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Production of Dark Chocolate Using Degla Beida Date Sugar Enriched with Spirulina

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Dedication

﴿ وَقُل رَّبِّ ارْحَمْهُمَا كَمَا رَبَّيَانِي صَغِيرًا﴾

All praise is due to Allah, whose guidance illuminated my path, whose mercy strengthened my determination, and whose blessings enabled me to complete this humble work.

I dedicate the fruit of my efforts and this academic achievement to those who have been the greatest source of support and inspiration throughout my journey:

To my beloved father, Moussa, whose sacrifices, wisdom, and unwavering encouragement have been the foundation of my success. May Allah reward him abundantly and grant him health, happiness, and a long life.

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﴿ رَبِّ أَوْزِعْنِي أَنْ أَشْكُرَ نِعْمَتَكَ الَّتِي أَنْعَمْتَ عَلَيَّ وَعَلَىٰ وَالِدَيَّ﴾

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﴿ وَمَا تَوْفِيقِي إِلَّا بِاللَّهِ ۚ عَلَيْهِ تَوَكَّلْتُ وَإِلَيْهِ أُنِيبُ﴾

I dedicate this humble work to all of you with profound gratitude and appreciation, praying that it may contribute, even modestly, to the advancement of knowledge and serve as a source of benefit to others.

Mouna Dehbi

Dedication

﴿ وَقُل رَّبِّ زِدْنِي عِلْمًا﴾

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الاهداء

بسم الله الرحمن الرحيم

(فَرحينَ بِمَا أتاهُمُ اللَّهُ مِن فَضْلِهِ)

هذه اللحظة لا تورث ولا تشترى، لقد دفعت ثمنها عمري وشبابي، سهري، خوفي، ودموعي، هي لهفة الوصول، ودمعة الحزن، فرحة العمر هذه هي النهاية السعيدة لبدايات أعظم وأصبح حلمي حقيقة.

اهدي تخرجي

الى نفسي الطموحة ,التي بدات بطموح و انتهت بنجاح.

الى من احمل إسمه بكل فخر ,الى من حصد الأشواك في طريقي ليمهد لي درب العلم ,الى من كان سندي بعد الله ,الى من علمني أن الإرادة تصنع المعجزات ,الى من غرس في قلبي حب العلم و رافقني في كل خطوة (أبي الغالي)

لى من اوصاني الله بها خيرا ,وجعل الجنة تحت قدامها ,الى من اطعمتني كما تطعم الطير صغارها ,الى من بفضل دعائها تحقق نجاحي,الى بلسم جراحي ,الى وطني الكبير الذي لا تحده حدود الجغرافيا (أمي الغالية)

إلى ضلعي الثابت وأمان ايامي

(اخوتي)

لكل من كان عوناً وسنداً في هذا الطريق ...للأصدقاء الأوفياء ورفقاء السنين

لأصحاب الشدائد والأزمات.

إلى من أفاضني بمشاعره ونصائحه المخلصة.

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ملاك زرقي

Production of Dark Chocolate Using Degla Beida Date Sugar Enriched with Spirulina

Abstract

The growing demand for healthier and sustainable food products has encouraged the exploration of natural alternatives to refined sugar and the incorporation of functional ingredients with high nutritional and therapeutic value. In this context, the present study aimed to develop a functional chocolate formulation using sugar extracted from the local date cultivar Degla Beida as a natural sweetening agent, combined with Spirulina (Arthrospira platensis) as a source of bioactive compounds. The study also sought to evaluate the physicochemical and functional properties of the developed product. Water-soluble sugars were extracted from Degla Beida dates collected from the Reguiba region, El Oued Province (Southeastern Algeria), using aqueous extraction followed by freeze-drying. Physicochemical and biochemical analyses were performed to determine total sugars, reducing sugars, proteins, and total phenolic compounds using standard analytical methods. Subsequently, spirulina-enriched chocolate formulations were prepared, and their physicochemical, microbiological, and functional properties were evaluated.the obtained results showed that the extraction yield of Degla Beida date sugar reached 42.8%. Chemical characterization revealed high contents of total sugars (60.62 ± 0.26%) and reducing sugars (59.65 ± 2.01%), together with appreciable levels of proteins (12.35

± 1.04%) and total phenolic compounds (29.58 ± 1.63%). Physicochemical analyses indicated a moisture content of 20%, a pH value of 6.36, a mineral content of 2.8%, and an electrical conductivity of 21.9 µS/cm, demonstrating suitable technological characteristics for chocolate manufacturing. The extracted date sugar contributed to improved texture, structural stability, sweetness, and sensory acceptability of the final product. Microbiological analyses confirmed the absence of pathogenic microorganisms, indicating satisfactory microbiological quality and product safety. Furthermore, antioxidant evaluation of the developed chocolate using the DPPH assay revealed a moderate radical-scavenging activity with an IC_{50} value of 1.47 ± 0.19 mg/mL. In addition, the spirulina-enriched chocolate exhibited promising antibacterial activity against pathogenic bacteria, highlighting its potential as a functional food with added health-promoting benefits. These findings demonstrate that the combination of Degla Beida date sugar and Spirulina can be successfully used to produce a functional chocolate with enhanced nutritional value, antioxidant capacity, antibacterial potential, and desirable technological and sensory properties.

Keywords: Functional chocolate, Spirulina platensis, Degla Beida,date sugar

الملخص

أدى الطلب المتزايد على المنتجات الغذائية الصحية والمستدامة إلى تشجيع البحث عن بدائل طبيعية للسكر المكرر وإدماج مكونات وظيفية ذات قيمة غذائية وعلاجية مرتفعة .وفي هذا السياق، هدفت هذه الدراسة إلى تطوير تركيبة شوكولاتة وظيفية باستخدام سكر مستخلص من صنف التمور المحلي الدقلة البيضاء كمُحلٍّ طبيعي، مع إضافة سبيرولينا (Arthrospira platensis)كمصدر للمركبات الحيوية الفعالة .كما سعت الدراسة إلى تقييم الخصائص الفيزيائية والكيميائية والميكروبيولوجية للمنتج المطور .تم استخلاص السكريات الذائبة في الماء من ثمار الدقلة البيضاء المجمعة من منطقة الرقيبة بولاية الوادي)جنوب شرق الجزائر (باستخدام الاستخلاص المائي متبوعًا بالتجفيف بالتجميد .وأُجريت تحاليل فيزيائية وكيميائية وحيوية لتحديد محتوى السكريات الكلية والسكريات المختزلة والبروتينات والمركبات الفينولية الكلية باستخدام طرق تحليلية معيارية .بعد ذلك، تم تحضير تركيبات شوكولاتة مدعمة بالسبيرولينا وتقييم خصائصها الفيزيائية والكيميائية والميكروبيولوجية والوظيفية,أظهرت النتائج أن مردود استخلاص سكر الدقلة البيضاء بلغ .42.8%كما بينت التحاليل الكيميائية احتواء المستخلص على نسب مرتفعة من السكريات الكلية (60.62 ± 0.26%)والسكريات المختزلة (59.65 ± 2.01%)، إلى جانب محتوى معتبر من البروتينات (12.35 ± 1.04%)والمركبات الفينولية الكلية .(29.58 ± 1.63%)وأظهرت التحاليل الفيزيائية والكيميائية أن المستخلص يتميز بنسبة رطوبة بلغت 20%، وقيمة أس هيدروجيني (pH)قدرها 6.36، ومحتوى معدني قدره 2.8%، وموصلية كهربائية بلغت 21.9ميكروسيمينز/سم، مما يدل على امتلاكه خصائص تكنولوجية مناسبة لصناعة الشوكولاتة .كما ساهم سكر التمور المستخلص في تحسين القوام والثبات البنيوي والحلاوة والتقبل الحسي للمنتج النهائي .وأكدت التحاليل الميكروبيولوجية خلو المنتج من الكائنات الحية الدقيقة الممرضة، مما يدل على جودة ميكروبيولوجية مرضية وسلامة المنتج . كما أظهر تقييم النشاط المضاد للأكسدة باستخدام اختبار DPPHقدرة متوسطة على تثبيط الجذور الحرة، حيث بلغت قيمة IC_{50} 1.47 ± 0.19ملغ/مل .بالإضافة إلى ذلك، أظهرت الشوكولاتة المدعمة بالسبيرولينا فعالية واعدة ضد البكتيريا الممرضة، مما يعزز إمكاناتها كغذاء وظيفي ذي فوائد صحية إضافية .وتؤكد هذه النتائج إمكانية الاستخدام المشترك لسكر الدقلة البيضاء والسبيرولينا في إنتاج شوكولاتة وظيفية تتميز بقيمة غذائية محسنة وقدرة مضادة للأكسدة ومضادة للبكتيريا، إلى جانب خصائص تكنولوجية وحسية مرغوبة.

الكلمات المفتاحية: الدقلة البيضاء، سكر التمور، الشوكولاتة الوظيفية، السبيرولينا (Spirulina (platensis

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Introduction

Sugar is one of the most widely consumed food ingredients worldwide, being incorporated into a wide variety of food products and beverages. Despite its important role in providing sweetness and enhancing the sensory characteristics of foods, excessive sugar consumption negatively affects dietary quality because it supplies high amounts of energy without providing essential nutrients. Furthermore, excessive intake of sugar has been associated with an increased risk of obesity, diabetes mellitus, hypertension, cardiovascular diseases, dental caries, and other health disorders related to elevated blood glucose levels (WHO, 2019).

Chocolate is among the most popular confectionery products consumed worldwide across all age groups, and its global demand continues to increase. According to consumption statistics, Switzerland ranks first globally in chocolate consumption, followed by Austria, reflecting the strong cultural and dietary association between these populations and chocolate products (Zugravu and Otelea, 2019). Beyond its desirable sensory attributes, chocolate has attracted considerable scientific interest because of its nutritional and functional properties, particularly those of dark chocolate.

Cocoa was first cultivated approximately 5,300 years ago in Central America by the Mayo-Chinchipe civilization (Fanton et al., 2021). Cocoa beans are recognized as a rich source of phenolic compounds and natural antioxidants, with polyphenols representing nearly 10% of their dry weight. Flavonoids such as catechins, anthocyanins, and proanthocyanidins are among the major bioactive compounds present in cocoa products (Zugravu and Otelea, 2019). Owing to these compounds, chocolate is considered a functional food with potential health benefits, including the prevention of several chronic and degenerative diseases such as diabetes, obesity, and Alzheimer's disease (Araujo et al., 2016; Gianfredi et al., 2018).

Dark chocolate contains substantially higher levels of polyphenols and flavonoids than milk and white chocolate, reaching nearly five times greater concentrations. In contrast, milk and white chocolate contain considerably higher amounts of fat and sugar, approximately 30 g/100 g and 52 g/100 g, respectively (Fanton et al., 2021). Consequently, dark chocolate represents an excellent matrix for the incorporation of functional ingredients aimed at enhancing its nutritional and health-promoting properties.

In response to growing health concerns regarding refined sugar consumption, increasing

attention has been directed toward natural sweeteners as healthier alternatives. Among these, date sugar has gained particular interest because of its content of natural sugars, dietary fiber, minerals, and antioxidant compounds, as well as its relatively lower glycemic impact compared with refined sugar. Several studies have investigated the use of date sugar as a sugar substitute in different food products and demonstrated its potential to improve nutritional quality while reducing the negative health effects associated with refined sugar consumption (Pandey, 2021). However, the incorporation of date sugar into functional chocolate formulations remains insufficiently explored, particularly when combined with other bioactive ingredients.

Spirulina (Arthrospira platensis) is a microalga recognized for its exceptional nutritional profile and diverse health-promoting properties. It contains high-quality protein, essential amino acids, polyunsaturated fatty acids, vitamins, minerals, and powerful antioxidant compounds. Previous studies have reported that Spirulina exhibits antioxidant, anti-inflammatory, and immunomodulatory activities, in addition to its beneficial effects on cholesterol and triglyceride levels and cardiovascular health. These properties are mainly attributed to bioactive compounds such as phycocyanin, β-carotene, and xanthophylls, which help reduce oxidative stress and protect cells from damage.Despite the growing interest in developing healthier chocolate products, there remains a need to formulate dark chocolate that combines the technological and sensory advantages of chocolate with the nutritional benefits of natural sweeteners and bioactive ingredients. Although date sugar has been previously studied as a natural alternative to refined sugar, limited information is available regarding its combined use with Spirulina in dark chocolate and the resulting effects on product quality, nutritional value, antioxidant capacity, antibacterial activity, and consumer acceptability. This gap highlights the importance of investigating the feasibility of developing a functional dark chocolate enriched with these natural ingredients while maintaining desirable physicochemical, microbiological, and sensory characteristics.

Therefore, this study aims to develop a functional dark chocolate by replacing refined sugar with date sugar as a natural sweetener and fortifying it with Spirulina (Arthrospira platensis). The study further seeks to evaluate the effects of date sugar and Spirulina incorporation on the physicochemical, microbiological, and sensory properties of the chocolate, as well as their contribution to enhancing its nutritional value, antioxidant potential, and antibacterial activity.

Part One

Chapter I: CHOCOLAT

I.1.Generality:

Chocolate is one of the most widely desired foods in the world. Initially, it was considered a luxury product; however, it is now also regarded as having medicinal properties **(BOUBAAYA,D,et al 2023)**

I.2.Definition of chocolate:

Chocolate is widely considered a promising functional food because of its rich content of polyphenols, particularly flavonoids, which are associated with positive health effects **(Delgado et al., 2018).** As a primary product derived from cocoa, chocolate contains compounds recognized for their antioxidant properties, which are especially beneficial for cardiovascular health. However, the extent of these benefits varies depending on the type of chocolate consumed, such as dark, milk, or white chocolate **(Amaya & Pabón, 2017).**

I.3.Main Types of Chocolate:

Chocolate exists in several varieties, each differing in composition, flavor, and cocoa content. Pure unsweetened chocolate, commonly known as baking chocolate, mainly consists of cocoa solids and cocoa butter in varying proportions. However, most commercially consumed chocolate is sweetened with sugar and combined with other ingredients.

I.3.1.Milk Chocolate:

Milk chocolate is a sweetened form of chocolate that contains milk powder or condensed milk. Regulations in the United Kingdom and Ireland require milk chocolate to contain at least 20% dry cocoa solids, while the minimum cocoa solid content in the European Union is 25%.**(Mahale Rajeshwari Bhausaheb.2021)**

I.3.2.White Chocolate:

White chocolate has a texture similar to milk and dark chocolate but does not contain cocoa solids, which are responsible for the characteristic brown color of chocolate. According to the United States Food and Drug Administration (FDA), white chocolate is produced from cocoa butter, milk solids, sweeteners, and other approved ingredients, without the addition of nonfat cocoa solids. **(JORA2022)**

I.3.3.Dark Chocolate:

Dark chocolate is manufactured by combining cocoa mass with sugar and fat. The FDA refers to it as “sweet chocolate” and requires a minimum chocolate liquor content of 15%, whereas European standards specify at least 35% cocoa solids. Generally, a higher cocoa

content results in a more bitter taste. Semisweet chocolate contains relatively low amounts of sugar, while bittersweet chocolate includes additional cocoa butter and vanilla with less sugar than semisweet varieties. Both types are commonly used interchangeably in baking applications. Properly stored dark chocolate can maintain its quality for up to two years. Although dark chocolate is often associated with health benefits, current scientific evidence remains insufficient to confirm significant effects on blood pressure or overall health. **(JORA 2022)**

I.3.4.Unsweetened Chocolate:

Unsweetened chocolate, also called bitter or baking chocolate, is composed entirely of pure chocolate liquor without added sugar or flavoring agents. It is produced from roasted and ground cocoa beans, giving it an intense and rich chocolate flavor. This variety is mainly used in baking and confectionery products where additional ingredients are incorporated. Raw chocolate generally belongs to the dark chocolate category and typically contains at least 75% cocoa.

Improperly tempered or poorly stored chocolate may develop whitish discolorations known as chocolate bloom. This occurs when sugar or fat separates from the chocolate surface during storage. Although bloom affects appearance and texture, the chocolate remains safe for consumption. **(Mahale Rajeshwari Bhausaheb.2021)**

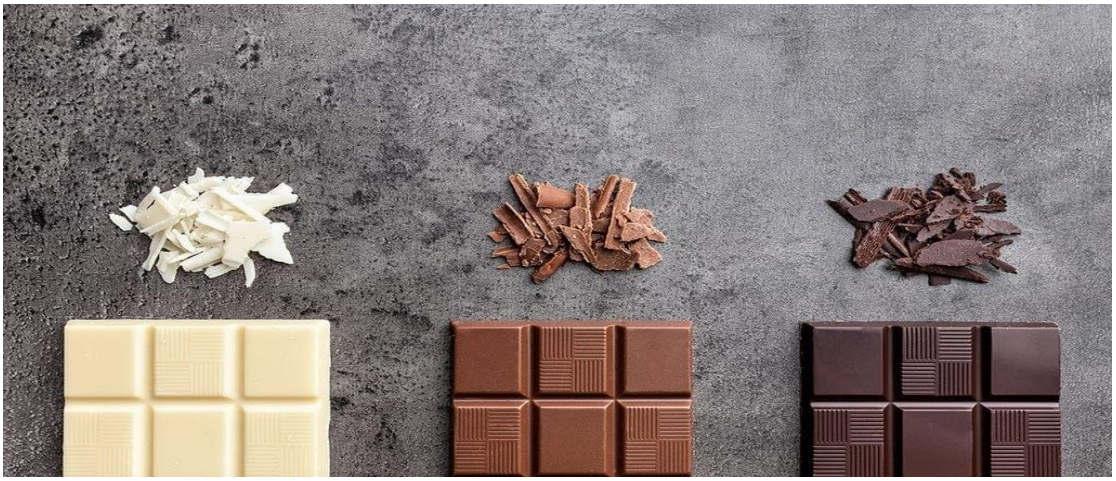

Figure 1: Type of chocolat (JORA 2022)

I.4.Composition of Chocolate :

I.4.1. Cacoa :

Cocoa is the primary raw material used in chocolate production. Cocoa derivatives are obtained through various processes such as extraction, pressing, and grinding. These derivatives may undergo chemical treatment and can be blended with sugars or other ingredients depending on industrial applications.

Cocoa beans undergo several key post-harvest stages that strongly influence the quality of the final product. Among the most important is fermentation, which is essential for removing the pulp surrounding the beans. During fermentation, a natural microbial ecosystem develops, mainly consisting of yeasts, lactic acid bacteria, and acetic acid bacteria. These microorganisms produce ethanol and organic acids, which diffuse into the beans and trigger complex biochemical reactions that contribute to the development of cocoa's characteristic properties.

