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**Evaluation of the phytochemical, biological, and sensory
properties of *Stevia rebaudiana* in functional food
formulations**

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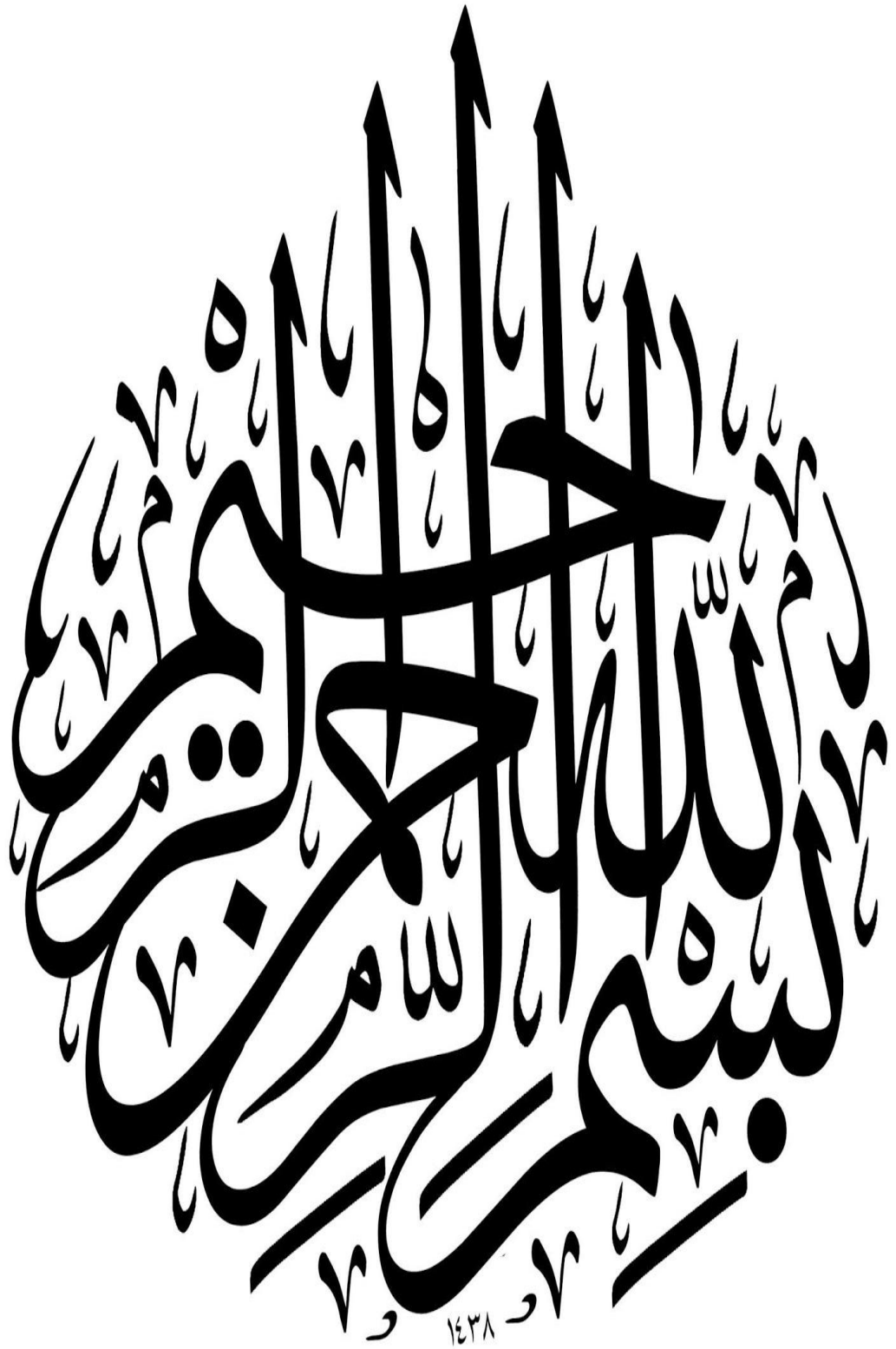
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Dedications

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To the light that guided my path and to the one from whom I drew my strength and pride...

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To the one whose prayers and sacrifices accompanied me throughout my life,

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Dedications

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Table of contents

Table of contents

Thanks	V
Dedications	VI
Table of contents	IX
List of Figures	XI
List of Tables	XIV
List of Abbreviations	XVI
Abstract	XVII
Résumé	XVIII
Introduction	1
Theoretical Part	5
Chapter I: General Overview of <i>Stevia rebaudiana</i>	6
I.1. <i>Stevia rebaudiana</i> Bertoni	7
I.2. Botanical and Agronomic Characteristics of <i>Stevia rebaudiana</i>	9
I.3. Uses of <i>Stevia rebaudiana</i>	12
I.4. Composition of <i>Stevia rebaudiana</i> leaves	13
I.5. Metabolism of Steviol Glycosides	24
I.6. Biological and Pharmacological Effects of Steviol Glycosides	26
I.7. Stevia and Glycemic Index	31
I.8. Limitations of Stevia	33
Conclusion	33
Chapter II: Biological Activities Of <i>Stevia Rebaudiana</i>	35
II.1. Polyphenols: Classification and Chemical Structure	36
II.2. Polyphenolic Compounds in <i>Stevia rebaudiana</i>	40

II.3. Antioxidant Mechanisms of Polyphenols	41
II.4. Antidiabetic Mechanisms of Polyphenols	43
II.5. Functional and technological applications of Stevia in food systems	44
Conclusion	47
Practical Part	48
Chapter III: Materials and methods	49
III.1. Plant material	50
III.2. Preparation of <i>Stevia rebaudiana</i> extract	50
III.3. Dosage of phenolic compounds	53
III.4. Physicochemical Analysis of Total Sugars in <i>Stevia rebaudiana</i> Extract	54
III.5. The biological activities of the plant	55
III.6. Sensory Evaluation of Functional Food Formulations	59
Chapter IV: Results and discussion	64
IV.1. Extraction Yield	65
IV.2. Phenolic Compound Content	66
IV.3. Total Sugar Content in <i>Stevia rebaudiana</i> Extract	68
IV.4. Results of Biological Activity Assays	69
IV.5. Sensory Evaluation Results and Discussion	76
General Conclusion	100
References	103
Annex	120

List of Figures

Figure titles	Page
Theoretical Part	
Chapter I: General Overview of <i>Stevia rebaudiana</i>	
Figure I.1: Leaves and flowers of stevia (<i>Stevia rebaudiana</i>)	7
Figure I.2: APG III Classification	10
Figure I.3: Biosynthetic conversion of ent-kaurenoate into steviol.	19
Figure I.4: Chemical structure of steviol.	19
Figure I.5: Basic structure of steviol glycosides and formation of rebaudioside A.	20
Figure I.6: Simplified schematic representation of the biosynthetic pathway and intracellular localization of steviol glycosides in <i>Stevia rebaudiana</i> .	23
Figure I.7: Metabolic pathway of steviol glycosides in humans.	25
Figure I.8: The possible mechanisms of the antihyperglycemic action of <i>Stevia rebaudiana</i> .	27
Figure I.9: Proposed mechanism of the antihypertensive effect of stevioside through inhibition of Ca^{2+} influx in vascular smooth muscle cells.	30
Chapter II: Biological Activities Of <i>Stevia Rebaudiana</i>	
Figure II.1: Representative chemical structures of polyphenolic compounds showing aromatic rings and hydroxyl groups.	37
Figure II.2: Basic flavonoid skeleton (C6-C3-C6) and main flavonoid subclasses.	38
Figure II.3: Chemical structures of hydrolyzable and condensed tannins.	39
Figure II.4: Main structural determinants of flavonoid antioxidant activity, including hydroxyl groups and conjugated double bonds.	40
Figure II.5: Schematic representation of the Nrf2/ARE signaling pathway and its role in antioxidant defense against oxidative stress.	42
Practical Part	

Chapter III: Materials and methods	
Figure III.1: Different stages of <i>Stevia rebaudiana</i> plant preparation.	50
Figure III.2: Preparation of the hydroethanolic extract of <i>Stevia rebaudiana</i> from powdered leave	52
Chapter V: Results and discussion	
Figure IV.1: Percentages of DPPH inhibition by <i>Stevia rebaudiana</i> extract	69
Figure IV.2: IC ₅₀ Results of the DPPH Radical	70
Figure IV.3: Ferric reducing antioxidant power (FRAP) expressed as A _{0.5} values (mg/mL).	71
Figure IV.4: IC ₅₀ values of protein denaturation inhibition of <i>Stevia rebaudiana</i> extract compared to diclofenac	73
Figure IV.5: IC ₅₀ values of α -amylase inhibitory activity of <i>Stevia rebaudiana</i> extract.	75
Figure IV.6: The sensory results related to sweetness evaluation are presented	77
Figure IV.7: The sensory results related to taste evaluation are presented	80
Figure IV.8: The sensory results related to bitterness evaluation are presented	82
Figure IV.9: The sensory results related to aftertaste evaluation are presented	84
Figure V.10: The sensory results related to aroma evaluation are presented	87
Figure IV.11: The sensory results related to overall acceptability evaluation are presented.	89
Figure IV.12: The sensory results related to colour evaluation are presented.	91
Figure IV.13: The sensory results related to aroma evaluation are presented.	93
Figure IV.14: The sensory results related to taste evaluation are presented.	94
Figure IV.15: The sensory results related to texture evaluation are presented.	96
Figure IV.16: The sensory results related to overall acceptability evaluation are presented.	97

List of Tables

Table titles	Page
Theoretical Part	
Chapter I: General Overview of <i>Stevia rebaudiana</i>	
Table I.1: Composition proximale des feuilles de <i>Stevia rebaudiana</i> (g/100 g de matière sèche).	14
Table I.2: Mineral composition of dried <i>Stevia rebaudiana</i> leaves (mg/100 g dry weight basis).	14
Table I.3: Main phytochemical compounds identified in <i>Stevia rebaudiana</i> leaves and their reported presence in the literature.	15
Table I.4: monoterpenes and sesquiterpenes identified in the essential oil of <i>Stevia rebaudiana</i> leaves are presented.	16
Table I.5: Fatty acid composition of <i>Stevia rebaudiana</i> leaf oil and classification according to saturation level.	17
Table I.6: Essential and non-essential amino acid composition of <i>Stevia rebaudiana</i> leaves (g/100 g dry matter).	17
Table I.7: Vitamin content (mg/100 g) of dried <i>Stevia rebaudiana</i> leaves.	18
Table I.8: Main steviol glycosides identified in <i>Stevia rebaudiana</i> leaves	21
Table I.9: Relative sweetening power of major steviol glycosides compared with sucrose Adapted from Ceunen	24
Table I.10: Glycemic Index Values of Selected Foods .Values adapted from international glycemic index tables	31
Table I.11: Presents a comparison between steviol glycosides and sucrose in terms of sweetness, glycemic effect, caloric value, insulin response, cariogenicity, and acceptable daily intake.	32
Chapter II: Biological Activities Of <i>Stevia Rebaudiana</i>	

Table II.1: Major polyphenolic compounds identified in <i>Stevia rebaudiana</i> leaves	40
Practical Part	
Chapter III: Materials and methods	
Table III.1: Sensory evaluation form used for orange juice formulations.	61
Table III.2: Sensory evaluation form used for Madeleine cake formulations.	62
Chapter V: Results and discussion	
Table IV.1: Crude extract yield from <i>Stevia rebaudiana</i> (leaves).	65
Table IV.2: Phenolic, flavonoid, and tannin content of <i>Stevia rebaudiana</i> extract	66
Table IV.3: Total sugar content of <i>Stevia rebaudiana</i> extract	68

List of Abbreviations

Abbreviation	Full Term
ARE	Antioxidant Response Element
BHA	Butylated Hydroxyanisole
BHT	Butylated Hydroxytoluene
CAT	Catalase
DPPH	2,2-Diphenyl-1-picrylhydrazyl
DNA	Deoxyribonucleic Acid
EFSA	European Food Safety Authority
FRAP	Ferric Reducing Antioxidant Power
GC-MS	Gas Chromatography–Mass Spectrometry
GI	Glycemic Index
GPx	Glutathione Peroxidase
GSH	Reduced Glutathione
GST	Glutathione S-Transferase
HO-1	Heme Oxygenase-1
IC ₅₀	Half Maximal Inhibitory Concentration
IL-1 β	Interleukin-1 Beta

MEP	Methylerythritol Phosphate
NF- κ B	Nuclear Factor Kappa B
NQO1	NAD(P)H Quinone Oxidoreductase 1
Nrf2	Nuclear Factor Erythroid 2–Related Factor 2
ORAC	Oxygen Radical Absorbance Capacity
RAAS	Renin–Angiotensin–Aldosterone System
ROS	Reactive Oxygen Species
SOD	Superoxide Dismutase
TNF- α	Tumor Necrosis Factor Alpha
UGTs	UDP-Glycosyltransferases
WHO	World Health Organization

Abstract

The increasing prevalence of obesity, diabetes, and other metabolic disorders associated with excessive sugar consumption has encouraged the search for natural low-calorie sweeteners with additional functional properties. In this context, the present study aimed to investigate the phytochemical composition, biological activities, and sensory applicability of *Stevia rebaudiana* in functional food formulations. Hydroethanolic extraction (70% ethanol) was selected due to its efficiency in extracting polar bioactive compounds, yielding an extraction recovery of 24.2%. The obtained extracts revealed a low total sugar content (0.23 ± 0.02 mg GE/g extract), along with the presence of phenolic compounds (2.91 ± 0.11 mg GAE/g), flavonoids (0.87 ± 0.003 mg QE/g), and tannins (1.088 ± 0.042 mg CE/g). Antioxidant activity evaluation showed a moderate reducing power ($A_{0.5} = 1.49 \pm 0.23$ mg/mL) and free radical scavenging activity with an IC_{50} value of 4.84 ± 0.22 mg/mL for the DPPH assay. Biological evaluation also demonstrated promising antidiabetic and anti-inflammatory activities through α -amylase inhibition ($IC_{50} = 4.69 \pm 0.05$ mg/mL) and inhibition of protein denaturation ($IC_{50} = 17.1 \pm 0.37$ mg/mL), respectively. The functional applicability of stevia was further investigated in orange juice and madeleine cake formulations based on partial or total sucrose replacement, including formulations enriched with marine collagen. Sensory evaluation was performed to assess sweetness, bitterness, aroma, aftertaste, taste, texture, and overall acceptability in order to evaluate the feasibility of using stevia in different food matrices. The results showed that

partial substitution of sucrose with stevia maintained relatively acceptable sensory properties, whereas complete replacement negatively affected bitterness, aftertaste, aroma, and overall acceptability. Marine collagen contributed additional nutritional and textural value, although its sensory masking effect remained limited. Overall, the study highlights the promising potential of *Stevia rebaudiana* as a natural low-calorie sweetener with functional and bioactive properties. However, further formulation optimization remains necessary to improve sensory quality and consumer acceptance of stevia-based functional foods.

Keywords:

Stevia rebaudiana, Antioxidant activity, Antidiabetic activity, Sensory evaluation, Functional foods.

Résumé

L'augmentation continue des taux d'obésité, de diabète et des troubles métaboliques liés à la consommation excessive de sucres a encouragé la recherche d'édulcorants naturels hypocaloriques possédant des propriétés fonctionnelles supplémentaires. Dans ce contexte, cette étude avait pour objectif d'évaluer la composition phytochimique, les activités biologiques et l'application sensorielle de *Stevia rebaudiana* dans des produits alimentaires fonctionnels. L'extraction hydroéthanolique (70 % d'éthanol) a été choisie en raison de son efficacité dans l'extraction des composés bioactifs polaires, avec un rendement d'extraction de 24,2 %. Les extraits obtenus ont montré une faible teneur en sucres totaux ($0,23 \pm 0,02$ mg GE/g d'extrait), ainsi que la présence de composés phénoliques ($2,91 \pm 0,11$ mg GAE/g), de flavonoïdes ($0,87 \pm 0,003$ mg QE/g) et de tanins ($1,088 \pm 0,042$ mg CE/g). L'évaluation de l'activité antioxydante a montré une activité réductrice modérée ($A_{0.5} = 1,49 \pm 0,23$ mg/mL), ainsi qu'une activité de piégeage des radicaux libres avec une valeur IC_{50} de $4,84 \pm 0,22$ mg/mL dans le test DPPH. L'évaluation biologique a également montré une activité antidiabétique et anti-inflammatoire prometteuse à travers l'inhibition de l'enzyme α -amylase ($IC_{50} = 4,69 \pm 0,05$ mg/mL) et l'inhibition de la dénaturation des protéines ($IC_{50} = 17,1 \pm 0,37$ mg/mL), respectivement. L'application fonctionnelle de la stévia a également été évaluée dans des formulations de jus d'orange et de madeleine basées sur le remplacement partiel ou total du saccharose, y compris des formulations enrichies en collagène marin. Une évaluation sensorielle a été réalisée afin d'étudier la douceur, l'amertume, l'arôme, l'arrière-goût, le goût,

la texture et l'acceptabilité globale dans le but d'évaluer la possibilité d'utiliser la stévia dans différentes matrices alimentaires. Les résultats ont montré que le remplacement partiel du saccharose par la stévia permettait de maintenir des propriétés sensorielles relativement acceptables, tandis que le remplacement total affectait négativement l'amertume, l'arrière-goût, l'arôme et l'acceptabilité globale. Le collagène marin a également contribué à renforcer la valeur nutritionnelle et texturale des produits, bien que son effet dans le masquage sensoriel soit resté limité. Globalement, cette étude met en évidence le potentiel prometteur de *Stevia rebaudiana* comme édulcorant naturel hypocalorique possédant des propriétés fonctionnelles et bioactives. Cependant, une optimisation supplémentaire des formulations reste nécessaire afin d'améliorer la qualité sensorielle et d'augmenter l'acceptabilité des consommateurs vis-à-vis des produits alimentaires fonctionnels à base de stévia.

Mots-clés : *Stevia rebaudiana*, activité antioxydante, activité antidiabétique, aliments fonctionnels, évaluation sensorielle.

المخلص:

إنّ التزايد المستمر في معدلات السمنة ومرض السكري والاضطرابات الأيضية المرتبطة بالاستهلاك المفرط للسكريات شجّع على البحث عن مُحلّيات طبيعية منخفضة السعرات الحرارية تمتلك خصائص وظيفية إضافية. وفي هذا السياق، هدفت هذه الدراسة إلى تقييم التركيب الكيميائي النباتي، والأنشطة البيولوجية، والتطبيق الحسي لنبات *Stevia rebaudiana* في منتجات غذائية وظيفية، تم اختيار الاستخلاص الهيدروإيثانولي (70% إيثانول) نظرًا لفعاليته في استخلاص المركبات الحيوية القطبية، حيث بلغت مردودية الاستخلاص 24.2%. وأظهرت المستخلصات المتحصل عليها انخفاضًا في محتوى السكريات الكلية (0.02 ± 0.23 mg GE/g extract)، إلى جانب احتوائها على مركبات فينولية (0.11 ± 2.91 mg GAE/g)، وفلافونويدات (0.003 ± 0.87 mg QE/g)، وتانينات (0.042 ± 1.088 mg CE/g). كما أظهر تقييم النشاط المضاد للأكسدة قدرة اختزالية متوسطة (1.49 ± 0.23 mg/mL A0.5)، بالإضافة إلى نشاط لاقتناص الجذور الحرة بقيمة IC_{50} بلغت 4.84 ± 0.22 mg/mL في اختبار DPPH، كما أظهر التقييم البيولوجي نشاطًا واعدًا مضادًا للسكري ومضادًا للالتهاب من خلال تثبيط إنزيم α -amylase ($IC_{50} = 4.69 \pm 0.05$ mg/mL) وتثبيط تمسخ البروتينات ($IC_{50} = 17.1 \pm 0.37$ mg/mL) على التوالي، كما تم تقييم التطبيق الوظيفي للستيفيا في تركيبات عصير البرتقال والمادلين اعتمادًا على الاستبدال الجزئي أو الكلي للسكر، بما في ذلك تركيبات مدعمة بالكولاجين البحري. وتم إجراء تقييم حسي لدراسة الحلاوة، والمرارة، والرائحة، والطعم، والقوام، والتقبل العام بهدف تقييم إمكانية استخدام الستيفيا في مصفوفات غذائية مختلفة. وأظهرت النتائج أن الاستبدال الجزئي للسكر بالستيفيا حافظ على خصائص حسية مقبولة نسبيًا، في حين أثار الاستبدال الكلي سلبيًا على المرارة والطعم اللاحق والرائحة والتقبل العام. كما ساهم الكولاجين البحري في تعزيز القيمة الغذائية والقوامية للمنتجات، رغم أن تأثيره في إخفاء الطعم اللاحق ظل محدودًا، وبصفة عامة تُبرز هذه الدراسة الإمكانيات الواعدة لنبات *Stevia rebaudiana* كمُحلّ طبيعي منخفض السعرات الحرارية يمتلك خصائص

وظيفية وحيوية. ومع ذلك، تبقى الحاجة قائمة إلى تحسين الصياغة الغذائية من أجل رفع الجودة الحسية وزيادة تقبل المستهلك للمنتجات الغذائية الوظيفية المعتمدة على الستيفيا.

الكلمات المفتاحية: *Stevia rebaudiana*، النشاط المضاد للأكسدة، النشاط المضاد لداء السكري، الأغذية الوظيفية، التقييم الحسي.

Introduction

Introduction

In recent decades, the increasing prevalence of metabolic disorders such as obesity, type-diabetes, and cardiovascular diseases has become a major global health concern. These 2 conditions are strongly associated with dietary habits, particularly the excessive consumption ,of refined sugars and high-calorie foods (**World Health Organization, 2020**). Consequently increasing attention has been directed toward healthier food alternatives capable of reducing caloric intake while maintaining acceptable sensory quality. Two important nutritional parameters commonly used to evaluate the health impact of foods are caloric value and glycemic index (GI). Caloric value reflects the amount of energy provided by food, whereas GI describes the effect of carbohydrate-containing foods on postprandial blood glucose levels (**Atkinson et al., 2021**). Diets rich in high-calorie and high-GI foods are closely linked to metabolic disorders, including insulin resistance and type-2 diabetes.

In this context, natural sweeteners have attracted considerable scientific and industrial interest. ,Among them, *Stevia rebaudiana* (Bertoni), a perennial plant belonging to the Asteraceae family is considered one of the most promising alternatives to conventional sugar. Stevia leaves contain diterpene glycosides such as stevioside and rebaudiosides, which are responsible for their intense sweetness and are widely used as sugar substitutes in foods, beverages, and pharmaceutical products (**Sreedhar et al., 2018**).

In addition to their sweetening properties, stevia extracts are also recognized for several biological activities, including antioxidant, antidiabetic, antihypertensive, and anti-inflammatory effects. These biological properties are largely associated with the presence of .polyphenolic compounds such as flavonoids and tannins (**Lemus-Mondaca et al., 2012**) Polyphenols are known for their ability to neutralize reactive oxygen species and reduce oxidative stress, while some studies have also demonstrated their potential to inhibit ;carbohydrate-digesting enzymes involved in glycemic regulation (**Pandey & Rizvi, 2009 McDougall et al., 2005**).

Several food products incorporating stevia, including beverages, dairy products, and bakery formulations, have been developed in recent years. Among bakery products, madeleine cakes represent an interesting food matrix for evaluating sugar substitution, since sucrose plays an important role not only in sweetness, but also in texture, colour development, moisture retention, and overall sensory quality during baking.Previous studies have demonstrated that stevia can reduce sugar and caloric content while maintaining satisfactory technological and nutritional properties (**Sreedhar et al., 2018**) .

Introduction

However, despite these advantages, the application of stevia in food systems remains limited by certain sensory drawbacks, particularly bitterness and licorice-like aftertaste, which may reduce consumer acceptance (**Prakash et al., 2014**)

To overcome these limitations, different formulation strategies have been proposed, including the incorporation of proteins and functional ingredients. Proteins such as whey proteins and collagen peptides may improve texture, enhance physicochemical stability, and reduce undesirable flavour perception. In addition, interactions between proteins and polyphenols may contribute to the stabilization of bioactive compounds and improve the functional quality of food products (**Ozdal et al., 2013**).

Accordingly, the development of functional beverages combining stevia and protein fortification represents a promising approach for producing low-calorie and low-glycemic-index products with improved biological and sensory properties. Based on this background the present study focuses on the formulation and sensory evaluation of orange juice sweetened with stevia extract and enriched with collagen protein as a potential functional beverage with enhanced nutritional and technological value.

Despite the growing use of *Stevia rebaudiana* as a natural sugar substitute, its incorporation into food products still faces sensory challenges, particularly bitterness and aftertaste, which may affect consumer acceptance. Therefore, developing low-calorie and low-glycemic-index functional beverages with improved sensory quality remains an important challenge

To what extent can a stevia-based beverage fortified with collagen protein improve the nutritional and sensory quality of a low-glycemic-index functional product?

The main objective of this study is to develop and evaluate functional food formulations based on stevia extract, including orange juice fortified with collagen protein and madeleine cake formulations, as potential low-calorie and low-glycemic-index products.

- ✓ To investigate the biological and functional properties of polyphenols present in *Stevia rebaudiana*.
- ✓ To study the antioxidant and antidiabetic mechanisms associated with stevia polyphenols.
- ✓ To evaluate the technological and sensory properties of stevia in food systems.
- ✓ To formulate different orange juice samples using sugar, stevia extract, and collagen protein.
- ✓ To formulate madeleine cake samples using partial or total sugar substitution with stevia extract and evaluate their sensory acceptability.

Introduction

- ✓ To compare the sensory characteristics of the prepared formulations, including sweetness, bitterness, aroma, texture, and overall acceptability .
- ✓ To assess the potential of stevia and collagen incorporation in improving the nutritional and functional quality of beverage formulations.



Theoretical Part

Chapter I: General Overview of *Stevia rebaudiana*

Chapter II: Biological Activities Of *Stevia Rebaudiana*

Chapter I

General Overview of *Stevia rebaudiana*

Chapter I: General Overview of *Stevia rebaudiana*

The increasing global prevalence of metabolic disorders such as obesity and diabetes, combined with growing consumer awareness of the potential health risks associated with synthetic sweeteners, particularly the risk of cancer, has significantly intensified the search for safe, low-calorie natural alternatives. This has led to the identification of several intensely sweet plant-derived compounds, among which are steviol glycosides extracted from *Stevia rebaudiana*, a zero-calorie natural sweetener. This chapter provides a detailed overview of the *Stevia rebaudiana* plant, covering its origin, classification, botanical description, global uses, chemical composition, and pharmacological effects, with particular emphasis on steviol glycosides.

I.1. *Stevia rebaudiana* Bertoni

Stevia rebaudiana Bertoni is a branched, bushy shrub belonging to the family Asteraceae (Soejarto, 2002). Commonly known as sweet leaf, sugar leaf, or sweet herb (Shaffert et Chetobar, 1994), it is native to the Amambay region in northeastern Paraguay and also occurs in neighboring parts of Brazil and Argentina. Today, its cultivation has spread to other regions of the world, including Canada and parts of Asia and Europe (Amzad-Hossain et al., 2010; Gardana et al., 2003). The general appearance of *Stevia rebaudiana*, including its leaves and flowers, is shown in Figure (1) (Amarakoon, 2021).



Figure I.1: Leaves and flowers of *stevia* (*Stevia rebaudiana*) (Amarakoon, 2021).

Morphologically, it is a small perennial shrub reaching up to 65 cm in height, with oppositely arranged lanceolate to oblanceolate leaves that are serrated above the middle. The

leaf epidermis bears trichomes of two distinct size classes: large (4–5 μm) and small (2.5 μm) (Shaffert & Chetobar, 1994).

For centuries, this plant has been used as a natural sweetener and for medicinal purposes (Shaffert & Chetobar, 1994). It is worth noting that among the approximately 230 species within the genus *Stevia*, only *S. rebaudiana* and *S. phlebophylla* are capable of producing steviol glycosides (Brandle & Telmer, 2007). The commercial value of *Stevia* lies in these glycosides; the most important of which are stevioside and rebaudioside A, the compounds responsible for its sweet taste (Prakash et al., 2008).

These compounds do not contribute to caloric intake, as they are not metabolized by the human body, and are approximately 100–300 times sweeter than sucrose (table sugar) (Goyal et al., 2010).

In addition to glycosides, *Stevia* leaves contain other important phytochemical constituents, including flavonoids, alkaloids, chlorophylls, water-soluble xanthophylls, hydroxycinnamic acids (such as caffeic acid and chlorogenic acid), water-soluble oligosaccharides, free sugars, amino acids, lipids, essential oils, proteins, and trace elements (Escutia et al., 2019).

I.1.1. Origin and History of *Stevia rebaudiana*

Stevia rebaudiana is a plant native to South America, particularly Paraguay and Brazil, where it has been used for centuries by the indigenous Guarani people. The Guarani referred to the plant as “Ka-a He-e,” meaning “sweet herb,” and traditionally used its leaves to sweeten beverages such as mate and various herbal preparations (Lewis, 1992; Ramesh et al., 2006).

The genus *Stevia* was named in honor of the Spanish botanist and physician Pedro Jaime Esteve (Petrus Jacobus Stevus, 1500–1556). The species *rebaudiana* was first described by the Swiss botanist Moises Santiago Bertoni in the late nineteenth century. In 1899, Bertoni initially classified the plant as *Eupatorium rebaudianum* in recognition of the Paraguayan chemist Ovidio Rebaudi, who was among the first scientists to study its sweet constituents. Later, after further botanical observations, the plant was reclassified under its current scientific name, *Stevia rebaudiana* Bertoni (Seidemann, 1976).

Scientific interest in stevia increased significantly during the twentieth century following the discovery of its intensely sweet compounds. In 1931, French chemists Bridel and Lavielle successfully isolated stevioside and rebaudioside, the glycosides mainly responsible for the

sweet taste of the plant. These compounds attracted attention because of their high sweetening power and low caloric value (Lewis, 1992).

Since then, stevia has gained considerable importance as a natural sweetener. Japan was among the first countries to develop the commercial cultivation and industrial use of stevia extracts, particularly after restrictions were imposed on certain artificial sweeteners during the 1970s. Over time, the use of stevia expanded worldwide due to the increasing demand for natural, low-calorie sweeteners and growing interest in healthier dietary alternatives.

Today, *Stevia rebaudiana* is cultivated in several countries across Asia, South America, and other regions of the world. In addition to its use as a sweetener, the plant has attracted scientific interest because of its biological properties, including antioxidant, antidiabetic, and anti-inflammatory activities, which are mainly associated with its steviol glycosides and phenolic compounds (Ramesh et al., 2006).

I.2. Botanical and Agronomic Characteristics of *Stevia rebaudiana*

I.2.1. Classification of *Stevia rebaudiana*

Stevia rebaudiana Bertoni belongs to the family Asteraceae, also traditionally referred to as Compositae. Asteraceae is considered one of the largest families of flowering plants, comprising more than 1,600 genera and approximately 25,000 species worldwide. Members of this family are predominantly herbaceous, although shrubs, trees, and climbing species are also represented (Judd et al., 2016).

The genus *Stevia* includes approximately 200–230 species distributed mainly throughout North and South America. The taxonomy of this genus is considered relatively complex due to morphological similarities and difficulties in species delimitation among several taxa (Soejarto, 2002).

Stevia rebaudiana is classified within the most recent botanical classification of angiosperms established by the Angiosperm Phylogeny Group (APG). To date, this represents the most important botanical classification framework. The third version, APG III, was published in 2009. According to the Angiosperm Phylogeny Group III classification, *Stevia rebaudiana* is placed within the Asteraceae family (APG III, 2009). This taxonomic hierarchy is illustrated schematically in Figure (2) below

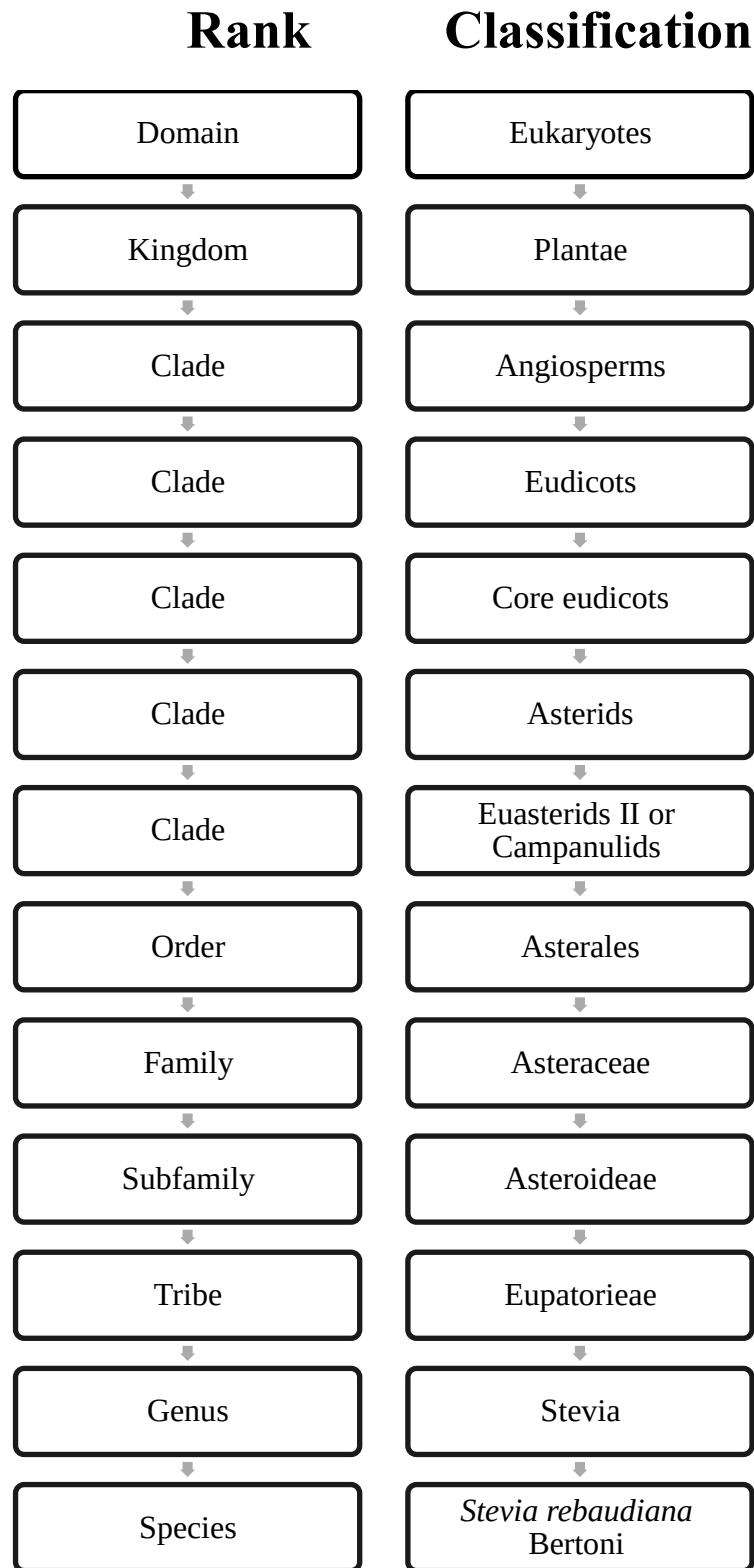


Figure I.2: APG III Classification

I.2.2. Botanical Description of *Stevia rebaudiana*

Stevia rebaudiana is a perennial shrub belonging to the Asteraceae family. Although the genus *Stevia* includes approximately 200 species, *S. rebaudiana* is considered the most economically important due to the presence of intensely sweet steviol glycosides in its leaves (Lemus-Mondaca et al., 2012).

The plant generally reaches a height of 60–80 cm and possesses a well-developed root system. Its stems are slender, semi-woody, pubescent, and highly branched. The leaves are sessile, approximately 3–5 cm long, with an oblong to elliptical shape and serrated margins. They are covered with fine hairs, giving them a soft texture (Lemus-Mondaca et al., 2012).

