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Assessment of Microbiological Quality and Potential Health Risks from Contaminant Microorganisms in Packaged and Bulk Flours

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Dedication

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For holding firm when everything was shaky,

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For believing, despite my doubts,

In the promise of tomorrow.

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List of abbreviation

BPW: Buffered Peptone Water

DM: Dry Matter

EFSA: European Food Safety Authority

FAO: Food and Agriculture Organization

GMP: Good Manufacturing Procedures

GHP: Good Hygiene Practice

GN: Nutritive agar

HK: Hektoen

HACCP: Hazard Analysis and Critical Control Points

IFAD: International Fund for Agricultural Development

ISO: International Organization for Standardization

MYP: Mannitol Egg Yolk Polymyxin agar

SFB: Bouillon Selenite Cystine

SS: *Salmonella-Shigella* Agar

UFC: Colony-forming unit

VRBL: Violet Red Bile Lactose Agar

WHO: World Health Organization

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General Introduction

1. Introduction

Cereals are food grains belonging to the grass family, which includes severalof the most widely consumed plant species in the world. Cereal products occupy a vital place in the agricultural system worldwide. They represent an essential food source for humanity, which form the basis of the diet of billions of people around the world (Salma et al.,2005; FAO, 2007).

Cereals such as wheat, oats, maize and rice are classified within the *Poaceae* (or *Gramineae*) family and are divided into several subfamilies. Wheat and oats belong to the *Pooideae*, while maize is classified in the*Panicoideae*, and rice in the *Oryzoideae* (Wu et al., 2019). Summer cereals are typically planted in the spring, whereas winter cereals are planted in the fall (Lelaalhe-Tidjani, 2022). The classification of cereals is crucial for understanding their agronomic characteristics and their use in human and animal nutrition. They are also distinguished based on their growth cycle, with winter cereals like wheat and oats, and summer cereals like maize and rice (Lelaalhe-Tidjani, 2022).

Cereals and their derived products represent a key sector of the agri-food industry. According to FAO, the forecast for 2024/25 global coarse grain utilization in Africa has been lowered by 3.2 million tons to 1 531 million tones, reflecting a 1.0 percent decrease from2023/24. As for Algeria, cereal production is around 3.5 million tons in 2024 (FAO, 2025).

Among these cereals, wheat (*Triticum aestivum*), corn (*Zea mays)*, rice (*Oryza sativa)* and oats (*Avena sativa*) occupy a prominent place, both in terms of their production and consumption. These cereals are transformed into flour, a versatile product used in the manufacture of various food such as bread, pasta, cakes, as well as many industrial products (Surget et Barron, 2005).

The term "flour without any qualifier" also known as "wheat flour" refers to exclusively to the powdered product obtained from a batch of wheat (*Triticum aestivum*, *ssp. vulgare*) that is healthy, fair and marketable, prepared for milling and industrially pure (Jeantet et al., 2007). The products of the milling of other cereal grains (rice, millet, corn, buckwheat) or legumes (peas, lentils, broad beans), cleaned and industrially pure, will be designated by the word flour followed by a qualifier indicating the name of the cereal or legume grain used in the composition either in an isolated state or as a mixture (International Organization for Standardization, 2013).

The classification of flours is based on the ash or mineral content. From type 45 to 150, we move from the whitest flour (low flour extraction rate) to the most "pricked", rich in grain envelopes (high flour extraction rate). This differentiation is based mainly on the notion of purity or whiteness, and does not correspond to a notion of technological value even if working the doughs is easier with white flour than with brown and wholemeal flours (Romain et al. 2007) (Tab.1)

Table 1: Types of flour (Guinet, 2006)

Type	**Ash content in % DM**	**Humidity (%)**	**Average extraction rate corresponding**
45	Less than 0.5	15.5%	67
55	From 0.5 to 0.6	15.5%	75
65	From 0.62 to 0.75	15.5%	78
80	0.75 to 0.9	15.5%	80_85
110	1.00 to 1.20	15.5%	85_90
150	More than 1.4	15.5%	90_98

DM: Dry Matter

The type number indicating the weight in grams of the mineral residue contained in these 100 grams of flour. There are a certain number of well-defined types of flour.

T45: White flour used for baking.

T55: Flour used for country bread.

T65: White flour is used to make country bread, or any other so-called tradition generally from organic farming, it does not contain ascorbic acid (vitamin C).

T80: Half-wholemeal flour commonly used in organic bakeries is used to make half-wholemeal bread.

T110: Wholemealflour.

T150: Whole meal flour is used to make wholemeal bread.

Today's cereals are fully automated; a handful of men are enough to operate a modern cereal. Some cereals have an integrated laboratory to test the technological qualities of wheat or other cereal. To obtain the desired flour, each miller develops a milling diagram, i.e. "a machine adjustment program" that allows the flour to be produced according to the characteristics of the wheat received and the desired flour. The transformation of cereals into flour involves several crucial steps. First, the grain undergoes cleaning or remove impurities such as dirt, straw and stones. Next, wheat is often moistened to facilitate the separation of the floury endosperm from its husks, which improves the quality of the final flour. The milling process itself consists of three main steps; grinding, crushing and reduction. Grinding uses fluted rollers to separate the endosperm from the husk, while crushing and reduction use smooth rollers to reduce the semolina into finer particles. After each step, sifting is performed to classify the products by size, ultimately resulting in flour (Posner& Hibbs, 2005).

On the nutritional level flour, regardless of its type, is an essential source of nutrients in the human diet. It is primarily composed of complex carbohydrates, mainly starch, which accounts for 60 to 80% of its composition and serves as the primary energy source in many diets worldwide(FAO, 2023). Proteins are also present, with varying levels depending on the type of grain; for instance, wheat flour contains gluten, a protein responsible for the elasticity and cohesion of dough, whereas corn and rice flours are naturally gluten-free, making them suitable for individuals with celiac disease(USDA, 2023).Flour is also rich in dietary fiber, particularly when it comes from whole grains, such as oat flour, contributing to intestinal transit regulation, satiety, and metabolic health(FAO, 2023). In terms of micronutrients, flour provides B vitamins, including thiamine (B1), riboflavin (B2), and niacin (B3), which play a crucial role in energy metabolism(Codex Alimantarius, 2022). It also contains essential minerals such as iron, magnesium, zinc, and phosphorus, which support various biological functions, including oxygen transport, muscle contraction, and bone health(USDA, 2023).

However, the nutritional value of flour largely depends on the refining process: refined flours lose a significant portion of their fiber, vitamins, and minerals, unlike wholemeal flours, which retain the bran and germ of the grain(FAO, 2023). Additionally, some flours are artificially enriched with micronutrients to compensate for these losses(Codex Alimentarius, 2022). The nutritional composition also varies depending on the grain type: oat flour is particularly known for its beta-glucan content, which helps reduce blood cholesterol levels, while rice flour is lighter and more easily digestible(USDA, 2023).

Lastly, cultivationharvesting, and storage conditions also influence the nutritional and microbiological quality of flour (FAO, 2023).

Flours, whether wheat, corn, rice or oat, display critical microbiological dangers that can compromise nourishment security (Dupont, 2020). These dangers basically came from defilement by pathogens such as *Escherichia coli* and *Salmonella*, which can be show in grains indeed some time recently preparing (Martin, 2018). Ponders appear that between 10% and 30% of flour tests may contain toxin-producing strains of *E. coli*, competent of causing genuine flare-ups (Leroy and Simon, 2021).The nature of the item, frequently seen as secure due to its dry state, is deceiving; in reality, flour could be a negligibly handled agrarian item that can harbor practical microorganisms for a few months beneath encompassing conditions (Nguyen, 2019). Furthermore, defilement can happen at different stages, counting gathering, capacity and preparing, where inappropriate hones can present pathogens (Kumar, 2022). Flours utilized in unbaked items, such as a crude cookie dough, pose a specific hazard since they don't experience adequate warm treatment to dispense with these pathogens (Benoit, 2023). To moderate these dangers, it is pivotal to receive through nourishment security measures, such as isolating flours from ready-to-eat nourishments and entirely taking after sterile hones when taking care of them (Giraud, 2017).

Many foodborne illnesses were linked to contaminated wheat, most notably infections brought on by *Salmonella spp*. And *Escherichia coli O157:H7*, two bacteria of serious public health concern. In addition to hemorrhagic colitis, which can be diagnosed by cramping in the lower abdomen and bloody diarrhea, *E.coli O157:H7* can induce hemolytic uremic syndrome (HUS), which can be fatal and result in acute renal failure, particularly in children and the elderly (Forghani& Oh, 2013). However, being infected by *Salmonella* causes salmonellosis, which usually manifests as fever, diarrhea and abdominal pain. Septicemia or reactive arthritis are serious systemic consequences that can occur, especially in immunocompromised people. Due to these infections' prolonged survival in low-moisture conditions, such as flour, the product is a silent but enduring vector (Beuchat et al., 2013). The urgent need to acknowledge flour as a raw product that necessitates safe handling procedures and sufficient cooking to prevent these infections has also been highlighted by outbreaks connected to the consumption of raw flour, such as in raw cookie dough, cake batter, or inadequately baked goods (Ribera et al., 2021).

In addition to *Salmonella* species and *Escherichia coli O157:H7*, other harmful bacteria –like *Bacillus cereus*, *Listeria monocytogenes* and *Clostridium perfringens*-that are linked to particular diseases can also contaminate flour. There are two types of food poisoning syndromes caused by the spore-forming bacterium *Bacillus cereus*, which is commonly isolated from cereal-based products like flour. The emetic type causes nausea and vomiting because of cereulide toxin, while the diarrheal type causes cramping in the abdomen and watery diarrhea. Despite being frequently associated with ready-to-eat foods, *Listeria monocytogens*can contaminate flour during post-processing, particularly in humid environments and cause listeriosis, a dangerous invasive infection that can cause meningitis, septicemia or miscarriage in pregnant women (Swaminathan &Gerner-Smidt, 2007).Moreover, another spore-former, *Clostridium perfringens*, can grow in flour-based preparations that are not adequately preserved and result in clostrisial food poisoning, which shows up as diarrhea and abdominal pain a few hours after consumption (McClane et al., 2006). Although they are not as typically found in flour as *Salmonella* or *E.coli*, these pathogens highlight the broad range of microbiological risks connected to this widely consumed basic material.

Moreover, molds such as *Aspergillus*, *Penicillium* and *Fusarium* are moreover of concern since they can deliver mycotoxins that influence nourishment security(Pitt & Hocking, 2009).Contamination can occur at different stages of the production chain, including harvest, transportation and storage.

The origins of microbial contamination of wheat, rice, maize and oat flours are diverse and can occur at different stages of production. For wheat, contamination can originate from harvest and storage conditions, where the presence of humidity, insects, rodents or birds in silos can play a key role (Bhatt et al., 2010). Cereals in general, including maize and oats, can be contaminated by pathogenic bacteria during their growth, which can survive heat treatment, or be introduced after this treatment by contaminated ingredients such as sugars or dehydrated fruits (Sperandio et al., 2017). In addition, approximately 25% of cereals consumed worldwide are contaminated with mycotoxins, such as aflatoxins and zearalenone, often present in maize and other cereals (Bennett &Klich, 2003). Finally, anthropogenic activities, such as contribute to microbiological contamination of fecal origin (WHO, 2018). Regarding rice, although sources do not specifically mention microbial contamination, cereals in general are subject to the same types of contamination as wheat and corn. Harvest and storage conditions, as well as contaminants introduced after processing, can affect the microbiological quality of rice flours (Kumar et al., 2019).

The primary objective of assessing the microbiological quality of cereal flours is to ensure food safety and prevent health risks associated with their consumption. Due to exposure to various environmental factors during cultivation, harvesting, and storage, cereals can become contaminated by a variety of microorganisms. Studies have shown that commercially available corn flours can contain high microbial loads, including mesophilic aerobic organisms, total coliforms, and molds (N'Gogan, 2018).

There is substantial scientific and socioeconomic interest in investigating the microbiological quality of flours. From a scientific perspective, such research helps identify the origins of microbial contamination at every stage of the production process, from harvest to storage. It also assists in evaluating microbiological hazards associated with organisms that may cause serious foodborne illnesses, such as Salmonella species, Escherichia coli, and Bacillus cereus. Effective prevention and control measures require the identification of contamination sources and the factors that promote microbial growth, including pH, temperature, and humidity (Sperber et al., 2007).

Given that flours are essential ingredients in numerous food products, contamination can lead to foodborne illnesses, negatively impacting public health and increasing healthcare costs (WHO, 2015). This research reinforces consumer confidence by ensuring flour safety, which is critical to maintaining and expanding the agri-food industry (Grunert, 2005). Additionally, producers can use the study's findings to improve their production, processing, and storage practices, thereby reducing contaminationrelated losses and improving profitability (FAO, 2017). Microbial contamination can also lead to product recalls, financial losses, and damage to a brand's reputation. This study supports businesses in avoiding such outcomes by identifying key contamination points, thus promoting economic stability in the sector (Hussain and Dawson, 2013). Finally, the study's recommendations will benefit small-scale flour producers, who are often less equipped to manage microbiological quality, by promoting safer and more competitive practicesultimately strengthening the local economy (IFAD, 2019). This research aligns with a global effort to enhance flour quality while addressing public health and economic development concerns.

Several research hypotheses may be developed to guide the investigation into the microbiological quality of rice, wheat, corn, and oat flours:

General hypothesis: The microbial contamination of rice, wheat, corn, and oat flours varies according to their origin, processing methods, storage, and distribution conditions.

These variations affect both their nutritional value and compliance with food safety regulations. Strict adherence to food safety standards and good hygiene practices is essential to ensure regulatory compliance and reduce microbial contamination (Prof. A. TantaouiElaraki, 2006).

A systematic approach to evaluating the microbiological quality of rice, wheat, oat, and corn flours forms the foundation of this study. Microbiological analysis was conducted to identify the presence and types of microorganisms in the samples. Results were expressed as colony-forming units (CFU/g) for each category of microorganism. The findings were then evaluated in light of international food safety guidelines (Codex Alimentarius, EFSA regulations) to determine their compliance(FAO/WHO, 2003; FAO/WHO, 2020).

This proposal is organized into several key sections to ensure a coherent and comprehensive presentation of the research. The introduction establishes the study's context by outlining the microbiological concerns related to flour and defining the research objectives. The materials and methods section describes the sampling and microbiological testing protocols used. The results section presents the findingsoften illustrated through tables and charts—highlighting microbiological differences between packaged and bulk flours. The discussion interprets these findings in relation to existing literature, examines their implications for food safety, and offers future research directions. Finally, the conclusion summarizes the main results, their practical implications, and recommendations for reducing microbiological risks. Each section contributes to a thorough understanding of the subject and a scientifically grounded presentation of the study.

Materials and Methods

1. Sampling and Technical equipment

1.1. Sampling

This research was conducted to identify contaminant micro-organisms in different types of flour and to evaluate their microbiological quality. Eight flour samples: wheat, corn, rice, and oat were obtained from various markets located in Guelma Province. Two categories of flour were selected for the study:

- **Packaged flours**: Four samples (wheat, corn, rice, and oat) were purchased from local supermarkets.
- **bulk flours**: Four samples (wheat, corn, rice, and oat) were purchased from vendors at the local Guelma market. These samples were collected under standard hygienic conditions and analyzed on the same day to prevent any deterioration due to storage.Each sample was collected using a sterile spatula and placed in sterile bags, then transported to the Microbiology Laboratory at 8 Mai 1945, Guelma University under controlled conditions at 4 °C.

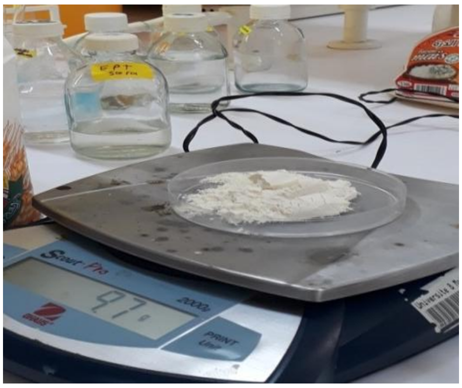


Figure 1: Wheat flour

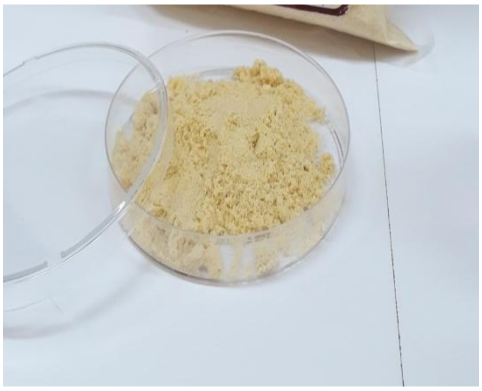


Figure 2: Corn flour

Figure 3: Rice flour

Figure 4: Oat flour

1.2 Technical equipment

The equipment required for carrying out our experimental protocol includes on the one hand for microbiological analyses requiring equipment such as Bunsen burner, petri dishes, test tubes, culture media (VRBL, PCA, Nutrient Agar, Sabouraud Chloramphenicol, Chapman, Mac Conkey, SS, Hektoen, Selenite Cystine Broth), Bain Marie, incubator thermostatically controlled at 37°C, 44°C and 25°C, Autoclave, Pasteur pipette, balance, platinum loop, spatula and sterile bottles.

2. Microbiological analysis

The microbiological analysis of flour aims to detect the presence or absence of microorganisms (bacteria and fungus) that may alter its sanitary quality and affect its safety for consumption.

Currently, the microbiological control of flour is based on techniques for detecting and enumerating potential contaminants, allowing the assessment of microbial load and verification of compliance with applicable standards.

The work is limited to the selective search for certain bacteria to the detriment of others depending on the availability of culture media and reagents. In this study, we carried out a systematic count of the germs indicating contamination such as:

- **Yeasts and Molds.**

●**Mesophilic aerobic germ*;***

●**Total and Fecal Coliforms*;***

●***Staphylococcus aureus*;**

●***E. coli*;**

●***Bacillus cereus*;**

●***Salmonella* and *Shigella*;**

2.1 Preparation of culture medium

The different culture media used for microbiological analyses were prepared according to the manufacturers' instructions. The characteristics of these culture media used and the details of their preparation are presented in **Appendix 1**.

2.2 Preparation of the stock suspension and decimal dilutions

The preparation of the stock suspension and decimal dilutions was carried out in accordance with standard NF EN ISO 6887-1, which defines the general rules for the preparation of the stock suspension and decimal dilutions for microbiological examination.

2.2.1 Stock suspension

10g of flour are weighed aseptically (around the Bunsen burner) using a balance and then placed in an Erlenmeyer flask. A volume of 90ml of buffered peptone water is added. The mixture is homogenized for 2 minutes to obtain the stock suspension corresponding to the 10^{-1} dilution. This solution is left to stand for 30 minutes to dissolve and allow the microorganisms to revive at room temperature. From this suspension, a series of decimal dilutions is then carried out.