The flavor profile of cocoa is further developed through a sequence of processing steps, including fermentation, drying, and roasting. These stages enhance the formation and transformation of volatile compounds responsible for the characteristic aroma and taste of cocoa and chocolate products.

Cocoa is also rich in a wide range of bioactive compounds, including methylxanthines such as theobromine and theophylline, as well as various amines and alkaloids. It additionally contains phenolic compounds, sulfur-containing compounds, and volatile aromatic molecules such as terpenes and alcohols. These constituents play a crucial role in defining the sensory attributes of cocoa, particularly its aroma, flavor complexity, and overall quality**(Chévez-Vera, H. D.,et al 2021).**

Figure 2 : Cacoa paste (Amraoui,A.M., et al 2023)

I.4.2. Sugar :

In recent years, attention has increasingly focused on the ingredients of chocolate and the role of each component, particularly sugar, which does not only provide sweetness but also contributes to the bulk, texture, and overall sensory properties of chocolate.

In chocolate manufacturing, sugar cannot be fully replaced by high-intensity sweeteners combined with bulking agents without affecting the final quality, as this leads to a loss of the characteristic texture that is largely dependent on sugar. Similarly, reducing or replacing sugar significantly disrupts the overall balance of the formulation.

Replacing sugar by increasing cocoa content may improve some nutritional aspects, but it also significantly alters the flavor, since chocolate is a complex system of fine particles dispersed within a fat matrix, and any change in particle size or distribution directly affects mouthfeel and texture.

Chocolate texture is also influenced by several factors such as fat content, particle size distribution, and moisture level. Since sugar represents a large portion of chocolate formulation, reducing it leads to noticeable changes in the final structure.

In addition, some sugar substitutes do not provide the same functional properties and may result in undesirable textures such as stickiness or loss of smoothness. Therefore, rheological properties such as viscosity and flow behavior play an important role in determining chocolate quality and sensory perception.

Overall, any modification of chocolate formulation, especially sugar reduction, requires precise control of ingredients to maintain the characteristic texture, taste, and overall sensory quality of chocolate. **(Ma,K.K.,Ziegler,et al 2024)**

Figure 3 : Sugar (Ma,K.K.,Ziegler,et al 2024)

I.4.3. Cacoa butter:

Cocoa butter is the main ingredient in the manufacturing of confectionery products such as chocolate. It is a pale yellow fat with a unique aroma and chocolate taste. It is an important component and the only continuous fat phase that aids in the dispersion of other ingredients in chocolate [3, 20]. The role of cocoa butter in chocolate making includes providing texture, flavor, and mouthfeel. Cocoa butter has a melting point of 27 to 35 °C. Its composition consists of palmitic, stearic, and oleic fatty acids, giving it a smooth melting texture when consumed**(Loke, Y. H.,et al 2024).**

Figure 4 : cacoa butter (Amraoui,A.M,et al 2023)

I.4.4. Soy Lecithin :

Lecithin is one of the oldest and most widely used emulsifiers in the chocolate industry. Soy lecithin was first patented in Germany in 1931. Lecithin (E322) mainly consists of a lipid mixture rich in phospholipids, which account for more than 50% of its composition, and it may originate from either plant or animal sources.

Phospholipids are essential components of biological membranes and are naturally present in foods of animal, marine, and plant origin. Commercial lecithin can therefore be obtained from several sources, including eggs, milk, fish, and plant seeds.

Today, vegetable lecithins are the most commonly used emulsifiers in the food industry because of their low toxicity and high nutritional value. In the chocolate industry, soy lecithin derived from soybean seeds is the most widely used type. However, lecithins extracted from other seeds, such as sunflower and rapeseed, are also being explored for use in the production of various food products**.(Toker,O.S. et al 2024)**

Figure 5 : Soy Lecithin (Toker,O.S. et al 2024)

I.4.5. Sweeteners:

Consuming large amounts of sugar increases the number of calories in the body, which can eventually lead to health problems such as diabetes, weight gain, and sudden spikes in blood glucose levels due to its rapid absorption. High blood glucose levels may result in diabetes and hyperglycemia.

Natural sweeteners are derived from various plant and animal sources, such as honey, a natural sweetener produced by bees from flower nectar. Natural sweeteners generally have a lower glycemic index, which is why many people prefer them over refined sugar. Common natural sweeteners include honey, dates, coconut sugar, maple syrup, molasses, and agave nectar**(Pandey,P 2021).**

I.5.Benefits of Chocolate :

Chocolate consumption has increased worldwide in recent years, with dark chocolate gaining particular popularity due to its high cocoa content and its potential health benefits compared to milk chocolate. In contrast, milk chocolate may be linked to less desirable effects because of its high sugar levels. Therefore, dark chocolate—rich in cocoa, flavonoids, and theobromine, and low in sugar—is more commonly associated with positive health outcomes than other types of chocolate, including a reduced risk of cardiovascular diseases. Additionally, cocoa has been shown to contribute to improved blood pressure regulation and better insulin sensitivity **(Latif, 2013; Montagna et al., 2019).**

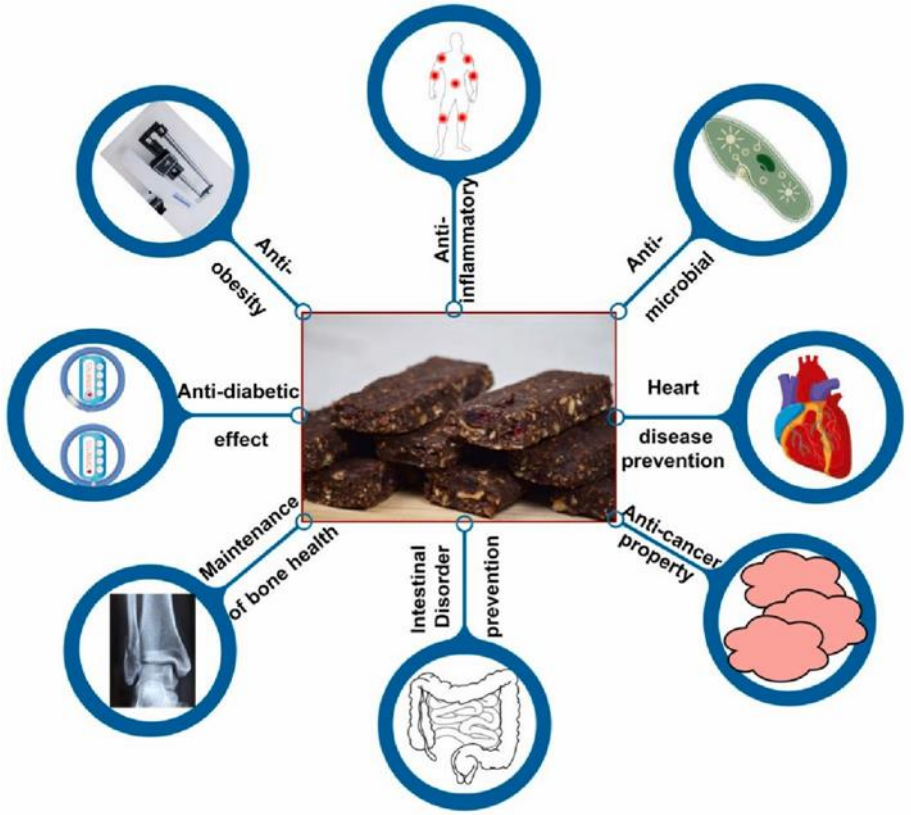

Figure 6 : Health benefits of fortified dark chocolate (Samanta, S., et al 2022).

I.6.Echnological Features of Chocolate Production:

I.6.1. Cocoa Bean Fermentation Stage:

Chocolate production begins with the fermentation of cocoa beans, a process that is mostly spontaneous and naturally occurring. Its main purpose is to remove the pulp surrounding the beans to facilitate drying. This stage is essential for the formation of volatile compounds such as alcohols, esters, and organic acids, which contribute to the characteristic cocoa flavor. Temperature and pH strongly influence microbial activity, enzyme function, and the biochemical transformations occurring within the beans. **(Tuigunov,D et al , 2025)**

I.6.2.Anaerobic Fermentation Stage:

At the beginning of fermentation, cocoa beans are in an acidic environment with high sugar content and limited oxygen availability. These conditions allow yeasts and lactic acid bacteria to become active. These microorganisms break down the sugars and organic acids in the pulp, producing ethanol, organic acids, and aromatic compounds responsible for cocoa flavor development. During this stage, the pH gradually decreases due to bacterial activity. **(Tuigunov,D et al , 2025)**

I.6.3.Aerobic Fermation stage :

In this stage, acetic acid bacteria oxidize ethanol into acetic acid in the presence of oxygen, leading to a temperature increase of approximately 45–50 °C. As fermentation progresses, the population of these bacteria declines due to high temperatures. Heat-resistant bacteria from the genus Bacillus may appear and can negatively affect flavor and aroma quality if fermentation conditions are not properly controlled**. (Tuigunov,D et al , 2025)**

I.6.4. Cocoa Bean Drying Stage:

After fermentation, cocoa beans are dried to reduce moisture content and improve storage stability. Drying can be carried out using sun drying, solar dryers, ovens, or microwave systems. Drying duration and temperature significantly affect bean quality and the final sensory properties of chocolate. Proper drying helps reduce undesirable compounds while preserving cocoa quality. **(Raj,T.A.A et al 2023)**

I.6.5. Cocoa Bean Roasting Stage:

Roasting aims to develop the characteristic flavor and aroma of chocolate while making the beans microbiologically safe and more brittle for easier grinding. This process is typically carried out using hot air at temperatures ranging from 130 to 150 °C for 15 to 45 minutes. It contributes to the development of the final color and flavor of chocolate**. (Raj,T.A.A et al 2023)**

I.6.6. Cocoa Bean Grinding Stage:

After roasting, cocoa beans are ground using specialized mills to obtain a smooth paste known as cocoa mass or cocoa liquor. This mass is the primary raw material used in the production of different types of chocolate and cocoa-based products. **((Raj,T.A.A et al 2023).**

I.6.7. Conching Stage:

Conching is one of the most important stages in chocolate manufacturing. During this process, the chocolate mass is continuously mixed and heated while fats and emulsifiers are added. This improves texture and fluidity, reduces viscosity, and helps remove undesirable volatile compounds, thereby enhancing the final flavor of chocolate. The efficiency of this process depends on factors such as duration, temperature, and aeration**. (Raj,T.A.A et al 2023)**

I.6.8. Tempering Stage:

This stage aims to control the crystallization of cocoa butter to obtain chocolate with good gloss, stable texture, and an appropriate melting profile. The process begins by heating the chocolate to melt all fat crystals, followed by gradual cooling with stirring to form stable crystals, and finally reheating slightly to preserve only the desired crystal form before molding . **(Samanta,S.et al 2022)**

I.6.9. Cooling, Packaging, and Storage Stage:

After completing all previous stages, the chocolate is gradually cooled and then packaged using materials that protect it from light, moisture, and oxygen. It is recommended to store chocolate at temperatures between 18 and 20 °C with low humidity to maintain its quality and sensory properties throughout its shelf life. **(Samanta,S.et al 2022)**

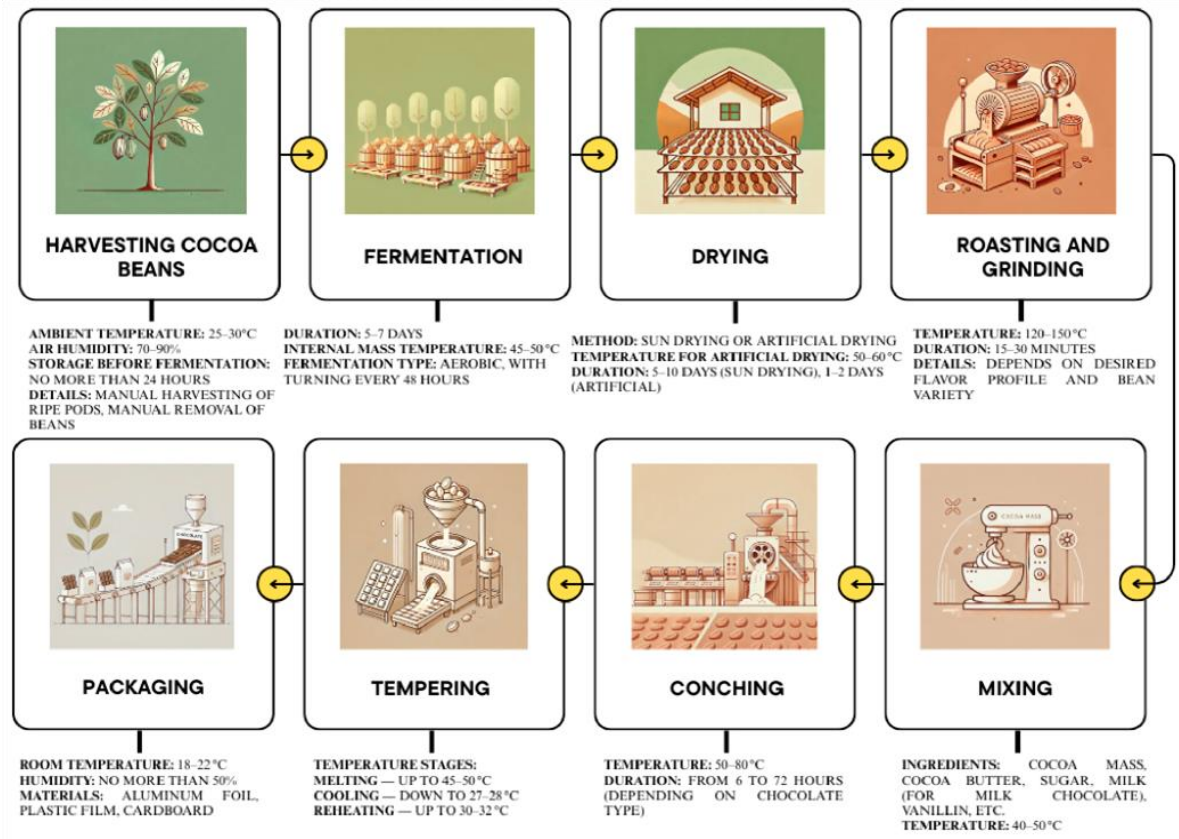

Figure 7 : Technological process of chocolate production (Tuigunov,D et al , 2025)

I.7. Date as sweatener :

I. 7.1.Definition of dates :

The fruit of the date palm (Phoenix dactylifera L.) has served as a vital food source for more than 6000 years, particularly in arid and semi-arid regions such as the Middle East and North Africa **(Ashraf and Hamidi-Esfahani, 2011; Khwaldia et al., 2023).**

Dates are considered highly valuable due to their rich nutritional composition and economic importance, which gives them strong potential as a food resource **(Khan and Khan, 2019)**. The palm tree begins to bear fruit after approximately five years and can remain productive for up to 60 years, with an average annual yield of 400–600 kg per tree **(AlShwyeh and Almahasheer, 2022).**

Beyond their nutritional value, dates also hold deep cultural and religious significance, particularly in Islam, where the date palm is frequently mentioned in the Holy Quran **(Roumani et al., 2024).**

In Islamic tradition, the Prophet Muhammad (peace be upon him) compared the date palm to a believer and encouraged the consumption of dates for their health benefits. He also stated that eating seven Ajwa dates in the morning protects a person from poison or magic on that day (reported by Al-Bukhari).

Figure 8 : fruits of deglet beida (Ben-Salah;2021).

I.7. 2. Classification of dates :

According to **(Benkreif et Bennouri al., 2024),** date fruits show a wide variation in texture. Based on this characteristic , they are classified into three main groups:

I.7.2.1.Dry dates :

These are firm fruits with a moisture content below 20%. They are rich in sucrose and have a floury texture, such as Degla-Beida and Mech-Degla.

I.7.2.2.Soft dates :

These contain at least 30% moisture and are mainly composed of invert sugars (glucose and fructose), such as Ghars and Litima.

I.7.2.3. Semi-soft dates :

These have an intermediate moisture content ranging from 20% to 30%, such as Deglet-Nour and Hamraia.

I.8.Nutritional value of dates :

Dates are characterized by _easily digestible sugars and provide a high level of energy to the consumer. They are rich in_ several vitamins, such as vitamin A, which enhances vision and is essential for growth and reproduction. They also contain vitamins B1 and B2, which are important for the nervous system; their deficiency may lead to fatigue, exhaustion, memory loss, and nervous tension. In addition, dates contain vitamin C, which helps prevent scurvy. All of these vitamins are essential for various biological reactions in the body.

Moreover, dates are a good source of minerals such as calcium (Ca), phosphorus (P), potassium (K), and magnesium (Mg) **(Mahmoud, 2017).**

I.9.Biochemical Composition of Dates :

I.9.1. Main components of date pulp

- **Water:**

The moisture content in date pulp varies depending on the cultivar, ripening stage, and climatic conditions. Generally, it ranges from 30% to 80%. The following table shows the moisture content of some date varieties **(Khalifa,H.S et al 2024)**

- **Sugars:**

Sugars are the predominant component of dates, representing between 60% and 80% of the dry weight of the pulp. This proportion varies according to cultivar, climate, and ripening stage. Analytical studies have identified three main sugars: sucrose, glucose, and fructose, in addition to small amounts of other sugars such as galactose and xylose **((Khalifa,H.S et al 2024)**

❖ **Dietary fibers:**

Dates are rich in dietary fiber, which accounts for about 2% to 6% of the dry weight in low-quality dates and may reach up to 10% in high-quality varieties. The fruit cell wall is composed of several types of fibers, including pectin, cellulose, hemicellulose, and lignin. These fibers are known to help prevent certain health disorders such as appendicitis, varicose veins, and hemorrhoids **.(Mihoubi,M., et al 2025)**

❖ **Phenolic compounds:**

Like other plant-based products, dates contain a variety of phenolic compounds, including cinnamic acids, flavones, flavanones, and flavonols, which contribute to their color and aroma. These compounds also play an important role in human health by helping to reduce blood pressure, enhance immune function, and provide anti-inflammatory effects **(Farhi soumia ,2021)**

I.9.2. Secondary components of date pulp

❖ **Proteins:**

Dates contain a relatively low protein content, ranging from 2.5% to 7.5% of dry weight. Although these proteins are of good nutritional quality, their quantity is too low for dates to be considered a primary protein source. Moreover, these proteins may affect juice extraction by causing turbidity and contributing to browning and sediment formation during ripening **. (Khalifa,H.S et al 2024)**

❖ **Lipids:**

The lipid content in dates ranges from 0.43% to 1.9%, depending on the variety and stage of ripening. **(Khalifa,H.S et al 2024)**

❖ **Mineral elements:**

Dates are considered rich in essential minerals, particularly potassium, magnesium, phosphorus, and calcium. Their mineral content ranges between 1.10% and 3.69% of the dry weight **.(Mihoubi,M., et al 2025)**

I.10.Valorisation of date

Dates constitute a highly valuable raw material for the production of a wide range of value-added products intended for both the food and nutraceutical industries. In recent years, bioprocessing technologies have attracted increasing attention due to their significant potential in the utilization and valorization of date by-products **(Younas et al., 2020**). Date fruit residues are considered one of the major agri-food wastes generated in many Arab countries **(Sifour et al., 2017).**

Traditional date processing remains widely practiced in oasis regions, where artisanal transformation activities play an important economic role. The development and expansion of these activities could contribute to the creation of new markets and support the growth of the date palm sector **(Djafri et al., 2020).** According to **Harrak and Boujnah (2012),** date processing can be divided into two principal categories:

Technological processing: industrial techniques applied for date transformation and preservation.

