



People's Democratic Republic of Algeria
وزارة التعليم العالي والبحث العلمي
Ministry of Higher Education and Scientific Research
جامعة أوبكر بلقايد-تلمسان
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Faculty of Natural and Life Sciences, and of Earth and Universe Sciences

Department of Biology

MEMOIR

Presented by

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In order to obtain the

Master's Degree

in biological Sciences, Option : Immunology

Topic

Evaluation of the antioxidant and anti-inflammatory effect
of kefir in patients with irritable bowel syndrome.

Defended on 18/06/2026 before the jury composed of:

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Academic year 2025/2026

Acknowledgements

First and foremost, I would like to express my deepest gratitude to Almighty God for granting me the strength, patience, perseverance, and determination to successfully complete this work.

I would like to extend my sincere appreciation and heartfelt thanks to **Dr. Laissouf Ahlem**, my supervisor, for her trust, continuous support, availability, valuable guidance, and constructive advice throughout the realization of this dissertation. Her expertise and encouragement have been essential to the completion of this work.

I would also like to express my sincere gratitude to **Dr. Nowari Wafaa** and **Dr. Bouali Wafaa** for their valuable contributions, insightful remarks, and the time they devoted to evaluating this work.

Dedication

I dedicate this modest work to my beloved parents, mom and dad, who have always been an inexhaustible source of love, support, sacrifice, and encouragement. No words can fully express my deep gratitude for their constant efforts, patience, and the trust they have always placed in me throughout my academic journey. May God protect them and reward them for everything they have done for me.

To my three brothers, Ilyes, Mustapha, and Adel, for their affection, support, and the precious moments we have shared. I wish them much success and happiness in their lives.

To my grandparents, both paternal and maternal, whose tenderness, advice, and prayers have always been a source of motivation and serenity.

To my uncles Mohamed and Rachid, my maternal uncles Rachid and Abdelhak, and my aunt Souhila, for their kindness, encouragement, and support throughout my studies.

To all my family, who have surrounded me with love and trust.

I also dedicate this work to all those who, directly or indirectly, contributed to the completion of this thesis.

With all my affection and gratitude.

To my dear friends, Marwa, Merieme, and Ikhlasse,

I dedicate this work to you in recognition of your sincere friendship, constant presence, moral support, and valuable help throughout my academic journey. Your encouragement, understanding, and the memorable moments we shared have been a great source of comfort and motivation during both challenging times and moments of success.

Thank you for always being by my side and for making this journey more meaningful and enjoyable

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Abriviation:

(IBS) : Irritable bowel syndrome

(IBS-C) : Irritable bowel syndrome constipation-predominant

(IBS-D) : Irritable bowel syndrome diarrhea-predominant

(IBS-M) : Irritable bowel syndrome mixed

(IBS-U) : Irritable bowel syndrome Unclassifiable

(OS): Oxidative stress

(ROS): reactive oxygen species

(NO₂): nitrogen dioxide

(CO): carbon monoxide

DNA:Deoxyribonucleic Acid

ATP:Adenosine Triphosphate

(NO): nitric oxide

(RNS): reactive nitrogen species

(H₂O₂): hydrogen peroxide

(O₂•⁻): superoxide radicals

(•OH): hydroxyl radicals

(¹O₂): singlet oxygen

(8-OH-dG): 8-hydroxy-2'-deoxyguanosine

MDA: malondialdehyde

LDL: low-density lipoproteins

SOD: Superoxide dismutase

CAT: Catalase

GPx: Glutathione peroxidase

GSH: reduced glutathione

(Ca): calcium

(Mg): magnesium

(Na): sodium

TLRs: Toll-like receptors

(ENS): enteric nervous system

(CNS): central nervous system

ENS's: Enteric Nervous System

(HPA): hypothalamic-pituitary-adrenal

CRH: Corticotropin-Releasing Hormone

(MC): mast cells

(DC): dendritic cells

LT: lymphocytes T

LB: lymphocytes B

Th1: T helper 1 cell

Th2: T helper 2 cell

(5-HT): serotonin.

IgA: immunoglobulin A

(LPDC): Intrinsic dendritic cells

TNF- α : tumour necrosis factor α

IL-10 : interleukin 10

IL-17 : interleukin 17

IL-6 : interleukin 6

IL-1 : interleukin 1

CD4+ T :Lymphocyte T CD4 positif

(EC): enterophilic cell;

TAC: total antioxidant capacity

(IBS-QoL): Irritable bowel syndrome quality of life

(IBSSS): Irritable bowel syndrome severity score

DC: dendritic cell

INF- γ : tumour necrosis factor γ

ER α : Estrogens receptors α

ER β : Estrogens receptors β

(SH-SY5Y): neuroblastoma

KEPS: kefir exopolysaccharides

KE: kefiran

NF- κ B: nuclear factor kappa B

MAPK:Mitogen-Activated Protein Kinase

DSS: dextran sulfate sodium

LAB: lactic acid bacteria

BUN: Blood Urea Nitrogen

CRP : protéine C-réactive

Résumé :

Le syndrome de l'intestin irritable (SII) est un trouble fonctionnel digestif multifactoriel caractérisé par des douleurs abdominales, des ballonnements, des troubles du transit intestinal et un inconfort digestif, associés à une dysbiose intestinale, une inflammation de faible intensité et un stress oxydatif.

Cette étude vise à évaluer l'effet de la consommation de kéfir sur les manifestations cliniques et les marqueurs biologiques du SII.

Avant consommation de kéfir, les sujets présentaient des symptômes digestifs marqués (douleurs abdominales, ballonnements et troubles du transit), associés à une élévation du MDA et de la CRP, ainsi qu'à une diminution du GSH et de la catalase, traduisant un déséquilibre oxydatif et inflammatoire.

Après consommation de kéfir, une amélioration clinique a été observée avec une réduction des symptômes digestifs. Sur le plan biologique, on note une diminution du MDA et de la CRP, ainsi qu'une augmentation du GSH et de la catalase, indiquant une amélioration du stress oxydatif et de l'inflammation. Les paramètres rénaux (urée et créatinine) restent stables, tandis que la vitamine D augmente légèrement.

Ces résultats suggèrent que le kéfir pourrait améliorer les symptômes du SII et exercer un effet bénéfique sur le stress oxydatif et l'inflammation, constituant ainsi une approche nutritionnelle complémentaire prometteuse.

Mots-clés : Syndrome de l'intestin irritable, kéfir, probiotiques, dysbiose intestinale, stress oxydatif, inflammation, MDA, GSH, catalase.

Abstract

Irritable bowel syndrome (IBS) is a multifactorial functional digestive disorder characterized by abdominal pain, bloating, altered bowel habits, and digestive discomfort, associated with gut dysbiosis, low-grade inflammation, and oxidative stress.

This study aimed to evaluate the effect of kefir consumption on the clinical manifestations and biological markers of IBS.

Before kefir consumption, subjects presented with marked digestive symptoms (abdominal pain, bloating, and altered bowel habits), associated with elevated MDA and CRP levels, as well as decreased GSH and catalase levels, reflecting an oxidative and inflammatory imbalance.

After kefir consumption, clinical improvement was observed, with a reduction in digestive symptoms. Biologically, a decrease in MDA and CRP was noted, along with an increase in GSH and catalase, indicating an improvement in oxidative stress and inflammation. Renal parameters (urea and creatinine) remained stable, while vitamin D levels increased slightly.

These results suggest that kefir could improve IBS symptoms and have a beneficial effect on oxidative stress and inflammation, thus representing a promising complementary nutritional approach.

Keywords: Irritable bowel syndrome, kefir, probiotics, gut dysbiosis, oxidative stress, inflammation, MDA, GSH, catalase.

ملخص

تُعد متلازمة القولون العصبي اضطرابًا وظيفيًا هضميًا متعدد العوامل، يتميز بآلام البطن والانتفاخ واضطرابات العبور المعوي والشعور بعدم الارتياح الهضمي، ويرتبط بخلل توازن الميكروبيوتا المعوية، والتهاب منخفض الشدة، والإجهاد التأكسدي.

هدفت هذه الدراسة إلى تقييم تأثير استهلاك الكفير على المظاهر السريرية والمؤشرات البيولوجية لدى المرضى المصابين بمتلازمة القولون العصبي.

قبل استهلاك الكفير، أظهر المرضى أعراضًا هضمية واضحة تمثلت في آلام البطن والانتفاخ واضطرابات العبور المعوي، وكانت مصحوبة بارتفاع مستويات المالمون ثنائي الأدهيد والبروتين المتفاعل س، وانخفاض مستويات الجلوتاثيون المختزل وإنزيم الكاتالاز، مما يعكس وجود اختلال في التوازن التأكسدي والالتهابي.

بعد استهلاك الكفير، لوحظ تحسن سريري تمثل في انخفاض الأعراض الهضمية. وعلى المستوى البيولوجي، سُجل انخفاض في مستويات المالمون ثنائي الأدهيد والبروتين المتفاعل س، مع زيادة مستويات الجلوتاثيون المختزل وإنزيم الكاتالاز، مما يشير إلى تحسن حالة الإجهاد التأكسدي والالتهاب. كما بقيت المؤشرات الكلوية، والمتمثلة في اليوريا والكرياتينين، مستقرة، في حين ارتفع مستوى فيتامين د بشكل طفيف.

تشير هذه النتائج إلى أن الكفير قد يساهم في تحسين أعراض متلازمة القولون العصبي ويُظهر تأثيرًا إيجابيًا على الإجهاد التأكسدي والالتهاب، مما يجعله مقاربة غذائية تكميلية واعدة.

الكلمات المفتاحية: متلازمة القولون العصبي، الكفير، البروبيوتيك، خلل توازن الميكروبيوتا المعوية، الإجهاد التأكسدي، الالتهاب، المالمون ثنائي الأدهيد، الجلوتاثيون المختزل، إنزيم الكاتالاز،

General Introduction

General Introduction :

Irritable bowel syndrome (IBS) is a common gastrointestinal disorder characterized by chronic abdominal discomfort, pain, and changes in bowel habits, as well as extraintestinal manifestations such as fatigue, musculoskeletal pain, gastroesophageal reflux, depression, and anxiety. Despite a prevalence of approximately 10% in the general population, its fundamental etiopathogenesis remains unclear. Current theories favor a multifactorial etiology, attributing a predominant role to psychological stress. The currently accepted models for IBS are based primarily on the gut-brain axis, visceral hypersensitivity, central sensitization, neuroendocrinology, dysbiosis, motility disorders, and persistent low-grade post-infectious mucosal inflammation **(Shiloh Plaut et al., 2026)**.

According to the Rome IV criteria, IBS can be classified into four subtypes: constipation-predominant (IBS-C), diarrhoea-predominant (IBS-D), mixed (IBS-M), and no unclassified (IBS-U) (2). Although IBS does not present with any identifiable organic pathology in the intestines, it impairs patients' quality of life and work productivity and may also lead to disability **(Cheng et al., 2024)**. Low-grade mucosal inflammation, including mast cell activation, is partly responsible, as demonstrated by various surrogate markers of the inflammatory mechanisms studied. In individual studies, alterations have been observed in IBS patients in mast cells, T lymphocytes, B lymphocytes, and mucosal cytokine production. However, no study has demonstrated a significant difference in the number of plasma cells, neutrophils, or eosinophils. Some research has suggested a link between mast cell abnormalities and the severity and frequency of symptoms, as well as associated fatigue and depression. However, studies have been limited by a lack of comparability of controls and by the fact that most of them were conducted in highly selected groups of patients with IBS **(Shulman et al., 2014)**.

Oxidative stress (OS) is defined as an imbalance between the production of reactive oxygen species (ROS) and the antioxidants of the body's defense system **(Marrocco et al., 2017)**. ROS are molecules composed of at least one oxygen atom and containing at least one unpaired electron. From a biochemical perspective, they are highly reactive compounds that interact with cellular organelles.

Kefir is a fermented drink renowned for its probiotics, which live in symbiosis with other microorganisms present in the kefir grains. Its consumption is associated with numerous nutraceutical benefits, including anti-inflammatory, antioxidant, anticancer, antimicrobial, antidiabetic, antihypertensive, and cholesterol-lowering effects. Furthermore, kefir can be

General Introduction :

incorporated into various substrates, thereby enabling the production of new functional drinks and product diversification (**Nor Farahin Azizi, 2021**).

The objective of our study is to evaluate the effect of kefir supplementation in patients with irritable bowel syndrome (IBS) by examining its impact on redox imbalance, intestinal inflammation, and alterations in the gut microbiota, as well as on the improvement of associated clinical symptoms.

Chapter 01

The Role of Oxidative Stress, Low-Grade Inflammation, and Gut Microbiota Dysbiosis in the Pathophysiology of Irritable Bowel Syndrome.

Chapter 01: The Role of Oxidative Stress, Low-Grade Inflammation, and Gut Microbiota Dysbiosis in the Pathophysiology of Irritable Bowel Syndrome:

Irritable bowel syndrome (IBS) remains a complex functional gastrointestinal disorder. Recent studies have described a multitude of exogenous factors act together to gradually alter the metabolic balance of intestinal mucosal cells, particularly their oxidative status, independently of any genetic predisposition. Although current biomarkers can distinguish IBS subtypes from other functional gastrointestinal disorders, most lack specificity and reflect clinical, biochemical and inflammatory imbalances. As irritable bowel syndrome (IBS) can also be caused by abnormalities in signaling pathways involving gastrointestinal secretion and stimulation of the autonomic nervous system, the hypothesis of oxidative stress in its pathophysiological mechanism is not immune to these factors. Regarding the role of oxidative stress in IBS, the results of previous research are contradictory: some animal models and clinical studies have shown significant oxidative imbalances at the systemic and local levels, but without providing conclusive evidence of a direct link between oxidative stress and IBS (Balmus et al., 2020).

Table 01: Subtype of IBS (adapted from Sabate et al., 2026)

Subtypes	Criteria
Predominant diarrhoea (IBS-D)	Type 1-2 stools <25% of the time Type 6-7 stools ≥25% of the time
Predominantly constipated (IBS-C)	Type 1-2 stools ≥25% of the time Type 6-7 stools <25% of the time
Alternating diarrhoea and constipation/mixed (IBS-M)	Type 1-2 stools ≥25% of the time Type 6-7 stools ≥25% of the time
Unclassifiable (IBS-U)	Does not meet the criteria for IBS-D, IBS-C or IBS-M

1. Intestinal hyperpermeability

Some authors have reported increased intestinal mucosal permeability and impaired tight junctions in some patients with irritable bowel syndrome (IBS). Studies have suggested that interactions between factors such as diet, gut microbiota, the epithelial barrier, and the mucosal immune system may induce pain by stimulating afferent nerves through inflammation processes. These factors are considered triggers of altered intestinal permeability (Soares et al., 2014).

Chapter 01: The Role of Oxidative Stress, Low-Grade Inflammation, and Gut Microbiota Dysbiosis in the Pathophysiology of Irritable Bowel Syndrome:

2. Interaction of the brain-gut axis

Another concept in the pathophysiology of irritable bowel syndrome is alterations in the brain-gut axis; several studies have demonstrated brain-gut interactions, and distension of specific regions of the colon has been associated with the activation of brain areas involved in pain perception (Lee et al., 2014).

