

**DISSERTATION FOR THE DEGREE OF DOCTOR OF
PHILOSOPHY (Ph.D.)**

**“Evaluation of the enhanced bee product and silver
nanoparticles.”**

By

Ayaz Mukarram Shaikh

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Head of Doctoral School:
Prof. Dr. Szilvássy Zoltán

Supervisor:
Prof. Dr. Kovács Béla
(Head of Food Science Programme)

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Author
Ayaz Mukarram Shaikh
Ph.D. Candidate

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in **Food Science**

Written by: **Ayaz Mukarram Shaikh** (Ph.D. candidate)

Name of Ph.D. school: Doctoral School of Nutrition and Food Sciences

Head of Ph.D. school: Prof. Dr. Szilvássy Zoltán

Supervisor: Prof. Dr. Kovács Béla

Review committee:

	Name	Sc. Degree	Signature
Chairman:	_____	_____	_____
Member:	_____	_____	_____
Secretary:	_____	_____	_____
Opponents:	_____	_____	_____
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Abbreviations

Abbreviation	Full Form
2θ	2-Theta
Å°	Angstrom
AgNPs	Silver Nanoparticles
cm	Centimeter
ELISA	Enzyme-Linked Immunosorbent Assay
EORTC QLQ-C30	European Organization for Research and Treatment of Cancer - Quality of Life Questionnaire
EQ-5D	EuroQol Five-Dimension Scale
FAO	Food and Agriculture Organization
FTIR	Fourier Transform Infrared Spectroscopy
g/L	Gram per liter
g mL ⁻¹	Grams per square meter
HDL	High-Density Lipoprotein
HMF	Hydroxymethylfurfural
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometer
LDL	Low-Density Lipoprotein
MFA	Multiple Factor Analysis
mg	Milligram
mg/kg	Milligrams per kilogram
mg/L	Milligrams per liter
mg/mL	Milligrams per milliliter
mL	Milliliter
mL h ⁻¹	Milliliter per hour
ml/min	Milliliter per minute
mM	Millimolar
mm ² s ⁻¹	Square millimeter per second

m/m	Mass per cent concentration
µg	Microgram
µL	Microliter
µm	Micrometer
nm	Nanometer
PCA	Principal Component Analysis
PUFA	Polyunsaturated Fatty Acids
SDGs	Sustainable Development Goals
SF 36	Short Form (36) Health Survey
SPSS	Statistical Package for the Social Sciences
TEM	Transmission Electron Microscopy
VOS	Visualization of Similarities
v/v	Volume per volume
w/v	Weight per volume
w/w	Weight per weight
WoS	Web of Science
XRD	X-ray Diffraction

1. INTRODUCTION

In recent years, researchers have focused on the use of medicinal plants for the treatment of ailments on common health-related issues (Hosseinzadeh et al., 2015). The most dominant tree in the nut category globally is the walnut (*Juglans regia L.*). It belongs to the *Juglandaceae* family and is commonly known as an English, Persian, white, or common walnut (Nael Abu Taha & Al-wadaan, 2021). This species originated in the Old World. It evolved in a region spanning from the western Himalayan to the Balkans and was planted in the European continent as early as 1000 BC (Fernandez-Lopez J & Aleta N, 2000). Currently, walnuts are grown for commercial purposes in the US, northern Africa, southern and western South America, eastern Asia, and Europe. China is the leading producer globally, followed by the United States, some parts of Iran, some parts of Turkey, Romania, Ukraine, some parts of France, and northern and southern parts of India (Martínez et al., 2010). The Walnut tree (*Juglans regia L.*) is a crucial source of food, pharmaceuticals, phototherapeutic compounds, raw materials for the wood industry, and biomass. It has been classified as "the magic tree" since antiquity (Mukarram, Wandhekar, Ahmed, Pandey, et al., 2024; Mukarram, Wandhekar, Ahmed, Várallyay, et al., 2024).

Walnut trees produce walnuts as fruits. The two species are the English, Persian walnut and black walnut. It is renowned for its nutritional attributes, texture, and health advantages. Whole walnuts are commonly consumed, but they are also incorporated into many culinary preparations such as ice cream, cakes, pastries, energy bars, and salads (Mukarram, Wandhekar, Ahmed, Pandey, et al., 2024) The nutritional composition includes proteins, vitamins, minerals, and a substantial amount of antioxidants, including phenolic compounds, lipids predominantly consisting of unsaturated fatty acids, phytosterols, and tocopherols (Gunduc & El, 2003). Walnuts are rich in essential dietary fats, specifically polyunsaturated fatty acids (PUFAs), encompassing the important omega-6 and omega-3 fatty acids. Walnuts have been consumed since antiquity because of the longstanding idea that they can enhance brain health, hereby promoting cognitive well-being (Zhao et al., 2021).

Similarly, nanotechnology has attracted interest because of its numerous applications that encompass the manipulation of materials using diverse processes to produce substances with specific desired characteristics. In recent years, substantial research has been conducted on nanoparticles owing to their remarkable features and varied applications. Silver nanoparticles have been developed as multifaceted nanomaterials with wide

applications in technology and medicine. Silver nanoparticles are silver particles with a typical size between 1 and 100 nanometers (Sharma et al., 2009). Because of their diminutive size and substantial surface-area-to-volume ratio, they exhibit distinctive physical, chemical, and biological features. Their distinctive physicochemical characteristics, including a high surface area-to-volume ratio and notable optical, electrical, antioxidant, and antibacterial activities, render them indispensable across multiple domains.

Silver nanoparticles exhibit high antibacterial efficacy against a diverse array of microorganisms, including bacteria, viruses, and fungi. Their capacity to disrupt bacterial cell membranes, inhibit enzyme function, and induce oxidative stress renders them potent antimicrobial agents. Formulations based on AgNPs nanoparticles been used in wound dressings, medical devices, cardiovascular implants, orthopedic implants, dental applications, catheters, and antimicrobial coatings to inhibit infections and facilitate wound healing (Catauro et al., 2004; Furno et al., 2004; Kim et al., 2007). AgNPs synthesized using different techniques have been thoroughly studied and examined for efficacy against over 650 pathogens, bacteria, and fungi (Dakal et al., 2016). As COVID-19 came, people started focusing on their health with high priorities, and at the same time, people realized that eating healthy food and maintaining a healthy diet plays an important role in fighting against any of the diseases that have a significant impact in bringing use of healthy and functional foods, as well as continuous innovation is needed to develop new foods, methods, processes, and integration of different fields in food science (Valoppi et al., 2021). Several products have been used globally to cure COVID-19, including widely honey (Ali & Kunugi, 2021; Soares et al., 2022). Honey has been consumed for generations as both a food and medicinal food/supplement because of its various health benefits and advantages. These advantages are ascribed to its abundant makeup, which includes carbohydrates, minerals, proteins, vitamins, and polyphenols. Research conducted by Penn State University examined 50 years of data and determined that climate variables, including temperature and precipitation, substantially affected honey output. Research indicates that ongoing climate change may lead to a reduction in pollinator resources in locations such as the Great Plains, negatively affecting honey production. Moreover, the primary factors contributing to the decline of bee populations are habitat destruction, diseases and pests, pesticide exposure, climate change, and demographic factors. Agricultural development and urbanization have resulted in considerable habitat loss, diminishing the availability and

variety of floral supplies and nesting sites for bees (Goulson et al., 2008; Goulson et al., 2015; Kaluza et al., 2018).

One of the reasons for raising this topic was to integrate food science with nanotechnology and to solve real-life issues and create something insightful that should also address the sustainable development goals (SDGs) given by the United Nations. We aim to achieve the SDGs, as shown below in the Figure 1.



Figure 1. Sustainable development goals aimed through research (source: author)

1.1 Background of the Study

After facing the biggest disaster of the COVID-19 pandemic, everyone has understood that continuous innovation is the only key to tackling global food and agriculture issues. Keeping this in mind, we decided to solve local issues related to food and agriculture to integrate nanotechnology and to create an impact through innovation and science.