The flowers are small, white, and mainly insect-pollinated. The seeds are very small (around 3 mm long) and exhibit a relatively low germination rate, which represents an important limitation for large-scale cultivation. Consequently, *S. rebaudiana* is commonly propagated through stem cuttings, although tissue culture techniques are increasingly used for rapid and uniform multiplication (Goettemoeller & Ching, 1999; Soejarto, 2002).

Stevia rebaudiana grows best under subtropical climatic conditions in well-drained soils. The plant is sensitive to frost and develops optimally at temperatures ranging from 20 to 24°C. Adequate soil moisture is essential for optimal growth, and several harvests can be obtained annually under suitable cultivation conditions (Lemus-Mondaca et al., 2012).

I.2.3. Climatic and Cultivation Requirements of *Stevia rebaudiana*

Stevia rebaudiana grows naturally under warm subtropical conditions and develops optimally at temperatures ranging from 20 to 30 °C (OMAFRA, n.d.; Ceunen & Geuns, 2013). The plant is highly sensitive to frost and low temperatures, which can negatively affect growth and survival.

The plant prefers well-drained sandy loam soils rich in organic matter and slightly acidic to neutral in pH. Due to its shallow root system, stevia requires soils capable of retaining sufficient moisture while avoiding waterlogging conditions that may promote root diseases.

Stevia is sensitive to drought and requires regular moisture for optimal growth and leaf production. Adequate rainfall or irrigation is therefore essential to maintain good vegetative development (Ceunen & Geuns, 2013). The plant is classified as a long-day species, and extended photoperiods delay flowering while promoting vegetative growth and the accumulation of steviol glycosides in the leaves. In contrast, shorter days induce earlier flowering and accelerate the reproductive cycle (Ceunen & Geuns, 2013).

Flowering generally occurs between 58 and 100 days after planting, depending on environmental and cultivation conditions (Zaman et al., 2015). Leaves are preferably harvested shortly before flowering, when stevioside and rebaudioside A concentrations are at their highest levels. Harvesting at this stage is therefore important to obtain better sweetener quality and higher concentrations of active compounds.

Stevia rebaudiana can be propagated through seeds, stem cuttings, layering, or tissue culture techniques. However, stem cuttings remain the most widely used propagation method because they produce genetically uniform plants with stable glycoside yields, although they require intensive labour. In temperate and cold regions, stevia is often cultivated as an annual crop to avoid winter damage.

I.3. Uses of *Stevia rebaudiana*

I.3.1. Traditional Uses

Stevia rebaudiana has traditionally been used by the indigenous Guarani people of Paraguay and Brazil, mainly for sweetening beverages such as mate. In traditional medicine, the leaves were also employed for different purposes, particularly in the management of diabetes and hypertension (Soejarto, 2002). Preparations were generally made as infusions or decoctions depending on the intended use.

In addition to its sweetening effect, stevia has been associated with several traditional benefits, including support of oral hygiene, reduction of dental plaque, improvement of digestion, and maintenance of skin cleanliness. Antimicrobial and antifungal activities have also been reported in relation to the phytochemical composition of the plant (Wilkens et al., 2008). Traditional knowledge concerning these uses has mainly been transmitted orally across generations, highlighting the ethnobotanical importance of the plant in South American traditional medicine (Wagner, 2012; Soejarto, 2002). However, some traditional claims still require further scientific confirmation.

I.3.2. Current Uses as a Natural Sweetener

Today, *Stevia rebaudiana* is widely used as a natural sweetener and food additive because of its strong sweetening power and low caloric value. The leaves contain the highest concentrations of steviol glycosides, especially stevioside and rebaudioside A, which are responsible for the sweet taste (Penner et al., 2004).

Stevia is commercialized in several forms, including dried leaf powder, aqueous extracts, and purified sweeteners. The leaves are also used in herbal teas and different food preparations (Midmore & Rank, 2002). Due to the increasing demand for natural low-calorie sweeteners, the food and beverage industry currently represents the main commercial market for stevia products (Barbet & Massin, 2015).

I.3.3. Cosmetic Uses

Stevia leaves are sometimes incorporated into cosmetic and personal care products such as toothpaste, facial masks, and skin-care preparations. These applications are mainly related to the antioxidant and cleansing properties of the plant, as well as its potential role in maintaining skin condition (Midmore & Rank, 2002).

I.4. Composition of *Stevia rebaudiana* leaves:

Before discussing the detailed chemical composition of *Stevia rebaudiana* leaves, it is important to note that the quantitative composition of the plant may vary according to environmental conditions and cultivation practices, whereas its qualitative composition generally remains relatively stable. Factors such as soil characteristics, climatic conditions, sunlight exposure, irrigation, harvesting stage, and post-harvest processing can significantly influence the concentration of bioactive compounds, particularly steviol glycosides, phenolic compounds, and minerals. Nevertheless, the overall chemical profile of the plant remains relatively consistent (Lemus-Mondaca et al., 2012; Ceunen & Geuns, 2013).

I.4.1. Proximate composition:

Previous studies reported that carbohydrates constitute the major component of *Stevia rebaudiana* leaves, followed by proteins, whereas lipids are generally present in lower amounts. Moisture, crude fiber, and ash contents may vary depending on cultivation and environmental conditions (Tadhani & Subhash, 2006; Mishra et al., 2010).

The approximate values of the main proximate components of *Stevia rebaudiana* leaves reported in previous studies are presented in Table I.1.

Table I.1: Composition proximale des feuilles de *Stevia rebaudiana* (g/100 g de matière sèche).

References			
Composant	Mishra et al. (2010)	Tadhani & Subhash (2006)	Serio (2010)
Glucides	52	35.2	53
Protéines	10	20.4	11.2
Lipides	3	4.34	5.6

I.4.2. Mineral Composition :

The leaves of *Stevia rebaudiana* contain essential minerals, including macronutrients such potassium (K), calcium (Ca), magnesium (Mg), phosphorus (P), sodium (Na), sulfur (S), and chlorine (Cl), as well as micronutrients such as iron (Fe), zinc (Zn), copper (Cu), manganese (Mn) chromium (Cr), cobalt (Co), selenium (Se), molybdenum (Mo), and iodine (I) (Adotey et al., 2009 Szefer & Nriagu, 2007; Jentzer, 2015). These minerals play vital roles in the ,growth and; maintenance of body functions, participate in the formation of cells and tissues and are essential for maintaining overall health (Szefer & Nriagu, 2007).

Table I.2: Mineral composition of dried *Stevia rebaudiana* leaves (mg/100 g dry weight basis).

Minerals	Goyal et al. (2010)	Savita et al., (2004)	Tadhani & Subhash (2006a)	Kaushik et al. (2010)	Abou-Arab et al. (2010)
Calcium	544	464	1550	722	17.7
Phosphorous	318	11	350	ND	ND
Sodium	89.2	190	160	32.7	14.93
Potassium	1780	1800	2510	839	21.15
Iron	3.9	ND	36.3	31.1	5.89
Magnesium	349	349	ND	ND	3.26
Zinc	1.5	ND	6.39	ND	1.26

ND: Not determined.

I.4.3. Phytochemical Composition :

The phytochemical composition of *Stevia rebaudiana* leaves is rich and diverse. It mainly includes steviol glycosides, phenolic compounds, flavonoids, terpenes, as well as phytosterols and other secondary metabolites. These compounds are responsible for the biological and functional properties of the plant, particularly its antioxidant, antidiabetic, and antimicrobial activities (Lemus-Mondaca et al., 2012; Ceunen & Geuns, 2013).

The main phytochemical compounds identified in *Stevia rebaudiana* leaves and their reported presence in the literature are presented in Table (I.3) .

Table I.3: Main phytochemical compounds identified in *Stevia rebaudiana* leaves and their reported presence in the literature.

Phytochemical group	Main identified compounds	Reported presence	References
Diterpene glycosides	Stevioside, Rebaudioside A, Rebaudioside C	High	Ceunen & Geuns (2013)
Phenolic compounds	Chlorogenic acid, caffeic acid	Present	Lemus-Mondaca et al. (2012)
Flavonoids	Quercetin, Kaempferol, Apigenin	Present	Tadhani & Subhash (2006)
Tannins	Condensed tannins	Moderate	Rengassamy (2015)
Saponins	–	Moderate	Rengassamy (2015)
Phytosterols	β -sitosterol, stigmasterol, campesterol	Present	Bridel & Lavielle (1984)
Terpenes and volatile compounds	Limonene, Caryophyllene	Present	Kaushik et al. (2010)
Alkaloids	Traces of alkaloids	Weakly reported	Tadhani & Subhash (2006)

"The references cited in the table are derived from peer-reviewed scientific studies on the phytochemical composition of *Stevia rebaudiana*."

I.4.4. Essential Oil Composition

According to the AFNOR standard (1986), essential oils are products obtained from plant materials by water or steam distillation and separated from the aqueous phase by physical processes. Their volatile chemical composition is responsible for their characteristic aromatic and biological properties.

The chemical composition of the essential oil extracted from *Stevia rebaudiana* leaves has been investigated using hydrodistillation followed by GC-MS analysis. Studies have shown that the oil is mainly composed of monoterpenes and sesquiterpenes, which are considered the major volatile constituents of the plant (Hossain et al., 2010; Sharma & Kaur, 2016). The relative proportions of these compounds may vary according to environmental conditions, geographical origin, cultivation practices, and extraction methods. Several studies have also reported moderate antioxidant and antimicrobial activities associated with these volatile compounds.

The main monoterpenes and sesquiterpenes identified in the essential oil of *Stevia rebaudiana* leaves are presented.

Table I.4: monoterpenes and sesquiterpenes identified in the essential oil of *Stevia rebaudiana* leaves are presented (Rengassamy, 2015)

Sesquiterpene	Monoterpenes
β -caryophyllene α -humulene caryophyllene oxide Spathulenol α -cadinol β -guaiene	"terpinen-4-ol α -terpineol linalol

I.4.5. Fatty Acid and Amino Acid Composition :

In addition to steviol glycosides and phenolic compounds, *Stevia rebaudiana* leaves contain various nutritional constituents, including fatty acids and amino acids, which contribute to the nutritional and functional value of the plant. Fatty acids are important structural and metabolic components involved in several biological functions, while amino acids participate in protein synthesis and various physiological processes.

- Previous studies have reported the presence of both saturated and unsaturated fatty acids in stevia leaves, including essential fatty acids of nutritional interest (Tadhani & Subhash, 2006). The main fatty acids identified in *Stevia rebaudiana* leaf oil are presented in Table (I.5).

Table I.5: Fatty acid composition of *Stevia rebaudiana* leaf oil and classification according to saturation level (Tadhani & Subhash, 2006).

Classification	Fatty acid	Content (g/100 g oil)
Saturated	Palmitic acid(C16)	27.51
	Stearic acid (C18)	1.18
Unsaturated	Palmitoleic acid (C16-1)	1.27
	Oleic acid (C18-1)	4.36
Polyunsaturated	Linoleic acid (C18-2)	12.40
	Linolenic acid (C18-3)	21.59

- Previous studies have reported the presence of several essential and non-essential amino acids in *Stevia rebaudiana* leaves, contributing to their nutritional value (Abou-Arab et al., 2010). The amino acid composition of stevia leaves is presented in Table (I.6)

Table I.6: Essential and non-essential amino acid composition of *Stevia rebaudiana* leaves (g/100 g dry matter) (Abou-Arab et al., 2010).

Essential amino acids	Value (g/100 g d.m.)	Non-essential amino acids	Value (g/100 g d.m.)
Arginine	0.45	Aspartate	0.37
Lysine	0.70	Serine	0.46
Histidine	1.13	Glutamic acid	0.43
Phenylalanine	0.77	Proline	0.17
Leucine	0.98	Glycine	0.25
Methionine	1.45	Alanine	0.56
Valine	0.64	Cysteine	0.40
Threonine	1.13	Tyrosine	1.08
Isoleucine	0.42		

I.4.6. Vitamin Composition

Vitamins are essential organic compounds involved in numerous metabolic and physiological functions. Among them, water-soluble vitamins such as the vitamin B complex and vitamin C play important roles in antioxidant protection and cellular metabolism (Latham, 2002; Halliwell et al., 1987).

Previous studies have shown that *Stevia rebaudiana* leaves contain several water-soluble vitamins. Kim et al. (2011) reported that folic acid (vitamin B9) was the predominant vitamin in stevia leaf extracts, followed by vitamin C and vitamin B2.

The content of the main water-soluble vitamins identified in dried *Stevia rebaudiana* leaves is presented in Table (I.7).

Table I.7: Vitamin content (mg/100 g) of dried *Stevia rebaudiana* leaves (Kim et al., 2011)

Vitamins	Content (mg/100 g)
Vitamin B1	0.00 ± 0.00
Vitamin B2	0.43 ± 0.02
Vitamin B3	0.00 ± 0.00
Vitamin B6	0.00 ± 0.00
Vitamin B9	52.18 ± 0.21
Vitamin C	14.98 ± 0.07

I.4.7. Steviol Composition

Glycoside

I.4.7.1. Definition and Occurrence:

Steviol glycosides are diterpene glycosides naturally present in *Stevia rebaudiana* Bertoni and are responsible for the characteristic sweet taste of the plant. These compounds are mainly found in the leaves, while lower amounts occur in stems and flowers, and they are generally absent from the roots (Soejarto, 2002; Brandle & Telmer, 2007). Chemically, they consist of a steviol aglycone linked to one or more sugar units such as glucose or rhamnose (Crammer & Ikan, 1986; Kinghorn, 1987).

I.4.7.2. Biosynthesis of Steviol:

Steviol is synthesized in chloroplasts through the methylerythritol phosphate (MEP) pathway, which is involved in terpenoid biosynthesis. This pathway shares several steps with gibberellin biosynthesis until the formation of ent-kaurenoic acid. In *Stevia rebaudiana*, a hydroxylation at carbon 13 converts ent-kaurenoic acid into steviol, differentiating steviol biosynthesis from gibberellin formation (Brandle & Telmer, 2007; Ceunen & Geuns, 2013).

The main step involved in steviol biosynthesis from ent-kaurenoic acid is presented in Figure I.3.

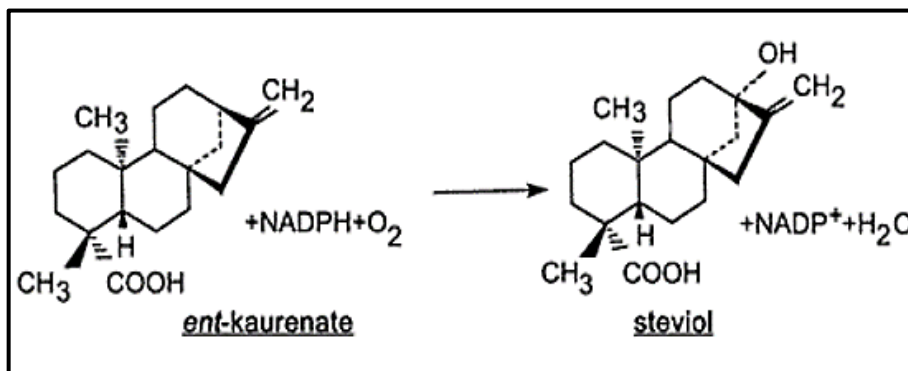


Figure I.3: Biosynthetic conversion of ent-kaurenoate into steviol (adapted from Brandle et al., 2002).

I.4.7.3. Chemical Structure of Steviol Glycosides

Steviol glycosides possess a diterpene skeleton derived from steviol, with sugar moieties attached mainly at carbon positions C13 and C19 (Chatsudthipong & Muanprasat, 2009). Figure I.4 shows the chemical structure of steviol and its carbon numbering system.

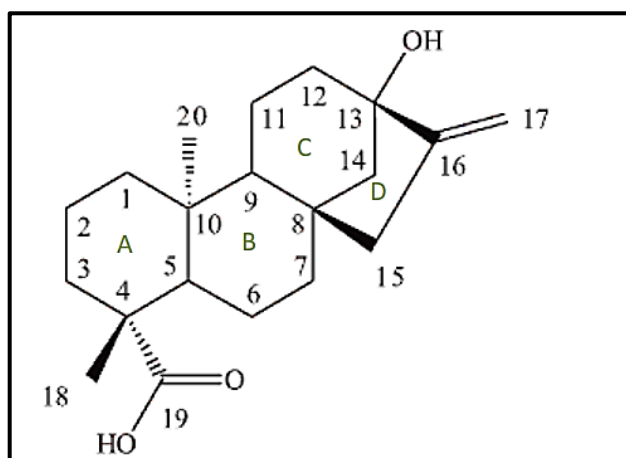


Figure I.4: Chemical structure of steviol and carbon numbering system (adapted from Chatsudthipong & Muanprasat, 2009).

The sugar attachment at C13 occurs through an O-glycosidic bond, while the linkage at C19 is formed through an ester bond. Differences in the number and arrangement of sugar units lead to the formation of several glycosides with different sweetness intensities and aftertaste properties (Crammer & Ikan, 1986; Kinghorn, 1987). The structural organization of steviol glycosides and the formation of rebaudioside A are illustrated in Figure I.5.

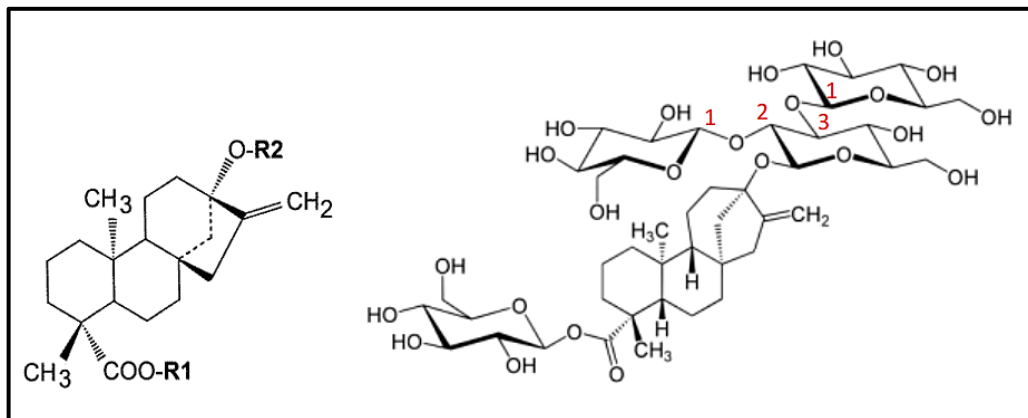


Figure I.5: Basic structure of steviol glycosides and formation of rebaudioside A (adapted from Ceunen & Geuns, 2013).

I.4.7.4. Main Types of Steviol Glycosides

More than forty steviol glycosides have been identified in *Stevia rebaudiana* leaves. Stevioside and rebaudioside A are the most abundant compounds and contribute mainly to the sweetening power of the plant. Other important glycosides include rebaudiosides B–F, steviolbioside, and dulcoside A (Kennelly, 2002; Starratt et al., 2002; Ceunen & Geuns, 2013). The principal steviol glycosides identified in *Stevia rebaudiana* leaves and their structural characteristics are summarized in Table I.8.

Table I.8: Main steviol glycosides identified in *Stevia rebaudiana* leaves

Name	R1	R2	Molecular Formula	References
Steviol	H	H	C ₂₀ H ₃₀ O ₃	Mine et al. (2004)
Steviolbioside	H	β -Glc- β -Glc (2 →1)	C ₃₂ H ₅₀ O ₁₃	Kohda et al. (1976)
Stevioside	β -Glc	β -Glc- β -Glc (2 →1)	C ₃₈ H ₆₀ O ₁₈	Bridel & Lavieille (1931)
Rebaudioside A	β -Glc	β -Glc- β -Glc (2 →1) β -Glc (3→1)	C ₄₄ H ₇₀ O ₂₃	Kohda et al. (1976)
Rebaudioside B	H	β -Glc- β -Glc (2 →1) β -Glc (3→1)	C ₃₈ H ₆₀ O ₁₈	Kohda et al. (1976)
Rebaudioside C	β -Glc	β -Glc- α -Rha (2→1) β -Glc (3→1)	C ₄₄ H ₇₀ O ₂₂	Sakamoto et al. (1977a)
Rebaudioside D	β -Glc- β - Glc (2→1)	β -Glc- β -Glc (2 →1) β -Glc (3→1)	C ₅₀ H ₈₀ O ₂₈	Sakamoto et al. (1977b)
Rebaudioside E	β -Glc- β - Glc (2→1)	β -Glc- β -Glc (2 →1)	C ₄₄ H ₇₀ O ₂₃	Sakamoto et al. (1977b)
Rebaudioside F	β -Glc	β -Glc- β -Xyl (2 →1) β -Glc (3→1)	C ₄₃ H ₆₈ O ₂₂	Starratt et al. (2002)
Dulcoside A	β -Glc	β -Glc- α -Rha (2→1)	C ₃₈ H ₆₀ O ₁₇	Kobayashi et al. (1977)

I. 4.7.5. Biosynthesis of steviol glycosides

The biosynthesis of steviol glycosides occurs mainly in the leaves of *Stevia rebaudiana*, where thesesweet compounds are synthesized, transported, and accumulated (**Brandle &**

Telmer, 2007; Morine, 2015). The biosynthetic pathway begins in the chloroplasts through the 2-C-methyl-D-erythritol-4-phosphate (MEP) pathway, the same metabolic route involved in terpenoid and gibberellin biosynthesis. Starting from pyruvate and glyceraldehyde-3-phosphate, the pathway leads to the formation of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), which are the main precursors of diterpenes (**Hedden & Thomas, 2012**).

Subsequently, a series of condensation and cyclization reactions results in the formation of ent-kaurene, which is then oxidized and hydroxylated to produce steviol, the aglycone precursor of steviol glycosides (**Brandle & Telmer, 2007**). Unlike the gibberellin pathway, steviol biosynthesis involves a specific hydroxylation at carbon 13, which directs the pathway toward steviol formation rather than plant hormone synthesis (**Ceunen & Geuns, 2013**).

The glycosylation of steviol occurs mainly in the cytosol through the action of UDP-glycosyltransferases (UGTs), which catalyze the attachment of glucose, rhamnose, or xylose residues at positions C13 and C19 of the steviol skeleton. Several UGT enzymes have been identified in *Stevia rebaudiana*, particularly UGT85C2, UGT74G1, and UGT76G1, which play key roles in the synthesis of stevioside, rebaudioside A, and other steviol glycosides (**Brandle & Telmer, 2007; Guleria & Yadav, 2013**).

Intermediate glycosides such as rubusoside participate in sequential glycosylation reactions leading to the formation of major compounds including stevioside, rebaudioside A, rebaudioside D, and rebaudioside M (**Yadav & Guleria, 2012**). As additional sugar residues are incorporated, steviol glycosides become increasingly hydrophilic and are finally transported through the endoplasmic reticulum and Golgi apparatus before accumulating in the vacuoles of leaf cells (**Morine, 2015**). The main stages involved in steviol glycoside biosynthesis and intracellular transport are illustrated in Figure I.6.

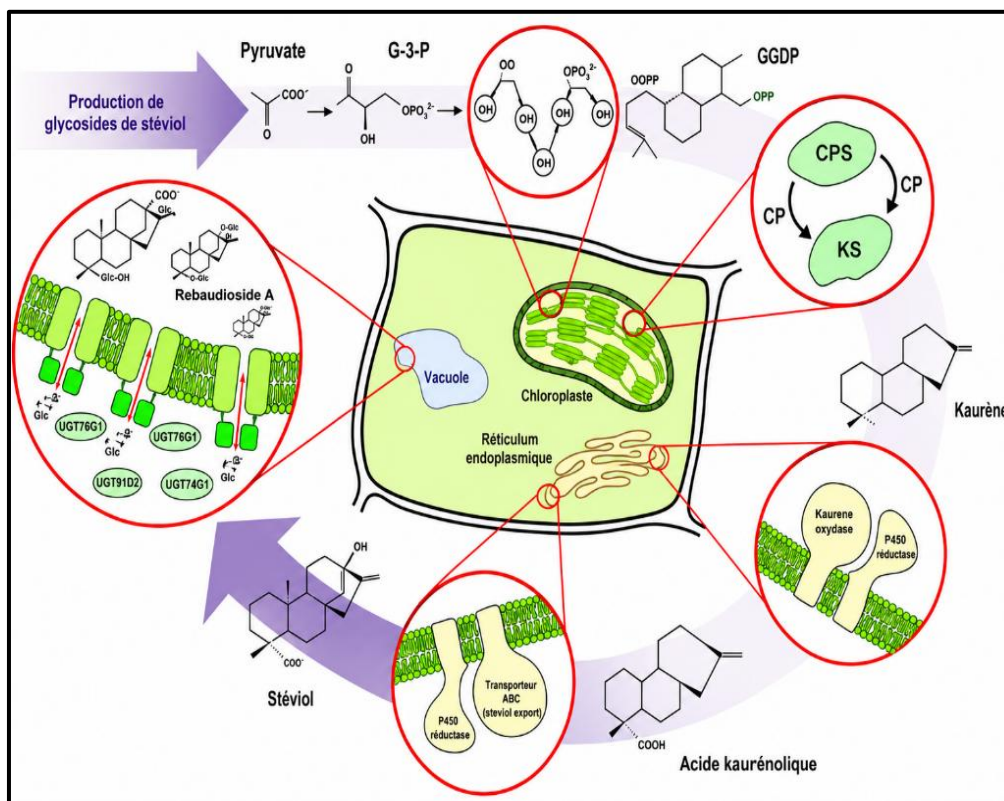


Figure I.6: Simplified schematic representation of the biosynthetic pathway and intracellular localization of steviol glycosides in *Stevia rebaudiana*. Source: (Ceunen et al., 2012).

The composition and concentration of steviol glycosides are influenced by genetic and environmental factors, including cultivar, photoperiod, temperature, and agronomic practices. Previous studies demonstrated that short-day conditions promote higher steviol glycoside accumulation compared to long-day conditions, particularly in upper leaves (Rengassamy, 2015).

I.4.7.6. Sweetening Power of Steviol Glycosides

The sweet taste of *Stevia rebaudiana* leaves is mainly attributed to the presence of steviol glycosides, which possess a sweetening power significantly higher than that of sucrose. Sweetening power refers to the relative sweetness intensity of a compound compared to sucrose, which is conventionally assigned a value of 1. It is generally evaluated through sensory analyses using aqueous sucrose solutions as a reference (Lemus-Mondaca et al., 2012).

The sweetening intensity of steviol glycosides depends mainly on their chemical structure, particularly the degree of glycosylation, the number and type of sugar residues, and the position of glycosidic linkages. These structural variations influence not only sweetness intensity but also taste quality and aftertaste characteristics (Ceunen & Geuns, 2013; Kinghorn, 2002).

The principal steviol glycosides identified in *Stevia rebaudiana* leaves and their relative sweetening powers are presented in Table I.9.

Table I.9: Relative sweetening power of major steviol glycosides compared with sucrose
Adapted from **Ceunen & Geuns (2013)** and **Kinghorn (2002)**.

Compound	Relative Sweetening Power
Stevioside	150 - 250
Rebaudioside A	200 - 300
Rebaudioside B	150
Rebaudioside C	30
Rebaudioside D	221
Rebaudioside E	174
Rubusoside	114
Dulcoside A	30
Steviolbioside	90
Rebaudioside F	200

I.5. Metabolism of Steviol Glycosides

The leaves of *Stevia rebaudiana* contain ent-kaurene diterpene glycosides, mainly stevioside and rebaudiosides, which are responsible for the intense sweet taste of the plant. These compounds are highly water-soluble and may exhibit sweetness levels up to 300 times greater than sucrose, while providing little or no caloric value. Steviol glycosides are widely used as natural sweeteners and are considered safe for human consumption within approved intake levels (**Soejarto et al., 1982; Megeji et al., 2005; Geuns et al., 2007**).

The metabolic fate of steviol glycosides is generally similar in humans and experimental animals. Previous studies demonstrated that these compounds follow a common pathway involving intestinal hydrolysis, absorption, hepatic conjugation, and urinary excretion (**Geuns, 2003; Geuns et al., 2007**). The metabolic pathway of steviol glycosides in humans, including intestinal hydrolysis, hepatic glucuronidation, and urinary excretion, is illustrated in Figure I.7.

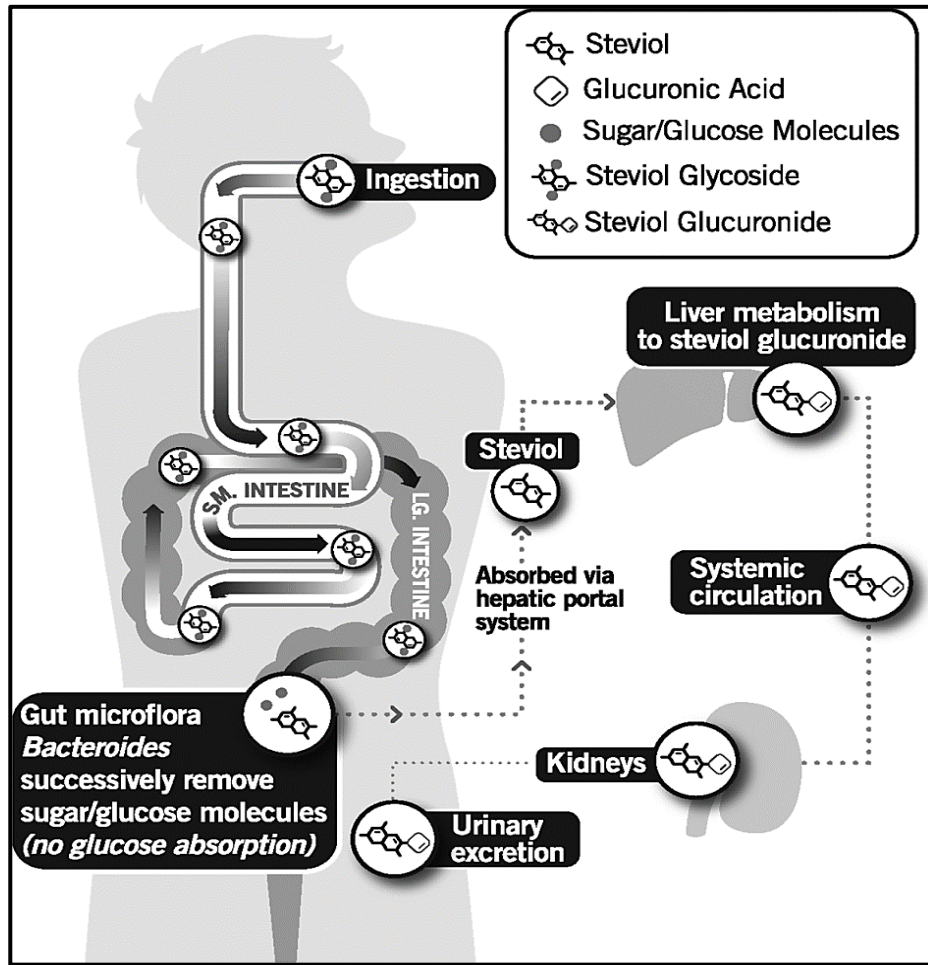


Figure I.7: Metabolic pathway of steviol glycosides in humans. Adapted from Gardana et al. (2003).

I.5.1. Hydrolysis by Intestinal Microbiota

Steviol glycosides are resistant to hydrolysis by gastric juice and digestive enzymes in the upper gastrointestinal tract. Consequently, they reach the colon largely unchanged, where they are hydrolyzed by intestinal microbiota. Rebaudioside A is first converted into stevioside, which is subsequently degraded into glucose and steviol (Koyama et al., 2003; Wingard et al., 1980).

The released glucose is mainly utilized by colonic bacteria and therefore contributes minimally to blood glucose levels. Steviol represents the final aglycone product generated during this metabolic process (Koyama et al., 2003).

I.5.2. Formation and Absorption of Steviol

All steviol glycosides are considered metabolically equivalent because they ultimately yield steviol after microbial hydrolysis. This metabolic equivalence allows toxicological and

safety data obtained for stevioside to be extrapolated to rebaudioside A and related steviol glycosides (**Koyama et al., 2003**).

Following its formation in the colon, steviol is absorbed through the intestinal wall and transported to the liver. Although some interindividual variability has been reported, the overall metabolic profile of steviol glycosides is considered comparable between humans and laboratory animals (**Wingard et al., 1980**).

I.5.3. Hepatic Glucuronidation and Excretion

In the liver, steviol undergoes glucuronidation to form steviol glucuronide, which is released into the bloodstream and subsequently filtered by the kidneys. The conjugated metabolite is then excreted mainly in the urine, whereas only small amounts are eliminated through feces (**Chatsudthipong & Muanprasat, 2009**).

In vitro studies demonstrated that stevioside is highly stable under gastric and enzymatic digestive conditions and cannot be hydrolyzed by digestive enzymes alone. Hydrolysis occurs primarily through the action of intestinal microflora, which convert stevioside into steviol before its final excretion as steviol glucuronide (**Hutapea et al., 1997; Chatsudthipong & Muanprasat, 2009**).

I.6. Biological and Pharmacological Effects of Steviol Glycosides

I.6.1. Anti-obesity Effect

Obesity is a major metabolic disorder associated with excessive fat accumulation and increased risk of diseases such as diabetes, hypertension, and cardiovascular disorders (**Stephen et al., 2010**). High consumption of sugar-rich foods and beverages is considered one of the main factors contributing to weight gain and obesity.

Stevia rebaudiana contains steviol glycosides, mainly stevioside and rebaudioside A, which provide negligible caloric value and are approximately 200–300 times sweeter than sucrose (**Soejarto et al., 1982; Megeji et al., 2005**). Due to their intense sweetness and low calorie content, stevia-based sweeteners are widely used as sugar substitutes in foods and beverages.

The replacement of sucrose with stevia may help reduce total caloric intake and support weight management programs. Some experimental studies, mainly in animal models, have reported that steviol glycosides may reduce body weight gain and decrease cravings for sweet foods (**Curry & Roberts, 2008; Jain et al., 2007**). Therefore, stevia is considered a promising natural sweetener for obesity prevention and dietary management.

I.6.2. Anti-hyperglycemic effect

Several pharmacological studies conducted on diabetic rats have shown that aqueous, methanolic, and ether extracts of *Stevia rebaudiana* leaves may exert antihyperglycemic activity. In particular, benzo-acetonic extracts administered at doses of 200 and 400 mg/kg for 10 days produced a significant reduction in blood glucose levels in diabetic rats (**Shivanna et al., 2013**).

In addition, *Stevia* leaves and their polyphenolic compounds have been associated with increased serum insulin levels and protective effects on hepatic and renal tissues in experimental diabetic models (**Himanshu et al., 2011**). Several mechanisms have been proposed to explain these effects, including improvement of mitochondrial respiration and inhibition of gluconeogenesis pathways (**Ferreira et al., 2006**).

Clinical investigations in humans have also suggested a potential antihyperglycemic effect of *Stevia*. In a study conducted on human volunteers, postprandial blood glucose levels were significantly lower in subjects receiving *Stevia* extract compared to the control group (**Curi et al., 1986**). More recent reviews have supported the potential role of steviol glycosides in glucose regulation and metabolic health, although additional clinical studies are still required to confirm these effects in humans (**Chatsudthipong & Muanprasat, 2009; Ceunen & Geuns, 2013**). The possible mechanisms involved in the antihyperglycemic effect of *Stevia rebaudiana* are illustrated in Figure II.8.