Biotechnological processing: industrial applications based on biological and fermentation processes.

I.10.1. Date Sugars

Date sugar is produced by grinding dates and mixing them with hot water to facilitate the extraction of sugars. A diffusion-based extraction process is generally preferred because it enhances sugar recovery while reducing the extraction of undesirable compounds. The obtained juice is subsequently concentrated under vacuum at low temperatures (40–45°C) until reaching a Brix value of approximately 30–35°. The final product may vary in color from light brown to bright yellow depending on the degree of purification and decolorization applied during processing **(Benkrif et Bennouri al.,2024)**

CHAPTER II: SPIROLINA

II.1.Overview :

Microalgae are photosynthetic microscopic organisms characterized by remarkable biological diversity and rapid growth in a wide range of aquatic environments, including freshwater and marine ecosystems. They comprise both eukaryotic microalgae and prokaryotic cyanobacteria, such as Spirulina (Arthrospira spp.), which are commonly classified as blue-green microalgae. **(Su, M et al 2023;Novoveská,L et al 2023)**

In recent decades, microalgae have attracted considerable scientific and industrial interest due to their high nutritional and biochemical value, as they are rich sources of proteins, essential amino acids, polyunsaturated fatty acids, vitamins, minerals, pigments, and bioactive compounds . **(Su, M et al 2023;Yu, B et al 2024)**

In addition, their ability to efficiently convert solar energy and carbon dioxide into biomass makes them promising candidates for sustainable biotechnological applications . **(Novoveská,L et al 2023;Yu, B et al 2024)**

Consequently, microalgae are extensively utilized in various sectors, including human nutrition, nutraceuticals, pharmaceuticals, cosmetics, wastewater treatment, and biofuel production, highlighting their significant role in the development of sustainable bioeconomy strategies . **(Su, M et al 2023;Yu, B et al 2024)**

II.2. Definition of Spirulina :

Spirulina is a microscopic photosynthetic organism belonging to the cyanobacteria group, commonly known as blue-green algae, and it is distinguished by its characteristic spiral morphology. It is classified as a prokaryote and is considered among the earliest life forms to have developed photosynthetic capability, thereby representing a primitive lineage from which higher plants are believed to have evolved. Spirulina typically inhabits extreme environments, particularly alkaline aquatic systems, where it demonstrates a remarkable capacity to survive and proliferate under conditions that are unfavorable for most other microorganisms. **(M. Zhang et al,2011)**

This microorganism is widely distributed across different geographical regions, including alkaline and freshwater ecosystems in Africa, the Americas, and Asia, and has historically served as a nutritional resource for ancient populations such as the Aztecs and certain African communities. The most commonly cultivated and consumed species, Spirulina platensis and Spirulina maxima, are extensively utilized as dietary supplements due to their high content of proteins, vitamins, minerals, and various bioactive constituents.

Although frequently referred to as an alga, Spirulina is, in fact, a prokaryotic organism with features closely resembling those of eubacteria. It exhibits a filamentous, helical structure that may undergo morphological variations in response to environmental factors. Moreover, Spirulina is capable of adapting to nutrient-limited conditions through physiological mechanisms such as nitrogen fixation, thereby playing a significant role in ecological nutrient cycling.

Owing to its exceptional nutritional profile and diverse biological properties, Spirulina has attracted considerable attention in the fields of nutrition and healthcare, as well as in various industrial sectors, including food production, pharmaceuticals, and cosmetics. Consequently, it is increasingly regarded as a promising and sustainable resource with potential applications in addressing global challenges related to health, nutrition, and environmental sustainability. **(M. Sabart, D. Pobel, E. Briand et al,2010)**

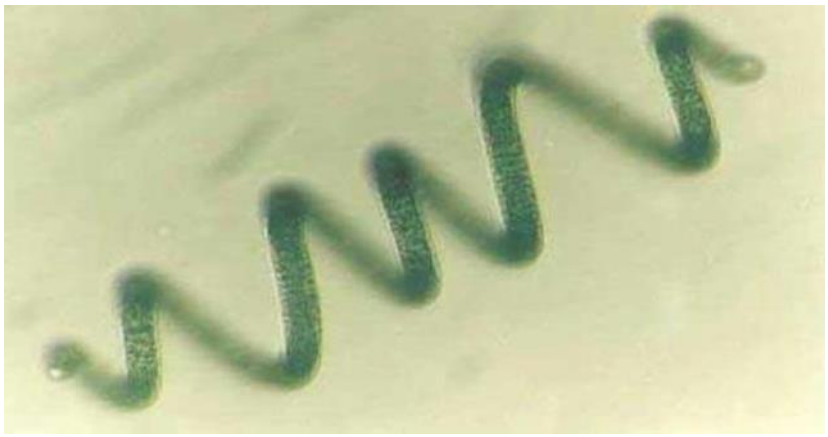

Figure 09: Spirolina (Jourdan, J.P. 2012)

II.3.Classification of Spirolina

Spirulina, is a filamentous photosynthetic cyanobacterium belonging to the group of blue-green microalgae. Although the commercial term “Spirulina” is widely used, the species exploited for nutritional and biotechnological applications are taxonomically classified within the genus Arthrospira. This microorganism is characterized by its helical multicellular filaments, high protein content, and remarkable adaptability to alkaline aquatic environments.**(Gentscheva G et al , 2023)**

The taxonomic classification of Arthrospira platensis is presented as follows (Table 1)

Table 1 : classification of Arthrospira platensis

Taxonomic Rank	Classification
Domain	Bacteria
Phylum	Cyanobacteria
Class	Cyanophyceae
Order	Oscillatoriales
Family	Microcoleaceae
Genus	Arthrospira
Species	*Arthrospira platensis*

Spirolina is considered one of the most important cyanobacterial species due to its nutritional, pharmaceutical, and biotechnological value. It has gained considerable scientific interest because of its richness in proteins, essential amino acids, vitamins, minerals, pigments, and bioactive compounds with antioxidant and therapeutic properties.**(Gentscheva G et al , 2023)**

II.4.Spirolina cultivation

Large-scale cultivation of Spirulina was initiated approximately three decades ago, primarily in Mexico and China, and subsequently expanded to other regions worldwide due to the relative simplicity of its cultivation requirements. Industrial production is predominantly carried out in open raceway pond systems, where paddle wheels are employed to ensure continuous mixing and proper circulation of the culture medium. The principal commercial producers of Spirulina include the United States, Thailand,

India, Taiwan, China, Bangladesh, Pakistan, Myanmar, Greece, and Chile. Globally, Spirulina is widely recognized for its significant nutritional potential.**(Nicoletti, M ,2016)**

Microalgae possess a high capacity for rapid proliferation under favorable culture conditions. These environmental parameters not only influence growth kinetics but also affect cellular composition, as well as the quantity and characteristics of the bioactive compounds synthesized.

The growth of microalgae can be divided into five distinct phases

II.4.1. Lag phase

During this initial stage, cells undergo an adaptation period to the new culture conditions. Cellular activity is primarily directed toward metabolic adjustment, 8resulting in minimal growth.

II.4.2.Acceleration phase

Cells accumulate sufficient intracellular components and complete DNA replication. The population begins to increase through vegetative reproduction, whereby each parent cell divides into two genetically identical daughter cells, each containing half of the original cellular content, which subsequently continue dividing.

II.4.3. Exponential phase

This phase is characterized by a constant and maximal growth rate. Environmental conditions are optimal, allowing for rapid cell division. Both the average cellular composition and population dynamics remain stable and uniform.

II.4.4.Stationary phase

Growth rate declines due to the depletion of essential nutrients or limiting factors such as light, nitrogen, phosphorus, or carbon. However, cells can survive by utilizing stored intracellular reserves to sustain metabolic functions. During this phase, certain compounds, particularly lipids and carbohydrates, may continue to accumulate. The rate of cell division becomes equal to the rate of cell death, resulting in a stable cell concentration.**(Derdouri et al,2022)**

II.4.5.Decline phase

In this final stage, most cells exhaust their intracellular reserves and are no longer capable of generating sufficient energy to maintain cellular processes, leading to cell death. The rate of cell mortality significantly exceeds that of cell reproduction. Some microalgal species can enter a dormant state as a survival mechanism under unfavorable environmental conditions.**(Derdouri et al,2022)**

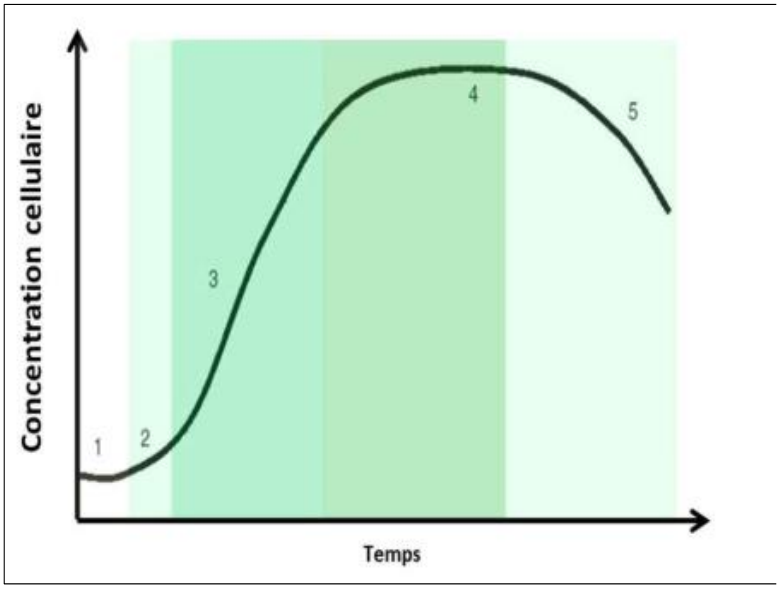

Figure 10: Theoretical growth curve of a microalgal population as a function of time. (Clément-Larosière, 2012)

II.5.Nutritional Components of Spirulina

II.5.1.Proteins

The principal protein fraction in Spirulina consists of phycobiliproteins, mainly phycocyanin, allophycocyanin, and phycoerthrin. Among these, phycocyanin is the predominant component, representing a substantial proportion of the total protein content, estimated at nearly 50% of the dry biomass. Structurally, phycocyanin is composed of α and β subunits that assemble into higher-order oligomers such as trimers and hexamers. Each subunit contains approximately 160–180 amino acids, with molecular weights ranging from 12–19 kDa for the α subunit and 14–21 kDa for the β subunit. Additionally, phycocyanin incorporates three chromophoric groups responsible for its characteristic light absorption, with a maximum absorption peak at 620 nm. Functionally, it exhibits multiple biological activities, including antioxidant, anti-inflammatory, and antitumor effects, and contributes to enhanced protein bioavailability.**(Nielsen CH et al, 2010)**

II.5.2.Polysaccharides

Spirulina is also rich in structurally diverse heteropolysaccharides, containing about 6% sulfate groups. These polysaccharides are primarily composed of monosaccharides such as D-mannose, D-glucose, D-galactose, L-rhamnose, and glucuronic acid, accounting for approximately 14–16% of the dry weight. In addition, minor constituents including xylose, arabinose, and fucose are present. These compounds play a crucial role in strengthening the

antioxidant defense system by scavenging free radicals, decreasing malondialdehyde (MDA) concentrations, and enhancing serum insulin levels. Consequently, they contribute to the reduction of oxidative stress and the improvement of glycemic regulation.**(Bhattacharyya S et al , 2012)**

II.5.3.Lipids

Despite its relatively low lipid content (6–9%), Spirulina is considered a valuable source of essential unsaturated fatty acids, notably gamma-linolenic acid (GLA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA). It is distinguished as the only autotrophic organism known to contain significant amounts of GLA. This fatty acid plays an important role in lowering blood lipid levels, regulating arterial pressure, and reducing cholesterol concentrations, thereby contributing to the prevention and management of cardiovascular diseases. Moreover, the antioxidant properties of these lipids support their application in the cosmetic industry, particularly in skin depigmentation and anti-aging formulations.**(Capelli B et al ,2010)**

II.5.4.Chlorophyll

Spirulina is characterized by a high chlorophyll content, exceeding that found in most terrestrial plants. Chlorophyll is a biologically active pigment with notable deodorizing, therapeutic, and anti-inflammatory properties. Its molecular structure consists of a porphyrin ring coordinated with a central magnesium ion, which confers structural similarity to heme. Being lipid-soluble, chlorophyll is widely utilized in pharmaceutical, food, and cosmetic applications. In Spirulina, it represents approximately 1–2% of the dry biomass and serves as an important indicator for assessing algal biomass quality and productivity.**(Singh, K et al , 2025)**

II.5.5.Other constituents

Spirulina is characterized by an exceptional nutritional profile, comprising a wide range of essential nutrients and biologically active compounds, which positions it among the most nutrient-dense natural foods. It is particularly rich in B-complex vitamins, as well as vitamins E and K, and provides significant amounts of minerals such as calcium—reported to exceed that of whole milk by approximately 180%—and iron, with concentrations reaching up to 5100% higher than those found in spinach.

In addition, Spirulina contains a variety of phytochemicals, including β-carotene at levels substantially greater than those in carrots (approximately 3100%), along with phycocyanin and

the antioxidant enzyme superoxide dismutase (SOD). These bioactive compounds exhibit pronounced anti-inflammatory and antioxidant properties, which play a crucial role in its health-promoting effects. Notably, a daily intake of only 3 g of Spirulina has been shown to provide antioxidant activity exceeding that of five servings of fruits and vegetables.

Collectively, these characteristics highlight Spirulina as a highly effective functional food, capable of addressing nutritional deficiencies while supporting overall health. Its complex biochemical composition further emphasizes its value as a dietary supplement and underscores its therapeutic potential across diverse applications. **(Singh, K et al , 2025)**

II.6.Main Applications of spirolina

Spirulina is of considerable interest across a wide range of fields due to its diverse applications.

II.6.1. Human Nutrition

Spirulina is widely recognized as a functional food and nutraceutical ingredient in human nutrition, and is increasingly incorporated into dietary supplements and fortified food formulations. It is characterized by a high nutritional density, particularly its elevated protein content (approximately 60–70% dry weight) and a well-balanced profile of essential amino acids, alongside significant amounts of bioavailable micronutrients such as iron, magnesium, potassium, and B-complex vitamins. In addition, it contains essential fatty acids, notably gamma-linolenic acid (GLA), which contribute to its nutritional and metabolic relevance in human diets .**(Gogna N, et al. 2023)**

From a biochemical perspective, spirulina exhibits high digestibility due to the absence of cellulose-based cell walls, which enhances intestinal nutrient absorption. It also contains major bioactive compounds, including phycocyanin, carotenoids, and phenolic derivatives, which are strongly associated with antioxidant activity and the modulation of oxidative stress pathways. These compounds contribute to its functional properties, particularly in reducing reactive oxygen species and supporting cellular protection mechanisms . **(Gogna N, et al. 2023)**

Current scientific evidence also highlights the role of spirulina in supporting physiological performance and metabolic function. Studies report potential benefits in improving exercise performance, enhancing endurance capacity, and reducing exercise-induced oxidative stress, which supports its use among physically active individuals and athletes. Furthermore, its immunomodulatory and anti-inflammatory properties have been documented through the stimulation of innate immune responses and regulation of cytokine production . **(Calella P, et al. 2022)**

Overall, spirulina is considered a safe and highly nutritious functional food ingredient when produced under controlled conditions that ensure the absence of contaminants such as heavy metals and cyanotoxins. Its nutritional and bioactive profile supports its application in preventive nutrition and health-oriented dietary strategies across different population groups . **(Gogna N, et al. 2023;Calella P, et al. 2022)**

II.6.2. Medical Applications

Clinical studies have demonstrated that spirulina can serve as an adjunct therapeutic agent in the management of various diseases , owing to its significant biological activities. These include anticancer properties, hypoproteinemic effects, and protective roles against obesity and diabetes .**(Demisu, D.G. et al , 2018)**

Spirulina supplementation, particularly in capsule form, has been shown to reduce blood lipid levels, decrease leukocyte counts following radiotherapy and chemotherapy, and enhance immune function . Furthermore, spirulina exhibits multiple pharmacological activities, including the stimulation of immune defenses against pathogenic bacteria, fungi, and RNA viruses—such as influenza and coronaviruses—while also contributing to the prevention of inflammatory diseases and cellular oxidative stress.**(Ragusa, I et al , 2021)**

II.6.3.Cosmetic Applications

Spirulina has gained considerable interest in cosmetic applications due to its rich content of bioactive compounds with dermatological benefits. It contains potent antioxidants, including carotenoids and vitamin E, which protect the skin against oxidative stress and environmental damage. Furthermore, spirulina-derived peptides enhance skin hydration, elasticity, and firmness by stimulating collagen biosynthesis. Its polysaccharides and sulfur-containing compounds exhibit notable anti-inflammatory properties while also improving moisture retention. In addition, carotenoids and lutein contribute to the inhibition of melanogenesis, thereby reducing hyperpigmentation and promoting skin radiance. Spirulina polysaccharides have also demonstrated photoprotective activity, exhibiting sun protection factor (SPF) values ranging from 10 to 15.**(Lympaki F et al , 2022)**

Part Two : Experimental part

Chapter I: Materials and methods

This study aims to explore the potential use of sugar extracted from the local date variety "**Degla Beida**" as a natural ingredient in chocolate production, enriched with Spirulina as a functional source rich in bioactive compounds.