2.2.2 Decimal dilutions

Decimal dilution consists of reducing the microorganisms' density of the flour, first to 1/10, then to 1/100, and so on until the microbial concentration of the stock suspension is reduced by a factor of 10^n. A quantity of 1ml of stock suspension is taken and then introduced into a first test tube containing 9 ml of previously prepared and sterilized distilled water. Then another 1 ml is taken from the first tube and introduced into a second tube also containing 9 ml of sterile distilled water. This operation continues in this manner until the desired dilution is obtained (for packaged flour: dilution up to 10^{-3}, for bulk flour: dilution up to 10^{-6}). This operation consists of reducing the load of the stock suspension (Fig.5)

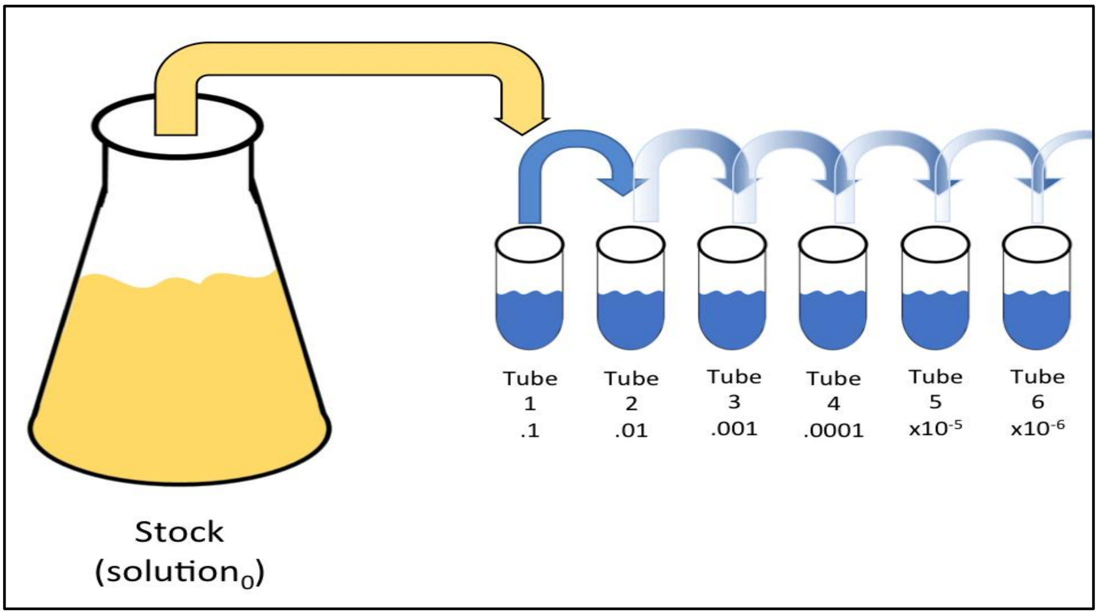


Figure 5: Decimal dilution diagram

3. Enumeration and Investigation of Microorganisms in Flour

3.1. Seeding and Incubation

Two types of seeding were performed during this experiment. These were mass seeding, which involved VRBL and PCA media, and surface seeding by spreading, which involved Chapman, SS, Hektoen, Sabouraud Chloramphenicol and nutrient agar media.

3.2. Detection and Enumeration ofTotal and Fecal Coliforms

- **Principle**

VRBL medium was used to count the total number of coliforms (bioMérieux, Paris, France). The precipitation of bile acids surrounding the colonies and the red hue of the pH indicator (naturel red) indicate the acidification caused by lactose fermentation. Gram positive bacteria are inhibited when bile salts and crystal violet are present at the same time. According to NF V08-050, NF V08-060, and NF ISO 4832(2006), only pink red colonies were counted (Fig.6).

- **Procedure**

_ Ensure uniformity 90ml of buffered peptone waterwith 10g of the flour. In sterile saline (0.85%), make serial dilutions (to $10^{-1}10^{-6}$).

_ The pour plate method fill a sterile petri dish with 1ml of the chosen dilution after cooling to 45to 50°C, add 10 to 15ml of melted VRBL agar and stir gently.

_ To limit surface growth, cover with 5ml of extra VRBL agar after allowing to settle.

_ For 24 hours, incubate at 37 and 44°C.

- **Reading of results**

Common colonies of total and fecal coliforms: because lactose ferments, it turns red or pink. Surrounded by bile precipitation, which produces a reddish halo. 1to 2 mm diameter following a 24 hours incubation period. For plates count only plates with 25 to 250 colonies are retained.

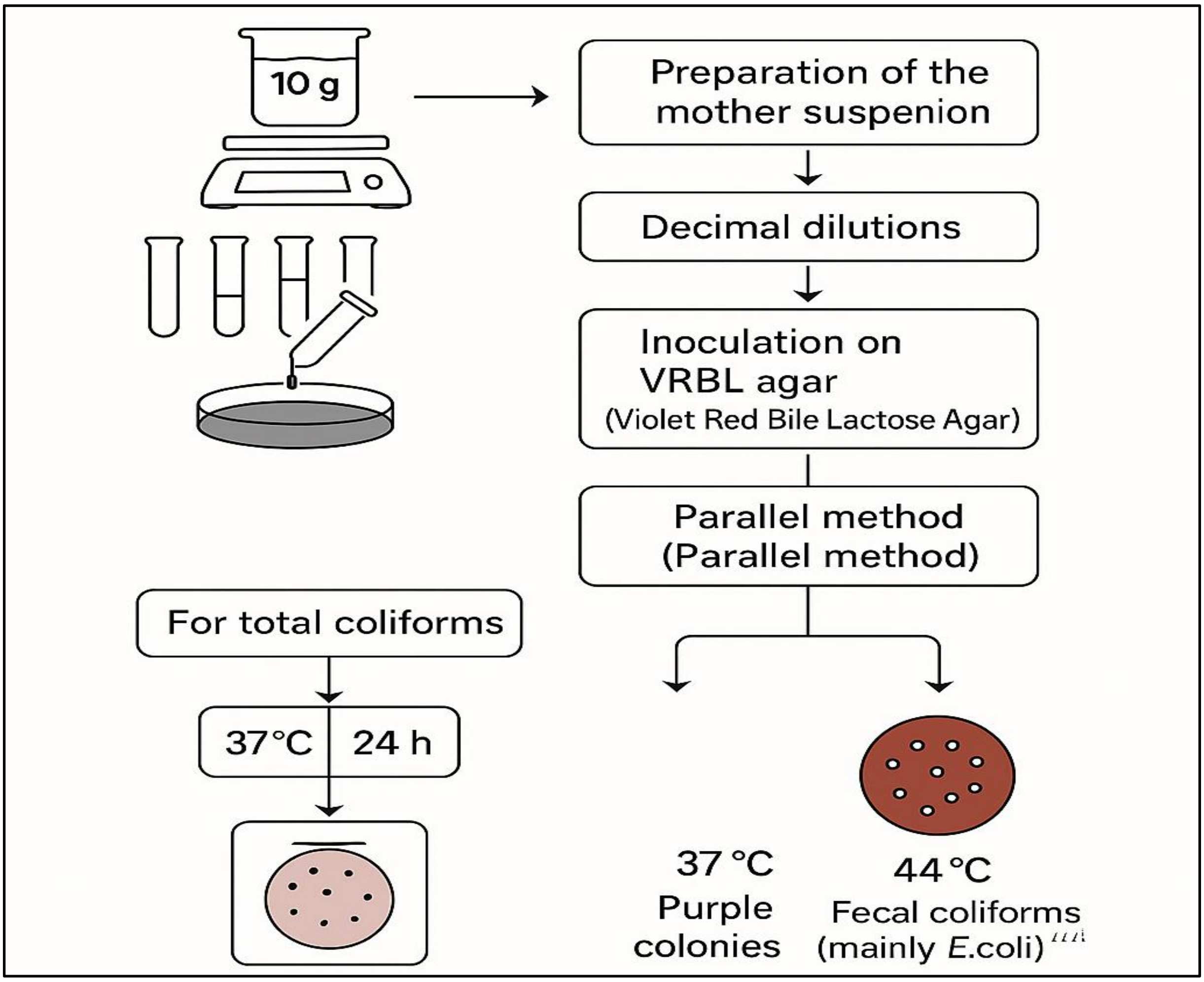


Figure 6: Protocol for the detection and counting of Total and Fecal Coliforms.

3.3 Detection and Enumeration ofMesophilic Aerobic Germ(FMAT)

- **Principle**

The mesophilic aerobic flora at 30°C (or also microorganisms at 30°C) is a technical indicator that attempts to represent the total microbial load of a food (previously, this parameter was known as total flora). It is not a specific taxonomic group but rather the set of bacteria, yeast, and molds capable of growing aerobically (in the presence of oxygen) on culture media defined by analysis standard.

Total microbiota is measured at two different temperatures to target both psychrophilic microorganisms (at 20°C) and those that are more mesophilic (at 37°C) (Merabet, 2011).Their enumerationprovides information on the product's microbial load and interprets the degree of contamination (Rodier, 2005).

- **Procedure**

_ Mix solid materials (such as 10g of flour) with 90 ml of buffered peptone water. Use straight or dilute as necessary for liquid. In sterile saline (ISO 4833-1,2013), make successive decimal dilutions (10^{-1} to 10^{-6}).

_ Pour plate method fill a sterile petri dish with 1ml of dilution and then 15ml of melted PCA that has been chilled to 45-50°C.Gently stir and allow solidify (Fig.7);

_ Plates should be incubated for 72hours at 30°C (mesophilic range) (FDA BAM,2020)

- **Reading of results**

To ensure counts accuracy, count colonies on plates containing 25–250 CFU (for pour plates).

$$\mathrm{CFU/g} = \frac{\text{colony count}}{\text{Planting volume} \times \text{Dilution factor}}$$

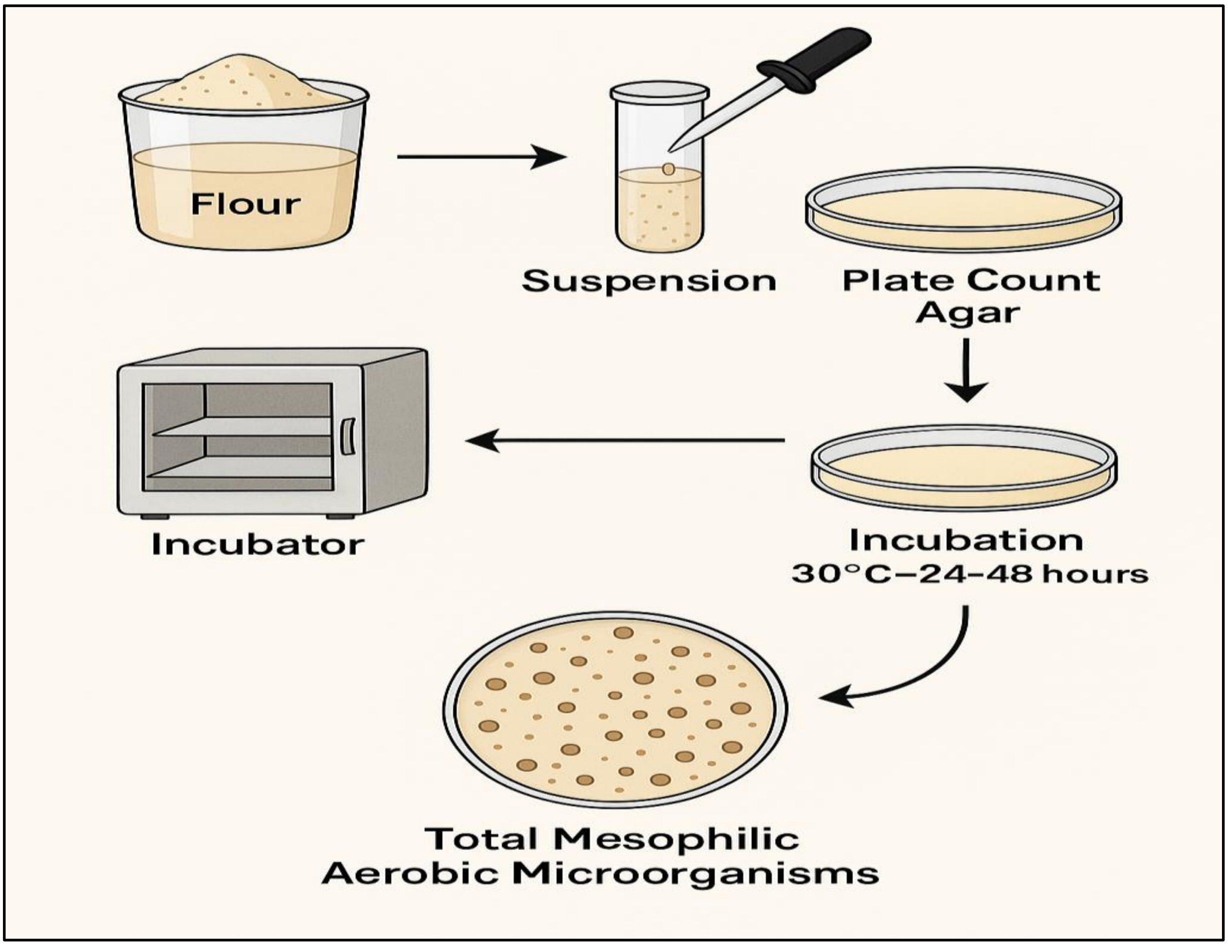


Figure 7: Protocol for the detection and counting of FMAT

3.4. Detection and Enumeration of E. coli

- **Principle**

E. coli and other Enterobacteriaceae are frequently isolated and identified using MacConkey agar, a selective and differential medium. The following guarantees its selectivity: Crystal violet and bile salts both prevent Gram positive bacteria from growing. As the only source of carbohydrates, lactose enables the separation of lactose negative bacteria from lactose positive (fermenting) bacteria. Lactose fermenting colonies turn pink or crimson when stained with neutral red, a pH indicator that indicates an acidic ph. As a lactose positive(Lac+) bacteria, E. coli undergoes intensive fermentation to generate pink to crimson colonies with bile precipitate. Lactose negative (Lac-) and colorless are characteristics of some pathogenic bacteria, including E. coli O157:H7.

- **Procedure**

- Mix 10 g of wheat with 90 ml of buffered peptone water (BPW);

- If required, make decimal dilutions ($10^{-1}$to 10^{-6});
- use a sterile glass loop to spread 0.1ml of each dilution onto MacConkey agar.;
- Incubate for 18 to 24 h at 37°C

- **Reading**

Common colonies of *E. coli* lactose fermentation cause a pink to vivid crimson coloration.Smooth, spherical, and occasionally encircled a hazy halo (bile salt precipitate) is its morphology. Atypical colonies: colorless (strains that do not ferment, such as O157:H7).Count the usual colonies, which range from 25 to 250 per plate. To confirm the results, we use catalase, oxidase test and API 2OE.

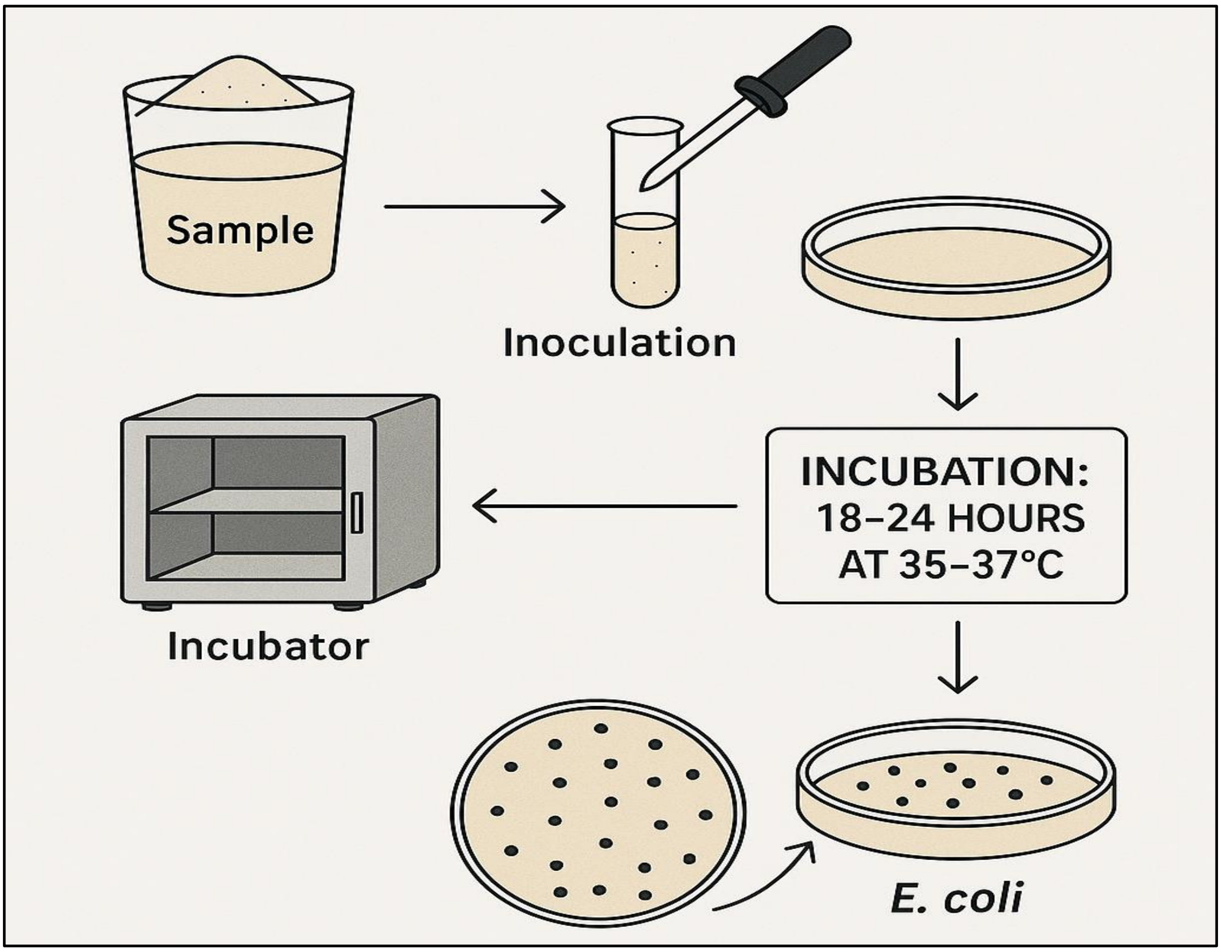


Figure 8: Protocol for the detection and counting of *E.coli*

3.5. Detection and Enumeration of *Staphylococcus aureus*

- **Principle**

Staphylococci are gram positive cocci, widely distributed in nature (air, water, soil) and often living commensally on the skin and mucous membranes of humans and animals. The genus *Staphylococcus* consists of several species, the main ones being: *Staphylococcus aureus*, *staphylococcus epidermis*, *staphylococcus saprophyticus*, and *staphylococcus intermedis*(Bouteldja et al., 2016).

- **Procedure**

Chapman's medium (selective medium) is characterized by its high sodium chloride concentration, which allows for the selective isolation of *staphylococcus.* Fermentation of mannitol is indicated by the yellowing of the colored indicator (phenol red) around the colonies.

Isolation: from the stock solution and using a sterile platinum loop, add 0,1ml of the chosen dilution and inoculate onto a chapman's medium plate. Incubate at 37°C for 24 hours (Fig.9).

- **Reading of results**

Staphylococcus aureus colonies are surrounded by a yellow halo due to mannitol attack. chapman medium only provides guidance for identifying the species *Staphylococcus aureus.*

However, this is only a presumptive step, and confirmation by specific tests remains mandatory (Joffin and Leyrol,2001).

The presence of *staphylococcus aureus* is confirmed by catalase test, and Galerie API staph.

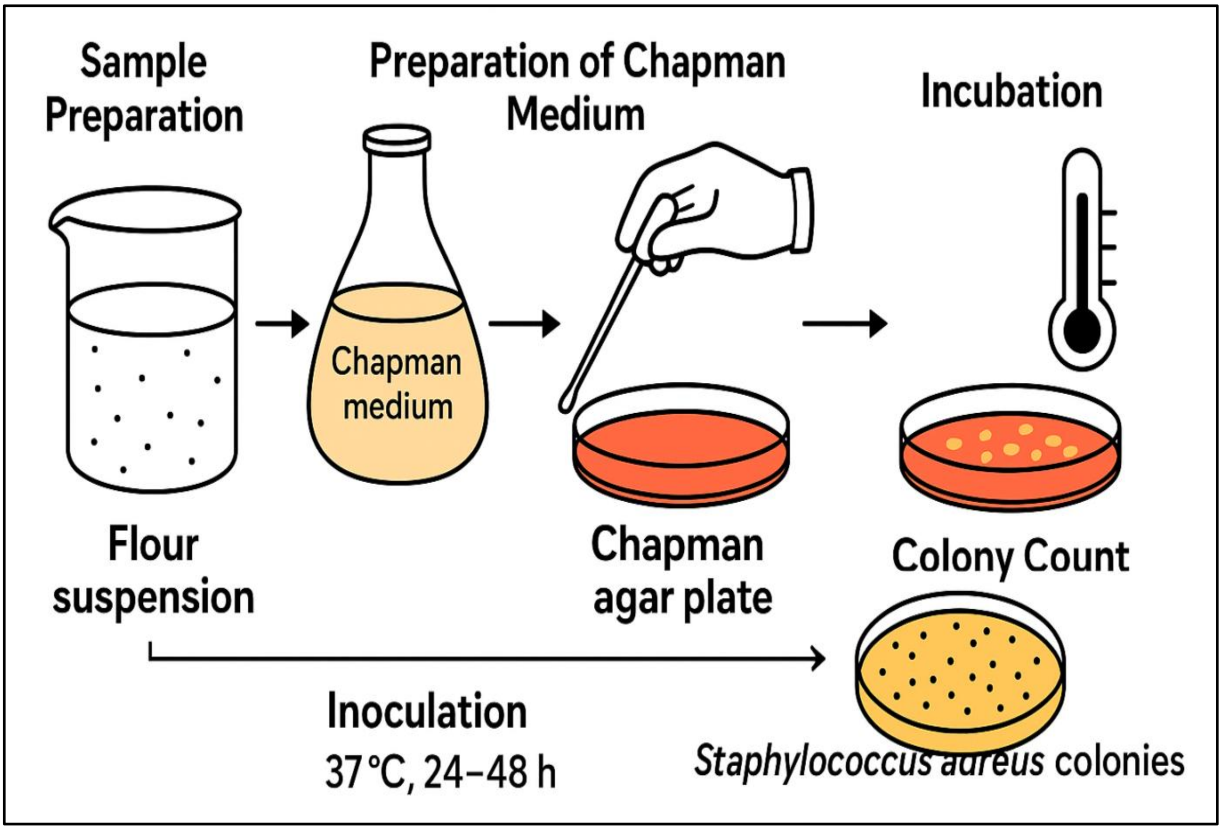


Figure 9: Protocol for the detection and counting of *staphylococcus aureus.*

3.5.1. Biochemical identification and Complementary tests

3.5.1.1. Oxidase test

Oxidase is an enzyme is an enzyme that is sought after in systematic bacteriology. Oxidase is believed to be associated with cytochrome oxidase in the IV enzyme complex's respiratory chain (Carbonnelle and Kouyoumdjian, 1998). Gram negative bacteria are identified with this technique.