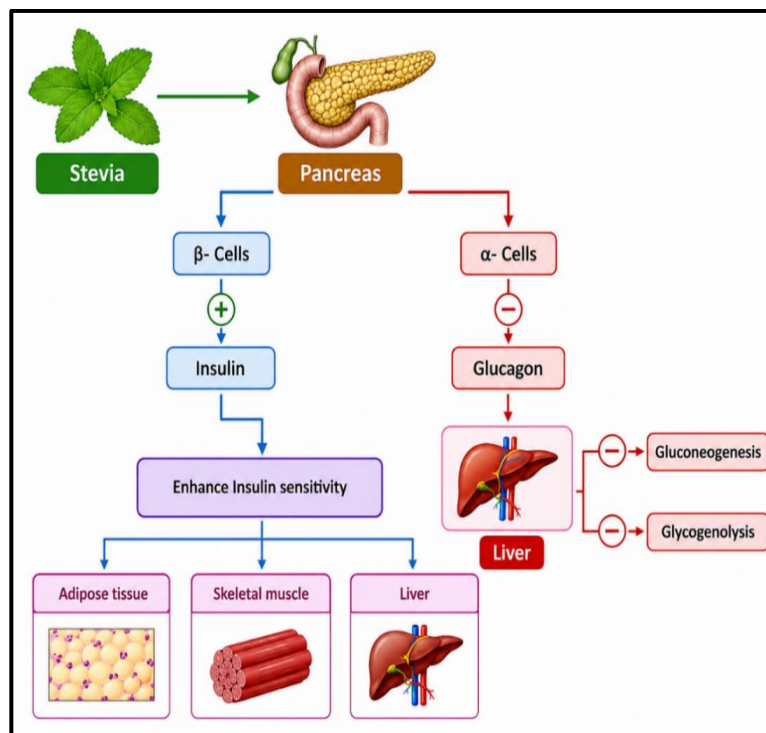


Figure II.8: The possible mechanisms of the antihyperglycemic action of *Stevia rebaudiana*.

Adapted from **Gandhi et al. (2018)**.

I.6.3. Antioxidant Effect

Antioxidants are compounds capable of neutralizing free radicals, whose excessive accumulation may induce oxidative stress involved in the development of several chronic diseases (**Devasagayam et al., 2004**). Interest in natural plant-derived antioxidants has increased due to concerns regarding the safety of synthetic antioxidants such as BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene) (**Shahidi & Ambigaipalan, 2015**).

Aqueous and ethanolic extracts of *Stevia rebaudiana* have shown notable antioxidant potential. Several studies reported that these extracts possess inhibitory activity against hydroxyl radicals, nitric oxide, and superoxide anions (**Hajhashemi & Geuns, 2013**). This antioxidant activity is mainly attributed to the high content of phenolic compounds and flavonoids present in *Stevia* leaves (**Tadhani et al., 2006**). In addition, aqueous leaf extracts demonstrated protective effects against cellular oxidative stress (**Ferrazzano et al., 2015**). Different in vitro methods are commonly used to evaluate the antioxidant capacity of *Stevia* extracts, including DPPH radical scavenging assay, ABTS assay, ferric reducing antioxidant power (FRAP), and oxygen radical absorbance capacity (ORAC). The antioxidant activity varies according to the extraction solvent, with methanolic and acetonic extracts generally exhibiting higher activity than aqueous extracts (**Lemus-Mondaca et al., 2012**).

One study demonstrated that *Stevia* leaf extract inhibited hydroperoxide formation in sardine oil, with an antioxidant activity comparable or superior to that of DL- α -tocopherol and green tea extract. This activity is related to the ability of *Stevia* compounds to donate electrons or hydrogen atoms, thereby contributing to the neutralization of reactive oxygen species (**Lemus-Mondaca et al., 2012**). Overall, *Stevia rebaudiana* extracts represent a promising source of natural antioxidants with potential applications in the prevention of oxidative damage and the development of functional foods and health supplements.

I.6.4. Anticariogenic Effect

Dental caries is a chronic infectious disease resulting from the action of oral bacteria that ferment dietary carbohydrates, mainly sucrose, glucose, and fructose, into organic acids. The accumulation of these acids leads to progressive demineralization of tooth enamel and subsequent cavity formation. *Streptococcus mutans* is considered the principal cariogenic bacterium, followed by *Lactobacillus casei* and *Streptococcus sanguinis*.

Unlike nutritive sweeteners such as sucrose, steviol glycosides from *Stevia rebaudiana* are non-nutritive sweeteners that contribute minimally to acid production because they are not readily fermented by oral microorganisms (**Ramarao et al., 2017**). In addition, several studies have reported inhibitory effects of steviol glycosides on the growth and acid production of cariogenic

bacteria, particularly *S. mutans*, which may reduce dental plaque formation and caries risk **.(Ramarao et al., 2017; Wu et al., 1998).**

Steviol glycosides may also stimulate salivary secretion, thereby contributing to acid neutralization, removal of food debris, and enamel remineralization **(Ferrazzano et al., 2015)** ,Furthermore, animal studies demonstrated that sucrose induced dental caries in rat pups whereas stevioside did not produce similar cariogenic effects **(Das et al., 1992).**

Overall, steviol glycosides represent promising non-cariogenic alternatives to conventional sugars and may contribute to oral health preservation when combined with adequate oral hygiene practices.

I.6.5 .Antihypertensive Effect

Hypertension is a major risk factor for cardiovascular, renal, and cerebrovascular diseases **(Oparil et al., 2018)**. Several studies have investigated the potential antihypertensive effects of steviol glycosides from *Stevia rebaudiana*.

Proposed mechanisms include reduction of sodium reabsorption in renal tubules, promotion of natriuresis and diuresis, and vasodilation, which may contribute to blood pressure regulation **(Chan et al., 2000; Hajhashemi & Geuns, 2013)**. Some studies also suggested a possible involvement of the renin–angiotensin–aldosterone system (RAAS) **(Thines, 2001)**.

Clinical results remain inconsistent. A two-year study reported significant reductions in systolic ,and diastolic blood pressure in hypertensive patients treated with stevioside **(Hsieh et al 2003)**. However, other studies found no clinically significant effect of rebaudioside A on blood pressure in healthy adults **(Maki et al., 2008)**. Animal studies have also shown variable results depending on dose and treatment duration **(Melis, 1995; 1997)**.

Despite these variations, steviol glycosides are generally recognized as safe. The European Food Safety Authority (EFSA) established an acceptable daily intake of 4 mg/kg body weight/day expressed as steviol equivalents **(EFSA, 2010)**. Overall, steviol glycosides represent a promising natural alternative for blood pressure management, although further clinical studies are still needed to confirm their efficacy.The proposed mechanism involved in the antihypertensive effect of stevioside is illustrated in Figure II.9.

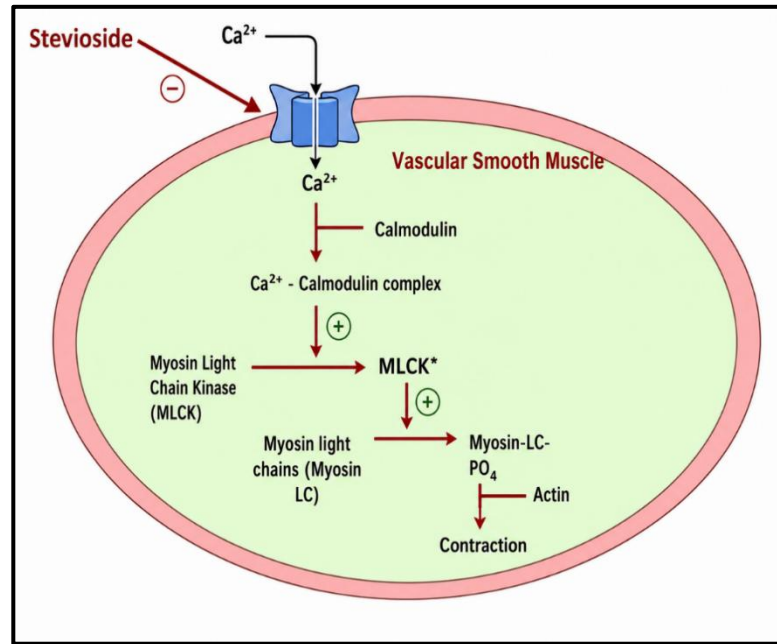


Figure II.9: Proposed mechanism of the antihypertensive effect of stevioside through inhibition of Ca^{2+} influx in vascular smooth muscle cells. Adapted from **Gandhi et al. (2018)**.

I.6.6. Anti-inflammatory Effect

Chronic inflammation is a persistent pathological condition associated with the developmen of several diseases, including obesity, cardiovascular disorders, autoimmune diseases, and inflammatory bowel disease (IBD) (**Fleit, 2014**). Proinflammatory cytokines such as tumor necrosis factor-alpha ($\text{TNF-}\alpha$), interleukin-1 beta ($\text{IL-1}\beta$), and nitric oxide (NO) play important roles in the inflammatory response.

Several studies have suggested that steviol glycosides from *Stevia rebaudiana*, particularly stevioside and steviol, may exert anti-inflammatory effects. **Boonkaewwan et al. (2006)** reported that stevioside inhibited the activation of nuclear factor kappa B ($\text{NF-}\kappa\text{B}$) and reduced the release of inflammatory mediators in lipopolysaccharide-stimulated THP-1 cells. In addition, steviol has been reported to inhibit inflammatory responses induced by phorbol ester .treatment (**Mizushina et al., 2005**)

Experimental studies also suggested that stevioside may contribute to tissue recovery by modulating inflammatory signaling pathways (**Bunprajun et al., 2012**). Furthermore, steviol and related compounds showed inhibitory effects on intestinal chloride secretion and intestinal hypermotility, which may be beneficial in inflammatory bowel disorders and diarrhea-associated conditions (**Shiozaki et al., 2006; Pariwat et al., 2008**).

Overall, these findings suggest that steviol glycosides may possess potential anti-inflammatory .properties through the modulation of inflammatory mediators and signaling pathways. However, further clinical studies are still needed to confirm these effects in humans.

I.7. Stevia and Glycemic Index

I.7.1. Definition of Glycemic Index (GI)

The glycemic index (GI) is a physiological classification system that ranks carbohydrate-containing foods according to their effect on postprandial blood glucose levels. It is determined by comparing the blood glucose response produced by a test food containing 50 g of available carbohydrates with that produced by the same amount of glucose used as a reference (GI = 100) (Jenkins et al., 1981). Foods with a low GI produce a slower increase in blood glucose and are generally associated with better glycemic control. Several factors can influence GI, including the type of carbohydrate, food processing, cooking methods, and the presence of dietary fiber, proteins, and fats (Augustin et al., 2015). Because steviol glycosides are non-nutritive sweeteners that are not metabolized to produce glucose and do not raise blood sugar levels, they have no measurable GI value. When used as a substitute for sucrose (GI $\approx$ 65-68), stevia can significantly reduce the glycemic load of foods and beverages, making it a valuable tool for glycemic management in diabetic and weight-control diets.

Table I.10: Glycemic Index Values of Selected Foods .Values adapted from international glycemic index tables (Atkinson et al., 2008).

Food	Glycemic Index (Glucose = 100)
Apples	36-40
Spaghetti	42-49
Carrots	39-47
Bananas	48-54
Brown rice	50-55
Potato chips	54-56
Honey	55-61
Oatmeal	55-60
Sucrose (table sugar)	65-68
White rice	64-72
White bread	70-75
Watermelon	72-76
Baked potato	78-85
Glucose	100

I.7.2. Effect of *Stevia* on Blood Glucose

▪ Metabolism and Glycemic Response

Several clinical and experimental studies have reported that steviol glycosides, the principal sweet compounds of *Stevia rebaudiana*, exert minimal effects on blood glucose levels. Unlike sucrose, steviol glycosides are not metabolized for energy production and do not cause significant increases in postprandial glycemia or insulin secretion, (Geuns, 2003; Gardana et al., 2003).

After ingestion, steviol glycosides are not absorbed in the upper gastrointestinal tract. They are metabolized by intestinal microbiota into steviol, which is subsequently converted into steviol glucuronide in the liver and excreted in the urine (Ferri et al., 2006). This metabolic pathway explains their negligible caloric value and low glycemic impact.

I.7.3. Comparison with Sucrose

Human studies have suggested that replacing sucrose with stevia may help reduce postprandial blood glucose levels and lower total caloric intake (Gegersen et al., 2004; Anton et al., 2010). In contrast to sucrose, which rapidly increases blood glucose and insulin levels, steviol glycosides provide intense sweetness with virtually no calories and minimal influence on glycemic response (Prakash et al., 2008). Therefore, stevia is considered a promising low-glycemic alternative to conventional sugar, particularly for individuals requiring glycemic control. To better illustrate the metabolic and functional differences between sucrose and steviol glycosides, a comparative summary is presented in Table I.11.

Table I.11: Presents a comparison between steviol glycosides and sucrose in terms of sweetness, glycemic effect, caloric value, insulin response, cariogenicity, and acceptable daily intake.

Parameter	Sucrose	Steviol Glycosides
Caloric value	≈ 4 kcal/g	0 kcal/g
Sweetness relative to sucrose	1	150-300 times sweeter
Glycemic index (glucose = 100)	65-68	≈ 0 or negligible effect
Effect on blood glucose	Increases postprandially	No increase; may slightly reduce
Insulin response	Increases insulin secretion	No effect or slight reduction
Fermentability by oral bacteria	Yes (cariogenic)	No (non-cariogenic)
Acceptable daily intake (ADI)	None established	4 mg/kg body weight/day (as steviol equivalents)

Adapted from Geuns (2003), Anton et al. (2010), Prakash et al. (2008), EFSA (2010), and Atkinson et al. (2021).

I.8. Limitations of Stevia

Despite its numerous nutritional and therapeutic advantages, the use of *Stevia rebaudiana* is still associated with certain limitations related mainly to sensory properties and stability during food processing and storage. These factors may influence consumer acceptance and industrial applications of steviol glycosides.

I.8.1. Bitterness and Aftertaste

Despite their high sweetening power, steviol glycosides may present undesirable sensory properties, particularly bitterness and a licorice-like aftertaste. These effects are more pronounced with stevioside, whereas rebaudioside A and rebaudioside M generally exhibit a cleaner and more pleasant taste (Prakash et al., 2008; DuBois & Prakash, 2012).

The bitter aftertaste is related to the interaction of steviol glycosides with sweet and bitter taste receptors (Hellfritsch et al., 2012). To improve sensory quality, several approaches have been developed, including enzymatic modification of glycosides and blending stevia with other sweeteners or flavor-masking agents (Prakash et al., 2014).

I.8.2. Stability Issues

Steviol glycosides are generally stable under normal food-processing conditions and are resistant to moderate heat. However, prolonged exposure to high temperatures or extreme pH conditions may reduce their stability and sweetness intensity (Kroyer, 2010; Jookan et al., 2012).

In acidic beverages and liquid formulations, degradation may occur during long storage periods. Therefore, purified or modified steviol glycosides are often used to improve stability and sensory quality in food products (Prakash et al., 2008; Lobete et al., 2016).

Conclusion

Stevia rebaudiana is an important natural source of steviol glycosides, which are widely used as low-calorie sweeteners with minimal effects on blood glucose levels. In addition to its sweetening properties, stevia contains several bioactive compounds that contribute to its nutritional and biological value.

Many studies have reported potential beneficial effects of stevia, including antioxidant, antihyperglycemic, antihypertensive, and anticarcinogenic activities. However, some limitations, such as bitterness and stability issues, may affect its use in food products.

Overall, stevia represents a promising natural alternative to sucrose for food, nutritional, and health applications.

Chapter II

Biological Activities Of *Stevia* *Rebaudiana*

Chapter II: Biological Activities Of *Stevia Rebaudiana*

Stevia rebaudiana is not only recognized as a natural non-caloric sweetener, but also as a valuable source of bioactive compounds, particularly polyphenols such as flavonoids, tannins, and phenolic acids. These compounds are known for their important biological properties, especially their antioxidant and antidiabetic activities (Goyal et al., 2010; Shahidi & Ambigaipalan, 2015).

Polyphenols exert their biological effects through several mechanisms, including free radical scavenging, metal chelation, modulation of carbohydrate-digesting enzymes, and regulation of glucose metabolism (Hanhineva et al., 2010). In addition to their health-promoting effects, stevia polyphenols also contribute to the functional and technological properties of food systems. This chapter focuses on the classification, structure, and biological activities of polyphenols in *Stevia rebaudiana*, with particular emphasis on their antioxidant, antidiabetic, and technological applications.

II.1. Polyphenols: Classification and Chemical Structure

II.1.1. Definition and Biosynthesis

Polyphenols, also known as phenolic compounds, constitute one of the largest groups of secondary metabolites naturally present in plants. They are characterized by one or more aromatic rings bearing hydroxyl groups and are mainly synthesized through the shikimate and phenylpropanoid pathways (Rezzik, 2016).

These compounds play important roles in plant physiology and defense. In particular, they help protect plants against pathogens such as fungi and bacteria and contribute to protection against ultraviolet (UV) radiation due to their ability to absorb solar energy (Sarni-Manchado, 2006).

II.1.2. General Classification of Polyphenols

Polyphenols are generally classified into three major groups: flavonoids, phenolic acids, and tannins. Flavonoids represent the most abundant and diverse class, while phenolic acids mainly include hydroxybenzoic and hydroxycinnamic acids. Tannins are subdivided into hydrolyzable and condensed tannins (Shahidi & Ambigaipalan, 2015; Dai & Mumper, 2010).

II.1.3. General Chemical Structure of Polyphenols

Polyphenols are structurally characterized by aromatic rings substituted with hydroxyl (-OH) groups. Their structural diversity depends on the number of aromatic rings, the degree of hydroxylation, and the presence of additional groups such as methoxy or glycosyl moieties. These structural variations are largely responsible for the diversity of their chemical and biological properties (**Rice-Evans et al., 1997**). The general chemical structure of polyphenols is illustrated in Figure II.1

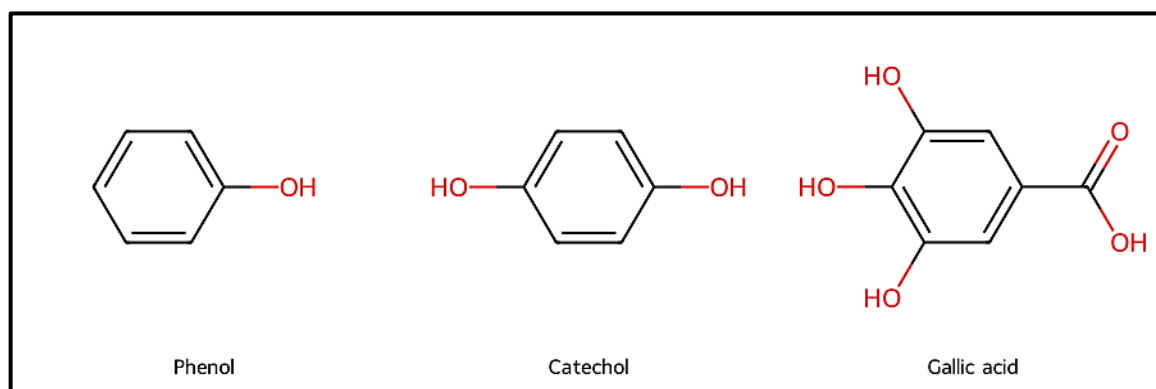


Figure II.1: Representative chemical structures of polyphenolic compounds showing aromatic rings and hydroxyl groups. Adapted from **Rice-Evans et al. (1997)** and **Dai & Mumper (2010)**

II.1.4. Flavonoids

Flavonoids are among the most important subclasses of polyphenols and are widely distributed in plants. They contribute to the coloration of flowers, fruits, and leaves and participate in plant defense against environmental stress. In addition, flavonoids are recognized for several biological activities, particularly their antioxidant properties (**Harborne, 1998; Crozier et al., 2009**).

II.1.4.1. Chemical Structure of Flavonoids

Flavonoids possess a common C6–C3–C6 carbon skeleton composed of two aromatic rings (A and B) connected by a three-carbon bridge forming a heterocyclic ring (C). According to the oxidation state and substitution pattern of the central ring, flavonoids are classified into different subclasses such as flavonols, flavones, flavanols, and anthocyanins (**Harborne, 1998**). The general chemical structure of flavonoids and their main subclasses are illustrated in Figure II.2.

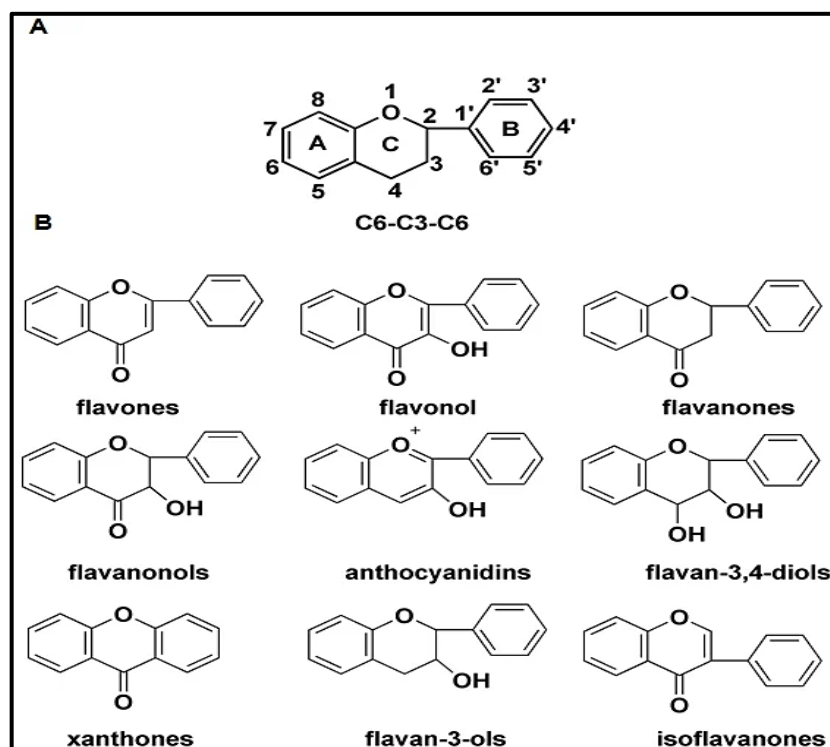


Figure II.2: Basic flavonoid skeleton (C6–C3–C6) and main flavonoid subclasses.

Adapted from Crozier et al. (2009)

II.1.5. Tannins

Tannins are high-molecular-weight polyphenolic compounds characterized by their astringent taste and their ability to bind proteins. They contribute to plant defense by limiting microbial growth and deterring herbivores. Due to these properties, tannins have also been used in traditional medicinal and industrial applications (Crozier et al., 2009).

II.1.5.1. Classification of Tannins

Tannins are divided into two principal groups: hydrolyzable tannins and condensed tannins. Hydrolyzable tannins are mainly esters of gallic or ellagic acid, whereas condensed tannins, also called proanthocyanidins, are polymers of flavan-3-ol units such as catechin (Shahidi & Ambigaipalan, 2015; Dai & Mumper, 2010).

The main structural differences between hydrolyzable and condensed tannins are illustrated in Figure II.3.

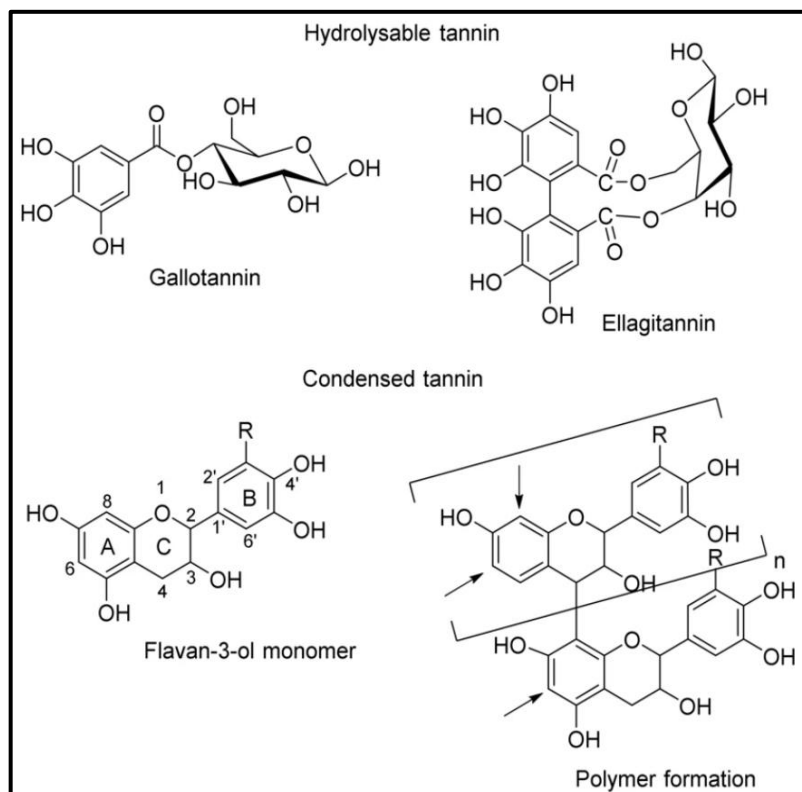


Figure II.3: Chemical structures of hydrolyzable and condensed tannins. Adapted from **Shahidi & Ambigaipalan (2015)**.

II.1.6. Structure–Activity Relationship (SAR)

The biological activity of polyphenols, especially their antioxidant capacity, is closely related to their chemical structure. Their antioxidant mechanisms mainly involve hydrogen atom transfer (HAT) and single electron transfer (SET), through which polyphenols may neutralize free radicals (**Heim et al., 2002**).

Several structural factors influence antioxidant activity, including the number and position of hydroxyl groups, the presence of conjugated double bonds, and the degree of glycosylation. In general, flavonoids containing multiple hydroxyl groups and a double bond between C2 and C3 exhibit stronger antioxidant activity, whereas glycosylation may reduce activity due to steric effects (**Rice-Evans et al., 1996**).

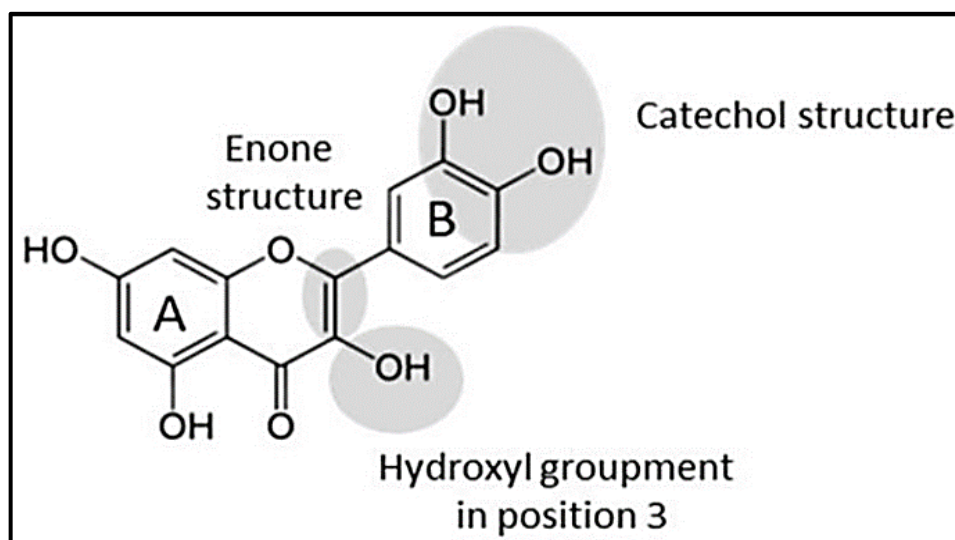


Figure II.4: Main structural determinants of flavonoid antioxidant activity, including hydroxyl groups and conjugated double bonds. Adapted from Heim et al. (2002) and Rice-Evans et al. (1996)

II.2. Polyphenolic Compounds in *Stevia rebaudiana*

II.2.1. Major Polyphenols Identified in *Stevia*

Several polyphenolic compounds have been identified in *Stevia rebaudiana* leaves, mainly flavonoids, phenolic acids, and tannins. The principal flavonoids include quercetin, kaempferol, rutin, and apigenin derivatives, while chlorogenic and caffeic acids are the predominant phenolic acids. Chlorogenic acids belong to the hydroxycinnamic acid family and are recognized for their strong antioxidant properties. Condensed tannins (proanthocyanidins) are also present in lower concentrations (Lemus-Mondaca et al., 2012; Kim et al., 2011).

The major polyphenolic compounds identified in *Stevia rebaudiana* leaves are summarized in Table II.1.

Table II.1: Major polyphenolic compounds identified in *Stevia rebaudiana* leaves

Polyphenol class	Main compounds identified
Flavonoids	Quercetin, kaempferol, rutin, apigenin
Phenolic acids	Chlorogenic acid, caffeic acid
Tannins	Proanthocyanidins

Adapted from Lemus-Mondaca et al. (2012) and Kim et al. (2011).

II.2.2. Contribution of Polyphenols to Biological Activities

Stevia rebaudiana leaves contain several bioactive polyphenolic compounds, mainly flavonoids, phenolic acids, and tannins. The principal flavonoids identified include quercetin, kaempferol, rutin, and apigenin derivatives, while chlorogenic and caffeic acids are the predominant phenolic acids (Kim et al., 2011; Lemus-Mondaca et al., 2012). It is important to note that steviol glycosides, such as stevioside and rebaudioside A, are diterpene glycosides and do not belong to the polyphenol family. Nevertheless, both polyphenols and steviol glycosides contribute to the biological and functional properties of *Stevia rebaudiana* (Goyal et al., 2010).

Polyphenols from stevia exhibit significant antioxidant activity through their ability to neutralize reactive oxygen species and chelate metal ions, thereby reducing oxidative stress (Shahidi & Ambigaipalan, 2015). In addition, several studies suggest that these compounds may contribute to anti-inflammatory effects by reducing the production of pro-inflammatory mediators. Polyphenols may also exert antidiabetic effects through the inhibition of carbohydrate-digesting enzymes such as α -amylase and α -glucosidase, which can help reduce postprandial blood glucose levels (Chatsudthipong & Muanprasat, 2009; Hanhineva et al 2010.).

II.3. Antioxidant Mechanisms of Polyphenols

II.3.1. Free Radical Scavenging and Metal Chelation

Polyphenols exert their direct antioxidant activity primarily through electron or hydrogen atom donation, which allows them to neutralize reactive free radicals and reduce oxidative damage. This antioxidant effect mainly occurs through two mechanisms: Hydrogen Atom Transfer (HAT) and Single Electron Transfer (SET) (Rice-Evans et al., 1997). In addition, polyphenols are able to chelate transition metal ions such as iron ($\text{Fe}^{2+}/\text{Fe}^{3+}$) and copper (Cu^{2+}), thereby limiting the formation of highly reactive hydroxyl radicals ($\bullet\text{OH}$) through Fenton and Haber–Weiss reactions (Prior et al., 2005).

Beyond their direct radical-scavenging activity, polyphenols can also modulate the Nrf2/ARE (Nuclear factor erythroid 2–related factor 2) signaling pathway. Activation of this pathway stimulates the expression of endogenous antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), which enhance cellular defense against oxidative stress (Halliwell & Gutteridge, 2015). Together, these mechanisms contribute significantly to the protection of biological systems against oxidative damage.

II.3.2. Activation of Cellular Defense Pathways (e.g., Nrf2/ARE)

Beyond their direct antioxidant activity, polyphenols can activate endogenous cellular defense systems, particularly the Nrf2/ARE (Nuclear factor erythroid 2–related factor 2 / Antioxidant Response Element) signaling pathway. Under oxidative stress conditions, activated Nrf2 translocates to the nucleus and binds to ARE sequences, leading to the expression of several antioxidant and detoxifying enzymes, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and heme oxygenase-1 (HO-1) (Liao et al., 2016; Saha et al., 2020).

Activation of the Nrf2/ARE pathway by dietary polyphenols contributes to the reduction of oxidative stress, inflammation, and cellular damage. In addition, this pathway has been associated with protective effects against chronic diseases such as diabetes and cardiovascular disorders (Saha et al., 2020). These findings suggest an important role of polyphenols in maintaining cellular redox balance and enhancing antioxidant defense mechanisms. The Nrf2/ARE signaling pathway involved in cellular antioxidant defense is illustrated in Figure II.X.

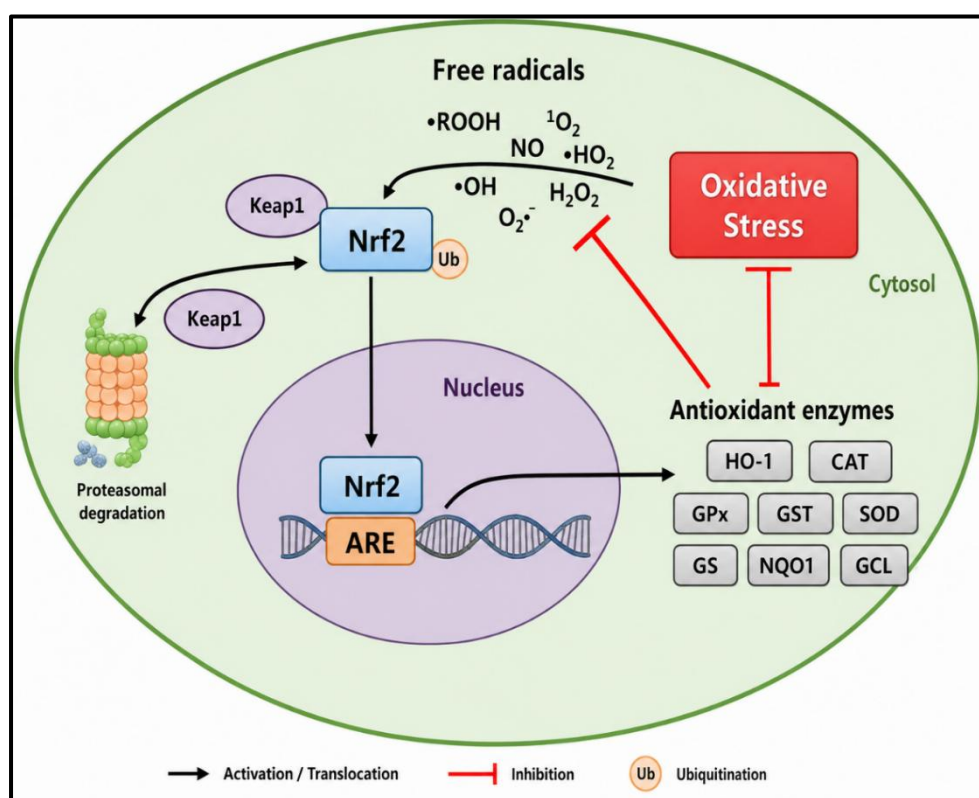


Figure II.5: Schematic representation of the Nrf2/ARE signaling pathway and its role in antioxidant defense against oxidative stress. Adapted from Saha et al. (2020).

II.3.3. Antioxidant Properties of *Stevia rebaudiana* Polyphenolss

Stevia rebaudiana leaves contain several bioactive polyphenolic compounds, mainly phenolic acids and flavonoids, including chlorogenic acid, caffeic acid, quercetin, and kaempferol (Lemus- Mondaca et al., 2012; Kim et al., 2011). These compounds are largely responsible for the antioxidant properties of stevia and contribute to its potential health benefits. In addition to their nutritional value, stevia polyphenols are considered promising natural antioxidants for food and pharmaceutical applications (Papaefthimiou et al., 2024).

The antioxidant activity of stevia polyphenols is mainly related to their ability to neutralize reactive oxygen species through hydrogen or electron donation and through metal-chelating mechanisms (Shahidi & Ambigaipalan, 2015). Antioxidant capacity is commonly evaluated using in vitro assays such as DPPH, ABTS, FRAP, and ORAC, which are widely used to estimate the radical-scavenging and reducing activities of plant extracts (Prior et al., 2005).

Oxidative stress results from an imbalance between reactive oxygen species production and antioxidant defense systems and is associated with several chronic diseases, including diabetes and cardiovascular disorders. By reducing oxidative damage and modulating antioxidant pathways, polyphenols may contribute to cellular protection against oxidative stress (Halliwell & Gutteridge, 2015).

However, the reported antioxidant activity of *Stevia rebaudiana* extracts may vary depending on extraction conditions, solvent polarity, plant origin, and the analytical methods used. For this reason, recent studies have highlighted the importance of integrating data from different antioxidant assays in order to obtain more reliable and comprehensive evaluations of stevia antioxidant potential (Papaefthimiou et al., 2024).

II.4. Antidiabetic Mechanisms of Polyphenols

Polyphenols play an important role in the regulation of glucose homeostasis through several mechanisms acting at different stages of glucose metabolism.