Date samples were collected from the Reguiba region in El Oued Province, located in southeastern Algeria. The research focuses on physicochemical and microbiological control and evaluation of chocolate prepared using this natural sugar and enriched with Spirulina. This approach seeks to promote locally sourced, healthier alternatives in line with global trends toward sustainability and reducing reliance on refined sugar.

I.1.Representation of the study area:

Reguiba **(figrue 12)** is a municipality in El Oued Province, Algeria, established under the 1984 administrative division that defined municipal boundaries and structures. It is located about 30 km north of the provincial , bordered by Hamraia to the north, Tegzout to the south, Guemar and Sidi Aoun to the east, and Sidi Khalil, Tendla, and Djamaa to the west the municipality spans roughly 1,965.5 km² **(Zouiouche, F. Z., & Berhamoun, M. (2017).**

The climate of Reguiba is arid desert, with long, extremely hot summers and mild winters. Rainfall is sparse and irregular, and the region experiences high annual evaporation, which adversely affects water availability and agriculture, especially where modern irrigation or water conservation methods are lacking. Summer highs reach 40–47°C, while winter highs range from 18–25°C. Winter lows may fall to 5–10°C in January, and summer temperatures can exceed 30°C. Annual rainfall is generally low, seldom exceeding 100 mm**(Climate-Data.org, n.d.)**.

The municipality is primarily agricultural (occupying 60% of its area), alongside commercial and pastoral activities. It is a major producer of potatoes, dates, tobacco, and various vegetables, ranking second in cultivated land in El Oued Province. Following the construction of the sidi Imran–Reguiba road, it is positioned to act as a link between Oued Souf and Oued Righ **(Zouiouche, F. Z., & Berhamoun, M. (2017).**

The Reguiba region (El Oued Province, Algeria) was selected as a primary site for date palm sample collection due to its distinctive geomorphological and climatic characteristics. The area is located within a low-lying sedimentary basin on the northern edge of the Grand Erg Oriental, where aeolian formations are present but vary spatially across the region. The landscape includes a variety of landforms such as plains, sebkhas, and sand accumulations.

The soil is mainly of Quaternary origin and is strongly influenced by wind activity. It generally exhibits a light texture, ranging from sandy to sandy-loam in some areas, with

moderate to high salinity levels. These environmental conditions are favorable for date palm cultivation, particularly Phoenix dactylifera L.

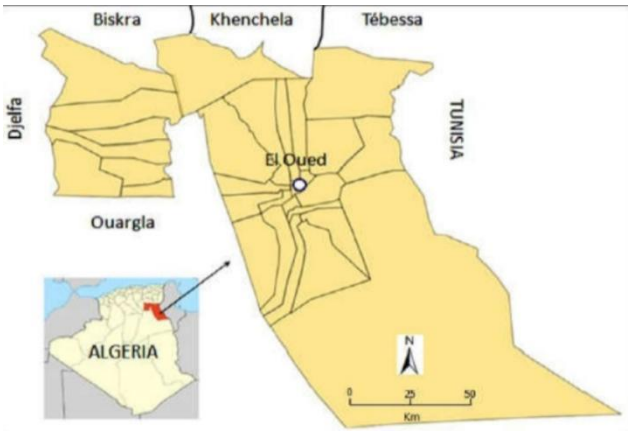

Figure 11 : Geolocation of the study area (Mayouf et al., 2014)

I.2. Collection of the sample :

Samples were collected from the Reguiba region during the autumn season, in October 2026, to ensure optimal ripeness and an accurate representation of the physico-chemical properties of the studied variety. The geographic coordinates of the collection site are :Aures GPS : 33°33'48.9"N 6°42'13.0"E

The selected cultivar is Degla Beida, which belongs to the species Phoenix dactylifera L.The primary objective of the sampling process was to extract sugars. This variety was selected for its distinctive nutritional and chemical properties. It is characterized by a high content of antioxidants and phenolic compounds, which contribute to its health benefits. It is also known for its high sugar content, making it a rich source of energy. In addition, it contains significant amounts of dietary fiber and essential minerals, enhancing its nutritional value. Owing to this composition, it is considered a primary raw material for the production of date sugar, which is used as a healthier alternative to white sugar.

Particularly **glucose** and **fructose**, in order to evaluate their potential use as natural sweeteners in chocolate production.

Figure 12 : Geographical location of Aures Neighborhood Reguiba, El Oued Province, southeastern Algeria (Google Maps, 2026).

I.3. Extraction of the Water-soluble sugar:

The preparation method was performed according to **(Mayouf et al., 2014)**, with some modifications as follows:

Step 01: Preparation of date juice :

1.The dates are washed with distilled water to remove solid impurities such as sand and clay particles.

2.The dates were cut into small pieces, and an exact amount of 200 grams was weighed for the sugar extraction process.

3.A mixture of 200 g of dates and 250 mL of distilled water was prepared, then heated at 50 °C for one hour.

Figure 13 : Preparing date juice

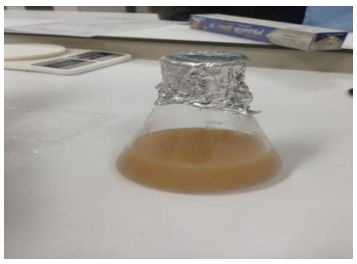

Figure 14: date juice (Degla Bayda)

4. The extraction process was repeated under the same conditions using 150 mL of distilled water to obtain a higher yield of date juice.

2.Step 02: Centrifugal sediment separation:

The collected extracts were centrifuged at 4000 rpm for 20 minutes to remove insoluble precipitates and clarify the liquid phase

Figure 15 : Centrifugal extraction

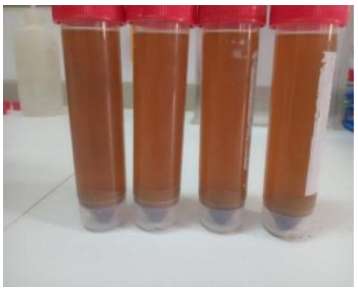

Figure 16 : extraction

3.Step 03:Sugar preparation:

The frozen extract is then freeze-dried (lyophilized) for 72 hours under reduced pressure to remove water while preserving the chemical structure of the sugars.

Figure 17 : lyophilizer

Figure 18 : sugar (Degla Beida) obained after freezing

I.4. Composition of the sugar extracts :

The sugar extract was analyzed to determine its total sugar, protein, and polyphenol content using colorimetric assays.

Solutions were prepared by dissolving 10 mg of each sugar extract in 100 mL of distilled water, and these solutions were subsequently stored at 4°C.

I.4.1. Quantification of Total Sugar Using Dubois Method:

Principle: The total sugar content was measured using a colorimetric method. (**Dubois et al**. **1956**) In this procedure, concentrated sulfuric acid breaks down glycosidic bonds, leading to dehydration of the carbohydrate units and the formation of furfural derivatives.these intermediates subsequently react with phenol in a condensation step, generating orange-yellow chromogens with a peak absorbance at 492 nm.

Materials and Reagents: A 5% (w/v) aqueous solution of phenol (Sigma-Aldrich, PL037) was prepared. Sulfuric acid (80%, Honeywell, 30743) and an aqueous glucose solution (100 µg/mL, Sigma-Aldrich, G8270) with appropriate dilutions were also used. Test sample consisted of DB at 0.01%. A water bath (SMCLAB QUIMICA, DK-420) and a UV/Vis spectrophotometer (JENWAY, 6705) were employed for the assays.

Operating mode:

1. Preparation of 5% Phenol Solution: Dissolve 1 g of phenol in 20 mL of distilled water, stirring until completely dissolved.

2. Preparation of 100 µg/mL Glucose Stock Solution: Dissolve 10 mg of glucose in 100 mL of distilled water to obtain the stock solution.(table 2)

Table 2: Preparation of the glucose by the Dubois method.

Concentartion(%)	White	0.001	0.002	0.005	0.008	0.01
Distilled water (uL)	200	180	160	100	40	0
Glu 100 ug/ml (ul)	0	20	40	100	160	200

3. Pipette 200 µL of the sample or standard into glass test tubes and place them in an ice bath. Add 200 µL of 5% aqueous phenol and mix using a vortex. Then, add 1 mL of 80% sulfuric acid (preheated to 90°C) and maintain this temperature for 30 minutes. After incubation, let the samples sit at room temperature for 30 minutes, protected from light. Measure the absorbance at 490 nm to quantify the results.

I.4.2.Protein determination by the Bradford method :

Principle: The protein content was determined using a colorimetric assay (**Bradford**.**1976**). This method relies on the binding of Coomassie Brilliant Blue G-250 dye to certain amino acids in proteins, mainly arginine, along with tryptophan, tyrosine, histidine, and phenylalanine. When the dye binds to the protein, it causes a change in the dye's absorbance maximum, which can be measured spectrophotometrically to quantify the protein-dye complex.

Materials and Reagents: Bovine serum albumin (BSA; Sigma-Aldrich, Cat. No. A2153) was prepared at a concentration of 100 µg/mL and serially diluted using distilled water. Test sample Degla Beida ,was prepared at 0.01% (w/v) and analyzed using a UV-Vis spectrophotometer (JENWAY 6705 UV/Vis).

Operating mode:

1.Bradford Reagent Preparation: To prepare the reagent, 100 mg of Coomassie Blue is first dissolved in 50 mL of 95% ethanol. The solution is stirred for 2 hours using a magnetic stirrer while being protected from light. Subsequently, 100 mL of 85% orthophosphoric acid is added, and the volume is brought up to 1 liter with distilled water. The resulting solution is then filtered through filter paper to obtain a clear reagent. When stored at 4°C, the prepared reagent remains stable for up to two weeks.

2.Preparation of 0.01% BSA Stock Solution: a 0.01% bovine serum albumin (BSA) stock solution was prepared by dissolving 10 mg of BSA in 100 mL of distilled water.(table 3)

Table 3: Preparation of the BSA by the Bradford method.

Concentartion(%)	white	0.0005	0.001	0.002	0.005	0.008	0.01
Distilled water (uL)	800	760	720	640	400	160	0
BSA 100 ug/ml (uL)	0	40	80	160	400	640	800

3.Pipette 500 µL of either the sample or the standard into assay tubes, then add 500 µL of Bradford reagent. Mix thoroughly by vortexing for 5 seconds to obtain a uniform solution. Incubate the mixture at room temperature in the dark for 30 minutes. Subsequently, record the absorbance at 595 nm using a spectrophotometer.

I.4.3.Determination of neutral sugars:

Principle: The quantification of neutral sugar components is carried out using the colorimetric method reported by **Monsigny et al. (1988)**. This approach is based on the

complete hydrolysis of polysaccharide glycosidic bonds using concentrated sulfuric acid under heat. The released monosaccharide units are then dehydrated to generate furfural derivatives, which subsequently react with resorcinol in an acidic environment to form a brown-orange colored complex. This method is recognized for its high sensitivity and allows accurate determination of neutral sugar content in the analyzed sample.

Materials and reagents: Resorcinol (Sigma-Aldrich, 398047) and a 0.6% (w/v) aqueous resorcinol solution; 80% sulfuric acid (Honeywell, 30743); glucose (Sigma-Aldrich, G8270) prepared as a 100 µg/mL aqueous standard with the required serial dilutions; distilled water;.; a water bath (Julabo, TW12); and a spectrophotometer (Shimadzu 1800).

Operating mode:

1.**Resorcinol solution preparation (0.6%)**: to prepare a 0.6% resorcinol solution, dissolve 6 mg of resorcinol in 1 mL of Milli-Q water. The solution can then be stored at 4 °C , where it remains stable for up to four weeks.

2.**Glucose stock solution preparation (100 µg/mL):** Prepare the glucose stock solution at 100 µg/mL by dissolving 10 mg of glucose in 100 mL of distilled water. This stock can then be further diluted as needed to obtain working solutions for the assay.(table 4)

Table 4: preparation of the glucose calibration using the Monsigny method.

Concentartion(%)	white	0.001	0.008	0.005	0.008	0.01
Distilled water (uL)	200	180	160	100	40	0
Glc 100 ug /ml (uL)	0	20	40	100	160	200

3. Aliquots of 200 µL of either the sample or standard are placed into a test tube, then 200 µL of resorcinol solution and 1 mL of H_2SO_4 are added. The mixture is shaken and incubated in a dry water bath at 80°C for 30 minutes. After cooling at room temperature for 30 minutes in the dark, the absorbance is recorded at 450 nm using a spectrophotometer.

I.4.4.Determination of total phenolic compounds :

Principle: The amount of phenolic compounds is determined using the colorimetric assay described **(Singleton et al.1999)**. In this technique, the Folin-Ciocalteu reagent, which contains sodium phosphomolybdate and sodium tungstate, oxidizes the polyphenols in the sample, generating molybdenum and tungsten oxides. The reduction of these metal ions increases absorbance at 765 nm, allowing for the quantification of the sample's phenolic content.

Materials and Reagents: The assay utilized Folin-Ciocalteu reagent (Sigma-Aldrich, F9252) and sodium carbonate (Sigma-Aldrich, S7795). A 10% (w/v) aqueous sodium carbonate solution was prepared to serve as the alkaline buffer. Gallic acid (Sigma-Aldrich, G7384) was used as the standard. Test sample included Degla Beida each at 0.01%, and measurements was performed using a UV-Vis spectrophotometer (JENWAY, 6705 UV/Vis).

Operating Procedure :

i. Prepare a 1/10 dilution of Folin's reagent by mixing one part of the reagent with nine parts of distilled water.

ii. To make a 20% sodium carbonate solution, dissolve 20 g of sodium carbonate in distilled water and adjust the final volume to 100 mL.

iii. Prepare a 0.1% (w/v) gallic acid stock solution by accurately weighing 100 mg of gallic acid and dissolving it in distilled water to a total volume of 100 mL.(table 5)

Table 5: Preparation of the gallic acid calibration using the Singleton method.

Concentartion (%)	white	0.0005	0.001	0.0015	0.002	0.0025	0.003	0.0035
Distilled water (uL)	100	95	90	85	80	75	70	65
Acide gallic 100 ug/ml (uL)	0	5	10	15	20	25	30	35

iv. And 100 µL of the sample and 500 µL of Folin's reagent into the assay tubes, mix gently, and let the mixture stand for 3 minutes. Then, add 2 mL of sodium carbonate solution, vortex for 10 seconds, and incubate the tubes in the dark at room temperature for 1 hour. Finally, record the absorbance at 765 nm.

I.5. Physico-chemical characteristics :

I.5.1. Humidity level :

Principle: This method determines the moisture content of a sample by measuring the loss in mass after drying. The observed mass reduction reflects not only water removal but also the evaporation of other volatile components, including alcohols, oils, organic solvents, aromatic compounds, and certain thermal decomposition products. The sample is dried in an oven at 110 °C for 6 hours, after which the difference in mass is used to estimate its moisture content**(Belibi et al., 2014).**

Materials and reagents : An analytical balance was used for accurate sample weighing, along with a drying oven (POL-EKO SP.K.) maintained at 110 °C. Heat-resistant aluminum or glass containers were employed to hold the samples, and metal tongs were used to safely handle the hot containers.

Operating mode:

i. The moisture content of the samples was assessed using a constant-temperature oven-drying method in accordance with the standard procedure. Initially, the samples were weighed, then dried in an oven at 110 °C for 6 hours. After drying, they were placed in a desiccator to cool while preventing moisture uptake, and subsequently reweighed.

ii. The moisture content was calculated from the difference between the sample mass before and after the drying process.

I.5.2. Mineral :

Principle: This method is relies on burning the sample in a muffle furnace at a high temperature (500 °C) to completely eliminate organic matter. After burning, inorganic (ash), which , is weighed, and This weight is used to estimate the total mineral content or to prepare the sample for furthre analysis, such as atomic absorption spectroscopy**(AFNOR, 1982).**

Materials and reagents: A muffle furnace maintained at 500 °C was utilized to incinerate the samples and eliminate organic matter.

Operating procedure:

i. Approximately 0.5 g of sugar was precisely weighed and transferred into a clean, dry ceramic crucible.

ii. The crucible was then placed in a muffle furnace set at 500 °C for 3 hours, until the sample was completely converted into gray, white, or black ash.

iii. After incineration, the crucible was carefully removed using metal tongs and placed in a glass desiccator to cool while preventing moisture uptake. The residual ash was then accurately weighed.

I.5.3. pH:

Principle : This analysis aims to measure the pH of a sugar solution extracted from dates. The pH serves as an indicator of the chemical nature of the medium (acidic, neutral, or alkaline), allowing evaluation of the properties and quality of the extracted sugar. The measurement is carried out using a pH meter equipped with a glass electrode, which provides a direct digital reading after the signal stabilizes in the diluted sugar solution**(Pakaleet al,. 2018)**

Materials and reagents: A digital pH meter (Consort C3010) fitted with a glass electrode was used for precise pH measurement.

Operating procedure:

i. The pH meter's glass electrode was rinsed with distilled water and gently dried using clean filter paper to eliminate any residues that could influence measurement accuracy.

ii. The electrode was then immersed in the extracted solution, and the reading was allowed to stabilize on the display before the final pH value was accurately recorded.

I.5.4. Conductivity :

Principle: The electrical conductivity of the solution was measured by immersing a probe into the sample. The probe contains two electrodes that pass a low electrical current through the solution. Dissolved ions in the solution move in response to this current, and the solution's ability to conduct current (i.e., its conductivity) is recorded by the instrument. Electrical conductivity depends on the concentration of ions in the sample; the higher the concentration, the higher the conductivity. After the reading stabilized, the value was displayed directly on the instrument screen in microsiemens per centimeter (µS/cm)**(Radiometer Analytical, 2004).**

Materials and reagents: A conductivity meter (Consort C3010) with a probe containing two electrodes was used to measure the conductivity of the sugar solution.

Operating mode: The conductivity meter was switched on, and the probe was cleaned before being immersed in the liquid sample. Once the reading stabilized, the electrical conductivity was recorded in µS/cm.

