarcina*, etc.) can lead to the formation of a pigmented bacterial film on the surface of the cut, with varying colors (whitish, greenish, etc.) that interfere with the product's original color.

I.4.1.2 Tenderness

Tenderness refers to how easily meat can be sliced or chewed, making it a crucial characteristic (Soltner, 1979). Both connective tissue and myofibrils contribute to meat tenderness. Connective tissue evolves slowly over time due to its high mechanical resistance and stability, primarily because of its collagen component.

Muscle fibers undergo various transformations after an animal's death, initially increasing in toughness with the establishment of rigor mortis, followed by tenderization during maturation. Tenderization is rapid in the first few days and then slows down, approaching a limit (Coibion, 2008).

The optimal tenderness conservation period depends on the storage temperature. It is around 8 days at 6°C, 14 days at 2°C, and 16 days at 0°C (Coibion, 2008; Lamoise et al., 1984).

I.4.1.3 Flavor

Flavor refers to the combined olfactory and gustatory sensations experienced during food consumption (Rosset and Linger, 1978; Coibion,2008). It depends on various chemical compounds released during cooking (Coibion,2008).

Raw meat has a subtle flavor mainly related to mineral salts and precursor substances of flavor. The lipid fraction of meat, classified into two categories, is responsible for flavor:

- Volatile compounds (aroma and odor) include sulfur compounds, alcohols, esters, aliphatic hydrocarbons, etc.

- Non-volatile compounds (taste) consist of nucleotides, certain amino acids, and creatinine. These precursors develop during meat maturation (Coibion,2008).

Flavor is influenced by several factors such as species, breed, age, sex, rearing method, and post-mortem evolution (Rosset, 1988).

I.4.1.4 Juiciness

Juiciness refers to the amount of muscle juice released by the muscle when pressed or chewed (Dassenoy,2003). It is generally accepted that the sensation of juiciness involves two components. The initial impression is determined by the amount of water released at the beginning of chewing, directly dependent on the water retention capacity of the product. The second, more prolonged component results from the stimulation of saliva by lipids. This latter component leaves a more lasting impression than the first (Geay et al., 2002).

I.4.2 Technological Qualities

The technological qualities of meats are defined as their suitability for processing. Among the most common processing methods are cooking, salting and drying. Salting and drying (Foury,2005).

I.4.2.1 Water Retention Capacity

Water retention capacity is the ability of meat to hold its own water firmly. Retain its own water (Huff-Lonergan, 2010). In meat, water retention is an important characteristic for several reasons: appearance of the raw product, exudations, thawing losses, cooking losses, juiciness of the cooked product (Apple,2013).

Indeed, it is essential to take this parameter into account, as it influences the profitability of the processing sector and, in turn, the quality of the product. Profitability of the processing sector and, more importantly, the organoleptic qualities of the meat. What's more, this parameter is often considered byquality criteria (Huff-Lonergan et Lonergan, 2005; Cheng et Sun, 2008).

I.4.2.2 pH

Although it is actually a chemical parameter, pH is usually classified among the technological characteristics because it has a very significant influence on water retention capacity and the ability to preserve and process meats (Huff-Lonergan, 2010).

The intramuscular pH value measured in vivo is close to 7. In the hours following slaughter, a drop in pH is observed within the muscle tissue due to the accumulation of lactic acid produced by the breakdown of intramuscular glycogen. When glycogen reserves are depleted, a stabilization of pH is observed. This is the ultimate pH or final pH, the value of which is close to 5.5 (Wu et al., 2014).

final value reached is considered a crucial factor in determining meat quality and consequently strongly influences meat preservation ability: for example, a high pH, above 6, promotes the development of spoilage microorganisms responsible for taste, odor, and color alterations in meat, as well as pathogenic microorganisms (Jeleníková et al., 2008; Huff-Lonergan, 2010).

The final value is mainly related to one factor: the amount of glycogen present in the muscle before slaughter. However, the factors influencing glycolytic reaction kinetics are much more numerous and complex. The rate of glycogenolysis varies from one species to another, and even within species (Shackelford et al., 1994).

pH evolution is not uniform throughout the carcass: it varies from one muscle to another, and even from one place to another within the same muscle. These variations between species and muscles are related to the metabolic type of muscle fibers. Furthermore, the rate of glycogenolysis is directly influenced by temperature. Therefore, it is essential to measure both pH and carcass temperature simultaneously to avoid any interpretation errors (Clinquart et al., 2000).

I.4.3 Nutritional Quality

Meat is a source of proteins and essential polyunsaturated fatty acids. However, these molecules promote lipid rancidity. On average, meat contains about 20 grams of protein per 100 grams of fresh tissue, which corresponds to nearly one-third of the recommended daily nutritional intake (Bauchart et al., 2008). Consequently, the pursuit of good nutritional quality by increasing the content of polyunsaturated fatty acids, which are presumed to be healthier than saturated fatty acids, conflicts with the technological and organoleptic qualities of these products.

Furthermore, this potential source of polyunsaturated fatty acids could diminish during cooking. Given the numerous disadvantages associated with unsaturated fatty acids, it seems unwise to try to increase their content in meat. After all, humans can find these compounds in large quantities in other food sources such as vegetable oils.

I.4.4 Hygienic Quality

Hygienic quality can be compromised by the proliferation of harmful bacteria and/or the production of toxic compounds in meat. These defects are strongly influenced by the post-mortem evolution kinetics of pH and the oxidation of polyunsaturated fatty acids. The decrease in pH has a bacteriostatic effect; however, pH has less of an impact on microbial growth than on the direction of microbial development. A high ultimate pH promotes the growth of putrefactive bacteria and hinders the penetration capacity of salt into the meat (Coibion, 2008).

I.4.5 Functional Quality

Meat must meet essential criteria expected by consumers beyond strictly nutritional aspects, such as its ability to be preserved, which translates into the shelf life of the food after purchase under specific storage conditions, convenience of use through easy storage (refrigeration), and ease of preparation that is quick and simple (Touraille, 1994).

I.5 Transformation of Muscle into Meat

Muscle is a tissue in living animal or human organisms characterized by its ability to contract, while meat refers to all foods of animal origin made from muscle tissues and intended for consumption, especially by humans (Denoyelle, 2008). Various factors influence the course of these transformations: intrinsic factors related to the muscle, such as its type, which defines the composition and nature of the enzymatic equipment involved in these transformations, as well as age, sex, and species, which also impact the typing characteristics of muscles; extrinsic factors include the technologies used, particularly the thermal regime imposed on carcasses and meat (Valin, 1988).

The transformation of muscle into meat occurs in three phases: the panting phase, the rigor mortis or post-mortem stiffness phase, and the maturation phase (BENAÏSSA, 2014). At the end of these three phases, meat develops organoleptic characteristics (color, tenderness, and flavor) that are crucial during consumption in its raw state (Oueslati et al., 2018).

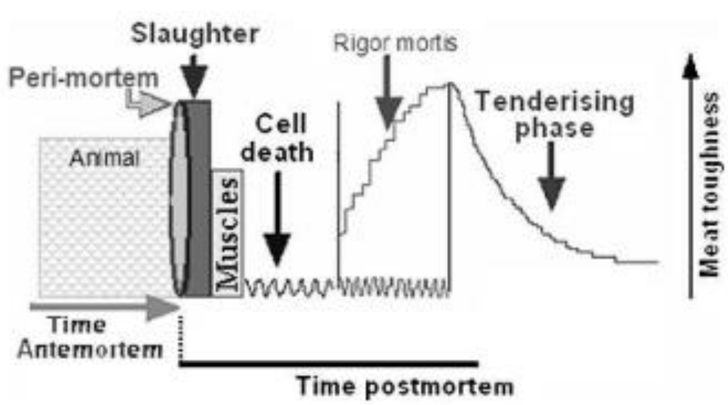

Figure 02 Stages in the transformation of muscle into meat (Ouali et al.,2006).

I.5.1The Panting Phase

The panting phase occurs immediately after slaughter. Panting begins with the cessation of blood circulation, which stops the supply of oxygen and exogenous energy substrates (glucose, amino acids, and fatty acids) (Dognon et al., 2018). Cellular oxidation capacity decreases rapidly, and only anaerobic reactions (primarily glycolysis) persist (Lonergan et al., 2010; Pearce et al., 2011).

The transformation of glycogen into lactic acid continues as long as the nervous system remains active (MALTIN et al., 2003).

I.5.2 Cadaveric Rigidity

Cadaveric rigidity, also known as rigor mortis, gradually sets in after the panting phase (Guérin C and Thapon J-L., 2007). During this phase, the muscle becomes progressively stiff and inextensible in the hours following the animal's death. The rigor process is characterized by a latency phase and a rapid contraction phase (Smili, 2014). This phenomenon results from the depletion of adenosine triphosphate (ATP), the compound that allows living muscle to maintain its elasticity and provides the energy needed for muscle work (Coibion L., 2008). The solidification of fat due to the decrease in carcass temperature also contributes to increasing meat firmness (Guérin C and Thapon J-L., 2007).

I.5.3 Maturation Phase

Maturation is the phase of favorable tenderness evolution resulting from the degradation of certain elements of muscle fibers (rupture of Z lines and elongation of sarcomeres) or connective tissue by proteases (Lonergan et al., 2010; Pearce et al., 2011). Texture changes in the muscle occur during this phase (Valin C, 1988). Meat texture is defined by the state and organization of the cytoskeleton (muscle structural proteins, myofibrillar proteins, and collagen) (Coibion L, 2008). Due to the depletion of muscle energy reserves in the moments following death, only hydrolytic phenomena remain, gradually disorganizing the different muscle structures and making the meat tender. The depletion of muscle energy reserves and acidification of the environment create favorable conditions for protein denaturation (Chriki, 2013).

Protein denaturation can result in changes in conformation leading to unmasking of groups, modifications in solubility properties, and increased sensitivity to proteolytic enzymes (Coibion, 2008).

Chapter II:

BACTERIOLOGY OF MEATS

II.1 Bacteriology of Meats

The bacterial microflora of meat and meat products mainly consists of saprophytic germs or hygiene indicators, and pathogenic flora responsible for diseases and food poisoning (Cartier, 2007; Ghafir and Daube, 2007).

II.1.1 The Saprophytic Germs or Hygiene Indicators

Many bacteria are counted as indices or indicators. Exceeding a given threshold can have multiple origins and meanings. The main ones are described below.

Saprophytic germs constitute the bulk of the contamination microflora in meat and meat products. Among the saprophytic bacteria isolated from meat, list in order of importance first *Pseudomonas, Acinetobacter*, and *Micrococcus*; then, *Enterobacteria and Flavobacterium*; finally, *Bacillus, Mycobacterium, Lactobacillus, Alcaligenes, Serratia, Streptococcus, Aeromonas, Corynebacterium, Arthrobacter*, and *Clostridium* (Fournaud, 1982). Among saprophytic bacteria, hygienists also consider *Escherichia coli*, fecal coliforms, and *enterococci* in general. These bacteria are considered to come directly from the digestive tract. However, *E. coli* remains currently the only and safest test germ to use in public hygiene (Fournaud, 1982).

II.1.1.1Total Aerobic Bacteria

Total aerobic bacteria do not constitute a specific bacterial family. They are microorganisms that form countable colonies after multiplication under defined laboratory conditions (McEvoy et al., 2004; Pearce and Bolton, 2005; Smith et al., 2005; Hutchison et al., 2006).

The sources of contamination of foodstuffs by total aerobic bacteria are varied: the environment, the animal (flora present in the intestine, on the skin, fleece, mucous membranes), cross-contamination with other carcasses or food items, contamination by the handler. In raw or processed food after treatment, it is normal to find a low quantity of these bacteria. They can include *enterobacteria, Bacillus, staphylococci, Pseudomonas,* lactic acid bacteria, or other potentially pathogenic agents. Their presence beyond defined limits may indicate a hygiene deficiency in manufacturing processes, or, beyond 10^7 cfu/g, a state of putrefaction. It can also result from storage at excessively high temperatures (Ghafir and Daube, 2007).

II.1.1.2 *Pseudomonas*

The genus Pseudomonas consists of Gram-negative bacilli, straight or slightly curved, with a size ranging from 0.5 to 1.0 µm by 1.5 to 5.0 µm, aerobic, oxidase-positive, non-sporulating, and generally mobile via one or more polar flagella. Some produce water-soluble fluorescent pigments or pyoverdine, a yellow-green color that acts as a siderophore. Most species are psychrotrophic.

They can grow between 4°C (or lower) and 43°C (Euzeby, 2007). *Pseudomonas* is ubiquitous and belongs to the γ subclass of Proteobacteria, capable of living in diverse ecological niches. While not highly virulent, several strains are opportunistic pathogens for humans and agents of spoilage in meats, fish, and dairy products. The most frequently encountered species in humans are *Pseudomonas aeruginosa, P. fluorescens, P. putida,* and *P. stutzeri* (Euzeby, 2007).

Pseudomonas are the main psychrotrophic bacteria found in meats, milk, and to a lesser extent, plant products. Present in food, refrigeration allows their multiplication and the production of proteolytic and lipolytic enzymes responsible for spoilage. Their presence in slaughterhouses, especially in cold rooms, is a constant source of meat contamination. *Pseudomonas* is primarily used as an indicator of spoilage in fresh meats and milk (Labadie et al., 1996).

II.1.1.3 *Enterobacteria*

Enterobacteriaceae or enterobacteria belong to a family of short Gram-negative rods, ranging from 0.3 to 1.0 µm by 1.0 to 6.0 µm, some of which are mobile via peritrichous flagella and others are non-motile. Non-sporulating, they multiply in the presence and absence of oxygen. They have a respiratory and fermentative metabolism, producing acids and often gas during the fermentation of glucose and other carbohydrates (Ghafir and Daube, 2007).

This is a biochemically and genetically related group, exhibiting great heterogeneity in terms of ecology, hosts, and pathogenic potential for humans, animals, insects, and plants. This family includes several genera and species of intestinal origin pathogens (*Shigella, Salmonella, Yersinia,* and pathogenic strains of *E. coli*). It also includes many genera naturally occurring in the environment, including on plants, without being of fecal origin or associated with foodborne diseases (Ray, 2001; Euzeby, 2007).

II.1.1.4 Coliforms Total and Fecal

Total coliforms are aerobic or facultative anaerobic Gram-negative bacteria, non-sporulating, rod-shaped, and may be motile or non-motile (Cardinal, 2003). These microorganisms possess the enzyme β-galactosidase, which hydrolyzes lactose at 37°C to produce red colonies on a suitable medium.

On the other hand, the coliform group has been used since the late 19th century as an indicator of fecal pollution (Archibald, 2000). Fecal coliforms or thermotolerant coliforms are a subgroup of total coliforms capable of fermenting lactose at a temperature of 44°C (Edberg et al., 2000).

II.1.1.5 *Acinetobacter*

Acinetobacter are Gram-negative bacilli, strict aerobes, non-sporulating, sometimes encapsulated, non-motile, catalase-positive, and oxidase-negative. They grow easily on ordinary media and are present in large numbers in the flora of spoiled or fresh foods such as poultry carcasses and meat from butchery animals (Guiraud et al., 2012).