II. 4.1. Modulation of Carbohydrate Digestion (Initial Intestinal Phase)

Polyphenols can inhibit key carbohydrate-digesting enzymes, particularly α -amylase and α -glucosidase. By reducing the breakdown of complex carbohydrates into simple sugars these compounds slow glucose release and help reduce postprandial blood glucose levels (Tundis et al., 2010 ; Xiao et al., 2020). This mechanism is similar to that of acarbose, a commonly used antidiabetic drug, although polyphenols may present fewer gastrointestinal side effects (McDougall et al., 2005).

II.4.2. Regulation of Glucose Absorption (Secondary Intestinal Phase)

In addition to their effects on digestive enzymes, polyphenols may reduce intestinal glucose absorption by modulating glucose transporters such as sodium-glucose linked transporter 1 (SGLT1) in enterocytes. This action contributes to improved glycemic regulation (Song et al., 2018).

II.4.3. Systemic Metabolic Regulation (Cellular Phase)

At the cellular level, polyphenols may improve glucose metabolism through several pathways. They can enhance insulin signaling in insulin-sensitive tissues such as skeletal muscle and adipose tissue, thereby promoting glucose uptake (Zhang et al., 2019). Polyphenols may also reduce hepatic gluconeogenesis, partly through the regulation of enzymes involved in glucose production, including phosphoenolpyruvate carboxykinase (PEPCK) (Ríos et al., 2015).

In addition, some polyphenols activate AMP-activated protein kinase (AMPK), an important regulator of cellular energy metabolism (Liu et al., 2020). They may also protect pancreatic β -cells against oxidative stress and reduce the formation of advanced glycation end-products (AGEs), which are associated with diabetic complications (Bansal et al., 2018; Yeh et al 2017). Overall, these mechanisms contribute to the potential antidiabetic effects of dietary polyphenols (Hanhineva et al., 2010).

II.5. Functional and technological applications of *Stevia* in food systems

The use of *Stevia rebaudiana* as a natural non-caloric sweetener has increased considerably in the food industry. In addition to their sweetening capacity, steviol glycosides possess several technological properties that support their application in beverages, dairy products, bakery products, and confectionery (JECFA, 2008; Prakash et al., 2008).

This section highlights the role of stevia as a functional food ingredient, with emphasis on its effects on glycemic index, bioavailability, stability during food processing, and sensory properties influencing consumer acceptance.

The following subsections focus on the functional role of stevia as a natural sweetener, together with its glycemic relevance, stability, and sensory properties in food applications.

II.5.1. Functional Foods and Natural Sweeteners

Functional foods are foods that provide health benefits beyond basic nutrition and may contribute to the prevention or management of chronic diseases. Increasing awareness of the relationship between diet and health has stimulated the demand for functional ingredients particularly natural sweeteners that can replace added sugars while supporting metabolic health (Goyal et al., 2010).

Excessive sugar consumption is associated with obesity, type-2 diabetes, and cardiovascular diseases. As a result, interest in natural non-caloric sweeteners has increased considerably. Natural sweeteners include bulk sweeteners, such as erythritol and xylitol, and high-intensity sweeteners, among which steviol glycosides from *Stevia rebaudiana* are the most widely used. These compounds provide little to no calories and exhibit a sweetness intensity approximately 200–300 times greater than sucrose (Prakash et al., 2008).

Beyond their sweetening capacity, stevia extracts have been associated with several biological properties, including antioxidant, antihyperglycemic, and antihypertensive effects. Therefore the incorporation of stevia into food products may reduce sugar and caloric content while supporting the development of functional food formulations aimed at improving metabolic health (JECFA, Goyal et al., 2010 ;2008).

II.5.2. Glycemic Index, Bioavailability, and Stability

Steviol glycosides from *Stevia rebaudiana* are considered low-calorie sweeteners with minimal effects on blood glucose levels and glycemic response. Unlike sucrose, steviol glycosides are not metabolized as an energy source and therefore do not significantly increase postprandial glycemia. For this reason, stevia-based sweeteners are widely used in products intended for diabetic and low-glycemic diets (Anton et al., 2010; Tey et al., 2017).

The bioavailability of steviol glycosides differs from that of conventional sugars. After ingestion, these compounds are not hydrolyzed in the upper gastrointestinal tract but are metabolized by intestinal microbiota into steviol, which is subsequently converted into steviol glucuronide in the liver and excreted mainly in urine (Geuns, 2003; Gardana et al., 2003). This metabolic pathway contributes to the low caloric value and limited glycemic impact of stevia sweeteners.

Steviol glycosides also exhibit good technological stability under various food processing conditions. Studies have shown that stevioside remains relatively stable at temperatures up to approximately 120 °C and within a pH range of 2–10. However, degradation may occur under highly acidic conditions or prolonged exposure to elevated temperatures (Geuns, 2008). In

,addition stevia sweeteners show good compatibility with beverages and other food ingredients supporting their application in different food systems.

II.5.3. Sensory Properties and Stability

II.5.3.1.Sensory Properties and Taste Improvement Strategies

One of the main limitations of stevia sweeteners is the bitter and licorice-like aftertaste associated mainly with stevioside, the predominant steviol glycoside in *Stevia rebaudiana* leaves. This sensory characteristic may reduce consumer acceptance in some food products (De Roode et al., 2015). For instance, stevia-sweetened beverages have been reported to exhibit bitterness, metallic taste, and lower overall sensory acceptance compared with sucrose-sweetened products (Rocha & Bolini,2015).

Several approaches have been developed to improve the sensory quality of stevia sweeteners. Enzymatic modification of steviol glycosides can produce glycosylated derivatives with reduced bitterness and improved sweetness quality (Ye et al., 2013; Yu et al., 2015). In addition, microencapsulation techniques using carriers such as maltodextrin or inulin have shown promising effects in masking undesirable aftertaste (Chranioti et al., 2016). Selective breeding strategies have also been used to develop stevia cultivars with a higher rebaudioside A content, resulting in extracts with better organoleptic properties and lower bitterness(Yadav et al., 2011; Kaplan & Turgut,2019).

Interactions between steviol glycosides and food proteins may also influence sensory perception and product stability. Such interactions can contribute to improved texture, reduced bitterness perception, and enhanced stability in protein-enriched beverages and other multiphase food systems. These properties are particularly relevant in the development of functional beverages containing proteins such as collagen (Yang et al., 2022).

II.5.3.2. Stability of Steviol Glycosides

Steviol glycosides exhibit relatively good stability under common food processing condition. Studies have shown that stevioside remains stable at moderate heating temperatures and over a broad pH range, making it suitable for beverages, dairy products, and bakery applications (Geuns, 2008). However, degradation may occur under highly acidic or alkaline conditions or after prolonged exposure to elevated temperatures (Jookan et al., 2012).

The stability of stevia sweeteners in food systems is also influenced by their interactions with other ingredients, including organic acids, vitamins, and proteins. These interactions may affect both sensory quality and physicochemical stability, which should be considered during the formulation of functional food products (Geuns, 2008; Yang et al., 2022).

Conclusion

Stevia rebaudiana is not only an important natural non-caloric sweetener but also a valuable source of bioactive polyphenolic compounds with diverse biological activities. The polyphenols identified in stevia, particularly flavonoids, phenolic acids, and tannins, contribute significantly to its antioxidant and antidiabetic properties through mechanisms involving free radical scavenging, metal chelation, modulation of glucose metabolism, and activation of cellular antioxidant defense pathways.

In addition to their biological effects, stevia extracts possess several functional and technological properties that support their application in food systems. Their low glycemic impact, good stability under common processing conditions, and potential use in functional foods make stevia a promising ingredient for the development of healthier food products. Despite some sensory limitations such as bitterness and aftertaste, different technological approaches have been developed to improve consumer acceptance and product quality.

Practical Part

Chapter III: Materials and methods

Chapter V: Results and discussion

Chapter III

Materials and methods

Chapter III: Materials and methods

III.1. Plant material

The studied *Stevia rebaudiana* Bertoni specimens were obtained from Ténès, Chlef province, northwestern Algeria (36° N, 1° E; approximately 200 km west of Algiers), a region with a semi-arid Mediterranean climate. Dr Khaled Laiche, an agronomy specialist at the University of El Oued, confirmed the botanical identity of the leaf material that arrived in El Oued province after greenhouse acclimatisation in Chlef (figure III.1).

Following harvesting, the plant parts (leaves and roots) were carefully cleaned to remove impurities. The leaves were then dried in an oven at 40°C until complete dryness was achieved. Subsequently, the dried leaves were ground into a fine and homogeneous powder. Finally, the obtained powder was stored in airtight glass containers at room temperature until use, according to **Ghezal & Remili (2024)**. The different stages of cultivation, acclimatization and drying of *Stevia rebaudiana* plant material are illustrated in Figure III.1.



Figure III.1: Different stages of *Stevia rebaudiana* plant preparation.

III.2. Preparation of *Stevia rebaudiana* extract

The hydroethanolic extract of *Stevia rebaudiana* leaves was prepared according to the method described by **Shukla et al. (2020)** with minor modifications.

Chapter III: Materials and methods

Ten grams (10 g) of the powdered plant material were accurately weighed and transferred into a 250 mL Erlenmeyer flask. The extraction solvent consisted of a mixture of 70 mL of ethanol (96% purity) and 30 mL of distilled water, yielding a final ethanol concentration of 70% (v/v). The solvent mixture was added to the flask containing the plant powder, and the mixture was thoroughly homogenized using a magnetic stirrer. The flask was sealed with aluminum foil and left for maceration at room temperature ($25^{\circ}2 \pm C$) for 24 hours under continuous stirring at 300 rpm to ensure efficient extraction of steviol glycosides and polyphenols. After the maceration period, the mixture was filtered through Whatman No. 1 filter paper to separate the solid residue from the liquid extract. The filtrate was collected in a pre-weighed round-bottom flask and concentrated under reduced pressure using a rotary evaporator at $45^{\circ}C$ until dryness. The obtained dry extract was stored in a sealed, light-protected container at $4^{\circ}C$ until further analysis.



Powdered *Stevia rebaudiana* leaves
before extraction



Hydroethanolic extraction of *Stevia rebaudiana*

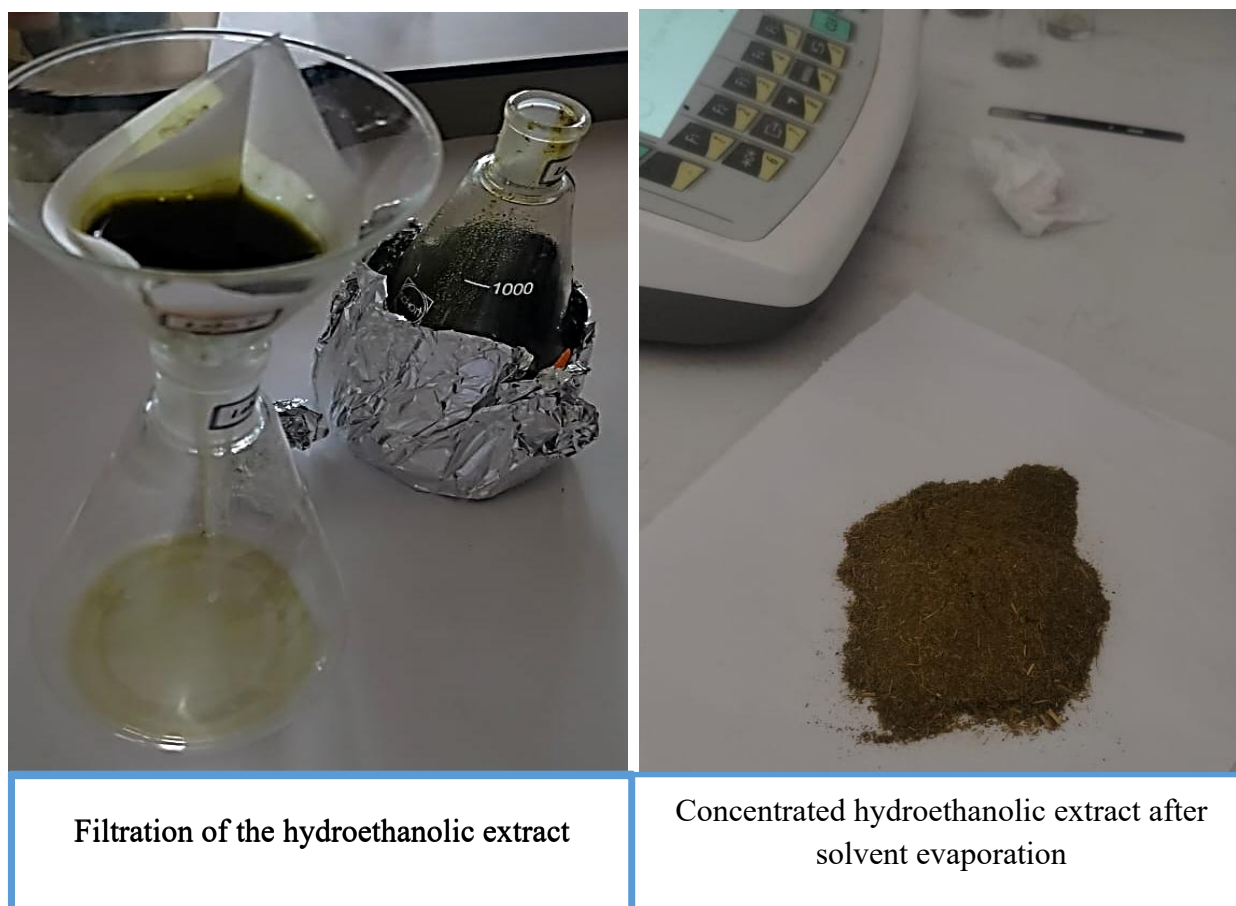


Figure III.2: Preparation of the hydroethanolic extract of *Stevia rebaudiana* from powdered leave

III.2.1. Determination of Crude Extract Yield

The extraction yield of the crude hydroethanolic extract was calculated using a gravimetric method. The yield (R) was defined as the percentage of the mass of the dry extract (Me) relative to the mass of the initial plant material (Mv). The calculation was performed using the following equation:

$$R (\%) = \frac{Me}{Mv} \times 100$$

All measurements were performed in triplicate, and the results were expressed as mean $\pm$ standard deviation. (Harborne, 1998)

III.3. Dosage of phenolic compounds

This quantitative determination aims to measure the total phenolic, total flavonoid, and total tannin content in the hydroethanolic extract of *Stevia rebaudiana* leaves, quantitative analysis was performed using standard spectrophotometric methods (Shahidi & Ambigaipalan, 2015).

3.1.Dosage of Total Polyphenols:

A) Principle :

The total phenolic content was determined using the Folin–Ciocalteu method, which is based on the reduction of phosphomolybdic–phosphotungstic acid complexes, resulting in a blue coloration measurable at 760 nm (Singleton et al., 1999).

B) Protocol :

A volume of 0.2 mL of extract was mixed with 1 mL of diluted Folin–Ciocalteu reagent (1:10). After 5 minutes, 0.8 mL of sodium carbonate (7.5%) was added. (Singleton et al., 1999) The mixtures were incubated for 30 minutes at room temperature in the dark (protected from light). Absorbance was then measured at 760 nm using a spectrophotometer. Results were expressed as mg GAE/g extract.

A calibration curve was established using gallic acid solutions with concentrations ranging from 0.005 to 0.02 mg/mL .

III.3.2. Dosage of Total Flavonoids

A) Principle:

The total flavonoid content was assessed using the aluminum chloride (AlCl_3) colorimetric method, where flavonoids form a yellow complex measurable at 420 nm (Chang et al., 2002).

B) Protocol:

0.75 milliliter of extract was mixed with 0.75 mL of 2% aluminum chloride. Following incubation for one hour at room temperature in darkness, absorbance was recorded at 420 nm by means of a UV-Vis spectrophotometer. Results were expressed as mg QE/g extract (Chang et al., 2002).

Quercetin solutions (ranging from 0.01 to 0.04 mg/mL) served as the standard reference.

III.3.3. Dosage of Total Tannins :

A) Principle:

Total condensed tannins were determined using the acidified vanillin–HCl method described by **Broadhurst and Jones (1978)**. The assay is based on the reaction between vanillin and flavan-3-ol units of condensed tannins in acidic medium, producing a red-colored complex measured spectrophotometrically at 500 nm

B) Protocol :

A volume of 0.75 mL of vanillin solution (4% in methanol) was mixed with 0.4 mL of concentrated hydrochloric acid (HCl). Then, 0.1 mL of either the sample extract or catechin standard solution was added. The reaction mixture was incubated at room temperature for 15 min, after which the absorbance was measured at 500 nm. Condensed tannin content was expressed as mg catechin equivalents per gram of extract (mg CE/g extract) using a calibration curve prepared with catechin (0–0.5 mg/mL) as the reference standard (**Broadhurst and Jones, 1978; Tlili, 2015**).

III.4. Physicochemical Analysis of Total Sugars in *Stevia rebaudiana* Extract

A) Principle :

This method determines total sugars using concentrated sulfuric acid. Monosaccharides are dehydrated to furfural derivatives (C₅H₆O₂), which then condense with phenol to produce orange-yellow colored complexes. The color intensity is proportional to the monosaccharide concentration. Absorbance is measured at 490 nm using a spectrophotometer. (**Dubois et al., 1956**)

B) Protocol :

A volume of 0.2 mL of the plant extract was mixed with 0.2 mL of 5% phenol solution. Subsequently, 1 mL of concentrated sulfuric acid (95–98%) was rapidly added to the mixture. The reaction was allowed to proceed for 10 minutes, followed by incubation at room temperature for 20 minutes to ensure complete color development. The absorbance was then

measured at 490 nm using a UV–Visible spectrophotometer. All measurements were performed in triplicate (Masuko et al., 2005).

A standard calibration curve was prepared using glucose solutions at concentrations ranging from 0 to 200 µg/mL. The absorbance values at 490 nm were plotted against glucose concentration to obtain a linear regression equation.

Calculation of Results: A standard calibration curve was prepared using glucose solutions at concentrations ranging from 0 to the concentration of total sugars in the sample was determined using the calibration curve equation:

$$C = (A - b) / a$$

where:

C = (Concentration of sugars) µg/mL

A = absorbance of the sample

a = slope of calibration curve

b = intercept

The total sugar content was then calculated as:

$$\text{Total sugars (mg/g extract)} = (C \times V) / m$$

where:

V = volume of extract (mL)

m = mass of extract (g)

Results were expressed as mg glucose equivalents per gram of extract (mg GE/g extract).

III.5. The biological activities of the plant

The biological activities of *Stevia rebaudiana* have attracted considerable scientific interest due to the presence of bioactive compounds such as polyphenols, flavonoids, and steviol glycosides. In this study, antioxidant, antidiabetic, and anti-inflammatory activities were evaluated using different in vitro assays.

III.5.1. Evaluation of Antioxidant Activity of *Stevia rebaudiana* extract

This study aims to evaluate the antioxidant activity of a hydroethanolic extract of *Stevia rebaudiana* leaves using two complementary spectrophotometric methods: the DPPH radical scavenging assay and the FRAP (ferric reducing antioxidant power) assay for measuring reducing power. These two methods allow the estimation of both the extract's free radical scavenging capacity and its reducing power.

- **DPPH radical scavenging assay:**

- A) Principle:**

The DPPH assay is based on the reduction of the stable free radical DPPH (2,2-diphenyl-1-picrylhydrazyl), which exhibits a deep violet color. In the presence of antioxidant compounds, DPPH is reduced to a yellow-colored diphenylpicrylhydrazine, resulting in a decrease in absorbance measured at 517 nm. The extent of this decrease reflects the radical scavenging activity of the sample (**Brand-Williams et al., 1995**).

- B) Protocol:**

The scavenging activity of the extracts was assessed following the method of **Alia et al. (2021)** with minor modifications. For each extract at various concentrations (0–0.07 mg/mL), 0.75 mL of extract was mixed with 0.75 mL of DPPH solution (0.1 mM in methanol). After a 15-minute incubation at room temperature, the absorbance was read at 517 nm. The percentage inhibition was calculated as described by **Chaouche et al. (2013)**.

$$\% \text{Inhibition} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

For the reference antioxidant (ascorbic acid), the concentrations used were between 0 and 0.07 mg/mL.

The IC₅₀ value, defined as the concentration required to inhibit 50% of the DPPH radicals, was determined from the plot of inhibition percentage versus extract concentration.

- **The ferric reducing antioxidant power (FRAP) assay:**

A) Principle:

The principle of this method lies in evaluating the reducing capacity of extracts toward ferric iron (Fe^{3+}) present in the ferricyanide complex, converting it to ferrous iron (Fe^{2+}) (**Mouffouk, 2019**). The amount of Fe^{2+} generated is indirectly measured by monitoring the increase in cyan-blue color density within the reaction mixture at a wavelength of 700 nm. The $\text{FeCl}_3/\text{K}_3\text{Fe}(\text{CN})_6$ system provides the necessary sensitivity for the semi-quantitative estimation of antioxidant concentrations that participate in the redox reaction (**Mouffouk, 2019**).

B) Protocol:

The ferric reducing antioxidant power (FRAP) of the extracts was assessed using a slightly modified version of the protocol described by **Bougandoura and Bendimerad (2013)**. In test tubes, 1 mL of extract at varying concentrations (0–0.7 mg/mL) was mixed with 1.25 mL of 1% (w/v) potassium ferricyanide ($\text{K}_3\text{Fe}(\text{CN})_6$) solution and 1.25 mL of 0.2 mol/L phosphate buffer (pH 6.6). The mixture was shaken and then incubated in a water bath at 50°C for 20 minutes. The reaction was terminated by adding 1.25 mL of 10% trichloroacetic acid ($\text{C}_2\text{HCl}_3\text{O}_2$), followed by centrifugation at 3000 rpm for 10 minutes. An aliquot of 1.25 mL of the resulting supernatant was combined with 1.25 mL of distilled water and 0.25 mL of 0.1% (w/v) ferric chloride ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) solution. The absorbance of the reaction mixture was measured at 700 nm against a blank prepared by substituting the extract with distilled water. Increased absorbance indicated greater reducing power of the tested extracts. A calibration curve was constructed using ascorbic acid as a standard, with absorbance measured under identical conditions (**Haddoudi et al., 2014**).

III.5.2. Evaluation of the anti-inflammatory activity

This evaluation aims to measure the anti-inflammatory activity of a plant extract through its ability to prevent protein denaturation. This method is considered a simple in vitro model that can be used to screen compounds that may possess anti-inflammatory properties (**Sakat et al., 2010**).

A) Principle:

Protein denaturation is characterized by the loss of tertiary and secondary protein structures under stressful conditions such as heat, resulting in increased turbidity measurable via

spectrophotometry. Inflammatory processes are frequently associated with protein denaturation.

Anti-inflammatory compounds are able to stabilize protein structure and prevent denaturation. In this assay, bovine serum albumin (BSA) or egg albumin is subjected to heat-induced denaturation, and the protective effect of the plant extract is assessed by measuring the reduction in turbidity at 660 nm. A lower absorbance value corresponds to greater inhibition of protein denaturation (Mizushima & Kobayashi, 1968; Sakat et al., 2010).

B) Protocol:

The anti-inflammatory activity of extract *Stevia rebaudiana* was evaluated following the protocol of Williams et al. (2008) with a modification of the number of concentrations to five. Briefly, 0.75 mL of BSA was added to 1 mL of plant extract. The mixture was first incubated at 37 °C for 15 min, then at 70°C for 5 min. The reaction was stopped by adding 0.75 mL of buffer solution (pH 6.4). A blank control (50% ethanol instead of extract) was prepared under identical conditions. Turbidity was measured at 660 nm, and results were compared to diclofenac and prednisolone standards. The percentage of anti-inflammatory activity is calculated according to the following equation:

$$(\%) = [Ac - At / Ac] \times 100$$

Ac: Absorbance du contrôle.

At: Absorbance d'extrait ou standard

III.5.3. anti-diabetic activity:

● **α -Amylase Inhibitory Activity of Methanolic Extract *Stevia rebaudiana* – Starch–Iodine Microplate Assay:**

The α -amylase inhibition assay is an in vitro method that measures the ability of a substance to inhibit the enzymatic activity of α -amylase, which hydrolyzes starch into simpler sugars. The assay is commonly used to screen for potential antidiabetic compounds that delay carbohydrate digestion. (Khadayat et al., 2020).

A) Principle:

The starch–iodine assay measures α -amylase activity by the loss of blue colour when starch is hydrolysed. Retention of colour indicates enzyme inhibition (**Chakrabarti et al., 2014**). This is because α -amylase breaks down starch into simpler compounds that do not react with iodine. When the enzyme is inhibited, undigested starch accumulates and reacts with iodine to form a blue-black complex.

B) Protocol:

The starch substrate was prepared by dissolving 200 mg of soluble starch in 25 mL of 0.4 M NaOH, heating at 100 °C for 5 min, cooling, adjusting the pH to 7.0, and bringing the final volume to 100 mL with distilled water (**Granados-Guzmán et al., 2022**). A stock solution of extract *Stevia rebaudiana* (40 mg/mL) was prepared in 1% DMSO (in distilled water), and two-fold serial dilution yielded the final concentrations of 40, 20, 10, 5, and 2.5 mg/mL (**Khadayat et al., 2020**). In each microplate well, 40 μ L of starch substrate was mixed with 20 μ L of the extract (or acarbose as positive control). The mixture was pre-incubated at 37 °C for 3 min. The enzymatic reaction was initiated by adding 20 μ L of α -amylase (3 U/mL in phosphate buffer pH 6.9 containing 6.7 mM NaCl) and incubated at 37 °C for 15 min (**Chakrabarti et al., 2014**). The reaction was terminated by adding 80 μ L of 0.1 M HCl, followed by 100 μ L of iodine-potassium iodide (IKI) solution (2.5 mM). Absorbance was measured at 630 nm using a microplate reader (**Granados-Guzmán et al., 2022**). The percentage inhibition was calculated as $[(A - B) / A] \times 100$, where A is the absorbance of the negative control (without inhibitor) and B is the absorbance of the sample with EXT-A. All tests were performed in triplicate, and results were expressed as mean $\pm$ standard deviation (**Khadayat et al., 2020**).

III.6. Sensory Evaluation of Functional Food Formulations

Sensory evaluation is an essential tool in food science for assessing consumer perception and acceptability of food products (**Stone & Sidel, 2004; Lawless & Heymann 2010**).In the present study, sensory analysis was carried out to evaluate the effect of stevia extract and collagen protein incorporation on the organoleptic characteristics of different functional food formulations. Particular attention was given to attributes such as sweetness, bitterness, aroma, texture, appearance, and overall acceptability (**Stone et al., 2012**).

The following sections describe the sensory evaluation protocols applied to the different formulated products.

● Sensory Panel

Sensory evaluation was performed by 15 untrained volunteer panelists aged between 20 and 45 years. The samples were evaluated independently according to the defined sensory attributes and the corresponding hedonic scales under the same evaluation conditions.

III.6.1. Orange Juice Formulations

A. Principle:

The sensory evaluation was performed to compare the organoleptic properties of orange juice formulations containing sucrose, *stevia* extract, and collagen protein. The evaluated attributes included sweetness, bitterness, aroma, texture, and overall acceptability (Lawless & Heymann, 2010).

B. Preparation of Samples

Five orange juice formulations were prepared under the same experimental conditions:

Commercial hydrolyzed marine collagen peptides (Naticol®, fish-derived collagen peptides) were used as a functional ingredient in the orange juice formulations at a concentration of 1 g/100 mL.

- ☐ Sample 1 (Control): 100 mL orange juice.
- ☐ Sample 2: 100 mL orange juice + 10 g white sugar.
- ☐ Sample 3: 100 mL orange juice + 10 g white sugar + 1 g marine collagen peptides.
- ☐ Sample 4: 100 mL orange juice + 100 mg stevia extract.
- ☐ Sample 5: 100 mL orange juice + 100 mg stevia extract + 1 g marine collagen peptides.

C. Experimental Procedure

Fresh orange juice was prepared and distributed into separate containers. The appropriate amounts of sucrose, stevia extract, and collagen protein were then added according to each formulation. All mixtures were manually homogenized until complete dissolution of the ingredients was achieved.

All samples were prepared on the same day and stored under refrigerated conditions prior to serving. To minimize bias during sensory evaluation, the samples were coded and presented randomly to the panelists. The sensory assessment was based on sweetness, bitterness, aroma,

texture, and overall acceptability, according to sensory evaluation principles described by **Stone and Sidel (2004)**. The use of stevia as a natural sweetener in beverage formulations was based on previous studies highlighting its suitability for the development of low-calorie functional foods (**Prakash et al., 2008**).

D. Sensory Evaluation Forms

The sensory evaluation of orange juice formulations was performed using a 5-point hedonic scale based on sweetness, taste, bitterness, aftertaste, aroma, and overall acceptability (**Stone & Sidel, 2004**).

Table III.1: Sensory evaluation form used for orange juice formulations.

Sample	Sweetness (1-5)	Taste (1-5)	Bitterness (1-5)	Aftertaste (1-5)	Aroma (1-5)	Overall Acceptability (1-5)
S1						
S2						
S3						
S4						
S5						

Hedonic scale: 1 = Very poor (Extremely disliked); 2 = Poor (Disliked); 3 = Neutral; 4 = Good (Liked); 5 = Excellent (Extremely liked).

Panelists were instructed to evaluate each sample independently according to the indicated sensory criteria.

III.6.2. Madeleine Cake Formulations

A. Principle

The Madeleine formulations were prepared to evaluate the effect of partial or total sucrose replacement with stevia sweetener on the sensory quality of bakery products. Particular attention was given to sweetness, bitterness, texture, aroma, and overall acceptability (**Prakash et al., 2008; Goyal et al., 2010**).

B. Preparation of Samples

Three Madeleine formulations were prepared under similar experimental conditions:

□ Sample A: two eggs, approximately 10 g sucrose, 0.4 g stevia sweetener, 150 g wheat flour, 10 g baking powder, lemon zest, vanilla, approximately 150 mL mixture of butter and vegetable oil, and one-quarter cup of milk.

□ Sample B: two eggs, approximately 130 g sucrose, 150 g wheat flour, 10 g baking powder, lemon zest, vanilla, approximately 150 mL mixture of butter and vegetable oil, and one-quarter cup of milk.

□ Sample C: two eggs, approximately 1 g stevia sweetener, 150 g wheat flour, 10 g baking powder, lemon zest, vanilla, approximately 150 mL mixture of butter and vegetable oil, and one-quarter cup of milk.

C. Experimental Procedure

Eggs and sweeteners were first mixed until homogenization. The lipid phase consisting of butter and vegetable oil was then incorporated, followed by milk, vanilla, and lemon zest. Wheat flour and baking powder were gradually added until a homogeneous batter was obtained. The batter was distributed into Madeleine molds and baked under controlled conditions until complete cooking and development of the characteristic golden colour. After cooling at room temperature, the samples were subjected to sensory evaluation based on sweetness, bitterness, aroma, texture, appearance, and overall acceptability according to sensory evaluation principles described by **Stone and Sidel (2004)**.

D. Sensory Evaluation Forms

The sensory evaluation of Madeleine cake formulations was carried out using a 5-point hedonic scale based on colour, aroma, taste, texture, and overall acceptability (Stone & Sidel, 2004).

Parameter	Sample A	Sample B	Sample C
Colour	1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐	1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐	1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐
Aroma	1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐	1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐	1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐

Chapter III: Materials and methods

Taste	1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐	1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐	1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐
Texture (softness)	1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐	1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐	1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐
Overall acceptability	1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐	1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐	1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐

Table III.2: Sensory evaluation form used for Madeleine cake formulations.

Evaluation scale: 1 = Dislike very much; 2 = Dislike; 3 = Acceptable; 4 = Like; 5 = Like very much.

Panelists were instructed to evaluate each sample independently according to the indicated sensory criteria.

Chapter IV

Results and discussion

Chapter IV: Results and discussion

IV.1. Extraction Yield

The initial quantitative assessment consisted of determining the crude extract yield from *Stevia* leaves. The results obtained from the extraction yield calculations are presented in Table:

Table IV.1: Crude extract yield from *Stevia rebaudiana* (leaves)

Parameter	Value
Plant material	<i>Stevia rebaudiana</i> (leaves)
Me (Mass of dry extract (g))	2,42
M (Mass of dry plant powder (g))	10
Solvent system	Hydroethanolic (70% ethanol + 30% distilled water, v/v)
R (Extraction yield (%))	24.2%

Thus, the hydroethanolic extract of *Stevia rebaudiana* leaves demonstrated an extraction yield of 24.2%. This indicates that the amount of extracted dry matter obtained using a 70% ethanol solution corresponded to 24.2% of the initial dry weight of the plant powder. This extraction yield is comparable to values reported in previous studies on hydroethanolic extracts of *Stevia rebaudiana* leaves. **Shukla et al. (2012)** reported extraction yields ranging between approximately 20% and 30% depending on solvent composition and extraction conditions. Similarly, **Formigoni et al. (2018)** observed relatively high recovery yields when using ethanol–water mixtures for the extraction of steviol glycosides and phenolic compounds. The relatively high extraction yield obtained in the present study can be attributed to the effectiveness of the hydroethanolic solvent system (70% ethanol + 30% water). This solvent mixture combines the ability of ethanol to extract non-polar to moderately polar compounds with the ability of water to extract polar compounds (**Alvarez-Rivera et al., 2020**). In addition, *Stevia* leaves are naturally rich in extractable compounds such as steviol glycosides, chlorophyll, and phenolic constituents, which contribute to the overall mass of the dry extract obtained (**Kaushik et al., 2010**). It is important to note that extraction yields may vary according to several factors, including the extraction method, solvent composition, temperature conditions, drying procedure, particle size, and extraction duration (**Celhay, 2013**).

IV.2. Phenolic Compound Content

Chapter IV: Results and discussion

The phytochemical composition of *Stevia rebaudiana* hydroethanolic extract was evaluated for total phenolics, flavonoids, and tannins. TPC was determined using the Folin-Ciocalteu method (Singleton et al., 1999) with a gallic acid calibration curve: $y = 13.954x + 0.0094$ ($R^2 = 0.9902$), expressed as mg GAE/g. TFC was determined using the aluminum chloride method (Chang et al., 2002) with a quercetin curve: $y = 10.081x + 0.0147$ ($R^2 = 0.993$), expressed as mg QE/g. Tannins were determined using a method adapted from Tlili (2015) with a catechin curve: $y = 0.1363x + 0.0024$ ($R^2 = 0.9869$), expressed as mg EC/g. The obtained results are summarized in Table (IV.2) below.

Table IV.2: Phenolic, flavonoid, and tannin content of *Stevia rebaudiana* extract

Compounds	Polyphenol (mg of GAE/g of extract)	Flavonoid (mg QE/g of extract)	Tannin (mg EC/g of extract)
Extract of <i>S. rebaudiana</i>	2.91 ± 0.11	0.87 ± 0.003	1.088 ± 0.042

The present study evaluated the phytochemical composition of *Stevia rebaudiana* through total phenolic content (TPC), total flavonoid content (TFC), and total tannin content. The obtained values were 2.91 mg GAE/g for TPC, 0.87 mg QE/g for TFC, and 1.088 ± 0.042 mg CE/g for tannins, indicating generally low concentrations of bioactive compounds.

The TPC value is lower than those reported in several studies, where values ranging from 30 to 60 mg GAE/g have been observed under optimized extraction conditions (Shukla et al., 2023; Al-Kahtani et al., 2024). Even lower values (2.38 mg GAE/g) have been reported using acetone (Alshawwa et al., 2022), while much higher values (63.01 mg GAE/g) were obtained from a different chemotype (a plant variety with a unique chemical profile) (Bugliani et al., 2022). However, such variation is expected due to differences in solvent composition, extraction parameters, and plant origin (Kumar & Tripathy, 2025).

Similarly, the TFC value (0.87 mg QE/g) is relatively low compared to literature values, such as 2.31 mg QE/g reported by El-Hadary et al. (2021), and higher values ranging from 5 to 9 mg QE/g or even up to 70 mg QE/g under optimized extraction conditions (Goyal et al., 2023; Shukla et al., 2023). These differences confirm the strong influence of extraction efficiency and environmental factors (Yang et al., 2024).