This study focuses on tackling the issue of population decline of bees due to climate change, the need for new products in the bee product category, conducting sensory analysis of developed bee products, and further integrating nanotechnology to develop silver nanoparticles and their future applications.

The objectives of this research were:

- understanding the medicinal plant, green walnut (*Juglans Regia L.*) and its literature review study;
- development of bee feed technology, evaluation and development of green walnut bee products;
- development and characterization of silver nanoparticles using green walnut (*Juglans Regia L.*);
- conducting sensory analysis and understanding consumer acceptance perspectives.

In simple terms, this study provides detailed information about the benefits of green walnut (*Juglans Regia L.*), how green walnut can be used in bee feed development, and how we can design a bee product that can be helpful in reducing the blood sugar level in humans and the development of silver nanoparticles (since the use of nanotechnology is at its peak), including the sensory analysis of the green walnut bee product from an international perspective.

The graphical representation of the thesis is given below for better understanding.

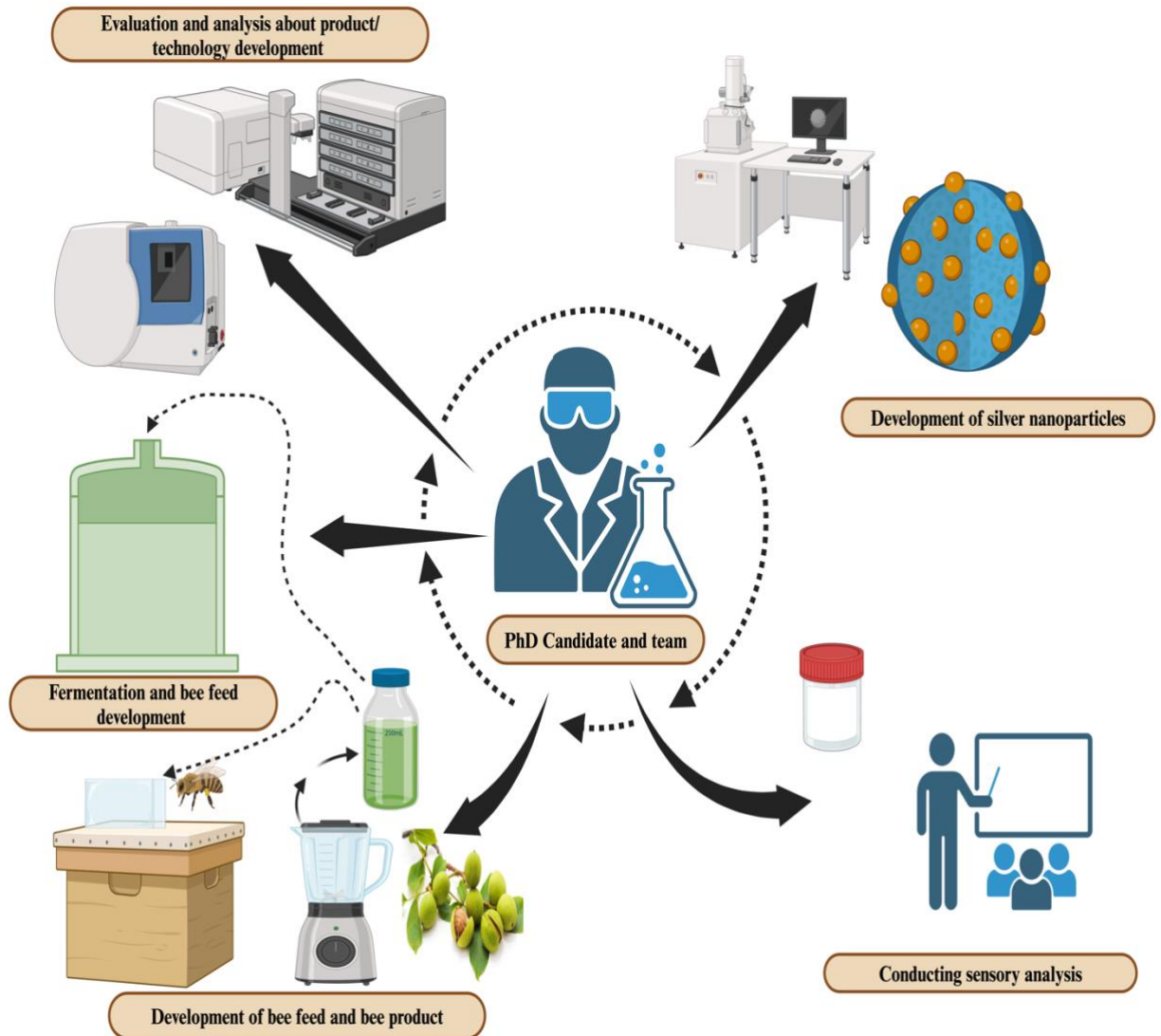


Figure 2. Graphical representation of PhD dissertation (source: author)

2. LITERATURE

2.1 About green walnut (*Juglans Regia L.*)

Ayurveda and several other medical disciplines have widely employed plant components. Walnut trees are becoming increasingly popular globally because of their natural origins, elevated phytochemical content, and lack of adverse health consequences compared to modern therapies. Medicinal plants play a crucial role in the delivery of healthcare and welfare services to humans. Walnuts, scientifically designated as *J. regia L.*, have a longstanding history of application for their therapeutic benefits in addressing diverse diseases. The specified plant components contain phytochemicals with therapeutic properties, such as flavonoids, alkaloids, and polyphenolic compounds (Sharma, 2022). The lifespan of this plant ranges from 100 to 200 years, with certain species attaining up to 1000 years (Caudullo et al., 2016).

Wild populations of *J. regia* have been identified in the Caucasus, Middle East, East and South Asia, as well as in the temperate and semi-arid mountainous areas of Central Asia. The exceptional morphological and physiological diversity of these forests, along with the existence of numerous wild relatives of cultivated species, highlights their importance as worldwide biodiversity hotspots.

Walnut trees (*Juglans regia*) are deciduous trees that can attain heights of 20–30 m. Juvenile trees possess smooth, grey bark; however, as they mature, their bark develops fissures. The pinnate alternate leaves measure 20–45 cm in length and consist of five to seven leaflets. Although monoecious, male flowers discharge their pollen prior to the opening of female flowers, resulting in insufficient fruit set. Male flowers manifest as catkins on twigs from the preceding season, while female flowers emerge in late spring as pistillate spikes comprising two to five blooms at the tips of the stems of the current season (Habibi et al., 2022). Green walnuts are conventionally used in alternative medicine for the management of diabetes, cancer, inflammation, and cardiovascular diseases (Zhang et al., 2022). They are widely recognized for their antioxidant, anti-inflammatory, and antibacterial properties (Aryapak & Ziarati, 2014). Diverse civilizations have employed unripe walnuts in their medicinal practices throughout most of their history. Walnut leaves have been used to enhance diabetes management and to reduce blood glucose levels. Green husks are regarded for their potential in combating several diseases, including cancer, bacterial infections, inflammation, and pain relief. The ancient application of green walnuts in

medicine reflects the various medicinal properties of walnut trees and their components (Gandev, 2007).

This thesis sought to thoroughly investigate the global medicinal applications of green walnuts and emphasize their integration as vital components in contemporary medical treatment, showcasing diverse traits and therapeutic properties. Another purpose was to understand the global perspective of the utilization of green walnuts. This study further examined the categories of scientific data related to the traditional use of green walnuts to investigate their phytochemicals, pharmacological properties, and biological implications post-extraction using several models. Furthermore, the present work examines the recent technologies, challenges, and prospects of green walnut utilization, particularly in applications such as bee feed development, the synthesis of silver nanoparticles, and the production of bee goods.

