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Physico-chemical characterization and biological properties of prickly pear, and evaluation of the stability of its derived products

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DEDICATION

I would like to express my gratitude to Almighty God for granting me health and courage to complete this work.

I would like to thank my dear parents who have always believed in me and in my abilities, without them, I would not have been able to accomplish such work.

To my husband, who has always been there to support and encourage me.

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Abstract

This study aimed to valorize the prickly pear fruit, due to its abundant presence in our country and its numerous virtues, focusing on its seeds and juice. Various tests were conducted, including factor-by-factor extraction and response surface methodology, to optimize the extraction conditions for a better antioxidant yield. The optimum conditions for the prickly pear seed powder were: an acetone concentration of 59.03%, a sample/solvent ratio of 0.54 g/20 ml, a microwave power of 762.23 W, and an irradiation time of 198.52 s. The quantification of polyphenols, flavonoids, and condensed tannins, as well as the evaluation of antioxidant, anti-inflammatory, and antimicrobial activities, were carried out. The results showed that *OFI* seeds are rich in bioactive compounds, with polyphenols (905.71±0.50 mg GAE/100g DM), flavonoids (50.77±0.08mg QE/100g DM), and condensed tannins (98.99±8.19mg CE/100g DM). The extracts exhibited significant antioxidant activities, such as strong DPPH inhibition (248.40±1.06mg GAE/100g DM) and high reducing power (382.56±7.70mg GAE/100g DM). Notably, the extract also displayed anti-inflammatory effects with an impressive inhibition rate of 86 ± 0.42%, the antimicrobial test demonstrated efficacy against certain pathogenic bacteria like *Bacillus cereus, Enterococcus faecalis, Staphylococcus aureus,* and *Micrococcus luteus,* and all tested fungi except the genus *Penicillium* sp. Furthermore, to improve the stability of unpasteurized prickly pear juice fortified with the hydro-soluble seeds extract, physicochemical analyses and antioxidant activities such as pH, BI, TA, Brix°, and FRAP, TPC, TFC, DPPH were conducted. Regarding the physicochemical properties, no detectable difference was found between enriched and control samples throughout the storage period. In addition, the enriched samples exhibited the highest content of phenolic compounds and total flavonoids; likewise, the enriched juice had a higher antioxidant capacity. Microbial analysis revealed the absence of microorganisms, even though the juices were unpasteurized. The hydro-soluble extract of prickly pear seeds improved the stability and nutritional value of fruit juice, preserving its physicochemical, phytochemical, and microbiological quality during storage. These findings emphasize the potential of prickly pear as a valuable source of bioactive compounds and highlight its significance in various applications.

Keywords: *Opuntia ficus indica,* Juice quality, seeds, Storage, antioxydant, Microbiological Analyses, Response Surface Methodology.

Résumé

Cette étude visait à valoriser le fruit de la figue de barbarie en raison de sa présence abondante dans notre pays et de ses nombreuses vertus, en mettant l'accent sur les graines et le jus de ce fruit. Plusieurs tests ont été réalisés, notamment l'extraction facteur par facteur et la méthodologie de surface de réponse, afin d'optimiser les conditions d'extraction pour obtenir un meilleure rendement d'antioxydants. Les conditions optimales pour la poudre de graines de figue de barbarie étaient : une concentration d'acétone de 59.03 %, un rapport échantillon/solvant de 0.54 g/20 ml, une puissance micro-ondes de 762.23 W et un temps d'irradiation de 198.52 s. La quantification des polyphénols, des flavonoïdes et des tannins condensés, ainsi que l'évaluation des activités antioxydante, anti-inflammatoire et antimicrobiennes, ont été réalisées. Les résultats ont montré que les graines de *OFI* sont riches en composes bioactifs, avec des polyphénols (905.71±0.50 mg GAE/100g MS), des flavonoïdes (50.77±0.08 mg QE/100g MS) et des tannins condensé (98.99±8.19 mg CE/100g MS). Les extraits ont présenté des activités antioxydante significatives, telles qu'une forte inhibition du DPPH (248.40±1.06 mg GAE/100g MS) et un pouvoir réducteur élevé (382.56±7.70 mg GAE/100g MS). De plus, l'extrait a également présenté un effet anti-inflammatoire avec un taux d'inhibition impressionnant de 86±0.42%, le test antimicrobien, a démontré une efficacité contre certaines bactéries pathogènes telles que *bacillus cereus, enterococcus faecalis, staphylococcus aureus* et *micrococcus luteus*, ainsi que tous les champignons testé a l'exception du genre *penicillium* sp. De plus, pour améliorer la stabilité du jus de figue de barbarie non pasteurisé enrichi en extrait hydrosoluble de graines, des analyses physico-chimiques et des activités antioxydante telles que pH, IB, Brix°, et AT, FRAP, CPT, CFT, DPPH ont été réalisées. Concernant les propriétés physico-chimiques, aucune différence détectable n'a été observée entre les échantillons enrichis et les échantillons témoins pendant toute la période de stockage. De plus, les échantillons enrichis présentaient la plus forte teneur en composes phénoliques et en flavonoïdes totaux ; de même, le jus enrichi présentait une capacité antioxydante plus élevée. L'analyse microbiologique a révélé l'absence de micro-organismes, même si les jus n'étaient pas pasteurisés.

Mots-cles : *opuntia ficus indica*, qualité du jus, graines, stockage, antioxydant, analyse microbiologique, méthodologie de surface de réponse.

الملخص:

هدفت هذه الدراسة إلى تسليط الضوء على قيمة ثمرة التين الشوكي، نظرًا لوجودها بوفرة في بلادنا وفوائدها العديدة مع التركيز على البذور وعصير الثمرة، وقد تم إجراء اختبارات متنوعة، كاستخلاص العوامل الناتجة ومنهجية سطح الاستجابة، لتحسين ظروف الاستخلاص للحصول على إنتاجية أفضل لمضادات الأكسدة. كانت الظروف المثلى لمسحوق بذور الصبار هي: تركيز الأسيتون بنسبة 59.03%، نسبة العينة إلى المذيب 0.54 غرام/20 مل، قدرة الميكروويف 762.23 واط، ووقت الإشعاع 198.52 ثانية، فتم إجراء تحديد كمية البوليفينولات والفلافونويدات والتانينات المكثفة، بالإضافة إلى تقييم الأنشطة المضادة للأكسدة والمضادة للالتهابات والمضادة للميكروبات. أظهرت النتائج أن بذور "*Opuncia ficus indica*" غنية بالمركبات الحيوية، مع تفوق البوليفينولات (905.71 ±50.0 ملغGAE / 100غ) والفلافونويدات (50.77 ± 0.08 ملغQE / 100غ) والتانينات المكثفة (98.9 ± 8.19 GAE/ 100غ)كما أظهرت الاستخلاصات، أنشطة مضادة للأكسدة ملحوظة مثل: قوة احتجاز DPPH (248.4 ± 15.06 ملغGAE / 100غ) وقدرة تخفيضية عالية (382.56 ± 7.70 ملغGAE / 100غ) إضافة لذلك، أظهر استخلاص التأثيرات المضادة للالتهابات معدل قمع مبهر بنسبة ,86±0.42% .وأظهر اختبار مضادات الميكروبات،كفاءة ضد بعض البكتيريا الممرضة مثل: باسيليوس سيريوس، إنتيروكوكوس فايساليس، ستافيلوكوكوس أوريوس، مايكروكوكوس لوتيوس وجميع الفطريات المختبرة باستثناء جنس البنسيليوم. وعلاوة على ذلك، لتحسين استقرار عصير التين الشوكي غير المبستر المحسن بمستخلص البذور القابل للذوبان في الماء.تم إجراء تحاليل فيزيكوكيميائية وأنشطة مضادة للأكسدة مثل: الرقم الهيدروجيني , درجة بريكس , المواد الصلبة القابلة للذوبان, الحموضة القابلة للتحليل ,مؤشر التحمير, البوليفينولات ,الفلافونويدات ,قوة تقليل الحديد الثلاثي,DPPH. وبالنسبة للخواص الفيزيكوكيميائية، لم يتم العثور على أي اختلاف قابل للكشف بين العينات المحسنة والعينات الغير مُحسنة طوال فترة التخزين. كما أظهرت العينات المحسنة أعلى محتوى للمركبات الفينولية والفلافونويدات الكلية؛ وبالمثل، كان للعصير المحسن قدرة أعلى على مكافحة الأكسدة. أظهر التحليل الميكروبي عدم وجود ميكروبات، على الرغم من أن العصائر لم تكن مبسترة. منح مستخلص بذور التين الشوكي القابل للذوبان في الماء استقرار وقيمة غذائية لعصير الفاكهة، محافظًا على جودتها الفيزيكوكيميائية والفيتوكيميائية والميكروبيولوجية أثناء التخزين. وفي الأخير، تؤكد هذه النتائج إمكانية استغلال التين الشوكي كمصدر قيم للمركبات الحيوية وتسلط الضوء على أهميتها في مجالات متنوعة.

الكلمات المفتاحية: التين الشوكي، جودة العصير، البذور، التخزين، مضادات الأكسدة، تحليل ميكروبي، منهجية سطح الاستجابة.

Abbreviations list

OFI	*Opuntia Ficus Indica*
QE	Quercetine equivalent
CE	Catechine Equivalent
TA	Tirable acidity
BI	Browning index
TSS	Total solid soluble
FRAP	Ferric reducing power
TFC	Flavonoid Compound
CT	condensed tannin
TPC	Total flavonoid compound
DM	Dry mater
RSM	Response Surface Methodology
ANOVA	Analysis Of Variances
BSA	Bovine Serum Albumin
ROS	reactive oxygen species
DW	Dry weight
OD	Optical density
DPPH	2,2-Diphenyl-1-picrylhydrazyl
PDA	Potato Dextrose Agar.
MH	Muller Hilton
Rpm	Rotation per minute
Re	Ratio of edible part
MAE	Microwave assisted extraction
TAC	Total Antioxidant Capacity
DMSO	Diméthylsulfoxyde

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ChapterIII

INTRODUCTION

I. Introduction

Recently, people have become more interested in the health benefits of different foods because they are paying more attention to what they eat and how it affects their health **(Missaoui *et al.*, 2020).** This surge in interest is fueled by a desire to understand the active phytochemicals present in food and their potential contributions to disease prevention. Moreover, guided by the principles of green chemistry, the exploration of eco-friendly methods to harness bio-wastes and extract bioactive compounds for the creation of innovative food and functional **(Paul *et al.*, 2010).**

In this context, *OFI*, also known as cactus pear, stands out as an important option in the search for sustainable and nutritious food sources. **(Cota-Sánchez *et al.*, 2016; Özcan *et al.*, 2023).** With its remarkable adaptability to challenging cultivation conditions, *OFI* has garnered global interest, positioned not only as a source of nourishment for humans and animals but also as a repository of bioactive constituents with potential health-enhancing attributes. The various components of the cactus pear, including its pulp, seeds, present a multitude of possibilities.

Originating from arid and semi-arid regions of Mexico and South America, *OFI* flourishes in challenging climates, including the arid and semi-arid regions **(El-Sayed *et al.*, 2014).** Despite its abundance, the utilization of cactus pear, particularly its fruit and seeds, remains largely underexplored, often limited to seasonal consumption.

Notably, the seeds are gaining prominence importance, particularly in countries such as Mexico, Argentina, Spain, Algeria, Morocco and Tunisia, where their potential has been recognized for applications ranging from cosmetics to cooking due to their high content of nutritional values **(Li *et al.*, 2023).** These seeds are also rich in diverse phytochemical compounds, including polyphenols and flavonoids, endowing them with natural antioxidant capabilities **(Bellumori *et al.*, 20123).**With a focus on the pivotal role of seeds in fruit valorization and their inherent antioxidant potency, this study is poised to explore the potential of cactus pear in depth.

As a crucial crop with economic and ecological significance, cactus pear boasts an array of bioactive molecules, most notably phenolic, fruit juices, as vehicles for these bioactive compounds, offer a conduit to enhancing human nutrition and well-being. Various preservation techniques have been explored to extend the shelf life of these juices, with the integration of natural additives being a particularly promising avenue **(Rodrigues *et al*., 2022).**

The extraction process, an initial step in isolating antioxidants, is influenced by factors such as microwave power, solvent type, sample/solvent ratio and extraction duration. By leveraging advanced techniques like response surface methodology, optimal conditions can be pinpointed to maximize the yield of target compounds **(Mandal *et al*., 2007).**

In light of these considerations, this study aims to promote the exploration and utilization of cactus pear, structured into three primary segments, encompassing, extraction optimization, phytochemical characterization of the seeds fruit, and evaluation of unpasteurized prickly pear juice stability fortified with the hydro soluble seeds extract, this research endeavors to shed light on the untapped potential of this remarkable botanical resource.

Bibliographic part

I. General information on the prickly pear

OFI Fruits are considered health-promoting foods due to the diversity of bioactive molecules found in these fruits **(Karacabey *et al*., 2021).**

The prickly pear grown in a wide range of environments, giving lead to major differences in plant survival and development, and harvest potential. The ecological success of *opuntia*, specifically *OFI*, is largely due to the peculiarity of their daily pattern of carbon uptake and water loss **(salehi *et al*., 2019).**

The *opuntia* fruits are relevant sources of phytochemicals with proven biological activities and high added-value in the food nutraceutical industry **(Barba *et al*., 2017; Mena *et al*., 2018)**. They are rich in ascorbic acid, betalains (betanin and indicaxanthin), phenolic acids (piscidic acid and hydroxybenzoic acid derivatives), flavonoids (isorhamnetin, kaempferol, and quercetin glycosides), and carotenoids (mainly lutein) **(García *et al*., 2019; Gómez *et al*., 2019).** It's also a popular edible plant that possesses considerable nutritional value and exhibits diverse biological actions including antioxidant, anti-inflammatory and antidiabetic activities, **(Jung *et al*., 2016).**

Different colors for prickly pear fruits are offered depending on varieties based on betalains covering a broad spectrum from yellow to purple with pigment contents of 66–1140 mg/kg fruit pulp **(Ferreira *et al*., 2023)** Increased consumption of grains, fruits and vegetables is related to a reduced risk of chronic diseases diabetes, cardiovascular and neurodegenerative diseases **(Mazzoni *et al*., 2023).**

I.1. Historic and geographic distribution

The prickly pear is native to arid and semi-arid regions of Mexico **(El-Sayed, *et al*., 2014)**, where it has been used by humans for around 6,500 years BC and was a fundamental component of the diet of indigenous populations **(Radi *et al*., 2023).** It thrives in various regions such as Africa, Australia, the Mediterranean Basin, and parts of Asia. It was dispersed worldwide, including to America, by the end of the 15th century **(Elhadi *et al*., 2011).**

Its cultivation dates back to ancient Mesoamerican civilizations, particularly the Aztec culture. It is believed that plants of this genus were brought to Spain by Christopher Columbus and subsequently spread throughout European and African countries in the Mediterranean basin. In the seventeenth century, cactus pear was introduced to Australia from Brazil to produce natural red dyes found in the cochineal scale insect (*Dactylopius opuntiae*), which resides on the cladodes. This dye was considered suitable for the Australian context **(Chávez *et al*., 2009).**

Figure 1: Geographic distribution of cactus prickly pear, **(Chávez *et al*., 2009)**

I.2. Taxonomic classification

In 1978, Miller classified this plant into the genus *Opuntia* within the Cactaceae family. The Cactaceae family comprises approximately 130 genera and 1600 species. However, the taxonomy of species within the *Opuntia* genus remains a topic of debate. Few studies have been conducted on this subject, and the high degree of variability and the lack of genetic information on the plant have hindered the systematic classification of *Opuntia* within the Cactaceae family **(Chauhan *et al.*, 2010).** Differentiating between various cultivars is based on factors such as the shape and quality of the fruits, as well as the flowering period and fruit maturity **(Yahia *et al*., 2011).**

The scientific classification of this species is presented in **Table 1.**

Table 1: Botanical classification of the prickly pear **(Yahia *et al*., 2011).**

Class	Name	Common Name
Kingdom	Plantae	
Subkingdom	Tracheobionta	Prickly pear
Division	Magnoliophyta	
Subclass	Magnoliopsida Caryophyllidae	Figue de barbarie
Order	Caryophyllales	
Family	Cactaceae	
Genus	*Opuntia*	
Subfamily	*Opuntioideae*	
Species	*Opuntia ficus indica*	

I.3. Botanical description

The prickly pear, also known as cactus, is a substantial plant that can reach a height of up to 5 m **Figure 1.** The peels, seeds, and pads are often regarded as by-products or even waste **(Tamer *et al*., 2014).**

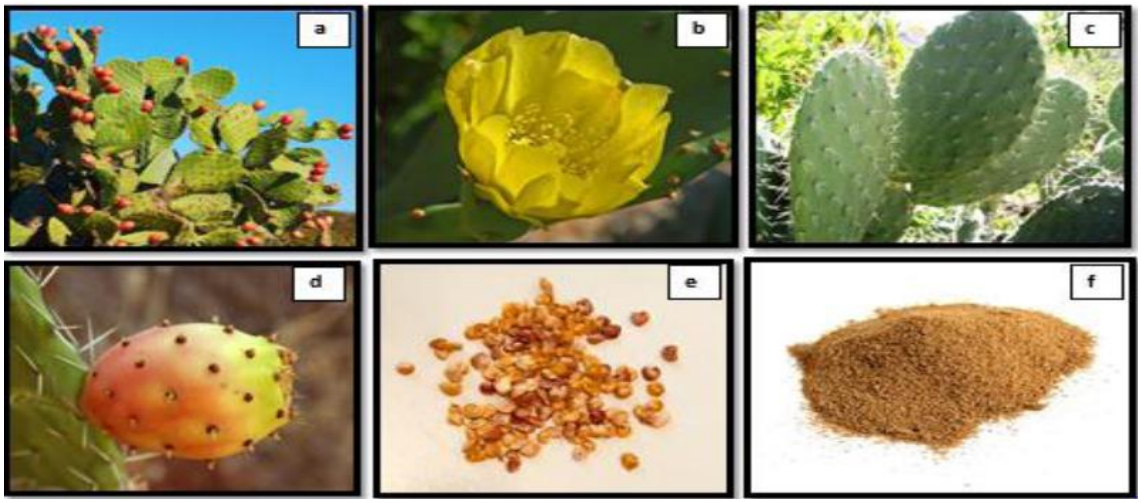

Figure 2 : a) The prickly pear cactus, b) the flower, c) the cladodes, d) the fruits, e) the seeds f) the seeds powder seeds, f) the seed powder

The prickly pear comprises multiple components, with each part delineated below:

-**The seeds**, enclosed within mucilage in the endocarp, are rich in polyunsaturated fatty acids, particularly linoleic and linolenic acids, known for their diverse health benefits **(Chahdoura *et al*., 2014)**. Additionally, the seeds boast the

highest protein levels compared to other fruit parts **(Albergamo *et al.*, 2022**). According to **(Bouaouich *et al.*, 2023).** The protein reserves of the seed are albumins. However, attention has focused mainly on the oils contained in these seeds. The extraction of these oils generates a cake which constitutes up to 90% of the weight of the raw material. This residue is very rich in cellulosic fibers. The other constituent polysaccharides are very rare, even non-existent. **(Nounah *et al.*, 2021).**

-**The cladode**, commonly referred to as "Nopalitos," is flattened, elliptical, or ovoid, green in color, and covered with small spines, varying in size between 17 and 38 cm long by 12 to 26 cm wide and 1.1 to 3.1 cm thick **(Bellumori *et al.*, 2023).**

-**The root system** aids in ground water absorption in low-consistency soils, with robustness to colonize challenging environments and improve the possibility of nitrogen-fixing microorganisms **(Chougui *et al.*, 2013).**

-**The flowers**, hermaphroditic and yellow or orange, are positioned on the sun-exposed side **(Amrane-Abider *et al.*, 2021).**

-**The fruit**, displaying variability in color, size, and shape, is juicy, smooth, sweet, and rich in sugar and vitamin C **(Kartika *et al.*, 2019).**

I.4. Interests and uses

The prickly pear is known for its many interests and uses. *OFI* is a vegetable that has a lot of functional potential **(Iftikhar *et al.*, 2023)**, as its consumption contributes to the intake of dietary fiber, minerals **(Flores *et al.*, 2015),** phenolic compounds **(Martins *et al.*, 2023)** ascorbic acid, and other antioxidant compounds **(Du Toi *et al.*, 2018),** which are related to hypoglycemic, antiobesogenic hypolipidemic and anti-inflammatory effects **(Gouws *et al.*, 2020),** The plant is used to treat heat stroke, sunburn, yellow fever, renal problems, and gastritis **(Socorro *et al.*, 2017)** The leaves prepared in infusion are used as anti-inflammatory. In the form of a poultice, they relieve skin irritations or make swellings disappear. The fruits are used as diuretics **(Fonnegra *et al.*, 2012)**. Fresh

stems are used to treat inflammation **(Ammam *et al*., 2023)**. Fresh stem is used to treat gallbladder, liver, diabetes, back pain, fractures**, (Fonnegra *et al*., 2012).**

Furthermore, *OFI* has gained attention in the food and beverage industry **(Ramadan *et al*., 2021).** Its unique flavor, vibrant color, and nutritional properties make it suitable for various products, including juices, jams, jellies, and even alcoholic beverages. Additionally, the fruit's high content of antioxidants and dietary fiber adds to its appeal **(Martins *et al*., 2023).**

Prickly pear seeds are also of significant industrial interest. They are a rich source of oil, which is extracted for various purposes. The oil is valued for its high content of essential fatty acids, including linoleic and linolenic acids, as well as its unsaponifiable matter. These properties make it valuable for use in cosmetics, skincare products, and pharmaceutical formulations **(Zine *et al*., 2013).**

Moreover, the cladodes, the flattened stems of the prickly pear cactus, have their own industrial applications. They are rich in dietary fiber and contain mucilage, making them appropriate for use in the food industry as a thickening agent or a natural dietary supplement. The cladodes are also explored for their potential as a source of bioactive compounds with antimicrobial and antioxidant properties **(Shoukat *et al*., 2023).**

The prickly pear can be used also as fodder for animals and a fresh or processed vegetable for human consumption **(Tamer *et al*., 2014).** The adaptation of the prickly pear to desert and semi-desert conditions allows it to constitute a crop of undeniable ecological and socio-economic interest. Indeed, it constitutes a shield against desertification and soil erosion. It is also grown for land regeneration. It does not require specialized cultural practices or the addition of fertilizers **(Neffar *et al*., 2012).**

The prickly pear offers various ecological advantages that contribute to its environmental significance. One notable ecological benefit is the clarifying property of its leaves, which can help purify turbid waters. The leaves have the ability to filter out impurities and sediment, aiding in the clarification of water sources and

promoting water quality **(Choudhary *et al.*, 2019).** Moreover, the prickly pear cactus plays a crucial role in combating erosion. Its extensive root system helps stabilize the soil, preventing erosion caused by wind or water. By anchoring the soil and reducing runoff, the cactus helps maintain the integrity of ecosystems and prevents the loss of fertile topsoil (**Monteiro *et al.*, 2023).** In addition to erosion control, the prickly pear cactus serves as a natural barrier against fires. Its succulent and water-filled tissues act as a defense mechanism, making it highly resistant to fire. During wildfires, the cactus can act as a firebreak, slowing down the spread of flames and protecting surrounding vegetation **(Stavi *et al.*, 2022).**

I.5. Prickly pear seeds

Prickly pear seeds, offer significant health and beauty benefits. Rich in essential fatty acids and antioxidants like vitamin E, these seeds are primarily used to produce valuable oil known for its moisturizing and anti-aging properties for the skin. As a dietary supplement, the seeds provide a natural source of fiber, protein, and antioxidants, supporting digestion, reducing oxidative stress, and potentially lowering cholesterol. Their use in the food industry to enrich health and wellness products also highlights their versatility and nutritional value (**Kang *et al.*, 2014).**

I.6. Antioxidant and biochemical components of *OFI*

Antioxidants are bioactive compounds that mitigate cellular damage resulting from oxidative stress by neutralizing or scavenging free radicals. Oxidative stress occurs when there is an imbalance between the production of (ROS) and the body's ability to detoxify or repair the resulting damage. Antioxidants exert their protective effects by donating electrons or hydrogen atoms to unstable free radicals, thereby interrupting chain reactions and reducing the potential for cellular and molecular damage **(Graham *et al.*, 2011).** *OFI* contains different types of antioxidants distributed throughout its various parts as shown in **Table 2**. The table below represents the predominant antioxidants in OFI fruit and their role.

Table 2: The major antioxidants in *OFI* and their role

Antioxidant	Role
Polyphenols	Polyphenols are plant compounds and they help to protect cells from oxidative damage and have been associated with various health benefits, such as reducing inflammation. They are present in the *Opuntia* fruit, cladodes, seeds and flowers **(Iftikhar *et al*., 2023).** .
Flavonoids	These compounds found in many plants and can contribute to protection against cardiovascular diseases and other conditions related to oxidative stress. They exist in the *Opuntia* fruit, cladodes, seeds and flowers **(Eden *et al.*, 2023).**
Carotenoids	These natural pigments, including betacyanins and betaxanthins, give prickly pear its red and yellow colors and temperature reduction. They act as antioxidants and have demonstrated anti-inflammatory properties. They are found in the *Opuntia* fruit, cladodes, and flowers **(Lahmidi *et al*., 2023).**
Beta-carotene	This compound is a precursor to vitamin A and also acts as an antioxidant. It plays an essential role in protecting the skin, eyes, and other tissues from damage caused by free radicals and are found in the *Opuntia* fruit, cladodes, and flowers **(Wannes *et al*., 2021).**
Vitamin C	Prickly pear is an excellent source of vitamin C, a potent antioxidant that helps neutralize free radicals in the body, thereby contributing to cellular health and the immune system. It is present not only in the fruit but also in the cladodes **(Al-Mushhin *et al*., 2021).**
Vitamin E	Vitamin E is a fat-soluble antioxidant that helps protect cell membranes against oxidative damage. It can also contribute to skin health and is present in the seeds **(El Mannoubi *et al*., 2023).**

The chemical composition of different parts of prickly pear depends on several factors, including species, cultivar, climatic conditions, maturity status, and postharvest treatment. The table below illustrates the biochemical composition of each part of the, **(Silva *et al*., 2021)**.

Table 3: Biochemical composition (g/100g) of *OFI* different parts **(Silva *et al.*, 2021).**

Component	*Opuntia ficus indica*			
	Pulp	Seed	Peel	Cladode
Moisture	87 - 94.4	18.0	90.30	94.0
Ash	0.24 - 4.03	10.37	0.29	1.08
Protein	0.08-1.03	3.67	0.14	0.30
Cruds protein		4.78		
Lipids	0.04-0.97	3.00-16.3	0.04-2.43	0.37-1.83
Crude lipids	0.40	5.00		
Total fiber	0.43-5.37	54.2	0.65	2.7
Cruds fiber	1.37 - 4.28	12.47	0.96	5.97
Carbohydrates	92.5			5.63
Starch	4.55	5.35	7.12	0.71
Vitamin c	5.17-33.0			
Ascorbic acid	17.2-29.0		59.8	1.83
Magnesium	1.05-25.0	8.07	1.47	94.1
Sodium	0.06-1.29	0.44	0.11	1.47
Potasium	11.1-158	64.4	9.48	224
Calcium	0.69-40.9	17.3	1.52	177
Zinc	0.07-1.63	4.16	0.13	0.37
Manganese	0.10-4.89	0.83	0.19	0.06

II. General information on fruits juice

II.1. Definition

Fruit juices, known for their fresh flavor, taste, and aroma, are highly valued for their health benefits and nutritional value **(Kaddumukasa *et al.*, 2017)**. It defined as the edible liquid obtained from the parenchyma by physical, mechanical or other appropriate processes (**Codex Alimentarius, 1992).**

There are different types of juice; among these types:

-Fruit juice: The liquid extracted from fruits, often through pressing or squeezing**.**

-Fruit drink: A beverage that contains a percentage of fruit juice but is typically diluted with water and may include added sugars or sweeteners.

-Fruit nectar: A beverage made by blending fruit pulp or puree with water and sometimes sweeteners.

- Smoothies: Thick, creamy drinks made by blending fruit with yogurt, milk or juice.

- Fruit Infusions: Drinks made from fruit infused in water, creating a light, flavored option (**Codex alimentarius, 1992).**

II.2. Juice deterioration

The quality of juices can be adversely affected by various factors, such as temperature, light, and microbiological contaminations significantly alter the physicochemical parameters and storage stability of the juices, leading to the deterioration of their organoleptic and physicochemical qualities and chemical changes (enzymatic and non-enzymatic reactions, and chemical interactions). As a result, consumers may reject the product **(Kaddumukasa *et al.*, 2017).** The main pathways of the rapid deterioration of fresh fruit juice are summarized in diagram below:

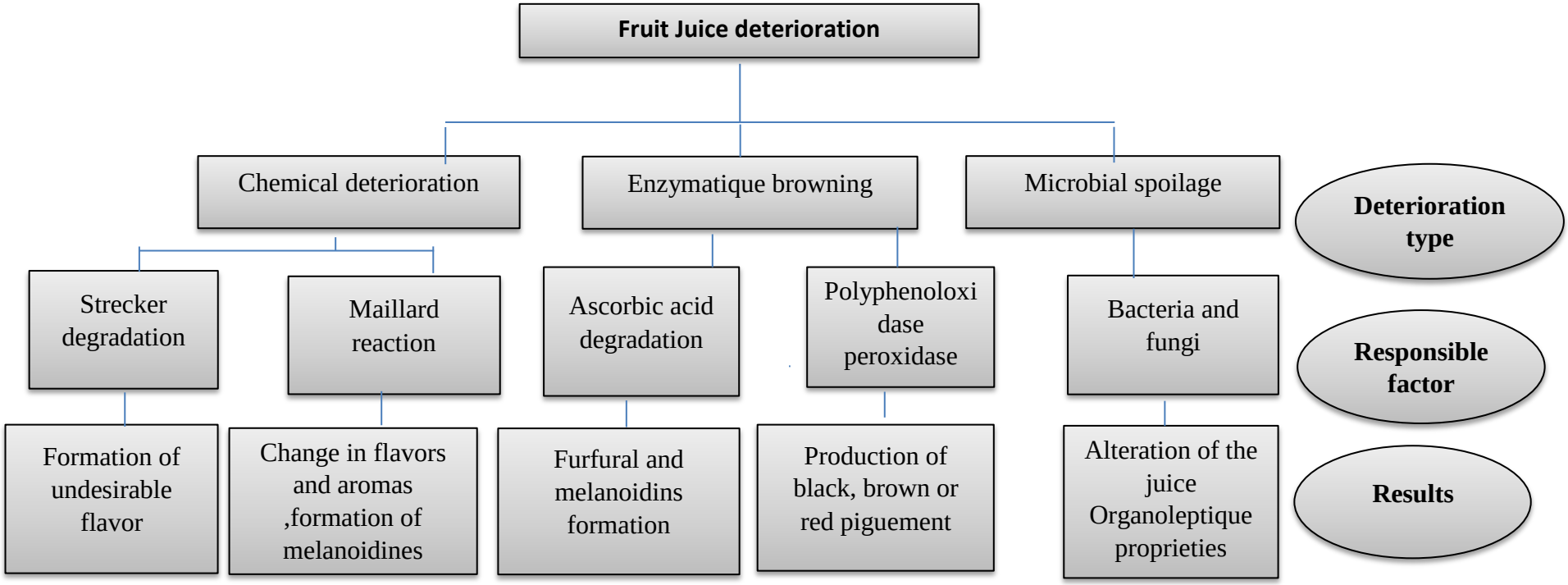

Figure 3: The main deterioration routes of fresh fruit juice during storage **(Kaddumukasa *et al*., 2017).**

The preservation of fresh fruit juice during storage is a complex process influenced by various factors, however different methods can be used to preserve juices and maintain its quality for example:

-Thermal treatments: including pasteurization, sterilization, and ultra-high temperature processing, have been used to reduce microbial counts to safe levels, eliminating health risks and ensuring food safety ,but this treatment can lead to the degradation of vitamins and nutriments **(Aghajanzadeh *et al*., 2023).**

-Cold treatments: including refrigeration and freezing although it may result in changes in taste and bacterial load **(Rodríguez *et al*., 2017)**.

-High-Pressure Processing (HPP): Applying high pressure to eliminate bacteria, yeasts, and molds without heat, preserving colors and nutrients, this treatment can cause microbial resistance, alteration of texture and taste **(Xia *et al*., 2023; Tribst *et al* ., 2022).**

-Dehydration: this method consists of removing water content to inhibit the growth of microorganisms and extend shelf life, but can cause alteration of taste and aroma **(Nono *et al*., 2002)**.

-Adding Preservatives: The addition of preservatives, whether, natural or chemical, such as citric acid or ascorbic acid, to inhibit microbial growth, is also a common method. However, these treatments often compromise the nutritional quality of the product, resulting in undesirable flavors, oxidative degradation, and losses of vitamins **(Wong *et al*., 2023).**

Consumers are increasingly seeking of fruits juices that have undergone various treatments for preservation can exhibit several undesirable effects affecting their quality **(Sevindik *et al*., 2021).** The demand for healthy, fresh fruit juice has prompted the utilization of natural agents to protect and enhance the juices quality. This is accomplished with the goal of creating juice products that are safe, nutritious, and environmentally sustainable **(Zeeshan *et al*., 2019).**

II.3. Juice fortification

Fortification or enrichments is the process of adding one or more nutrients into a food **(Kiros *et al*., 2016).**

Includes various methods aimed at enhancing the nutritional content of juice:

-Vitamin and Mineral Enrichment: The process of increasing the content of essential vitamins and minerals in a food product to enhance its nutritional value this can involve adding specific nutrients that may be deficient in the original product **(Barnokhon *et al.*, 2022).**

-Fortification with nutrient powders: The addition of powdered forms of essential nutrients, including vitamins and minerals, to a food product. Nutrient powders are often used in fortification to provide a concentrated and easily dispersible form of key nutrients, contributing to the overall nutritional profile of the food **(Nguyen *et al*., 2016).**

-Protein Fortification: The process of increasing the protein content in a food product, typically through the addition of protein-rich ingredients or supplements. This fortification strategy is employed to enhance the protein quality of the food, making it a more substantial source of this essential macronutrient **(Khulal *et al*., 2021)**

-Fortification with Functional Ingredients: The incorporation of specific functional ingredients into a food product to provide health benefits beyond basic nutrition. These ingredients may include bioactive compounds, antioxidants, or other substances that contribute to physiological well-being, offering a dual purpose of both nutritional enhancement and potential health promotion **(Ahmad *et al*., 2022).**

-Fiber Enrichment: The process of increasing the fiber content in a food product by adding additional sources of dietary fiber. Fiber enrichment aims to improve the product's digestive and nutritional qualities **(Tomic *et al*., 2017; Cerniauskiene *et al*., 2014).**

III. Extraction enhanced by microwave

The first works using microwaves to extract organic compounds were published by **Ganzler *et al*., 1986.** Since then, microwave-assisted plant extraction has been the fruit of numerous research and patents. Microwave assisted extraction this relatively recent method combines the use of microwaves, with the conventional method of solvent extraction **(Destandau *et al*., 2022).** The energy delivered to the medium is absorbed and converted into thermal energy **(Chavez *et al*., 2013).** Microwaves increase the temperature of the solvent and the plant, increasing the extraction kinetics. Initially, due to the increase in temperature and pressure within the sample, the extractable substances separate from the substrate, allowing the solvent diffusing into the substrate to solubilize them **(Nithya *et al*., 2023).**

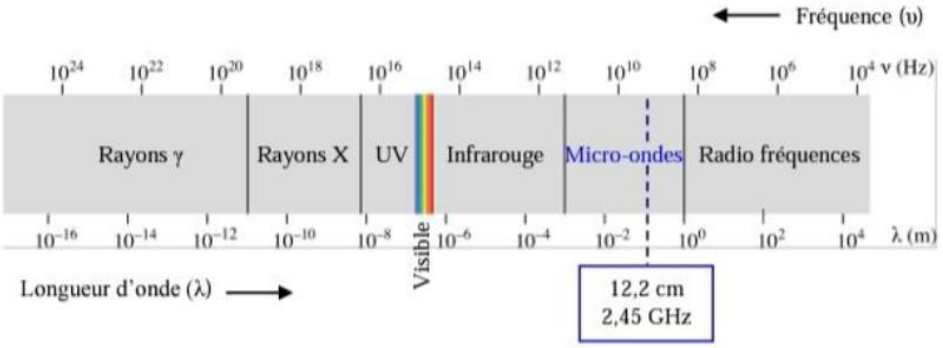

Figure 4: Representation of the electromagnetic spectrum and the placement of domestic microwave ovens.

This method offer advantages, such as the increase in the possibility of reducing the quantities of toxic solvents used, that of reducing the operating costs **(Chaari *et al*., 2024)**, the energy required and the consumption of solvents, which makes a more environmentally friendly technology **(Ajila *et al*., 2010).**

IV. Application of Response Surface Methodology in extraction processes

Response Surface Methodology is a statistical and mathematical technique used for optimizing processes and experimental designs. It is commonly employed in various fields, including chemistry, engineering, and manufacturing **(Petchimuthu *et al.*, 2023).**

RSM involves creating a mathematical model that represents the relationship between multiple independent variables (factors) and the response variables of interest. The goal is to find the optimal combination of factor levels that maximizes or minimizes the response variable, depending on the objective of the study **(Setyani *et al*., 2023).**To implement RSM, a series of experiments were conducted with different combinations of factor levels. The data obtained from these experiments are then used to fit a mathematical model, typically a polynomial equation that describes the relationship between the factors and the response variables. **(Gao *et al*., 2023).** Once the mathematical model is developed, various statistical techniques are applied to analyze and interpret the data. These techniques include analysis of variance (ANOVA), which assesses the significance of the factors, and their interactions, as well as graphical tools such as response surface plots and contour plots that visualize the relationship between the factors and the main advantages of RSM are its ability to identify the optimal factor levels, determine the significance of factors, and explore the interactions between factors. It allows for efficient experimentation by reducing the number of experiments required compared to traditional one-factor-at-a-time approaches **(Azizi *et al*., 2023).**

RSM has many advantages; its primary benefits stem from the efficiency it offers in optimizing processes, allowing researchers to achieve optimal results with fewer experiments, thus translating to significant cost savings in terms of time, resources, and materials **(Veza *et al*., 2023).** RSM's ability to unveil interaction effects between variables provides crucial insights into system behavior, and its visual representation tools, such as response surface plots, facilitate a clearer understanding of relationships between factors **(Susaimanickam *et al*., 2023).** Moreover, RSM includes model validation methods, ensuring the accuracy of generated mathematical models, and allows for robustness testing, with its widespread applicability, reduction of experimental error, time-saving capabilities, and scientific rigor, RSM emerges as a versatile and indispensable methodology for designing, optimizing, and understanding complex processes across various domains **(El-taweel *et al*., 2023).**

Experimental part

Chapter I

I. Optimization of *OFI* seeds extract

I.1. Sampling

I.1.1. Sampling Zone

The fruits were harvested in Hassenaoua, exactly (Ain Hamra), department of Bordj Bou Arreridj (Latitude: 36°15′44’81″S, Longitude: 4°79′13’85″W) towards the end of August in 2019; 2020 and 2021, This fruit known as Hendi, was in maturity phase characterized by nuanced color of yellow-orange and purple, the fruits were picked carefully with gloves because they were very thorny. The fruit has an elongated form, with Re =33%.

I.1.2. Sample preparation

About 10kg of fruits were stripped of their thorns, washed, and then peeled to recover the seeds, the cohesion between the seeds is ensured by the mucilage and the fibers contained in the pulp, the fruits were then kneaded with an electric mixer (SEB 500 Watt) to facilitate the separation of seeds from the pulp with a colander and running water to eliminate all the mucilage, the seeds were arranged in a single layer on a plate and exposed to sunlight (with a daily temperature of 30°C) for about one day and then ground in an electric grinder (SEB 500W) to obtained powder, the latter was passed through standard sieve 0.02Um and stored in an airtight box until use.

I.1.3. Climatic conditions of the exchange region

The climate is Mediterranean, cold in winter with temperatures between 2°C and 12°C, and very hot in summer with temperatures between 25°C and 40°C, with peaks that can exceed 45°C. Precipitation averages 30 to 50 mm per month from November to February and is almost absent in summer, with less than 5 mm per month.

I.2. Extraction process (MAE)

A domestic microwave oven (CMW-A2602, condor, Algeria) with cavity dimensions of 22.5 cm × 37.5 cm × 38.6 cm and 2450 kHz was used. The oven contained a digital control system for irradiation time and microwave power (the

microwave was linearly adjustable from 100 to 1000W). The latter was modified in order to condensate into the sample the vapors generated during extraction.

A different amount of seed powder was placed in a round bottom flask of 250 mL (medium neck of 45 mm) containing water/acetone at different percentage (v/v). The extraction was carried under different power of microwave, after variable time. The samples extracts were separated by centrifugation at 3000 rmp (SIGMA 3-30ks) for 15 min at the end they were stored at 4°C until use.

I.3. Preliminary experiment (Factor by factor extraction)

The initial step of the preliminary experiment involved assessing the individual influences of each parameter while maintaining a constant relationship among them to streamline the overall experimental process. Parameters such as solvent type (water, ethanol, methanol, and acetone), solvent concentration, extraction power, irradiation time, and sample/solvent ratio were selected based on prior research (**Yolmeh *et al*., 2014; Liu *et al* ., 2013**), given their significant impact on extraction yield. Variables that were not directly studied were held constant at specific values (500W for extraction power, 0.5g/20 ml for sample/solvent ratio, and a 50% water/acetone mixture) during this phase. The outcomes of these controlled conditions were used to enhance precision in subsequent studies using the Response Surface Methodology (RSM).