The tannin content (1.088 mg CE/g) falls within the lower range of reported values, but remains comparable to certain studies reporting values around 0.9 mg CE/g (Khan et al., 2022). Higher

values (5–11 mg CE/g) have been reported when using optimized extraction methods (**Bender et al., 2023**), further emphasizing the importance of extraction conditions.

The coherence between TPC, TFC, and tannin values suggests that flavonoids and tannins represent fractions of the total phenolic compounds (approximately 30% and 37%, respectively), confirming internal consistency of the results. The relatively low levels observed in this study can be attributed to several interrelated factors.

First, the choice of 70% ethanol may not be optimal for extracting all phenolic subclasses. Some polyphenols, glycosylated flavonoids, and hydrolysable tannins dissolve better in highly polar solvents such as pure water or methanol, while condensed tannins dissolve better in concentrated ethanol. Therefore, a 70% ethanol mixture may not efficiently extract all compound types (**Kumar & Tripathy, 2025**). Second, non-optimized extraction conditions (insufficient time or inappropriate temperature) may lead to incomplete extraction or thermal degradation of sensitive compounds, particularly tannins and flavonoids (**Bender et al., 2023**). Third, biological and environmental factors naturally influence the abundance of these compounds across *Stevia* varieties and geographical origins, including soil type, climate, and water stress (**Yang et al., 2024**). Fourth, for flavonoids specifically, the absence of purification steps (e.g., chlorophyll and lipid removal) may interfere with colorimetric measurements, and enzymatic degradation during preparation (polyphenol oxidase) may alter sensitive compounds (**Chang et al., 2002**). Finally, studies reporting much higher values (31–70 mg QE/g) used advanced extraction techniques such as ultrasound-assisted extraction or high-temperature pressurized methods, which were not applied in the present work (**Shukla et al., 2023**).

Overall, these findings indicate that the low phytochemical content does not reflect the absence of bioactive compounds, but rather the influence of experimental conditions, in agreement with recent literature. Nevertheless, as highlighted above, low concentrations do not necessarily imply weak biological activity. Even at low levels, phenolic compounds may exert significant antioxidant or anti-inflammatory effects, particularly through synergistic interactions between different constituents in the crude extract. Therefore, the phytochemical profile obtained in this study should be interpreted as a basis for further biological investigations rather than as a measure of poor bioactivity.

IV.3. Total Sugar Content in *Stevia rebaudiana* Extract

Chapter IV: Results and discussion

Total sugar content in *Stevia rebaudiana* extract was determined using the spectrophotometric phenol-sulfuric acid method according to **Dubois et al. (1956)**. A glucose calibration curve was established, showing a good linear relationship described by the equation $y = 4.0656x + 0.78$, with a coefficient of determination $R^2 = 0.9758$, where y is the absorbance and x is the glucose concentration (mg/mL). The sugar content of these extracts was calculated from the linear regression equation of the calibration curve (mg/mL). Results were expressed as milligrams of glucose equivalent per gram of dry extract (mg GE/g).

Table IV.3: Total sugar content of *Stevia rebaudiana* extract

Extract	Total Sugars (mg GE/g of extract)
Extract of <i>S. rebaudiana</i>	0.23±0.02

The analysis shows that the *Stevia rebaudiana* extract contains 0.23 ± 0.02 mg glucose equivalents of total sugars per gram of extract, indicating a relatively low level compared to aqueous extracts that recorded higher values (3–10 mg/g) (**Lemus-Mondaca et al., 2023**) and exceeded 5 mg/g (**Goyal et al., 2023**). These differences are attributed to water being more effective in extracting polar sugars than alcoholic solvents (**Shukla et al., 2023**).

Our result agrees with the master's thesis of **Beroual & Berima (2024)** on *Stevia rebaudiana* leaves, where sugars ranged between 0.25 and 0.46 mg/mL depending on the extraction method ($p > 0.05$, indicating no statistically significant differences between methods), and our value lies at the lower end of the range. This consistency across different extraction methods further supports the reliability of our findings.

A broader comparison with the literature reveals that the sugar content in *Stevia* varies dramatically depending on sample state and extraction conditions. For instance, **Arora & Jood (2015)** reported that fresh *Stevia* leaves contain up to 15.63% total sugars, while dried leaf powder contained only 1.78% under similar conditions. This large difference (approximately 8–9 fold) highlights the impact of sample drying on sugar recovery, as heat treatment may caramelize or degrade certain sugars. Moreover, when compared to our value (0.023%), the difference is even more pronounced, reflecting the additional influence of solvent choice (70% ethanol vs. water).

Therefore, the recorded low value reflects the influence of extraction conditions (solvent type, time, temperature, and sample state) and not the true sugar composition of the plant.

IV.4. Results of Biological Activity Assays

IV.4.1. Antioxidant Activity of *Stevia rebaudiana* extract

● DPPH radical scavenging assay:

The antioxidant activity of the extract was evaluated using the DPPH assay according to **Brand-Williams et al., (1995)**. This method measures the ability of compounds to scavenge the stable DPPH radical, which has a violet color that turns yellow upon reduction. The reaction involves hydrogen or electron donation from antioxidants to DPPH, monitored by a decrease in absorbance at 517 nm (**Guediri, 2022**). Ascorbic acid was used as a standard, and results were expressed as percentage inhibition (I%). Based on this principle, the DPPH assay was performed on the hydroalcoholic extract of *Stevia rebaudiana* leaves to assess its antioxidant capacity. The IC₅₀ value was determined from the inhibition curve presented in the following figure (IV.1).

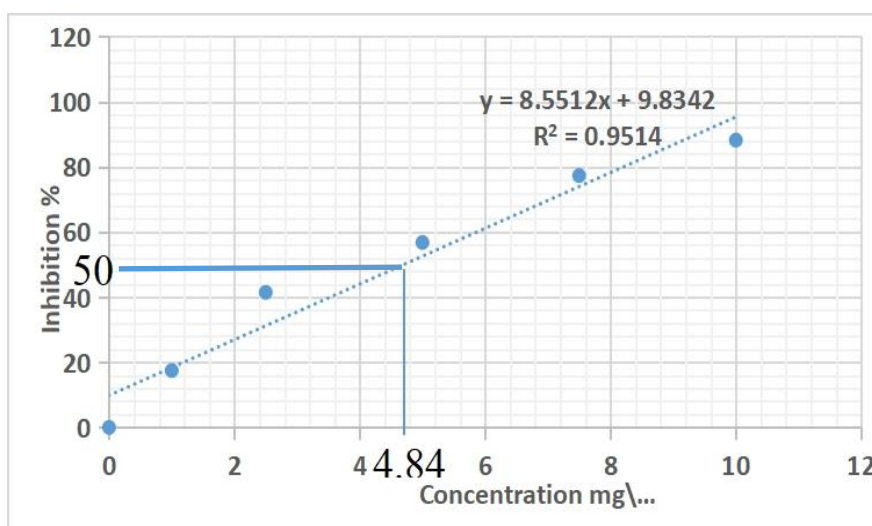


Figure IV.1: Percentages of DPPH inhibition by *Stevia rebaudiana* extract

The IC₅₀ value (concentration required to inhibit 50% of DPPH radicals) was determined from the inhibition curve; lower IC₅₀ values indicate stronger antioxidant activity.

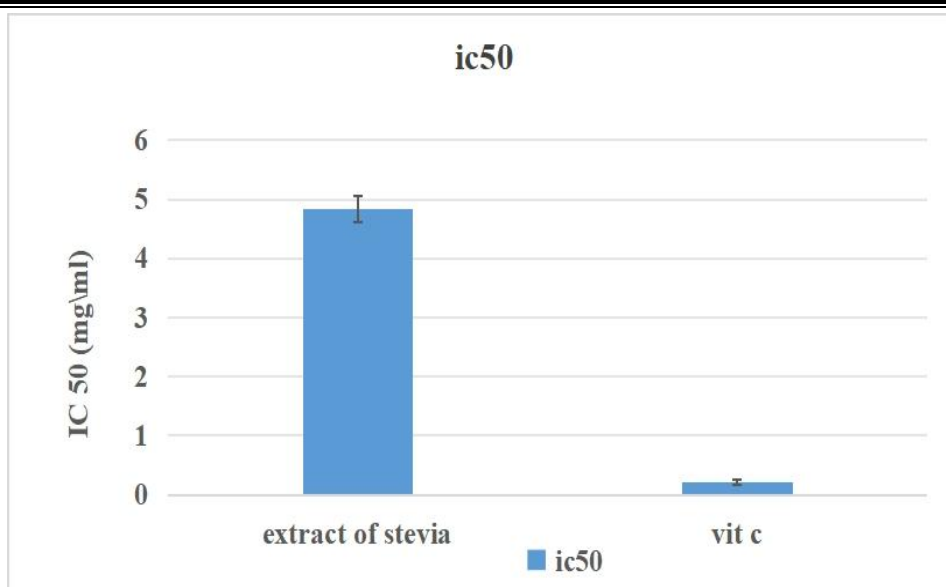


Figure IV.2: IC₅₀ Results of the DPPH Radical

The antioxidant activity of the hydroethanolic extract of *Stevia rebaudiana* leaves was evaluated using the DPPH assay, and the IC₅₀ value was determined to be 4.84 ± 0.22 mg/mL. This value reflects the ability of the extract to scavenge free radicals, with lower IC₅₀ values indicating stronger antioxidant activity. The relatively low standard deviation also indicates good reproducibility of the measurements.

When compared with the reference antioxidant, ascorbic acid exhibited a much lower IC₅₀ value of 0.21 ± 0.04 mg/mL, confirming its higher efficiency as a pure antioxidant compound. This difference is expected, as ascorbic acid is a well-known standard with strong radical scavenging capacity. Similar observations have been reported in studies using the same DPPH protocol on other plant species. For instance **Khan et al., (2021)** reported an IC₅₀ value of approximately 5.2 mg/mL for *Mentha spicata* extracts, which is very close to the value obtained in the present study and indicates a moderate antioxidant activity.

In addition, previous studies on *Stevia rebaudiana* have reported comparable values. For example, **Abou-Arab and Abu-Salem (2010)** reported an IC₅₀ value of 4.2 mg/mL for a 70% ethanolic extract, while **Muanda et al. (2011)** obtained 3.9 mg/mL under similar conditions. These values are in the same range as the present result, supporting its reliability.

On the other hand, more recent studies on *Stevia rebaudiana* have reported significantly lower IC₅₀ values. For instance, a recent study reported an IC₅₀ of approximately 67.95 µg/mL (i.e.,

0.067 mg/mL) for stevia leaf extracts, indicating a higher antioxidant activity under optimized conditions (Nuryandani et al., 2024).

The relatively higher IC₅₀ value obtained in this study can be explained by several factors, including the use of hydroethanolic extraction conditions that may not be fully optimized, differences in solvent polarity, and the relatively low concentration of phenolic compounds. In addition, variations in plant origin, drying conditions, and extraction parameters are known to significantly influence antioxidant activity (Shukla et al., 2012; Alvarez-Rivera et al., 2020).

● The ferric reducing antioxidant power (FRAP) assay:

The antioxidant activity of the studied extracts was evaluated using the FRAP (Ferric Reducing Antioxidant Power) assay, which is based on the reduction of ferric ions (Fe^{+3}) to ferrous ions (Fe^{+2}) by antioxidant compounds. This method is simple, rapid, and reproducible (Benzie & Strain, 1996). The increase in absorbance is directly proportional to the reducing power of the sample. The obtained absorbance values were analyzed using linear regression derived from the calibration curve (Figure IV.3).

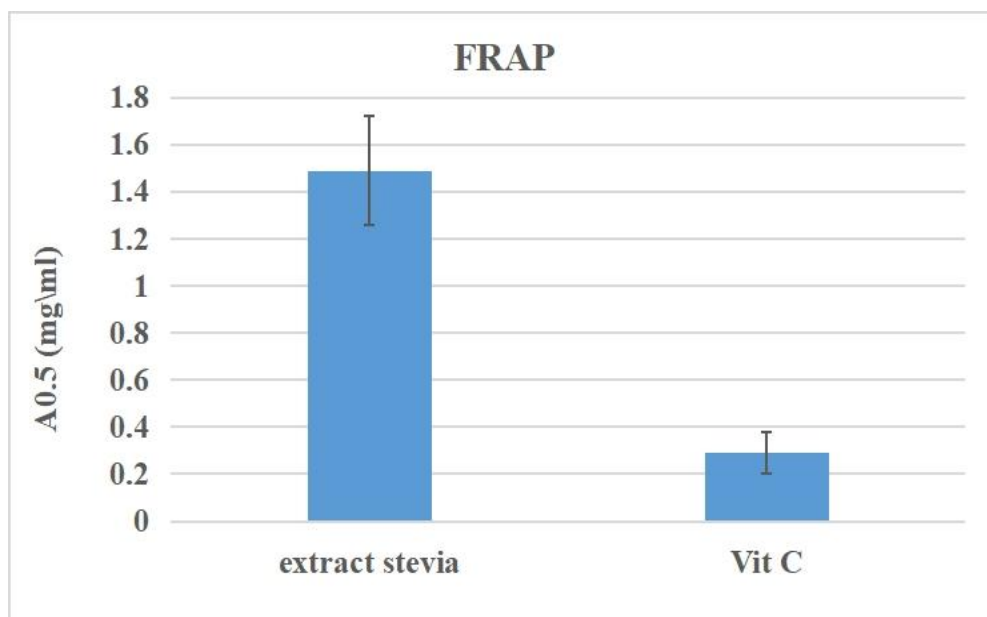


Figure IV.3: Ferric reducing antioxidant power (FRAP) expressed as A_{0.5} values (mg/mL).

The antioxidant activity of the hydroethanolic extract of *Stevia rebaudiana* leaves was evaluated using the FRAP assay. The A_{0.5} value, which corresponds to the concentration required to reach an absorbance of 0.5, was found to be 1.49 ± 0.23 mg/mL. This value reflects

the ability of the extract to act as an electron donor and reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), which is a key indicator of reducing power. The relatively low standard deviation also shows that the results are consistent and reproducible.

When compared to ascorbic acid, which showed an $\text{A}_{0.5}$ of 0.29 ± 0.09 mg/mL, the extract exhibited a lower reducing capacity. This difference is expected, since ascorbic acid is a pure compound with strong antioxidant properties, while plant extracts contain a mixture of different compounds that may act with varying efficiency.

Similar behavior has been observed in other plant extracts evaluated using the same method. For example, a comprehensive study conducted on fifteen genetic varieties (genotypes) of *Orthosiphon aristatus* (Batubara et al., 2020) showed that its reducing capacity in the FRAP assay ranged between 0.07 and 1.60 $\mu\text{mol TE/g DW}$. Although these values are expressed in a unit completely different from ours (mg/mL), they confirm the key concept we are discussing, which is that the antioxidant activity of plant extracts can vary significantly (approximately 23-fold in that study) depending on the chemical composition, which is itself influenced by genetic and environmental factors.

In addition, A recent systematic review demonstrated that the antioxidant activity of *Stevia rebaudiana* is strongly associated with its phenolic and flavonoid composition. Moreover, this activity may vary significantly depending on the extraction method and solvent used (Papaefthimiou et al., 2023).

Previous studies on *Stevia rebaudiana* have reported comparable reducing activity values expressed in the same unit (mg/mL). For instance, Abu Arab and Abu Salem (2010) reported an $\text{A}_{0.5}$ value of 4.2 mg/mL for a 70% ethanolic extract, while Muanda et al. (2011) obtained a value of 3.9 mg/mL under similar extraction conditions. The $\text{A}_{0.5}$ value obtained in the present study (1.49 mg/mL) was lower than these reported values, suggesting a relatively stronger ferric reducing activity and supporting the reliability of the obtained results.

The stronger reducing activity observed in the present study may be attributed to differences in extraction conditions, solvent polarity, plant origin, drying conditions, extraction time, and sample preparation (Papaefthimiou et al., 2023). In addition, the phenolic content of the extract (2.91 mg GAE/g) may also have contributed to the observed antioxidant activity.

Overall, the obtained results confirm the presence of antioxidant compounds capable of reducing ferric ions, supporting the potential of *Stevia rebaudiana* as a natural source of bioactive molecules.

IV.4.2. Anti-inflammatory activity of *Stevia rebaudiana* extract

The anti-inflammatory activity of the hydroethanolic extract of *Stevia rebaudiana* leaves was evaluated using the protein denaturation assay based on BSA (Bovine Serum Albumin). This method relies on the ability of bioactive compounds to inhibit heat-induced protein denaturation, a process associated with inflammatory reactions (Sakat et al., 2010). In this study, BSA was thermally denatured in the presence of the extract, and the inhibitory effect was expressed as a percentage of inhibition. Higher inhibition values indicate stronger protection against protein denaturation. The anti-inflammatory activity was finally expressed as IC₅₀ values.

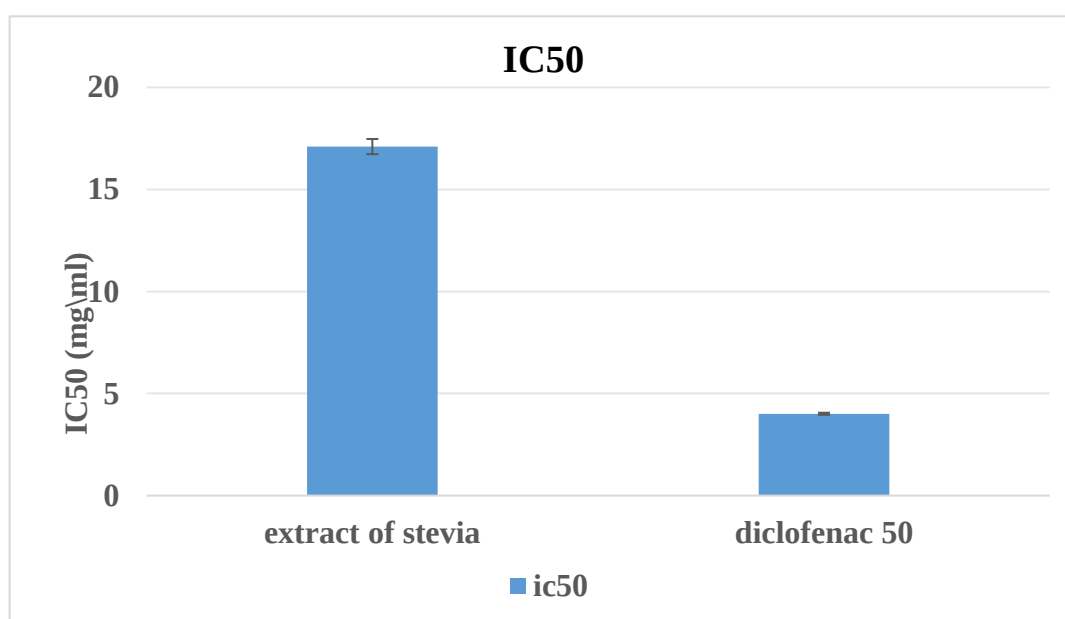


Figure IV.4: IC₅₀ values of protein denaturation inhibition of *Stevia rebaudiana* extract compared to diclofenac

The results of the protein denaturation assay using BSA (Bovine Serum Albumin) showed that the hydroethanolic extract of *Stevia rebaudiana* leaves exhibited an IC₅₀ value of 17.1 ± 0.37 mg/mL, indicating its ability to inhibit heat-induced protein denaturation, although the effect can be considered moderate. The relatively low standard deviation reflects good reproducibility and consistency of the results.

When compared with the reference drug diclofenac, which showed a much lower IC₅₀ value (4.01 ± 0.05 mg/mL), the extract appears less effective. This is expected, as diclofenac is a pure compound with strong anti-inflammatory activity, while plant extracts contain a mixture of compounds with varying biological effects.

Similar studies using the same assay on other plant species have reported lower IC₅₀ values. For example, *Myrtus communis* showed an IC₅₀ value of 60.35 µg/mL (i.e., 0.060 mg/mL), indicating much stronger anti-inflammatory activity (Alam et al., 2023).

Regarding *Stevia rebaudiana*, direct comparison with previous studies remains difficult because anti-inflammatory activity has been expressed using different evaluation systems, including percentage inhibition and IC₅₀ values. Nevertheless, it is well established that the anti-inflammatory activity of plant extracts is closely related to their phenolic and flavonoid composition (Sakat et al., 2010).

The relatively higher IC₅₀ value obtained in this study may be explained by several factors, including the use of a hydroethanolic extraction method (70% ethanol), which may not be optimal for extracting all active compounds, as well as the relatively low content of phenolic compounds (2.91 mg GAE/g) and flavonoids (0.87 mg QE/g) in the extract. Other factors such as plant origin, environmental conditions, and sample preparation are also known to influence biological activity.

Overall, the results suggest that *Stevia rebaudiana* extract exhibits measurable anti-inflammatory activity, although its effectiveness remains lower compared to standard drugs and some other plant extracts.

IV.4.3. Anti-diabetic activity of *Stevia rebaudiana* extract

The α -amylase inhibitory activity of *Stevia rebaudiana* hydroethanolic extract was evaluated using the starch-iodine method. In this assay, blue color persistence indicates enzyme inhibition, whereas its disappearance confirms starch hydrolysis. Higher inhibition percentages thus reflect stronger α -amylase inhibitory activity (Chakrabarti et al., 2014). Inhibiting α -amylase delays carbohydrate digestion and helps control postprandial blood glucose levels (Khadayat et al., 2020). Accordingly, the ability of the *Stevia extract* to inhibit this enzyme suggests a potential antidiabetic effect. Based on this protocol, the assay was applied, and the results were expressed as percentage inhibition and IC₅₀ values.

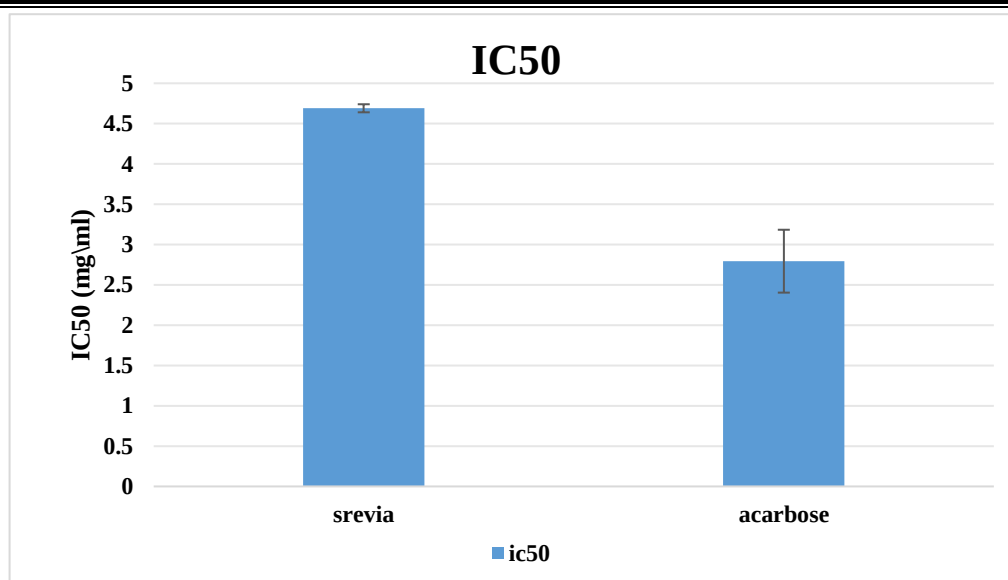


Figure IV.5: IC₅₀ values of α -amylase inhibitory activity of *Stevia rebaudiana* extract.

The α -amylase inhibition assay showed that the hydroethanolic extract of *Stevia rebaudiana* exhibited an IC₅₀ value of 4.69 ± 0.05 mg/mL, indicating a noticeable ability to inhibit the enzymatic hydrolysis of starch. The low standard deviation reflects good reproducibility and consistency of the obtained results.

When compared with acarbose, the reference antidiabetic drug, which showed an IC₅₀ value of 2.79 ± 0.39 mg/mL, the extract exhibited a lower inhibitory activity. However, the relatively close values (approximately 1.7-fold difference) suggest that the extract possesses a measurable inhibitory effect against α -amylase.

Similar studies using the same assay have reported inhibitory activities in medicinal plants rich in phenolic compounds. For example, **Moein et al. (2022)** reported that purified *anthocyanin* extracts exhibited an IC₅₀ value of 1.14 ± 0.003 mg/mL, reflecting a strong inhibitory effect, which is expected for purified compounds compared to crude extracts.

Regarding *Stevia rebaudiana*, **Singla et al. (2019)** reported that the aqueous leaf extract showed an IC₅₀ value of approximately $8.63 \mu\text{g/mL}$ (0.00863 mg/mL) against α -amylase, which is lower than the value obtained in our study, indicating a much stronger activity. The authors attributed this activity to the presence of steviol glycosides and phenolic compounds in the extract. Compared with these studies, the IC₅₀ value obtained in the present work (4.69 mg/mL) is relatively higher, indicating lower inhibitory activity.

Overall, the obtained results suggest that the hydroethanolic extract of *Stevia rebaudiana* possesses measurable α -amylase inhibitory activity (IC₅₀ = 4.69 mg/mL) and may represent a

potential natural source of bioactive compounds with antidiabetic properties, although its activity remains lower than that of aqueous extracts reported in some studies.

This difference may be explained by several factors, including the extraction solvent used (70% ethanol vs. aqueous extraction), extraction conditions, plant origin, and variations in the concentration of bioactive compounds such as steviol glycosides and phenolics.

IV.5. Sensory Evaluation Results and Discussion

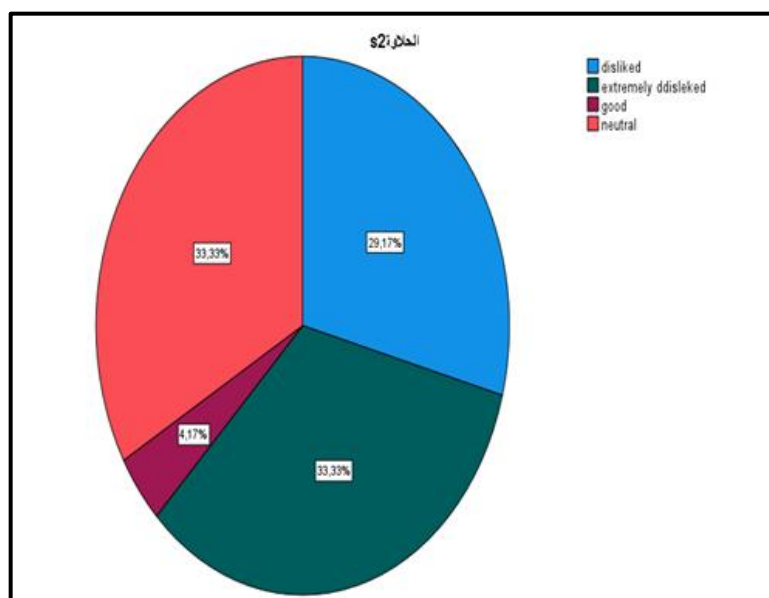
This section presents the sensory evaluation results of the formulated orange juice and Madeleine cake samples. Particular attention was given to the effect of stevia and collagen incorporation on organoleptic properties and overall consumer acceptability.

IV.5.1. Orange Juice Formulations

Different orange juice formulations were prepared using sucrose, stevia extract, and collagen protein in order to evaluate their influence on sensory characteristics and consumer acceptability. The sensory attributes assessed included sweetness, taste, bitterness, aftertaste, aroma, and overall acceptability.

IV.5.1.1. Sweetness Evaluation

The sensory results related to sweetness evaluation are presented in Figure IV.6.



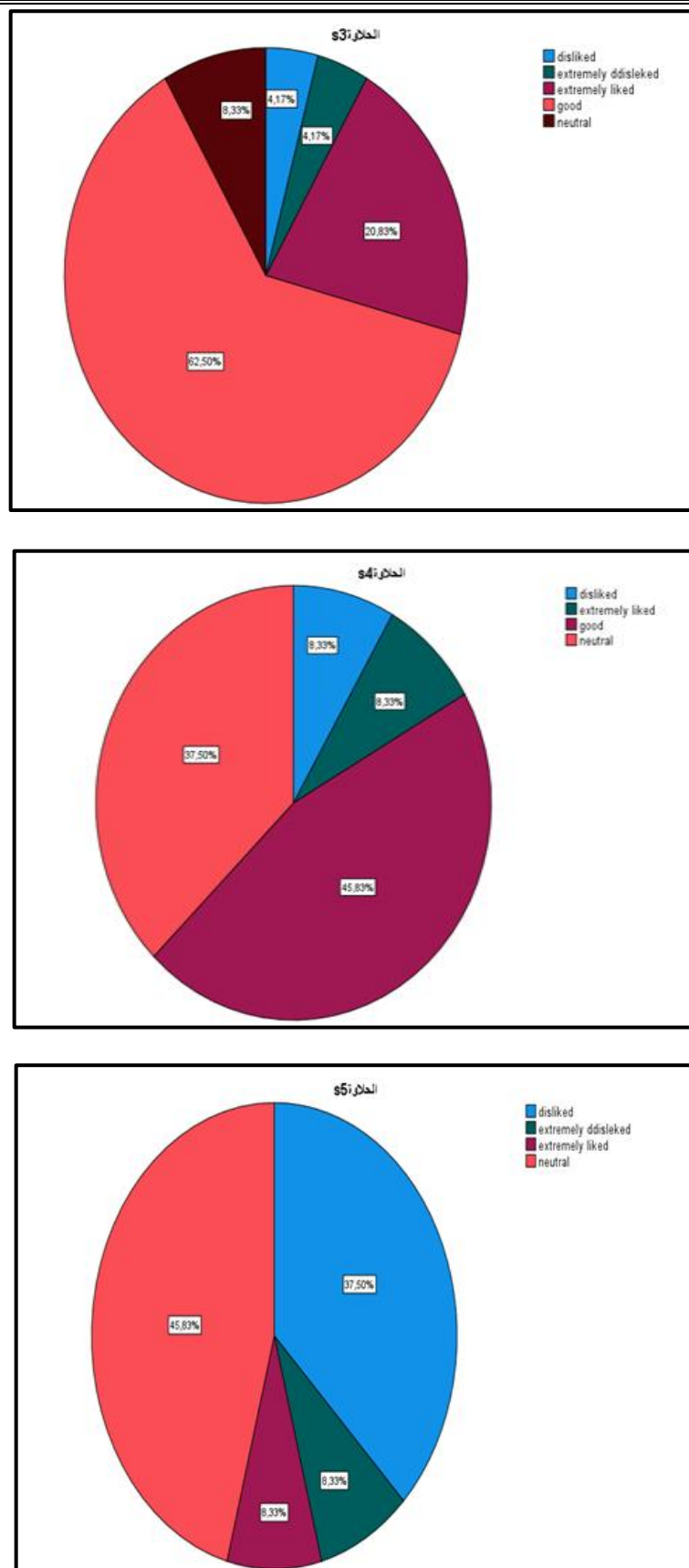


Figure IV.6: The sensory results related to sweetness evaluation are presented

Chapter IV: Results and discussion

The sensory evaluation of sweetness revealed noticeable differences among the formulated orange juice samples. As expected, Sample S3 (sucrose-sweetened) received the highest sweetness acceptance scores.

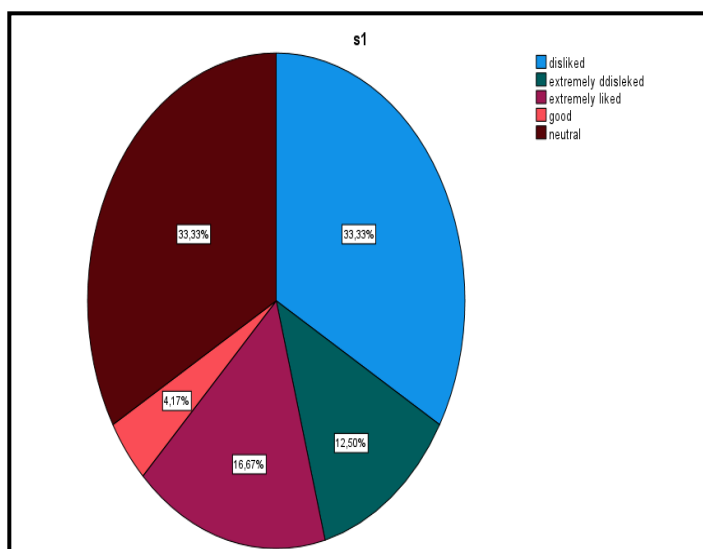
Sample S4, sweetened with stevia extract alone, showed moderate acceptability, with approximately 83.4% of panelists providing positive or neutral evaluations (41.7% positive, ~41.7% neutral), indicating that stevia can maintain acceptable sweetness in orange juice.

In contrast, Sample S5, containing both stevia extract and collagen protein, exhibited lower sweetness acceptance, which may be associated with the characteristic aftertaste of stevia and differences in formulation composition among the samples.

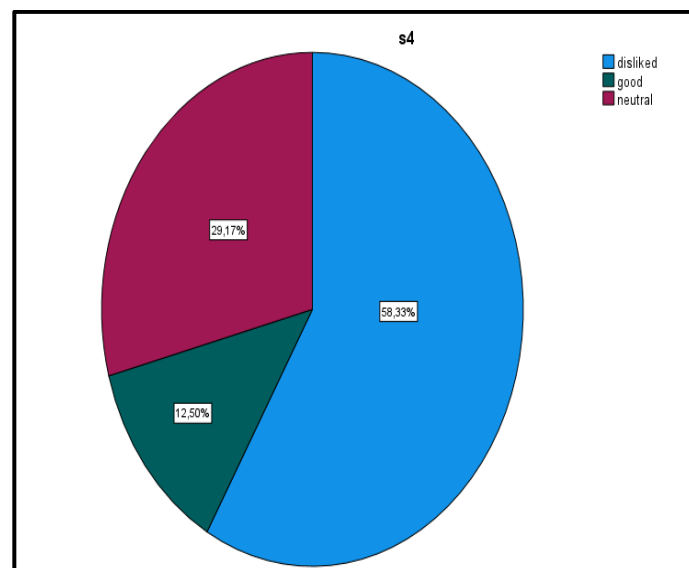
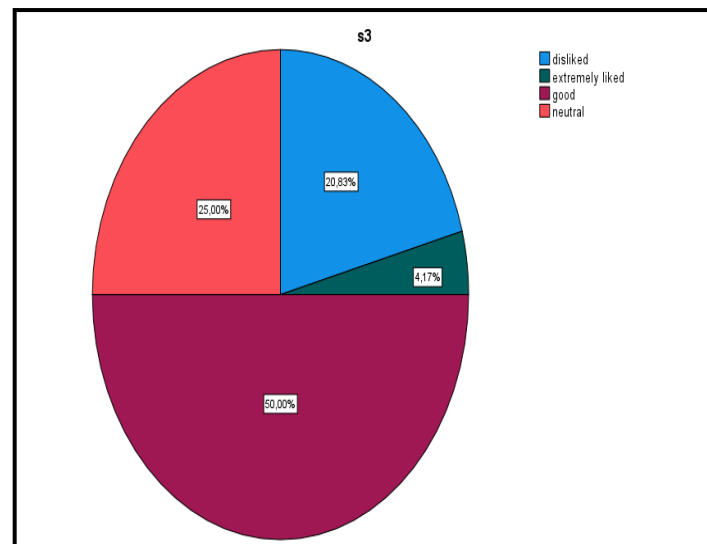
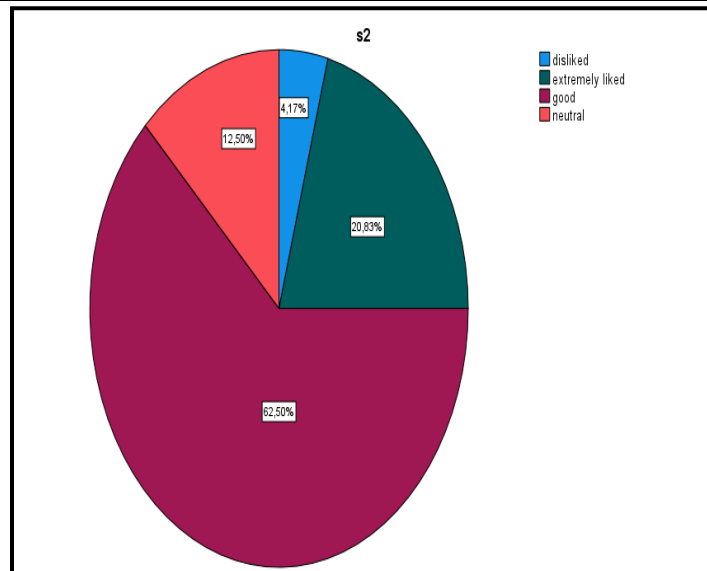
Overall, certain stevia-containing formulations maintained acceptable sweetness and sensory acceptability. Similar observations have been reported in previous studies on stevia-sweetened beverages (**Prakash et al., 2008**).

