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Entitled

Biological effects of tiger nut tubers *Cyperus esculentus* L. associated or not with probiotic bacteria.

Effets biologiques des tubercules de souchet comestible *Cyperus esculentus* L. associés ou non aux bactéries probiotiques.

Defended on……

The Jury

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Dedication

I dedicate this work to my family, who have given me much more than mere instruction. Thanks to their constant support, wisdom, and unwavering love, they have instilled in me the principles and determination necessary to reach my current position. Their support and presence have been sources of inspiration and strength throughout my journey. Without their help, none of what I have accomplished would have been possible. This work represents everything they have passed on to me.

To my parents

I wish to express my deep gratitude and warmly thank you for all the invaluable support, unwavering affection, wise recommendations, and exemplary patience you have shown me throughout my studies. You have been a constant support and a source of inspiration, encouraging me to follow my aspirations and put forth my best effort to achieve my life goals. It is thanks to your help that I have managed to overcome obstacles and progress with confidence.

This work is, to a large extent, a reflection of your sacrifices and the enormous commitment you have constantly shown towards me. On these pages, you will discover the result of your hard work and the expression of my sincere love, as well as my infinite gratitude for everything you have offered me.May God keep and protect you, you who are the authentic source of my inspiration and strength.

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To my sisters and brother

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Noussaiba belmadani

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Abstract

This study assesses the biological impact of tiger nut (*Cyperus esculentus* L.) tuber extract associated or not to the probiotic strain *Lasticaseibacillus rhamnosus* SL42. The tiger nut flour was used to make dietary cookies for people with diabetes. In the first part of this thesis, the flour's bioactive components were identified using physicochemical, rheological, and functional analysis. Our findings revealed an abundance of lipids, proteins, mineral salts, and phenolic compounds. Following that, cookies made from 100% tiger nut flour with no sugar added, enriched or not with the strain imbedded in honey syrup (5%), were made. The panelists certified this formulation following extensive organoleptic analyses after one and 15 days of storage at 25°C. A SEM study of the cookies soaked in syrup revealed that SL42 adhered to the surface of the cookies, and the microbial count confirmed its survival at an appreciable load after 21 days of storage. Thus, our cookies met international standards and were suitable for diabetic patients. The second part of this thesis investigated the therapeutic impact of tiger nut extract on vulvovaginal candidiasis (VVC) in an *in vivo* model of Wistar rats. An intimate gel containing the hydro-ethanolic extract and the beads of the probiotic strain SL42, was proposed. Preliminary microbiological study indicated the minimum inhibitory concentration of tiger nut extract to be employed in the gel. Histological results revealed that SL42 alone and the intimate gel both had good antifungal activity against *Candida albicans* 10231, regenerating healthy epithelial tissue after 7 days of therapy. The inclusion of our SL42 probiotic isolate had a cooperative effect on maintaining the vaginal mucosa's homeostasis. More in-depth research is required to demonstrate the long-term efficacy of both formulations.

Key words: *Cyperus esculentus* L., biological effect, *Lacticaseibacillus rhamnosus* SL42, cookies, physicochemical analysis, vulvovaginal candidiasis.

Résumé

Cette étude évalue les effets biologiques de l'extrait de tubercule de souchet comestible (*Cyperus esculentus* L.) associé au non à la souche probiotique *Lacticaseibacillus rhamnosus* SL42. Dans la première partie de cette thèse, la farine de souchet a été utilisée pour fabriquer des biscuits diététiques pour les diabétiques. Les composants bioactifs de la farine ont été identifiés à l'aide d'analyses physico-chimiques, rhéologiques et fonctionnelles. Nos résultats ont révélé une abondance de lipides, de protéines, de sels minéraux et de composés phénoliques. Par la suite, des biscuits fabriqués à partir de farine de souchet à 100 % sans sucre ajouté, enrichis ou non avec la souche SL42 incorporée dans un sirop de miel (5 %), ont été confectionnés. Les panélistes ont certifié cette formulation après une analyse organoleptique approfondie à un et 15 jours de stockage à 25°C. Une étude SEM des biscuits imbibés dans le sirop a révélé que SL42 adhérait à la surface des biscuits, et le comptage microbiologique a confirmé sa survie à une charge appréciable après 21 jours de stockage. Ainsi, nos biscuits répondaient aux normes internationales et pourraient être adaptés aux diabétiques. La deuxième partie de cette thèse a étudié l'impact thérapeutique de l'extrait de souchet sur la candidose vulvo-vaginale (CVV) dans un modèle de rats Wistar *in vivo*. Un gel intime contenant l'extrait hydro-éthanolique et les microcapsules de la souche probiotique SL42 a été proposé. Une étude microbiologique préliminaire a calculé la concentration minimale inhibitrice de l'extrait de souchet à utiliser dans le gel. Les résultats histologiques ont révélé que SL42 seule et le gel intime avaient tous deux une bonne activité antifongique contre *Candida albicans* 10231, régénérant un tissu épithélial sain après 7 jours de thérapie. L'inclusion de notre isolat probiotique SL42 dans la composition du gel a eu un effet synergétique sur le maintien de l'homéostasie de la muqueuse vaginale. Des recherches plus approfondies seraient nécessaires pour démontrer l'efficacité à long terme des deux formulations.

Mots-clés : *Cyperus esculentus* L., effet biologique, *Lacticaseibacillus rhamnosus* SL42, biscuits, analyse physico-chimique, candidose vulvovaginale.

ملخص

تقيّم هذه الدراسة التأثير البيولوجي لمستخلص درنات نبات حب العزيز (*Cyperus esculentus* L.) المرتبط أو غير المرتبط بالسلالة البروبيوتيك *Lacticaseibacillus rhamnosus* SL42. تم استخدام دقيق حب العزيزلصنع بسكويتات غذائية للأشخاص المصابين بالسكري .في الجزء الأول من هذه الأطروحة، تم تحديد المكونات النشطة بيولوجيًا للدقيق باستخدام التحليل الفيزيائي الكيميائي، والتحليل اللزوجي، والتحليل الوظيفي .كشفت نتائجنا عن وفرة من الدهون والبروتينات والأملاح المعدنية والمركبات الفينولية .بعد ذلك، تم صنع بسكويت من دقيق حب العزيز بنسبة 100% بدون إضافة سكر، سواء كان مرفقا أو لا بالسلالة المدمجة في شراب العسل .(5%) شهاد أعضاء اللجنة بهذه التركيبة بعد تحليلات حسية مكثفة بعد يوم واحد و15 يومًا من التخزين عند 25 درجة مئوية .أظهرت دراسة SEM للبسكويت المنقوع في الشراب أن SL42 التصق بسطح البسكويت، وأكدت العد الميكروبي بقائه بكمية ملحوظة بعد 21 يومًا من التخزين . وهكذا، فإن بسكويتاتنا تفي بالمعايير الدولية وكانت مناسبة للمرضى المصابين بالسكري .الجزء الثاني من هذه الأطروحة بحث التأثير العلاجي لمستخلص حب العزيزعلى داء المبيضات الفرجيني المهبلي (VVC) في نموذج حيواني من فئران ويستار .تم اقتراح جل حميمي يحتوي على المستخلص الهيدرو-إيثانولي وكرات السلالة البروبيوتيك .SL42 أشارت الدراسة الميكروبيولوجية الأولية إلى الحد الأدنى من التركيز المثبط لاستخراج حب العزيزالذي سيتم استخدامه في الهلام .أظهرت النتائج النسيجية أن SL42 بمفرده والهلام الحميمة كلاهما كان لهما نشاط مضاد للفطريات جيد ضد*Candida albicans* 10231 ، مما أدى إلى تجديد الأنسجة الظهارية الصحية بعد 7 أيام من العلاج .كان لإدراج عزلتنا البروبيوتيك SL42 تأثير تعاوني في الحفاظ على توازن الغشاء المخاطي المهبلي .تتطلب الأبحاث الأكثر عمقًا لإثبات الفعالية طويلة الأمد لكلتا التركيبين.

الكلمات المفتاحية: حب العزيز، التأثير البيولوجي، *Lacticaseibacillus rhamnosus* SL42، البسكويت، التحليل الفيزيائي الكيميائي، داء المبيضات الفرجاني المهبلي.

Abbreviations in General Use

SL42: *Lacticaseibacillus rhamnosus*

BD: Bulk Density

WAI: Water Absorption Index

OAC: Oil Absorption Capacity

XRD: X-ray Diffraction Analysis

IC: Ion chromatography

IC_{50}: Inhibitory Concentration 50%

FRAP: Ferric Reducing Antioxidant Power

DPPH: 2,2-Diphenyl-1-picrylhydrazyl

SEM: Scanning Electron Microscope

VVC:Vulvovaginal candidiasis

RVVC: Recurrent vulvovaginal candidiasis

MIC: Minimum Inhibitory Concentration

DMSO: Dimethyl sulfoxide

MFC: Minimal fungicidal concentration

UV-HPLC: Analysis of the extract using High Performance Liquid Chromatography coupled to UV detection .

CFU: Colony Forming Unit

MPO: Myeloperoxidase activity

PGE2: Myeloperoxidase and Prostaglandin

ELISA: Enzyme-Linked Immunosorbent Assay

MFC: Minimum Fungicidal Concentration

3,4 di-HBA : 3,4 di-hydroxybenzoic acid

PG: Pyrogallol

PHG : Phloroglucinol compounds

CADE: Caffeic Acid Dimethyl Ether

CA:*Candida albicans*

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General Introduction

General introduction

For centuries, plants have served not only as food for humans but also as remedies to treat various diseases. The abundant phytological resources in Africa help meet the nutritional and therapeutic needs of the populations. Nevertheless, a large part of these resources remains unknown, underutilized, or even unused, despite their attractive characteristics that could solve serious malnutrition problems in our regions. In 2016, the number of undernourished individuals worldwide reached 815 million. Sub-Saharan Africa remains the region with the highest rate of undernourishment, reaching 22.7% **(FAO *et al*., 2017).** Moreover, the use of plants in the fields of therapy and nutrition promotes a holistic view of health, taking into account not only physical manifestations but also mental and emotional balance. Methods such as aromatherapy and meditation in natural settings demonstrate that interaction with vegetation can also lead to benefits for psychological well-being.

In Algeria, the use of medicinal plants dates back many years. This practice draws its inspiration from the experiences of communities and traditional Arab medicine. In this context, we focused on studying of the tiger nut tuber (*Cyperus esculentus* L.).

Tiger nut is a tuber that develops on the roots of a sedge plant. The ancient Egyptians cultivated tubers in the Nile Valley **(Fabunmi *et al.*, 2016).** *Cyperus* tubers have been found in tombs in Egypt **(Kizzie-Hayford *et al.*, 2021).**

According to various sources, tiger nuts were imported to Spain from Africa. Tiger nut is a sweet tuber that can be edible. These tubers are also called "earth almond" and "yellow nut sedge" **(Onuoha *et al.*, 2017).** The pericarp of the nut has notable robustness and is usually immersed in water before consumption **(Asare *et al.*, 2020).**

According to studies, the tuber is recommended for individuals suffering from dyspepsia, dysentery, and bloating **(Ahaotu *et al.*, 2020).** The high presence of oleic acid in tiger nut has a positive impact on cholesterol, and thus stimulates blood cholesterol levels. Regarding cholesterol, it stimulates blood content and slows down heart attacks and thrombosis **(Onyibe *et al.*, 2021).** Tiger nut helps prevent constipation and reduces the risk of colorectal cancer **(Benavides *et al.*, 2016).**

Currently, scientists are increasingly interested in women's health, particularly focusing on the vaginal microbiome. Because the vagina harbors a vast microsystem that contains billions of types of microorganisms **(Witkin and Forney, 2020).** In the majority of reproductive and healthy women, the vaginal flora is primarily dominated by beneficial *Lactobacillus* species, which are believed to strengthen defenses against the invasion and colonization by pathogenic organisms **(Melgaço *et al.*, 2018).**

Besides bacterial vaginosis (BV), which has been the subject of numerous studies, vulvovaginal candidiasis (VVC) has garnered significant interest. It is a multifactorial infectious condition affecting the lower female reproductive tract, primarily caused by *Candida albicans*, and causing pathological

inflammation **(Farr *et al.*, 2021).** In a study that gathered 649 different cases of patients suffering from VVC in China, scientists observed that *Candida albicans* remained the predominant microorganism in VVC cases **(Pang *et al.*, 2022).**

The conventional treatment recommended for vaginal infections caused by *C. albicans* is antifungal therapy include azoles. However, the prolonged use of antibiotics increases relapse rates, likely due to the inability to restore the normal balance dominated by *Lactobacillus*.
Interest in the therapeutic use of probiotics has increased in recent years. It is recommended to use *Lactobacillus* species in general as a new approach, this strategy gaining popularity thanks to the accumulation of evidence demonstrating their effectiveness in restoring normal microbial function and preventing urogenital infections **(Amabebe and Anumba, 2018).**
It is also impossible to completely abandon the traditional use of medicinal plants, despite the advancement of synthetic drugs, given that plant extracts have considerable clinical importance in the management of infections caused by resistant microbial strains **(Abedini, 2013).**
In this thesis, we analyzed the biological impact of the edible tiger nuts associated or not to probiotics. The present thesis is structured into two experimental chapters preceded with three theorical chapters which reflected a concise literature review on the subject.

The first Chapter resumed an abridged ethnobotanical synthesis about the tubers, their physico-chemical constitution, and their presumed beneficial effects on health. The second one was dedicated to the biotechnological aspect of biscuit as it was primordial for a better comprehending of the first experimental part. The last theorical chapter involved the clinical and microbiological aspects of vulvoaginal candidiasis, serving as glossary for chapter V.

Chapter IV and V were the experimental part of the thesis. In Chapter IV, we examined the chemical composition of tiger nut, highlighting its active principles and the potential health benefits they can bring. Subsequently, we designed a recipe of dietary cookies, including edible tiger nut flour and *Lacticaseibacillus rhamnosus* SL42, with the aim of developing a product that is both delicious and health-beneficial for people with diabetes. The cookie could offer a nutritious option while facilitating the integration of probiotics into everyday diets.

In Chapter V, we developed an intimate gel containing tiger nut extract enriched with probiotics, with the aim of analyzing the synergistic impacts of this combination on the vaginal mucosa infected by *C. albicans*.

Chapter I: General information On nutsedge *Cyperus esculentus* L.

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In this section, a literature search on the anatomy, growth and development physiology, ecology, and life-history characteristics of *Cyperus esculentus* will deepen our understanding of this species' life cycle and help us identify disruptors to its development.

I.1. History, origin, and worldwide presence

According to **Ahmed and Hussein (2014)**, the edible nutsedge *Cyperus esculentus* L. was discovered in ancient Egypt. The discovery of dried nutsedge tubers in 6000-year-old Egyptian tombs provides strong evidence for the hypothesis that nutsedge cultivation began in Egypt. The name of edible nutsedge varies according to the country where it is produced or consumed. Several names are summarized in Table 1, ranging from tiger nut in Africa to حب العزيز (Hab el Aziz = grains of Al-Aziz) in Egypt (a name inspired by the name of the Fatimid ruler who was known as a fan).

Table 1: Vernacular names for Cyperus esculentus L. (Ibrahim *et al*., 2016).

Country	Languages	Common names
Senegal	Wolof	Ndir
Mali	Bambara	Tiongon
Ivory Coast	Baoulé	Atadjo
Guinea Conakry	Malinké	Toki
Ghana	---	Atadwe
South Africa	Zulu	Zulu nut
Nigeria	Haoussa	Aya
	Yoruba	Ofio
Spain	Spanish	Chufa
Middle East	Arabic	Habelaziz

Yellow nutsedge is widespread throughout the world but is most common in wet or swampy areas of subtropical, temperate and subarctic regions. Originally from the eastern Mediterranean basin, *C. esculentus* is now an invasive species on all continents except Antarctica, which is most often found in warmer zones. However, since around the middle of the 20th century, the species seems to have developed in the colder climates of temperate regions, as far north as the 50th parallel in the northern hemisphere. Its range extends

northwards into Europe. Since the 1970s, it has been a problem in southern and eastern Africa and in Central and North America (**Ahmed and Hussein, 2014)**.

Ter Borg *et al.* (1998) summarize the geographical distribution of the four wild varieties, based on Kükenthal's 1935 data and a morphological study of 1000 herbarium specimens (**Dodet, 2006).** The esculentus variety is found throughout Africa, southern Europe and occasionally in the northeastern United States and neighboring Canada. The leptostachyus variety is found in the colder regions of North and South America. The var. macrostachyus thrives in the warmer regions of America, while the var. hermanii is restricted to a limited area in the western United States.

In France, populations of *C. esculentus* come from a variety of origins and reflect the diversity of this species.

Three categories of populations can be distinguished: populations that may be spontaneous and localized in the Mediterranean region, probably sensitive to cold (var. esculentus, originally from Africa, already reported in southern Europe by Kükenthal in 1935); some very localized populations invading horticultural farms that have imported plant material, probably from Spain (var. sativus), and populations found mainly along the banks of major western rivers, imported from North America and perhaps exported by the extraction of Loire sands (var. leptostachyus).

With the exception of the first two varieties mentioned above, which are restricted to limited, well-defined geographical areas, all samples from French populations distributed throughout mainland France have been identified by Ter Borg (**Ter Borg *et al.*, 1998)** as belonging to the leptostachyus variety. Given that the esculentus variety was originally the only one in Europe, and that it remains located around the Mediterranean, the expansion of the species' range cannot be due to a simple northward shift. New populations are therefore more likely to have originated in the USA, where the leptostachyus variety is most widespread. In Loir-et-Cher, this variety was introduced as early as 1947 by mixing gladiolus bulbs imported from the USA. The presentation of var. leptostachyus has probably been introduced several times **(Ter Borg *et al*., 1998; Dodet, 2006).**

I.2 Taxonomy and morphological description of Tiger Nutsedge

Cyperus esculentus var *sativus* (**L. 1753)** is the scientific name for yellow nutsedge. It is a genus of plants in the order Poales, family Cyperaceae, genus *Cyperus*, species esculentus and variety sativa. French synonyms for souchet include pois sucré, noix tigrée, souchet comestible or tubéreux, amande de terre and souchet sultan. In Spanish, it's called “Chufa”; in English, several names such as yellow nutsedge, tigernut or earth almond, refer to tiger nut because of its resemblance to grasses or the edible nature of its tubers (**Dodet, 2006**). Tiger nut is commonly referred to in Dioula as “tchogon”.

Cyperus esculentus is a species of plant with a folded pre-foliage and triangular cross-section. The leaf blade is unfolded and hairless. The plant measures between 20 and 70 cm in height. According to **Dodet (2006),** the leaves are extremely long, thin, 4 to 8 mm wide and pointed. It can be recognized by the broad V-shaped

part of the blade and its yellowish-green hue. Seven (7) rays emerge from its base. According to **GEOMAR international (2002),** spikelets are elongated, acuminate, mulched and composed of several flowers. Roots are elongated, small and about one millimeter in diameter. They have rhizomes that end in spherical, oval or elongated tubers. Yellow nutsedge is grown for its tuberized rhizomes. Its tubers are ochre to brown, with white, slightly mealy, sweet flesh. **Figure 1** illustrates this description.

I.3.Classification

The taxonomic tree of the nutsedge plant is as follows **(Dodet, 2006):**

Domain Biota

Kingdom Plant

Phylum Spermatophyte

Subphylum Angiosperm

Class liliopsida (equisetopsida)

Superorder lilianae

Order Cyperales

Family Cyperaceae

Subfamily scirpoideae

Genus *Cypercus*

Species *Esculentus*

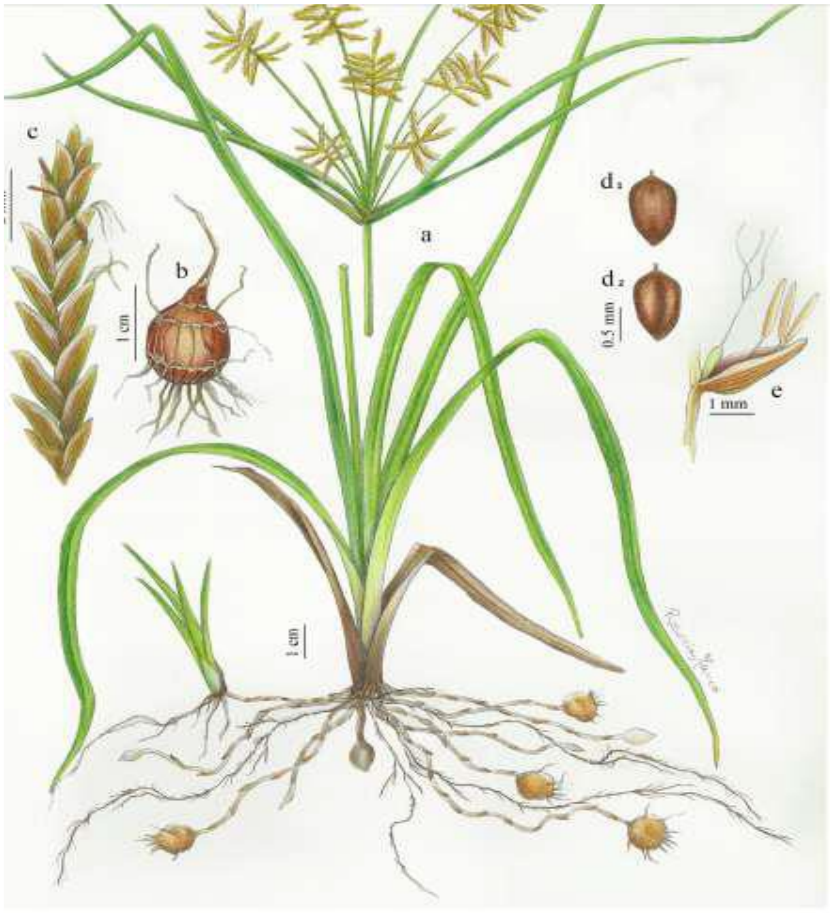


Figure 1:Cyperus esculentus plant (Follak *et al*., 2016).

(a) plant in flower; (b) mature tubers; (c) spikelet; (d1) achene: dorsal view; (d2) achene: ventral view; (e) flower details, ventral view.

I.4. Nutsedge plant agronomy

Yellow nutsedge requires a mild climate and sandy soil for cultivation. According to **Bamishaiye *et al*. (2011)**, nutsedge reproduces using seeds and wind pollination. Yellow nutsedge is a grass that grows in wetlands. Planted in April, it is harvested in November. The planting period is divided into a main period (April-July) and a secondary period (September-November). Yellow nutsedge tubers are mixed with stones, animal dung and other foreign matter when harvested from the fields (**Ayeh-Kumi *et al*., 2014). Figure 2** shows a field of nutsedge.

Figure 2: Cyperus esculentus field (Anonymous, 2017).

I.5. Yellow nutsedge production

The production process is divided into different stages: burning, loosening, sieving and winnowing (**Figure 3**). In the pre-harvest period, farmers carry out superficial burning of the above-ground biomass in the fields. This is followed by the collection of debris and other devoured residues. After lifting and improving the soil, sieving and winnowing are carried out **(Haoua *et al.*, 2018).** At harvest time, they are dried raw or after washing. Drying takes place in the sun for several weeks. Tubers must be regularly stirred to ensure even drying. According to **Abano and Amoah (2011),** drying plays a crucial role in the lifespan of tubers by preventing bacterial infection and rotting. Well-dried tubers can be stored for over a year. It is necessary to keep them dry and rehydrate them by soaking without losing their crispness.

Figure 3:Harvesting stages in Niger (Haoua *et al.*, 2018).

I.6 Edible nutsedge tuber

I.6.1. Morphology

The tubers of *C. esculentus* L. are the result of the thickening, over several internodes, of the distal end of certain stolons (**figure 4**). These stolons are small whitish or brownish rhizomes, 5-20 cm long, which appear either as tubers or seedlings. They turn brown with age. These tubers vary in shape and size on the same plant, from sub-spherical to elongated and elliptical, measuring from 5 to 17 mm in diameter. They are divided by African farmers into male tubers (elongated forms) and female tubers (spherical forms). When they are fresh, their shapes are regular, but when the cells gather, indentations form, where numerous microorganisms and deposits settle (**Arranz *et al.*, 2006**). An adult tuber is composed of two distinct parts: the body of the tuber itself, which generally consists of 3 to 4 internodes filled with reserves, and a distal part, called "the apical cone," which includes several internodes separated from each other and whose leaf scales persist in the form of a cone that covers the tuber (**Onovo et Ogaraku, 2007; Salma *et al.*, 2006).**

I.6.2. Tuber anatomy

In cross-section, the tuber of Cyperus esculentus L. is composed of two main parts:

Figure 4: Tuber formation (Anonymous, 2018).

From the outside to the inside, the cortical zone is bounded by an epidermis with tangentially elongated cells and covered by a relatively thick cuticle layer.

It includes a fibro-collenchymatous sheath and a parenchymatous zone. The cortical fibers form a protective layer composed of 3 to 5 cells with a very narrow lumen. One can observe a collenchyma of 2 to 3 layers of cells with thick walls beneath this sclerenchyma layer. The underlying collenchyma zone consists of massive polyhedral cells.

The central cylinder, which is extremely small, is separated from the cortical zone by one to three clusters of small cells elongated vertically and with thick radial walls. The structure of the fundamental parenchyma is similar to that of the cortical parenchyma. The liber-wood bundles of the central cylinder are greatly reduced in number and importance. In these bundles without sclerotic formation, the wood occupies a central position and is surrounded by a phloem with poorly developed elements.

The rootlets form by relying on the small cells that separate the cortical zone from the central cylinder, which is the zone of carbohydrate reserves. All parenchymal cells exhibit lipid inclusions, to varying degrees. Their density decreases in two directions: from the periphery to the center and from both ends to the center. The embedding of protein reserves in the cortical zone **(Follak *et al.*, 2016).**

I.6.3. The physicochemical characteristics of *Cyperus esculentus* L. tubers

Cyperus esculentus L contains an abundance of vitamins, minerals, carbohydrates, lipids, and proteins. Its protein percentage can fluctuate between 5 and 13%. The carbohydrate content varies from 38.43% to 69%, while the starch content ranges from 25% to 31%. Crude cellulose reaches a maximum of 11.9%. Even though tiger nut is a tuber, it contains a fat content that can reach 44.52% **(NDIAYE, 2020).**

Most of the tubers of *Cyperus esculentus* L. are yellowish in color and exhibit varied tails and sizes. Sometimes, the dark yellow tubers are considered mutants. In comparison with Côte d'Ivoire and some common staple foods in Africa, tigernut has a high concentration of proteins and minerals. It is recommended to consume tigernut tubers along with part of the available food for individuals suffering from nitrogen deficiency. Active compounds such as sterols, polyterpenes, and alkaloids are also present in tigernut plants **(Koffiet *et al*., 2011).** Raw tubers exhibit high concentrations of minerals (mg/100g): 896.3 mg (K), 267.8 mg (P), 104.9 mg (Mg), 26.07 mg (Ca), 15.8 mg (Na), 4.51 mg (Fe), and 2.03 mg (Zn) according to the characterization. According to the research, the actual levels of C (12.6 mg), E (80.32 mg), and B (B1, B2, B3, B5, B6, B9, and B12) vitamins are also negligible, with respective contents in mg/100g of 0.23; 0.37; 1.8; 1.5; 0.58; 0.21; and 0.004 mg. The protein content is estimated at 5%, carbohydrates at 63.83%; the fleshy fraction at 24.72% and fiber at 32.8% **(Ndiaye and Ndoye, 2021)**.

Table 2: Physical and Chemical Composition of *Cyperus esculentus* Tubers (Koffi *et al*., 2005).

Measured parameters	Composition (%)
Humidity	8.3
Ashes	2.17
Cellulose	11.9
Cell walls (NDF)	28.20
Lignocellulose (ADF)	13.81
Lignin	3.32
Proteins (N*6.25)	6.04
Fat	24.70
Total sugars	20.2
Starch	32.0

Table 3: Physical Characteristics of *Cyperus esculentus* Tubers (Koffi *et al*., 2005).

Measured parameters	Characteristics
Length of oblong, ovoid, ellipsoid tubers (mm)	13,82 ± 1,3
Large diameter oblong, ovoid, ellipsoidal tubers (mm)	8 ± 0,8
Small diameter oblong, ovoid, ellipsoidal tubers (mm)	4,6 ± 0,4
Diameter of round tubers (g)	5,1 – 6,3
Mass (g)	0,81 ± 0,22
Masse (100 tubers) (g)	81 ± 0,3
Mass (small black tubers) (g)	0,22 ± 0,06
Mass (100 small black tubers) (g)	22 ± 0,07
Diameter (small black tubers) (mm)	0,3 ± 0,03

I.6.4. The food use of tubers of *Cyperus esculentus* L.

Products made from tiger nuts (flour, oil, and milk with or without added sugar) have high concentrations of bioactive compounds that are good for the health of the eyes. While flour would be suitable for dietary recommendations for people with hyperglycemia, tiger nut milks showed improved protein digestibility. Therefore, flour may be a great component for functional food compositions that are suitable for diabetics **(Hernández-Olivas *et al*., 2022).**
Tiger nut, which has been suggested as a treatment for type 2 diabetes **(Ademosun *et al*., 2015),** has been shown in multiple studies to have anti-inflammatory and anti-apoptotic properties to prevent testicular dysfunction **(Adelakun *et al*., 2020)** enhance male arousal **(Allouh *et al*., 2015)** lessen diarrheal symptoms in albino rats **(Shorinwa *et al*., 2020**), and lessen inflammation and oxidative stress in the liver with atherosclerosis . Alkaloids, quercetin, vitamins, steroids, zinc, and other substances found in tiger nuts are probably the cause of the outcomes. Tiger nuts thus provide the possibility to be turned into useful foods in addition to being food (**figure 5**) and industrial material **(Achoribo and Ong, 2017).**

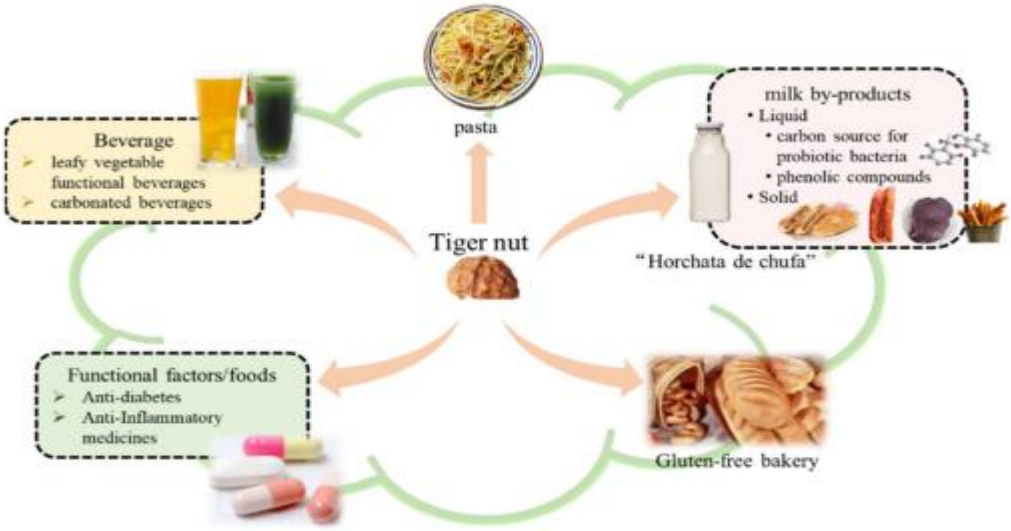


Figure 5 : Applications of the whole tuber of the tiger nut in the food Industry (Achoribo and Ong, 2017).

I.6.4.1. Tiger nut milk

Spanish people continue to drink "horchata de chufa," a plant milk (**figure 6**) made from tiger nuts. Particularly for those who suffer from lactose intolerance and milk allergies, plant milk offers numerous health benefits over animal milk. The tiger nut milk is produced by the steps of wet milling, filtration, addition of ingredients, sterilization, homogenization, aseptic packaging and refrigeration, respectively. It contains phenolic compounds, unsaturated fatty acids and biologically active substances (**Yali Yu *et al*., 2022).**

I.6.4.2. Gluten-Free Bakery Products

The prevalence of celiac illness is rising, which is driving up demand for gluten-free products. However, producing high-quality gluten-free bread is a significant issue.

Figure 6 : Chufa (Tiger Nut) (Poiret, 2017).

Cereal flours, starches, proteins, hydrocolloids, and emulsifiers are typically combined to create gluten-free bread in order to enhance its organoleptic qualities and shelf life. A novel ingredient for gluten-free products was the tiger nut. When combined with chickpea flour, tuber flour can be used as an emulsifier and shortening in gluten-free bread, giving the finished products good baking qualities in terms of color, hardness, and volume.

A further study found that biscuits prepared with tiger nut powder had superior shape, cross-sectional area, hardness, and surface appearance when compared to biscuits made with simply corn flour **(Yali Yu *et al.*, 2022).**

Figure 7: tigernut flour (Anonyme, 2012).

I.6.4.3. Tigernut oil

Lipid content in tiger nuts ranges from 22.14 to 44.92%. Olive oil, which is regarded as the best fat for human consumption, is comparable to the lipid profiling **(Touria *et al.*,2022).** In addition to the oil, it typically contains phenols, potassium, calcium, magnesium, vitamin C, and vitamin E. When compared to other vegetable oils, this oil's antioxidants give it greater oxidation stability **(Nina *et al.*, 2020).** Additionally, it includes tannins, saponins, and alkaloids, all of which have anti-inflammatory and antibacterial properties (**Vega-Moralesa *et al.*,2019**). Vegetable oils' sensory qualities are influenced by volatile molecules (**Lasekan *et al.*, 2012**) have discovered 75 odor-active chemicals in roasted nut oil, including n-propyl-9,12-octadecadienoate, ethyl hex decanoate, and 5 hydroxymethyl-furfural, which contribute to the oil's pleasant flavor.

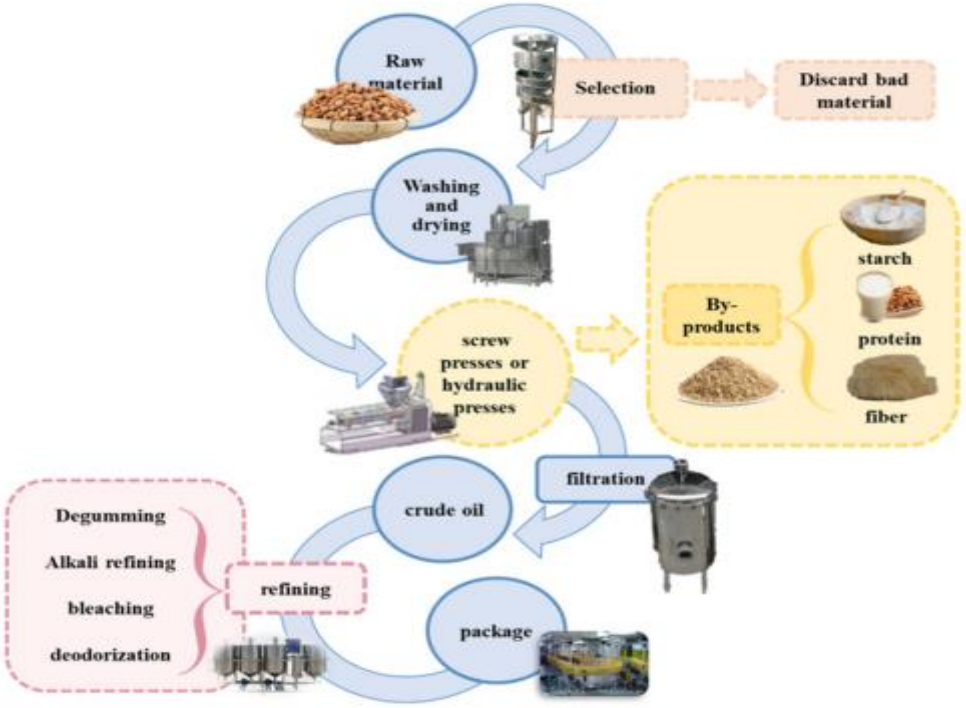


Figure 8: The preparation of tiger nut oil in food industry and by-products (Yali Yu et al., 2022).

Animal fat in meat products can be substituted with tiger nut oil **(Carvalho *et al.*, 2019**). It was used in the manufacturing of beef and deer burgers as an alternative to animal fat. Deer patties with 3 g/100 g of tiger nut oil added exhibited textural and sensory characteristics comparable to those of burgers with pork fat as a lipid source **(Vargas-Ramella *et al.*, 2020**). A healthier meat product with high levels of unsaturated fatty acids and low levels of total fat and saturated fatty acids was produced when beef patties were made entirely using tiger nut oil emulsion instead of beef fat (**Barros *et al.*, 2020**). When animal fat was substituted with burger fat, the researchers noticed a notable reduction in fat content. The researchers found that using animal fat substitute in burgers significantly reduced their fat level. The altered batches might be marketed as "low fat" burgers, which would greatly increase their acceptance among consumers.

I.6.4.4. Tiger nut starch

The main ingredient in plant reserves, starch, is a rich, non-toxic, renewable, and reasonably priced foodstuff. It accounts for about 80–90% of all the polysaccharides found in human food **(Masina *et al.*, 2017**). An appropriate plant for industrial starch, production is the tiger nut, which has the advantages of high yield, easy culture, and most importantly, easy starch, extraction **(Santos and Francisco, 2020).** The oval shape of the particles, hygroscopicity, and adhesion are some of the characteristics of soy starch, that are similar to those of corn starch, tomato starch, and sweet potato starch **(Builders *et al.*, 2013; Fathoni *et al.*, 2020).**

The viscosity characteristics of tiger nut starch are close to those of native starch, but its gel texture characteristics (hardness, elasticity, cohesion, and chewiness) are higher than those of traditional corn and sweet potato starch. Moreover, it is more stable during freezing and thawing than corn starch. It can be used in both cold beverages and frozen foods and offers numerous applications. Thus, it has potential commercial value and has been used in the food and pharmaceutical sectors.

According to **Menek *et al*. (2012)**, water chestnut starch gels exhibit clear, smooth, adhesive, and cohesive characteristics, making them an ideal option for use as a thickener, binder, and humectant in food products, such as soups and dessert powders. Moreover, it exhibits a strong gelling capacity and stability upon thawing in the hydrated state, making it ideal for the production of jellies, cold beverages, and candies **(Li *et al.*, 2017)**.

Starch is one of the most commonly used pharmaceutical ingredients and is classified as GRAS (Generally Recognized as Safe) by the World Health Organization. The compatibility and high binding capacity of tiger nut starch comply with pharmaceutical standards, with good swelling power, dense water absorption, and binding efficiency with medications **(Builders *et al*., 2013)**. According to **Akonor *et al*. (2019)**, nut starch can serve as an excipient, binder, filler, and lubricant in the manufacture of pharmaceutical preparations.

I.6.4.5. Tiger nut protein

Once the oil has been extracted, the flours, which are by-products of the oil extraction process, are used for animal feed or disposed of directly, generating a great deal of waste and environmental pollution. Yellow nutsedge proteins are beneficial for people with diabetes or digestive disorders and play an essential role in preventing heart disease **(Adejuyitan, 2011).** So, once the Tiger Nut oil has been extracted, the protein extracted from the meals can be converted into health products.

I.6.4.6. Composition and nutritional evaluation of tiger nut protein

The chufa protein has a molecular weight ranging from 5.5 to 88 kDa. The proteins present in tiger nut are as follows: According to **Agboola *et al.* (2017)**, there is 47.5% gluten, 31.8% albumin, 4.7% globulin, and 3.8% prolamin. Moreover, other studies have revealed varied results regarding the composition, with tiger nut protein containing the highest amount of gluten (approximately 82-91%) **(Idoia *et al*., 2015)**. According to **Asare *et al.* (2020)**, this disparity may be due to the fact that the proximate composition of tiger nut is primarily influenced by its geographical origin. The functional properties of tiger nut proteins are influenced by the different components, and it is essential to find suitable protein components for the manufacture of various products in order to optimally utilize tiger nut proteins.

From now on, plant proteins can be considered as functional ingredients or biologically active components rather than as essential nutrients. The functional characteristics of proteins are often closely linked to their

amino acid profile, making amino acid composition the potential quality of a plant protein. The profile of essential amino acids and the digestibility of tiger nut proteins were evaluated by researchers to assess the nutritional value of tiger nut proteins. The amino acids present in tiger nut protein are 18 types, of which 46.03% are essential amino acids, which far exceeds the value indicated in the WHO/FAO model (36%), higher than soy protein (41.3%) and close to egg protein (48.8%). Lysine is, according to the literature, the first amino acid that limits the consumption of certain nuts (Brazil nuts, macadamia nuts, and almonds) and certain cereals (rice, white flour, corn, for example). However, the concentrations of lysine in tiger nuts are much higher.

With a concentration of 15.4% of essential amino acids, it surpasses the 13.3% of soy **(Jing *et al*., 2013)**. Thus, regarding the concentration of lysine amino acids, the proteins of cereals and tiger nuts are nutritionally complementary. Methionine is the first limiting amino acid in tiger nut protein. If the score of the first limiting amino acid is considered as the amino acid score of the proteins, the amino acid score of tiger nut proteins is 78.9, which is higher than that of soy proteins (51.4) and lower than that of egg proteins (97,2).

I.6.4.6. Pasta with tiger nuts

It is becoming increasingly common to use ingredients based on non-durum wheat to improve the nutritional and functional qualities of pasta. Wheat flour was combined with tiger nut to create composite fresh pasta. The qualities of the fibers, fats, and minerals of the product have been significantly improved thanks to the addition of tiger nut powder. The addition of 30% tiger nut powder can ensure an increase in the fiber content of the products, thus exceeding 3%. Nevertheless, it is necessary to strengthen the gluten structure to reduce losses during cooking and improve the firmness of the pasta **(Albors *et al*., 2016).**

Fresh egg pasta can be filled by adding powder (20 and 40%) and xanthan gum (1%), which allows for a robust gluten network to be obtained. An important modification of the rheological and structural properties of the pasta was demonstrated with an optimal cooking time of two minutes **(Martin *et al*., 2018).** Uncooked pasta exhibits excellent water absorption and a high swelling index, which reduces loss during cooking. Cooked pasta exhibits satisfactory hardness, elasticity, color, and sensory characteristics **(Martin *et al*., 2018).**

I.6.5. Health benefits of Tiger nuts

I.6.5.1. Prebiotic property

Prebiotics are a type of plant fiber that helps to support the beneficial bacteria that are already present in the big intestine. Tiger nuts are a resistant starch food, which is only one of their many notable advantages. In contrast to ordinary starch, resistant starch (RS) enhances insulin sensitivity, lowers blood sugar, lowers the risk of colon cancer, decreases appetite, and improves the health of your digestive system **(Malashree *et al*., 2021).**

RS is a plant fiber that has recently gained recognition as a health food for both people and animals. It is used to describe the percentage of fiber's starch and starch derivatives that are resistant to digestion as they go through the digestive system. The starch's inherent qualities have been shown to improve gut microbial populations and activate cell signaling pathways linked to anti-inflammatory, anti-diabetic, and anti-obesity effects. By escaping digestion in the small intestine, RS has few interactions (**figure 9**) with other components of the upper gastrointestinal tract. It undergoes fermentation in the large intestine, producing short-chain fatty acids (SCFA), carbon dioxide, methane, hydrogen, and organic acids like lactic acid. But compared to lactulose and other indigestible oligosaccharides, RS is thought to produce just a small amount of these gasses **(Malashree *et al*., 2021).**

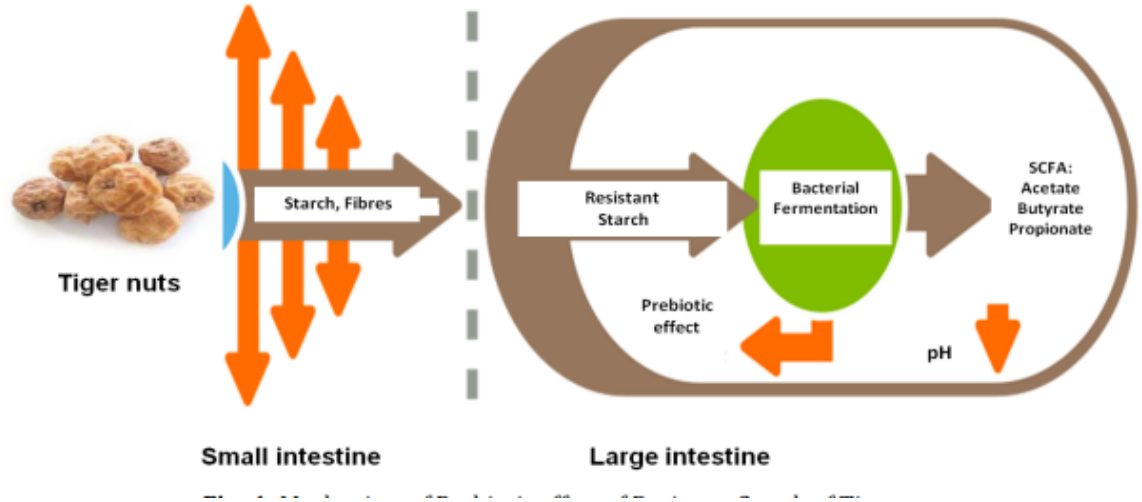


Figure 9 : Mechanism of Prebiotic effect of Resistant Starch of Tiger nut (Malashree *et al*., 2021).

I.6.5.2. Regenerative, digestive and antioxidant properties

Research has shown that nutsedge tubers are beneficial for tissue repair, muscles, blood flow, body growth and bone strength, as they are rich in minerals (phosphorus, potassium, calcium, magnesium). Indigestion, flatulence, and diarrhea are often treated with nutsedge, as it contains enzymes **(Ndiaye, 2020).**

Cyperus esculentus has been reported to help prevent blood clots and activate blood circulation (Adejuyitan et al., 2009), it reduces the risk of coronary heart disease, arteriosclerosis (**Gambo and Da'u, 2014),** Yellow nutsedge extract plays an essential role in preventing heart disease **(Maduka and Ire, 2018).**

Tiger nutsedge is responsible for the prevention and treatment of urinary tract infections and bacterial infections, helping to reduce the risk of cancers (colon cancer) **(Adejuyitan *et al*., 2009**), it recommended for those with heavy digestion, flatulence and dysentery **(Gambo and Da'u, 2014**), Consumption of Tiger tubers contributes greatly to the prevention and treatment of urinary tract infections as well as other bacterial infections **(Maduka and Ire, 2018).**

Due to the presence of phenolic compounds and vitamin E, nutsedge tubers have antioxidant properties that also help reduce anxiety. Nutsedge has anti-inflammatory virtues. When nutsedge oil is administered to male

wistar rats treated with streptozotocin STZ to cause liver damage, there is a significant increase in the antioxidant activity of enzymes such as glutathione peroxidase, glutathione reduction and superoxide dismutase. According to **Ndiaye (2020),** Tiger Nuts oil helps improve lipid profile and antioxidant capacity.

I.6.5.3. Antidiabetic property

Yellow nutsedge tubers have numerous anti-diabetic properties. These tests involve raw tubers, Tiger Nuts oil and Tiger Nuts milk. According to Chevallier's research, nutsedge has the capacity to regulate diabetes. As a result, a reduction in blood glucose concentration was observed in studies carried out by Chukwuma and colleagues, compared with controls. This reduction in blood glucose concentration is attributed to the high presence of arginine, which promotes the release of the hormone insulin **(Ndiaye, 2020).**

Sugar-free nutsedge can be used to treat diabetes due to its high content of carbohydrates, such as the higher sucrose and starch, as well as arginine, which stimulates the production of insulin hormones (**Gambo et Da'u, 2014).**

Additionally, research indicates that RS of tiger nuts can increase the release of gut-secreted hormones with anti-obesity and glucose tolerance properties, such as GLP-1 (glucagon-like peptide 1) and PYY (peptide YY). While PYY aids in lowering hunger and restricting food intake, GLP-1 lowers blood sugar levels by increasing insulin production. Therefore, elevated GLP-1 and PYY are crucial in the way that RS affects the buildup of body fat (**Adejuyitan, 2009).**

I.6.5.4. Cholesterol-lowering properties

The use of tigernut oil helps lower cholesterol levels, reduce the risk of coronary heart disease and weight gain. In albino rats, the administration of Tiger Nut oil leads to a reduction in cholesterol levels and an increase in HDL-C **(Ndiaye, 2020).** Compared with other soft drinks, tiger Nuts milk is not only a pleasant drink, it's also extremely healthy. It helps lower cholesterol levels by reducing the amount of "bad" cholesterol present in low-density lipoproteins (LDL). The presence of vitamin E also plays a role. It is also dedicated to the fight against cholesterol. This is due to its antioxidant action against harmful fats **(Gambo and Da'u, 2014).**

I.6.5.5. Anti-arthritic and anti-atherogenic properties

The anti-arthritic properties of nutsedge oil are similar to those of sodium diclofenac, a drug used to combat this condition. Nutsedge oil therefore had a greater effect on reducing swelling than Diclofenac sodium over a short period of time. This demonstrates the effectiveness of Nutsedge in treating arthritis **(Ndiaye, 2020).**

I.6.5.6. Antimicrobial property

The significant presence of phytochemical compounds in tiger nut tubers endows these tubers with antimicrobial properties. Thus, the in vitro antimicrobial activity of the aqueous extract of tiger nut tubers revealed significant effects on *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Bacillus subtilis*, and *Staphylococcus aureus* **(Ndiaye, 2020).**

Chapter II:

Biscuit Technology

Chapter II: Biscuit technology

II.1. Definition

"Biscuit" comes from the Latin "bis coctus" and, later, the French word "bescuit," which means "twice cooked." Products that were baked and then dried in a slow oven are referred to by this name; they are often prepared from wheat flour. In the United States, the goods are referred to as "cookies and crackers," even though the name "biscuits" is used extensively in many other nations. (**Divyasree Arepally *et al.*, 2020)**

Biscuits are bakery products, generally made from wheat flour, fat, and sugar. They are made in a variety of shapes and sizes and can contain dried fruits, nuts, and food coloring (**figure 10**). It is a dry product, generally golden brown in color and with a crunchy texture.

Figure 10: Different types of sweet cookies (Anonyme, 2018).

Biscuits have a lengthy history; the Romans, Persians, and Egyptians all manufactured various types of "twice baked" cuisine in antiquity.They served as an inexpensive source of sustenance for sailors, soldiers, and the impoverished. For European sailors traveling to Asia, Africa, Australasia, and the Americas, biscuits were a staple diet.(**Divyasree Arepally *et al.*, (2020)**

Biscuits are wholesome, come in a variety of sweet and savory varieties, and have a long shelf life. They are also consumed right out of the package. In addition to being made for children, the elderly, and people with particular needs like gluten-free foods, biscuits come in a variety of functional forms and are enhanced with calcium, iron, and vitamins.

Worldwide, biscuits are consumed, and several varieties—such as soda crackers, snack crackers, hard sweet biscuits, short biscuits, and cookies—are well-known in many nations. On the other hand, every nation has its own unique variety of bread.

Additionally, a lot of unique products are well-liked in their own country: England: Cream crackers, rich tea, and digestive
France: The Petit Beurre
Lebkuchen, Germany
India: Glucose
Italy: Frollini biscuits and breakfast

II.2. Classification of Biscuits

The diversity of productions and the variety of components that can be used in different manufacturing processes do not allow for an official classification of biscuits. But one can consider a classification based on the consistency of the dough before baking.**(BENKADRI, 2010).**

- The types of sweet and savory dry biscuits are made from hard or semi-hard doughs: snacks, shortbread, petit beurre, etc. This is an egg-based production that represents about 60% of biscuit consumption.
- Soft doughs are intended for the pastry industry. These include both dry biscuits (savoiardi, ladyfingers) and soft items (genoise, madeleines, cakes, macarons). These biscuits are distinguished by their high content of eggs and fats.
- Pasta containing a large amount of milk or water is low in fat. They are wafers.
- The quality of the biscuits can be influenced by various elements such as the quality and level of the ingredients used, the manufacturing conditions such as kneading, resting, and molding the dough, as well as the baking and cooling of the biscuits. (**BENKADRI,2010).**

II.3. The categories of biscuits

In general, biscuits can be divided into four groups based on their methods and recipes: cookies (including filled cookies), short-dough biscuits, hard sweet and semi-sweet biscuits, and crackers. Specific mixing, shaping, and baking procedures are needed for each category and product type.

For each of the major biscuit categories, an example of the product's formulation and manufacturing method is provided. Please take note that all of the recipes, formulations, and process details provided are merely suggestions, and their application is contingent upon the availability of local ingredients and manufacturing tools. In each instance, during commissioning, the recipes and procedure will need to be modified to accommodate the specific ingredients and machinery available. (**Divyasree Arepally *et al.*, (2020)**

II.3.1. Crackers

Crackers (**figure 11**) are perceived by some as savory biscuits, by others as unsweetened, savory, and crunchy biscuits, and by others as unsweetened, savory, and crispy biscuits.The wheat flours used for making crackers most often have a higher protein content and are more resilient than the flours used for biscuits and cookies **(Miller, 2016).**

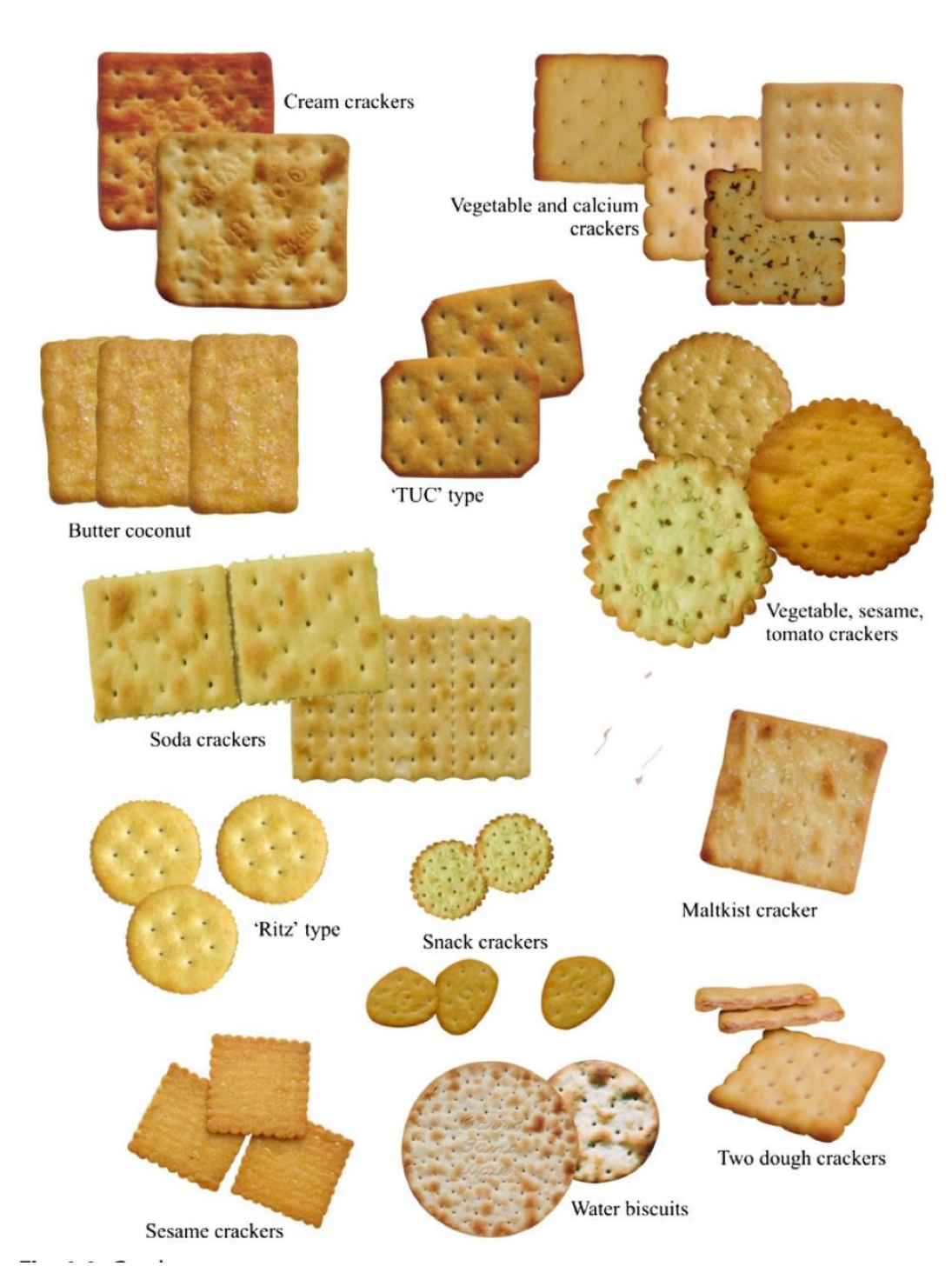


Figure 11: Crackers (Divyasree Arepally *et al*., 2020).

II.3.2. Shortcrust biscuits

The most common biscuit and cookie worldwide is made from short dough. They also come in a wide variety of ingredients, sizes, shapes, and flavors. As a general rule, short pasta is made from low-gluten soft wheat flours that contain 9% or less protein, and the formulas are rich in water, sugar, and fat.**(Miller, 2016).** The gluten in short pasta is not developed during kneading for various reasons: The development of gluten is delayed by the high levels of sugar and fat; the water level is not high enough to properly hydrate the gluten, preventing it from developing **(Miller, 2016).**

II.3.3. Long Shelf-Life Cakes, Snack Cakes

A range of snack cakes are made in tunnel ovens that run continuously. These consist of snack cakes baked individually in tins or pans, Korean "pies" made of soft deposited doughs baked on a steel band, and layer cakes baked in a single, continuous sheet that is later chilled, sliced, and cut into individual items (**Figures 12, 13**).

Cakes are made from comparatively low viscosity, soft batters.

• Some cakes, called layer cakes, are baked using whole batter sheets that are placed on steel bands.

• Pans that are moved through the oven on chain tracks are used to bake some snack cakes.

• Korean "pies" are baked as placed cookies on steel bands (**Divyasree Arepally *et al.*, 2020).**

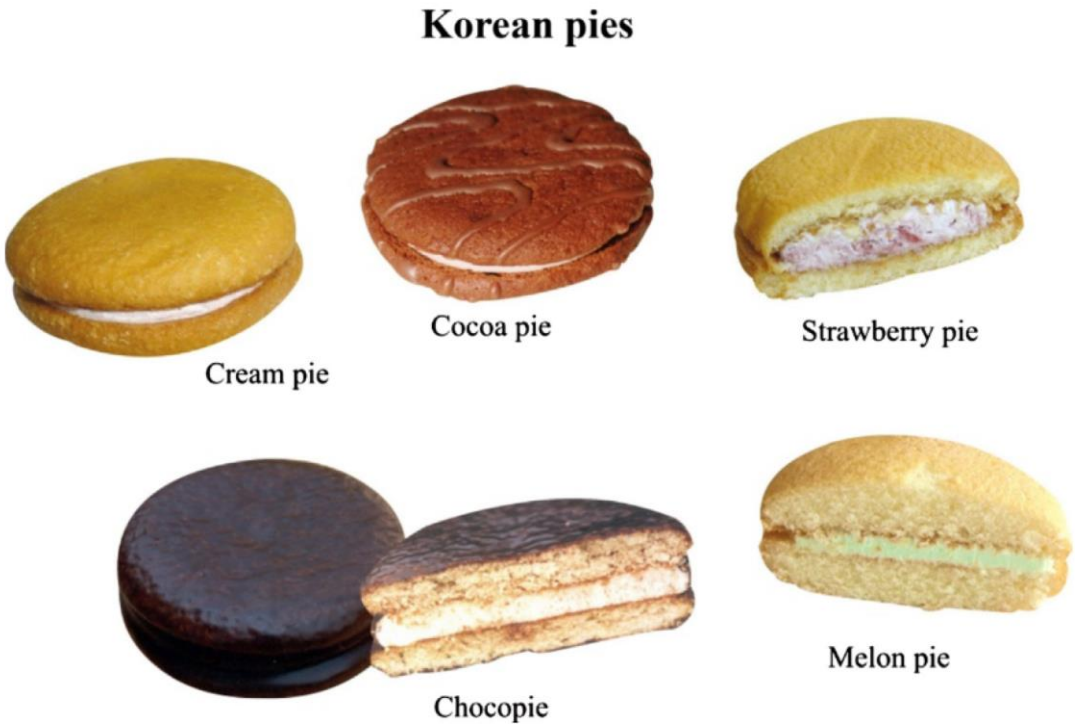


Figure 12: Korean pies (Divyasree Arepally *et al*., 2020).

Figure 13 : Snack cakes (Divyasree Arepally *et al*., 2020).

II.3.4. Cookies

Cookies contain a large amount of fat and sugar, as well as a low water content, which results in a short and crumbly dough with an almost non-existent gluten network **(Cauvain, 2016).**

The majority of cookies contain less than 5% moisture. Their size, shape, formulation, preparation method, and flavor are very different. The consistency can range from crispy and hard to soft and fluffy.**(Miller, 2016).**

The sensory properties sought by the consumer in terms of visual appearance (crust color and crunchiness), taste (sweetness, mouthfeel, etc.), and smell depend on the quality and quantity of the ingredients used in the cookie dough, as well as its preservation characteristics. **(Diguer and Ammouche, 2020).**

According to **Sykes and Davidson (2020),** there are different types of cookies:

Chocolate chip cookies **(Figure 14)**

Figure 14 : Chocolate chip cookies (Sykes et Davidson, 2020).

Maryland-type cookies (**Figure 15**)

Figure 16 : Maryland-type cookies (Sykes et Davidson, 2020).

Oat flour cookies (**Figure 16**)

Figure 17 : Oat flour cookies (Sykes et Davidson, 2020).

Coconut cookies (**Figure 17**)

Figure 18: Coconut cookies (Sykes et Davidson, 2020).

Peanut cookies (Figure 18)

Figure 19: Peanut cookies (Sykes et Davidson, 2020).

II.3.5. Dietary Biscuits

Individuals with diabetes face a disruption in glucose metabolism. After a meal, the blood glucose level rises and then subsequently falls. Individuals with diabetes must monitor their carbohydrate intake. That's why cakes for diabetic patients should contain low amounts of low molecular weight sugars (carbohydrates) and

the labeling must clearly specify the nature and quantity of these carbohydrates **(Manely, 2001).** Sugar is replaced by polyols such as mannitol or polydextrose. **(Gallagher *et al.*, 2003; Ansari et Kumar, 2012).**

II.4. The main ingredients

Depending on the type of cookies or cakes desired, which require various doughs and different molding techniques, the quantities of ingredients and molding play a crucial role **(Denis, 2011).**

Flours, sugars, and fats are the essential components for biscuit production. To these components, it is possible to add several small components for leavening, flavor, and texture. **(Davidson, 2018).**

II.4.1. Flour

Bakery products are primarily based on wheat flour. In addition to its great abundance, its frequent use is attributed to the dough's ability to capture gas, thereby facilitating its development during baking. According to Table 4, flour is a complex compound that includes various components (proteins, lipids, sugars...) which contribute directly or indirectly to the structure and aeration of the dough. **(Lassoued, 2009).**

Depending on the required processing and texture criteria, biscuit producers use flours from soft and hard wheats. Products made solely from durum wheat flour generally have increased density, increased thinness, and increased hardness. They also require a higher amount of water to make a workable dough, and the use of more energy in the cooking process. **(Cauvain, 2016).**

Table 4 : Composition of Wheat Flour (Lassoued, 2009).

Constituents	% dry matter of the flour
Water	14
Proteins	7-15
Starch	63-72
No-amylaceous polysaccharides	4.5-5
Lipids	1.2

According to **Cauvain (2016),** the main criteria for flours for biscuits and crackers are as follows:

- **Humidity:** it must not exceed 14% to prevent the development of mold during storage.
- **Proteins**: for shortcrust and semi-sweet biscuits 8-9.5% and for crackers 9.5-10.5%

- **Starch degradation:** flours with a limited water absorption capacity are generally preferred, and it is therefore better to have a low percentage of damaged starch.
- **Water absorption**: it can be evaluated in the same way as for bread production, however, for cookie dough, it is more crucial to consider water absorption.

II.4.2. Sucre

Sugars play a crucial and significant role in the majority of cookies. Besides their sweet flavor, they are compounds that transform and optimize the composition and aroma of the biscuits. **(Manley, 2011).**

Sugars influence the dimensions, hue, strength, surface coating, and sweet flavor of items. When heated, sugar dissolves to create a structure similar to that of glass. This leads to an open texture when the gluten has not formed. Crispy biscuits have characteristics that complicate their consumption due to their high sugar content: for example, ginger biscuits contain up to 34% sugar, while 19% for digestives degrade more quickly. **(Cauvain, 2016).**

The biscuits also contain other reducing sugars in syrup form, such as glucose syrup, malt extracts, and honey. In the presence of amino acids, reducing sugars cause the Maillard reaction, which gives the biscuit its color **(Davidson, 2018).**

The nutritional value for 100g of sugar is described in **Table 5**.