I.3.1. Effect of solvent nature

The extraction time was fixed at 200s, microwave power at 500W, and ratio at 0.5 g/20ml; samples were extracted using different solvents: 50% acetone (v/v), 50% ethanol (v/v), 50% methanol (v/v), and water. The choice of solvent for extraction plays a crucial role in determining the efficiency and selectivity of the extraction process. Different solvents have varying polarities and interactions with the target compounds, which can affect the extraction efficiency and the profile of extracted compounds.

I.3.2. Effect of solvent concentration

The effect of solvent concentration was investigated using the selected best solvent from the initial step. The samples were extracted using different concentrations of the solvent, namely 20, 40, 60, 80, and 100% (v/v), while keeping the extraction time, temperature, and ratio fixed at 200 seconds, 500W, and 0.5 g/20 ml, respectively.

I.3.3. Effect of sample/solvent ratio

Samples were extracted using the best solvent and the best solvent concentration. The extraction procedure was repeated by varying the sample/solvent ratio 0.2, 0.4, 0.6, 0.8, 1, 1.2 g/20ml, while fixing the extraction time and the microwave power at 200s and 500W.

I.3.4. Effect of extraction time

The effect of extraction time on the samples was investigated using the previously determined optimal solvent, solvent concentration, and ratio, the extraction time was varied from 120 seconds to 200 seconds, with intervals of 20 seconds (60, 80, 100, 120, 140, 160, 180, and 200 s), while keeping the microwave power fixed at 500W.

I.3.5. Effect of microwave power

The effect of microwave power on the extraction process was investigated using the optimal combination of solvent, solvent concentration, ratio, and extraction time. The samples were subjected to extraction at different microwave power levels ranging from 100 to 1000W, specifically at 100, 300, 500, 700, 900, and 1000W.

I.4. Response Surface Methodology

I.4.1. Experimental design

Four independent variables was employed for optimization with respect to four important reaction variables the extraction solvent concentration x1, ratio x2, irradiation time x3 and microwave power x4, while response variable were TPC and antioxidant activity.

I.4.2. Experimental procedure

The optimization of TPC and DPPH extraction conditions was determined using the RSM by employing a Box-Behnken design with four levels to evaluate the combined effect of four independent variables: solvent concentration, ratio, power, and irradiation time, designated as x1, x2, x3, and x4, respectively. These variables are displayed in **Table 8**. The coded value of 0 represents the central point of the variables and was repeated for experimental error. Factorial points were coded as ±1 these parameters were studied to optimize two responses: the TPC and DPPH, according to the following formula:

$$\mathbf{N = 2k\ (k–1) + Cp}.$$

Where: **N**: is the number of experiments. **k**: is the number of factors. **Cp**: is the number of central points.

A total of 27 experiments were conducted to estimate the mathematical model for the investigated responses **(Bezerra *et al*., 2008).**

I.4.3. Model verification

The optimal conditions for the extraction of TPC and DPPH, depending on the solvent composition, power, and extraction time, were obtained using the predictive equations of RSM. Experimental and predicted values were compared to determine the validity of the model. The RSM allows modeling the studied responses in the form of a second-degree polynomial equation presented below:

$$\boldsymbol{Y = B_0 + \sum_{i=1}^{K} BiXi + \sum_{i=1}^{K} Biix^2 + \sum_{i\geq 1}^{K} BijXiXj}$$ Where:

Y: represents the studied response (in our case, Y represents TPC, DPPH).
B_O: is a constant.

Bi, **B**ii, and **B**ij**:** are coefficients for the linear, quadratic, and interaction terms respectively. **xi** and **xj** represent the coded independent variables.

I.5. Statistical analysis

All assays were conducted in triplicate, and the results are expressed as the mean. The influence of factors on the yield of TPC and DPPH in the experiment

was statistically evaluated using analysis of variance (ANOVA) with the least significant difference test. JMP software (Version 14) was used to construct the Box-Behnken design experimental plan for the analysis of all results.

Chapter II

II. Study of the antioxidants compounds and biological proprieties of the prickly pear seeds

II.1. Determination of antioxidant substances

II.1.1. Total phenolic compounds

The TPC of the juice samples was determined by the method using the Folin-Ciocalteu reagent **(Adesegun *et al.*, 2007).** An aliquot of 100 µl of the extract was mixed with 800 µl of Folin-Ciocalteu (10%) and 400 µl of sodium carbonate (7%). After 30 min of incubation at room temperature, the absorbance was measured at 760 nm against the blank. The result was expressed in mg (GAE) per 100g DM of seeds by referring to the calibration curve.

II.1.2. Total flavonoids content

The TFC of the juice samples was determined by a colorimetric method **(Ayoola *et al.*, 2008).** 2 ml of juice was added to 2 ml of aluminum trichloride reagent $AlCl_3$ (2% in pure methanol). The absorbance was recorded at 420 nm after 10 min incubation at room temperature against the blank. The result was expressed in mg (QE) per 100gDM of seeds by referring to the calibration curve.

II.1.3. Determination of condensed tannins

The Determination of condensed tannins was based on the butanol/HCl mixture **(Porter *et al.*, 1994).** 400 µl of extract was mixed with 2 ml of ferrous sulphate acid solution (7.7 mg ferric ammonium sulphate $Fe^2 (SO_4)^3$ dissolved in 50 ml (n-butanol; HCl 3;2(v.v.)). After incubation at 95 °C for 15 minutes, the absorbance was measured at 530 nm. Results were expressed as mg cyanidin-3-glucoside equivalents/100 g DM.

II.2. Evaluation of antioxidant activity

II.2.1. DPPH radical scavenging capacity

The DPPH radical scavenging capacity was evaluated according to the method described by **Brand-Williams *et al.* (1995).** A volume of 200 µl of the sample was added to 1 ml of a methanolic solution of DPPH (60 µM). Absorbance was measured at 517 nm after 30 min incubation at room temperature and in the dark.

The result was expressed in mg galic acid equivalent (GAE) per 100g DM of seeds by referring to a calibration curve.

II.2.2. Ferric reducing antioxidant power

The ferric-reducing antioxidant power was evaluated according to the method described by **Oyaizu (1986).** A volume of 2.5 ml of the juice sample was mixed with 2.5 ml of phosphate buffer (0.2M; pH 6.6) and 2.5 ml of potassium ferricyanide (1%). After 20 min incubation at 50°C, 2.5 ml of trichloroacetic acid solution (10%) was added. A volume of 2.5 ml of the reaction mixture was diluted with distilled water (v/v) and then added with 500 µl of ferric chloride solution (0.1%). The absorbance was measured at 700 nm and the result was expressed in mg (GAE) per 100g DM of seeds referring to a calibration curve.

II.2.3. Hydrogen peroxide scavenging test

Hydrogen peroxide scavenging activity was determined by the method of **(Ruch *et al.*, 1989).** In hemolysis tubes, 50 µl of extract was added to 1ml of H_2O_2 (40mM) in phosphate buffer and 1450µl phosphate buffer solution (0.1mM, pH 7.4), the mixture was incubated for 10 minutes and the absorbance was read at 230nm. The control was prepared in the same way except that the extract was replaced by acetone 60%. The hydrogen peroxide scavenging activity was calculated using the following formula

$$\textbf{Scavenging activity (\%)} = [(Ac-At) / Ac] \times 100$$

Where Ac and At were absorbance of the control and the sample (DM of seeds), respectively.

II.2.4. β-carotene bleaching test

The method described by **(Kartal *et al.*, 2007).** β-carotene/linoleic acid emulsion was prepared by dissolving 2 mg of β-carotene in 1 ml of chloroform, then adding 25 µl of linoleic acid and 200 mg of tween 40. The chloroform is completely evaporated in a rotavapor, 100 ml of oxygen-saturated distilled water is added and the resulting emulsion is stirred vigorously. To 2.5 ml of the previous mixture (β-carotene/linoleic acid emulsion) 350 µl of extract were added, three

replicates were made. Test tubes were incubated in the dark at laboratory temperature. Two control tubes were also prepared using the same procedure, one containing a reference antioxidant BHT dissolved in methanol (2 mg/ml) (positive control) and the other without antioxidant (negative control) where the sample is replaced by 350 µl of 60% acetone. The kinetics of emulsion discoloration in the presence and absence of antioxidant was monitored at 490 nm at regular time intervals for 48 hours all assays were performed in triplicate. The relative antioxidant activity (RAA) of the extracts is calculated according to the following equation:

$$\boldsymbol{RAA}\% = \frac{\boldsymbol{Abs48h(sample)}}{\boldsymbol{Abs48h(BHT)}} \times \mathbf{100}$$

Where **RAA** and Abs were the relative antioxidant activity and absorbance of sample (DM of seed) after 48 hours, respectively.

II.3. Anti-inflammatory activity

Inflammation is a response to pathogens and tissue injury. Cellular damage or pathogen-associated molecular patterns (PAMPs) expressed by microbes are recognized by immune cells (macrophages, leukocytes, neutrophils, and mast cells), which are thus drawn to the site of injury. These cells then release various inflammatory mediators, which include cytokines, histamine, nitric oxide, leukotrienes and prostaglandins **(Tavares *et al*., 2023).**

II.3.1. Inhibition BSA denaturation

Anti-inflammatory activity was determined in vitro by heat denaturation of BSA according to the method described by **Kandikattu *et al*. (2013).** A volume of 1 ml of the extract was mixed with 1 ml of bovine serum albumin solution (0.2%) prepared in Tris-HCl buffer (50 mM, pH 6.6). The tubes were then heated to 37 °C for 15 min and then to 72 °C for 5 min. The absorbance was measured at 660 nm using a UV-visible spectrophotometer after cooling to room temperature. The experiment was performed in triplicate. (VOLTARENE®) was used as standard. The protective effect of the samples against BSA denaturation was presented as inhibition percentages calculated according to the following formula.

$$I\% = \frac{Ac-As}{Ac} \times 100$$ Where

I: The inhibition percentage

AS: absorbance of the test sample

AC: absorbance of control

II.3.2. Statistical analysis

The results (n=3) were subjected to a two-factor analysis of variance. Mean values were compared using Fisher's test ($p<0.05$). All statistical analyzes are carried out using Infostat®.

II.4. Correlation between antioxidant activities, inhibition percentage and bioactive compounds

The correlation is a statistical measure that evaluates the relationship between two variables **(Sedgwick, 2012).**

In this study the correlation between bioactive compounds and antioxidant activities, also the inhibition percentage was investigated, the Pearson correlation coefficient was used to calculate the correlation, which measures the strength and direction of the linear relationship between two continuous variables. The Pearson correlation coefficient varies from -1 to 1**(Sedgwick, 2012),** where:

1 indicates a perfect positive correlation: as the value of one variable increases, the value of the other variable also increases.

-1 indicates a perfect negative correlation: as the value of one variable increases, the value of the other variable decreases.

0 indicates no linear correlation between the variables.

II.5. Antimicrobial activity

II.5.1. Antibacterial activity

This is an in vitro method to assess the antibacterial power of the compounds. The technique used was the well method **(Mouas *et al.*, 2017)**. The bacterial strains chosen for this study are pathogenic bacteria involved in.

Table 4: Bacterial strains used and their pathogenic powers

Strains	GRAM	Pathogenic power
Bacillus cereus	+	- Food contamination (vegetable origin such as rice, spices)
Enterococcus faecalis	+	- Chronic intestinal inflammation - Bladder and prostate infections
Proteus mirabilis	-	- Urinary tract infections - Cysts and acute pyelonephritis
Salmonella typhimirium	-	-Typhoid fever - Gastroenteritis
Pseudomonas aeruginosa	-	- Septicemia, infant meningitis, surgical wound infections, gastroenteritis - Abdominal pain and bloody diarrhea
Esherichia coli	-	Septicemia, infant meningitis, surgical wound infections, gastroenteritis - Abdominal pain and bloody diarrhea
Staphylococcus aureus	+	- Hospital-acquired infections - Abscesses, wound infections, septicemia, pneumonia, food poisoning - Potentially fatal infections in humans
Micrococcus liteus	+	- Bacteremia - Infections associated with ventricular shunts - Cases of abscesses and Pneumonia

These strains were provided to us by the microbiology and phytopatology laboratory at Mohamed El Bachir El Ibrahimi University (BBA). They were maintained by subculturing on nutrient agar medium conducive to their growth in the dark for 24 hours at 37°C.

II.5.2. Antifungal activity

The antifungal activity of the dried extract was determined against five fungi: *Phytophthora infestans, Aspergillus parasiticus, Penicillium* sp*, Trichoderma* sp*, Fusarium* sp, and two yeasts: *Candida albicans, Candida glabrata.*

II.5.3. Preparation of culture media

-Mueller-Hinton this is the culture medium used to study antibacterial activity because it is the most commonly used medium for antimicrobial susceptibility testing.

-**Sabouraud agar** for the isolation and maintenance of yeasts and studying their sensitivity to the extracts.

II.5.4. Sterilization of equipment

Distilled water, test tubes used in the preparation of bacterial solutions, and wattman filter paper discs (6 mm in diameter) wrapped in aluminum foil were sterilized in an autoclave at 121°C for 15 minutes.

II.5.5. Preparation of OFI seeds extract dilutions

OFI extracts were dissolved in (DMSO) to prepare different concentrations through successive dilutions. The concentration of the dry extract stock solution was 110 mg/ml. The solutions were prepared with agitation and refrigerated until use**.**

Table 5: Different concentrations of acetone extract and corresponding DMSO (mg/ml)

C	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8
ES	110	55	27.5	13.75	6 .87	3.43	1.71	0.85
VOL	400	200	100	50	25	12.5	6.247	3.123
DMSO	0	200	300	350	375	387.5	393.753	396.877

II.5.6. Standardization and preparation of the inoculum

After thawing the preserved microbial strains and letting them reach room temperature, a few well-isolated and identical colonies are picked from young pre-cultures of each bacterial strain to be tested, using a platinum loop. The loop was then dipped into sterile physiological water to release the bacteria. The bacterial suspension was thoroughly homogenized, and its opacity should be equivalent to 0.5 McFarland or an (OD) of 0.08 to 0.10 at 625 nm. The inoculum can be adjusted by adding either more culture (colonies) if it is too weak or sterile physiological water if it is too dense.

II.5.7. Inoculation

Petri dishes containing MH medium (for bacteria) and PDA medium (for fungi) or Sabouraud medium (for yeasts) were aseptically inoculated by streaking

the entire surface of the medium with a sterile swab that has been soaked in the microbial suspension (using 24-hour-old bacteria).The swab was streaked back and forth over the entire agar surface in tight zigzag patterns. The procedure was repeated three times, rotating the plate each time and ensuring the swab was rotated as well. Finish the inoculation by swabbing around the periphery of the medium **(Athamena *et al.*, 2010).**

II.5.8. Well diffusion method

After the plates have dried, wells are created using sterile Pasteur pipettes. The cavities thus formed are filled with agar, and then the extract was added to each well (approximately 25 µL per well, with each well having a different concentration). The plates are sealed with parafilm and allowed to cool **(Athamena *et al.*, 2010; Bouyahya *et al.*, 2017).**

II.5.9. Incubation

The Petri dishes were incubated for 24 hours at 37°C for bacteria and 48 hours at 35°C for yeast and fungi. The results were evaluated by measuring the diameter, in mm, of the zone of inhibition. The experiment was repeated three times for each extract and each bacterial species, and the experimental results were expressed as the mean of the obtained values ± the standard deviation.

II.5.10. Interpretation

The interpretation was done by measuring the diameters of the inhibition zones around the discs using a caliper. The results were expressed as the diameter of the inhibition zone and could be represented by symbols based on the sensitivity of the strains to the extracts.

The bacteria were classified into the following categories **(Mouas *et al.*, 2017).**

Non-sensitive (-) or resistant: diameter < 8 mm.
Sensitive (+): diameter between 9 and 14 mm.
Highly sensitive (++): diameter between 15 and 19 mm.
Extremely sensitive (+++): diameter > 20 mm.

II.6. Statistical analysis

The results (n=3) were subjected to a two-factor analysis of variance. Mean values were compared using Fisher's test ($p<0.05$). All statistical analyzes are carried out using Infostat®.

Chapter III

III. Evaluation of the stability of enriched prickly pear juice

III.1. Sampling

The sample was collected from the same region described in chapter I, About 10kg of prickly pear were stripped of their thorns, washed, and then peeled to recover the pulp, the fruits were then kneaded with an electric mixer (SEB 500 Watt) to facilitate the separation of seeds from the pulp with a colander and running water to eliminate all the mucilage the pulp recuperated was packed and frozen at -18°C until use, the fruits pulp was centrifuged (SIGMA 3-30KS) at 3000 rpm for 10 min. The filtrate recovered, constituting the juice, was split into two batches. The first batch was enriched with the hydrosoluble extracts of prickly pear seeds (100 mg/l), while the second batch was considered as a control. Both lots were stored at 10, 20, and 30°C. Samples for analysis were taken after 2, 4, 6, 8, 10, and 12 days.

A volume of 20 mL of the seeds extract was passed through a rotary evaporator to evaporate the solvent. After 1 hour, the recovered sample was dried in an oven at 40°C. After 48 hours, a dry extract of the seeds (hydrosoluble extract), was obtained, which would be used for fortifying the *OFI* juice.

III.2. Determination of physicochemical parameters

III.2.1. Hydrogen Potential

The measurement of hydrogen potential (pH) was performed using a pH meter that has been calibrated before the experimental process.

III.2.2. Titratable acidity

The determination of the TA consists in placing in a beaker 10 ml of sample (juice) with a few drops of color indicator (0.1% phenolphthalein in pure ethanol). The reaction mixture was titrated with 0.1N NaOH solution until obtained a persistent pink color. The results were calculated according to the following equation:

$$\mathbf{TA}\ (\%) = \frac{\mathbf{N_{NaOH}\ x\ V_{NaOH}\ x\ M_{citric\ acid}}}{\mathbf{V_{sample}\ x\ 3\ x\ 10}}$$

N_{NaOH}: molar concentration of N_{aOH},
V_{NaOH}: volume of N_{aOH},
$M_{citric\ acid}$: molar mass of citric acid,
V_{sample}: volume of sample.

The division by 3 because citric acid is triacid (requires three molecules of NaOH to neutralize one molecule of citric acid); while the division by 10 is to express the results relative to 100 ml of juice **(Méndez *et al*., 2015).**

III.2.3. Total soluble solid

The total soluble solids in a solution were measured with a refractometer. After placing a drop of juice on the surface of the glass plate, the value indicated represents the degree of brix expressed in percentage (%).

III.2.4. Browning index

The browning index was determined according to the method reported by **Meydav *et al.* (1977)**. The samples were centrifuged (824×g, 18°C, 20 min); the recovered supernatants were diluted with ethanol (v/v) and then filtered through Whatman N°2 paper. The absorbance was measured at 420 nm.

III.3. Determination of antioxidant substances

III.3.1. Total phenolic compounds

The TPC of the juice samples was determined by the method using the Folin-Ciocalteu reagent **(Adesegun *et al*., 2007).** An aliquot of 100 µl of the extract was mixed with 800 µl of Folin-Ciocalteu (10%) and 400 µl of sodium carbonate (7%). After 30 min of incubation at room temperature, the absorbance was measured at 760 nm against the blank. The result was expressed in mg (GAE) per 100 ml of juice by referring to the calibration curve.

III.3.2. Total flavonoids content

The TFC of the juice samples was determined by a colorimetric method **(Ayoola *et al*., 2008).** 2 ml of juice was added to 2 ml of aluminum trichloride reagent $AlCl_3$ (2% in pure methanol). The absorbance was recorded at 420 nm after 10 min incubation at room temperature against the blank. The result was expressed in mg (QE) per 100 ml of juice by referring to the calibration curve.

III.4. Evaluation of antioxidant activity

III.4.1. DPPH radical scavenging capacity

The DPPH radical scavenging capacity was evaluated according to the method described by **Brand-Williams *et al*. (1995).** A volume of 200 µl of the sample was added to 1 ml of a methanolic solution of DPPH (60 µM). Absorbance was measured at 517 nm after 30 min incubation at room temperature and in the dark. The result was expressed in mg gallic acid equivalent (GAE) per 100 ml of juice by referring to a calibration curve.

III.4.2. Ferric reducing antioxidant power

The ferric-reducing antioxidant power was evaluated according to the method described by **Oyaizu (1986).** A volume of 2.5 ml of the juice sample was mixed with 2.5 ml of phosphate buffer (0.2 M; pH 6.6) and 2.5 ml of potassium ferricyanide (1%). After 20 min incubation at 50 °C, 2.5 ml of trichloroacetic acid solution (10%) was added. A volume of 2.5 ml of the reaction mixture was diluted with distilled water (v/v) and then added with 500 µl of ferric chloride solution (0.1%). The absorbance was measured at 700 nm and the result was expressed in mg (GAE) per 100 ml of juice referring to a calibration curve.