IV.5.1.2. Taste Evaluation

The sensory results related to taste evaluation are presented in Figure V.7.



Chapter IV: Results and discussion



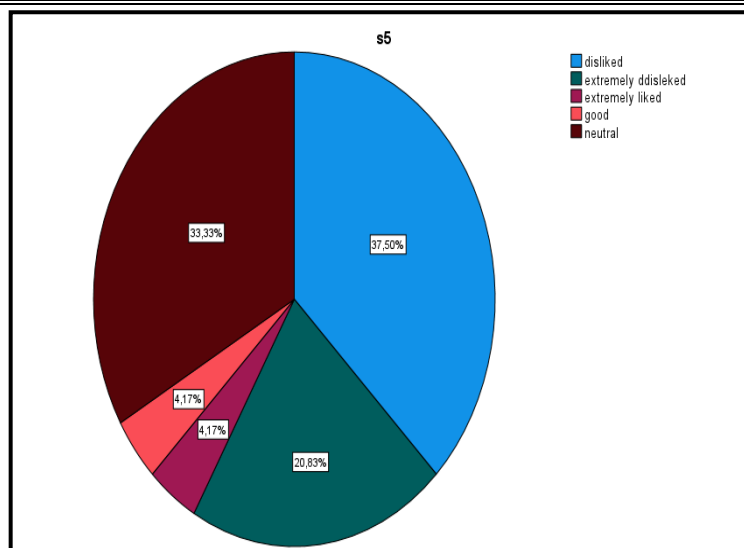


Figure IV.7: The sensory results related to taste evaluation are presented .

The sensory evaluation of taste revealed noticeable differences among the formulated orange juice samples. Sample S3 (sucrose-sweetened) showed the highest taste acceptability, with approximately 50% of panelists providing positive evaluations.

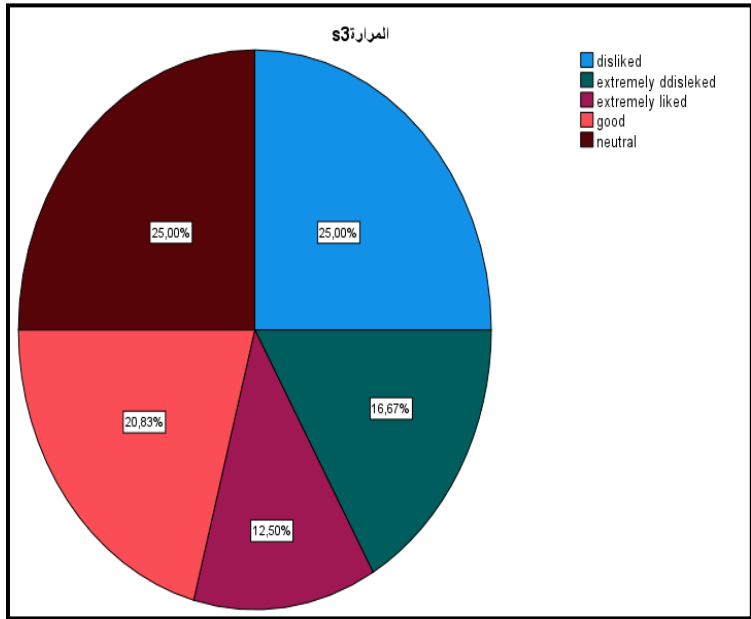
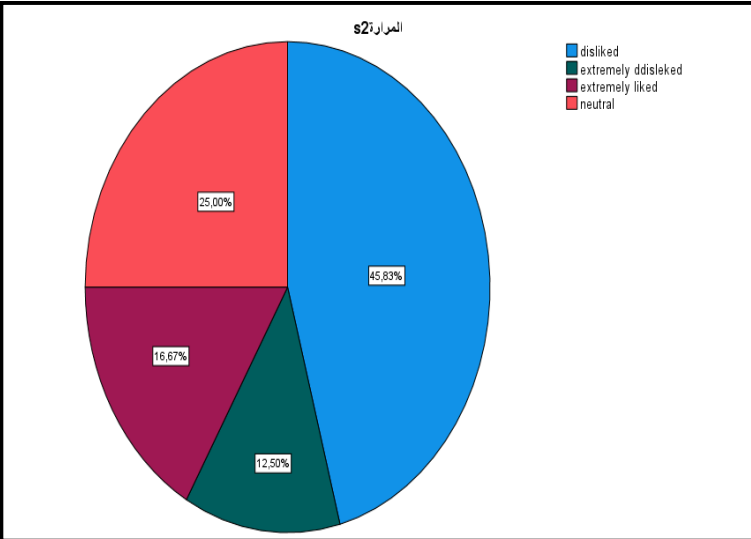
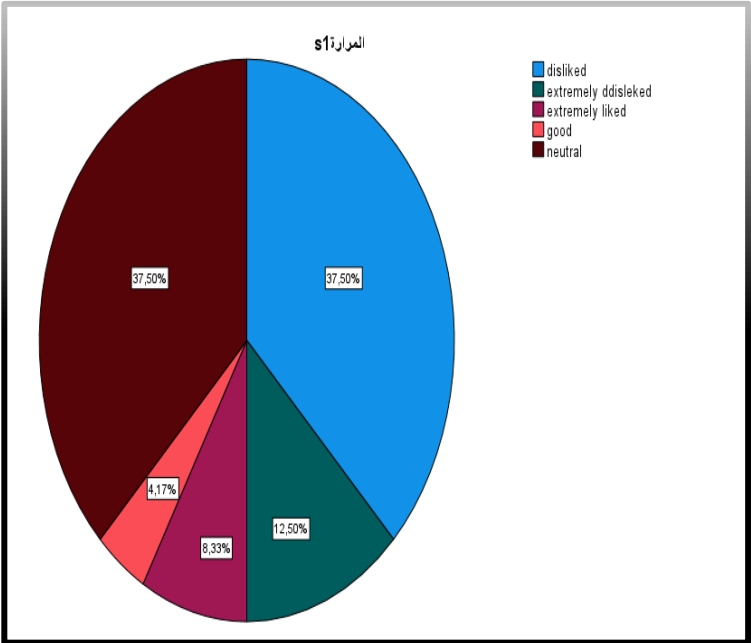
Sample S4, formulated with stevia extract alone, also showed relatively acceptable taste perception, with approximately 87.5% of panelists providing positive or neutral evaluations (29.2% positive, 58.3% neutral), indicating moderate consumer acceptance.

In contrast, Sample S5, containing both stevia extract and collagen protein, exhibited lower taste acceptability, with approximately 20.8% of panelists expressing negative evaluations, which may be related to the characteristic aftertaste of stevia and differences in formulation composition among the samples.

Overall, formulation composition significantly influenced taste perception and sensory acceptability of the orange juice samples. Similar observations have been reported in previous studies on stevia-sweetened beverages (**Prakash et al., 2008**).

IV.5.1.3. Bitterness Evaluation

The sensory results related to bitterness evaluation are presented in Figure V.8.



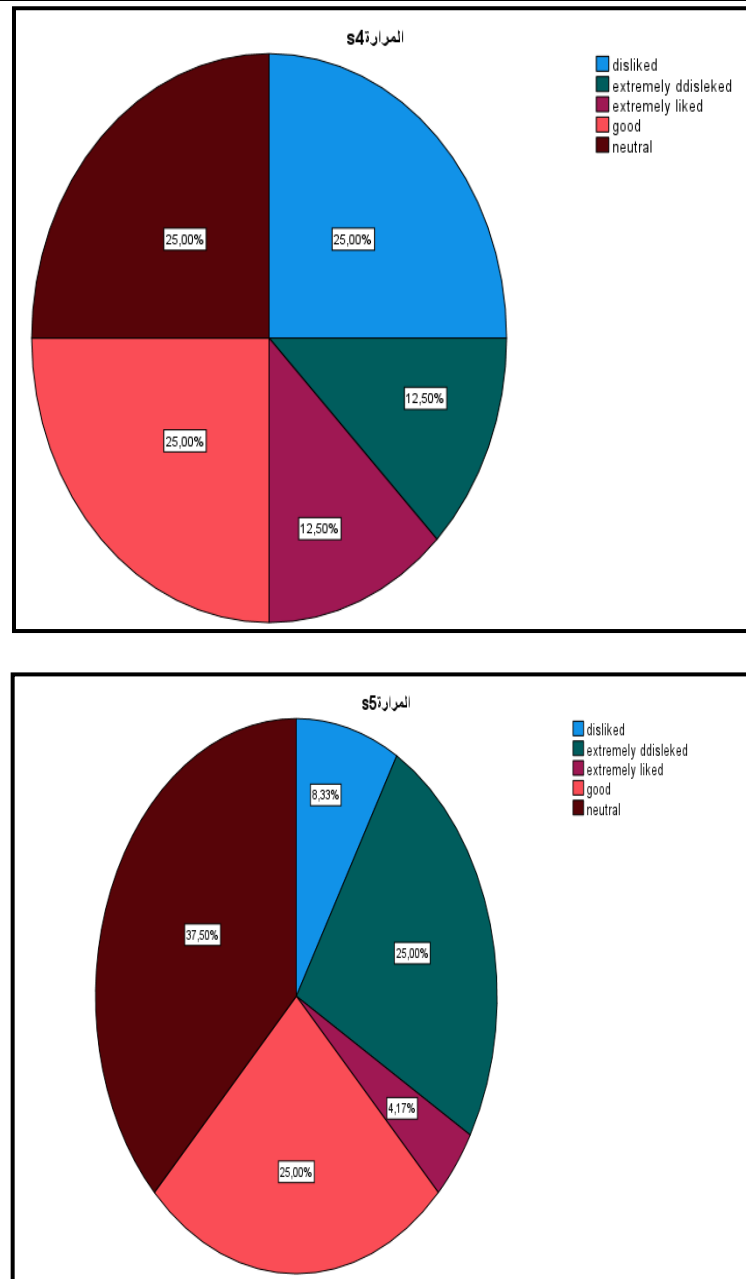


Figure IV.8: The sensory results related to bitterness evaluation are presented.

The sensory evaluation of bitterness revealed noticeable differences among the formulated orange juice samples. Sample S3 (sucrose-sweetened) showed the lowest perceived bitterness and the highest acceptability.

Sample S4, formulated with stevia extract alone, showed moderate bitterness perception, with approximately 33.3% of panelists providing positive evaluations and 25% providing neutral evaluations.

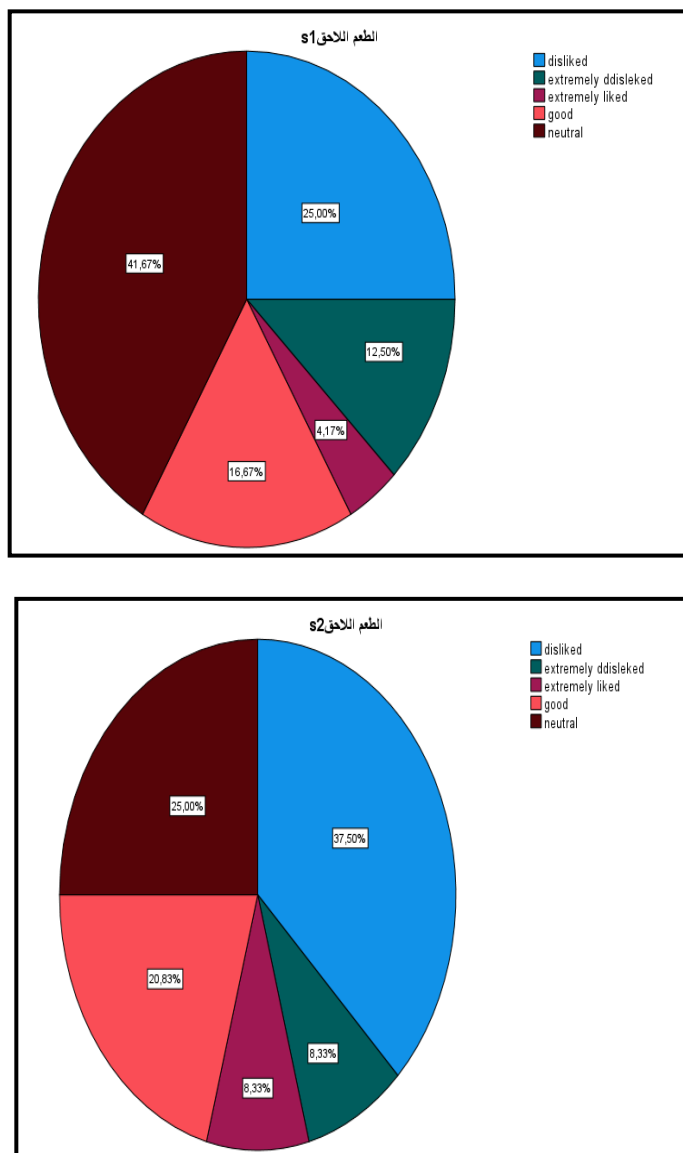
In contrast, Sample S5, containing both stevia extract and collagen protein, exhibited lower bitterness acceptability, which may be related to the characteristic bitter aftertaste of stevia and differences in formulation composition among the samples.

Chapter IV: Results and discussion

Overall, bitterness perception varied according to formulation composition, particularly in samples containing stevia. Similar observations have been reported in previous studies on stevia-sweetened beverages (Prakash et al., 2008).

IV.5.1.4. Aftertaste Evaluation

The sensory results related to aftertaste evaluation are presented in Figures IV.9.



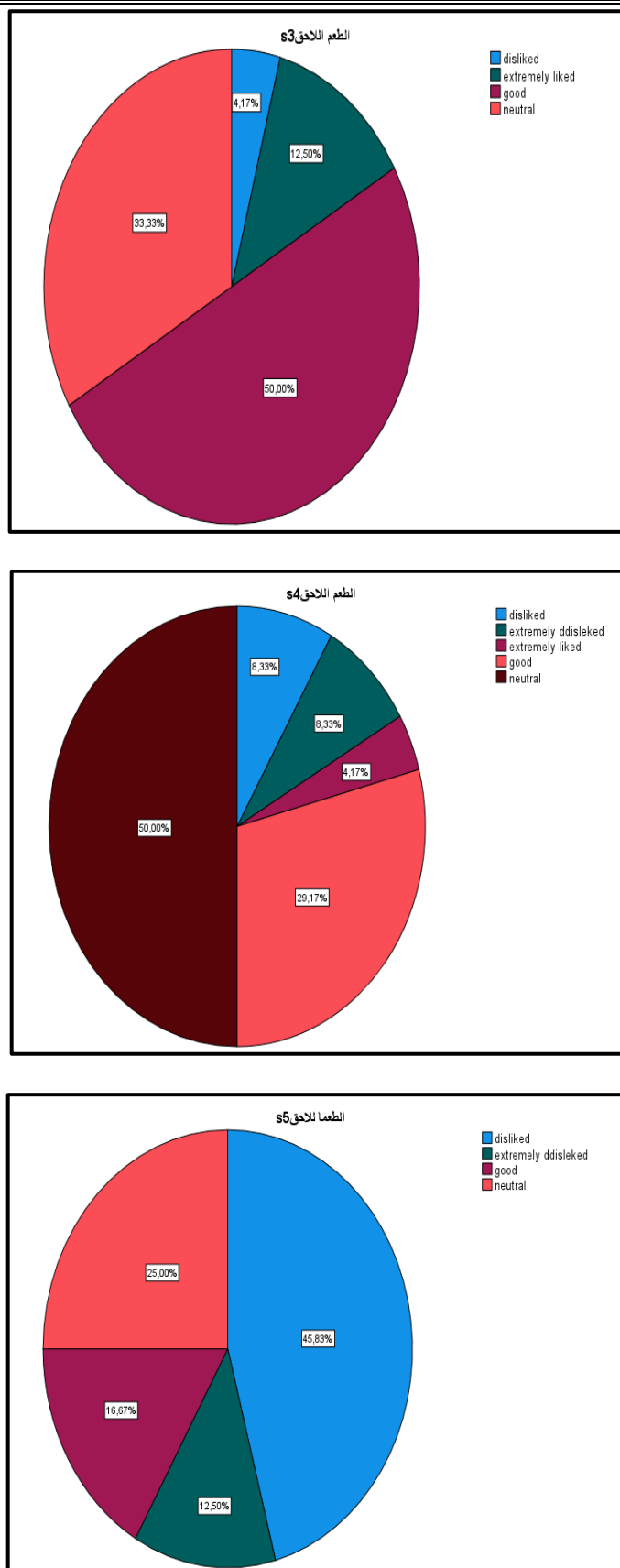


Figure IV.9: The sensory results related to aftertaste evaluation are presented.

Chapter IV: Results and discussion

The sensory evaluation of aftertaste revealed noticeable differences among the formulated orange juice samples. Sample S3 (sucrose-sweetened) showed the most acceptable aftertaste profile, with 50% of panelists providing positive evaluations.

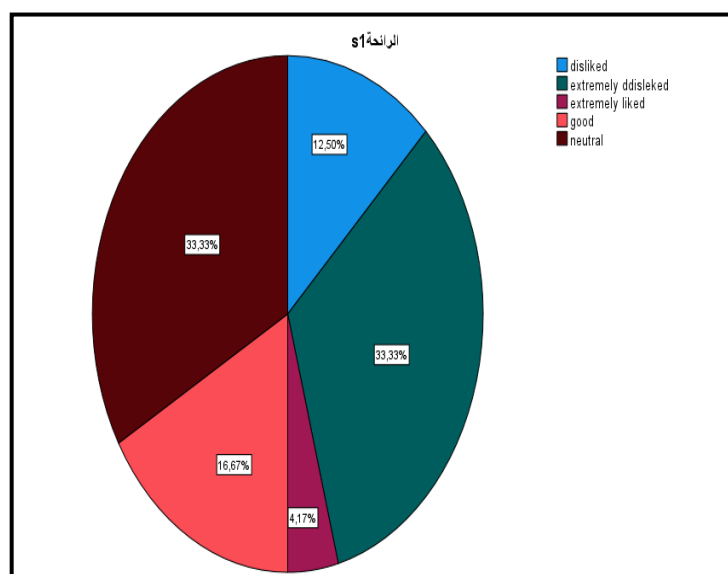
Sample S4, formulated with stevia extract alone, showed moderate aftertaste acceptability, with approximately 33.3% of panelists rating the aftertaste positively and 50% providing neutral evaluations.

In contrast, Sample S5, containing both stevia extract and collagen protein, exhibited lower aftertaste acceptability, with approximately 45.8% of panelists expressing negative evaluations, which may be related to the persistence of stevia aftertaste and differences in formulation composition among the samples.

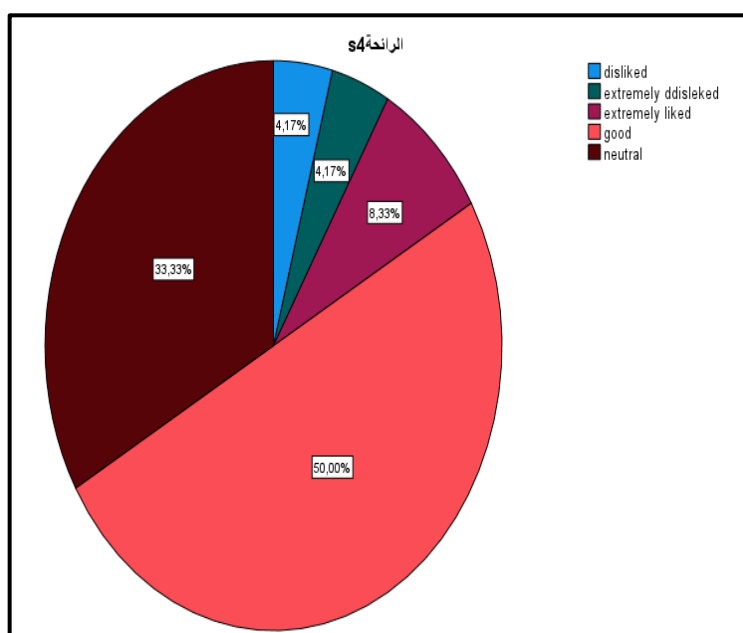
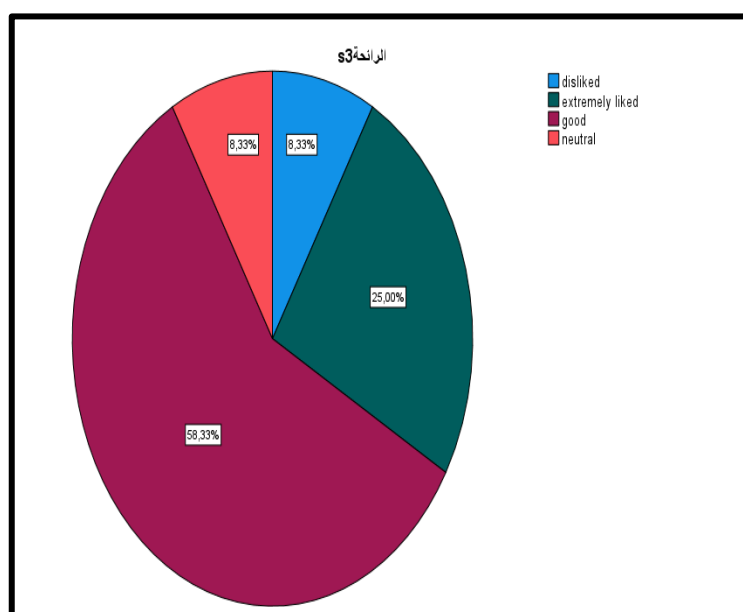
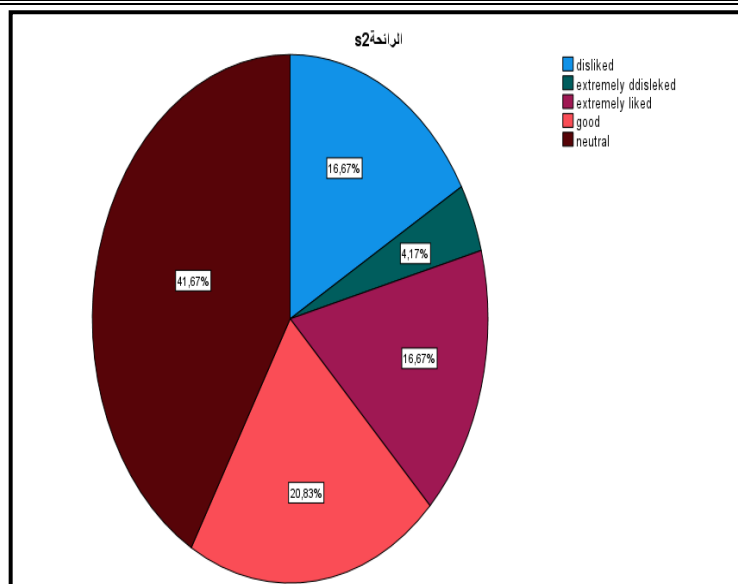
Overall, aftertaste perception varied according to formulation composition, particularly in stevia-containing samples. Similar findings have been reported in previous studies evaluating stevia-sweetened beverages (**Prakash et al., 2008**).

IV.5.1.5. Aroma Evaluation

The sensory results related to aroma evaluation are presented in Figures IV.10.



Chapter IV: Results and discussion



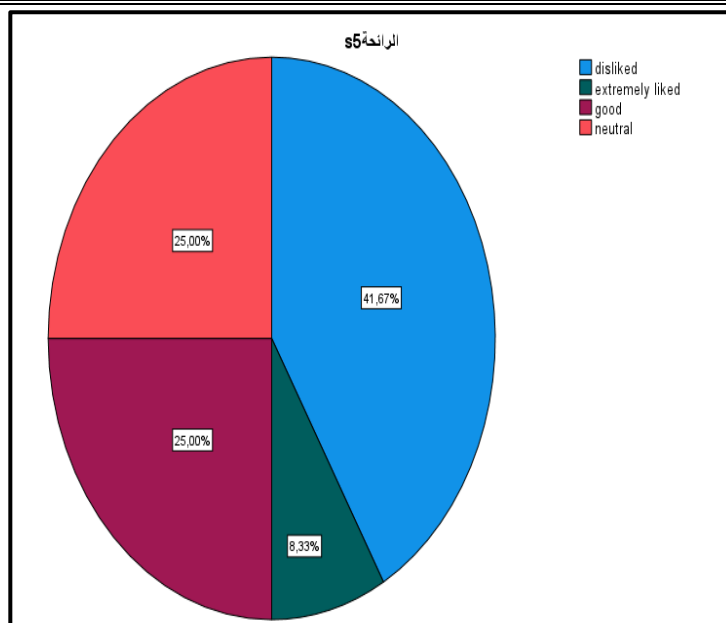


Figure V.10: The sensory results related to aroma evaluation are presented.

The sensory evaluation of aroma revealed noticeable differences among the formulated orange juice samples. Sample S3 (sucrose-sweetened) presented the highest aroma acceptability, with approximately 58.3% of panelists providing positive evaluations.

Sample S4, containing stevia extract alone, showed moderate aroma acceptability, with approximately 33.3% of panelists providing positive evaluations and 50% giving neutral evaluations.

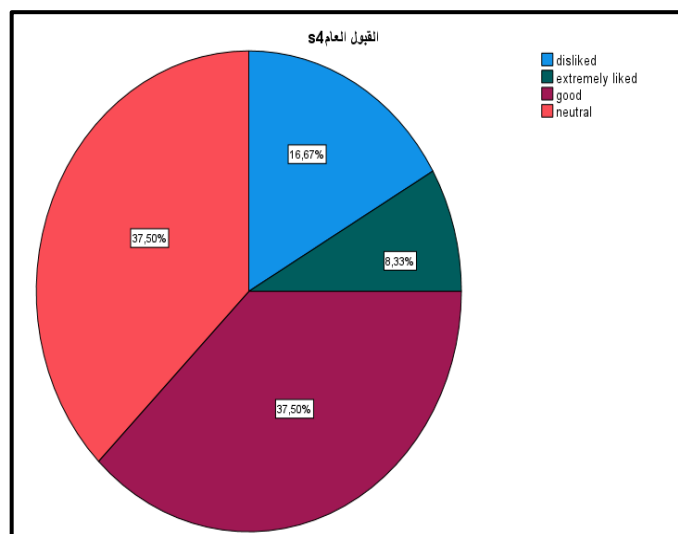
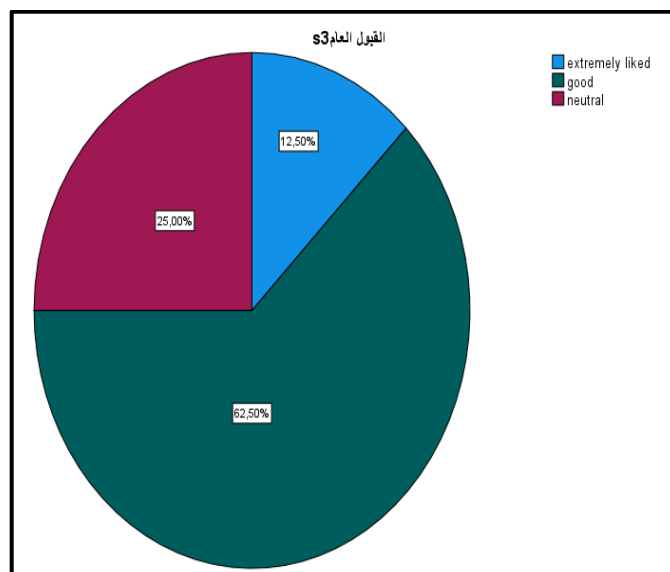
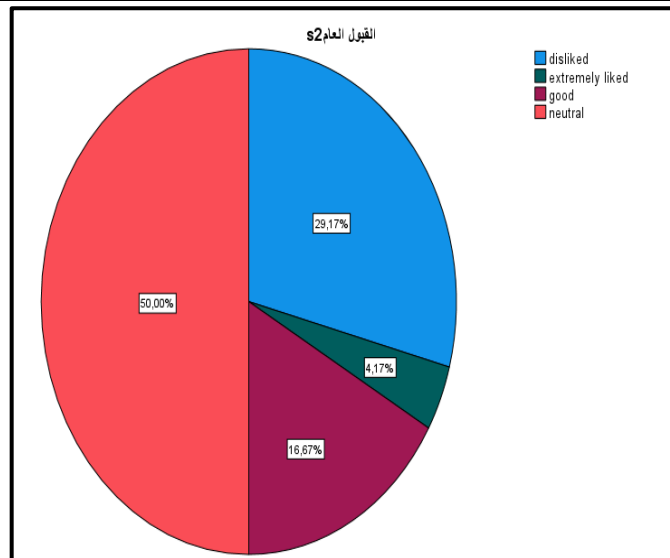
In contrast, Sample S5, formulated with both stevia extract and collagen protein, exhibited lower aroma acceptability, with approximately 41.7% of panelists expressing negative evaluations, which may be associated with differences in formulation composition and sensory perception among the samples.

Overall, aroma perception varied according to formulation composition among the tested samples. Similar observations have been reported in previous studies on stevia-based beverages (Prakash et al., 2008).

IV.5.1.6. Overall Acceptability

The sensory results related to overall acceptability evaluation are presented in Figure IV.11.

Chapter IV: Results and discussion



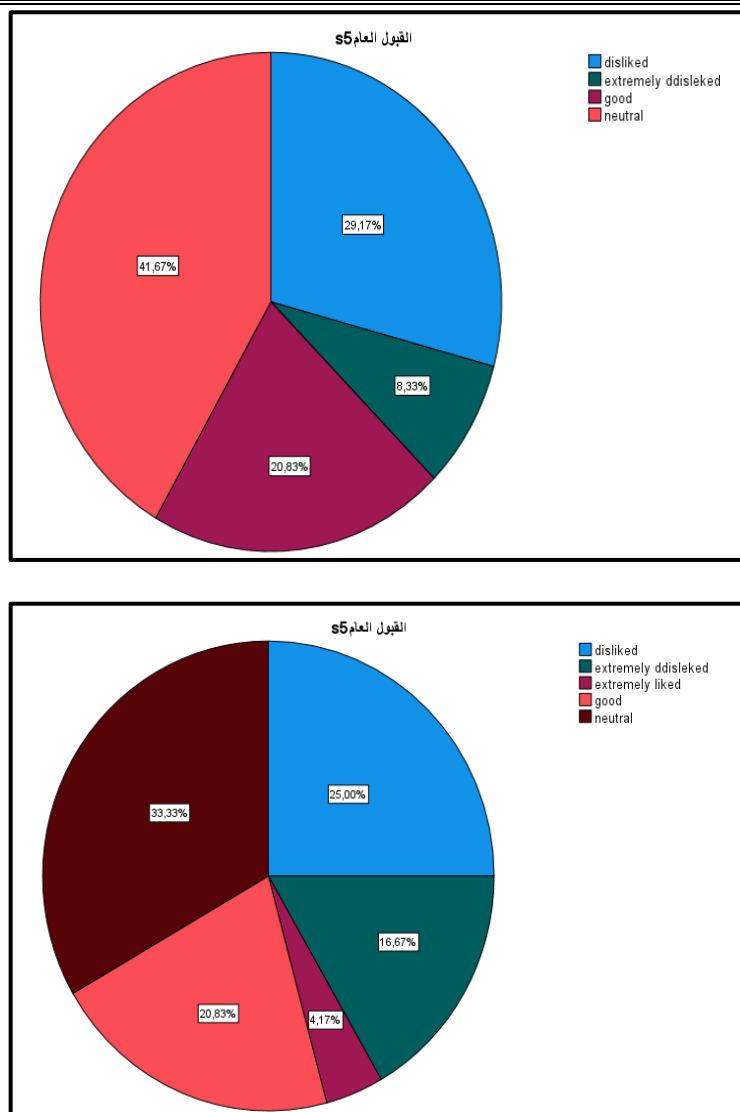


Figure IV.11: The sensory results related to overall acceptability evaluation are presented.

The sensory evaluation of overall acceptability revealed noticeable differences among the formulated orange juice samples. Sample S3 (sucrose-sweetened) showed the highest overall acceptability, with approximately 62.5% of panelists giving positive evaluations.

Sample S4, formulated with stevia extract alone, showed moderate overall acceptability. Around 37.5% of panelists expressed positive opinions, while a similar proportion provided neutral evaluations, indicating an acceptable sensory profile for most participants.

In contrast, Sample S5, containing both stevia extract and collagen protein, exhibited lower overall acceptability, with approximately 45.8% of panelists expressing negative evaluations. This reduction may be associated with differences in formulation composition and sensory perception among the samples.

Overall, the results suggest that formulations containing stevia alone maintained a relatively acceptable level of consumer acceptance compared with the other tested formulations. Similar observations have been reported in previous studies on stevia-based beverages (**Prakash et al., 2008**).

IV.5.1.7. General Discussion of Orange Juice Sensory Results

Overall, the sensory evaluation results showed that formulation composition significantly influenced the acceptability of the orange juice samples. As expected, the sucrose-sweetened sample (S3) showed the highest sensory acceptance for most evaluated attributes.

Among the stevia-containing formulations, Sample S4 (stevia extract alone) maintained a relatively acceptable sensory profile, suggesting that stevia can be incorporated as a natural sweetener in orange juice while maintaining acceptable consumer acceptance.

In contrast, Sample S5, containing both stevia extract and collagen protein, exhibited lower sensory acceptance, particularly for bitterness, aftertaste, aroma, and overall acceptability. This reduction may be associated with the characteristic aftertaste of stevioside and differences in formulation composition among the samples.

Furthermore, the acidic nature of orange juice may have contributed to the persistence of bitterness and aftertaste perception (**Belitz et al., 2009**).

Overall, the results support the potential use of stevia as a natural sugar substitute in functional orange juice formulations, although sensory optimization remains necessary. Similar observations have been reported in previous studies on stevia-sweetened beverages (**Prakash et al., 2008; Gandhi et al., 2018**).

IV.5.2. Madeleine Cake Formulations

Different madeleine cake formulations were prepared using sucrose and stevia extract in order to evaluate the effect of sugar substitution on sensory characteristics and consumer acceptability. The evaluated sensory attributes included colour, aroma, taste, texture, and overall acceptability.

IV.5.2.1. Colour Evaluation

The sensory results related to colour evaluation are presented in Figure IV.12.