- **Principle**

This test finds the phenyldiamine oxidase enzyme in bacteria that have been cultured in agar medium. N-dimethylparaphenediamine is a reagent that this enzyme can oxidize. When the enzyme is present, this colorless reagent releases a pinkish red substance that turns black when exposed to air. Phenylendiamine oxidase is a pinkish substance that is a colorless reagent.(fig.10)

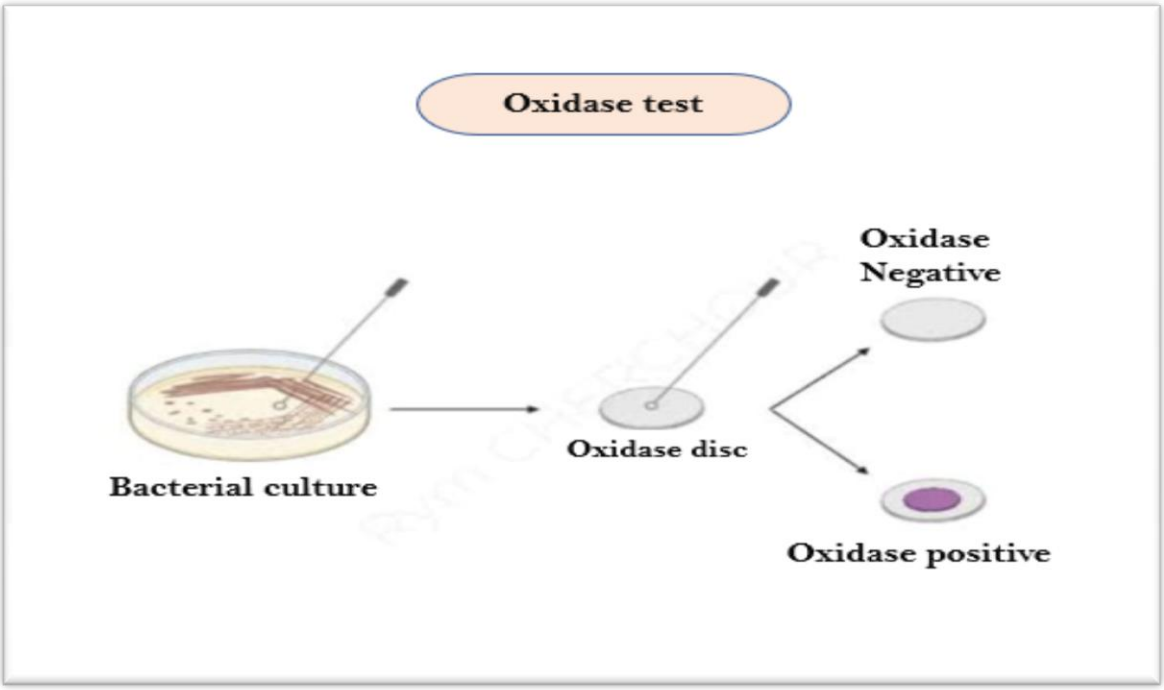


Figure 10: Oxidase test.

- **Procedure**

  There are two types of the reagent:

Either in solution lay a square of filter paper on a glass slide and immerse it in recently made reagent solution. Or as a disc that has already been impregnated with the reagent. In both situations, use the tip of Pasteur pipette a device that prevents that reagent from oxidizing to crush a colony of germs to be examined on this paper.

- **Reading of results**

- If the colony turns purple, the organism possesses oxidase; the test is positive.

-If the colony remains colorless, the organisms does not possess oxidase; the test is negative.

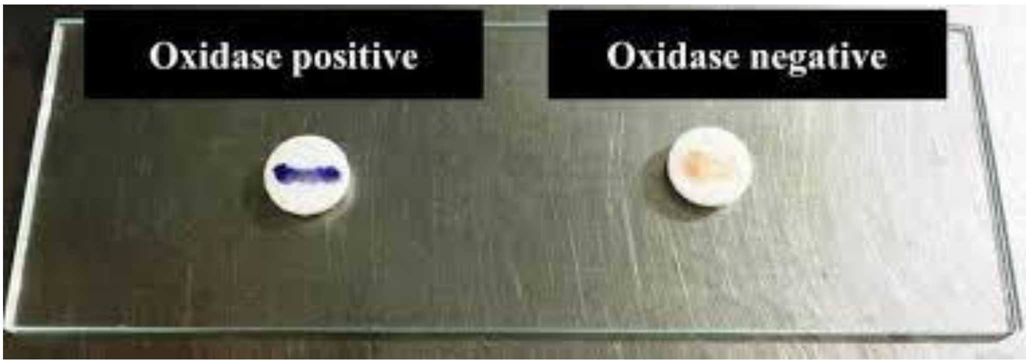


Figure 11: Oxidase results.

3.5.1.2. Catalase test

Catalase is a metabolic product toxic to bacteria. To do this, a drop of 30% hydrogen peroxide(H_2O_2) is added to the colony placed on a microscope slide. Bubble production (gas release) is observed when the reaction is positive. This test is used to differentiate bacteria of the Micrococaceae (*Staphylococcus*) catalase(+) family from those of the Streptococaceae catalase (-) family. (Delarras,2003).

- **Principle**

This enzyme allows the breakdown of hydrogen peroxide into water and free oxygen, which is released in gaseous from according to the following reaction:

$H_2O_2 \rightarrow H_2O + 1/2O_2$

This test is the basis for the identification of Gram-positive bacteria.

- **Procedure:**

We placed a drop of hydrogen peroxide (H_2O_2) on a microscope slide and added a dose of bacteria taken from the strain's agar medium (Fig.12).

- **Reading of results**

If gas bubbles (oxygen) appear, the test is considered positive (Delarras,2003).

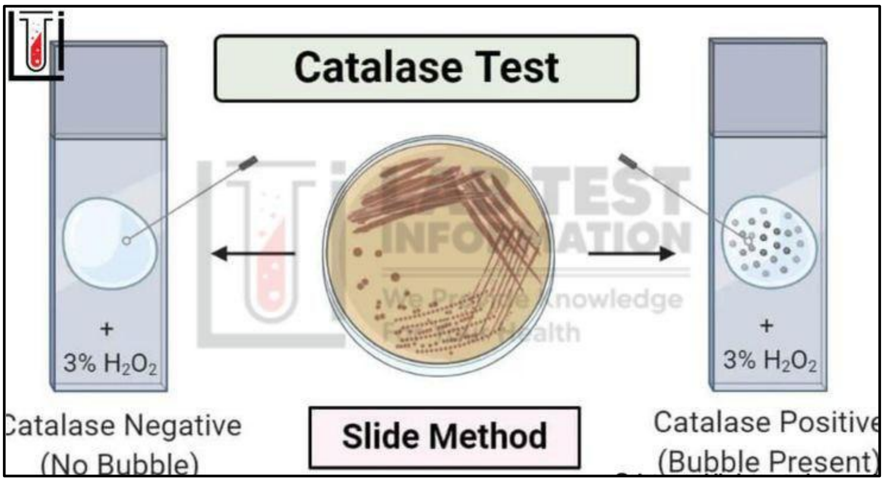


Fig.12: Catalase results.

3.5.1.3. API Staph Gallery

- **Principle**

A standardized biochemical technique for identifying *Staphylococcus aureus* and coagulase negative staphylococci (CNS) is the API Staph system (bioMérieux).

Based on 20 biochemical tests (fermentation, and substrate hydrolysis). Uses wells that have been rehydrated with a bacterial solution and contain dried substrates. After 18 to 24 hours of incubation at 35 to 37°C, identification is determined by reading colorimetric reaction.

- **Procedure**

  _ Suspension in 0.5 McFarland's volume of sterile saline (0,85% NaCl) in 5ml;

  _ Pour the suspension into the API Staph wells.;

  _ Fill the anaerobic wells with mineral oil (nitrate reduction test);

  _ Incubation;18 to 24 hours at 35 to 37°C (48 h for certain slow- moving species).

- **Reading**

Examine the reference table and the colorimetric reaction. To identify the results, enter them into the API web

Figure 13: The API Staph Gallery**.**

3.5.1.4. API 20 E Gallery

- **Principle**

The 20 microtubes of dehydrated biochemical substrates in the API 20 E strip are used to test for a variety of metabolic processes including enzymatic reaction, amino acid decarboxylation, and fermentation of carbohydrates. These substrates get hydrated when the strip is inhected with a bacterial suspension, and depending on the bacterial metabolism, they undergo color changes following incubation. These outcomes produce a numerical code that, when compared to a database, can be used to identify the organisme(Aryal,2020).

- **Procedure**

-Make a suspension of one isolated colony(from a pure culture) in sterile distilled water.

-Consider the API 20E biochemical chemicals. The biochemical test strip API 20 E is marketed commercially. When bacteria interact with them, they will produce a veriety of colors that can be used to identify the species.

-Fill these chambers to the full with the bacterial suspension using a Pasteur pipette.

-Fill the ADH, LDC, ODC, H2S, and URE compartements with sterile oil.

-After adding a few drops of water, place the API test strip inside the tray and shut it.

-Put a date, organism's identification number, and your intials.

-Incubate the tray at 37°C for 18 to 24 hours(Mount sinai lab manual,2020).

- **Reading**

Each tube's color shift after incubation is interpreted. Certain tests necessitate the inclusion of reagents, such as ferric chloride for the TDA(tryptophan deaminase) test and kovac's reagent for the indole (IND) test.

A seven dight numerical code is generated by scoring and grouping each positive or negative reponse into groups of three. To identify the bacterial species, this code is subsequently input into the APIweb TM identification program or compared to the API 20 E database.

Figure 14: the API 2O E Gallery.

3.6. Detection and Enumeration of Yeasts and Molds

- **Principle**

The count is carried out on Sabouraud Chloramphenicol medium. The inoculation is done on the surface and the cultures are then incubated at 37°C for 5 days (Fig.15).

- **Procedure**

- Fromthestocksuspension or decimal dilution, aseptically transfer 1 ml of the product to be analyzed into sterile Petri dishes;
- Pour approximately 15 ml of SabouraudChloramphenicol Agar into each Petri dishes, melted then cooled and maintained at 47±2°C in a water bath;
- Mix thoroughly using back-and-forth movements and in a figure-8 shape to thoroughly homogenize the agar and the inoculums;
- Allow the mixture to solidify on a horizontal benchtop for 15 minutes;
- incubate the dishes with the lids down at 25°C for 5 days.

- **Reading of results**

For colony counting, distinguish between yeasts and molds according to their macroscopic appearance: molds are always pigmented colonies, with a more or less swollen, velvety appearance, and yeasts are colonies resembling those of bacteria, can have regular or irregular edges, convex or flat shapes and are often opaque. For plates count only plates with 10 to 150 colonies are retained.

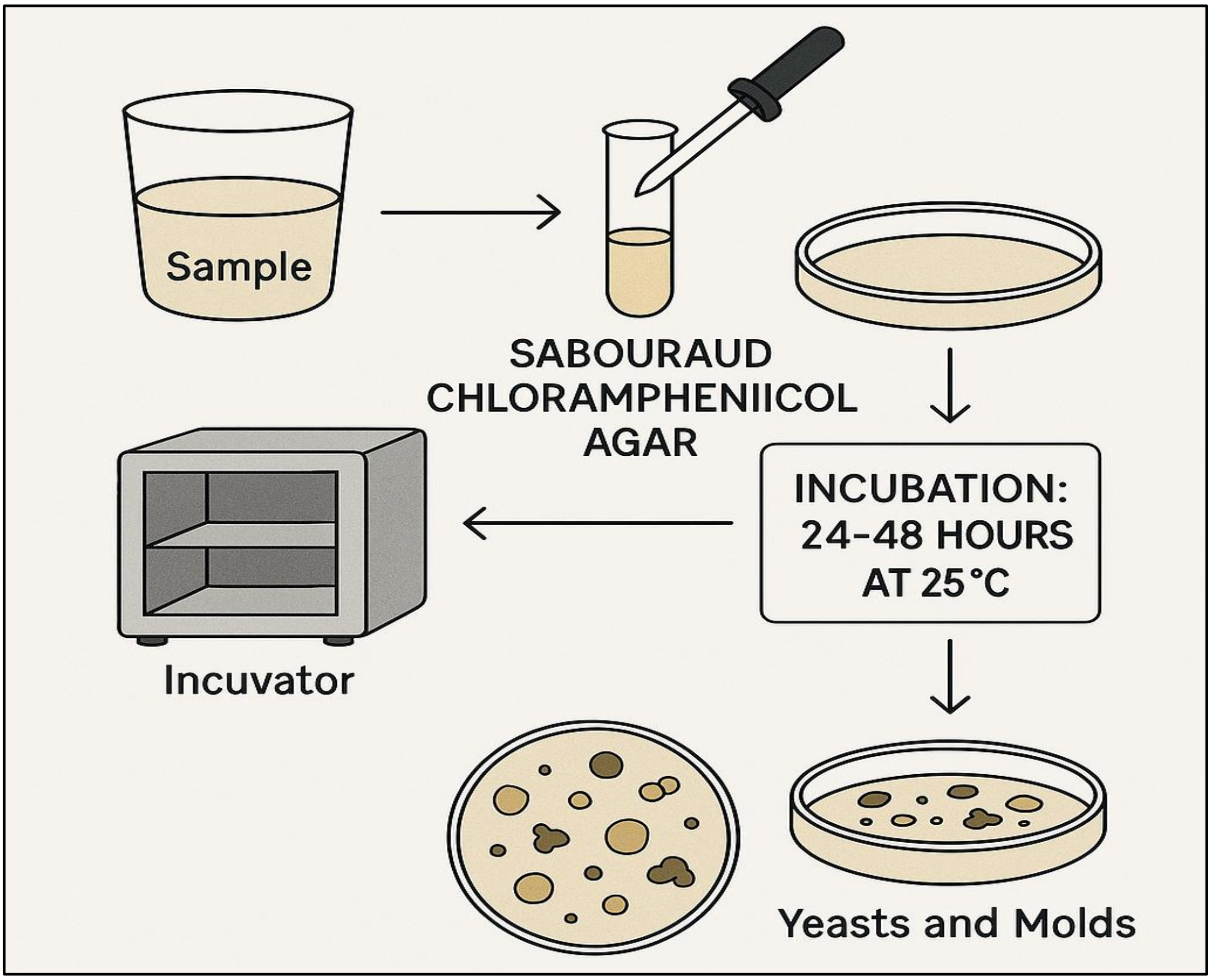


Figure 15: Protocol for the detection and counting of yeasts and molds.

3.7. Bacillus cereusdetection

- **Principle**

Bacillus cereus is a sporulated, ubiquity found, Gram positive bacterium that is frequently found in foods, including cereal products. It can result in two different kinds of food intoxication: diarrheal and vomiting or vomiting. The detection is based on starting in a selective environment such as MYP agar (Mannitol Egg Yolk Polymyxin agar).

Concurrent suppression of the flower by polymyxine B. the lecithinase of *B. cereus* hydrolyzes the lecithine (found in yellow eggs) to generate an opaque precipitate. The lack of mannitol fermentation results in rose pourpre colonies without a yellow halo. This method is widely recognized and validated by international standards such as ISO 7932:2020 for the analysis of food stuffs.

- **Procedure**

  -The preparation of the sample is an essential first step in the analytical process. For solid foods, 90ml of buffered peptone water are used to homogenize 10 grams that have been aseptically collected.

  -The surface approach is an alternative technique that involves spreading 0.1ml of the dilution onto the MYP agar that has already solidified. To create cross referencing plates in both situations, it is advised to make at least two distinct dilutions.

  -Following solidification, the plates are incubated in an aerobic environment for 24 to 48 hours at 37°C.

- **Reading**

The plates are inspected under the proper lighting after the incubation period. Because mannitol fermentation is not present, typical *B. cereus* colonies are round, slightly domed,3 to 5 mm in diameter, and purple in color. Lecithinase activity is indicated by the opaque halo that surrounds the colonies, which is the results of the hydrolysis of lecithin in the egg yolk. Plates with between 15 and 150 colonies are used for counting. It can be required to confirm colonies for regulatory analyses. Additional biochemical tests, like API 20E Gallery.

Note: Nutrient agar was used instead of Mannitol Egg Yolk Polymyxin (MYP) agar due to limited resources (see fig.16)

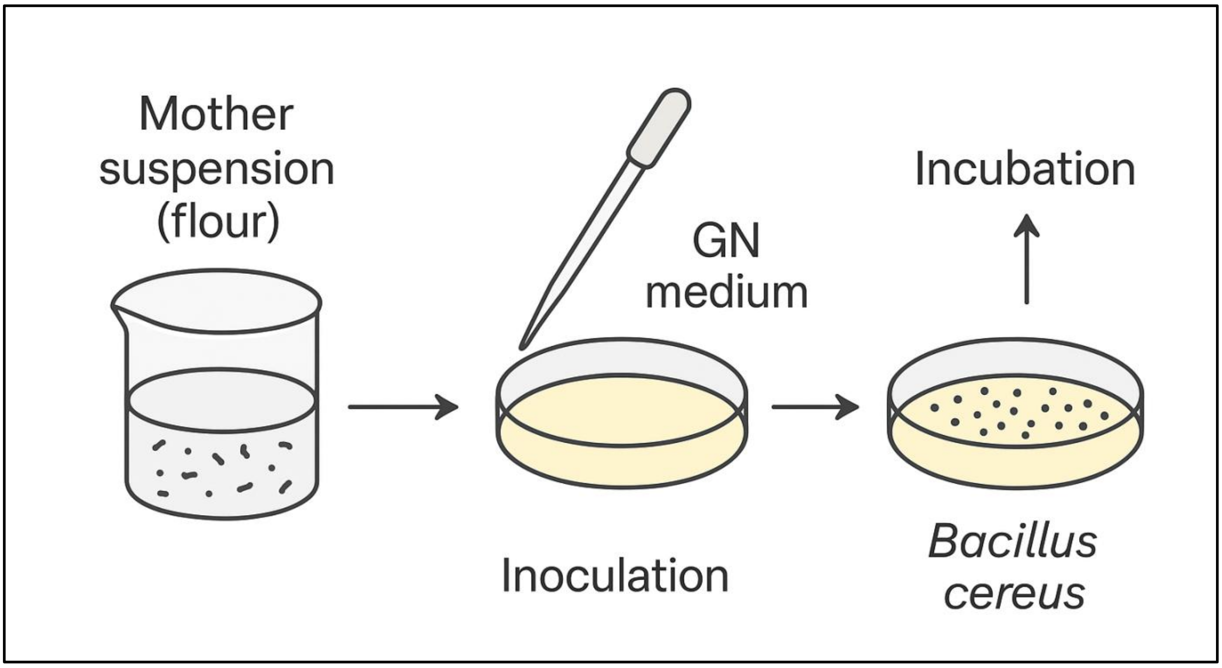


Figure 16: Protocol for detection and counting of Bacillus cereus

3.8 Detection of*Salmonella*and*Shigella*

3.8.1 Detection of*Salmonella*

- **Principle**

Salmonella are Gram negative, facultatively anaerobic, rod shaped, enteric bacteria, mostly motile with peritrichous flagella and producing hydrogen sulfide. They grow at 37°C to 48 hours on SS medium, forming small, smooth, regularly contoured colonies, pigmented green with a black center.