2.2 Use of Green Walnut at a Global Perspective

The pharmacological properties of green walnuts enable their inclusion in the repertoire of natural therapeutic treatments and active application in traditional medicine. The United Nations Food and Agriculture Organization has prioritized walnut because of their distinctive health benefits and nutritional value, emphasizing their significance for human nutrition (Gandev, 2007). *Juglans regia* L. has pharmacological properties in various components, including the shell, seed, leaves, bark, green husk, and fruits (Milind & Deepa, 2011). It contains many chemical components including ascorbic acid, gallic acid, terpenoids, polyphenols, quercetin, and sitosterol. Green walnut has anti-inflammatory, diuretic, anticancer, laxative, anti-diabetic, antimutagenic, antifungal, antioxidant, antiseptic, antibacterial, antiallergic, astringent, and antiulcer properties (Berkey et al., 2020; Pan et al., 2019). Green walnuts have been consumed by humans since ancient times and are found in many regions of the world. Green walnuts are prevalent in France, Italy, and Turkey, where they constitute fundamental elements of numerous regional cuisines. These immature walnuts are attractive for their distinctive flavor and versatility, and they are essential to the gastronomic heritage of the region. Green walnuts are gaining global recognition.

2.3 Botanical Profile and Impact

Walnut (*Juglans spp.*) is a member of the *Juglandaceae* family, which includes seven genera and approximately 60 monoecious tree species (Thakur, 2011). It belongs to the order Fagales and is a diploid species with a somatic chromosome count of $2n = 32$. The genus *Juglans* has 21 species (Sharma, 2022). Figure 3. presents real-time photographs of walnut trees and their components cultivated at the campus of the Faculty of Agriculture, Food Science, and Environmental Management at the University of Debrecen. The taxonomic classification of *Juglans regia* is schematically illustrated in the below Figure 3.

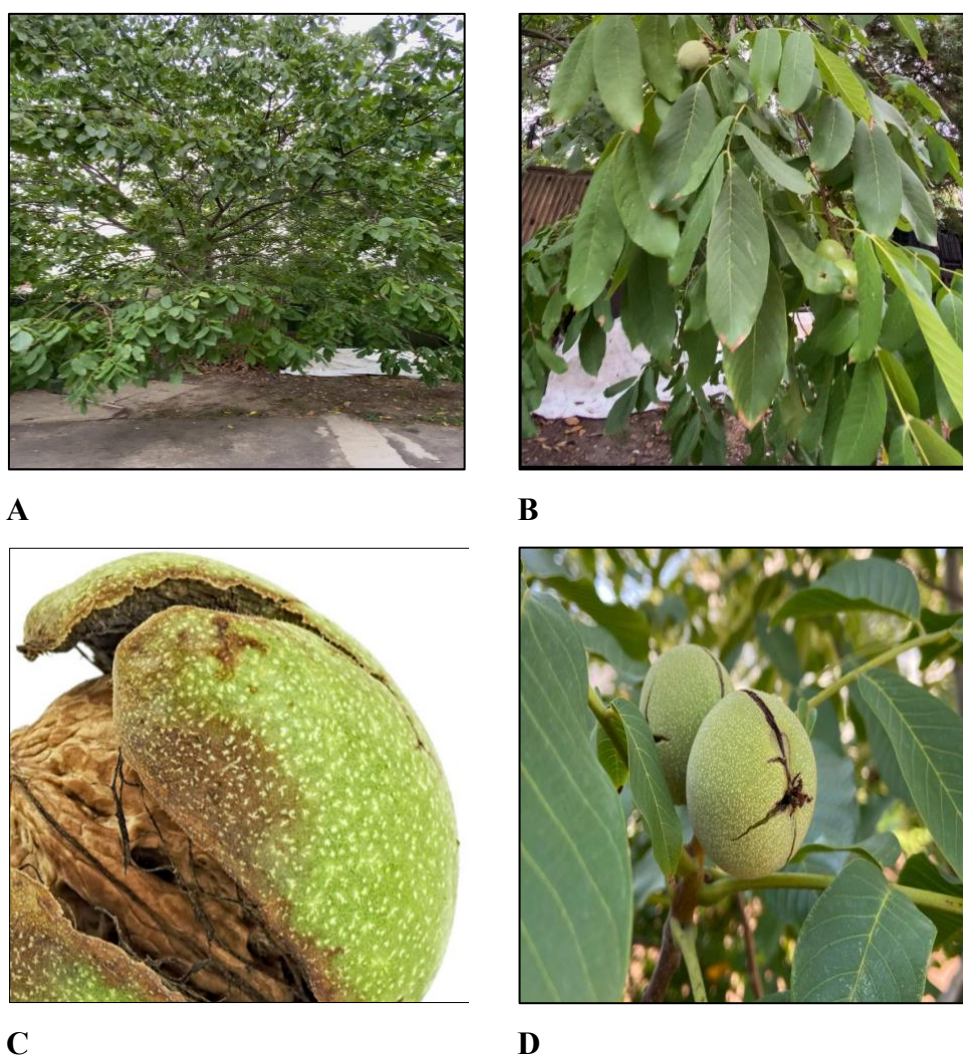


Figure 3. Real-time images of (A) green walnut tree, (B) leaves, (C) husk, and (D) green walnut fruit. (Source: author, Location: Faculty of Agriculture, Food Science, and Environmental Management at the University of Debrecen).

Walnuts are monoecious, with male and female flowers located at distinct sites on the tree (Chand L. et al., 2017). Male flowers emerge laterally from simple buds on one-year-old wood, whereas female flowers often form clusters of one to three from mixed buds, with flowering occurring at the terminal position of the current season's leafy shoot, and can be categorized into three bearing groups—terminal, middle, and lateral—based on the position of leafy shoot emergence (Germain, 1989). The taxonomic profiles are shown in Figure 4.

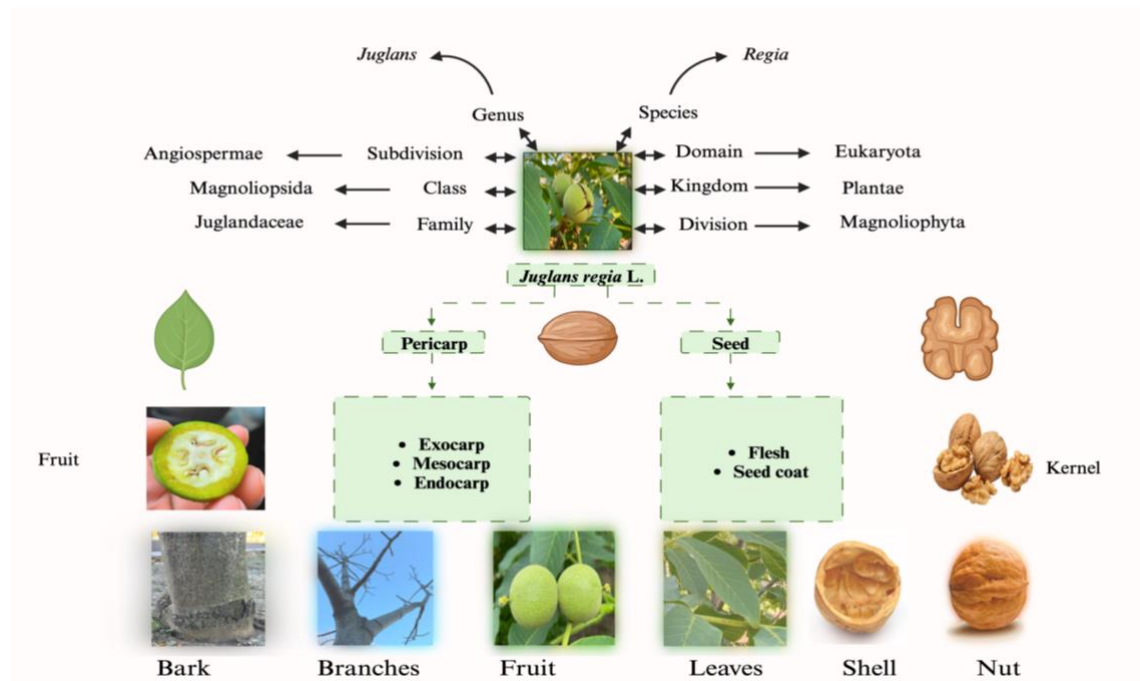


Figure 4. Taxonomic classification of *Juglans regia* .L (Sharma, 2022; Žalac H. et al., 2023) (Source: author)