In the same context, several authors have reported that Acinetobacter is considered among the main surface microflora found immediately after slaughter on carcasses, along with the following bacterial species: *Micrococcus, Pseudomonas, Moraxella, Staphylococcus, Streptococcus, Bacillus, Brochothrix thermosphacta, Lactobacillus, Flavobacterium, Kurthia, Enterobacteriaceae,* and *Corynebacterium.*

II.1.2 Pathogenic Germs

Pathogenic bacteria that contaminate meats and minced meats, and are responsible for foodborne illnesses, generally include *Salmonella spp*., *Listeria monocytogenes, Campylobacter jejuni, Clostridium botulinum, Clostridium perfringens, Bacillus cereus, Staphylococcus aureus, Yersinia enterocolitica, Aeromonas hydrophila, Shigella,* and recently*, enterohemorrhagic Escherichia coli or E. coli* O157:H7 (Dennai et al., 2001; Heredia et al., 2001).

II.1.2.1 *Escherichia coli*

Escherichia coli belongs to the family *Enterobacteriaceae*. They are short, motile rods with peritrichous flagella, Gram-negative, facultative anaerobes, non-sporulating bacteria. They can ferment several sugars, but their characteristic fermentation of lactose with gas production sets them apart (Salifou et al., 2013).

Multiplication at 44°C, production of indole, and the presence of ß-glucuronidase activity are also characteristic of Escherichia coli. E. coli is serotyped based on its 173 somatic antigens (O), 56 flagellar antigens (H), and 80 capsular antigens (K) (Feng, 2001; Eslava et al., 2003). Being the predominant bacterial species in the intestine and feces, the presence of E. coli in food and water is considered an indication of fecal contamination and therefore a possible presence of fecal origin pathogenic microorganisms (Ghafir et al., 2007).

The main disease they cause in humans is hemorrhagic colitis or EHEC, with serotype O157 being well known. Besides hemorrhagic colitis, EHEC can cause diarrhea, hemolytic uremic syndrome, and thrombotic thrombocytopenic purpura (Feng, 2001; Ray, 2001).

II.1.2.2 *Salmonella*

Since the first observations reported by Eberth in 1880 until today, the genus Salmonella has remained of considerable importance in veterinary and medical fields, both due to economic losses from animal diseases and the high incidence in humans (typhoid fever and *Salmonella* foodborne infections) (Bornert, 2000).

Salmonella belongs to the family *Enterobacteriaceae*. *Salmonella* bacteria are straight Gram-negative bacilli, non-sporulating, with a size of 0.7 to 1.5 µm wide and 2.0 to 5 µm long, facultative anaerobes. The bacilli are generally motile with peritrichous flagella. They typically produce acids and gas from glucose and use citrate as their sole carbon source. These bacteria grow at temperatures between 8°C and 45°C but are heat-sensitive.

They are mesophiles, capable of growing at temperatures between 5.2°C and 47°C, optimally between 35 and 37°C, at pH levels between 4.5 and 9, and water activity (aw) above 0.93 (Fosse et al., 2006). Within the subspecies *Salmonella enterica enterica*, there are more than 2400 different serotypes, some of which are potentially pathogenic to humans. These serotypes are ubiquitous and can be found in the digestive tract of humans, domestic and wild animals, pets, and particularly poultry for *S. enteritidis*. Regarding beef, *S. dublin* is also often implicated, which can be harbored in the digestive tract of cattle and humans.

Salmonella infections from meats can be serious due to both the number of affected individuals and the severity of symptoms. Ingesting 10^1 to 10^{11} Salmonella cells can trigger an infection characterized by a fever of 39°C to 40°C, abdominal pain, nausea, vomiting, and a diarrheal syndrome with liquid and foul-smelling stools (AFSSA, 2002).

II.1.2.3 *Staphylococcus aureus*

Staphylococcus aureus is a microorganism of the *Micrococcaceae* family. It is Gram-positive cocci, measuring 0.5 to 1 µm in diameter, often arranged in clusters, non-sporulating, and coagulase-positive. This species is a facultative aerobe, but it prefers aerobic metabolism. It is a mesophilic organism, capable of multiplying between 4°C and 46°C, optimally at 37°C, with a pH range of 5 to 9, an optimum of 7.2 to 7.6, and a water activity (aw) of 0.86 in aerobic conditions and 0.90 in anaerobic conditions. It is a halophilic and xerophilic microorganism because it can grow even in the presence of salt and sugar and survives in dehydrated foods: its growth is possible up to a salt concentration of 18% in aerobic conditions (Fosse et al., 2006; Bailly et al., 2012).

Contamination of meats is possible during butchering, udder removal, and especially whenever there is direct contact between humans and the carcass. Symptoms (nausea, vomiting, diarrhea) can occur in consumers after ingesting food containing toxins.

II.1.2.4 *Yersinia enterocolitica*

The genus *Yersinia* comprises 11 species belonging to the *Enterobacteriaceae* family. These are Gram-negative bacilli, non-sporulating, facultative anaerobes that ferment glucose. Smaller than most other enterobacteria, they often appear as coccobacilli when multiplying at 37°C. This genus includes four well-characterized pathogenic species: Yersinia pestis, responsible for bubonic and pneumonic plague, *Y. pseudotuberculosis*, pathogenic to rodents and occasionally humans, *Y. ruckeri* causing diseases in freshwater fish, and *Y. enterocolitica*, an intestinal pathogen. *Y. pseudotuberculosis* and *Y. enterocolitica* are the two foodborne pathogens. *Y. enterocolitica* is psychrotrophic, meaning it can multiply at temperatures below 4°C. However, its optimal growth temperature is 28-30°C (Krauss et al., 2003; Robin-Browner et Hartland, 2003).

II.1.2.5 *Campylobacter*

Campylobacter is a genus of slender, Gram-negative bacilli that are curved and spiral-shaped, sometimes resembling an "S." They are non-spore-forming and typically have a single polar flagellum that is not sheathed, located at one or both ends, giving them their characteristic tapered appearance. They range in size from 0.2 to 0.9 µm in diameter and from 0.5 to 5 µm in length (ASPC, 2012).

Campylobacter species have a respiratory-type metabolism and are microaerophilic, meaning they thrive in environments with reduced oxygen levels. Some strains can occasionally multiply in aerobic or anaerobic conditions. They are unable to oxidize or ferment sugars and test positive for the oxidase enzyme (Ghahir and Daube, 2007).

All *Campylobacter* species multiply at 37°C, but *Campylobacter* thermophiles (*such as C. jejuni, C. coli,* and *C. lari*) have optimal growth at 42°C and do not multiply at temperatures below 25°C

II.1.2.6 *Clostridium sulfito-reducteurs*

Clostridium sulfito-reducteurs and *Clostridium perfringens* are bacteria that reduce sulfites to black sulfides. They are Gram-positive bacilli and strict anaerobes. *Clostridium sulfito-reducteurs are* commensal bacteria of the intestine or saprophytes in soil; they can be used as a test for possibly old fecal contamination due to the spores' resistance to environmental conditions (Poumeyrol and Popoff, 2006).

II.1.3 Other micro-organisms

II.1.3.1 Yeasts

Their presence in food is relatively limited, but some of them have been reported in meat. These include *Saccharomyces, Candida,* and *Trichospora* (Serge, 2007; Fernandes, 2009).

II.1.3.1. Molds

Filamentous fungi (or molds) are heterotrophs, aerobic, and generally acidophilic. The genera *Aspergillus, Penicillium*, and *Mucor* are more frequently encountered in meat. Overall, since meat is a favorable substrate for germ development, their multiplication can lead to serious hygiene consequences (Fernandes, 2009; Guiraud et al., 2012).

II.1.4 Origin of Bacterial Contamination in Meats

The origins of microbial contamination in meat are varied and exhibit differing levels of significance. Various factors contribute to the occurrence of this contamination. Microorganisms can be classified as endogenous or exogenous depending on the source of contamination (Goudiaby, 2005).

II.1.4.1 Endogenous Origin

Animals engaged in food production are the primary source of microbial contamination, with their digestive, respiratory systems, and skin harboring these microorganisms. The digestive, respiratory systems, and skin of animals serve as reservoirs for these microorganisms. These components constitute the primary contamination sources for carcasses (Cartier, 2004).

Healthy animals, whether they are living or deceased, function as natural reservoirs of microorganisms due to the microbiota they host, potentially leading to surface contamination on animal carcasses. These microorganisms are present on the epidermis, within the gastrointestinal and mammary systems, as well as in the respiratory (upper) and urogenital (lower) tracts (Cartier, 2004).

The population of microorganisms found on the epidermis of cattle is primarily composed of *staphylococci, streptococci, coliforms*, and *enterobacteria*, which may include pathogenic strains like *Escherichia coli* O157:H7 and *Salmonella enteritidis*. The hides may harbor between 10^6 to 10^{10} bacteria per square centimeter (Bacon et al., 2000; Cartier, 2007). These cutaneous microorganisms can originate from the animal itself (either saprophytic or pathogenic flora) or from external sources like soil or fecal material. Interestingly, certain fecal-derived microorganisms may be more abundant in dried fecal matter sticking to the hair during the dry season (with 12% of samples containing *Salmonella*) compared to the feces alone (where only 4.4% of samples show *Salmonella*) as evidenced by Sofos et al.'s (1999) research on *Salmonella*. The microorganisms present on the skin have the potential to transfer onto the surface of carcasses either directly through contact between the hide and the carcass (during the dressing process) or indirectly through various vectors (equipment, personnel, air, etc.).

- The digestive flora consists of resident saprophytic germs and transient pathogenic bacteria (*Salmonella, Enterobacter, Escherichia coli* O157:H7, etc.) primarily found in the rumen and colon (mostly anaerobes ranging from 10^{10} to 10^{11} germs/g of ruminal content and ranging from 10^{6} to 10^{11} germs/g of colon content (Yokoyama & Johnson, 1988; Hirsch, 1999). Germs from the digestive contents can contaminate carcass surfaces indirectly (feces soiling hides) or directly (feces at the anal margins or from digestive reservoir perforation during evisceration) (Leyral & Vierling, 1997).

- The udder is normally sterile (excluding the teat canal) except in cases of mastitis (*staphylococci, streptococci, enterobacteria,* etc.), which can be clinical or subclinical. Contaminated milk discharge, natural (untreated and non-dried dairy cow), through pressure or incision of the udder, can increase the bacterial load on the skin during udder excision.

- Germs present in the respiratory tract (mostly *Pasteurella*) primarily affect the rhino pharynx and trachea, with the lungs typically devoid of microorganisms.

- Regarding the urogenital tract, the uterus and bladder are normally free of germs, with germs physiologically found in distal urinary and genital pathways (10^{3} germs/mL) (Woolcock, 1991).

II.1.4.2 Exogenous Origin

II.1.4.2.1 Equipment

Equipment, including machines, tools, and work supports that come into contact with the carcass, represents a potential source of contamination. Among the most important are knives (present at all stations but with increased risk during bleeding, skinning, rectum bagging, and evisceration), hide chains (skinning), saws (for splitting and halving), piercers, tongs, hooks, and lift platforms (especially those at the evisceration station). All these tools can serve as vectors of germs between contaminated elements and the carcass, for example, between "dirty" operations (e.g., skin incisions preceding dressing) and "clean" ones (subcutaneous incisions during dressing) performed without cleaning the same equipment (Cartier, 2007).

II.1.4.2.2Environment

Various elements of the environment (buildings and premises, air and dust, water, pests such as rodents, birds, and insects, waste) can be sources of carcass contamination. Poorly maintained and/or difficult-to-clean premises, and/or contamination during slaughter (via outlets: hides, digestive tracts, udders) are definite sources of contamination, promoting increased bacterial pressure in the environment and thus increasing the risk of carcass contamination (Cartier, 2007).

The ground is an important source of microorganisms. It contains microscopic algae, bacteria, and fungi (Leyral & Vierling, 1997).

Polluted air (germs, dust, condensation) can serve as a vector and allow the deposition of contaminants and germs on carcasses. Indeed, a study by Rahkio and Korkeala (1997) showed a significant correlation (r=0.86) between the level of air contamination by bacteria and the superficial contamination of carcasses. Similarly, water used during slaughter (post-evisceration rinsing, splitting) and for cleaning can, if unfit for consumption, be a primary source of contamination or serve as a vector for carcass contamination (especially when splashing onto carcasses from the ground).

In the same context, Andjongo (2006) reported that water can be a source of germ multiplication, especially in humid areas not regularly cleaned. On the other hand, insects and rodents, by hosting germs, are sources of both primary and secondary contamination.

II.1.4.2.3 Methods

Non-compliance with certain work methods can lead to carcass contamination. Within the slaughter chain, factors such as animal stress before slaughter, regurgitation of stomach contents into the lungs, transfer of hide contaminants into bleeding wounds during rinsing, crossing paths of skinned and unskinned carcasses, contact between carcasses, and contact between the outer side of hides and carcasses can all contribute to an increase in bacterial surface contamination of carcasses (Salifou et al., 2012). Although some contamination may be inevitable during various stages of the slaughter process, it is still possible to limit them by following certain work methods.

II.1.4.2.4 Workers

The Workers includes all individuals involved in the slaughter chain, from unloading animals and bringing them into the slaughter corridor to dispatching carcasses and quartering. Any operator could carry pathogenic germs in the intestinal, cutaneous, or buccopharyngeal regions (Salifou et al., 2012). Personnel are also a significant potential source of germs (normal skin flora with 10^2 to 10^5 germs/cm^2 (dry or moist skin are) (Cartier, 2007), lack of personal hygiene [hand contamination with fecal germs considering an average of 10^{11} germs/g of feces in humans], carriers of *salmonella* or *Staphylococcus aureus*) (Vallotton, 2004).

II.1.5 Meat Alterations

The degradation of meat by bacteria, through their proteolytic and lipolytic activities targeting protein and lipid compounds, contributes to altering the organoleptic qualities of meat. This process produces low molecular weight substances responsible for the appearance and odor of spoiled meat. Meat spoilage is a progressive phenomenon (Cartier, 1997).

II.1.6 Types of Meat Contamination

Microbiological studies conducted on meat have confirmed the presence of various microorganisms on both fresh and minced meat (Dennaï et al., 2001).

II.1.6.1 Deep Contamination

Meat can be contaminated deeply in vivo. This type of contamination is not very common because sick animals are systematically eliminated. However, there are still apparently healthy animals. Contaminations during slaughter and carcass preparation can occur from the environment, skin (hide), instruments, handlers, and fecal matter. Among these causes, fecal matter is the most feared (Kamoun, 1993).

II.1.6.2 Surface Contamination

Surface contamination of carcasses is much more significant than deep contamination and mainly comes from the animal itself (hair, excrement), the slaughter environment (floor, handlers), cutting workshops, and storage chambers (Kamoun, 1993). The surface microflora of carcasses can be reduced if good hygiene practices are followed in slaughterhouses during processing (Cartier, 2007).