*Salmonella*is divided into two major groups: minor and major, which are highly pathogenic (Khemis,2013).

- **Procedure**

-Perform enrichment in tubescontaining 9ml of Selenite Cystine Broth medium. Add 1ml of the stock solution and incubate at 37°C for 24 hours;

-inoculation is done by surface inoculation or by streaking with a platinum loop after pouring the *Salmonella* agar into the petri dishes. Incubate at 37°C for 24 hours;

-after incubation, a reading will be taken on the plates containing the SS agar, knowing that *Salmonella* appears as medium sized colonies, green in color, generally with a black center (Lebres, 2002).

3.8.2 Detection of*Shigella*

- **Principle**

Shigellosis (also known as bacillary dysentery) is caused by the Gram negative, non-spore forming, non-motile bacteria in the genus *Shigella*. With an infectious dose as low as 10 to 100 cells, these gut infections are extremely contagious. Due to its high infectivity and clinical significance, accurate identification of *Shigella* in contaminated samples especially food relies on selective media designed to isolate it effectively. In complex samples like food,Hektoen Enteric (HE) agar was created specially to separate and distinguish *Shigella* from other Enterobacteriaceae(ISO 21567:2017)(fig.17).

- **Procedure**

- Perform enrichment in tubes containing 9ml of Selenite Cystine Broth medium. Add 1ml of the stock solution and incubate at 37°C for 24hours;
- Seeding is done by spreading on the surface or by streaking with a platinum loop after pouring the Hektoen agar into the petri dishes. Incubate at 37°C for 24 hours.
- after incubation, a reading will be taken on the plates containing the Hektoen agar.

- **Reading**

After 24 hours of incubation, the colonies typically measure between 1to 2 mm in diameter. They exhibit a deep blue green to jade green coloration, with even edges and a smooth surface texture. Notably, there is a total absence of hydrogen sulfide(H_2S) production, as indicated by the lack of a black center.

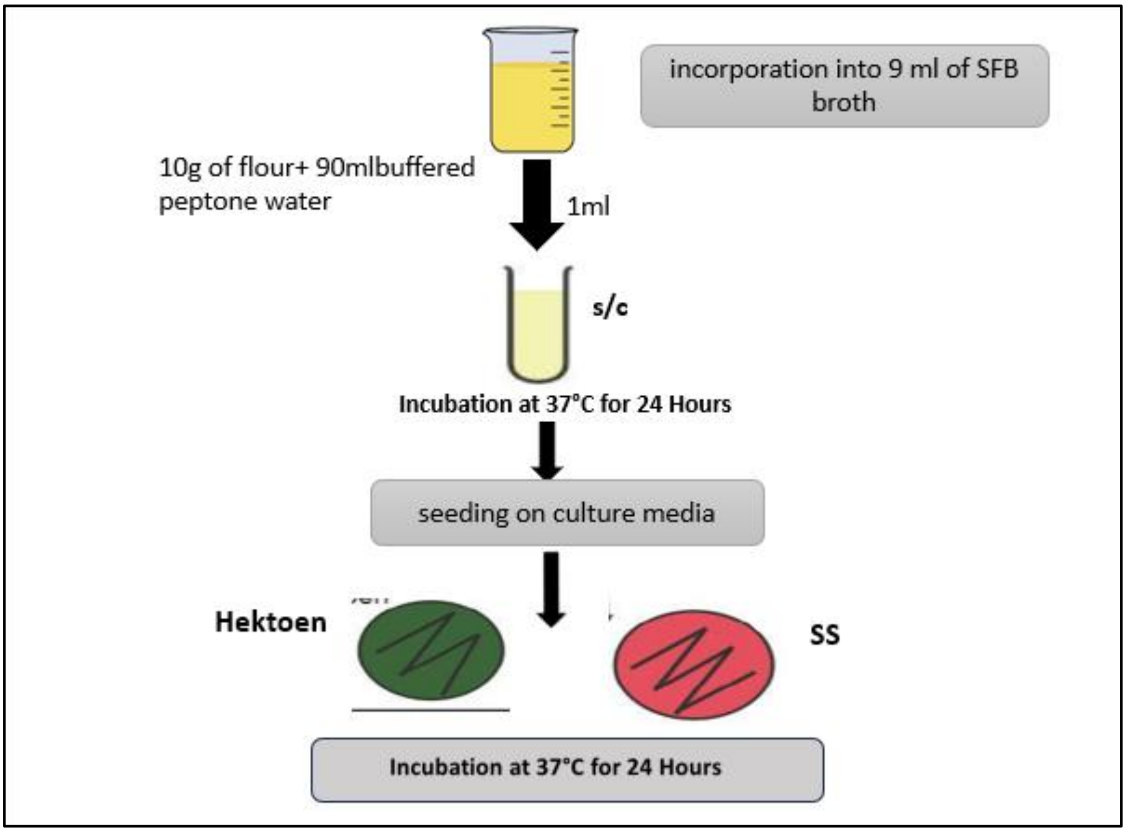


Figure 17: Protocol for detection and counting Salmonella and Shigella

Results and Discussion

1. Microbiological analyses results

The results of microbiological analyses of different types and categories of flour purchased from supermarkets and markets located in Guelma Province are summarized in the tables and figures below.

1.1. Total and Fecal coliforms count

The results of total and fecal coliform enumeration indicate that these microorganisms are absent in all types of packaged flour (wheat, corn, rice and oat) (Fig. 18). In contrast, bulk flours, particularly oat flour, exhibited a significant presence (Fig.19), with a count of 1.7×10^3 CFU/g. This finding represents a clear non-compliance with both Algerian and international microbiological standards, which stipulate the absence of total and fecal coliforms in 1 to 10 grams of sample for products intended for human consumption.

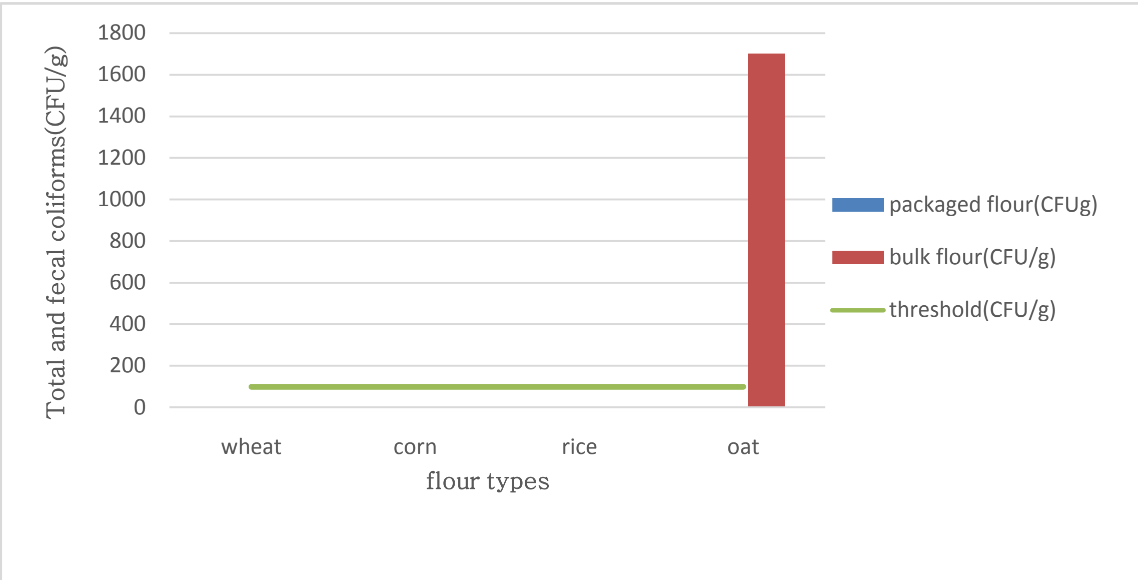


Figure 18: Enumeration of Total and fecal Coliforms in Packaged and Bulk Flours.

Figure 19: Growth of Total and fecal Coliforms on selective media from bulk Oat Flour Sample

1.2. Total Aerobic Mesophilic counts

Total Mesophilic Aerobic Flora (FTAM) is one of the primary indicators used in microbiological analyses to assess the hygienic quality of flour. Enumerationresultsrevealed the presence of significant bacterial contamination in all four types of packaged flours, with levels exceeding the upper countable limit defined by ISO 4833-1 (30 to 300 CFU/plate), thereby preventing accurate enumeration. According to international (Codex Alimentarius) and Algerian (N.M.1593-2007) microbiological standards, the maximum acceptable concentration of mesophilic aerobic microorganisms in flour ranges from 10^5 to 10^6 CFU/g. Therefore, the findings for the packaged flours clearly indicate non-compliance with these standards. In contrast, bulk wheat and corn flours showed minimal bacterial growth, with colony numbers below the countable threshold, making accurate enumeration impossible but still indicating conformity with regulatory limits. Bulk rice flour exhibited a viable count of 1.47×10^3 CFU/g, falling within acceptable standards. Similarly, bulk oat flour presented a comparatively elevated count of 1.26×10^3 CFU/g, which also remains within permissible limits(Fig.20, 21 and 22).

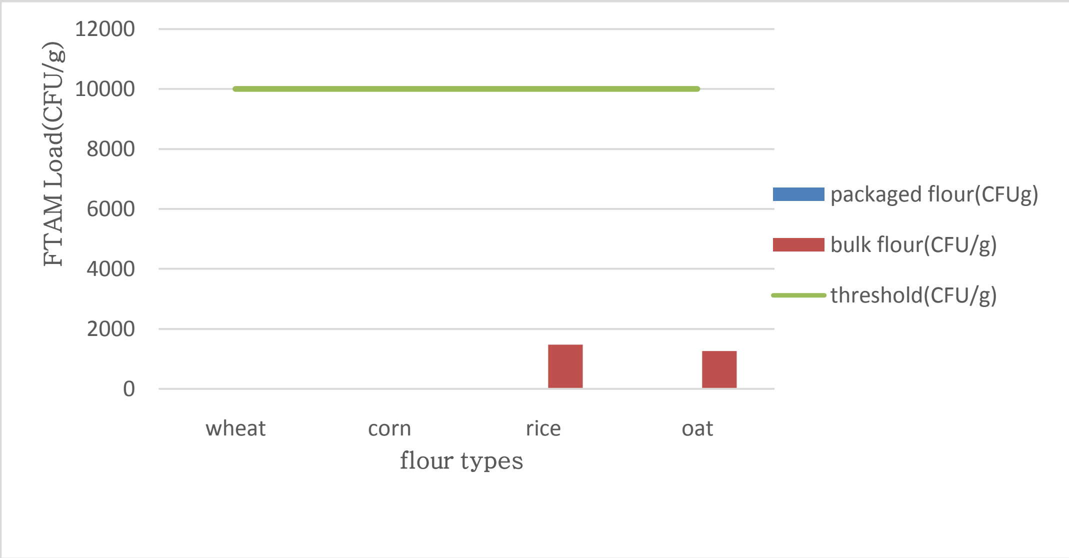


Figure 20: Enumeration of FTAM in Packaged and Bulk Flours.

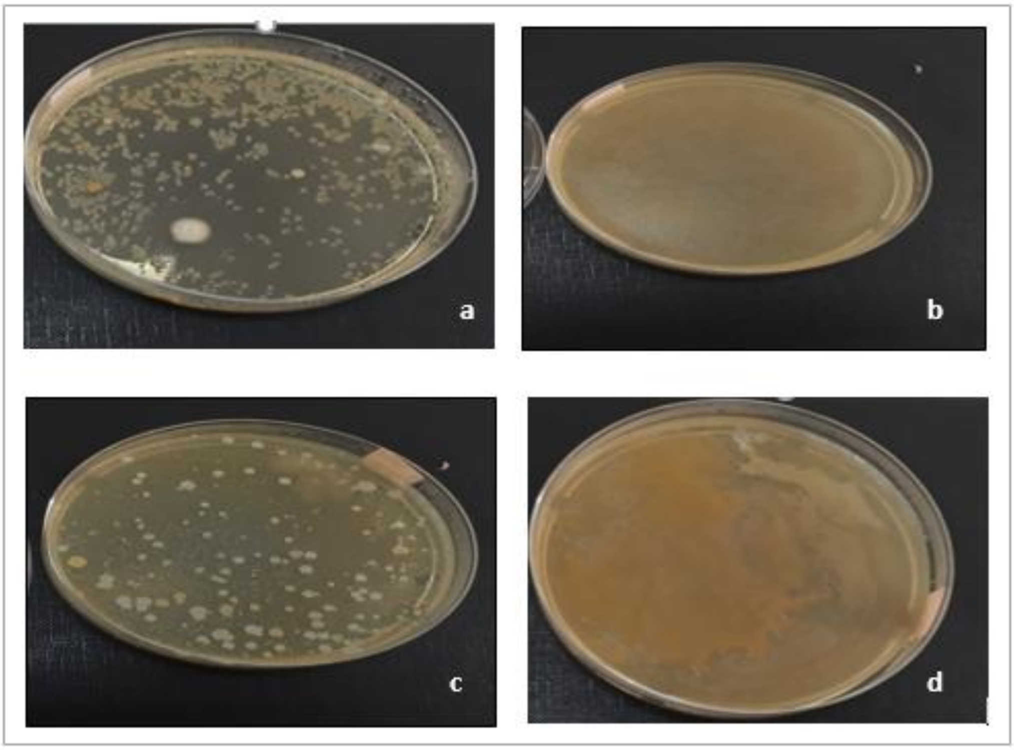


Figure 21: Macroscopic Observation of FTAM for Packaged Flour Count on PCA Agar

(a) wheat; (b) corn; (c) rice; (d) oat

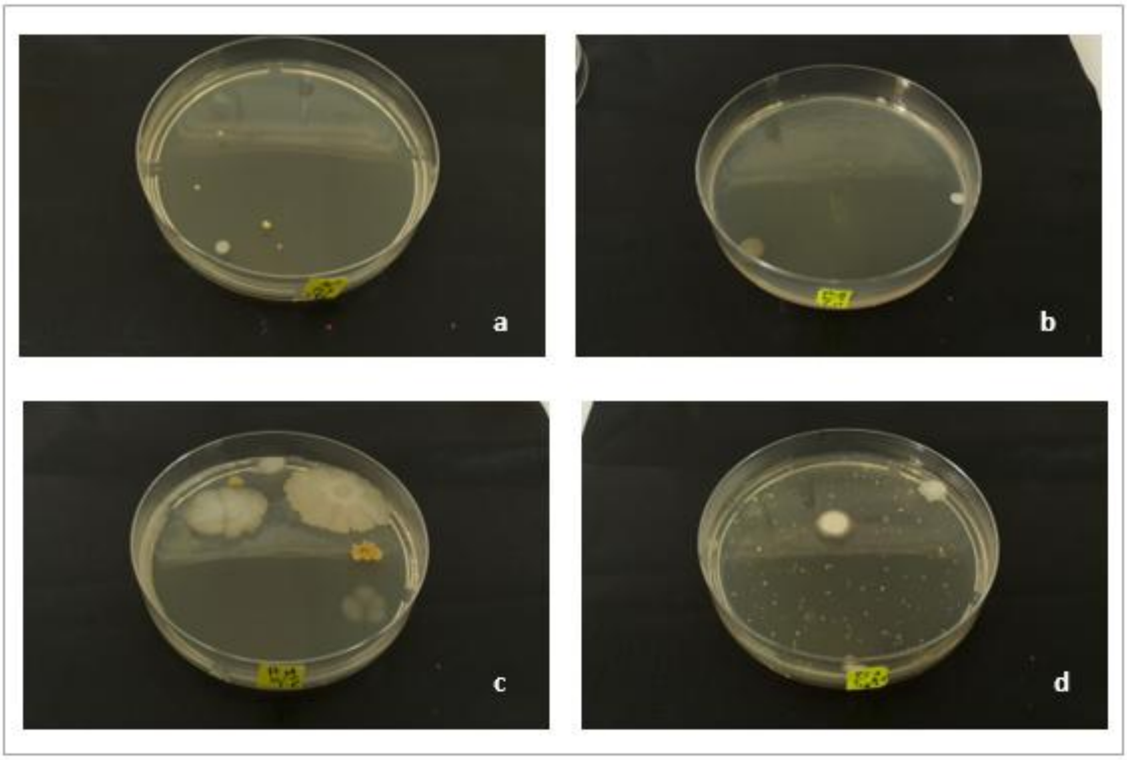


Figure 22: Macroscopic Observation of FTAM for bulk Flour Count on PCA Agar

(a) wheat; (b) corn; (c) rice; (d) oat.

1.3. Enumeration results of *E. coli* in Packaged and Bulk Flour Samples

After 24 hours of incubation at 37°C on MacConkey agar plates, no *Escherichia coli* colonies were detected in either of the flour samples analysed both bulk and packaged. These results indicate the absence of *E. coli* contamination under the tested conditions.

1.4. Results of *Staphylococcus aureus* Enumeration in Flour Samples

The *Staphylococcus aureus* counts, as presented in fig.23, varied across the four types of flour analysed (wheat, corn, rice, and oat), in both bulk and packaged forms. In packaged flours (fig.24), the highest bacterial load was observed in corn flour at 2.18×10^4 CFU/g, a level considered unacceptable according to international and Algerian microbiological standards. Wheat flour showed a count of 3.8×10^3 CFU/g, which is classified as unsatisfactory. In contrast, no *S. aureus* colonies were detected in rice flour, indicating full compliance with the standards. Packaged oat flour exhibited a very low number of colonies, below the quantification threshold (<15 colonies/plate), making enumeration unreliable but still within acceptable limits.

Bulk flour samples generally showed lower contamination. In bulk wheat flour, *Staphylococcus aureus*was detected at levels well below the count threshold. However, bulk rice flour (fig.25) presented a count of 1.2×10^4 CFU/g, which exceeds the acceptable limit. No *S. aureus* was detected in bulk corn and oat flour, both of which complied with microbiological standards requiring counts below 10^4 CFU/g.

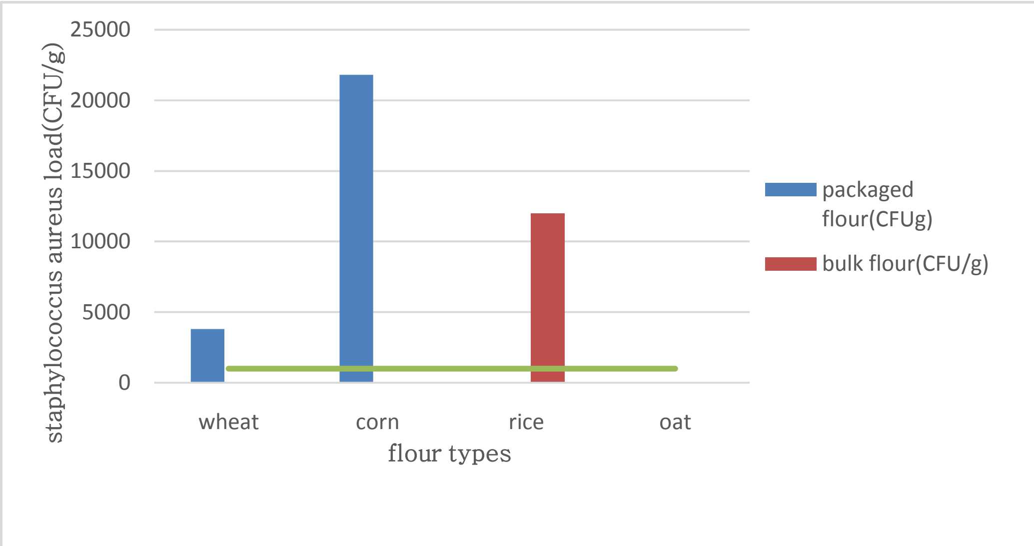


Figure 23: Staphylococcus aureus counts (CFU/g) in packaged and bulk flours (Wheat, Corn, Rice, and Oat).