Chapter IV: Results and discussion

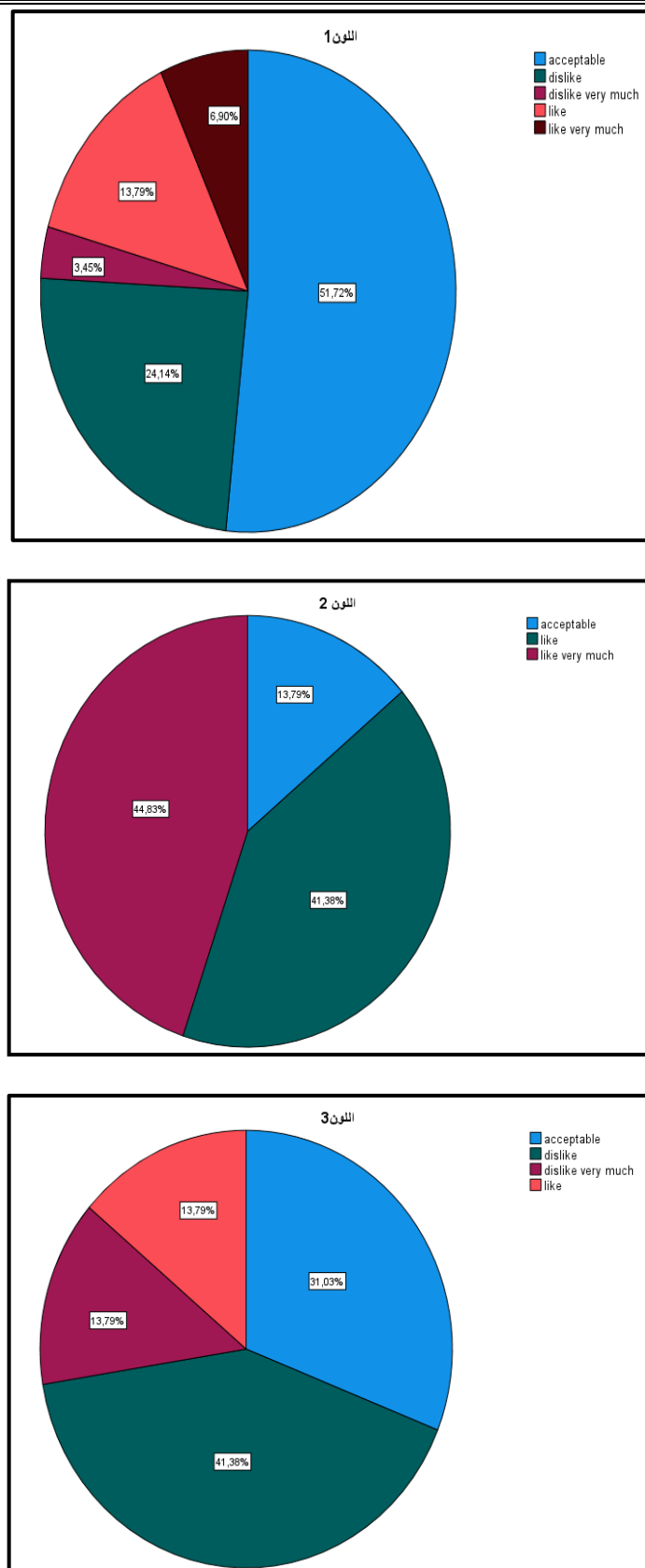


Figure IV.12: The sensory results related to colour evaluation are presented.

Chapter IV: Results and discussion

The sensory evaluation of colour revealed noticeable differences among the formulated madeleine cake samples. Sample B (sucrose-sweetened) showed the highest colour acceptability, with approximately 44.8% of panelists expressing positive evaluations.

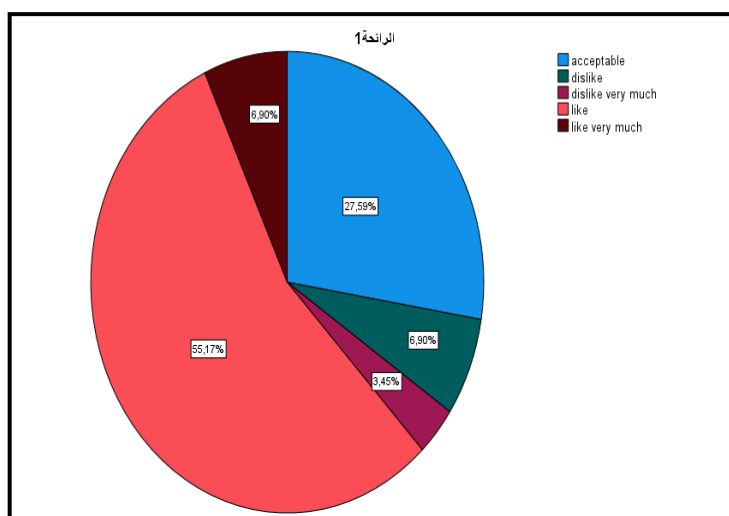
Sample A, containing both sucrose and stevia, also showed relatively good colour perception, with approximately 51.7% of panelists rating the colour as acceptable.

In contrast, Sample C, formulated with stevia alone, exhibited lower colour acceptability, with approximately 41.4% of panelists expressing negative evaluations, which may be related to the lower caramelisation and browning effect associated with stevia compared to sucrose during baking.

Overall, sugar substitution influenced colour perception and visual acceptability of the madeleine cake samples.

IV.5.2.2. Aroma Evaluation

The sensory results related to aroma evaluation are presented in Figure IV.13.



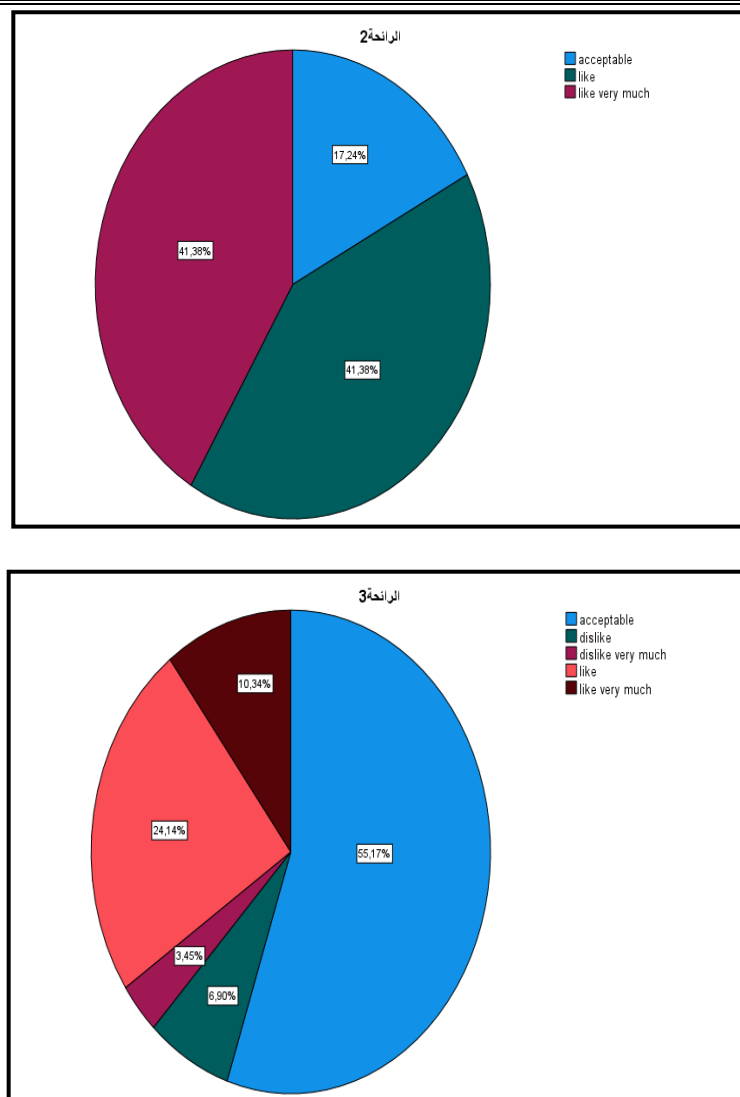


Figure IV.13: The sensory results related to aroma evaluation are presented.

The sensory evaluation of aroma revealed noticeable differences among the formulated madeleine cake samples. Sample B (sucrose-sweetened) showed the highest aroma acceptability, with approximately 41.4% of panelists providing positive evaluations and 41.4% giving neutral evaluations, indicating a relatively good level of aroma acceptance.

Sample A, containing both sucrose and stevia, also showed relatively good aroma perception, with approximately 55.2% of panelists providing positive evaluations.

In contrast, Sample C, formulated with stevia alone, exhibited lower aroma acceptability, with approximately 55.2% of panelists expressing negative evaluations. This reduction may be related to the characteristic aroma associated with stevia and the absence of sucrose, which normally contributes to pleasant baked flavour development during baking.

Overall, the results suggest that sugar substitution influenced aroma perception and sensory acceptability of the madeleine cake samples.

IV.5.2.3. Taste Evaluation

The sensory results related to taste evaluation are presented in Figure IV.14.

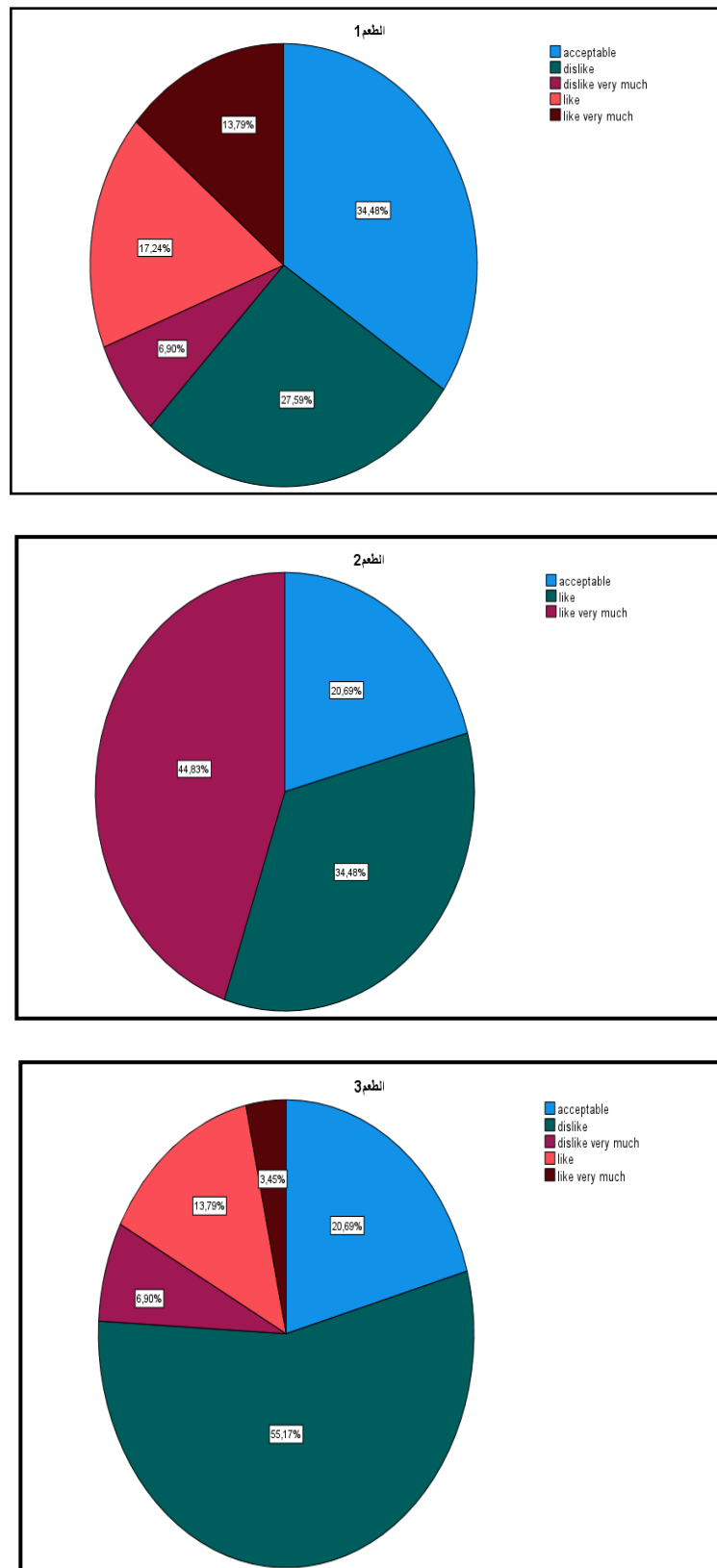


Figure IV.14: The sensory results related to taste evaluation are presented.

Chapter IV: Results and discussion

The sensory evaluation of taste revealed noticeable differences among the formulated madeleine cake samples. Sample B (sucrose-sweetened) showed the highest taste acceptability, with approximately 44.8% of panelists providing positive evaluations and 34.5% giving neutral evaluations.

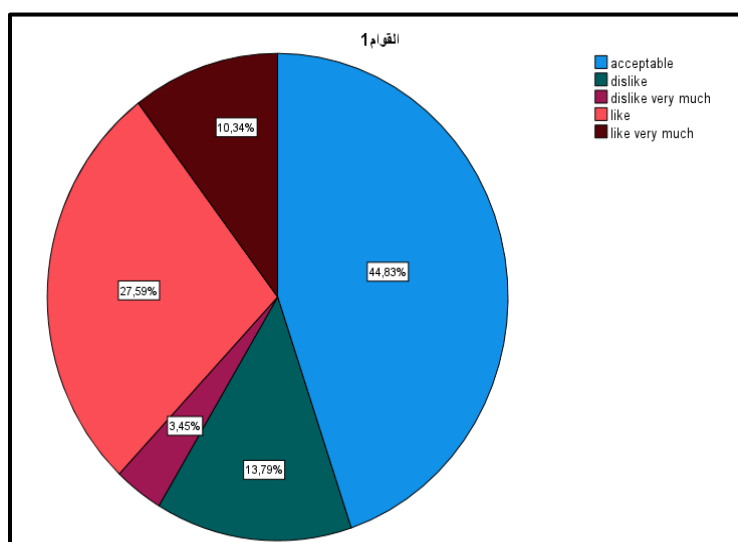
Sample A, containing both sucrose and stevia, also showed relatively good taste perception, with approximately 34.5% of panelists considering the taste acceptable and 34.5% giving neutral evaluations.

In contrast, Sample C, formulated with stevia alone, exhibited lower taste acceptability, with approximately 55.2% of panelists expressing negative evaluations. This reduction may be related to the characteristic aftertaste and bitterness associated with stevia when used as the sole sweetener.

Overall, the results suggest that sugar substitution significantly influenced taste perception and sensory acceptability of the madeleine cake samples.

IV.5.2.4. Texture Evaluation

The sensory results related to texture evaluation are presented in Figure IV.15.



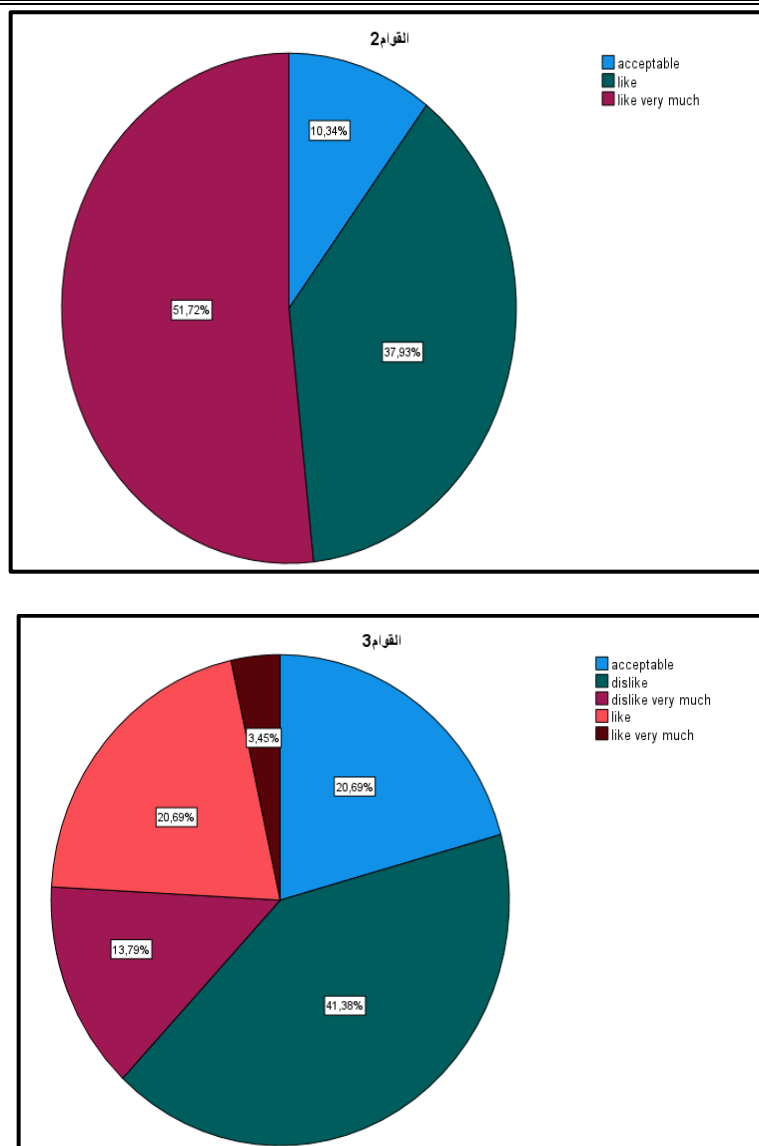


Figure IV.15: The sensory results related to texture evaluation are presented.

The sensory evaluation of texture revealed noticeable differences among the formulated madeleine cake samples. Sample B (sucrose-sweetened) showed the highest texture acceptability, with approximately 51.7% of panelists providing positive evaluations and 37.9% giving neutral evaluations.

Sample A, containing both sucrose and stevia, also showed relatively good texture perception, with approximately 27.6% of panelists providing positive evaluations and 44.8% giving neutral evaluations.

In contrast, Sample C, formulated with stevia alone, exhibited lower texture acceptability, with approximately 41.4% of panelists expressing negative evaluations. This reduction may be related to the absence of sucrose, which normally contributes to moisture retention and desirable texture development in baked products.

Chapter IV: Results and discussion

Overall, the results suggest that sugar substitution influenced texture perception and sensory acceptability of the madeleine cake samples.

IV.5.2.5. Overall Acceptability

The sensory results related to overall acceptability evaluation are presented in Figure IV.16.

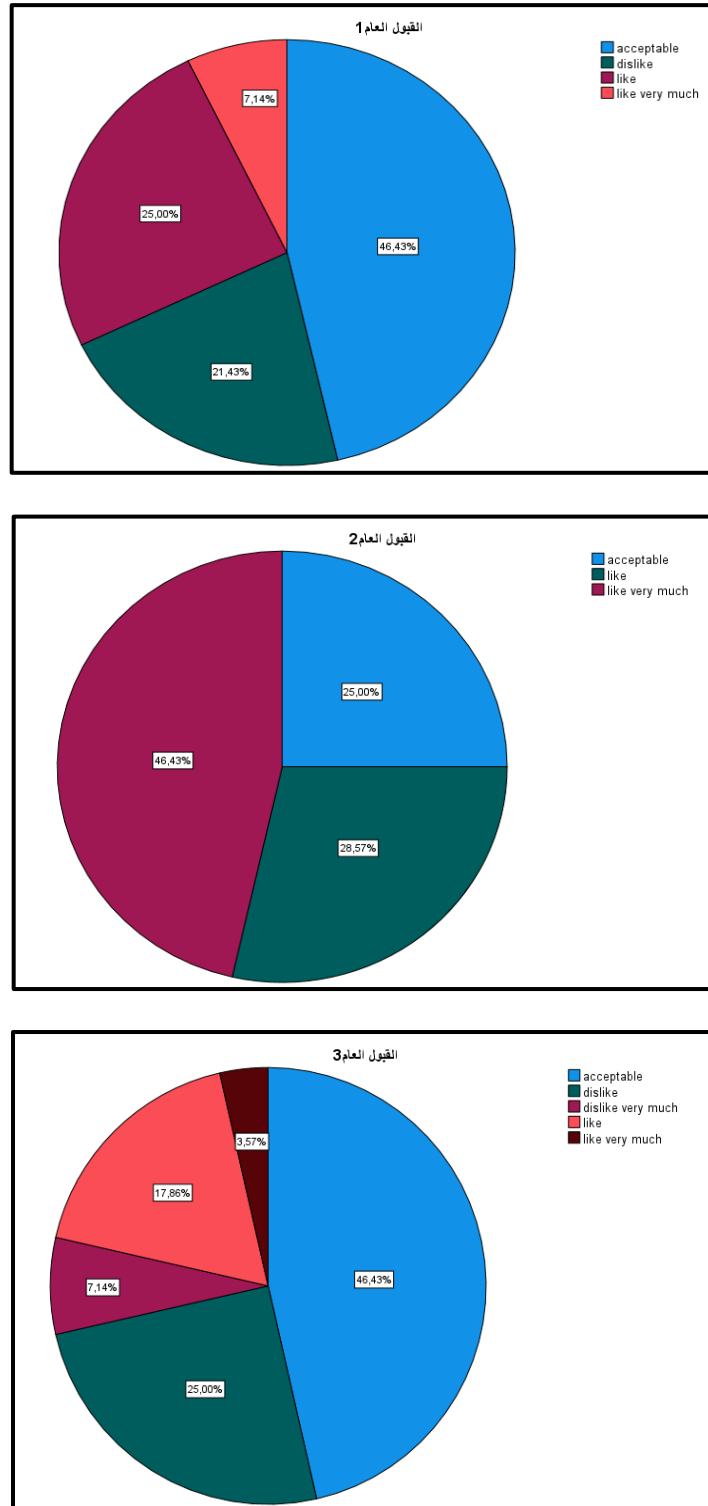


Figure IV.16: The sensory results related to overall acceptability evaluation are presented.

The sensory evaluation of overall acceptability revealed noticeable differences among the formulated madeleine cake samples. Sample B (sucrose-sweetened) showed the highest overall acceptability, with approximately 46.4% of panelists providing positive evaluations and 28.6% giving neutral evaluations.

Sample A, containing both sucrose and stevia, also showed relatively good overall acceptability, with approximately 25% of panelists expressing positive opinions and 46.4% giving neutral evaluations, indicating an acceptable sensory profile for most participants.

In contrast, Sample C, formulated with stevia alone, exhibited lower overall acceptability, with approximately 25% of panelists expressing negative evaluations (while the remaining 75% gave positive or neutral responses). This reduction may be related to the sensory changes associated with complete sugar substitution by stevia.

Overall, the results suggest that partial replacement of sucrose with stevia maintained a relatively acceptable level of consumer acceptance, whereas complete substitution reduced sensory acceptability of the madeleine cake samples.

IV.5.2.6. General Discussion of Madeleine Sensory Results

Overall, the sensory evaluation results showed that sugar substitution significantly influenced the acceptability of the madeleine cake samples. As expected, the sucrose-sweetened sample (B) showed the highest sensory acceptance for most evaluated attributes.

Sample A (sucrose + stevia) maintained a relatively acceptable sensory profile, suggesting that partial sugar substitution with stevia can reduce sugar content while preserving acceptable sensory quality.

In contrast, Sample C (stevia alone) exhibited lower sensory acceptance, particularly for aroma, taste, texture, and overall acceptability. This reduction may be related to the characteristic aftertaste of stevia and the absence of sucrose, which normally contributes to flavour development, browning, moisture retention, and texture formation during baking.

Overall, partial sugar substitution with stevia appears more suitable for madeleine cake formulations than complete replacement. Similar observations have been reported in previous studies on stevia in bakery products (Goyal et al., 2010; Prakash et al., 2008).

IV.5.3. General Conclusion of Sensory Evaluation

Overall, the sensory evaluation results demonstrated that formulation composition significantly influenced consumer acceptability of both orange juice and madeleine cake

Chapter IV: Results and discussion

samples. Sucrose-sweetened formulations showed the highest sensory acceptance, while stevia-containing formulations maintained relatively acceptable sensory profiles, particularly when stevia was used in partial combination with sucrose (Sample S4 in orange juice and Sample A in madeleines).

Complete sugar replacement (Sample S5 in orange juice and Sample C in madeleines) adversely affected bitterness, aftertaste, aroma, and overall acceptability. In contrast, partial substitution preserved consumer acceptability while reducing sugar content.

Marine collagen showed potential nutritional value, particularly regarding protein and textural properties, although its influence on sensory masking remained limited.

Overall, formulation optimization is essential to improve sensory quality and consumer acceptance of functional products containing stevia. Stevia shows promising potential as a natural low-calorie sweetener, especially when used in partial substitution of sucrose.

General Conclusion

General Conclusion

This study aimed to explore the potential of *Stevia rebaudiana* as a natural low-calorie sweetener in the development of healthier and more balanced functional food products, particularly in light of the increasing prevalence of obesity and diabetes associated with excessive consumption of added sugars. Stevia is considered a promising natural alternative to sucrose due to its high sweetening power and near-zero caloric content, making it suitable for dietary regimens intended for diabetic, overweight, and obese individuals.

The study focused on evaluating the phytochemical and biological properties of stevia extract, in addition to investigating its sensory acceptability when incorporated into different food models such as orange juice and madeleine cake.

The results showed that stevia extract contains bioactive compounds, particularly phenolics and flavonoids, which contributed to its antioxidant activity, as well as its promising antidiabetic and anti-inflammatory properties. These findings highlight the functional importance of stevia not only as a sugar substitute, but also as a natural source of bioactive compounds that may help reduce oxidative stress and certain metabolic disorders associated with obesity and diabetes.

The study also demonstrated that replacing sucrose with stevia significantly affected the sensory properties of the investigated food products. Sucrose-sweetened formulations received the highest acceptability scores, whereas stevia-containing formulations maintained relatively acceptable sensory quality, particularly under partial sugar substitution. In contrast, complete sugar replacement resulted in lower sensory acceptability due to increased bitterness and the characteristic aftertaste associated with certain steviol glycosides.

Furthermore, the incorporation of marine collagen contributed to enhancing the functional aspect of the products through protein enrichment, reflecting the possibility of developing functional foods combining natural sweetening and added nutritional value. The study also revealed that the effect of functional ingredients varied according to the type of food matrix, whether in acidic beverages or bakery products, highlighting the importance of formulation optimization depending on product characteristics.

Overall, this study confirms the promising potential of stevia in the development of low-sugar functional food products that may contribute to reducing conventional sugar consumption and supporting dietary strategies aimed at preventing obesity and diabetes. It also emphasizes the importance of further research to improve the sensory properties of stevia-based products,

General Conclusion

particularly through the development of effective strategies to reduce bitterness and aftertaste, or by combining stevia with other natural sweeteners (such as erythritol) or low amounts of sucrose, in order to produce healthier foods that meet modern consumer demands and current trends in functional nutrition.

References

References

References

1. Abou-Arab, A. E., Abou-Arab, A. A., & Abu-Salem, M. F. (2010). Physico-chemical assessment of natural sweetener stevia (*Stevia rebaudiana* Bertoni) leaves and juice. *Journal of American Science*, 6(5), 269–281.
2. Adotey, J., Chon, S. H., & Kim, H. S. (2009). Analysis of mineral and trace element compositions in *Stevia rebaudiana* Bertoni leaves. *Journal of Food Composition and Analysis*, 22(7), 680–685.
3. Alvarez-Rivera, G., Bueno, M., Ballesteros-Vivas, D., Cifuentes, A., & Ibáñez, E. (2020). Green extraction of bioactive compounds from natural sources. In E. Ibáñez & A. Cifuentes (Eds.), *Green Extraction Techniques in Food Analysis* (pp. 29–63). Comprehensive Analytical Chemistry. <https://doi.org/10.1016/bs.coac.2020.02.001>.
4. Amzad-Hossain, M., Al-Raqmi, A. H., Al-Mijizy, Z. H., Weli, A. M., & Al-Riyami, Q. (2010). Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of *Stevia rebaudiana* Bertoni. *Journal of Medical Sciences*, 10(5), 132–137.
5. Angiosperm Phylogeny Group. (2009). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society*, 161(2), 105–121. <https://doi.org/10.1111/j.1095-8339.2009.00996.x>.
6. Anton, S. D., Martin, C. K., Han, H., Coulon, S., Cefalu, W. T., Geiselman, P., & Williamson, D. A. (2010). Effects of stevia, aspartame, and sucrose on food intake, satiety, and postprandial glucose and insulin levels. *Appetite*, 55(1), 37–43. <https://doi.org/10.1016/j.appet.2010.03.009>.
7. Arora, S., & Jood, S. (2015). Effect of drying treatments on nutrient composition and phytochemical constituents of *Stevia rebaudiana* leaves. *International Journal of Food Sciences and Nutrition*, 66(2), 166–172. <https://doi.org/10.3109/09637486.2014.979603>.
8. Association Française de Normalisation. (1986). *Huiles essentielles: Recueil de normes françaises* (3rd ed.). AFNOR.
9. Atkinson, F. S., Brand-Miller, J. C., Foster-Powell, K., Buyken, A. E., & Goletzke, J. (2021). International tables of glycemic index and glycemic load values 2021: a systematic review. *The American Journal of Clinical Nutrition*, 114(5), 1625–1643. <https://doi.org/10.1093/ajcn/nqab233>.

References

10. Augustin, L. S., Kendall, C. W., Cordova, R., Ciudad-Mulero, M., Courten, B. D., Ferrari, G., ... & Jenkins, D. J. (2015). Glycemic index, glycemic load and glycemic response: An International Scientific Consensus Summit from the International Carbohydrate Quality Consortium (ICQC). *Nutrition, Metabolism and Cardiovascular Diseases*, 25(9), 795-815. <https://doi.org/10.1016/j.numecd.2015.05.005>.
11. Barbet, J., & Massin, M. (2015). Global market trends for natural sweeteners: The rise of *Stevia rebaudiana* in the food and beverage industry. *International Food Marketing & Technology*, 29(2), 14–18.
12. Batubara, I., Wijaya, S. H., & Julistiono, H. (2020). Ferric reducing antioxidant power (FRAP) and phytochemical profiling of fifteen genotypes of *Orthosiphon aristatus*. *Biocatalysis and Agricultural Biotechnology*, 25, Article 101590. <https://doi.org/10.1016/j.bcab.2020.101590>.
13. Belitz, H. D., Grosch, W., & Schieberle, P. (2009). *Food chemistry* (4th ed.). Springer-Verlag. <https://doi.org/10.1007/978-3-540-69934-7>.
14. Bender, C., da Silva, R. R., & Soares, L. A. (2023). Optimization of tannin extraction and phenolic profile from medicinal plants: Influence of extraction parameters and thermal degradation. *Phytochemical Analysis*, 34(3), 289–301. <https://doi.org/10.1002/pca.3211>.
15. Benzie, I. F. F., & Strain, J. J. (1996). The Ferric Reducing Ability of Plasma (FRAP) as a measure of “antioxidant power”: The FRAP assay. *Analytical Biochemistry*, 239(1), 70–76. <https://doi.org/10.1006/abio.1996.0292>.
16. Boonkaewwan, C., Toskulkao, C., & Tiaworanant, S. (2006). Anti-inflammatory effects of stevioside on LPS-stimulated RAW 264.7 cells and THP-1 cells. *Biological and Pharmaceutical Bulletin*, 29(1), 78–83. <https://doi.org/10.1248/bpb.29.78>.
17. Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, 28(1), 25–30. [https://doi.org/10.1016/S0023-6438\(95\)80008-5](https://doi.org/10.1016/S0023-6438(95)80008-5).
18. Brandle, J. E., & Telmer, P. G. (2007). Steviol glycoside biosynthesis. *Phytochemistry*, 68(14), 1855–1863. <https://doi.org/10.1016/j.phytochem.2007.02.010>.
19. Bridel, M., & Lavieille, R. (1931). Le principe à saveur sucrée du Kaà-hêé (*Stevia rebaudiana* Bertoni): II. Les produits d'hydrolyse diastasique du stéviolside. *Journal de Pharmacie et de Chimie*, 14, 154–161.

References

20. Bugliani, R., Cincinelli, A., & Martellini, T. (2022). Phytochemical and chemotype variations in *Stevia rebaudiana* Bertoni cultivated in Europe. *Biochemical Systematics and Ecology*, 102, Article 104421. <https://doi.org/10.1016/j.bse.2022.104421>.
21. Bunprajun, T., Tocharus, C., Suksamrarn, A., & Toskulkao, C. (2012). Stevioside modulates inflammatory signaling pathways and protects against muscle wasting in experimental models. *Phytomedicine*, 19(12), 1095–1102. <https://doi.org/10.1016/j.phymed.2012.07.004>.
22. Celhay, F. (2013). *Evaluation of extraction parameters affecting the yield and quality of plant polyphenols* [Doctoral dissertation, University of Bordeaux]. Institutional Repository.
23. Ceunen, S., & Geuns, J. M. (2013). Steviol glycosides: Chemical diversity, metabolism, and function. *Journal of Natural Products*, 76(6), 1201–1228. <https://doi.org/10.1021/np400203b>.
24. Chan, P., Tomlinson, B., Chen, Y. J., Liu, J. C., Hsieh, M. H., & Cheng, J. T. (2000). A double-blind placebo-controlled study of the effectiveness and tolerability of oral stevioside in human hypertension. *British Journal of Clinical Pharmacology*, 50(3), 215–220. <https://doi.org/10.1046/j.1365-2125.2000.00260.x>.
25. Chang, C. C., Yang, M. H., Wen, H. M., & Chern, J. C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis*, 10(3), 178–182. <https://doi.org/10.38212/2221-6618.2748>.
26. Chaouche, T. M., Haddouchi, F., Ksouri, R., & Atik-Bekara, F. (2013). Evaluation of antioxidant activity of different extracts from various parts of some Algerian medicinal plants. *Journal of Medicinal Plants Research*, 7(34), 2533–2540. <https://doi.org/10.5897/JMPR2013.4475>.
27. Chatsudthipong, V., & Muanprasat, C. (2009). Stevioside and related compounds: Therapeutic applications beyond sweetness. *Pharmacology & Therapeutics*, 121(1), 41–54. <https://doi.org/10.1016/j.pharmthera.2008.09.004>.
28. Crammer, B., & Ikan, R. (1986). Sweet glycosides from the Stevia plant. *Chemistry in Britain*, 22(10), 915–917.
29. Crozier, A., Clifford, M. N., & Ashihara, H. (Eds.). (2009). *Plant secondary metabolites: Occurrence, structure and role in the human diet*. John Wiley & Sons.
30. Curi, R., Alvarez, M., Bazotte, R. B., Botion, L. M., Godoy, J. L., & Bracht, A. (1986). Effect of *Stevia rebaudiana* on glucose tolerance in normal volunteers. *Brazilian Journal of Medical and Biological Research*, 19(6), 771–774.