II.1.7 Consequences of Microbial Contamination on Meat Quality

II.1.7.1 Putrefaction

Post-mortem contamination flora of meat causes deterioration of meat, resulting in putrefaction. Depending on the origin and progression, there are two types of putrefaction: a foul smell observed in high-fat muscle masses and superficial putrefaction caused by psychrotrophic aerobic bacteria. These alterations manifest in various ways:

II.1.7.2 Viscosity

Viscosity is due to bacteria such as *Pseudomonas, Streptococcus, Leuconostoc, Bacillus, Micrococcus, Lactobacillus,* and sometimes yeast and mold. Minced and piece meats are more susceptible to putrefaction than carcasses. To limit the development and action of aerobic bacteria responsible for spoilage and putrefaction, vacuum packaging or modified atmosphere packaging is widely used with strict adherence to cold chain protocols. These packaging methods create an unfavorable environment for the proliferation of putrefactive bacteria, allowing the meat to be marketed fresh for several days (Pierre, 1998).

II.1.7.3 Color Modifications

Color modifications can include meat discoloration due to *Lactobacillus, Leuconostoc,* yeast, or pigmentation caused by bacteria such as *Pseudomonas, Chromobacterium, Bacillus, Flavobacterium, Micrococcus*, or yeast *(Rhodotorula*) and mold (*Cladosporium herbarum, Penicillium)*. Color changes result from pigment synthesis or transformation of endogenous pigments in the food (myoglobin) (Pierre, 1998).

II.1.7.4 Organoleptic Changes

Organoleptic changes manifest as rancidity in oxidized fats due to exposure to air (oxygen), resulting in a rancid taste and odor and releasing compounds responsible for undesirable appearance (color), texture, and flavor (odor and taste) under the action of microorganisms such as *Pseudomonas, Acinetobacter, Alcaligenes, Aspergillus, Rhizopus, Flavobacterium, Clostridium* (Pierre, 1998).

II.1.8 Food Poisoning

The main cause of food poisoning is the consumption of contaminated, poorly prepared, and inadequately refrigerated foods. Types of food poisoning include:

- Food poisonings caused by preformed toxins in food during bacterial growth (*Staphylococcus aureus, Clostridium botulinum*),

- Foodborne infections caused by active or live pathogens (such as *Salmonella, Shigella)* present in the food in high numbers,

- Actual food poisonings caused by microorganisms such as *Clostridium perfringens, Bacillus* cereus present at high levels in the food (10^8 to 10^{10} bacteria/g).

Histamine intoxications are caused by consuming foods containing decarboxylation amines from the degradation of amino acids by non-specific germs.

CHAPTER III:

MATERIALS AND METHODS

PRAT 01:

BACTERIOLOGY METHODS

III.1 Objective

The aim of this study was to evaluate the generally microbial hygiene indicator contamination of beef meat sold in different butcher shops, The number of samples analyzed is for 3 days per butcher shop, which means 15 samples for the 5 butcher shops. located in the region Municipality of Laghouat (Algeria). For this reason, 15 samples of beef meat be taken and subjected to indicator counts:

- Total mesophilic aerobic flora (TMAF).
- thermo-tolerant coliforms.
- Staphylococci.

III.2 Period and laboratory of the study

The analysis period took place from 10/01/2024 to 11/02/2024. Samples were randomly collected from 05 butcheries shops located in the Laghouat. The analyses were conducted out on the same day at the laboratory of the Faculty of Natural and Life Sciences of Amar Telidji University (FNLS).

III.3 Materials and Methods

III.3.1 Materials

III.3.1.1 Equipments (see Appendix 1).

III.3.1.2 Consumable Product (see Appendix 2).

III.4 Microbiological analysis

The microbiological analyses were conducted on 15 samples of beef meat purchased from various randomly selected points of sale in the city of Laghouat. Once purchased, they are placed in sterile bags containing all the information (butchery, location, etc.). The samples are then placed in a cooler containing ice packs and transported to the laboratory of the Faculty of Natural and Life Sciences at the University of Amar Telidji University (FNLS).

III.4.1 Decimal dilutions and culture medium

III.4.1.1 Physiological Saline Solution

- **Physiological water 9%**

To prepare the solution, begin by measuring precisely 9 grams of sodium chloride (NaCl). Pour the NaCl into an Erlenmeyer flask, then add 1 liter of distilled water to the container. Stir the mixture gently until the NaCl completely dissolves. Once dissolved, they are placed into Reagent bottles and test tubes, then sterilized autoclave the container to ensure sterilization.

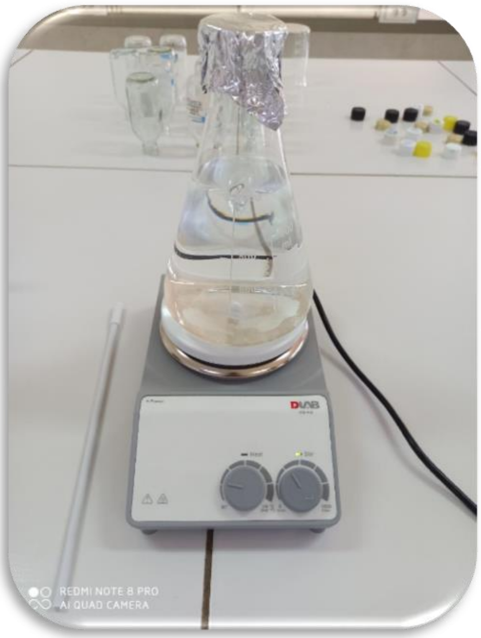

Figure 3. Preparation of Physiological Saline Solution (Personal Photo 2024).

III.4.2 Preparation of the culture suspension

10 g of meat were aseptically weighed using a balance and introduced into a sterile flask containing 90 ml of sterile physiological saline solution. The mixture was homogenized by agitation using a vortex for 1 to 2 minutes. The prepared suspension was left for 15 minutes at room temperature before performing decimal dilutions and inoculating the media. Finally, we obtained a mother suspension .

III.4.3 Preparation of Decimal Dilutions

A series of dilutions was performed from the mother solution. Using an automatic pipette, 1 ml of the mother solution was withdrawn and introduced into the first test tube containing 9 ml of sterile physiological saline solution to obtain dilution 10^{-2}. The process was continued until dilution 10^{-6} was achieved.

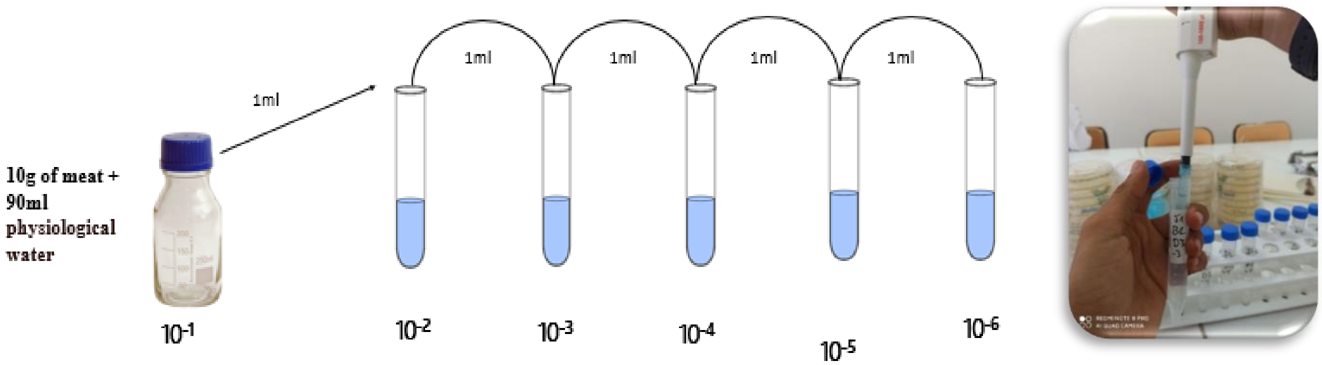

Figure 4. Diagram of the technique for preparing decimal dilutions (Personal Photo 2024).

III.5 Bacteriological Analysis

We prepared 25 Petri dish from every agar leaving us with a total of 75 Petri dish a day. Then we divided our Petri dishes to three germs where each group has 5 Petri dishes from each growth medium (5 PCA Petri dishes,5 Baird Parker Petri dishes,5 VRBL Petri dishes).

III.5.1 Total Mesophilic Aerobic Flora

Glucose yeast extract agar, known as "Plate Count Agar" or PCA by Anglo-Saxons, is used in food bacteriology for enumerating aerobic bacteria in food products, animal feed, and environmental samples. It is also employed for the enumeration of psychotropic microorganisms.

- **Preparation PCA Agar**
  - Suspend 23.5 g grams in 1000 ml distilled water.
  - Boil until it dissolves completely.
  - Using a funnel pour the medium in 250ml reagent bottles.
  - add masking tape and write down the name of the agar on it and then autoclave them.
  - Sterilize by autoclaving at (121 °C) for 15 minutes.

- Cool to 45-50 °C.

Figure 5. Preparation of PCA agar (Personal Photo 2024).

III.5.1.1 Detection and enumeration

The method and procedure for enumerating total mesophilic aerobic flora are conducted according to (EN ISO 4833-2). From the prepared inoculum, take 0.1 ml of the bacterial suspension; place the suspension on the surface of poured and solidified Plate Count Agar in each Petri dish. Using a Glass Pasteur pipette to spread the bacterial suspension evenly over the surface of the Plate Count Agar. Allow the Petri dishes to dry for a few minutes, containing all the information (butchery, day, The dilution used, etc.). the prepared plates are incubated upside down in an oven set at 30°C for 48 hours.

The counting and detection of mesophilic aerobic flora involve microorganisms forming countable colonies after multiplication under defined laboratory conditions .The colonies that appear are then counted. Each plate selected must contain no more than 300 colonies and at least 30 colonies (EN ISO 4833-2). The results are expressed in colony-forming units per gram (CFU/g).

III.5.2 Thermo-Tolerant Coliforms

The Violet Red Bile Lactose (VRBL) agar is a selective medium used for the detection and enumeration of coliforms and thermotolerant coliforms in food products.

The fermentation of lactose results in acidification, indicated by the pH indicator (neutral red) turning red, and the precipitation of bile salts around colonies. The simultaneous presence of crystal violet and bile salts ensures the inhibition of Gram-positive bacteria.

- **Preparation VRBL Agar**

Since (VRBL) cannot be autoclaved, all necessary equipment must be sterilized beforehand and prepared in a sterile area near a Bunsen burner while wearing sterile gloves. As a result:

- Suspend 45.03 grams in 1000ml distilled water.
- Heat to boiling to dissolve the medium completely.
- Using a funnel pour the medium in 250ml reagent bottles.
- add masking tape and write down the name of the agar on it and then autoclave them.
- Cool to 45-50 °C. – Store the bottles in room temperature.
- DO NOT AUTOCLAVE. Avoid excessive or prolonged heating during reconstitution.
- Cool to 45-50 °C.

Figure 6. Preparation VRBL Agar (Personal Photo 2024).

III.5.2.1 Detection and enumeration

The method and procedure for enumerating total mesophilic aerobic flora are conducted according to (ISO 4832). The method involves pipetting 1 ml of decimal dilution using an Automatic Micropipette into a sterile Petri dish, with successive dilutions used 15ml of cooled Violet Red Bile Lactose (VRBL) are poured into each Petri dish. The inoculum is carefully mixed into the culture medium by circular and back-and-forth movements or in a figure-eight shape on a, horizontal surface. After solidification. containing all the information (butchery, day, the dilution used, etc.) the prepared plates are incubated upside down in an oven set at 44°C for 84 hours.

The counting and detection of Violet Red Bile Lactose involve microorganisms forming countable colonies after multiplication under defined laboratory conditions. The colonies that appear are then counted. Each plate selected must contain no more than 300 colonies and at least 30 colonies (ISO 4832). The results are expressed in colony-forming units per gram (CFU/g).

III.5.3 *Staphylococcus aureus*

Baird-Parker (BP) agar is recommended for the detection and enumeration of coagulase-positive *staphylococci*. Its use is recommended for the detection of *Staphylococcus aureu*s in food.

Staphylococcus aureus is characterized by the formation of black, shiny, convex colonies surrounded by a lightening halo of the egg yolk. in order to stimulate the growth of *Staphylococcus aureus* without destroying the selectivity. The tellurite additive is toxic to egg yolk clearing strains other than *Staphylococcus aureus* and imparts a black color to the colonies. The egg yolk additive, in addition to being an enrichment, aids in the identification process by demonstrating lecithinase activity (egg yolk reaction). Glycine and lithium chloride have inhibitory action for organisms other than *Staphylococcus aureus*.

- **Preparation BP Agar**
  - Suspend 63.5g grams in 1000 ml distilled water.
  - Boil until it dissolves completely.
  - Using a funnel pour the medium in 250ml reagent bottles.

- add masking tape and write down the name of the agar on it and then autoclave them.
- Sterilize by autoclaving at (121 °C) for 15 minutes.
- Cool to 45-50 °C.

Tellurite additive: add 1% of tellurite and 5% egg yolk for every 90ml of Baird Parker medium (JORA, 2004).

III.5.3.1 Detection and Enumeration

The method and procedure for enumerating *Staphylococcus aureus* are conducted according to (NI ISO 6888-1). From the prepared inoculum, take 0.1 ml of the bacterial suspension; place the suspension on the surface of poured and solidified Baird-Parker in each Petri dish. Using a Glass Pasteur pipette to spread the bacterial suspension evenly over the surface of the Baird-Parker. Allow the Petri dishes to dry for a few minutes. Containing all the information (butchery, day, the dilution used, etc.) the prepared plates are incubated upside down in an oven set at 37°C for 84 hours.

The counting and detection of Baird-Parker involve microorganisms forming countable colonies after multiplication under defined laboratory conditions. The colonies that appear are then counted. Each plate selected must contain no more than 150 colonies and at least 15 colonies (NI ISO 6888-1). The results are expressed in colony-forming units per gram (CFU/g).

III.6 Expression of Results

According to (JORA, 2004), the mathematical formula used for enumerating the sought microorganisms, and the result obtained is expressed in cfu/g (colony-forming units per gram).

$$N=\frac{\sum C}{V.(N1+0.1N2)d}$$

N: The number of microorganisms per gram of product

C: Total sum of counted colonies.

n1: Number of counted plates in the first dilution.

n2: Number of counted plates in the second dilution.

d: Dilution factor from which the initial counts were obtained.

III.7 Physicochemical Analysis

The control of meat is essentially done by determining the pH. The latter plays an essential role and serves as an indicator of the progress of post-mortem events. The progressive acidification of the muscle with the drop in muscular pH is caused by the accumulation of lactic acid and the release of H+. The two most important parameters of this pH drop are the speed and the amplitude. The former essentially depends on the speed of ATP hydrolysis and therefore on the ATP-as activity of myosin, while the latter mainly depends on the amount of glycogen stored in the muscle at the time of slaughter (Bendall, 1973).