The fruiting characteristics are linked to branching density, placement of flowering buds on annual shoots, and age of the fruiting shoot bearer (Solar et al., 2003). Each walnut variety group varied in the number of branches on its one-year-old shoot. Bearing habits also affect the architecture and yield of trees. Lateral bearing is correlated with precocity and increased yield (Laurens et al., 2000). Lateral bud fruitfulness is regarded as the primary determinant of yield and can be influenced by selective breeding (Akca & Ozongun, 2004). It is essential to choose types that exhibit favorable characteristics, such as lateral bearing, dense branching, reduced height, early fruiting, substantial nut size, and an improved nut-kernel ratio. The size of germplasm collections, degree of variation within these collections, and accessibility to biologists and breeders are critical factors influencing their use in crop enhancement initiatives.

2.4 Current Research on *Juglans regia* L. (Green walnut).

2.4.1 Literature review using AI Tool - ResearchRabbit

Recently created software and online techniques were employed to identify significant study findings for green walnuts using the Web of Science (WoS) and consensus (an AI-driven search engine) databases to locate literature and essential findings accessible online across many databases. ResearchRabbit, an AI-driven search engine, was used to identify gaps and examine the specified authors, collaborations, summaries, and other relevant details. The following terms were employed to investigate discoveries on green walnuts: “Green Walnut, *Juglans regia* L., Medicinal Benefits of Green Walnut, Therapeutic Applications, Culinary Uses, Ecological Applications.” Significant correlations were found among articles published over the specified timeframe, indicating that publications were disseminated from 2011 to 2023 and from 2007 to 2023 (Figures 5–8). Approximately 40 papers pertained to its therapeutic applications and other uses, whereas approximately 40 studies focused on its morphology and characterization.

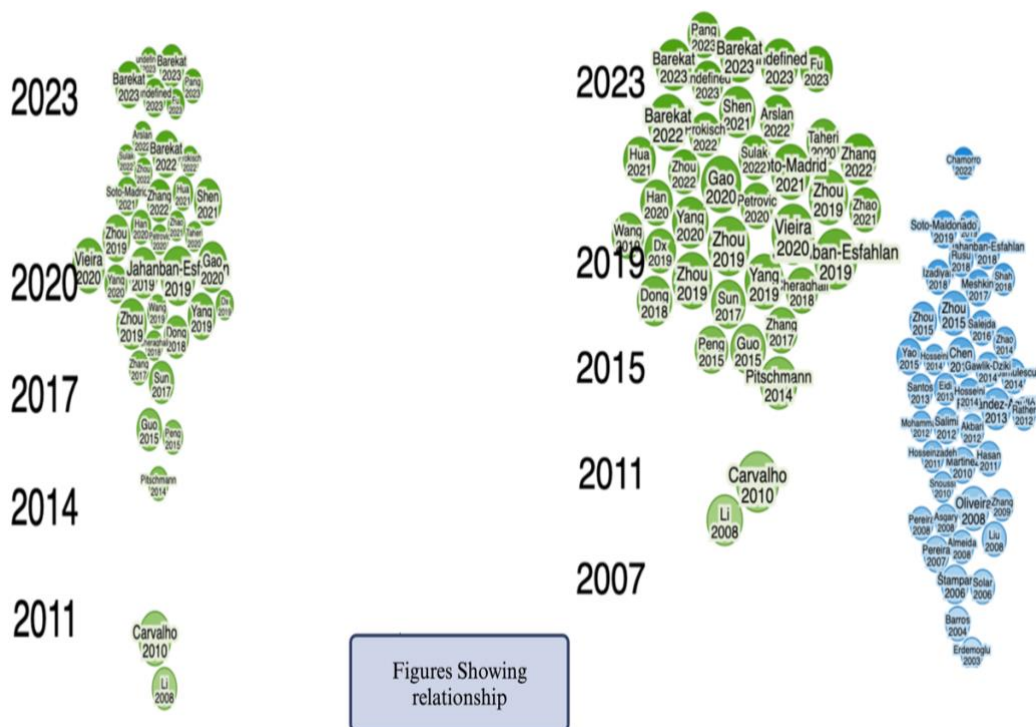


Figure 5 & 6. Combined insights into the scientific research on *Juglans regia* L. from 2000 to 2023. **Green color:** Number of Scopus- and WoS-indexed articles (2011–2023), indicating the availability of scientific papers (40 in total) that explore its medicinal and other uses. **Blue color:** Similarities and thematic matches among papers (2000–2023), based on properties and characteristics of *Juglans regia* L.

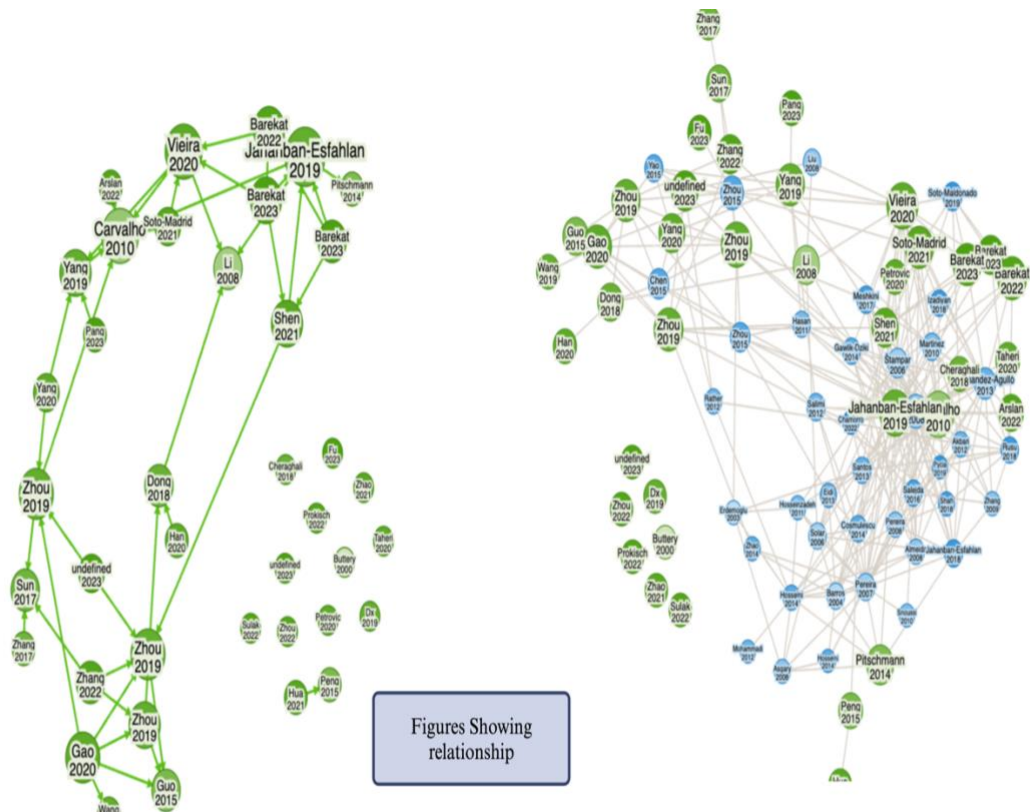


Figure 7-8: Network visualization of published articles on *Juglans regia L.* from 2011 to 2023 (40 articles), highlighting author collaborations and research focus. The green network (WoS Indexed Network, Scopus) shows contributions by different authors and their publication trends, while the blue network illustrates connections among researchers and thematic clusters related to the properties, characteristics, and applications of *Juglans regia L.*