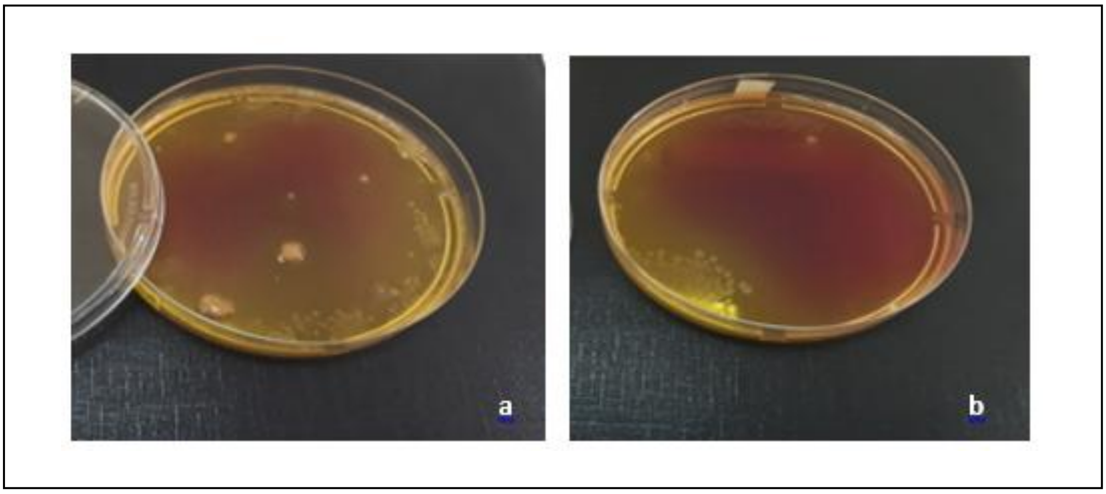


Figure 24: Macroscopic Profile of *Staphylococcus aureus* on Chapman agar for packaged flour samples (a: wheat Flour, b: corn Flour)

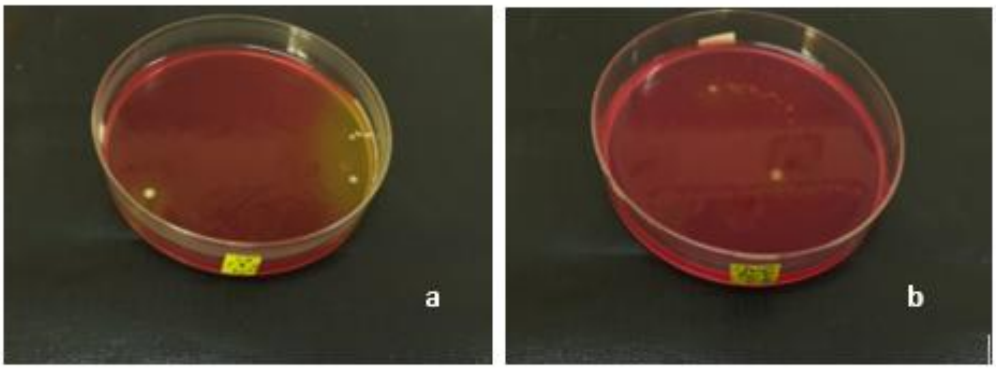


Figure 25: Macroscopic profile of *Staphylococcus aureus* on Chapman agar for bulk flour samples (a: wheat flour , b: rice flour).

1.5. *Salmonella* and *Shigella* Detection in Flour Samples

After 24 hours of incubation at 37°C on Hektoen and SS agar plates, no *Salmonella* or *Shigella* colonies were detected in either the packaged or bulk flour samples.

1.6. Bacillus cereus Detection in Flour Samples

The plating of the different dilutions of the tested flours on nutrient agar (GN) did not allow the isolation of *Bacillus cereus*. This bacterium typically forms large colonies with irregular edges, a dry or slightly granular texture, and a dull grayish-white appearance on GN. These typical morphological characteristics were not observed in any of the tested samples.

1.7. Yeasts and Molds

1.7.1. Microscopic and macroscopic profiles of Yeasts and Molds in Flour Samples

Microscopic observation of the flour samples allowed the visualization of various microbial structures. The key microscopic characteristics observed are summarized in the following tables, and representative examples are shown in Figures.

For yeasts, the microscopic examination revealed the presence of small, round to oval cells, often appearing in clumps, which are characteristic of yeast(fig.26). Some slightly elongated forms were also observed, suggestive of budding yeast. These structures, mostly spherical or oval in shape, support the identification of yeast-like cells (tab.02)

Table 02: Microscopic profile of Yeasts in different types of flour (Packaged and Bulk).

Flour type	Flour category	Morphology	Yeast code
Packaged	**wheat**	Mostly spherical or oval structure (yeast like cells).	**Y1**
	corn	The small, round cells and clumps are very reminiscent of yeast. The slightly elongated shapes.	**Y2**
	rice	Round to oval cells grouped in clusters.	**Y3**
	oat	**-**	**-**
Bulk	**wheat**	**-**	**-**
	corn	**-**	**-**
	rice	**-**	**-**
	oat	Round, oval budding cells.	**Y4**

- : Absent

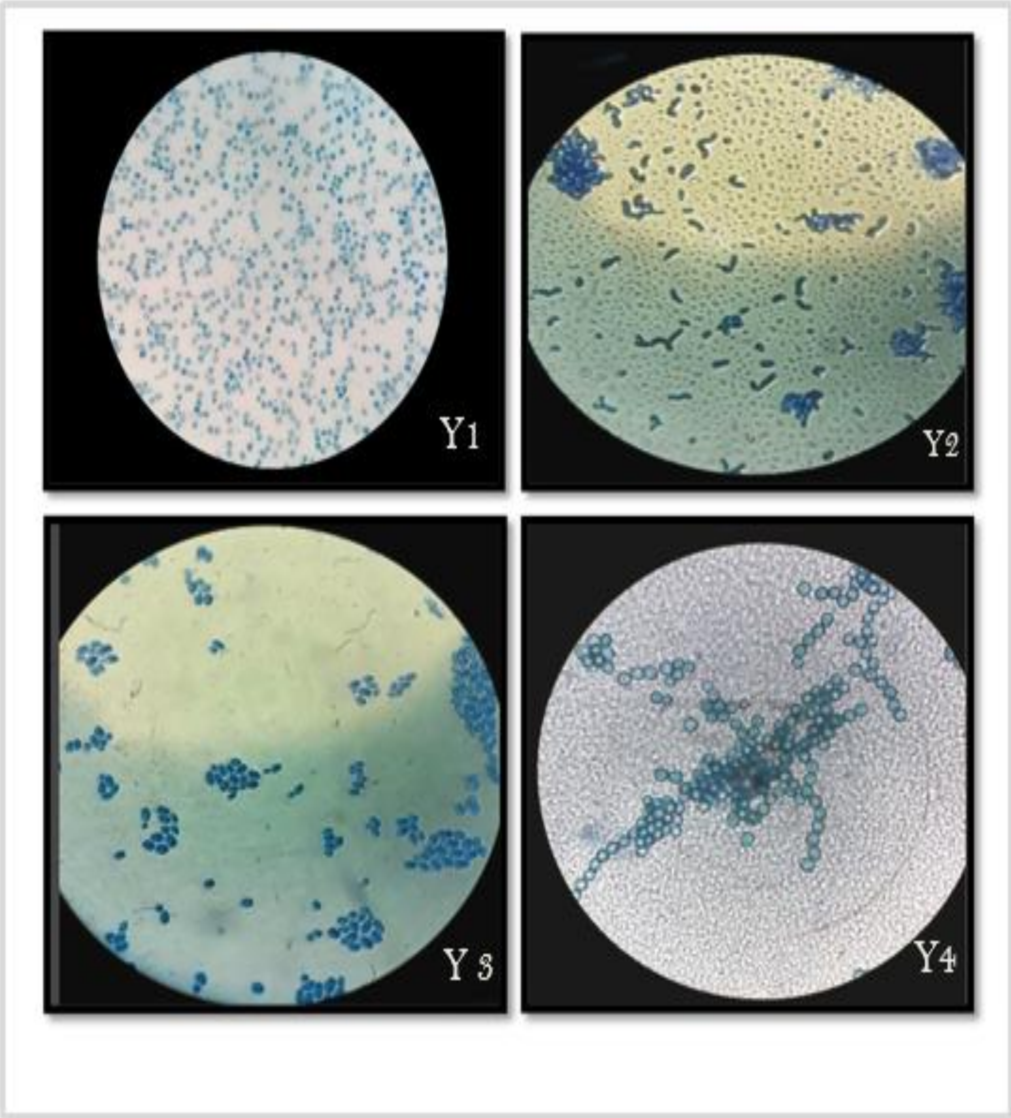


Figure 26: Yeast structures observed under light microscopy(magnification x100) in flour samples.

For molds, table 03 and figure 27 presents the main microscopic features observed in the fungal species isolated from the different flour samples. These characteristics include cell shape, hyphal structures, spore types, and other diagnostic elements.

Table 03:Microscopic profile of Molds in different types of flour (Packaged and Bulk).

Flour type	Flour category	Morphology	Molds code
Packaged	Wheat	Filamentous fungus that has brown, geniculate conidiophores and septate, branched hyphae. Its enormous, muriform (multicellular) conidia, are particularly prominent in the top center.	M1
	Corn	Its hyphae are hyaline and septate. Long chains of tiny, spherical, green conidia are produced by uniseriate or biseriate phialides	M2
	Rice	It produces chains of green or bleu green circular conidia through brush like conidiophores and septate, branched hyphae.	M3
	Oat	has septate, brown hyphae and dark, sometimes bent conidiophores that produce branching chains of oval to lemon-shaped brown conidia	M4
Bulk flour	Wheat	Root like rhizoids and aseptate hyphae. Long sporangiophores terminate in sporangia with a dome shaped columella at the base that contain numerous oval sporangiosphores.	M5
	Corn	septate, hyaline hyphae. Phialides produce chains of round conidia,	M6
	Rice	shows broad, aseptate (coenocytic) hyphae and long, unbranched sporangiophores. These end in large, round sporangia filled with sporangiospores	M7
	Oat	Septate hyphae. Globose conidial heads at the tips of straight conidiophores.	M8

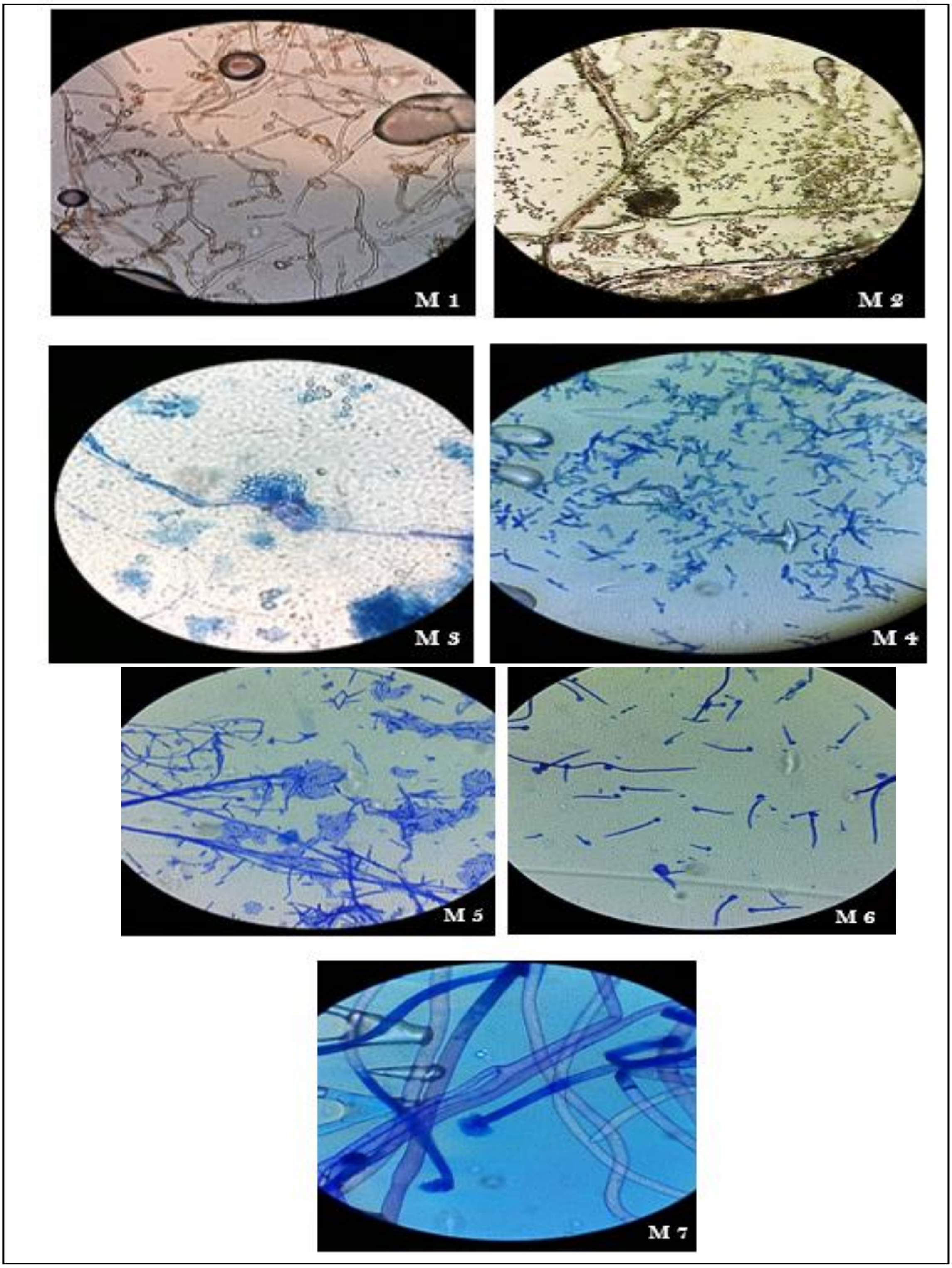


Figure 27: Microscopic Structures of Fungal Species Isolated from Flour Samples

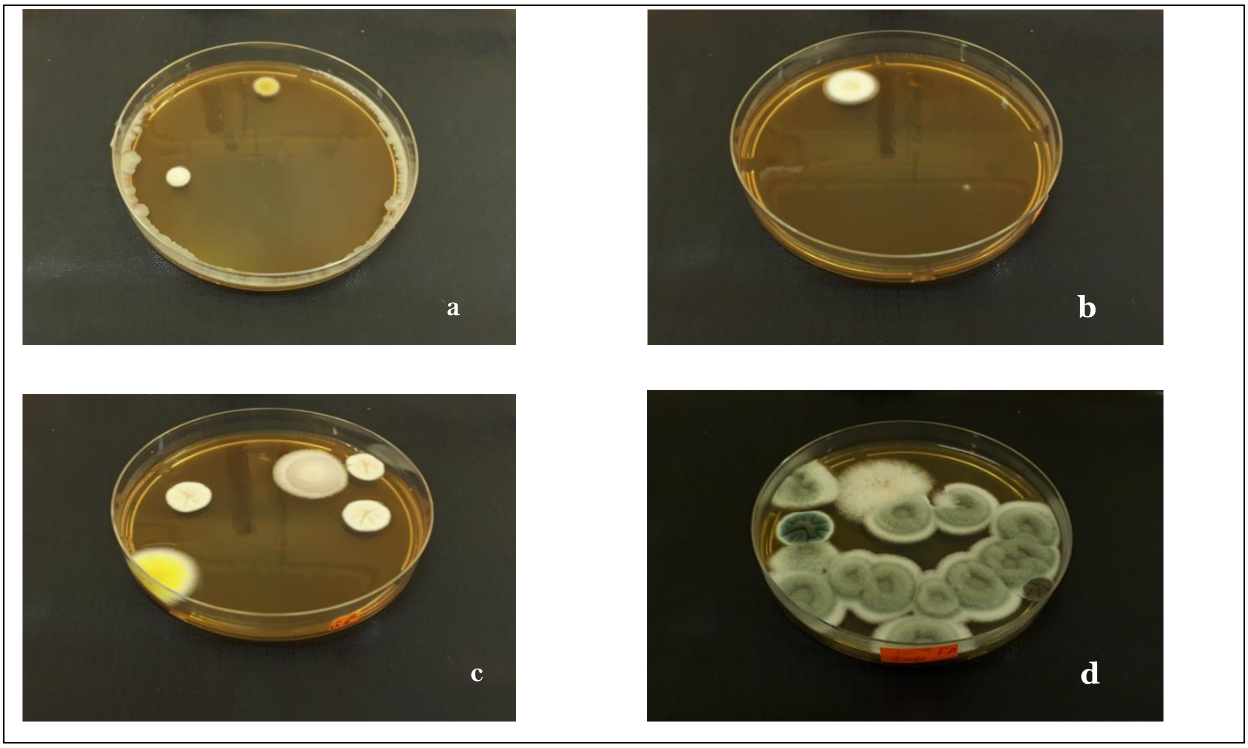


Figure 28: Macroscopic observation of yeasts and molds for packaged flour in Sabouraud Chloramphenicol agar.(a: wheat, b: Corn, c: Rice, d: Oat.

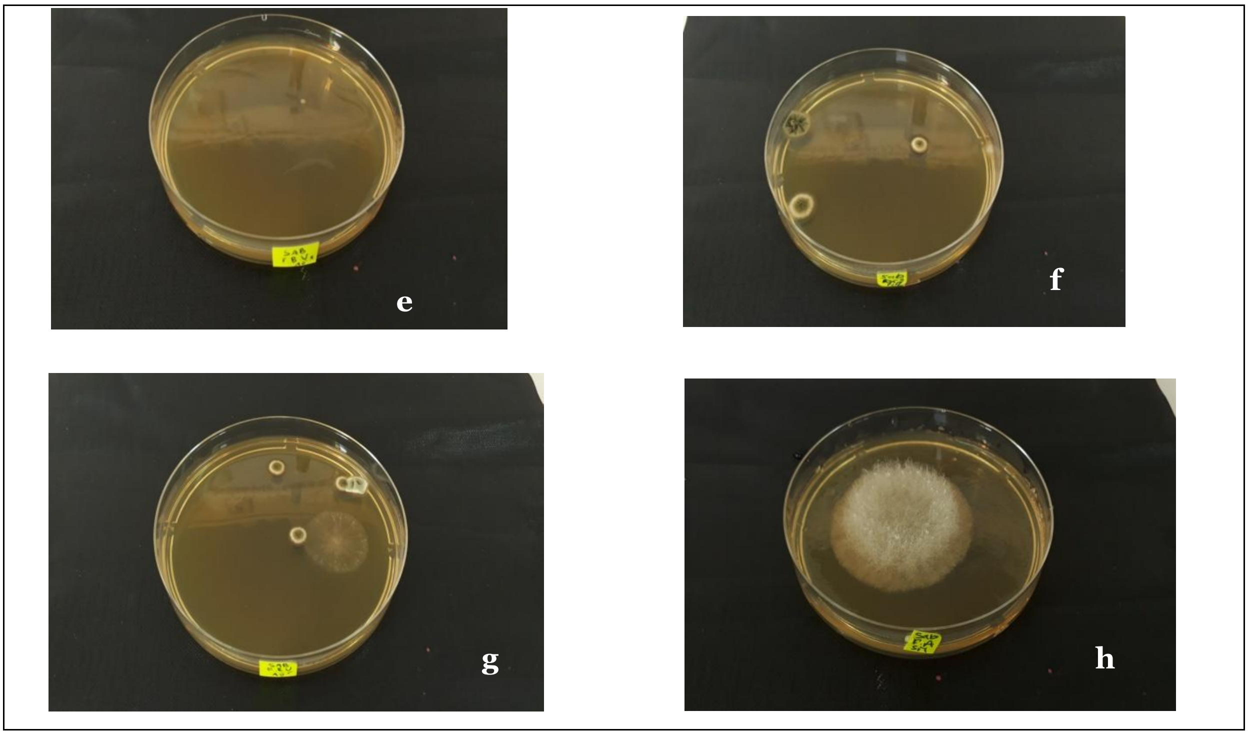


Figure 29: Macroscopic observation of yeasts and molds for bulk flour in Sabouraud Chloramphenicol agar.(e: wheat, f: Corn, g: Rice, h: Oat)

The yeasts and molds isolated from packed flours **(Fig.28)** and bulk flours **(Fig.29)** grown on Sabouraud agar supplemented with chloramphenicol are observed macroscopically in the aforementioned pictures. The variety of the extant fungal flora can now be highlighted thanks to these selective media. We see a more varied fungal growth in packaged flours, including filamentous molds that are occasionally colored (particularly greenish or blackish colonies indicating the presence of *Aspergillus* or *Penicillium* and white, smooth, and shiny yeast colonies. In the oat sample (d), we specifically observe a high level of contamination with a thick growth of molds. Though some (like wheat, figure h) have a well-developedwhite colony of mold with a cottony feel, bulk flours often exhibit a more constrained growth in the number of colonies. The storage, packing, and hygienic conditions unique to each variety of flour may be the cause of these variations. Overall, prior to microscopic identification, the macroscopic appearance permits an initial taxonomic orientation.