The pH determination is carried out for all meat samples using 10 g of meat. The pH is obtained using a (pH spear) Meter previously calibrated with two buffers (pH=4, pH=7) by inserting the electrode into the homogenate. The pH reading is taken directly from the device's scale to within 0.01 pH unit. The operation is repeated three times.

The results are expressed as the arithmetic average of the three values obtained for each sample (Rejsek, 2002).

Figure 7. pH measurement (Personal Photo 2024).

III.8 Statistical Analysis

The results were processed using Excel 2016 software; Are indicated on average and standard deviation and then converted to decimal Log to normalize the distribution; the student's t-test is used to compare the observed means with the theoretical values indicated by the standard.

PRAT 02:

QUESTIONNAIRE METHODS

III.1 Methodology

The survey we conducted primarily aimed to gather all information related to the conditions prevailing in the various costumers under investigation (hygiene of premises, working equipment, personnel, and appearance of meat intended for sale).

The objective is to comprehend individuals' behaviors and preferences about the acquisition and consumption of beef from certain butchers at the community level of laghouat, with the aim of enhancing service quality or more effectively satisfying client requirements to determine the quality of service and the level of consumption culture for beef sold by butchers in Laghouat, with a focus on health and hygiene. The data could also be used to develop the quality of service and to know the sources of contamination of meats as well as to know the shortcomings of knowledge of the consumer of the region and to discern models in the behavior of customers and strive to improve them.

This comprehension may assist vendors in enhancing their products and services and formulating marketing strategies that more effectively focus on their desired audience.

III.2 Questionnaire Design and Pre-Survey

The questions were formulated after compiling literature on this topic. Notes from the individuals interviewed were taken into consideration. Some questions were added, modified, and others were removed. As a result, the final questionnaire became clearer, more understandable, and adapted to our work needs.

III.3 Conduct of The Survey

Our survey was conducted between 01/03/2024 and 25/04/2024 we used two methods The first is by preparing questions in Google Form and sharing the link, The second method was a paper survey distributed at different points in the region of Laghouat. Participants of both sexes were selected based on their consent to participate in the study. The inclusion criteria were age and responsibility for purchasing in the household. Once we contacted the participants, we created an environment of trust to facilitate data collection. After explaining to the participants, the purpose and content of the work, we provided them with a guarantee of confidentiality and anonymity regarding the information collected and intended exclusively for scientific use and research purposes. Each subject is interviewed for 10 to 15 minutes. Each question is well explained to the subject so that the meaning is understood. The questionnaire is thus completed on-site by means of a direct interview with the subject.

III.3 Designing a Questionnaire

The questionnaire an Arabic-language used is written in a vertical format on a page (see Appendix 3) consisting of 17 questions to gather information

information on the following points:

1-Demographic and social variables.

2- Consumer behavior.

It contains closed, semi-closed questions. The questionnaire consists of several components: Subject characteristics, consumer purchasing behavior, estimation of consumers' knowledge and level of information and perceptions about the quality of beef meat.

III.4 Statistical Analysis

The responses from the questionnaire were evaluated through the use of Google Forms and Excel 2016, as well as the statistical software SPSS version 20.

Chapter IV:

RESULTS AND DISCUSSION

<u>PRAT 01:</u>

BACTERIOLOGY RESULTS

IV.1 Total Mesophilic Aerobic Flora Results

After 48 hours of incubation at 30°C, microbial colonies appear on the surface of the plate count agar or PCA (figure 7).

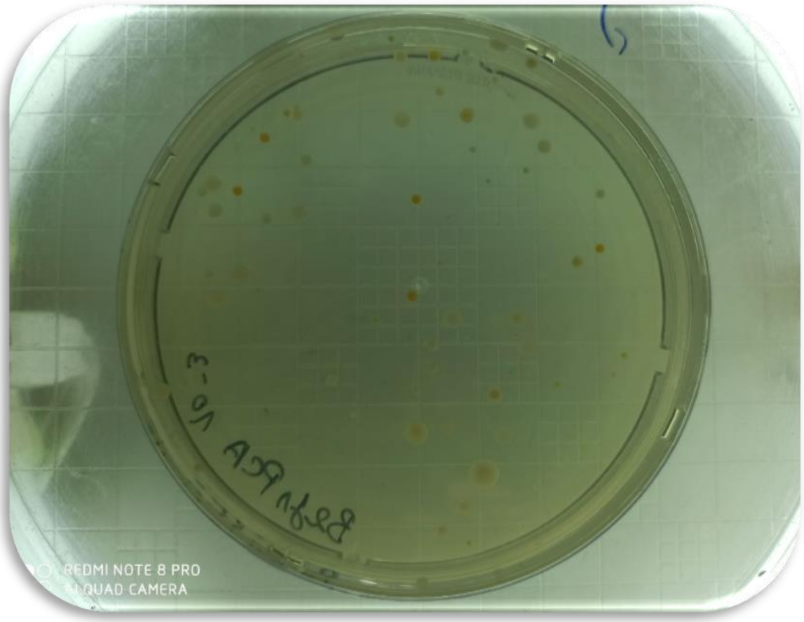

Figure 8. Petri dishes of TAMF colonies on PCA agar. Photographed after 84h incubation at 30°C (Personal Photo 2024).

The total mesophilic aerobic flora is considered an important indicator for assessing the hygienic and sanitary quality of meat in general. It serves as an indicator of spoilage microorganisms contaminating the food. A high number of total mesophilic aerobic flora indicates that the meat is of poor microbiological quality, which could affect its shelf life. The total mesophilic aerobic flora includes all revivable microorganisms (bacteria, yeasts, molds, etc.) and provides an idea of the overall microbial load in a food product (APHA, 2001).

The table 2 and figure 9 depict the microbial load of total mesophilic aerobic flora after enumeration of colony-forming units (CFU) from 15 samples taken from five butcher shops (three samples per butcher shop, n=3) in the city of Laghouat (Microbial load expressed in CFU/g and Log10 CFU/g). The table also shows that the acceptability threshold for total flora in meat is 10^6 CFU/g (6Log10 CFU/g) according to national regulations (JORA, 1998).

According to the table 2 and figure 9, samples from butcher shop 5 show a highly significant difference ($p<0.01$) above the mean values of total mesophilic aerobic flora and the acceptability threshold set by regulations. These mean values have exceeded the acceptable limit of 6Log10 CFU/g, rendering the quality of the meat from this butcher shops not satisfactory. As for butcher shop 1,2,3 and 4, no significant difference ($p>0.05$) was observed below the mean value of total mesophilic aerobic flora and the acceptability threshold, while our findings revealed no statistically significant distinction, indicating that can be classified as mediocre, suggesting that enhancements are warranted through improved food handling practices. This elevated result of butcher shop 5 may be attributed to non-compliance with hygiene practices during preparation: from slaughter, washing, transportation to the butchers, to inadequate hygiene practices by sellers at the point of sale, and the absence of cold storage for products as well as exposure of these products to open air or ambient temperature. Additionally, butchers who do not adhere to various hygienic preparation and handling processes, and do not wear specific attire, gloves, masks, and head coverings, may directly or indirectly contaminate the product for sale.

Table 2. Result of the count and interpretation of the presence of total aerobic mesophilic flora.

Butchers	Number of samples	CFU/g ± Standard deviation	Log_{10} CFU/g ± Log_{10} Standard deviation	Acceptability threshold in CFU/g	Acceptability threshold In Log_{10} CFU/g	P (Probability)	Significance thresholds
Butcher 1	3	$6.96x10^7$± $9.71x10^8$	7.31±1.00	10^6	6	0.087	NS
Butcher 2	3	$4.67x10^7$± $8.00 x10^7$	6.38±1.57	10^6	6	0.696	NS
Butcher 3	3	$3.02x10^7$± $3.13x10^7$	7.14±0.31	10^6	6	0.071	NS
Butcher 4	3	$1.40x10^8$± $2.41x10^8$	6.65±1.82	10^6	6	0.56	NS
Butcher 5	3	$2.57x10^2$± $29.74 x10^4$	6.38±0.16	10^6	6	0.014	**

- **Significance thresholds meaning**

– NS: not significant.
– *: $p < 0.05$: significant difference.
– **: $p < 0.01$: highly significant difference.
– ***: $p < 0.001$: very highly significant difference.

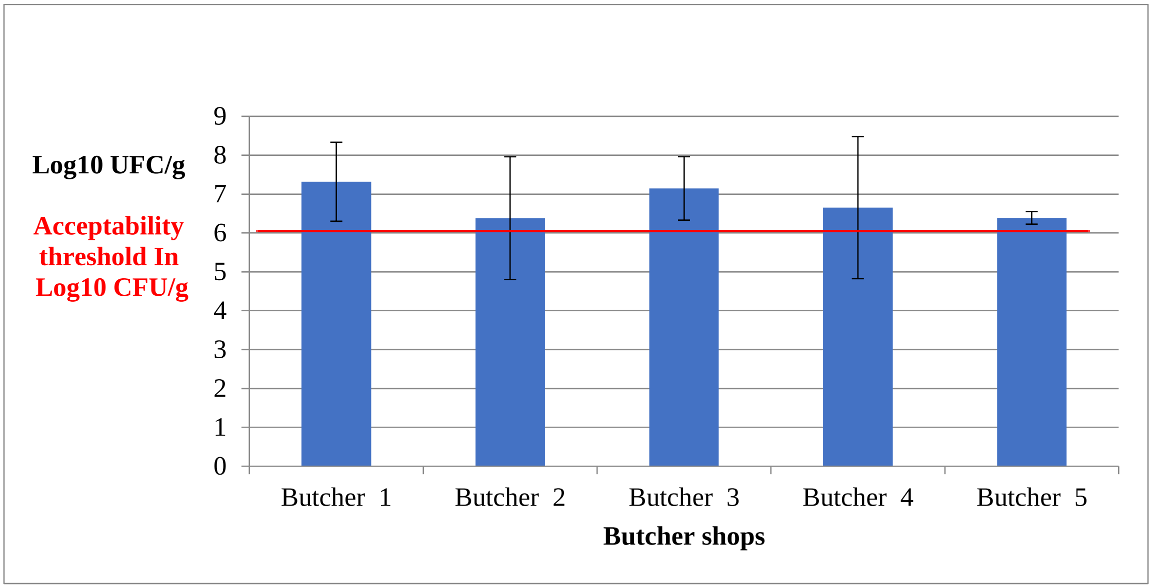

Figure 9. histograms representing the Distribution of total aerobic mesophilic flora by level of contamination.

IV.2 Thermo-Tolerant Coliforms Results

After 48 hours of incubation at 44°C, microbial colonies appear on the bottom of VRBL agar (figure 9)**.**

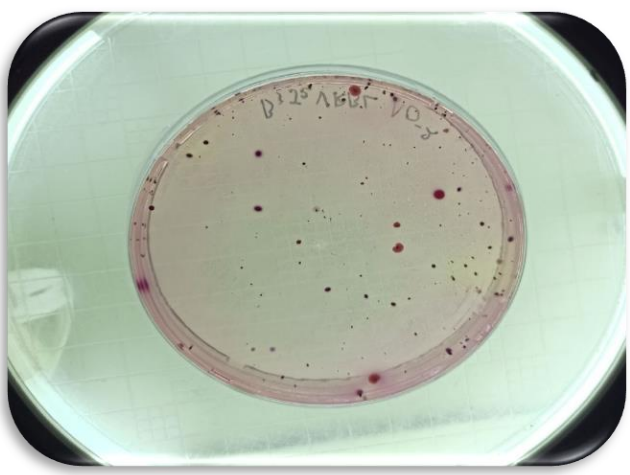

Figure 10. Petri dishes of thermo-tolerant coliforms colonies on VRBL agar. Photographed after 84h incubation at 30°C (Personal Photo 2024).

The presence of coliforms indicates fecal contamination, which can be explained by the lack of good hygiene practices throughout the meat production chain. A high number of coliforms reflects inadequate hygiene during the production and handling of raw materials, surfaces in contact with meat, and employees. Meanwhile, a high number present on carcass surfaces and cutting pieces is highly undesirable and primarily suggests fecal contamination, indicating potentially serious danger (ERIBO and JAY, 1985)

Samples from five butcher shops (three samples per butcher shop, n=3) in the city of Laghouat (Microbial load expressed in CFU/g and Log10 CFU/g). The table 2 also shows that the acceptable threshold for thermotolerant coliforms in meat is $3x10^2$ CFU/g (3 Log10 CFU/g) according to national regulations (JORA, 1998).

The thermos-tolerant coliforms (TTC) indicates that the environment of preparation, handling, or transportation was unsanitary, either in the slaughterhouse or in these butcher shops, and the meat was contaminated with fecal matter (ERIBO and JAY, 1985). The origin of coliforms present on meat may be at the beginning of the chain, namely, at the slaughterhouses where cross-contamination occurs. A defect during evisceration (rupture of viscera), as well as numerous contacts of carcasses with contaminated surfaces (tables, bags, knives, etc.), can also be the cause of fecal contamination.

According to the table 3 and figure 11, Samples analyzed from all butcher shop show a no significant difference (p>0.05) was observed below the mean counts of thermo-tolerant coliforms (TTC) and the acceptable threshold set by regulation, and these means are below the acceptable limit, rendering the quality of meat from these all butcher shops satisfactory. This good quality of meat sold in these all butcher shops may be due to thorough cleaning and disinfection of surfaces in contact with meat after each stage of preparation, handling, or sale in the butcher shop, as well as good personal hygiene practices.

Table 3. Result of the count and interpretation of the presence of thermo-tolerant coliforms.

Butchers Shops	Number of samples	CFU/g ± Standard deviation	Log_{10} CFU/g ± Log_{10} Standard deviation	Acceptability threshold In CFU/g	Acceptability threshold In Log_{10} CFU/g	P (Probability)	Significance thresholds
Butcher 1	3	$6.15x10^4$± $3.90x10^4$	3.90±0.91	$3*10^2$	3	0.126	NS
Butcher 2	3	$2.54x10^2$± $3.53\ x10^2$	2.39±0.53	$3*10^2$	3	0.118	NS
Butcher 3	3	8.8010^3± $7.91x10^3$	2.98±1.73	$3*10^2$	3	0.148	NS

Butcher 4	3	$2.60x10^3$± $2.16x10^3$	2.05±0.55	$3*10^2$	3	0.260	NS
Butcher 5	3	$5.20x10^2$± $4.00\ x10^2$	2.33±0.57	$3*10^2$	3	0.116	NS

- **Significance thresholds meaning**

– NS: not significant.
– *: $p < 0.05$: significant difference.
– **: $p < 0.01$: highly significant difference.
– ***: $p < 0.001$: very highly significant difference.