2.4.2 Literature review using VOS-Viewer

A wide range of scientific literature exists within the field of science and is stored in many databases such as Google Scholar, PubMed, Scopus, and Web of Science (WoS). The authors of this review chose to employ Web of Science because it is a venerable, authoritative, and widely used database that includes citations and research papers from around the world, encompassing material from thousands of journals (Birkle et al., 2020). WoS is a significant archive of literature and a data repository that includes peer-reviewed studies. The interface is simple and user-friendly, streamlining the execution of bibliographic literature reviews and data visualization via programs such as the VOSviewer. The authors used the following terms in WoS: ("green walnut" OR "unripe

improving memory, supporting learning abilities, and protecting against age-related cognitive decline. Cardiovascular benefits: promoting heart health and lowering the risk of cardiovascular disease. Gut health promotes a balanced gut microbiome. The green husk of walnut possesses potent antioxidant capabilities and serves as a source of natural antioxidants (Noshirvani et al., 2015). Walnuts contain flavonoids and ellagic acid, which may affect blood cholesterol levels and exhibit cardioprotective properties (Savage, 2001). The extract of green husks and powder serves as a natural additive and therapeutic agent (Salejda et al., 2016). Essential fatty acids, including linoleic acid found in walnuts, help mitigate the risk of cardiovascular diseases by elevating HDL and decreasing LDL levels (Amaral et al., 2003).

Juglans regia L. (green walnut) contains several bioactive components recognized for their nutritional significance and potential beneficial effects on human health. These bioactive compounds, mainly belonging to polyphenols, include flavonoids, phenolic acids (such as gallic and ellagic acids), phytosterols, tocopherols, carotenoids, and fatty acids (Ali & Zeb, 2024; Croitoru et al., 2019)

2.6 Bioactive Components of *Juglans regia L.*

2.6.1 Major Bioactive Compounds in Green Walnut

The primary bioactive compounds in *Juglans regia* includes those- Polyphenols Encompassing flavonoids, phenolic acids (e.g., gallic acid, ellagic acid), and tannins. These compounds exhibit antioxidant, anti-inflammatory, and anti-cancer potential (Croitoru et al., 2019) Fatty Acids are Particularly rich in polyunsaturated fatty acids (PUFAs), including omega-3 (linolenic acid) and omega-6 (linoleic acid), beneficial for cardiovascular health. Phytosterols and Tocopherol are Linked to cholesterol management and antioxidative activities, respectively (Ali & Zeb, 2024) Carotenoids Known for their antioxidative properties and roles in enhancing visual health. Green walnut kernels are rich in protein, fiber, omega-3 fatty acids, vitamin E, phytosterols, and polyphenols, among other nutrients and bioactive compounds (Greve et al., 1992). Beneficial lipids, including omega-3 fatty acids, promote cardiovascular health and cognitive functions. Proteins are excellent source of essential amino acids. Besides enhancing digestive health, fiber may also aid in lowering cholesterol levels (Elmadfa & Kornsteiner, 2009).

2.6.2 Health Benefits of Bioactive Compounds in Green Walnut through different parts (Kernel and leaves)

They are extensively used in traditional medical practices to address many conditions, including hemorrhoids and venous insufficiency. Walnut leaf extracts possess keratolytic, hypotensive, antifungal, calming, and antiscrofulous effects (Qa'dan et al., 2005). The pharmacological and therapeutic attributes of phenolic compounds found in walnut leaves render them very valuable (Nour et al., 2016). The bioactive compounds in green walnut contribute significantly to various health-promoting properties, positioning them as key ingredients in the development of functional or novel food products. The rich polyphenolic content, including gallic and ellagic acids, effectively neutralizes reactive oxygen species (ROS), thereby reducing oxidative stress implicated in chronic diseases such as cardiovascular disorders and diabetes (Croitoru et al., 2019). In terms of Anti-inflammatory Effects Polyphenols and fatty acids present in walnuts can mitigate inflammation, a common factor in chronic diseases. Omega-3 and omega-6 fatty acids, along with phytosterols and tocopherols, contribute to the improvement of lipid profiles, significantly reducing cardiovascular risk (Ali & Zeb, 2024) which protects cardiovascular health. Polyphenolic compounds and dietary fibers found in green walnuts may improve glycemic control, representing potential for dietary interventions in diabetes. The dietary fibers and polyphenolic compounds can positively influence the gut microbiota composition, thus enhancing overall gastrointestinal health.

2.6.3 Husk

The pericarp of green walnuts comprises numerous beneficial components, including juglone, phenolic acids, vitamin C, tannins, and flavonoids (Oliveira et al., 2008). Juglone possesses antioxidant, anti-inflammatory, and anti-cancer properties. Tannins exhibit antioxidant and anti-inflammatory properties. Flavonoids, as antioxidants, may offer protection against chronic illnesses (Meshkini & Tahmasbi, 2017). Phenolic acids also exhibit antioxidant properties. Green walnuts are an abundant source of vitamin C, which supports immune function and collagen synthesis (Fernández-Agulló et al., 2013) as seen in Table 1. and Figure 10. below.

2.6.4 Shell

Walnut shells contain several bioactive components such as tannins, phenolic compounds, pyrolygineous acids, and naphthoquinones (Jahanban-Esfahlan et al., 2019) Naphthoquinones exhibit antioxidant effects. The shell has astringent and antioxidant

properties, which can be attributed to the presence of tannins. The antioxidant capacity of walnuts was partially ascribed to phenolic compounds. The features of pyrroligneous acids, including their anti-inflammatory, anticancer, and antioxidant effects, were augmented as illustrated (Zhai et al., 2015) in the Figure.4

2.6.5 Shoot

The health benefits of walnut trees can be partially attributed to the bioactive compounds present in their shoots. The abundant polyphenolic compounds present in shoots have been associated with anti-inflammatory, anticancer, and antioxidant properties (Solar et al., 2003). It has been identified that immature walnut shoots contain flavonoids, quinones, and phenolic acids. These compounds have been shown to possess antioxidant properties and several health-enhancing attributes. Shoots from walnut trees are used to address several ailments and problems, including diabetes, cancer, inflammation and cardiovascular disorders (Cheniary et al., 2013). Table 1 and Figure 10. Notably, the data presented specifically refer to the green parts of the walnut plant, which include shoots and unripe nuts, known for their rich bioactive composition

Table 1. Therapeutic potential of *Juglans regia* L. (Green walnut).

Parts of Green walnut	Name of bioactive compounds	Roles /Benefits	Experiment conducted country, doses	References
Shell	phenolic compounds, dihydroxytetralone hexoside, pyroligneous acids, juglone, tannins	anti-inflammatory properties and Antioxidant, anticancer	An experiment was conducted in Serbia to ascertain the bioactive components and produce green walnut liquor.	(Jahanban-Esfahlan et al., 2019; Kazemipour et al., 2008; Medic et al., 2021)
Root	hydrojuglone derivative 1, hydrojuglone derivative 5, hydrojuglone derivative rhamnoside, dihydroxytetralone hexoside, polyphenolic compounds, alkaloids, 1,4-naphthoquinone, juglone, α -hydrojuglone, flavonoids,	Dandruffs, skin disorders, Hair fall	Research conducted in China and India focused on the extraction of bioactive substances from walnut tree roots and identified their different applications in the medical area. Infuse tender roots in mustard oil for 2–3 months, then apply the oil to the scalp to help reduce hair loss and dandruff.	(Allaie et al., 2018; Hassan et al., 2013; Medic et al., 2022; Medic et al., 2021; Raja et al., 2017)
Husk	flavonoid, phenolic acids, vitamin C, 1,4-naphthoquinone, juglone, tannins, citric acid, malic acid,	Protective effects on the liver and kidneys antioxidant capabilities,	Experiments to identify bioactive chemicals from green walnut husk were conducted in Hungary and the United	(Anderson K. J. et al., 2001; Fernández-