Based on **microscopic** and **macroscopic** observations following inoculation on Sabouraud agar supplemented with chloramphenicol, the fungal isolates were classified into two groups: **yeasts** and **filamentous fungi (moulds)**.

- **Yeasts Identified :**

- **Y1**: *Candida* sp.
- **Y2**: *Pichia* sp.
- **Y3**: *Saccharomyces cerevisiae*
- **Y4**: *Saccharomycopsis* sp.

These yeasts are commonly associated with cereal-based products. Their presence may originate from the natural microbiota of the grains or from post-harvest contamination during processing or storage.

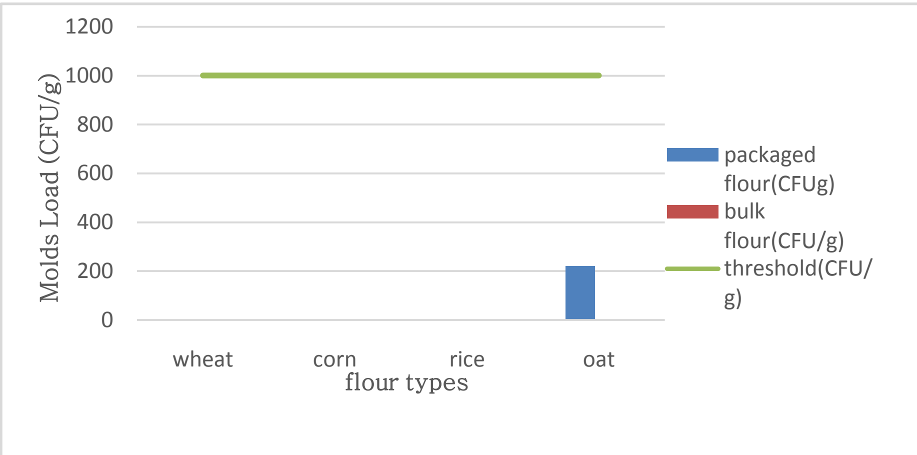
- **Molds Identified :**

- **M1**: *Alternaria alternata*
- **M2**: *Aspergillus fumigatus*
- **M3**: *Penicillium* sp.
- **M4**: *Cladosporium* sp.
- **M5**: *Rhizopus* sp.
- **M6**: *Aspergillus* sp. (unspecified species)
- **M7**: *Mucor* sp.

These molds are frequently encountered on stored plant-based materials. Under favorable environmental conditions (notably high humidity and temperature), some of them are capable of producing mycotoxins, which may pose a risk to food safety.

1.7.2 Yeasts and molds counts

The yeast and mold loads evaluated in four types of flour (wheat, corn, rice, and oat), both in bulk and packaged forms, showed variable values depending on the type of flour. In packaged flour samples, yeasts and molds were detected in all types (wheat, corn, rice, and oat), but their numbers remained well below the count threshold (<10 colonies/plate), preventing reliable quantification of the microbial load. An exception was oat flour, which showed a high mold load with a value of 2.2×10^2 CFU/g. Nonetheless, all four types of packaged flour comply with international standards, which require microbial loads to be below 10^3 CFU/g.



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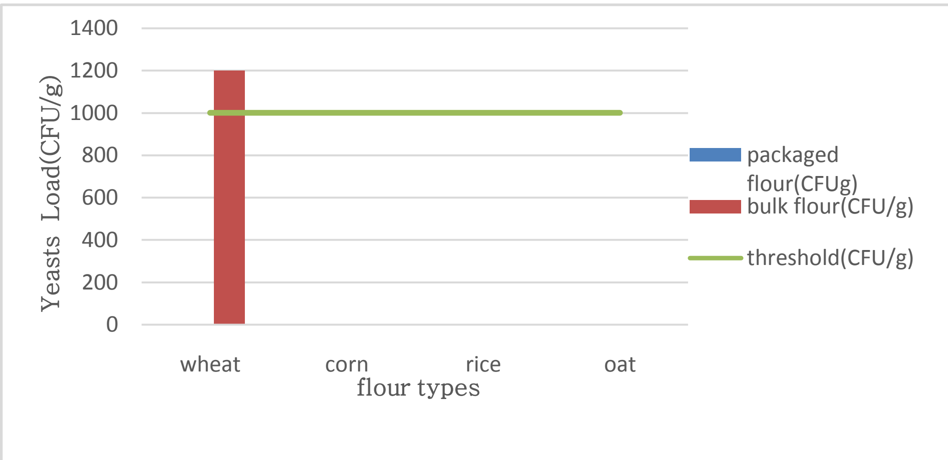

Figure 30: Microbial counts on Sabouraud chloramphenicol agar (a: molds; b: yeast)

In contrast, wheat flour exhibited a high yeast load with a value of 1.2×10^3 CFU/g, exceeding the international standard and therefore considered non-compliant. For bulk flour, the presence of yeast and mold colonies was also observed; however, their counts remained well below the threshold (10 colonies/box), indicating compliance with international standards. Only one value exceeded the recommended limits, highlighting the importance of regular monitoring

1.8. Other contaminants detected in flour samples

Following incubation of the various bulk and packaged flour samples (corn, rice, oat, and wheat) on different culture media (GN, Chapman, MacConkey, SS, and Hektoen) microscopic examination (tab.04; tab.05 and fig.31) was performed to characterize the bacterial isolates. Gram staining allowed for the differentiation between **Gram-positive** and **Gram-negative** bacteria, as well as the observation of **cell morphology** (cocci, bacilli) and cellular arrangements (clusters, chains, pairs).

1.8.1. Packaged flour

Table 04: Morphological features and Gram reaction of bacteria isolated from packaged flours

Flour type	Media	Microscopic profile	Strain code
Wheat flour	Chapman	Round (cocci), Gram positive, resembling bunches of grapes.	**S1**
	SS	Rod shaped, gram negative, found in clusters	**S2**
	H	Bacilli morphology, gram negative	**S3**
Corn flour	Chapman	Round, Gram positive, resembling bunches of grapes	**S4**
Rice flour	Chapman	Cocci shaped, resembling grape like bunches, Gram positive.	**S5**
	Nutritive agar	Bacilli morphology, Gram negative	**S6**
Oat flour	Chapman	Coccishaped, resembling grape like bunches, Gram positive.	**S7**

1.8.2. Bulk flour

Table 05: Morphological features and Gram reaction of bacteria Isolated from bulk flours

Flour type	Media	Microscopic profile	Strain code
Wheat flour	**Chapman**	Gram-positive cocci, colonies appear smooth, round, bulging and yellow pigmented, arranged in clusters (like "grape clusters")	**S9**
Corn flour	**Chapman**	Gram-positive cocci, grouped in clusters shaped like grapes	**S10**
	H	Gram-negative bacilli, the colonies are round and iridescent, isolated or in pairs	**S11**
Rice flour	**Chapman**	Gram-positive cocci, colonies appear smooth, round, bulging and yellow pigmented, arranged in clusters (like "grape clusters")	**S12**
	H	Gram-negative bacilli, the colonies are round and iridescent, isolated or in pairs	**S13**

Flour type	Media	Microscopic profile	Strain code
Oat flour	**Chapman**	Gram-positive cocci, arranged in clusters (like "grape clusters")	**S14**
	Nutritive agar	Gram-negative bacilli, the colony shape is circular, white, raised and entire, isolated or in pairs	**S15**
	Mac conckey	Gram-negative, rod-shaped bacilli, isolated or in pairs	**S16**
	SS	Gram-negative bacilli, colonies are large, white and opaque, isolated or in pairs	**S17**

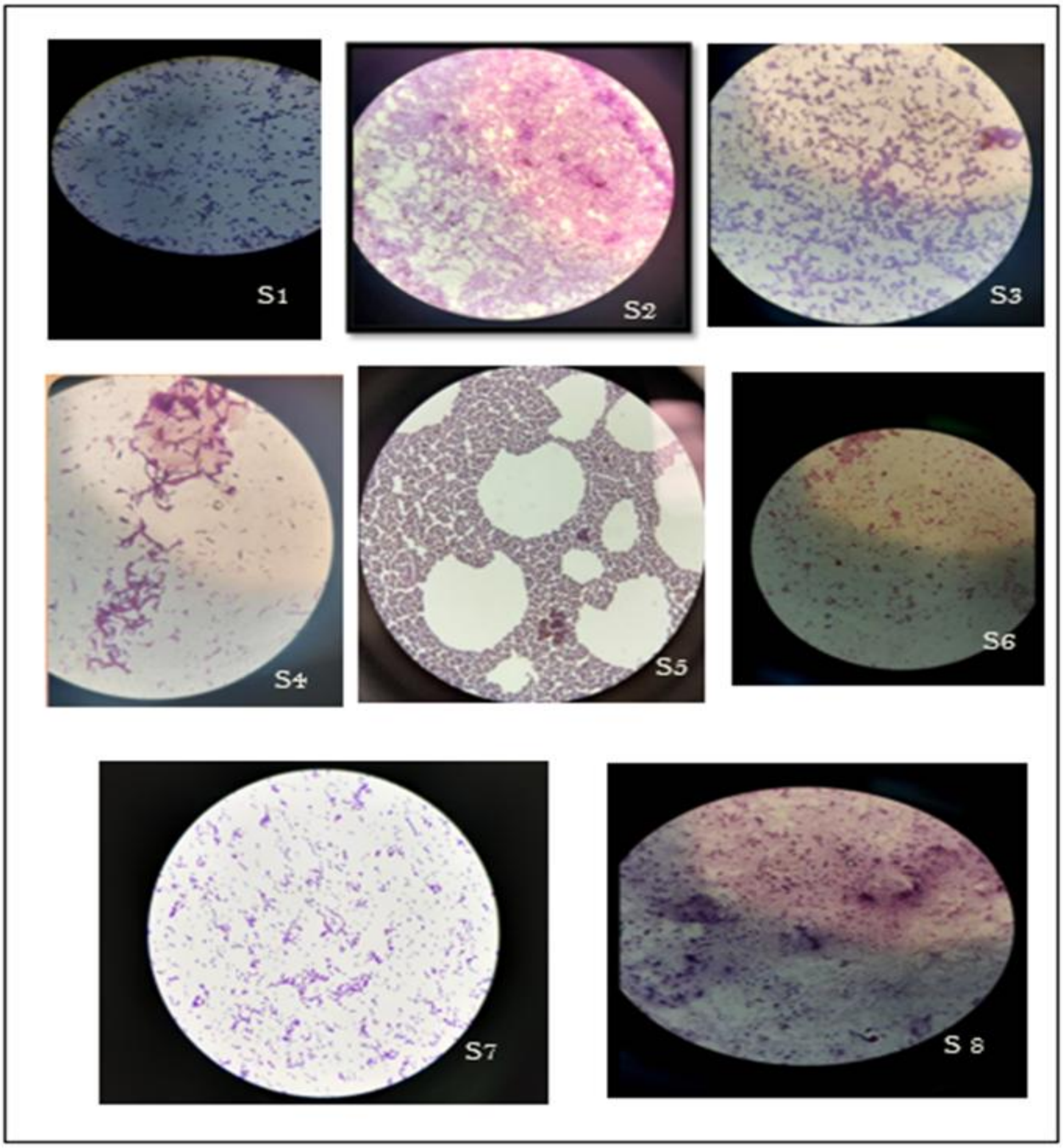

Figure 31: Microscopic view of bacterial isolates from packaged flour after Gram staining (×100 objective lens).

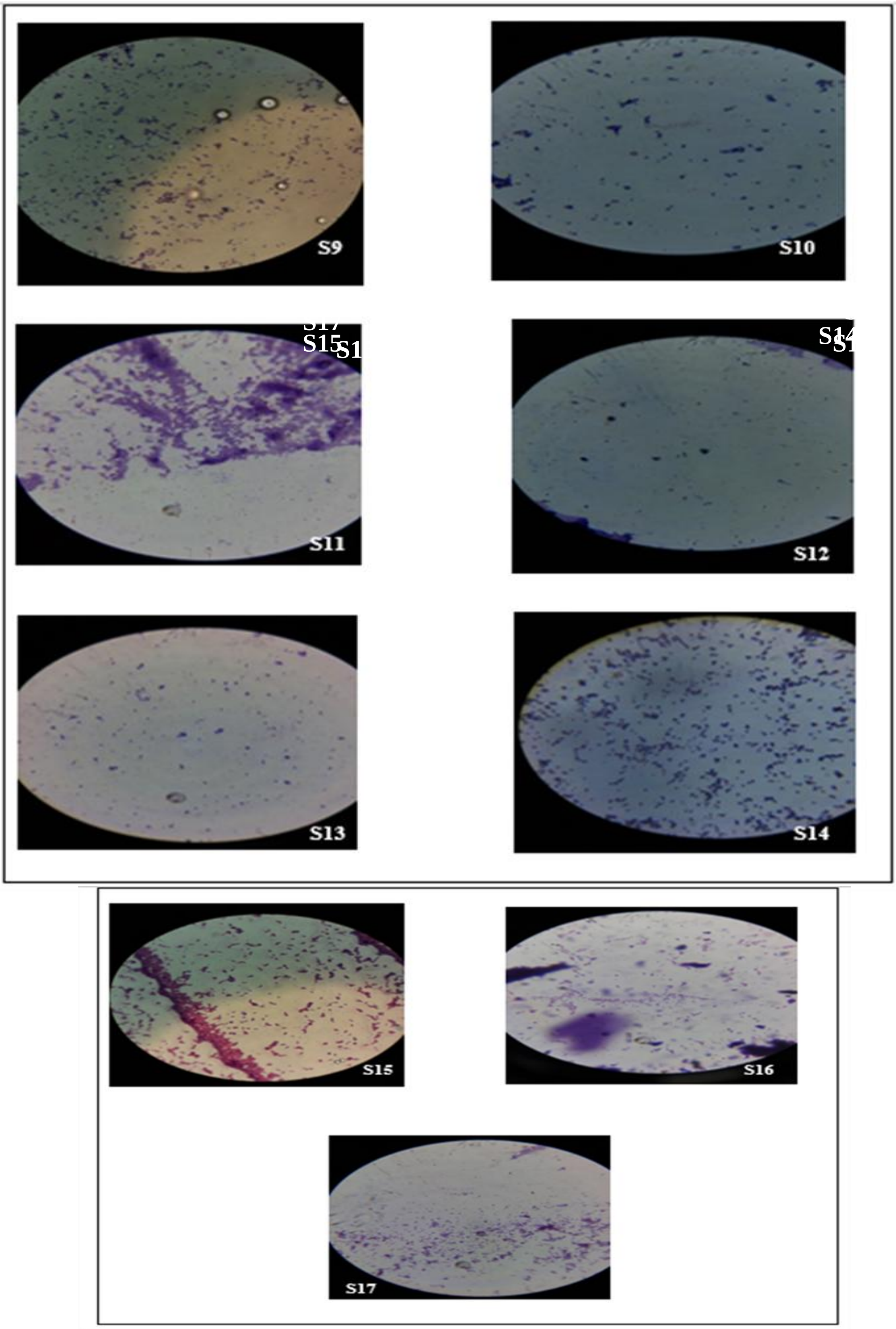

Figure 32: Microscopic view of bacterial isolates from bulk flour after Gram staining (×100 objective lens).

Macroscopic examination included the observation of colony morphology, such as color, texture, shape, edge, elevation, and opacity allowing an initial differentiation of the bacterial populations present in each sample, as illustrated in the figure below.

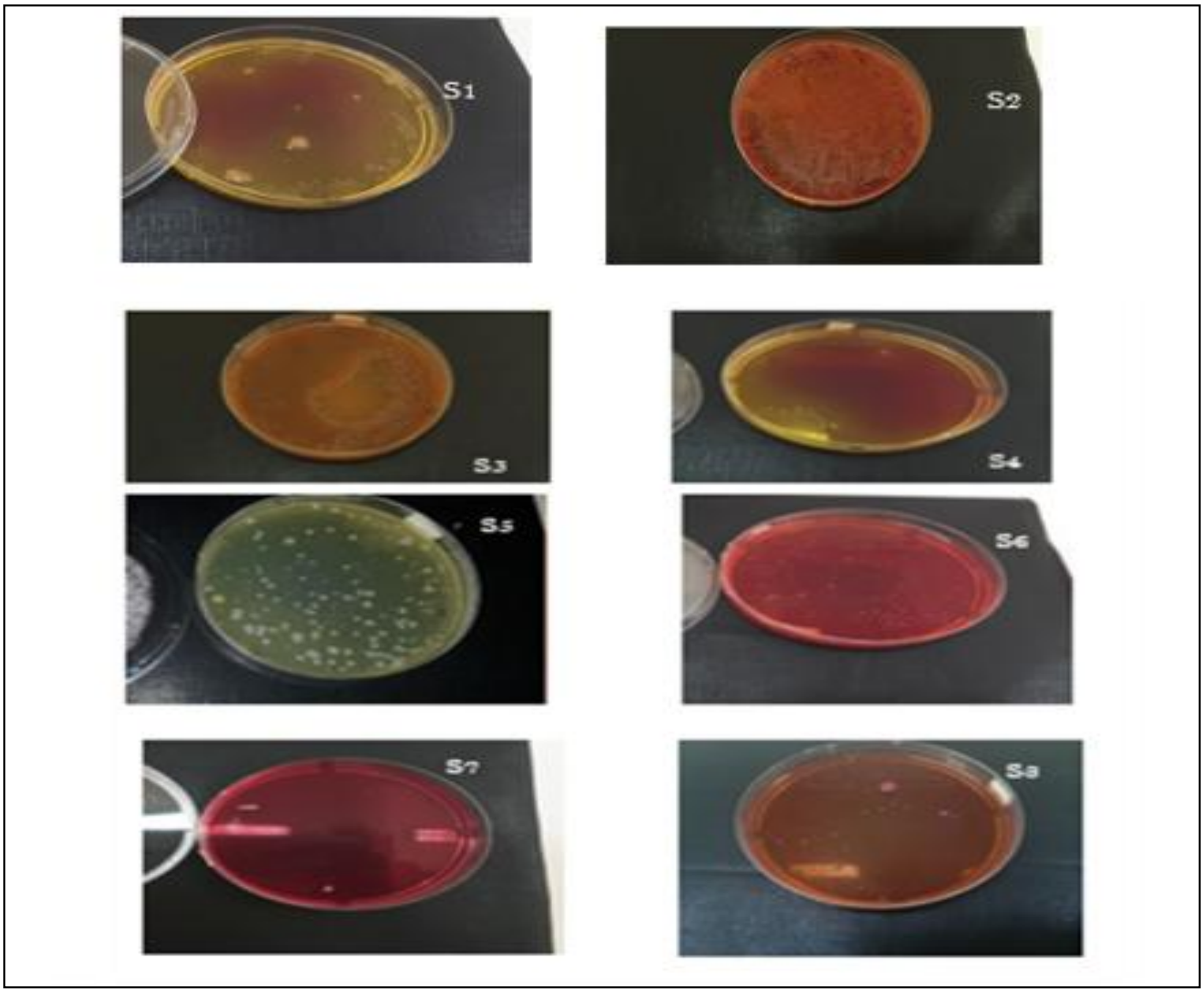

Figure 33: Macroscopic profile of bacterial colonies isolated from packaged flour samples.