References

31. Curry, L. L., & Roberts, A. (2008). Subchronic toxicity and weight management properties of steviol glycosides in animal models. *Food and Chemical Toxicology*, 46(7), S11–S20. <https://doi.org/10.1016/j.fct.2008.05.003>.
32. Dai, J., & Mumper, R. J. (2010). Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules*, 15(10), 7313–7352. <https://doi.org/10.3390/molecules15107313>.
33. Das, S., Das, A. K., Murphy, R. A., Punwani, I. C., Nasution, M. P., & Kinghorn, A. D. (1992). Evaluation of the cariogenic potential of the intense natural sweeteners stevioside and rebaudioside A. *Caries Research*, 26(5), 363–366. <https://doi.org/10.1159/000261469>.
34. Devasagayam, T. P., Tilak, J. C., Bloor, K. K., Sane, K. S., Ghaskadbi, S. S., & Lele, R. D. (2004). Free radicals and antioxidants in human health: Current status and future prospects. *Journal of Association of Physicians of India*, 52, 794–804.
35. DuBois, G. E., & Prakash, I. (2012). Non-caloric sweeteners, sweetness modulators, and sweetener enhancers. *Annual Review of Food Science and Technology*, 3(1), 353–380. <https://doi.org/10.1146/annurev-food-022811-101236>.
36. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, 28(3), 350–356. <https://doi.org/10.1021/ac60111a017>.
37. EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS). (2010). Scientific Opinion on the safety of steviol glycosides for the proposed uses as a food additive. *EFSA Journal*, 8(4), 1537. <https://doi.org/10.2903/j.efsa.2010.1537>.
38. Escutia, M. S., Sanchez-Nava, A., & Castillo, R. (2019). Phytochemical characterization and nutritional value of *Stevia rebaudiana* Bertoni leaves: A review. *Food Chemistry*, 280, 142–150.
39. European Food Safety Authority (EFSA). (2010). Scientific opinion on the safety of steviol glycosides for the proposed uses as a food additive. *EFSA Journal*, 8(4), 1537. <https://doi.org/10.2903/j.efsa.2010.1537>.
40. Ferrazzano, G. F., Cantile, T., Alcidi, B., Coda, M., Ingenito, A., Zarrelli, A., Di Fabio, G., & Pollio, A. (2015). Is *Stevia rebaudiana* Bertoni a cariogenic agent or a phytotherapeutic resource for oral health? *Molecules*, 20(2), 2614–2631. <https://doi.org/10.3390/molecules20022614>.
41. Ferreira, E. B., de Assis Rocha Neves, F., da Costa, M. A., do Nascimento, O., & Bracht, A. (2006). Comparative effects of Stevia extracts on mitochondrial respiration and

References

- gluconeogenesis in diabetic rat livers. *Phytomedicine*, 13(8), 537–543. <https://doi.org/10.1016/j.phymed.2005.09.004>.
42. Ferri, L. A. F., Alves-Do-Prado, W., Yamada, S. S., Gazola, S., Batista, M. R., & Bazotte, R. B. (2006). Investigation of the antihypertensive effect of oral crude stevioside in spontaneously hypertensive rats. *Phytomedicine*, 13(3), 178–185. <https://doi.org/10.1016/j.phymed.2005.01.006>.
43. Fleit, H. B. (2014). Chronic inflammation. In *Pathobiology of human disease* (pp. 222–234). Elsevier.
44. Gardana, C., Scaglianti, M., & Simonetti, P. (2003). Evaluation of steviol and its glycosides in *Stevia rebaudiana* leaves and commercial sweeteners by ultra-high-performance liquid chromatography-mass spectrometry. *Journal of Chromatography A*, 1005(1-2), 143–150.
45. Gardana, C., Simonetti, P., Canzi, E., Zanchi, R., & Pietta, P. (2003). Metabolism of stevioside and rebaudioside A by group microflora. *Journal of Agricultural and Food Chemistry*, 51(22), 6618–6622. <https://doi.org/10.1021/jf030361g>.
46. Geuns, J. M. (2003). Stevioside. *Phytochemistry*, 64(5), 913–921. [https://doi.org/10.1016/S0031-9422\(03\)00426-6](https://doi.org/10.1016/S0031-9422(03)00426-6).
47. Geuns, J. M., Buyse, J., Vankeirsbilck, A., & Temme, E. H. (2007). Metabolism of stevioside by healthy volunteers. *Food and Chemical Toxicology*, 45(12), 2420–2424. <https://doi.org/10.1016/j.fct.2007.06.019>.
48. Ghezal, A., & Remili, F. (2024). Optimization of drying methods for *Stevia rebaudiana* leaves and their effect on phytochemical content. *Algerian Journal of Natural Products*, 12(2), 45–53. <https://doi.org/10.1016/j.ajnp.2024.01.002>.
49. Goettemoeller, J., & Ching, A. (1999). Seed germination in *Stevia rebaudiana*. In *Perspectives on new crops and new uses* (pp. 510–511). ASHS Press.
50. Goyal, S. K., Samsher, & Goyal, R. K. (2010). Stevia (*Stevia rebaudiana*) a bio-sweetener: A review. *International Journal of Food Sciences and Nutrition*, 61(1), 1–10. <https://doi.org/10.3109/09637480903193049>.
51. Granados-Guzmán, G., Castro-Ríos, R., de la Luz Salazar-Cavazos, M., & Waksman-Minsky, N. (2022). Optimization of a starch-iodine microplate assay for screening alpha-amylase inhibitors from plant extracts. *Phytochemical Analysis*, 33(3), 441–448. <https://doi.org/10.1002/pca.3102>.

References

52. Gregersen, S., Jeppesen, P. B., Holst, J. J., & Hermansen, K. (2004). Antihyperglycemic effects of stevioside in type 2 diabetic subjects. *Metabolism*, 53(1), 73-76. <https://doi.org/10.1016/j.metabol.2003.07.013>.
53. Guleria, P., & Yadav, S. K. (2013). Identification and characterization of UDP-glycosyltransferases involved in steviol glycosides biosynthesis from *Stevia rebaudiana*. *Gene*, 532(1), 101–109. <https://doi.org/10.1016/j.gene.2013.09.025>.
54. Hajhashemi, S., & Geuns, J. M. (2013). Steviol glycosides, total phenolics, flavonoids and antioxidant capacity in different organs of *Stevia rebaudiana* grown in Iran. *Journal of Medicinal Plants Research*, 7(34), 2521–2529.
55. Halliwell, B., & Gutteridge, J. M. C. (2015). *Free radicals in biology and medicine* (5th ed.). Oxford University Press.
56. Halliwell, B., Wasil, M., & Grootveld, M. (1987). Biologically significant scavenging of the myeloperoxidase-derived oxidant hypochlorous acid by ascorbic acid. *FEBS Letters*, 213(1), 15–17. [https://doi.org/10.1016/0014-5793\(87\)81454-9](https://doi.org/10.1016/0014-5793(87)81454-9).
57. Hanhineva, K., Törrönen, R., Bondia-Pons, I., Pekkinen, J., Kolehmainen, M., Mykkänen, H., & Poutanen, K. (2010). Impact of dietary polyphenols on carbohydrate metabolism. *International Journal of Molecular Sciences*, 11(4), 1365–1402. <https://doi.org/10.3390/ijms11041365>.
58. Harborne, J. B. (1998). *Phytochemical methods: A guide to modern techniques of plant analysis* (3rd ed.). Chapman & Hall.
59. Hedden, P., & Thomas, S. G. (2012). *The gibberellins: Biosynthesis and signal transduction*. John Wiley & Sons.
60. Heim, K. E., Tagliaferro, A. R., & Bobilya, D. J. (2002). Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships. *The Journal of Nutritional Biochemistry*, 13(10), 572–584. [https://doi.org/10.1016/S0955-2863\(02\)00208-5](https://doi.org/10.1016/S0955-2863(02)00208-5).
61. Hellfritsch, C., Brockhoff, A., Stähler, F., Meyerhof, W., & Hofmann, T. (2012). Human psychophysical and taste receptor responses to steviol glycosides. *Journal of Agricultural and Food Chemistry*, 60(27), 6782-6793. <https://doi.org/10.1021/jf301297n>.
62. Himanshu, M., Sharma, S., & Singh, R. (2011). Protective effect of polyphenolic compounds from *Stevia rebaudiana* on hepatic and renal tissues in experimental diabetes. *Journal of Diabetes and Its Complications*, 25(4), 265–271.
63. Hsieh, M. H., Chan, P., Sue, Y. M., Liu, J. C., Liang, T. H., Huang, T. Y., Tomlinson, B., Chow, M. S., Kao, P. F., & Chen, Y. J. (2003). Efficacy and tolerability of oral stevioside

References

- in patients with mild essential hypertension: A two-year, randomized, placebo-controlled study. *Clinical Therapeutics*, 25(11), 2797–2808. [https://doi.org/10.1016/S0149-2918\(03\)80334-X](https://doi.org/10.1016/S0149-2918(03)80334-X).
64. Hutapea, A. M., Toskulkao, C., Buddhasukh, D., Wilairat, P., & Glinsukon, T. (1997). Digestion of stevioside, a natural sweetener, by various digestive enzymes. *Journal of Clinical Biochemistry and Nutrition*, 23(3), 177–186. <https://doi.org/10.3164/jcbtn.23.177>.
65. Jain, P. K., Sharma, N., & Jain, A. (2007). Anti-obesity and weight management programs using non-nutritive sweeteners in animal models. *Journal of Ethnopharmacology*, 112(2), 345–351.
66. Jenkins, D. J., Wolever, T. M., Taylor, R. H., Barker, H., Fielden, H., Baldwin, J. M., ... & Hockaday, T. D. (1981). Glycemic index of foods: a physiological basis for carbohydrate exchange. *The American Journal of Clinical Nutrition*, 34(3), 362–366. <https://doi.org/10.1093/ajcn/34.3.362>.
67. Jentzer, A. (2015). *Minerals and trace elements in sweet plants: Focus on Stevia rebaudiana*. Éditions Lavoisier.
68. Jooker, E., Amery, R., Vanculen, S., Geuns, J., & Meesschaert, B. (2012). Stability of steviol glycosides in several food matrices. *Journal of Agricultural and Food Chemistry*, 60(42), 10606–10612. <https://doi.org/10.1021/jf302621r>.
69. Judd, W. S., Campbell, C. S., Kellogg, E. A., Stevens, P. F., & Donoghue, M. J. (2016). *Plant systematics: A phylogenetic approach* (4th ed.). Sinauer Associates.
70. Kaushik, R., Narula, P., Sahu, A., Gala, M. K., & Sharma, P. K. (2010). Nutrient composition of *Stevia rebaudiana* Bertoni leaves and its exploitation as a sweetener in value-added products. *Journal of Food Science and Technology*, 47(6), 661–664. <https://doi.org/10.1007/s13197-010-0109-1>.
71. Kennelly, E. J. (2002). Sweet and non-sweet constituents of *Stevia rebaudiana*. In A. D. Kinghorn (Ed.), *Stevia: The genus Stevia* (pp. 68–85). Taylor & Francis.
72. Khan, S. A., Ahmad, R., & Aslam, M. (2022). Quantitative determination of tannins and antioxidant potential of hydroalcoholic and aqueous extracts of *Stevia rebaudiana*. *Journal of Herbal Medicine*, 32, Article 100523. <https://doi.org/10.1016/j.hermed.2022.100523>.
73. Kim, I. S., Yang, M. R., Lee, O. H., & Kang, S. N. (2011). Antioxidant activities of hot water extracts from various parts of *Stevia rebaudiana* Bertoni. *Microbiology and Biotechnology Letters*, 39(3), 200–205.

References

74. Kinghorn, A. D. (1987). Biologically active compounds from plants with medicinal and sweetening properties. *Journal of Natural Products*, 50(6), 1009–1024. <https://doi.org/10.1021/np50054a001>.
75. Kinghorn, A. D. (Ed.). (2002). *Stevia: The genus Stevia*. Taylor & Francis.
76. Kobayashi, M., Horikawa, S., Degrandi, I. H., Ueno, J., & Mitsuhashi, H. (1977). Dulcoside A and B, new diterpene glycosides from *Stevia rebaudiana* Bertoni. *Phytochemistry*, 16(9), 1405–1408. [https://doi.org/10.1016/S0031-9422\(00\)88792-0](https://doi.org/10.1016/S0031-9422(00)88792-0).
77. Kohda, H., Kasai, R., Yamasaki, K., Murakami, K., & Tanaka, O. (1976). New sweet diterpene glucosides from *Stevia rebaudiana*. *Phytochemistry*, 15(6), 981–983. [https://doi.org/10.1016/S0031-9422\(00\)84392-7](https://doi.org/10.1016/S0031-9422(00)84392-7).
78. Koyama, E., Kitazawa, K., Osumi, T., Izawa, O., Kakegawa, K., Fujino, A., & Ui, M. (2003). In vitro metabolism of steviol glycosides, stevioside and rebaudioside A, by human intestinal microflora. *Food and Chemical Toxicology*, 41(3), 359–374. [https://doi.org/10.1016/S0278-6915\(02\)00236-1](https://doi.org/10.1016/S0278-6915(02)00236-1).
79. Kroyer, G. T. (2010). *Stevia rebaudiana* Bertoni—Sweetener and antioxidant. *LWT - Food Science and Technology*, 43(3), 407–410. <https://doi.org/10.1016/j.lwt.2009.09.009>.
80. Kumar, S., & Tripathy, S. (2025). Advanced extraction techniques and solvent selection strategies for polyphenols and bioactive compounds from medicinal plants. *Phytotherapy Research*, 39(1), 112–128. <https://doi.org/10.1002/ptr.7891>.
81. Latham, M. C. (2002). *Human nutrition in the developing world* (FAO Food and Nutrition Series No. 29). Food and Agriculture Organization of the United Nations.
82. Lemus-Mondaca, R., Vega-Gálvez, A., & Ah-Hen, K. (2023). Chemical composition and sensory properties of aqueous extracts of *Stevia rebaudiana* Bertoni leaves as a function of processing methods. *Food Chemistry*, 401, Article 134120. <https://doi.org/10.1016/j.foodchem.2022.134120>.
83. Lemus-Mondaca, R., Vega-Gálvez, A., Zura-Bravo, L., & Ah-Hen, K. (2012). *Stevia rebaudiana* Bertoni, source of a high-potency natural sweetener: A comprehensive review on the biochemical, nutritional and functional aspects. *Food Chemistry*, 132(3), 1121–1132. <https://doi.org/10.1016/j.foodchem.2011.11.140>.
84. Lewis, W. H. (1992). Early uses of *Stevia rebaudiana* (Asteraceae) leaves by Guaraní Indians. *Economic Botany*, 46(3), 336–340.

References

85. Lobete, M. M., Fernandez, P. A., & Busto, M. D. (2016). Stability and sensory optimization of steviol glycosides in acidic functional beverages. *Food Chemistry*, 203, 412-420. <https://doi.org/10.1016/j.foodchem.2016.02.090>.
86. Maki, K. C., Curry, L. L., Reeves, M. S., Toth, P. D., McKenney, J. M., Farmer, M. V., Schwartz, S. L., Lubin, B. C., Booplan, R., Dicklin, M. R., Carakostas, M. C., & Harrison, A. R. (2008). Chronic consumption of rebaudioside A, a steviol glycoside, in men and women with normal and low-normal blood pressure. *Food and Chemical Toxicology*, 46(7), S47–S53. <https://doi.org/10.1016/j.fct.2008.04.030>.
87. McDougall, G. J., Shpiro, F., Dobson, P., Smith, P., Blake, A., & Stewart, D. (2005). Different polyphenolic components of soft fruits inhibit alpha-amylase and alpha-glucosidase. *Journal of Agricultural and Food Chemistry*, 53(7), 2760–2766. <https://doi.org/10.1021/jf0489926>.
88. Megeji, N. W., Kumar, J. K., Singh, V., Kaul, V. K., & Ahuja, P. S. (2005). Introducing *Stevia rebaudiana*, a natural zero-calorie sweetener, into Indian agriculture. *Current Science*, 88(5), 801–804.
89. Melis, M. S. (1995). Chronic administration of aqueous extract of *Stevia rebaudiana* in rats: Renal effects. *Journal of Ethnopharmacology*, 47(3), 129–134. [https://doi.org/10.1016/0378-8741\(95\)01262-J](https://doi.org/10.1016/0378-8741(95)01262-J).
90. Melis, M. S. (1997). Effects of stevioside on blood pressure and renal function of rats. *Life Sciences*, 61(11), 1063–1068. [https://doi.org/10.1016/S0024-3205\(97\)00619-3](https://doi.org/10.1016/S0024-3205(97)00619-3).
91. Midmore, D. J., & Rank, A. H. (2002). *A new agricultural commodity for Australia: Stevia (Stevia rebaudiana)*. Rural Industries Research and Development Corporation.
92. Minne, V., De Smet, L., & Van Der Eycken, J. (2004). Isolation and structural elucidation of new trace diterpene aglycones from *Stevia rebaudiana* Bertoni. *Chromatographia*, 59(5-6), 349–353.
93. Mishra, P., Singh, R., Kumar, K., & Prakash, D. (2010). Nutritional and phytochemical evaluation of *Stevia rebaudiana* Bertoni leaves. *International Journal of Food Sciences and Nutrition*, 61(1), 60–67. <https://doi.org/10.3109/09637480903383671>.
94. Mizushima, Y., Akihisa, T., Ukiya, M., Hamasaki, Y., Murakami-Nakai, C., Kuriyama, I., Takahashi, N., Joki, S., Yoshida, H., & Sakaguchi, K. (2005). Structural analysis of steviol as an inhibitor of inflammatory responses induced by phorbol ester. *Biochemical Pharmacology*, 69(11), 1573–1583. <https://doi.org/10.1016/j.bcp.2005.03.013>.
95. Morine, S. (2015). *Intracellular transport and vacuolar accumulation of secondary metabolites: Focus on steviol glycosides* (Doctoral dissertation). University of Illinois.

References

96. Ontario Ministry of Agriculture, Food and Rural Affairs. (n.d.). *Stevia: A potential new crop for Ontario*. OMAFRA. <https://www.omafra.gov.on.ca>.
97. Oparil, S., Acelajado, M. C., Bakris, G. L., Berlowitz, D. R., Cífková, R., Dominiczak, A. F., Grassi, G., Jordan, J., Kreutz, R., Rodgers, A., & Whelton, P. K. (2018). Hypertension. *Nature Reviews Disease Primers*, 4(1), 1–22. <https://doi.org/10.1038/nrdp.2018.14>.
98. Papaefthimiou, D., Papanikolaou, A., & Xanthopoulou, A. (2023). Phytochemical profile, antioxidant activity, and health benefits of *Stevia rebaudiana* Bertoni: A systematic review. *Phytotherapy Research*, 37(5), 1902–1921. <https://doi.org/10.1002/ptr.7750>.
99. Pariwat, P., Chatsudthipong, V., Muanprasat, C., & Toskulkao, C. (2008). Inhibitory effects of steviol and related compounds on intestinal chloride secretion and intestinal hypermotility. *European Journal of Pharmacology*, 580(1-2), 232–239. <https://doi.org/10.1016/j.ejphar.2007.10.053>.
100. Penner, M. H., Kim, H. J., & Reynolds, M. (2004). Characterization of steviol glycosides in commercial sweetener formulations. *Journal of Agricultural and Food Chemistry*, 52(24), 7351–7356. <https://doi.org/10.1021/jf040213q>.
101. Prakash, I., Campbell, M., & Chaturvedula, V. S. P. (2014). Catalytic enzymatic modification of steviol glycosides. *Molecules*, 19(8), 12210–12225. <https://doi.org/10.3390/molecules190812210>.
102. Prakash, I., DuBois, G. E., Clos, J. F., Wilkens, K. L., & Fosdick, L. E. (2008). Development of rebiana, a natural, non-caloric sweetener. *Food and Chemical Toxicology*, 46(7), S75–S82. <https://doi.org/10.1016/j.fct.2008.05.004>.
103. Price, M. L., Van Scoyoc, S., & Butler, L. G. (1978). A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. *Journal of Agricultural and Food Chemistry*, 26(5), 1214–1218. <https://doi.org/10.1021/jf60219a031>.
104. Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry*, 53(10), 4290–4302. <https://doi.org/10.1021/jf0502698>.
105. Ramarao, P., Sharma, S., & Singh, R. (2017). Non-cariogenic properties of steviol glycosides and their inhibitory effects on *Streptococcus mutans*. *Journal of Oral Health and Dental Management*, 16(3), 145–151.

References

106. Ramesh, K., Singh, V., & Megeji, N. W. (2006). Cultivation of *Stevia rebaudiana* (Bertoni): A comprehensive review. *Advances in Agronomy*, 89, 137–177. [https://doi.org/10.1016/S0065-2113\(05\)89003-0](https://doi.org/10.1016/S0065-2113(05)89003-0).
107. Rengassamy, M. (2015). Phytochemical screening and quantitative estimation of secondary metabolites in *Stevia rebaudiana* leaves. *International Journal of Pharmacognosy and Phytochemical Research*, 7(4), 720–725.
108. Rezzik, S. (2016). *Phytochemical study and biological activities of some medicinal plant extracts* (Doctoral dissertation / Master thesis reference).
109. Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine*, 20(7), 933–956. [https://doi.org/10.1016/0891-5849\(95\)02227-9](https://doi.org/10.1016/0891-5849(95)02227-9).
110. Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1997). Antioxidant properties of phenolic compounds. *Trends in Plant Science*, 2(4), 152–159. [https://doi.org/10.1016/S1360-1385\(97\)01018-2](https://doi.org/10.1016/S1360-1385(97)01018-2).
111. Ruiz-Ruiz, J. C., Moguel-Ordoñez, Y. B., & Segura-Campos, M. R. (2015). Biological activity of *Stevia rebaudiana* Bertoni and their relationship to health. *Critical Reviews in Food Science and Nutrition*, 55(10), 1332–1343. <https://doi.org/10.1080/10408398.2012.688998>.
112. Sakat, S., Juvekar, A. R., & Gambhire, M. N. (2010). In vitro antioxidant and anti-inflammatory activity of Methanolic extract of *Oxalis corniculata* Linn. *International Journal of Pharma and Bio Sciences*, 1(2), 1–14.
113. Sakamoto, S., Kuroyanagi, M., Inayama, S., & Osawa, T. (1977a). Rebaudioside C, a new sweet diterpene glycoside from *Stevia rebaudiana* Bertoni. *Chemical and Pharmaceutical Bulletin*, 25(4), 844–846. <https://doi.org/10.1248/cpb.25.844>.
114. Sakamoto, S., Kuroyanagi, M., Inayama, S., & Osawa, T. (1977b). Structures of rebaudioside D and E, novel sweet diterpene glycosides from *Stevia rebaudiana* Bertoni. *Chemical and Pharmaceutical Bulletin*, 25(12), 3437–3439. <https://doi.org/10.1248/cpb.25.3437>.
115. Sarni-Manchado, P., & Cheynier, V. (2006). *Les polyphénols en agroalimentaire* [Polyphenols in the food industry]. Lavoisier.
116. Savita, S. M., Sheela, K., Sunanda, S., Shankar, A. G., & Ramakrishna, P. (2004). *Stevia rebaudiana* – A functional component for food industry. *Journal of Human Ecology*, 15(4), 261–264. <https://doi.org/10.1080/09709274.2004.11905703>.

References

117. Seidemann, J. (1976). *Stevia rebaudiana* Bertoni: Eine Übersicht über die Inhaltsstoffe und die Verwendungsmöglichkeiten der süßen Pflanze [An overview of the constituents and uses of the sweet plant]. *Die Nahrung*, 20(4), 437–443. <https://doi.org/10.1002/food.19760200414>.
118. Serio, L. (2010). *Analyse nutritionnelle et composition proximale de Stevia rebaudiana* (Technical Report). Université de Toulouse.
119. Shaffert, E. E., & Chetobar, E. A. (1994). Structure and development of trichomes in *Stevia rebaudiana* Bertoni. *Acta Biologica Cracoviensia. Series Botanica*, 36, 15–22.
120. Shahidi, F., & Ambigaipalan, P. (2015). Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects – A review. *Journal of Functional Foods*, 18, 820–897. <https://doi.org/10.1016/j.jff.2015.06.018>.
121. Sharma, N., & Kaur, R. (2016). Chemical composition, antioxidant and antimicrobial activities of essential oil of *Stevia rebaudiana* Bertoni leaves from India. *Journal of Essential Oil Bearing Plants*, 19(3), 600–609. <https://doi.org/10.1080/0972060X.2016.1171068>.
122. Shiozaki, K., Awale, S., & Kadota, S. (2006). Effect of steviol glycosides on intestinal hypermotility and diarrhea-associated conditions in experimental models. *Biological and Pharmaceutical Bulletin*, 29(8), 1630–1635.
123. Shivanna, N., Naika, M., Khanum, F., & Kaul, V. K. (2013). Antioxidant, anti-diabetic and renal protective properties of *Stevia rebaudiana* Bertoni in alloxan-induced diabetic rats. *Journal of Diabetes and Its Complications*, 27(2), 103–113. <https://doi.org/10.1016/j.jdiacomp.2012.10.001>.
124. Shukla, S., Mehta, A., & Bajpai, V. K. (2012). Optimization of hydroethanolic extraction of steviol glycosides from *Stevia rebaudiana* leaves using response surface methodology. *Journal of Food Science and Technology*, 57(6), 2105–2115.
125. Shukla, S., Mehta, A., & Bajpai, V. K. (2020). Extraction of bioactive compounds from *Stevia rebaudiana* leaves using green technologies and evaluation of their antioxidant potential. *Industrial Crops and Products*, 145, Article 112110. <https://doi.org/10.1016/j.indcrop.2020.112110>.
126. Shukla, S., Mehta, A., Bajpai, V. K., & Shukla, S. (2023). Green extraction techniques for extraction of bioactive compounds from *Stevia rebaudiana* leaves and evaluation of their phytochemical and functional potential. *Industrial Crops and Products*, 195, Article 116420. <https://doi.org/10.1016/j.indcrop.2023.116420>.

References

127. Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in Enzymology*, 299, 152–178. [https://doi.org/10.1016/S0076-6879\(99\)99017-1](https://doi.org/10.1016/S0076-6879(99)99017-1).
128. Soejarto, D. D. (2002). Botany of *Stevia rebaudiana*. In A. D. Kinghorn (Ed.), *Stevia: The genus Stevia* (pp. 18–39). Taylor & Francis.
129. Soejarto, D. D., Kinghorn, A. D., & Farnsworth, N. R. (1982). Potential intense natural sweeteners of plant origin. III. *Stevia rebaudiana*. *Journal of Natural Products*, 45(5), 590–599. <https://doi.org/10.1021/np50023a011>.
130. Starratt, A. N., Kirby, C. W., Pocs, R., & Brandle, J. E. (2002). Rebaudioside F, a diterpene glycoside from *Stevia rebaudiana*. *Phytochemistry*, 59(4), 367–370. [https://doi.org/10.1016/S0031-9422\(01\)00472-5](https://doi.org/10.1016/S0031-9422(01)00472-5).
131. Stephen, J., Sharma, S., & Singh, R. (2010). Obesity and its metabolic complications: The role of dietary carbohydrate modification. *Nutrition Research Reviews*, 23(2), 241–253.
132. Szefer, P., & Nriagu, J. O. (Eds.). (2007). *Mineral components in foods*. CRC Press. <https://doi.org/10.1201/9781420003987>.
133. Tadhani, M. B., Patel, V. H., & Subhash, R. (2006). In vitro antioxidant activities of *Stevia rebaudiana* leaves and stem. *Journal of Food Composition and Analysis*, 19(6–7), 542–547. <https://doi.org/10.1016/j.jfca.2005.10.004>.
134. Tadhani, M. B., & Subhash, R. (2006a). Mineral composition of dried *Stevia rebaudiana* Bertoni leaves. *Journal of Cell and Tissue Research*, 6(1), 1815–1821.
135. Thines, M. (2001). Involvement of the renin–angiotensin–aldosterone system in the mechanism of action of stevioside. *Phytomedicine*, 8(5), 360–366.
136. Wagner, H. (2012). Ethnomedicine and phytotherapy in South America: The history of sweetening plants. *Journal of Ethnopharmacology*, 140(3), 451–458. <https://doi.org/10.1016/j.jep.2012.01.023>.
137. Wilkens, M., Alarcón, R., & Urrea, C. (2008). Antimicrobial and antifungal activities of *Stevia rebaudiana* Bertoni leaves crude extracts. *Brazilian Journal of Microbiology*, 39(4), 701–705. <https://doi.org/10.1590/S1517-838220080004000021>.
138. Wingard, R. E., Brown, J. P., Enderlin, F. E., Dale, J. A., Hale, R. L., & Seitz, C. T. (1980). Intestinal degradation and elimination of the intense sweetener stevioside in rats and humans. *Experientia*, 36(5), 519–521. <https://doi.org/10.1007/BF01965773>.

References

139. Wu, C. D., JX, C., & Kinghorn, A. D. (1998). Inhibitory effects of steviol glycosides on the growth and acid production of cariogenic bacteria. *Journal of Dental Research*, 77, 124–131.
140. Yadav, K., & Guleria, P. (2012). Metabolic engineering of steviol glycosides biosynthetic pathway: Current status and future prospects. *Plant Biotechnology Reports*, 6(4), 287–296. <https://doi.org/10.1007/s11816-012-0226-5>.
141. Zaman, M. M., Chowdhury, M. A., & Rahman, M. H. (2015). Effect of harvesting intervals on biomass yield and steviol glycoside content of *Stevia rebaudiana* Bertoni. *Bangladesh Journal of Agricultural Research*, 40(2), 265–275. <https://doi.org/10.3329/bjar.v40i2.24563>.
142. Ghezal, A., & Remili, A. (2024). Étude in vitro des activités biologiques des extraits d'*Artemisia absinthium* [Mémoire de master, UFM Constantine 1].
143. Shukla, S., Mehta, A & ,Bajpai, V. K. 2022) Optimization of hydroethanolic extraction of stevio glycosides from *Stevia rebaudiana* leaves using response surface methodology. *Journal of Food Science and Technology*.2115-2105 ,(6)57 ,
144. Shahidi, F. and Ambigaipalan, P. (2015) Phenolics and Polyphenolics in Foods, Beverages and Spices: Antioxidant Activity and Health Effects—A Review. *Journal of Functional Foods*, 18, 820-897.
145. Harborne, J. B. (1998). **Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis** (3rd ed.). London: Chapman & Hall.
146. Singleton, V.L., Orthofer, R. and Lamuela-Raventos, R.M. (1999) Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent. *Methods in Enzymology*, 299, 152-178.
147. Chang, C., Yang, M., Wen H., and Chern. J, “Estimation of total flavonoid content in propolis by two complementary colorimetric methods”, *J. Food Drug Anal*, 10(3). 178-182. May 2002.
148. Price, M.L., Van Scoyoc, S. and Butler, L.G. (1978) A Critical Evaluation of the Vanillin Reaction as an Assay for Tannin in Sorghum Grain. *Journal of Agricultural and Food Chemistry*, 26, 1214-1218.
149. Trease, G.E. and Evans, W.C. (1989) *Pharmacognosy*. 13th Edition, Baillière Tindall, London.

References

150. Tlili, M. L. (2015). Contribution à la caractérisation physico-chimique et biologique des extraits de *Pergularia tomentosa* issue de quatre sites sahariens différents (Sahara septentrional) (Doctoral dissertation, Université Kasdi Merbah-Ouargla)
151. Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. (1956) Colorimetric Method for Determination of Sugars and Related Substances. *Analytical Chemistry*, 28, 350-356.
152. Masuko, T., Minami, A., Iwasaki, N., Majima, T., Nishimura, S.-I. and Lee, Y.C. (2005) Carbohydrate Analysis by a Phenol-Sulfuric Acid Method in Microplate Format. *Analytical Biochemistry*, 339, 69-72.
153. Nielsen, S.S. (2010) *Food Analysis*. 4th Edition, Food Science Text Series, Springer, USA, 602.
154. Brand-Williams, W., Cuvelier, M.E. and Berset, C.L.W.T. (1995) Use of a Free Radical Method to Evaluate Antioxidant Activity. *LWT-Food Science and Technology*, 28, 25-30.
155. Benzie, I. and Strain, J. (1996) The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power: The FRAP Assay". *Analytical Biochemistry*, 239, 70-76.
156. Alia, F., Chouikh, A., Djahra, A. B., Bousbia Brahim, A., Nani, S., & Tliba, A. (2021). Comparative study of some physicochemical and biological properties of effect host species variation on the relationship Saharan parasitic plant *Cistanche violacea* (Desf.) Beck. *Notulae Scientia Biologicae*, 13(4), 11054. <https://doi.org/10.15835/nsb13411054>.
157. Chaouche, T. M., Haddouchi, F., Ksouri, R., Medini, F., & Atik-Bekara, F. (2013). In vitro evaluation of antioxidant activity of the hydro-methanolic extracts of *Juniperus oxycedrus* subsp. *oxycedrus*. *Phytothérapie*, 11(4), 244-249 .
158. Mouffouk, C., Mouffouk, S., Dekkiche, S., Hambaba, L., & Mouffouk, S. (2019). Antioxidant and Antibacterial Activities of the species *Silene inflata* Sm.: Biological activities of *S. inflata*. *PSM Biological Research*, 4(2), 74–86. Retrieved from
159. Bougandoura, N. and Bendimerad, N. (2013) Evaluation de l'activité antioxydante des extraits aqueux et méthanolique de *Satureja calamintha* ssp. *nepeta*(L.) Briq. *Nature & Technologie*, 9, 14-19.

References

160. Haddoudi, M., Mellouk, H., Bejjany, B., Dani, A., & Digua, K. (2014). Valorisation du marc du café: Extraction de l'huile et évaluation de son activité antioxydante. *Les Technologies de Laboratoire*, 8 .(36)
161. Sakat, S., Juvekar, A.R. and Gambhire, M.N. (2010) In vitro Antioxidant and Anti-Inflammatory Activity of Methanol Extract of *Oxalis corniculata* Linn. *International Journal of Pharmacy and Pharmaceutical Sciences*, 2, 146-155.
162. Williams, S.E., Shoo, L.P, Isaac, J.L., Hoffman, A.A. and Langham, G. (2008) Towards an integrated framework for assessing the vulnerability of species to climate change. *PLoS Biology*, 6, 2621-2626. doi:10.1371/journal.pbio.0060325
163. Chakrabarti, R., Singh, B., Vanchhawng, L., & Thirumurugan, K. (2014). Screening of nine herbal plants for in vitro α -amylase inhibition. *Screening*, 7(4), 84-89.
164. Granados-Guzmán, G. et al. (2022)n, G., Alanis, B., Castro-Ríos, R., Waksman, N., & Salazar-Aranda, R. (2022). ASSESSMENT OF α -AMYLASE INHIBITION ACTIVITY BY AN OPTIMIZED AND VALIDATED IN VITRO MICROSCALE METHOD. *Química Nova*, 45. <https://doi.org/10.21577/0100-4042.20170919>
165. Khadayat, K., Marasini, B. P., Gautam, H., Ghaju, S., & Parajuli, N. (2020). Evaluation of the alpha-amylase inhibitory activity of Nepalese medicinal plants used in the treatment of diabetes mellitus. *Clinical Phytoscience*, 6, 1-8.
166. Amarakoon, S. (2021). *Stevia rebaudiana*: A review on agricultural, chemical and industrial applications. *Journal of Nature and Applied Research*, 1(1), 14–27.
167. Brandle, J. E., Richman, A., Swanson, A. K., & Chapman, B. P. (2002). Leaf ESTs from *Stevia rebaudiana*: A resource for gene discovery in diterpene synthesis. *Plant Molecular Biology*, 50(4–5), 613–622. <https://doi.org/10.1023/A:1019838228478>
168. Chatsudthipong, V., & Muanprasat, C. (2009). Stevioside and related compounds: Therapeutic benefits beyond sweetness. *Pharmacology & Therapeutics*, 121(1), 41–54. <https://doi.org/10.1016/j.pharmthera.2008.09.007>
169. Ceunen, S., & Geuns, J. M. C. (2013). Steviol glycosides: Chemical diversity, metabolism, and function. *Journal of Natural Products*, 76(6), 1201–1228. <https://doi.org/10.1021/np400203b>

References

170. Broadhurst, R.B., & Jones, W.T. (1978). Analysis of condensed tannins using acidified vanillin. *Journal of the Science of Food and Agriculture*, 29(9), 788–794. DOI: 10.1002/jsfa.2740290908

Annex

Annex:

Sensory evaluation procedure and experimental samples of orange juice and madeleine formulations:

Sensory Evaluation Form – Orange Juice Samples

Sample	Sweetness الحلاوة (1-5)	Taste الطعم (1-5)	Bitterness المراوة (1-5)	Aftertaste الطعم اللاحق (1-5)	Aroma الرائحة (1-5)	Overall Acceptability القول العام (1-5)
S1						
S2						
S3						
S4						
S5						

يتم التقييم على مقياس (5-1) من حيث:

- 5 = Excellent (Extremely liked) ممتاز (مقول جداً)
- 4 = Good (Liked) جيد (أعجبني)
- 3 = Neutral (معتدل) متوسط (معتدل)
- 2 = Poor (Disliked) ضعيف (أبغضت)
- 1 = Very poor (Extremely disliked) سيء جداً (أبغضت بشدة)

Sensory Evaluation Form – Madeleine Samples

Sample	Sweetness الحلاوة (1-5)	Taste الطعم (1-5)	Bitterness المراوة (1-5)	Aftertaste الطعم اللاحق (1-5)	Aroma الرائحة (1-5)	Overall Acceptability القول العام (1-5)
S1						
S2						
S3						
S4						
S5						

يتم التقييم على مقياس (5-1) من حيث:

- 5 = Excellent (Extremely liked) ممتاز (مقول جداً)
- 4 = Good (Liked) جيد (أعجبني)
- 3 = Neutral (معتدل) متوسط (معتدل)
- 2 = Poor (Disliked) ضعيف (أبغضت)
- 1 = Very poor (Extremely disliked) سيء جداً (أبغضت بشدة)



Annex

Experimental Procedures for Phytochemical and Antioxidant Analyses of *Stevia rebaudiana* Extract:

