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Theme

Effect of Spirulina supplementation on metabolic syndrome in mice

Presented by: **BECHKOURA Nour El Houda**

BOUACHA Chaima

SABER Merwan

Board of examiners:

President: Dr. BOUMAZA Awatif	**M.C.B**	**University of Guelma**
Examiner: Dr. ABDAOUI Wissem	**M.C.B**	**University of Guelma**
Supervisor: Dr. BRAIK Asma	**M.C.B**	**University of Guelma**

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Abstract

Metabolic syndrome is a cluster of health conditions, including hyperlipidemia, hyperglycemia, insulin resistance, and obesity that can lead to serious diseases. In our study, we investigated the effect of spirulina, an edible microalgae, rich in protein and antioxidants, on metabolic syndrome in mice. Before administering spirulina to the mice, Phytochemical analyses and antioxidant activity using DPPH and FRAP assays were conducted. The metabolic syndrome (MetS) was induced by feeding mice with a high-fat and high-fructose diet. Concomitantly, spirulina (1000 mg/kg/days) was administered orally during for 28 days. After sacrificing the mice, glycemic and lipid profile in addition to liver transaminases were evaluated. Oxidative stress parameters were also assessed. The results showed that spirulina exhibits *in vitro* antioxidant potential. In addition, spirulina significantly increases the levels of reduced glutathione (GSH) and improves catalase (CAT) and glutathione peroxidase (GPx) activities. Additionally, spirulina also shows a slight decrease in blood glucose levels and improves the entry of glucose into the tissues. It also reduces triglyceride levels and contributes to a reduction in liver body weight percentage and body mass index while maintaining weight gain. Although, the duration of four weeks was not sufficient to induce significant MetS changes in mice but there were signs of developing these conditions, and spirulina shows a positive effect against these features.

Key words: Metabolic syndrome; Hyperglycemia; Dyslipidemia; Spirulina; Blue-green algae; Antioxidant activity.

Résumé

Le syndrome métabolique (MetS) est un ensemble de problèmes de santé, réunissant notamment l'hyperlipidémie, l'hyperglycémie, la résistance à insulinique et l'obésité, qui peuvent entraîner des maladies graves. Dans notre étude, nous avons étudié l'effet de la spiruline, une microalgue comestible, riche en protéines et en antioxydants, sur le syndrome métabolique chez la souris. Avant d'administrer la spiruline aux souris, des analyses phytochimiques ainsi que l'activité antioxydante par les tests DPPH et FRAP ont été réalisées. Le syndrome métabolique a été induit en alimentant les souris avec un régime riche en graisses et en fructose. Parallèlement, de la spiruline (1 000 mg/kg/jours) a été administrée par voie orale pendant 28 jours. Après avoir sacrifié les souris, les profils glycémique et lipidique ainsi que les transaminases hépatiques ont été évalués. Les paramètres du stress oxydatif ont également été évalués. Les résultats ont montré que la spiruline présente un potentiel antioxydant *in vitro*. De plus, la spiruline augmente significativement les niveaux de glutathion réduit (GSH) et améliore les activités catalase (CAT) et glutathion peroxydase (GPx). De plus, la spiruline entraîne également une légère diminution de la glycémie et améliore l'entrée du glucose dans les tissus. Il réduit également les niveaux de triglycérides et contribue à une réduction du pourcentage de poids corporel du foie et de l'indice de masse corporelle tout en maintenant la prise de poids. Bien que la durée de quatre semaines n'ait pas été suffisante pour induire des changements significatifs dans le MetS chez la souris, il y avait des signes de développement de ces conditions, et la spiruline montre un effet positif contre ces caractéristiques.

Mots clés : Syndrome métabolique ; Hyperglycémie; Dyslipidémie; Spiruline ; Algues bleu-vert; Activité antioxydante.

ملخص

متلازمة التمثيل الغذائي (متلازمة الأيض) هي مجموعة من الحالات الصحية، بما في ذلك ارتفاع نسبة الدهون في الدم، وارتفاع السكر في الدم، ومقاومة الأنسولين، والسمنة التي يمكن أن تؤدي إلى أمراض خطيرة. في دراستنا، قمنا بدراسة تأثير السبيرولينا، وهي طحالب دقيقة صالحة للأكل، غنية بالبروتين ومضادات الأكسدة، على متلازمة التمثيل الغذائي لدى الفئران. قبل إعطاء السبيرولينا للفئران، أجريت التحليلات الكيميائية النباتية ونشاط مضادات الأكسدة باستخدام فحوصات DPPH و FRAP .تم إحداث متلازمة التمثيل الغذائي (MetS) عن طريق تغذية الفئران بنظام غذائي غني بالدهون وعالي الفركتوز. في الوقت نفسه، كانت تعطى السبيرولينا (1000 ملغم / كغم / يوم) عن طريق الفم لمدة 28 يوما. بعد قتل الفئران، تم تقييم نسبة السكر في الدم والدهون بالإضافة إلى الانزيمات الكبدية. كما تم تقييم معلمات الإجهاد التأكسدي. وأظهرت النتائج أن السبيرولينا تظهر نشاط مضادات الأكسدة. بالإضافة إلى ذلك، تزيد السبيرولينا بشكل كبير من مستويات الجلوتاثيون المنخفض (GSH) وتحسن أنشطة الكاتالاز (CAT) والجلوتاثيون بيروكسيديز (GPx) بالإضافة إلى ذلك، تظهر السبيرولينا أيضًا انخفاضًا طفيفًا في مستويات الجلوكوز في الدم وتحسن دخول الجلوكوز إلى الأنسجة. كما أنه يقلل من مستويات الدهون الثلاثية ويساهم في تقليل نسبة وزن الجسم ومؤشر كتلة الجسم مع الحفاظ على زيادة الوزن. على الرغم من أن مدة أربعة أسابيع لم تكن كافية لإحداث تغييرات كبيرة الخاصة بمتلازمة التمثيل الغذائي لدى الفئران ولكن كانت هناك علامات على تطور هذه الظروف، وتظهر السبيرولينا تأثيرًا إيجابيًا ضد هذه الميزات.

الكلمات المفتاحية: متلازمة التمثيل الغذائي. ارتفاع السكر في الدم. عسر شحميات الدم؛ سبيرولينا؛ طحلب اخضر مزرق؛ النشاط المضاد للأكسدة.

Summary

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List of abbreviations

A

AACE: American Association of Clinical Endocrinologists.

AG: Gallic acid.

ALAT: Alanine aminotransferase.

$AlCl_3$: Aluminum chloride.

ANSES : Agence nationale de sécurité sanitaire de l'alimentation de l'environnement et de travail.

AOPP: Advanced oxidation protein products.

APOA5 : Apolipoprotein A5.

APOC3 : Apolipoprotein C3.

ASAT: Aspartate aminotransferase.

AUC: Area under the curve.

B

BMI: Body mass index.

BP: Blood pressure.

C

C: Control.

Ca: Calsuim.

Cat: Catalase.

CD: Cafeteria diet.

CDHF: Choline deficient high fat.

Cr: Chromium.

CRP: Creative protein.

CProt: Protien concentration.

Cp: The concentration of the inhibitor.

CVD: Cardio vascular deseases.

D

DO: Optical density.

DTNB: 5,5 -dithio-bis-2-nitrobenzoicacid.

DPPH: 2,2 -diphenyl -1- picrylhydrazyl.

DW: dry weight.

E

EGIR: European group for the study of insulin resistance.

Eq: Equivalence.

F

FAO: The food and agriculture organization.

FBG: Fasting blood glucose.

FD: Dilution factor.

Fe: Iron.

$FeCl_3$: Ferric chloride.

$FeSO_4$: Ferrous sulfate.

FRAP: Ferric reducing power.

G

GCCG: Oxidized glutathione.

GR: Glutathione reductase.

GPX: Glutathione peroxidase.

GSH: Glutathione.

GST: Glutathione-S-transferases.

H

HCl: Hydrochloric acid.

HDL: High-density lipoprotein.

HDL-C: High-density lipoprotein Cholesterol.

HFD: High fat diet

$HgCl_2$: Mercury chloride.

H_2O_2: Hydrogen peroxide.

I

IC_{50}: Concentration for inhibiting 50%

IDF: International federation of diabetes.

IGT: Impaired glucose tolerance.

IIMSAM: International institution for use the Microalgae Spirulina Against Malnutrition.

IR: Insulin resistance.

K

K: Potasuim.

$K_3Fe(CN)_6$: Potassium ferricyanide.

KCl : Chlorure de potassium.

KI : iodure de potassium.

L

L: Length of tank used.

LBW: Liver body weight.

LDL: Low-density lipoprotein.

LDL-R: low-density lipoprotein Receptor.

LPL: Lipoprotein lipase.

M

MEPP: Mitochondrial energy production pathway.

MetS: Metabolic syndrome.

MDA: Malondialdehyde.

Mg: Magnesium.

IIMSAM: The International Institution for use the Microalgae Spirulina Against Malnutrition.

Mn: Manganese.

MS: Metabolic syndrome.

MS+SP: Metabolic syndrome and spirulina.

MUFAs: Monounsaturated fatty acids.

N

NaCl: Sodium chloride.

NaOH: Sodium hydroxide.

NCDs: non-communicable diseases.

NCEP-ATP III: National Cholesterol Education Program's Adult Treatment Panel III

NMR: Nuclear magnetic resonance spectroscopy.

O

$^{\bullet}O_2$: Super oxide anion radical.

OGTT: Oral glucose tolerance tests.

P

P: phosphorus.

PAMELA: Pressioni Arteriose Monitorate E Loro Associazioni.

PUFAs: Polyunsaturated fatty acids.

Q

QRC: Quercetin.

R

RBP4: Retinol-binding protein.

ROS: Reactive oxygen species.

Rpm: round per minute.

S

SEM: Standard error of the mean.

SFAs: Saturated fatty acid.

SOD: Superoxide dismutase.

SP: Spirulina.

SPPH: Spirulina platensis Protease Hydrolysate.

SSBs: Sugar sweetened drinks.

T

TG : Triglycerides.

T2D : Type 2 diabetes.

TBA: Thiobarbituric acid

TCA: Trichloroacetic acid.

TRx: Thioredoxin.

TRxR: Thioredoxin Reductase.

U

UA : Uric acid.

UV radiation : Ultraviolet radiation.

V

VLDL: Very low-density lipoprotein.

VS: Supernatant volume.

VT: Total volume of the reaction medium.

W

WC: Waist circumference.

WD: Western diet.

WHO: World Health organization.

Z

Zn: Zinc.

Introduction

The consumption of an unhealthy diet and living a lazy lifestyle, along with a lack of physical activity, has resulted in a widespread prevalence of overweight and obesity globally. These two factors contribute to the prevalence of chronic non-communicable diseases (NCDs), such as cardiovascular diseases and type 2 diabetes mellitus, which are the leading causes of mortality worldwide **(Castro-Barquero et al., 2020)**.

Today, metabolic syndrome or syndrome X is recognized as a significant public health challenge in both developed and developing countries **(Rigo et al., 2009)**. MetS is a significant risk factor for cardiovascular disease, stroke, and diabetes **(Grundy, 2016)**. Furthermore, the criteria for MetS, which include hypertension, insulin resistance, hyperlipidemia, hyperglycemia and abdominal obesity, are considered a cluster of cardiovascular risk factors. In at least one patient with metabolic syndrome, three of these components must be present: elevated blood pressure (BP), hyperglycemia, reduced levels of high-density lipoprotein (HDL), increased waist circumference, and hypertriglyceridemia **(Nolan et al., 2017)**. There are several definitions of MetS provided by different organizations, such as the International Federation for Diabetes (IDF) and the National Cholesterol Education Program's Adult Treatment Panel III (NCEP-ATP III) **(Hajian-Tilaki et al., 2014)**. According to the IDF, the global prevalence of MetS is approximately 25%, considering race, age, and sex as influencing factors **(O'Neill & O'Driscoll, 2015)**. The global prevalence of metabolic syndrome is higher in countries with higher income levels **(Noubiap et al., 2022)**.

In light of the widespread occurrence and seriousness of MetS, therefore, it is imperative to investigate new therapies aimed at reducing the damage caused by MetS and spirulina remains one of them.

Spirulina, also known as *Arthrospira platensis*, is a photosynthetic filamentous cyanobacterium with microscopic dimensions that exhibits high biomass productivity, boasting the highest rate of CO_2 fixation **(Singh et al., 2016)**.

It has been used since ancient times due to its exceptional nutritional profile. It is rich in proteins, accounting for nearly 60-70% of its dry weight, and also contains all the essential amino acids. Furthermore, it contains high levels of carotenoids (6,25%) **(Zahroojian et al., 2013)**, essential fatty acids (linoleic, gamma-linolenic, and palmitic acid), vitamins E, C, and selenium **(kalafati et al., 2010)**. Due to its concentrated nutrition, Spirulina has become one of the nutraceutical foods for managing various health issues in recent years **(Deng & Chow, 2010)**.

It has been shown that a number of bioactive peptides isolated from this cyanobacterium exhibit antibacterial, antiviral, anticancer, immunomodulatory, antiallergic, and antihypertensive properties **(Ovando et al., 2018)**. Moreover, phenolic phytochemicals from Spirulina, such as phycobiliprotein C-phycocyanin, have potent anti-inflammatory and antioxidant properties **(machu et al., 2015)**.

The objective of our thesis is to study the effect of Spirulina supplementation on a high fat and fructose diet-induced metabolic syndrome in mice.

This thesis has two main parts:

The first part is a bibliographic review that contains theoretical notions on spirulina and metabolic syndrome.

The second part describes the methodology based on the phytochemical analysis of spirulina followed by the evaluation of the effect of spirulina on metabolic syndrome in mice through in vivo study. In this section, obtained results are analyzed and discussed and the conclusion is also presented at the end of the thesis.

Part one: Review

Chapter 1: Metabolic syndrome

1. Definition

The definition of metabolic syndrome has been modified several times over the years because it is challenging to establish universal criteria for it. Metabolic syndrome (MetS) is a term used to describe a set of metabolic abnormalities, mostly consisting of hypertension, central obesity, insulin resistance, and dyslipidemia. Virtually all of the disorders related to MetS are associated with a pro-inflammatory state caused by altered glucose metabolism. Type 2 diabetes (T2D) and cardiovascular diseases (CVDs) are indeed among the consequences strongly associated with MetS. The pathogenesis of MetS involves a combination of genetic and acquired factors that influence the final pathway of inflammation **(Ambroselli et al., 2023)**.

The first definition of MetS was introduced by the Swedish researcher Kylin in 1923. It was described as a syndrome characterized by hypertension, hyperglycemia, and hyperuricemia **(Levesque & Lamarche, 2008)**.

Later, Himsworth divided subjects into insulin-sensitive and insulin-resistant groups, adding important information connected with the pathophysiological background of MetS **(Sarafidis & Nilsson, 2006)**.

During the subsequent years, various names have been attributed to the clustering of the components of Metabolic Syndrome, such as syndrome X **(Reaven, 1988)**, the insulin-resistance (IR) syndrome **(DeFronzo & Ferrannini, 1991)** and the deadly quarte **(Kaplan, 1989)**. However, the first comprehensive description of the MetS dates back to the late 1980s by Reaven who originally defined syndrome X namely, individuals with a combination of impaired glucose tolerance (IGT) and hyperinsulinemia together with high triglycerides (TG), high low-density lipoproteins (LDL), low high-density lipoproteins (HDL) levels, and hypertension **(Reaven, 1988)**.

A year later, Kaplan integrated this definition by incorporating the concept of visceral adiposity as an additional crucial component of the cluster **(Pasternak, 2003)**.

In 1999, the World Health Organization (WHO) Diabetes Group centered the definition on the presence of IR, which is described as IGT or high plasma insulin levels or T2D. In order to make a positive diagnosis, two additional risk factors need to be present in patients affected by obesity, hypertension, and dyslipidemia **(Table 1) (Kurian et al., 2016)**.

In the same year, the European Group for the Study of Insulin Resistance (EGIR) attempted to streamline the WHO definition by removing the microalbuminuria criterion and prioritizing the concept of central obesity over general obesity **(Balkau & Charles, 1999)**.

Table 01: World Health Organization definition of the metabolic syndrome **(Alberti & zimmet, 1999)**.

The patient must have 1 of the following:	
Diabetes mellitus Fasting plasma glucose	Fasting plasma glucose ≥ 7 mmol/L (126 mg/dL) or 2-h post glucose load ≥ 11,1 mmol/L (200 mg/dL)
Impaired glucose tolerance	Fasting plasma glucose < 7 mmol/L (126 mg/dL) and 2-h post-glucose load ≥ 7,8 mmol/L (140 mg/dL) and < 11,1 mmol/L (200 mg/dL)
Impaired fasting glucose	Fasting plasma glucose ≥ 6,1 mmol/L (110 mg/dL) and < 7 mmol/L (126 mg/dL) and (if measured) 2-h post glucose load < 7,8 mmol/L (140 mg/dL)
Insulin resistance	Glucose uptake below lowest quartile for background population under investigation under hyper insulinemic, euglycemic conditions
Plus any 2 of the following:	
Waist-to-hip ratio	> 0,9 in men, > 0,85 in women; BMI > 30; or both
Triacylglycerols	≥ 1,7 mmol/L (150 mg/dL); HDL cholesterol < 0,9 mmol/L (35 mg/dL) in men, < 1,0 mmol/L (39 mg/dL) in women; or both
Blood pressure	≥ 140/90 mm Hg (revised from ≥ 160/90 mm Hg)
Microalbuminuria	urinary albumin excretion rate ≥ 20 µg/min or albumin-to-creatinine ratio ≥ 30 mg/g

Other major criteria were proposed in 2001 by the National Cholesterol Education Program-Adult Treatment Panel III (ATP III). These criteria do not require the demonstration of IR as a mandatory criterion, but instead require the presence of at least three out of five factors to establish the diagnosis **(Table 2) (Alberti et al., 2009)**.

Other groups have also proposed their own definitions, and in 2005, the International Diabetes Federation (IDF) tried to standardize the classifications of MetS **(Levesque & Lamarche, 2008)**.

Table 02: Adult Treatment Panel III definition of the metabolic syndrome **(jama, 2001)**.

Any 3 of following:	
Fasting glucose	≥ 6.1 mmol/L (110 mg/dL)
Waist circumference	Men: > 102 cm (40 in) Women: > 88 cm (35 in)
Triacylglycerols	≥ 1.7 mmol/L (150 mg/dL)
HDL cholesterol	Men: < 1.036 mmol/L (40 mg/dL) Women: < 1.295 mmol/L (50 mg/dL)
Blood pressure	≥ 130/85 mm Hg

There are other criteria for MetS that have been proposed by the American Association of Clinical Endocrinologists (AACE). However, the AACE definition differs slightly from the definition given by ATP III and other organizations. According to the AACE, MetS is a prediabetic condition characterized by insulin resistance and the presence of at least one of the following symptoms present in **(table 3) (Kassi et al., 2011)**.

The diagnosis of MetS is based on measuring several simple parameters, including waist circumference (WC), blood pressure, HDL cholesterol (HDL-C), TG levels, and blood glucose. Metabolomic approaches, such as NMR spectroscopy and chromatographic techniques, can be employed to identify various metabolites that are associated with the presence of MetS or its individual components. Consequently, these molecular biomarkers could be utilized to define and predict the condition of patients with MetS **(Ambroselli et al., 2023)**.

Table 03: Diagnostic criteria for the metabolic syndrome according to (AACE) **(Almeda-Valdés et al., 2009)**.

American Association of Clinical Endocrinologists (AACE)	
Overweight/obesity BMI	≥ 25 kg/m^2
Elevated triglycerides	≥ 150 mg/dL
Low HDL cholesterol	Men < 40 mg/dL Women < 50 mg/dL
Elevated Blood pressure	≥ 130/85 mmHg
2-Hour post glucose challenge	> 140 mg/dL
Fasting glucose	between 110 and 126 mg/dL
Other risk factors	Family history of type 2 diabetes, hypertension or CVD, Polycystic ovary syndrome, Senderaty lifestyle, advancing age and Ethnic groups having high risk for type 2 diabetes or CVD.

2. Epidemiology of metabolic syndrome

Metabolic syndrome is a significant public health concern, given its high and steadily growing prevalence and incidence. In fact, it has even been characterized as a pandemic in recent years **(Scuteri et al., 2015)**.

In the study conducted over a one-year period (January 2022–February 2023), the prevalence of metabolic syndrome among participants aged between 30 and 79 years old was 63,3%.

The prevalence of metabolic syndrome was slightly higher in males compared to females, with 52,5% of men and 47,5% of women being affected by the condition.

Moreover, individuals over 60 demonstrated a higher incidence of metabolic syndrome, indicating that age is a risk factor for the condition **(Rus et al., 2023)**.

This information fluctuates according to various factors such as age, gender, ethnic group, genetic predisposition, socio-economic status and educational level. In advanced nations like the United States and Europe, metabolic syndrome affects an about one-third and one-quarter of the population respectively. However, in developing countries with a predominance of young adults, the prevalence is considerably lower. Nevertheless, the increase in the standard of living and the aging of the population have significantly contributed to the increase in the prevalence of metabolic syndrome **(Claire Mayer, 2019)**.

2.1. Metabolic Syndrome Prevalence in Algeria

The occurrence of MetS in Algeria is subject to which criteria were used. The prevalence is approximately 34,0% depending on NCEP-ATP III criteria (the National Cholesterol Education Program Adult Treatment Panel III), while for IDF criteria (International Diabetes Federation), it is approximately 31,5% **(Ngwasiri et al., 2023)**.

2.2. Gender-Specific Insights

Prevalence of MetS in women in Algeria:

29,1% (NCEP-ATP III) and 28,3% (IDF). The proportion of MetS in women aged 35-44 years and 45-54 years was 35,1% and 38,2% respectively, while in women aged ≥ 65 years, MetS increased to 43,3% and 38,3% respectively.

Prevalence of MetS in men in Algeria:

12,0% (NCEP-ATP III) and 44,3% (IDF). The prevalence of MetS in men with physical inactivity was 2,67 and that was 1,30 among those who consumed fruit every day, both findings when the IDF definition was used **(Ngwasiri et al., 2023)**.

3. Risk factors

Metabolic syndrome is a common risk factor for CVD and diabetes. These conditions include abnormal blood sugar, high blood pressure, high TG levels, low HDL cholesterol levels and obesity **(Alberti et al.,2009)**.

3.1. Abdominal Obesity

Abdominal obesity plays an important role in the emergence of metabolic syndrome and can be considered one of the symptoms of adipose tissue dysfunction **(Claire Mayer, 2019)**.

Body mass index (BMI) is not sufficient to diagnose obesity. On the other hand, measuring the waist circumference of overweight or obese people can detect excess fat, which is associated with the onset of metabolic syndrome. Therefore, for the same BMI, a person's risk of developing metabolic diseases will not be the same whether the obesity is male (more than 40 inches) or female (more than 35 inches) **(John Hopkins Medicine, 2024)**.

3.2. Dyslipidemia

Atherogenic dyslipidemia is characterized by elevated levels of LDL and TG, along with decreased levels of cardioprotective HDL. This combination plays a crucial role in the metabolic syndrome, where insulin resistance drives its pathogenesis and contributes to the development of atherosclerosis and CVD **(Ahima, 2024)**.

Indeed, the process of hypercholesterolemia is characterized by the buildup of LDL in the plasma, which is caused by genetic changes in the LDL receptor (LDLR) or apolipoprotein B-100, a crucial component of atherogenic lipoproteins. Hypertriglyceridemia, on the other hand, is caused by the condensation of chylomicrons or very low-density lipoprotein (VLDL) or both in the plasma, coupled with a decrease in HDL levels. These condensations occur as a result of mutations in apolipoprotein A5 (APOA5), which leads to increased breakdown of triglycerides, and the inhibition of lipoprotein lipase (LPL) and hepatic lipase by apolipoprotein C-III (APOC3), contributing to elevated triglyceride concentrations **(Silva Figueiredo et al., 2017)**.

3.3. High blood pressure

Worldwide, high blood pressure is a major risk factor for heart and kidney diseases. Negative consequences of high blood pressure are not limited to people with high blood pressure. People with prehypertension are more likely to have high blood pressure and have a higher risk of heart disease than people with high blood pressure **(Appel, 2013)**.

One of the necessary criteria for diagnosing the MetS is the presence of high blood pressure. The data indicates that individuals with MetS often experience hypertension **(Chimonas et al., 2010)**.

In the Pressioni Arteriose Monitorate E Loro Associazioni (PAMELA) study, elevated blood pressure was found to be the most common component of the MetS in patients, with a prevalence of over 80%. Additionally, participants with MetS exhibited higher levels of home, office, and ambulatory blood pressure when compared to those without the syndrome **(Katsimardou et al., 2020)**.

3.4. Diabetes type 2

In general, people with MetS suffer from high blood sugar. Blood sugar can be defined as a prediabetic state or diabetes, depending on its value, and both are thought to be risk factors for MetS.

T2D is often associated with MetS and obesity. In patients with type 2 diabetes, insulin resistance begins to occur, which is compensated by excess insulin (hyperinsulinemia). Thus, a diagnosis of diabetes can be made if the fasting blood sugar is between 5,55 and 6,94 mmol/L or the blood sugar after 2 hours is between 7,77 and 11,1 mmol/L **(Claire Mayer, 2019)**.

4. Role of nutrition in the development of metabolic syndrome

Nutrition has an intricate and complicated function in the development of MetS. Observational studies have revealed that MetS can be influenced by various nutrients, including macronutrients (saturated fatty acids, sugars), micronutrients (antioxidant, vitamins….), polyphenols (flavonoids, resveratrol) and other compounds (fucoxanthin, policosanol) **(Kern & Mitmesser, 2018)**.

4.1. Saturated fatty acid

Worldwide dietary standards recommend limiting the amount of saturated fatty acids (SFAs) consumed as part of an ideal approach to prevent cardiovascular diseases. Furthermore, these dietary guidelines clearly prohibit the use of foods high in saturated fat, such as full-fat and reduced-fat dairy products **(Houston, 2017)**.

These recommendations were originally based on studies conducted in the early twentieth century that showed a positive relationship between dietary saturated fat content and CVD. However, more recent studies assessing the impact of saturated fat intake on metabolic health have yielded inconclusive and widely variable results.

These disparities are most likely related to the heterogeneity of SFAs. The carbon length and structure of SFAs differ greatly depending on their dietary source. Thus, the significance of dietary SFA chemical heterogeneity is more nuanced than previously acknowledged, and the physiological impact of dietary SFAs depends not only on the dietary source and food matrix, but also on the type(s) and composition of SFAs **(Unger et al., 2019)**.

4.1.1. Source of fatty acid

The saturated fatty acids are most commonly found in animal products and their derivatives. They are abundant in solid animal fats at ambient temperatures, such as pork fat,

duck fat, and lard. There are also saturated fatty acids in butter, non-emulsified dairy products such as whole milk and certain yogurts, cheeses, and fresh cream. In addition, saturated fatty acids can be found in some plant-based products, such as coconut and palm oil. Finally, certain commercial vegetable oils contain high levels of saturated fatty acids **(leleux, 2022)**.

A study discovered that a Western dietary pattern, which includes a lot of refined carbohydrates, processed meat, fried meals, and red meat, is linked to a higher risk of having metabolic syndrome. After further investigation, the researchers discovered that people who consumed the most meat were more likely to acquire metabolic syndrome **(harrison, 2015)**.

It is crucial to follow nutritional recommendations to maintain a balanced and healthy diet, with a good distribution of fatty acids, not too much, not too little saturated fat, and a good balance of polyunsaturated fat **(Nutrition-Sante.pdf)**.

4.1.2. Daily intake of saturated fatty acid

The ANSES (Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail) recommends a saturated fatty acid intake of less than or equal to 12% of the total energy intake. It is important to note that the daily intake of saturated fatty acids varies according to age, gender, and physical activity **(Leleux, 2022)**.

4.2. Sugar

Sugar-sweetened drinks (SSBs) include caloric sweeteners such sucrose, high-fructose corn syrup and fruit juice concentrates. A meta-analysis of data from 11 prospective cohort studies comprised 19,431 people with MetS and 310,819 with T2D. Individuals who drank one to two servings of SSB per day had a greater risk of developing MetS and T2D compared to those who consumed none or less than one serving per month **(Malik et al., 2010)**.

A longitudinal study of over 88,000 women (17 A 24-years) indicated that consuming ≥ 2 SSBs/day increased the incidence of coronary heart disease by 35% compared to occasional users **(Kern & Mitmesser, 2018)**.

4.3. Micronutrients

4.3.1. Antioxidant vitamins

The primary mechanisms linking energy excess and obesity to insulin resistance and the ensuing metabolic diseases are systemic inflammation and oxidative damage. Antioxidant substances have the ability to control oxidative stress and may even be able to prevent health issues linked to oxidative damage **(Avignon et al., 2012)**.

For instance, evidence from both *in vitro* and *in vivo* studies suggests that vitamin A, in the form of retinol, not only affects immunological function but also reverses chronic inflammation by reducing the amount of adipocytokines **(García, 2012)**, and in persons with T2D or in insulin-resistance states such as non-alcoholic fatty liver disease and metabolic syndrome **(Esteve et al., 2009)**.

Animal studies have shown that retinol-binding protein (RBP4), the transport protein for retinol, is negatively correlated with insulin sensitivity **(Wu et al., 2009 ; Dakshinamurti, 2015)**.

Diets high in antioxidants, such as vitamin C, E, and β-carotene, have been linked to a lower incidence of CVD and have positive effects on glucose metabolism and the prevention of diabetes, according to epidemiological research **(Kern & Mitmesser, 2018)**.

4.4. Polyphenols

4.4.1. Flavonoids

Flavonoids are a type of polyphenol that are frequently present in fruits, vegetables, legumes, herbs, and tea. Extensive research has been conducted on the anti-inflammatory and antioxidant properties of flavonoids. Prospective studies indicate that there is an inverse associations between the occurrence or mortality of CVD and the intake of flavonoids **(Hertog et al., 1995)**. Similarly, a systematic review reveals the positive effects of foods rich in flavonoids on CVD indicators, including reduced blood pressure and improved endothelial function **(Hooper et al., 2008)**, Examples of such foods include cocoa, chocolate, grapes, and black tea **(Davì et al., 2010)**.

5. Metabolic disruption associated with metabolic syndrome

5.1. Inflammation

The inflammatory state associated with metabolic syndrome is unique in that it is not accompanied by infection or autoimmunity, and there are no severe tissue lesions. It is defined as “chronic low-level inflammation”, and several studies have shown a link between inflammation and metabolic syndrome. Inflammation can manifest as an acute phase with increased C-reactive protein (CRP) or a systemic phase with increased plasma levels of proinflammatory cytokines IL-6 and TNF-α **(Figure 01) (Claire Mayer, 2019)**.

5.2. Insulin-resistance

Insulin resistance is a medical condition in which the body's cells do not respond correctly to insulin, a hormone produced by the pancreas. Insulin is responsible for the absorption of glucose (sugar) from the blood by cells to provide energy. When cells are insulin resistant, glucose accumulates in the blood, which can lead to a variety of health issues, including T2D **(Figure 01) (Ahima, 2024)**.

Before the current definition of metabolic syndrome, it was observed that risk factors such as dyslipidemia, hypertension, and hyperglycemia were frequently observed in insulin-resistant individuals. Insulin resistance is considered a characteristic of metabolic syndrome, but it is more strongly linked to metabolic complications of obesity when associated with other metabolic perturbations such as inflammation, mitochondrial dysfunction (diminished mitochondrial number and density, as well as mitochondrial oxidation of fatty acids and glucose), hyperinsulinemia, lipotoxicity, and dyslipidemia **(Claire Mayer, 2019)**.

5.3. Oxidative stress

Reactive oxygen species (ROS) generation and the body's antioxidant defenses are imbalanced in metabolic syndrome, resulting in oxidative damage to cells and tissues. Key components of metabolic syndrome, such as insulin resistance, dyslipidemia, endothelial dysfunction, and inflammation, are associated with oxidative stress. T2D, CVD, and other metabolic syndrome-related issues have been linked to heightened oxidative stress within the syndrome **(Figure 01) (Ahima, 2024)**.

The diseases associated with MS are also characterized by the downregulation of antioxidant systems, including the depletion of GSH concentration and the decrease in the activity of detoxifying enzymes such as superoxide dismutase (SOD), catalase (Cat), glutathione peroxidase (Gpx), glutathione reductase (GR), and the couple constituted by thioredoxin (Trx) and thioredoxin reductase (TrxR) **(Rani et al., 2016 ; Vona et al., 2019)**. In addition, oxidative stress plays a crucial role in inflammatory and apoptotic processes, cellular growth, and function. During the early stages of atherosclerosis, oxidative stress plays a role in the oxidation of LDL **(Claire Mayer, 2019)**.

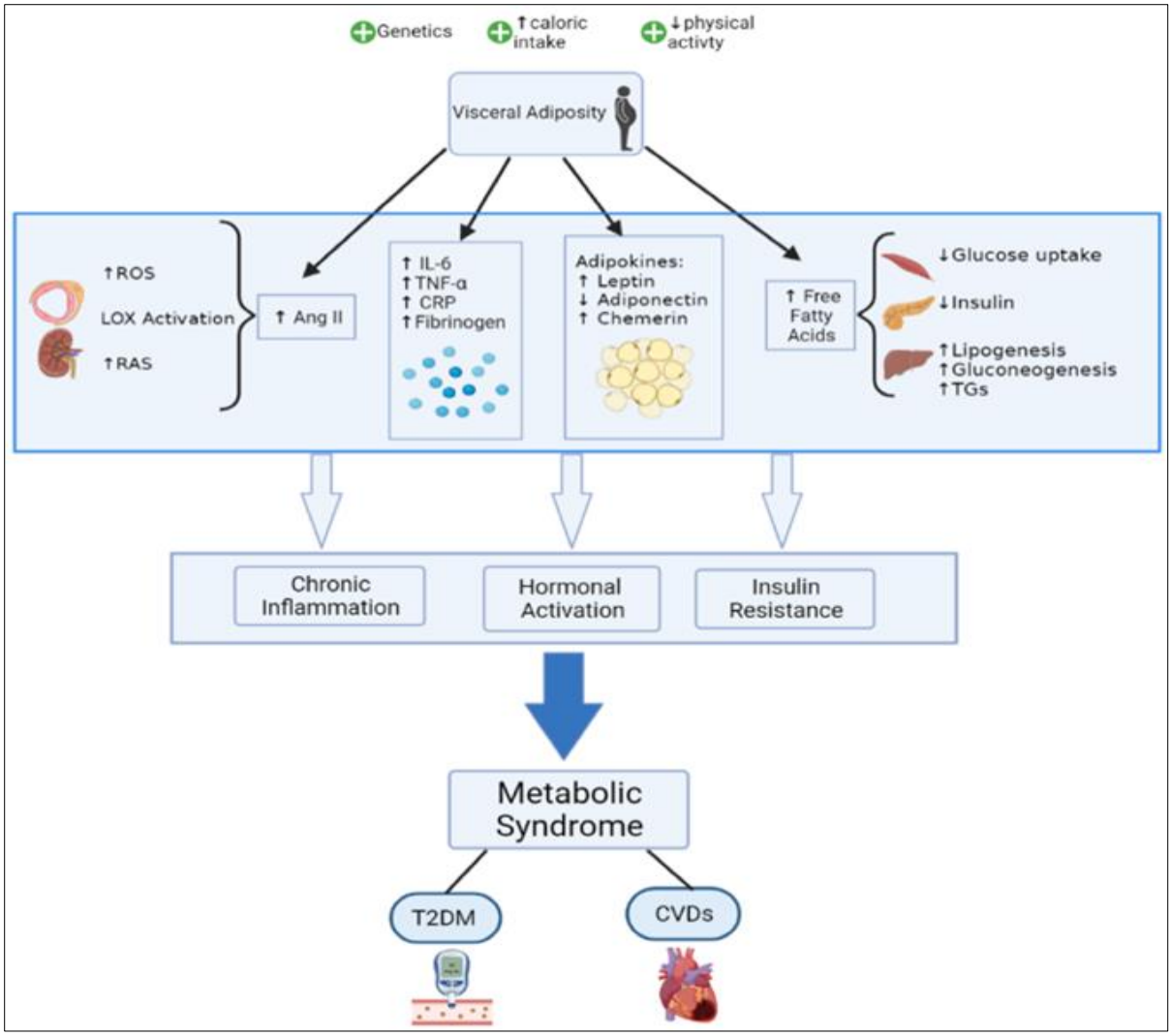

Figure 01: Mechanisms highlighting MetS pathophysiology **(Fahed et al., 2022)**.

6. Metabolic syndrome and associated diseases

Metabolic syndrome criteria include obesity, dyslipidemia, high blood pressure, and changes in glucose metabolism **(Mendrick et al., 2018)**. Metabolic syndrome can cause a variety of major health conditions, including T2D, dyslipidemia and CVD.