Table 5: Composition of Sugar (Diguer and Ammouche, 2020).

Ingredient	%
Proteins	0%
Carbohydrates	99.6%
Lipids	0%
Vitamin	0%

II.4.3. Fat content

One of the most essential components of cookies is fat. It provides structure, additional flavor quality, and extra taste to the product. In the absence of fat, the cookies would not be distinguishable from those we currently find. The fat in a biscuit can be described in various ways: it can be referred to as butter, animal fat, vegetable fat, or vegetable oil (including varieties designated as palm oil, sunflower oil, etc.) **(Atkinson, 2011).**

By increasing the fat concentration in the recipes, you get softer products, but it can also alter other attributes of the cookies. For example, higher fat content in the shortcrust pastry causes movement during the baking process, leading to more delicate cookies with larger diameters **(Cauvain, 2016).**

According to **Davidson (2018),** crackers and hard sweet biscuit doughs, which are rolled and cut, have a fat content of 10 to 22% of the flour by weight. Rotary-molded doughs can contain 17 to 30% fat, and cut and deposited cookie doughs can contain 25%-60% fat.

Table 6 below presents the nutritional value for 100 grams of vegetable fat:

Table 6 : Nutritional value of vegetable fat (Diguer and Ammouche, 2020).

Lipids	82%
Carbohydrates	0.2%
Water	16%
Miscellaneous	Including minerals, vitamins, and various additives (emulsifier, acidifier, preservative,

II.5. Other ingredients

II.5.1. Milk

Due to its remarkable taste and nutritional content, milk is a traditional ingredient in baking. The presence of proteins and reducing sugars (lactose) in dairy products plays a crucial role in the Maillard reaction, which gives biscuits a golden brown hue on the surface. However, due to its impact on surface coloration, it is rarely used in small quantities. It is uncommon to use fresh milk in biscuit making, as dry powders are easier to handle and store **(Manley, 2011).**

II.5.2. Salt

In the majority of bakery products, table salt (NaCl) accounts for about 2% of the total weight of the flour. It is a flavor exfoliant that reduces withdrawals and delays the yeast process. It also tends to restrict the availability of water, thereby contributing to its optimization for preservation. The salt that dissolves in the water generates ionic bonds with the flour proteins, which optimizes its ability to absorb water. Salt also contributes to the color of the crust, which remains pale when it is absent. **(Menasra, 2020).**

II.5.3. Water

According to **Ndangui (2015),** water plays a crucial role in the creation of the dough. It hydrates the flour and gives its components the necessary movement to carry out chemical reactions. During the kneading

process, the multiplication of contacts between the starch granules and water causes the dispersion of water molecules within the flour particles, thereby promoting their interactions.

A dough suitable for baking generally contains between 0.6 and 0.8 grams of water per kilogram of dry flour, of which about half is "non-freezable" water, used by the protein network. Water plays a crucial role by intervening in three stages of the kneading process:

Water facilitates the dissolution of soluble compounds, thus ensuring the uniformity and strength of the dough. For example, salt that dissolves in water generates ionic bonds with the proteins in the flour, which are crucial for the formation of the dough.

The rheological characteristics of the dough (cohesion, consistency, viscoelasticity...) are largely influenced by water: the energy required to deform the dough decreases significantly as the volume of water in the mixture increases.

It acts as a plasticizer: its reduced molecular weight facilitates the mobility of macromolecules (proteins, etc.) by increasing the free volume and reducing viscosity.

II.6. The manufacturing of biscuits

The dough represents the transition between flour and the biscuit, and its characteristics determine the final industrial success. Indeed, the structure of the dough is of great importance in the biscuit manufacturing process. Therefore, a dough that is too rigid or too soft will not be properly processed on the appropriate forming equipment and will not produce a satisfactory product **(Benkadri, 2010).**

Biscuit manufacturing consists of four major processes mixing, forming, baking and packin

II.6.1. Mixing

The process of mixing involves combining all the ingredients in the proper ratio to make dough.After that, these ingredients are fed into mixers, which mix them and prepare the dough for shaping. The main ingredients are sugar, oil, flour, and extra ingredients depending on the desired outcome. It is possible to mix in one, two, or three steps.

- **One stage or all in One**

is a kind of mixing that involves adding all of the ingredients and water at once. The mixing process is continued until the dough is ready. This kind of mixing is typically used for firm dough.

- **Two - Stage mixing**

Creaming: All ingredients, with the exception of flour, are added to water and combined for 4 to 5 minutes. The second step involves mixing flour and chemicals to create a uniform dough.

- **Three stages mixing**

The first stage involves mixing fat, sugar, and other ingredients like milk, chocolate, malt, honey, etc. with a portion of water to create a cream; the second stage involves mixing salt, chemicals, and flavorings with colors with water; and the third stage involves adding flour and water to the prepared cream and mixing until a satisfactory dough is created. The mixing process has the following characteristics that have been watched for better results.

Several mixing devices (**figure 19**) are available based on the kind of dough that needs to be worked with.

Among them are:

- Horizontal mixer that works well with rotary-molded biscuit dough and hard, sweet dough.

Figure 20 : Horizontal mixer (Multi-Disciplinary Training, 2016)

Centre, Gandhi Darshan Rajghat

- Vertical mixer (**figure 20**), mostly used for making crackers, particularly for the sponge and dough method.

Figure 21: Vertical mixer. (Multi-Disciplinary Training 2016)

- A planetary mixer (**figure 21**) that works very well with soft dough, primarily for wire-cut or deposited products, although it can also be used with rotary mold dough.

Figure 22: Planetary mixer (Multi-Disciplinary Training, 2016)

- A popular tool in the bread industry, the spiral mixer (**figure 22**) works well with rotary moulded dough.

Figure 23 : Spiral mixer (Multi-Disciplinary Training, 2016)

Our experts must carefully consider the type, size, and performance of the mixers because each of these mixing systems has pros and cons.

II.6.2. Kneading

The mixing of flour and other ingredients is facilitated by the kneading process. It can provide information regarding the rheological characteristics of the dough. Indeed, the mixture is subjected to a mechanical process that generates the necessary energy to create several interactions between the components of the dough. This leads to changes in consistency that alter the technological capacity of the dough. **(Benkadri, 2010).**

There are three categories of kneading:

- **Traditional kneading**: which guarantees a mechanical work often inadequate for contemporary flours with rather resistant gluten.
- **Intensive kneading:** which produces overcooked pasta and a large loaf of bread, but white.
- **Improved kneading:** garantissant un équilibre optimal entre le développement de la pâte et la préservation de son aspect, sa saveur et ses senteurs **(Saadoudi, 2019).**

II.6.3. Fermentation

The kneaded biscuit dough is placed in a tank at 25 degrees Celsius for a variable resting period to facilitate fermentation. Generally, the dough is subjected to fermentation at 25 and 32 °C for a specific duration. Fermentation gives rise to a dough that is extensible, homogeneous, and resistant to gases. **(Menasra, 2020).**

II.6.4. Rolling

The most common technique for making biscuit dough sheets is rolling. This requires the production of a thickened dough, whose thickness is reduced using various rotary rollers **(Menasra, 2020).**

II.6.5. The segmentation

Cutting is the procedure that follows lamination. We cut the rolled sheets on a forming machine. It is clearly appreciated that each piece of dough has identical weight and measurements. **(Saadoudi, 2019).**

II.6.6. Cooking

Baking represents an essential phase during which the raw dough is converted into a light, porous, easily digestible, and delicious product, thanks to the heat. To achieve the required quality characteristics, baked cereal products require a specific cooking procedure. During the baking process, the main interactions observed include volume increase, crust formation, enzyme functions, protein coagulation, and partial gelatinization of starch in the mixture **(Lara *et al.*, 2011).**

Generally, the baking time for cookies is less than 10 minutes. To ensure reproducible results, it is essential that the hot oven door remains closed for an extended period during the loading process and that the headspace (the distance between the dough and the top of the oven) is minimal. **(Manley, 2001).**

According to **Cauvain (2016),** when the room temperature increases, the following processes occur:

- The fat melts and the undissolved sugar dissolves in the water of the dough, the viscosity of the dough is reduced and it begins to flow.
- Ammonium salts quickly lose carbon dioxide at temperatures up to about 60°C; the released ammonia dissolves in the dough water and is lost during the rest of the operation.

- Sodium bicarbonate begins its production of carbon dioxide either through thermal degradation or by reacting with the acids present in the dough.
- Due to the decrease in its internal viscosity and the gas pressure generated by the leaven and water, the dough expands.

- The baking process leads to a late browning of the surface, with yellow hues initially appearing when the temperature exceeds 110°C. At higher temperatures, the formation of red hues is observed, which result in a brown tint on the surface of the biscuit.

II.6.7. Cooling

For sweet biscuits, it is essential to cool them, as they are particularly soft and pliable as soon as they come out of the oven and harden when they are cold. The slight reduction in the moisture of the cookies is also advantageous for their quality and longevity **(Menasra, 2020).**

For various reasons, it is necessary to cool any hot and cooked product before packaging:

- The products cannot withstand the hot sealing packaging process.
- The packaging material may shrink around a hot product or the quality of the products would deteriorate.
- The normal method of cooling the products is to place them on an open conveyor and transfer them a certain distance from the oven.
- The products cool naturally in the ambient atmosphere of the factory; in some cases, it is necessary to provide air to facilitate the cooling process **(Manley, 1998).**

II.6.8. Packaging and Storage

The packaging represents the final stage of the biscuit production process. The biscuits that come out of the oven or the secondary processing phase must have an adequate shape (**figure 23**) and appearance and be in ideal condition for use **(Manley, 2011).**

According to **Davidson (2018)**, the functions of packaging are:

- Present the biscuits in an attractive manner for potential consumers.
- Display the type of biscuit, its weight, its ingredients, and its manufacturer.
- Preserve the freshness and flavor of the cookies for a long shelf life.
- Provide an effective barrier against moisture and foreign odors
- Resist the infiltration of fats and oils
- Protect against visible light and UV rays
- Protect cookies from damage during transportation

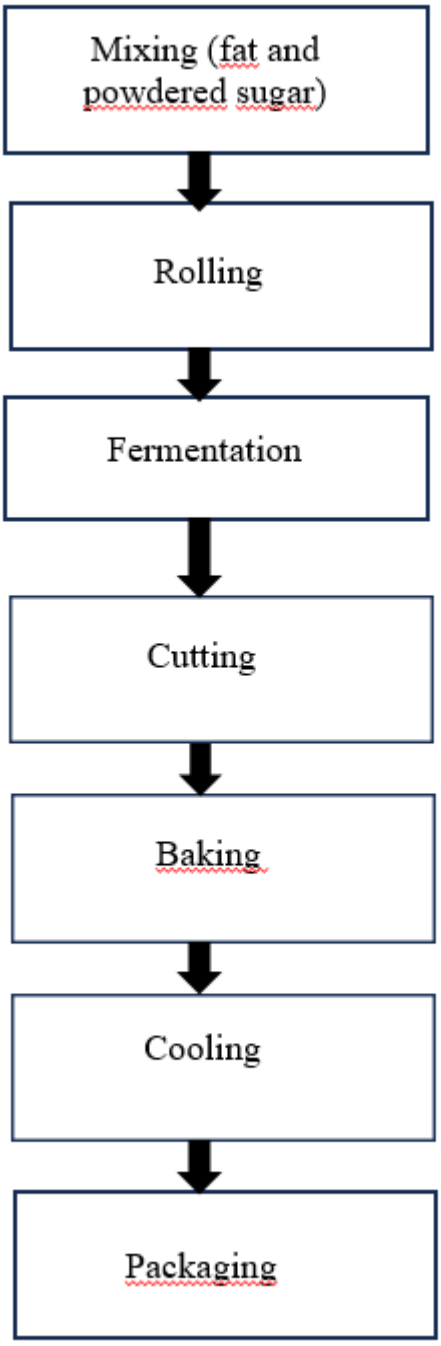


Figure 24 : Organizational chart for the adopted biscuit manufacturing (Yadav *et al*., 2012).

II.7.1. Criteria for Biscuit Quality

The requirement for faster and more accurate processing is growing along with consumer demand. In order to stay up, new testing technologies have had to be developed. Traditional testing now encompasses a greater variety of flour quality criteria that could impact the creation of Products in the baking sector are standardized. Additional product attributes that influence cookie geometry,

such as cookie density, flour kinds, flour treatments, and additives, can be described using conventional techniques like the AACCI (Cereal and Grains Association) methodologies. (**Bosc-Bierne *et al.*, 2022**)

The quality characteristics of food include taste, aroma, texture, color, and nutrient content. In the majority of situations, these characteristics deteriorate as soon as another substance or ingredient is introduced into the biscuit **(Menasra, 2020).**

II.7.2. The texture

It is mainly determined by the amount of moisture, fats, and the types and concentrations of structural carbohydrates (cellulose, starches, pectins...), as well as the proteins present. Moreover, a significant event in the creation of texture is identified.

The rheological characteristics of the dough depend on the behavior and interactions between these components, as well as the solubility of the gas in the continuous phase. The significant expansion generates a low density, which leads to the formation of biscuits with high porosity. The development of the porous structure in the oven regulates the physical dimensions of the biscuit, while the evaporation of water during its baking is essential to control its mass and moisture content. The resistance of the biscuit to deformation, also known as hardness and firmness, is a textural property that plays a crucial role in baked goods, as it is strongly linked to the perception of its freshness. In this context, texture is a crucial quality criterion, where the goal is to create a soft and tender crumb (**Safir, 2020).**

II.7.3. The color

An essential organoleptic component in determining whether or not the consumer would accept the finished product is cookie color. The Maillard reaction, caramelization, and browning reactions are the causes. The quantity of accessible sugar determines both reactions, which in turn depends on the quantity of Amylase activity, accessible damaged starch, and, of course, product formulation. (**Bosc-Bierne *et al.*, 2022**)

II.7.4. Taste, flavor, and aroma

The attributes of taste are salty, sweet, bitter, and sour. The volatile aroma components are produced under the effect of heat, oxidation, non-enzymatic activity on proteins, fats, and carbohydrates (example: Maillard reaction) (**Safir, 2020).**

II.7.5. Shape

The product's diameter and thickness mostly dictate the shape of the cookie. Two key properties—dough elasticity and extensibility—are controlled to achieve the desired form. The dough's ability to stretch without breaking is known as extensibility.

The ability of the dough to maintain its shape. The dough's elasticity is its propensity to regain its original shape following deformation. Both of these attributes are impacted by the protein's quality. (**Bosc-Bierne *et al.*, 2022**)

II.7.6. Stickness

The dough's stickiness is its ability to stick to surfaces. Numerous processing issues could result from this, including more extensive cleaning procedures and cookie forming issues. Water leaking from the dough, which happens as a result of damaged dough, is the primary source of this problem. starch to retain the previously absorbed water throughout subsequent mixing or processing stages. or the lack of protein that could absorb the water that was leaking. To retain the proper consistency and stickiness of the product, more protein is needed when the amount of damaged starch increases. (**Bosc-Bierne *et al.*, 2022**)

II.7.7. Blisters & cracks

These are seen as flaws in the finished product brought on by the volume of water that evaporates during baking. The strength of the protein network and the amount of water in the finished dough have an impact on them (**Bosc-Bierne *et al.*, 2022**).

II.8. The nutritional quality of biscuits

Cookies contain a large amount of carbohydrates, fats, and calories, but they lack fiber, vitamins, and minerals, making them harmful for daily use. Moreover, biscuits contain only 6 to 7% protein **(Farzana et Mohajan, 2015).**

Usually, biscuits are made up of flour, sugar, fat, water, salt, and baking powder. This variety of biscuit composition gives them an attractive nutritional value. Dry biscuits contain a majority of cereal materials at around 72% and starch at 51.5%. They contain a good amount of protein and fiber. The difference between dry biscuits and other cereal products lies in their low water content: 1 to 5% compared to 15 to 30% for cakes and 35 to 40% for breads: 1 to 5% compared to 15 to 30% for cakes and 35 to 40% for breads.

Due to their low water content, dry biscuits have a high energy density. It is estimated that dry biscuits contain a lipid content of 12% **(Saadoudi, 2019).**

The nutritional value of different biscuits is determined in **Table 7**.

Table 7: Average Nutritional Value of Some Biscuits (Saadoudi, 2019, Menasra, 2020).

Nutrients (g/100g)	Cookies	Chocolate biscuit	Petit beurre biscuit	Dry biscuit	Jam-filled biscuit	Cheese biscuit	Biscuit without cheese
Proteins	6	6.9	8.2	9	5	11.8	8.4
Carbohydrates	29	60.4	75	69.2	45	49.1	62.9
Lipids	27	24	10.9	12	5	28.1	19.6
Fiber	-	3.1	2.2	3.2	-	3.1	3.3

ChapterIII:

Vulvovaginal candidiasis.

ChapterIII: Vulvovaginal candidiasis.

III.1. Vaginal flora

Around 90% of the vaginal flora is made up of lactobacilli, which play a protective role in the genital flora, while the remaining 10% is made up of various bacteria (aerobic, anaerobic, gram+) and fungi. Throughout a woman's life, the composition of her vaginal flora constantly evolves. Indeed, there will be great variability between the four groups of lactobacilli over time.

During a woman's life, fluctuations in the Lactobacillus population are influenced by estrogen. From birth to infancy, we can already observe a significant evolution in vaginal flora. *Lactobacillus* colonization is recorded in the first few weeks of life. This may have occurred as a result of exposure to estrogen during pregnancy (**Bloch, 2013**; **Contet-Audonneau Schmutz, 2001)**.

III.2. Imbalances in vaginal flora

III.2.1. Condidosis

III.2.2. Etiology

Yeasts of the *Candida* genus is regularly present as commensals on skin and mucous membranes and can be ubiquitously detected in both animate and inanimate nature, depending on the species. Of the more than 150 known *Candida* species, only a limited number appear as regular infectious agents in humans. *Candida albicans* remains the most important pathogen in both superficial and invasive (or systemic) candidiasis. Depending on local epidemiology, non-albicans *Candida* species are detected with varying frequency.

III. 3. Vulvovaginal candidiasis (VVC)

VVC is an infection caused by *Candida* fungi on the vaginal mucosa. In some cases, it can become pathogenic. It manifests as leukorrhea, vulvar hyperemia, intense pruritus, dysuria and dyspareunia, and affects around 75% of women at least once in their lives. Candidiasis is the result of an imbalance between the microbiota and these yeasts. In addition, around 138 million women worldwide may suffer from recurrent vulvovaginal candidiasis (RVVC). Contributing factors include diabetes mellitus, pregnancy, antibiotics and steroids, hormone replacement therapy, use of hormonal contraceptives, immunosuppressive diseases and hygiene habits (**Janaina *et al*., 2021**).

Nearly 20-30% of healthy asymptomatic women may have this yeast in their vaginal tract at any time in their lives if tested by culture, but over 60% if tested by NAAT methods (**Farr *et al.*, 2021**, **Drell *et al*.,2013**).

Uncomplicated or sporadic VVCs include mild to moderate clinical signs and symptoms diabetes mellitus such as a thick cottage cheese-like discharge, pain, erythema, vaginal and vulvar pruritus, burning and/or edema, as well as external dyspareunia and dysuria **(Ullmann *et al.*, 2012).**

Symptoms of vaginitis in women, particularly those of childbearing age, are often caused by vulvovaginal candidiasis (VVC). Approximately 9% of women with symptoms have developed VVCR (at least 3 episodes of symptomatic VVCR in less than a year). Candida albicans is generally responsible for VVC. Other varieties of Candida are found in 10-20% of CVVRs and are less resistant to current treatments. The resistance of C. albicans to azole antifungals is also increasing, making it very difficult to control.

III.3. Role of vaginal epithelial cells in the pathogenesis of VVC

III.3.1. Vaginal epithelial cells: more than just a barrier

The vaginal mucosa not only acts as a physical barrier, but also plays an active role in the host's immune defense against invasion of underlying tissues by potentially toxic pathogens, such as Candida spp. To accomplish this, vaginal epithelial cells (VECs) display a series of receptors called pattern recognition receptors (PRRs) on their surface. These receptors enable VECs to recognize and bind to specific pathogen-related molecular patterns (PAMPs) secreted by or presented on the surface of human pathogens, such as C. albicans. (**Ardizzoni *et al.*, 2021**, **Zheng *et al.*, 2015, Fazeli *et al.*, 2005**). More specifically, VECs locate microbial PAMPs using various PRRs (C-type lectin receptors (CLRs), nucleotide oligomerization domain receptors (NLRs) and RigI-helicase receptors). Recognition of *C. albicans* is influenced by TLR2, TLR4, Dectin-1 and the non-classical PRR type A ephrin receptor 2 (**D'Enfert *et al.*, 2021**).

Antimicrobial peptides are also released by epithelial cells in response to pathogens: for example, iron-binding compounds such as neutrophil gelatinase-bound lipocalin, lactoferrin and calprotectin have broad-spectrum antimicrobial activity and antifungal properties, preventing the proliferation of microorganisms requiring iron for growth. **(Linhares *et al.*, 2019)** VECs also release antimicrobial factors such as mannose-binding lectin (MBL), beta-defensins and cathelicidins. Indeed, it is common for women suffering from a lack of MBL, caused by genetic variations, to be more vulnerable to RVVC **(Babula *et al.*, 2003).**

The exfoliation and lysis of epithelial cells releases glycogen into the vaginal lumen. α-Amylase breaks down the glycogen to produce small carbohydrates that promote the proliferation of lactobacilli, essential for vaginal health (**Amabebe *et al.*, 2018).** Finally, the rapid regeneration process of mucosal squamous epithelial cells has a protective effect, preventing the adhesion and invasion of *C. albicans*, which must attach to the mucosa to cause vaginal infections. In this way, in addition to their role as a physical barrier, VECs are also seen as initial hazard detectors and responsible for adaptive defense responses against pathogens, such as C. albicans, as well as a link between the innate and adaptive immune systems (**Netea *et al.*, 2015).**

III.3.2. VEC (Vaginal Epithelial Cell) response to *C. albicans*

It is crucial for C. albicans to attach to the surface of the vaginal mucosa if it is to remain in the host, either as a commensal microorganism or as a pathogen. This is a complex, dynamic and multifactorial process, involving an intimate interaction between fungal cell wall elements and epithelial surface proteins.

VECs are constantly exposed to C. albicans, as this fungus is the essential component of the healthy vaginal mycobiota.

Importantly, VECs have proven their ability to distinguish between commensal and pathogenic forms of C. albicans. Furthermore, host innate immunity is influenced by both Candida morphology and fungal load. Indeed, in healthy women, VECs can tolerate the presence of C. albicans yeast and a small amount of fungal filaments. However, when the hyphal load is high, VECs become sensitive to the fungus, triggering a severe inflammatory response. **(Ardizzoni *et al.*, 2021, Moyes *et al.*, 2010, Moyes *et al.*, 2011, Moyes *et al.*, 2015**, **Nikou *et al.*, 2019**, **Roselletti *et al.*, 2019)** Passive forces are responsible for the initial interaction between the fungus and the vaginal epithelium, i.e. Van der Waals and hydrophobic forces. Subsequently, the binding of fungal adhesins to specific receptors on surface epithelial cells and to elements of the host's extracellular matrix reinforces and stabilizes adhesion. **(Moyes *et al.*, 2015**, **Nikou *et al.*, 2019**, **Zhang *et al.*, 2022**). C. albicans has a wide range of adhesins to facilitate its adhesion to receptors, including the well-identified agglutinin-like protein (Als) family. This family is made up of eight glycosylphosphatidylinositols linked to the 1,6-glucans of the fungal cell wall (Als1p-Als7p and Als9p). Consistent expression of the ALS1-5 and ALS9 genes was observed in an experimental model of oral candidiasis (**Green *et al.*, 2004)** On the other hand, the ALS1, ALS2, ALS3 and ALS9 genes have often been observed in clinical samples of vaginal fluid. (**Cheng *et al.*, 2005).** These results suggest that various Als family proteins have specific functions in different regions of the mucosa.

After Candida has adhered to the vaginal mucosa, the dimorphic transition from yeast to hyphal form gives rise to additional adhesins linked to hyphal morphotypes, namely Als3p, Hwp1p (hyphal wall protein 1) and Ssa1p. The interaction of these proteins with E-cadherin, an essential component of intercellular connections, and with a heterodimeric receptor complex composed of epidermal growth factor receptor (EGFR) and Her2 (EGFR/Her2) enhances fungal adhesion to epithelial cells. **(Richardson, 2018 ; Liu and Filler, 2011).**

Research has evolved towards targeting virulence factors to combat Candida infections. Specifically, in recent years, Als3p has been seen as a potential target for vaccines against C. albicans infection, and an NDV-3 vaccine that targets the N-terminus of Als3p is recently in clinical trial (**Bu 2022**).

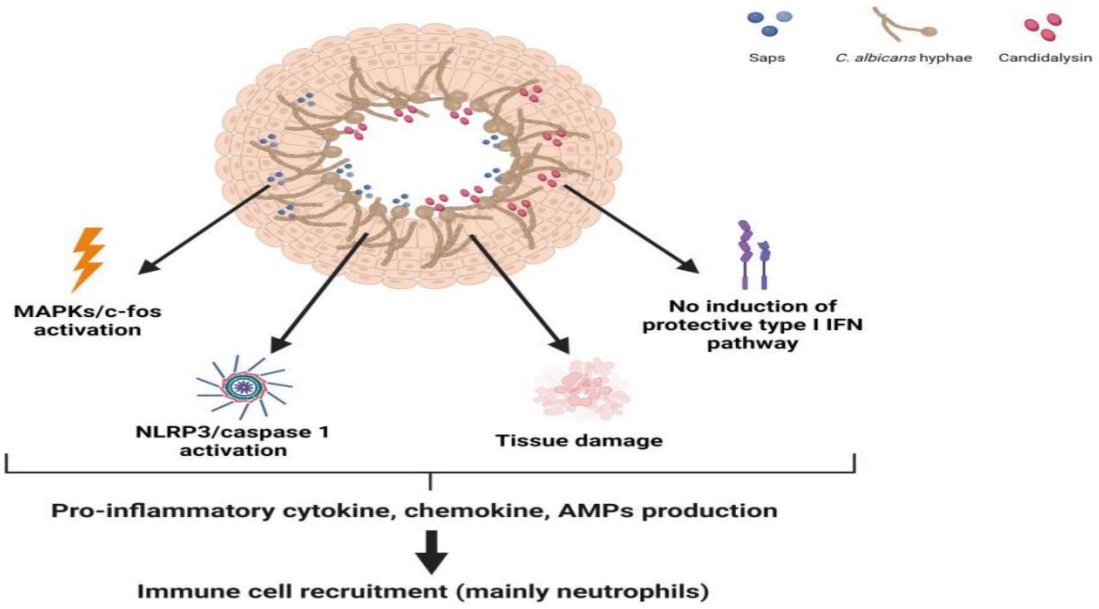
When adherent, Candida hyphae can enter epithelial cells by two different mechanisms: endocytosis and active penetration. The former is caused by the interaction of C. albicans' Ssa1p and Als3p proteins with cadherins and EGFR/Her2 **(Richardson, 2018, Liu and Filler, 2011**, **Phan *et al.*, 2007, Zhu *et al.*, 2012)** The second occurs when the tip of the growing hypha pushes the epithelial cell membrane and causes cell damage by releasing numerous virulence factors, such as candidalysin, a cytolytic peptide toxin, secretory aspartyl proteinases (SAPs), lipases and phospholipases.

Identification of filamentous forms of Candida by VECs leads to regular activation of the three mitogen-activated protein kinase (MAPK) pathways (p38, JNK and ERK1/2), which leads to c-fos activation via p38 and the release of antimicrobial peptides such as alarmins and pro-inflammatory cytokines (GM-CSF, G-CSF, IL-6, IL-1α, IL-1β, IL-36γ and CCL20), which play a key role in the recruitment of innate immune

cells, notably neutrophils and macrophages. (**Moyes *et al.*, 2015** ; **Nikou *et al.*, 2019** ; **Roselletti *et al.*, 2019** ; **Naglik *et al.*, 2017, Winkle *et al.*, 2016 ; Pellon *et al.*, 2020 ; Wang *et al.*, 2021).** Various recent investigations have demonstrated that candida lysin and SAPs, as well as the intracellular damage-sensitive molecular complex known as the NLRP3 inflammasome, and VECs all play a key role in VVC immunopathology. Indeed, candidalysin and SAPs have been found to intensely and persistently activate the NLRP3-caspase-1 pathway **(figure 24)** to produce pro-inflammatory cytokines, notably IL-1β (the main effector of the inflammasome), and to recruit neutrophils, which (in turn) appear unable to suppress fungal infection and, in fact, contribute more to the perpetuation of the chronic inflammatory **(Roselletti *et al.*, 2019)**. This is discussed in more detail in the next section.

Type I IFN signaling, produced by vaginal epithelial cells in response to *Candida* spp. infections, has been shown to increase epithelial resistance to infection and decrease pro-inflammatory responses. Sala and colleagues conducted an interesting study that examined a group of VVC and *C. albicans* colonizing vaginal isolates to determine the in vitro phenotypes related to either group. It should be emphasized that the isolates did not differ in their genetic profile or behavior (growth rate and filamentation) according to culture media.

However, they did adopt distinct behaviours when interacting with VECs. More specifically, C. albicans isolates from VECs induced greater fungal shedding of epithelial cells and detachment than those from healthy women. Notably, VVC-associated isolates also failed to stimulate type I IFN. According to this study, VVC isolates may present an intrinsically higher pathogenic potential due to their ability to stimulate various epithelial responses, including the type I IFN pathway. Figure shows a schematic representation of the main effects of *C. albicans* hyphae-ECV interaction.



<u>Figure</u> 25: Schematic illustration of the major effects of *C. albicans* hyphae-VEC interaction in VVC.

III.4.1. Conventional approaches to treating uncomplicated VVC

Uncomplicated candidiasis accounts for up to 90% of all cases (**Workowski, 2015**). For the majority of cases of uncomplicated VVC, the primary treatment relies on the use of azole antifungals which, by inhibiting the fungal enzyme CYP51, prevent the accumulation of toxic fungal sterols (**Pappas *et al.*, 2016; Sobel *et al.*, 2004**). A 2020 study reveals no disparity in the clinical efficacy of VVC treatment between oral and vaginal azole drugs. However, it is important to consider that oral administration of drugs is often associated with potentially more serious systemic side effects than topical application. Thus, it is possible that further research will reveal disparities in the use of these two methods (**Denison *et al.*, 2020**). In general, the duration of treatment with topical azoles is 3 days, and symptoms should disappear within 2 to 3 days. Clotrimazole, miconazole and butoconazole are topical antifungal agents, but they can have side effects such as itching and burning (**Nyirjesy *et al.*, 2021; Nyirjesy and Sobel, 2003**). Thus, in line with the 2021 recommendations of the Centers for Disease Control and Prevention, it is recommended to treat uncomplicated VVC using short-course topical preparations. This approach appears to be more advantageous for patients than oral azoles, as agents used topically will not cause systemic side effects.

Abdominal pain, headaches and nausea can be caused by oral azoles. In addition, there is a risk of drug interactions with other medications for patients. Nevertheless, it should be borne in mind that topical agents may cause burning or irritation, but compared with the side effects of oral azoles, these do not appear to be significant. Fluconazole is administered orally in a single dose of 150 g of the drug. However, in pregnant women, fluconazole may cause side effects such as hepatotoxicity, interactions with cytochrome P450 and possible adverse effects on the fetus. In addition, VVC returns after 6 months in around 50% of patients treated with fluconazole (**Mendling, 2015**).

According to the American Society of Infectious Diseases, patients are recommended to use topical and oral azoles at the same time (**Pappas *et al.*, 2009**).

III.4.2. Unconventional approaches to treating uncomplicated VVC

Probiotics appear to be a controversial alternative to azoles. Exogenous lactobacilli have demonstrated their ability to impede *C. albicans* biofilm formation in in vitro studies (**Matsubara *et al.*, 2016**). In 2021, Stabile and colleagues conducted a trial involving 40 women divided into two groups. Patients in the first group received treatment with topical clotrimazole for 6 days, while patients in the second group received oral treatment with live strains of Saccharomyces cerevisiae, melatonin and Lactobacillus idophilus GLA-14 (Unilen Microbio+). 85% of women were infected with *C. albicans*, and *C. glabrata* was the cause in the remaining women. Unilen Microbio+ demonstrated greater efficacy than topical treatment with clotrimazole alone (90% vs. 80%). There were no side effects in any patient during treatment. Moreover, flare-ups were twice as frequent in the group treated with clotrimazole alone as in the group treated with the preparation (**Stabile *et al.*, 2021**). However, recommendations from the Centers for Disease Control and Prevention clearly underline the lack of evidence for the use of probiotics in the treatment of VVC.