I.6. Quality control of chocolate :

I.6.1. Chocolat preparation using sugar dates :

I.6.1.A. Ingredients :

- Cocoa butter (40 g)
- Cocoa powder (20 g) (Dada Noir)
- Sugar DB extcat (1 g)
- Spirulina (0.3 g) (obtained Biological farm Al-kiram)

I.6.2.B. Preparation Steps :

1. Introduce the **cocoa butte**r into the conche and warm it to 45 °C.
2. 2.Gradually incorporate the **sugar (DB) extcat** while stirring.
3. Then gradually add the **cocoa powder** while maintaining thorough mixing.
4. Proceed with conching , keeping the temperature within **45–60 °C**, until the mixture becomes uniform and smooth.
5. Perform tempering following this thermal profile:

- Warm to 45 °C
- Lower the temperature to 27 °C
- Raise it again to 31 °C

6. Transfer the mixture into the molds.
7. Top the surface with **spirulina** flakes for added nutrition and visual appeal.
8. Let the mixture cool and fully set in the molds.

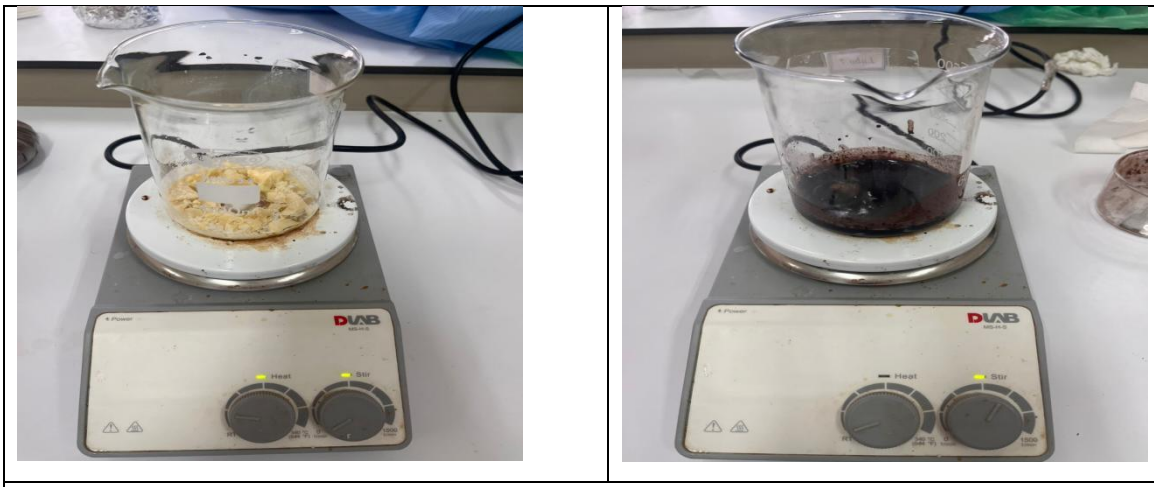

Figure 19 : Chocolat preparation

I.6.2. Physicochemical analysis :

I.6.2.1. Organoleptic characterization :

The sensory attributes of a product are essential in shaping its perception before use or consumption, as well as its assessment during actual use or ingestion **(Jacquemain, 1961)**. All formulations were subjected to sensory evaluation following the procedures outlined **(Mishra et al. 2014 and Chen et al. 2016).**

Sensory characteristics : The characteristics evaluated included physical appearance (opaque or glossy), color and odor, as well as structural defects (presence or absence of visible separation or imperfections). Homogeneity and overall product appearance were also assessed in order to evaluate its organoleptic quality.

I.6.2.2 pH :

The pH was measured using the same method described on page 38.

I.6.2.3 Conductivity :

The Conductivity was measured using the same method described on page 38.

I.6.2.4 Centrifugation stability :

Principal: assesses the resistance of formulations to phase separation under accelerated gravitational force, which simulates long-term storage conditions in a shortened time **(Ostrosky et al., 2015).**

Materials and reagent :

centrifuge , chocolat sample

Operating mode :

After 24 hours of preparation, the formulations were centrifuged at 3000 rpm for 30 minutes at ambient temperature. Their appearance, uniformity, and organoleptic properties were then evaluated macroscopically **(Ostrosky et al., 2015).**

I.6.2.5 Freeze–thaw cycle stability :

Principal :

The freeze–thaw stability test evaluates the resistance of a formulation to repeated temperature changes between 4 ± 2 °C and 45 ± 2 °C (75% ± 5% RH), which may induce structural and physicochemical changes such as phase separation or component redistribution **(Ostrosky et al., 2015).**

Materials and reagent : Prepared Formulations, refrigeration unit (4 ± 2 °C), incubator (45 ± 2 °C, 75% ± 5% relative humidity).

Operating mode:

The chocolate sample was subjected to alternating conditions of 4 ± 2 °C for 24 hours, followed by 45 ± 2 °C with 75% ± 5% relative humidity for another 24 hours, representing one complete cycle. Sensory properties, pH, and electrical conductivity were measured before and after the test **(Ostrosky et al., 2015).**

III.6.2.6 Solubility :

Principal : Solubility represents the maximum concentration of a solute that can be dissolved in a solvent at a specified temperature and pressure, resulting in the formation of a saturated solution. The dissolution process is largely influenced by the physicochemical similarity between the solute and solvent, according to the principle “like dissolves like” (similia similibus solvuntur), whereby polar substances preferentially dissolve in polar media, while non-polar substances exhibit greater solubility in non-polar solvents (Silberberg, 2012; Zumdahl & Zumdahl, 2014).

Materials and reagent :

Chocolate sample, Distilled water , Ethanol

Operating mode:

A measured quantity of chocolate sample was dispersed separately in distilled water and ethanol at room temperature with continuous agitation. Solubility was evaluated by visual inspection of the resulting mixtures. The sample did not exhibit complete dissolution in either solvent under these conditions. The aqueous dispersion was then subjected to heating in a water bath with constant stirring, which promoted the dissolution of the sample. The physical appearance of the mixtures and the degree of dissolution were subsequently assessed by macroscopic examination.

I.6.3.Microbiological Quality Control of chocolate

I.6.3.1.Total viable microbial count (TVMC)

Principle

Microbiological analysis was conducted to evaluate the safety and quality of the formulated chocolate. The procedure involved inoculating specific growth media with chocolate samples and incubating them under controlled conditions. Any viable microorganisms present proliferate into visible colonies, quantified as Colony Forming Units (CFU).

The obtained microbial load will be compared with the limits specified in the Algerian Official Journal standards for food quality.

Materials and reagents

- **Culture Media:** Mueller-Hinton Agar (MHA) was utilized for bacterial screening, while Sabouraud Dextrose Agar (SDA) was employed for the isolation of yeasts and molds.
- **Reagents and Diluents:** Tween 20,sterile physiological saline (0.9% and 0.45%) served as the primary diluent for sample preparation.
- **Sample:** Chocolate samples (2 g per analysis unit).
- **Laboratory Hardware:**
    - **Sample Processing:** Micropipettes (1000 µL) with sterile tips for precise volume transfers and a vortex mixer for sample homogenization.

- **Inoculation:** Sterile Petri dishes, test tubes, and sterile glass beads for uniform surface distribution (spread plate technique).

- **Equipment & Sterilization:** All media and glassware were sterilized via autoclaving (121°C for 15–20 minutes) prior to use. Incubation was conducted in temperature-controlled environments at 25°C, 30°C, and 37°C to facilitate various microbial growth profiles.

❖ **Operating mode**

Step 01: preparation of original solutions

1. A 2 g portion of chocolate was weighed and supplemented with 1 mL (1000 µL) of Tween 20.
2. For bacterial analysis, 1 g of chocolate was aseptically transferred into 9 mL of sterile physiological saline solution (0.9% NaCl) to obtain the initial suspension.
3. For fungal analysis, the same procedure was applied using 9 mL of sterile physiological saline solution (0.45% NaCl).
4. The mixtures were thoroughly vortexed for 15 seconds until a homogeneous suspension was obtained.

Step 02 : Preparation of serial decimal dilutions

1. One milliliter of the initial suspension was transferred into a sterile tube containing 9 mL of diluent to obtain a 10^{-1} dilution. For bacterial analysis, sterile physiological saline (0.9% NaCl) was used, while for fungal analysis, sterile physiological saline (0.45% NaCl) was applied. The tube was vortexed for 15 seconds until a homogeneous suspension was obtained.

2. One milliliter of the 10^{-1} dilution was then transferred into a second sterile tube containing 9 mL of the appropriate diluent to obtain a 10^{-2} dilution, followed by vortexing for 15 seconds to ensure homogeneity.

3. The procedure was repeated in the same manner to obtain successive dilutions up to 10^{-3}.

Step 03: Inoculation and Plating

1. 100 µL were aseptically taken from each dilution tube.
2. Each dilution was inoculated in triplicate onto the center of sterile Petri dishes containing Mueller–Hinton agar (for bacterial counts) and Sabouraud dextrose agar (for molds and yeast counts).
3. The inoculum was evenly distributed over the agar surface using sterile glass beads. The Petri dishes were gently rotated to ensure uniform spreading, and the beads were subsequently removed into a sterile container.
4. The plates were inverted and incubated: bacterial cultures were incubated at 30–37 °C for 24–48 hours, while yeasts and molds were incubated at 25 °C for up to 7 days.
5. The total bacterial and fungal count of the chocolate sample was determined using the plate count method **(Spencer and Spencer, 2001 ; Da Silva et al., 2018).**

I.6.3.2. Detection of the pathogen bacteria

❖ **Principle**

Pathogenic bacteria detection and microbial enumeration were based on enrichment, serial dilution, and selective culture techniques to determine the presence or absence of pathogenic microorganisms as well as the total microbial load in chocolate samples after incubation on appropriate culture media.

❖ **Materials and reagents**

- Culture media: Nutrient Broth, MacConkey Broth, MacConkey Agar, Cetrimide Agar, Tryptic Soy Broth (TSB), Baird–Parker Agar (BPA), Selenite–Cystine Broth, Hektoen Enteric Agar (HEA).
- Reagents and diluents: sterile physiological saline solution (0.9% NaCl), sterile physiological saline solution (0.45% NaCl);
- Samples: 2 g of chocolate samples (10^{-1} initial dilution).
- Equipment : sterile Petri dishes, sterile test tubes, sterile inoculating loops, sterile swabs, micropipettes (100 µL and 1000 µL) with sterile tips; equipment: vortex mixer, rotary shaker incubator, incubators (30–35 °C, 37 °C, 42–44 °C).

❖ **Operating mode**

- Identification of Escherichia coli

One milliliter of the 10^{-1} sample dilution was inoculated into a Petri dish containing 10 mL of nutrient broth (Fig. 2) and incubated at 30–35 °C for 18–24 hours.

After incubation, 1 mL of the enriched broth was transferred into 100 mL of MacConkey broth medium and incubated at 42–44 °C for 24–48 hours. Subsequently, a subculture was performed on selective MacConkey agar using the sterile swab plating technique, followed by incubation at 30–35 °C for 18–72 hours **(Cobzaru et al., 2025).**

- Identification of Pseudomonas aeruginosa

One milliliter of the 10^{-1} sample dilution was inoculated into a Petri dish containing 10 mL of nutrient broth and incubated at 30–35 °C for 18–24 hours. Following incubation, subcultures were performed on selective Cetrimide agar (Fig. 7) using the sterile swab plating technique, and the plates were incubated at 30–35 °C for 18–72 hours **(Cobzaru et al., 2025).**

- Identification of Coagulase-positive staphylococci

A 100 µL aliquot of the saline suspension from each sub-sample was inoculated into 5 mL of Tryptone Soya Broth (TSB) supplemented with 6.5% sodium chloride and incubated overnight at 37 °C in a rotary shaker. Subsequently, one loopful from each enriched culture was streaked onto selective Baird–Parker agar (BPA) plates and incubated overnight at 37 °C. Colony morphology and coloration were then examined for presumptive identification **(Safdar et al., 2003; Sanders, 2012).**

- Identification of Salamonella

A 100 µL aliquot of the previously prepared saline suspension was inoculated into 5 mL of Selenite–Cystine Broth for pre-enrichment and incubated overnight at 37 °C in a rotary shaker. The following day, a loopful from each culture was streaked onto selective Hektoen Enteric Agar (HEA) plates and incubated overnight at 37 °C. Colony morphology and pigmentation were then examined for presumptive identification **(Safdar et al., 2003; Sanders, 2012)**

I.6.4. Bioactivity of the chocolate

II.6.4.1.Evaluation of antioxydant activity :

Principle : The DPPH free radical scavenging assay is a commonly used method for evaluating the antioxidant activity of extracts. Antioxidants act by donating hydrogen atoms to the DPPH radical, which is initially purple in color, leading to its reduction into a yellow-colored compound. The antioxidant capacity is determined by monitoring the reduction in DPPH absorbance at 517 nm. This technique is widely recognized as an important tool for assessing antioxidant activity**(Chebrou el al., 2022).**

Materials and reagents: Ethanol (Biochem Chemopharma), distilled water, L-ascorbic acid (Sigma-Aldrich), 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma-Aldrich), and a UV-Visible spectrophotometer (SHIMADZU 1800).

Procedure:

The radical scavenging method is widely used and is typically the first approach applied to evaluate the antioxidant activity of a compound **(Shahidi and Zhong., 2015).**

1.Preparation of DPPH solution : 9.858 mg of DPPH is dissolved in 100 mL of ethanol with continuous stirring. The solution is protected from light by covering it with aluminum foil and used immediately.

2.Preparation of ascorbic acid solution : 10 mg of ascorbic acid is dissolved in 100 mL of distilled water and used as a positive control.table (table 6)

Table 6 : preparation of ascorbic acid .

Concentartion	0.1	0.08	0.04	0.02	0.008	0.004
distilled water	0	200	400	800	920	940
Ascorbic acid	1000	800	600	200	80	60
DPPH	1000	1000	1000	1000	1000	1000

3. Preparation of sample solution : 10 mg of chocolate is dissolved in 10 mL of ethanol.(table 7)

Table 07: Preparation of sample solution .

Concentartion	0.1	0.8	0.4	0.1	0.05	0.01
distilled water	0	200	600	900	950	990
Chocolate	1000	800	400	100	50	10
DPPH (uL)	1000	1000	1000	1000	1000	1000

of the reaction mixture In each test tube, 1 mL of the sample solution is mixed with 1 mL of DPPH solution. The mixture is incubated in the dark for 30 minutes.The absorbance is measured at 517 nm using a spectrophotometer. Ascorbic acid was employed as the standard in this test, and the inhibition percentage was calculated using the following equation **(Gheraissa., 2022).**

Percentage Inhibition: (A0 – A1) / A0 × 100

A0: Absorbance of the control.

A1: Absorbance of a test or standard sample.

The concentration of extract/control for 50% inhibition (IC50) is determined by plotting the percentage inhibition against the concentration **(Tukappa et al., 2015).**

I.6.4.2.Evaluation of antibacterial activity :

Principle: This method consists of placing a sterile disc impregnated with a chocolate extract onto a bacterial lawn during its early growth phase. The area where bacterial growth is inhibited is then measured, allowing the determination of the inhibition zone diameter, which indicates the antibacterial activity of chocolate . The antibacterial potential of aqueous chocolate extracts was evaluated using the disc diffusion technique**(Ait EL hadj & Haddar., 2020).**

❖ **Materials and reagents:**

filter paper (Wattman's), Physiological saline solution , Muller Hinton agar , Nutrient agar (nutritive agar), petri dishes, Pasteur pipette, Staphylococcus aureus , Escherichia coli ATCC25922 , Gentamicin(GMN) .

❖ **Operating mode:**

The antibacterial activity of chocolate samples was evaluated using the agar diffusion method,as described by (Ojagh et al., **2010)**. The antibacterial effect was tested against two bacterial strains: Escherichia coli (ATCC 25922) and Staphylococcus aureus. Bacterial cultures were stored at 4 °C and subcultured every 24 hours on nutrient agar at 37 °C. The pathogenic strains were activated by incubation in nutrient agar (Merck, Germany) at 37 °C for 18 hours. Subsequently, 200 µL of the bacterial suspension was evenly spread onto nutrient agar plates (Merck, Germany). Chocolate samples were dissolved in physiological saline solution (0.9% sodium chloride) to obtain test solutions. Sterile discs were then impregnated with the chocolate solutions and placed on the inoculated agar surface. Petri dishes were incubated at 37 °C for 24 hours. Antibacterial activity was assessed by measuring the diameter of inhibition zones (mm), which appeared as clear areas around the discs**(Mahcene.Z., 2021).**

I.6.5.Sensory analysis :

I.6.5.1.Organolyptic analysis :

The sensory evaluation of chocolate was performed to assess its organoleptic quality. The characteristics considered included appearance (opaque or glossy), color and odor. Structural integrity was also examined by checking for the presence or absence of visible defects, such as phase separation or surface imperfections. In addition, homogeneity and overall product appearance were evaluated to determine the general sensory quality of the samples.

Chapter II: Results and discussion

This chapter presents the results of the extracted sugar from a single date variety (Deglet Beida), together with an evaluation of its physico-chemical properties and it application in chocolat production.

II.1. Extraction yields:

Aqueous extracts of the *Phoenix dactylifera* L. cultivar (Deglet Beida) collected from Reguiba (El Oued Province, Algerian Sahara) was subjected to freeze-drying using a lyophilizer. This process removed water by sublimation, producing a concentrated dry extract rich in water-soluble compounds, mainly sugars.

The Deglet Beida (DB) extcat variety recorded a moderate extraction yield of 42.8%, reflecting its moderate content of extractable compounds compared to the other studied date varieties. This yield can be explained by its intermediate textural and chemical characteristics, as it is less soft than moist varieties and less dry than semi-dry varieties, which influences the solvent's ability to penetrate the plant matrix and extract bioactive compounds. In addition, the sugar, fiber, and phenolic compound contents of this variety play an important role in determining extraction efficiency. These findings are consistent with those reported by **Bouhlali et al. (2022)**, who highlighted that variations in the physicochemical properties among date varieties directly affect extraction yield and the recovery efficiency of bioactive compounds.

II.2.Chemical compositions of date extract :

The total sugar, polyphenol, and protein contents were determined in the aqueous extract of the Deglet Beida (DB) date variety, as presented in Table (8). The results showed that this variety is characterized by a high sugar content, with total sugars reaching 60.62%, while reducing sugars accounted for 59.56%, reflecting the richness of this cultivar in soluble carbohydrate compounds commonly associated with ripe date fruits.

Regarding the other bioactive compounds, the polyphenol content reached 29.58%, whereas proteins were recorded at12.35%. These values indicate that Deglet Beida contains important nutritional and functional components that may contribute to its biological and nutritional value. This chemical composition may be attributed to the genetic characteristics of the cultivar, as well as the effects of ripening stage and extraction conditions.

These findings are consistent with previous studies reporting that date fruits are rich sources of sugars and phenolic compounds, although the concentrations may vary depending on the cultivar, maturity stage, and processing conditions. The relatively high sugar content of

Deglet Beida also highlights its potential use in food industries and functional food products as a natural source of energy and bioactive compounds **(Al-Harrasi et al., 2020).**

Table 8 : chemical composition of deglet beida sugar

Compound	Deglet Beida (%)
Total sugars	**60.62±0.26**
sugars	**59.65±2.01**
Proteins	**12.35±1.04**
Polyphenols	**29.58 ±1.63**

II.3. Physico-chemical characteristics :

Table 9 : Physicochemical characteristics of Deglet Beida (DB) date variety.

Parameter	Value
Humidity level	20%
PH	6.36
Conductivity	21.9µS/cm
Mineral	2.8%

II.3.1 Humidity level :

Moisture content is an important parameter for assessing the physical quality and shelf life of date fruits. The analysis of the Deglet Beida (DB) variety showed a moisture level of 20%, which falls within the range typically reported for dry date cultivars. Based on moisture content, date fruits are generally categorized into soft (>30%), semi-dry (20–30%), and dry (<20%) types (Al-Farsi & Lee, 2008). The obtained value positions Deglet Beida at the lower limit of the semi-dry category, close to the dry type, indicating its naturally low moisture content and good storage stability. During the Tamr stage, moisture decreases further while total soluble solids and reducing sugars increase, enhancing resistance to microbial growth and improving overall storage stability (Al-Hooti et al., 1997).

II.3.2 PH :

The measured pH value of the Deglet Beida (DB) date fruit was 6.36, indicating a slightly acidic condition tending toward neutrality.

This range (approximately 6–7) is commonly reported for mature date fruits and is associated with a reduction in organic acid content during advanced ripening stages, as demonstrated in a study on the physicochemical properties of several Algerian date varieties,

which reported pH values close to neutrality across most examined cultivars (Bezghouche & Selatnia, 2013).

This relatively low acidity may contribute marginally to the stability of sugars by limiting acid-related hydrolytic reactions. Consequently, it may help maintain the balance between sucrose and reducing sugars such as glucose and fructose, which can, in turn, influence sensory attributes such as perceived sweetness and overall product quality during storage.

II.3.3 Mineral:

The total mineral content of Deglet Beida (DB) was determined using Electrothermal Vaporization (ETV), resulting in a value of 2.8%.

This value is slightly higher than the range commonly reported in the literature (0.5–2.5%), which may be attributed to the dry nature of this variety.

Dry date cultivars are generally characterized by higher apparent mineral concentrations due to substantial moisture loss during maturation, which increases the relative proportion of minerals on a fresh weight basis (Al-Hooti et al., 1997). Date fruits are notably rich in essential minerals, including potassium, calcium, magnesium, iron, phosphorus, and zinc, all of which contribute significantly to human health and overall physiological well-being (Al-Shahib & Marshall, 2003).

II.3.4 Conductivity :

Electrical conductivity (EC) was determined in the aqueous extract of Deglet Beida (DB) in order to assess its total dissolved ionic content. The measured EC value of 21.9 µS/cm indicates the presence of dissolved minerals such as potassium, sodium, and calcium, suggesting a balanced ionic profile and a satisfactory mineral composition. Ripe date fruits at the Tamr stage are known to contain essential minerals, including potassium, calcium, and phosphorus, which contribute significantly to their nutritional value and functional properties (Al-Shahib & Marshall, 2003). In addition, the recorded EC value may be related to soluble compounds that enhance the suitability of Deglet Beida for various food processing applications, particularly in the production of date pastes and extracts.

II.4. Production of chocolate :

Following the preparation stages, Figure 20 illustrates the final chocolate product obtained.

Figure 20 : Final chocolate product

II.4.1 Physicochemical analysis of chocolate :

II.4.2 Organoleptic characterization :

The sensory properties of the chocolate formulation were evaluated by a semi-trained panel using a five-point hedonic scale, focusing on five attributes: color, homogeneity, odor, gloss, and hardness. As presented in the radar chart, color achieved the highest score (4/5), indicating an attractive visual appearance, which is a key determinant of consumer acceptance in chocolate products (Afoakwa, 2010). Homogeneity and odor also received favorable scores, suggesting a uniform product structure and a characteristic cocoa aroma. These attributes are strongly influenced by the quality of raw ingredients and the efficiency of the conching process (Beckett, 2009). In contrast, gloss and hardness showed moderate ratings, which may be linked to the crystallization behavior of cocoa butter, particularly the formation of stable Form V crystals that are responsible for desirable shine and snap in chocolate (Lonchampt & Hartel, 2004). Overall, the formulation demonstrated an acceptable sensory profile consistent with quality expectations for chocolate-based products**.**

Figure 21 : results of the Organoleptic characterization.

II.4.3 PH :

The pH of our chocolate sample was 7.43, indicating a slightly alkaline character. This value was higher than those reported by Bengoechea et al. (2008) and Ait Chitt et al. (2016), who reported pH values of 6.8 and 6.2, respectively.

The pH measurement is one of the most important parameters in food quality control. Indeed, pH influences the stability, preservation, and sensory properties of food products. In addition, pH plays an essential role in the effectiveness of acidity regulators used as preservatives **(Amiot et al., 2002).**

II.4.4 Centrifugation stability :

Centrifugation testing revealed slight sedimentation of the sample at the bottom of the tube after treatment, indicating partial physical instability of the system.figure

This behavior can be attributed to differences in the density of the components and to a relatively poor homogeneity between the lipid phase and the solid particles constituting the chocolate matrix.

This phenomenon reflects the system's susceptibility to internal component redistribution under high mechanical stress, without reaching complete phase separation.

These findings are consistent with those reported by Rivas et al. (2016), who demonstrated that cocoa butter–rich systems may exhibit structural changes and rearrangement within the fat network when subjected to strong physical stresses, particularly under conditions of reduced fat phase stability.

Figure 22 : Centrifugation stability test of the chocolate sample

II.4.5 Freeze–thaw cycle stability :

Table 10 : Physicochemical parameters of the formulation before and after freeze–thaw cycle testing.

parameter	Before Freeze–Thaw	After Freeze–Thaw
PH	7.43	7.21
Electrical conductivity (uS/cm)	27	27

The results revealed a slight decrease in pH, from 7.43 before the test to 7.21 following the freeze–thaw cycle. This variation remains limited and may be attributed to minor physicochemical rearrangements within the chocolate matrix, induced by thermal stress and water redistribution. Studies focusing on the behavior of complex lipid systems have indicated that freeze–thaw cycles may promote the release of trace amounts of organic acids within the aqueous phase, resulting in a slight decline in pH values without compromising the overall chemical equilibrium of the formulation (Afoakwa, 2016). It is worth noting that both values fall within the neutral range, suggesting that this decrease does not pose any significant risk to product stability or sensory quality.

In contrast, electrical conductivity remained entirely stable at 27 µS/cm both before and after the test, indicating that the ionic balance and internal organization of the system were not substantially affected by the freeze–thaw conditions. The stability of electrical conductivity is considered a reliable indicator of the chemical integrity of the formulation, as it reflects the absence of any significant changes in dissolved ion concentration or in the nature of intermolecular interactions within the matrix (Afoakwa, 2016; Lonchampt & Hartel, 2004).

Taken together, these results indicate that the formulation exhibits acceptable physicochemical stability under thermal stress conditions, suggesting its suitability for

preserving its essential characteristics during storage and transportation, where temperature fluctuations may occur.

II.4.6 Solubility :

Table 11 : Solubility of Chocolate in Different Solvents

Solvent	Solubility
Distilled water	Soluble ,It becomes soluble at elevated temperatures
Ethanol	Insoluble

The findings revealed that chocolate failed to dissolve completely in either distilled water or ethanol at ambient temperature, a result that aligns with its complex physicochemical composition. Chocolate comprises cocoa butter, which constitutes a non-polar lipid fraction, in addition to sugars and proteins as polar constituents. This dual nature renders complete dissolution in a single solvent thermodynamically unfavorable, in agreement with the "like dissolves like" principle **(Silberberg, 2012; Zumdahl & Zumdahl, 2014).**

When the aqueous dispersion was subjected to heating, complete dissolution was achieved. This can be attributed to the fact that elevated temperatures enhance molecular kinetic energy, decrease solvent viscosity, and disrupt intermolecular interactions, collectively facilitating the solubilization process **(Chang, 2010).**

Regarding ethanol, its failure to dissolve chocolate, even partially, may be explained by its semi-polar character, which proves insufficient to overcome the cohesive forces governing the lipid fraction within the chocolate matrix **(Beckett, 2008).**

II.5 Microbiological Quality Control of chocolat

Microbiological quality control tests were performed on the chocolate sample to assess its hygienic quality and safety for consumption by detecting the presence of bacteria and fungi. The results are presented in Tables.

II.5.1 Total bacterial count

The enumeration of total aerobic mesophilic bacteria was performed according to ISO 4833-1:2013. Serial dilutions were prepared and appropriate plates containing 30–300 colonies were selected for counting. Two consecutive dilutions (10^{-1} and 10^{-2}) were considered for calculation.

CFU counts were determined at different time points during storage (1, 7 and 14 days) to evaluate the microbial stability of the product.

The total count was calculated using the ISO formula:

$$N = \frac{\Sigma C}{(n_1 + 0.1n_2) \times d}$$

Where:

ΣC: is the sum of colonies counted on selected plates.

n_1 and n_2 :are the number of plates at two successive dilutions.

d :is the lowest selected dilution.

II.5.1.1 Total Bacterial Count 24 hours after chocolate preparation

According to the results presented in the table 12 , the total viable count was estimated at **1.9×10^3 CFU/g.**

The microbiological analysis performed 24 h after chocolate preparation revealed a relatively low bacterial load in the analyzed samples. Bacterial colony counts in the original suspension ranged from 30 to 140 colonies, while lower counts were observed in the diluted samples.

The detected colonies were predominantly white and circular in appearance, although some irregular forms were also observed in certain dilutions.

The calculated Colony Forming Unit value (1.9×10^3 CFU/g) indicated moderate microbial contamination, which may be attributed to environmental contamination during preparation or handling procedures.

This value is higher than **m (10^3 CFU/g)**, the threshold below which the microbiological quality of the product is considered satisfactory, but remains below **M (10^4 CFU/g),** the threshold above which the product is considered microbiologically unacceptable. Therefore, the microbial load falls within the acceptable range. This interpretation was made in accordance with the microbiological criteria established in the Algerian Official Journal (2017).

Chocolate is generally characterized by low water activity, high sugar content, and the presence of phenolic compounds naturally found in cocoa, all of which contribute to inhibiting microbial growth. These findings are consistent with the observations reported by Beckett (2008), who stated that chocolate products usually exhibit limited microbial proliferation due to unfavorable conditions for bacterial development. Similarly, Othman et al. (2007) reported that cocoa phenolic compounds possess antimicrobial activity against several pathogenic microorganisms.

Table 12 .Bacterial count and colony morphology after 24 h of chocolate preparation

Dilution		Total bacterial count	Color and shape
Original	P1	140	White,circular
	P2	30	White,circular
	P3	74	White,circular
10^{-1}	P1	49	White,circular
	P2	131	White,circular
	P3	79	White,circular
10^{-2}	P1	78	White,circular
	P2	33	White,circular
	P3	250	White,irregular
10^{-3}	P1	121	White,irregular
	P2	76	White,circular
	P3	45	White,circular

P:Plate

II.5.1.2 Total Bacterial Count 7days after chocolate preparation

After 7 days of storage, the microbiological results showed a clear increase in bacterial growth compared with those obtained after 24 hours (Table 13). The initial sample exhibited heavy, non-countable growth (>300 CFU), with colonies covering the entire surface of the culture medium.

At the 10^{-1} dilution, colony counts ranged from 200 to 256 CFU, the mean colony count was calculated as 222 CFU. while a progressive decrease was observed at higher dilutions (10^{-2} and 10^{-3}). The colonies were mainly white and circular, although some irregular and flower-like morphologies were also observed, indicating microbial diversity during storage.

Thus, the bacterial concentration in the original sample was estimated to be $\mathbf{2.22 \times 10^3}$ colony-forming units per milliliter (CFU/g).

This increase in microbial load may be attributed to contamination resulting from storage conditions or repeated handling of the product. In addition, inadequate control of temperature

and humidity may have supported the survival and proliferation of certain microorganisms despite the low moisture content of chocolate.

Jay *et al*. (2005) reported that improper storage conditions can lead to increased microbial contamination even in foods with low water activity. Similarly, bacterial or fungal contamination may occur due to errors in packaging or handling.

Table 13 . Bacterial count and colony morphology after 7days of chocolate preparation

Dilution		Total bacterial count	Color and shape
Original	P1	Non-countable	Confluent growth (growth over the entire surface)
	P2	Non-countable	Confluent growth (growth over the entire surface)
	P3	Non-countable	Confluent growth (growth over the entire surface)
10^{-1}	P1	200	White,circular
	P2	256	White,circular
	P3	210	White,flower-shaped
10^{-2}	P1	14	White,circular
	P2	32	White,irregular
	P3	19	White,irregular
10^{-3}	P1	6	White,circular
	P2	0	White,circular
	P3	3	White,circular

P:Plate

II.5.1.3 Total Bacterial Count 14 days after chocolate preparation

Microbiological analysis after 14 days of storage revealed a marked reduction in bacterial counts compared with the results obtained on day 7. Most plates showed almost complete absence of microbial growth, with values below 1 CFU/g, while only a very limited number of colonies were observed in the 10^{-1} dilution, ranging between 1 and 4 colonies.

Bacterial growth was below the countable range (<30 colonies per plate), therefore the bacterial concentration was considered too low for accurate enumeration. An estimated count of 1.67×10^{1} CFU/mL was obtained (Table 14)

The few colonies detected were white and circular in morphology, whereas no visible growth was observed on several plates. This decrease in bacterial load may be attributed to increasingly unfavorable storage conditions for microbial survival.

The low water activity of chocolate, combined with the osmotic pressure induced by its high sugar content, likely contributed to the progressive inhibition of microbial growth. In addition, cocoa contains phenolic compounds with known antibacterial and antifungal properties.

These findings are consistent with those reported by Afoakwa (2010), who stated that chocolate products generally exhibit high microbiological stability during storage due to their low moisture content and protective matrix.

Overall, the chocolate maintained satisfactory microbiological quality after 14 days of storage.

Table 14 . Bacterial count and colony morphology after 14 days of chocolate preparation

Dilution		Total bacterial count	Color and shape
original	P1	0	/
	P2	0	/
	P3	0	/
10^{-1}	P1	1	White,circular
	P2	4	White,circular
	P3	0	/
10^{-2}	P1	0	/
	P2	0	/
	P3	0	/
	P1	0	/

10^{-3}	P2	0	/
	P3	0	/

II.5.2 Total fungal count

II.5.2.1 Total fungal count 24h after chocolate preparation

The microbiological analysis performed 24 hours after chocolate preparation (Table15) revealed a very low fungal load in the analyzed samples. As presented in Table 1, no fungal colonies were detected in the original suspension for all three replicates (P1, P2, and P3), resulting in CFU values below the detection limit (<1 CFU/g). Only a single fungal colony was observed in sample P1 at the 10^{-1} dilution. This colony exhibited a green, cottony appearance, which is characteristic of environmental molds belonging to the genera Aspergillus or Penicillium. No fungal growth was detected at the 10^{-2} and 10^{-3} dilutions in any replicate.

However, because molds generally require several days to develop visible colonies, these results should be considered preliminary and may not reflect the final fungal load of the product The isolated colony was most likely due to accidental environmental contamination during sample preparation or handling rather than generalized contamination of the product, as confirmed by the absence of growth in replicates P2 and P3 at the same dilution **(Mossel *et al*., 2002).**

Chocolate is generally characterized by low water activity, high sugar content, and the presence of natural phenolic compounds from cocoa, all of which contribute to inhibiting fungal growth. These results are consistent with those reported by Stephen T. Beckett (2008), who stated that chocolate products usually exhibit limited microbial proliferation because of their unfavorable physicochemical conditions. In addition, the incorporation of spirulina (Arthrospira platensis) may have enhanced this inhibitory effect. According to Avigad Belay (2002), spirulina contains several bioactive compounds, including phycocyanin and antioxidant peptides, which possess antimicrobial and antifungal properties.

Table 15 . Fungal count and colony morphology after 24h of chocolate preparation

Dilution		**Total fungal count**	**Color and shape**
Original	P1	0	/
	P2	0	/
	P3	0	/
	P1	1	Green,cottony

10^{-1}	P2	0	/
	P3	0	/
10^{-2}	P1	0	/
	P2	0	/
	P3	0	/
10^{-3}	P1	0	/
	P2	0	/
	P3	0	/

II.5.2.2 Total fungal count after 7days of chocolate storage

After 7 days of storage at +4 °C , the fungal load remained remarkably stable, as shown in Table 16. A single white, cottony fungal colony was detected only in sample P1 of the original suspension. No fungal growth was observed in any of the other samples or dilutions (10^{-1}, 10^{-2}, and 10^{-3}).

The white cottony morphology observed in sample P1 is consistent with molds belonging to the genera Mucor or Rhizopus, which are common environmental contaminants frequently encountered in food-processing environments (Pitt and Hocking, 2009). The difference in colony appearance compared with the green colony observed after 24 hours suggests that the two isolates likely represent distinct fungal species resulting from separate contamination events.

The absence of any significant increase in fungal load between 24 hours and 7 days demonstrates the excellent microbiological stability of the product during storage. This stability may be attributed to the combined effect of the low water activity of the chocolate matrix and the potential antimicrobial activity of both date sugars and spirulina-derived bioactive compounds. James M. Jay et al. (2005) reported that foods with low water activity possess a natural resistance to microbial proliferation, even under suboptimal storage conditions.

Furthermore, the fungal counts obtained at both sampling times remained significantly lower than the maximum permissible levels specified in the Algerian Official Journal (CFU/g should be between 10 2 and 10 3). Therefore, the formulated chocolate met the national microbiological quality requirements and can be considered microbiologically safe for consumption.