6.1. Diabetes type 2

Individuals with metabolic syndrome are more likely to develop T2D due to insulin resistance and elevated blood sugar levels **(Kurian et al., 2016)**.

6.2. Lipid disorders

Dyslipidemia caused by MetS can lead to atherosclerosis, the accumulation of plaque in the arteries that increases the risk of heart disease and stroke **(Ahima, 2024)**.

6.3. Cardiovascular Disease

There is a strong correlation between MetS and cardiovascular diseases (CVD). Elevations in serum uric acid (UA) have a linear dose-response relationship and are associated with a higher risk of metabolic syndrome. Research indicates that elevated serum UA levels are associated with a greater frequency of metabolic syndrome and an increased risk of cardiovascular disease **(WHO, 2021)**.

6.4. Hepatic Steatosis

MetS is frequently associated with non-alcoholic fatty liver disease, characterized by the buildup of fat in the liver and can progress to more serious liver disorders **(Almeda-Valdés et al., 2009)**.

Metabolic syndrome can affect blood vessels all over the body, thereby increasing the risk of peripheral artery disease and other circulatory problems. Although there are no licensed medications for this condition, numerous treatments target linked disorders, which may increase the risk of drug-drug interactions **(Mendrick et al., 2018)**.

7. Experimental models for metabolic syndrome induction

There are many animals which can be used as model for studying and treating the MetS. The induction of the MetS is possible by genetic modification or by following diet rich on carbohydrates or fats or combination between the two, the genetic model of MetS is made by modification in certain genes like the leptin gene and the leptin receptors gene these models develops MetS faster than other animal models.

The diet-induced MetS model exhibit to special diet like the high-carbohydrate diet or high fat diet or the combination between the two, this simulates more the human lifestyle **(Fuchs et al., 2018 ; Wong et al., 2016)**.

Mostly for the high carbohydrate diet the fructose and saccharose were given to the animals with different proportion in drinking water, concerning high fat diet can include lard and soy oil with different proportion **(Table 4) (Gunawan et al., 2021)**. The duration required to induce MetS by special diet ranges from 3 to 48 weeks **(Wong et al., 2016 ; Fuchs et al., 2018)**.

Table 04: Composition of HCD and HFD **(Nadkarni et al., 2013)**.

	HCD	HFD
Weight content (g/kg)		
Milk proteins	140.0	170.0
Starch	622.4	436.6
Sucrose	100.3	71.1
Soy oil	40.0	225.0
Minerals	35.0	35.0
Vitamins	10.0	10.0
Cellulose	50.0	50.0
Choline	2.3	2.3
Energy content (%)		
Protein	14.7	14.4
Carbohydrate	75.9	42.9
Fat	9.4	42.8
Energy density (kJ/g)	15.95	19.82
Food quotient	0.946	0.847

Rodents such mice and rats are the most utilized as MetS experimental models. The most common diet-induced MetS model use animals like: male Wistar rat, male Sprague Dawley rats and male C57BL/6 j mice **(Wong et al., 2016)**.

For the mutagenic animal models used like: ob/ob mouse, DB/DB mouse, kk mouse, Zucker rats and Goto - kakiza ki rats (Fuchs et al., 2018)

Other animals can be used like dogs, cats, rabbits and pigs **(Fuchs et al., 2018 ; Hidayati et al., 2020)**.

Chapter 02: Spirulina

1. Definition

Spirulina is a blue green algae, edible filamentous bacteria that belong to the cyanobacteria phylum. It is oxygenic photosynthetic, lives in fresh and salt water. It is called spirulina due to the spiral shape of its filaments. Arthrospira platensis is rich in many nutrients, especially proteins, which can reach up to 70% of its composition. Spirulina is also rich in vitamins, minerals and bio-active molecules. It is used as a very common algae-supplement and provides many biological properties such as antioxidant, anti-inflammatory, immunomodulatory, antibacterial, antiviral, anti-allergic and anti-cancer **(Karkos et al., 2011 ; Calella et al., 2022 ; Chaouachi et al., 2024)**.

There have been many studies that have investigated the ability of spirulina to treating various diseases such as cardiovascular diseases, obesity, hyperglycemia, hypertension, inflammatory diseases and tumors. Furthermore, there have been many *in vivo* studies examining the beneficial effects of spirulina on brain health from various perspectives **(Karkos et al., 2011 ; Sorrenti et al., 2021)**.

2. History of spirulina

The spirulina has been used for hundreds of years as a source of food or dietary supplement. The Aztecs including the Mayas, Toltecs and Kanembueating used to eat the blue green algae which they extracted from Lake Texcoco (Mexico). The Spanish chroniclers noticed the Aztec fishermen while they extracted a blue green mass from the lakes, and they ate it as dried cakes. They used to call it Tecuitlatl **(García et al., 2017)**.

In 1815, spirulina was discovered for the first time by two Spanish scientists Hernando Cortez and Conquistadors. Cortez saw the Aztec people eating spirulina and observed the beneficial effect of spirulina on flamingos for surviving by consuming this blue-green algae **(Soni et al., 2017)**.

Before 1962, spirulina was considered to be a type of algae. However, in that year, it was reclassified as a member of the prokaryotic kingdom, and the name “cyanobacteria” was suggested for it. The modern age and the biotechnology development of the blue green algae began in the early 1940s and gained popularity since the University of Stanford held the first algal mass culture reunion. Since then, there have been many suggestions of methods and technologies to culture this cyanobacterium for exploiting it commercially regardless of its presence in nature **(García et al., 2017)**.

In 1969, the first spirulina factory (SOSA TOXCOCO) was created by the French in Mexico **(Soni et al.,2017)**.

In 1974, the World Food Conference held by the United Nations declared the spirulina as the best food for the future.

The International Institution for the use of Microalgae Spirulina Against Malnutrition (IIMSAM) initiated a revised draft resolution on the use of spirulina to fight hunger, malnutrition and achieve sustainable development **(García et al.,2017)**.

In 2008, the Food and Agriculture Organization (FAO) submitted a project to exploit these algae as a substitute for high quality food with less damage to the environment because it can be cultured in uncultivated areas **(García et al, 2017)**.

Now, spirulina is industrialized and used as a dietary supplement in different forms. It can be used as a food supplement in agriculture, aquarium and poultry industries **(Hameed & Shemiss, 2023)**.

3. Description and taxonomy

3.1. Description

Spirulina is multicellular and filamentous blue-green microalgae that reproduce by binary fission. It takes many forms, such as rod or disk shaped **(Figure 02)**. It lives with bacteria that fix nitrogen from the air in a symbiotic relationship. Spirulina is photosynthetic and autotrophic. Among the pigments it contains are chlorophyll a, carotenoids. Phycocyanin gives it a blue color, and phycocythrin is responsible for the pink or red color of bacteria **(Habib et al., 2008)**.

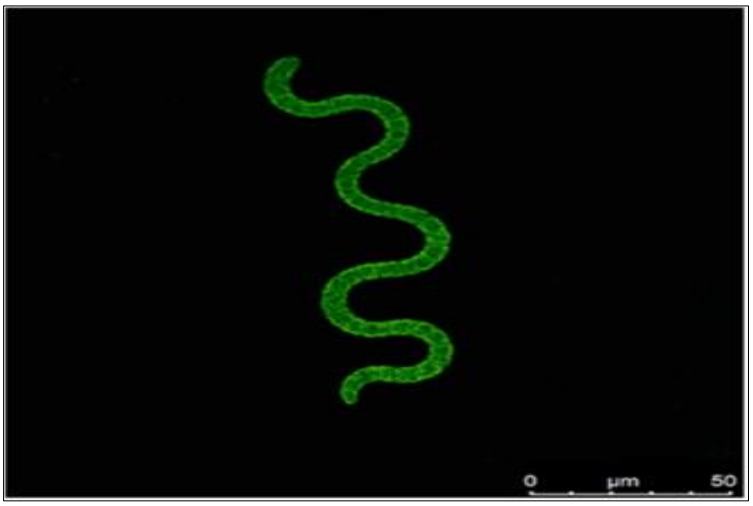

Figure 02: Confocal microscopic image of 5-day-old Spirulina **(Athiyappan et al., 2024).**

Spirulina has a cell wall similar to that of gram-negative bacteria, and it contains peptidoglycan, a lysozyme sensitive heteropolymer that confers shape and osmotic protection to the cell. The floating mats result from the gas filled vacuoles and the helical shape of the filaments which is characteristic of the genus. It is found only in a liquid environment or culture medium **(Habib et al., 2008)**.

3.2. Taxonomy

spirulina is a genus of cyanobacteria (blue-green algae). Its classification in detail is as follows **(AlFadhly et al., 2022)**:

Domain: Bacteria.

Kingdom: Eubacteria.

Phylum: Cyanophycea.

Class: cyanophyceae.

Sub-class: Oscillatoriophycideae.

Order: Osellatorlales.

Family: Oscillatorlaceae.

Genus: Arthrospira.

Species: A. platensis.

4. Culture media

Zarrouk's media is the best media because it contains the most important elements that spirulina needs, such as carbon and nitrogen. These elements are provided by sodium bicarbonate and urea. The high pH is maintained by sodium chloride **(Athiyappan et al., 2024)**.

There are three types of spirulina cultivation systems: open systems, closed systems, and hybrid systems. The first and the third are usually used on a large scale. The difference between the three systems is shown in **(Figure 3) (Athiyappan et al., 2024)**.

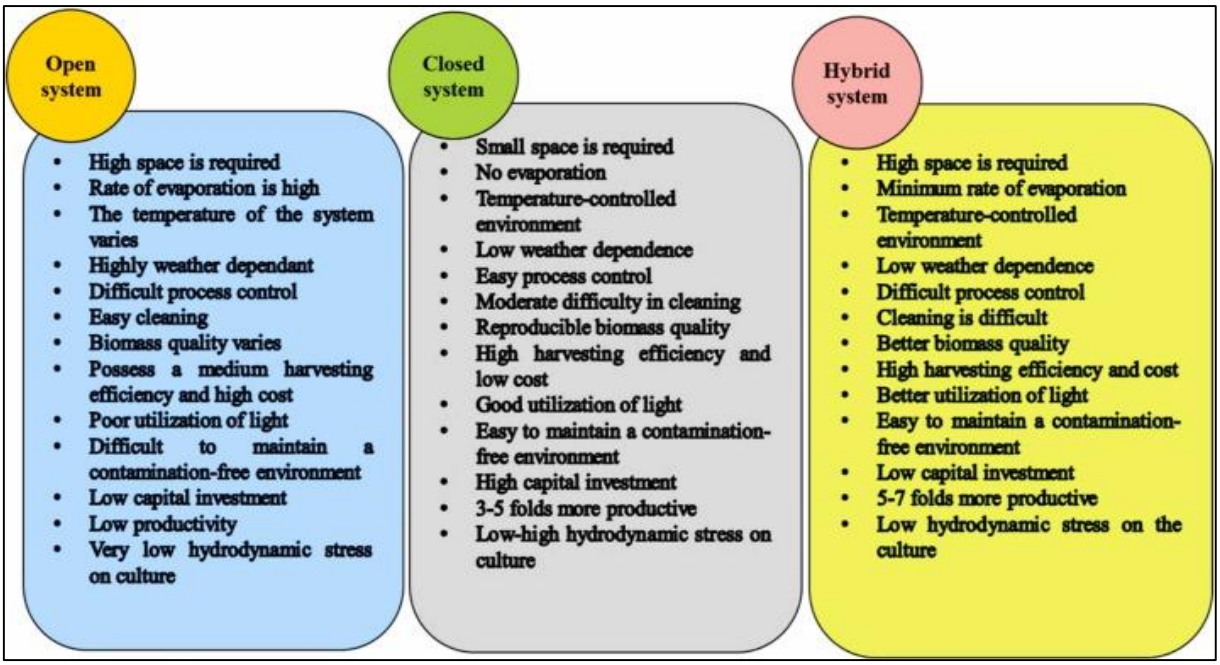

Figure 3: Comparison between Spirulina production in an open system, closed system, and hybrid system **(Athiyappanet et al., 2024)**.

5. Composition of spirulina

5.1. Proteins

Spirulina is a rich source of protein, and its ratio differs depending on the condition of the culture, reaching up to 60% of its dry weight (DW). The protein of this blue green algae contains essential amino acids such as methionine, lysine, threonine, valine, isoleucine and phenylalanine. The large proportion of spirulina proteins, about 60%, is represented by phycobilisomes, which consist of C-phycocyanin and A-phycocyanin. The proteins of this micro-algae have a high digestibility (83-90%) due to the fragile murein membrane cell of spirulina cells **(Fais et al., 2022)**.

5.2. Carbohydrates

The percentage of carbohydrates in spirulina can be around 15 to 25% of its dry weight. This small amount of sugars makes it low in calories **(Manet, 2016)**.

Spirulina contains sugars such as glucose, xylose, mannose and galactose, as well as glycogen and it is free of cellulose. There is a polysaccharide with high molecular weight present in spirulina called immolina. The principal sugar in this type of polysaccharide is rhamnose, representing between 49,7% and 52,3% of the total sugars generated by spirulina. The proportion of total carbohydrates in spirulina is about 17,25% of its total biomass (1,22 g/L), of which 2,59% is polysaccharides such as rhamnosamine (9,7%), glucosamine (1,9%),

and glycogen (0,5%). There is a small quantity of sugars like glucose, fructose, glycerin, sorbitol, mannitol and sucrose. The sugars present in the wall of the spirulina are similar to the sugars present in the wall of gram-negative bacteria. They contain glucosamine, glucosamine bound to the peptide and muramic acid **(AlFadhly et al.,2022)**.

5.3. Lipids

The lipid content in spirulina range between 7-16% of its dry weight **(Fais et al.,2022)**.

Spirulina has a high quantity of polyunsaturated fatty acids (PUFAs) and contains γ-linolenic acid (ALA), linoleic acid, docosahexaenoic acid, arachidonic acid and licosapentaenoic acid. For the monounsaturated fatty acids (MUFAs), they are present in low concentration. γ-linolenic acid and palmitic acid have the highest content of MUFAs while myristic acid has the lowest content at 0,2% **(AlFadhly et al.,2022)**.

According to **(matos et al.,2018)**, the amount fatty acids in spirulina can vary due to many reasons, including the growing conditions.

5.4. Vitamins

There are many circumstances that can influence the amount of vitamins in spirulina, such as culture conditions, harvesting methods, and drying methods **(AlFadhly et al.,2022)**.

Spirulina contains all the vitamins including the B group vitamins, **(Edelmann et al., 2019)** found that the amounts of vitamins B2 and B12 were respectively 36,3 and 2,4-0,6 ug/g. Folic acid is present in low quantities in dry spirulina **(AlFadhly et al.,2022)**.

Spirulina is an excellent source of β-carotene, which is then transformed into vitamin A. Dried spirulina contains 5,8mg/g of β-carotene. Consuming up to 2g of spirulina per day provides the daily requirement of vitamin A. According to **(Seyidoglu et al., 2017)** dried spirulina contains 5mg/100g of vitamin E, 2,2mg/100g of vitamin k, 0,005 mg/100g of biotin and 0,1mg/100g of pantothenic acid. According to **(Stanic-Vucinic et al., 2018)**, 3g of dried blue-green algae contain 0,8mg of vitamin C, 1200 IU of vitamin D, 9µg of vitamin B1, 75µg vitamin K and 11,250 IU of vitamin A.

5.5. Enzymes

There are many enzymes present in spirulina, but the most important for the antioxidant activity is superoxide dismutase (SOD), which superoxide that is present in high ratio in spirulina (216 000 IU in 10 g). This catalyst plays a crucial role in scavenging the free radicals that cause oxidative stress **(Proy, 2019)**.

5.6. Minerals

Spirulina is an interesting source of different minerals such as iron, calcium, potassium, copper, sodium, phosphor, boron, molybdenum, zinc, magnesium and manganese. The content of iron in spirulina is very high, it is ten times higher the other iron rich foods with high absorbance (60%). The quantities minerals in 100 g dried spirulina can be as follows: 700mg of Ca, 800 mg of P, 1400 mg of K, 0,28 mg of Cr, 100 mg of Fe, 400mg of Mg, 5 mg of Mn, and 3 mg of Zn **(AlFadhly et al.,2022)**.

5.7. Pigments

Spirulina is a blue-green algae rich in pigments, which are responsible for its color, including phycocyanin and chlorophyll. It also contains carotenoids **(Sguera, 2008 ; Benucci et al., 2023)**.

5.7.1. Phycocyanin

Phycocyanin is a water-soluble protein extracted from cyanobacteria and some crypto monads. It is the principal pigment in spirulina and its spectral properties. Determine its division into phycocyanin A and C **(Liu et al., 2022)**.

Phycocyanin is located in the phycobilisomes, protein complexes that act as antennas for the photosynthetic system and are attached to thylakoids. Phycocyanin A contains 14 % of the total iron in spirulina **(Fernandes et al., 2023)**.

The amount of phycocyanin is 0,301mg/g. Phycocyanin C represents about 20% of the dried weight of spirulina **(AlFadhly et al.,2022)**.

Phycocyanin has many biological properties, such as anti-inflammatory activity that can prevent many diseases caused by inflammation. It has high antioxidant potential. According to research and studies, phycocyanin has the ability to scavenge many free radicals, including hydroxyl radicals (•OH), alkoxyl radicals (RO•), superoxide anions ($O_2^{\bullet-}$), and singlet oxygen ($^1O_2$). Additionally, phycocyanin plays an anti-cancer role by inhibiting tumor cell proliferation **(liu et al.,2022 ; Fernandes et al.,2023)**.

5.7.2. Carotenoids

Carotenoids are lipid soluble pigments present in many plants and microorganisms. Their color ranges from yellow to red. They cannot be produced by the human body, so they must be obtained through diet. Carotenoids have health effects against diseases caused by inflammatory stress, such as cardiovascular diseases, certain types of cancer, and age-related diseases **(Park et al., 2018)**.

The total carotenoid content is (400-650mg/100 g), including beta carotene (100-250mg/100g), Xanthophylls (250-470mg/100g) and Zeaxanthin (125-200mg/100g) **(AlFadhly et al., 2022)**.

5.7.3. Chlorophylls

Chlorophyll is a molecule consisting of a porphyrin ring bound to a magnesium ion with a hydrocarbon. It plays a crucial role in photosynthesis and is mainly present in green plants **(Park et al.,2018)**.