3. Risk factors for irritable bowel syndrome:

3.1 Power supply

Diet appears to play an important role in the pathogenesis of irritable bowel syndrome (IBS), as most patients are able to identify the foods that trigger their symptoms. They adapt their diet by limiting their consumption of these foods that they perceive as problematic (Soltani et al., 2019; Lenhart et al., 2022). Some even go so far as to avoid entire food categories without consulting a health professional, which can increase their risk of malnutrition (Hayes et al., 2014). In general, certain diets, such as Western diets high in sugar and fat and ultra-processed foods, are associated with IBS (Buscailet et al., 2017; Schnabel et al., 2018).

3.2 Environmental pollution

Recent studies have suggested potential correlations with gastrointestinal disorders such as IBS. Indeed, increased concentrations of certain pollutants present in the atmosphere, such as nitrogen dioxide (NO₂) and carbon monoxide (CO), have been associated with a higher prevalence of IBS in children (Marynowski et al., 2015; Tan et al., 2019; Vignal et al., 2021).

Exposure to air pollution may cause changes in the composition of the gut microbiota (Mutlu et al., 2018). One hypothesis is that the resulting dysbiosis could be associated with the development of IBS (Nget et al., 2024).

3.3 Stress

Stress may be involved in the pathogenesis. More specifically, significant and traumatic events during early childhood have been linked to the development of IBS (Ju et al., 2020). Studies show that trauma in early childhood or childhood can sensitise and predispose an individual to the onset of functional gastrointestinal symptoms later in life.

Chapter 01: The Role of Oxidative Stress, Low-Grade Inflammation, and Gut Microbiota Dysbiosis in the Pathophysiology of Irritable Bowel Syndrome:

Patients with IBS have stress reactions that are ten times greater than those of healthy individuals, manifesting as intestinal symptoms. This is particularly reflected in phenomena related to intestinal motility, visceral perception and sensitivity, and hormone levels associated with the hypothalamic-pituitary axis (**Chang, 2011; Daugey, 2024**).

3.4 female sex and neuroticism

Neuroticism is a personality trait associated with negative emotions and emotional instability, including irritability, anxiety, depression, worry, guilt, loneliness, and other negative feelings. High neuroticism, which is more prevalent in adult women, has been correlated with poorer health, including worsening symptoms of irritable bowel syndrome (IBS) such as diarrhea, constipation, and abdominal pain (**Cong et al., 2022**). Neuroticism has also been associated with weakened immunity, i.e., a reduced ability of the body to respond to physical attacks (such as infections) by activating an appropriate immune response, which is essential for maintaining good health, preventing and curing disease, and improving quality of life (**Verster et al., 2023**).

3.5 Age

The risk of developing IBS is inversely proportional to age (**Enck et al., 2016**). The older a person is, the lower their risk of being diagnosed with IBS, because if they present with typical IBS symptoms, these are more often associated with a gastrointestinal disease (cancer, inflammatory bowel disease (IBD), celiac disease) (**Enck et al., 2016**); (**Lacy and Patel, 2017**). Several studies have determined that in people under 50 years of age, the risk of developing IBS is higher (**Lacy and Patel, 2017**).

3.6 Psychological profile

It is now well established that virtually all intestinal functions are sensitive to stress and that the digestive system reacts differently depending on emotional and psychological experiences. Thus, the discomfort caused by the digestive disorders of irritable bowel syndrome (IBS) can lead to or aggravate stress and anxiety, creating a vicious circle that is difficult to break on one's own. A survey of patients with IBS revealed that 44% of them also suffered from anxiety and 84% were affected by depression (**Solmaz et al., 2003; Nicholl et al., 2007**).

4. Oxidative stress

Oxidative stress is an imbalance between the production of free radicals, primarily resulting from oxidation reactions, and the body's antioxidant defense systems. When these free radicals accumulate, they can damage cellular macromolecules, such as DNA, proteins, and lipids, thereby promoting the development of various pathologies (**Cabello-**

Chapter 01: The Role of Oxidative Stress, Low-Grade Inflammation, and Gut Microbiota Dysbiosis in the Pathophysiology of Irritable Bowel Syndrome:

Verrugio et al., 2018). To limit these harmful effects, cells possess protective mechanisms that reduce the formation of free radicals and prevent oxidative damage. These mechanisms rely on the combined action of enzymatic and non-enzymatic antioxidants (**Sharma et al, 2012**).

4.1 Definition of a free radical

Free radicals are molecules or atoms with one or more unpaired electrons in their outer shell, which makes them energetically and kinetically unstable. Because of this instability, they rapidly reach a stable state by reacting with another molecule, either by losing or gaining an electron. Thus, as electron acceptors or donors, these free radicals are extremely reactive towards other molecules and have a very short lifespan (**Koechlin-Ramonatxo 2006**). They are products of normal cellular metabolism. Most reactive oxygen species (ROS) are generated in cells by the mitochondrial respiratory chain. ATP is synthesised by reduction of molecular oxygen to water through a sequence of proton and electron transfer reactions. During this process, some electrons escape prematurely, leading to the formation of reactive oxygen species (ROS). Recently, it has become clear that under hypoxic conditions, the mitochondrial respiratory chain also produces nitric oxide (NO), which represents another reactive species known as a reactive nitrogen species (RNS) (**Fu et al., 2014**); (**Biswas et al., 2017**). A radical's ability to be either reducing or oxidising depends on the compound that allows it to stabilise. In biology, free radicals are primarily oxidising agents and are therefore considered potentially toxic.

4.1.1 Endogenous Sources

Free radicals produced within cells originate primarily from specific organelles, particularly mitochondria, peroxisomes, and the endoplasmic reticulum, where oxygen metabolism is highly active. In addition to these sources, several oxidase enzyme systems also contribute to their production, including acyl-CoA oxidase, D-aspartate oxidase, and xanthine oxidase, all of which play important roles in the production of these reactive species.

4.1.2. Exogenous Sources

Free radicals can also originate from external factors such as contaminated water, pesticides, or certain medicinal substances. After entering the body through various routes,

Chapter 01: The Role of Oxidative Stress, Low-Grade Inflammation, and Gut Microbiota Dysbiosis in the Pathophysiology of Irritable Bowel Syndrome:

these compounds are metabolised and can lead to the production of free radicals (Pham-Huy et al., 2008; Phaniendra et al., 2015).

4.2 Properties of some free radicals (oxidants)

ROS (Reactive Oxygen Species) are molecules composed of at least one oxygen atom and containing at least one unpaired electron. From a biochemical perspective, they are highly reactive compounds that interact with cellular organelles (Jopkiewicz, 2018). Examples of ROS include hydrogen peroxide (H_2O_2), superoxide radicals ($\text{O}_2^{\bullet-}$), hydroxyl radicals ($\bullet\text{OH}$), and singlet oxygen ($^1\text{O}_2$). They are primarily produced as byproducts of oxygen metabolism, but certain external factors can contribute to their increase (Pizzino et al., 2017). These factors include exposure to the external environment (ultraviolet radiation, chemicals such as benzene and phenols), certain diseases (cancers, infections), and certain medications (cyclosporine, gentamicin) (Wanget et al., 2019).

4.3 Oxidative Damage

4.3.1 Nucleic Acids

Studies show that mitochondrial DNA is more vulnerable than nuclear DNA because it is located near the sites of reactive oxygen species (ROS) production (Kauppila, 2015). The hydroxyl radical ($\bullet\text{OH}$) can interact with DNA by damaging purine bases, pyrimidine bases, and deoxyribose (Biswas et al., 2017). A typical example is the reaction of guanine with $\bullet\text{OH}$, which leads to the formation of 8-hydroxy-2'-deoxyguanosine (8-OH-dG). This molecule, instead of pairing with cytosine, associates with adenine, thus causing mutations. These genetic alterations can disrupt the expression of cellular signals and are implicated in aging and cancer development (Haleng et al., 2007).

4.3.2. Proteins

Amino acids have varying sensitivities to reactive oxygen species (ROS). Among them, the most vulnerable are histidine, proline, tryptophan, cysteine and tyrosine. When a free radical attacks an amino acid, certain residues undergo oxidation, leading to the formation of carbonyl groups.

Most of these alterations are irreversible and can cause major functional disruptions, such as loss of enzymatic activity or the inability of a ligand to recognise its receptor (Haleng et al., 2007).

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4.3.3 Lipids

Lipid peroxidation is the process of oxidation of polyunsaturated fatty acids, leading to their degradation (Ayala, 2014). When free radicals attack polyunsaturated membrane lipids, they produce malondialdehyde (MDA), which is considered a reliable marker of this phenomenon. The oxidation of membrane lipids alters the fluidity of membranes, disrupting normal cell function.

This process also affects lipoproteins, particularly LDL (low-density lipoproteins). Once oxidised, these LDLs are no longer recognised by their specific receptors and accumulate in macrophages. This leads to the formation of foam cells, which play a key role in the development of atherosclerosis (Peluso et al., 2012).

5. Antioxidants

To protect itself from the harmful effects of reactive oxygen species (ROS), the body has a complex network of antioxidant mechanisms. An antioxidant is defined as any substance capable, even at low concentrations relative to an oxidisable substrate, of delaying or preventing the oxidation of that substrate (Biswas, 2016).

Antioxidants are divided into two types:

5.1. Preventive antioxidants

This group mainly includes antioxidant enzymes that are directly involved in neutralising ROS and reactive nitrogen species (RNS). Among the most important are:

Superoxide dismutase (SOD): converts superoxide anion into hydrogen peroxide. Catalase (CAT): breaks down hydrogen peroxide into water and oxygen, Glutathione peroxidase (GPx): reduces lipid peroxides and hydrogen peroxide, Glutathione reductase (GRx): regenerates reduced glutathione, which is essential for maintaining the antioxidant system.

5.2. Antioxidant scavengers

These antioxidants, also known as chain-breaking antioxidants, intercept free radicals to stop them from spreading. They include:

Coenzyme Q10, uric acid, reduced glutathione (GSH), polyphenols (found in fruit, vegetables, tea, wine, etc.)

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In addition, there are essential micronutrients that boost the body's antioxidant capacity, such as calcium (Ca), magnesium (Mg) and sodium (Na) **(Haleng et al., 2007; Pham-Huy et al., 2008; Wang et al., 2017; Goff, 2018)**.

The fact that oxidative stress is involved in many common physiological pathways, as well as in frequent pathologies such as gastrointestinal, nutritional, neurological and psychiatric disorders, is not new in research **(Mesika et al., 2019)**. As irritable bowel syndrome (IBS) can result from deficits in signalling pathways involving both gastrointestinal secretion and neurovegetative stimulation, IBS is no exception to the oxidative hypothesis regarding its pathological mechanisms. In a recent review of animal models of IBS, Wang et al. **(Wang et al., 2017)** noted that the underlying pathogenic mechanisms of IBS remain ambiguous, although increased epithelial permeability, inflammation, visceral hypersensitivity, and altered brain-gut interaction are considered to play a key role.

Irritable bowel syndrome (IBS) has been characterised as a simple mild inflammatory imbalance, as a functional antioxidant system is synonymous with a functional anti-inflammatory system. The correlation between the antioxidant system and IBS has been confirmed by studies in animal models.

Based on these observations, it appears that although there are differences in serum oxidative markers between patients with irritable bowel syndrome and healthy controls, these variations are not directly correlated with digestive symptoms or quality of life.

(Oranet al., 2014) suggest that irritable bowel syndrome (IBS) and oxidative stress are associated through micro-inflammatory processes. They point out that oxidative stress is defined as the effect of an overload of antioxidant defences by reactive oxygen species (ROS), but attribute to the latter a pro-inflammatory mediating role exacerbated by their excessive production and insufficient hydrolysis.

(Oranet al., 2014) The presence of NO in blood samples from patients with irritable bowel syndrome (IBS) is an indicator of increased inflammation and lipid peroxidation. The authors explain the link between the deleterious events associated with oxidative stress and the inflammatory processes that may occur, demonstrating that ROS overproduction is due to inflammatory signalling from the colonic cells of these patients. **(Mete et al., 2013)**.

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Figure 1. Factors affecting oxidative stress levels.

(Sara Jarmakiewicz-Czaja et.,2023)

The intestinal mucosa incorporates a complex enteric immune system, rich in various immune cells. In patients with irritable bowel syndrome (IBS), this system appears to be imbalanced, with increased expression of pro-inflammatory factors and activation of immune cells (Chen et al., 2022). In those with IBS-D, the levels of 5-HT and 5-HT₃ receptors in the mucosa are significantly higher than in healthy subjects, indicating a dysfunction of the serotonergic system (Fu et al., 2019). An increase in the expression of Toll-like receptors (TLRs) is also observed in IBS patients (Dlugosz et al., 2017). Present in intestinal epithelial and immune cells, these TLRs interact with neuronal and immune receptors to regulate mucosal homeostasis (Assas et al., 2014). They detect microbial components of commensal and pathogenic bacteria, promoting immune tolerance to the former and defence against the latter (Kivit et al., 2014). Nevertheless, the precise mechanisms by which the gut microbiota modulates local immunity remain debated and poorly understood.

Intestinal dysbiosis stimulates the mucosal immune system, altering the epithelial barrier and inducing visceral hypersensitivity and alterations in gastrointestinal motility in patients with irritable bowel syndrome (IBS). These dysfunctions cause abdominal pain, diarrhea or exacerbation of symptoms. The gut microbiota and the brain are interconnected, influencing each other to regulate gastrointestinal nervous, endocrine and

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immune functions, thereby contributing to the development and progression of IBS (Cheng et al., 2024).

The digestive system is colonised by a large commensal flora that participates in digestion by balancing the luminal pH, synthesising vitamins and metabolites, and contributing to the metabolism of enteric cells (Ford et al., 2017). Dysbiosis is an imbalance in the bacterial flora with a decrease in bacterial diversity and an imbalance between different strains, leading to a disruption of physiological functions. This imbalance can occur as a result of (Rahmoun et al., 2016) A Western diet (too high in fat and low in fibre, refined foods). Frequent use of antibiotics. Gastroenteritis (10% of IBS cases). Exposure to air pollutants, including smoking, pesticides and chronic stress, which promote it.

6. Immune activation and inflammation

The role of low-grade inflammation in irritable bowel syndrome (IBS), a common disorder of the gut-brain axis, remains poorly defined. Its association with symptom severity, as well as the effects of first-line treatments (Tack et al., et al 2026) Another pathophysiological hypothesis suggests that inflammatory cells may play a role in the pathogenesis of irritable bowel syndrome (IBS) symptoms. Low-grade mucosal inflammation, particularly mast cell activation, is partly responsible, as shown by various surrogate markers of inflammatory mechanisms studied. In individual studies, alterations have been observed in patients with IBS in mast cells, T lymphocytes, B lymphocytes and mucosal cytokine production. However, no studies have demonstrated a significant difference in the number of plasma cells, neutrophils or eosinophils. Some research has suggested a relationship between mast cell abnormalities and symptom severity and frequency, as well as associated fatigue and depression. However, studies have been limited by a lack of comparability of controls and by the fact that most have been conducted in highly selected groups of patients with IBS (Shulman et al., 2014).