Table 16 . Fungal count and colony morphology after 7 days of chocolate preparation

Dilution		Total fungal count	Color and shape
original	P1	1	White ,cottony
	P2	0	/
	P3	0	/
10^{-1}	P1	0	/
	P2	0	/
	P3	0	/
10^{-2}	P1	0	/
	P2	0	/
	P3	0	/
10^{-3}	P1	0	/
	P2	0	/
	P3	0	/

1. Detection of the pathogen bacteria

The microbiological analyses revealed the complete absence of pathogenic bacteria in all chocolate samples analyzed at the different sampling times. No colonies characteristic of the targeted pathogenic microorganisms were detected on the selective culture media used, indicating that their levels were below the detection limit of the method. These findings are inconsistent with those reported by **(Souvik Roy., et al., 2026)**, who detected pathogenic bacteria in chocolate samples. In contrast, the results of the present study demonstrated the absence of detectable pathogenic microorganisms, suggesting satisfactory microbiological quality and safety of the analyzed product (Tables 17, 18)

Table 17 .Detection of the pathogen bacteria after 7 day .

Parameter	Samples					Standard		Method
	1	2	3	4	5	m	M	J.O.A 75 of 2017
Escherichia Coli	Abs	Abs	Abs	Abs	Abs	Abs	Abs	
Pseudomonas aeruginosa	Abs	Abs	Abs	Abs	Abs	Abs	Abs	ISO 22717
Staphylococci Positive Coagulase	Abs	Abs	Abs	Abs	Abs	10^{2}	10^{3}	J.O.A 68 of 2014
Salmonella	Abs	Abs	Abs	Abs	Abs	Abs	Abs	J.O.A 44 of 2017

Table 18 .Detection of the pathogen bacteria after 14 day

Parameter	Samples					Standard		Method
	1	2	3	4	5	m	M	J.O.A 75 of 2017
Escherichia Coli	Abs	Abs	Abs	Abs	Abs	Abs	Abs	
Pseudomonas aeruginosa	Abs	Abs	Abs	Abs	Abs	Abs	Abs	ISO 22717
Staphylococci Positive Coagulase	Abs	Abs	Abs	Abs	Abs	10^{2}	10^{3}	J.O.A 68 of 2014
Salmonella	Abs	Abs	Abs	Abs	Abs	Abs	Abs	J.O.A 44 of 2017

II.5.2 Bioactivity of the chocolate :

II.5.2.1 Evaluation of antioxydant activity :

The values from the DPPH assay provided results for antioxidant activity in terms of the percentage of free radical inhibition, as shown in Table 19

Table 19 : Antioxidant Activity of chocolate Extract Assessed by DPPH Assay.

Composition	IC50 (mg/ml)
extract	**1.47 ±0.19**
Ascorbic acid	**0.044 ±0.005**

The DPPH assay results demonstrated that the chocolate extract possesses antioxidant activity through its ability to scavenge free radicals. The antioxidant capacity was expressed as IC50 values, which represent the concentration required to inhibit 50% of DPPH radicals. As presented in the table, the chocolate extract exhibited an IC50 value of 1.47 ± 0.19 mg/mL, whereas ascorbic acid, used as the positive control, showed a significantly lower IC50 value of 0.044 ± 0.005 mg/mL

These findings indicate that the chocolate extract exhibits moderate antioxidant activity compared with ascorbic acid. Since lower IC50 values correspond to stronger radical scavenging activity, the superior antioxidant effect of ascorbic acid can be attributed to its high efficiency in neutralizing reactive free radicals. Nevertheless, the observed antioxidant activity of the chocolate extract may be related to the presence of bioactive compounds such as polyphenols, flavonoids, and spirulina, which are capable of donating hydrogen atoms or electrons to stabilize DPPH radicals.

Therefore, the obtained results suggest that the chocolate could serve as a natural source of antioxidants and may contribute to reducing oxidative stress through its free radical scavenging potential**(Jaćimović et al. 2022)**

II.5.2.2 Evaluation of antibacterial activity :

The antibacterial activity of chocolate against bacteria (E. coli and Staphylococcus aureus) was evaluated using the disk diffusion method, and the obtained results are presented in (Table 20)

The results demonstrated that the chocolate exhibited antibacterial activity against both tested bacterial strains, although the inhibition zones remained relatively moderate compared with the positive control (Gentamicin).

Table 20 : results antibacterial activity of chocolate.

	Concentration	chocolate	Gentamycine	
			C-	C+
E.coli	0,5	4.83±2.02	0	2.7±0.1
	0.25	3.3±1.47		
Staphylococcus aureus	0.5	4.5±2.20	0	2.33±0.2
	0.25	4.23±1.5		

For E. coli, the highest inhibition zone was observed at the concentration of 0.5, with a value of 4.83 ± 2.02 mm, whereas lower inhibition diameter was recorded at concentration of 0.25. Similarly, against Staphylococcus aureus, the chocolate showed inhibitory effects with inhibition zones ranging from 4.23 ± 1.5 mm to 4.5 ± 2.20 mm. In contrast, the negative control showed no inhibitory activity, while Gentamicin exhibited stronger antibacterial effects against both bacterial strains.

The antibacterial activity observed in the chocolate may be attributed to the presence of bioactive compounds naturally found in cocoa, particularly polyphenols, flavonoids, tannins, and other phenolic constituents and spirulina, known for their antimicrobial properties. These compounds are capable of disrupting bacterial cell membranes, altering membrane permeability, and interfering with essential microbial metabolic processes, thereby inhibiting bacterial growth.

In addition, the variability in inhibition diameters between concentrations and bacterial strains could be explained by differences in bacterial cell wall structure. Gram-positive bacteria such as Staphylococcus aureus are generally more sensitive to phenolic compounds than Gram-negative bacteria like E. coli, whose outer membrane acts as an additional barrier limiting the penetration of antimicrobial agents. Similar findings have been reported in previous studies investigating the antimicrobial activity of cocoa and dark chocolate against pathogenic bacteria **(Katz et al., 2011; Jędrusek-Golińska et al., 2020)**

Conclusion

CONCLUSION

In the context of the growing global interest in functional foods with enhanced nutritional and health-promoting properties, the present study proposed an innovative approach based on the incorporation of two high-value natural resources, namely Degla Beida date sugar and Spirulina (Arthrospira platensis), into the formulation of functional chocolate. The study focused on the extraction and characterization of natural sugars obtained from dates, as well as the evaluation of the physicochemical, nutritional, and functional quality of the final product.

The obtained results demonstrated that sugar extracted from Degla Beida dates possesses promising nutritional and technological properties due to its high content of soluble sugars and phenolic compounds associated with antioxidant activity. These characteristics support its potential use as a natural alternative to refined sugar in chocolate manufacturing. Furthermore, the incorporation of spirulina significantly enhanced the nutritional value of the formulated chocolate because of its richness in proteins, vitamins, minerals, essential fatty acids, and bioactive pigments such as phycocyanin. The developed chocolate also maintained acceptable sensory and physicochemical properties in terms of texture, taste, and homogeneity.

In addition, the study highlighted a functional synergism between date-derived sugar and spirulina bioactive compounds, which contributed to increasing the antioxidant potential and nutritional density of the final product. This interaction provides the developed chocolate with an innovative functional dimension, transforming it from a conventional confectionery product into a value-added food with potential health-promoting properties. The findings therefore emphasize the importance of valorizing local agricultural resources through the development of sustainable and nutritionally enriched food products adapted to current consumer demands.

Moreover, this work opens promising perspectives for the future application of microalgae and natural date-derived ingredients in modern food industries, particularly within the field of functional and health-oriented foods. Future investigations may focus on optimizing formulation parameters, evaluating rheological and storage stability properties, and exploring the biological activities and health effects of spirulina-enriched chocolate through advanced biochemical and clinical studies. Additional research may also contribute to expanding the use of date sugar and spirulina in other innovative food matrices with high nutritional and functional potential.

Overall, this study demonstrates that the integration of date sugar and spirulina represents not only a technological alternative to conventional sweeteners, but also an innovative nutritional strategy based on the sustainable valorization of natural local resources for the development of healthier and functionally enriched food products.

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Annex

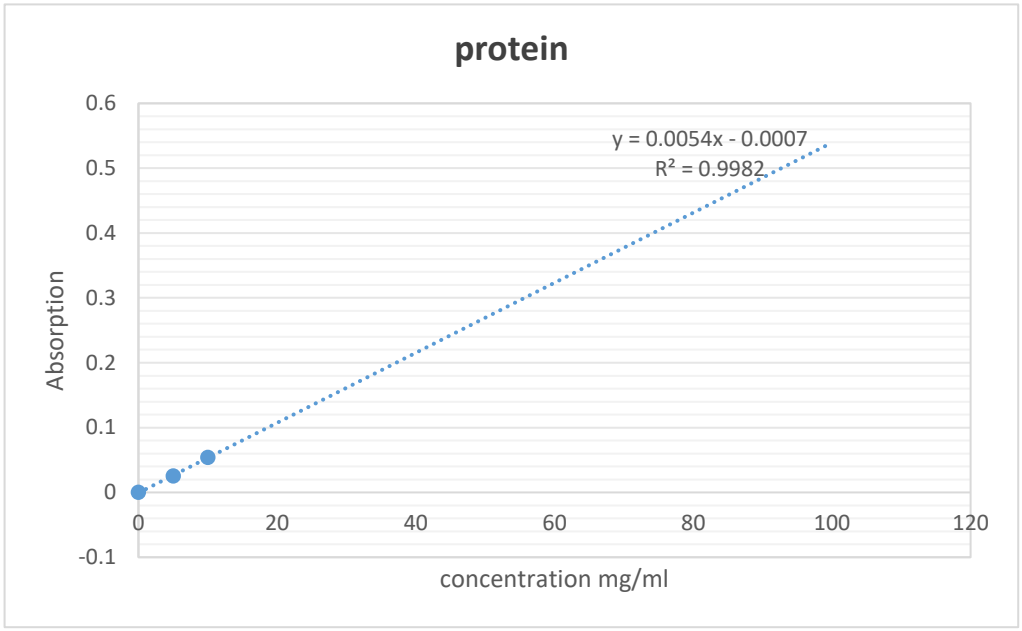


Figure 23 : Calibration Curve for protein

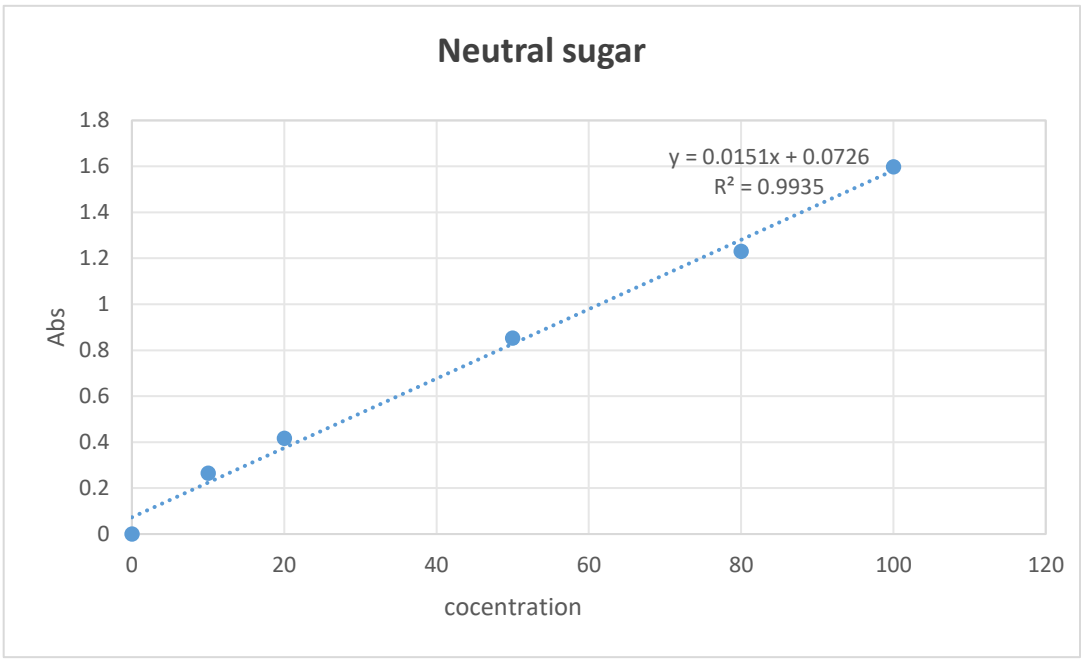


Figure 24 : Calibration Curve for Neutral Sugars

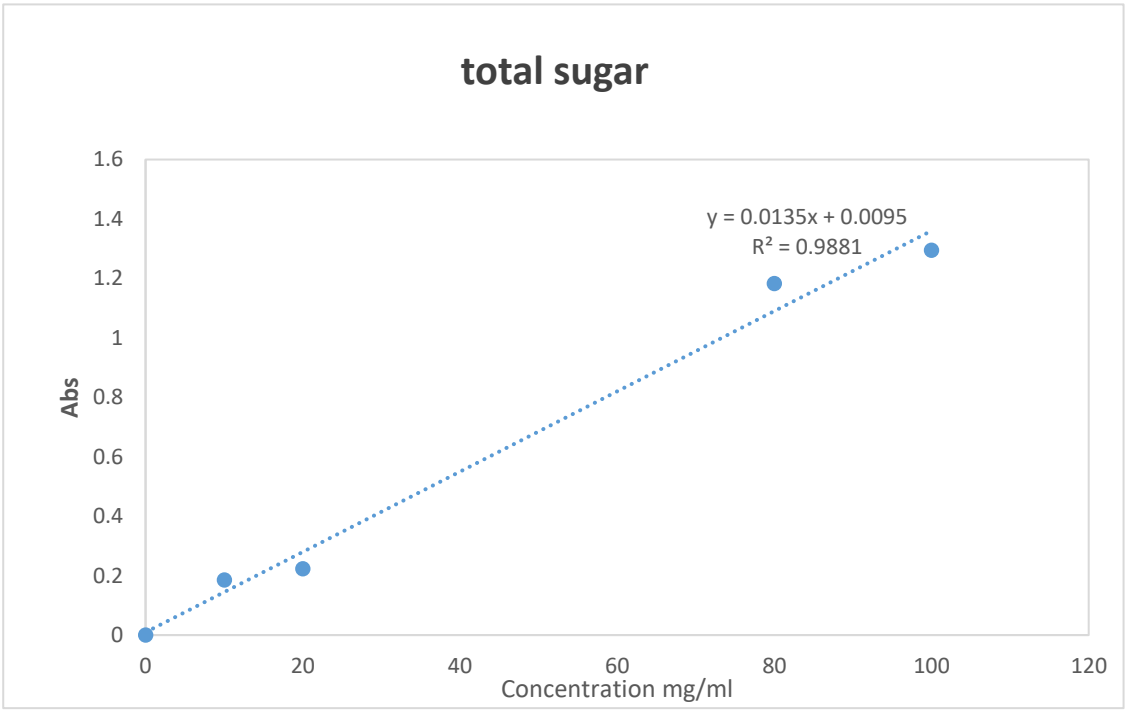


Figure 25 : Calibration Curve for Total Sugars.

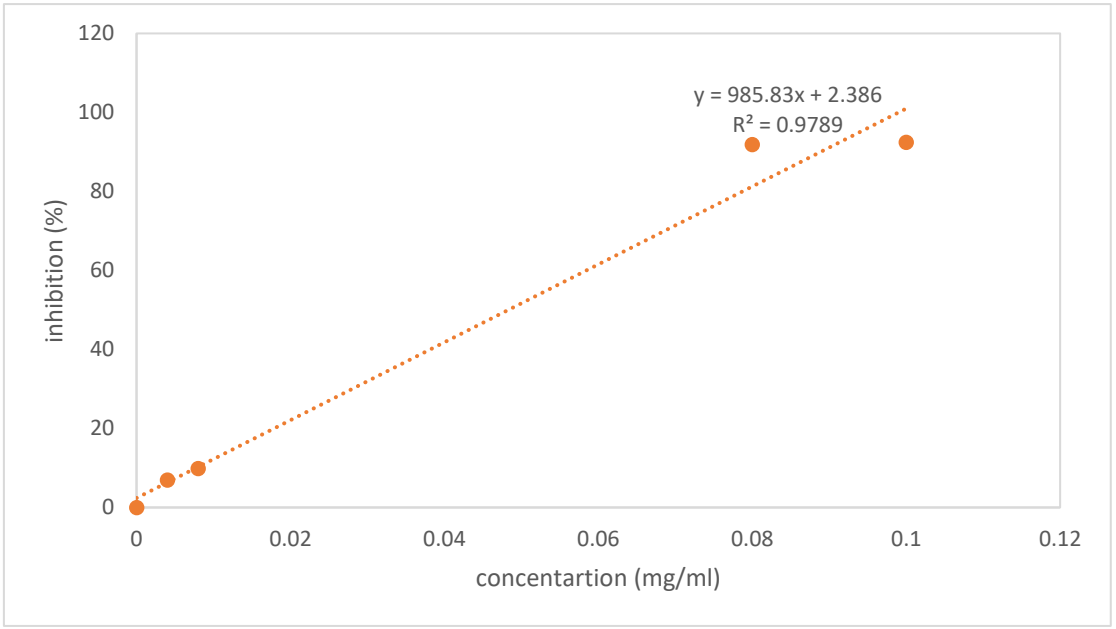


Figure 26 : Calibration Curve of ascorbic acid for the DPPH

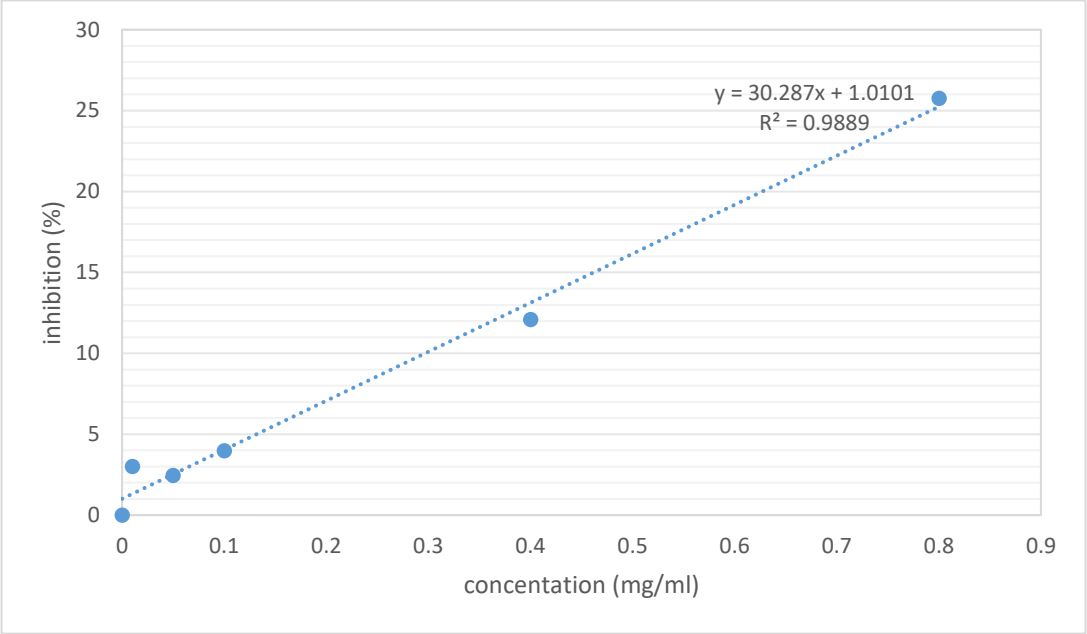


Figure 27 : Calibration Curve for Inhibition percentage % chocolate concentration (mg /ml)