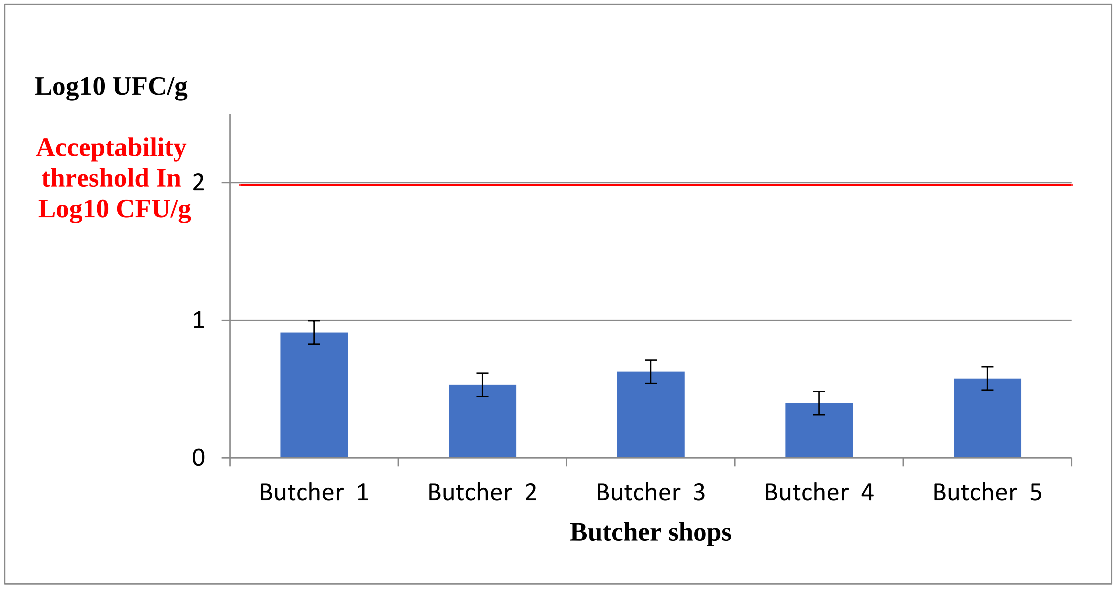

Figure 11. histograms representing the Distribution of thermo-tolerant coliforms by level of contamination.

IV.3 *Staphylococcus aureus*

After 48 hours of incubation at 37°C, microbial colonies appear on the surface of BP agar (figure 1).

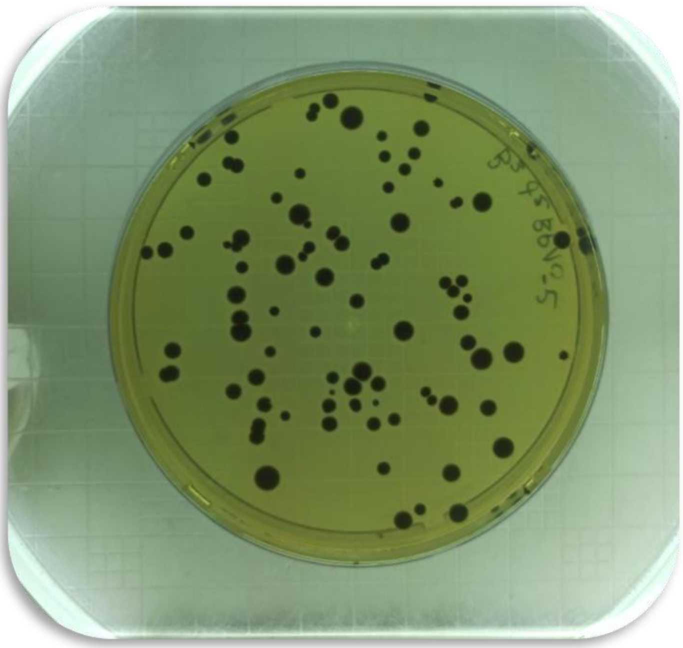

Figure 12. Petri dishes of *Staphylococcus aureus* colonies on BP agar. Photographed after 84h incubation at 37°C (Personal Photo 2024).

Staphylococci are commonly found on the skin (especially if it's injured and infected) and in the upper respiratory tracts of both humans and animals, and they can easily contaminate food in all its forms. *Staphylococci* do not form spores but can contaminate food during preparation, processing, and handling (SIRIKEN, 2004).

The table 4 and figure 13 show the microbial load of *staphylococci* after enumerating colony forming units (CFU) from 15 samples collected from five butcher shops (three samples per butcher shop, n=3) in the city of Laghouat (Microbial load expressed in CFU/g and Log10 CFU/g). The table... also indicates that the acceptable threshold for Staphylococci in meat is 10^2 CFU/g (2 Log10 CFU/g) according to national regulations (JORA, 1998).

Based on the table 4 and the figure 13, samples from butcher shop 3 and butcher shop 5 show a highly significant difference ($p<0.05$) between the mean counts of *staphylococci* and the acceptable threshold set by regulation, and these means have exceeded the acceptable limit of

2Log10 CFU/g, rendering the quality of meat from these two butcher shops unacceptable. As for butcher shop 2, very highly significant difference ($p<0.05$) was observed above the mean count of *staphylococci* and the acceptable threshold; however, the quality of meat from this butcher shop in this case is unacceptable and toxic. These findings may be associated with meat contamination by *S. aureus*, which is due to poor food safety practices during meat handling or directly from infected food-producing animals. In other studies, conducted in Croatia (Kozacinski et *al*., 2006), the United States (Thapaliya et *al*., 2017) and Ethiopia (Tsehayneh et *al*., 2017), unacceptable contaminations by *S. aureus* were found in 57%,67.8% and 54.45%, of beef samples, respectively. these researchers concluded that *S. aureus* contaminations reported in uncertified establishments could be reduced through hygiene education, regular training of food handlers, hygienic control of equipment, surfaces, and utensils, and by maintaining the cold chain.

Samples analyzed from butcher shop 1 and butcher shop 4 show a no significant difference ($p<0.05$) between the mean counts of *staphylococci* and the acceptable threshold set by regulation, and these means are below the acceptable limit, rendering the quality of meat from these two butcher shops while our findings revealed no statistically significant distinction, indicating that the quality is not hazardous, it falls short of being deemed satisfactory. Instead, it can be classified as mediocre. These results are likely due to the adherence to hygiene rules by the personnel handling the meat and especially the health status of the butchers, namely the health of the pharynx-larynx and the health of the skin, as these two locations are the most significant sources of *staphylococci*. In a survey conducted in Nigeria, counts of S. aureus in samples of beef (Adesiji et *al*, 2011). Lower rates of S. aureus in samples of beef were recorded with 28% and 14.6% in Ankara (Özdemir et *al*., 2016).

Table 4. Result of the count and interpretation of the presence of *Staphylococcus aureus.*

Butchers	Number of samples	CFU/g ± Standard deviation	Log_{10} CFU/g ± Log_{10} Standard deviation	Acceptability threshold in CFU/g	Acceptability threshold In Log_{10} CFU/g	P (Probability)	Significance thresholds
Butcher 1	3	$7.00 \times 10^{1} \pm 2.65 \times 10^{1}$	1.82±0.15	10^2	2	0.125	NS
Butcher 2	3	$2.76 \times 10^{4} \pm 8.97 \times 10^{3}$	4.42±0.16	10^2	2	1.27574×10^{-05}	***
Butcher 3	3	$3.97 \times 10^{4} \pm 3.66 \times 10^{4}$	4.25±1.85	10^2	2	0.010	**
Butcher 4	3	$1.01 \times 10^{4} \pm 1.65 \times 10^{4}$	2.82±1.42	10^2	2	0.120	NS
Butcher 5	3	$2.61 \times 10^{4} \pm 2.17 \times 10^{4}$	4.05±0.91	10^2	2	0.017	**

- **Significance thresholds meaning**

– NS: not significant.
– *: $p < 0.05$: significant difference.
– **: $p < 0.01$: highly significant difference.
– ***: $p < 0.001$: very highly significant difference.

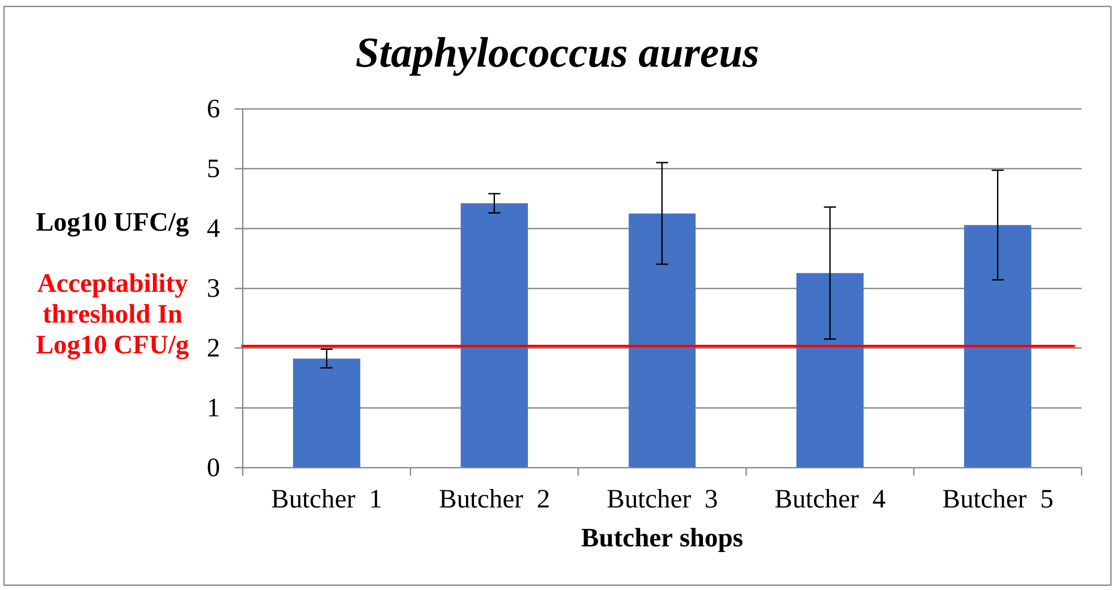

Figure 13. histograms representing the Distribution of *Staphylococcus aureus* by level of contamination.

IV.4 pH Measurement for Beef Meat Across all Samples

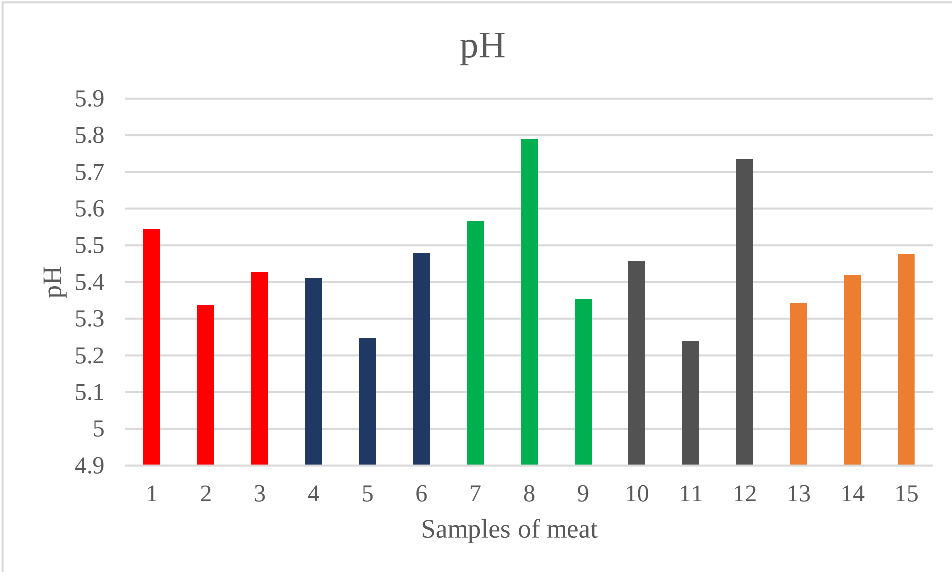

Figure 14. histograms representing the Physicochemical analysis of pH measurement for beef meat across all samples.

Based on the pH measurement histogram, it's evident that the samples exhibit variable values. Specifically, five samples (1,6,7,8,12) demonstrate a higher pH compared to the others.

Consumption of meat also involves physicochemical monitoring, which includes controlling certain parameters such as pH and electrical conductivity. pH values above 6 promote the proliferation of bacteria responsible for spoilage. These bacteria are believed to be the cause of the appearance of a greenish color on the surface of all meat samples (Newton et *al*., 1981; Dokmanovic et *al*., 2014).

According to Lawrie (1998), the pH value for reed meats always stabilizes at a minimum value referred to as the ultimate pH, which typically falls between 5.6 and 5.8.

The acidity increases during storage. Meat with a pH of 6 spoils more rapidly than that with a pH of 5.3. This indicates that acidity has a bacteriostatic effect on the growth of microorganisms (Lawrie, 1998).

Table 5. analysis of pH measurement for beef meat across all samples.

Butchers	pH value		
Butcher 1	Day 1	5.543333	0.103619675
	Day 2	5.336667	
	Day 3	5.426667	
Butcher 2	Day 1	5.41	0.119737367
	Day 2	5.246667	
	Day 3	5.48	
Butcher 3	Day 1	5.566667	0.218352416
	Day 2	5.79	
	Day 3	5.353333	
Butcher 4	Day 1	5.456667	0.249005429
	Day 2	5.24	
	Day 3	5.736667	
Butcher 5	Day 1	5.343333	0.0669162
	Day 2	5.42	
	Day 3	5.476667	

PRAT 02:

QUESTIONNAIRE RESULTS

IV.1 Questionnaire Results

IV.1.1 Demographic and Social Variables

IV.1.1.1 According to the Gender in the survey

The results of the survey on gender in the Laghouat region are represented by (Figure 15, Table 6).

Table 6. Gender Frequency and Percent.

		Frequency	Percent
Valid	Man	72	45.0%
	Woman	86	53.8%
	Total	160	100.0%

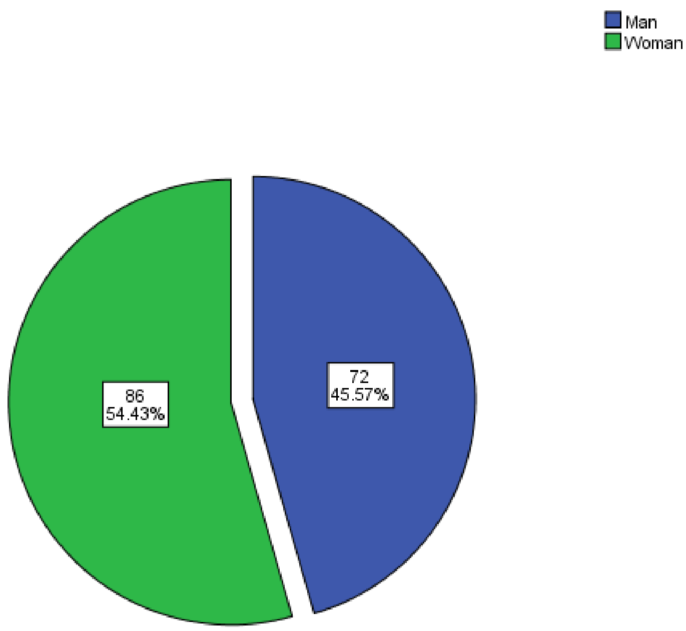

Figure 15. Gender Frequency and Percent.