III.4.3. Treatment of complicated VVC

III.4.3.1. Treatment of recurrent VVC

VVCR is a persistent, difficult-to-treat and destructive condition affecting women worldwide. VVCR is defined in the U.S. as three or more episodes of symptomatic VVC in less than a year (**Workowski *et al.*, 2021**). RVVC, on the other hand, is defined by European guidelines and Infectious Diseases Society of America guidelines as four or more symptomatic episodes of VVC within a one-year period (**Donders *et al.*, 2022**). Current treatment protocols confirm that the diagnosis of RVVC is established with at least three symptomatic episodes of VVC (**Nyirjesy *et al.*, 2021**). The global prevalence rate of the disease is 3871 per 100,000 women, and 372 million women are affected throughout their lives (**Denning *et al.*, 2018**). RVVC consists of induction with a topical antifungal or oral fluconazole 150 mg for 10 to 14 days, followed by administration of oral fluconazole 150 mg for 6 months. Resistance to fluconazole is seen in women with RVVC, but it's important not to rule out misuse of the drug before resistance can be diagnosed (**Donders *et al.*, 2022; Lírio *et al.*, 2019**). Fluconazole is a long-term treatment with high cost and side effects, with recurrence of symptoms in around 50% of women a few months after the end of treatment (**Lírio *et al.*, 2019**).

Switching to mechanical hormonal contraception, treating the sexual partner and using topical gentian violet are also suggested (**Cooke *et al.*, 2022**). Clotrimazole, miconazole, terconazole and vaginal boric acid can be used in the topical treatment of RVVC, as can nystatin. However, azoles appear to be more effective than nystatin according to the literature (**Philips *et al.*, 2004**). The fact that these methods have limited efficacy suggests the importance of developing new, more effective therapies for RVVC that can be used long-term without significant side-effects. In April 2022, the US Food and Drug Administration (FDA) approved the use of oteseconazole (VT-1161) for the treatment of RVVC in women without reproductive potential (**Martens *et al.*, 2022**). Oteseconazole may be a first-line drug for women with RVVC, as it prevents vaginal recolonization for a longer period than current treatment options, particularly in women infected with *C. glabrata*, as well as in those with poorly controlled diabetes mellitus in whom the response to fluconazole is impaired (**Sobel *et al.*, 2021).**

III.4.3.2. Treatment of vulvovaginal candidiasis in diabetics

Another complex VVC situation is related to diabetes. The group at higher risk of VVC includes patients confronted with the disease and those at higher risk of developing it **(Farr *et al.*, 2021).** The various causes are associated with increased blood glucose levels in vaginal cells. It is linked to increased fungal adhesion and binding, and also decreases the ability of neutrophils to perform effective phagocytosis of these cells (**Yano *et al.*, 2019; Donders *et al.*, 2022).**

In addition, these patients are at increased risk of non-albicans *Candida* species infections, particularly those caused by *C. glabrata* and characterized by a less effective response to antimycotic therapy (**Farr *et al.*, 2021).** This may be due to the fact that some patients with diabetes receive SGLT2 inhibitors (such as dapagliflozin and canagliflozin), which are thought to increase the number of VVC episodes (**Farr *et al.*, 2021).** It is likely that this is related to the mechanism of action of these drugs, which is based on reducing blood glucose levels by lowering the renal threshold for glucose and increasing its urinary excess, which may favor more frequent infections.

III.4.3.3. Treatment of VVC in pregnant women

The occurrence of VVC is greatly influenced by patients' current hormonal status (**He *et al.*, 2015)**. High estrogen levels, such as pregnancy and hormone replacement therapy or the use of hormonal contraceptives are all risk factors for VVC (**Denning *et al.*, 2018; Willems *et al.*, 2020**). The incidence of VVC is estimated to increase significantly during pregnancy compared with non-pregnant women, but precise statistics are lacking **(He *et al.*, 2015).** The treatment of VVC during pregnancy poses a challenge, as on the one hand, it is essential to avoid infection-related complications, and on the other, it is necessary to modify treatment regimens appropriately to ensure the safest treatment (**Chew *et al.*, 2016).**

The use of azoles in the treatment of VVC in pregnant women is largely consistent with current guidelines **(Farr *et al.*, 2021).** Local administration of azoles is generally advised **(Farr *et al.*, 2021; Edwards *et al.*, 2019).** The Centers for Disease Control and Prevention has developed guidelines including a limited statement recommending the use of topical azoles for 7 days, and no further in-depth recommendations have been given. On the other hand, the British Association for Sexual Health and HIV has described different treatment regimens that can be used, and the specific management of acute and recurrent VVC in pregnant women has been highlighted. In the case of acute VVC, the authors suggest the daily administration of 500 mg clotrimazole in the pessary for a period of 7 days. Alternatively, they recommend a 7-day course of reduced-dose clotrimazole (200 mg) or clotrimazole 10% cream 5 mg. Other possible treatments include econazole 150 mg in pessaries, miconazole in cream (5 g of 2% agent, daily for 7 days) or capsules (1200 or 400 mg daily for 7 days). Then, in the case of CVVR, recommendations state that the only recommended management is topical imidazole for 10 to 14 days and 500 mg clotrimazole in small amounts once a week for maintenance **(Edwards *et al.*, 2019**). According to the latest recommendations of the German research group mentioned above, the most advantageous treatment regimen is in line with the above-mentioned schemes and involves local administration of clotrimazole. In particular, the authors highlight the importance of such management during the first trimester of pregnancy, in order to avoid the oral use of azole and the potential fetal malformations that this entails **(Farr *et al.*, 2021**).

Despite the results of the meta-analysis on the incidence of complications following fluconazole treatment, which showed that such a treatment regimen did not significantly increase the risk of various congenital malformations, they suggested the need for further study into a possible correlation between cardiac malformations and fluconazole exposure (**Alsaad *et al.*, 2015**).

Various national and international recommendations suggest the use of boric acid as an alternative to azole-based treatment in non-pregnant women. Nevertheless, such a treatment regimen may be linked to a higher risk of various congenital malformations in pregnant women, and it therefore seems that it should be avoided (**Mittelstaedt *et al.*, 2021**).

III.5. The role of probiotics in the treatment of VVC

In gynecology, the main basis of probiotic action is the antimicrobial capacity of lactobacilli. The vaginal flora is mainly composed of Lactobacillus crispatus, but other species can also be found, such as Lactobacillus gasseri, Lactobacillus iners, Lactobacillus vaginalis and some twenty other species (**Jovana Tanasijevic, 2023**). Probiotics are used to improve the clinical and microbiological efficacy of treatment with azole molecules for C. albicans vaginal infections. According to the study, it was found that the microbial balance of the vaginal ecosystem was restored, which is essential for reducing falls. All patients showed a statistically significant reduction in pruritus, burning, edema, erythema, dyspareunia and vaginal secretions. Research suggests that this medical device could be beneficial in preventing recurrent episodes (**Kovachev *et al*., 2014)**

III.6. Essential oils and medicinal plants to treat VVC

There are a large number of antifungal agents available, but recurrences are still frequent due to the failure to eliminate the causative factors and the increasing resistance of *Candida* to antifungal agents. Plants and essential oils (**table 8**) are highly recommended for treating resistant and recurrent candidiasis. There are many plants and essential oils with antifungal activity, but they are still little used due to the lack of clinical studies. It is difficult to predict whether an essential oil can be used on mucous membranes without the risk of adverse cutaneous effects. Antifungal herbs and essential oils should therefore be recommended more frequently, but in the absence of in-depth clinical studies on these treatments, it is important that pharmacists remain vigilant and cautiously recommend these herbal treatments (**Jovana Tanasijevic, 2023**).

Table 8: Practical use of essential oils (Jovana Tanasijevic, 2023).

Chemical family	Essential oils	Properties	Precautions for use	Contraindications
Phenols	Cinnamon leaves (eugenol) Savory (carvacrol) Thyme with thymol-carvacrol	Phenols are the molecules with the strongest bactericidal and fungicidal activity.	Dermocaustic (avoid application on the skin) Limited treatment duration Combine a hepatoprotective essential oil	Liver failure Cirrhosis Hepatitis Liver Cancer Pregnant woman child

			during the treatment (lemon essential oil, rosemary, milk thistle)	
Monoterpene alcohols	Tea tree (terpinen-4-ol) Thyme with linalool Ho wood (linalool) Rosewood (terpineol) Lavender (linalool)	Bactericidal and fungicidal activity (less powerful than phenols)		
Ketones	Sage (thujone)	Immunostimulants and antifungal	Neurotoxic Lowers the epileptogenic threshold Abortive → Respect the maximum dosages	Pregnant women with epilepsy Children under 12 years old
Monoterpene esters	Lavender (linalool)	Calmants and analgesics		
Ether-oxide	Niaouli Laurel (1-8 cineole)	Antifungal Anti-inflammatory	Always use diluted on the skin to avoid allergies.	
Aldehydes	Cinnamon (Cinnamaldehyde)	Anti-infectives Immunostimulants Tonics	Do not apply to mucous membranes	Dermocaustic

Chapter IV:

Functional cookies made with 100% tiger nut flour.

Chapter IV: Functional cookies made with 100% tigernut flour.

IV.1. Introduction

The human need for food is not just a choice for him, but rather an essential part of his body. By nature, humans eat different types of food, whether animal or vegetable. Among these plants, we mention the edible stem plant which is rich in vitamins, antioxidants, mineral salts, dietary fibers and proteins. It is considered one of the ancient plants that was famous for its use in many fields such as food and traditional medicine, thanks to the nutrients and important components that are present in it. The *Cyperus esculentus* L., also known as tiger nuts, terrestrial almonds in Africa, chufa in Spain and "Hab Alaziz" in Algeria and in many Arab countries.

The use of tiger nuts dates back to more than 3000 years and already mentioned in writings of ancient Egypt. It was introduced to Europe by the Arabs from the 19th century, it is especially in the kitchens that the use of the tiger nuts is traditionally reserved. It can be eaten cooked or grilled. In several countries, it is reduced to powder and comes into the composition of porridge or biscuits. In Spain, France, Dominican Republic, Mexico, Panama and USA, Horchata de chufa is the nutritious product of milky aspect, obtained mechanically by aqueous extraction using pressure from the tiger nuts **(Roselló-Soto *et al*., 2019).**

Cookies are the most popular cereal products consumed around the world due to various reasons, their taste quality, their affordable cost, their availability in different varieties, as well as their long shelf life. Without sugar or salt, with protein, high in insoluble fiber or gluten-free. Dietary cookies are labeled "healthy", "light" or "thin"... this range is constantly expanding and multiplying nutritional promises **(Simanca-Sotelo *et al*., 2021).**

According to a recent study published in May 20122, 70.31% of Algerians said that they have become accustomed to paying attention to their diet. A custom acquired during the Covid-19 pandemic that prompted the ordinary Algerian citizen to question the composition (list of ingredients) contained in an industrialized product. Moreover, 48% believe that the negative health effects of sweetened and industrialized foods are now greater **(Ziar *et al*., 2022).**

Dietetic cookies are very limited in number in the Algerian market. The little that exists comes from the efforts of some non-governmental industries. In this study, dietary cookies were made and where wheat flour was 100% replaced by a dietary flour. The aim of this work was to use tiger nut flour to make healthy-cookies" with or without probiotic bacteria. In this work, we determine the physico-chemical properties of the edible tiger nut flour that will be used for making the biscuit. Thus, physical and sensory analyses on cookies without sugar or enriched with bee honey would also be carried out.

IV.2. Materials and Methods

IV.2.1. Raw materials

Purchased from herbalists in Southern Algeria, the plant material is made up of organic tiger nut tubers, which are native to the Ouaddaï region of Eastern Tchad.

IV.2.2. Reducing tiger nuts to flour

Tiger nut flour was made from tubers that were the most uniform in size, weight, and maturity. Figure 2 shows the steps involved in getting the flour. The dried tubers underwent flow drying before being ground into 300 μm particles in a spray mill. Polythene plastic bags were used to package the flour.

IV2.3 Chemical description of raw tiger nut flour

According to the approved procedures of the American Association of Cereal Chemists International, the flour's moisture, protein, fat, sugar, fiber, mineral, and ash contents (%) were calculated using the following techniques: 44–15.02, 46–30.01, 30–10.01, 32–45-01, 40–70.01, and 08–03.01 (**AACCI, 2010)**.

The contents of glucose, fructose, and sucrose were examined using ion chromatography (IC). At the very least, every chemical and reagent utilized was analytical reagent grade. We bought D (+)-sucrose monohydrate, D (−)-fructose, and D (+)-glucose anhydrous from Fluka (Switzerland). Utilizing deionized water from RiOsTM type I simplicity 185 (Millipore Waters, USA) with a resistivity of 18.2 MΩ cm, all aqueous solutions were made. Sunnyvale, California, USA's Dionex Instrument DX-500 IC system was used for chromatographic separations. The apparatus included an ED40 electrochemical detector with a thin-layer amperometric cell and a GP40 gradient pump. The cell was composed of a platinum counter electrode in IPAD mode and a gold working electrode with a diameter of 1 mm. An analytical column (250 mm × 2 mm ID) and a guard column (50 mm × 2 mm ID) made up the CarboPac PA 10 column set used for the separations. 25 μL was the injection volume for the sample. A flow rate of 1 mL min−1 was observed. Inside a temperature controller were the columns. Data collection, processing, and chromatographic system control were carried out with Dionex's PeakNet 6.0 software.

In 25 milliliters of water, precisely 0.25 grams of uncooked flour were dissolved. A membrane filter with a pore size of 0.22 μm was used to filter sample solutions. The sample was directly injected into the chromatographic apparatus for the sugar analysis after being 100 times diluted with water (**Suksom *et al.*, 2015).**

IV2.4. Rheological characteristics

IV2.4.1. Density of bulk (BD)

The **Wang and Kinsella (1976).** method was used to measure the bulk density. A 25 mL graduated cylinder containing 10 grams of flour was gently tapped ten times from 5-8 cm above the benchtop. Test flour's ultimate volume was calculated and expressed in grams per milliliter.

IV.2.4.2. Index of water absorption (WAI)

The approach previously published for cereals **(Stojceska *et al.*, 2008)** was used to determine WAI. For 30 minutes, the powdered flour was suspended in water at room temperature, stirred gently, and then centrifuged for 15 minutes at 3000 × g. A pre-weighed evaporating dish was used to decant the supernatants. WAI was the weight of gel per unit weight of the initial dry solids after the supernatant was removed.

IV.2.4.3. Absorbance capacity of oil (OAC)

Lin et al.'s approach **(1974)** was used to calculate the oil absorption capacity. A mixture of 0.5 g flour and 10 mL refined oil was vortexed for 10 minutes in a centrifugal tube that had been previously weighed. For 25 minutes, the tubes were centrifuged at 3000 × g. The oil was emptied and the centrifuge tubes were weighed following ten minutes of inversion.

IV.2.4.4. Foaming capacity

According to **Awolu (2017)** A soluble solution of souchet is prepared at 1% (1g of souchet in 100 ml of deionized water), and it is then adjusted to a pH of 7.4 using NaOH 1,0N and HCI 1,0N. A 100 ml (V_1) concentrated suspension was mixed for three minutes with the aid of a very fast mixer. Afterward, the volume of mousse is recorded (Vf) in a 250 ml graduated cylinder.

IV.2.4.5. The dispersibility (Dis)

Ten grams of edible soy farine are placed in a graduated cylinder, and water is added until the graduation reaches 100. The mixture is then vigorously stirred and allowed to rest for three hours. Finally, the particle volume is noted (**Awolu, 2017)**

IV.2.4.6. Yield of gluten

The AACCI Method 38-10.01 (**AACCI, 2010)** was followed for performing the hand-washing procedure. A suitable quantity of water (12–15 mL) was combined with aliquots of 25 g flour, and the dough was kneaded until it was smooth and firm. It was then let to rest for two hours to allow the gluten structure to form. The wet gluten was weighed after the dough was cleaned until just the dark gluten ball was left.

Wet gluten yield= (weight of wet gluten obtained/weight of flour) ×100

IV.2.4.7. Diffraction analysis of X-rays (XRD)

Using an automatic multifunctional X-ray diffractometer (D8 Advance, Bruker, Germany), two milligrams of flour were measured (**Miranda *et al.*, 2019**). With a scanning range of 4–60° 2θ and a scan speed of 2.0°/min, the X-ray generator was operated at 40 kV and 40 mA.

IV.2.5. Functional properties of the flour

Aqueous (using 100% ultrapure water), and hydro-ethanolic (30:70; water:ethanol, v/v) tiger nut flour's extracts were subjected to the following analysis:

IV.2.5.1. Content of polyphenols

Two milliliters of 7.5% saturated Na_2CO_3 and 2.5 milliliters of 10% Folin-Ciocalteu reagent were combined with each extract (500 µL) **(Roselló-Soto *et al.*, 2018)** A UV spectrophotometer (UV-1800, Shimadzu Corporation, Kyoto, Japan) was used to measure the absorbance at 760 nm after the reaction was carried out for 15 minutes at 50°C in a water bath. Gallic acid equivalents (GAE, mg/100 g extract) were used to present the results (y = 0,0287x - 0,1374; R2 = 0,9712).

IV.2.5.2. Content of flavonoids

10% $AlCl_3$ and 0.3 mL of 5% $NaNO_2$ have been combined with one milliliter of the extract. 2.5 mL of distilled water and 2 mL of 1M NaOH were added after 6 minutes. Using a UV-VIS spectrophotometer, the color intensity of flavonoids **(Roselló-Soto *et al.*, 2018)** was determined at 510 nm.

Quercetin equivalent (QE) was used to compute the total flavonoid content, which was then represented as mg/g (y = 0,0431x - 0,0682; R2 = 0,9987).

IV.5.3. Antioxidant activity

The **Brand-Williams *et al.* (1995)** technique involves the use of a spectrophotometer (DU ® 730, Beckman Coulter Inc., Brea, CA, USA). Using Trolox, a calibration line with values ranging from 0 to 200 mg/L was created for the evaluation of antioxidant activity in both techniques. IC 50 and EC 50 values (mg/mL) were used to showcase the findings.

IV.2.5.4. Anti-microbial activity

The antimicrobial activity was tested by growing 100 µL of the microbial suspensions of *Pseudomonas aeruginosa ATCC 27853, Staphylococcus aureus ATCC 33862, Bacillus subtilis ATCC 6051, Bacillus cereus ATCC 10876, Escherichia coli ATCC 25922,* and *Candida albicans ATCC 10231* in triplicate on Mueller-Hinton agar (Difco) plates. With the use of sterile forceps, sterile filter paper disks impregnated with tiger nut flour extracts (100 mg/mL: hydroethanolic or aqueous extracts) were deposited on the inoculation medium's surface. Amoxicillin (80 mg/mL) served as the positive control, while a sterilized disk served as the negative

control. The findings were assessed and reported as the millimeter-wide inhibitory zone diameters that developed around the extract-containing discs during a 24-hour incubation period at 37 °C.

IV.2.5.5. Sugar-free cookies enriched with probiotics

Cookies were made (table 9) by mixing the following ingredients and baked 15min/170°C:

The same baked preparation was soaked in a syrup made from 5% (w/v) honey containing the probiotic isolate SL4 to make dietary cookies based on probiotic bacteria.
The bacteria, in lyophilized form, were added at a final concentration of 1×10^{7} CFU/mL to each cookie enriched with honey syrup. To ensure that the edible tiger nut flour and bee honey do not exert an inhibitory action on the SL4 isolate, growth tests in the presence of bee honey and fermentability tests of the tiger nut flour were conducted beforehand.

Table 9: Types and quantities of ingredients added in the production of edible tiger nut cookies.

The ingredients	The quantities
Edible tiger nut flour	100g
Butter	50g
Skim milk powder	20g
Sodium bicarbonate	1g
Sel	1g
Water	20 ml

IV.2.5.6. Microencapsulation of the probiotic bacteria

The spray-dried powder of *L. rhamnosus* SL42 was made using the method reported by **Sompach *et al.* (2022),** with minor modifications. Sucrose was used as the encapsulant formulation solution for spray-dried microcapsules, at a concentration of 5% (w/w) in deionized water. The sucrose solution was stirred for 15 minutes at 600 rpm using an overhead stirrer (RW 20 digital, IKA, Taufkirchen, Germany). The concentrated cells were dispersed in the solution and agitated for another 30 minutes. The feed suspension was spray-dried using a tiny spray drier (BÜCHI Labortechnik AG, Flawil, Switzerland) and constantly swirled with a magnetic stirrer to preserve homogeneity.

The spray-dried microcapsules were dried at a constant feed flow rate of 7 mL/min and an inlet air temperature of 130°C. The aspiration rate was kept at around 35 m3/h, while the flow meter spraying flow rate was 475 L/h. The spray-dried microcapsules were then placed in a zip-lock polyethylene bag and packed

with aluminum foil laminate bags made of three layers of polyethylene terephthalate, aluminum foil, and polyethyle.

IV.2.5.7. The fermentability of edible tiger nut flour

2% of tiger nut flour was sterilely added to the MRS broth or to the water. peptone and the media are inoculated with the SL4 strain at a concentration final of 1 $\times 10^7$ CFU/mL. A count on MRS-cys agar medium was performed at 0h and after 24h of incubation at 37°C in anaerobiosis.

IV.2.5.8. Growth of the SL4 isolate in the presence of 5% bee honey

in order to prepare cookies enriched with probiotic bacteria, bee honey (honey forest eucalyptus bee (Chlef) at 5% was used to make a honey syrup containing the SL4 isolate. The growth of the SL4 isolate in the presence of honey has been verified as follows: the 5% bee honey (**Riazi and Ziar, 2008)** was added sterilely in MRS broth or peptone water, and both media are inoculated with the strain SL4 at a final concentration of 1 $\times 10^7$ CFU/mL. A count on medium MRS agar was performed at 0h and after 24 hours of incubation at 37°C in anaerobiosis.

IV.2.5.9. Appearance and texture analysis

We measured the diameter and thickness using a caliper at two distinct points for each cookie (figure 18), then we determined the average for each. We evaluated the fracturability by applying an identical force to the central point of the cookie. For each batch, an average of 6 cookies was rated, while their weight was determined using a digital scale. The formula used to calculate the spread ratio is as follows: diameter of the cookies divided by the height of the cookies **(Zoulias et al., 2000).** Commercial cookies made from wheat flour were used as a reference.

IV.2.5.10. Survival of the probiotic bacteria

The survival of the SL4 isolate was monitored during the 4 weeks of storage at ambient temperature of the cookies produced. A cookie is weighed and dissolved in 250 mL of sterile isotonic solution. Dilutions were performed allowing for enumeration. This last one was performed on MRS-cys agar medium at day 0 and after 7,15, and 21 days storage.

IV.2.5.11. Scanning Electronic Microscopy analysis of cookies

Through the use of scanning electron microscopy (FEG-SEM-LV SU-5000, Hitachi, Milexia, 91190-Saint-Aubin, France), the morphological characteristics of the cookies, the produced syrup, and SL42 adhesion were illustrated. Each cookie had two parts: one was fixed (**figure 25**) for high resolution observations (SE(L) detector, 2keV, 30 spot size, 5mm WD) and the other was directly observed using the ambient configuration (50 Pa, UVD detector, 10keV, 30 spot size, Peltier stage system at -15°C). Magnifications

ranging from 100 to 25,000 X were employed. Samples were submerged in a 2% glutaraldehyde and 0.1 M sodium cacodylate buffer solution (pH 7.4) for fixing. A diamond pen was used to cut the 1 cm square microscope glass slide onto which the culture contained in the syrup was placed.

Following a 70% ethanol cleaning, a 5-minute plasma cleaner, a 10-minute ethanol 100% sonication bath, and a 5-minute MilliQ water rinse, glass slides were coated with 0.1%M polylysine (Merck Sigma Aldrich) for five minutes. After being treated in the fixative solution for an hour at room temperature, the samples were stored at 4 °C for the night. Samples were washed twice in a 0.2 M sodium cacodylate solution (pH 7.4) for 10 minutes each after the fixative solution was removed. After soaking in ethanol at different concentrations (50, 70%, 90%, 100%, and anhydrous 100%), the samples were subjected to progressive dehydration before being critical-point dried under CO 2 (Leica EM300, slow 20 exchange cycles, 2min delay). Samples were mounted on aluminum stubs (15 mm and 32mm diameter) with carbon adhesive discs (LFG France), and was coated with Au/Pd (Quorum SC7620, 5 Pa of Ar, 3 x 180 seconds of sputtering at 3.5.

Figure 26: Scanning electron microscopy (FEG-SEM-LV SU-5000, Hitachi, Milexia, 91190-Saint-Aubin, France).

IV.2.5.12. Sensorial analysis

IV.2.6. Statistical analysis

Statistical analyses were performed using EXCEL to determine the means and standard deviations as well as the percentages of each criterion in the hedonic test. The results of the sensory analysis were carried out using analysis of variance (ANOVA) to determine if there are significant differences ($p<0.05$) in the average degree of appreciation between the two types of formulated cookies (sugar-free or with honey and probiotics).

IV.3. Results and discussion

IV.3.1. Physico-chemical parameters of the flour

When comparing various tiger nut flour varieties, the centesimal composition of the flour (**Table 10**), which has a water content of 8.3%, is thought to be lower than that found by **Oldele *et al.* (2002)** (3.5 – 3.75%) and comparable to that observed by **Yapi and *al.* (2021)** (8.03 – 8.6%).

At 3.04 percent, the ash content was greater than the values found in the literature, which were 1.6-2.4% by **Yapi *et al.* (2021)** and 2.17% by **Bankoffi *et al.* (2005).** According to **Bankoffi *et al.* (2005),** the high ash level (**table 10**) suggests a high mineral content (2.26%), including sodium, calcium, iron, magnesium, phosphorus, and zinc, which are more prevalent in C. esculentus tubers than in regularly consumed cereals. Ash content shows that the tuber is contaminant-free and safe to consume **(Sidohounde *et al.*, 2018).**

Tiger nut flour has a fat content of 26.54% (**table 10**). The computed value is within this plant's particular range. Compared to our investigation, **Oladele *et al.* (2007)** discovered a fat content that ranged from 32.13 to 35.43%. Conversely, **Nina *et al.* (2019)** discovered lower figures between 19% and 22%. But when compared to ordinary millet (7.6%), quinoa (6.3%), cajan peas (1.80%) (**Okpala and Mammah, 2001)**, and wheat flour (3.10%) (Akubor and Badifu, 2004), the fat content of tiger nut flour is thought to be extremely high.

Together, tiger nuts are nutrient-dense, with a lipid content similar to that of olive oil and a high concentration of minerals, especially potassium and phosphorus **(Fabunmi *et al.*, 2016).** Its importance in a balanced diet is explained by its crude fat content, which also decreases cholesterol and the risk of heart disease, among other advantages **(Anand *et al.*, 2015).**

On a dry basis, the protein, fat, and fiber contents were high and in line with **Nwosu *et al*.'s research (2022). Adegunwa *et al*. (2017)** also reported similar findings, obtaining protein, fat, and carbohydrate levels of 6.73, 32.75, and 46.07%, respectively. Likewise, **Nwaoguikpe (2010)** found that the yellow tiger nut has protein, fat, and carbohydrate levels of 7.15, 32.13, and 46.99%, respectively.

Table 10: Chemical composition (%) of tiger nut flour

	Water content	Ash	Lipids	Sugars	Fiber	Proteins	Minerals
Tiger nut flour	**8.3**	**3.04**	**26.5**	**55.6**	**15.5***	**5.8**	**2.26**

* g/100g

Ion chromatography is a practical analytical technique that has several uses in food analysis. It enables food scientists to measure a wide range of important analyte groups, including sugars, that are vital to human health. IC's data are extremely accurate and precise, and they may be utilized to fully monitor the makeup of food goods **(Muntean, 2024).**

Data revealed that tiger nut flour had a considerably higher concentration of sucrose than glucose, and fructose **(figure 26).**

Recent studies on tiger nuts have not paid much attention to the high sucrose content of defatted tiger nut meal (**Marchyshyn *et al.*, 2021).** Unfortunately, a major barrier to the proper use of tiger nuts is the paucity of research on their sucrose. They are discarding a potentially useful resource **(Zhang *et al.*, 2023).** Tiger nut meal may develop as a new Non-centrifugal sugar (NCS) resource due to its high sucrose concentration, which would raise its value. **(Zhang *et al.*, 2023).**

Furthermore, because it contains a number of non-sucrose components, such as fructose, phenols, flavonoids, minerals, and vitamins, unrefined sugar, like that found in tiger nuts, may be regarded as a health food **(Zhu *et al.*, 2020).** Numerous studies have demonstrated the advantages of unprocessed sugars, including cytoprotective, antioxidant, immunological-stimulating, and anticancer effects **(Lee *et al.*, 2018; Takahashi *et al.*, 2016; Zidan and Azlan, 2022).**

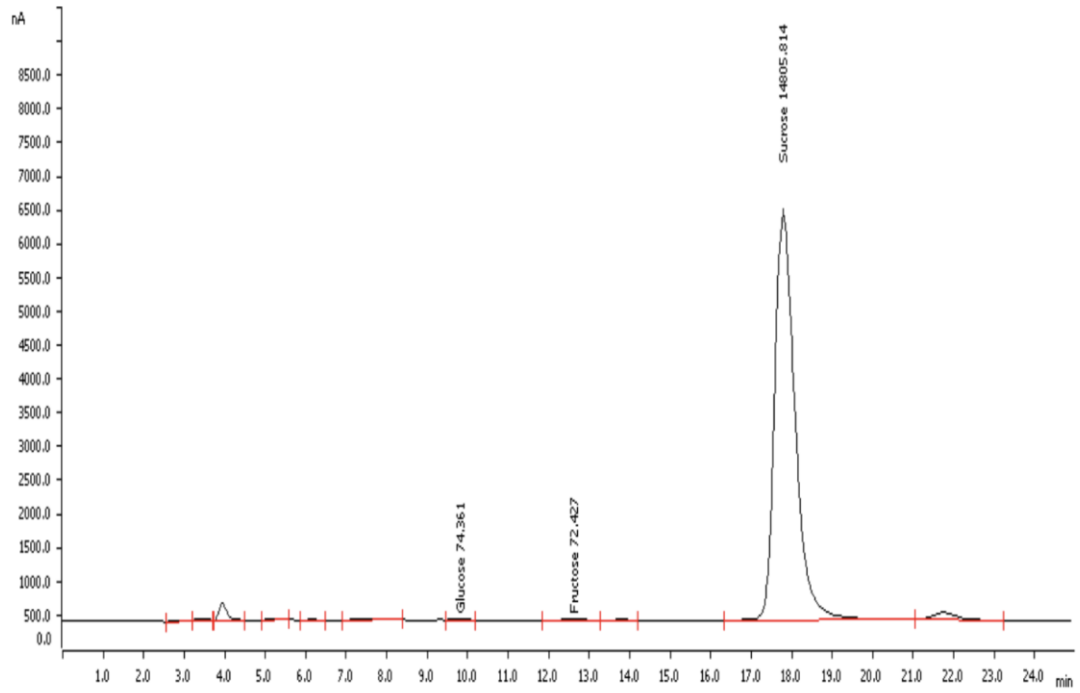


Figure 27: Ion chromatogram of principal tiger nut's sugars.

IV.3.2. Rheological values of organic tiger nuts flour

The bulk density of the tiger nuts flour was 0.74 g/mL (**Table 11**), which was greater than that of **Yapi *et al.*, (2021)** (0.5 to 0.54 g/mL) and Oladele and **Aina (2007**) (0.55 g/mL), but comparable to that of **Nina *et al.*, (2019)** (0.76 g/mL). The breakdown of complex compounds, such as starch, is summarized by the bulk density value **(Menasra, 2020)**. However, as it indicates that the product may be easily packaged for cost-effective use, an appreciated value may be beneficial when creating functional foods. **(Nina *et al.*, 2019).**

The dispersibility of Tiger Nut flour was approximately 68% (**Table 11**), which is almost the same as the 72.5% figure for the same type of flour reported by **Adejuyitan *et al.* (2009).** Additionally, a value of 64% for cajan pea flour (*Cajanus cajan*) was reported by **Ohizua *et al.* (2017).** The ability of a flour's individual molecules to disperse and homogenize in a medium is known as its dispersibility. It is described as a flour-moisturizing quality by **Gaiani *et al.* (2011).**

Table 11 : Technological attributes of tiger nut flour.

	Bulk density (g /mL)	Dispersibility (%)	Water absorption index (g/mL)	Oil absorption capacity (mL/g)	Foaming capacity (%)	Wet gluten yield (%)
Tiger						

nut flour	**0.74**	**68**	**0.086**	**2.2**	**12**	**0**

Tiger nut flour's water absorption capacity (**Table 11**) was 0.08 g/mL, which was less than the 1.74 g/mL found in **Nina *et al. (*2019)** research. This number is thought to be a crucial technical factor in regulating the dough's consistency. In the presence of liquid water, it exhibits its hydration capacity and is mostly dependent on moisture and the pace at which the starch is damaged. The starch's molecular makeup and chemical makeup also affect its ability to absorb water. The low percentage of A chains and the high percentage of B1 and B2 chains of amylopectin cause its poor solubility in starch **(Chung *et al.*, 2008).**

Tiger nut flour (**Table 11**) had an oil absorption capacity of 2.2 mL/g, which was more than that of **Yapi *et al.* (2021**) (1.67 to 1.88 mL/g), **Oladele and Aina (2007)** (1.13 mL/g), and **Nina *et al. (*2019**) (1.75±0.02 mL/g). Since it stops rancidity from developing, flour's oil absorption capacity (AHC) is a crucial component in food preservation. **(Diallo *et al.*, 2015).**

Table 11 showed that the edible strain flour had a moisture capacity of 12%, which was greater than 4.72±1.34% reported by **Nina *et al.* (2019).** However, it was comparable to 11.07% determined by **Oladele and Aina (2007**). High quantities of protein and starch could be the result of a high foaming capacity **(Menasra, 2020).**

In order to preserve a consistent texture and structure during processing and storage, food items like cakes, bread, meringues, crackers, ice cream, and various other baked goods need have foaming qualities **(Nawaz *et al.*, 2015).**

The gluten-free status of tiger nut flour is confirmed by our reported result. Gluten-free flours include white and brown rice, maize, oats, millet, amaranth, quinoa, chickpeas, and tiger nuts, according to **Culetu *et al.*, (2021).** Their nutritious qualities make them a popular ingredient in gluten-free cereal food items. Hard wheat flour has 17.35 to 32.20% wet gluten **(Zilic, 2014).**

A-type starches are found in cereals, B-type starches are found in tubers, and C-type starches are found in legumes and some seeds, according to XRD patterns. The C-type pattern varies depending on the type of legume and blends A and B polymorphs in different ratios (**He and Wei, 2017).**

Figure 27 displayed the XRD pattern of the whole tiger nut flour. The X-ray diffraction pattern at 2θ is 8.1◦, 16.9◦, 18.6◦, and 22.4◦, suggesting that the tiger nut starch in the flour has a C–type polymorphism structure. **Chung *et al*. (2008)** The C-type pattern was discovered to be represented by the bean starch signals at 5.6, 15.0, 18.0, 20.0, and 23.0° 2θ. According to some researchers, the diffraction patterns observed in C-type starch are a hybrid of the A- and B-type patterns. With unique properties and uses, C-type starch is more complex than A- and B-type starches. Proteins, starch, lipids, and minerals can all create conjugates that are linked to the degree of starch crystallinity and XRD signals. (**Gani *et al.*, 2016; He and Wei, 2017).**

In general, amorphous and crystalline peaks can be noted. The three crystalline peaks that occurred in the range 10° to 25° 2θ represented well the semicrystalline characteristic of B- type tiger nut starch **(Manek *et al*., 2012).**

B chains are classified as resistant starches (RS), which are starch fractions that ferment in the large intestine but do not break down in the small intestine. In addition to having a prebiotic impact, the byproducts of RS fermentation can lower cholesterol and the risk of colon cancer **(Ziar *et al*., 2014).**

Tiger nut flour's structure has a weak single diffraction peak at 19.2° and sharp diffraction peaks at 34.5° and 35.3°, suggesting the existence of long arrangement structure.

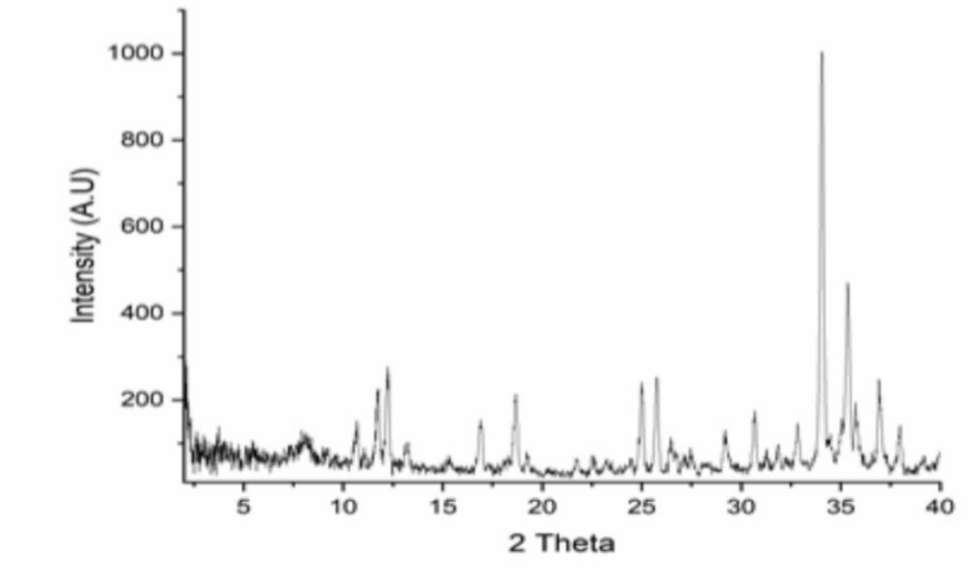


Figure 28: XRD diffraction of the whole organic tiger nut flour.

IV.3.3 Functional qualities of tiger nut extracts

With recorded values of 111.82 and 68.5 mg EGA/100g DW, respectively, the aqueous extract's total polyphenol content (**Figure 28**) was greater than that of the ethanolic extract (water 30v/ethanol 70v).

Phenolic compounds from tubers seem to be more easily extracted by water than by organic solvents, suggesting that flour has a higher concentration of water-soluble phenolic compounds. According to **Djikeng *et al.* (2022)**, methanolic extracts have a total polyphenol concentration ranging from 18.31 to 300.44 mg EGA/100 g. **Oladele *et al.* (2014)** observed similar findings (95.2 - 388.5 mg/100 g).

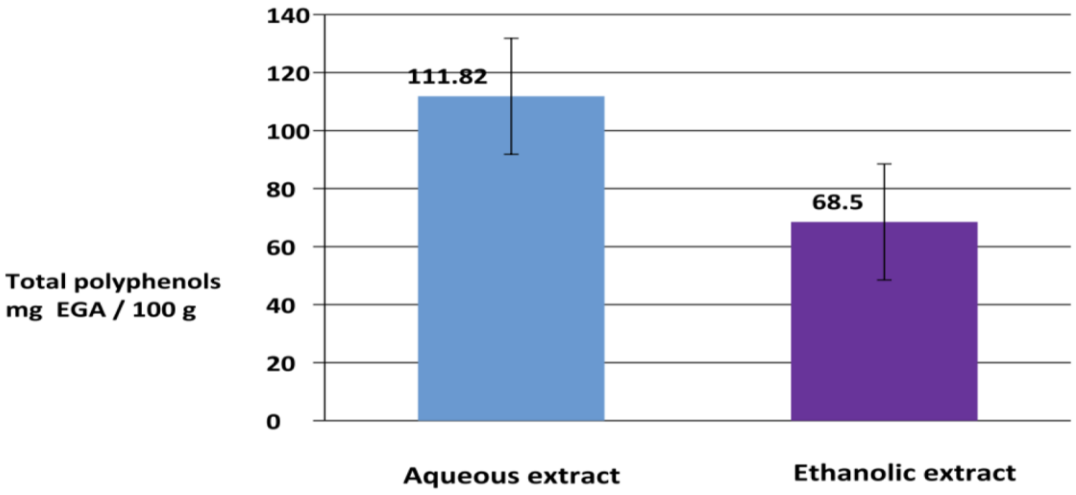


Figure 29 : Total polyphenol contents in aqueous and ethanolic extracts of tiger nut.

Achoribo and Ong (2019) used an ethanolic extract to find lower levels, ranging from 3 to 12 mg EGA/100 g. The differences noted in the literature may be attributed to plant species, extraction method, extraction solvent type, and environmental factors (climate, storage site, harvest period, temperature, etc.) (**Hsu *et al.*, 2006).**

The aqueous extract (280 mg EQER/100g DW) contains slightly more flavonoids (**figure 29**) than the ethanolic extract (198.18 mg EQER/100g DW). **Willis *et al.* (2017)** found similar amount of flavonoids (220.68 mg EQER/100g DW).

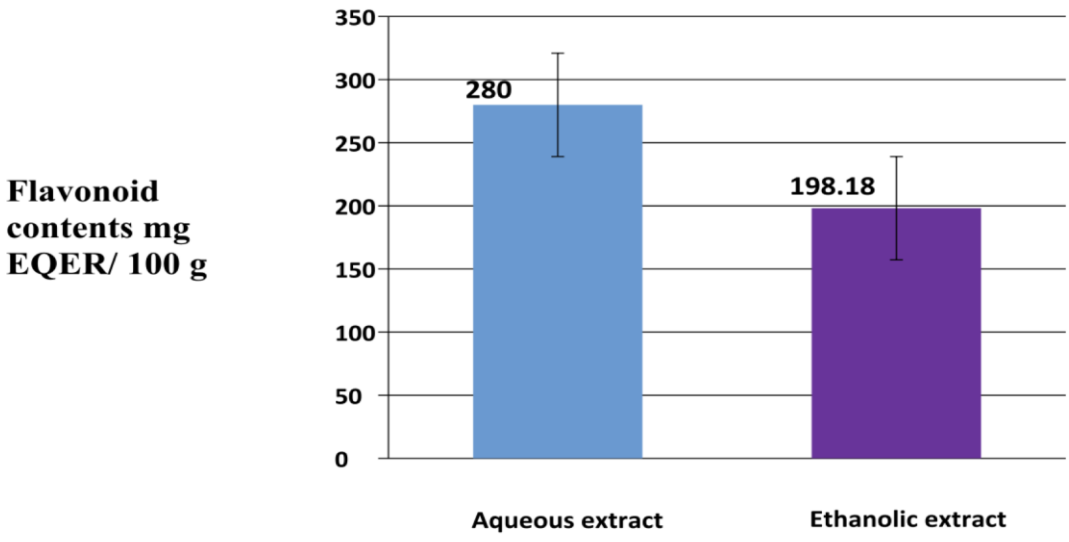


Figure 30: Flavonoid contents in aqueous and ethanolic extracts of tiger nut.

The permeability of flavonoid components across the intestinal epithelium and their solubility after soaking in water was enhanced.

According to the DPPH method, the aqueous and ethanolic extracts of C. esculentus exhibit antioxidant properties, as shown in **Figure 30**, with corresponding IC 50 values of 49.96 and 6.22mg/mL. The flavonoids' basic antioxidant properties are mostly attributed to their hydroxyl groups, which can also readily change into their O-methyl, O-glycosyl, O-sulfat, or O-acyl forms. The antioxidant and other biological functions of flavonoids are typically inhibited, even though a portion of sugar linked to the molecules may make them more soluble in water and bioavailable **(Slámová *et al.*, 2018).**

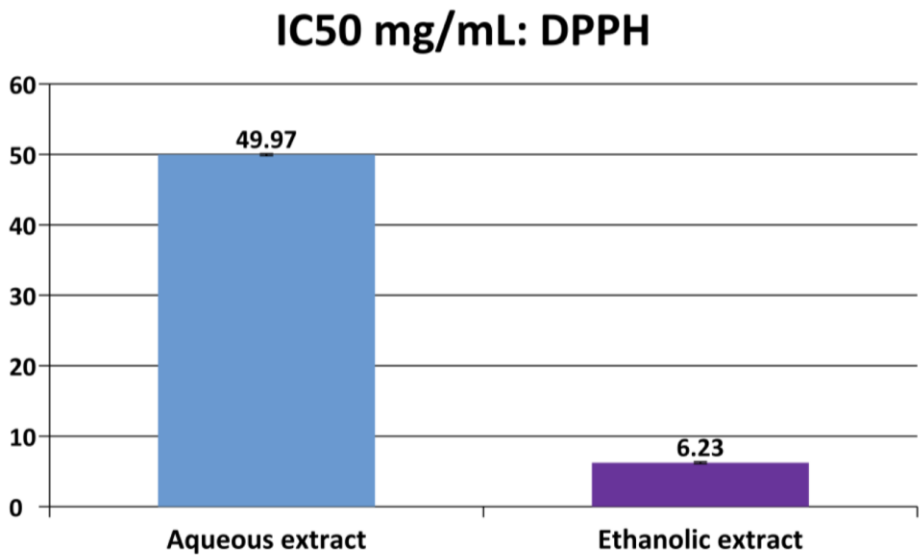


Figure 31 : IC $_{50}$ Values of tiger nut extracts as calculated with the DPPH method.

With corresponding EC $_{50}$ values of 211.5 and 12.15 mg/mL, the aqueous and ethanolic extracts of *C. esculentus* exhibit antioxidant properties, according to the results of the FRAP (Ferric Reducing Antioxidant Power) technique **(Figure 31**). In contrast to the water extract, the ethanolic extract significantly inhibits iron ions.

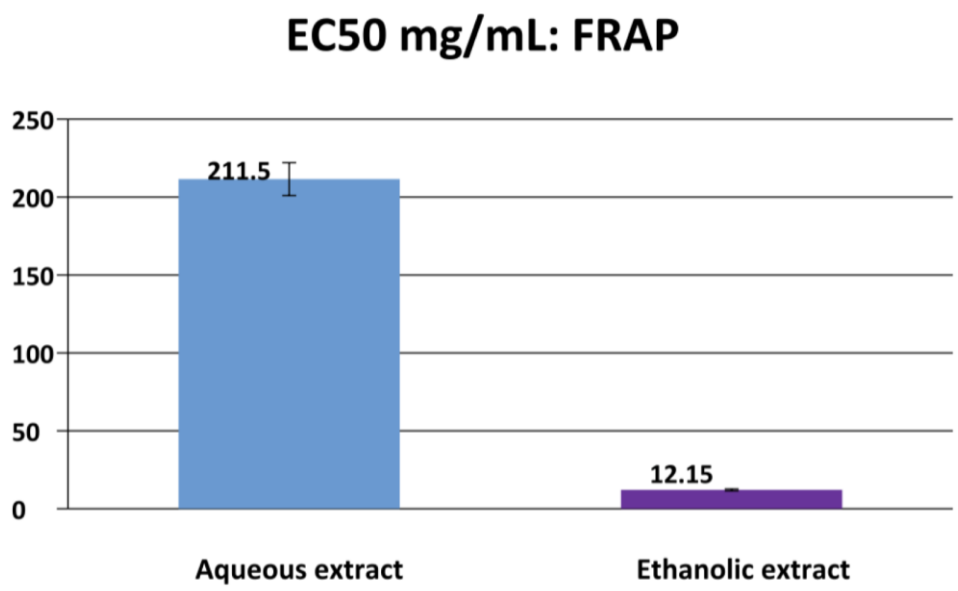


Figure 32 : EC $_{50}$ Values of tiger nut extracts as calculated with the FRAP method.

Nwosu *et al.* (2022) found that the ethanolic (pure) extract of tiger nuts had EC $_{50}$ values of around 0.53 mg/mL, while ascorbic acid, which is a benchmark for antioxidant activity expressed in TROLOX, had EC $_{50}$ values of 0.07 mg/mL.

Plant pharmacological activity is highly dependent on the extraction technique used **(Abubakar & Haque, 2020).** A common method for recovering and isolating bioactive compounds and assessing their in vitro activity is solvent extraction **(Truong *et al.*, 2019).**

The antibacterial activity of the tiger nut aqueous and ethanolic extracts at 100 mg/mL demonstrated a broad range of activity against potentially harmful microorganisms. Using a caliper, the inhibition zones surrounding the discs impregnated with the different extracts were assessed. The findings are displayed in **Table 12**. Both ethanolic and aqueous extracts demonstrated inhibitory effects on the pathogens under evaluation. In fact, the 30 v water/70 v ethanol extract, with the exception of Bacillus species, has an inhibitory activity that is often two times greater than that provided by the water-soluble components.

In a recent study by **Nwosu *et al.* (2022),** the authors used a cold ethanolic extract of tiger nuts at 100 mg/mL to report 10 mm for *Shigella* sp, 14 mm for *Salmonella* sp and *Staphylococcus aureus*, and 16 mm for *Escherichia coli.*

The majority of *Cyperus esculentus* L. extracts are known to have antibacterial properties, and the plant is known to have a wide range of active chemicals. In **2009, Prakash and Ravagan** made a variety of tiger nut extracts with acetone, 50% ethanol, and chlorophorm, among other solvents. Using the disc diffusion method, their antibacterial properties against *Citrobacter freundii, Salmonella sp., Klebsiella pneumoniae,*

Proteus vulgaris, Pseudomonas aeruginosa, Escherichia coli, and *Staphylococcus aureus* have been proven. With regard to *S. aureus, Klebsiella pneumoniae,* and *Proteus vulgaris*, the acetone extract exhibited the strongest inhibitory action.

Table 12 : The antibacterial activity of the tiger nut aqueous and ethanolic extracts at 100 mg/mL

Pathogens	**Inhibition zone (mm)**	
	Aqueous extract	**Hydro-ethanolic extract (30v/70v)**
Candida albicans	10 ± 0.18b	25 ± 0.36a
Escherichia coli	11 ± 0.02b	23 ± 0.10a
Bacillus cereus	12 ± 0.11a	16 ± 0.10a
Staphylococcus aureus	17 ± 0.30b	24 ± 0.20a
Pseudomonas aeruginosa	08 ± 0.22b	22 ± 0.20a
Bacillus subtilis	11 ± 0.08b	19 ± 0.04a

* The results are means of three independent replicates (n = 3) ± SD.

a-b : Significant differences in the same row.

IV.3.3.1. Description of cookies following the international standards

We were able to make sugar-free dietary cookies with 100% organic tiger nut flour. The pictures in **Figure 32** depict the general look of the baked cookies as well as the macroscopic differences between the SL42 probiotic strain-containing honey-bee syrup and sugar-free dietary cookies.

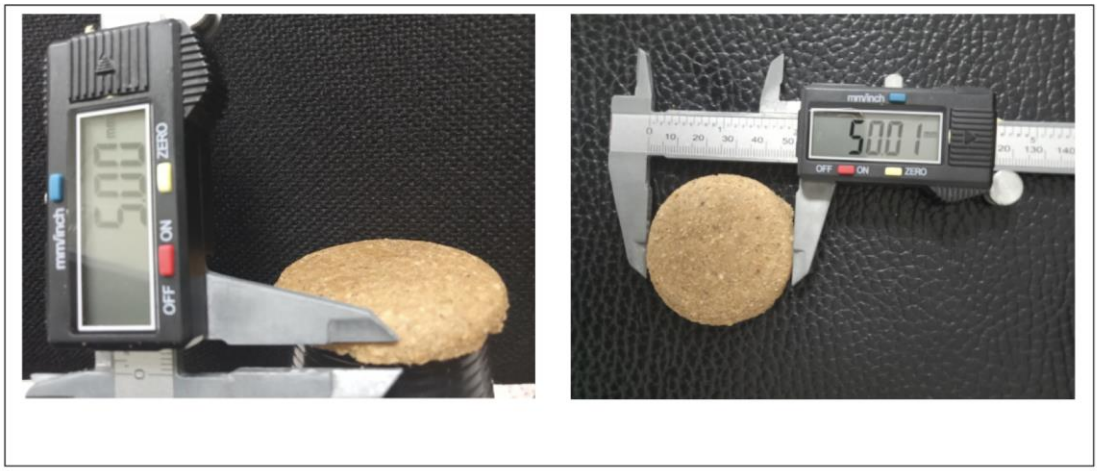


Figure 33: General appearance of the baked cookies.

According to **Table 13** 's results, the sugar-free tiger nut cookie and the one containing honey and the probiotic SL42 strain differ significantly in terms of weight, diameter, thickness, and spread ratio.

Table 13: Physical standards * of tiger nut cookies compared to the commercial one.

Parameter	Control tiger nut cookie (CC)	Tiger nut cookie with honey-syrup (CH)	Commercial cookie made of wheat flour
Weight (g)	12.33±0.81b	12.66±0.51b	11.7±0.4a
Diameter (mm)	50.66±0.82a	50.5±0.54a	53.85±0.1a
Thickness (mm)	5.16±0.4a	5.33±0.51ab	5.71±0.02b
Spread ratio	9.81±2.05b	9.47±1.05a	9.43±0.1a
Hardness (N)	97.5±0.3a	98.3±0.4b	99.5±0.5c

* Mean values ± SD of 6 cookies.

a-c : Significant differences in the same row.

The quality of the flour used in cooking and the dough's absorption ability are assessed based on two factors: the spreading ratio and diameter. According to **Adéola and Ohizua (2018)**, biscuits with a larger spreading ratio are more appealing. It should be about 10 in the case of cookies.

A comparison between the commercial Delight © cookies made with wheat flour and cocoa and the tiger nut cookies showed that the latter "fulfilled" the technological requirements in terms of thickness, weight, and diameter. Additionally, tiger nut cookies were less likely to shatter **(Figure 33).** The crystalline structure of the starch and the denatured proteins have been connected to cookies' resilience to cracking **(Chauhan *et al.*, 2015).** This is the main issue that manufacturers have while determining the best packaging to provide more protection.

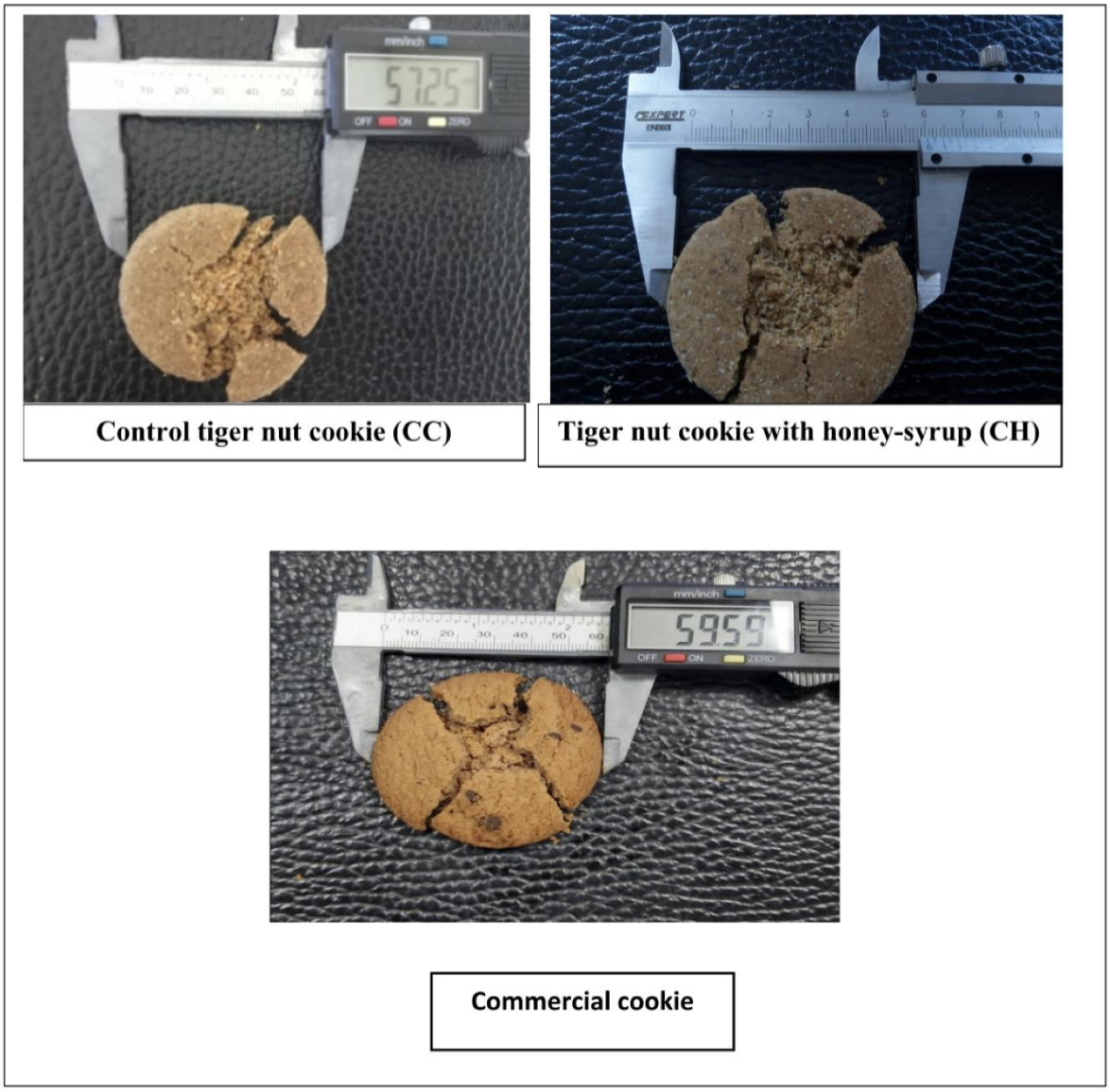


Figure 34: Assessment of the cookie's vulnerability to break (mm).

IV.3.3.2. The probiotic strain SL42 growth's capacity in the presence of tiger nut flour or honey- bee

The SL42 probiotic strain shown good development capacity **(Figure 34)** in the presence of honey-bee (5%, w/v) and tiger nut flour (up to 2% without inhibiting impact) in cultural assays. To rule out any potential negative effects on the probiotic bacterium, these experiments were crucial.

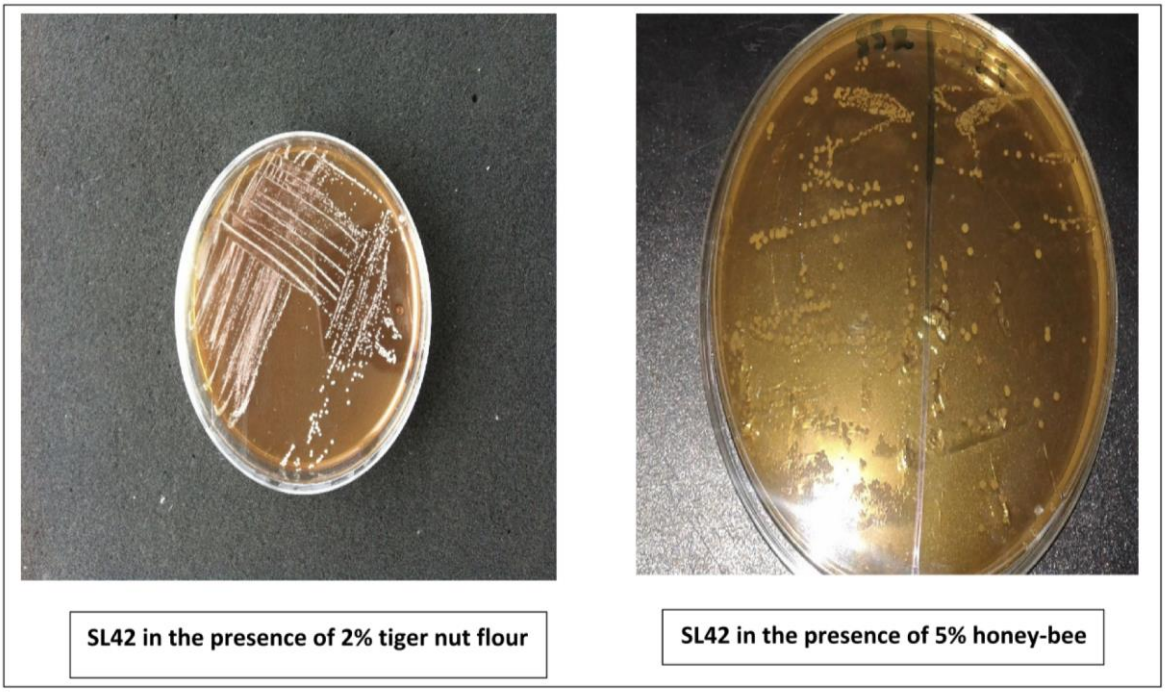


Figure 35: SL42's growth tests in the presence of tiger nut flour (0-2%, w/v) or honey-bee (0-5%, w/v).

IV.3.4. General evaluation of both tiger nut cookies

The total sensory analysis values of probiotic-enriched honey syrup cookies and sugar-free cookies made with tiger nut flour after one day and fifteen days of storage at 25°C are displayed in **Tables 14 and 15,** respectively. The results of the first storage day showed that cookies enhanced with honey syrup (CH) outperformed sugar-free (CC) cookies in terms of general acceptability and sensory quality indicators like aroma, color, and taste. The preference for sugar-free cookies has changed after 15 days of storage.

According to multiple panelists, the first day's difference was centered on the excessively dark color and overwhelming sweetness of the cookies enhanced with honey syrup. The scent criterion was added to the distinctions between the two cookie varieties after they had been stored for 15 days. Our participants gave sugar-free cookies higher ratings at that time period.

SL42 with 5% honey-bee present SL42 with 2% tiger nut flour present **Babiker et al. (2021)** showed in a recent study that the recipe for tiger nut biscuits may be made with or without wheat flour.

Table 14 : Sensory properties of cookies* made of 100% tiger nut flour and sugar-free or wit honey syrup enriched with probiotics, stored at 25°C (1 st tasting day: D= 1 day).

Quality parameter	Control tiger nut cookie (CC)	Tiger nut cookie with
Taste	5.5±2.01a	6.1±1.86b
Color	3.35±1.53a	3.75±1.58b
Aroma	3.7±1.17a	3.85±1.03a
Texture	1.45±0.60a	1.45±0.82a
Overall acceptability	3.5±1.32a	3.66±1.32a

* Mean values ± SD of 100 cookies.

a-b : Significant differences in the same row.

Table 15: Sensory properties of cookies* made of 100% tiger nut flour and sugar-free or with honey syrup enriched with probiotics, stored at 25°C (2 nd tasting day: D=15 days).

Quality parameter	Control tiger nut cookie (CC)	Tiger nut cookie with honey-syrup (CH)
Taste	5.4±2.25a	5.55±1.63a
Color	3.75±0.91b	3.15±1.26a
Aroma	3.95±1.19b	3.3±1.12a
Texture	1.35±0.74a	1.45±0.68a
Overall acceptability	3.61±1.27b	3.36±1.17a

* Mean values ± SD of 100 cookies.

a-b : Significant differences in the same row.

IV.3.5. CIE system analysis's results

Cookies' hue influences people's initial acceptance of food products. After one and fifteen days of storage at 25°C, respectively, the trichromatic analysis findings for free-sugar tiger nut cookies vs those containing honey and probiotics are shown in **Tables 16 and17**
Given that it affects consumers' approval of the product, color is the most crucial aspect of food presentation. The preparation of foods and their derivatives can result in a variety of reactions that alter color.

Table 16: The color characteristics of cookies* made of 100% tiger nut flour and sugar-free or with honey syrup enriched with probiotics, stored at 25°C (1 st tasting day: D=1 day).

Parameter	Control tiger nut cookie (CC)	Tiger nut cookie with honey-syrup (CH)
L*	62.25±4.66a	61.75±4.94a
a*	6.95±0.88a	7.1±0.85b
b*	26.5±4.61b	23.5±4.62a

* Mean values ± SD of 30 cookies.

a-b : Significant differences in the same row.

L* : Lightness ; a* : Redness ; b* : Yellowness

Table 17 : The color characteristics of cookies* made of 100% tiger nut flour and sugar-free or with honey syrup enriched with probiotics, stored at 25°C (2 nd tasting day: D=15 days).

Parameter	Control tiger nut cookie (CC)	Tiger nut cookie with honey-syrup (CH)
L*	62.75±4.66a	61.75±4.43a
a*	7.11±0.48a	7.17±0.96a
b*	25.25±4.72b	23.25±3.35a

* Mean values ± SD of 30 cookies.

a-b : Significant differences in the same row.

L* : Lightness ; a* : Redness ; b* : Yellowness

The most frequent ones are ascorbic acid oxidation, browning (such the Maillard reaction), and color degradation. Food color changes can be inferred indirectly using L*, a*, and b* coordinates since they are quicker and simpler to evaluate than chemical analysis.
The dark color of the cookies was reflected in their low L*, or brightness, which ranged from 61.75 to 62.75. Similar findings were obtained by Simanca-**Sotelo *et al.* (2021)** in their investigation of dietary cookies prepared with yacon flour (L* = 61.7). **Chauhan *et al.* (2015)** determined that L*=65.2 for cookies made with wheat flour. It was demonstrated that there was a negative correlation between a cookie's protein content and brightness, suggesting that the Maillard process is crucial to color formation.

In the present investigation, dark cookies with a chromatic characteristic a* of about 7 were produced by baking raw (non-peeled) tiger nut flour cookies.

On the other hand, sugar-free cookies (25-26) seem to have a higher b* chromatic characteristic than cookies with honey and probiotics.

For wheat flour cookies, **Chauhan *et al.* (2015**) measured chromatic features a* and b*, which came out to be 6.3 and 21.8, respectively.

The impact of drying mode and maturity on flour color is frequently examined using the CIE 1976 trichromatic measurement (L*, a*, b*). The flour's shine is indicated by the L* values. The flour becomes clearer as the value of L* increases. Although brown flour made from tiger nut tubers is common and expected.

As a result, Algerian consumers typically avoid dark brown dishes.

The acceptance of a certain cuisine is greatly impacted by cultural factors. Another way that browning during cookie baking could produce this color is.

Another possible explanation for the color development is the Maillard reaction, which happens when the product's proteins and carbohydrates combine to produce a brown hue. This development is also influenced by the oven's humidity level, cooking temperature, and cooking time **(Saadoudi, 2019).**

IV.3.6. Sensorial analysis results

The cookie's modest initial moisture content and water activity make it typically considered microbiologically stable. Texture, particularly the loss of hardness and crustiness, and occasionally lipid oxidation are the main changes that affect its quality during storage.
Taste and flavor were the first attributes assessed. 50% of panelists thought the flavor of the control sugar-free cookies was good ($P<0.05$) after a day of storage, as opposed to 65% for cookies with honey. Indeed, according to our panelists, both varieties of cookies tasted like the product, with no unfavorable associations noted **(figure 35).**

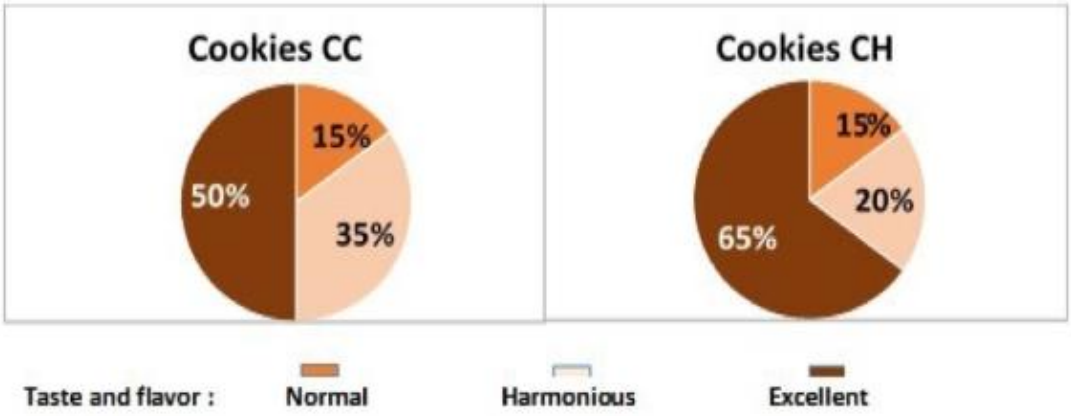
At the second tasting session, the preference for sugar-free cookies were constant at 55% ($P >0.05$), whereas cookies with honey added were more than harmonious (45%) or excellent (45%) (**figure 36).**

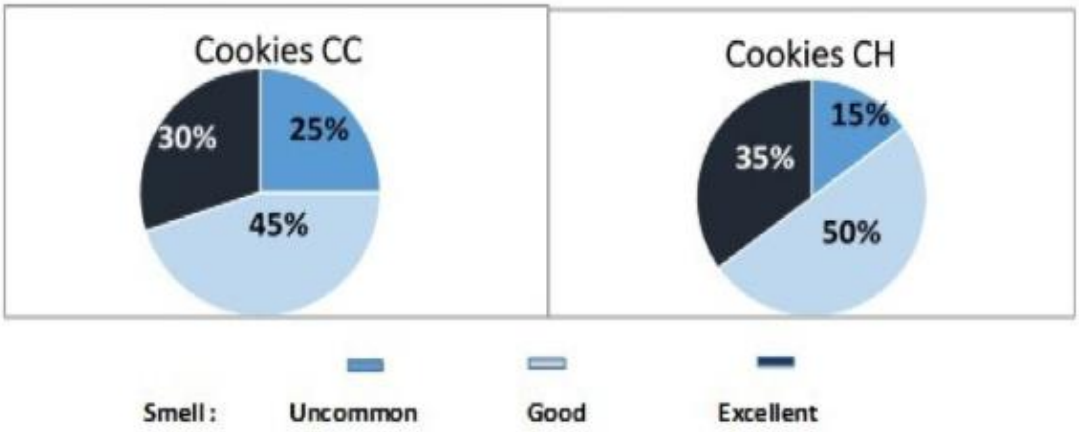
Its unique scent produced the same results as its flavor and taste. Our sugar-free and honey-enriched cookies smelled good or great, like freshly made goods, according to 75% and 85% of our panelists ($P<0.05$). The panelists' favorable evaluations (85 to 95%) persisted after 15 days of storage since our cooked cookies maintained or even enhanced their delightful aroma. This can be largely because of the packaging type that was selected.

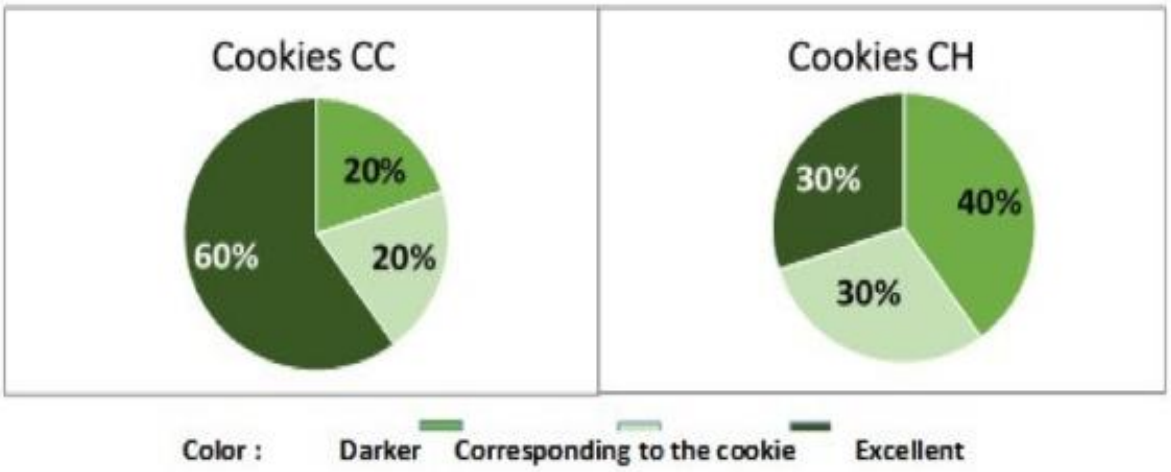
Although this variation was anticipated after storage, our tasters perceived the honey syrup-soaked cookies as darker (D1/D15: 40%/25%) than the sugar-free cookies (D1/D15: 20%/10%). This could possibly be attributable to the composition of the polyfloral honey utilized. This could also be attributable to the type of polyfloral honey utilized. Furthermore, our cookies had a 60% color match to the desired product, with no negative connotations.

Honey-enriched cookies had a softer texture after one day of storage ($P< 0.05$). However, disparities in hardness values between the two cookie groups diminished afterward, resulting in equivalent notices ($P>0.05$) at the 15 th day (**figures 36 and 37**). The texture changes in cookies have been linked to changes in moisture content and interactions between the various components found in the cookie matrix. These texture alterations have a significant impact on the final product's quality and acceptance **(Romani *et al.*, 2016).**

Our cookies were rated as "crispier" by 50-55% of our tasters and "well baked " by 30-35%. Only 10 to 15% of our tasters complained that our cookies were "too soft or hard" after 15 days of storage.


Cookies CC
15%
50%
35%
Cookies CH
15%
20%
65%
Taste and flavor :
Normal
Harmonious
Excellent


Cookies CC
30%
25%
45%
Cookies CH
35%
15%
50%
Smell :
Uncommon
Good
Excellent


Cookies CC
20%
60%
20%
Cookies CH
30%
40%
30%
Color :
Darker
Corresponding to the cookie
Excellent

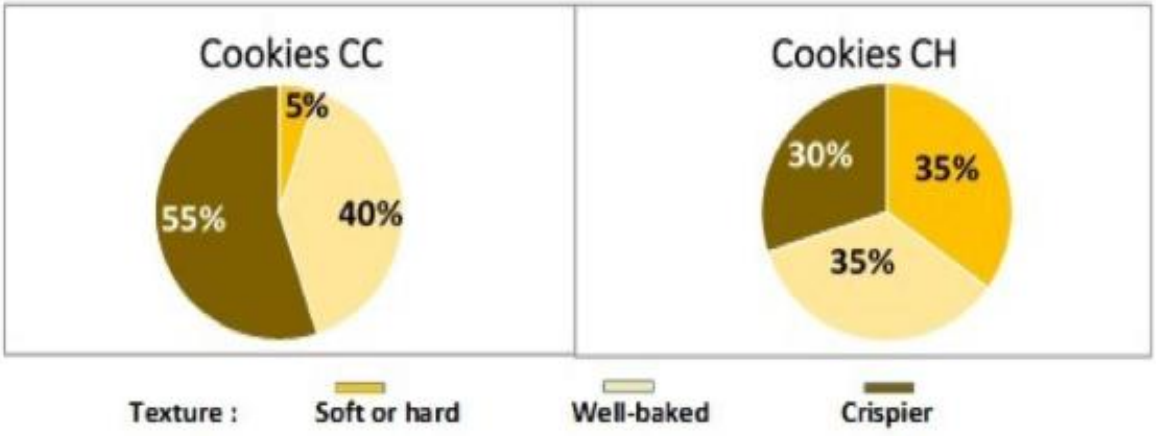

Cookies CC
5%
55%
40%
Cookies CH
30%
35%
35%
Texture :
Soft or hard
Well-baked
Crispier

Figure 36: Panelists satisfaction level with the taste and flavor, smell, color, and texture of sugar- free (CC) or honey-enriched (CH) tiger nut cookies (1 st tasting, D = 1 day).

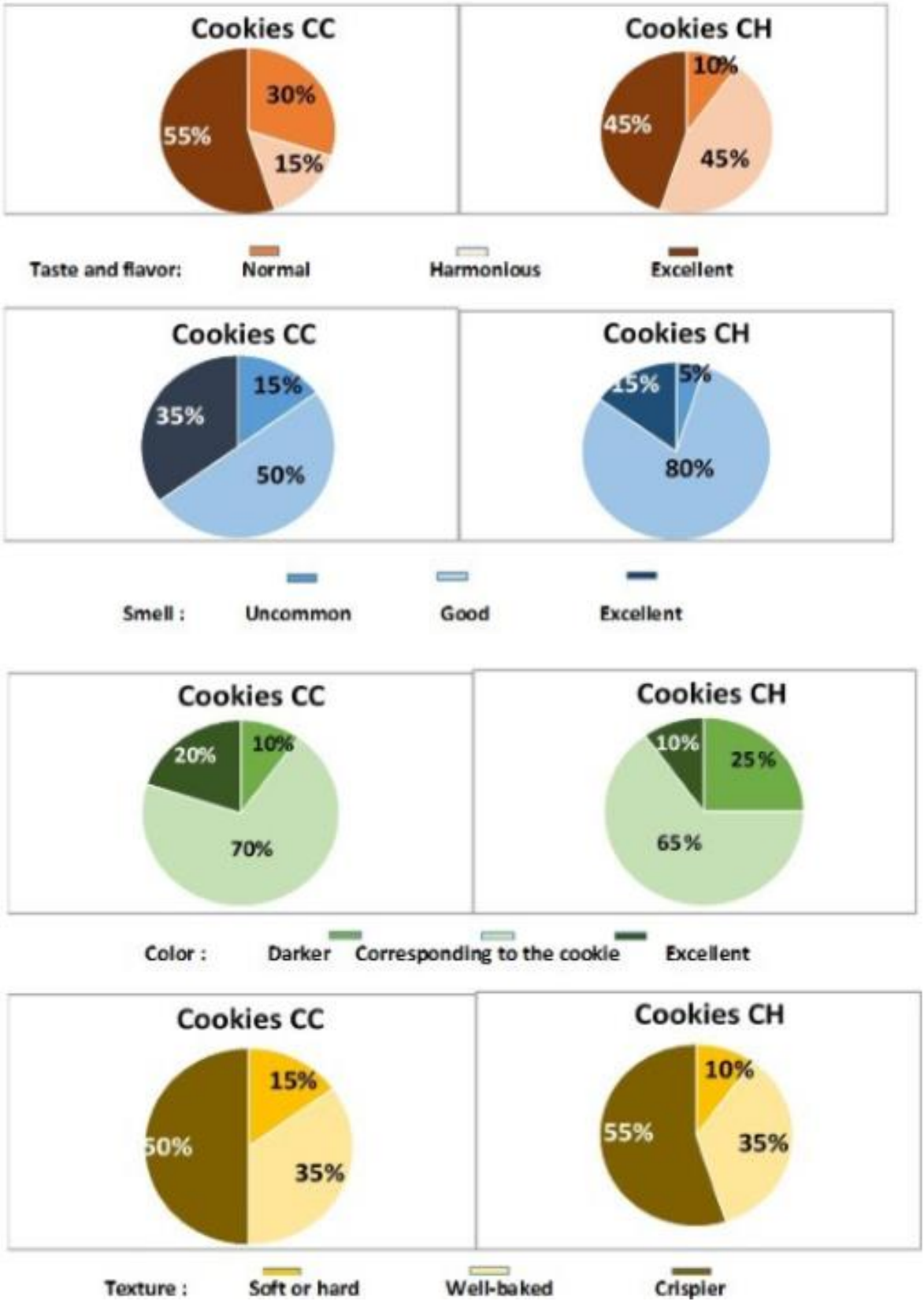


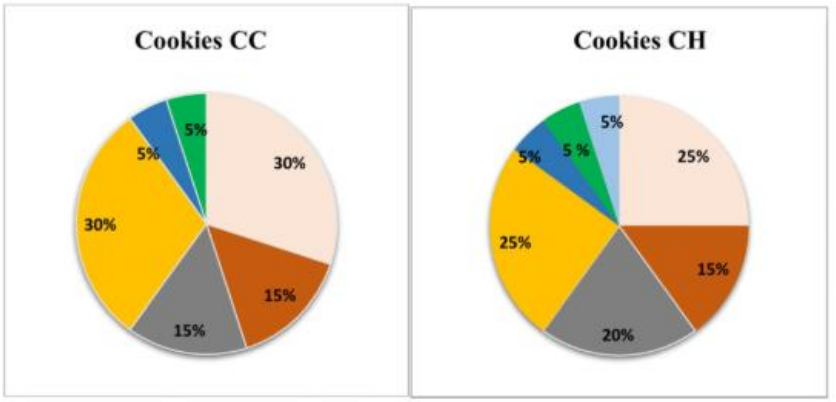
Figure 37: Panelists satisfaction level with the taste and flavor, smell, color, and texture of sugar- free (CC) or honey-enriched (CH) tiger nut cookies (2 nd tasting, D = 15 days).

The hedonic test enabled us to distinguish between sugar-free cookies and those fortified with honey and probiotics. It is a good test of appreciation for founding an opinion on the general acceptability of a food product.

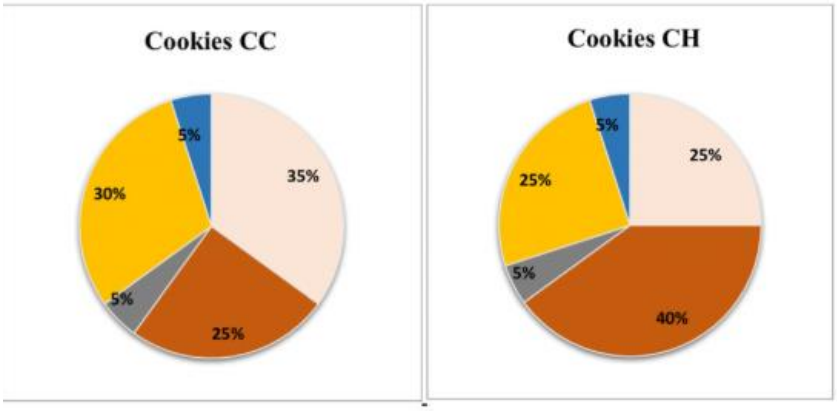
Sensory examination of our fresh stored cookies (1 day storage) found that the majority of tasters preferred the cookies made with tiger nut flour. Only 5% of our panel "dislike slightly" the baked cookies (see **figure 37**).

After 15 days of storage, this general dissatisfaction decreased (**figure 37**). Actually, the sugar-free cookie and the one with honey bee syrup were rated as "extremely" and "very much" by 35 and 25% of our panelists, respectively. The findings of the sensory analysis ought to be connected to a series of biological processes that take place throughout the production and preservation of cookies.

Color, texture, flavor, and scent all change as a result of these reactions. The Maillard reaction and the product's altered moisture content are mostly to blame for this.



(a) First tasting: D=1day



(b) Second tasting: D= 15days

Figure 38: Hedonic test done one sugar-free (CC) or honey-enriched (CH) tiger nut cookies (a: 1 st tasting, D = 1 day; b: 2 nd tasting, D = 15 days). Nine-point scale was used:

Like extremely ; like very much ; like moderately ; like slightly ; indifferent ;
dislike slightly ; dislike moderately ; dislike very much ; dislike extremely .

IV.3.7. SL42 survived in stored cookies

The probiotic strain SL42's bacterial load on a single cooked cookie is shown in **Table 18.** After being remixed in honey syrup, this bacterium has been exposed to lyophilized conditions, which already give it stability while stored at room temperature. Our results show that the probiotic isolate has a high capacity for survival in a moderately dry environment. From the initial amount in each cookie, its load did not decrease significantly (P >0.05) and stayed at the suggested level **(Ziar *et al.*, 2012).** 6.43 Log CFU/one cookie was the load after 21 days of storage.

The trend of incorporating probiotics into food has already permeated the bakery industry, as several bakeries now provide probiotic-enriched bars, bread, biscuits, muffins, even crepe mix. After baking, probiotics are commonly added to bakery items to ensure their long-term vitality. Probiotic formulations might be added to chocolate or icing cream after baking, or they could be sprayed over the product right before packaging.

when both methods work, adding the probiotic directly to the product when creating the dough is definitely more beneficial. This guarantees that germs are dispersed uniformly and eliminates the need for a last step. Additionally, a greater variety of baked goods, both sweet and salty, can incorporate probiotics thanks to this technique.

The business LALLEMEND BAKING UPDATE conducts tests using the bacterium B.
subtilis Rosell-179, which can generate spores. According to the producer, this bacterium fits
all of the criteria for successful inclusion into bread formulation **(Anonymous, 2022).**

While adding health-promoting ingredients, like probiotics, may increase a product's value, its appearance, flavor, texture, scent, and other organoleptic qualities shouldn't suffer as a result.

Table 18: Survival of the SL42 probiotic strain (Log CFU/one cookie*) in cookies.

Storage period	Tiger nut cookie with honey-syrup (CH)
Day 0	7.15±0.43a
Day 1	6.93±0.96a
Day 7	6.66±0.44a
Day 15	6.55±0.35a
Day 21	6.43±0.05a

*one cookie = 12 g.

a: non-significant differences in the same column.

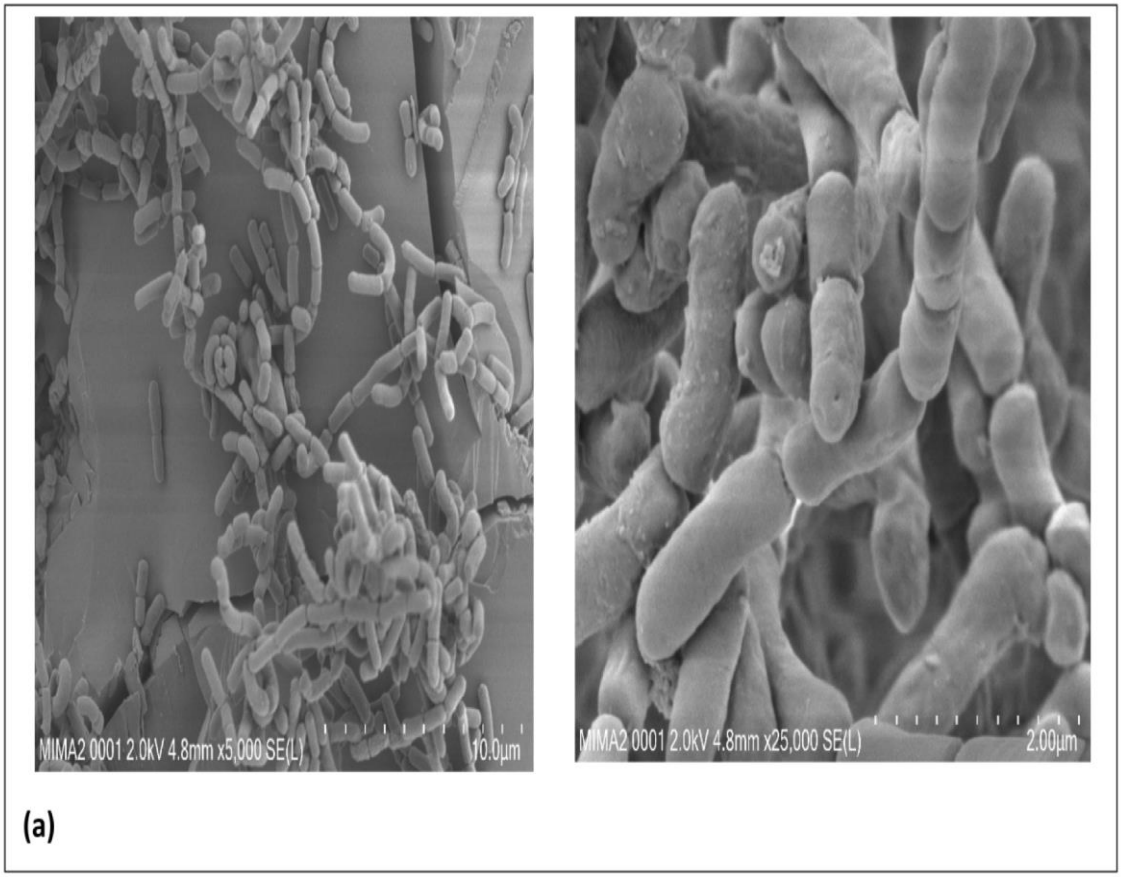
IV.3.8. SEM results

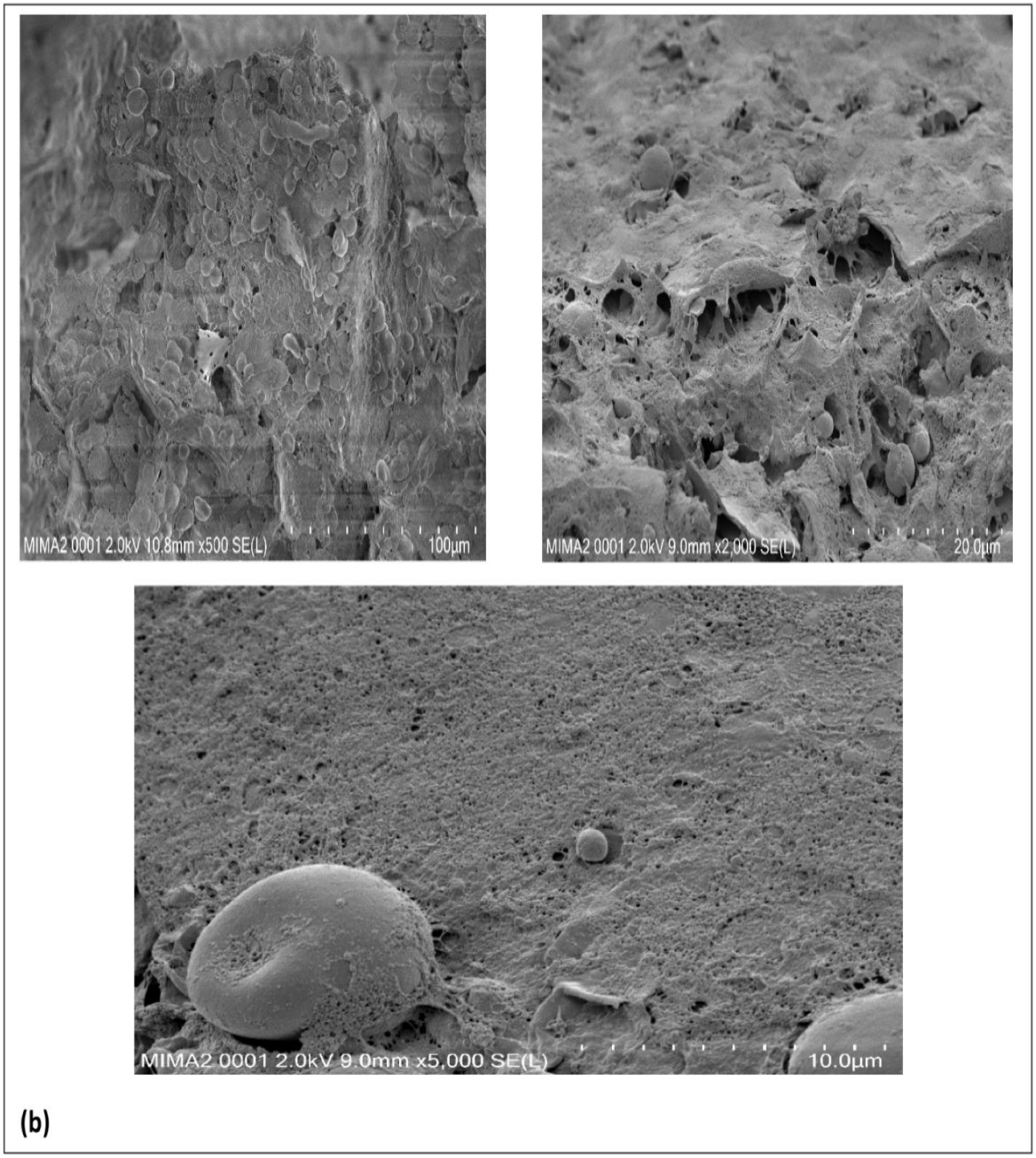
The findings are displayed in **Figure 38.** The morphological characteristics of the tiger nut cookies were obviously impacted by the syrup.

Figure 38a, b illustrates how the shape of the starch granules changed from round, smooth, and fracture-free in control cookies (CC) to granules of starch with fracture and more severe adhesion in honey-bee syrup enriched cookies (CH). The following cookies (**figures 38c and d**) are mostly irregular flaky structures with threads on their surfaces, with a small quantity of tiger nut starch in the form of spherical granules. Generally speaking, adding syrup has resulted in aggregates with holes and larger particle sizes.

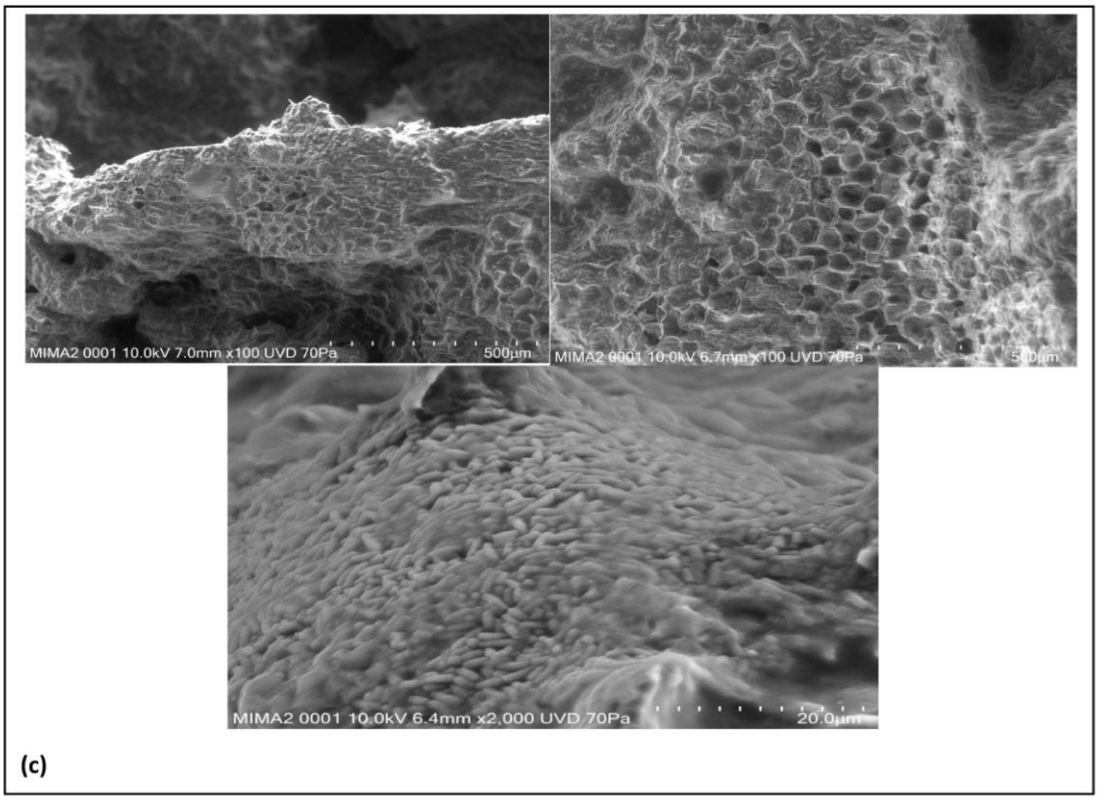
A rough surface, significant morphological breakdown, and the adhesion of other materials to the starch could be the results of the partial gelatinization of starch granules subjected to high temperatures and moisture **(Liang *et al*., 2021).**

Small spherical structures were more susceptible to damage, and the technique often produced more severe starch breaking at small particle sizes. The amount of non-starchy components, like proteins, may also have an impact on how much the different forms of starch break **(Li *et al.*, 2024).**




MIMA2 0001 2.0kV 10.8mm x500 SE(L)
100µm
MIMA2 0001 2.0kV 9.0mm x2,000 SE(L)
20.0µm
MIMA2 0001 2.0kV 9.0mm x5,000 SE(L)
10.0µm

(b)



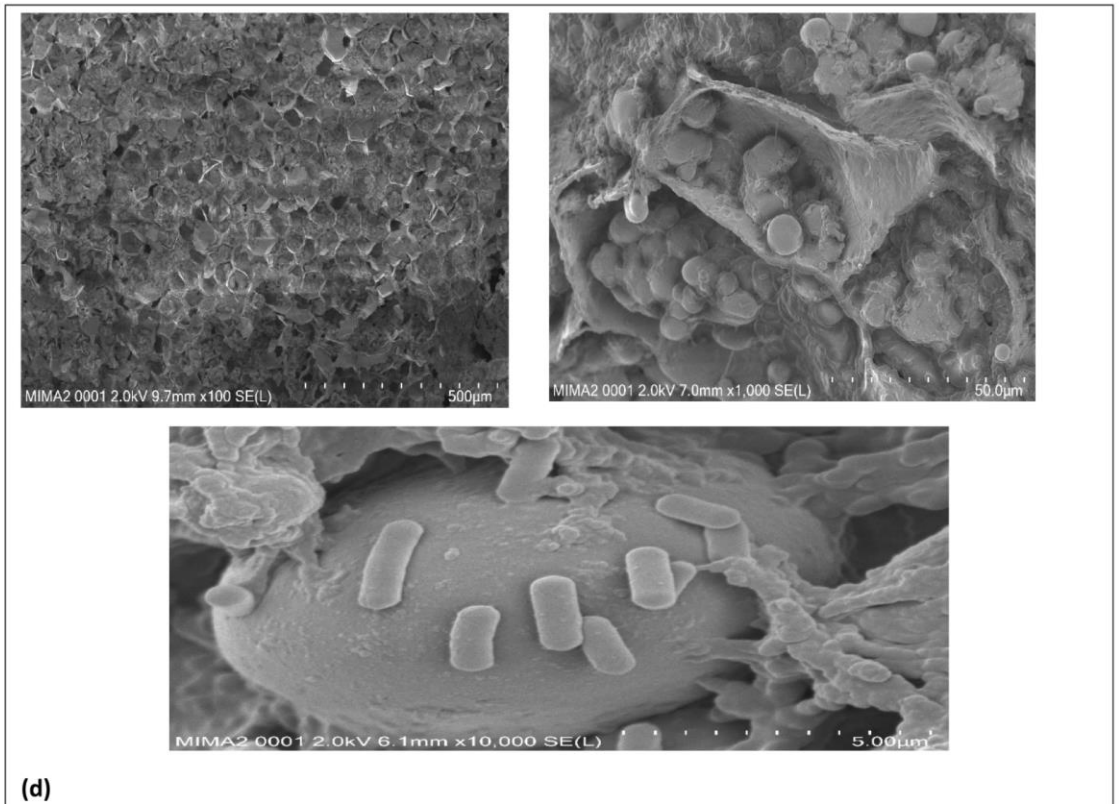


Figure 39: SEM analysis of (a): fixed honey-bee syrup containing probiotic strain SL42; (b): fixed control cookies free of sugar (CC); (c): unfixed cookies (environmental mode) enriched with honey-bee syrup containing SL42 at the 7th day storage.

However, the addition of honey-bee syrup caused SL42 to stick to the cookie surface (**figs. 38c and d)**, indicating a good ability to tolerate a temperature of 25°C and low humidity in stored cookies, as previously demonstrated by MRS-cys culture **(table 18).**

Conclusion

Cookies with improved nutritional characteristics can appeal to health-conscious consumers. Future study should focus on improving sensory qualities, such as using natural flavors, different flour types, and baking procedures. Natural preservatives and sophisticated packaging can help extend product shelf-life. Conducting sensory evaluations and market studies to connect items with customer preferences can help determine the commercial potential of cookies. Future research can help build commercially viable, nutritionally enriched cookies that fulfill consumer expectations, have a longer shelf-life, and have appealing sensory properties.

Chapter V:

A natural intimate gel based on hydro-ethanolic extract of *Cyperus esculentus* L. and probiotic bacteria.

Chapter V: A natural intimate gel based on hydro-ethanolic extract of *Cyperus esculentus* L. and probiotic bacteria.

V.1. Introduction

Vulvovaginal candidiasis (VVC) is an infection caused by fungi of the genus Candida on the vaginal mucosa. In some cases, it can become pathogenic. It manifests itself as leucorrhoea, vulvar hyperaemia, intense pruritus, dysuria and dyspareunia, and affects around 75% of women at least once in their lives. Candidiasis is the result of an imbalance between the microbiota and these yeasts. In addition, around 138 million women worldwide may suffer from recurrent vulvovaginal candidiasis (RVVC). Contributing factors include diabetes mellitus, pregnancy, antibiotics and steroids, hormone replacement therapy, use of hormonal contraceptives, immunosuppressive diseases and hygiene habits **(Denning *et al*., 2018).**

Nearly 20-30% of healthy asymptomatic women may have this yeast in their vaginal tract at any time in their lives if tested by culture, but over 60% if tested by Nucleic Acid Amplification Tests, NAAT methods (**Disha and Haque, 2022).**

Uncomplicated or sporadic VVC includes mild to moderate clinical signs and symptoms such as a thick cottage cheese-like discharge, pain, erythema, vaginal and vulvar pruritus, burning and/or oedema, as well as external dyspareunia and dysuria. Symptoms of vaginitis in women, particularly those of childbearing age, are often caused by vulvovaginal candidiasis (VVC). Approximately 9% of women with symptoms have developed VVCR (at least 3 episodes of symptomatic VVCR in less than a year). *Candida albicans* is generally responsible for VVC. Other varieties of *Candida* sp are found in 10 to 20% of CVVRs and are less resistant to current treatments. The resistance of C. albicans to azole antifungals is also increasing, making it very difficult to control **(Denning *et al.*, 2018)**.

There has been an increase in research into probiotics, and these are now considered to be sources of new therapeutic strategies for a number of ailments. It is also impossible to eliminate the traditional use of medicinal plants despite the development of synthetic drugs, because plant extracts have significant clinical value in the treatment of infections with resistant microbial strains (**Abedini, 2013).**

Our aim was to propose an effective and natural solution for combating VVC. An intimate gel based on tiger nut extract and microencapsulated strain of SL42, was studied as a novel treatment for candidiasis in wistar rat.

V.2. Materials and Methods

V.2.1. Animals

A total of 48 uncoupled female wistar rats (180-200 g), free of specific pathogens were purchased from Institut Pasteur (Algiers, Algeria) and distributed into treatment groups of 8 rats. Rats were maintained in Laboratoire des Micro-organismes Bénéfiques, des Aliments Fonctionnels et de Santé (LMBAFS) at Abdelhamid Ibn Badis University (Mostaganem, Algeria). Rats were housed in cages with controlled temperature (21 ± 2°C) and humidity (30 ± 5%), and a 12/ 12 h light/dark cycle. A standard laboratory diet and free access to tap water were supplied. Care of the animals was in accordance with the guidelines defined in the NIH Guide for the Care and Use of Laboratory Animals. The study received the approval of the Institutional Animal Care and Use Committee of LMBAFS laboratory, Abdelhamid Ibn Badis University (SNV-SA-LMBAFS-CE-2022-090). Animals were allowed to acclimatize for two weeks.

V.2.2. Candida albicans

Strain of *C. albicans* ATCC 10231 (CA), supplied by the LMBAFS Laboratory, was cultured aerobically on Sabouraud Dextroxe agar (SDA, Biomérieux, France) supplemented with 50 mg/mL chloramphenicol (28°C for 48 h). One colony of fungal cells was collected and suspended in Nutrient broth (NB) supplemented with 2% glucose, and adjusted to 5×10^8 yeasts/mL.

V.2.3. Tiger nut extract preparation, MIC and MFC Determination

The solvent was chosen according to literature and confirmed in Chapter IV: the results of the antimicrobial activities, which determined the highest inhibitory zone for *C. albicans.*

After macerating the tiger nut flour in 70 mL of 96% ethanol and 30% water for 72 days, the solvent was evaporated to complete dryness using a standard Buchi rotary-evaporator. The resulting dry extracts were re-suspended in 5 mL of DMSO. To determine the real concentration of each extract, 1 mL of the previous homogenization of the respective extracts was removed, completely oven-dried, and then weighed to determine the amount of extract per mL of final solution.

For MIC testing, the inoculum size was adjusted to $1–5 \times 10^5$ *Candida* cells/mL and NB supplemented with 2% glucose was employed. The MIC endpoints were determined photometrically after 28 h at 37°C. The MIC is determined using a control for fungal growth, a negative control (10% DMSO), a positive control (Econazole 200 mg), and tiger nut extract and fractions. The tests were carried out in triplicate. Each well was treated with 100 µL of NB, and 0.1 mL of the sample solution was added to column well 12, which contained the medium in the sample. After blending, the solution was transferred to well 11. To make a series of serial dilutions with different concentrations in each well (1000, 500, 250, 125, 62.5,

31.25, 15.625, 7.812, 3.906, 1.953 µg/mL). Serial dilution was performed step by step toward the smallest column. The MICs were determined as the lowest concentrations that inhibited *C. albicans* growth.

Minimal fungicidal concentration "MFC" was determined according to **Canton *et al*. (2004).** Briefly, MFCs were evaluated by transferring 0.1 mL from all clear MIC wells (no growth seen in microdilution trays) onto SDA plates. The MFC was the lowest drug concentration that killed ≥ 99.9% of cells.

V.2.4. Analysis of the extract using High Performance Liquid Chromatography coupled to UV detection (UV-HPLC)

HPLC system, or high-performance liquid chromatography analysis was performed on

a Thermo Scientific Dionex UltiMate 3000 Rapid Separation LC (RSLC) system (Thermo Fisher Scientific Inc., MA, USA) that was connected to a rapid separation diode array detector (DAD-3000RS), quaternary rapid separation pump (LPG-3400RS), and Ultimate 3000RS auto sampler (WPS-3000). An Acclaim® C18 (4.6 x 250 mm; 5µm) column (Dionix, USA) was used to separate phenolic compounds. It was kept at 30°C using a temperature-controlled column compartment (TCC-3000). Dionix Chromeleon software was used for data collecting, peak integration, and calibrations (Version 6.80 RS 10).

A solution of acetic acid at pH 3.0 (solvent B), methanol (solvent C), and acetonitrile (solvent A) made up the mobile phase. This gradient elution program was used to run the system: 0 min at 5% A/95% B; 10 min at 10% A/80% B/10% C; 20 min at 20% A/60% B/20% C; and 30 min at 100% A. To equilibrate the column, a 5-min post-run was conducted under initial conditions. The injection volume was 20 µL, and the flow rate was maintained at 1 mL/min throughout the analysis. The wavelength program was adjusted for UV detection in order to track phenolic chemicals at the wavelengths where they have the highest absorbance: The diode array detector was set at an acquisition range of 200 nm to 700 nm, and the wavelengths were held at λ 280 nm for 18.0 min, λ 320 nm for 6 min, and λ 380 nm.

V.2.5. Incorporation of the probiotic strain SL42 into xanthan gum and preparation of the tiger nut extract-intimate gel.

To create SL42 beads, 2.5 g of xanthan gum (COSMO NATUREL, France) was dissolved in 50 mL of water at 80°C. Then, 2.2 g of bacterial cells, 0.8 g of potassium lactate, and 500 mg of MFC tiger nut hydro-ethanolic extract were mixed into the xanthan solution. Following that, the solution was aseptically poured into 10 mL of organic sesame oil (fresh-pressed from the herbalist), 2.5 mL cocoglucoside (COSMO NATUREL, France), 17.5 mL cocobetaine (COSMO NATUREL, France), and 10 mL glycerin (COSMO NATUREL, France) using sterile syringe fitted on a 27.5 G needle.

After 20 min of agitation at 200-400 rpm, 6 drops of E-vitamin (COSMO NATUREL, France) were added into the emulsion.

pH measurements were performed in triplicate, using a pH meter at room temperature (25 ± 0.2 °C), and at vaginal physiologic temperature (37 ± 0.2 °C).

V.2.6. Antifungal effect of the gel

The gel was also compared to its ingredient the hydro-ethanolic extract regarding the antifungal effect on Potato dextrose agar (PDA) agar. Agar well diffusion method was used **(Daoud *et al*., 2015).** One mL of fresh fungi culture was pipetted in the center of sterile Petri dish. Molten cooled PDA was then poured into the Petri dish containing the inoculum and mixed well. Upon solidification, wells were made using a sterile cork borer (6 mm in diameter) into agar plates containing inoculums. Then, 100 µL of the extract or the gel was added to respective wells. The plates were placed in the refrigerator for 30 min to let the extracts diffusion well into the agar. Then, the plates were incubated at 28°C for 18h. Antimicrobial activity was detected by measuring the zone of inhibition.

V.2.7. Establishment of a VVC model in rats

A previously reported rat model of VVC (**Menon *et al*., 2021**) was implemented here with te following modifications:

The murine VVC study accounted for a total of 9 days. Briefly, rat were administered with 0.2 mg of β-estradiol 17- valerate dissolved in 100 µL sesame oil by subcutaneous injection (**figure 40**) to maintain a pseudoestrus state, 72 h prior to inoculation (day −3) and on day 3. Estrogenized rat were intravaginally inoculated by introducing 20 µl of *C. albicans* blastoconidia (5.5×10^5 CFU/20 µL) into the vaginal lumen (day 0), and the infection was allowed to progress until day 6 (the negative control rats were injected with the same volume of NB). Interventions were administered intravaginally twice on days 2 and 4.

Wistar rats treated with œstrogenic substances (day 6) were randomly divided into 6 groups:

- Two control groups infected or not with two separate vulvovaginal infections of *C. albicans*;
- Two treatment groups: treated with *Lactobacillus rhamnosus* Lcr35 (Gynophilus®, France) or Econazole nitrate 1% (emulsion, EG laboratories, France), 0.5 mL applied once a day/7 days **(figure 41).**

- 

Figure 40: Hormonal stress injection administered subcutaneously.

Figure 41: Drugs used in this study.

- Three prevention groups: 2.2 g *L. rhamnosus* SL42 in xanthan beads embedded or not in the intimate gel containing hydro-ethanolic tiger nut extract, 30/70; v/v at a concentration of 125µg/mL (Preliminary MIC tests on *C. albicans* determined this concentration of MFC as minimal fungicidal concentration), or the hydro-ethanolic tiger nut extract at MFC in DMSO: 0.5 mL applied once a day/7 days).

V.2.8. VVC confirmation

Gram staining of vaginal swabs from all rats was conducted on the fifth day after inoculation and evaluated under light microscopy. Vaginal tissues biopsied from several infected rats were fixed in 4% paraformaldehyde, paraffin-embedded, sectioned, and stained with hematoxylin-eosin (HE) to confirm inflammation. Rats with VVC exhibited indications of irritation and erythema, as well as thick white vaginal secretions. Meanwhile, the existence of yeast and hyphae was confirmed using microscopy. Each rat's vaginal wash was cultured on SDA with chloramphenicol for 48 h at 28°C. During the vaginal infection, the number of yeasts in each animal was counted and expressed as colony-forming units (CFU)/mL.

V.2.9. *L. rhamnosus* Lcr35 preparation

One vaginal tablet was dissolved in sterile normal saline to prepare a drug solution of 5×10^8 CFU *L. rhamnosus* Lcr35 /50 µL upon use.

V.2.10. Efficacy of treatment

The efficacy of treatment was assessed by counting *C. albicans* (CFU) in vaginal wash and detecting hyphae or yeast in vaginal secretions using Gram staining under light microscopy in the treated and untreated 7-day groups. Pathogen-negative samples showed no identifiable CFU, hyphae, or yeast in light microscopy analysis.

V.2.11. Sacrifice and organ removal

Animals were anesthetized with 30 mg/kg of phenobarbital sodium, ~100 µL of vaginal wash fluid was collected to determine the vaginal fungal burden, while the vaginal tissues were fixed in formaldehyde in order to make Haematoxylin and Eosin-sections for histological analysis. Other dissected tissues were stored in Eppendorf tubes at -80°C under nitrogen gas until the day of the biochemical analysis.

V.2.12. MPO activity and PGE2 in vaginal tissues

Commercial ELISA kits were employed to measure MPO activity and PGE2 production. Vaginal tissue lysate was produced by homogenizing the tissue in lysis buffer (Krishgen Biosystems, CA, USA), which contained phosphatase and protease inhibitors. The supernatant was added to a reaction mixture containing 1.6 mM tetramethyl benzidine and 0.1 mM hydrogen peroxide, incubated at 37°C, and the absorbance at 650 nm was monitored over time. The MPO activity and PGE_2 ELISA analyses were carried out according to **Zhang *et al*. (2018).**

V.2.13. Statistical Analysis

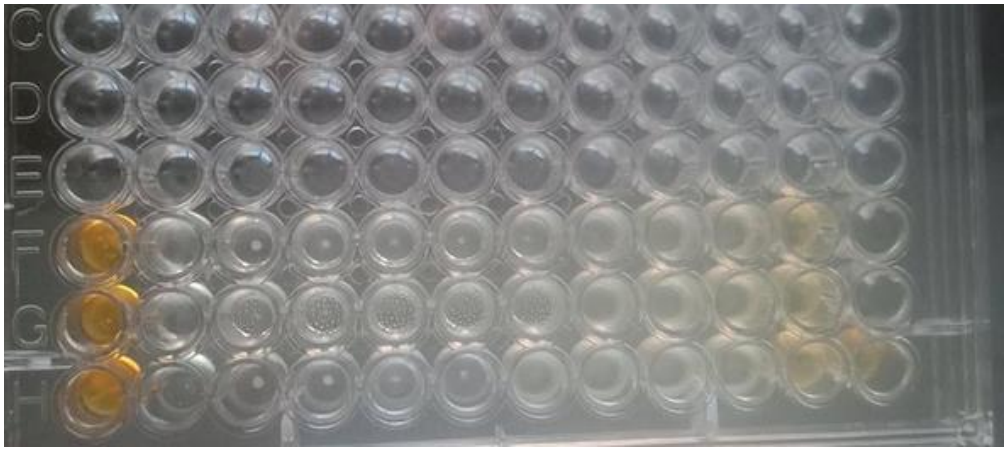
All data were analyzed using statistical analysis software SPSS 11.0. The results represent the mean standard deviation (n = 6 rats) of three independent experiments. Quantitative variables were tested for normal distribution and data were compared using a single-factor analysis of variance (ANOVA) using Windows software (SPSS Inc., Chicago, IL, United States). Fisher's LSD test was used to evaluate differences between two groups. A $P < 0.05$ was taken to indicate statistical significance.

V.3. Results

V.3.1. MCI and MFC from the microdilution tray assay

To determine the MIC, antifungal activity testing was performed using the broth microdilution method. This method is cost-effective, requires minimal sample, and is not affected by medium thickness.

The results of the antifungal testing using the microdilution method can be seen in **Figure 41**. The MIC values for the ethanol extract at 24, 48, and 72 h was 62.5 µg/mL, indicating strong activity in inhibiting *Candida albicans*. Econazole has an MIC value of 64 µg/mL at 24, 48,



	1 mg/mL	500 µg/mL	250 µg/mL	125 µg/mL	62.5 µg/mL	31.25 µg/mL	15.625 µg/mL	7.812 µg/mL	3.906 µg/mL	1.953 µg/mL	DMSO Negative	Econaz positive
F	-	-	-	-	-	-	+	+	+	+	-	+
G	-	-	-	-	-	-	-	-	+	+	-	+
H	-	-	-	-	-	+	+	+	+	+	-	+

Figure 42: Antifungal MIC measurement results.

and 72 h. This indicates that Econazole and our tiger nut extracts have almost equal antifungal activities.

Tiger nut extract and fractions had an MFC value of 125 µg/mL as determined by SDA plate cultures. This demonstrates that the tiger nut hydro-ethanolic extracts and fractions exhibit fungicidal properties at that concentration. Econazole has fungicidal activity against Candida albicans, having an MFC value of 64 µg/mL. The efficiency of an antifungal drug in preventing growth is determined by the type of fungus, the concentration, and the time of contact. The results of the tiger nut extract fractions are equivalent to

Econazole. This could be attributed to the high quantity of secondary metabolite chemicals in these samples, which are powerful enough to suppress *Candida albicans* proliferation.

V.3.2. Phenolic compounds revealed by HPLC/UV detection

HPLC/UV chromatograph is still one of the best instrument for the separation of organic compounds through the use of ultra-violet detection. In accordance with the present study, compounds of hydro-ethanolic extract of tiger nut has been identified with compounds name, retention time, peak area and its bioactive activities through HPLC-UV evaluation.

The hydro-ethanolic extract displayed 12 biologically active compounds, and these biologically active compounds names, retention time, peak area and bio-active activities were presented in **Table 19** and elucidated in **figure 42** The bioactive compounds are 3,4 di-hydroxybenzoic acid (3,4 di-HBA) with a retention time of 1.10 and a peak area of 0.12. The 3,4 di-HBA has been revealed as a potent inhibitor of the sn-glycerol-3-phosphate oxidase system of *Trypanosoma brucei brucei in vitro* and have significant trypanocidal activity *in vivo*, as well as a strong protective effect on myocardial infarction **(Grady *et al*., 1986; Vincent *et al*., 2024).** Gallic acid (GA) with a retention time of 2.28 and a peak area of 0.19. GA, a naturally existing phenolic molecule, can mediate a variety of therapeutic effects including anti-inflammation, anti-obesity, and anti-cancer activity. More recently, GA has been found to inhibit cancer growth *via* a variety of biological processes, including migration, metastasis, apoptosis, cell cycle arrest, angiogenesis, and oncogene expression **(Jiang *et al*., 2022).** 2,4 di-hydroxybenzoic acid (2,4 di-HBA) with a retention time of 9.93 and a peak area of 1.71. The 2,4-diHBA prevented renal fibrosis and dysfunction **(Widmeier *et al*., 2019).** Catechol with a retention time of 11.00 and a peak area of 2.90. Catechol, or 1,2-dihydroxybenzene, has been hypothesized to modulate glucose metabolism, particularly in the context of type 2 diabetes **(Knezevic *et al*., 2021).**

Phenolic substances in plants play a critical role in stress responses and operate as reactive species scavengers, helping to maintain redox homeostasis inside cells.

3-hydroxybenzoic acid (3-HBA) has a retention time of 18.25 and a peak area of 2.33. 3-HBA is a promising organic compound for its ability to disrupt biofilm formation in clinical and virulent bacterial isolates **(Pathoor *et al.*, 2024).** Resorcinol has a retention time of 18.37 and a peak area of 0.36. Resorcinol-based compounds are now safer options among commonly used skin-whitening agents, and their efficacy has been confirmed in cellular and clinical contexts, including moderate-to-severe melasma cases **(Beaumet *et al*., 2024).** Tyrosol (Tyr) has a retention time of 18.42 and a peak area of 0.21. Tyr is a promising molecule for the development of an effective neuroprotective agent for use in ischemic stroke **(Plotnikov *et al*., 2021)**. Pyrogallol (PG), with a retention time of 21.16 and a peak area of 0.49. Recently, PG was combined to azole drugs to eradicate effectively *Candida glabrata* isolates **(Yao *et al*., 2021).** Acetyl-phloroglucinol compounds (PhG), with a retention time of 25.80 and a peak area of 48.64. PhG, recognized as a phloroglucinol

derivative, was the main phenolic compound detected in our tiger nut extract. Ebracteolatain A is an acetyl-phloroglucinol compound found in different plants, and has been reported to have antitumor activity on breast cancer **(Li *et al*., 2023**).

Caffeic acid is a polyphenol chemical that we eat on a regular basis, most commonly in the form of coffee. It offers numerous health benefits. Caffeic acid may affect cancer, diabetes, atherosclerosis, Alzheimer's disease, and bacterial and viral infections **(Pavlíková, 2022).** However, its derivative Caffeic Acid Dimethyl Ether (CADE) significantly inhibits alcohol-induced hepatic steatosis *in vivo* **(Lu *et al*., 2022).** CADE was detected in our tiger nut extract with a retention time of 35.30 and a peak area of 20.30. It was the second main phenolic compound detected in tiger nut extract.

Folates as vitamins are required for a number of processes that regulate gene and protein expression, as well as lipid, chlorophyll, and lignin biosynthesis in plants. Under our analytical conditions, folic acid was detected at retention time of 46.38 and registered a little peak area of 0.11. From the panoply of health benefits that are inherent in this vitamin, **Wang *et al*. (2019)** have found that folic acid supplementation significantly reduced the risk of stroke in patients with cardiovascular disease.

β-Carotene, the precursor of vitamin A, has strong antioxidant effects. This has focused the attention on β-carotene dietary supplements in healthcare and therapy for chronic diseases and cancer kinds. In our tiger nut extract, β-Carotene was the last flavonoid detected at retention time of 46.55 and registered a little peak area of 0.12.

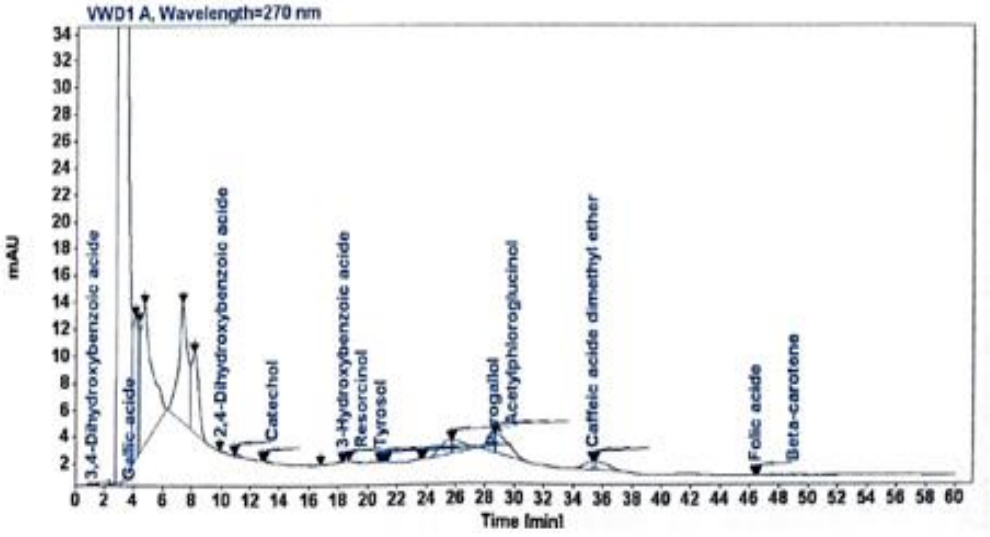


Figure 43 : HPLC/UV chromatogram of yellow nutsedge extract (*Cyperus esculentus* L.).

Table 19: Bioactive compounds identified in the hydro-ethanolic tiger nut extract.

	Name of the compounds	Retention time	Peak area
1	3,4 di-hydroxybenzoic acid (3,4 di-HBA)	1.10	0.12
2	Gallic acid (GA)	2.28	0.19
3	2,4 di-hydroxybenzoic acid (2,4 di-HBA)	9.93	1.71
4	Catechol, or 1,2-dihydroxybenzene	11.00	2.90
5	3-hydroxybenzoic acid (3-HBA)	18.25	2.33
6	Resorcinol	18.37	0.36
7	Tyrosol (Tyr)	18.42	0.21
8	Pyrogallol (PG)	21.16	0.49
9	Acetyl-phloroglucinol compounds (PhG)	25.80	48.64
10	Caffeic Acid Dimethyl Ether (CADE)	35.30	20.30
11	Folate	46.38	0.11
12	β-Carotene	46.55	0.12

However, polyphenols content in tiger nut flour represents a good alternative as net suppliers of carotenoids and vitamin E **(Hernández-Olivas *et al*., 2022).**

V.3.3. The intimate gel elaborated in this study

Figure 43 shows the macroscopic appearance of the intimate gel formulation, highlighting important characteristics such as its semi-solid consistency, fluidity, homogeneity, and white-color.

pH of the gel was found to be 5.5 at 25°C and 6.3 at 37°C. No precipitate was observed at room temperature for a month when the gel was stored in glass sealed recipient. So, refrigeration was defined as not mandatory for the storage of this product.

Figure 44: Macroscopic appearance of the intimate gel.

V.3.4. The intimate gel effect on *C. albicans in vitro*

The *in vitro* anticandidiasis properties of the gel compared to the hydro-ethanolic extract have been assessed in this study. The results revealed that both were efficiently suppressing the growth of *C. albicans* (CA) with variable potency. As stated in **Figure 44,** the intimate gel had the maximum zone of inhibition against CA of 23.5 ± 1.5 mm, whereas hydro-ethanolic extract of tiger nut showed a maximum zone of inhibition of 17.5 ± 1.2 mm. These results consolidated those obtained in chapter IV: antimicrobial section. Furthermore, the gel contains the protected strain of SL42, previously assigning a good effect on CA ATCC 10231 **(Keddar *et al.*, 2023)**, that could explain the superiority of the gel regarding its ingredient the hydro-ethanolic extract.

It has been reported previously that the extracts from several plants and spices possessed significant ($P < 0.05$) antibacterial and antifungal activities against wide range of food spoilage bacteria (Gram-positive and Gram-negative), as well as yeast and mold **(Liu *et al.*, 2017).**

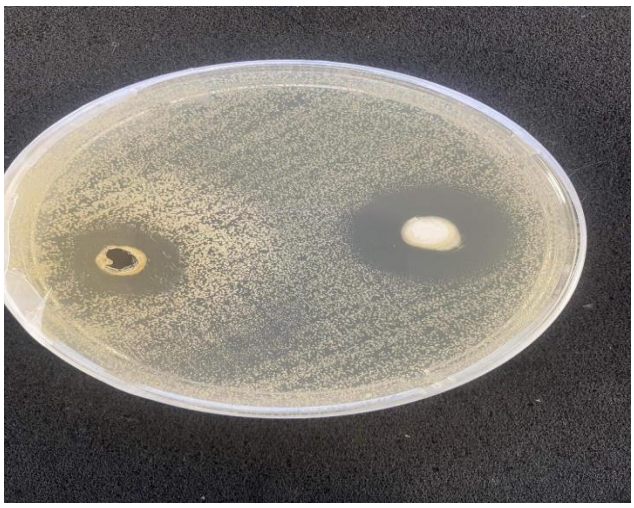


Figure 45: Anticandidiasis effect of the intimate gel compared to the tiger nut hydro-ethanolic extract on PDA agar.

V.3.5. VVC establishment

First, we assessed the succeed of the VVC model in Wistar rat. **Table 20** elucidated the establishment of the fungal burden by quantifying the colony formation at the first and second inoculation (negative control rats: group 6, were free of *C. albicans* and are not represented in the table). The mean CFU/mL for *C. albicans* at the 1st day was 1.97 ± 2.77 10^3 on average in all groups, and was followed by a sharp increase in vaginal *Candida* burden to 102.94 ± 3.78 10^3 on average at the 2nd inoculation.

In all the VVC model rats that were infected with *C. albicans*, the vaginal swabs were tested positive for *C. albicans*, with the epithelial cells being infiltrated by the hypha and neutrophils, as confirmed by HE staining **(figure 45)**.

All Model rats presented abnormal vaginal discharge and tissues welling, as well as severe inflammation of the epithelium following *Candida* infection.

V.3.6. Using the intimate gel or probiotics to treat the VVC model

V.3.6.1. CA counting

The antifungal effects of intravaginal *L. rhamnosus* Lcr35, *L. rhmanosus* SL42, the hydro-ethanolic tiger nut extract, the intimate gel, or the Econazole nitrate 1% emulsion, were assessed individually on the fungal burden by quantifying the colony formation (**table 20**).

Table 20: *Candida albicans* cells after two inoculations in all infected groups.

After 1st inoculation of *Candida albicans* (UFC/mL×10^3)						After 2nd inoculation of *Candida albicans* (UFC/mL×10^3)					
Positif control	Group (1)	Group (2)	Group (3)	Group (4)	Group (5)	Positif control	Group (1)	Group (2)	Group (3)	Group (4)	Group (5)
3	2	4	2	0	0	110	117	98	112	122	107
4	0	1	0	0	4	89	132	70	127	114	111
5	3	1	6	3	0	130	136	85	90	108	125
4	0	0	2	2	0	67	120	79	86	100	94
4	0	1	4	2	4	80	79	99	100	102	104
5	3	0	2	0	0	88	120	80	90	113	122

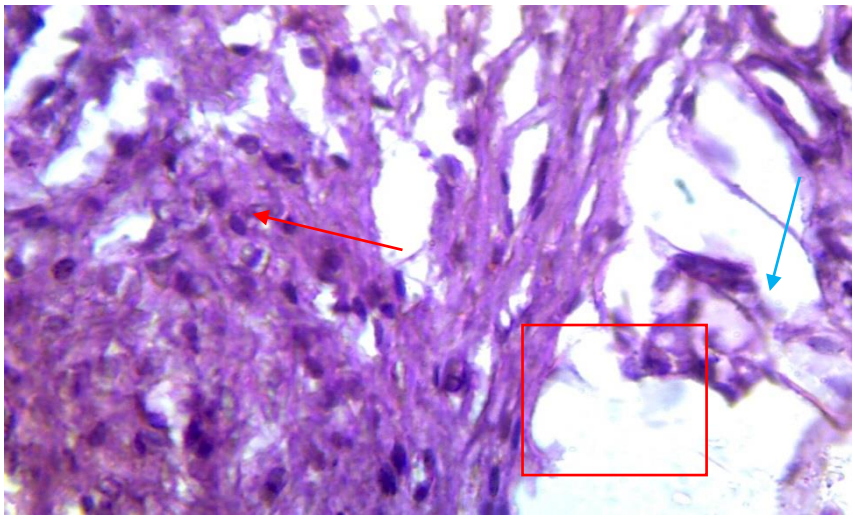


Figure 46: Results of *Candida albicans* invasion in our VVC model as demonstrated by HE staining (day 5). Damaged vaginal epithelium (red box), neutrophils infiltration (red arrows) and hyphae (blue arrows). Scale bar =50µm.

Control rats were free of *C. albicans*. The mean CFU/mL for *C. albicans* was 50.00 ± 1.17 10^3 in the VVC model group at Day 15 (one day after the elapsed treatment period). One day after the treatment, the mean CFUs in the Lcr35 (7.00 ± 4.22 10^3) and SL42 (00.22 ± 3.11 10^3) groups, were significantly lower than those in the VVC model group (**table 21** ; $P < 0.001$). There were significant differences in the CA mean in the Econazole nitrate 1% (2.00 ± 2.87 10^3), the probiotic, and the intimate gel groups (00.77 ± 4.22 10^3) registered one day after 7 days' treatment ($P < 0.001$). The tiger nut extract (11.10 ± 0.33 10^3) was the less effective among the others treatment groups of our established VVC illness.

Table 21: The quantification of fungal burden and number of the uninfected animals in all rat groups.

	Control	VVC model	Econazole nitrate 1%	Lcr35	SL42	Tiger nut extract	Intimate gel
Day one after 7 days' treatment (day 15)	0.0±0.0 CFU/mL (10^3); 6/6 uninfected rats	50.00±1.17 CFU/mL (10^3); 0/6 uninfected rats	2.00±2.87 CFU/mL (10^3); 5/6 uninfected rats	7.00± 4.22 CFU/mL (10^3); 5/6 uninfected rats	00.22±3.11 CFU/mL (10^3); 5/6 uninfected rats	11.10± 0.33 CFU/mL (10^3); 3/6 uninfected rats	00.77±4.22 CFU/mL (10^3); 5/6 uninfected rats

V.3.6.2. Histological findings

HE staining was performed to evaluate the probiotic properties of the two *Lactobacillus* strains, Econazole nitrate 1%, the tiger nut extract, or the intimate gel on the vaginal tissues following VCC establishment. Histological study of the vagina in the control group, as shown in **Figure 46,** revealed the presence of a typical structure in the vaginal wall. This structure comprised layers of non-keratinized squamous epithelial cells arranged in a stratified way, resting on a stroma of connective tissue rich in blood vessels and fibroblasts.

The establishment of candidiasis in the rat vagina (**Figure 47**) has resulted in various histological changes. HE staining revealed serious damage to the vaginal mucosa, with epithelial cell exfoliation and inflammatory cell infiltration, following infection with *C. albicans*.

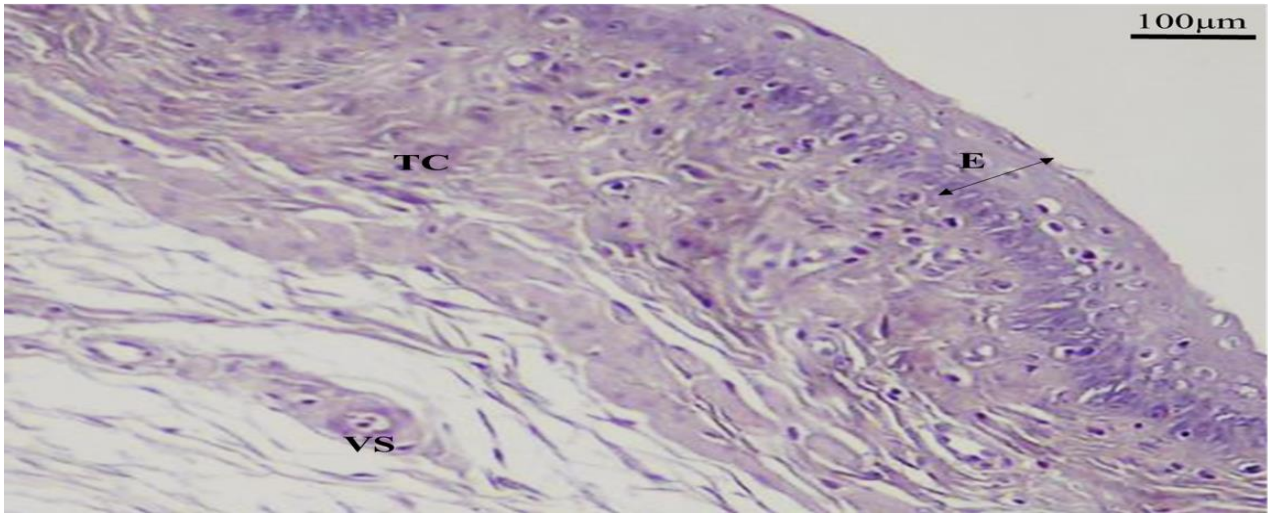


Figure 47: Histological section of the vaginal tissue in the control group. VS: blood vessels, TC: connective tissue, E: epithelium.

The lining epithelium was thickened and associated with increased inflammatory infiltration into the connective tissue, a large layer of keratin was dissolved with the presence of necrotic tissue debris penetrated by *Candida* yeasts and hyphae. This invasion involved the penetration of *Candida* yeasts, hyphae and neutrophils into both the epithelial layer and the underlying connective tissue.

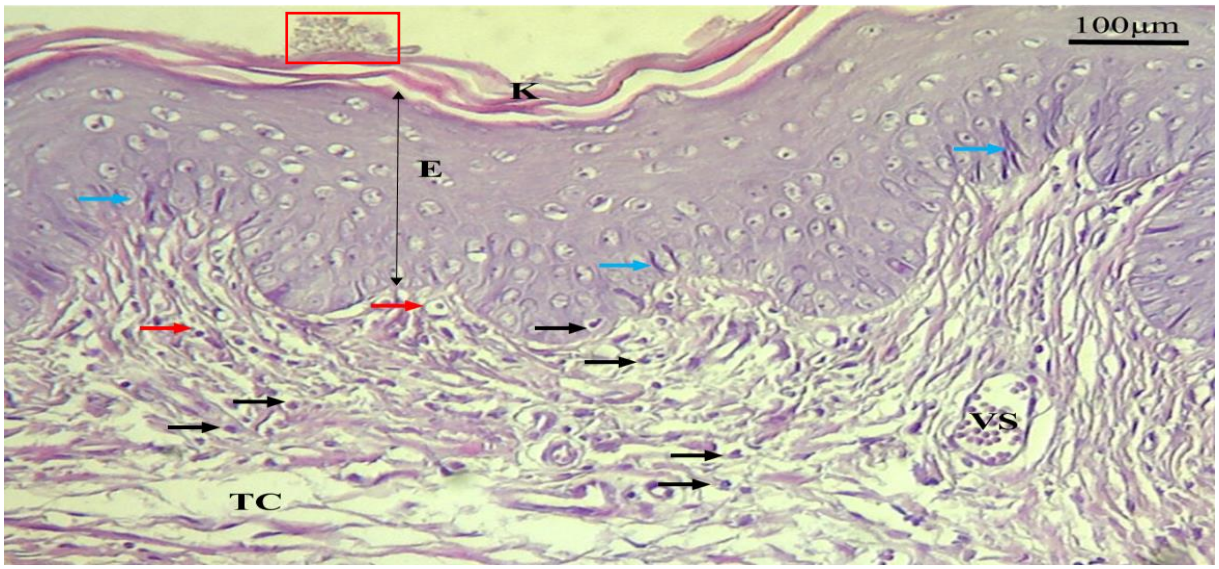


Figure 48: Histological section of the vaginal tissue in VVC model. VS: blood vessels, TC: connective tissue, E: epithelium, K: keratin, neutrophil infiltration of (black arrows), exfoliated vaginal epithelial cells (red box), Candida yeasts (red arrows)

Treatment with ECONAZOLE EG 1% (**Figure 48**) revealed a reduction in the diameter of the vaginal mucosa compared with the infected group (VVC model), with the presence of a thin layer of keratin (K). In addition, a total neutralization of *Candida* yeasts and hyphae was observed by neutrophils present in the underlying connective tissue. A moderate amount of neutrophils infiltrated in both the epithelial layer and the underlying connective tissue.

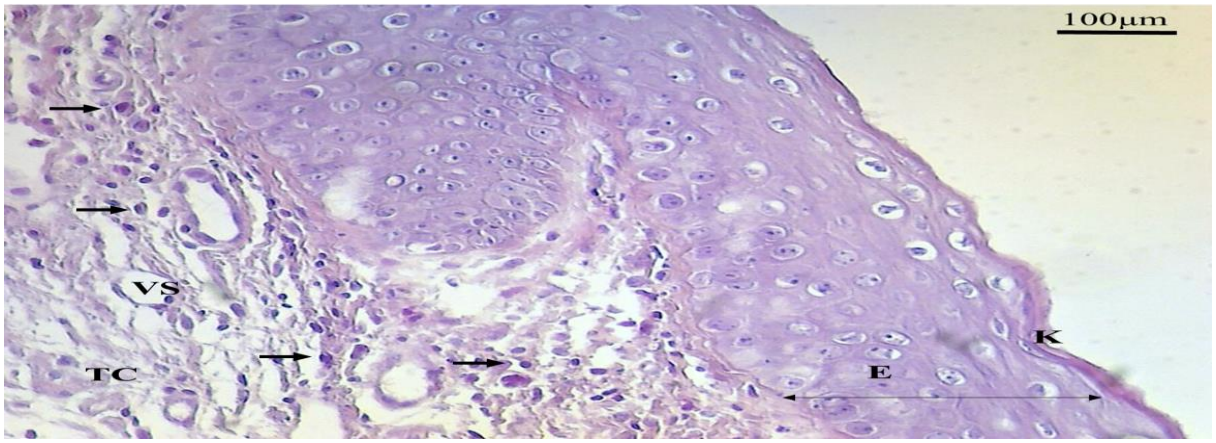


Figure 49: Histological section of vaginal tissue from Candida-infected group treated with ECONAZOLE EG 1%.VS: blood vessels, TC: connective tissue, E: epithelium, K: keratin, neutrophil infiltration of (black arrows).

Compared to the VVC model animal's mucosa, the rats treated with the reference probiotic strain from Gynophilus® for 7 days showed a significant alleviation of inflammation and damage to the vaginal epithelial mucosa.

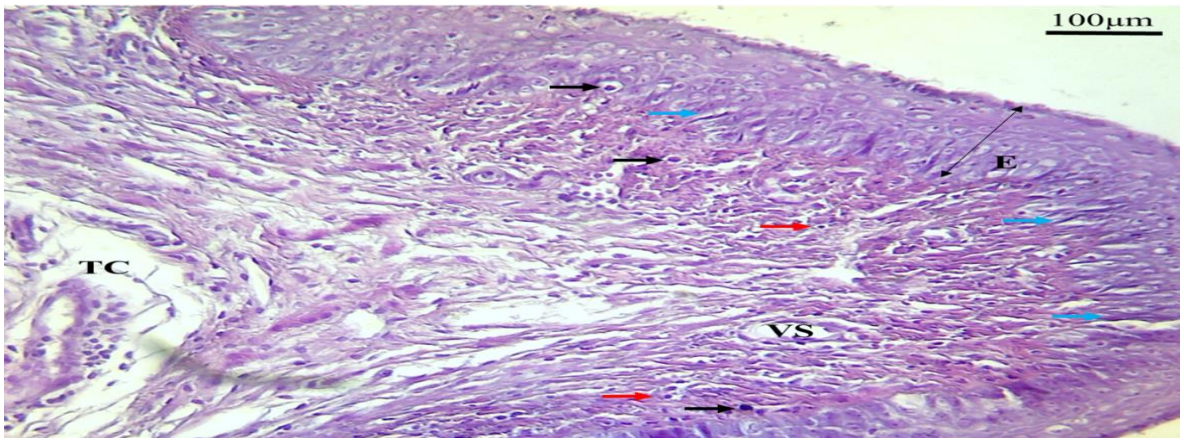


Figure 50: Histological section of group vaginal tissue infected with Candida and treated with the reference probiotic strain of Lcr35. VS: blood vessels, TC: connective tissue, E: epithelium, neutrophil infiltration of (black arrows), Candida yeasts (r

Treatment with Lcr 35 (**Figure 49**) revealed the presence of non-keratinized stratified squamous epithelium with a thickness similar to control, a less *Candida* yeast infiltration and a few hyphae in the epithelium, combined to a moderate amount of neutrophils infiltrated in both the epithelial layer and the underlying connective tissue.

In contrast, microscopic observation of the tiger nut extract-treated group (**Figure 50**) showed a thickening of the epithelial layer with the appearance of keratin (K), and persisting *Candida* yeasts, hyphae, and neutrophils infiltration in the epithelium and underlying connective tissue.

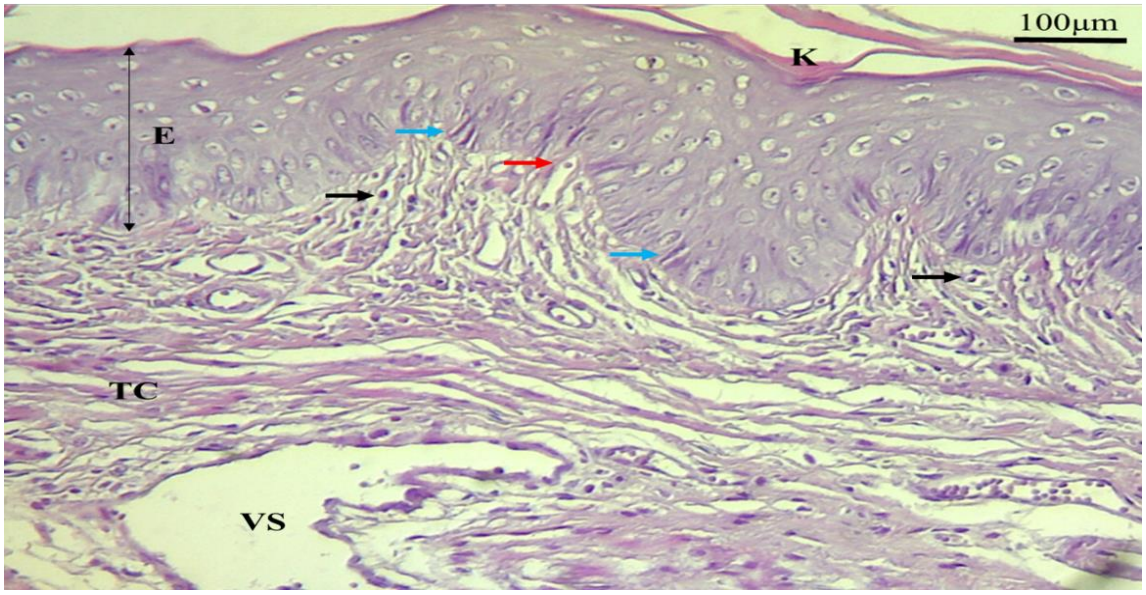


Figure 51 : Histological section of Candida-infected group vaginal tissue treated with tiger nut extract. VS: blood vessels, TC: connective tissue, E: epithelium, K: keratin, neutrophil infiltration of (black arrows), *Candida* yeasts (red arrows) and hyph

Furthermore, treatment with our probiotic strain SL42 (**Figure 51**) led to a reduction in lesions and inflammation of the vaginal epithelial mucosa, resulting in a restoration of vaginal architecture close to its normal state, although some inflammatory cells persisted on the surface of the mucosa and in the *lamina propria*. Several damaged tissues were observed with a complete restoration of a healthy vaginal mucosa.

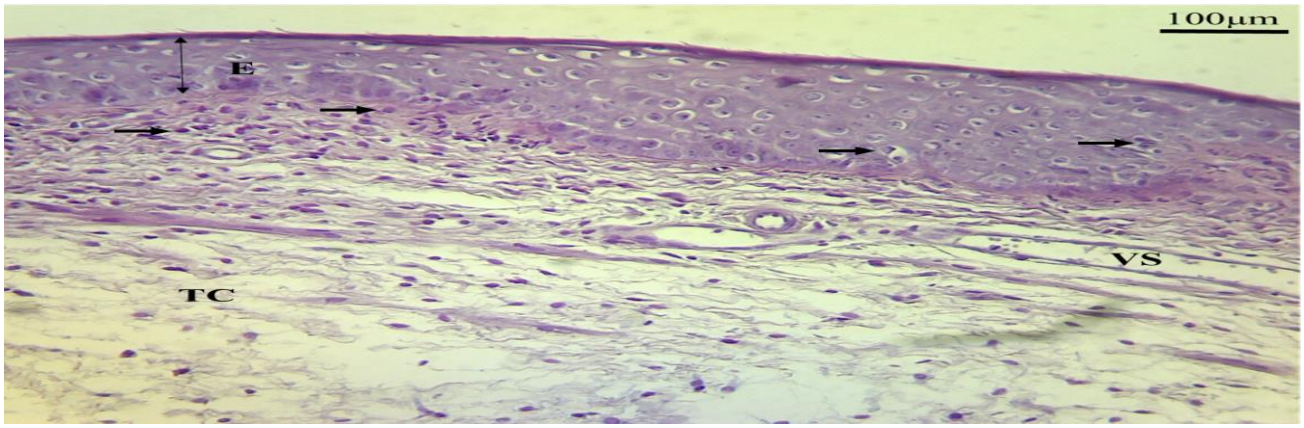


Figure 52: Histological section of group vaginal tissue infected with *Candida* and treated with SL42.VS: blood vessels, TC: connective tissue, E: epithelium, neutrophil infiltration of (black arrows).

The last treatment group was that treated with our elaborated intimate gel. Its application on rats (**Figure 52**) demonstrated improvements in a wide range of *Candida* yeast damage. Specifically, the thickness of the epithelium decreased compared to the infected and untreated groups, without achieving the diameter of the control group. Additionally, a moderate neutrophil infiltration of the underlying connective tissue, and isolated cases of hyphae presence were observed in the vaginal tissue.

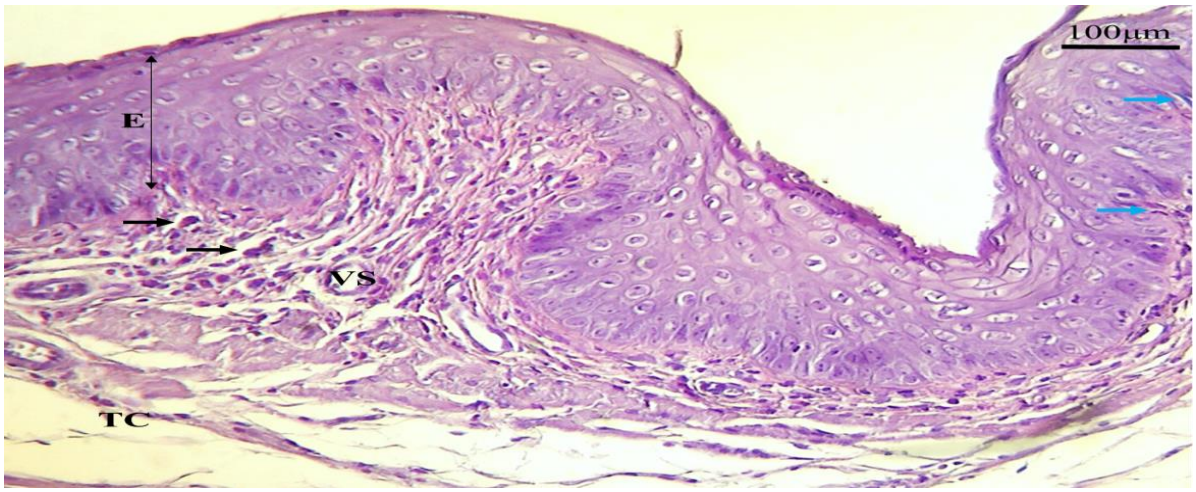


Figure 53: Histological section of group vaginal tissue infected with *Candida* and treated with the intimate gel. VS: blood vessels, TC: connective tissue, E: epithelium, neutrophil infiltration of (black arrows), and hyphae (blue arrows).

The future of VCC treatment could lie in phytotherapy. Will the use of herbal medicine one day become the norm in the treatment of VCC? Can herbal medicine replace traditional pharmacotherapy?

VVC is a significant clinical issue affecting a growing number of patients. Due to the easy access to the most common azole medications without the need for a specialist's prescription, their isolated use and abuse by patients are multiplying, which contributes to the increase of strains resistant to current treatments.

Fluconazole is typically used as the first-line medication for women because of its wide range of action **(Varadarajan *et al.*, 2015).** Its side effects are mainly at the gastrointestinal level, such as vomiting, nausea, and diarrhea. However, seizures, dizziness, and insomnia are also noted (**Govindarajan *et al*., 2024).**

Research unequivocally demonstrates the positive efficacy of herbal remedies, and highlights other effects such as the suppression of mitochondrial dehydrogenase activity. Comparisons of their intervention with traditional pharmaceutical treatment also support the alternative technique: herbal preparations prove to be more effective than frequently used medications. A striking example is curcumin where a complete cure of VVC was observed in 66% of women treated with turmeric cream, while the use of clotrimazole led to a cure rate of 48.9% **(Abouali *et al*., 2019).**

According to literature, the present study is the first *in vivo* temptation to examine the effect of tiger nut *Cyperus esculentus* L extract on VVC. The novelty is incorporating a promising probiotic strain embedded in a naturel intimate gel.

Different phytotherapeutic approches against candidiasis could also be found in literature, such as the use of garlic, *Zataria multiflora* **(Farshbaf-Khalili *et al*., 2016**), *Ageratina pichinchensis* **(Romero-Cerecero *et al*., 2017),** and *Calendula officinalis* **(Saffari *et al*., 2017).**

In parallel and in the lay literature, the use of probiotics to treat and prevent VVC seems to be a practice that dates back a long time. The scientific argument in favor of the exogenous replacement of *Lactobacillus* is based on the hypothesis that these species have a notable protective impact *in vivo*, by reducing the virulence of *Candida* organisms that colonize the vagina, and strengthening the immune defense systems of vaginal epithelial cells. It is also assumed that women suffering from VVC, particularly recurrent VVC, have a reduced amount of protective *Lactobacillus* species present *in situ*, a hypothesis that is completely unproven, unlike women affected by bacterial vaginosis.

Probiotics play a crucial role in the management of female vaginitis. The production of various antifungal compounds and lactic acid by lactobacilli inhibits the growth of pathogenic microorganisms, while stimulating the immune system through competitive adhesion to achieve the treatment effect for VVC **(Vicariotto *et al*., 2014).**

Our results demonstrated the effectiveness of the individual probiotic strains as well as the elaborated intimate gel containing the tiger nut hydro-alcoholic extract and the protected strain of SL42 in managing a rapid recovery and a complete healing of the vaginal tissues. This protective anti-fungal effects of the intimate gel on the vaginal mucosa in VVC rats is partially associated with its capacity to inhibit adhesion

and invasion by the fungal pathogen. The main flavonoids detected in the tiger nut extract acetyl-phloroglucinol compounds (PhG) and caffeic acid dimethyl ether (CADE), could be almost responsible of its fungicidal activity.

Our results are also similar to those of **Somayeh Saleki1 *et al*. (2018)** who found that the alternative treatment of vulvovaginal candidiasis with *Lactobacillus* species had similar effects to the conventional treatment with clotrimazole alone. Consequently, both therapies (clotrimazole and clotrimazole + probiotics) acted similarly on the symptoms of vulvovaginal candidiasis. Whereas in the study conducted by **Kovachev and Vatcheva-Dobrevska (2015),** the authors revealed a significantly higher clinical efficacy in the group treated with an antifungal combined to a probiotic compared to the group treated with the antifungal alone.

V.3.6.3. Myeloperoxidase and Prostaglandin PGE_2 activities in vaginal tissues

.MPO is a lysosomal protein widely expressed in neutrophils and contributes to the antimicrobial effects of neutrophil activation (**Soubhye, 2016)** by converting hydrogen peroxide (H_2O_2) into the highly toxic antimicrobial compound hypo chlorous acid (HOCl), amplifying their capacity for destroying pathogens and regulating inflammation **(Buchan *et al*., 2019).**

MPO activity was assessed in this study as a biochemical indicator of the level of neutrophil infiltration in the vaginal tissues of mice infected with Candidiasis (**Trifonova, 2007).**

MPO activity was measured from vaginal tissue lysates. MPO activity in the untreated infected group VVC model (570 ± 9.2 %) was almost five times higher ($P< 0.001$) than in the other treated groups at **(Table 22).** However, MPO activities in the infected group treated with the intimate gel (89 ± 1.88 %) were lower than the other treated groups: 90 ± 1.44 % ($P> 0.05$) in the SL42 group, 95 ± 3.2 % ($P> 0.05$) in that treated with the drug emulsion, 101 ± 0.70 %

Table 22 : Myeloperoxidase activity % in vaginal tissues

Rat groups	MPO activity (%)
Control	99 ± 2.4
Infected non treated (VVC model)	570 ± 9.2***
Infected + treated with 1% econazole nitrate	95 ± 3.2
Infected + treated with SL42	90 ± 1.44
Infected + treated with Tiger nut extract	108 ± 0.24*
Infected + treated with Lcr35	101 ± 0.70
Infected+ treated with the intimate gel	89 ± 1.88

** P≤0.05 compared to control group; *** P≤0.001 compared to control group*

(P> 0.05) in the Lcr35 group, and 108 ± 0.24 % (P< 0.05) in the tiger nut extract. In general, all the treated groups registered MPO values similar to that of the control (P< 0.05).

MPO is believed to have a role in antimicrobial defense, as pathogens create virulence factors that target it. However, the specific site of action remains unknown. MPO, in addition to strengthening antimicrobial defence, is an essential inflammatory regulator. The entrance of neutrophils at the wound site initiates the anti-inflammatory response by delivering MPO to consume H_2O_2 and reduce inflammation **(Buchan *et al.*, 2019).**

PGE2 is a putative prognostic indicators of disease severity and pharmacologic agents aimed at reducing PGE2 concentration may be therapeutic options **(Nash *et al.*, 2016).** That why, this lipid signaling molecule involved in pain and inflammation, was also quantified in vaginal tissues. The **Table 23** illustrated the values of PGE2 in all infected, treated and naïve rats.

Elevated PGE2 level (806 ± 4.2 pg/mL) was registered in our VVC model. In fact, *Candida albicans* utilizes arachidonic acid (AA) released during the course of infection (Candidiasis) from phospholipids of infected host cell membranes and synthesizes extracellular prostaglandin(s) which play an important role in hyphae formation and host cell damage. However, **Nash *et al.*, 2016** showed that elevated concentrations of PGE2 were detected only in *C. albicans* biofilm.

Prostaglandins have been known to be potential molecules to mediate cross talk between host and pathogen resulting in down-regulation of host immunological responses. Prostaglandins also play an important role in the persistence of fungal pathogen in immunocompetent hosts resulting in chronic infections **(Mishra *et al.*, 2014).** Likewise, and in recent years, increasing evidences have defined that PGE2 might potentiate tissue restoration and repair following injuries **(Cheng *et al.*, 2021).** This could be the reason for the PGE2 levels in all treated groups, which were 8–9 times lower than those in the VVC-untreated group.

The intimate gel demonstrated its advantage over all other therapies in regulating PGE2, as was indicated for the MPO activity.

Table 23: Prostaglandin PGE2 activity pg/mL in vaginal tissues

Rat groups	**PGE_2 activity (pg/mL)**
Control	54 ± 5.07
Infected non treated (VVC model)	806 ± 4.2 ***
Infected + treated with 1% econazole nitrate	89 ± 2.30*

Infected + treated with SL42	89 ± 1.40*
Infected + treated with Tiger nut extract	95 ± 2.66*
Infected + treated with Lcr35	82 ± 0.33*
Infected+ treated with the intimate gel	74 ± 1.09

** P≤0.05 compared to control group; *** P≤0.001 compared to control group*

V.3.7. Conclusion

This research demonstrated the biological impact of the tiger nut extract *Cyperus esculentus* L. in association with our probiotic strain *L. rhamnosus* SL42, in the context of treating vulvovaginal candidiasis. Candidal vulvovaginitis, primarily induced by *Candida albicans* is a common and generally recurrent infection that affects many women. Unfortunately, conventional treatment often relies on antifungals that can be prone to resistance and present various side effects.

The results obtained in this study indicate that the extract of "*Cyperus esculentus* L." associated with the probiotic bacteria could be an alternative treatment for vulvovaginal candidiasis due to its antimicrobial, antioxidant, and anti-inflammatory properties, by reducing the fungal load and improving the balance of the vaginal microbiota. The introduction of probiotics inhibits the excessive proliferation of *Candida albicans*. This synergistic effect between the tiger nut extract and the probiotic could offer a powerful, more natural, and less invasive therapy for the fight against vulvovaginal candidiasis.

On the other hand, even though our results are positive and promising, it is important to conduct larger-scale clinical studies, including randomized trials, to prove the efficacy and safety of this method in order to better understand the effect of *Cyperus esculentus* extract combined with probiotics on the pathophysiology of vulvovaginal candidiasis.

In summary, the results of our study shed light on a new sustainable therapeutic approach against vulvovaginal candidiasis. This therapeutic approach could be an alternative to reduce dependence on conventional antifungal treatments.

The microbiome and its potential impacts on other *Lactobacillus* species were not evaluated. For a more comprehensive evaluation of the stability of the gel, future studies following international guidelines will allow for longer-term assessments and provide more robust data on the formulation’s stability over time.

General conclusion

General conclusion

A significant number of medicinal plants have not been investigated for their potential health benefits. Herbal medication is an important component in all conventional health systems in developed and developing countries. Most plants are used as raw materials to produce new herbal drugs, which account for a significant proportion of the world drug market. *Cyperus esculentus* L. (Tiger nut) is produced as food for livestock and can be consumed as raw, drinks, baked, grated or used for the production of ice creams. Tiger nuts are tubers that grow on the roots of sedge plants. The tubers were cultivated in the Nile valley by ancient Egyptians.

Tiger nuts are sweet nutty tubers that can be eaten. These tubers are also known as ‘earth almond’ and ‘yellow nut sedge’.

Research has shown that for individuals who suffer from dyspepsia, dysentery and gassiness, the tuber is recommended. A high concentration of oleic acid in tiger nut has a beneficial impact on cholesterol and thus activating blood content, inhibiting thrombosis and heart attacks. Tiger nut averts constipation and minimizes the threat of colon cancer. In China, tiger nut milk is used as a heart stimulant, liver tonic, relief of severe stomach ache, aid regular menstrual flow and a cure to gum and mouth ulcers. The nut contains substantial quantities of water-soluble flavonoids, glycosides and relatively high antioxidant capacity. This could explain why the traditional Algerian pharmacopeia recommends water extract for treating hyperglycaemia and high pressure.

Tiger nuts have been underused due to a lack of information on their health benefits. Despite the mentioned benefits, oral mastication of the soaked tuber can result in uneasiness, especially for older people with teeth problems. Nevertheless, the inclusion of tiger nut products such as cookies, alternatively to the direct consumption of the soaked tuber, could be taken into account in the development of functional foods for diabetic patients.

In the first phase of our study, we investigated the physicochemical, rheological, and functional qualities of the organic flour that will be used to make the cookies.
The second part of the paper described new sugar-free cookies that included or not the microencapsulated probiotic strain *Lacticaseibacillus rhamnosus* SL42 from our laboratory collection. We proposed the formulation of functional cookies with 100% non-peeled organic flour and no wheat flour or sugar addition, with the goal of establishing a new probiotic carrier and a healthy food product.

1. The physicochemical composition of the edible tiger nut extract revealed a notable richness in sugars, proteins, and lipids, as well as in mineral salts. These nutritional components not only contribute to therapeutic virtues but also to the potential it presents as a source of nutrients. Furthermore, the presence of polyphenolic compounds indicates its appeal as a natural antioxidant,

which can be crucial for preventing various pathologies. As a result, the edible tiger nut stands out for its nutritional and healing properties.

2. Next, we succeeded in developing a new cookie formulation with 100% tiger nut flour without the addition of table sugar. The physicochemical analysis of the cookies revealed that the cookies made with tiger nut flour not only meet the standards and requirements of consumers but also have a higher nutritional density compared to other formulations.

3. The sensory analyses conducted showed that the biscuits have satisfactory organoleptic characteristics. Indeed, it was found that the biscuits have an acceptable color, a pleasant mouthfeel, and a good aroma.

4. The addition of honey syrup enriched with our probiotic isolate *Lacticaseibacillus rhamnosus* SL42 not only enhanced the taste of our cookies but also enriched their nutritional values. The enriched cookies were appreciated and praised by the panelists for their nutritional value on one hand, and their unparalleled taste on the other. The results of the SEM analyses demonstrated the adhesion and the survival of our isolate after 21 days of storage.

The second part of this thesis demonstrates the involvement of the tiger nut gel containing the probiotic strain *Lacticaseibacillus rhamnosus* SL42 as a possible treatment for women affected by VVC, especially for those who are resistant to medications, who experience side effects, or who have contraindications to the use of antifungal treatments.

1. We successfully induced vulvovaginal candidiasis in female Wistar rats by hormonal stress and two inoculation attempts. The results of the vaginal lavage following the second inoculation demonstrated a significant presence of *Candida* in the vaginal cavity of the rats. The examination of MPO levels in vaginal tissues confirmed the local inflammatory response of vaginal tissues to *Candida albicans.*
2. The UV-HPLC results concerning the hydro-alcoholic extract of tiger nut "*Cyperus esculentus* L." highlighted the richness of the extract in phenolic compounds, which possessed a strong antimicrobial potential.
3. Subsequently, we worked on developing a high-end intimate gel, based on tiger nut extract and the probiotic isolate considered as an effective remedy against vulvovaginal candidiasis.
4. The histological results showed the effectiveness of using an intimate gel in the management of VVC, which has particularly notable effects on the physiological and morphological changes of the vaginal mucosa.
5. The promising results of this study highlighting the potential of treating VVC with a natural intimate gel based on hydro-alcoholic extract of tiger nut and *Lacticaseibacillus rhamnosus* SL42 in clinical practice must be validated by well-designed clinical studies with larger samples and long-term follow-up.

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