The spirulina chlorophyll content ranges between 1300 mg and 1700 mg/100g **(AlFadhly et al 2022)**.

5.8. Phenolic compounds

Phenolic compounds are bioactive molecules characterized by the presence of a benzene ring and at least one hydroxyl substituent. They can be derived from plants and microorganisms like spirulina. Most of these molecules have biological functions such as antioxidants and antimicrobial activity **(Asnake et al., 2021 ; Bortolini et al., 2022)**.

Spirulina contains phenols and flavonoids such as gallic acid and quercetin. The blue-green algae phenols have multiple biological abilities such as anticancer, antiaging, cardio protective, hepatoprotective and antimutagenic effects **(Asnake et al.,2021 ; AlFadhly et al.,2022)**.

6. Biological activities of spirulina

6.1. The antioxidant activity of spirulina

The imbalance between antioxidants and free radicals causing oxidative stress can lead to many diseases, disorders and cellular aging. To handle this imbalance, the body needs more antioxidants, which can be found in different food sources and supplements **(Hajam et al., 2022)**.

Since spirulina contains many components, including vitamins such as vitamin C, minerals such as selenium, pigments such as phycocyanin, it gives spirulina its high antioxidant potential.

Spirulina activates antioxidant enzymes, scavenges free radicals, inhibits lipids peroxidation, and increases the activity of catalase and superoxide **(Chen et al., 2018)**.

6.2. Anti-inflammatory activity of spirulina

The inflammation is an immune biological response of the body. It can be induced by many factors and can lead to serious diseases at the level of many organs such as brains, liver and intestinal tract **(Chen et al., 2018)**.

A number of studies, including in *vivo* and in *vitro* and in human, have reported the anti-inflammatory effect of spirulina. The two components, phycocyanin and β-carotene have the ability to inhibit the formation of pro-inflammatory cytokine formation and have auto inflammatory activity **(Deng & Chow, 2010)**.

7. Spirulina and metabolic syndrome

The metabolic syndrome is a cluster of many components including hypertension, hyperglycemia and hyperlipidemia. It can lead to serious diseases such the diabetes and cardiovascular diseases.

Spirulina can be used as a tool to manage different MetS components. The antioxidants of spirulina have a positive effect on obesity and lipid metabolism. The phycocyanin content in the blue green algae can reduce the blood pressure **(Bobescu et al., 2020)**.

Many studies aimed to investigate the effect of Spirulina on MetS. For this they used animal models and gave them different doses to come up with positive or negative results. Other studies were conducted on patients with MetS to investigate the effect of the blue-green algae on MetS.

A study on Zucker fatty rats, shows that administration of 28,5 mg / kg of body weight of spirulina for 12 weeks decreases the weight of the liver and adipose tissue without changing levels of hepatic enzymes like catalase and GSH, GSSG, SOD, GPX **(Vidé et al., 2019)**.

Another study was conducted on mice fed with a high fat diet. It was found that daily consumption of spirulina (A.maxima) at 150 mg or 450 mg of body weight can reduce body weight, blood fasting glucose, lipid concentration, and subcutaneous and visceral adipose tissue **(Ramos-Romero et al., 2021)**.

In addition, mice with induced MetS were given a standard diet plus 5% spirulina. These animals showed positive signs against MetS, such as reducing dyslipidemia **(Fujimoto et al., 2012)**.

Another study was conducted on horses aged between 8-14 years, of both sexes with equine MetS. They were daily administered 500 g of tested spirulina for three months. This supplementation accelerated weight loss and improved insulin resistance **(Nawrocka et al., 2017)**.

A group of female crossbred sows were fed a Western diet (WD). Half of these animals were supplemented daily with 20 g of spirulina. The WD group showed metabolic disorders, such as an increased cholesterol concentration and indications of liver damage. The low dose of micro-algae partially affected the metabolic disorders by normalizing plasma insulin levels **(Lugarà et al., 2022)**.

For patients with MetS, the daily consumption of 1g-19g per day of spirulina for half to six months can have a positive effect on MetS, such as reducing high blood pressure **(Yousefi et al., 2019)**.

Part two: Materials and Methods

The experiments conducted in this study, specifically the phytochemical analysis of our sample "spirulina" was carried out in the Biochemistry laboratory. Additionally, animal experiments were conducted to investigate the effect of spirulina supplementation on metabolic syndrome in mice at the university's pet store, located at the University of 8 May 1945 on Guelma.

1. Material

1.1. Description of spirulina

Commercial spirulina **(figure 04)** in form of capsules were bought and used in this study, the powder inside the capsules were weighed at sensitive balance and then was dissolved in specific solvent.

Figure 04: Commercial spirulina tested in the study.

1.2. Solubility

Depending on the chemical test performed, dissolve and extract the maximum amount of the desired component. For chemical screening or antioxidant tests, water or other organic solvents can be used to dissolve spirulina and extract the maximum amount of the desired component. Ethanol and water were used for phytochemical screening, and ethanol was used to measure the antioxidant activity.

2. Methods

The goal of phytochemical screening of spirulina is to identify the secondary metabolites present in spirulina, such as alkaloids, terpenoids, tannins, saponins, flavonoids, etc. These secondary metabolites often exhibit interesting biological and medicinal properties.

Otherwise, the aim of the following methods is to evaluate its quality and composition in addition to the antioxidant's potential of the blue-green algae by using tow assays DPPH and FRAP.

2.1. Phytochemical screening of spirulina

2.1.1. Preparation of extract

The preparation of the spirulina extract is conducted by mixing 1g of spirulina powder with 1ml of ethanol. The resultant solution is filtered through filter paper and stored for 24 hours before utilization.

2.1.2. Saponosids test

The detection of saponins is performed by mixing spirulina with distilled water (2,5mg/ml), shake the solution in a glass test tube for 15 minutes. The presence of saponins was indicated by the formation and persistence of 1 cm layer of foam after 15 minutes **(Saklani et al., 2019)**.

2.1.3. Terpenoids test

The presence of triterpenoid is evaluated using chloroform, sulfuric acid. A few drops of sample were added into a test tube containing 2 ml chloroform. Subsequently, 3 drops of sulfuric acid were added into the test tube. The positive results can be seen from the color change of the sample: initially, the color will turn into red **(Shankar Mane & Chakraborty, 2018)**.

2.1.4. Alkaloids test

2 ml of concentrated Hydrochloric acid (HCl) was added to 2 ml spirulina platensis extract. Then few drops Mayer's reagent was added. Presence of green color or white precipitate indicates the presence of alkaloids **(Shankar Mane & Chakraborty, 2018)**.

For the Mayer's test preparation: dissolve 1,3g of HgCl2 in 60 ml of distilled water. On the other hand, dissolve 5g of KI in 10 ml of distilled water. Then, mix the two solutions together and adjust the total volume to 100ml.

2.1.5. Tannins test

The presence of tannins is evaluated by mixing 1 ml of ferric chloride (5% FeCl3) with 1 ml of the spirulina extract. The formation of a dark blue or greenish-black color indicates the presence of tannins **(Shankar Mane & Chakraborty, 2018)**.

2.1.6. Flavonoids test

The presence of flavonoids is evaluated by mixing 1 ml of 2N sodium hydroxide (NaOH) with 2 ml of spirulina extract. The formation of a yellow color indicates the presence of flavonoids **(Shankar Mane & Chakraborty, 2018)**.

2.1.7. Phenols test

The presence of phenols is evaluated by mixing a few drops of ferric chloride (10%), 2 ml of distilled water, and 1 ml of the Spirulina extract. The formation of a blue or green color indicates the presence of phenols **(Shankar Mane & Chakraborty, 2018)**.

2.1.8. Carbohydrates test

The presence of carbohydrates is evaluated by mixing the spirulina extract with 3 ml of alpha naphthol in alcohol. Sulphuric acid is carefully added to the side of the test tubes. The formation of a violet ring at the junction of the two liquids indicates the presence of carbohydrates **(Rahoof Iqbal, 2020)**.

2.1.9. Reducing sugars test

The presence of reducing sugars is evaluated by adding drops of Fehling solution A and B to the spirulina extract, and heating the mixture for 2 minutes. The appearance of a reddish-brown color indicates the presence of reducing sugars **(Rahoof Iqbal, 2020)**.

2.1.10. Glycosides test

The presence of glycosides is evaluated by mixing 3 ml of chloroform with ammonium solution (10%) and 2 ml of the spirulina extract. The formation of a pink color indicates the presence of glycosides **(Mane and Chakraborty, 2018)**.

2.2. Dosage of polyphenols, flavonoids and phycocianins

2.2.1. Dosage of polyphenols

Concept

To estimate the total polyphenols in spirulina, the Folin - Ciocalteu method is adopted **(Li et al., 2007)**.

Folin-ciocalteu reagent is a heteropolyacid compound and contains the necessary acids to carry out this process " phosphomolybdic/phosphotungstic acid" **(Agbor et al., 2014)**.

In this process electrons are transferred from the phenolic compound towards phosphomolybdic / phosphotungstic acid complexes resulting in a blue complex. The latter can be determined by spectroscopy at around 765 nm **(Ainsworth & Gillespie, 2007)**.

Protocol

1 ml of reagent Folin - Ciocalteu diluted 10 times, 200 µl of spirulina ethanolic extract solution. The solution is mixed and incubated for 5 minutes and 800 µl of sodium carbonate (7,5%) were added. The mixture was incubated again for 90 minutes in darkness and at room temperature. The same steps are performed for the blank where spirulina ethanolic extract is replaced with ethanol. The dilution ranges of gallic acid 0,025, 0,05, 0,075, 0,1, 0,125, 0,150, 0,175 and 0,2 is used to obtain the calibration curve and the dilutions are subject to the same examination protocol. Absorbance measurements are performed by spectrophotometry (JENWAY 630S) at 765 nm. Each measurement is performed in triplicate times.

Results expression

Results are expressed as milligrams equivalent of gallic acid per gram of spirulina (mg Eq AG/g SP).

2.2.2. Dosage of flavonoids

Concept

The method used for flavonoids dosage is developed by **(Bahorun et al., 1996)**. To measure the total amount of flavonoids, present in the spirulina extract.

During this process the 4,5 carbons of flavonoids are broken down using 2 % of aluminum chloride ($AlCl_3$) This reaction produces a yellow compound that absorbs light at 430nm **(Tine et al., 2019)**.

Protocol

1 ml of the methanol solution of 2 % $AlCl_3$ was added to 1 ml of spirulina ethanolic extract (1 mg/ml). After stirring, the mixture was incubated for a few minutes at room temperature in darkness, the same steps are repeated for the blank replacing the spirulina ethanolic extract with 1 ml of ethanol. Optical density is measured at 430 nm. To prepare the calibration range a stock solution of quercetin was prepared at 0,1 mg/ml (reference flavonoid) from which dilutions (0,005, 0,01, 0,025, 0,05, 0,075, 0,1 mg / ml) were made. Each measurement was performed in triplicate.

Results expression

Results are expressed in milligrams equivalent of quercetin per gram of spirulina (mg Eq QRC/g SP).

2.2.3. Dosage of phycocyanin

Concept

The bursting in this method is carried out using different concentrations around the cell wall using water extraction **(Roumaissa et al., 2022)**.

Protocol

4% of spirulina suspension using water is prepared in darkness. The obtained solution is centrifuged (9000 rpm) for 15 minutes at 4°C. The supernatant was diluted with water (100 times), then the absorbance was measured at 615 nm, 652 nm, 620 nm and 280 nm.

Results expression

The percentage (%) of phycocyanin = 1,873 × (Abs620 – 0,474×Abs652) = f/C.

2.4. Evaluation of the antioxidant activity of spirulina

2.4.1. DPPH radical inhibition power

Concept

The DPPH **(**2, 2 –diphenyl-1-picrylhydrazyl) is synthetic radical present in oxidant state with intense purple color **(Molyneux,2003)**. It became yellow when it is reduced by the protons of antioxidant molecule **(Figure 05)**. Whenever the extract was rich in antioxidants molecules, the yellow color of DPPH became more and more intense. Measurement of the color absorbance was carried out. Antioxidant standards (vitamin C, Quercetin) are used to compare antioxidant activity.

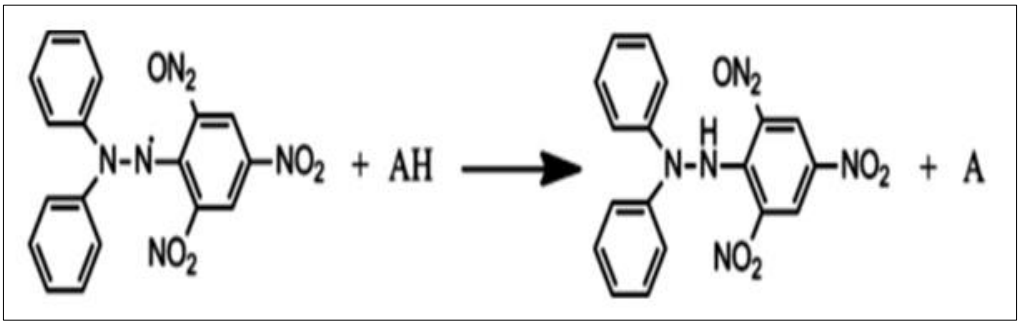

Figure 05: DPPH free radical, AH antioxidant molecule, A new free radical was formed **(Sirivibulkovit et al., 2018)**.

Protocol

The measurement of antioxidant activity of the spirulina was done using the modified protocol of **(Koleva et al., 2002)**.

Different concentrations of spirulina extract (0,001 - 1 mg/ml) were added to 1,5 ml of DPPH ethanolic solution (4×10^{-5}M). The mixture was kept in darkness and at ambient temperature during 30 minutes. The standard going through the same procedures by replacing the spirulina extract with the standard. Absorbance were estimated by spectrometry at 517 nm. All procedures are done in triplicate.

Results expression

The percentage of the inhibition of DPPH radical is calculated using this formula:

Percentage of inhibition = (Abs Control - Abs Sample / Abs Control) x 100

Using this formula, IC_{50} values which expresses the concentration that inhibited 50% of the free radicals, are calculated.

2.4.2. Ferric reducing power FRAP

Concept

The reducing power of a phenolic extracts is based on the conversion of Fe^{3+} present in potassium ferricyanide $K_3Fe\ (CN)_6$ into Fe^{2+} in Prussian bleu. It reveals the presence of donating electrons ability **(figure 06)**.

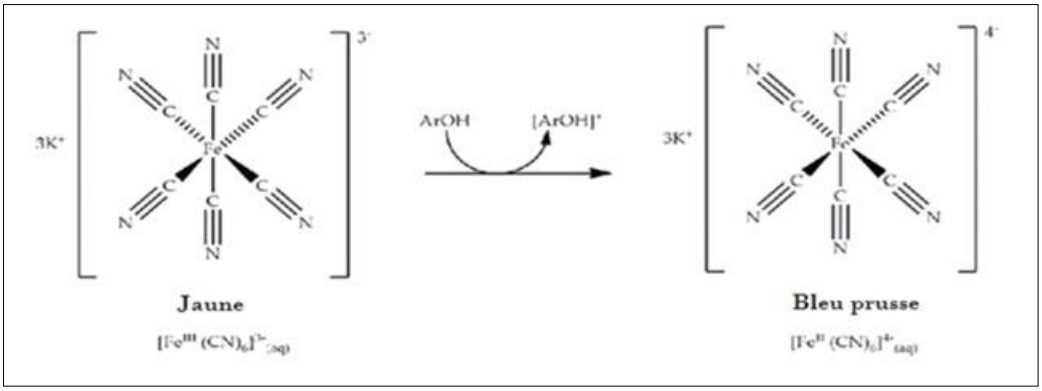

Figure 06: Mechanism of the reduction of the potassium ferricyanide **(Bibi Sadeer et al., 2020)**.

Protocol

To 500 µl of samples (ethanolic extract of spirulina, quercetin and vitamin C) at different concentrations (0,1-1 mg/ml), 1 ml of phosphate buffer (0,2 M pH= 6,5). 1ml of potassium ferricyanide ($K_3Fe\ (CN)_6$ 1%) was added and then incubated at 50° for 20 minutes. After

Adding 1 ml of trichloroacetic acid (TCA 10%) for stopping the reaction, tubes are centrifuged at 3000 rpm for 10 minutes.

After, 1,5 ml of supernatant was added to 1,5 ml of distilled water and 0,1 of FeCl (0,1%). After agitation, the mixture is incubated for 10 minutes and the absorbance was read at 700 nm.

The absorbances were converted to mmol /mg of spirulina, quercetin or vitamin C using a standard curve performed with different concentrations of $FeSO_4(5H_2O)$ prepared under the same conditions.

Results expression

The results are expressed by calculate the reducing power of the spirulina extract (mM fell /mg P, Q) (mg FeII / mg antioxidant).

Chapter 02: Animal Experimentation

1. Materials

The study was conducted on 22 male mice, their weight was between 19g and 31 g. They were provided from the Pasteur institute of Algiers, Algeria. The animals have been adapted to the university's pet store conditions. They were hosted in polypropylene cages with a stainless-steel cover and a covered bottom filled with sawdust, which is changed three times a week. Mice have been maintained with a standard diet of pellets and the current water from the faucet Circadian cycle.

Mice were weighed, and their size and abdominal circumference were measured once every three days.

2. Methods

2.1. Experimental protocol

After a period of adaptation, the mice were divided into four groups and treated during a period of 28 days following this protocol:

Control group (C): Mice were fed a standard diet and had free access to water.

Spirulina group (SP): water dissolved spirulina at a dose of 1000 mg/kg/day *via* gastric gavage, for a duration of 4 weeks (28 days).

Metabolic syndrome group (MS): Mice were provided with a solution containing 60% fructose in drinking water as well as fat and carbohydrate diet (composed of 60% beef tallow and 40% soybean oil) over a duration of 4 weeks.

Metabolic syndrome treated group (MS+SP): Mice were provided with a solution containing 60% fructose in drinking water, as well as fat and carbohydrate diet (composed of 60% beef tallow and 40% soybean oil). In addition, spirulina (1000 mg/kg/day) was administered *via* gastric gavage during 4 weeks.

2.2. Fasting blood sugar and Oral glucose tolerance test

Oral glucose tolerance test remains as the most common test for determination of glucose intolerance and diabetes in genetically engineered or dietary-induced rodent research **(Andrikopoulos et al., 2008)**.

Fasting blood glucose (FBG) determination and oral glucose tolerance tests (OGTT) were performed at week 2 and 4 after overnight fasting using blood from tail vein with VITAL Check

glucometer. For OGTT, mice were given intraperitoneally a glucose load of 2 g/kg body weight (as 40 % glucose solution *via* oral gavage). Blood glucose concentrations were measured at 30, 60, 90, and 120 min after oral glucose administration **(Zhuhua et al., 2015 ; Sok et al., 2018)**.