IV.1.1.2 Acording to age in the survey

The results of the survey on age in the Laghouat region are represented by (Figure 16, Table 7).

Table 7. Age Frequency and Percent.

		Frequency	Percent
Valid	18-24	99	61.9%
	25-34	22	13.8%
	35-44	19	11.9%
	>55	19	11.9%
	Total	160	100.0%

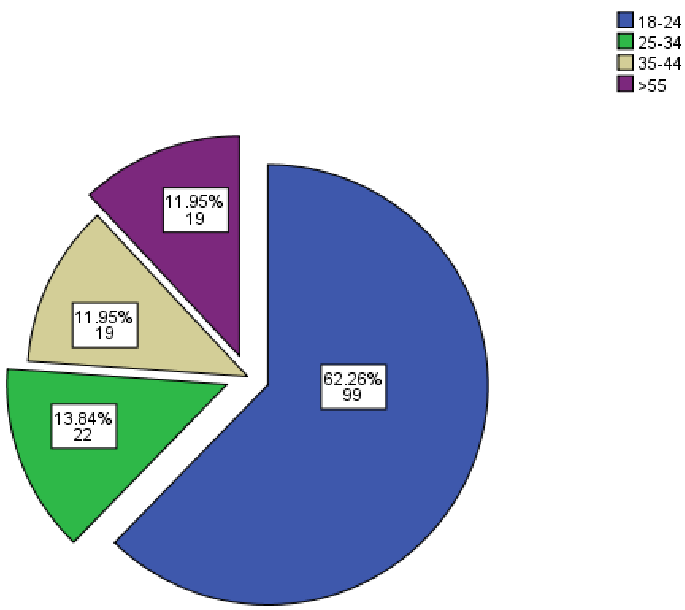

Figure 16. Age Frequency and Percent.

IV1.1.3 Educational level in the survey

The results of the survey on educational level in the Laghouat region are represented by (Figure 17, Table 8).

Table 8. Level Frequency and Percent.

		Frequency	Percent
Valid	university	107	66.9%
	non-university	52	32.5%
	Total	160	100.0%

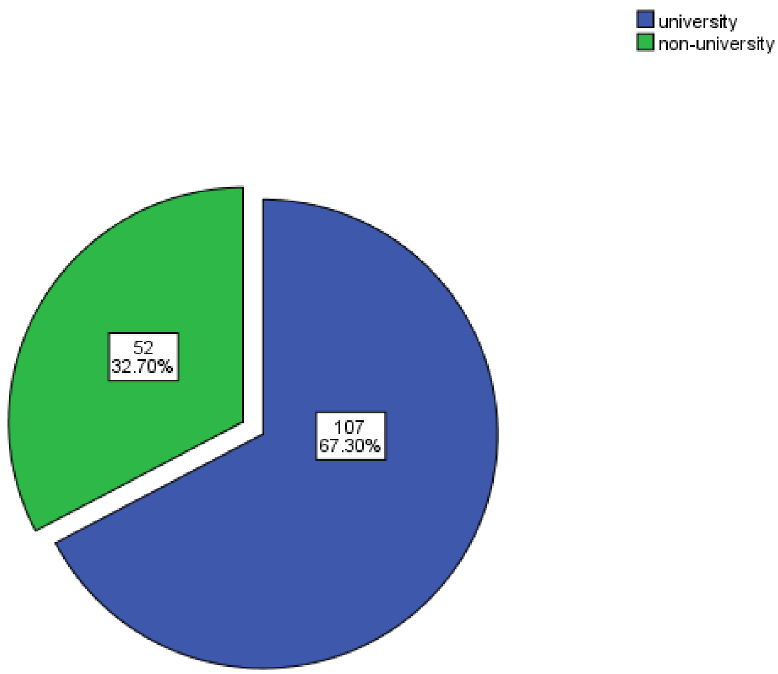

Figure 17. Level Frequency and Percent.

IV.1.2 Consumer Behavior.

IV.1.2.1 consume Beef meat in surveys

The results of the survey on consuming beef meat in the Laghouat region are represented by (Figure 18 and Table 9).

Table.9 Frequency and percent representing if a consumer consuming Beef.

		Frequency	Percent
Valid	yes	83	51.9%
	no	75	46.9%
	Total	160	100.0%

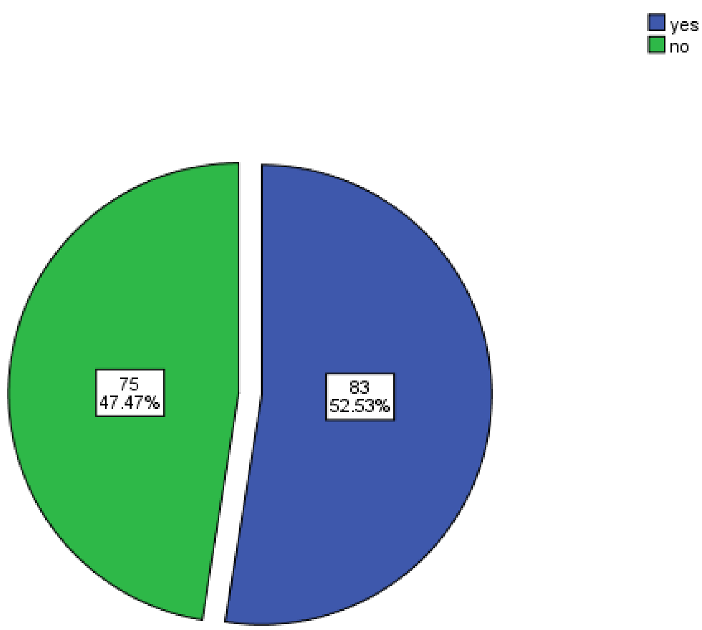

Figure 18. Frequency and percent representing if a consumer consuming Beef.

IV.1.2.2 average beef consumption in surveys

The results of the survey on average beef consumption in the Laghouat region are represented by (Figure 19 and Table 10).

Table10. Frequency and percent representing average beef consumption.

		Frequency	Percent
Valid			
	Several times a week	6	3.8%
	Once a week	13	8.1%
	A few times a month	49	30.6%
	Rarely	86	53.8%
	Total	160	100.0%

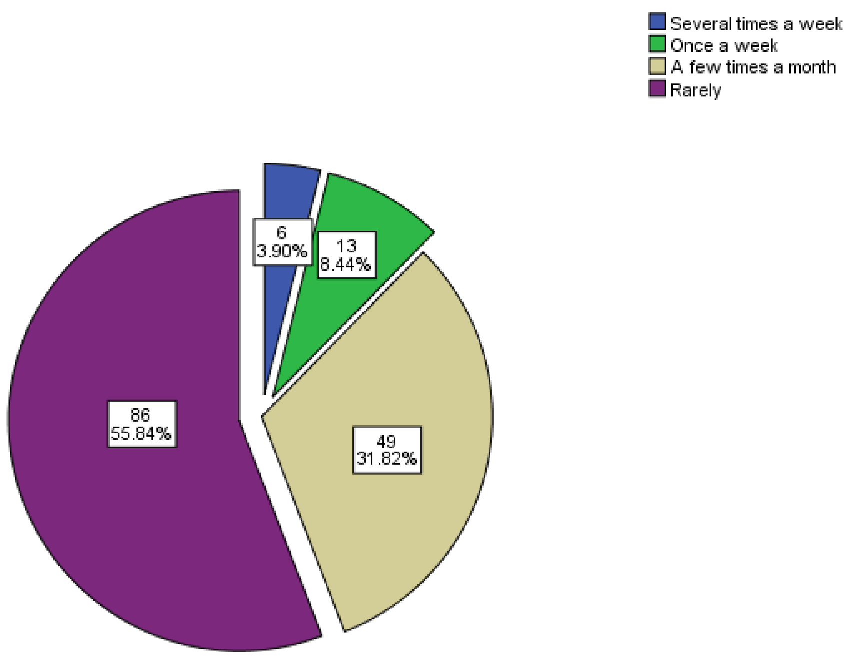

Figure 19. Frequency and percent representing average beef consumption.

IV.1.2.3 Rate of consumption of beef in recent years in surveys

The results of the survey on average beef consumption in recent years in the Laghouat region are represented by (Figure 20 and Table 11).

Table11. Frequency and percent representing average beef consumption in recent years.

		Frequency	Percent
Valid	Rise	12	7.5%
	Dropped	49	30.6%
	Stable	39	24.4%
	I stopped consuming	56	35.0%
	Total	160	100.0%

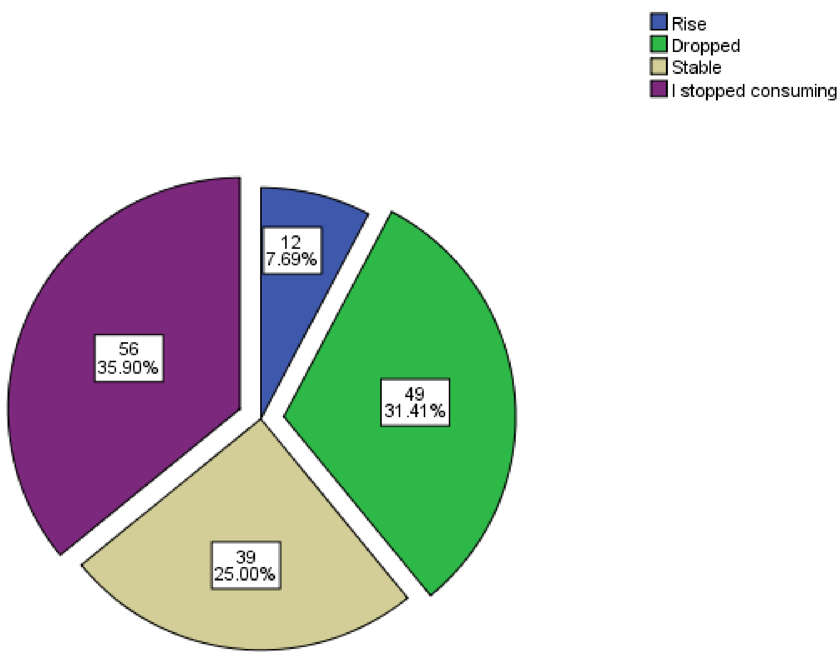

Figure 20. Frequency and percent representing average beef consumption in recent years.

IV.1.2.4 Survey About Buying beef usually

The results of the survey about buying beef, usually in the Laghouat region, are represented by (Figure 21 and Table 12).

Table12. Frequency and percent representing about buying beef usually.

		Frequency	Percent
Valid	Supermarket	5	3.1%
	Traditional butcher	140	87.5%
	Market	3	1.9%
	Buying cows for consumption	2	1.3%
	Total	160	100.0%

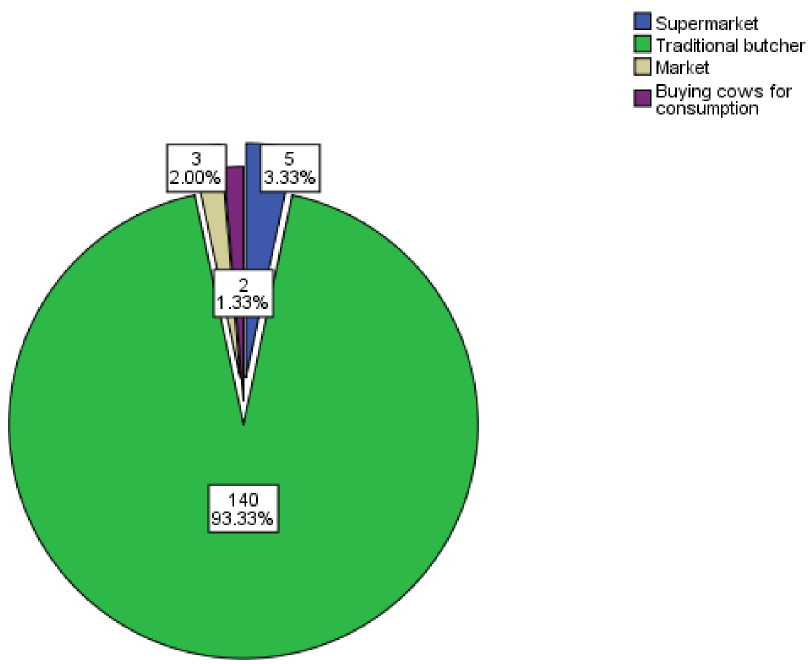

Figure 21. Frequency and percent representing about buying beef usually.

IV.1.2.5 Survey if the customer is willing to pay more for beef that meets quality

The results of the survey on customer willingness to pay more for beef that meets quality standards in the Laghouat region are represented by (Figure 22 and Table 13).

Table13. Frequency and percent representing Customer responses.

		Frequency	Percent
Valid	Yes	103	64.4%
	No	55	34.4%
	Total	160	100.0%

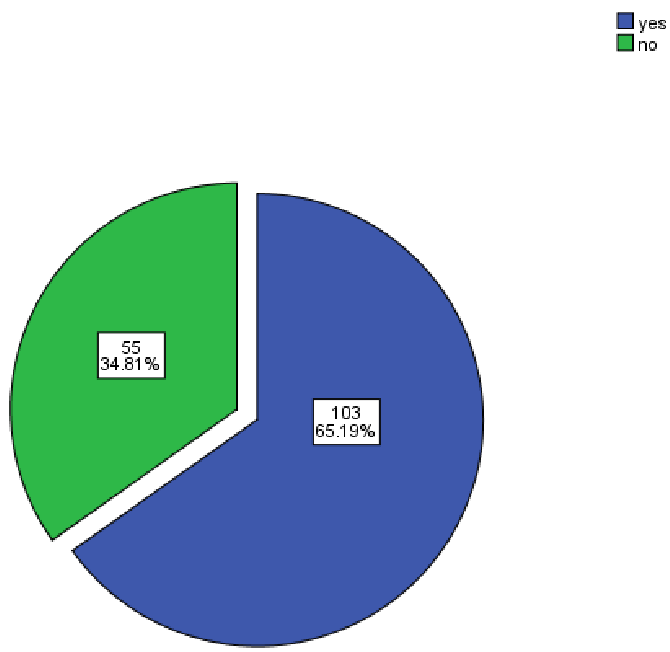

Figure 22. Frequency and percent representing Customer responses.

IV.1.2.6 Survey if the customer is interested in additional information about the beef you buy (e.g. breed of beef)

The results of the survey show that customers interested in additional information about the beef you buy (e.g, breed of beef) in the Laghouat region are represented by (Figure 23 and Table 14)

Table14. Frequency and percent representing Customer responses.