Clinical observations Recently, the issue of low-grade inflammation, or immune activation, and ecological imbalance that may trigger or exacerbate IBS has been raised (Rodriguez-Fandino et al., 2010). The importance of low-grade inflammation in the progression of IBS is now supported and confirmed by observations. In a subgroup of patients with IBS, increased innate immune activation has been observed in both the

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intestinal mucosa and the blood. The main feature of the disease is intestinal hyperpermeability, a condition in which the integrity of the blood-intestinal barrier is impaired, allowing intestinal fluids (such as immune cells and microbiota) to pass into the bloodstream, resulting in widespread low-grade systemic inflammation (**Ohman et al., 2010**).

These results suggest that patients with IBS have a higher systemic immune inflammation index. The progression of symptoms is accompanied by changes over time (**Talley et al., 2020**). Analysis of the studies reviewed revealed that patients with irritable bowel syndrome (IBS) had a higher frequency of immune activation than healthy subjects, with increased numbers of mast cells and lymphocytes, altered cytokine levels, and increased intestinal permeability (**Matricon et al., 2012**). An increase in innate immune activity in the intestinal mucosa and blood was also observed in the subgroup of subjects with IBS (**Ohman et al., 2010**).

7. Inflammatory signals transmitted via the brain-gut axis

The enteric nervous system (ENS), which innervates the intestine, comprises approximately 200 million neurons and is primarily responsible for intestinal motility. It interacts bidirectionally and closely with the central nervous system (CNS) via the gut-brain axis, justifying the ENS's designation as the 'second brain'. It modulates the motor, sensory and secretory functions of the digestive tract. The intestine and its microbiota produce more than four-fifths of endogenous serotonin, a key hormone for well-being. Any disturbance of this flora, such as intestinal dysbiosis, impacts the body and is implicated in neurodevelopmental disorders or neurodegenerative diseases (**Lecerf et al., 2021**).

Mental health disorders play a major role in the development and progression of irritable bowel syndrome (IBS), a multifactorial condition (**Spiller et al., 2000**). Early stress, one of the most common aggravating factors, can stem from family, work, or social contexts, or be linked to personality traits (**Tao et al., 2022**). Inflammatory susceptibility and reactive alterations of the hypothalamic-pituitary-adrenal (HPA) axis are central to the pathogenesis of IBS, often associated with negative emotions, with stress acting as the underlying process. Although the pathophysiological mechanisms are not fully understood, the data consistently confirm the crucial role of inflammatory mediators and HPA axis activation in stress-related disorders (**Yuan et al., 2023**).

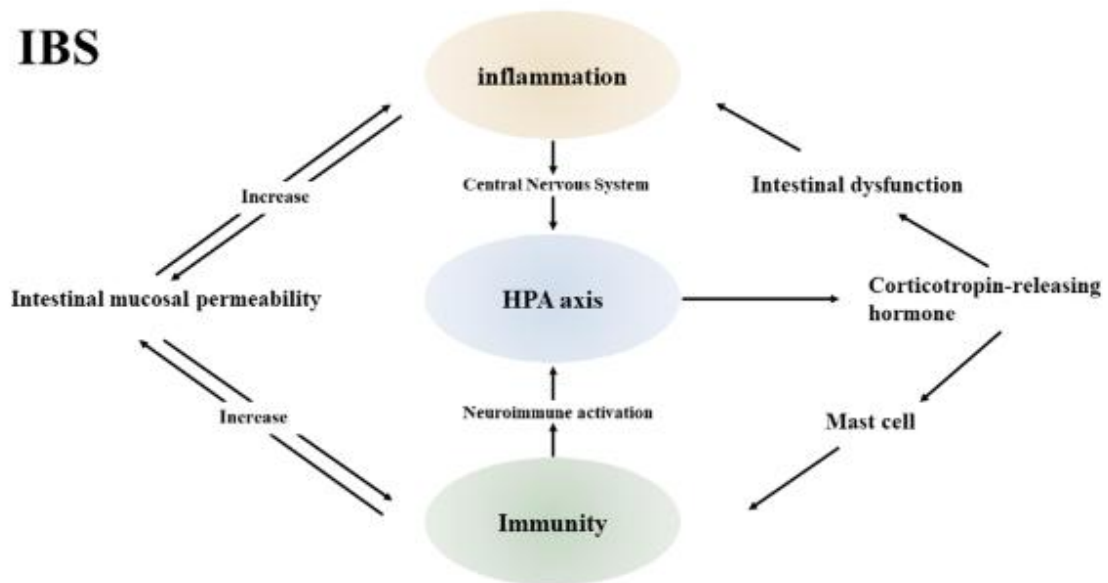


Figure 2: “Neuro-immuno-endocrine interactions in irritable bowel syndrome: role of inflammation, intestinal immunity and the HPA axis” (Yi Yuan et al., 2023).

Inflammation, immunity, and the HPA axis in irritable bowel syndrome (IBS). The inflammatory, immune, and HPA axes interact in IBS. Peripheral responses to environmental stressors or intestinal inflammation are integrated at the central nervous system level, triggering the HPA axis. The HPA axis coordinates the release of glucocorticoids by the adrenal glands, thus promoting gastrointestinal dysfunction. Activation of intestinal immune cells can disrupt the epithelial barrier function, thereby altering mucosal permeability and potentially inducing intestinal inflammation. This inflammation, in turn, increases intestinal mucosal permeability. The intestinal immune response is enhanced by mast cell activation via CRH. Intestinal immunity stimulates neuroimmune activation.

8. Immune cells involved in IBS

In patients with irritable bowel syndrome (IBS), the immune system of the intestinal lamina propria comprises various immune cells, including mast cells (MC), dendritic cells (DC), LT/LB, etc. Certain external or internal factors can cause these immune cells to produce an allergic reaction, which in turn induces an inflammatory response (Krystel-Whittemore et al., 2015).

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8.1 Mast cells (MC)

These are multifunctional cells involved in immune and inflammatory regulation. An increased number of MCs has been observed in the terminal ileum and rectosigmoid mucosa of patients with irritable bowel syndrome (IBS) (**Wang et al., 2004**). Mucosal biopsies from these patients showed increased release of mediators, among which the secretory products of MC serotonin (5-HT), histamine and trypsin-like enzymes were significantly higher than in controls (**Buhner et al., 2009**).

Furthermore, mast cell mediators have been shown to excite damaged visceral sensory nerves in rats, suggesting that mast cell infiltration may be associated with the visceral hypersensitivity observed in IBS (**Barbara et al., 2007**). Finally, MCs also promote the proliferation and polarisation of B lymphocytes into IgA-producing plasma cells, thereby contributing to the modulation of the immune response (**Merluzzi et al., 2010**).

8.2 Intrinsic dendritic cells (LPDC)

Are important antigen-presenting agents present in the intestinal chorion and are now considered essential for innate and acquired immunity (**Chirido et al., 2005**). Studies on LPDCs in patients with irritable bowel syndrome (IBS) or chronic inflammatory bowel disease (IBD) have shown that LPDCs significantly increase TNF- α secretion, that the expression of microbial recognition receptors is upregulated, and that dendritic cells produce more pathological cytokines (**Hart et al., 2005**). In addition, dendritic cells appear to be activated and participate in the immune response via the generation of IL-10 and the proliferation of CD4⁺ T lymphocytes (**Psarras et al., 2021**).

8.3 Enterochromaffin cells (EC)

Specialised in the intestine, they produce and release serotonin (5-HT) and ATP, two mediators capable of activating or modulating the intestinal nerve reflexes involved in visceral and painful sensations (**Linan-Rico et al., 2017**). 5-HT plays a central role in the pathophysiology of irritable bowel syndrome (IBS), influencing peristalsis, nociception, visceral inflammation, immune response and brain activity (**Mishima et al., 2021**). An experimental study using an animal model of early stress through maternal isolation showed that the expansion of intestinal stem cells and their differentiation into the secretory lineage led to EC proliferation, increased 5-HT production and visceral

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sensitisation. These findings suggest that early stress-induced IBS may be linked to modulation of 5-HT secretion by ECs.

9. Cytokines involved in IBS

Recent studies conducted on 90 patients with IBS (according to the Rome III criteria) and 90 matched healthy controls measured serum levels of inflammatory cytokines (TNF- α , IL-17), anti-inflammatory cytokines (IL-10), and biomarkers of oxidative stress (malondialdehyde [MDA] and total antioxidant capacity [TAC]). The results show that IBS patients have significantly higher levels of TNF- α , IL-17, and MDA, and lower levels of IL-10 and TAC compared to controls. The IBS-D subtype (diarrhea-predominant) is distinguished by a marked increase in pro-inflammatory cytokines and a decrease in IL-10, while the other subtypes show profiles closer to those of controls.

The authors emphasize that inflammatory cytokines are directly correlated with the severity of gastrointestinal symptoms, the IBS severity score (IBSSS), and quality of life (IBS-QoL), unlike oxidative stress biomarkers which, although altered, do not show a significant link with symptoms.

IBS, and particularly IBS-D, is associated with low-grade inflammation and immune imbalance, which contribute to symptomatology and a reduced quality of life. These results reinforce the idea that IBS is not solely a functional disorder, but that it involves measurable biological mechanisms (Razieh Choghakhoria et al., 2021).

Table 02: “Profile of inflammatory cytokines and biomarkers of oxidative stress in irritable bowel syndrome” (Razieh Choghakhoria et al., 2021).

Marker	Witnesses	Patients IBS	IBS-D subtype
TNF α (pro-inflammatory)	Normal	↑	↑ ↑
IL-17(pro-inflammatory)	Normal	↑	↑ ↑
IL-10(anti- inflammatory)	Normal	↓	↓ ↓
MDA (oxidative stress)	Normal	↑	↑
TAC (antioxidant)	Normal	↓	↓

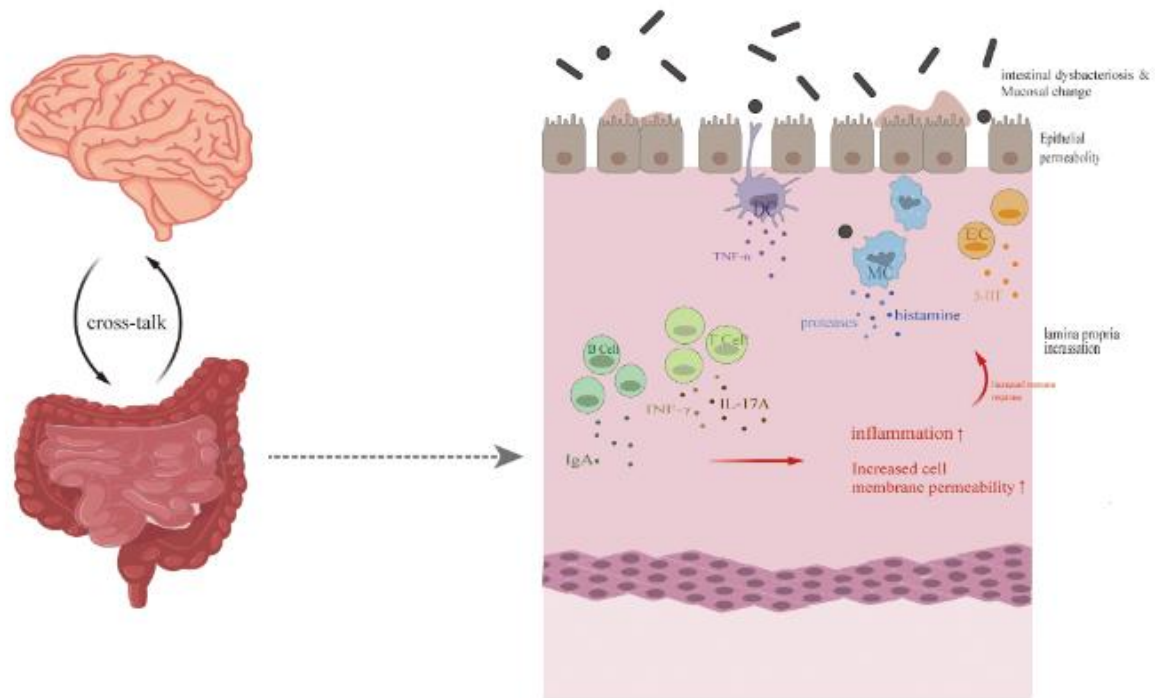


Figure 03: "Intestinal immune activation and mucosal barrier alteration in irritable bowel syndrome: role of dendritic cells, mast cells and pro-inflammatory cytokines" (Yi Yuan et al., 2023).

Presence of intestinal immune activation in irritable bowel syndrome. In the intestinal tissues of patients with irritable bowel syndrome, the alteration of the intestinal mucosal barrier due to dysbiosis can activate a pro-inflammatory dendritic cell phenotype, thereby stimulating an immune response by T and B lymphocytes and increasing cell permeability. DC: dendritic cell; EC: enterophilic cell; IL-17A: interleukin 17A; INF- γ : tumour necrosis factor γ ; IgA: immunoglobulin A; MC: mast cell; TNF- α : tumour necrosis factor α ; 5-HT: serotonin.

10. Hormones (Estrogens) Involved in IBS

Recent studies highlight the importance of sex hormones, particularly estrogens, in the pathophysiology of irritable bowel syndrome (IBS). The authors begin with the observation that IBS is more common in women, suggesting a hormonal influence. Estrogens, via their receptors (ER α and ER β), participate in the regulation of intestinal motility, visceral sensitivity, and the local immune response. Dysregulation of this signaling leads to an immune imbalance characterized by an increase in pro-inflammatory

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cytokines and a decrease in anti-inflammatory cytokines, promoting low-grade inflammation in the intestinal mucosa. This mechanism contributes to increased intestinal permeability, activation of local immune cells (mast cells, lymphocytes), and enhanced interaction with the gut microbiota, which exacerbates digestive symptoms such as abdominal pain, diarrhea, or bloating. The article highlights that sex differences in IBS are not solely due to psychosocial factors, but also to specific biological mechanisms involving steroid hormones. In conclusion, it proposes that dysregulation of estrogen signaling is a key factor explaining women's increased susceptibility to IBS and paves the way for sex-specific therapeutic approaches (Choghakhoria et al., 2021).

Table 03: Impact of estrogen signaling dysregulation on the pathophysiology of irritable bowel syndrome (Choghakhoria et al., 2021).