2.3. Sacrifice and organ harvesting

At the end of the protocol period, and after a 12-hour fasting with free access to water, the mice are weighed. Their size and circumference of their abdomen were measured. After sacrificing the mice, blood samples were taken from the beating heart and placed in dry tubes for biochemical tests. The livers were removed and immediately placed in a solution of sodium chloride (NaCl). They are then dried with absorbent paper and weighted. Additionally, liver samples were taken from each lot and placed in boxes containing formaldehyde 10% for histological examination. The remaining organs are preserved for testing the levels of antioxidants and oxidative stress biomarkers.

2.4. Relative Organ /Body Weight

Relative organ/body weight measurement sheds light on organ development and the possible impacts of MetS or spirulina therapy on liver size/weight **(Vidé et al., 2019)**. The formula used for calculation is:

Relative liver/body weight = Liver weight (g)/BW of rat on sacrifice day (g)

2.5. Biochemical analyses

The blood samples were placed in dry tubes and subsequently taken to the laboratory for biochemical tests. Lipid profile (serum total cholesterol and triglycerides levels) and transaminases activities were measured using commercial colorimetric assay kits using automatic analyzer Selectrapro S.

2.5.1. Lipid profile

A lipid profile is a blood test that measures the concentration of lipids in the blood. Typically, it encompasses several measurements: total cholesterol, LDL, VLDL, HDL and triglycerides. Elevated levels of lipids in the bloodstream can result in the accumulation of plaque in blood vessels and arteries, thereby inducing damage and elevating the likelihood of cardiovascular issues **(Cleveland Clinic, 2021)**.

2.5.2. Transaminases

The transaminase test, also known as the liver enzyme test, assesses the levels of two enzymes, aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT), in the bloodstream. These enzymes are primarily found in the liver and muscles, and their detection in blood samples can provide insight into any possible damage or illness affecting these tissues **(Thiébaux, 2024)**.

2.6. Oxidative stress biomarkers

To prepare the liver cytosolic fraction, 1g of liver was mixed then homogenized in 9 ml of phosphate buffer (0,1M, pH 7,4) containing 1,17% of chlorure de potassium (KCl), using ULTRA-TURRAX homogenizer at 10000 rpm for 10 minutes at 4°C to obtain the supernatant. This cytosolic fraction was fractionated and stored at -20°C for the evaluation oxidative stress parameters **(Figure 07)**.

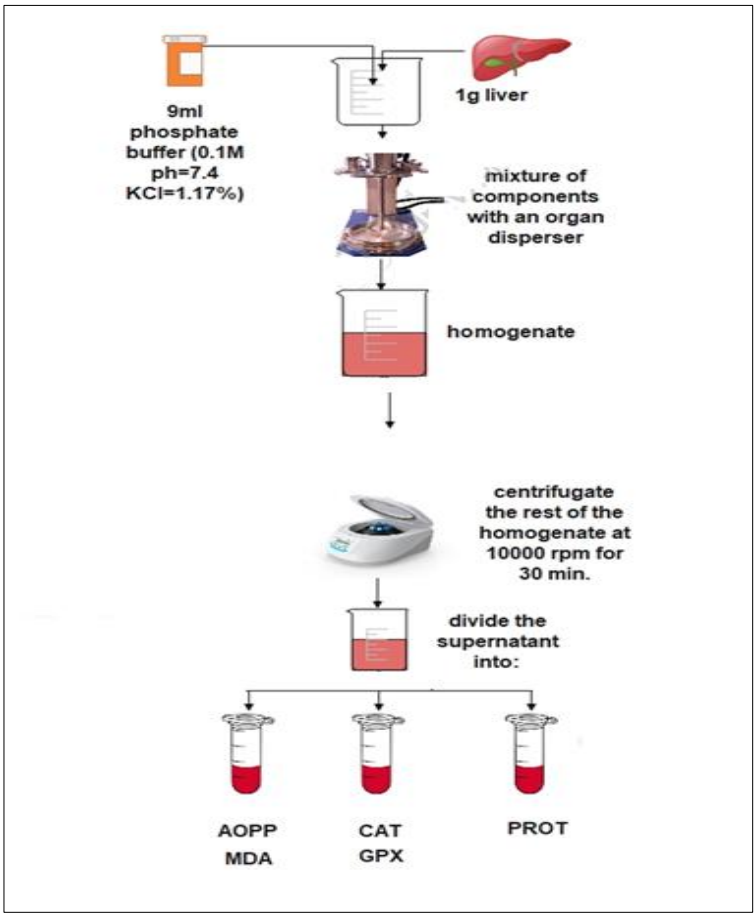

Figure 07: Preparation of the liver cytosolic fraction.

2.6.1. Malondialdehyde dosage

Concept

Malondialdehyde (MDA) is a commonly used as biomarker to estimate membrane lipid peroxidation. Prior to TBA (thiobarbituric acid) reaction, MDA undergoes a process of forming a complex that appears pink in color at 100°C. This compound can be extracted by an organic solvent, such as n-butanol, and its absorbance is measured to determine the MDA staining in the sample **(Chellouai et al., 2019)**.

Protocol

Determination of MDA level in liver tissue was performed using the method of **(Ohkawa et al., 1979)**.

250 µl TCA (20%) were added to 250 µl of cytosolic fraction. 500 µl TBA (0,67%) were then added. The mixture was heated at 100°C in a water bath for 10 minutes. After cooling, 2 ml of n-butanol was mixed then centrifuged at 3000 rpm for 15 minutes. The absorbance of the supernatant was measured at 532 nm.

Results expression

The level of MDA was expressed in nmol of MDA/g of liver tissue using a calibration curve produced from 1,1, 3,3-tetraethoxypropane which releases MDA during its acid hydrolysis.

2.6.2. Advanced oxidation protein products (AOPP)

Concept

Advanced protein oxidation products result from the interaction between plasma proteins and chlorinated oxidants **(Witko-Sarsat et al., 1996)**.

Protocol

200µl homogenate were added to 400µl phosphate buffer (0,1 M, pH 7,4). After incubation for 2 minutes, 50 µl iodure de potassium (KI) (1,16 M) were added, then 100 µl of acetic glacial. After agitation and centrifugation (3500 × g) for 15 minutes at 4°C, the absorbance of supernatant was read at 340 nm.

Results expression

The tissue AOPP content was calculated using the extinction coefficient and expressed as nmol/mg protein. It is determined using the following formula:

$$AOPP\ level\ = DO \times \frac{Fd}{\varepsilon \times L \times C\ protien}$$

***DO*:** Optical density of the sample at 340 nm

***Fd*:** Dilution factor (*Vt/Vs*) or *Vt*: Total volume of the reaction medium, *Vs*: Supernatant volume.

***ℰ*:** Molar extinction coefficient of AOPP (261 mM^{-1} cm^{-1}).

***L*:** Length of tank used (L = 1 cm).

***CP*:** Protein concentration (mg/g of tissue).

2.7. Antioxidant status

2.7.1. Reduced Glutathione dosage

Concept

The principle for determining reduced glutathione (GSH) is based on the reactivity of GSH's thiol groups with a specific reagent, such as DTNB (5,5'-dithio-bis-2-nitrobenzoic acid), to produce a chromophore thione. The resulting yellow color is then measured spectrophotometrically to determine the GSH content in a sample. The GSH test technique relies on precise chemical processes that allow for accurate detection of GSH, thus making it a reliable, straightforward, sensitive, and specific method for assessing this crucial biochemical parameter **(BEN SAAD et al., 2017)**.

Protocol

Estimation of the GSH was performed using the method described by **(Lahouel et al., 2004) (lahouel et al., 2004)**.

1 g of liver tissue was homogenized in 5% TCA and then centrifuged for 30 minutes at 1000 rpm. 1700 µl of phosphate buffer (0,1 M, pH 7,4) and 100 µl of DTNB (0,01 M) were added to 200 µl of the supernatant. The mixture was incubated for 5 minutes. Absorbance was measured at 412 nm against the blank which is prepared under the same conditions by replacing the homogenate with distilled water.

A standard curve of GSH was performed based on different concentrations (0,125 to 1 mM).

Results expression

The results are expressed in µmoles of glutathione/g of liver tissue.

2.7.2. Glutathione peroxidase GPX Dosage

Concept

The determination of GPX (glutathione peroxidase) is based on the enzyme's ability to reduce peroxides using its specific substrate, reduced glutathione (GSH). GPX is a selenoprotein that catalyzes the reduction of peroxides by utilizing GSH. This process involves the competition between the oxidation reaction of pyrogallol by O2 (resulting in a yellow color) and the reduction of peroxides by GPX using GSH as the substrate **(haleng et al.,2010)**.

GPX plays a crucial role in eliminating various radical forms and depends on selenium as a cofactor. GPX activity indicates the current levels of oxidative stress, and its interpretation should consider the balance with levels of glutathione reductase (GR) **(eurofins, 2023)**.

Protocol

200 µl homogenate were added to 400 µl GSH, 200 µl phosphate buffer, place the mixture in a water bath at 25°C for 5 minutes, then 200 µl H2O2 (1,3 mM) were added, after incubation for 10 minutes, 1000 µl TCA (1%) were added, after agitation and centrifugation (3000 rpm) for 10 minutes, 480 µl of the supernatant were removed and mixed with 2200 µl phosphate buffer and 320 µl (DTNB freshly prepared) (1 mM). After another of incubation for 5 minutes, the absorbance of mixture was read at 412 nm.

Results expression

$$GSH - Px = \frac{DOec \times DO\,et \times 5}{DOet \times cp} 0.04$$

DO ec: The enzyme activity of GPx in the presence of glutathione (GSH).

DO et: The enzyme activity of GPx in the absence of glutathione (GSH).

cp: The concentration of the inhibitor.

2.7.3. Catalase (CAT) Dosage

Concept

The principle of the catalase (CAT) assay is to measure the enzymatic activity of catalase, an enzyme found in many tissues. Catalase acts as a defense mechanism for cells against

oxidative stress by eliminating active derivatives of oxygen, such as hydrogen peroxide (H_2O_2), and accelerating their decomposition into water and oxygen. The enzymatic reaction occurs in two stages, where catalase reacts with hydrogen peroxide to break it down into water and oxygen. Catalase activity is measured by determining absorbance after a specific time in presence of its substrate H_2O_2.This assay assesses the functioning of this crucial enzyme in protecting cells against oxidative damage **(Université frères Mentouri Constantine)**.

Protocol

25 µl homogenate were added to 1ml phosphate buffer (0,1M, pH 7,4) and 950 µl H_2O_2 (0,019 M). The mixture was prepared in quartz tanks. The absorbance of the mixture was read at 240nm for 2 minutes (0, 1, 2).

Results expression

The result of the enzymatic activity is expressed in IU/g of proteins/g of tissue, and obtained in the formula below:

$$\textbf{CAT activity} = \left(\Delta T \times \log.\frac{DO1}{DO2}\right) \times \frac{FD}{\varepsilon \times L \times C\ proteins}$$

DO_1: Absorbance at 0 minutes.

DO_2: Absorbance at 1 minute.

Δ : Time interval in minutes.

FD: Dilution factor (Vt/Vs) where Vt: Total volume of reaction medium, Vs: Supernatant volume.

ε: Molar extinction coefficient of H_2O_2 (43,6 M^{-1} cm^{-1}).

L: Length of the cuvette used (L = 1 cm).

C protein : Protein concentration (mg/g of tissue).

2.7.4. Protein Dosage

Concept

In the biuret reaction, a chelate is formed between the Cu2+ ion and the peptide bonds of the proteins in alkaline solutions to form a violet-colored complex. The absorbance of this

complex is measured photometrically. The intensity of the color produced is proportional to the concentration of protein in the sample.

$$Cu2+ + \text{Serum protein} \xrightarrow[25\text{-}37^{o}C]{pH > 12} \text{Copper-protein complex}$$

Reagent composition

R1 Biuret reagent: Cupric sulfate 6 mmol/L, sodium-potassium tartrate 21 mmol/L, potassium iodide 6 mmol/L, sodium hydroxide 0,75 mol/L C R:34.

CAL Protein standard: Bovine serum albumin 7 g/dl (70 g/L). The concentration value is traceable to Standard Reference Material 927.

Protocol

1ml Buiret reagent, 20µl sample, mix and incubate for 5min. for the Cal standard is prepared with the same steps without the sample. Read the absorbance (A) of the samples and the standard at 540 nm against the reagent blank which contains just Bruit reagent.

Results expression

$$\frac{A\ \text{Sample}}{A\ \text{Standard}} \times C\ \text{Standard} = \text{g/dl total protein}$$

Samples with concentrations higher than 12 g/dl should be diluted 1:2 with saline and re-assayed. Multiply the results by 2. If the results are to be expressed as SI units, apply the following conversion: g/dl x 10 = g/L.

2.8. Statistics

All data were expressed as mean ± S.E.M, statistical analysis was performed with Graph Pad Prism 8,0 software. Differences between groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post-test. To statistically interpret the differences between the results of the biochemical parameters a $p<0,05$ was considered statistically significant.

Results and discussion

1. Results of phytochemical screening

This table **(table 05)** represents the results of phytochemical screening of the extract of our spirulina to estimate its components with potential therapeutic effects.

Table 05: Phytochemical screening results of spirulina.

Tests	Spirulina extract
Alkaloids	+
Terpenoids	+
Saponins	+
Flavonoids	+
Phenols	+
Carbohydrates	+
Tannins	+

The analysis has detected the presence of metabolites such as saponins, which contain anti-inflammatory effects **(Milgate & Roberts, 1995)**, Our results match those of **(kannan, 2014 ; Agustini et al., 2015)** and that find saponins in the spirulina extract. We detect also the presence of phenols and flavonoids, which have been reported for their effective antioxidants activity, pharmaceutical and medical applications **(S. Kumar & Pandey, 2013)** these results agree those of **(Shankar Mane & Chakraborty, 2018)**, but this result is incompatible with **(Yuniati et al., 2020)** who finds that Spirulina does not contain phenols.

We detect also the presence of alkaloids. They contain a variety of pharmacological potentials in modern medicine, and their effects include analgesic, anti-hyperglycemic, and anticancer properties **(Ng et al., 2015)**. Our result agree those of **(Ayoola et al., 2008 ; Nga et al., 2019)** they also found alkaloid in spirulina. Terpenoids were also present in our spirulina. Humans have traditionally utilized plant-derived terpenoids in the chemical, food, and medicinal industries. In addition, they have been increasingly employed in the production of

biofuel **(Tholl, 2015)**. Our result coincided with **(Shankar Mane & Chakraborty, 2018)**'s result in term of the terpenoids containment of spirulina. Finally, we found that spirulina contains carbohydrates also Tannins, as reported by **(Wu et al., 2005)**, whose study demonstrated the potential of these metabolites in terms of antioxidant activity. Tannins also possess significant antibacterial, antiviral, and anti-inflammatory properties. Instead, **(Hamouda Ali & Doumandji, 2017)** did not find tannins in their aqueous spirulina extract.

This difference in results is due to extraction conditions, culture conditions, experiment method or quality of spirulina.

2. Dosage of polyphenols

Polyphenols have antibacterial, anti-inflammatory, and antioxidant properties, making them valuable in biomedical fields for preventing infectious or chronic illnesses **(Guo et al., 2023)**.

In our spirulina extract, the concentration of polyphenols is 230,0 ± 65,2 mg/g. We obtained these results from an increasing concentration of gallic acid **(figure 08)**, as calculated using the formula y= 11,593 [gallic acid] + 0,08. The concentration of polyphenols was expressed as the equivalent of gallic acid/g spirulina (mg gallic acid Eq/g spirulina).

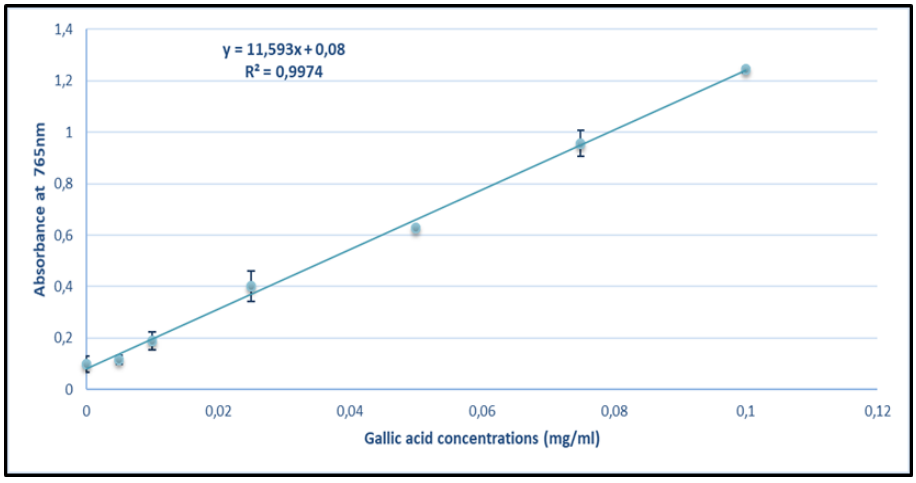

Figure 08: Calibration curve for the determination of polyphenols.

Our result is higher than **(Lafri et al., 2017)**, their results range from 4 to 22 mg/g for the Algerian strain and from 6 to 25 mg/g for the Tunisian strain, and also higher than **(Al-Dhabi & Valan Arasu, 2016)**, their result is estimated to be between 2,4 and 24,4 mg/ml.

These differences in proportions can be attributed to variations in region, extraction methods, and cultural factors.

3. Dosage of flavonoids

Flavonoids are secondary metabolites that can be found in fruits, vegetables, herbs, seeds, cereals, and are also present in micro-algae like spirulina **(Ballard & Maróstica, 2019 ; A. Kumar et al., 2022)**.

Flavonoids are natural antioxidants investigated to have significant biological properties such as antiviral, antifungal, anti-tumorigenic, antioxidant, and anti-inflammatory effects **(Rashid et al., 2019 ; Prasad et al., 2023)**.

Our result is obtained from a calibration curve derived from cross-concentrations of Quercetin (mg/ml) **(figure 09)**. In our spirulina extract, the concentration of flavonoids is 16,09 ± 3,06 mg/g, as calculated using the formula y= 32,362 [quercetin] + 0,01638, the concentration of flavonoids was expressed by equivalent of Quercetin /g spirulina (mg QRC Eq/g spirulina).