		Frequency	Percent
Valid	Yes	88	55.0%
	No	67	41.9%
	Total	160	100.0%

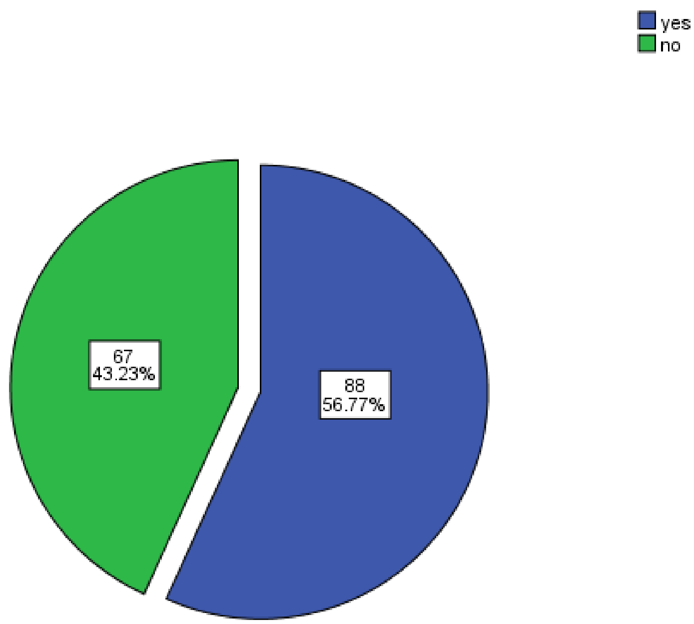

Figure 23. Frequency and percent representing Customer responses.

IV.1.2.7 Survey if the customer is paying attention to the cleanliness of the meat butcher when buying meat

The results of the survey show that customer is paying attention to the cleanliness of the meat The results of the survey show that customers are paying attention to the cleanliness of the meat butcher when buying meat in the Laghouat region, as represented by (Figure 24 and Table 15).

Table15. Frequency and percent representing Customer responses.

		Frequency	Percent
Valid	Yes	157	98.1%
	No	1	0.6%
	Total	160	100.0%

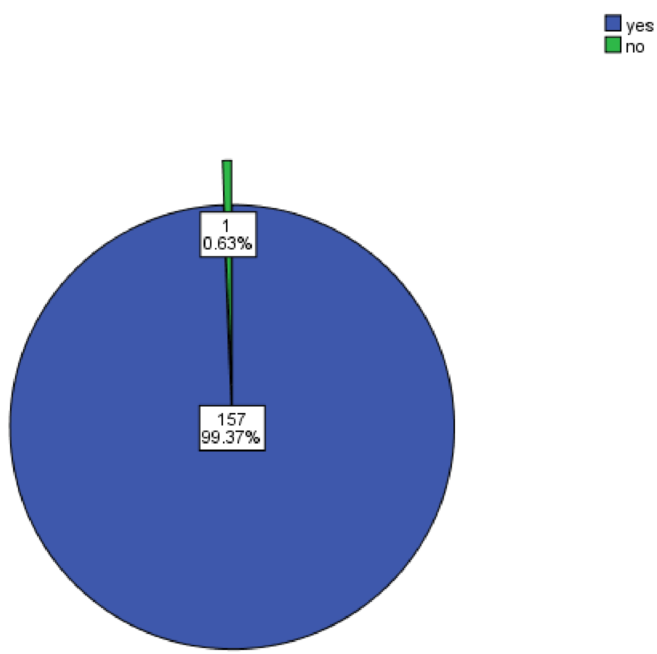

Figure 24. Frequency and percent representing Customer responses.

IV.1.2.8 Survey if the customer is interested in knowing who, how, when, and where cows were slaughtered

The results of the survey show that customers are interested in knowing who, how, when, and where cows were slaughtered in the Laghouat region, as represented by (Figure 26 and Table 16).

Table16. Frequency and percent representing Customer responses.

		Frequency	Percent
Valid	Yes	132	82.5%
	No	26	16.3%
	Total	160	100.0%

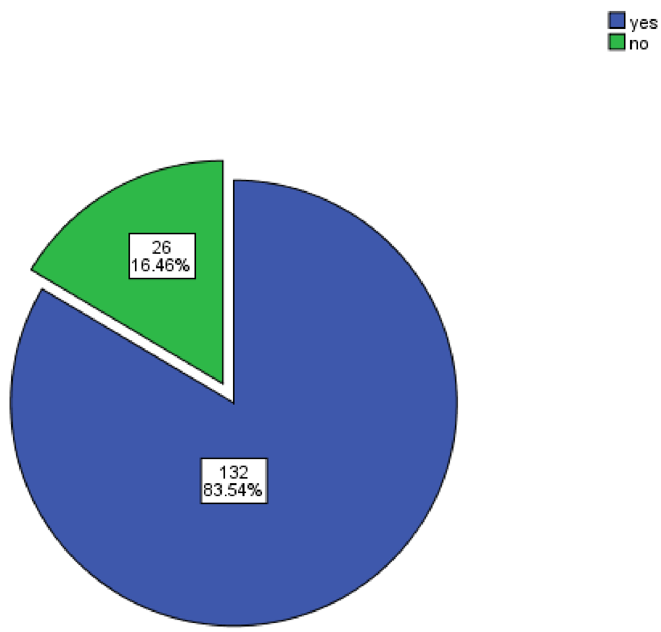

Figure 25. Frequency and percent representing Customer responses.

IV.1.2.9 Survey if the customer is concerned about the cleanliness of the vendor and work tools

The results of the survey show that the customer is concerned about the cleanliness of the vendor and work tools in the Laghouat region, as represented by (Figure 26 and Table 17).

Table17. Frequency and percent representing Customer responses.

		Frequency	Percent
Valid	Yes	157	98.1%
	No	1	0.6%
	Total	160	100.0%

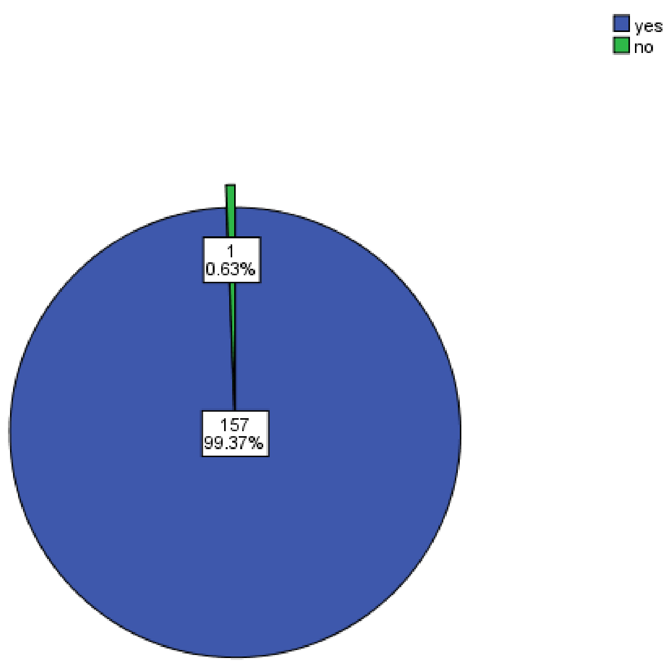

Figure 26. Frequency and percent representing Customer responses.

IV.1.2.10 Survey if the customer is making sure that the beef has undergone health surveillance (a health certificate)

The results of the survey show that the customer is making sure that the beef has undergone health surveillance (a health certificate) in the Laghouat region, but a large percentage also say the opposite, as they are represented in (Figure 27 and Table 18).

Table18. Frequency and percent representing Customer responses.

		Frequency	Percent
Valid	Yes	86	53.8%
	No	72	45.0%
	Total	160	100.0%

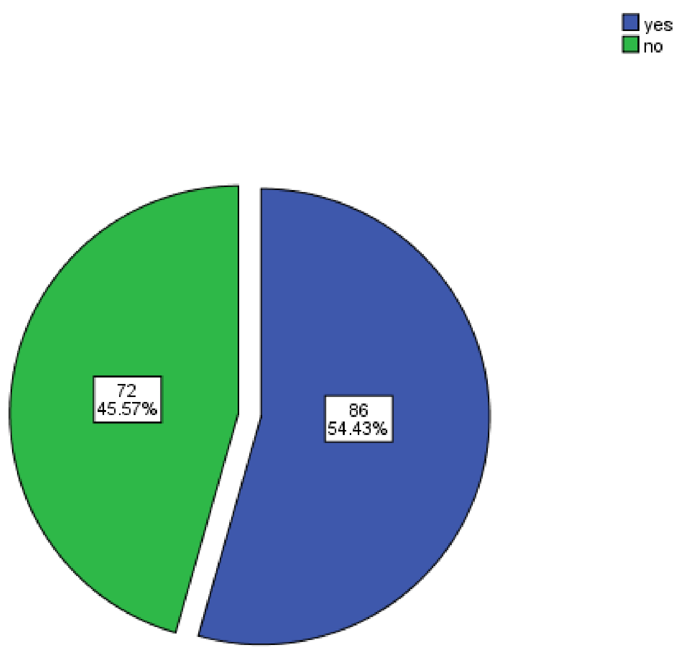

Figure 27. Frequency and percent representing Customer responses.

IV.1.2.11 Survey if the customer buys stamped meat when purchasing meat

The results of the survey show that the customer is does not buy stamped meat when purchasing meat in the Laghouat region, but a large percentage also say the opposite, as they are represented in (Figure 28 and Table 18).

Table19. Frequency and percent representing Customer responses.

		Frequency	Percent
Valid	Yes	72	45.0%
	No	85	53.1%
	Total	160	100.0%

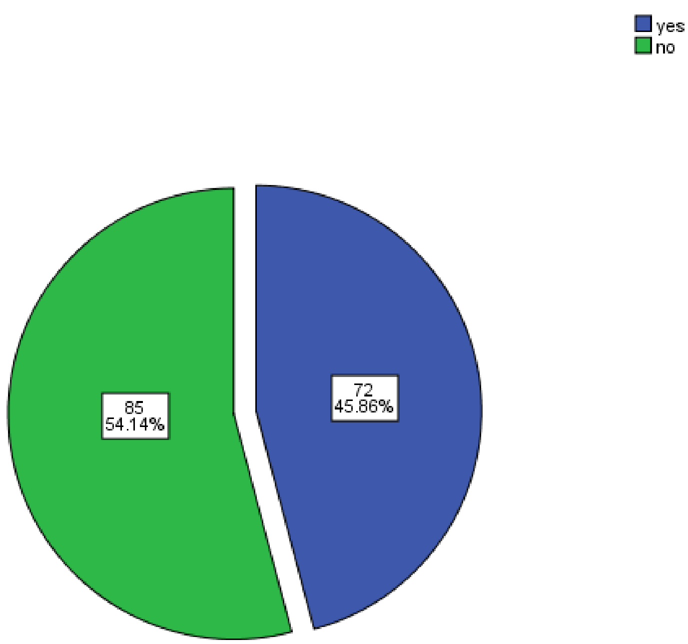

Figure 28. Frequency and percent representing Customer responses.

IV.1.2.12 Survey if the customer was exposed to food poisoning from beef

The results of the survey show that the customer was not exposed to food poisoning from beef in the Laghouat region, as represented by (Figure 29 and Table 20).

Table20. Frequency and percent representing Customer responses.

		Frequency	Percent
Valid	Yes	5	3.1%
	No	153	95.6%
	Total	160	100.0%

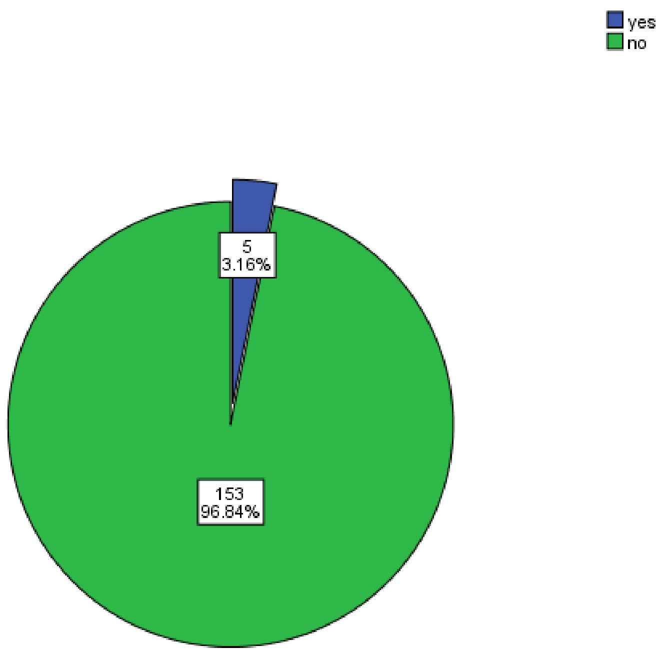

Figure 29. Frequency and percent representing Customer responses.

IV.1.2.13 Survey what criteria that the customer uses to evaluate the quality of the meat.

The results of the survey show the customer was not exposed to food poisoning from beef in the Laghouat region, as represented by (Figure 30 and Table 21).

Table 21. Frequency and percent representing Customer responses.

		Responses	
		Frequencies	Percent
Valid	Odor	160	100.0%
	Absence of Fat	43	26.9%
	Presence of Fat	24	15.0%
	Softness	17	10.6%
	Flavor	77	48.1%
	Juicer	12	7.5%
	Texture	54	33.8%
	Color	160	100.0%

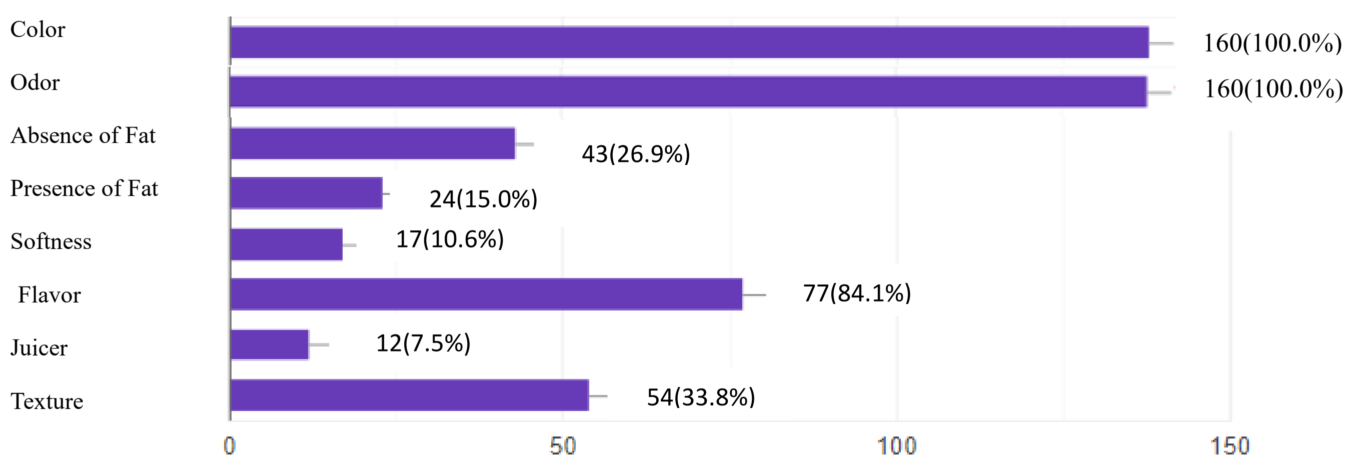

Figure 30. Frequency and percent representing Customer responses.