S3 S4

S6

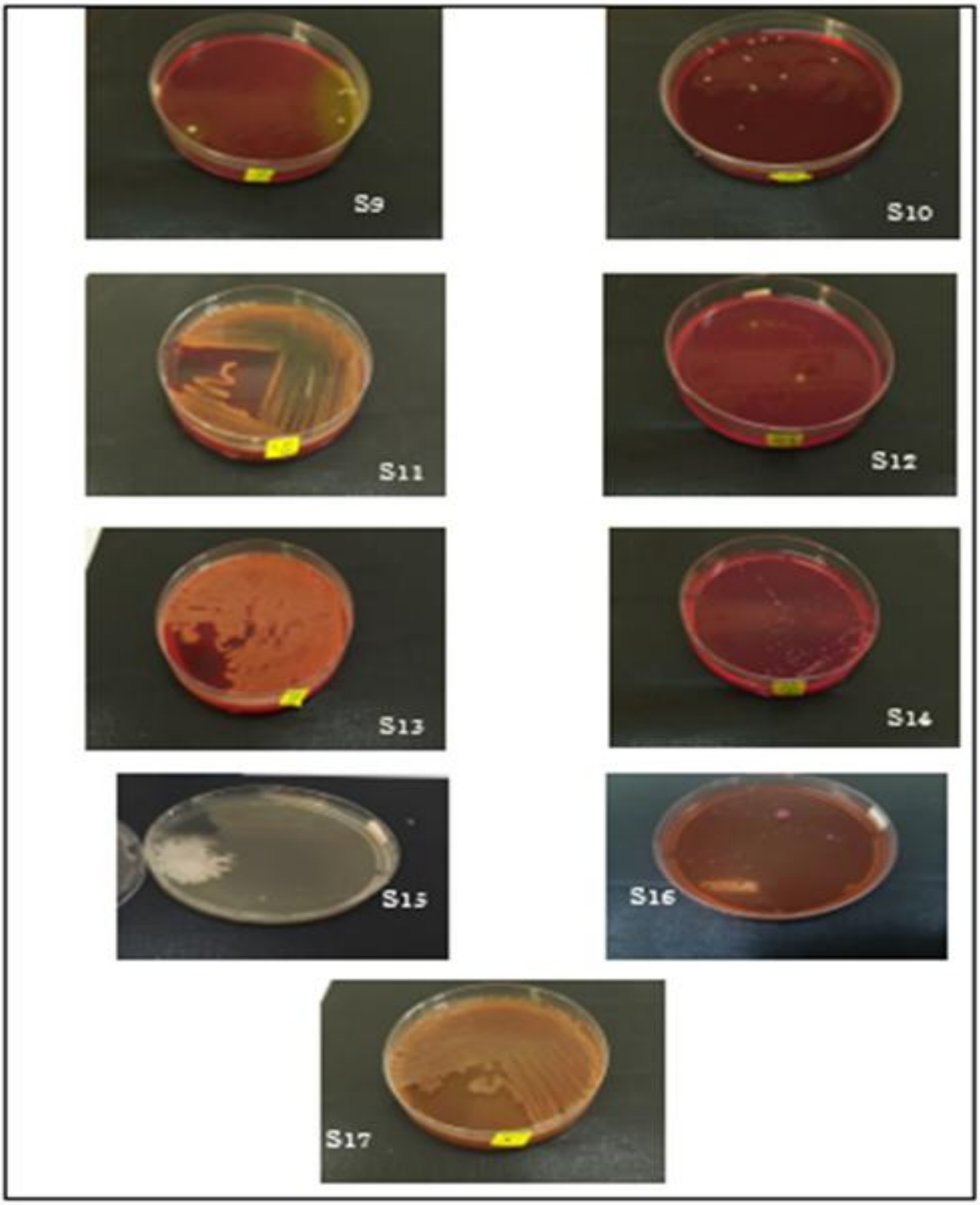

Figure 34: Macroscopic profile of bacterial colonies isolated from bulk flour samples

1.9. Metabolic profiling of isolated strains using API Systems

The metabolic characteristics of selected bacterial isolates were assessed using API 20 E and API Staph identification systems. The results revealed distinct biochemical profiles among the Gram-negative and Gram-positive strains. Isolates identified as **Gram-negative rods** exhibited variable enzymatic activities, including **glucose fermentation, citrate utilization**, and **indole production**, consistent with members of the Enterobacteriaceae family.

Gram-positive cocci showed diverse metabolic patterns, with several isolates demonstrating **positive mannitol fermentation, urease activity**, and other traits typically associated with **Staphylococcus spp.** The combination of these biochemical markers supported the presumptive identification of the isolates and highlighted the metabolic diversity present across the different flour samples.

The findings are summarized in tables 06 and 07, which present the key enzymatic reactions for each isolate. Representative API profile strips illustrating the metabolic reactions are shown in Figure 35, further supporting the biochemical diversity of the bacterial populations associated with the different types of flours.

1.9.1. API 20 E

Table 06: Biochemical profile of bacterial isolates from flour samples based on API 20 E System

Flour type	Flour category	Medium	S/c	ONPG	ADH	LDC	ODC	CIT	H_2S	URE	TDA	IND	VP	strain
Packaged	wheat	SS	S2	+	+	-	-	+	-	-	-	+	-	***Pantoea spp.***
		HK	S3	+	+	-	-	+	-	-	-	+	-	***Citrobacter freundii***
	rice	GN	S7	-	+	-	-	+	-	-	-	-	-	***Enterobacter cloacae***
	oat	Mac Conkey	S8	+	+	+	+	+	-	-	+	-	-	***Pantoea spp.***
Bulk	corn	HK	S11	+	+	-	-	+	-	-	-	+	-	***Enterobacter cloacae***
	rice	HK	S13	+	+	-	-	+	-	-	+	-	-	***Enterobacter cloacae***
	oat	SS	S17	+	+	-	+	+	-	+	+	-		***Citrobacter braakii***
		GN	S15	+	+	+	+	-	-	-	+	-	+	***Serratia liquefaciens***
		Mac Conkey	S16	+	+	+	+	+	-	-	+	+	-	***Citrobacter freundii***

Flour type	Flour category	Medium	Strain code	GEL	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA	strain
Packaged	wheat	SS	S2	-	+	+	-	+	+	+	+	+	+	***Pantoea spp.***
		HK	S3	-	+	+	-	+	+	+	+	+	+	***Citrobacter freundii***
	rice	GN	S7	-	-	+	-	+	+	+	+	+	+	***Enterobacter cloacae***
	oat	Mac Conkey	S8	-	-	-	-	+	+	+	+	+	+	***Pantoeaspp.***
Bulk	corn	HK	S11	-	+	+	-	+	+	+	+	+	+	***Enterobacter cloacae***
	rice	HK	S13	-	+	+	+	+	+	+	+	+	+	***Enterobacter cloacae***
	oat	SS	S17	+	+	+	-	+	+	+	+	+	+	***Citrobacter braakii***
		GN	S15	+	+	+	-	+	+	+	-	-	+	***Serratia liquefaciens***
		Mac Conkey	S16	-	+	+	-	+	+	+	+	+	+	***Citrobacter freundii***

Table 06 (Following): Biochemical profile of bacterial isolates from flour samples based on API 20 E System

Figure 35: Metabolic activity patterns revealed by API 20 E system for selected bacterial strains

1.9.2. API Staph

Table 07: Biochemical profile of bacterial isolates from flour samples based on API Staph System

Flour type	Flour category	Strain code	0	GLU	FRU	MNE	MAL	LAC	TRE	MAN	XLT	MEL	strain
packaged	wheat	S1	-	+	+	+	+	+	+	+	+	+	*S.aureus*
	corn	S4	-	+	+	+	+	+	+	+	+	-	*S. aureus*
	rice	S5	-	+	+	+	+	+	+	+	-	+	*S. xylosus*
	oat	S7	-	+	+	+	+	+	+	+	-	-	*S.xylosus*
bulk	wheat	S9	-	+	+	+	+	+	+	+	+	+	*S. aureus*
	corn	S10	-	+	+	+	+	+	+	+	+	+	*S.xylosus*
	rice	S12	-	+	+	+	+	+	+	+	+	+	*S.aureus*
	oat	S14	-	+	+	+	+	+	+	+	+	+	*S.lentus*

Table 07 (following): Biochemical profile of bacterial isolates from flour samples based on API Staph System

Flour type	Flour category	Strain code	NIT	PAL	VP	RAF	XYL	SAC	MDG	NAG	ADH	URE	strain
packaged	wheat	S1	+	-	-	-	+	+	-	+	-	+	*S.aureus*
	corn	S4	+	-	-	+	+	+	+	+	+	-	*S.aureus*
	rice	S5	+	-	-	-	+	+	-	+	+	-	*S.xylosus*
	oat	S7	+	-	-	+	+	+	-	+	-	+	*S.xylosus*
bulk	wheat	S9	-	-	-	-	-	+	-	+	+	+	*S.aureus*
	corn	S10	+	-	-	+	-	+	-	-	+	+	*S.xylosus*
	rice	S12	+	-	--	-	-	-	+	-	+	+	*S.aureus*
	oat	S14	-	-	-	+	+	+	-	+	-	+	*S.xylosus*

Figure 36: Metabolic activity patterns revealed by API Staphsystem for selected bacterial strains

Discussion

Packaged and bulk wheat, corn, rice, and oat flours microbiological analyses offer vital information about their hygienic quality and safety for human consumption. The findings show different levels of microbial contamination that are impacted by the composition of the raw materials, processing processes, packing methods, storage conditions, and hygiene habits. All of these elements work together to assess if the flour satisfies international (Codex Alimentarius) and Algerian (NM 1593-2007)standards. We may gain a better understanding of the origins of microbial flora, the influence of flour composition, and the efficacy of present techniques in reducing contamination risks by contrasting these results with those of flour microbiology research. This will identify areas in the production chain that require improvement.

The main source of microbial flora in flours is the raw material, which is made up of cereal grains. During production and harvest, grains being plant-based products are inherently vulnerable to environmental pollutants such soil, irrigation water, dust, and wildlife. If not well controlled, the chemical makeup of cereals which are high in starch, dietary fiber, and residual sugars creates the perfect habitat for microbial growth. According to Bhatt et al. (2010), bulk oat flour, for example, showed notable total and fecal coliforms(1.7×10^3 CFU/g), indicating fecal contamination that was probably introduced during harvest or stored under less-than-ideal conditions, like excessive humidity or rat exposure. The high mold load in packed oat flour (2.2×10^2 CFU/g) indicates that oat flour is more prone to microbial growth, particularly molds, due to its higher lipid and soluble fiber content than wheat or rice. This supports the findings of Sperandio et al. (2017). He stated that the nutritional profile of cereals with high fat content makes than vulnerable to fungal infestation. It is encouraging that *E.coli*, *Salmonella*, *Shigella*, and *Bacillus cereus* were not found in any of the samples; this suggests that the main pathogens were successfully controlled during the early stages of agricultural operations.

Although the data point to shortcoming in present procedures, processing strategies are essential for limiting microbial contamination. If grains are not fully cleaned or equipment is not properly disinfected, contamination may be introduced during the grinding, crushing, and sifting steps of the milling process. This was especially noticeable in packaged corn flour, which had an unsatisfactory *Staphylococcus aureus* load (2.18×10^4 CFU/g), suggesting that manufacturing may have contaminated people. As noted by Leroy and Simon (2021), who discovered that 10-30% of wheat samples were contaminated with pathogens as a result of inadequate processing hygiene, *Staphylococcus aureus*, a human-associated pathogen, signals hygiene lapses, such as inadequate handwashing or insufficient equipment sterilization. Bulk wheat and corn flours, on the other hand, showed less mesophilic development, presumably as a result of less post-milling processing, which reduces the possibility of secondary contamination. While still meeting regulations, the modest levels of 1.47×10^3 and 1.26×10^3CFU/g, respectively, in bulk rice and oat flours indicated market exposure to the environment. These results support those of Kumar et al. (2019), who pointed out that microbial loads in rice flour are influenced by poor processing and storage conditions.

Although packaging is intended to shield flours from secondary contamination, the findings show that this does not always guarantee high sanitary quality. Non-compliant mesophilic aerobic germ counts were found in packaged flours, which should have lower microbial burdens because of controlled settings. This could indicate contamination during the packing process or breaches in packaging integrity. Further evidence that packaging procedures may introduce contaminants if hygiene guidelines are not followed comes from the high *Staphylococcus aureus* loads in packaged wheat (3.8×10^3CFU/g) and corn flours. In contrast, Nguyen (2019) contended that microbial proliferation in dry products is considerably decreased by sealed packing. The higher yeast load in bulk flour (1.2×10^3CFU/g) was probably caused by the fact that bulk flours, which are kept in open containers at markets, are more susceptible to environmental elements like dust and humidity. Similar results were reported by N'Goran et al. (2018), who noted that inadequate ventilation and moisture exposure results in high mold counts in corn flours sold in open marketplaces. According to the FAO (2017), microbial loads might be considerably reduced by using airtight, food grade packaging and preserving cold chain conditions, particularly for oat flour.

Both intrinsic and external sources contribute to the microbial flora found in flours. The nutritional makeup of flours naturally affects how easily they can become contaminated. Microbial growth is exacerbated by extrinsic environmental conditions such excessive humidity during storage and transit. According to Sanogo et al. (2020)in their study on infant flours, the presence of thermotolerant coliforms in bulk oat flour indicates environmental contamination, potentially from water or equipment. The lack of *Shigella*, *salmonella*, and *Bacillus cereus* indicates the efficacy of fundamental farming methods, such as preventing fecal contamination during harvest.

Regarding yeasts and molds, fungal contamination remained generally low (<10 colonies/plate) in packaged flours, except for oat flour which showed a significant mold load ($2.2.10^2$ CFU/g), although still compliant with standards (<10^3 CFU/g). Conversely, in bulk flours, it was wheat flour which showed high yeast contamination ($1.2.10^3$ CFU/g), exceeding regulatory thresholds. These results are consistent with observations made in several African and Mediterranean studies. For example, Kone et al. (2020), in a study conducted in Bamako, noted yeast and mold non-compliance rates reaching 22 to 30% depending on the distribution channel (bakeries, retailers, markets). They observed higher contamination in products sold in bulk, linked to prolonged exposure to air, heat and repeated handling, which corroborates the high levels found in our sample of bulk wheat flour. Similarly, Zinedine et al. (2011) reported that flours from traditional mills in Morocco had significant contamination by *Aspergillus*, *Penicillium* and *Cladosporium*, with a prevalence of 81% for the genus *Aspergillus*. Our results confirm these observations, since *Aspergillus fumigatus* and *Penicillium sp.* were also identified in both packaged and bulk flours. The presence of these genera is of concern because several species, including *Aspergillus flavus* and *Penicilliumverrucosum*, are capable of producing heat-stable mycotoxins, such as aflatoxins or ochratoxins, which are dangerous to human health even at low doses. These observations are consistent with those of Kabak et al. (2006), who point out that molds in cereal products can produce toxins even at low humidity, under prolonged storage conditions. Finally, our identification of yeasts such as *Candida*, *Saccharomyces cerevisiae* and *Pichia* is comparable to the work of Abdel-Rahim et al. (2020), who identified the same genera in artisanal wheat flours in Sudan, suggesting post-harvest or storage contamination.

However, the identification of additional bacteria with API 20E, including *Enterobacter cloacae*, *Citrobacter freundii*, *Citrobacter braakii*, and *Serratia liquefaciens*, indicates that some opportunistic or environmental pathogens may still be present in some samples. These organisms may pose health risks, particularly to those with weakened immune systems, and are known to thrive in dry food matrices. Whether the microbial flora is inherent to the grain, acquired during milling, or introduced during packaging and distribution, their existence highlights the significance of monitoring in source and the identification of additional bacteria with API Staph including *S. aureus*, *S. lentus*, and *S. xylosus*, suggesting a range of contamination sources, most likely from human contact.

Cleanliness hones are vital over the flour generation chain, however the comes about highlight noteworthy holes. The tall *Staphylococcus aureus* loads in bundled corn and wheat flours, and bulk rice flour (1,2×10^4CFU/g), show human related defilement, likely from dishonorable taking care of by specialists or sellers. Giraud (2017) emphasized the significance of individual defensive hardware and normal gear cleaning to anticipate such defilement. The execution of Risk investigation and Basic Control Focuses (HACCP) frameworks seem address these issues by distinguishing basic control focuses, such as processing and building, where defilement dangers are most noteworthy. According to IFAD (2019), it is crucial to train operators on good manufacturing procedures (GMP), especially for small scale companies. According to Hussain and Dawson (2013), routine compliance inspections might guarantee adherence to microbiological requirements and avoid product recalls, which can harm a brand's reputation and result in financial losses.

The findings are given context by contrasting these results with those of other investigations. Similar to the higher yeast and mold loads in packaged wheat and oat flours here, JuhaneEnnadir et al. (2012) reported significant microbiological loads, especially molds. In Moroccan wheat flours as a result of poor storage. Nevertheless, their investigation found *Salmonella*, which was not found in, indicating improved management of the main infections in this area. In line with the considerable *Staphylococcus aureus* contamination in fermented cereal products as a result of inadequate handing. In contrast, the Journal of Food Protection (2022) found the *Bacillus cereus* was present in grain products because of its spore forming resilience, indicating that flour samples collected had undergone efficient heat treatments. These parallels draw attention to the general problem of limiting microbial contamination in flours, which is impacted by local customs an environmental factor.

The ensure flour safety, adherence to microbiological criteria is essential yet the results indicate noncompliance in multiple cases. The tight limits for mesophilic aerobic germs (<10^5-10^6CFU/g) and *Staphylococcus aureus* (<10^4CFU/g) specified by Algerian and codex Alimentarius standards were exceeded in bulk rice flour and packaged flours, Standards that demand total and fecal coliforms be absent from 1-10 g of samples are breached when these bacteria are found in bulk oat flour. These results are consistent with EFSA (2020), which emphasized the necessity of ongoing surveillance to mitigate microbiological hazards. As advised by FAO/WHO (2003), growers should improve processing methods, such as strict sorting and drying, and conduct regular testing to attain compliance. According to IFAD (2019), assistance for small scale manufactures could provide access to contemporary machinery and training, guaranteeing safer goods and stable economies.

The mix of raw materials, processing, packaging, storage, and hygiene procedures all influence the microbiological qualityof flour samples obtained from markets in Guelma. Significant concerns are highlighted by the substantial *Staphylococcus aureus* and others like *S. lentus*, *S. xylosus* loads, as well as total and fecal coliforms, in bulk oats flour, despite the encouraging absence of major pathogens (*Salmonella*, *Shigella*, *Bacillus cereus*). Stricter hygienic guidelines, better post-harvest procedures, and cutting-edge packaging are necessary to improve flour safety, adhere to legal requirements, and safeguard the public's health. These results highlight the need for all encompassing approach to reduce microbial in cereal products, adding to the larger conversation on food safety.

Conclusion

Conclusion

This study conducted a comparative microbiological assessment of packaged and bulk wheat, corn, rice and oat flours obtained from GuelmaProvince. The objective was to evaluate the hygienic and sanitary quality of these commonly consumed food products and ascertain their adherence to Algerian (NM 1593-2007) and international (Codex Alimentarius) requirements.

The research has demonstrated that cereal flours are not microbiologically inert, as evidenced by the initial hypothesis, laboratory results and discussion. The results demonstrate that microbial loads vary greatly among flour kinds and sources. Due to physical handling, dust, humidity and direct exposure to the air in local markets, bulk flours-especially oat and rice- often showed greater levels of contamination. Unexpectedly, certain packaged flours also have microbiological counts-such as *Staphylococcus aureus*, molds and yeasts- above permissible levels. These findings highlight shortcomings in equipment sanitation, package hygiene and post-harvest practices.

Important foodborne pathogens like *Shigella*, *Salmonella*, *Escherichia coli* and *Bacillus cereus* are not present in any of the samples, which is encouraging. It suggests that these high-risk pathogens were successfully kept out of the area by primary production and simple hygiene measures. However, the existence of coagulase-negative staphylococci (*S. lentus*, *S. xylosus*) and opportunistic bacteria (*Enterobacter cloacae*, *Citrobacter spp*.,*Serratia liquefaciens*), particularly in bulk and packaged oat and corn flours, indicates the continued risk of contamination for both humans and the environment.

In conclusion, microbiological analyses of flours samples show that microbiological purity varies and frequently falls short of food safety regulations. The ultimate microbial status of the product is primarily determined by the handling and ambient circumstances, even while the type of the cereal grain itself influences the microbial profile- oat flour, for example, is more susceptible to fungal development because of its high fat and beta-glucan content.

A thorough strategy is needed to improve flour safety:

_ At the agricultural level, through improving the drying, sorting and cleaning of grain;

_ At the level of processing, by guaranteeing the cleanliness of personnel, facilities and equipment;

_ and at the packaging level, food-grade, hermetic materials are used in sanitary conditions, by preventing bulk flours from being exposed to environmental pollutants at the market level.

To ensure flour safety and consumer protection, it is also essential to implement HACCP procedures, train operators on good practices (GMP/GHP), and conduct regular microbiological monitoring.

This study further advances our knowledge of the microbial ecology in wheat matrices and emphasizes the difficulties in ensuring microbiological safety for flour producers, especially in traditional and small-scale market settings. Through establishing similarities with other domestic and foreign research, it creates opportunities for more comprehensive regional evaluations and focused solutions.