Fatilab

Autorisation N°: 154 du 14/07/2009

مخبر التحاليل ومراقبة النوعية والمطابقة
LABORATOIRE D'ANALYSE ET DE CONTRÔLE DE LA QUALITÉ ET DE LA CONFORMITÉ

BULLETIN D'ANALYSE
Microbiologique

N°: 04182-26

Information Client:

Client: ZARGUI MALAK — **Code:** 2469
Adresse: El Oued
Tel: 06 64 31 13 44 / 06 96 73 41 52

Information Echantillon

Référence: E0647.02.26 — **Prélever** 19/04/2026
Dénomination: Chocolat — **Par:** Lui-même
Nature: Produit final — **Lieu :** /
Embalage: 20 g

Les résultats:

Echantillon reçu le: 19/04/2026 — **Lancer le:** 20/04/2026

Paramètre	Echantillons 1	2	3	4	5	Norme m	M	Méthode
Escherichia Coli	Abs	Abs	Abs	Abs	Abs	Abs	Abs	J.O.A 75 du 2017
Pseudomonas aéruginosa	Abs	Abs	Abs	Abs	Abs	Abs	Abs	ISO 22717
Staphylocoques à coagulase +	Abs	Abs	Abs	Abs	Abs	10^2	10^3	J.O.A 68 du 2014
Salmonella	Abs	Abs	Abs	Abs	Abs	Abs	Abs	J.O.A 44 du 2017

Observation: Ces résultats d'analyses ne concernent que l'échantillon reçu
NB: Ce bulletin est identique à la souche archivée chez le laboratoire qui ne contient aucun surcharge ou correction et dans le cas contraire la présente feuille sera annulée

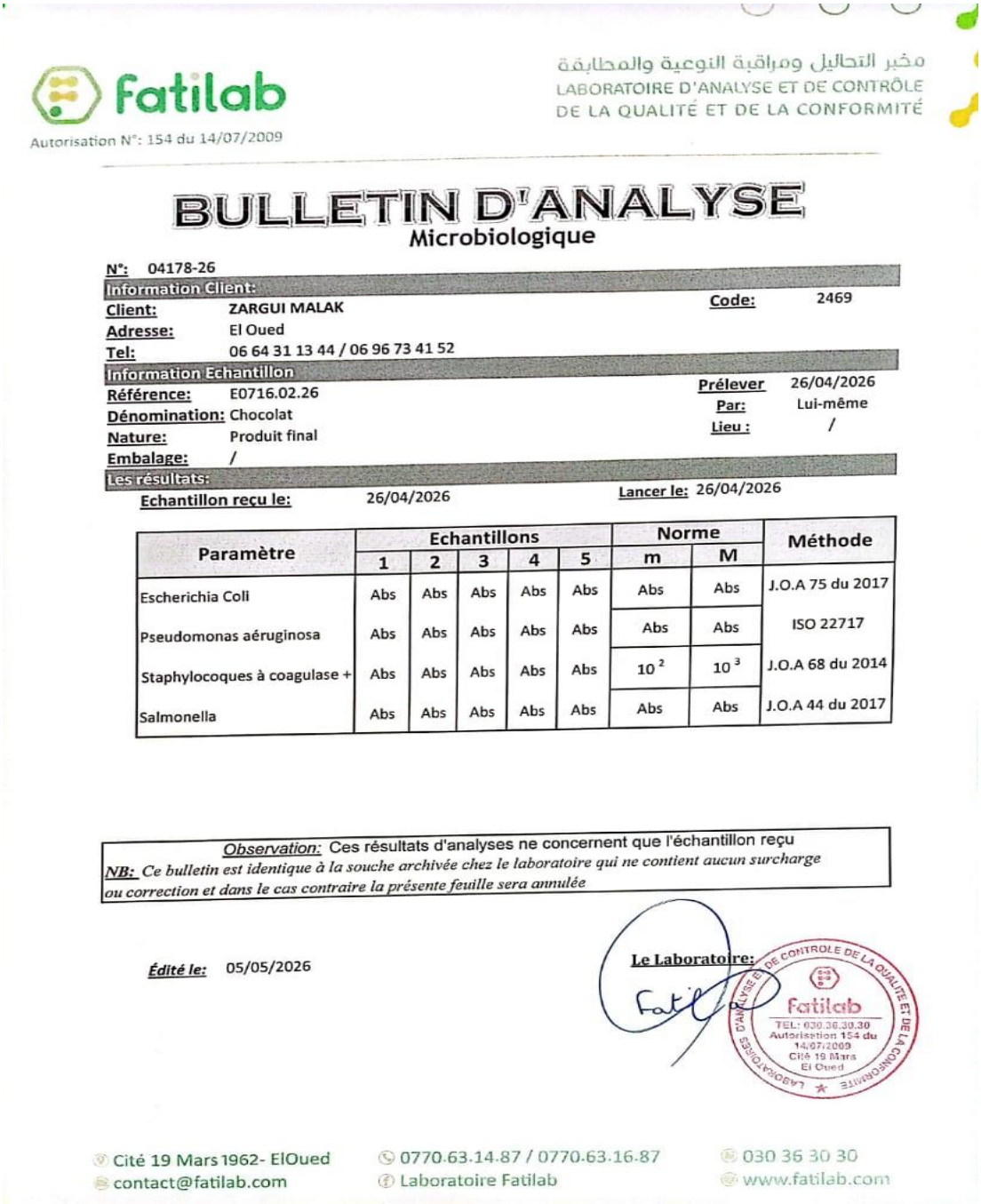
Édité le: 05/05/2026

Le Laboratoire:

fatilab
TEL: 030.36.30.30
Autorisation 154 du 14/07/2009
Cité 19 Mars El Oued

Cité 19 Mars 1962- ElOued — 0770.63.14.87 / 0770.63.16.87 — 030 36 30 30
contact@fatilab.com — Laboratoire Fatilab — www.fatilab.com

Figure 28 : Detection of the pathogen bacteria after 7 day



Fatilab
Autorisation N°: 154 du 14/07/2009

مخبر التحاليل ومراقبة النوعية والمطابقة
LABORATOIRE D'ANALYSE ET DE CONTRÔLE
DE LA QUALITÉ ET DE LA CONFORMITÉ

BULLETIN D'ANALYSE
Microbiologique

N°: 04178-26

Information Client:

Client: ZARGUI MALAK — **Code:** 2469
Adresse: El Oued
Tel: 06 64 31 13 44 / 06 96 73 41 52

Information Echantillon

Référence: E0716.02.26 — **Prélever** 26/04/2026
Dénomination: Chocolat — **Par:** Lui-même
Nature: Produit final — **Lieu :** /
Embalage: /

Les résultats:

Echantillon reçu le: 26/04/2026 — **Lancer le:** 26/04/2026

Paramètre	Echantillons					Norme		Méthode
	1	2	3	4	5	m	M	
Escherichia Coli	Abs	Abs	Abs	Abs	Abs	Abs	Abs	J.O.A 75 du 2017
Pseudomonas aéruginosa	Abs	Abs	Abs	Abs	Abs	Abs	Abs	ISO 22717
Staphylocoques à coagulase +	Abs	Abs	Abs	Abs	Abs	10^2	10^3	J.O.A 68 du 2014
Salmonella	Abs	Abs	Abs	Abs	Abs	Abs	Abs	J.O.A 44 du 2017

Observation: Ces résultats d'analyses ne concernent que l'échantillon reçu
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Cité 19 Mars 1962- ElOued — 0770.63.14.87 / 0770.63.16.87 — 030 36 30 30
contact@fatilab.com — Laboratoire Fatilab — www.fatilab.com

Figure 29 : Detection of the pathogen bacteria after 14 day

Figure 30 :Bacterial count and colony morphology after 24 H of chocolate preparation

Figure 32 : Bacterial count and colony morphology after 7 days of chocolate preparation

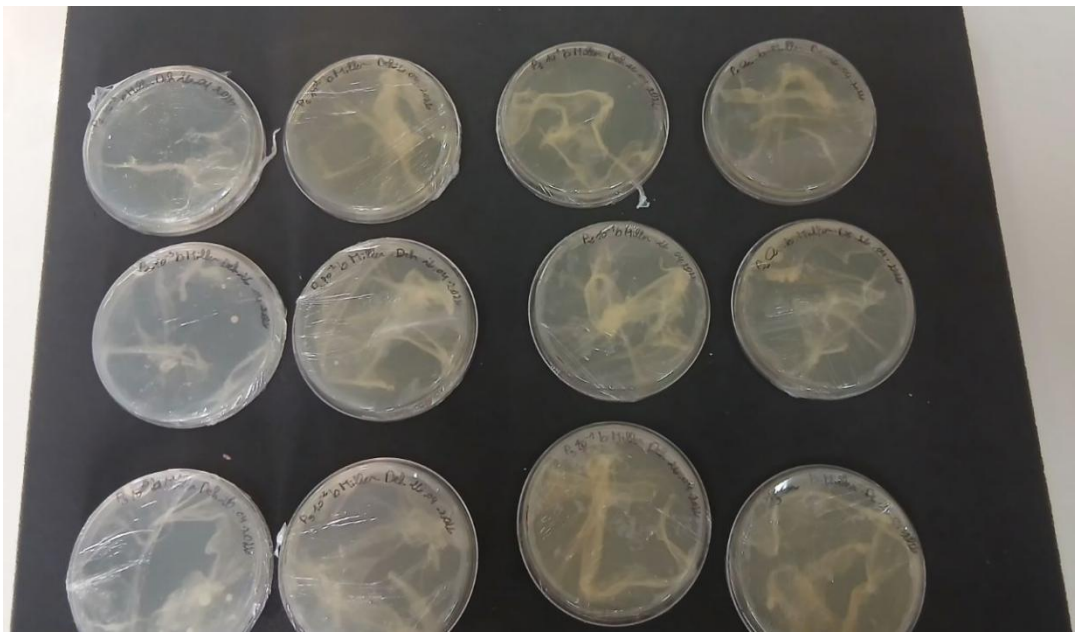

Figure 31 : Bacterial count and colony morphology after 14 days of chocolate preparation

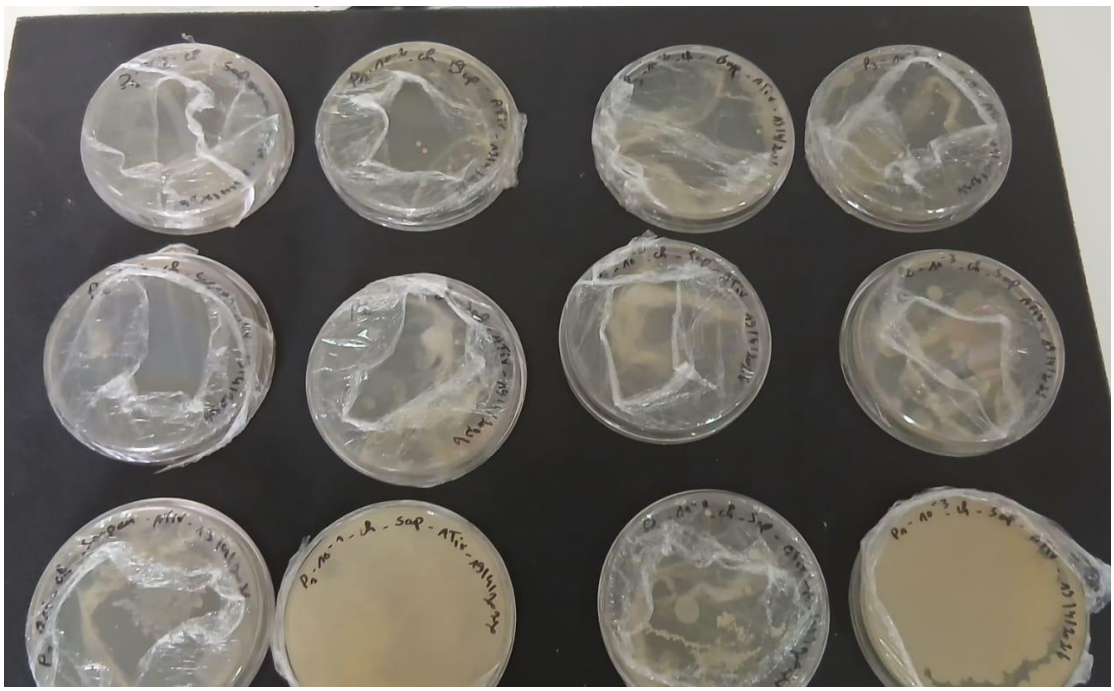

Figure 32: Fungal count and colony morphology after 1 days of chocolate preparation

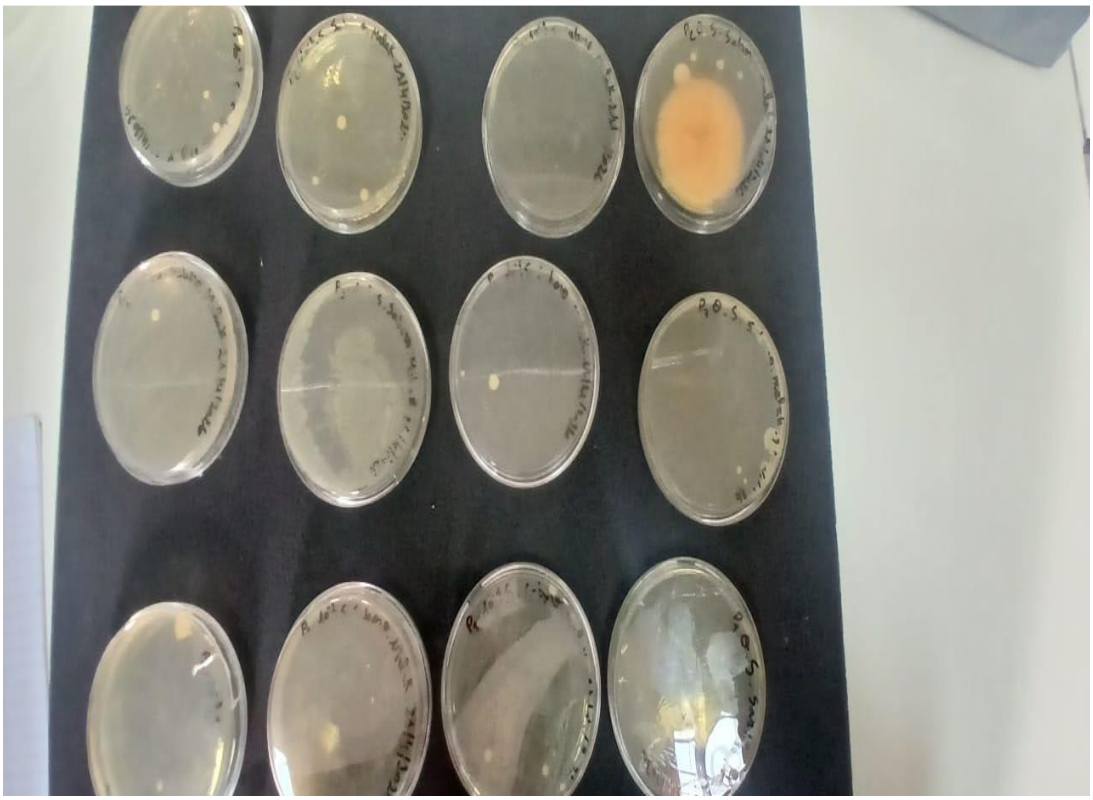

Figure 33 : Fungal count and colony morphology after 14 days of chocolate preparation

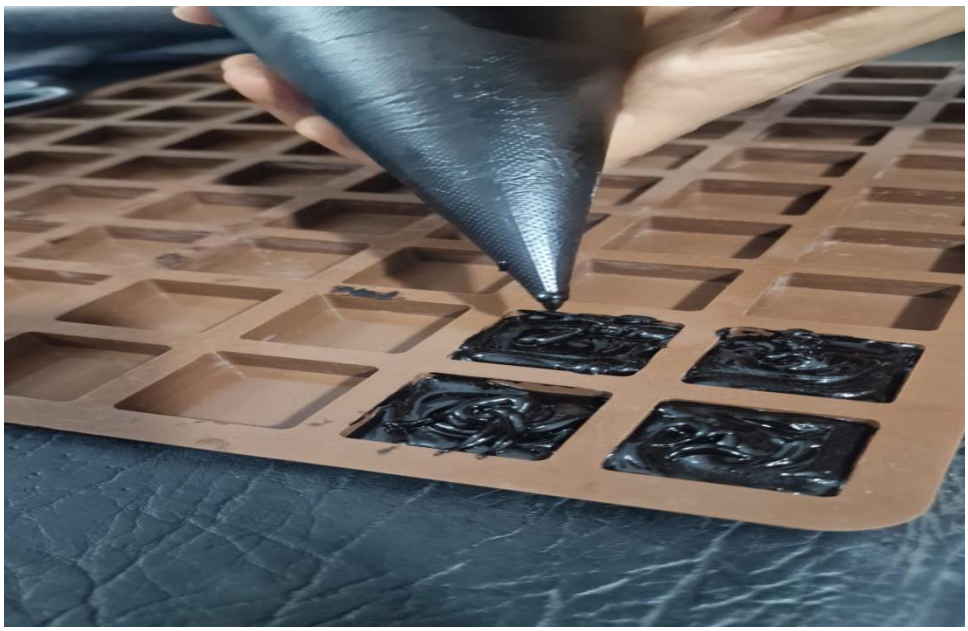

Figure 34 : Final chocolate product