IV.1.2.14 Survey about most important criteria for you when choosing beef (Rank them in order of priority)

The results of the survey show the most important criteria for you when choosing beef (Rank them in order of priority) in the Laghouat region, as represented by (Figure 31 and Table 22).

Table22. Frequency and percent representing Customer responses ranked in order of priority.

Age of the carcass

		Frequency	Percent
Valid	1.00	5	3.1%
	2.00	24	15.0%
	3.00	36	22.5%
	4.00	75	46.9%
	5.00	1	0.6%
	Total	160	100.0%

General hygiene

		Frequency	Percent
Valid	1.00	62	38.8%
	2.00	42	26.3%
	3.00	30	18.8%
	4.00	13	8.1%
	5.00	3	1.9%
	Total	160	100.0%

Quality

		Frequency	Percent
Valid	1.00	32	20.0%
	2.00	59	36.9%
	3.00	45	28.1%
	4.00	10	6.3%
	5.00	1	0.6%
	Total	160	100.0%

Price

		Frequency	Percent
Valid	1.00	52	32.5%
	2.00	20	12.5%
	3.00	35	21.9%
	4.00	42	26.3%
	5.00	1	0.6%
	Total	160	100.0%

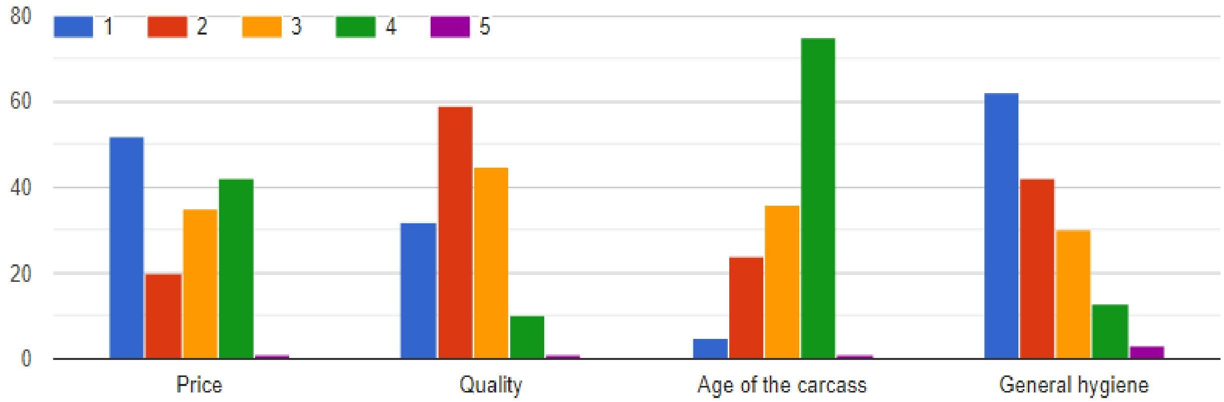

Figure 31. Frequency representing Customer responses ranked in order of priority.

CONCLUSION AND PERSPECTIVE

The beef meat market in Algeria has seen a significant increase in the importation of beef recently. This increase is attributed to the government's efforts to meet the growing demand for meat and to stabilize prices in a way that suits all levels of society, enabling citizens from various economic backgrounds to consume more meat.

However, the increased importation of beef in Algeria may lead to challenges related to microbial contamination due to the increased handling of meat, in addition to weak infrastructure for refrigeration and transportation. To mitigate these effects, it is necessary to tighten controls, improve inspections, and train workers. Local meat, on the other hand, faces similar challenges related to hygiene in slaughterhouses and poor storage practices. To improve its quality, it is important to enhance hygiene practices, train workers, and raise awareness about the importance of food safety.

Hough rich in nutrients, meat is a highly favorable breeding ground for most microbial to most microbial contaminations, which can lead to health risks for consumers. The objective of this study has enabled us to provide a quantified microbiological quality assessment of beef meat in laghaout region of the levels of contamination by spoilage indicator germs (total aerobic mesophilic flora: FAMT), faecal contamination (thermotolerant coliforms) and *Staphylococcus aureus* contamination.

Our study determined the number of certain indicator microorganisms affecting overall hygiene quality and showing the possibility of contamination by fecal origin germs and FAMT and *staphylococci* in retail beef sold in five butcher shops in the regain of laghouat.the Methodology is to carry out microbiological analyses of 15 beef meat samples taken from 5 selected butchers' shops on a 3 days period, in the regain of laghouat.

During our study, we noted that the total aerobic mesophilic flora count in the meat sample tested showed values above the acceptability threshold set by national regulations in one butcher's shop, indicating poor compliance with hygiene rules, while in the other four butcher's shops, the TAMF load was below the threshold set by the standard, indicating meats of good microbiological quality being sold.

Le nombre de coliformes thermotolérants dans les échantillons de viande provenant de cinq boucheries présentait des valeurs inférieures à la limite fixée par la norme nationale, la contamination fécale de la viande produite était limitée.

The microbial load of *staphylococci* in the meats sold in two butchers' shops was below the limit set by the national standard, indicating a limited level of contamination by these pathogenic germs; in contrast, the other three butchers' shops showed a *staphylococci* load above the standard, indicating that the meats produced in these shops are less contaminated by this type of germ.

A complementary study was conducted to obtain indices of consumer preferences for beef products. A questionnaire was developed for this purpose. The questionnaire included 16 questions to gather information on demographic and social variables also consumer behavior, perceptions of beef, and beef consumption preferences. The results show that the local consumer is somewhat familiar with these products and their behavior reflects the consumption habits of the region

Hygiene conditions at all stages (from farm to Table), compliance with international food safety systems, and the proper/efficient consumption of beef according to general hygiene rules are essential to producing quality beef and reducing microbial risks.

poor handling during meat preparation operations leads to very high levels of contamination. These contaminations can cause various serious health diseases and rapid meat spoilage, thus limiting its shelf life.

The comparative study showed that the market has unsatisfactory hygiene conditions due to:

- Non-adherence to slaughter and storage protocols

- Disruption of the cold chain process

- Suboptimal storage temperatures

- Poor hygiene of personnel, working equipment, and premises

- Unsuitable circumstances during the transportation, distribution, and commercialization of meat.

In perspective, it would be useful to control the microbiological quality of meats at various levels from slaughterhouses to butchers to better understand and identify the origins of contamination and microbiological contamination issues.

And it would be necessitates a thorough study in the future concerning this topic and the significance of recommending Configuring workers responsible for meat handling in good hygiene practice.

As well as conducting questionnaire with butcher workers on hygiene also helps to identify weaknesses, raise awareness among workers, improve public health, target training, enhance client confidence, comply with health standards, improve the working environment, reduce waste, improve work reputation and achieve quality in products.

Here are some recommendations for ensuring the safety of beef meat:

1. Good Hygiene Practices:

- Ensure all surfaces, utensils, and equipment used in the processing and handling of beef are clean and sanitized.

- Workers should wash their hands thoroughly with soap and water before and after handling meat.

- Implement regular cleaning schedules for the entire facility to prevent cross-contamination.

- Use separate cutting boards for raw meat and other meat.

2. Proper Storage:

- Store beef meat at appropriate temperatures (refrigerated at 0-4°C or frozen at -18°C or below) to inhibit microbial growth.

- Use airtight containers or vacuum-sealed packaging to prevent exposure to air and contaminants.

3. Safe Handling:

- Use separate cutting boards and utensils for raw and cooked meat to avoid cross-contamination.

- Ensure that beef is cooked to a safe internal temperature of at least 63°C (145°F) for whole cuts and 71°C (160°F) for ground beef.

4. Regular Inspections:

- Conduct regular inspections of the meat for any signs of spoilage, such as unusual odors, discoloration, or slimy texture.

- Implement routine checks for temperature control in storage areas.

5. Training and Education:

- Provide regular training for all staff on food safety practices, including personal hygiene, safe handling, and proper storage of meat.

- Educate staff on the importance of preventing cross-contamination and the risks associated with improper food handling.

6. Traceability and Documentation:

- Maintain accurate records of meat sources, handling procedures, and storage conditions.

- Implement a traceability system to quickly identify and address any potential contamination issues.

7. Control of Supply Chain:

- Source beef from reputable suppliers who adhere to strict safety standards and have good animal husbandry practices.

- Monitor and control the transportation conditions to ensure meat remains at safe temperatures throughout the supply chain.

8. Consumer Education:

- Educate consumers on proper meat storage, handling, and cooking practices at home to ensure they maintain safety standards after purchase.

- Provide clear labeling with storage and cooking instructions.

9. Effective Pest Control:

- Implement a robust pest control program to prevent contamination from rodents, insects, and other pests.

- Regularly inspect facilities for signs of pests and take immediate action to address any issues.

10. Implementing HACCP (Hazard Analysis Critical Control Point):

- Develop and implement a HACCP plan to identify and control potential hazards at critical points in the production process.

- Regularly review and update the HACCP plan to address new risks and ensure its effectiveness.

Implementing these comprehensive measures can further enhance the safety and quality of beef meat, protecting consumers and ensuring compliance with regulatory standards.

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APPENDICES

List of Appendices

Appendix 1. Equipments

Equipments	Quantity
Laboratory balance	1
Erlenmeyer flask 1000ml	2
Hot plate magnetic stirrer	2
Magnetic stir bar	2
Reagent bottles 250ml	15
Autoclave	1
Ice packs	1
Ice chest	1
Chopping board	1
Knives	1
Spatula	1
Screw caps test tubes	225
Test tube holder	3
Automatic Micropipette (100µl - 1000µl)	2
Pipette tips	300
Pipette tip Box	2
Incubators	3
Glass Pasteur pipette	200
Water bath	2
Cleaning brush	1
Colony counter	1
Rag	1
Permanent marker	1

pH meter	1
Vortex mixer	1

Appendix 2. Consumable Product

Thick aluminum foil and regular aluminum foil	30
Sterile gloves	15
Surgical masks	3
Detergent	Bottle
Petri dishes	225
10ml syringes	1
Samples	15
Nacl	18g
Plate Aount Agar	47g
Baird Parker Agar	127g
Violet Red Bile Lactose Agar	90g
Distilled water	2025ml
potassium tellurite	Ampoules

Appendix 3.

➢**الجنس:** ذكر ◯ أنثى ◯

➢**العمر:**

18-25 ◯ 25-34 ◯ 34-44 ◯ 44-55 ◯ سنة 55< ◯

➢**المستوى:** جامعي ◯ غير جامعي ◯

➢**هل تستهلك لحم البقر؟** نعم ◯ لا ◯

➢**ماهو معدل استهلاكك للحوم البقر؟**

عدة مرات في الاسبوع ◯ مرة واحدة في الاسبوع ◯ بضع مرات في الشهر ◯ نادر ◯

➢**في السنوات الاخيرة استهلاكك للحم البقر ؟** ارتفع ◯ انخفض ◯ مستقر ◯ توقفت عن الاستهلاك ◯

➢**من اين تشتري لحم البقر عادة ؟**

سوبر ماركت ◯ جزار تقليدي ◯ سوق ◯ شراء البقر من اجل الاستهلاك ◯

➢**هل انت مستعد لدفع المزيد بالنسبة للحم البقر الذي يلبي الجودة ؟** نعم ◯ لا ◯

➢**هل تهتم بسلالة البقر ؟** نعم ◯ لا ◯

➢**هل تهتم متى واين وكيف ذبح البقر ؟** نعم ◯ لا ◯

➢**هل تهتم بنظافة القصابة عند شراء اللحم ؟** نعم ◯ لا ◯

➢**هل تهتم بنظافة البائع ووسائل العمل ؟** نعم ◯ لا ◯

➢**هل تتاكد ان لحم البقر خضع للرقابة الصحية ؟** نعم ◯ لا ◯

➢**هل تشترط عند شراء اللحم ان يكون مختوم عليه ؟** نعم ◯ لا ◯

➢**هل تعرضت للتسمم الغذائي من لحم البقر ؟** نعم ◯ لا ◯

ماهي المعايير التي تعتمدها لتقييم جودة اللحم ؟ اللون ◯ الرائحة ◯ غياب الدهون ◯ وجود الدهون ◯ النعومة ◯ الطعم ◯ العصارة ◯ القوام ◯

➢**ماهي اهم المعايير بالنسبة لك عند اختيار لحم البقر رتب حسب الأولوية ؟**

	1	2	3	4
السعر	◯	◯	◯	◯
الجودة	◯	◯	◯	◯
عمر الذبيحة	◯	◯	◯	◯
النظافة بشكل عام	◯	◯	◯	◯

Q1➢Gender? man○ woman○

Q2➢Age? 18-24○ 25-34○ 35-44○ 45-55○ 55≤○

Q3➢Level? University○ Non-University○

Q4➢Do you consume beef? Yes○ No○

Q5➢What is your average beef consumption? Several times a week○ Once a week○

Several times a month○ Scarcely○

Q6➢In recent years, has your consumption of beef increased? Increased○ decreased○

stable○ stopped consuming○

Q7➢Where do you usually buy beef from? supermarket○ market○

traditional butcher○ Purchasing Cattle for Consumption○

Q8➢Are you willing to pay more for beef that meets quality? Yes○ No○

Q9➢Are you interested in additional information about the beef you buy (e.g. breed of beef)?
Yes○ No○

Q10➢Interested in knowing who, how, when and where cows were slaughtered?

Yes○ No○

Q11➢Do you pay attention to the cleanliness of the meat butcher when buying meat?
Yes○ No○

Q12➢Do you care about the cleanliness of the vendor and work tools? Yes○ No○

Q13➢Do you make sure that the beef has undergone health surveillance (health certificate)?
Yes○ No○

Q14➢Do you require the meat to be sealed at the time of purchase? Yes○ No○

Q15➢Did you get food poisoning from beef? Yes○ No○

Q16➢What are the criteria you rely on to evaluate the quality of meat?

Color○ Odor○ Absence of fat○ presence of fat○ softness○ Flavor○ Juicer○ Texture○

Q17➢For you, what are the most important criteria when choosing beef?

	1	2	3	4
Price	○	○	○	○
Qualit11y	○	○	○	○
Age of the carcass	○	○	○	○
General hygiene	○	○	○	○

Appendix 4.

Composition PCA agar	
Ingredients	**gram/liter**
Tryptone	5g
Yeast extract	2.5g
Glucose	1g
Bacteriological agar agar	12g
pH	at 25°C: 7.0 ± 0.2.

VRBL agar composition	
Ingredients	**gram/liter**
peptone	7.00g
Sodium Chloride	5g
Yeast extract	3g
Neutral Red	0.03g
Bile salts	1.5g
Violet Crystal	0.002g
Lactose	10g
Agar	15,00g

Baird Parker agar composition	
Ingredients	**gram / liter**
Pancreatic digestion of casein	10.0g
Meat extract	5.0g
Yeast extract	1.0g
Sodium pyruvate	10.0g
L-glycine	12.0g
Lithium chloride	5.0g
Agar	**20.0g**
pH	**7.2 ± 0.2**