This work ultimately provides a scientific basis for future regulations, technological advancements and public health initiatives aimed at reducing microbial hazards in cereal-based products- a crucial step in bolstering food security and guaranteeing the population receives healthy nourishment.

Practical recommendations

Implementing a variety of coordinated interventions is crucial to addressing the microbiological problems our study found and promoting the production of safer flour in GuelmaProvince. Improvements in post-harvest handling, such as promptly drying and cleaning grains to prevent microbial growth before storage and milling, should be the first focus of efforts. To prevent cross-contamination, especially with human-associated bacteria like *Staphylococcus aureus*, it is equally important to strengthen hygiene protocols at the processing level. This includes regular disinfection of milling equipment, proper sanitation of work surfaces and strict adherence to personal hygiene among workers. Modernizing packaging techniques will greatly lower environmental exposure and the danger of contamination by using food-grade, hermetically sealed materials and maintaining clean conditions throughout the packaging process. Better procedures, like utilizing closed containers, controlling storage humidity and minimizing direct human contact, should be promoted for vendors and local authorities to implement for flours sold in bulk, which are more vulnerable to contamination from open-air storage in markets. In addition, regular microbiological testing of flour batches at various supply chain points-production, distribution and sale-should be made mandatory by law in accordance with Algerian and Codex Alimentarius requirements. Supporting small-scale flour manufacturers and vendors might significantly increase the microbiological safety of their products. This support could include financial or technical aid to purchase improved equipment, as well as access to training in appropriate manufacturing and hygiene procedures.

Future research perspectives

The identification of microbial species using molecular approaches could be the focus of future research in order to better understand the existence of pathogenic or toxic strains that may not be discovered by traditional culture methods. To quantify mycotoxins, more research is advised, especially in oat and maize flours, which have been shown to have high mold loads. For a more comprehensive picture of flour safety across Algeria, longitudinal studies tracking microbial evolution during storage, trials assessing the efficacy of hygienic measures and more extensive comparison surveys across various locations would be helpful.

Finally, since consumer behavior might affect microbiological risks after a purchase, it would be helpful to research how consumers handle and store flour at home. All together, these pragmatic and scientific approaches serve as the cornerstone for a sustained enhancement of flour quality, safeguarding public health and modernizing the supply chain for cereal products.

REFRENCES

Abdel-Rahim, A. M., & El-Haroun, A. M. (2020). Microbiological and mycotoxin contamination of flour in Sudan. African Journal of Food Sciences, 14(6), 147-154

Adams, M. R., & Moss, M.O. (2008). Food Microbiology (3rd ed.). Royal Society of Chemistry.

Aryal, S., 2022.*API (Analytical Profile Index) 20E Test – Procedure, Uses and Interpretation.* Microbiology Info. https://microbiologyinfo.com/api-20e-test

Beuchat, L. R., Komitopoulou, E., Beckers, H., Betts, R. P., Bourdichon, F., Fanning, S., Joosten, H., & terKuile, B. H. (2013). Low-water activity foods: Increased concern as vehicles of foodborne pathogens. Journal of Food Protection, 76(1), 150-172. https://doi.org/10.4315/0362-028X.JFP-12-211

Benoit, H. (2023). Risks Associated with the Use of Raw Flour. Journal of Food Safety**.**

Clinical Microbiology Reviews (2019) - Bacillus cereus, a Volatile Human Pathogen.

Codex Alimentarius. (2022). International Food Standards. Retrieved from https://www.fao.org/fao-who-codexalimentarius

Delarras, Mr Kourichi Brahim 2003 : Mémoire en vue de l'obtention du diplôme de Master académique Caractérisation physico-chimique et microbiologique de l'eau de source de Ain Essebaine, willaya de Tiaret.

De Vuyst, L., et al., (2014). Microbialecology of sourdough fermentations: Diverse or uniform? Food Microbiol.

Doyle, M, P., & Buchanan, R. L (2013). Food Microbiology : Fundamentals and Frontiers (4th ed.). ASM Press.

Dupont, J. (2020). Microbiological Risks in Cereal Products. Agro-Food Publishing**.**

EFSA (2020). Microbiological risks in food: A review of emerging pathogens. European Food Safety Authority Journal**.**

European Regulation No 2073/2005 - Microbiological criteria for foodstuffs.

FAO. (2023). Food and Agriculture Organization of the United Nations. Retrieved from https://www.fao.org

FAOSTAT (2023). http://faostat.fao.org

FAO (2017). The future of food and agriculture: Trends and challenges. Food and Agriculture Organization of the United Nations.

FAO, 2007. Introduction à la gestion de conservation de l'eau, de la biomasse et de la fertilité des sols (GCES). FAO. Rome. (Consultéen 2020). http://www.fao.org/3t

FAO/WHO (2003). Codex Alimentarius: Food hygiene and safety standards. Food and Agriculture Organization of the United Nation.

FDA Bacteriological Analytical Manual, Chapitre 14 (2021) - Bacillus cereus.

FDA BAM (Ch.4, 2020) : Protocole pour l'isolement d'E. coli sur MacConkeydans les matrices alimentaires.

FDA BAM (2020). Enumeration of E. Coli and coliforms (cited indirectly in PMC 5923861).

Food Microbiology (2022) - Advances in Detection Methods for Bacillus cereus and Its Virulence Factors.

Forghani, F., & Oh, D. H. (2013). Hurdle strategies to control foodborne pathogens in dried foods. Journal of Food Science, 78(9), R1347-R1351. https://doi.org/10.1111/1750-3841.12246

Giraud, P. (2017). Hygiene Practices in the Kitchen. Hygiene Publishing.

Guinet., (2006). Technologie du pain français ; In , pain et nutrition P.P.I.S, (Ed) paris, P.75.

Grunert, K. G. (2005). Food quality and safety: consumer perception and demand. European Review of Agricultural Economics.

Hussain, M. A., Dawson, C. O (2013). Economic impact of food safety outbreaks on food businesses. Food Control.

ICMSF (International Commission on microbiological Specifications for Food). (2018). Microorganisms in Foods 8 : Use of Data for Assessing Process Control and Product Acceptance. Springer.

IFAD (2019). Supporting smallholder farmers in achieving food security. International Fund for Agricultural Development.

International Journal of Food Microbiology (2023). Optimization of Selective Media Components for Improved Recovery of Environmental Bacillus cereus Strain

International Organization for Standardization. (2013). *ISO 5526:2013 – Cereals, pulses and other food grains — Nomenclature*. https://www.iso.org/standard/50932.html

ISO 16649-2:2022 : Méthode de référence pour E. coli dans les aliments via MacConkey + confirmation β-glucuronidase.

ISO 21567 :2017. Microbiology of the food chain - Horizontal method for the detection of Shigella spp.

ISO 4833-1:2013. Microbiology of the food chain horizontal method for the enumeration of microorganisms, part 1: colony count at 30°C by the pour plate technique.

ISO 7932:2020 - Microbiology of the food chain - Horizontal method for the enumeration of presumptive Bacillus cereus - Colony-count technique at 30°C.

Jay, J.M., Loessner, M.J., & Golden, D.A. (2005). Modern Food Microbiology (7 th ed). Springer.

Journal of Food Protection (2022) - Comparative Evaluation of Selective Media for Detection and Enumeration of Bacillus cereus in Dairy Products.

JuhaneEnnadir et al., (2012). Qualité microbiologique des farines de blé consommées au Maroc. Canadian Journal of microbiology**.**

Kabak, B., Dpbsonm A. D. W., & Var, I, (2006). Strategies to prevent mycotoxin contamination of food and animal feed: A review. Critical Reviews in Food Science and Nutrition, 46(8), 593-619.

Kone, M. N., Sangaré, I., & Yao, K. M. (2020). Etude de la qualité microbiologique des farines panifiables dans la ville de Bamako. Revue Malienne de Science et de Technologie, 25, 87-96.

Kumar, P., & al. (2019). Microbiological quality of rice and its products. Journal of Food Science and Technology, 56(2), 538-546.

Kumar, R. (2022). Microbial Contamination in Agriculture. Agronomy Review.

Lelaalhe-Tidjani, (2022). Summer cereals in the Algerian Sahara. DSpace, Kasdi Merbah University Ouargla.

Leroy, M., & Simon, T. (2021). Study on the Microbiological Quality of Flours. International Journal of Microbiology, 45(2), 123-135.

Malete et al., (2013). Evaluation de la qualité hygiénique de tchaamessibu, une pate acide consommé a natitingou au nord bénin. Rev. Microbiol.Ind San et Environn. 7:228-244.

Martin, A. (2018). Pathogens in Grains: A Silent Threat. Public Health Review.

McClane, B. A., Uzal, F. A., Navarro, M. A., Miyakawa, M. F., & Robertson, S. L. (2006). Clostridium perfringens enterotoxins. Toxicon, 49(5), 655-682. https://doi.org/10.1016/j.toxicon.2006.01.015

Mount Sinai Hospital – Department of Microbiology, 2020. *API 20E Test Procedure.* Mount SinaiLaboratoryManua.

https://eportal.mountsinai.ca/Microbiology/manual/tech/tech04_02.pdf

NF EN ISO

4832. (2006). Microbiologie des aliment, méthode horizontal pour le dénombrement des coliforms, Méthode par comptage des colonies.

NF V08-050. (2009). Microbiologie des aliments. Dénombrement des coliforms présumés par comptage des colonies obtenues a 30°C.

NF VO8-060. (2009). Microbiologie des aliments. Dénombrement des coliformsthermotolérents par comptage des colonies obtenues a 44°C.

N'Goran-AW et al.,(2018). Qualité microbiologique des farines de Mais aux marches d'Abidjan, 6 (4): 476- 482.

Nguyen, L. (2019). Microbial Shelf Life of Dry Food Products. Nutrition Publishing

Pitt, J. I., & Hocking, A.D (2009). Fungi and Food Spoilage (3rd ed.). Springer.

Posner, E. S., & Hibbs, A. N. (2005).*Wheat Flour Milling* (2nd ed.). AACC International.

Prof. A. TantaouiElaraki (2006). Microbiologie des céréales et farines.

Ribera, M., Aragonés, L., & Rodríguez-Lázaro, D. (2021). Microbiological safety of flour: A potential source of foodborne pathogens. Applied Sciences, 11(8), 3646. https://doi.org/10.3390/app11083646

Rodier et al., (2005). Memoireprèsenté en vue de l'obtention du Diplome de Master Domaine: Sciences de la Nature et de la Vie Spécialité: Ecologie microbienne la recherche de Vibrio cholerae dans les eaux usées de Oued boumerzoug, Oued Rhumel et la STEP d'Ibn Ziad.

Sanogo Bougmaet al.,(2022). Evaluation de la qualité physico chimique et microbiologique de quelques farines infantiles locales vendues à Ouagadougou Burkina Faso. Pamji one health

https://www.one-health.panafrican-med-journal.com/content/article/9/25/full

Slama T, Minamba B, Birama S.C. & Adama C. (2015). Amélioration de la gestion de la fertilité des sols et celle des cultures dans les zones sahéliennes de l'Afrique de l'Ouest : une condition sine qua none pour l'augmentation de la productivité et de la durabilité des systèmes de culture à base de mil. Research Gate. P.26. https :www.researchgate.net/publication/237827087

Sperber, W. H., Doyle, M.P (2007). Compendium of the microbiological spoilage of foods and beverages. Springer.

Sperandio, D., & al. (2017). Microbial contamination of grains and its impact on food safety. Journal of Food Protection, 80(5), 761-771.

SURGET A. et BARRON C., (2005). Histologie du grain de blé. Industrie des céréales, n.145, pp.4-7

Swaminathan, B., &Gerner-Smidt, P. (2007). The epidemiology of human listeriosis. Microbes and Infection, 9(10), 1236-1243. https://doi.org/10.2903/j.micinf.2007.05.011

USDA. (2023). National Nutrient Database for Standard Reference. United States Department of Agriculture. Retrieved from https://fdc.nal.usda.gov

WHO (2018). Foodsafety. OrganisationMondale de la santé.

WHO (2015). Estimating the global burden of foodborne diseases. World Health Organization.

Wu, Y., et al. (2019). Phylogenetic relationships among grasses (Poaceae). Journal of Systematic and Evolution, 57(4), 341-354.

Zinedine, A., Soriano, J. M., Moltó, J. C., &Mañes, J. (2011). Presence of mycotoxins in Moroccancereals and beans. Food Control, 22(1), 1-5. https://doi.org/10.1016/j.foodcont.2010.06.002

Annexes

Appendix 1: CHARACTERISTICS AND PREPARATION OF CULTURE MEDIA

Table 1: Characteristics of culture media

Culture media	Type of sterilization	Quantity to collect	Selective medium for
VRBL	Not autoclavable	41.53g/L of distilled water	Total and fecal coliforms
PCA	Autoclavable at 121°C	17.5g/L of distilled water	Total aerobic flora
Chapman	Autoclavable at 121°C	111.02g/L of distilled water	*Staphylococcus aureus*
MacConkey	Autoclavable at 121°C	51.55g/L of distilled water	*Escherichia coli*
SS	Not autoclavable	52.0g/L of distilled water	*Salmonella/Shigella*
Hektoen	Not autoclavable	41.68g/L of distilled water	*Salmonella/Shigella*
GN	Autoclavable at 121°C	28.0g/L of distilled water	*Bacillus cereus*
Sabouraud Chloramphenicol	Autoclavable at 121°C	65g/L of distilled water	Yeasts and Molds
BPW	Autoclavable at 121°C	8.4g/L of distilled water	Preparation of stock solutions
SFB	Not autoclavable	23g/L of distilled water	Enrichment medium for *Salmonella and Shigella*

Appendix 2:

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9- Céréales et produits dérivés

Catégories des denrées alimentaires	Micro-organismes/ métabolites	Plan d'échantillonnage		Limites microbiologiques (ufc/g)	
		n	c	m	M
Farines et semoules	*Escherichia coli*	5	2	10	10^2
	Staphylocoques à coagulase +	5	2	10^2	10^3
	Bacillus cereus	5	2	10^3	10^4
	Moisissures	5	2	10^3	10^4
	Anaérobies sulfito-réducteurs	5	2	10^2	10^3
Céréales en grains destinées à la consommation en l'état et non à la transformation	Moisissures	5	2	10^3	10^4
	Anaérobies sulfito-réducteurs	5	2	10^2	10^3
Couscous et pâtes alimentaires	Moisissures	5	2	10^2	10^3
	Anaérobies sulfito-réducteurs	5	2	10^2	10^3
Pâtes précuites séchées (diouls, ktaef, rechta…)	Levures et moisissures	5	2	10^4	10^5
	Escherichia coli	5	2	10^2	10^3
	Staphylocoques à coagulase +	5	2	10^3	10^4
	Salmonella	5	0	Absence dans 25 g	
Pâtes fraîches (nature ou farcies)	*Escherichia coli*	5	2	10	10^2
	Staphylocoques à coagulase +	5	2	10^2	10^3
	Anaérobies sulfito-réducteurs	5	2	10^2	10^3
	Bacillus cereus	5	2	10^3	10^4
	Moisissures	5	2	10^4	10^5
	Salmonella	5	0	Absence dans 25 g	
Produits de biscuiterie	Germes aérobies à 30 °C	5	2	10^3	10^4
	Escherichia coli	5	2	3	30
	Moisissures	5	2	10^2	10^3
	Staphylocoques à coagulase +	5	2	10^2	10^3
	Salmonella (1)	5	0	Absence dans 25 g	

Abstract

This study evaluated the microbiological quality of eight flour samples (wheat, corn, rice, and oat), both packaged and sold in bulk, collected from markets in the Guelma region. The analysis focused on mesophilic aerobic bacteria, total and fecal coliforms, *E. coli*, *Staphylococcus aureus*, yeast and molds, Bacillus cereus, *Salmonella*, and *Shigella*. Results revealed the absence of major foodborne pathogens such as *E. coli*, *Salmonella*, *Shigella*, and *B. cereus*. However, several samples particularly some packaged and bulk flours exceeded acceptable microbiological limits for mesophilic flora and *S. aureus*. Oat flour showed the highest fungal contamination, likely due to its higher fat and fiber content. Variations in contamination levels were attributed to differences in raw material composition, hygienic practices during processing, packaging conditions, and environmental exposure. Although this study did not assess productions facilities or supply chains directly, the microbiological noncompliance observed in market ready products underscores the need for improved post-harvest handling, secure packaging, and regular microbiological monitoring. These findings support the recommendation for broader implementation of food safety systems such as Hazard Analysis and Critical Control points (HACCP) to enhance the microbial safety of cereal-based products in traditional and small-scale market settings.

Keywords: cereal flours; microbiological quality; packaged and bulk flours; pathogenic bacteria; Yeasts and molds.

Résumé

Cette étude a évalué la qualité microbiologique de huit échantillons de farine (blé, maïs, riz et avoine), conditionnés ou vendus en vrac, prélevés sur les marches de la region de Guelma. Les analyses ont porté sur les bactéries aérobies mésophiles, les coliforms totaux et fécaux, *E. coli*, *Staphylococcus aureus*, les levures, les moisissures, *Bacillus cereus*, *Salmonella* et *Shigella*. Les résultats ont montré l'absence de pathogènes majeur tells que *E. coli*, *Salmonella*, *Shigella* et *B. cereus*. Toutefois, plusieurs échantillons notamment certains conditionnés ou vendus en vrac ont dépassé les limites microbiologiques réglementaires pour la flore mésophile et *S. aureus*. La farine d'avoine a présenté la contamination fongique la plus élevée, probablement en raison de raison de sa teneur plus élevée en matières grasses et en fibres. Bien que cette étude ne porte pas directement sur les proceeds de production ou la chaîne d'approvisionnement, les non conformités microbiologiques observes dans des produits prêts à la consummation soulignent la nécessité d'améliorer la manutention post récolte, de garantir un emballage hermétique et d'effectuer un suivi microbiologiques régulier. Les résultats appuient la recommandation de renforcer la mise en oeuvre de systems de sécurité sanitaire des aliments tells que le système HACCP (Analyse des dangers et points critiques pour leur maîtrise), afin d'assurer la sécurité microbiologiques des produits céréaliers, notamment dans les circuits traditionnels et de petite echelle.

Mots clés: farines de céréales, qualité microbiologiques, farines conditionnés et vrac, bactéries pathogènes, levures et moisissures.

الملخص

هدفت هذه الدراسة إلى تقييم الجودة الميكروبيولوجية لثماني عينات من الدقيق (القمح، الذرة، الأرز، والشوفان)، سواء المعبأة أو المعروضة للبيع بالجملة، والمجمّعة من أسواق منطقة قالمة. شملت التحاليل الميكروبيولوجية البكتيريا الهوائية المتوسطة، القولونيات الكلية والبرازية، *الإشريكية القولونية* (*E. coli*)، *المكورات العنقودية الذهبية*(*Staphylococcus aureus*)، الخمائر، الفطريات، *العصوية الشمعية* (*Bacillus cereus*) (*Salmonella and shigella*). ظهرت النتائج غياب مسببات الأمراض الرئيسة مثل *E. coli, Salmonella, Shigella*، و*B. cereus*، إلا أن بعض العينات وخاصةً من الدقيق المعبأ والبيع بالجملةتجاوزت الحدود الميكروبيولوجية المسموح بها فيما يخص الفلورا المتوسطة و *S. aureus*. وقد سُجل أعلى مستوى من التلوث الفطري في دقيق الشوفان، ويُعزى ذلك إلى محتواه العالي من الدهون والألياف.

رغم أن هذه الدراسة لم تتناول ممارسات التصنيع أو سلسلة الإمداد بشكل مباشر، إلا أن عدم الامتثال الميكروبيولوجي في منتجات جاهزة للتسويق يبرز الحاجة إلى تحسينات في مناولة ما بعد الحصاد، وتغليف محكم، ورقابة ميكروبيولوجية دورية. وتدعم النتائج التوصية بتعزيز تطبيق أنظمة سلامة الأغذية، مثل نظام تحليل المخاطر ونقاط التحكم الحرجة (HACCP) ، بهدف تعزيز السلامة الميكروبيولوجية لمنتجات الحبوب، لا سيما في الأسواق التقليدية والصغيرة النطاق.

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الكلمات المفتاحية: دقيق الحبوب، الجودة الميكروبيولوجية، الدقيق المعبأ والسائب، البكتيريا المسببة للأمراض، الخميرة والعفن.