MINISTERE DE L'ENSEIGNEMENT SUPERIEUR ET DE LA RECHERCHE SCIENTIFIQUE

UNIVERSITE AKLI MOHAND OULHADJ – BOUIRA

FACULTE DES SCIENCES DE LA NATURE ET DE LA VIE ET DES SCIENCES DE LA TERRE

DEPARTEMENT DE BIOLOGIE

Réf : ……./UAMOB/F.SNV.ST/DEP.BIO/21

MEMOIRE DE FIN D'ETUDES

EN VUE DE L'OBTENTION DU DIPLOME MASTER

Domaine : SNV **Filière :** Sciences Biologiques
Spécialité : Microbiologie Appliquée

Présenté par :

LATRECHE Abir

Thème

Microbiological and physicochemical analysis of carbonated soft drink packaging type (Polyethylene terephthalate, Aluminum and Glass) case of Coca Cola.

Soutenu le : 14 / 07 / 2021 **Devant le jury composé de :**

Nom et Prénom	*Grade*		
Mr. KADRI N.	**MCA**	**Univ de Bouira**	**Président**
Mr. REMINI H.	**MCB**	**Univ de Bouira**	**Examinateur**
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Mme. MEDBOUA C.	**MCB**	**Univ de Bouira**	**Co-Promotrice**

Année universitaire: 2020/2021

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Analyses microbiologiques et physico chimiques d'une boisson gazeuse type d'emballage (Polyethylene terephthalate , Aluminium et Verre) cas de Coca Cola

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Dedication

Gratitude beam that smiles the clouds away and tints tomorrow with prophetic ray.

No gift on earth is greater, no treasure held above, the joy that comes from knowing a parent's endless support.

My brother! In spite of how it's tested it grows from year to year providing strength and comfort it always draws us near.

shedding light upon you like a bright and shining star and when all things are measured not one shall rise above, or be compared in value to a sister's endless love.

even though I might not say I appreciate all you do richly blessed is how I feel having sister and brothers in law like you,

To my nieces & nephews all hail the news, your favorite aunty has graduated and ready to Roll!

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List of abbreviations

Al: Aluminum

B°: Degrees Brix

CCP: Critical Control point

CFU: Colony forming unit

CO_2: Carbon Dioxide

CSD: Carbonated soft drink

DLA: Double Layer Agar

DWI: Drawn and Wall Ironed"

H_2O_2: Hydrogen peroxide

HACCP: Hazard Analysis and Critical Control Point

O_2: Oxygen

OJAR: Official Journal of the Algerian Republic

PCA: Plat Count Agar

P.E.T: PolyEthylene Terephthalate

pH: Potential of Hydrogen

RGB: Returnable Glass Bottle

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Introduction

The expression "Carbonated Soft Drink" refers to beverages that contain flavorings and/or fruit juices, as well as other technological or nutritional components along with carbonation, to improve the product's appeal and stability, and also to ensure that its organoleptic traits remain intact throughout a respectable storage period **(Taylor,2016).** The contemporary rigorous quality and legislative controls assess these aspects to upgrade the performance of all ingredients, in its formulation **(Bonnefoy et al., 2002)**.

Any foodstuff, must display an assurance towards any risk likely to affect the health and/or safety of the consumer, however, quality control is among the procedures that verifies their sustainability, the food industries are indeed mandated to call on various laboratories to perform testing for legislative purposes, that provide proof of conformity of their products **(Kotsanopoulos, & Arvanitoyannis,2017)**.

According to *Mr. Hamani,* chief executive office of the Algerian Drinking Industry, Algerian public consume an average of 37,5 liters per year of carbonated drinking in the recent years since 2012, 2,3 billion liters were sold across the nation **(Benali,2018).**

Due to its low pH, carbonated soft drink (CSD) is a hostile setting in which a vast portion of microorganisms perish **(NSA,2001)**. Spoilage yeasts, *Zygosaccharomyces bailii, Z. rouxii, Z. lentus and Z. bisporus* are most commonly reported yeasts in CSD, others may be detected such *Saccharomyces, Torulopsis, Brettanomyces (Dekkera), Candida, Kloeckera, Hansenula and Pichia* they can survive relatively high carbonation and can also grow when refrigerated and aerated **(Steels et al., 1999).** Molds as *Aspergillus, Penicillium, Mucor and Fusarium,* Enterobacteria such *Klebsiella, Citrobacter, Serratia* **(Lawlor et al. 2009)**. Two genera of aciduric bacteria Acetobacter *spp, Gluconobacter spp* **(Thompson, 2009).** Spore-forming bacteria of the genera *Bacillus* and *Clostridium* **(Back, 2005).**

The formation of clouds, particulates, taints, and excessive gas are all forms of spoilage effects **(Back et al., 1999)**. CSD infection usually originates from raw ingredients, bottles returned or environmental vectors, insects are considered as a vector for yeasts **(Lachance et al., 1995).**

It's not only the product, though, that promotes and boost profit. In securing the drink, container and packaging must ensure the adequacy of the planned outlets and offer optimum comfort to the customer **(Ghoshal, 2019)**. Throughout the last numerous decades packaging technology has been confronted with a succession of significant advances in the areas of refining and enhancement. Whether glass, polyethylene terephthalate (PET) or aluminum

containers, specialized expertise and distinctive facility layout are required **(Steen and Ashurst, 2006).**

In a nutshell: pliability, sustainability, productivity, cost-performance, minimal packaging, and product security are all crucial aspects constantly ascertaining the future in the packaging trade **(Lewis, 2005).** Overconsumption of carbonated soft drinks has a significant influence on human health, resulting in illnesses such as obesity, diabetes, dental and bone abnormalities, and others, particularly among youths **(Xavier et al., 2007).**

The inadvertent ingestion of yeast or mold spoiled beverage produces brief gastrointestinal distress, nausea, cramps, and diarrhea; if the immune system is feeble unremitting vomiting or diarrhea, increased abdominal soreness, fever and chills, if the CSD was spoiled due to bacteria, the symptoms depends on the pathogenicity of the bacteria and its toxic dose **(Rawat,2015).**

On this work outlines the major goal of our study in order to have a concise grasp of the packaging's influence on the microbiological and physicochemical quality of a carbonated soft drink: To assess which sort of CSD packaging is suitable for safe, risk-free consumption.

The first section is devoted to a review of the literature, describing the broader background of the principal ideas and techniques pertaining to our project. The experimental section is divided in two parts:

- The first narrows the microbiological and physicochemical analysis (the main objective).
- The second part highlights a marginal objective: observing the antimicrobial effect of a CSD examined brand of Coca Cola.

Literature review

I. Overview of carbonated soft drinks

I.1. Definition

Carbonated soft drinks are sweet, non-alcoholic effervescent refreshments that come in a variety of flavors **(Ockerman, 1978).** Carbonation is the process of dissolving carbon dioxide gas in water using pressure and temperature; the dissolved gas not only gives a distinct flavor, zest, and shimmer to the beverage, but it also plays a crucial role in suppressing or ravaging pathogenic bacteria **(Woodroof & Phillips, 1981).**

I.2.Types of carbonated soft drinks

The different types of soft drinks are defined as follows **(Atte yavo, 2017):**

- **Soda:** Is a carbonated drink that has been sweetened with fruit scents, vegetable aromatics, or fruit juice, and may have been acidified with citric, malic, or lactic acid, or sodium citrate.
- **Lemonade:** Is a carbonated, sweet, translucent, and colorless drink with scented or flavorful components derived from lemon and perhaps other hesperides (essential oils), acidulated under the same conditions as above.
- **Cola**: Is a beverage that differs from sodas in that it combines cola, caramel, caffeine, and phosphoric acid.
- **Bitter**: Is a sort of soda that has a bitter taste due to the inclusion of citrus extract.
- **Tonic:** Is a type of soda that can be hazy or clear and has a bitter aftertaste due to bitter extracts.

I.3.Coca Cola beverage

I.3.1. Denotation

Manufactured by The Coca-Cola Company in Atlanta, Georgia. When *John Pemberton* created it in the late 19th century as a medical elixir, it was purchased out by Mr. *Asa Griggs Candler*, whose marketing acumen led Coca Cola to the crest dominion of the soft-drink industry during the 20th century; the name was derived from the original ingredients, which were kola nuts, a caffeine source, and Coca leaves ; Cola's formula is a trade secret, despite the fact that a plethora of claimed formulas and attempted recreations have been published **(Eschner & Kat, 2017).**(Fig.1)

Fig.1: Coca cola commercial logo **(Web site 1)**

I.3.2. Components and nutritional values

All soft drinks have the same few ingredients. Other specialized ingredient combinations contribute to the diversity shown on the shelves (table I). They often contain water, sugar (8-12% w/v), carbon dioxide (0.3-0.6% w/v), acidulates (0.05-0.3% w/v), flavorings (0.1-0.5% w/v), colorings (0-70 ppm), chemical preservatives (lawful limitations), antioxidants (<100 ppm), and/or foamy agents (e.g., saponins up to 200 mg/mL) **(Geiger, 2001).**

Table I: Coca cola's components, role and amount **(Blanding, 2011)**

Component	Role
Carbonated water Amount : 6g/l Norm : 6–8 g/l	• CO_2 provides the beverage its effervescent character, also produces an anaerobic environment that prevents bacterial development **(Wareing, 2018).** • The carbonation process causes beverages to become more acidic, which helps to increase the shelf life of soft drinks **(Kregiel, 2015).**
Sweeteners Amount: 90g/l Norm : uninformed	• Often sucrose, derived from cane sugar; it enhances and preserves the flavor of beverages while also providing a pleasurable experience **(Kregiel, 2015).** • It is a key source of yeasts and molds that cause sugar syrup to deteriorate **(Misra et al., 2017)**
Caffeine Amount: 9.58mg/100ml Norm: 20mg/100ml	• A flavoring ingredient that acts as a central nervous system stimulant **(Griffiths & Vernotica, 2000).** • Increases stomach acid secretion **(Ramalakshmi & Raghavan, 1999)**.