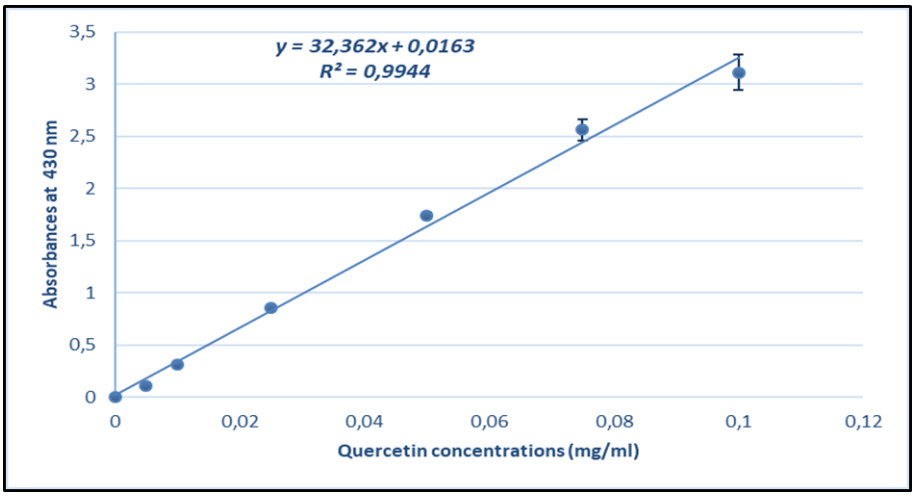

Figure 09: Calibration curve for the determination of flavonoids.

Our results were higher than those of **(Rahim et al., 2021 ; Yuliani et al., 2022)**, who respectively found 6,6 mg QE/g±1,12, 1,004 mg QE/g. In contrast, our results were lower than those of **(Biapa & Moukette, 2016 ; Ngu et al., 2022)**, who found 56,4 ± 6,47 mg QE/g and 83,41 ± 2,049 mg QE/g, respectively. The method of **(Bahorun et al., 1996)** showed significant efficiency in terms of flavonoid dosage in the ethanolic extract of spirulina. **(Biapa et Moukette,2016 ; Ngu et al.,2022)** found that the concentration of flavonoids in spirulina was much higher. Both our study and the study by **(Biapa et Moukette,2016)** used ethanol as the solvent to extract flavonoids in spirulina. The highest concentration of flavonoids in spirulina indicates the efficacy of ethanol as a solvent for extracting flavonoids. The differences in the

results regarding the total flavonoid content in spirulina may be attributed to the type of solvent used, the culture and harvesting circumstances or extracting method.

4. Dosage of phycocyanin

Phycocyanin has many medicinal benefits and is present in blue-green algae, such as spirulina platensis. It is frequently used as a dietary nutritional supplement. Furthermore, it has anti-inflammatory action **(Shih et al., 2009)**.

The results indicated the presence of phycocyanin, which was estimated at 0,188 ± 0,03 mg/ml, using the equation: Concentration of phycocyanin (mg/ml) = 1,873× (DO_{620} – 0,474× DO_{652}). The water method was used for extraction. This value is lower than the result found by **Izadi and Fazilati**, which is 0,422 mg/ml using sonication and 0,405 mg/ml using enzymatic means **(Izadi & Fazilati, 2018)**. It is also lower than the results obtained by **Silveira et al**. The highest amount of phycocyanin was extracted in the first 48 hours using 10 mM sodium phosphate buffer (pH 7,0), approximately 4,20 mg/ml. After that, water extraction yielded a phycocyanin content of 3,73 mg/ml. The most phycocyanin was extracted after 72 hours using water, with a content of 4,54 mg/ml **(Silveira et al., 2007)**.

Differences in results between our work and other studies may be due to variations in manipulation methods and extraction methods.

5. Evaluation of the antioxidant activity of Spirulina in vitro

5.1. Dosage of DPPH

The DPPH test is an easy, quick, popular, and affordable method for measuring antioxidant activity. This test involves converting the radicals of DPPH (intense violet) into stabilized molecules in the presence of an antioxidant **(Sirivibulkovit et al., 2018 ; Baliyan et al., 2022)**.

The percentage of DPPH inhibition was calculated and presented in **figure 10**.

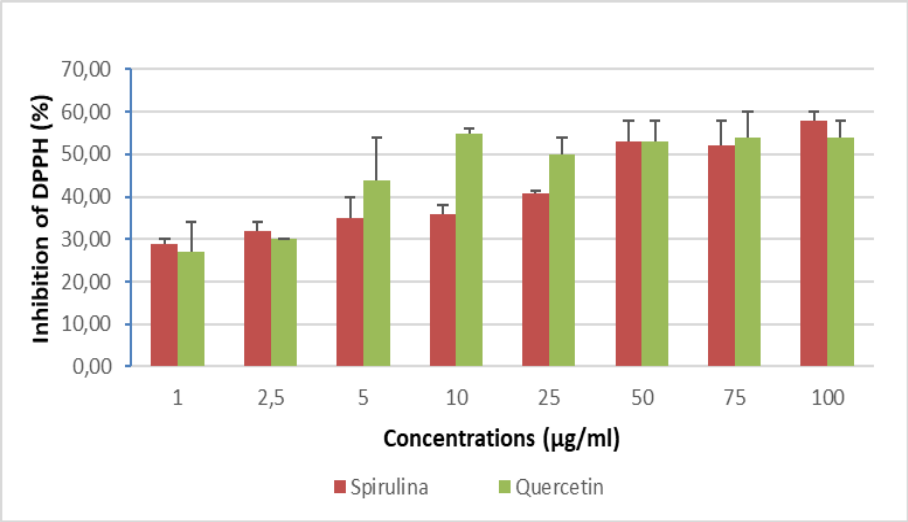

Figure 10: The percentage of inhibition the DPPH radicals by spirulina and quercetin.

The percentage of inhibition of DPPH increased considerably with the concentration of spirulina and quercetin. Spirulina gave the highest percentage at 58%, while quercetin was at 55%. The IC_{50} concentration for inhibiting 50% of DPPH radicals was higher with spirulina ethanolic extract at 0,0439 (mg/ml) compared to quercetin at 0,0250 (mg/ml).

Regarding our results, the IC_{50} of the spirulina extract was 0,0439 (mg/ml), which is lower than that reported by **(Aissaoui et al., 2017 ; Ngu et al., 2022)** at 0,0704±0,00076 and 0,107±0,00598 (mg/ml), respectively.

The difference in results may be due to the use of different solvents. **(Aissaoui et al.,2017)** used an aqueous solvent, and their EC_{50} values were 0,0704±0,00076 (mg/ml), which is higher than our result of 0,0439 (mg/ml) where ethanol was used as the solvent.

The organic solvents with high polarity, such as ethanol, exhibit higher abilities for the extraction of antioxidants when compared to other solvents like water **(Ngu et al., 2022)**.

The difference in the percentage of DPPH radical inhibition can be attributed to the concentration or quality of the spirulina used.

On the other hand, at the maximum concentration used spirulina exhibited a significant percentage of inhibition of the DPPH radical at 58% compared to Quercetin at 55%. These results indicate the significant antioxidant potential of spirulina, which is attributed to its rich content of bioactive molecules such as phycocyanin, chlorophylls, carotenoids, phenols, as well as vitamins E and C, and minerals. These compounds possess potent antioxidant functions.

5.2. Dosage of FRAP

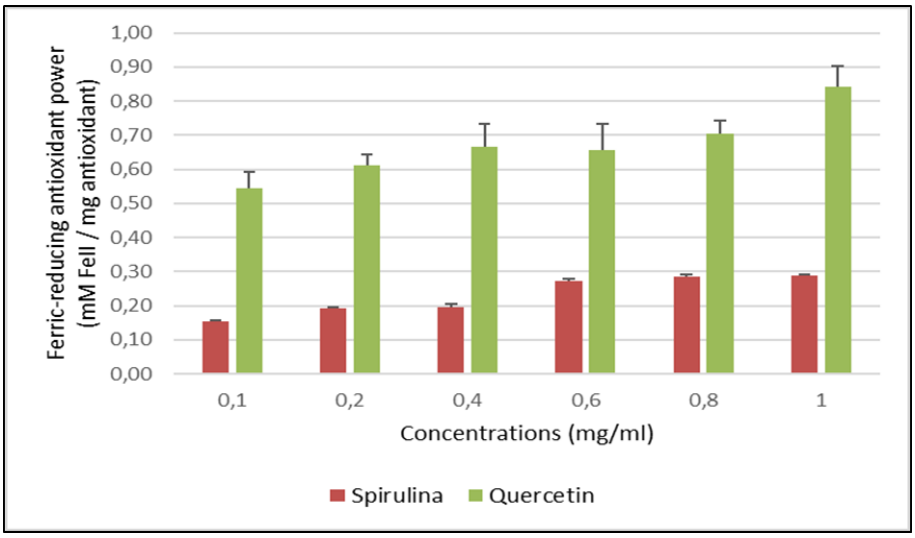

Figure 11: Antioxidant activity of spirulina and quercetin concentrations by the FRAP test.

The FRAP assay is a technique commonly employed to assess the antioxidant power of a substance. This is achieved by measuring the formation of a colorful ferrous-tripyridyl triazine complex at low pH levels, which occurs as a result of the reduction of ferric to ferrous ions. In order to determine the FRAP values, the absorbance change at 593 nm in test reaction mixtures is compared to those containing known concentrations of ferrous ions **(Benzie & Strain, 1996)**.

The **figure 11** indicate that the concentration of quercetin is much higher compared to the concentration in spirulina. In spirulina, the concentrations range between 0,16, 0,19, 0,20, 0,27, 0,29 and 0,29 mM FeII/mg antioxidant, while for quercetin, the concentrations are 0,54, 0,61, 0,67, 0,66, 0,70 and 0,8 mM FeII/mg antioxidant. These results were obtained using the **(Aparadh et al., 2012)** method. However, the value of spirulina's concentration is higher than the results obtained in the study by **(Wan et al., 2021)**, where ferrous sulfate was used to measure the total antioxidant capacity, which was found to be 0,3 mmol Eq FeSO4/g. Additionally, they used a kit (S0119, Beyotime, China) to measure the reduction of ferric ions by the antioxidant power.

Maybe our results don't agree with the results of **(Wan et al.,2021)** because they used a different method to determine the value of FRAP.

6. Effect of spirulina on different organe/body weight changes

6.1. LBW/BMI variations

The **table 06** shows that there is similarity in the liver body weight (LBW) percentage between all four groups: control group (4,5% ± 0,2 %), SP group (4,3% ± 0,2 %), MS group (4,7% ± 0,3 %) and MS+SP group (4,7% ± 0,4 %). The BMI values were respectively (3,5 ± 0,1), (3,3 ± 0,1), (3,6 ± 0,1) and (3,3 ± 0,2).

The BMI was slightly higher in the MS group (3,6 ± 0,1) and lower in the MS+SP group (3,3 ± 0,2) compared to the control group (3,5 ± 0,1).

The BMI slightly reduced in the MS+SP group (3,3 ± 0,2) compared to the MS group (3,6 ± 0,1).

Table 06: Variation in liver body weight percentage (LBW%) and Body Mass Index (BMI) between the groups.

Results were analyzed using a one-way ANOVA followed by Tukey post hoc test. The results are expressed as Mean ± SEM. P≤0,05 indicates a significant change, while p≥0,05 indicates no significant change. ***a****: groups vs control group.* ***b****: groups vs MS group.*

Groups	C	SP	MS	MS+SP
LBW (%)	4,5±0,2	4,3±0,2	4,7±0,3	4,3±0,4
BMI	3,5±0,1	3,3±0,1	3,6±0,1	3,3±0,2

The liver body weight percentage in the mice ranges from 3% to 5% **(Rogers & Dintzis, 2018)**. The similarity in the percentage of liver body weight indicates that there is no change in liver weight, which may be due to the absence of fat accumulation.

(Pak et al., 2012) found a significant difference in the liver weight index of male Wistar rats in the choline deficiency high-fat diet group (CDHF) (5,10%) compared to the control group (2,88%). Additionally, they observed a remarkable decrease in non-alcoholic rat models with choline deficiency high-fat diet who were given spirulina (6g/kg/day) (3,05%) compared to the CDHF group.

The increase in liver weight per body weight can be attributed to the accumulation of fats in the liver, which is associated with CDHF and leads to oxidative stress and inflammatory

reactions. On the other hand, the decrease in liver weight can be attributed to the antioxidant and anti-inflammatory activity of spirulina **(Pak et al., 2012)**.

BMI is statistical tool using for estimating the accumulation of fats in the body is measuring by dividing the body weight by the highest of the body **(Weir & Jan, 2024)**.

In our study, the MS group had a slightly higher BMI compared to the control group, indicating a mild accumulation of body fat in MS mice. However, the MS+SP group showed a reduction in BMI, which may be attributed to the investigation of spirulina, as it has been shown to significantly reduce body fats, BMI, body weight, and waist circumference **(Bohórquez-Medina et al., 2021)**.

In a study conducted by **(Diniz et al., 2021)**, rats were used for 8 weeks, and it was found that the BMI of the high-calorie diet group (0,66±0,010) was higher than that of the control group (0,52±0,01), but the difference was not significant. The HCD + SP group (25mg/body weight) (0,64±0,01) showed no difference compared to the HCD group. The increase in BMI in the HCD group may be due to excessive calorie intake.

The difference in BMI between **(Diniz et al.,2021)** and our study, particularly in the high-calorie diet group compared to the control group, may be attributed to the type or duration of the diet. Their study lasted for 8 weeks, while ours lasted for 4 weeks, which may not have been sufficient to induce obesity.

6.2. Body weight variations

After analyzing the **figure 12**, we observed an increase in weight variation in the MS group from 28,8 ± 5,1 g to 31,4 ± 4,6 g, but this increase was not statistically significant when compared to the control group from 29,7 ± 2,5 g to 32,1 ± 1,3 g.

On the other hand, the MS+SP group showed a decrease in body weight from 28,7 g ± 5,1g to 26,6 ± 5,5 g compared to the control group.

In comparison to the MS group, which experienced a slight increase in body weight from 28,8 ± 5,1 g to 31,4 ± 4,6 g, the MS+SP group showed a slight reduction in body weight from 28,7 ± 5,1 g to26,6 ± 5,5 g.

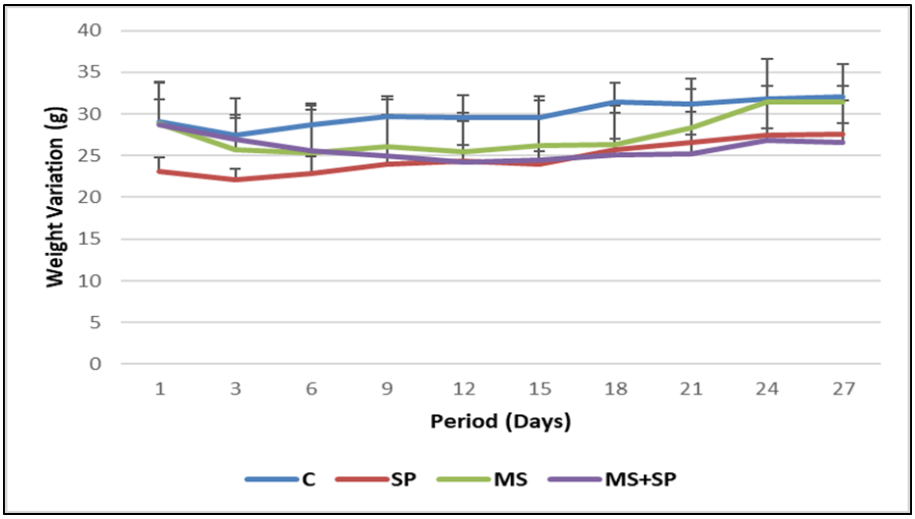

Figure 12: Body weight variation in defferent groups measuring each three day during the experiment diet duration.

Results were analyzed using a one-way ANOVA followed by Tukey post hoc test. The results are expressed as Mean ± SEM. P≤0,05 indicates a significant change, while p≥0,05 indicates no significant change. ***a****: groups vs control group.* ***b****: groups vs MS group.*

Obesity is characterized by the abnormal accumulation of fat in the body and can lead to serious health problems such as diabetes mellitus and cardiovascular diseases **(Panuganti et al., 2024)**.

The similarity in body weight variation between the MS group and the control group, with final body weights of 31,4 ± 4,6 g and 32,1 ± 1,3 g respectively, suggests that the high-fat and fructose diet used may not have been sufficient in terms of duration, composition or quantity to induce obesity in the mice. This indicates that there was no excessive fat accumulation in the body.

Compared to the initial and final body weight of the MS+SP group, there was a slight decrease in body weight, possibly due to the anti-obesity properties of spirulina. Spirulina has been shown to reduce obesity in mice by inhibiting the accumulation of lipids and reducing the expression of adipogenic proteins like C/EBPα and P2, as well as lipogenic proteins such as SREBP, ACC, and FAS **(Seo et al., 2018)**.

Our results were compared to the study conducted by **(Seo et al.,2018)**, who used male ICR mice. They found that the weight of the high-fat diet (HFD) group significantly increased from 26 g to 45 g in 6 weeks. However, when the HFD group was treated with spirulina maxima, there was a significant reduction in body weight starting from the first week. This suggests that spirulina maxima is effective in reducing body obesity.

7. Effect of spirulina on biochemical parameters

7.1. Glycemia

We observed in the histograms **(figure 13)** that the concentration of fasting serum glucose in the mice of the MS group (1,40 ± 0,10 g/L) was higher than that of the control group (1 ± 0,05 g/L), but there was no significant statistical difference. The concentration of blood serum in the MS+SP group (1,11 ± 0,20 g/L) also showed no significant difference compared to the control group.

On the other hand, there was no significant increase in the MS group (1,4 ± 0,10 g/L) compared to the MS+SP group (1,11 ± 0,20 g/L).

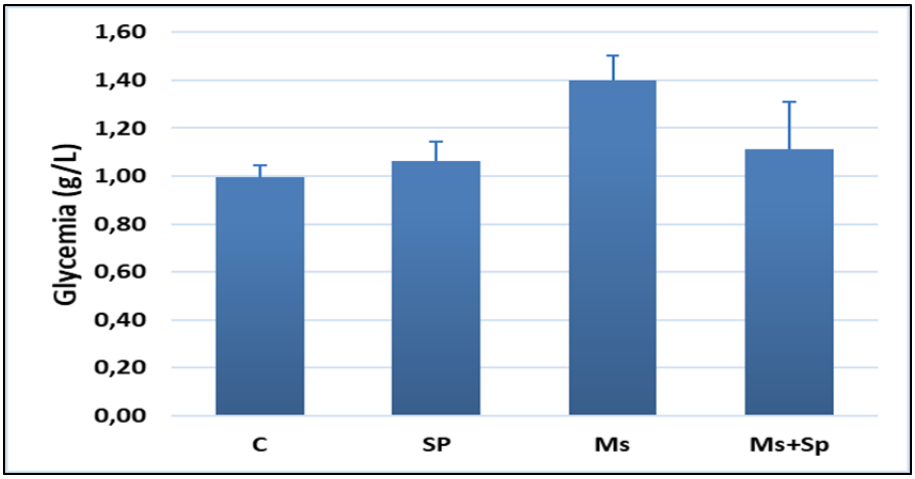

Figure 13: Fasting blood glucose levels in the different groups.

Results were analyzed using a one-way ANOVA followed by Tukey post hoc test. The results are expressed as Mean ± SEM. P≤0,05 indicates a significant change, while p≥0,05 indicates no significant change. ***a****: groups vs control group.* ***b****: groups vs MS group.*

The glucose blood levels can be considered an indicator of the patient's condition. Increasing levels are called hyperglycemia, which can lead to serious health problems **(Mouri & Badireddy, 2024)**.

The MS group exhibited a slight increase in blood glucose levels (1,40 ± 0,10 g/L) compared to the control group (1,00 ± 0,05 g/L), which can be attributed to the high fat and high fructose diet administered to the mice in the MS group for a duration of 28 days. Conversely, the MS + SP group showed a reduction in blood glucose levels, possibly due to spirulina's hypoglycemic properties, such as its ability to stimulate insulin secretion by the pancreas **(Hatami et al., 2021)**.

The lack of statistically significant changes in blood glucose levels among the groups ($p \geq 0,05$) could be attributed to the insufficient duration or amount of the diet composition to induce hyperglycemia.

Our findings agree with those of **(Vidé et al., 2019)**, who observed no statistically significant difference in blood glucose levels between the Zucker fatty rats (2,20 ± 0,74 g/L) (a model exhibiting various hepatic metabolic alterations) and the Zucker plus spirulina group (2,16 ± 0,61 g/L).

In contrast, **(Souza et al., 2022)** reported that rats consuming a hypercaloric diet did not demonstrate a significant difference in blood glucose levels compared to the standard group (91,4 ± 3,1 mg/dL vs. 86,9 ± 4,5 mg/dL). The hypercaloric group with different spirulina concentrations also did not exhibit a significant difference in blood glucose levels (86,2 ± 2,6, 89,7 ± 4,8 and 85,6 ± 2,4 mg/dL).

7.2. Oral glucose tolerance test (OGTT) evaluation

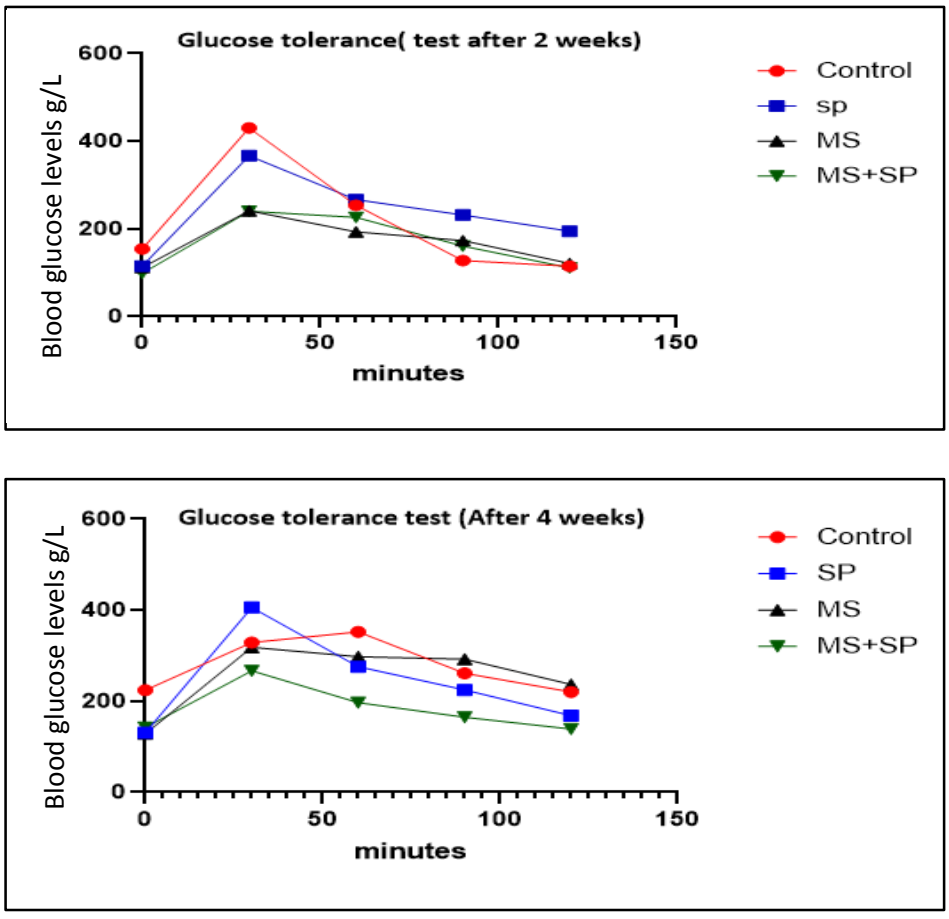

Figure 14: Blood glucose levels evaluation during the oral glucose tolerance test after two weeks and four weeks.

Results were analyzed using a one-way ANOVA followed by Tukey post hoc test. The results are expressed as Mean ± SEM. P≤0,05 indicates a significant change, while p≥0,05 indicates no significant change.

After two weeks on the diet, the first oral glucose tolerance test (OGTT) was conducted in this study **(figure 14)**. There was no significant statistical change between the groups (p=0,5585). After 120 minutes, the control group, MS group, and MS+SP group had almost returned to their initial glucose concentrations before glucose was introduced via gavages. However, the SP group showed an increase in glucose concentration after 120 minutes.

After four weeks, the second OGTT **(figure 14)** was performed. There was no significant statistical difference (p=0,2817) between the groups. The blood glucose concentrations before and after the glucose gavage administration at 120 minutes were similar in the control group, SP group, and MS+SP group. However, in the MS group, the blood glucose concentration rises after 120 minutes compared to the initial concentration. We can conclude that the blood glucose concentration was higher in the second OGGT of the MS group after 120 minutes compared to when the mice were fasting. This suggests that the glucose is unable to enter the cells due to the weak tissue response to insulin stimulation, resulting in insulin resistance **(Freeman et al., 2024)**.

In contrast, the glucose blood levels remained stable in the MS+SP group, which may be attributed to spirulina's potential to improve insulin secretion from the pancreas.

(Vidé et al., 2019) conducted a study using Zucker fatty rats to examine the effects of spirulina and silicon-enriched spirulina. This animal model is characterized by obesity, insulin resistance, hyperlipidemia, and increased liver weight associated with steatosis. The researchers found that in the second OGGT, the blood glucose concentration at the beginning and end of the test (after 180 minutes) was similar in the Zucker rats + SP and Zucker rats + SP + silicon groups, and both groups showed an increase in blood glucose levels.

7.3. Lipid profile evaluation

After analyzing the **figure 15**, it is clear that the concentrations of cholesterol in the MS group (1,33 ± 0,06 g/L) and the MS+SP group (1,31 ± 0,08 g/L) are higher compared to the control group (0,92 ± 0,06 g/L). These differences are statistically significant.

On the other hand, the concentration of triglycerides is higher in the control group (0,64 ± 0,05 g/L), but there is no statistically significant change in the MS group (0,49 ± 0,07 g/L) and the MS+SP group (0,45 ± 0,05 g/L).

When comparing the MS group (1,33 ± 0,06 g/L) to the MS+SP group (1,31 ± 0,08 g/L), there is no significant decrease in blood cholesterol levels. Additionally, there is a slight decrease in triglyceride levels in the MS+SP group (0,45 ± 0,05 g/L). However, these differences are not statistically significant (p>0,05) for both parameters.

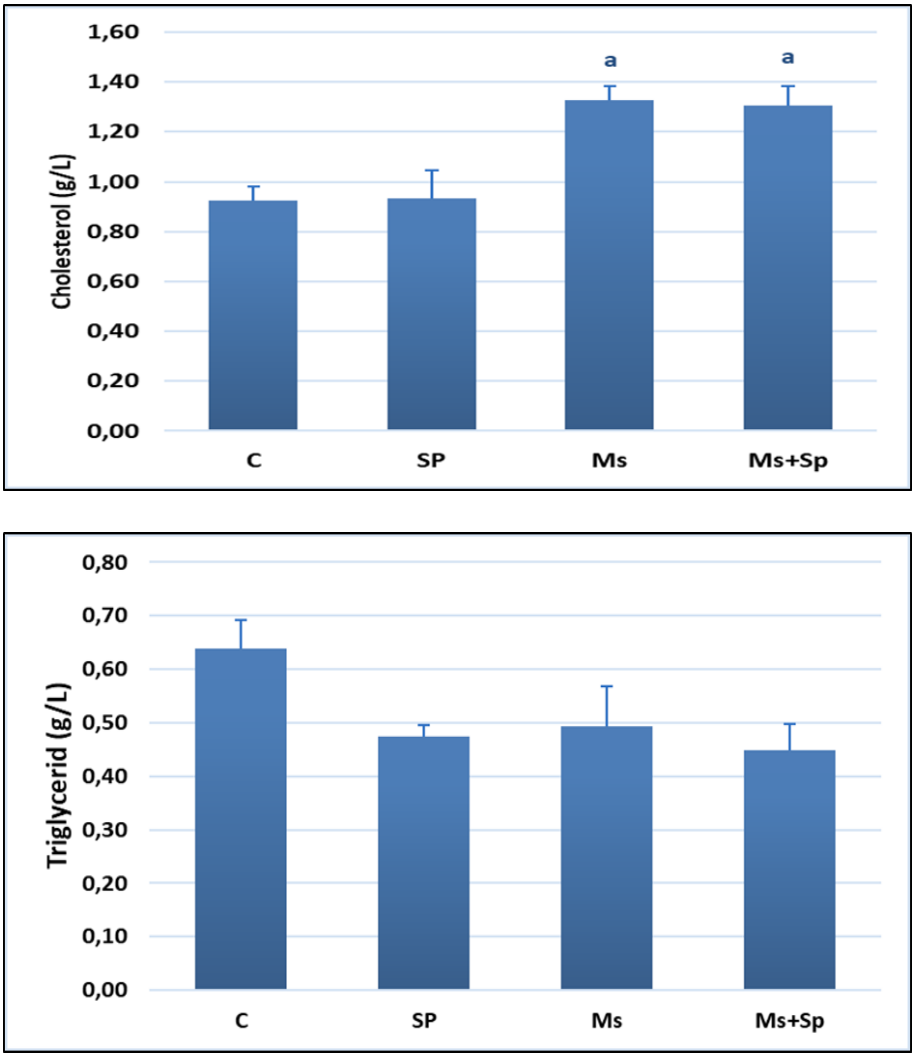

Figure 15: Lipid profile (Cholesterol and triglycerides) in the different groups.

Results were analyzed using a one-way ANOVA followed by Tukey post hoc test. The results are expressed as Mean ± SEM. P≤0,05 indicates a significant change, while p≥0,05 indicates no significant change. ***a****: groups vs control group.* ***b****: groups vs MS group.*

Cholesterol is a lipophilic molecule that plays significant roles, such as being a precursor for vitamin D and steroid hormone production **(Huff et al., 2024)**.

Triglycerides refer to three fatty acids combined with one glycerol molecule, and they serve as the main form of fat storage and energy in the body **(Washabau, 2013)**.

Elevated levels of cholesterol and triglycerides in the bloodstream, known as hyperlipidemia, are strongly associated with cardiovascular diseases **(Lee & Siddiqui, 2024)**.

The significant increase in cholesterol levels was observed in the MS group (1,33 ± 0,06 g/L) compared to the control group (0,92 ± 0,06 g/L). This could be attributed to the high-fat diet given to these mice, which consisted of 60% animal tallow blended with 40% soybean oil. The higher fat content, particularly saturated fats, accounted for 52,59 % of the total fatty acids in beef tallow. Saturated fats are known to elevate blood cholesterol levels, especially LDL cholesterol, which is associated with serious cardiovascular disorders **(Gershuni, 2018)**.

The MS + SP group did not show a significant decrease in cholesterol levels, suggesting that spirulina may not have a hypocholesterolemia effect.

While a fructose-rich diet can lead to lipogenesis in the liver and the accumulation of triglycerides in the body **(Stanhope & Havel, 2008)**, in our study we found no significant increase in triglyceride levels in the MS group, despite using a high proportion of fructose in their drinking water (60%). This may be due to the mice not consuming enough water containing fructose or the duration of the study not being sufficient.

(Yj et al., 2018) demonstrated a significant increase in cholesterol levels in the high-fat diet group (1,545 ± 0,148 g/L), compared to the control group (1,298 ±0,099 g/L). In contrast, our results showed a significant decrease in cholesterol levels (1,193 ± 0,101 g/L) in the high-fat diet group treated with spirulina, compared to the high-fat diet group (1,545 ± 0,148 g/L). Additionally, the levels of triglycerides significantly increased in the high-fat diet group (1,64 ± 0,372 g/L), while spirulina demonstrated an interesting decrease in triglyceride levels in the high-fat diet group treated with spirulina (0,92 ± 0,273 g/L).

The results of **(Yj et al.,2018)** indicate a significant increase in triglyceride levels in the high-fat diet group, along with a significant decrease in cholesterol and triglyceride levels in the treated group. These findings could be attributed to the type of spirulina used or the dosage administered. Specifically, the lower dose (150 mg/kg per day) demonstrated a better hypo-lipidic impact compared to the higher dose (450 mg/kg per day).

7.4. Hepatic transaminases assessment

From **Figure 16**, we observed that the ASAT level in mice with MS (392,0±67,8 U/L) increased compared to the control group (321,9±16,8 U/L), but this increase is not significant. We also observed that the ASAT level in the MS+SP group (386,3±39,8 U/L) is not significantly different from the control group, but there is still an increase.

In comparison with mice in the MS and MS+SP groups, the ASAT rate in the MS+SP group (386,3 ± 39,8 U/L) is not significantly different, but a small decrease is observed as well.

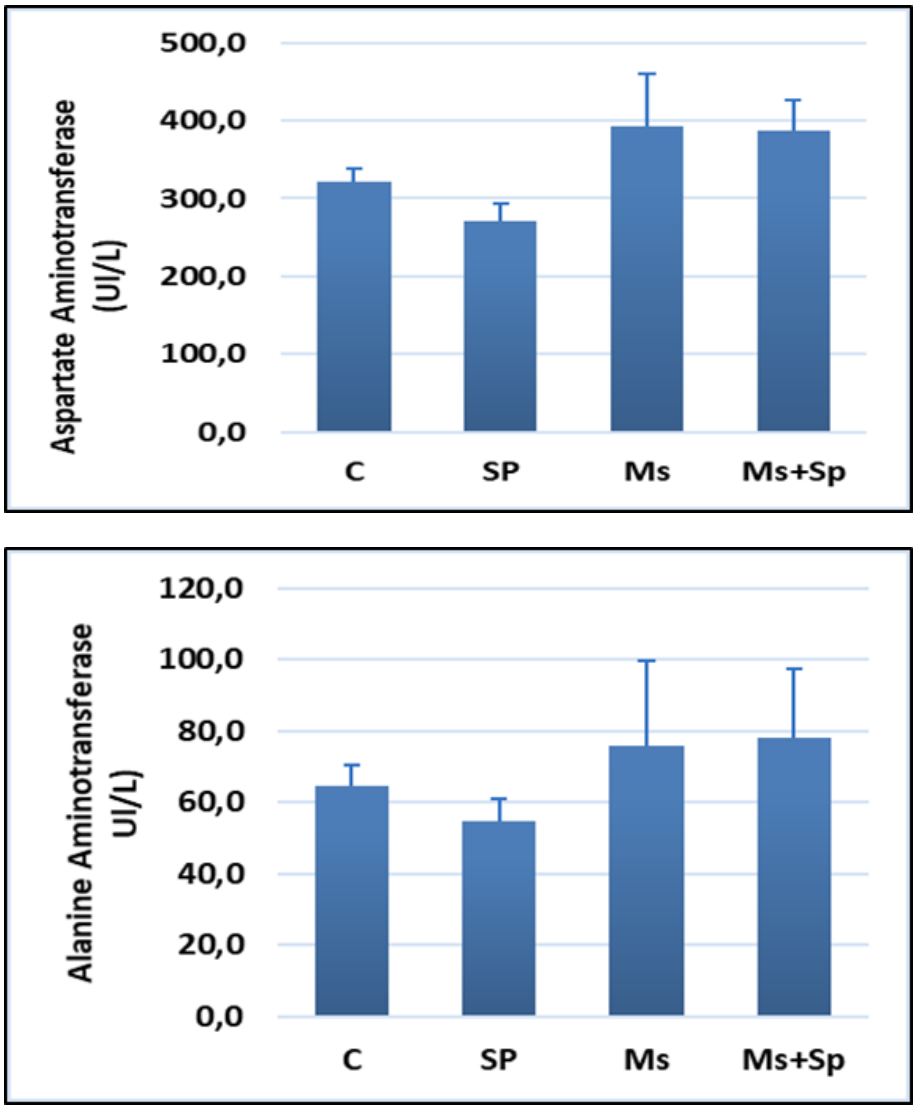

Figure 16: Transaminase levels in the blood in the different groups.

Results were analyzed using a one-way ANOVA followed by Tukey post hoc test. The results are expressed as Mean ± SEM. P≤0,05 indicates a significant change, while p≥0,05 indicates no significant change. ***a****: groups vs control group.* ***b****: groups vs MS group.*

For the second parameter: ALAT **(Figure 16)**, first compare the control group with the others. It is observed that the level of ALAT in mice of the MS group (75,8±24,0 U/L) is higher compared to the mice of the Control group (64,5±6,1 U/L), but this difference is not statistically significant. Additionally, a non-significant increase is observed between the control group and MS+SP (78,2±19,4 Ul/L), although this increase is noteworthy.

Second, when comparing the MS group with the MS+SP group, we observe that the ALAT rate is slightly elevated, although not significantly.