	<p>Jjglone, polyphenols, α-hydrojuglone, hydrojuglone-β-D-glucopyranoside, , Juglanin B, p-hydroxymetoxybenzobijuglone, Regiolone,, Dihydroxytetralone hexoside, Dihydroxytetralone galloyl hexoside, Trihydroxytetralone derivative Hydrojuglone rutinoside, Hydrojuglone rhamnoside, Hydrojuglone pentoside, Hydrojuglone derivative 5, Hydrojuglone derivative pentoside 1,2 & 3, Hydrojuglone derivative rhamnoside, Hydrojuglone hexoside derivative, Bisjuglone 5-Hydroxy-2,3-dihydro-1,4-naphthalenedione, 4,5,8-Trihydroxynaphthalene-5-D-</p>	<p>anti-inflammatory effects, anticancer attributes, and enhancement of immunological function.</p>	<p>States. Husk extract exhibiting antibacterial action at concentrations ranging from 1.25 to 5 mg/mL.</p>	<p>Agulló et al., 2013; Medic et al., 2022; Medic et al., 2021; Rahimi et al., 2011)</p>
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	glucopyranoside, 1,4,8-Trihydroxynaphthalene-1-D-glucopyranoside			
Leaves	Juglone, galactosidase, arabinoside, xyloside, rhamnoside, 1,4-Naphthoquinone, Juglone Phenolic acids, tannins, essential fatty acids, ascorbic acid, flavonoids, caffeic acid, para-coumaric acid, Hydrojuglone-β-D-glucopyranoside, Hydrojuglone rutinoid, Hydrojuglone rhamnoside, Hydrojuglone pentoside, Hydrojuglone derivative 1 & 2, Hydrojuglone derivative pentoside 1 & 2, Hydrojuglone hexoside derivative, Hydrojuglone dihexoside derivative, 5-Hydroxy-2,3-dihydro-1,4-	Anti-diabetic effect, anti-cancer effect, hepato-protective activity and Anti-ageing activity, Anti-oxidant activity, lipid-lowering effect, antihypertensive effect, anti-microbial effects, gastroprotective activity, hypercholesteraemic activity	In the regions of Europe, Asia, Iran and United states the study was conducted on identification of various bioactive compounds from the walnut tree leaves and its application in the food and medical field through several clinical trials. 400ppm leaves extract having the antioxidant activities	(Al-Snai, 2019; Medic et al., 2022; Noumi et al., 2011; Rahimipanah et al., 2010; Verma et al., 2013)

	naphthalenedione, Dihydroxytetralone hexoside			
Bark	flavonoids, Polyphenols, 1,4-Naphthoquinone, Juglone, α -Hydrojuglone, Hydrojuglone- β -D-glucopyranoside, Hydrojuglone rutoside, Hydrojuglone rhamnoside, Hydrojuglone pentoside, Hydrojuglone derivative 1,2,3,4,5 & 6, Hydrojuglone derivative pentoside 1,2 & 3, Hydrojuglone derivative rhamnoside, Dihydroxytetralone hexoside, Procyanidin dimer 2 (+) Catechin, Ellagic acid derivative	Prevent tooth ache and tooth decay, antimicrobial activity, antioxidant activity, antifungal activity, platelet aggregation, antiseptic	Study regarding extraction of bioactive compounds from the walnut tree bark and its assessment conducted in Pakistan. Dried bark twigs referred as toothbrushes	(Bhatia et al., 2006; Medic et al., 2022; Medic et al., 2021; Mohammadi et al., 2011)
Kernel	Hydrojuglone- β -D-glucopyranoside Ellagitannin, Pedunculagin	Antiproliferative effects, chemopreventive properties, Hypolipidemic effects, antioxidant	Experiments were undertaken in Hungary and Africa to investigate the therapeutic potential and molecular mechanisms of the bioactive compounds present in green walnut kernels. Numerous dose and	(Colaric M. et al., 2005; Greve L. C. et al., 1992; Martínez et al., 2010;

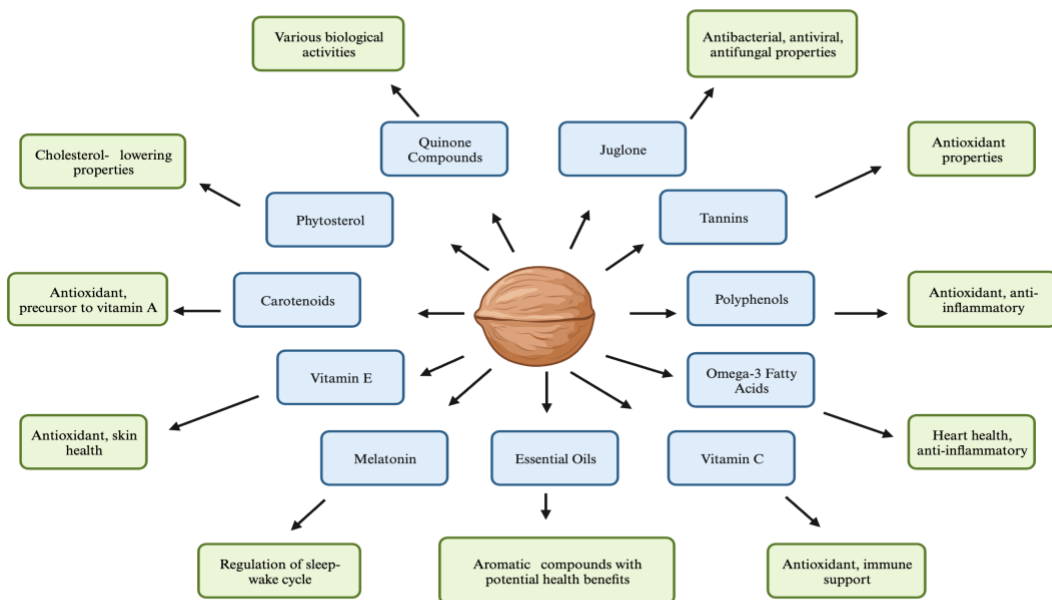
		activity, antibacterial characteristics	<p>clinical investigations have been conducted for medical and nutritional purposes.</p> <p>Five grammes of fruit kernel consumed with 500 mL of boiling milk has been identified as a treatment for constipation and a cognitive enhancer.</p>	<p>Medic et al., 2022; Medic et al., 2021; Zarghami Moghaddam P. et al., 2017)</p>
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2.4.6 Bark

Walnut bark contains several bioactive components including polyphenolic compounds, flavonoids, and alkaloids, which have demonstrated medicinal effects (Taha & Al-wadaan, 2011). The bark has historically been used to address skin ailments, stimulate liver function, alleviate constipation, and improve digestion (Thakur, 2011). These bioactive compounds have been associated with antioxidant, antibacterial, antiviral, anticancer, anti-inflammatory, and cognitive enhancing properties (Bhatia et al., 2006). Barks are included in dental products to improve brush preparation and oral hygiene. Walnut bark extract has been used as a natural hair coloring and antibacterial ingredient in contemporary cosmetic formulations (NirmlaDevi et al., 2011).

2.4.7 Root

The roots of walnut trees contain bioactive compounds that offer numerous health advantages. Its roots have been utilized in traditional medicine and are rich in phytochemicals, including alkaloids, flavonoids, and polyphenolic substances (Raja et al., 2017). Walnut roots possess antioxidant capabilities and may serve as a possible source of bioactive substances. These bioactive molecules exhibit potential antifungal and antibacterial activities (Allaie et al., 2018) as shown in Table 1. and Figure 10.