	• Caffeine's mutagenic impact on microorganisms may explain its antimicrobial action **(George et al., 2008).**
Acidity regulator Amount: 0.57g/l Norm :0.7g/l	• It enhances the sweetness of sugar. • Promotes the flow of saliva in the mouth. The acids act as mild preservatives by lowering the pH of the product, which slows the growth of bacteria and molds that would otherwise grow quickly in the sugar-rich beverage **(Murphy, 1983).** • Phosphoric acid bears a drastic flavor when compared to other flavors such as citric acid or tartaric acid **(Taylor, 2016).**
Colorants	• Caramel color, in addition to giving outstanding reddish to brown colors, may improve the foaming properties, mouthfeel, and flavor of soft drinks. • Caramel color has an emulsifying action with flavor oils in soft drink concentrates, which aids in the elimination of some forms of "floc." (**Wang et al., 2015)**
Natural flavoring	• Cola's original formula included cocaine. Following the legislative amendment, the beverage is still flavored with a Cola leaf extract, and the cocaine derived from the leaves is marketed for medical purposes. • Yet, a 2015 research discovered and quantified 58 fragrance components in popular colas, indicating large quantities of chemicals related to cinnamon, vanilla, nutmeg, orange, and lemon hesperides in Coke **(Lorjaroenphon & Cadwallader., 2015).**

I.3.3. Manufacturing process

The manufacturing process begins with sugar, flavoring, and beverage base. The finalized goods will be packed in PET bottles, glass bottles, or metal cans. Coca Cola is manufactured and distributed via a franchising model. The Coca Cola Company solely manufactures syrup concentrate, which it sells to bottlers worldwide. The bottlers make the finished drink by combining the syrup with filtered water and sweeteners, carbonating it, and

then packaging it in cans and bottles, which they then sell and distribute to retail stores, vending machines, restaurants, and food service distributors **(Shachman, 2004).**

Coca Cola drinks are subjected to stringent quality controls and inspection procedures to guarantee that they satisfy the highest international standards. In summary, these procedures entail the following phases **(Fig.2) (Shakil,2020):**

1- **Water treatment:** To eliminate all contaminants, pure water is exposed to advanced filtration, softening, and disinfection processes.
2- **Syrup:** Sugar is combined with the proper drink concentration to create 'Syrup,' the basic component of the soft drink.
3- **Carbonation**: To give the beverages their famed 'Fizziness,' the liquid is saturated with carbon dioxide at a low temperature and under high pressure.
4- **Filling**: Automated apparatus pours the mixture into sterile bottles in precisely determined amounts, while another cans, caps, or seals them.
5- **Labelling**: The containers are then transferred to another machine that adds labels and bar codes before being automatically examined to ensure they fulfil all standards.
6- **Packaging**: After final inspection, bottles and cans are moved to machines that pack them in cartons or boxes before placement on wooden pallets.
7- **Transportation and Storage:** Trucks transport packaged beverages to storage facilities where they await delivery to consumers.

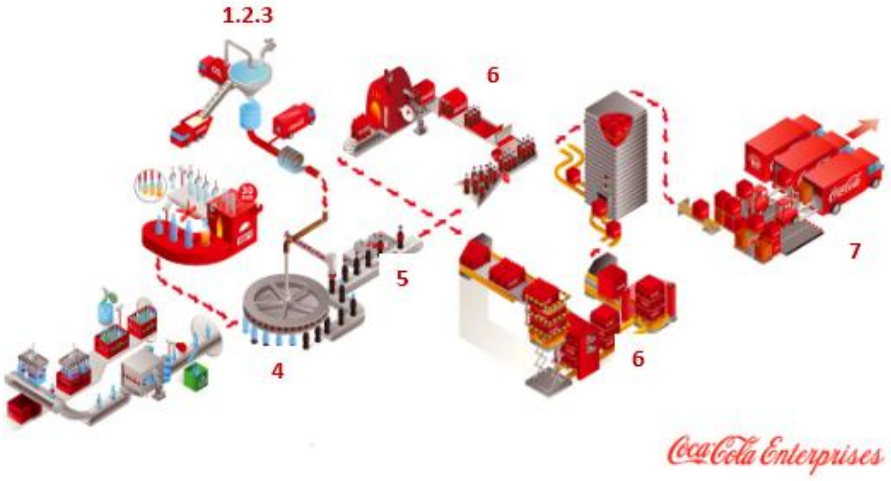


Fig. 2: The bottling process of Coca-Cola beverage **(Shakil, 2020)**

I.3.4. Health issues related to the overconsumption

The fact that soft drinks generate energy with minimal nutritional value, displace other food sources, and are related to numerous important health conditions such as diabetes adds to the case for limiting soft drink intake **(Vartanian et al., 2007).** Phosphoric acid alone has been identified as a risk factor for hypocalcemia **(Biggs et al., 2017)**. Caffeine is linked to persistent insomnia, anxiety, depression, and gastrointestinal issues **(Pallarés et al., 2013).** The consumption of carbonated soft drinks raises the chance of having a cardiovascular condition, thus it have been related to esophageal cancer, obesity, and hyperactivity, among others **(Kharde et al., 2013).**

I.3.5. Auto-quality control

- **Organization of auto-control**

It is the totality of actions taken by the firm to ensure the best quality of its products (Fig.3). These measurements are situated at all levels of the manufacturing process, which is based on the HACCP (Hazard Analysis Critical Control Point) approach **(Nahemiah et al., 2014).**

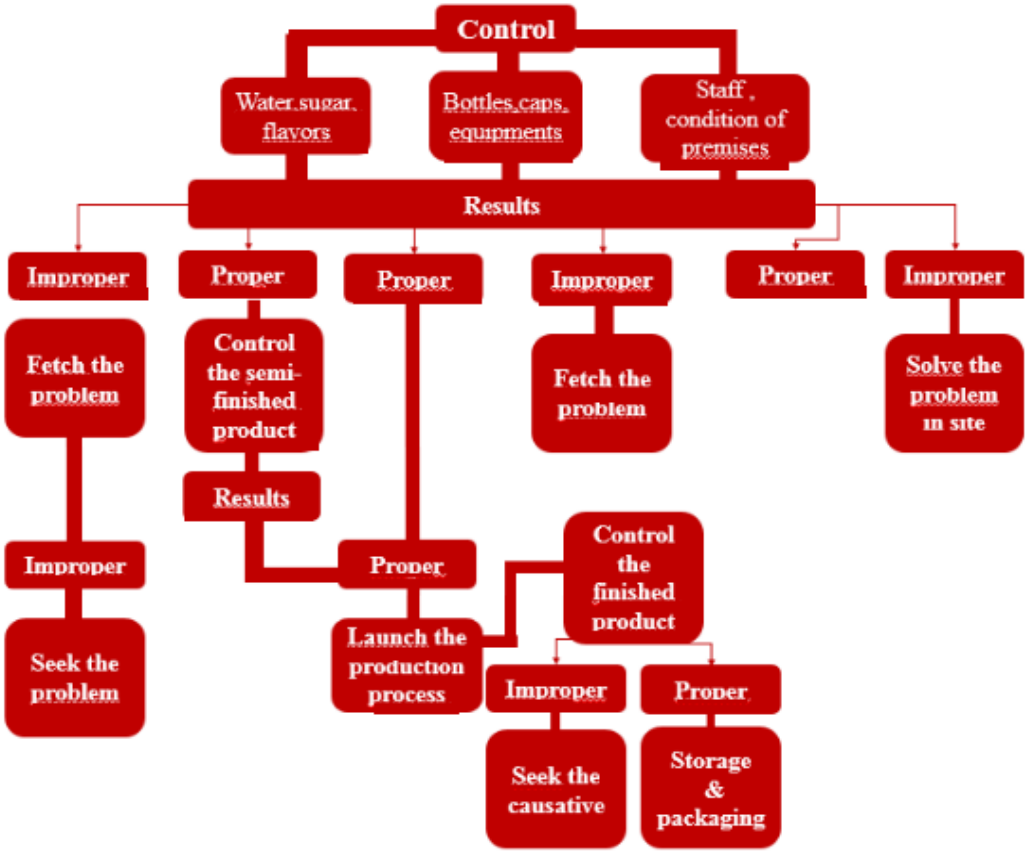


Fig. 03: Quality control flow chart **(Lekbir, 2009)**

The completed product, packaging, raw materials, water, and sugar must meet the criteria outlined in the interministerial order dated January 24, 1998, pertaining to the microbiological specifications of certain products **(Lekbir, 2009).**

Following the microbiological control and risk analysis of each phase of the production process, a critical procedure follows:

- **Determination of critical control points (CCP) for mastery:**

This entails deciding which of the identified risks are essential control points. The implementation of a decision tree particular to the HACCP process, which shows a logical reasoning approach, greatly facilitates the identification of a CCP in the (HACCP) system **(Romain et al., 2006).**

II. Functional performance for carbonated beverage packaging

Despite its main flaws of weight and brittleness, all early CSDs were packaged in glass, which remains the performance benchmark for product protection until now, today, a significant fraction of all drinks are packed in some type of plastic container, plastic-laminated paperboard, or other flexible packaging, the majority of which have only made a substantial contribution to the markets since the final part of the twentieth century, metal cans are still a viable option to other forms of packaging with a few exceptions, carbonated beverage packaging is confined to glass, metal cans, and PET **(Baughan & Attwood, 2010).**

II.1. Definition

The words "Package," "Packaging" and "Packing'' are vital to differentiate **(Glossary of Packaging Terms, 1988):**

- **The package:** Is the physical unit containing the product.
- **The packaging:** For one or more of the following purposes; the confinement, safety, preservation, communication, usefulness and performance of packaging is taken to mean the enclosing of goods, additionally, it's a discipline as in "Packaging Technologist."
- **Packing:** Is the act of enclosing a solitary object (or numerous objects) in a package or container.

II.2. Packaging / food interactions

Food packaging is seldom inert; material transfers can occur as a result of contact between the container and the content (Fig.4), these events have the potential to affect food quality, degrade package mechanical characteristics, and generate toxicological issues, packaging and food can interact in three ways: permeation, sorption, and migration (**Konkol, 2004).**

a-Migration:

Refers to the transfer of substances from packaging to food. It is measured in milligrams per kilogram of food or milligrams per square meter of packaging-contact surface (**Berlinet, 2006).**

b-Permeation:

It is defined as the flow of gases through the packaging, such as O_2 to the food, CO_2 to the outside of the packaging, and volatiles from the outside to the food. This phenomenon must be minimized in order to prevent the growth of bacteria in food, the loss of carbonation in soft drinks, and the loss of smells or taste in the completed product. Indeed, the organoleptic characteristics of foods are the consequence of a balance between volatile chemicals that are likely to be transmitted from the product to the outside (aroma loss) and substances that are likely to flow from the outside to the food (product contamination) (**Konkol, 2004; Zaki, 2008).**

c- Scalping:

When a product comes into touch with its packaging, molecules from the food can be transferred to the packaging **(Zaki, 2008)**. The absorption of food ingredients through the packaging wall, followed by their penetration into the polymer, is referred to as sorption or scalping, it can cause fragrance loss and structural changes in the polymer, which can cause it to age **(Bach Campa, 2011).**

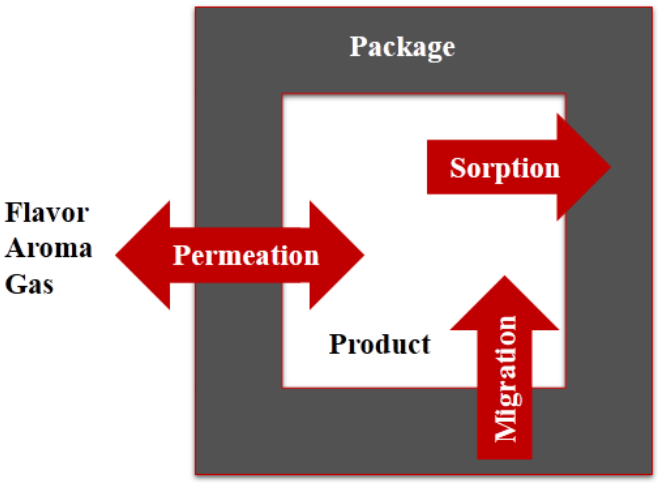


Fig. 04: Schematic illustration of food-packaging interactions**.**

II.3. Packaging levels

There are four distinct levels of product packaging, each of them plays an important role in the protection during shipping and storage (**Virginillo, 2011**).:

- **Primary package:**

The one in direct touch with the goods included. Initial and generally primary protective barrier (metal cans, cartons, glass and plastic bottles) are provided. It's often what consumers buy at retail stores**.**

- **Secondary package:**

A secondary package, such as a corrugated case or box, contains several primary packages. It is the physical delivery carrier and is progressively intended to be used in retail stores for primary package exhibition, it is referred to as shelf ready**.**

- **Tertiary package:**

Is composed of many secondary packages, the most typical of which is a stretch-wrapped pallet of corrugated cases.

- **Quaternary package**:

Is commonly used in interstate and international trade to assist the handling of tertiary shipment, this is often a metal container up to 40 meters long that can accommodate numerous pallets and is multimodal in nature, meaning it can be transported by enormous cranes to or from ships, railways, and flatbed trucks **(Fellows, 2009).**

II.4. Packaging's impact

Packaging is crucial in preserving CSDs from most degradation. As previously stated, CSDs can't exist as a product unless they are packed in a container that retains CO_2, therefore gas retention is a necessary protective feature (Fig.5). However, no container has been created that can protect the product from the effects of heat exposure and aging, despite the fact that the complicated nature of the aging process of drinks may be exacerbated by O_2 intrusion and the impacts of light **(Fellows, 2009).**

a-Aluminum can:

Made from aluminum (Al) alloys in two or three-piece cans using the "drawn and wall ironed" (DWI) technique ,because two-piece cans lack the side seam seen in three-piece containers, they are less prone to liquid and CO_2 leaks the danger of leakage is relatively low if the single end is attached and sealed correctly, and cans probably give the best CO_2 retention of any container type the possibility of leaking as a consequence of can corrosion and eventual pin holing owing to the acids is a possible exception **(Robertson, 2016).**

Can sealing is also important, and applying the end in such a way that no gas or liquid leaks occurs necessitates seaming machinery calibrated to extremely fine tolerances, with frequent quality checks to offer the necessary confidence **(Yam, 2010)**

b-Glass bottles:

Glass containers are checked throughout the manufacturing process, and any faulty glass that is undetected by such testing is likely to fail during the filling of carbonated beverages. As a result, leakage at the contact between the top and the bottle body is the most frequent cause of glass container failure. Because of the rigidity of glass, every molding fault of the neck rim

that creates an uneven surface for the closure becomes a possible source of leakage, the closure must thus be pressure-resistant, and the options are metal or plastic with a composite liner to form the seal between the closure and the bottle body **(Theobald, 2006).** Glass bottles provide the utmost quality test to prevent loss of carbonation **(Ghose & Nair, 2013).**

c- Plastic:

PET bottles are now manufactured in two steps: an amorphous preform is formed first by injection molding and then stretch–blow-molded to create a biaxial orientated, semi crystalline yet transparent bottle, modern PET bottle resins are often polyesters, with isophthalic acid being the most prevalent comonomer **(Bashir et al., 2002).** All polymers are permeable to gases, and most CSDs makers specify a maximum CO_2 loss of 15% over 26 weeks for a 1.5 or 2.0 L bottle. Smaller bottles, with a less favorable surface area: volume ratio, are likely to lose 15% of their CO_2 over a period of 10–12 weeks **(Steen, 2008).**

Packaging Type	Advantages	Disadvantages
Glass bottles	• Can be either single trip or returnable • Considering the cost factor, returnable bottles are preferred more than the single-trip bottles.(Ghose 2013) • Excellent barrier properties; Supporters claim the "feel" of the product creates a favorable impression in terms of quality; glass is synonymous with "class". (Smye Holland 2013)	• Glass containers have the disadvantage of being breakable and sometimes heavy. (Ghose 2013)
Aluminum cans	• Beverage cans are convenient, unbreakable and above all, light weight. • They are easy to open. • Because of metal good thermal conductivity, can cools beverages faster than other packaging. • Good printability. (Draskovic 2009; Liew 2005)	• Cans cannot be resealed, cans have only a one-time purpose, as is demonstrated with carbonated drinks and tinned food products. Products in cans is often said to leave an 'aftertaste of metal'.(Smye Holland 2013)
PET bottles	• PET is transparent, unbreakable, and lightweight. • Low-cost production capability. (Smye Holland 2013) • The lightness of PET enables easier and more cost-efficient transport – especially when delivered to brand owners as preforms, which are then blown into full-size containers on their own premises. (Smye Holland 2013, Steen 2008)	• Lower gas barrier characteristics compared to glass and aluminum. • Shorter shelf life. (Draskovic 2009)

Fig.05: An overview of perceived advantages and disadvantages by packaging type for CSDs **(Noha, 2016).**

II.5. Key contribution of carbonation

The initial preparation of a product to prevent or remove contamination is critical, but once within the container, the packaging plays an important role in both retaining CO_2 and protecting the contents from further contamination, the presence of CO_2 in carbonated drinks significantly decreases the risk of spoiling for the following reasons **(Damar & Balaban, 2011):**

- Carbon dioxide is a metabolite of many species of yeast, and its presence at pressure will, in many cases, decrease the activity of these organisms to the point where they halt to proliferate **(Stratford,2006).**
- The presence of CO_2 creates a blanket of inert gas in the product's headspace, with little or no O_2, this blanket both reduces the danger of infection and inhibits the development of organisms that require oxygen (e.g., molds) **(Petruzzi,2017).**
- When carbon dioxide is dissolved in water, it generates a weak acid, and its presence lowers the pH of the product. This decrease in pH will improve the efficacy of any chemical preservatives applied to the product **(Shankar et al., 2019).**

III. Indices of failure and spoilage of CSDs

Loss of carbonation and oxidation or acid hydrolysis of essential flavor oils are the two primary deteriorative processes in carbonated beverages, the first is primarily determined by the package's ability to provide a barrier to gas penetration, while the latter may be avoided to a significant part by using high-quality flavorings and antioxidants, as well as de-aerating the mix prior to carbonation **(Robertson, 2016**). It all could be summarized as in table II **:**

Table II: Failure Benchmark Synopsis **(Fellows, 2009).**

Failures indices	Features	Determined by
Physical	-Loss of contents -Loss of carbonation	-Net weight -Carbon dioxide level
Physicochemical	-Taste deterioration -Change of appearance -Presence of contaminants	-Organoleptic assessments -Unacceptable visual appearance -Analytical techniques
Microbiological	-Presence of unwanted microorganisms	-Microbial count or gross effects
Packaging	-Damage or deformation	-Visual inspection

III.1. Physicochemical decay:

The physical retention of liquid content and CO_2 in most CSDs is only a problem when the container is ruined or near the end of its shelf life, and the decaying effects of O_2, heat and light **(Narasimhan et al., 2001)**

- **O_2:**

Effect on CO_2: Dissolved O_2 will cause a misleading measurement of the CO_2 level, but this is generally within the tolerance of most analytical techniques in use, more importantly, it is likely to cause the phenomena of "fobbing," as nucleation sites are formed as a direct result of air in the product. When the pressure is removed, the product gushes uncontrolled out of the container **(Gleizes et al., 1980).**

Effect on components: The sensitivity of the terpene hydrocarbon components to oxidation, make essential oils much vulnerable to oxidation, and can induce taste deterioration and quickly render the finished product undesirable **(Ashurst & Hargitt, 2009).**

- **Light:** Direct sunlight generally causes a wide range of soft drink components to degrade quickly the most visible impact of exposing soft drinks to light is generally color fade or loss; however, color fade or loss may also signal taste degradation; natural tastes are especially susceptible to the effects of light (and O_2) because they include several terpene chemicals that degrade quickly **(Morata, 2021).**
- **Heat and Aging:** Most carbonated beverage products have a shelf life of at least 6 months in temperate markets, but just 3 or 4 months in hot climates **(Fellows, 2009).**

III.2 Microbial spoilages:

CSD microbial deterioration is generally marked by visual changes and off-flavors (table III) **(Juvonen et al., 2011).** When the microbial concentration is greater than 10^5 CFU/mL, spoiling occurs visually **(Stratford, 2006).** To evaluate the microbial quality of CSD the concentration must be between 10 to 10^2 CFU/ml **(OJAR, 2017).** To spoil soft drinks, microorganisms must be able to thrive in the presence of CO_2, as well as sustain acidic conditions and the presence of chemical preservatives **(Stratford, 2006, Lawlor et al, 2009).** Although these variables put selection pressure on acidophilic and anaerobic bacteria, the inclusion of essential oils in some soft drinks, particularly citrus essential oils, may impart antimicrobial benefits, but it does not appear to be an effective barrier to microbial development **(Beuchat & Golden, 1989).**

Table III: Examples of metabolites and quality changes associated with common spoilage microbes **(Juvonen et al., 2011.)**