III.5. Antimicrobial activity

III.5.1. Preparation of dilutions

From the initial suspension (prickly pear juice), decimal dilutions were carried out under aseptic conditions.

III.5.2. Detection and enumeration of total and fecal coliforms

A volume of 1 ml of sample was placed in empty petri dishes prepared for this purpose and numbered. Then approximately 20 ml of medium (VRBG) was poured. The tests were carried out in duplicate. A series of plates were incubated at 37°C for 24h. This will be used to detect total coliforms , the other series was incubated at 44°C for 48 hours for the detection of fecal coliforms.

III.5.3. Research and enumeration of yeasts and molds

1ml of juice was brought into a sterile and numbered petri dish. Then approximately 15 ml of medium (sabauraud) was poured. The medium was homogenized with the sample by 8-shaped movements. The tests were carried out in duplicate. The boxes were incubated at 25°C for 05 days.

III.5.4. Detection and enumeration of total and fecal coliforms

A volume of 1 ml of the juice sample was placed in empty petri dishes prepared for this use and numbered. Then about 20 ml of medium (VRBG) was poured in. The tests were carried out in duplicate. A series of dishes were incubated at 37°C. For 24 h. this will be used for the search for total coliforms, the other series was incubated at 44°C, for 48 hours for the search for fecal coliforms.

III.5.5. Search and enumeration of yeasts and molds

1 ml of juice was brought to a sterile and numbered petri dish. Then about 15 ml of medium (sabauraud) was poured. Homogenization of the medium with the sample was made by 8-shaped movements. The tests were carried out in duplicate. The dishes were incubated at 25°C for 5 days.

III.6. Statistical analysis

The results (n=3) were subjected to a two-factor analysis of variance. Mean values were compared using Fisher's test ($p<0.05$). All statistical analyzes are carried out using Infostat®.

Results and Discussion

Chapter I

I. Optimizing *OFI* seeds extract processing

I.1. Preliminary tests

Same letters in the same column refers to means not statistically different according to ANOVA and Tukey's test.

Table 5: Results of single-factor experiments for microwave assisted extraction from *OFI* seed

The variable factors	TPC yield (GAE/100gDM)		DPPH yield (GAE/100gDM)
Solvant type	Water 100%	113.29 ^{c} ±0.002	26.98^{c} ±0.01
	Ethanol 50%	128.58 ^{b} ±0.006	24.22 ^{b} ±1.2
	Methanol 50%	128.15^{b} ±0.001	23.76 ^{b} ±0.03
	Acetone 50%	152.87 ^{a} ±0.04	21.04^{a} ±0.7
Solvent concentration	20%	83.42^{b} ±0.008	30.12^{b} ±0.09
	40%	86.89^{b} ±0.004	28.59^{b} ±0.5
	60%	100.27 ^{a} ±0.002	23.06 ^{a} ±1.03
	80%	119.32 ^{a} ±0.001	11.58 ^{a} ±0.2
	100%	51.85^{c} ±0.09	30.84^{c} ±0.09
Ratio	0.2g	87.08 ^{b} ±0.007	40.75^{b} ±0.02
	0.5g	110.11^{a} ±0.004	5.09^{a} ±0.07
	0.7g	88.06 ^{b} ±0.06	11.35 ^{b} ±0.9
	0.8g	85.71 ^{b} ±0.03	18.20 ^{b} ±1.01
	0.9g	15.78^{c} ±0.04	32.15^{c} ±1.04
	1.2g	20.34^{c} ±0.1	24.04^{c} ±0.3
Irradiation time	60s	123.30 ^{c} ± 0.01	21.12^{c} ±1.03
	80s	124.65^{c} ±0.02	17.01^{c} ±0.008
	100s	116.64^{c} ±0.03	13.22^{c} ±0.3
	120s	126.18^{c} ±0.09	22.59 ^{c} ±1.02
	140s	133.68 ^{b} ±0.4	11.66 ^{b} ±0.06
	160s	146.48 ^{b} ±0.01	10.15 ^{b} ±0.5
	180s	163.34 ^{a} ±0.003	9.57^{a} ±0.2
	200s	142.30 ^{b} ±0.008	17.87^{b} ±0.4
Microwave power	100W	87.23^{c} ±0.001	27.47^{c} ±0.01
	300W	90.21^{c} ±0.02	28.20^{c} ±1.02
	500W	113.03 ^{b} ±0.05	12.06 ^{b} ±0.06
	700W	136.15^{a} ±0.002	33.88^{a} ±0.2
	900W	138.72^{a} ±0.001	29.03^{a} ±0.5
	1000W	140.12 ^{a} ±0.09	28.56^{a} ±0.03

I.1.1. Effect of solvent type

The choice of solvent type has a significant impact on the extraction of bioactive compounds from *OFI* seeds. In this study, different solvents including acetone, ethanol, methanol, and water were compared.

Among the solvents tested, acetone showed the highest levels of TPC in *OFI* seeds, with a value of 152.87±0.04mg GAE/100g DM of seed. Additionally, acetone exhibited strong antioxidant activity; with a value of 21.04±0.7mg GAE/100g DM measured using the DPPH assay. These results were consistent with a previous study by **Bachir Bey *et al*. (2013),** which also found acetone to be the most effective solvent for phenolic extraction from *OFI* seeds. Ethanol was the second most efficient solvent, yielding TPC levels of 128.58±0.006 mg GAE/g and antioxidant activity of 24.22±1.2 mg GAE/g. On the other hand, extraction using methanol and water showed relatively weaker results compared to acetone and ethanol, indicating lower solubility and extraction efficiency for phenolic compounds from *OFI* seeds **(Segneanu *et al*., 2013).**

Based on these findings, acetone was selected as the optimal solvent for the extraction of bioactive compounds from *OFI* seeds, considering its superior performance in terms of TPC levels and antioxidant activity.

I.1.2. Effect of solvent concentration

The concentration of acetone used as a solvent in the extraction process significantly influences the recovery of total phenolic content TPC and antioxidant activity **(Tamjid *et al*., 2023)**. It was observed that using pure acetone (100% concentration) resulted in the weakest extraction results for both TPC and antioxidant activity. This could be attributed to the absence of polarity and the low solubility of antioxidants in pure acetone. Similar findings were reported in previous studies, such as the extraction of polyphenols from grape seed using aqueous ethanol solution **(Zehentbauer *et al*., 2014).** On the other hand, when acetone concentrations ranging from 20% to 80%, higher TPC and antioxidant activity values were obtained, specifically, concentrations of 80% acetone showed

the highest results, with TPC 119.32±0.001mg GAE/100g DM and antioxidant activity 11.58±0.2mg GAE/100g DM. These results align with the findings of **Zhao *et al.* (2006),** who reported that 80% acetone was more effective than 80% ethanol, 80% methanol, or water for phenolic extraction from barley.

A concentration of 80% acetone appears to be favorable for achieving higher yields of bioactive compounds. The polarity and solubility properties of acetone in this range contribute to enhanced extraction efficiency.

I.1.3. Effect of the sample/solvent ratio

The sample/solvent ratio has a notable impact on the recovery of phenolic compounds and antioxidant activity in *OFI* seed extracts. The investigation of different ratios was based on their influence on phenolic recovery and antioxidant activity. The results demonstrated that using a ratio of 0.5g/20ml yielded the highest concentrations of TPC and antioxidant activity, with TPC concentration of 110.11±0.004 mg GAE/100 DM and an antioxidant activity of 5.09±0.07mg GAE/100 g DM. It was observed that the antioxidant activity increased as the ratio was changed from 0.2 to 0.6g/20ml. However, a ratio of 0.8g/20ml did not exhibit any significant effect on antioxidant activity or TPC. These findings align with the range of results reported by **Bachir bey *et al.* (2013)** that the optimal sample/solvent ratio concentration may vary depending on the specific plant material and extraction method used. Optimizing the ratio is crucial for achieving maximum phenolic recovery and antioxidant activity in *OFI* seed extracts. By selecting the appropriate ratio, researchers can enhance the extraction efficiency and obtain extracts with higher bioactive compound concentrations **(Tan *et al.*, 2011).**

I.1.4. Effect of irradiation time

The effect of extraction time on the TPC and antioxidant levels in *OFI* seeds was investigated within a range of 60 to 200 seconds. The results indicated that longer extraction times resulted in higher TPC and antioxidant levels, with concentrations ranging from 116.64±0.03 to 163.34±0.003mg GAE/100g DM for TPC and from 9.57±0.2 to 22.59±1.02mg GAE/g DM for antioxidants, respectively

(Chaari *et al*., 2024). This suggests that increasing the extraction time allows for a more efficient extraction of bioactive compounds from *OFI* seeds, leading to higher TPC and antioxidant activity. The longer extraction time provides more opportunity for the phenolic compounds to be released from the seed matrix and transferred into the solvent **(Spigno *et al*., 2007).** In the case of grape pomace, for example, **Pinelo *et al* (2005)** found that a temperature of 50°C was optimal for TPC extraction.

I.1.5. Effect of microwave power

The microwave power has a significant effect on the efficiency of TPC extraction and antioxidant activity from *OFI* seeds. Increasing the microwave power from 500 W to 1000 W, resulted in higher TPC and antioxidant activity levels. This indicates that higher microwave power enhances the extraction efficiency of bioactive compounds from *OFI* seeds **(Shang *et al*., 2020).** These findings align with the results reported by **Lasunon *et al*. (2021)**, who also used microwave extraction methods and observed an increase in TPC and antioxidant activity with higher microwave power. The increase in microwave power can contribute to more efficient and rapid extraction by promoting the release and diffusion of phenolic compounds from the seed matrix. The higher energy provided by the microwave power aids in breaking down the plant tissue, facilitating the extraction process and increasing the yield of bioactive compounds **(Lasunon *et al*., 2021).** Therefore, optimizing the microwave power level was crucial in achieving higher TPC and antioxidant activity in *OFI* seed extracts **(Shang *et al*., 2020).**

I.2. Optimization by RSM

The optimization of antioxidant extraction from *OFI* seeds was performed using RSM with the objective of maximizing the TPC and antioxidant activity. Preliminary tests were conducted to select the parameters to be investigated, including the choice of acetone as the extraction solvent, concentration ranging from 40 to 80 ml acetone/water, ratio ranging from 0.2 to 0.8 g/20ml, extraction time or irradiation time ranging from 140 to 200s, and microwave power from 500 to 1000W. **Table 6** present the results of TPC and antioxidant activity obtained from the experiments, as well as the corresponding predicted values based on the

Box-Behnken design. The predicted values were generated using the mathematical model developed through RSM. By comparing the experimental and predicted values, the accuracy and reliability of the model can be assessed, providing insights into the effectiveness of the optimization process.

The results in **Table 6** will help in determining the optimal combination of parameters that yield the highest TPC and antioxidant activity in *OFI* seeds.

Table 6: Results of the 27experemental essay

NBR	1/-1	X1	X2	X3	X4	Observed TPC	Predicted TPC	Observed DPPH	Predicted DPPH
1	+1	40	0.5	750	200	502.49	518.00	232.59	221.68
2	+1	60	0.2	750	200	386.76	360.35	159.76	138.68
3	-1	60	0.5	1000	140	504.69	557.23	51.92	76.86
4	+1	80	0.8	750	170	615.04	625.67	79.73	94.51
5	-1	80	0.2	750	170	426.89	443.12	154.54	160.86
6	-1	60	0.5	500	140	466.57	473.58	162.76	163.43
7	+1	60	0.8	1000	170	459.81	478.94	160.39	163.58
8	+1	60	0.8	750	200	502.12	512.03	181.65	179.19
9	-1	40	0.5	740	200	461.19	449.91	169.23	162.29
10	+1	80	0.5	750	140	497.38	493.35	177.1	173.05
11	-1	60	0.8	750	140	247.42	263.85	74.45	69.47
12	-1	40	0.8	750	140	260.29	283.96	78.52	76.88
13	+1	80	0.5	500	170	455.54	461.99	192.84	189.66
14	+1	60	0.2	1000	170	520.69	521.54	215.95	201.56
15	0	60	0.5	750	170	878.25	889.79	53.84	59.65
16	+1	60	0.2	500	170	398.57	404.52	171.62	166.22
17	+1	80	0.5	700	140	367.81	379.56	149.45	163.77
18	-1	60	0.2	750	140	444.09	458.54	172.36	201.02
19	-1	40	0.5	500	170	276.65	323.67	121.22	97.28
20	-1	60	0.5	500	200	269.26	218.97	88.12	78.52
21	+1	60	0.5	1000	200	568.44	529.78	175.09	175.48
22	+1	80	0.5	1000	170	595.61	558.92	183.13	186.29
23	0	60	0.5	750	170	834.37	832.53	42.96	44.53
24	+1	60	0.5	500	170	466.82	466.95	147.56	151.89
25	-1	40	0.2	750	170	492.52	482.29	169.84	174.18
26	0	60	0.5	750	170	486.19	882.29	74.1	74.18
27	-1	40	0.5	1000	170	468.15	482.29	178.61	174.18

The results of the study indicate that the TPC of *OFI* seeds ranged from 247.42 to 878.25mg GAE/100g DM, while the antioxidant activity varied between 42.96 to 221.68 mg GAE/g DM (as shown in **Table 6**).

Furthermore, the observed values plotted against the predicted values demonstrate that the model's predicted values align well with the measured responses. All the values for TPC and antioxidant activity fall within the confidence interval, indicating the accuracy and reliability of the models. This suggests that the developed models based on the experimental data effectively capture the variations in TPC and antioxidant activity, and can be used to predict and optimize the extraction process for maximum phenolic content and antioxidant activity in *OFI* seeds **(Pali *et al* ., 2023).**

The analysis of variance makes it possible to calculate a statistical parameter R^2 which is the ratio of the sum of the squares of the calculated responses (corrected to the mean) by the sum of the squares of the measured responses (corrected to the mean). In the present study, the R^2 values were 0.95 and 0.93 for the TPC and DPPH models respectively. In other words, the explanatory powers of the TPC and DPPH models were respectively 95 and 93% and only 5 and 7% of the variations of the two models have not been explained. When the coefficient of determination R^2 is very close to 1, the models are highly significant.

I.2.1. Response modeling

According to **Table 7,** the analysis of the variance of the model showed that the latter was very significant ($p<0.001$) and that the lack of adjustment was not significant ($p>0.05$); this indicates that the models were satisfactory, (in other words, these two models have strong powers of explanation of the experimental results). It should be noted that when the p-value of the lack of fit of a given model is significant, this model will be rejected **(Bachir Bey *et al*., 2014).**

Table 7: Regression coefficient, standard error, and Student's t-test results of response surface for antioxidant activity (mg GAE/100 g DM) and TPC

Source	Degrees of freedom	Sum of squar	Mean squar	F ratio	Prob. > F
TPC(mgGAE/100gDM)					
Model	14	282311.14	20165.1	16.5519	
Error	12	14619.50	1218.3		
Total Corrected	26	296930.64	1429.97	8.9428	<0.0001*
Lack of Fit	10	14299.696			
Pure Error	12	319.802	159.90		
Total Error	12	14619.498			0.1047
R^2	0.950765				
Adjusted R^2	0.893323				
TPC(mgGAE/100gDM)					
Model	14	46389.696	3313.55	11.0767	
Error	12	3589.756	299.15		
Total Corrected	26	49979.452			<0.0001*
Lack of Fit	10	3551.2888	355.129	18.4641	
Pure Error	2	38.4669	19.233		
Total Error	12	3589.7557			0.0524
R^2	0.928175				
Adjusted R^2	0.84438				

Source	Degrees of freedom	Sum of squar	Mean squar	F ratio	Prob. > F
DPPH(mgGAE/100gDM)					
Model	14	282311.14	20165.1	16.5519	
Error	12	14619.50	1218.3		
Total Corrected	26	296930.64			<0.0001*
Lack of Fit	10	14299.696	1429.97	8.9428	
Pure Error	2	319.802	159.90		
Total Error	12	14619.498			0.1047
R^2	0.950765				
R^2adj	0.893323				
DPPH(mgGAE/100gDM)					
Model	14	46389.696	3313.55	11.0767	
Error	12	3589.756	299.15		
Total Corrected	26	49979.452			<0.0001*
Lack of Fit	10	3551.2888	355.129	18.4641	
Pure Error	2	38.4669	19.233		
Total Error	12	3589.7557			0.0524
R^2	0.928175				
R^2adj	0.84438				

I.2.2. Linear effect

The concentration of the solvent with the same p-value <0.0001 for TPC, DPPH with a probability of P <0.0005 for TPC and with P <0.0067 for the DPPH. These factors influence in a highly significant way the values of TPC and DPPH. However, the Interaction effect, these results were consistent with the findings of **Thanh *et al.* (2017).** The current study's results show that the Solvent Concentration*Ratio interaction was significant for DPPH only with a p<0.0131. And was not significant for TPC. Concerning the other interaction combinations ((x1-x2), (x2-x3), (x1-x4), (x2-x4), (x3-x4)), there were no significant effect, p>0.05.

I.2.3. Quadratic effect

The linear effects of the concentration of the solvent and the ratio on TPC and DPPH were highly significant with p-values less than 0.0001 for the concentration of the solvent and p-values less than 0.0005 for the ratio for TPC, and p-values less than 0.0067 for the ratio concentration for DPPH. These factors have a significant impact on the values of TPC and DPPH **(Ilaiyaraja *et al.*, 2015).** In terms of quadratic effects, the concentration squared (concentration$^{\wedge 2}$) has a highly significant effect on TPC and DPPH with a p-value of 0.0001. However, the quadratic effects of other factors such as ratio squared, power squared, and time squared were not significant with p-values greater than 0.0005. These results highlight the influence and importance of solvent concentration compared to other factors, while the effects of extraction time and irradiation power are not significant (**Arvindekar *et al.*, 2016).** To determine the significance of the quadratic model, ANOVA analysis was conducted. The results of the analysis, as presented in **Table 9**, demonstrate the significance of the model with significant F values and p values. The determination coefficient (R^2) of 0.95 for TPC and 0.93 for antioxidant capacity indicates a good fit of the model. The adjusted determination coefficient (R^2adj) of 0.89 for TPC and 0.84 for antioxidant activity suggests that the adjusted model is also highly significant **(Ondrejovič *et al.*, 2012).** The regression coefficients from the experimental data and the adjusted values show a high degree of correlation between the observed and predicted values. Additionally, the low

coefficient of variation indicates a high degree of precision in the model **(Eyenga *et al*., 2020).**

Table 8: ANOVA table for the effect of acetone concentration, time, ratio, and power on TPC extraction and antioxidant activity (mg GAE/ 100g DM)

Terme	Estimation	Standard Error	t ratio	Prob. > |t|
Constant	482.28	20.15	23.93	<0.0001*
Concentration (60,80)	-98.86	10.07	-9.81	<0.0001*
Ratio (0.2,0.8)	-47.30	10.07	-4.69	0.0005*
Power (500,1000)	18.56	10.07	1.84	0.0902
Time (140,200)	15.88	10.07	1.58	0.1408
Concentration*Ratio	31.52	17.45	1.81	0.0960
Concentration*Power	-20.91	17.45	-1.20	0.2538
Ratio*Power	-11.20	17.45	-0.64	0.5328
Concentration*Time	-5.83	17.45	-0.33	0.7441
Ratio*Time	1.32	17.45	0.08	0.9410
Puissance*Time	0.65	17.45	0.04	0.9706
Concentration*Concentration	-133.12	15.11	-8.81	<0.0001*
Ratio*Ratio	-8.849	15.11	-0.59	0.5691
Puissance*Power	-28.97	15.11	-1.92	0.0793
Time*Time	23.60	15.11	1.56	0.1443
Terme	**Estimation**	**Standard Error**	**t ratio**	**Prob. > |t|**
Constant	482.28	20.15	23.93	<0.0001*
Concentration (60,80)	-98.86	10.07	-9.81	<0.0001*
Ratio (0.2,0.8)	-47.30	10.07	-4.69	0.0005*
Power (500,1000)	18.56	10.07	1.84	0.0902
Time (140,200)	15.88	10.07	1.58	0.1408
Concentration*Ratio	31.52	17.45	1.81	0.0960
Concentration*Power	-20.91	17.45	-1.20	0.2538
Ratio*Power	-11.20	17.45	-0.64	0.5328
Concentration*Time	-5.83	17.45	-0.33	0.7441
Ratio*Time	1.32	17.45	0.08	0.9410
Puissance*Time	0.65	17.45	0.04	0.9706
Concentration*Concentration	-133.12	15.11	-8.81	<0.0001*
Ratio*Ratio	-8.84	15.11	-0.59	0.5691
Power*Power	-28.97	15.11	-1.92	0.0793
Time*Time	23.60	15.11	1.56	0.1443

The four studied parameters, solvent concentration, time, microwave power and ratio on TPC extraction and antioxidant activity, were found to have positive linear effect. The quadratic effects of the solvent; microwave power and ratio, also the interaction between solvent-time influenced negatively antioxidants extraction.

The quadratic effects of the solvent; microwave power and ratio, also the interaction between solvent-time influenced negatively antioxidants extraction.

The quadratic effects of the solvent; microwave power and ratio, also the interaction between solvent-time influenced negatively antioxidants extraction. However interaction terms between solvent concentration-microwave power and time–ratio and quadratic effect of time for both TPC and antioxidant activity as well as interaction term between solvent concentration and time for TPC were found to have no effects.

I.2.4. Mathematical models

In the present study, a second-degree polynomial model was employed to describe the relationship between the factors (concentration of the solvent, irradiation power, extraction time, and ratio) and the extraction of bioactive substances (TPC and antioxidant activity). The significance of each coefficient and the intensity of interaction for each parameter were determined using p-values, with values below 0.05 indicating statistical significance and a stronger influence **(Zhang *et al*., 2016).**

The mathematical model developed in this study accurately captures the observed phenomenon. Based on the analysis, the optimal experimental conditions can be represented by the following relationship for TPC:

TPC = 482.28 - 98.864x1 - 47.30x2 - 133.12x12

Similarly, for DPPH, the optimal experimental conditions can be represented by:

DPPH = 174.183 - 47.247x1 - 16.337x2 - 25.16x1x2 - 42.687x12

Here, x1 represents the concentration of the solvent, x2 represents the irradiation power, and x12 represents the interaction between the two factors. The coefficients (a0, linear coefficients, interaction coefficients, and quadratic coefficients) provide information about the influence of each factor and their interactions on the extraction of TPC and DPPH.

By utilizing the mathematical model and the derived equations, researchers can determine the optimal conditions for maximizing the extraction of bioactive substances and fine-tune the extraction process accordingly **(Eyenga *et al*., 2020).**

I.2.5. Determination and experimental validation of the optimal conditions

In order to validate the predictive capacity of the model, the optimal conditions were determined by maximizing desirability using the JMP (14) prediction profiler **(Ye *et al.*, 2000).** The results of the maximized conditions were then used for an extraction test to TPC and antioxidant activity.

The optimal conditions identified for achieving the highest extraction of phenolic compounds from *OFI* seeds and maximizing antioxidant activity were as follows: acetone concentration of 59.03%, microwave power of 762.23W, ratio of 0.54g/20ml, and extraction time of 168.52s. Subsequently, under these optimal conditions, the experimental values for TPC and antioxidant activity were determined. The TPC value was measured as 905.74±0.50 mg GAE/100g DM while the antioxidant activity was determined as 248.40±1.06mg GAE/100g DM. These experimental results were found to be in agreement with the predicted values obtained from the model, which were 906 mg and 246mg GAE/100g DM, respectively.

The close alignment between the experimental and predicted values further supports the reliability and accuracy of the model in determining the optimal conditions for extracting phenolic from *OFI* seeds and achieving maximum antioxidant activity.

I.2.6. Spatial representation and analysis of response surface models

The 3D response surface is the graphical representation of the regression equation. It represents the relationship between the responses and the experimental levels of each variable and the type of interactions between two test variables **(Ye *et al.*, 2000).**

Figure 5 illustrates the three-dimensional representation of the effects of solvent concentration and ratio concentration on the extraction of TPC and DPPH from prickly pear seeds. It can be observed that the solvent concentration and the ratio exert a significant effect on the TPC content and the DPPH.

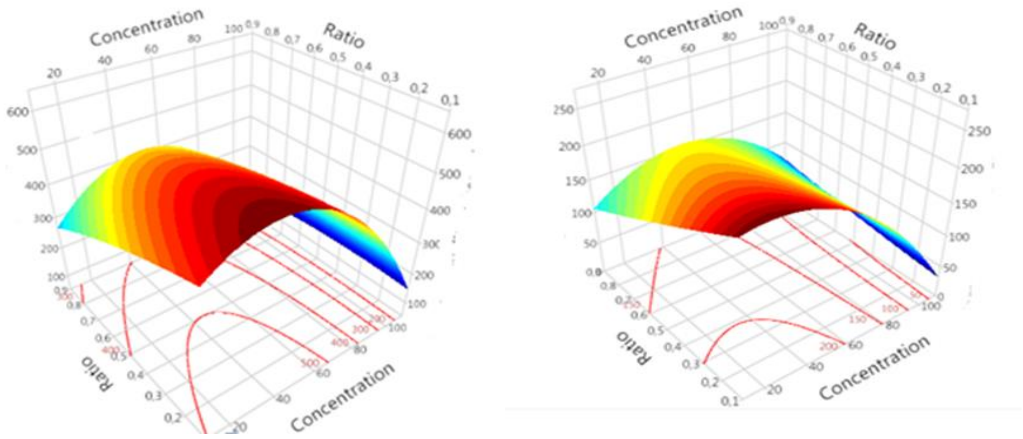

Figure 5: Effect of solvent concentration and solid liquid extraction of TPC and DPPH prickly pear seeds

Examination of the graphs in **Figure 5** showed the existence of the optimal levels of solvent concentration and ratio for TPC and DPPH. This is due to the quadratic and linear effect of the solvent concentration and the linear effect of the ratio. The estimate of the quadratic coefficients of the solvent concentration is statistically significant for the TPC and the DPPH.

The choice of solvent for extraction processes depends on various factors, including the solubility of the matrix, the interaction between the matrix and the solvent. It is crucial to ensure that the samples are fully submerged during extraction, requiring a sufficient volume of solvent **(Goti *et al.*, 2023).**

Polarity plays a significant role in the extraction of antioxidants. Increasing the concentration of acetone in the solvent reduces its polarity, favoring the extraction of less polar components **(Cheok *et al.*, 2012).** Moreover, higher acetone concentrations promote the degradation of cell membranes, improving solvent permeability in the solid matrix **(Pinelo *et al.*, 2005).**

Figure 6 represents the effects of solvent concentration and irradiation power on TPC and DPPH of prickly pear seeds. It can be noted that the solvent concentration and the potency exert a significant effect on the TPC content and the DPPH. In addition, examination of graphs shows the existence of the optimal concentration levels for TPC and DPPH. This is due to the quadratic and solvent

concentration effect on TPC and DPPH. Quadratic and linear effect of the power was not observed in the same way as for the interaction between the two variables.

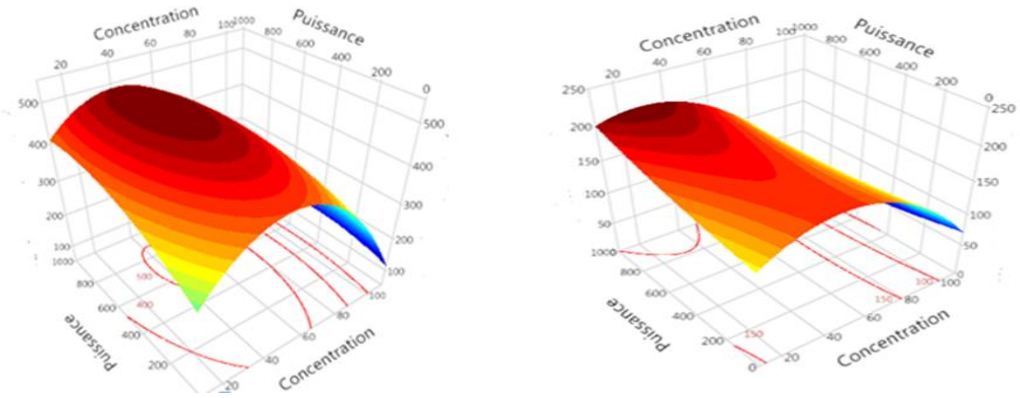

Figure 6: Effect of solvent concentration and power on the extraction of TPC and DPPH from prickly pear seeds

Extraction processes conducted at higher microwave power have been found to enhance the solubility of solutes and improve the diffusion coefficient, thereby promoting extraction efficiency **(Alupului *et al.*, 2012).**

The application of high microwave power helps to soften plant tissues and weaken interactions between phenols and proteins or polysaccharides, facilitating the diffusion of polyphenols into the solvent **(Garcia-Vaquero *et al.*, 2020).** However, it is important to note that the effect of using microwave power on phenolic extraction has limitations, the stability of these compounds begins to decrease, leading to adverse effects on their antioxidant activity, excessive heat can cause degradation or chemical transformations of the phenolic compounds, diminishing their overall antioxidant potential **(Alupului *et al.*, 2012).**

Figure 7 represent the three-dimensional graph showing the effects of solvent concentration and irradiation time on TPC extraction and DPPH, respectively

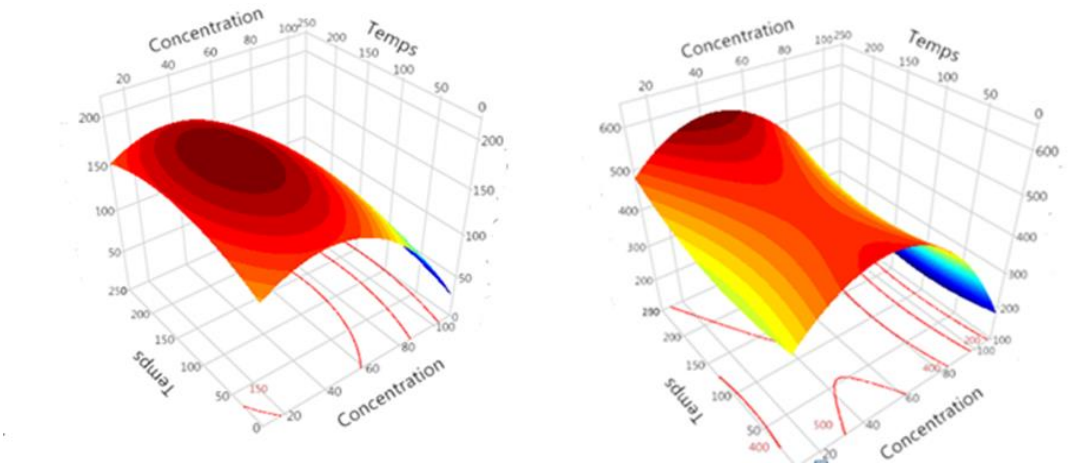

Figure 7: Effect of solvent concentration and time on the extraction of TPC and DPPH from prickly pear seeds

From these figures, it was observed that the concentration of the solvent and the extraction time exert a significant effect on the TPC content and the DPPH, The solvent concentration plays a crucial role in determining the efficiency of compound dissolution, with higher concentrations often resulting in enhanced extraction yields due to increased solubility of target compounds **(Spigno *et al*., 2007).** Additionally extraction time has a direct impact on the kinetics of compound release, as prolonged extraction periods allow for more complete diffusion of TPC and DPPH into the solvent **(Lasunon *et al*., 2021),** this is due to the quadratic effect of the solvent concentration and the irradiation time is slightly affected for DPPH. The estimation of the coefficients for the quadratic form of the solvent concentration was statistically significant; however the irradiation time was not statistically significant for the TPC and DPPH. However, the interaction between the two factors was not observed for both variables.

According to **Figure 8** represents the effect of power and irradiation time on the extraction of TPC and DPPH. It is observed that the power and the irradiation time slightly influence the extraction of the TPC and the DPPH.

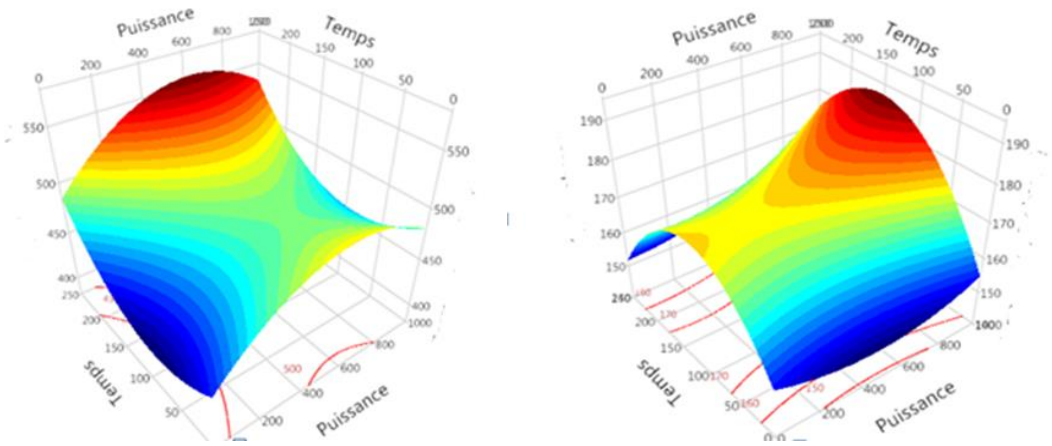

Figure 8: Effect of power and time on the extraction of TPC and DPPH from prickly pear seeds

Upon examining the graphs in **Figure 8**, it was evident that there were not optimal levels of concentrations for total phenolic content and DPPH. This was because there was an absence of the quadratic effect of power and extraction time on these parameters. The estimation of the quadratic coefficients for power and irradiation time was statistically insignificant for TPC and DPPH. Elongated extraction time allows for prolonged contact between the plant material and the solvent, facilitating the diffusion of phenolic compounds into the solvent. This can lead to increased extraction yields as more compounds have the opportunity to be released from the plant matrix **(Ozdemir *et al.*, 2023).** On the other hand, microwave power plays a crucial role in enhancing the extraction process by providing the energy necessary to break down cell walls and promote the release of phenolic compounds. Higher microwave power levels can result in more efficient extraction due to the increased thermal effects **(Lasunon *et al.*, 2021).**

According to **figure 9** represented the effects of the ratio and the power on the extraction of the TPC and the DPPH.

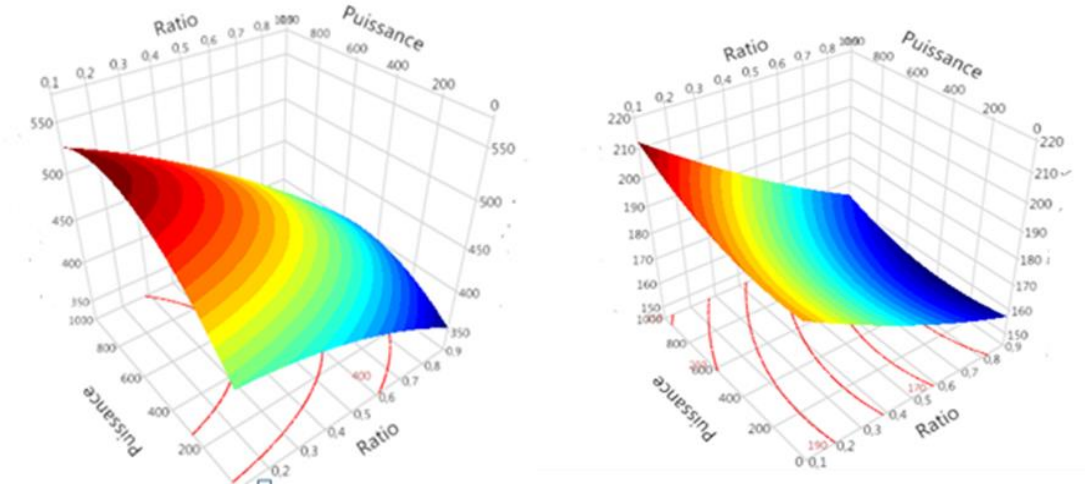

Figure 9: Effect of ratio and power on the extraction of TPC and DPPH from prickly pear seeds

From **figure 9**, the irradiation power influences the extraction of the TPC and the DPPH. However, the extraction ratio plays a crucial role in determining the concentration and yield of phenolic compounds and antioxidant activity in the final extract **(Ozdemir *et al*., 2023).** A higher extraction ratio, involving a larger amount of plant material relative to the solvent, can potentially lead to enhanced extraction yields due to a greater availability of phenolic compounds for dissolution **(Zahoor *et al* .,2023).** However, an excessively high ratio might also lead to reduced efficiency due to steric hindrance, and inadequate contact between the solvent and plant material. Conversely, a lower extraction ratio could result in incomplete extraction **(Tan *et al*., 2011).** Therefore, finding the optimal extraction ratio is essential to ensure a balance between the amount of phenolic compounds extracted and the efficiency of the extraction process from *Opuntia* deeded. However the ration does not affect the extraction of TPC and the DPPH. Examination of the graphs in **Figure 9** did not show the existence of optimal concentration levels for TPC and DPPH. This was due to the absence of the quadratic effect of ratio and power. The estimation of the quadratic coefficients of the ratio and irradiation power was not statistically significant for TPC and DPPH **(Lin *et al*., 2020).** However, the interaction between the two factors was not observed.

According to **figure 10** represented the effects of the ratio and temple on the extraction of the TPC and the DPPH,

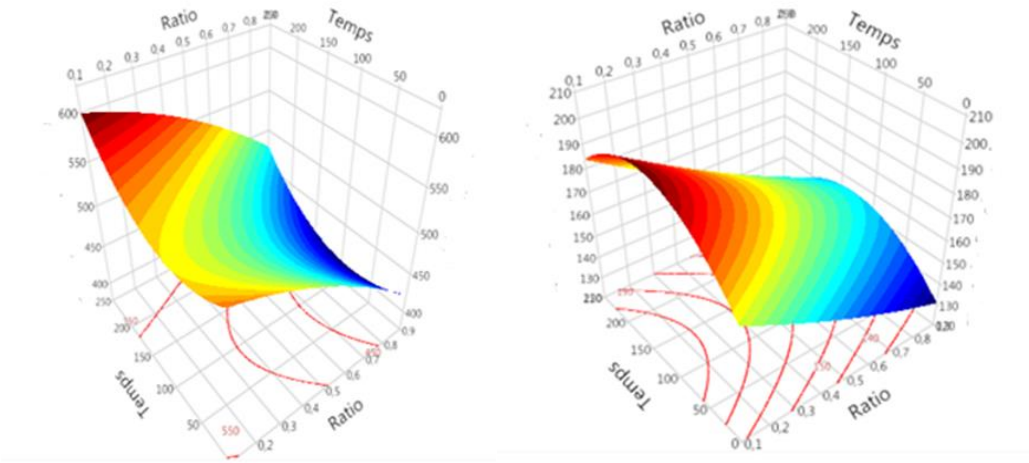

Figure 10: Effect of ratio and time on the extraction of TPC and DPPH from prickly pear seeds

The extraction of TPC and DPPH from prickly pear seeds was not significantly influenced by the ratio of seeds and the extraction time. This can be attributed to the absence of a quadratic effect of the ratio and extraction time. The estimation of the quadratic coefficients for the ratio and temperature was not statistically significant for both parameters. Furthermore, the interaction between these two factors was not observed for TPC and DPPH. The ratio determines the concentration of bioactive compounds in the extraction mixture, impacting the yield of TPC and DPPH. Longer extraction times allow for a more thorough diffusion of these compounds into the solvent, potentially leading to higher extraction yields. Finding the right balance between the ratio of seeds to solvent and the extraction time is crucial for maximizing the recovery of valuable TPC and DPPH from *OFI* seeds **(Barros *et al.*, 2023).**

Chapter II

II. Study of the antioxidants compounds and biological proprieties of the prickly pear seeds

II.1. Determination of bioactive compounds

The results of the determination of antioxidant activities, including total phenolic compounds, flavonoids, and condensed tannins, in prickly pear seed extracts are summarized in **Table 12** These compounds were determined spectrophotometrically using different procedures.

Table 9: Contents of total phenolic compounds, flavonoids and condensed tannins in prickly pear seeds

Dosages	Content
TPC (mgGAE/100g)	905.71±0.50
Flavonoïdes (mg QE/100g)	50.77±0.08
Condensed tannins (mg CE/100g)	98.99±0.19

II.1.1. Polyphenols

Based on the absorbance values of the extract reacted with the Folin-Ciocalteu reagent showed in **Table 9**, the TPC was determined to be 905.71±0.50 mg GAE/100g DM. This value was double the concentration reported by **Cardador-Martinez *et al*. (2011)** for the same species in cultivars of Mexican origin, which ranged from 337 to 460 mg GAE/100g. The difference in the reported values could be attributed to variations in the type of solvent used or differences in storage conditions. The solubility of phenolic compounds can be influenced by the polarity of the solvent, the degree of polymerization of the polyphenols, as well as interactions with other plant compounds and the formation of insoluble complexes. A similar observation was made by **Chaalal *et al*. (2013)** for ground seeds of *OFI*, where they found different results for red and yellow species (298.29 mg GAE/100g and 316.46 mg GAE/100g, respectively). This variability in total phenolic content could be attributed to the ripening degree of the fruits.

II.1.2. Flavonoids

The assay results obtained for *OFI* seeds **Table 11** showed a flavonoid content of 50.77±0.08 mg EQ/100g DM. Our results demonstrated a higher flavonoid

content compared to the study conducted by **Cardador-Martinez *et al*. (2011) and Khatabi *et al*. (2016)** for prickly pear seeds, which reported values ranging from 46 to 50 mg EQ/100g DM. For the whole fruits of *OFI*, the flavonoid content was found to be 17.81±0.10 ECa mg/kg for red fruits and 15.03±1.36 ECa mg/kg for yellow fruits. The variation in flavonoid content among studies could be attributed to factors such as fruit color or the year of harvest. Additionally, it should be noted that flavonoid content is often expressed using different equivalent standards (quercetin, rutin, catechin), and the choice of standards used can influence the final result in addition to the aforementioned factors.

II.1.3. Condensed Tannins

The determination of condensed tannins in prickly pear seeds was performed using a colorimetric method that involves the oxidative cleavage of proanthocyanidins with ferrous sulfate **(Vermerris *et al.*, 2007).** The results presented in **Table 11** showed a concentration of 98.99±8.19 mg/100g DM for condensed tannins in prickly pear seeds. This value was lower than the range reported by **Cardador-Martinez *et al*. (2011),** which was between 137 and 205 mg ECa/100g DM. Similar observations were made for the tannin content of seeds from other species, such as oranges, where the condensed tannin levels varied depending on fruit characteristics. Additionally, physico-chemical parameters, including heat and conductivity, can influence the degree of condensed tannins present in the samples **(Moulehi *et al*., 2012).**

Overall, the results of the antioxidant analyses indicate that prickly pear seeds are a rich source of bioactive compounds, including phenolic compounds, flavonoids, and condensed tannins, which contribute to their antioxidant properties. These findings highlight the potential health benefits of prickly pear seeds and their potential applications in the development of functional foods or nutraceutical products**, (Boudjouan *et al*., 2022).**

II.2. Antioxidant potential

The antioxidant potential of prickly pear seed extracts was evaluated using four different tests: DPPH antiradical activity, FRAP, H_2O_2 scavenging, and β-carotene bleaching. The results are summarized in **Table 12.**

Table 10: Values of DPPH, FRAP, H_2O_2 and β-carotene bleaching antioxidant activities of prickly pear seeds

Antioxydants activités	**Values**
DPPH (mg GAE/100g)	248.40±1.06
PR (mg GAE/100g)	382.56±1.70
H_2O_2(%)	62.01±0.57
β-carotene bleaching (%)	83.87±1.76

II.2.1. DPPH radical scavenging test

The DPPH assay is a commonly used method to measure antioxidant activity. It involves the reduction of a stable, purple-colored DPPH radical by natural antioxidants or reducing compounds, resulting in a color change to pale yellow **(Kartika *et al.*, 2019).** The evaluation of the anti-radical activity of prickly pear seed extracts showed a DPPH radical inhibition concentration of 248.40±15.06 mg GAE/100 g DM (**Table 12)**. This result was higher than the values reported by **Chaalal *et al.* (2013)** for cultivars of Algerian prickly pear seeds, ranging from 891.38 ± 6.75 mg EAA/100g to 114.699 mg AAE/100g for different varieties. **Yolmeh *et al.* (2014),** who studied the whole fruit of *OFI*, reported a higher value of 547.8 mg GAE/100 g DM. The variation in results can be attributed to the complexity and heterogeneity of chemical compounds among different species, which contribute to the total antioxidant capacity **(Zaghad *et al.*, 2019).**

II.2.2. Ferric reducing power (FRAP)

The reducing power of a compound can be used to measure its antioxidant activity. The FRAP assay measures the ability of an extract to donate electrons and reduce ferric ions (Fe^3+) to ferrous ions (Fe^2+) **(Al Juhaimi *et al.*, 2020).** The evaluation of the reducing power of prickly pear seed extracts showed a concentration of 382.56±7.70 mg GAE/100g DM (**Table 12)**. This result was higher

than the values reported by **Chaalal *et al.* (2013)** for ground prickly pear seeds, ranging from 18.6155±0.176 mg EAA/100g DM to 19.7816±0.1318 mg EAA/100g mg EAA/100g DM for different varieties. It falls within the range of results obtained by **Chougui *et al.* (2013)** for prickly pear seeds (32.3 mg EAA/100g and 51.3 mg EAA/100g). The presence of hydroxyl groups in phenolic compounds is likely responsible for the reducing power of the extracts, as they can act as electron donors. The reducing power is considered a significant indicator of potential antioxidant activity **(Bougandoura *et al.*, 2012).**

II.2.3. Hydrogen peroxide scavenging test

The H_2O_2 scavenging test evaluates the ability of extracts to neutralize hydrogen peroxide, a reactive oxygen species involved in oxidative stress **(Al Juhaimi *et al.*, 2020).** The results presented in **Table 12** show an average H_2O_2 scavenging activity of 62.01±2.57% for the tested prickly pear seed extracts. This result was lower than the values reported **by Chaalal *et al.* (2013)** for crushed prickly pear seeds, which ranged from 91.87±1.25% to 93.55±1.32% for different varieties. **Chougui *et al.* (2013)** also reported higher percentages of H_2O_2 scavenging (76% to 96%). The phenolic compounds present in the extracts act as electron donors and facilitate the conversion of H_2O_2 to H_2O. The scavenging effect of hydrogen peroxide increased with the phenolic content, indicating their contribution to the antioxidant activity.

II.2.4. β-carotene bleaching test

The β-carotene bleaching test is utilized to evaluate the potential of a sample to inhibit lipid peroxidation in vitro in this test; the oxidation of linoleic acid generates free radicals that subsequently oxidize the highly unsaturated β-carotene, resulting in the disappearance of its orange color. However, the presence of an antioxidant can neutralize the free radicals derived from linoleic acid, thereby preventing the oxidation and bleaching of β-carotene **(Dawidowicz *et al.*, 2010; Loucif *et al.*, 2020).**

The result of the β-carotene bleaching test **Table 12** indicates a relative antioxidant activity of 83.87±1.76%. The high percentage of inhibition observed in this study can be attributed to the polarity and chemical composition of the extract.

An extract that exhibits inhibition of β-carotene bleaching can be described as a free radical scavenger and a primary antioxidant **(Liyana *et al.*, 2006).** According to several authors, the combination of the linoleic acid inhibition assay with the β-carotene test serves as a mimetic model for lipid peroxidation in biological membranes **(Ferrari *et al*., 2006).**

II.3. Inhibition of BSA denaturation

The presence of flavonoids in different parts of *OFI* contributes to its anti-inflammatory activity by inhibiting important regulatory enzymes. Certain flavonoids have been identified as potent inhibitors of prostaglandin production, which are highly active pro-inflammatory molecules **(Murad *et al.*, 2023).** To evaluate the anti-inflammatory activity of the studied prickly pear seed extract, the BSA (Bovine Serum Albumin) protein denaturation method was employed. The protective effect of the seed extracts against thermal denaturation of BSA was expressed as a percentage of inhibition **Figure 12**, and the inhibition rate was found to be dose-dependent.

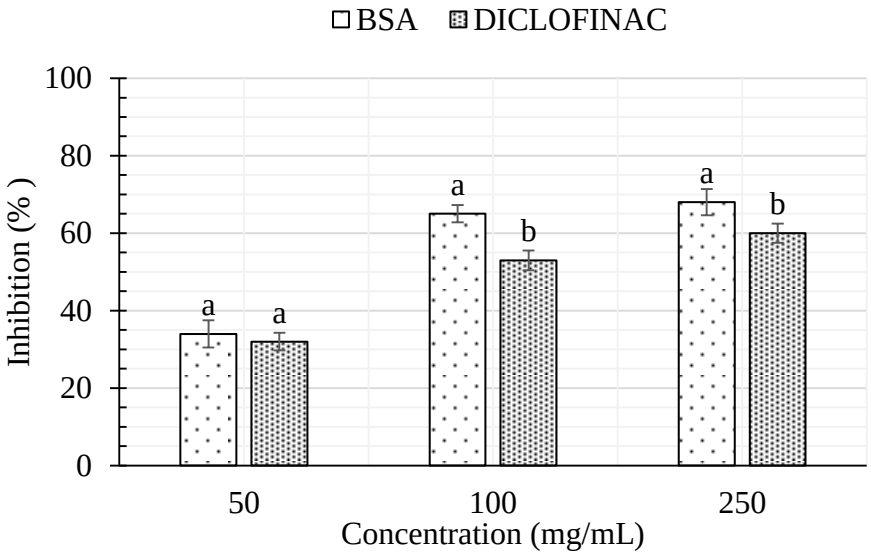

Figure 11: Percentage inhibition of heat-induced albumin denaturation by *OFI* extracts and diclofenac

According to **figure 11** the acetonique extracts of the seeds, at different concentrations (50, 100, and 250 mg/ml), exhibited a significant protective effect against the denaturation of BSA induced by heating at 72°C. The inhibition percentages were 34±0.002%, 65±0.003%, and 86±0.42%, respectively. These values were significantly higher ($p \leq 0.05$) than those of diclofenac, a non-steroidal anti-inflammatory drug, which showed inhibition percentages of 23±0.01%,53± 0.11%, and 60 ± 0.007% at the corresponding concentrations. , these results fall within the findings of **Youssef *et al*. (2021)** who found that the *opuntia* genus has an important anti-inflammatory effect.

Previous studies have reported that certain non-steroidal anti-inflammatory drugs, including diclofenac, salicylic acid, phenylbutazone, and indomethacin, not only inhibit the synthesis of pro-inflammatory prostaglandins but also possess a protective effect against thermally induced protein denaturation at physiological pH (pH: 6.2 to 6.5) **(Ramalingam *et al*., 2010; Sangeetha *et al*., 2011).**

II.4. Correlation between bioactive compounds and antioxidant activities

The correlations between antioxidant contents (TPC, TFC, CT) and antioxidant activities (DPPH, FRAP, H_2O_2 and β-carotene bleaching) of prickly pear seed powder extracts are presented in (**Table 11).**

Table 11: Correlation between antioxidant activities, inhibition percentage and bioactive compounds

	TPC	Flavonoïdes	Tannins
DPPH	0.92	0.58	0.71
FRAP	0.83	0.63	0.79
H_2O_2 %	0.67	0.45	0.63
β-carotene bleaching %	0.72	0.51	0.68
Inhibition %	0.88	0.72	0.78

Table 11 presents the correlations between various bioactive compounds and antioxidant activities measured by the DPPH, PR, and H_2O_2, the β-carotene as well as the inhibition percentage.

The correlations between the bioactive compounds and antioxidant activities reveal consistent positive associations. From **Table 11**, we observe significant positive correlations between TPC, Flavonoids, and Condensed tannins with antioxidant activities measured by the DPPH, PR, H_2O_2 tests, β-carotene bleaching and inhibition %. These results suggest that these bioactive compounds may significantly contribute to the antioxidant activity of the hydro-soluble extract; these findings were in good agreement with a previous study by **Fernández *et al*. (2010),** who stated that the antioxidant activity of prickly pear grown in Spain was positively correlated with the phenolic compound content of the extracts. Additionally**, Rocha-Guzman *et al*. (2007)** reported that the antiradical activity of seed acetone extracts and cactus pear fruit extract was highly correlated with the total polyphenol content. **Ali *et al*. (2021)** reported a high correlation between the extract of (*Caragana brachyantha Rech.f*), of South Africa and bioactive compounds.

Table 11 demonstrates that TPCs exhibit stronger correlations with the DPPH and PR tests, whereas Flavonoids display more moderate correlations. These differences may reflect the diverse antioxidant mechanisms present among the various bioactive compounds.

Furthermore, the positive correlation between the bioactive compounds and the percentage inhibition of BSA reinforces the idea that these compounds may also be involved in modulating antioxidant activity against free radicals, thus contributing to the protection of proteins against oxidative damage.

II.5. Antimicrobial activity

The evaluation of antibacterial and antifungal activity *OFI* extracts was estimated in terms of the diameter of the inhibition zone around the disks containing the extracts at various concentrations, against pathogenic bacteria, molds and yeasts.

II.5.1. Antibacterial Activity of hydro soluble Extract of *OFI* seeds

The following table presents the values in mm of the inhibition zones achieved with the studied strains.

Same letters in the same column refers to means not statistically different.

Table 12: Diameter of inhibition zones (in mm) of bacteria for the hydro soluble extract of prickly pear seeds in the antimicrobial test. Same letters in the same column refers to means not statistically different.

Acetonic extract concentration	Gram	Diameters of inhibition zones							
		C1	C2	C3	C4	C5	C6	C7	C8
Bacillus cereus	+	-	-	-	-	-	-	-	-
Enterococcus faecalis	+	15[a]	-	-	-	-	-	-	-
Micrococcus luteus	+	-	-	-	-	-	-	-	-
Pseudomonas Aeruginosa	+	15[a]	14[b]	12[c]	-	-	-	-	-
Escherichia coli	-	14[a]	-	-	-	-	-	-	-
Proteus mirabilis	-	15[a]	14[b]	-	-	-	-	-	-
Salmonella typhimurium	-	-	-	-	-	-	-	-	-

After 24-hour incubation at 37°C, the study revealed that the hydro soluble extract of *OFI* seeds did not exhibit any antibacterial activity *against Bacillus cereus, Micrococcus Luteus,* and *Salmonella Typhimurium* at all concentrations tested. These findings were not consistent with the study conducted by **Eleojo *et al.* *(*2019)** on prickly pear fruit and peel extracts, which reported an activity against *Pseudomonas Aeruginosa, E.coli,* and *Micrococcus luteus.* . Compound structure, type and prevailing concentration around the cell may affect the ability to permeate microbial cells resulting in varied microbial response to extract components **(Ciocan & Bara, 2007).**

However, at a concentration of 110 mg/ml, the *OFI* extract showed inhibition zones against *E. coli* (diameter = 14 mm), *Enterococcus faecalis* (diameter = 15 mm), and *Pseudomonas Aeruginosa* (diameter = 15 mm), as well as *Proteus mirabilis* (diameter = 15 mm). These findings suggest that higher concentrations of the extract may be necessary to observe significant antibacterial activity.

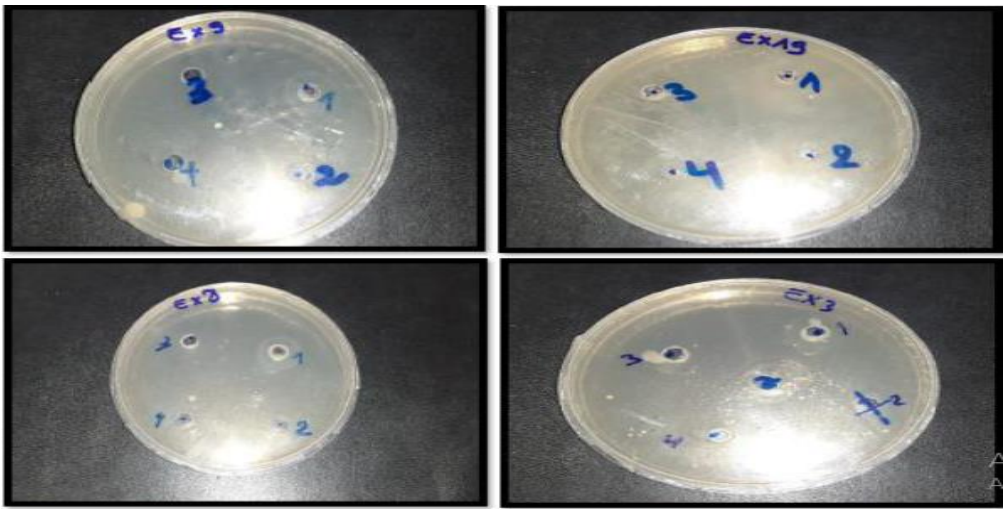

Figure 12: Inhibition zones of hydro soluble extract on some bacteria (*Escherichia coli, micrococcus luteus, Pseudomonas aeroginosa, Enterococcus Feacalus)*

According to **Figure 12**, concentrations C2 (55 mg/ml; diameter = 14 mm) and C3 (27.5 mg/ml; diameter = 12 mm) exhibited antibacterial activity against *Pseudomonas Aeruginosa*, while C2 (diameter = 14 mm) showed activity against *Proteus mirabilis*. These results are consistent with the findings of **Eleojo *et al.* (2019),** who found antimicrobial activity against *Pseudomonas Aeruginosa* and *Proteus mirabilis* at different concentrations of fruit and peel extracts. These results suggest that prickly pear seed extract may possess antimicrobial properties, albeit at specific concentrations. The lack of antibacterial activity may be attributed to the specific characteristics of the bacterial strains tested **(Eleojo *et al*., 2019).**

Our results were not in line with the findings of **Benattia (2017),** who studied prickly pear seeds. Their results showed that all *Opuntia* seed extracts were inactive against *E. coli, Pseudomonas aeruginosa,* and *Micrococcus luteus*. This discrepancy could be due to the differences in the chemical composition of the extract or the extraction method **(Ramírez *et al*., 2017).**

Previous studies have shown that variations in secondary metabolites, such as flavonoids and polyphenols, can influence the antimicrobial efficacy of plant extracts **(Cristani *et al*., 2007).** These compounds have the ability to penetrate the cell membranes of bacterial strains and interact with critical intracellular sites, leading to cell death **(Iftikhar *et al*., 2023).**

According to the scale for estimating antimicrobial activity, a bacterial strain is considered resistant to antibacterial agents when the inhibition diameter is less than 10 mm **(Koohsari *et al.*, 2015).** Thus, we can conclude that the strains studied in this work were sensitive to the hydro-soluble extract of *OFI* seeds. The optimal efficacy of an extract may not be attributed to a single active constituent but rather to the combined action (synergy) of different compounds present in the extract **(Essawi *et al.*, 2000).** Several studies have highlighted the higher sensitivity of Gram-positive bacteria compared to Gram-negative bacteria **(Turkmen *et al.*, 2007).** This difference could be attributed to variances in the outer layers of Gram-negative and Gram-positive bacteria. These findings align with our results.

II.5.2. Antifungal Activity of the hydro soluble extract

The in vitro antifungal activity of various extracts from prickly pear seeds was investigated using the diffusion method.

The table below presents the values in mm of the inhibition zones achieved with the studied strains. Same letters in the same column refers to means not statistically different.

Table 13: Diameter of inhibition zones (mm) of the hydro soluble extracts from prickly pear seeds against yeasts and fungi

Acetonic extract concentration	Diameters of inhibition zones							
	C1	C2	C3	C4	C5	C6	C7	C8
Candida albicans	Very sensitive.							
Candida glabrata	15^{a}	-	-	-	-	-	-	-
Phytophthora infestans	9^{a}	9^{a}	9^{a}	-	-	-	-	-
Aspergillus parasiticus	16^{a}	-	-	-	-	-	-	-
Penicillium sp	-	-	-	-	-	-	-	-
Trichoderma sp	12^{a}	10^{b}	10^{b}	10^{b}	9^{b}	9^{b}	8^{c}	
Fusarium sp	9^{a}	9^{a}	-	-	-	-	-	-

Table 13 showed the antifungal activity results for the acetonic extract .Exhibited activity against all tested fungi except for *Penicillium sp*. The obtained results were as follows: *Phytophthora infestans* (9 mm diameter), *Aspergillus parasiticus* (16

mm diameter), *Trichoderma* sp (12 mm diameter), and *Fusarium* sp (9 mm diameter). Based on the scale outlined by **Mutai *et al*. (2009),** a fungal strain is considered sensitive to various antimicrobial agents when the diameter of the inhibition zone falls between 9 and 19 mm.

Concerning the yeasts, there was activity against both tested strains, particularly *Candida albicans,* where the diameter was >20 mm, and a diameter of 15 mm for *Candida glabrata*. The inhibition zones increased with the concentrations of the hydro soluble. This sensitivity was likely related to the high concentrations of secondary metabolites (flavonoids, polyphenols) in the extract. These compounds can penetrate the cell membranes of fungal strains and enter the interior of the cell, interacting with critical intracellular sites such as enzymes and proteins, ultimately leading to cell death **(Elkady *et al.*, 2023).**

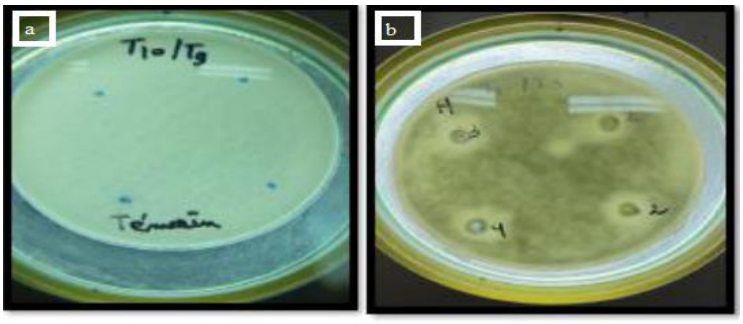

Figure 13: Effect of water-soluble extract on *Trichoderma* sp.: (a) control; (b) inhibition zone

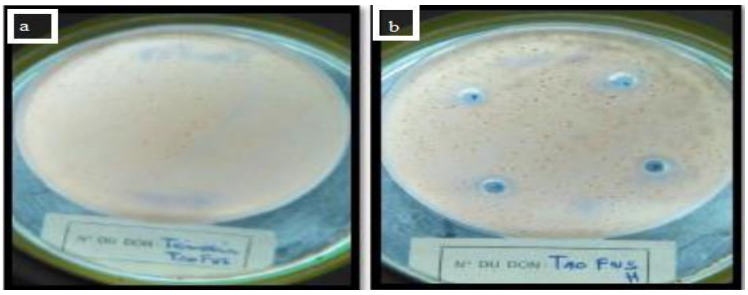

Figure 14: Effect of water-soluble extract on *Fusarium* sp.: (a) control; (b) inhibition zone.

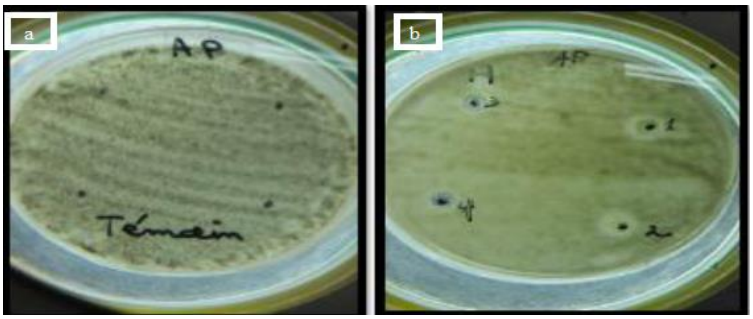

Figure 15: Effect of water-soluble extract on *Aspergillus parasiticus*.: (a) control; (b) inhibition zone.

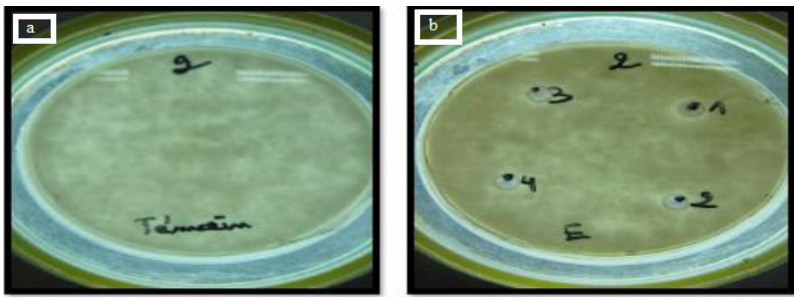

Figure 16: Effect of water-soluble extract on *Phytophthora infestans*.: (a) control; (b) inhibition zone.

Chapter III

III. Evaluation of the stability of enriched prickly pear juice

III.1. Evolution of physicochemical parameters

The parameters measured during the physicochemical analyzes of unpasteurized prickly pear juice (fortified and non-fortified) are pH, titratable acidity, Brix, and browning index.

III.1.1. Hydrogen potential

The results of the hydrogen potential of juice not enriched and enriched by the dry extract of prickly pear seeds, as well as their evolution during storage, are shown in **Figure 17.**

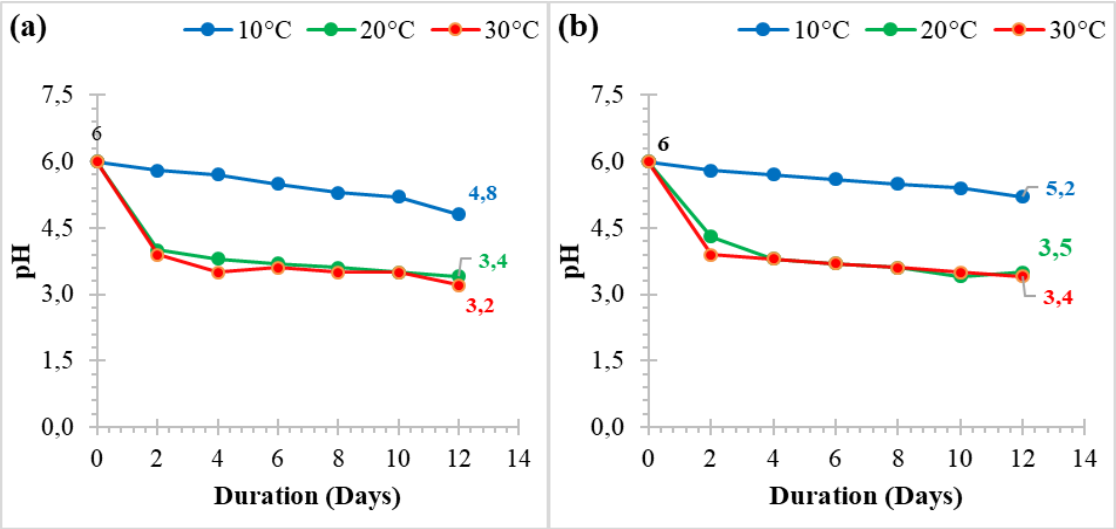

Figure 17: Evolution of the pH of fortified (a) and non-fortified enriched (b) prickly pear juice during storage

According to **Figure 17**, the pH value of prickly pear juice was 6, regardless of whether it is non-enriched or enriched. Therefore, enrichment does not have an effect on the pH value of the analyzed juice. This value was higher than that reported by **Hernández-Fuentes *et al.* (2015).** Who worked on Mexican prickly pear with pH values ranging between 2.74 and 3.54 .This difference may be attributed to variations in the region and the soil composition of the sampling area. The pH of prickly pear juice was higher compared to many other fruits. For instance, the pH values of pear nectars range between 3.56 and 3.91 **(Riu-Aumatell *et al.*, 2004)**.

During storage, the pH value of both non-enriched and enriched juice decreases significantly ($p<0.05$) after 2 days at 20 and 30°C. After 12 days of storage at 10, 20, and 30°C, the pH values of non-enriched samples were 1.2, 2.6, and 2.8, respectively, while the pH values of enriched samples were 0.8, 2.5, and 2.6 respectively. The decrease in pH after storage can be explained by the presence of microorganisms in the juice leading to fermentation, which produces organic acids such as lactic acid. These organic acids can lower the pH of the juice over time **(Jood *et al.*, 2012).** Additionally, certain natural chemical reactions can occur in the juice during storage, resulting in the formation of acids **(Franklin *et al.*, 2014).**

Statistical analysis revealed a significant difference between the juice stored at 10°C compared to those stored at 20 and 30°C. However, there was no significant difference observed between the pH values of juice stored at 20 and 30°C.

III.1.2. Titratable acidity

TA is a measure of the total concentration of acids primarily citric acid, lactic acid, tartaric acid and acetic acid in one volume of juice **(Sadler *et al.*, 2010).**

The results of the TA of unenriched juice and the enriched with the dry extract of prickly pear seeds, as well as their evolution during storage are shown in **Figure 18.**

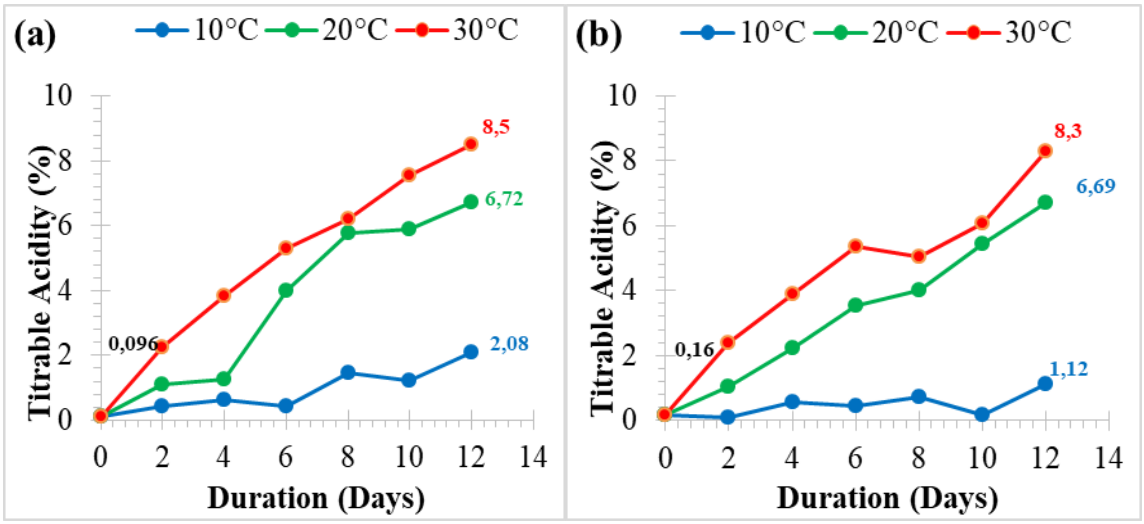

Figure 18: Evolution of titrable acidity of fortified (a) and non- enriched (b) prickly pear juice during storage

As can be seen from **Figure 18**, TA values after 12 days storage increased for both fortified and control juices to rich respectively 1.12 and 2.08% at 10°C, 6.72, 8.5% at 20°C and 6.69 and 8.3% at 30°C. In the same context, **(Ilkin *et al.*, 2020)** who worked on the fortified orange juice didn't found any statistical difference between the mean values of the samples during storage period.

Statistical analysis did not revealed the presence of a significant difference between the titratable acidity of juices stored at different temperatures.

III.1.3. Brix degree

The brix degree of a juice is a measure of its sugar content, with higher brix indicating a sweeter taste. **Figure 19** presents the results of the brix degree for both non-enriched and enriched juice with dry extract of prickly pear seeds, along with their changes over time during storage.

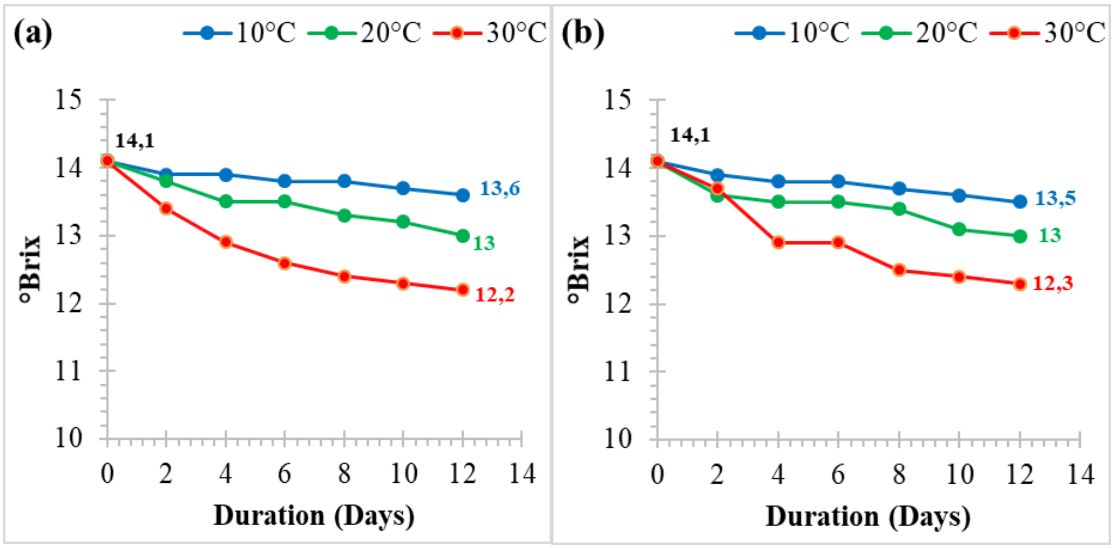

Figure 19: Changes in the brix degree of fortified (a) and non- fortified (b) prickly pear juice during storage

According to **figure 19** the brix value of both non-fortified and fortified juices was 14.1. This indicates that the enrichment process did not lead to a modification in the brix value. The obtained value falls within the range reported by **Chougui *et al.* (2013),** which was 15%.

During storage at 10, 20, and 30°C, the brix values of the unenriched and enriched juices decrease, reaching values of 13.6%, 13.0%, and 13.2% for the

unenriched juice, and 13.5%, 13.0%, and 12.3% for the enriched juice, respectively. The absorption of moisture during storage could be the reason for decreased in Brix° values **(Dangui, *et al.*, 2014),** or it could be due to the increase in the acidity **(Sadras *et al.*, 2013).** The same results were found by **Gao *et al.* (2018)** who reported a decrease in TSS values of navel orange fruits during storage.

Statistical analysis revealed that the interaction between storage duration and temperature does not have a significant effect on the brix value during storage.

III.1.4. Browning index

Enzymatic browning is the main reaction responsible for the discoloration of fruits and vegetables. It results from the oxidation of phenolic compounds present in the plant cell. **(Lee *et al.*, 2016).**

The results of the browning index of unenriched juice and enriched with the dry extract of prickly pear seeds as well as their evolution during storage are presented in **Figure 20.**

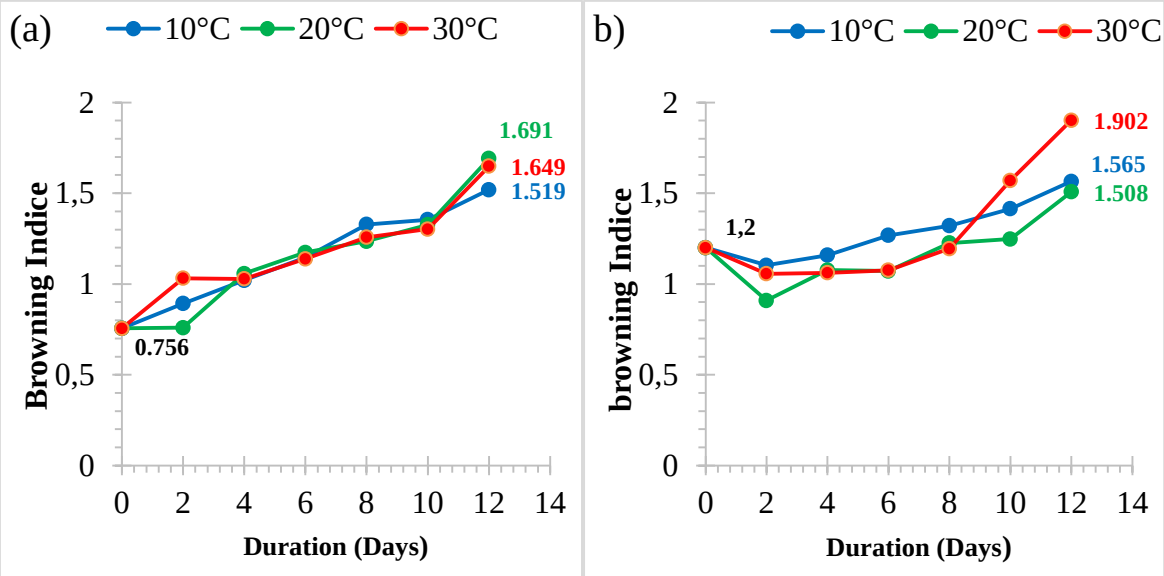

Figure 20: Evolution of the Browning index of fortified (a) and non- fortified (b) prickly pear juice during storage

From **Figure 20**, during storage at 10, 20 and 30°C, the values increased respectively to achieve 1.519±0.06, 1.649±0.11 and 1.691±0.06 for enriched juice and 1.508±0.03, 1.565±0.01 and 1.902±1.002 for control juice. **Touati *et al.* (2016)** noted same trend in different fruits nectars. This augmentation may be due to the

results of phenolic compound oxidation that occurred in the plant cell **(Inchuen *et al.*, 2010).**

Statistical analysis did not reveal a significant difference between the browning index of juices stored at different temperatures.

III.2. Evolution of antioxidant content

III.2.1. Total phenolic compounds

The results of the content of total phenolic compounds of juice not enriched and enriched with aqueous prickly pears extract seeds, as well as their evolution during storage, are shown in **figure 21**

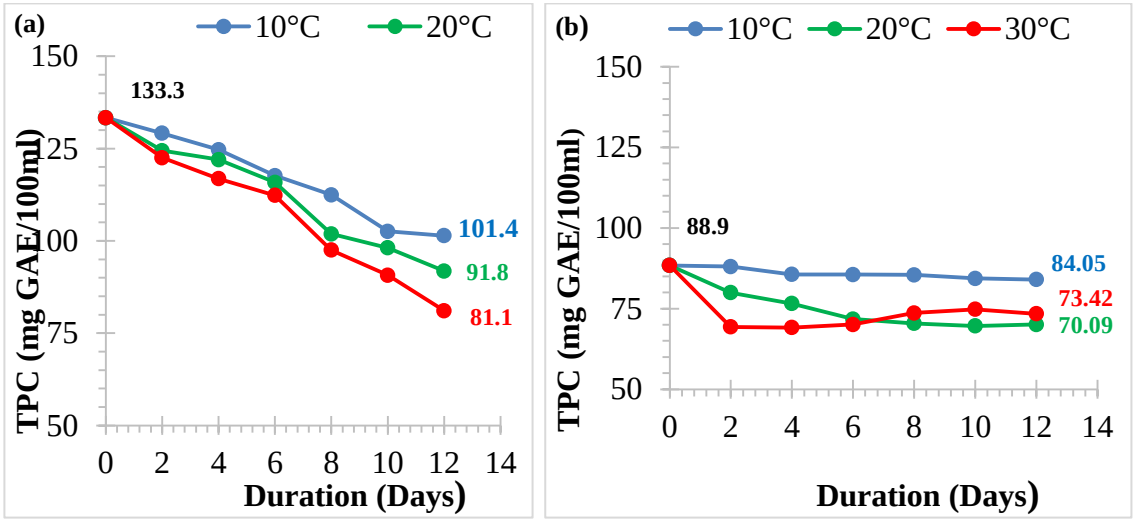

Figure 21: Evaluation the TPC of fortified (a) and non-fortified (b) prickly pear juice during storage

From **Figure 21** prior storage, the content of TPC in enriched juice was 133.3±3.4mgGAE/100ml against 88.39±4.2mgGAE/100ml in control one. Therefore, the enrichment of juice induced an increase of 50.80% in the yield of TPC. Statistical analysis revealed that there was a significant difference between juices at $P<0.05$. The results obtained were higher than the values (31.0 - 51.1mgGAE/100g) reported by **Palmeri *et al.* (2020).** However, our results were lower than those reported by **Socorro *et al.* (2015)** who declared that TPC values in the prickly pear juice were ranged from 630.9 to 880.6mgGAE/100ml. **Dehbi *et al.* (2014)** and **Chavez-Santoscoy *et al.* (2009)** reported values of 632.11±5.50µgGAE/g for the Marrocan *OFI.* L juice and 226.3µgGAE/g for

Mexican prickly pear juice, respectively. the content of TPC in enriched juice stored at different temperatures exhibited the same decrease tendency, which was important for juices stored at 30°C followed by those stored at 20 and 10°C to reach respectively values of 101.4±0.005, 91.8±0.02 and 81.1±0.005mgGAE/100ml The content of TPC in the control juice stored at 10 and 20°C decreased significantly to reach values of 84.05±1.001 and 70.09±1.003mgGAE/100ml, respectively. In the juice stored at 30°C, a fluctuation was noted; a decrease during the first two days of storage, then stability until the sixth day, followed by an increase until the tenth day, then a decrease to reach the value of 73.42±0.003mgGAE/100ml. This may be due to leaching losses favored by the breakdown of cellular structures occurring as a result of exposure to high temperatures **(Al Juhaimi *et al.*, 2005).** Several authors have found that TPC appears to exhibit stability during refrigerated storage while a decrease in ambient and high-temperature storage **(Touati *et al.*, 2014).**

III.2.2. Total flavonoids

TF content was determined using colorimetric methods. The results of TF content in both enriched and control juices before and during storage are presented in **Figure 22**.

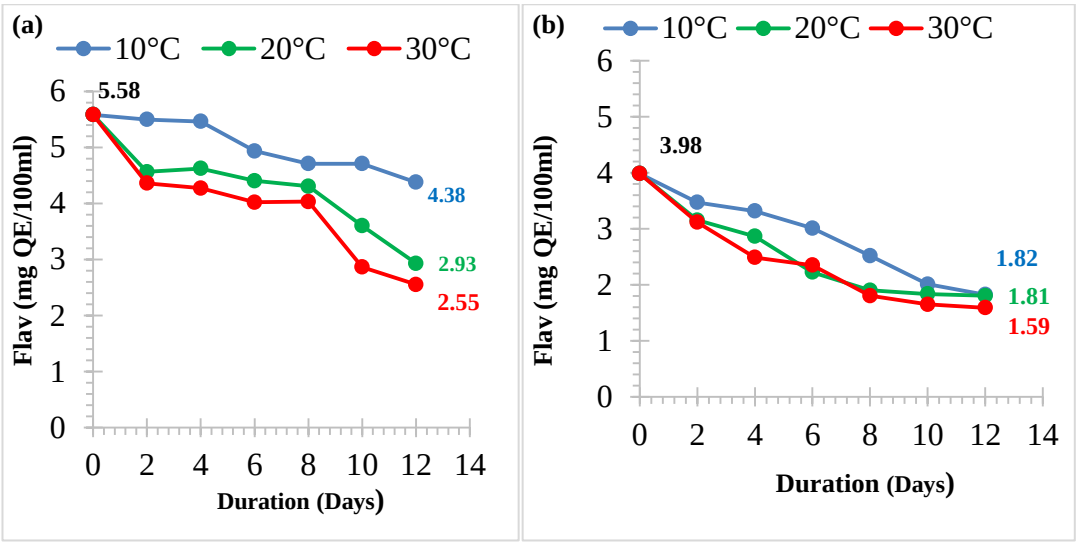

Figure 22: Evolution of the total flavonoids content of fortified (a) and non-fortified (b) juice during storage

From **Figure 22**, TF content were 5.58±0.07 and 3.98±1.003mgQE/100ml for enriched and control juices, respectively. This indicates that the enrichment process led to a 40.20% increase in TF yield. Statistical analysis revealed a significant difference in the TF content between the analyzed juices at a significance level of $P<0.05$. The obtained results were higher than those reported **by Zeghad et al. (2019)** who stated the value of 1.95mgQE\100g in prickly pear. On the other hand, our results were in concordance with values reported by **Palmeri et al. (2020)** who worked on prickly pear juice of different cultivars (4.7 and 5.7mgQE\100g) as TF in red and yellow cultivars. The TF content in enriched juice stored at 10°C during the first four days did not exhibit a significant decrease ($P<0.05$); however, prolonged storage induced a significant decrease which led to reach the value of 4.38±0.008mgQE/100ml. Samples stored at 20 and 30°C showed a significant decrease during the first two days; while during extensive storage, the TF content showed slight stability until the eighth day followed by a significant decrease to reach values of 2.93±0.05 and 2.55±0.02mgQE/100ml for juice stored at 20 and 30°C, respectively. Concerning the control juice stored at different temperatures, the trend of reduction in TF content was greater for samples stored at 30°C followed by those stored at 20 and 10°C to reach the values of 1.82±0.02, 1.81±0,001 and 1.59±0.003mgQE/100ml, respectively. These results were consistent with the literature **(Ogodo *et al.*, 2016; Ali *et al.*, 2013).** The decline in TF content may be attributed to the breakdown of cell structure which occurred during the storage period **(Ali *et al.*, 2013).** The decrease of TF content was lower in the enriched juice than the control one. This fact may be due to the addition of hydro-soluble prickly pear seeds extract

III.3. Evolution of antioxidant activity

Antioxidant contents have been reported to be the main responsible for foods TAC **(Touati *et al.*, 2016).** Therefore, TAC measurement could be a useful indicator of the quality deterioration of fruit juice during storage. For this purpose, DPPH and FRAP values of enriched and control juices were determined before and during 12 days of storage at 10, 20, and 30°C.

III.3.1. Anti-free radical activity

The measurement of anti-free radical activity by the DPPH radical is a commonly used method to assess antioxidant activity; it is based on the reduction of the DPPH radical by a transfer of hydrogen which results in the discoloration of the DPPH solution from purple to yellow **(Wong *et al.*, 2005).**

Figure 23 shows the results of the anti-free radical activity of unenriched prickly pear juice enriched with the dry extract of its seeds as well as their evolution during storage.

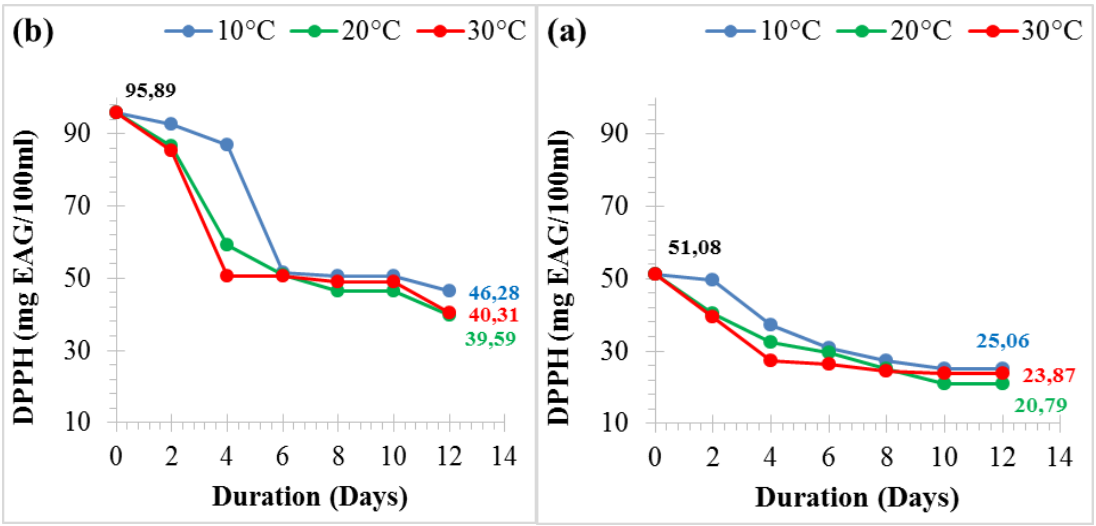

Figure 23: Evolution of the DPPH values of fortified (a) and non-fortified (b) due during storage

According to **Figure 23**, Prior storage, the antiradical DPPH activity results were 95.89±14.27 and 51.08±14.27mgGAE/100ml for the enriched and the control juices, respectively. Statistical analysis revealed a significant difference between the analyzed juices ($P<0.05$). The enrichment process led to an increment of 46.73% in the antiradical DPPH activity. The total antioxidant capacity of prickly pear juices evaluated using the DPPH assay has been widely reported in the literature**. Palmeri *et al.* (2020)** reported DPPH results ranging from 37.6 to 49.4mgGAE/100ml for prickly pear juices. **Smida *et al.* (2017)** also noted that the antiradical DPPH activity increased with an increase in the concentration of *OFI.*

As can be seen from figures 23 a and b, the enriched juice exhibited a significant decrease in antiradical DPPH activity after 6, 8 and 4 days of storage at 10, 20 and 30°C, respectively. Extended storage up to the tenth day was characterized by stability, and then followed by a decrease to reach values of 46.28±1.006, 39.59±0.003 and 40.31±0.07mgGAE/100ml for juice stored at 10, 20 and 30°C, respectively. This might be explained by the decrease of antioxidants which where deteriorated during storage as corroborated by **Tudora *et al*. (2015)** who reported that under high temperatures storage some biochemical changes occurred in the fruit's structure. Regarding the control juice, the antiradical DPPH activity results were stable during the first two days of storage at the temperature of 10°C ($P<0.05$); however, the prolonged storage induced a significant decrease to reach the value of 25.06±0.006mgGAE/100ml. For juice stored at 20°C, the evolution of antiradical DPPH activity showed a decrease reaching a value of 20.79±1.04mgGAE/100ml at the end of storage. Concerning juice stored at 30°C, the values of antiradical DPPH activity showed a decrease during the first four days, and then followed by stability until the end of storage with a value of 23.87±0.06mgGAE/100ml. These findings indicate a degradation of antioxidants during storage, which could be attributed to the effects of temperature and other storage conditions.

III.3.2. Ferric Reducing power

The reducing power test of juice can serve as an indicator of its antioxidant activity because the reducing activity is associated with the presence of reduce ones which shows antioxidant potential by breaking chain reactions by donating an electron **(Amrane *et al*., 2023).**

The results of the reducing power of unenriched prickly pear juice enriched with the dry extract of its seeds are shown in **Figure 24.**

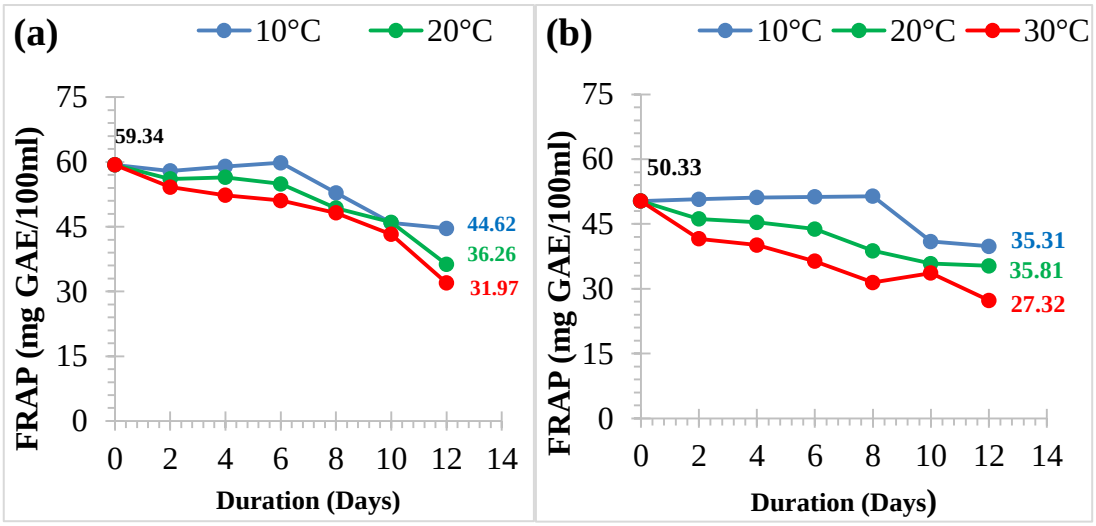

Figure 24: Evolution of the FRAP values of fortified (a) non-fortified (b) due during storage

From **Figure 24** the reducing power value of enriched prickly pear juice is 59.34 mg GAE /100ml. **Daramola. (2013)** recorded a reducing power between 80 and 100 mg GAE / 100ml of apple juice of different varieties. Compared to 50.33 mg EAG / 100ml for unenriched juice, **Xu *et al.* (2008)** noted a reducing power of 30.74 mg AAE/100ml of citrus juice. As a result, the enrichment of prickly pear juice with the aqueous extract of its seeds led to an increase in activity estimated at 17.90%. Statistical analysis revealed a significant difference in the activities of the two juices analyzed at the error threshold of $p < 0.05$.

During storage, the reducing power results fluctuated for the two juices analyzed (unenriched and enriched). However, after storage, the temperature of 10°C induced less loss of reducing activity compared to juices stored at 20 and 30°C. The reducing power values at the end of storage at 10, 20 and 30°C were respectively 39.83; 35.31 and 27.32 mg EAG /100ml for unenriched juice; and 44.62; 36.26 and 31.97 mg EAG / 100ml for the enriched juice, high values were reported for the samples stored at 10°C, a sign that after high temperatures storage some biochemical changes occurred in the fruits structure **(Tudora *et al.*, 2015).**

III.4. Microbiological analyzes

Factors that affect microbial colonization of juices are redox potential, pH, water activity, nutrients, temperature, antimicrobial agents and relative humidity **(Raybaudi-Massilia *et al.*, 2009).**

The change in the number of microorganisms in juices depends on their composition and the conditions of their storage conditions.

The results of the microbiological analysis of the juice without enrichment and juice enriched by the aqueous seeds extract stored at three temperatures (10.20 and 30°C) for 12 days are gathered in **table 14** and **15.**

Table 14: The results of the microbiological analysis of the juice without enrichment

Microorganisms sought	Storage Temperature	2 Days	6 Days	12 Dys	Norme
Total coliforms	10°C	_	_	_	< 10
	20°C	_	_	$4{,}5\times 10^3$	
	30°C	_	3×10^2	$3{,}9\times 10^2$	
Fecal coliforms	10°C	_	_	_	Absence
	20°C	_	_	_	
	30°C	_	_	_	
Yests and molds	10°C	_	_	_	10^4
	20°C	_	_	_	
	30°C	_	_	$10{,}2\times 10^3$	

Table15: The results of the microbiological analysis of the enriched juice

Microorganisms sought	Storage temperature	2 Days	6 Days	12 Dys	Norme
Total coliforms	10°C	_	_	_	< 10
	20°C	_	_	_	
	30°C	_	_	_	
Fecal coliforms	10°C	_	_	_	Absence
	20°C	_	_	_	
	30°C	_	_	_	
Yests and molds	10°C	_	_	_	10^4
	20°C	_	_	_	
	30°C	_	_	_	

Prior storage, as shown in **table 14** and **15**, the results obtained from the microbiological analysis of the juice studied reveal a total absence of contaminating germs (coliforms, yeasts and molds) for all the samples after two days of storage, which perfectly meets the standards required by **JORA (2017).** This results was similar to those found bay **(Garg *et al.*, 2021)** who worked on Indian goose berry fortified, **Asghar *et al.* (2018)** reported that unpasteurized juices such as apple, carrot, orange, and extracted sugar represent a high load of total coliforms.

III.4.1. Total coliforms

Prior to storage ; the microbiological analysis of the fortified and non-fortified prickly pear juices, as presented in **Table 14 and 15**, showed the absence of a total coliforms, yeasts, and molds in all samples. This indicates that the juices fall within the standards required by **JORA (2017)** regarding microbiological quality. These findings was similar t with the results of **Garg *et al.* (2021),** who studied enriched gooseberry juice in India, and **Al Amin *et al*. (2018),** who declared the absence of total coliforms in orange and apple juice samples. However, it important to highlight that **Asghar *et al*. (2018)** observed a significant presence of total coliforms in unpasteurized juices, including apple, carrot, orange, and sugar-extracted varieties., During the 12 days of storage, the enriched juice showed the absence of total coliforms, which can be attributed to the antimicrobial activity of the aqueous extract of *OFI* seeds, earlier research has shown the notable antibacterial efficacy of prickly pear seed extract against a range of bacterial strains **(Xiyu *et al*., 2020; Shimaa *et al*., 2022).** Similar results were found by **Al Amin *et al*. (2018),** who reported the absence of total coliforms in commercial pineapple and lemon juice samples On the contrary, the control juices exhibited the existence of total coliforms., with a count of 3×10^2 CFU/ml after 6 days of storage at 30 °C. This is consistent with the findings of **Lewis *et al.* (2006)** and **Rahman *et al*. (2011),** who reported the presence of total coliforms in juice samples. After 12 days of storage, the total coliform count increased to 4.5×10^3 CFU/ml and 3.9×10^2 CFU/ml for unriched juices stored at 20 and 30 °C, respectively, this can be due to the metabolic activities of microorganisms during storage can lead to the deterioration of juice samples and reduce their shelf life **(Adal *et al*., 2022).** Moreover, the low

pH of the juice can promote the growth of acid-tolerant bacteria, further more contributing to **s**poilage **(Algari *et al.*, 2016)**. According to **JORA (1998)** standards, the total coliform count in juice should be lower than 10 CFU/ml.

III.4.2. Fecal coliforms

After 6 days of storage of unenriched juice, the results show an absence of germ at all the studied temperatures, furthermore after 12 days of storage the faecal coliforms were absent at 10 20 and 30°C. For the enriched and unriched juice, which mean that the enriched juice is perfectly in good marketable and hygienic quality.

III.4.3.Yeasts and molds

According to the results obtained, the enriched juice remained devoid from the presence of yeasts and molds throughout the storage period at all temperatures (10, 20, and 30°C). This may be attributed to the high concentrations of secondary metabolites, such as flavonoids and polyphenols, present in the extract. These compounds can penetrate the cell membranes of fungal strains and interact with critical intracellular sites, leading to cell death **(Cristani *et al.*, 2007).** In the control juice, yeasts and molds were absent in samples stored at 10 and 20 °C. However, in samples stored for 12 days at 30°C, the number of yeasts and molds reached 10.27×10^3 CFU/ml, this phenomenon occurs due to the elevated temperature, which facilitates the proliferation of yeasts and molds. Specifically, a temperature of 30°C is regarded as optimal for their growth. **(Sevindik *et al.*, 2021; Hika *et al.*, 2021).** These findings align with the standards set by **JORA (2017),** which specify that the yeast and mold count should be lower than 10^4 CFU/ml.

In the present study, the unfortified juice containing more bacteria than yeast as claimed by **Aneja *et al,* (2014).** On the other hand, the enriched juice does not contain bacteria, yeast or mold. In study by **Al Amin *et al,* (2018)** detected a total absence of total coliforms in samples of orange, apple juices.

Besides **(Asghar *et al.*, 2018)** reported that the unpasteurized juices such as apple, carrot, orange and extract sugar representing a high load of total coliforms.

At pH values of 1.5, molds and yeasts are capable of this development, these ph. values ranging from 2.9 to 3.5, from 3 to 4 and from 3.6 to 4.5 allow the growth of lactic acid bacteria; acetic bacteria and enteric bacteria respectively are higher than that allowing the growth of yeasts **(Lawlor *et al*., 2009)**.

Conclusion

The purpose of this study was to valorize the fruit of the prickly pear. We are particularly interested in the seeds and juice of this fruit due to its numerous virtues and abundant presence in our country. Additionally, it is undervalued in Algeria despite its significant importance.

Several tests were conducted for this thesis work, starting with extraction optimization and then the physico-chemical analysis of the *OFI* seeds, subsequently, the tests included the quantification of total polyphenols, flavonoids, and condensed tannins, as well as the evaluation of antioxidant, anti-inflammatory, and antimicrobial activities; the stability of the juice over time was also assessed.

The response surface plots demonstrated that all four studied variables (acetone concentration, extraction time, microwave power, solvent concentration and the sample/solvent ratio) significantly influenced the total polyphenol content and antioxidant activity of the *OFI* extracts. The preliminary tests allowed for more precision, and the experimental values were found to be in agreement with the predicted values, confirming the suitability of the developed quadratic models, these results validate the predictability of the model for the extraction of total polyphenol content and antioxidant activity from prickly pear seeds under the experimental conditions used.

The results of the antioxidant analysis showed the richness of *OFI* seeds in bioactive compounds, particularly polyphenols (905.71±0.50 mg GAE/100g DW), flavonoids (50.77±0.08 mg QE/100g DM), and condensed tannins (98.99±8.19 mg CE/100g DM). This justifies the significant importance of these seeds.

The antioxidant potential of the tested extracts was evaluated through various mechanisms, including direct scavenging of free radicals using the DPPH methods reducing power, β-carotene bleaching test, and hydrogen peroxide scavenging. In vitro results revealed antioxidant activities for prickly pear seed extracts, with strong DPPH inhibition (248.40±1.06 mg GAE/100g DM),significant reducing power (382.56±7.70 mg GAE/100g DM), high percentage of β-carotene bleaching

(83.87±1.76%), and interesting capacity to scavenge hydrogen peroxide (62.01±2.57%).

Moreover, the extracts displayed notable anti-inflammatory effects, with an impressive inhibition rate of 86±0.42%, in the BSA denaturation assay. This suggests that prickly pear seeds could serve as a valuable source of compounds with potent anti-inflammatory properties.

Positive correlations were observed between the different antioxidant activity tests, the inhibition percentage, and the measured antioxidants. This indicates that the in vitro antioxidant capacities and the inhibition percentage have a direct relationship with the content of secondary metabolites in prickly pear seed extracts.

Through the study of the antibacterial activity of the hydrosoluble extract of prickly pear, the results showed remarkable effectiveness against *Bacillus cereus, Enterococcus faecalis, Staphylococcus aureus, and Micrococcus luteus*. The inhibition of growth varied depending on the bacterial species, concentration of the tested product, culture medium used, chemical compositions, phenolic components, and the sensitivity of Gram-positive bacteria compared to Gram-negative bacteria.

Regarding antifungal activity, the acetone extract exhibited activity against all tested fungi except the genus *Penicillium* sp.

To improve the stability of unpasteurized fruit juice, it was enriched with the hydrosoluble extract of *OFI* seeds. Throughout the storage period, no detectable differences in physicochemical properties were observed between the enriched and control samples

Additionally, the enriched samples had the highest content of phenolic compounds and total flavonoids, as well as a higher antioxidant capacity; furthermore, the seed extract effectively reduced the proliferation of microorganisms. These results demonstrate the effectiveness of enriching fruit juice with the hydrosoluble extract of prickly pear seeds to enhance nutritional value and improve stability during storage. Moreover, storing the juice at 10°C proved to preserve its physicochemical, phytochemical, and microbiological quality.

In order to continue this work and based on the results obtained, it would be interesting to set the following points as prospects.

-Studies of the in vivo anti-inflammatory effect of *OFI* seed.

-Study the acute and chronic toxicity of *OFI* seeds and the fortified juice made.

-Characterizes the *OFI* seeds, the fortified juice of the prickly pear, and the hydrosoluble extract by HPLC.

-Study the in vivo and in vitro bioavailability of enriched juice and *OFI* seeds.

-Put the industrial application of the hydrosoluble extract as a bio conservator in food products.

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