The ALAT and ASAT tests are crucial liver function tests used for the detection of liver cell injury or inflammation, monitoring of liver function, and differentiation between hepatic and extrahepatic causes of elevated liver enzymes. These enzymes are primarily located in liver cells, but they can also be found in other tissues, such as muscles **(Haukeland et al., 2008)**.

The MS group shows a higher concentration of ASAT, we can concluded that slight liver injury happened due to oxidative stress **(Allameh et al., 2023)**. Furthermore, the slight reduction in ASAT and ALAT levels in the MS + SP group can be attributed to the strong antioxidant function of spirulina, which is abundant in antioxidant compounds **(Han et al., 2021)**.

According to the findings of **(Hua et al., 2018)**, there is a significant increase in ALAT and ASAT concentration compared to the control group, indicating successful induction of liver dysfunction in the rat model after four weeks on a high-fat diet. However, the group that received a high-fat diet combined with only Spirulina platensis Protease Hydrolysate (SPPH) showed a remarkable decrease in ASAT and ALAT levels, possibly due to the effects of SPPH.

8. Effect of spirulina on oxidative stress parameters

8.1. Oxidative stress biomarkers

From the results obtained **(Figure 17)** in the first comparison with the control group, we observed that the MDA rate in mice with MS (37,4±6,0 nmole/g) is significantly higher compared to the control group (21,0 ± 2,7 nmole/g). In the MS+SP group (31,5 ± 4,0 nmole/g), we note an increase the MDA rate compared to control group but this difference is not statistically significant.

In the second comparison between the MS and MS+SP groups, we observed a slight decrease in the MDA rate. However, this decrease is not statistically significant.

Meanwhile, in **(Figure 17)**, we compare the AOPP rate between mice in the control group (55,8±4,4 mmole/mg prot) with the MS group (60,7 ± 5,5 mmol/mg prot), as we note an increase in the AOPP rate, but it is not statistically significant. Additionally, with the MS+SP group (50,5±6,9 mmol/mg prot), we observe that the AOPP rate is not statistically significant, but there is a decrease. Finally, with the SP group (88,9 ± 23,2 mmol/mg prot), we observe a significant increase in the AOPP rate.

Secondly, when we compare the MS group with the MS+SP group, we observed that the AOPP rate is slightly decreased, although not significantly.

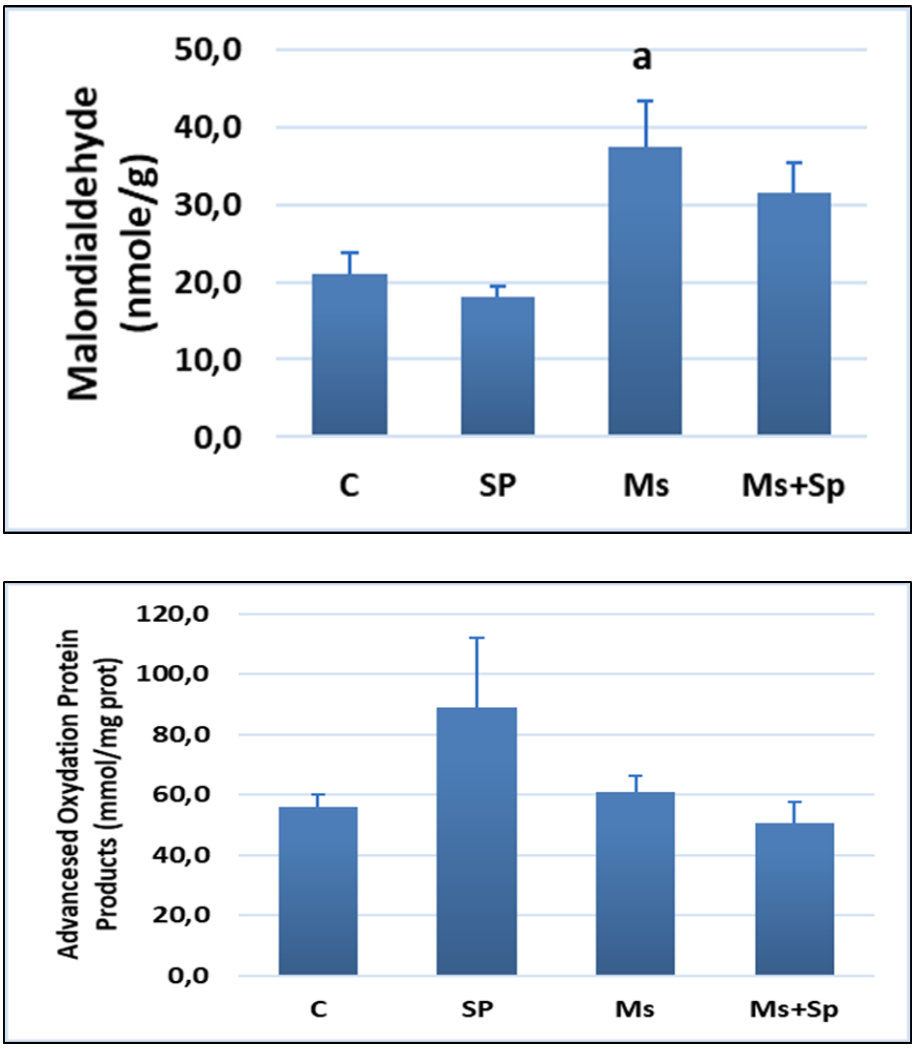

Figure 17: Variation of oxidative markers (MDA, AOPP) in different groups.

Results were analyzed using a one-way ANOVA followed by Tukey post hoc test. The results are expressed as Mean ± SEM. P≤0,05 indicates a significant change, while p≥0,05 indicates no significant change. ***a****: groups vs control group.* ***b****: groups vs MS group.*

Two different techniques are used to quantify oxidative stress in biological samples: the Advanced Oxidation Protein Products (AOPP) and Malondialdehyde (MDA) tests **(Taylor et al., 2015)**.

An organism's lipid peroxidation process is triggered by free radicals. One of the end products of polyunsaturated fatty acid peroxidation in the cells is malondialdehyde (MDA), Overproduction of MDA is caused by a rise in free radicals **(Gaweł et al., 2004)**.

Advanced oxidation protein products (AOPP) are the end products of free radicals acting on proteins and are proposed as a potential indicator of oxidative damage **(Skvarilová et al., 2005)**.

As noted previously, the levels of AOPP and MDA increased in the MS group. This could be attributed to the mice being exposed to oxidative stress, leading to the production of AOPP and MDA as byproducts of protein and lipid oxidation. The decrease in these levels in the MS+SP group can be explained by the presence of spirulina, which contains antioxidants that help combat oxidative stress. It is possible that the increase in AOPP levels in the SP group is a result of the elevated dose of spirulina consumed by the mice that became a prooxidant, leading their bodies to enter a state of oxidative stress. In contrast, the MS+SP group used this dose to inhibit free radicals.

Our findings align with those of **(Arrari et al., 2023)**, who observed that rats treated with a cafeteria diet-induced obesity and oxidative stress, and consumed spirulina, had lower levels of AOPP compared to rats that only consumed the cafeteria diet.

Additionally, our results correspond with those of **(Gargouri et al., 2018)** who found that the rate of AOPP decreased in the group exposed to oxidative stress and treated with spirulina, compared to the group treated with only oxidative stress.

8.2. Evaluation of antioxidant statute

8.2.1. evaluation of nonenzymatic antioxidant level

Based on the **(figure 18)**, it is observed that the GSH rate in the MS group (0,94±0,04 mole/g tissue) and the SP group (0,95±0,02 mole/g tissue) is significantly lower than that of the control group (1,73±0,10 mole/g tissue). Furthermore, there is no significant difference in the GSH rate between the MS+SP group (1,55±0,03 mole/g tissue) and the control group (1,73±0,10 mole/g tissue), but there is also a diminution.

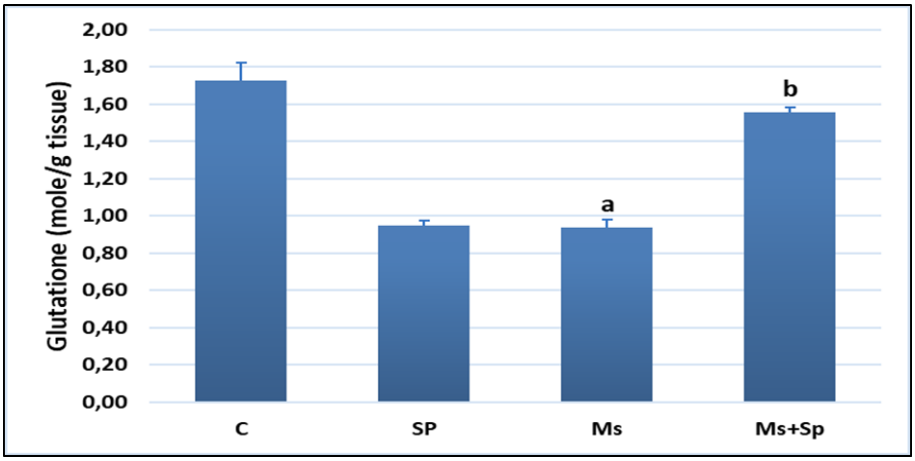

Figure 18: Variation of GSH levels in the different groups.

Results were analyzed using a one-way ANOVA followed by Tukey post hoc test. The results are expressed as Mean ± SEM. P≤0,05 indicates a significant change, while p≥0,05 indicates no significant change. ***a****: groups vs control group.* ***b****: groups vs MS group.*

Moreover, when comparing the MS group (0,94 ± 0,04 mole/g tissue) with the MS+SP group (1,55 ± 0,03 mole/g tissue), it is noted that the GSH rate is significantly lower.

Glutathione is the strongest intracellular antioxidant and, through and glutathione peroxidases (GPX), catalyzes the detoxification of several peroxides. Consequently, the ratio of oxidized to reduced glutathione (GSH: GSSG) functions as a typical indicator of the cell's ability to combat oxidative stress. An insufficiency of GSH exposes the cell to oxidative stress. GSH deficiency is linked to a variety of illnesses, including cancer, and neurological disorders... **(Fraternale et al., 2009)**.

The decrease in GSH rate indicates the presence of oxidative stress (free radicals), which may explain its decreased rate in the MS group (0,94 ± 0,04 mole/g tissue). In this case, the cells secrete GSH to inhibit free radicals. However, due to the antioxidants present in spirulina, it can effectively inhibit free radicals, which may explain why the cells did not secrete GSH and maintained its normal rate in the MS+SP group (1,55 ± 0,03 mole/g tissue). There was a significant difference observed between the GSH levels in the SP group (0,95 ± 0,02 mole/g tissue) and the control group (1,73 ± 0,10 mole/g tissue), indicating that the antioxidants in spirulina work effectively at a low dose without causing harm to the body.

Our findings contradict the results of **(Vidé et all., 2019)** who found no significant difference between the MS group (2,540 ± 356 mole/g tissue) and the MS+SP group (2,332 ±

619 mole/g tissue). This discrepancy may be due to the different dose of spirulina used in our study.

8.2.2. Evaluation of enzymatic antioxidant activities

From **Figure 19**, we observe that there is no significant difference in catalase rate among all groups. However, there is a noticeable difference in catalase rate for the control group (0,71 ± 0,19 UI/g prot/g tissu), which has a lower catalase rate compared to SP group (1,27 ± 0,14 UI/g prot/g tissu) and MS+SP group (1,25 ± 0,30 UI/g prot/g tissu).

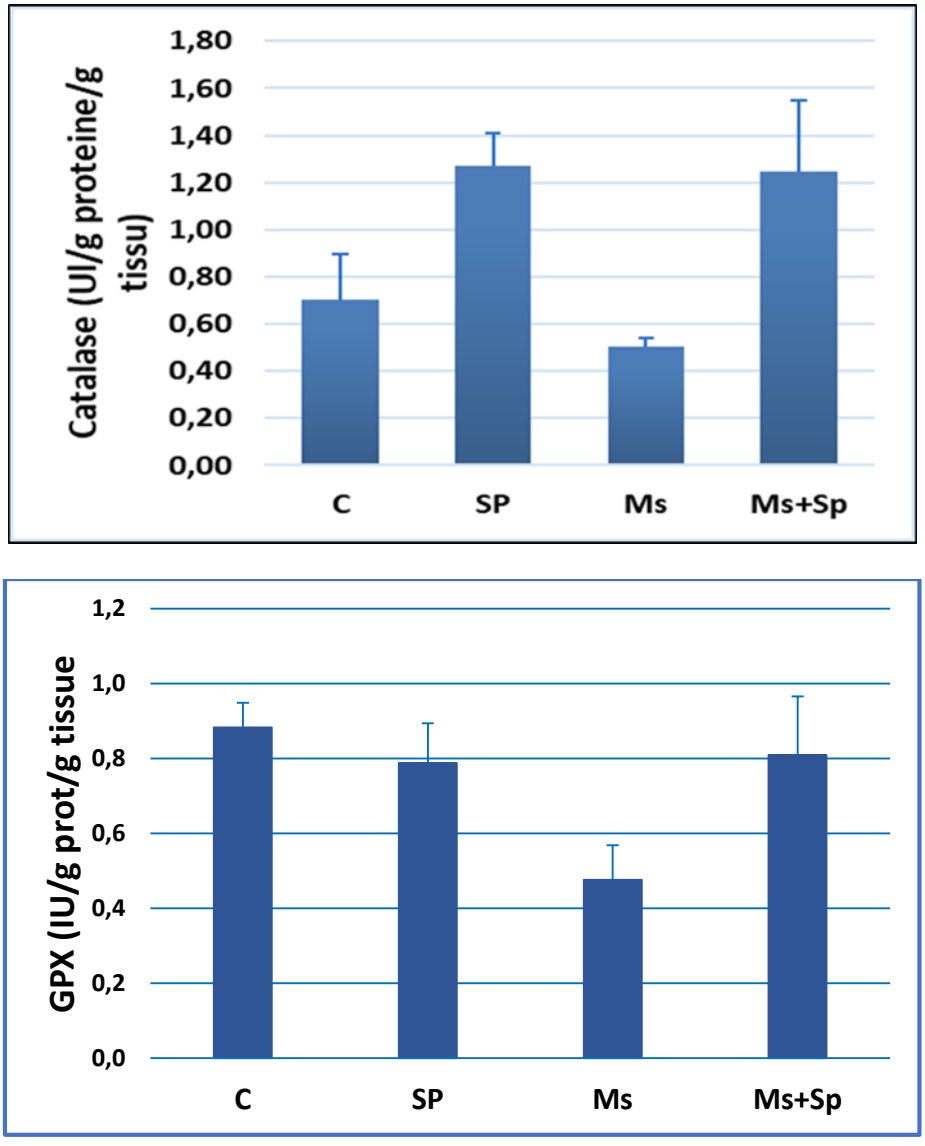

Figure 19: Variation in enzymatic antioxidant activities in the different groups.

Results were analyzed using a one-way ANOVA followed by Tukey post hoc test. The results are expressed as Mean ± SEM. P≤0,05 indicates a significant change, while p≥0,05 indicates no significant change. ***a****: groups vs control group.* ***b****: groups vs MS group.*

Additionally, we can observe a small difference in the catalase rate between MS group (0,51 ± 0,04 UI/g prot/g tissue) and the control group (0,71 ± 0,19 UI/g prot/g tissu).

Furthermore, from **Figure 19**, we notice that there is no significant difference in the GPX rate between different groups, except for MS group (0,5 ± 0,1 UI/g/g tissu), which is slightly lower than the others control (0,9 ± 0,1 UI/g/g tissu), MS+SP (0,8 ± 0,2 UI/g/g tissu) groups.

Secondly, when we compare the MS group with the MS+SP group, we observed that the GPX rate is slightly increased, although not significantly.

Catalase is a cellular antioxidant enzyme (hydrogen peroxide/hydrogen peroxide oxidoreductase) that protects against oxidative stress. It functions as a protective barrier for cells against the harmful effects of elevated hydrogen peroxide (H2O2) concentrations by accelerating the breakdown of H2O2 into molecular oxygen and water while preventing the generation of free radicals **(Shangari & O'Brien, 2006)**.

Glutathione peroxidase (GPX) plays a crucial role in the overall antioxidant defense mechanism, particularly in relation to the superoxide anion radical (•O2) that is continuously generated during normal bodily metabolism, specifically through the mitochondrial energy production pathway (MEPP) **(Ighodaro & Akinloye, 2018)**.

As we observed, the levels of the enzymes GPX and catalase are high in the MS+SP group, while the SP group may indicate evidence that Spirulina possesses antioxidant activity or the ability to stimulate the production of these enzymes.

Our results agree with the findings of **(vidé et al.,2019)**, which indicated that there is no significant difference among all groups and found that Spirulina has the capacity to increase the levels of antioxidants such as GPX for the MS+SP group.

Conclusion

Our study initially aimed to conduct *in vitro* analysis and phytochemical tests on spirulina. Subsequently, we initiated a therapeutic approach using spirulina to assess the potential benefits of this microalgae.

Male mice were subjected to a high-calorie diet (high in fat and fructose) for 4 weeks, which resulted in metabolic abnormalities. We observed a slight increase in body weight, elevated levels of cholesterol, glycemia, MDA, AOPP, and disturbances in transaminase levels. These findings suggest the development of metabolic syndrome in this model but steel the four weeks are not enough.

The phytochemical study of spirulina revealed the presence of various phenolic compounds, including flavonoids, polyphenols, saponins, tannins, terpenoids, alkaloids, and carbohydrates. The identification of these secondary metabolites suggests that spirulina may have interesting and important activities, such as the antioxidant effect observed in our study. This effect was observed during tests on both mice, including GSH, GPx and CAT tests.

When spirulina was administered daily to hypercaloric mice, interesting results were obtained. In fact, the metabolic evaluation of spirulina's effects supports previous studies that highlight its major properties, especially its antioxidant activity. Additionally, spirulina has been shown to have a role in reducing hypoglycemia, combating obesity, and lowering triglyceride levels.

Finally, when it comes to maintaining good health, it is important to consider both the quality and quantity of food that we consume. This is crucial in order to prevent the development of health issues that may be challenging to treat later on. It is worth noting that even spirulina, despite its beneficial properties, can become prooxydants when consumed in excessive amounts.

perspectives

First, in relation to our study, we would appreciate having more than four weeks to ensure that our protocol is completed thoroughly.

Secondly, considering the risks posed by metabolic syndrome to human health, it is crucial to increase public awareness among both men and women. This includes educating individuals about the symptoms of metabolic syndrome and effective preventive measures

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