Spoilage microbe	**Off flavors/odors**	**Visual spoilage**	**Metabolites**
Yeast	Bad drink, vinegar, sweet pineapple note, sweet butter, yeasty, aldehyde off-flavor,	Swollen packages, tainting, haze, clouds, particulates, surface films.	CO2, ethanol, acetic acid, diacetyl, acetaldehyde, acetoin, esters, 1,3- pentadiene, exocellular polysaccharides
Mold	Musty, stale	Mycelial mats, discoloration, swollen packages	Pectin degradation, formic acid, increase in pH due to metabolism of acids, gas

			production, gluconic acid
Bacteria	Cheesy notes, sour, Vinegar , antiseptic and smoky taints	Loss of CO2, ropiness, Turbidity , Haze, swollen packages, Ropiness , sometimes difficult to detecte .	CO2, gluconic acid, acetic acid, ethyl acetate, acetoin

III.2.1. Yeast:

Yeasts are the most common CSDs contaminants and spoilers because to their inherent presence in the components such as sugar and fruit juices, as well as their propensity to flourish in acidic conditions and carbonation levels exceeding 3.0 vol **(Stratford, 2006).** Although yeasts do not pose a health danger to consumers **(Ndagijimana et al., 2004),** they can harm the company's image and can result in significant financial losses **(Loureiro & Queirol, 1999).**

Wareing & Davenport (2005) recommended categorizing yeasts into four categories based on their likelihood of soft drink spoiling (table V), group 1 (high risk) yeasts are fermentative and preservative-resistant yeasts, whereas group 2 yeasts ruin soft drinks due to inadequacies in processing conditions, such as hygiene issues. Group 3 yeasts do not degrade soft drinks but serve as indications of poor sanitation, whereas group 4 yeasts may be isolated from soft drinks but are not common contaminants and do not grow in these beverages **(James & Stratford, 2003).**

Table V. Examples of yeast species found in soft drink factory environments **(Wareing &Davenport, 2005).**

Group	**Yeast species**
Fermentative & preservative resistant	*Dekkera anomala / D. bruxellensis D. naardenensis /Saccharomyces cerevisiae /Schizosaccharomyces pombe / Zygosaccharomyces bailii /Z. bisporus /Z. lentus /Z. rouxii*
Spoilage and hygiene indicators	*Candida davenportii / C. parapsilopsis / Debaryomyces hansenii /Hanseniaspora uvarum /Lodderomyces elongisporus / Pichia anomala / Membranifaciens /Saccharomyces bayanus /S. cerevisiae*

Hygiene Indicators	*Aureobasidium pullulans /Candida sake /C. solani /Clavispora lusitaniae /Cryptococcus albidus /Cryptococcus laurentii /Rhodotorula glutinis*
Aliens	*Kluyveromyces lactis /K. marxianus*

III.2.2. Molds ''Filamentous fungi'':

Conidia or spores of filamentous fungi, as well as mycelial debris, can contaminate the CSD environment**, Sato (2010)** studied filamentous fungus at a soft drink plastic cap factory and discovered 47 filamentous fungus species recovered from 52 swabs and air samples, the most polluted locations were the cover inspection room and the resin storage room**.** Although certain species, such as *Fusarium, Mucor, Rhizopus* **(Scholte et al., 2004),** *Byssochlamys***,** Alternaria,and other heat resistant molds, may thrive under anaerobic environments (**Pitt & Hocking, 1999**).

CSD spoiling by filamountus fungi can result in off-flavor. Fungal metabolism produces various enzymes (lipases, proteases, carbohydrases) that can cause beverage discolouration **(Juvonen et al., 2011),** and some fungus strains can create toxigenic chemicals such as aflatoxins **(Pitt & Hocking, 1999).**

III.2.3 Bacteria:

Acetic acid bacteria, spore-forming bacteria, and mesophilic aerobic bacteria are the primary types of bacteria linked with soft drink deterioration **(Back et al., 1999, Juvonen et al., 2011).**

- **Aciduric bacteria:**

Acetobacter, Gluconobacter, and Gluconoacetobacter are the primary threats for CSD spoiling among the acetic acid bacteria **(Juvonen et al., 2011).** They are common in nature, especially in environments rich in sugar and ethano**l (Back, 2005).** The majority of species thrive around pH 3.6–3.8, with some even growing at pH 3.0 **(Raspor & Goranovic 2008, Lawlor et al., 2009).** The ideal temperature for growth is 25–30 °C **(Back, 2005).** Their abundance in process settings is thought to signify inadequate hygiene **(Back, 2005, Raspor & Goranovic, 2008).** Many species can develop biofilm on industrial surfaces **(Back, 2005).**

Acetic bacteria may oxidize sugars, organic acids and other compounds of the beverage in the process, these products cause changes in viscosity, the presence of sediments, turbidity, packing distension, and taste alterations of the CSD **(Juvonen et al., 2011, Raspor &**

Goranovic, 2008). They are mainly a problem in beverages packed in oxygen-permeable containers(PET) (**Raspor & Goranovic, 2008).**

- **Pathogenic:**

Salmonella and *Listeria monocytogenes***,** which are pathogenic bacteria, do not survive in CSDs, pathogens rapidly lose viability when exposed to acidic and carbonated conditions **(Massa et al., 1998).** Although CSDs are insufficient substrates for their growth, their existence might be linked to the microbiological condition of the water used to produce these goods, pathogens such as *Salmonella and E. col*i are known to be intestinal bacteria that are often transferred through water **(Levantesi et al., 2012).**

- **Spore-forming bacteria:**

Due to low pH, *Bacillus* and *Clostridium* are generally suppressed in soft drinks. However, spores may survive **(Back, 2005).** *Clostridium butyricum* and *Clostridium sporogenes*, can degrade sugar syrups used in the beverage industry during syrup production or storage, resulting in a rancid off-flavor in the finished goods, even at pH levels of 3.6–3.8, these bacteria were active **(Hawthorne et al., 1991).**

Material & Methods

I. Material

Presentation of the internship site

The experimentation took place in three different locations ‘’Laboratory Espace Pasteur Bouira’’,’’ Hygiene laboratory of Bouira ‘’ for food quality control analysis, and the’’ university’s lab N°5’’, where the necessary equipment were under dispositive (table VI), starting from April 27th to May 31st. The samples were bought randomly from a Coca Cola deposit stock, located in Bouira, 90 bottles of different packaging types (30 glass bottles ,30 PET bottles and 30 cans /25cl), were stored at room temperature, for 30 days 5 samples of each type underwent microbiological and physicochemical analyses per 5 days.

Tab. VI: Used equipment and reagents.

Step	Reagents	Material
Media preparation Microbial analysis	Powder of PCA, Sabouraud Chloramphenicol. Distilled water	Micro balance accurancy 0.1g - 0.001mg. Flaskes, Petri-dishes, Bunsen burner Eye dropper , Spreader, Magnetic Hot Plate Stirrer ,Siring nylon filter ,Autoclave ,Incubator
Physicochemical analysis	Distilled water	pH metre, Refractometre, Beaker Hot plate ,Glass watch
Germs identification	Crystal violet Iodine, Alcohol Safarine, Steril water *Immersion* oil H_2O_2 Oxidase reagent	Sticky tape , Microscop ,Filter paper

II. Methods

II.1. Media preparation

1) Plat count agar

‘PCA’ is microbiological growth medium commonly used to assess or to monitor "total" or viable bacterial growth of a sample , it is available as a premixed powder .

- **Procedure**

Distilled water was combined with 65g of powder to brought up to volume 1.0L. Mixed thoroughly. On a hot plate stirrer set the temperature between 60 and 65 °C, gently heated and brought to boiling (Fig. 06). Distributed into flasks. Autoclaved for 15 min at 15 psi pressure–121°C.

2) Sabouraud Agar with Chloramphenicol

A growth agar for the cultivation of yeasts and molds. It is available as a premixed powder.

- **Procedure**

Add 20g of the powder (Fig. 06) to distilled water and bring volume to 1.0L. Mix thoroughly. Gently heat and bring to boiling on a hot plate stirrer . Distribute into flasks. Autoclave for 15 min at 15 psi pressure 121°C.

3) Preparation of antibiotic solution

The antibiotic inhibits the bacterial growth and select only the fungal flora:

- **Procedure**

Add 0.06 g powder of the antibiotic Chloramphenicol (fig.06) to distilled water and bring volume to 10.0mL. Mix thoroughly. Filter sterilize. Aseptically add sterile antibiotic solution to the prepared medium (fig.06). Mix thoroughly.

A. Sabouraud dried medium

B. Medium on hot plate stirrer

C. Antibiotic dried form

D. Versing the antibiotic to the meduim

Fig.06: Taken pictures representing the protocol of media preparation.

II.2. Microbial analysis

5 units of each packaging type (Fig.7) were tested for mesophilic aerobic germs and the fungal flora, the standard should be less than 10-10^2 CFU/ml **(Spencer, 2001)**:

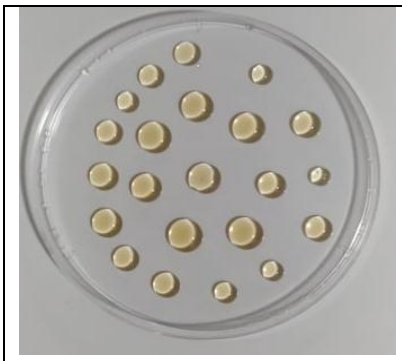
Fig.07: Samples collection

1) **Mesophilic aerobic flora**

- **Procedure**

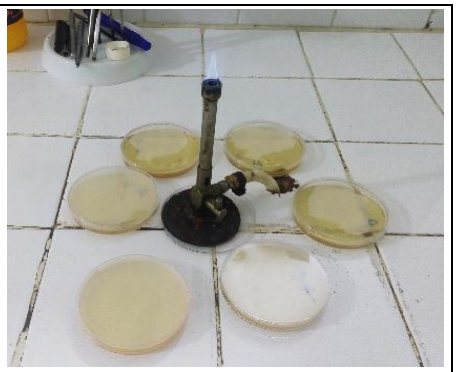
When the count is predictable to be less than $2.5x10^3$ CFU /ml , the dilution is neglected **(Yang, 2017):**

After labeling the petri-dishes , the Double-Layer Agar (DLA) technique is applied **(Spencer, 2001)**. Spread 1 ml of the sample over the entire surface of the petri -dish, drop wise (Fig.8). Pour 15 ml of PCA agar and stir in the eight motion's mouvement . Let harden,then add a second 5ml layer of agar (fig. 8). After solidifying flip'em (to avoid water drops condensation) , incubate at 30 ° C for 72 hours .



A. Drop-wise sample

B. Medieum pouring



C. Solidification

Fig.08: Taken pictures representing the protocol of DLA.

1) **Yeast and Molds**

- **Procedure**

Based on applying Spread Plate Method **(Spencer, 2001)**, on a petri dish that contains the medium, drop 0.2 ml of the sample dropwise, after forming a rake shape with the pipette, spread the inoculum on the surface (fig. 9). Incubate at 25°C for 5 days without flipping'em (the inoculum would leak off the plate).

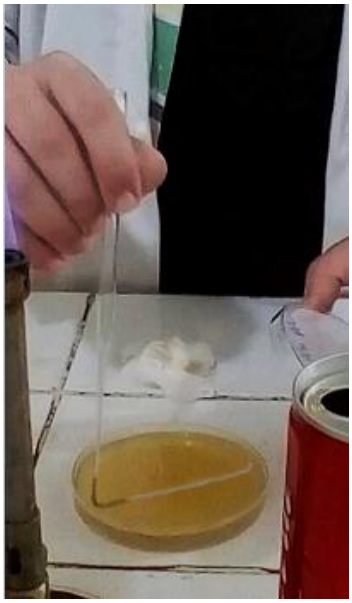


Fig. 09: Spreading the sample

II.3. Physicochemical analysis

A set of physicochemical criteria determines the quality of carbonated beverages. Because of the hideous capacities of the laboratory equipment, only two parameters were examined: Brix and pH .The aim of this analysis is to monitor them changings throughout the course of the research to see if the packaging type has an effect on them.

1) The assessment of pH

It is the measuring of a product's acidity or alkalinity. In our study, the pH is measured with a pH meter by putting the probe into the beaker containing 5 ml of the Coke sample, and the result is read directly on the device's screen.

2) Brix assessment

Brix is commonly used to indicate the amount of dissolved solids in a solution; It is especially used in the soft drinks industry to measure the sugar content of a beverage, syrup, or juice; It is proportion of dissolved sugar in a water solution represented in degrees Brix (°B) **(Shachman, 2004).**

- **Concept**

The refraction of light as it passes through a liquid may be measured using the Brix scale; The resultant refractometer measurement is assigned a value on it, allowing you to compare various concentrations in solution; Pure water with no suspended particles has a Brix value of zero, but water in solution containing sugars, minerals, or other substances refracts light to give a greater Brix value **(Shachman, 2004).**

a- Decarbonation

Before measuring the B° it's mandatory to decarbonate the Coca Cola sample; The primary reason is that CO_2 bubbles would scatter light substantially, altering the absorption spectra. Pour into a beaker 5 ml of Coca Cola on top of a hot plate and put a watch glass over it until it boils. keep boiling for more 5 minutes in order to expel the CO_2 that's in the solution while the glass watch keeps the CO_2 from the air out of the solution (fig.10).

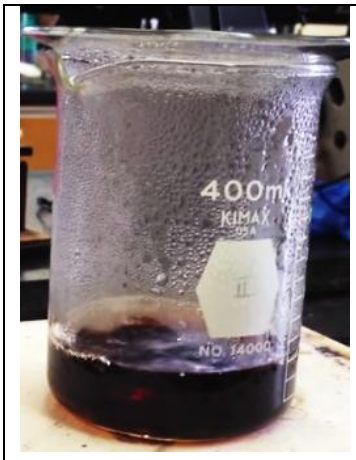
b- Calibration

By using distilled water, calibrate the refractometer. We place the refractometer on a flat surface. Open the daylight plate of the refractometer and deposit two drops of distilled water on the prism.

Close and push the daylight plate to equally distribute the water across the prism. Look through the eyepiece of the refractometer toward a light source. The circular area carries the index for your specific type of liquid. The line should intersect the index at zero. If it doesn't, tweak the refractometer's calibration screws until it does.

c- Sample deposit

After wiping the prism add 1 drop of decarbonized Coca Cola sample and observe the refraction index on the scale (fig. 11).



A. Decarbonation

B. Measuring the B°

Fig.11: Taken pictures representing the Brix measurement.

A query was proposed out of curiosity: 'The analysis of the same bottle for 10 days'. 9 bottles (3 Glass, 3 PET, 3 Aluminum) underwent the same process of microbial and physicochemical analysis as mentioned above, the only difference is the re-use of the same samples for 10 days (table VII)

Tab. VII: Schedule of proceeded analysis

Day 1	Day 3	Day 6	Day 9
3 PET bottles	3 PET bottles	3 PET bottles	3 PET bottles
3 Glass bottles	3 Glass bottles	3 Glass bottles	3 Glass bottles
3 Al bottles	3 Al bottles	3 Al bottles	3 Al bottles

II.4. Identification of the germs

1) Yeast and molds identification

Fungi are very diverse organisms and have different developmental stages so it is hard to visually identify most of them. The colonies must be stained with Lactophenol cotton blue to provide perfect microscopic identification. Due to the lack of reagents the identification relied only on macroscopic and blurry microscopic observation.

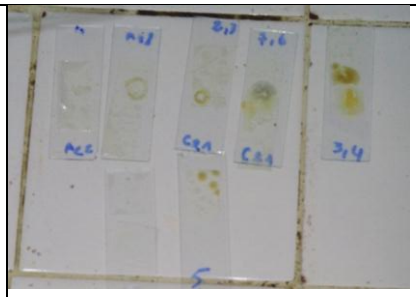
a- Yeast

With sterile inoculation loop, grab a colony, on a lamina mix it with a drop of physiologic water, cover with lamella (fig.12), and pass to microscopic observation from lower to higher magnifier X40, X100

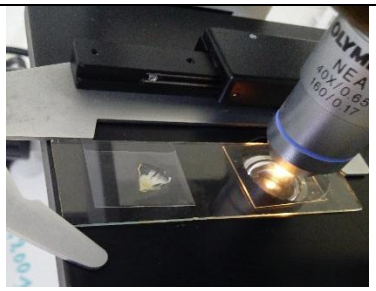
b- Mold

A loop of sticky tape, pressed against the mycelium. The tape is placed, sticky side down on the slide (fig. 12). Microscopic observation (Magnifier X10 - X40-X100)

A. Slide yeast sample



B. Mold slides



C. Microscopic observation

Fig.12: Taken pictures representing the fungal flora identification.

2) Bacteria identification

When identifying bacteria in the laboratory, the following characteristics are implemented: Gram staining, shape, presence of a capsule, bonding tendency, motility, respiration, growth medium... Some other tests are applicable as indicated in (Fig.13). Due to the unfortunate budget only 3 methods were applied:

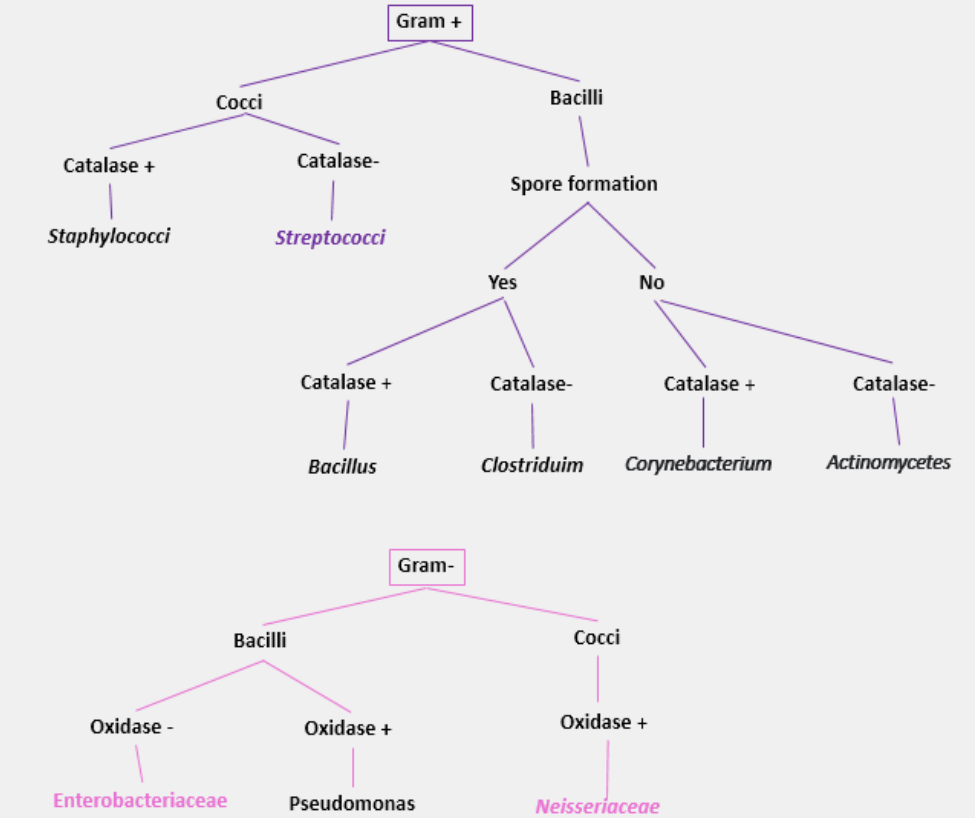


Fig. 13: Flowchart of biochemical tests used to identify Gram positive and gram negative bacteria **(Skerman, 1960)**

a- Gram staining

The staining method as demonstrated in table VIII distinguishes bacteria species based on cell wall structure. Gram-positive cells are distinguished by a thick peptidoglycan coating that stains blue to purple. Gram-negative cells are distinguished by a thin peptidoglycan coating that stains red to pink.

Table VIII: Gram staining procedure

Step	Reason
For 1 minute, flood an air-dried, heat-fixed smear of cells with crystal violet	Staining reagent
Wash the slide for 2 seconds in an indirect stream of tap water.	Remove the trace of the previous reagent

Soak the slide with Gram's iodine ,1 minute wait.	The mordant
Wash the slide for 2 seconds in an indirect flow of tap water	Remove the trace of the previous reagent
Apply alcohol to the slide. Wait 15 seconds or add drops to the slide one at a time until the decolorizing chemical flowing from the slide runs clean.	decolorizing agent
Flood the slide with safranin for 30 seconds to 1 minutes	Counter stain
Wash the slide in an indirect stream of tap water until there is no color in the effluent, then blot dry with absorbent paper.	
Examine the outcomes of the staining technique in the presence of oil immersion.	Observing with 100x Lens

b- Catalase test

- **Principal**

Catalase is the enzyme responsible for converting hydrogen peroxide (H_2O_2) into H_2O and O_2. H_2O_2 is a powerful oxidizing agent that may wreak havoc in a cell; as a result, any cell that uses O_2 or can exist in the presence of O_2 must have a means to eliminate the peroxide. One of these methods is to produce catalase. This test It is used to distinguish bacteria that produce the enzyme catalase, such as *Staphylococci*, from bacteria that do not produce catalase, such as *Streptococci*.

- **Procedure**

Use a loop to transfer a small amount of colony growth in the surface of a clean, dry glass slide. Place a drop of H_2O_2 in the glass slide. Observe for the evolution of oxygen bubbles.

c- Oxidase test

The oxidase test detects organisms that generate the cytochrome oxidase enzyme. Cytochrome oxidase is an electron transport chain enzyme that transfers electrons from a source molecule to oxygen. A chromogenic reducing agent, which is a chemical that changes color when oxidized, is included in the oxidase reagent. If the test organism produces cytochrome oxidase, the oxidase reagent will become blue or purple in 15 seconds.

- **Procedure**

Add 3rd of the weight of sterile water to the oxidase reagent using a pipette. Soak a strip filter paper with few drops of the solution**,** and then smear a speck of culture on it with a loop. A positive reaction is indicated by an intense deep-purple hue, appearing within 5-10 seconds and a negative reaction by absence of coloration.

Results & Discussion

I. Physicochemical analysis

All foodstuffs normally deteriorate during storage, deterioration in product quality may be the result of effects of changing physicochemical factors. The results of physicochemical analyzes carried out on the CSD studied are given below:

Table IX: The variation of the pH during storage period of all 99 bottles

	Day 1	**Day 40**
Glass	2.65	2.67
Aluminum	2.65	2.66
PET	2.65	2.65

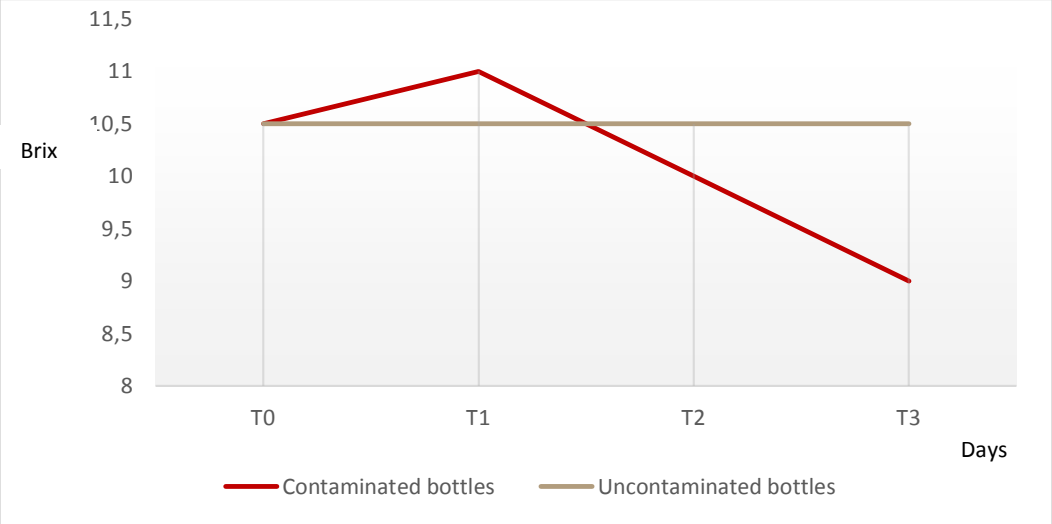


Fig.14 : Variation of brix ° in time of all 99 bottles.

The table IX illustrates how pH level changed throughout the course of 40 days of storage. It remain constant throughout storage, and the minor variation is neglected according to **Beldjenna (2019)**. Unless the CSD is contaminated, the Brix level remains constant, as indicated in the diagram of fig.20. T0=day 1, T1=day 7, T2=25, and T3=day 30, where T0=day 1, T1=day 10, T2=25, and T3=day 40, shows a noticeable increase followed by a significant decrease in B°, indicating that the sucrose was broken down and consumed by yeast primarily , as well as bacteria and mold as a source of energy **Bealing & Bacon (1953) .**

These findings indicates that the physicochemical quality of a CSD is unaffected by the packaging type.

II. Microbial analysis

The number of colonies (CFU/ml) appeared in the incubated plates is demonstrated in the table X:

Table X: The outcomes of the microbial analysis of the 90 (glass bottles, PET bottles and aluminum cans) (CFU/ml).

	Glass bottles		PET bottles		Al bottles	
	PCA	**Sabouraud**	**PCA**	**Sabouraud**	**PCA**	**Sabouraud**
Test 1	0-0-0-0-0	2-3-1-0-1	0-0-0-0-0	0-0-0-0-0	0-0-0-0-0	0-0-0-0-0
Test 2	0-0-0-0-0	1-1-1-1-0	0-0-0-0-0	1-2-0-0-0	0-0-0-0-0	0-0-0-0-0
Test 3	0-0-0-0-0	2-1-4-0-1	0-0-0-0-0	1-2-1-0-0	0-0-0-0-0	0-0-0-0-0
Test 4	0-0-0-0-0	1-2-0-0-0	0-0-0-0-0	1-1-0-0-0	0-0-0-0-0	0-0-0-0-0
Test 5	0-0-0-0-0	9-2-2-1-1	2-1-1-0-0	2-2-1-0-0	0-0-0-0-0	0-0-0-0-0
Test 6	10-0-0-0-0	1-0-1-0-0	1-0-0-0-0	2-0-1-0-0	0-0-0-0-0	0-0-0-0-0

The bottles branded from Coca Cola are of satisfactory quality, all numbers of sample units' values are lower than the microbial limit (10 CFU/ml), no spoilage is detected. For so**,** instead of three-class plan this study relied on the two-class plan since the standard is low (10 to 100 CFU / ml) **(Gunthier, 1999). Sebbak & Yahiaoui (2019)** results disagree with our findings, their study clue to that the quality was unacceptable due to bacterial contamination 58 CFU/ml.

The figure 15 depicts the proportion of bacterial and fungal contamination in three types of packaging. Aluminum cans yielded negative results: there was no contamination. Leaving the glass and PET containers to be compared. Both are prone to fungal contamination, but glass comprises a considerably higher frequency than PET. Both types have a low risk of bacterial contamination, although PET has a higher rate than glass. Despite its low value, it is brought into question.

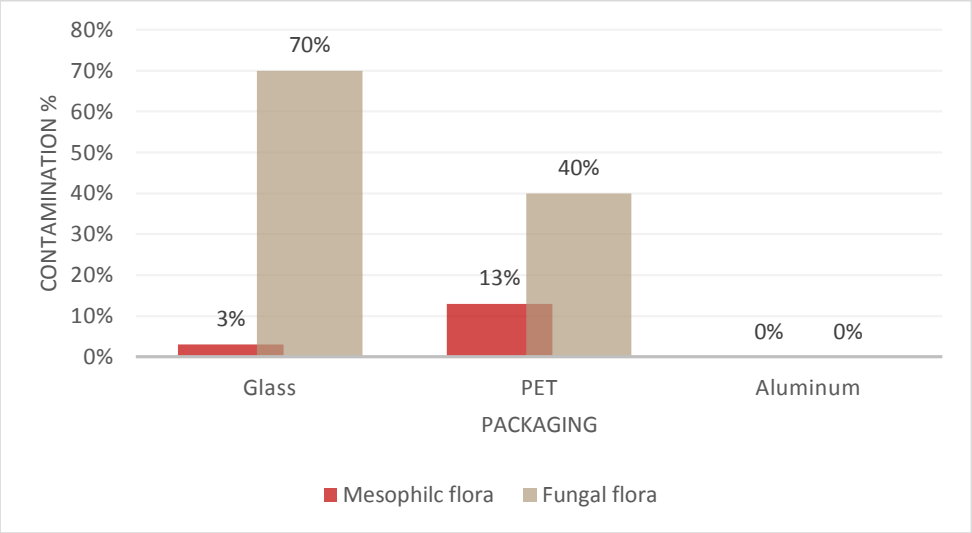


Fig.15: Bar chart of contamination rate by three packaging types.

Untarnished aluminum cans explain the intactness of the product; aluminum cans are oxygen-impermeable packaging. Probably also the high quality of the raw materials and compliance to sanitary procedures. Acetic acid bacteria may thus only thrive and degrade CSDs that have lost their anaerobicity due to carbonation loss or oxygen penetration via PET. PET bottles feature more surface roughness, hydrophobicity, and electrostatic charges than glass bottles due to cells adhering to the bottle surface, and often have higher bacterial counts.

Beverage nutrients are absorbed and concentrated on PET surfaces, allowing access to bacterial contamination. Adsorption of organic materials thus serves as the foundation for microbe adherence to bottle surfaces. **Jayasekara et al (2015)** showed a significant variance amongst bottles from the same water manufacturer, with up to 83 % of the entire microbial population adhering to the inside surfaces of those bottles. **Jones et al (2000)** on the other hand, found significantly lower amounts of adhesion. Using scanning electron microscopy, they discovered sparse cell adhesion to the surfaces of polyethylene terephthalate (PET) packaging.

Yeasts and molds can establish colonies in manufacturing plants at any level of the process owing to inadequate process cleanliness or spread from contaminated packaging. The presence of water and high acidity are prerequisites for fungal deterioration of carbonated soft drinks. Sugar is unquestionably a tonic for yeast development **(Stratford, 2006**).

For CSD manufacturing, most plant operations do not sterilize nonreturnable containers (PET or AL cans). Although plants may rinse particles (e.g., cardboard fibers) from empty bottles using chlorinated water, this should not be considered a sanitation process for packaging materials. Instead, the chlorine in the water is provided to maintain the feed line clean of biofilms that may slough off and contaminate bottles during the rinsing process.

Returnable glass bottles (RGB) however, serve as a substantial yeast reservoir in a CSD facility. Furthermore, leftover preserved product in RGB enables a natural selection and enrichment mechanism for preservative-resistant yeasts. Furthermore, the process of washing RGB generates huge amounts of condensation and standing water in a CSD facility, which promotes the growth of spoiling germs **(Kregiel, 2015).**

III. Germs identification

The results of monitoring the microbial quality of 9 bottles in10 days is shown in the table XI, the figurative pictures for table XII and table XIII are found in appendix section.

Table XI: Microbial results of coca cola same analyzed bottles for 10 days

Bottles		**Glass**				**PET**				**Aluminum**			
		D1	D3	D6	D9	D1	D3	D6	D9	D1	D3	D6	D9
PCA	B1	0	2	1	0	0	0	0	0	0	2	1	2
	B2	0	5	3	0	0	0	0	0	0	3	2	2
	B3	0	1	1	0	1	1	2	2	0	1	0	1
Sabouraud	B1	0	1	1	2	0	1	1	1	0	1	2	2
	B2	1	1	2	2	1	1	2	2	0	1	2	2
	B3	0	1	1	1	0	0	0	0	0	2	2	3

Table XII: Fungal flora in Coca Cola samples

Yeasts	**Molds**
Rhodotorula	*Aspergillus*
Saccharomyces bayanus	*Rhizopus*
Candida	*Cladosporium*
	Penicillium
	Fusarium
	Mucor

Table XIII: The mesophilic germs identification tests results

Shape	**Gram**	**Catalase**	**Oxidase**
Cocci in chain	Positive	Negative	Negative
Diplococci	Negative	Positive	Positive
Rod	Negative	Positive	Negative

Streptococci, Enterobacteriaceae and *Neisseria* are the groups we suspected their presence; they are involved as they are anaerobic bacteria. Most *streptococci* and *Enterobacteriaceae* are facultative anaerobes, and some are obligate (strict) anaerobes. Neisseria is generally considered to be an obligate aerobe; it can, however, grow in the absence of oxygen by anaerobic respiration.

The table XI depicts the results of the microbiological examination of 9 bottles of various packaging forms, on which the experiment was repeated for 10 days. The samples that gave negative findings on the first day did not sustain this result, as we detected the presence of several bacterial colonies the next day, and the bacterial load diminished with time.

The intensity of contamination varies depending on the containers type, and this is due to the closure system; for instance, aluminum bottles are prone to severe contamination after opening because they are difficult to close properly, despite the fact that we sealed them with sterile aluminum foil as we did with all samples. The PET bottle prevents the beverage from contamination once it has been opened. better than the other types.

Whether the fungal contamination emerged on the first day or after opening, the colonies grew at a typical rate. In regard to molds, the table XII illustrates the numerous fungal species that developed either before or after the opening.

Yeasts were present prior to the opening because the main source of contamination is in air particles especially that Coca Cola is an excellent environment for growth due to the acidic and sugary medium as table XII shows, the yeast's species identified are spoilage and hygiene indicators.

The table XIII present the bacterial diversity that were revealed after the drink was opened and contaminated. Because the bacterial load lessened, we can conclude that Coca Cola has an antibacterial effect on *Streptococcus as* **Radhakrishna (2012)** did**,** *Neisseria* and *Enterobacteriaceae,* this was validated by **Şeker et al (2015**) when they studied Antibacterial effect of coke by Cut Plug method. They found that Coca Cola has a strong antibacterial effect on *Bacillus cereus, Enterococcus faecalis, Escherichia coli, Escherichia coli O: 157 H: 7, Salmonella enteritidis, Yersinia enterocolitica.* **Munteanu (2020)** and **Tricoulet (2014)** proved the same clinically when she studied its effects on sick children.

Antagonisme can be put forth since fungi like *Penicillium* can suppress some bacteria, but further research is needed to prove this. the closing system have a key role in preserving the beverage's quality after opening and this was confirmed by the disparity of contamination intensity between the types of containers.

Conclusion & Perspectives

With the purpose of defining which form of packaging is the most trustworthy assurance of the microbiological and physicochemical quality of CSDs, we conducted comparative tests contrasting glass, PET, and aluminum containers.

The pH of Coca Cola drink held steady regardless of container type or microbiological contamination, according to our findings, Brix appears to be impacted by the presence of microbes that consume it, thus a beverage contaminants-free will sustain the Brix level.

The Outcomes of the microbiological analyses revealed that Coca Cola is of satisfactory quality; Nevertheless, the aim of our study is not limited to abide the established criteria, but that the presence of one colony is sufficient, we chose a two-class plant for this reason.

The results stated that the glass bottles are more susceptible to fungal contamination owing to its refillability; the flaw is in its inadequate rinsing, whereas the PET is more susceptible to bacterial contamination with aciduric bacteria due to the so-called permeability. Regarding aluminum, the risk is unsubstantial since it is impermeable and effectively retains CO2.

The procedure of monitoring the same samples for 10 days out of inquisitiveness.uncovered namely, a reduction in bacterial load, indicating that Coca-Cola has an antibacterial impact on *Enterobacteriaceae, Neisseriaceae*, and *Streptococcaceae*. Yet no antifungal correlation was observed, though molds: *Aspergillus Rhizopus; Cladosporium; Penicillium;Fusarium;Mucor,* which are sourced from the manufacturing plant, and yeasts: *Rhodotorula; Rhodotorula , Saccharomyces bayanus* & *Candida sp* which are usually sourced from poor quality sugar, deem the CSD as not only a suitable medium, thus a promoter for growth .

We finally assume that the packaging type has a significant impact on the quality of the CSD. The closure mechanism likewise tends to maintain the CSD integrity once unsealed. Unlike PET and glass, aluminum bottles are rated the safest. Coca Cola present an antibacterial effect.

The boldest measures are the safest. The firm FRUITAL COCA COLA, one of the market leaders in Algeria, applies HACCP systeme to assure the product's health and safety. However, critical control points at the rinsing, filling, and raw material management levels must be verified. Also, transportation and storage must adhere to safety standards, such as preventing direct sunlight and storing at a low temperature. High hopes on developping a zero flaws containers that are inert, impermeable, with an iconic closure systeme .

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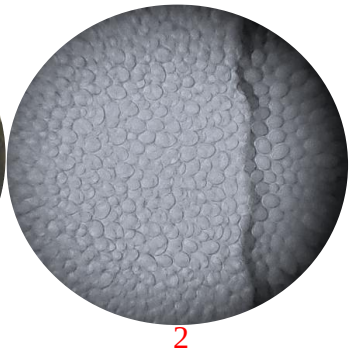
Web sites :

- **Web site 1** : https://www.coca-colacompany.com/

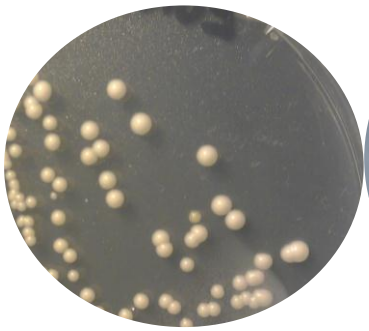
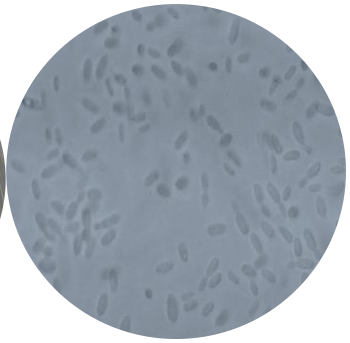
Photo Gallery

Appendix 01: Microbial results of fungal flora macroscopic (1) and microscopic aspect(2) (Yeasts at 40 x magnification /Molds at 10 x magnification)

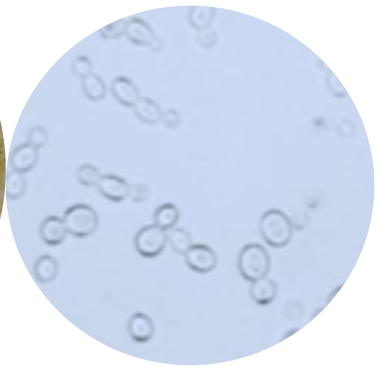
Yeasts:

1 ***Candida*** 2

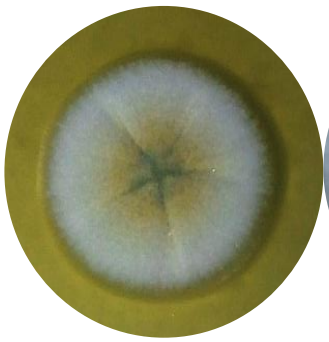
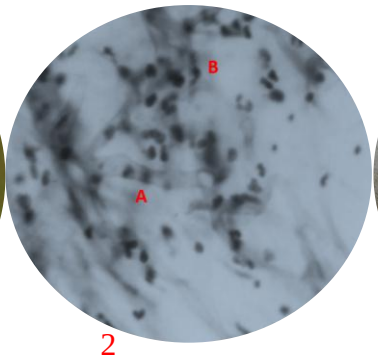



1 ***Saccharomyces bayanus*** 2

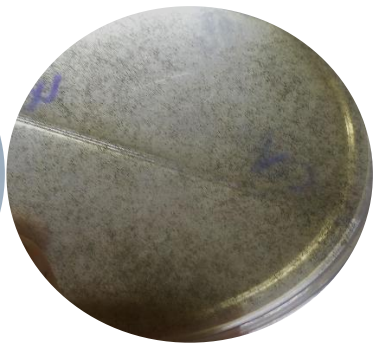
1 ***Rhodotorula*** 2

Molds :

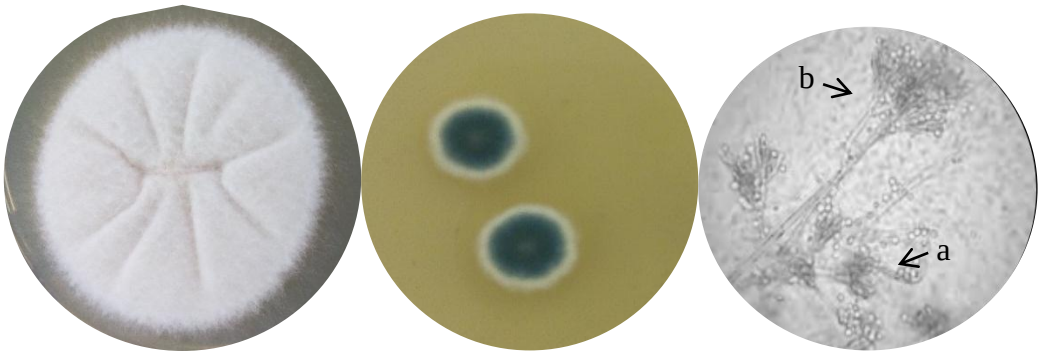



1 2

Mucor :A = Coenocytic mycelium /B=Destroyed sporangium with liberated spores.