Phytonutrient of green walnut and their functional properties

Figure 10. Schematic structure of phytonutrients and different properties of green walnut (source: author)

2.7 Green Walnut as Functional or Novel Food Ingredients

Walnuts contain several phenolic compounds with antioxidant effects. Due to their elevated antioxidant levels and nutritional significance, Persian walnuts are essential for human nutrition (Arcan & Yemenicioğlu, 2009). Incorporating walnuts and their products into the diet may minimize the risk of developing specific diseases including cancer, cardiovascular issues, and degenerative disorders. Walnut trees are medicinal plants, used to treat diabetes with their roots, fever, rheumatic pain, skin ailments with their leaves, and malaria and rheumatic pain with their blooms (Tsasi et al., 2016). It possesses the highest concentration of antioxidants among all nuts and seeds (Blomhoff et al., 2006). Owing to its elevated phenolic content, walnut extract possesses anti-inflammatory and anticancer properties (Valdebenito et al., 2017). It also serves as a blood purifier and possesses antiparasitic and anti-diarrheal properties (Tapsell et al., 2009). Numerous investigations have been performed (Table 2.) on various polyphenols and their bioactivities. The phytonutrients present in green walnuts are illustrated in Figure 10. The structure of the bioactive compounds is shown in Figure 11.

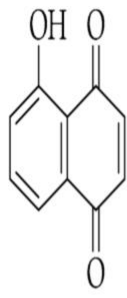
The potential of green walnuts in food innovation is substantial due to their nutritional richness and bioactive properties. Developing food products using green walnut extracts aligns with the concepts of functional foods or novel foods, characterized by their ability to provide additional health benefits beyond basic nutritional functions. Functional foods typically include natural or modified foods containing bioactive compounds, which, when consumed regularly in effective amounts, impart specific health benefits beyond basic nutrition. In contrast, novel foods refer to foods not significantly consumed in the EU before 1997, including ingredients derived from novel production processes or new sources. Regulatory considerations regarding health or nutritional claims associated with such foods are essential and must comply with established frameworks set by food regulatory authorities, such as the European Food Safety Authority (Authority, 2025)

Considering these advantages, we have utilized green walnuts to prepare feed for bees and their derived bee feed product, subsequently integrating these bee products into yogurt to conduct blind trials. The development criteria and regulations for functional foods involve numerous challenges, and the approval process for novel foods is also complex. Therefore, future consideration of our products will depend on securing additional funding and obtaining approvals from relevant regulatory authorities.

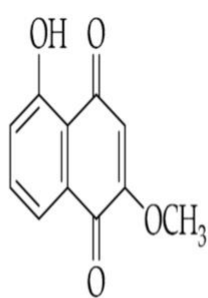
Table 2. Studies conducted worldwide on its bioactive and polyphenols.

Bioactivities on Human health	Bioactive Activity	Description	References
Blood sugar level control	Walnut kernels with polyphenolic extracts	Enhances the health status of individuals with type 2 diabetes and decreases insulin levels. Additionally, it contributes to a reduction in blood glucose levels. Decreases total cholesterol and triglyceride levels	(Fukuda et al., 2003; Jiang et al., 2002; Prokisch et al., 2022; Zhao et al., 2019)
Gut Health	Walnut polyphenol extracts that include omega-3 and omega-6 fatty acids	Regulating the gut microbiome Immunomodulation denotes the alteration or regulation of the immune system. Improve the composition of intestinal microbiota and stimulate the synthesis of secondary bile acids. Reduce the levels of LDL cholesterol.	(Bober et al., 2018; Sethi et al., 2018)
Anti-cancer	Juglone Walnut polyphenol extracts	Induce cell cycle arrest and programmed cell death (apoptosis) in human endometrial carcinoma cells. Inhibit the proliferation of Caco-2, MCF-7, and more cell types. Reduce the rate of prostate tumour proliferation by 30% to 40%. Reduce the probability of breast cancer development, diminish the growth rate of tumours.	(Figuerola et al., 2017; Park et al., 2020; Reiter et al., 2013; Zhang et al., 2019)

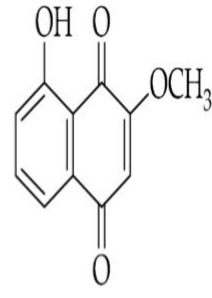
Cardiovascular activity	Ellagitannin Walnut kernels Ellagic acid	Prevents the onset of neurodegenerative diseases. Minimise the risk of cardiovascular disease and enhance blood lipid profiles. The augmentation of HDL-cholesterol and antioxidants, together with the diminution of total cholesterol and LDL, substantially prevents the onset of cardiovascular diseases. Mitigate the irritation.	(Badimon et al., 2019; Bechthold et al., 2019; Comstock et al., 2010; Estruch et al., 2013; Kurihara et al., 2019; Nergiz-Ünal et al., 2013)
Anti-oxidation	Ellagitannin-derived Walnut polyphenol extracts	Regulating the enzymatic activity. The antioxidant efficacy in terms of scavenging capacity for DPPH and ABTS ⁺ . Chemical agents that reduce electron count in a reaction, agents that eliminate unstable molecules with unpaired electrons, agents that provide hydrogen atoms. Improved blood cholesterol levels and endothelial function.	(Adams et al., 2010; Khalil et al., 2018; Pandareesh et al., 2018; Zhang et al., 2020)