Element	Normal role of estrogens	Effect in case of deregulation
Intestinal motility	Transit regulation	Disturbance →diarrhea /constipation
Visceral sensitivity	Pain modulation	Hypersensitivity → abdominal pain
Immune response	Pro/anti-inflammatory balance	low-grade inflammation
Intestinal barrier	Maintaining integrity	Increased permeability
Microbiota	Microbial balance	Dysbiosis worsening of symptoms

Irritable bowel syndrome (IBS) affects women more than men, with a prevalence approximately twice as high. Symptoms in women are often more intense (abdominal pain, bloating, alternating diarrhea and constipation) and strongly influenced by hormonal fluctuations (menstrual cycle, pregnancy, menopause). Stress and anxiety play a more pronounced aggravating role, and comorbidities such as fibromyalgia or migraines are more common. In men, IBS more often manifests as constipation, with a more consistent response to standard treatments (Heath et al., 2025).

Chapter 02: Therapeutic perspectives

Kefir is a highly popular and trendy fermented drink, renowned for its beneficial microorganisms and numerous health benefits. It includes milk kefir and water kefir, each of which is the subject of particular interest. Although milk kefir is better known, it is essential to fully understand both, their similarities and their differences. Their main health benefits, such as their probiotic properties, their protective effect on the gastrointestinal system, their role in regulating blood sugar levels, their antioxidant properties, antimutagenic and anticarcinogenic properties, their cholesterol-regulating and antimicrobial effects, as well as their ability to improve lactose digestion, are analysed and supported by recent in vivo and in vitro scientific data (**Lim et al., 2026**).

There are two types

1 Dairy kefir

For thousands of years, milk has been a staple of the human diet. To extend its shelf life, surplus milk has been subjected to fermentation processes designed to preserve its nutritional qualities (**Rattray et al., 2011**). Kefir, a traditional dairy drink, is produced by the fermentation of milk induced by the microbial diversity of kefir grains. This process, which generally takes place over more than 24 hours at room temperature, is carried out in various containers such as goatskins, wooden pots or earthenware jars (**Basaran et al., 2022**). Various types of ruminant milk (cow, sheep, goat, buffalo or camel) can serve as a substrate for this fermentation (**Peluzio et al., 2021**). Kefir production relies either on a traditional method or on an industrial process involving the inoculation of kefir grains into the milk, thereby ensuring the standardisation and reproducibility of the product (**Nejati et al., 2020**). The study of this complex process contributes to the elucidation of the microbiological dynamics of kefir and highlights its potential applications in promoting digestive health and general well-being

1.2 No-dairy kefir (water kefir)

Water kefir, also called sweet kefir or tibico (or tepache de tibico), has been gaining increasing interest in recent years. It is an excellent alternative for vegans and people with allergies or intolerances to dairy products, thanks to its richness in probiotics, prebiotics, and antioxidants (**Peluzio et al., 2021; Egea et al., 2022**). Its fermentation relies on water kefir grains cultivated in a sweetened aqueous medium, primarily based on brown sugar, supplemented with various substrates such as fruit juices (grape, pomegranate, apple, pineapple, melon), vegetables (onion, ginger, soy, carrot), or sweeteners (molasses, honey,

cane sugar) (**Kesenkaş et al., 2017; Guzel-Seydim et al., 2021**). This variability offers a wide range of variations, meeting the needs of consumers who exclude dairy products, particularly vegans (**Tzavaras et al., 2022**).

The fermentation process favors substrates rich in easily fermentable carbohydrates (glucose, fructose, sucrose), homogenized with grain biomass at approximately 25°C for 24 hours (**Corona et al., 2016**). To obtain a sparkling beverage, natural carbonation is added (**Destro et al., 2019**).

1.3 Kefir grains

Kefir grains cannot be obtained simply by combining pure cultures of their constituent microorganisms. However, studies have demonstrated the possibility of recreating their formation by reproducing the traditional milk fermentation process in bags made from animal hides (goat or sheep) (**Nielsen et al., 2014; Bourrie et al., 2016**).

This process involves inoculating intestinal microorganisms into pasteurized cow's milk placed in a goatskin bag, followed by agitated incubation for 48 hours at 24–26 °C. Subsequently, 75% of the coagulated milk is replaced with fresh milk every 12 hours. A water-insoluble gelatinous matrix then develops inside the pouch and spreads into the milk, forming a morphological and functional structure analogous to traditional kefir grains (**Nielsen et al., 2014; Kesenkas et al., 2017**).



Figure 04: Different methods of fermentation of non-dairy and dairy kefir (Azizi et al., 2021).

2. Nutritional Composition of Kefir

The nutritional composition of kefir varies depending on the milk composition, the starter culture used, the fermentation temperature, the fermentation time, and the storage conditions

Kefir is a good source of minerals such as calcium, phosphorus, magnesium, and potassium, and of B vitamins

Table 4: “Biochemical composition and nutritional characteristics of kefir” (Athira et al., 2026).

Components	Characteristic	Notes
Water	80-90 %	Main component
Proteins	2,7-3,5%	More digestible thanks to microbial proteolysis
Fats	2-3%	Depends on the type of milk used
Carbohydrates	11,6g/240 ml	Partially hydrolyzed lactose
Amino acids	Lysine; glutamic acid, phenylalanine	Improved profile compared to milk
Vitamins	B1, B2, B5, B12, C, A, K	Microbial synthesis during fermentation
Minerals	Calcium, phosphorus, magnesium	Trace elements: Zn, Cu, Mo
Fermentation products	Lactic acid, acetic acid, ethanol,	Responsible for aroma and taste
Probiotics	Lactobacillus kefiranofaciens lactococcus lactis	Beneficial for intestinal health
ph	4,2-4,6	Typical acidity of kefir

In addition, kefir contains small amounts of alcohol (0.5–2%) and lactic acid (0.8–1.0%). Lactic acid is formed during the fermentation phase. During this phase, 30% of the lactose in milk is hydrolyzed by the bacterial enzyme galactosidase and converted into glucose and galactose. The glucose produced is then converted into lactic acid by the kefir bacteria (Rosa et al., 2017).

According to the Turkish Codex Alimentarius Commission's notification on fermented dairy products, kefir must contain at least 2.7% protein and 0.6% milk solids, and the fat content must not exceed 10%. There is no specific regulation regarding the alcohol content (Koyu and Demirel, 2018).

3. Probiotics and Digestive Tract Protection

Regular kefir consumption can improve the composition of the gut microbiota and the intestinal lumen immune response (Rosa et al., 2017). The luminal surface of the digestive tract is colonised by a wide diversity of microbial communities, which regulate processes important for human health (Bellikci-Koyu et al., 2019; Kim et al., 2019). Milk

kefir, rich in probiotics, can combat pathogenic bacteria by binding to the epithelial cells of the digestive tract and releasing bacteriocins and acids that inhibit pathogen proliferation in the host's gut. It may also promote intestinal transit and the elimination of pathogenic bacteria, thus contributing to improved gut microbiota balance and proper digestive system function through colonisation by beneficial microbial populations **(Bellikci-Koyu et al., 2019; Garrote et al., 2010; Kim et al., 2019; Leite et al., 2013)**. A recent study **(Dahiya et al., 2023)** explained that milk kefir consumption can contribute to restoring the gut microbiota and mitigating antibiotic-induced intestinal dysbiosis, an imbalance of the gut microbiota responsible for adverse effects such as weight fluctuations, blood sugar spikes, and diarrhoea. Another study suggests that milk kefir may stimulate the secretion of immunoglobulin A (IgA) in the digestive system. IgA antibodies can strengthen immune defenses by neutralising pathogens and limiting their adhesion to epithelial cells of the gastrointestinal tract. This mechanism could potentially minimize gastrointestinal infections by inhibiting colonisation by pathogens **(Salari et al., 2022)**.

Water kefir, rich in probiotics, may contribute to improving gastrointestinal homeostasis and promoting gut health **(Hamida et al., 2021)**. Regular consumption of water kefir has demonstrated high efficacy in supporting intestinal homeostasis and modulating the immune system through the production of short-chain fatty acids, including acetate, butyrate, and propionate, from fermentation **(Calatayud et al., 2021)**.

4. Antioxidant Properties of Kefir

Kefir is recognised as a recommended food for mitigating oxidative stress and the damage it causes, thanks to its beneficial microorganisms and fermentation metabolites, which act as excellent free radical scavengers **(Fiorda et al., 2017)**. Kefir fermentation leads to the formation of antioxidants, including peptides from milk casein hydrolysate, amino acids such as tryptophan, cysteine, taurine, and tyrosine, vitamins A and E, carotenoids, superoxide dismutase, glutathione peroxidase, and catalase. These antioxidants can help to inhibit lipid degradation, muscle contraction and relaxation processes, cholesterol metabolism, and protein oxidation **(Chen et al., 2015; Dufresne & Farnworth, 2000; Liu et al., 2005; Ozcan et al., 2018)**. Several researchers have demonstrated increased antioxidant activity during milk fermentation **(Grishina et al., 2011; Satir & Guzel-Seydim, 2015; Yilmaz-Ersan et al., 2016)**.

The results showed that milk kefir contained approximately three times more phenolic compounds than unfermented milk. Their findings suggest that proteolysis induced by the microflora within targeted protein fractions triggered the release of amino acids (Liu et al., 2005 ;Yilmaz-Ersan et al., 2016).

Water kefir also exhibits an excellent antioxidant effect (Kumar et al., 2021) and highlighted the strong neuroprotective and antioxidant capacity of water kefir against hydrogen peroxide-induced oxidative stress in neuroblastoma (SH-SY5Y) cells, a model frequently used to study neurodegenerative diseases. These effects are mediated by the enhancement of superoxide dismutase and catalase activity and the regulation of apoptosis genes, thus helping to prevent oxidative stress. The antioxidant power of water kefir fermented in kiwi and pomegranate juice is attributed to a wide range of polyphenols, such as ellagic acid, anthocyanins and punicalagin isomers, which act as oxygen scavengers and prevent oxidative damage in cells (Randazzo et al., 2016).

5. Anti-inflammatory Properties of Kefir

Globally, the consequences of chronic neurological and inflammatory disorders are a leading cause of morbidity and mortality. In recent years, numerous ex vivo studies have confirmed the undeniable anti-inflammatory and immunomodulatory properties of kefir (Pereira et al., 2024; Ye et al., 2023). This therapeutic approach enhances anti-inflammatory agents while inhibiting pro-inflammatory cytokines. These results suggest the potential of kefir to mitigate the adverse effects associated with chronic neurological and inflammatory conditions (Lai et al., 2019; Ekici et al., 2021).

Further studies have revealed that isolated fractions and organisms of kefir promote the Th2 anti-inflammatory response while inhibiting the Th1 pro-inflammatory response (Pimenta et al., 2018). Kefir consumption may reduce blood glucose and improve the balance between pro- and anti-inflammatory cytokines, as indicated by the modulation of interleukin-1 (IL-1), IL-6 and TNF/IL-10 ratio levels (Hadisaputro et al., 2012).

In a recent study, the anti-inflammatory properties of new kefir exopolysaccharides (KEPS) and kefiran (KE) were evaluated in vitro and demonstrated a remarkable ability to attenuate lipopolysaccharide-induced IL-6 secretion. The impact of KEPS and KE was investigated by oral administration for one week to NF- κ B-luciferase transgenic mice (male and female) subjected to systemic injury. KE and KEPS were observed to strongly inhibit the expression of inflammatory signaling molecules (IL-6 and phosphorylated

MAPK). These compelling findings highlight the potential therapeutic efficacy of KEPS in attenuating inflammation by inhibiting NF- κ B/MAPK signaling pathways, thus suggesting a promising avenue for their use in the treatment of inflammatory diseases (Liao et al., 2023).

Similarly, a study was conducted to evaluate the anti-inflammatory properties of fermented kefir in a murine model of dextran sulfate sodium (DSS)-induced colitis. Kefir supplementation was reported to have a mitigating effect on the inflammatory cascade, as evidenced by a reduction in reticular edema, neutrophil accumulation, and an increase in autophagosome numbers. Furthermore, it increased acetate and propionate levels, thereby reducing intestinal damage associated with DSS-induced colitis. It was concluded that kefir may also promote the restoration of the epithelial barrier, thus contributing to the anti-inflammatory effect (Nascimento da Silva et al., 2023).

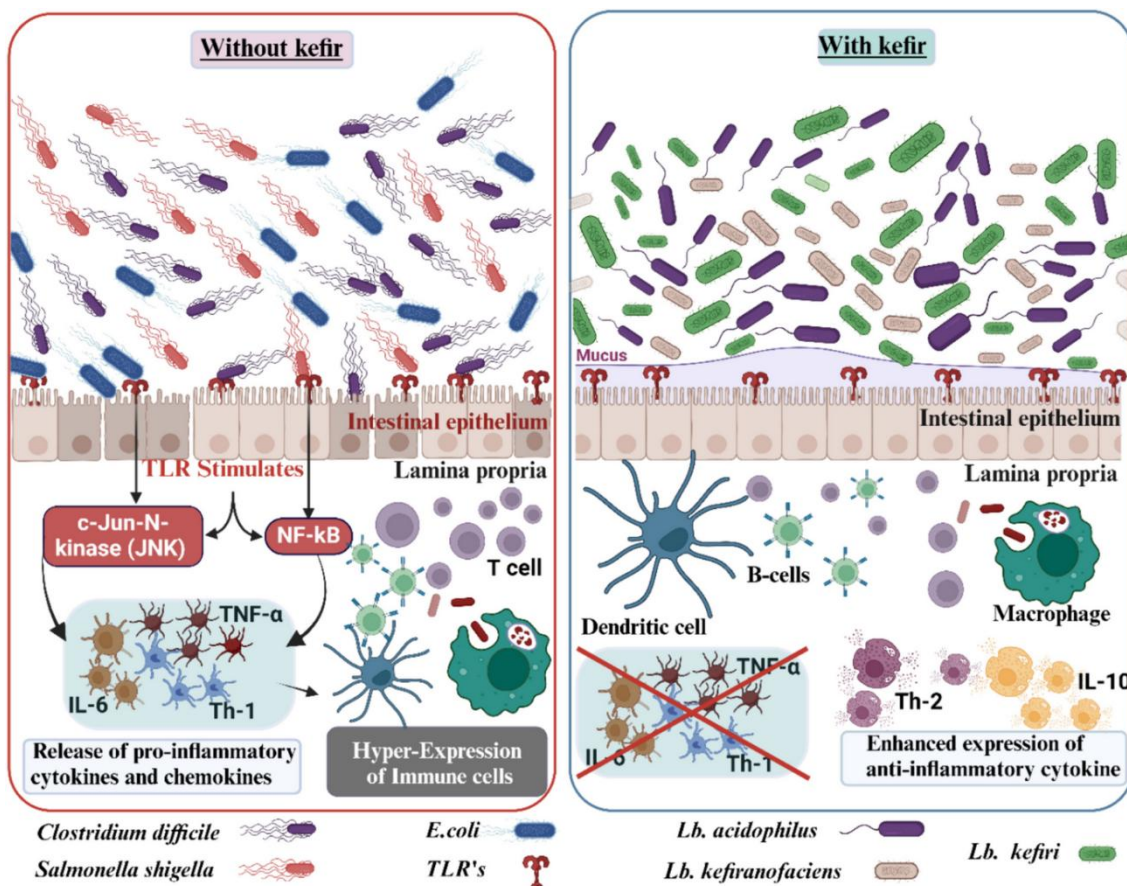


Figure 05: Kefir extract: a fermented plethora of symbiotic microbiota and health benefits (Jagan Mohan Rao Tingirikari et al., 2024).

The anti-inflammatory properties of kefir are explained by the increase of anti-inflammatory cytokines and the suppression of pro-inflammatory cytokines via an

interaction with TLR receptors present in the intestinal epithelium. TLR: Toll-like receptor; IL-6: interleukin-6; TNF- α : tumor necrosis factor alpha; Th-2: type 2 helper T cells; NF- κ B: nuclear factor kappa B; IL-10: interleukin-10.

6. Immunomodulatory and Antitumor Properties

According to (Yegin et al.,2022), the antitumor activity of kefir is optimised when fermentation is carried out over a period of 24 to 48 hours. In vitro studies have shown that kefir inhibits the proliferation of human breast cancer cells in a dose-dependent manner, with a reduction of up to 56% of tumor cells at a concentration of 2.5%. Furthermore, water kefir has demonstrated antimetastatic and antiangiogenic properties in murine models of breast cancer.

However, (Can et al.,2009) did not observe a significant effect of kefir on the efficacy of chemotherapy or on the quality of life of patients, but did note an improvement in sleep quality in adults undergoing chemotherapy.

Finally,(Peluzio et al.,2021) conducted a study on 28 overweight adults, demonstrating that kefir consumption altered serum zonulin concentration. This protein plays a key role in maintaining the integrity of tight junctions in the intestinal epithelium, thereby regulating paracellular permeability. Improved regulation of these junctions could help limit inflammatory processes and reduce the risk of associated pathologies.

7. Antimicrobial Properties of Kefir

Kefir-based beverages, produced by fermenting grains, have infection prevention potential due to a bactericidal effect on the gastrointestinal tract. This effect is primarily linked to the production of bioactive metabolites and peptides during fermentation, in conjunction with an acidic pH (Al-Mohammadi et al., 2021).

The antimicrobial activity of kefir relies on the presence of lactic acid bacteria (LAB), which compete with pathogenic microorganisms for nutrients. Furthermore, fermentation promotes the synthesis of organic acids (particularly lactic and acetic acids), carbon dioxide, acetaldehyde, bacteriocins, cathelicidin, and hydrogen peroxide. These compounds exert an inhibitory effect on a broad spectrum of pathogens (Abadi et al., 2023).

Finally, the proteolytic degradation of milk proteins in kefir also contributes to the antimicrobial effect, by generating peptides and other bioactive molecules capable of neutralising certain pathogenic bacterial strains (**Lima et al., 2018**).

In summary, kefir, a traditional fermented drink made from milk or plant-based substrates, is generating increasing interest as a functional food due to its rich microbial diversity and bioactive metabolites. Numerous studies have highlighted its antimicrobial, anti-inflammatory, anticancer, antioxidant, and antidiabetic properties, attributed in particular to kefir peptides, its immune and cytokine modulation capabilities, and its free radical scavenging action. Plant-based kefir offers a suitable alternative for people with lactose intolerance or those following a vegan diet. However, scientific gaps remain regarding the understanding of the symbiotic interactions between microorganisms and the kefir matrix, as well as the influence of microbial diversity on quality and functional properties. Future research should focus on optimising production parameters and conducting clinical trials to confirm its benefits and develop specific cultures capable of producing metabolites targeted to particular pathologies. Thus, kefir appears as a promising functional drink , but one that still requires in-depth investigations to fully exploit its therapeutic potential (**Jagan Mohan Rao Tingirikari et al., 2024**).

Materials and methodes

Objective

The main aim of our work is to investigate the relationship between irritable bowel syndrome, oxidative stress and immune inflammatory processes, whilst assessing the potential therapeutic effect of kefir in patients with this condition.

In this context, various anthropometric and clinical data are collected and analysed from patients, including age, weight, height and body mass index (BMI). Furthermore, information regarding dietary habits, eating patterns, family history and certain lifestyle factors is also taken into account.

The aim is thus to better understand the role of oxidative stress and the inflammatory immune response in the pathophysiology of irritable bowel syndrome, and to explore the potential of kefir as a complementary nutritional and therapeutic approach likely to improve patients' gut health and quality of life.

1. Type of study

This is a study that is both experimental and observational, aimed at assessing the relationship between irritable bowel syndrome, oxidative stress and immune inflammation, as well as the potential effect of kefir as a complementary therapeutic approach..

2. Study location

This study is being conducted at the PPABIONUT laboratory of the Faculty of Natural and Life Sciences (SNV/STU) at the University of Tlemcen, in collaboration with a hospital chu responsible for recruiting patients with irritable bowel syndrome

3. Study Population and Group Composition

The study population consists of patients with irritable bowel syndrome (IBS), recruited from the hospital setting.

Participants will be divided into 2 groups:

- Group 1 : patients with irritable bowel syndrome before consuming kefir
- Group 2: patients with irritable bowel syndrome after consuming kefir

Materials and methodes

For each participant, several anthropometric and clinical parameters are recorded, including:

Age,

Weight and height,

Body mass index (BMI),

Dietary habits and rhythm,

Family history and certain lifestyle factors.

Blood samples are taken to analyse various biological markers associated with oxidative stress and the inflammatory response. The parameters studied include malondialdehyde (MDA), reduced glutathione (GSH) and catalase activity, as well as levels of vitamin D, calcium and C-reactive protein (CRP). In addition, kidney function is assessed by measuring urea and creatinine levels.

The questionnaires, created using Google Forms and sent electronically to the twenty participants (see Appendices), were developed as part of the study entitled ‘Assessment of the effect of kefir consumption on digestive health and oxidative stress’. They were used to collect sociodemographic data (age, gender, weight, height, BMI, place of residence, occupation), as well as information on medical and family history and lifestyle habits (diet, physical activity, sleep, tobacco and alcohol consumption). The questionnaire also explores the presence and frequency of digestive disorders (abdominal pain, constipation, diarrhoea, bloating, postprandial discomfort), perceived stress levels and sleep quality. Finally, a section is devoted to knowledge of and consumption of kefir, including frequency, duration and perceived effects on digestion and symptoms.

3.1. MDA Assay

Plasma and erythrocyte malondialdehyde (MDA) is determined according to the method described by Draper and Hadley (1990), based on the reaction with thiobarbituric acid (TBA). This method is widely used for lipid peroxidation assessment due to its simplicity and high sensitivity.

After acid treatment and heating, the aldehydes produced by lipid peroxidation react with thiobarbituric acid to form a chromogenic MDA-TBA complex, composed of two TBA molecules and one MDA molecule. The absorbance of this complex is measured spectrophotometrically at 532 nm.

The MDA concentration is then calculated using the molar extinction coefficient of the MDA-TBA complex ($\epsilon = 1.56 \times 10^5 \text{ mol}^{-1} \cdot \text{L} \cdot \text{cm}^{-1}$ at 532 nm).

3.2. Determination of Reduced Glutathione

The measurement of reduced glutathione (GSH) in erythrocytes and plasma is performed using the colorimetric method with Ellman's reagent (DTNB) (Ellman, 1959). The reaction involves the cleavage of the 5,5-dithiodis-2-nitrobenzoic acid (DTNB) molecule by GSH, which releases thionitrobenzoic acid (TNB) according to the following reaction:

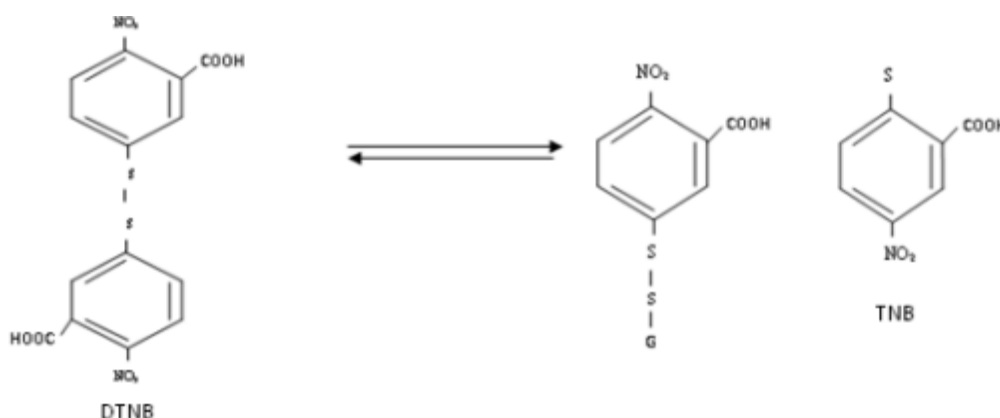


Figure 06: At an alkaline pH (8–9), thionitrobenzoic acid (TNB) exhibits an absorbance at 412 nm with an extinction coefficient of $13.6 \text{ mM}^{-1} \text{ cm}^{-1}$.

3.3. Determination of Catalase Activity

This enzymatic activity is measured by spectrophotometric analysis of the hydrogen peroxide decomposition rate (AEBI, 1974). In the presence of catalase, the decomposition of hydrogen peroxide leads to a decrease in the absorption of the H_2O_2 solution over time. The reaction medium contains 1 mL of supernatant (1/50 diluted lysate or plasma), 1 mL of H_2O_2 , and 1 mL of phosphate buffer (50 mmol/L, pH 7.0). After 5 min incubation, 1 mL of titanium dioxide sulfate (TiOSO_4) reagent (1.7 g in 500 mL of 2N H_2SO_4) is added. The reading is taken at 420 nm. The concentrations of the remaining H_2O_2 are determined from a standard range of H_2O_2 with phosphate buffer and TiOSO_4 reagent in order to obtain concentrations of 0.5 to 2 mmol/l in the reaction medium.

The calculation of one unit of enzyme activity is:

$$A = \log A_1 - \log A_2$$

A_1 is the initial H_2O_2 concentration

A_2 is the H_2O_2 concentration after incubation (after 5 min)

Specific activity is expressed in U/g Hb or U/ml.

3.4. C-Reactive Protein (CRP)

Is a biomarker of systemic inflammation, widely used in clinical practice to assess immune activity. In the context of irritable bowel syndrome (IBS), several studies have highlighted slightly elevated CRP levels compared to healthy controls, particularly in diarrhoeal and severe forms. Although these values generally remain below pathological thresholds and within the limits of biological normality, they nevertheless reflect low-grade inflammation. This subtle elevation suggests that the pathophysiology of IBS is not limited to a purely functional disorder, but includes a measurable immune dimension. Thus, the integration of CRP into the clinical and scientific analysis of IBS strengthens the hypothesis of subtle inflammatory activation, contributing to the understanding of the underlying mechanisms and the stratification of patients according to their inflammatory profile.

3.5. Creatinine Assay

Kinetic determination of creatine kinase following the recommendations of the IFCC and DGKC. Creatine kinase (CK) catalyses the reversible transfer of a phosphate group from phosphocreatine to ADP. This reaction is in addition to others catalysed by hexokinase (HK) and glucose-6-phosphate dehydrogenase (G6PDH). The rate of NADPH formation, determined by photometry, is proportional to the catalytic concentration of CK in the sample being tested.

3.6. Urea Assay

Urease catalyses the hemolysis of urea, present in the sample, into ammonia (NH₃) and carbon dioxide (CO₂). Ammonia ions react with salicylate and hypochlorite (ClO₂Na), in the presence of the catalyst nitroprussate, to form a green indophenol: The intensity of the color formed is proportional to the concentration of urea in the test; the decrease in the concentration of NAD⁺ in the method is proportional to the concentration of urea in the sample tested.

3.7. Vitamin D Assay

Vitamin D levels were measured by determining the concentration of 25-hydroxyvitamin D [25(OH)D] in serum, which is the best indicator of vitamin status.

After blood collection, samples were centrifuged at 3000 rpm for 10 minutes to collect the serum. This serum was then stored at -20°C until analysis.

The assay was performed using an enzyme-linked immunosorbent assay (ELISA), based on a specific antigen-antibody reaction.

The principle of the method relies on the binding of vitamin D present in the sample to specific antibodies fixed on an ELISA plate. After several washing steps to remove unbound substances, an enzyme conjugate was added. The enzymatic reaction produces a color whose intensity is proportional to the concentration of vitamin D in the sample.

Optical density is measured using a spectrophotometer at a wavelength of 450 nm. Vitamin D concentration is then determined from a calibration curve established with standards of known concentrations.

The results are expressed in ng/mL.

Statistical analysis

The results are presented as mean $\pm$ standard deviation. After verifying the normal distribution, the comparison of means between the two study groups (population with irritable bowel syndrome before and after kefir consumption) was performed using Student's t-test. Differences between means are significant when p is less than 0.05. All statistical calculations were performed using STATISTICA software.

Result and interpretation

Result and interpretation

Table 5: Characteristics of the studied population.

setting	Before consuming kéfir	After consuming kéfir
Number of patients (n)	30	30
Middle age (years)	39,8 ± 9,1	39,8 ± 9,1
IMC middle (kg/m ²)	27,4 ± 3,2	26,6 ± 3,0
Abdominal pain score (0–10)	7,2 ± 1,1	3,8 ± 1,0
Bloating (score 0–10)	7,8 ± 1,0	4,1 ± 1,2
Number of bowel movements / day	3,1 ± 1,2	1,9 ± 0,8
Stool consistency (Bristol score)	6,2 ± 0,7	4,3 ± 0,6
Perceived stress (score 0–10)	6,9 ± 1,3	4,0 ± 1,1
General fatigue (score 0–10)	6,4 ± 1,5	3,9 ± 1,2
Kefir consumption period	—	4 weeks

Each value represents the mean ± standard deviation. Comparison of means before and after kefir consumption.

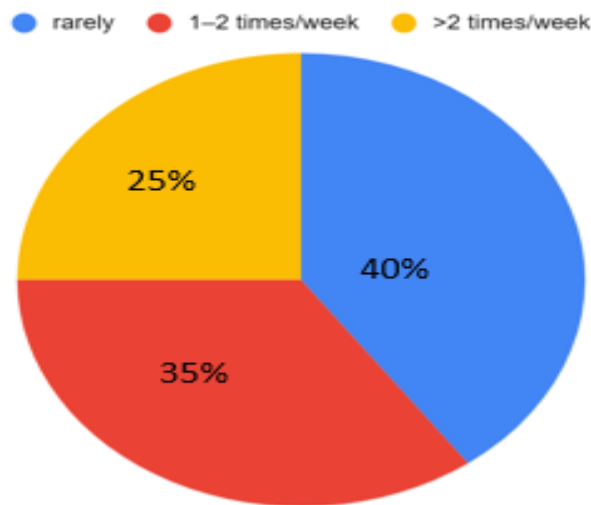
The study conducted on patients with irritable bowel syndrome who consumed kefir for four weeks highlights promising clinical results. The study population had a relatively homogeneous average age and a generally stable body mass index before and after the intervention.

Symptomatically, a notable improvement was observed after kefir consumption. The intensity of abdominal pain and bloating decreased significantly, while stool frequency and consistency gradually normalized. A significant reduction in the daily number of bowel movements was also noted.

Furthermore, scores for perceived stress and general fatigue showed a marked decrease after the intervention, reflecting an overall improvement in the patients' well-being and quality of life. Overall, patients who participated in the study showed a favorable clinical response, suggesting that kefir could have a beneficial and relevant effect in the management of irritable bowel syndrome.

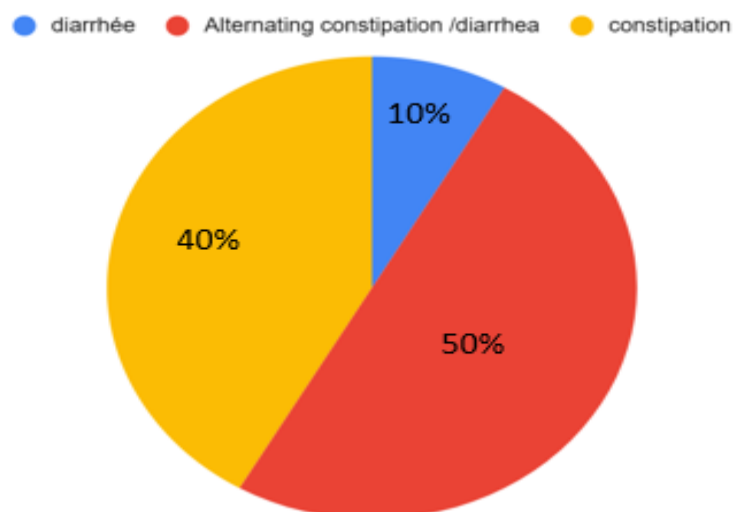
Result and interpretation

Figure 1: Frequency of abdominal pain among respondents



The diagram illustrates that all respondents experience abdominal pain to varying degrees: 40% rarely suffer from it, 35% once or twice a week, and 25% more than twice a week. From a scientific perspective, abdominal pain appears to be a key clinical indicator, reflecting both the chronicity of digestive disorders and their functional impact on quality of life.

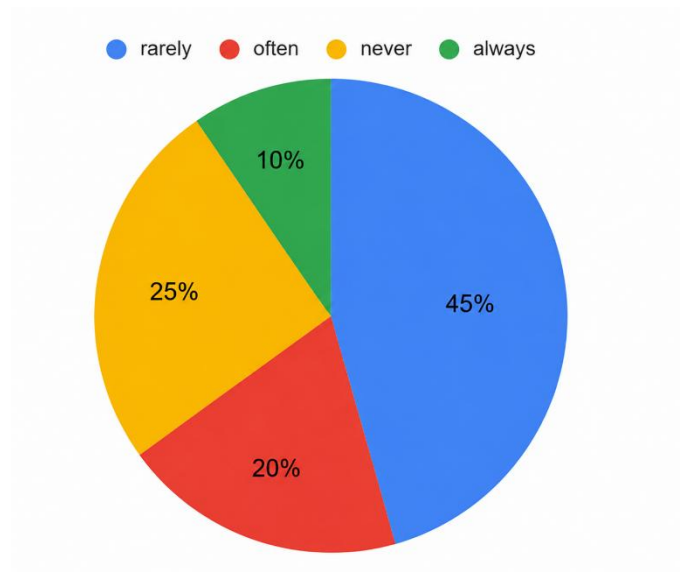
Figure 2: Distribution of respondents according to the type of digestive disorders



The diagram illustrates that all respondents present with types of digestive disorders to varying degrees: 50% suffer from alternating constipation/diarrhea, 40% have isolated constipation and only 10% report diarrhea

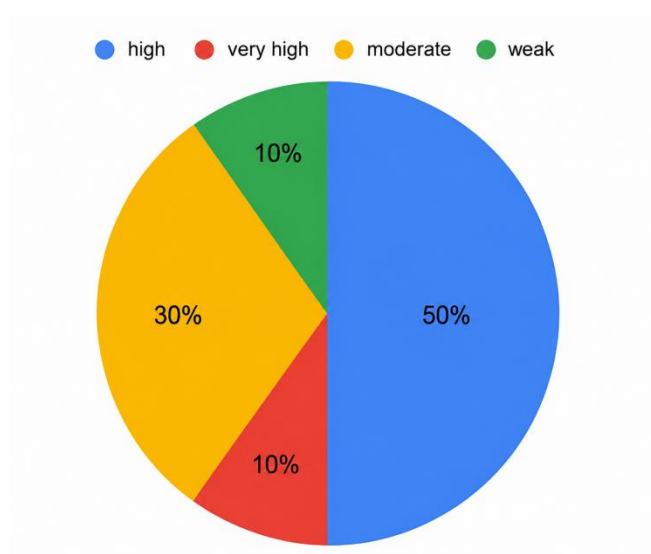
Result and interpretation

Figure 3: Frequency of postprandial discomfort among respondents



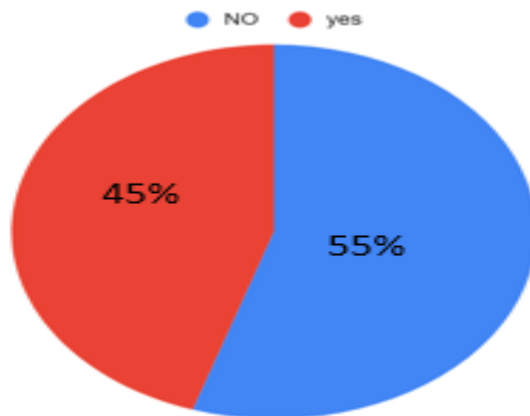
The diagram shows that of the 20 respondents, 45% reported experiencing discomfort after meals on a daily basis, while 20% experienced it often, 45% rarely, and 25% never. These results suggest that digestive discomfort after meals is a relevant clinical sign, which may be related to functional disorders (slow digestion, reflux, intestinal hypersensitivity) or specific dietary habits (consumption of fats, sugary or caffeinated drinks).

Figure 4: Descriptive study of self-assessed stress levels



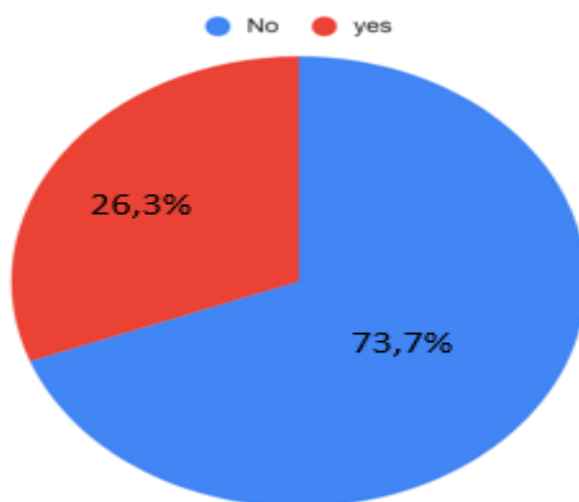
The diagram shows that of the 20 respondents, half (50%) report a high level of stress in their daily lives, while 30% describe it as moderate, 10% as low and 10% as very high.

Figure 5: Descriptive study of kefir awareness



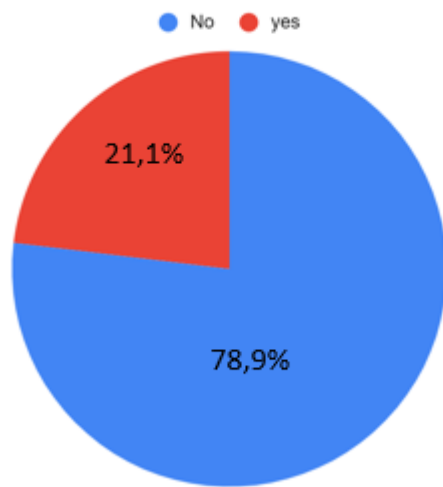
The figure shows that of the 20 respondents, 55% stated they were unfamiliar with kefir, while 45% had heard of it. This distribution highlights a relative lack of awareness of this fermented product within the sample. Despite its recognized role in digestive health due to its high probiotic content, these results suggest that kefir remains a food not widely integrated into local dietary habits.

Figure 6: Distribution of responses regarding the effects of kefir on health



The figure shows that of the 20 respondents, a large majority (73.7%) stated that they did not know the effects of kefir on health, while only 26.3% were aware of them.

Figure 7: Distribution of responses regarding kefir consumption



The figure shows that of the 20 respondents, a large majority (78.9%) had never consumed kefir, while only 21.1% had ever tasted it. This distribution highlights the low integration of this fermented product into their eating habits.

Plasma levels of malondialdehyde (MDA), reduced glutathione (GSH) and catalase in the studied population

Plasma levels of malondialdehyde (MDA), reduced glutathione (GSH), and catalase are shown in Figure 8,9,10 and . A highly significant decrease in malondialdehyde (MDA) activity was observed in the population after kefir consumption, along with a significant increase in glutathione (GSH) and catalase levels.

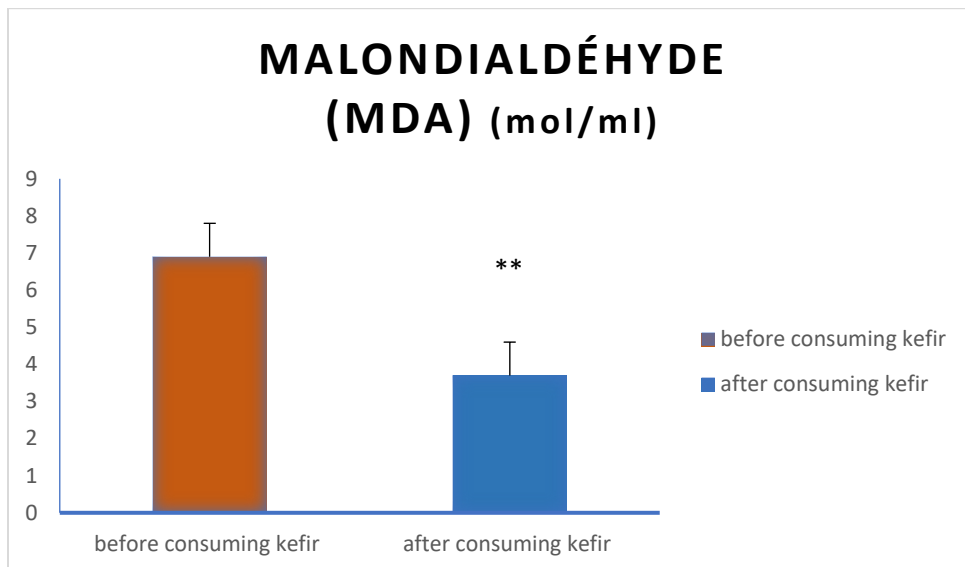


Figure 8: Plasma levels of malondialdehyde (MDA) levels

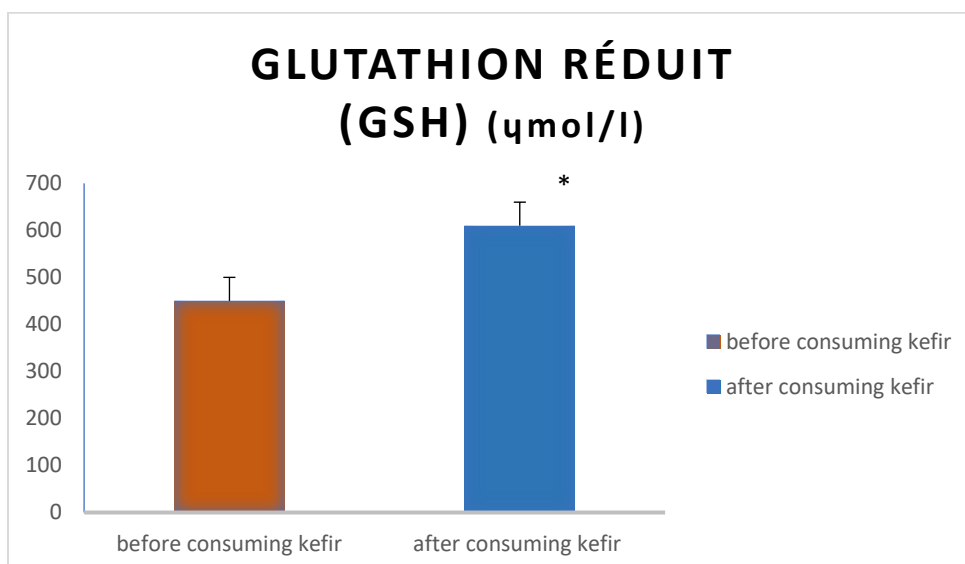


Figure 9: Reduced glutathione (GSH) levels

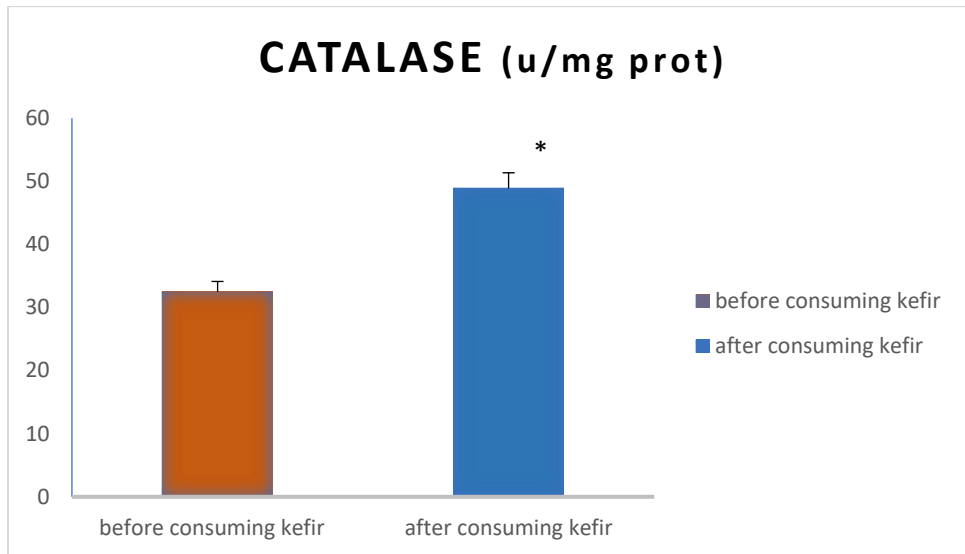


Figure 10: Plasma catalase levels

malondialdehyde (MDA), reduced glutathione (GSH), and catalase. Each value represents the mean $\pm$ standard deviation. Means were compared using Student's t-test. * $P < 0.01$.

Plasma levels of vitamin D, CRP, urea and creatinine in the study population

Plasma levels of vitamin D, CRP, urea, and creatinine are presented in Figure 11,12,13,14 and . A significant decrease in CRP was observed after kefir consumption, while creatinine and urea levels were not significantly decreased, and vitamin D levels were not significantly increased after kefir consumption in the studied population.

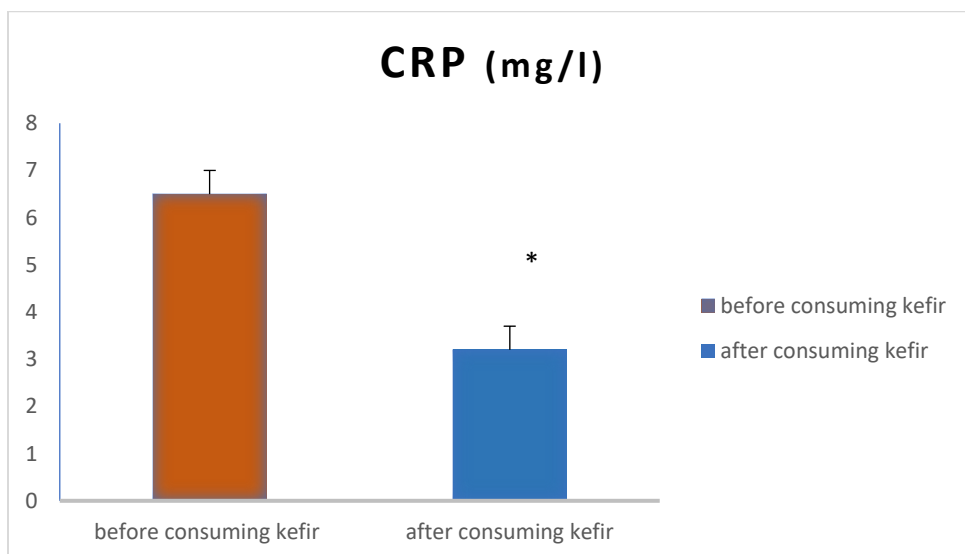


Figure 11 : Plasma CRP levels

Result and interpretation

The graph shows a significant decrease in CRP levels after kefir consumption. Before the intervention, the average concentration was approximately 6.5 mg/L, while after supplementation, it dropped to nearly 3.2 mg/L. This reduction reflects a decrease in systemic inflammation, indicating a notable anti-inflammatory effect of kefir. These results suggest an improvement in the participants' inflammatory and metabolic profile, confirming the potential role of kefir in modulating the immune response and reducing oxidative stress.

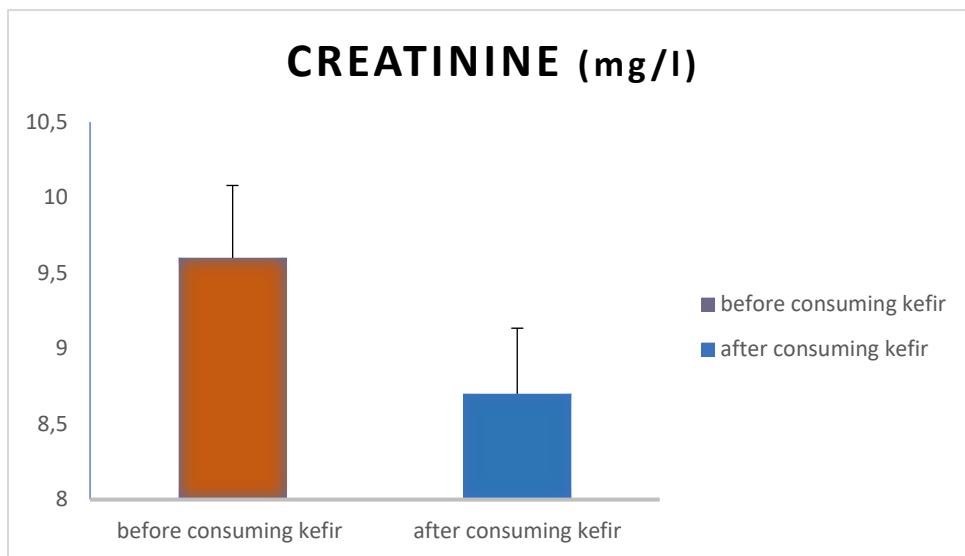


Figure 12: Plasma creatinine levels

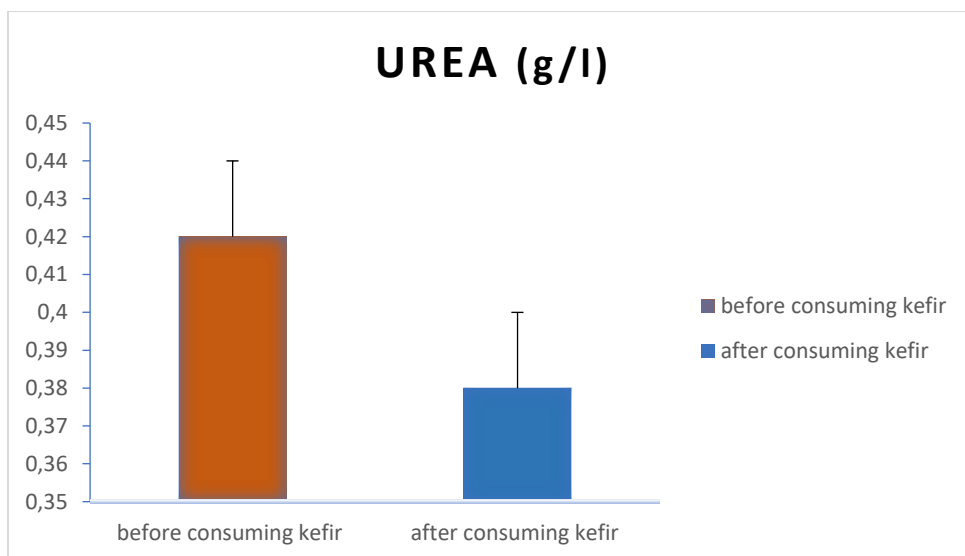


Figure 13: Plasma urea levels

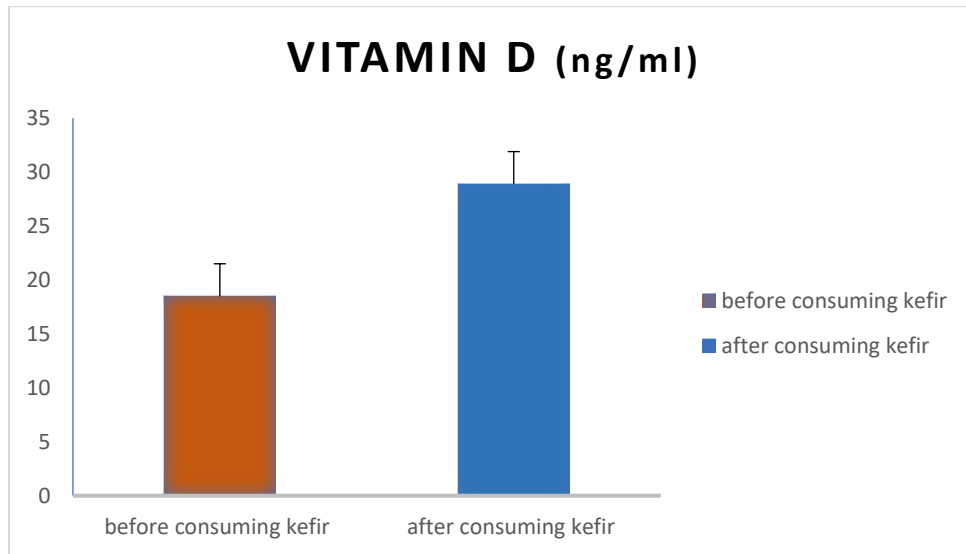


Figure 14: Plasma vitamin D levels

The graph highlights a non-significant increase in vitamin D concentrations after kefir consumption. Before the intervention, the average value was around 18.5 ng/mL, while after supplementation, it reached approximately 28.9 ng/mL, representing a substantial increase. This change reflects a notable improvement in the participants' micronutritional status and suggests the effectiveness of kefir in replenishing vitamin D stores.

CRP, creatinine, urea, and vitamin D: Each value represents the mean $\pm$ standard deviation. Means were compared using Student's t-test. * $P < 0.01$.

Discussion

Discussion:

Irritable bowel syndrome (IBS) is the most prevalent chronic functional disorder of the gastrointestinal system. It is characterized by altered intestinal motility, abdominal bloating, and visceral pain. Research conducted over the past few decades has highlighted dysbiosis of the gut microbiota in patients with IBS, suggesting a role for this disruption in the cause and maintenance of the symptoms observed in these patients **(Bourrie et al., 2016)**.

Several hypotheses have been proposed to explain the complex etiology of this disease, including alterations in the gut microbiota, low-grade inflammation, genetic predisposition, diet, and environment. A response to a triggering event on the mucosal surface stimulates communication through multiple pathways, which can lead to the development of visceral hypersensitivity and alterations in stool frequency and form **(Stern and Brenner, 2018)**.

Our study, conducted on patients with irritable bowel syndrome (IBS) after four weeks of kefir consumption, demonstrated favorable clinical effects. The results showed a significant improvement in digestive symptoms, characterized by a decrease in abdominal pain, bloating, and the number of daily bowel movements, with a progressive normalization of their frequency and consistency. Furthermore, a notable reduction in perceived stress and general fatigue was also observed, reflecting an improvement in the patients' well-being and quality of life. These results suggest that kefir could represent a promising complementary nutritional approach in the management of IBS.

Kefir is a fermented beverage known for its richness in probiotics that live in symbiosis with various microorganisms present in the kefir grains. Its consumption is associated with numerous beneficial health effects, including anti-inflammatory, antioxidant, antimicrobial, anticancer, antidiabetic, antihypertensive and cholesterol-lowering properties **(Nor et al., 2021)**

The results of this study highlight the high prevalence of digestive disorders among participants with irritable bowel syndrome, including abdominal pain and bowel movement irregularities, primarily characterized by alternating constipation and diarrhea. A significant proportion of participants also experienced frequent digestive discomfort after meals, reflecting intestinal hypersensitivity potentially influenced by dietary habits.

Discussion:

Furthermore, high stress levels were observed in the majority of respondents, confirming the major role of the gut-brain axis in exacerbating digestive symptoms and impairing patients' quality of life. The study also reveals limited knowledge and consumption of kefir among the studied population. The majority of participants are unaware of its potential effects on digestive health and do not consume it regularly. These results highlight the need to raise nutritional awareness about probiotic fermented foods, particularly kefir, which could represent a valuable complementary approach in the management of irritable bowel syndrome due to its potential beneficial effects on the gut microbiota, inflammation, and digestive comfort. Studies conducted by several researchers, including (Grishina et al., 2011) et (Yilmaz-Ersan et al., 2016), have shown that milk kefir has a phenolic compound content approximately three times higher than that of unfermented milk. Researchers attributed this increase to the fermentation process and the proteolytic activity of the lactic acid bacteria and yeasts present in the kefir grains, which promotes the release of bioactive peptides and amino acids with antioxidant activity.

Furthermore, (Liu et al ; Güven et al., 2005) et (Yilmaz-Ersan et al. 2016), evaluated the antioxidant activity of kefir using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, which measures the ability of compounds to neutralize free radicals. The results revealed a significant increase in antioxidant capacity during fermentation, with this activity maintained for at least three weeks of storage. These observations confirm that kefir is an important source of bioactive compounds that can contribute to reducing oxidative stress.

The results obtained highlight a significant beneficial effect of kefir on oxidative stress markers and antioxidant defenses in the participants. A substantial decrease in MDA concentrations was observed after the intervention, from approximately 6.9 nmol/mL before kefir consumption to nearly 3.7 nmol/mL after supplementation. This reduction reflects a mitigation of lipid peroxidation and suggests an improvement in cellular redox balance as well as better protection against oxidative damage.

Another study conducted by (Erdogan et al.,2019) evaluated the effect of natural kefir grains on the gut microbiota and their antioxidant potential in BALB/c mice. Biochemical analyses revealed a significant increase in total antioxidant status (TAS) in animals receiving a kefir-enriched diet. Furthermore, the low concentrations of

Discussion:

malondialdehyde (MDA), a marker of lipid peroxidation, revealed a reduction in oxidative stress, suggesting a protective and probiotic effect of kefir on the gut microbiota. The researchers concluded that kefir consumption improves the body's antioxidant defenses, thus confirming the significant antioxidant potential associated with this kefir formulation.

Concurrently, a significant increase in reduced glutathione (GSH) levels was recorded, with average concentrations rising from approximately 450 $\mu\text{mol/L}$ to 610 $\mu\text{mol/L}$ after the intervention, reflecting enhanced cellular antioxidant capacity and a restoration of defense mechanisms against free radicals. Furthermore, catalase concentrations showed a notable increase, rising from approximately 32.5 U/mg protein to 48.9 U/mg protein after kefir consumption, indicating improved activity of antioxidant enzyme systems. **(Ghoneum et al. ,2020)** conducted an experimental study to evaluate the effect of a kefir derivative on oxidative stress in aged mice. The animals received oral PFT supplementation for six weeks, and several biomarkers of oxidative stress were then analyzed in blood and tissues. The results showed a significant decrease in malondialdehyde (MDA), associated with an increase in reduced glutathione (GSH) levels and the activity of antioxidant enzymes, including catalase (CAT). The authors concluded that kefir exerts a protective effect against oxidative stress by enhancing cellular antioxidant defenses and reducing oxidative damage. Overall, these results suggest that kefir exerts a protective effect against oxidative stress due to its antioxidant and probiotic properties, supporting its potential as a complementary nutritional approach in the management of irritable bowel syndrome.

A significant decrease in C-reactive protein (CRP) levels was observed, with the average concentration dropping from approximately 6.5 mg/L before the intervention to nearly 3.2 mg/L after supplementation, reflecting a reduction in systemic inflammation and suggesting a notable anti-inflammatory effect of kefir. These results are consistent with several studies demonstrating the existence of low-grade chronic inflammation in patients with IBS. Indeed, **(Rodil Roth et al.,2024)** showed that IBS patients had significantly higher CRP concentrations than healthy control subjects, and that 18.1% of patients had values above the normal range. This increase in CRP is thought to be linked to persistent immune activation, intestinal dysbiosis, and altered intestinal permeability, which facilitates the passage of pro-inflammatory substances into the systemic circulation. Furthermore, several studies suggest that probiotics and fermented foods such as kefir can

Discussion:

reduce inflammatory markers through their modulating action on the gut microbiota and intestinal permeability.

Moreover, creatinine concentrations decreased from 9.6 mg/L to 8.7 mg/L. The results of this study are consistent with those reported by **(Hande Bakihan et al.,2026)**, who observed a decrease in serum creatinine concentrations after regular kefir consumption in subjects with gastrointestinal disorders. Similarly, **(Firouzi et al.,2018)** showed that probiotics and fermented foods contributed to the improvement of certain renal and metabolic parameters. The decrease in creatinine could be explained by an improvement in the participants' hydration status, a reduction in oxidative stress, and a beneficial modulation of the gut microbiota induced by kefir. Furthermore, the bioactive compounds in kefir possess antioxidant properties that may protect tissues against oxidative damage associated with chronic inflammation.

While urea levels have fallen from approximately 0.42 g/L to 0.38 g/L after the intervention. These changes indicate improved protein metabolism as well as enhanced renal function and nitrogenous waste elimination. These results are comparable to those reported by **(Zeki Erol et al.,2023)**, who demonstrated a reduction in blood urea nitrogen (BUN) and urea after kefir administration in an experimental model of irritable bowel syndrome. **(Firouzi et al.,2018)** also reported improved nitrogen metabolism parameters after probiotic supplementation.

This decrease in urea could be linked to the beneficial effect of kefir on the gut microbiota and its ability to reduce the production of toxic substances resulting from protein fermentation. The probiotics in kefir would thus contribute to improving intestinal metabolism and the elimination of nitrogenous waste.

In parallel, a significant increase in vitamin D concentrations was recorded, with average values rising from approximately 18.5 ng/mL to 28.9 ng/mL after kefir consumption, reflecting an improvement in the participants' micronutritional status. Overall, these results suggest that kefir could exert beneficial anti-inflammatory, metabolic, and nutritional effects thanks to its probiotic and bioactive properties, thus reinforcing its potential as a complementary nutritional approach in the management of irritable bowel syndrome and the improvement of patients' overall health.

Discussion:

These results are particularly important because several studies have shown that vitamin D deficiency is common in patients with IBS. **(Huang et al.,2022)**, in a meta-analysis published in the *Nutrition Journal*, showed that vitamin D supplementation significantly improved IBS symptoms as well as patients' quality of life. **(Yang Bin et al.,2022)** also reported that serum vitamin D concentrations were significantly lower in IBS patients compared to healthy subjects. The increase in vitamin D concentrations observed in this study could be related to improved intestinal absorption secondary to the restoration of gut microbiota balance induced by kefir. Indeed, probiotics can improve the integrity of the intestinal mucosa and promote micronutrient absorption.

General Conclusion

General conclusion:

This thesis aimed to investigate the potential effect of kefir on irritable bowel syndrome (IBS), a complex functional digestive disorder involving microbial, immune, inflammatory, and neurophysiological mechanisms. Through the analysis of available scientific data and the evaluation of several biological parameters, this study highlights the beneficial effects of kefir on the inflammatory, metabolic, nutritional, and oxidative status of the participants.

Irritable bowel syndrome (IBS) is a chronic functional digestive disorder characterized by several recurring gastrointestinal symptoms, including abdominal pain, cramps, bloating, a feeling of intestinal discomfort, and changes in bowel habits that may manifest as diarrhea, constipation, or alternating episodes of both. Patients may also experience excessive flatulence, a feeling of incomplete evacuation after defecation, and changes in stool consistency. These symptoms are often associated with factors such as stress, imbalances in the gut microbiota, and certain dietary habits.

The results obtained after regular kefir consumption showed a significant decrease in C-reactive protein (CRP) concentrations, reflecting a reduction in the low-grade systemic inflammation frequently associated with irritable bowel syndrome. A decrease in creatinine and urea concentrations was also observed, suggesting improved protein metabolism and better elimination of nitrogenous waste. Concurrently, the increase in vitamin D concentrations reflects an improvement in the nutritional and immune status of the participants.

Furthermore, this study highlighted a decrease in malondialdehyde (MDA) concentrations, a marker of oxidative stress and lipid peroxidation, accompanied by an increase in reduced glutathione (GSH) levels and catalase activity, two essential components of the antioxidant defense system. These results indicate that kefir possesses significant antioxidant activity, enabling it to limit cellular damage induced by free radicals and oxidative stress, which are implicated in the pathophysiology of IBS.

The observed effects could be attributed to the probiotic, anti-inflammatory, antioxidant, and immunomodulatory properties of kefir. Thanks to its richness in lactic acid bacteria, probiotic yeasts, and bioactive metabolites, kefir helps restore the balance of the gut microbiota, strengthen the intestinal barrier, and modulate the immune response. This

General conclusion:

overall action contributes to the improvement of digestive symptoms, the reduction of inflammation, and the optimization of the general condition of patients with irritable bowel syndrome. Thus, kefir appears as a promising complementary nutritional approach in the prevention and management of irritable bowel syndrome. However, despite the encouraging results observed in this study and in the scientific literature, further research is needed to better understand its mechanisms of action, identify optimal doses, and scientifically confirm its long-term therapeutic efficacy.

Perspectives

Perspectives:

The data obtained in this study open up several avenues of research regarding the use of kefir in the prevention and management of irritable bowel syndrome and other digestive and inflammatory conditions. Large-scale clinical trials would be valuable to confirm the therapeutic, anti-inflammatory, and antioxidant effects of kefir in humans. Further studies should also provide a better understanding of the interactions between kefir microorganisms, the gut microbiota, the immune system, and the gut-brain-microbiota axis.

Moreover, identifying the bioactive metabolites responsible for the beneficial effects of kefir represents an important research area for the development of new targeted therapeutic applications. Optimizing fermentation conditions and exploring the microbial diversity of kefir grains could also improve the nutritional, functional, and sensory properties of this fermented beverage.

The development of functional kefir-based drinks tailored to individuals with digestive, metabolic, or inflammatory disorders also presents a promising avenue. Furthermore, plant-based kefir could offer an attractive alternative for those with lactose intolerance or following a vegan diet. Finally, interdisciplinary research combining nutrition, microbiology, biotechnology, and medicine is essential to fully explore the therapeutic potential of kefir and promote its integration into preventive and complementary nutritional strategies.

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ANNEX

Questionnaire on Digestive Disorders, Kefir Consumption and Oxidative Stress

Study title: Evaluation of the Effect of Kefir Consumption on Digestive Health and Oxidative Stress

Dear Participant,

This questionnaire is part of a scientific research study aiming to evaluate the relationship between digestive disorders, dietary habits, stress, and kefir consumption.

Your participation is voluntary and all collected information will remain strictly confidential and will only be used for scientific purposes.

Please answer the following questions by ticking the appropriate box or completing the requested information.

1. **Participant name or code :**

2. **Age :**

_____ years

3. **Sex:**

☐ Male

☐ Female

4. **Weight:**

_____ kg

5. **Height:**

_____ cm

6. **Body Mass Index (BMI):**

Place of residence:

8. **Profession:**

9. **How long have you been suffering from digestive symptoms?**

☐ < 6 months

☐ 6–12 months

☐ 1–3 years

☐ > 3 years

10. **Have you ever been diagnosed with a specific digestive disease?**

☐ Yes

☐ No

11. **If yes, specify:**

12. Family history of digestive diseases:

- ☐ Yes
- ☐ No

13. Do you suffer from other chronic diseases (diabetes, hypertension, cardiovascular diseases, etc.)?

- ☐ Yes
- ☐ No

14. If yes, specify:

15. Number of meals consumed per day:

- ☐ 1 meal
- ☐ 2 meals
- ☐ 3 meals
- ☐ More than 3 meals

16. Consumption of dairy products (milk, yogurt, kefir, etc.):

- ☐ 1–2 times/week
- ☐ 3–5 times/week
- ☐ Daily

17. Consumption of fruits and vegetables:

- ☐ Rarely
- ☐ 1–2 portions/day
- ☐ 3–5 portions/day
- ☐ > 5 portions/day

18. Consumption of fatty, fried, or processed foods:

- ☐ Rarely
- ☐ 1–2 times/week
- ☐ 3–5 times/week
- ☐ Daily

19. Consumption of sugary or caffeinated beverages:

- ☐ Rarely
- ☐ 1–2 times/week
- ☐ 3–5 times/week
- ☐ Daily

20. Frequency of abdominal pain:

- ☐ Never
- ☐ Rarely
- ☐ 1–2 times/week
- ☐ > 2 times/week

21. Type of digestive disorder:

- ☐ Constipation
- ☐ Diarrhea
- ☐ Alternating constipation and diarrhea

22. Bloating:

- ☐ Never
- ☐ Rarely
- ☐ Often
- ☐ Always

23. Feeling of discomfort after meals:

- ☐ Never
- ☐ Rarely
- ☐ Often
- ☐ Always

24. Perceived stress level in daily life (self-assessment):

- ☐ Low
- ☐ Moderate
- ☐ High
- ☐ Very high

25. Sleep quality:

- ☐ Good
- ☐ Average
- ☐ Poor
- ☐ Very poor

26. Physical activity (frequency/week):

- ☐ None
- ☐ 1–2 times/week
- ☐ 3–4 times/week
- ☐ > 4 times/week

27. Smoking consumption:

- ☐ Yes
- ☐ No

28. Alcohol consumption:

- ☐ Yes
- ☐ No

29. Do you know kefir?

- ☐ Yes
- ☐ No

30. If yes, do you know its composition?

☐ Yes

☐ No

31. Do you know its possible health effects?

☐ Yes

☐ No

32. If yes, specify:

Have you ever consumed kefir?

☐ Yes

☐ No

33. If yes, for how long have you been consuming it?

☐ < 1 month

☐ 1–3 months

☐ 3–6 months

☐ > 6 months

34. Frequency of kefir consumption:

☐ Rarely

☐ 1–2 times/week

☐ 3–5 times/week

☐ Daily

35. After consuming kefir, how did you feel?

☐ No effect

☐ Better digestion

☐ Less abdominal pain

☐ Less bloating

36. Did you notice an overall improvement in your digestive disorders?

☐ Yes

☐ No

37. If yes, specify:

Annex :

Paramètre	Avant kéfir	Après kéfir
Nombre de patientes (n)	30	30
Âge moyen (ans)	39,8 ± 9,1	39,8 ± 9,1
IMC moyen (kg/m ²)	27,4 ± 3,2	26,6 ± 3,0
Score douleur abdominale (0–10)	7,2 ± 1,1	3,8 ± 1,0
Ballonnements (score 0–10)	7,8 ± 1,0	4,1 ± 1,2
Nombre de selles / jour	3,1 ± 1,2	1,9 ± 0,8
Consistance des selles (Bristol score)	6,2 ± 0,7	4,3 ± 0,6
Stress perçu (score 0–10)	6,9 ± 1,3	4,0 ± 1,1
Fatigue générale (score 0–10)	6,4 ± 1,5	3,9 ± 1,2
Durée consommation kéfir	—	4 semaines

Résumé:

Le syndrome de l'intestin irritable (SII) est un trouble fonctionnel digestif multifactoriel caractérisé par des douleurs abdominales, des ballonnements, des troubles du transit intestinal et un inconfort digestif, associés à une dysbiose intestinale, une inflammation de faible intensité et un stress oxydatif.

Cette étude vise à évaluer l'effet de la consommation de kéfir sur les manifestations cliniques et les marqueurs biologiques du SII.

Avant consommation de kéfir, les sujets présentaient des symptômes digestifs marqués (douleurs abdominales, ballonnements et troubles du transit), associés à une élévation du MDA et de la CRP, ainsi qu'à une diminution du GSH et de la catalase, traduisant un déséquilibre oxydatif et inflammatoire.

Après consommation de kéfir, une amélioration clinique a été observée avec une réduction des symptômes digestifs. Sur le plan biologique, on note une diminution du MDA et de la CRP, ainsi qu'une augmentation du GSH et de la catalase, indiquant une amélioration du stress oxydatif et de l'inflammation. Les paramètres rénaux (urée et créatinine) restent stables, tandis que la vitamine D augmente légèrement.

Ces résultats suggèrent que le kéfir pourrait améliorer les symptômes du SII et exercer un effet bénéfique sur le stress oxydatif et l'inflammation, constituant ainsi une approche nutritionnelle complémentaire prometteuse.

Mots-clés : Syndrome de l'intestin irritable, kéfir, probiotiques, dysbiose intestinale, stress oxydatif, inflammation, MDA, GSH, catalase.

Abstract

Irritable bowel syndrome (IBS) is a multifactorial functional digestive disorder characterized by abdominal pain, bloating, altered bowel habits, and digestive discomfort, associated with gut dysbiosis, low-grade inflammation, and oxidative stress.

This study aimed to evaluate the effect of kefir consumption on the clinical manifestations and biological markers of IBS.

Before kefir consumption, subjects presented with marked digestive symptoms (abdominal pain, bloating, and altered bowel habits), associated with elevated MDA and CRP levels, as well as decreased GSH and catalase levels, reflecting an oxidative and inflammatory imbalance.

After kefir consumption, clinical improvement was observed, with a reduction in digestive symptoms. Biologically, a decrease in MDA and CRP was noted, along with an increase in GSH and catalase, indicating an improvement in oxidative stress and inflammation. Renal parameters (urea and creatinine) remained stable, while vitamin D levels increased slightly.

These results suggest that kefir could improve IBS symptoms and have a beneficial effect on oxidative stress and inflammation, thus representing a promising complementary nutritional approach.

Keywords: Irritable bowel syndrome, kefir, probiotics, gut dysbiosis, oxidative stress, inflammation, MDA, GSH, catalase.

ملخص

تُعد متلازمة القولون العصبي اضطرابًا وظيفيًا هضميًا متعدد العوامل، يتميز بآلام البطن والانتفاخ واضطرابات العبور المعوي والشعور بعدم الارتياح الهضمي، ويرتبط بخلل توازن الميكروبيوتا المعوية، والتهاب منخفض الشدة، والإجهاد التأكسدي. هدفت هذه الدراسة إلى تقييم تأثير استهلاك الكفير على المظاهر السريرية والمؤشرات البيولوجية لدى المرضى المصابين بمتلازمة القولون العصبي.

قبل استهلاك الكفير، أظهر المرضى أعراضًا هضمية واضحة تمثلت في آلام البطن والانتفاخ واضطرابات العبور المعوي، وكانت مصحوبة بارتفاع مستويات المالمون ثنائي الألدheid والبروتين المتفاعل س، وانخفاض مستويات الجلوتاثيون المختزل وإنزيم الكاتالاز، مما يعكس وجود اختلال في التوازن التأكسدي والالتهابي.

بعد استهلاك الكفير، لوحظ تحسن سريري تمثل في انخفاض الأعراض الهضمية. وعلى المستوى البيولوجي، سُجل انخفاض في مستويات المالمون ثنائي الألدheid والبروتين المتفاعل س، مع زيادة مستويات الجلوتاثيون المختزل وإنزيم الكاتالاز، مما يشير إلى تحسن حالة الإجهاد التأكسدي والالتهاب. كما بقيت المؤشرات الكلوية، والمتمثلة في اليوريا والكرياتينين، مستقرة، في حين ارتفع مستوى فيتامين د بشكل طفيف.

تشير هذه النتائج إلى أن الكفير قد يساهم في تحسين أعراض متلازمة القولون العصبي ويُظهر تأثيرًا إيجابيًا على الإجهاد التأكسدي والالتهاب، مما يجعله مقاربة غذائية تكميلية واعدة.

الكلمات المفتاحية: متلازمة القولون العصبي، الكفير، البروبيوتيك، خلل توازن الميكروبيوتا المعوية، الإجهاد التأكسدي، الالتهاب، المالمون ثنائي الألدheid، الجلوتاثيون المختزل، إنزيم الكاتالاز.