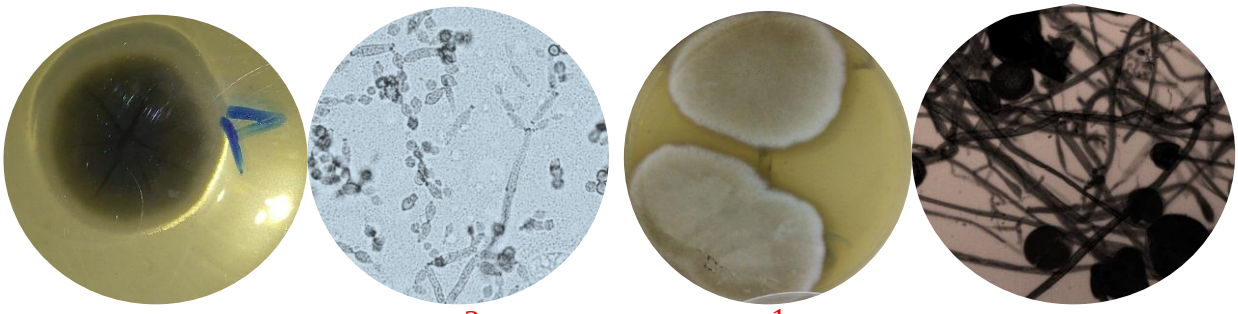



1 ***Fusarium*** 2

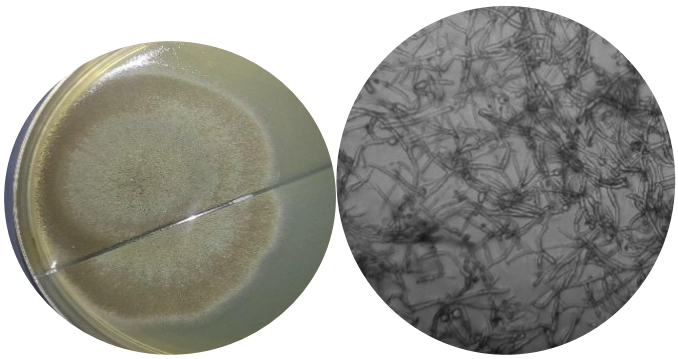


1 1 2

Penicillium a= conidia , b= conidiophore



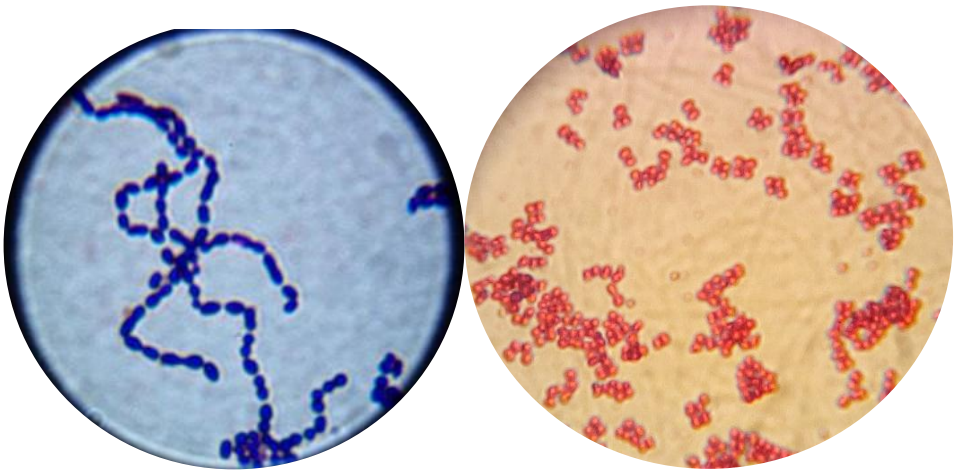
1 ***Cladosporium*** 2 1 ***Rhizopus*** 2



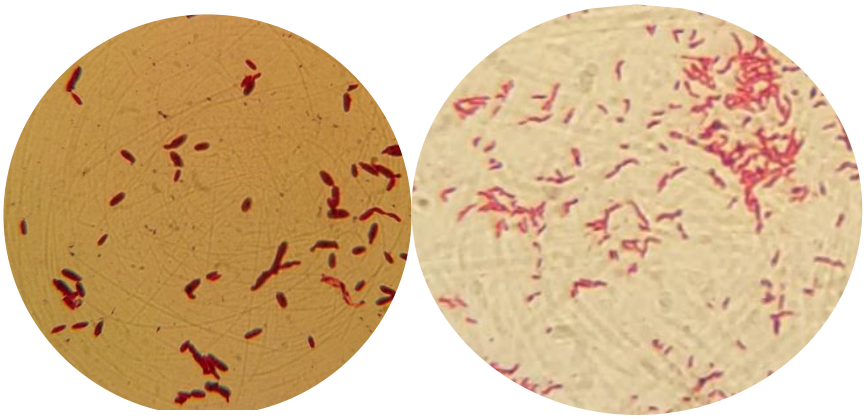
1 2

Aspergillus sp

Appendix 02 : Gram staining results (100 x magnification)

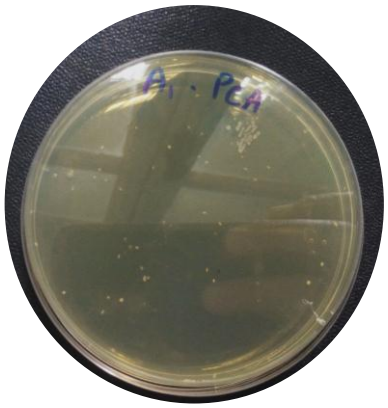


Streptococciaceae ***Niessieriaceae***

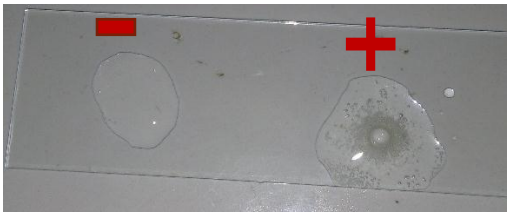


Enterobacteriaceae

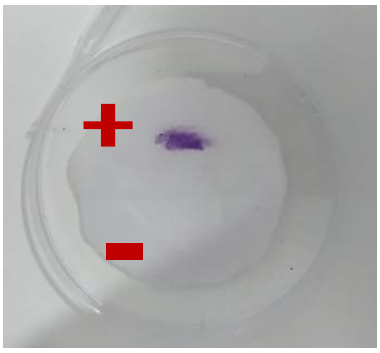
Appendix 04: Catalase , oxidase tests results and bacterial contamination



Bacterial colonies



Catalase test



Oxidase teste

Appendix 06: pH & Brix results

Brix screen scale **pH metre**

Abstract

We evaluated the physicochemical and microbiological quality of a CSD '' Coca Cola '' conditioned in all three forms of packaging. We performed a series of analyses by applying the DLA and spread methods and tracked the changes in pH and brix. The studies revealed that the pH is unaffected, but the sugar quantity is entirely reliant on mic robial infection. In terms of microbiological findings, the CSD is of satisfactory quality however we detected yeasts, molds, and aciduric bacteria Coca Cola even has an antibacterial impact on *Enterobacteriaceae, Neisseriaceae, and Streptococc aceae*. Therefore, we stated that aluminum is the safest type since it guarantees risk-free consumption when compared to glass and PET bottles. The species that are most likely to ruin the product in PET bottles vary from those that spoil the product in glass bottles. It has been hypothesized that the rate of bacterial contamination is higher in PET bottles, whereas the incidence of fungal contamination is higher in glass bottles. When it comes to protecting the CSD after they've been opened, PET outperforms glass and metal.

الملخص

قمنا بتقييم مقارن للجودة الفيزيائية والكيميائية والميكروبيولوجية للمشروب الغازي كوكا كولا معبا ضمن انواع العبوات الثلاث. أجرينا سلسلة من التحليلات عن طريق تطبيق تقنية الطبقة المزدوجة وتقنية الانتشار للزرع وتعقبنا التغيرات في الأس الهيدروجيني ومعدل السكر المنحل. كشفت الدراسات أن الأس الهيدروجيني لا يتأثر، لكن معدل السكر متعلق نسبيا بالعدوى الجرثومية. من حيث النتائج الميكروبيولوجية، فإن المشروب الغازي المدروس ذو جودة مرضية. الا اننا اكتشفنا الخمائر والعفن والبكتيريا الحمضية. للكوكا كولا ايضا تأثير مضاد للجراثيم من الاصناف التالية: *البكتيريا المعوية، السبحيات ونظيرات النيسرية*. حيث لاحظنا تناقصا في الحمل الكتيري. توصلنا الى أن الألومنيوم هو النوع الأكثر أمانًا لأنه يضمن استهلاكًا خاليًا من المخاطر عند مقارنته بالنوعين الاخرين. تختلف الاصناف الجرثومية التي من المرجح أن تتلف جودة المشروب الغازي حسب نوع العبوة. ان معدل التلويث البكتيري أعلى في زجاجات ع. ا. م.م. ح، في حين أن حدوث التلويث الفطري أعلى في الزجاجات. عندما يتعلق الأمر بحماية المشروب الغازي بعد فتحه، تتفوق عبوات ال ع. ا. م.م. ح على الزجاج والمعدن.

Résumé

Nous avons évalué la qualité physico-chimique et microbiologique du BG "Coca-Cola" conditionné dans les trois types d'emballage. Nous avons effectué une série d'analyses en appliquant des méthodes de gélose à double couche et de diffusion en surface, et suivi les changements de pH et de Brix. Les études ont montré que la valeur du pH n'est pas affectée est constante, mais le taux de Brix est complètement lié aux contamination microbiennes. Concernant la microbiologie du Coca Cola la qualité est satisfaisante, mais nous avons détecté la présence des levures, moisissures et des bactéries acidophiles. Coca Cola a même des effets antibactériens sur *Enterobacteriaceae, Neisseriaceae et Streptococcus*. Par conséquent, nous disons que l'aluminium est le type le plus sûr car il peut garantir une consommation sans risque par rapport aux bouteilles en verre et aux bouteilles en PET. Le type de produit le plus susceptible d'endommager la bouteille PET est différent du type de produit contenu dans la bouteille en verre. On suppose que le taux de contamination bactérienne dans les bouteilles en PET est plus élevé, tandis que le taux de contamination fongique dans les bouteilles en verre est plus élevé. Dans la protection du BG après ouverture, le PET est meilleur que le verre et le métal.