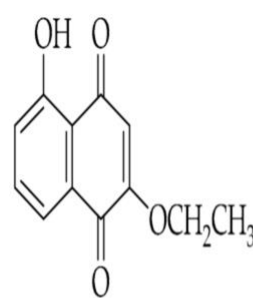
Juglone



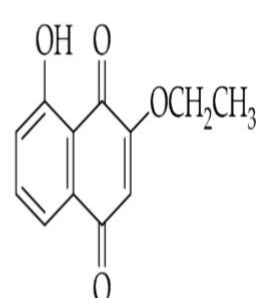
2-Methoxy juglone



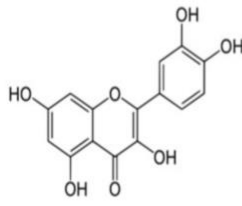
3-Methoxy juglone



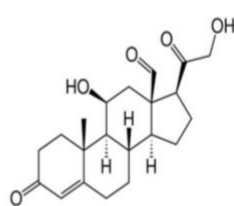
2-Ethoxy juglone



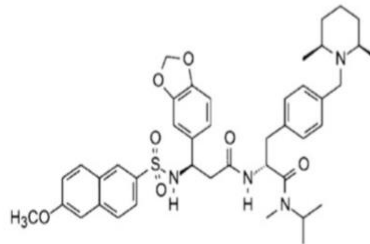
3-Ethoxy juglone



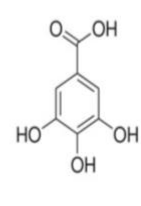
Quercetin



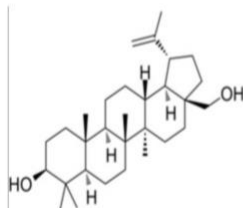
Steroids



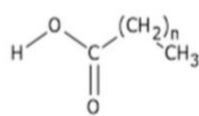
Kinins



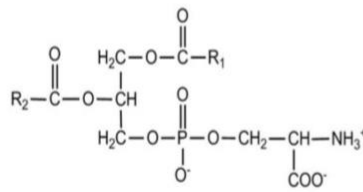
Gallic acid



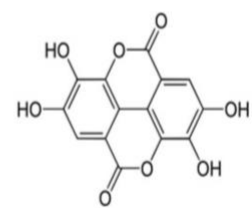
Triterpenes



Fatty Acid



Phosphatidylserine



Ellagic acid

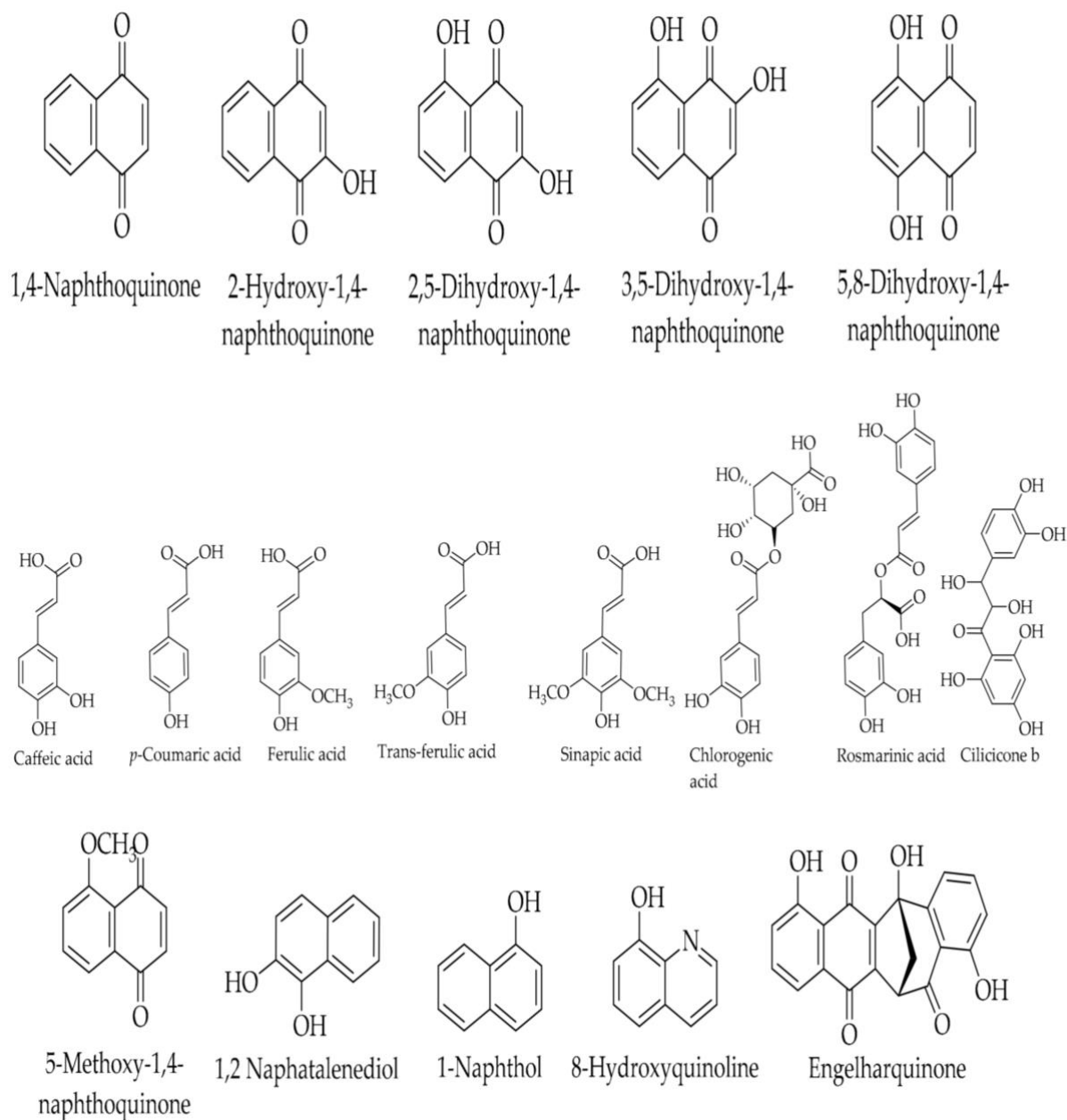


Figure 11. Structure of different bioactive compounds found in green walnut. (Source: author, Created using Biorender Design website, <https://www.biorender.com/>).

Different benefits are shown below with a nice schematic representation to better understand the benefits of green walnut (*Juglans Regia L*) in three different figures, individually (Figure 12. -15.).

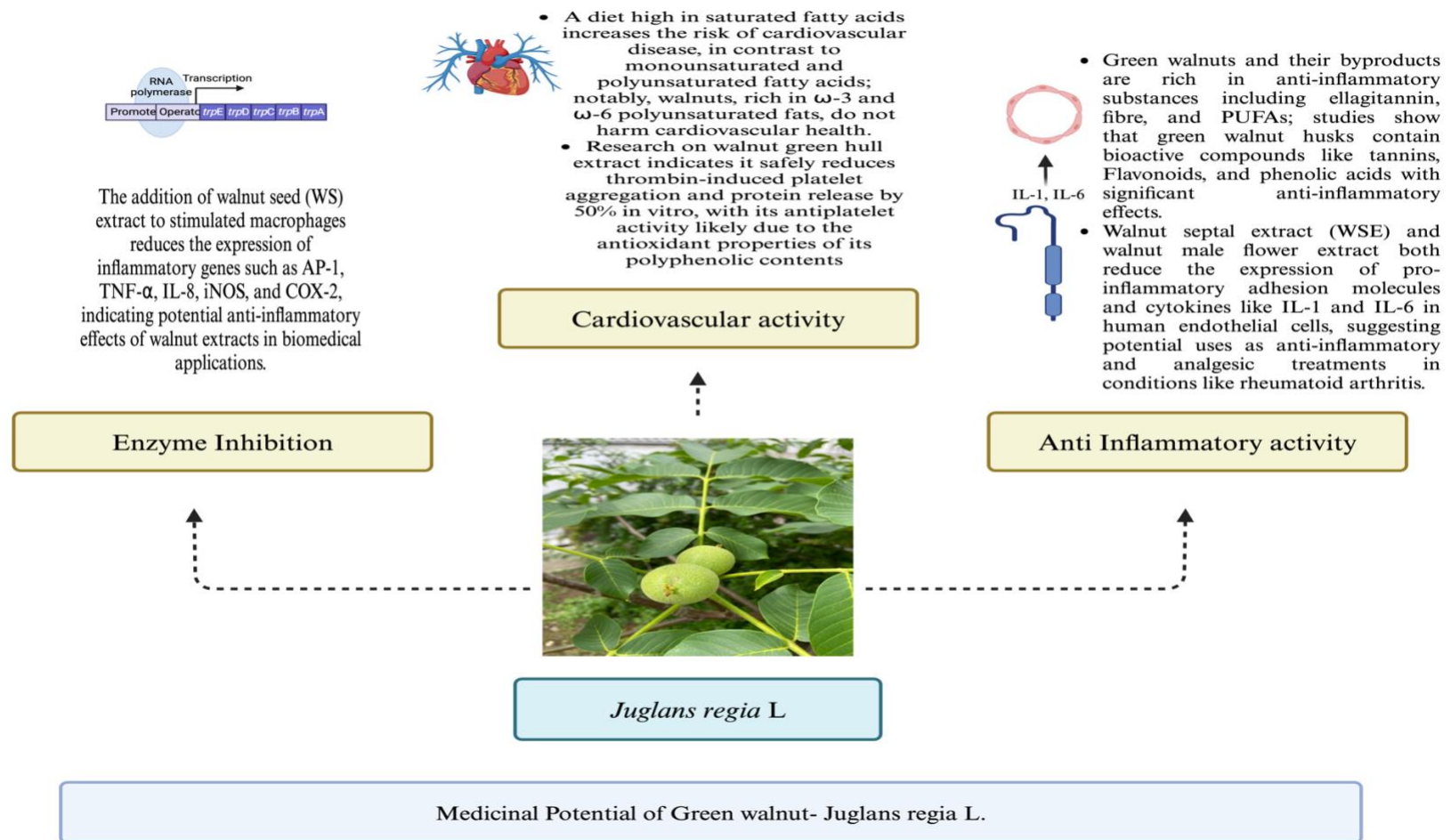


Figure 12. Different therapeutic benefits of green walnut (*Juglans Regia L.*) globally (source: author, Created using Biorender Design website, <https://www.biorender.com/>) (Ademiluyi et al., 2019; Hosseinzadeh et al., 2011; Meshkini & Tahmasbi, 2017; Salem et al., 2022; Simopoulos, 2002; Verma et al., 2013)

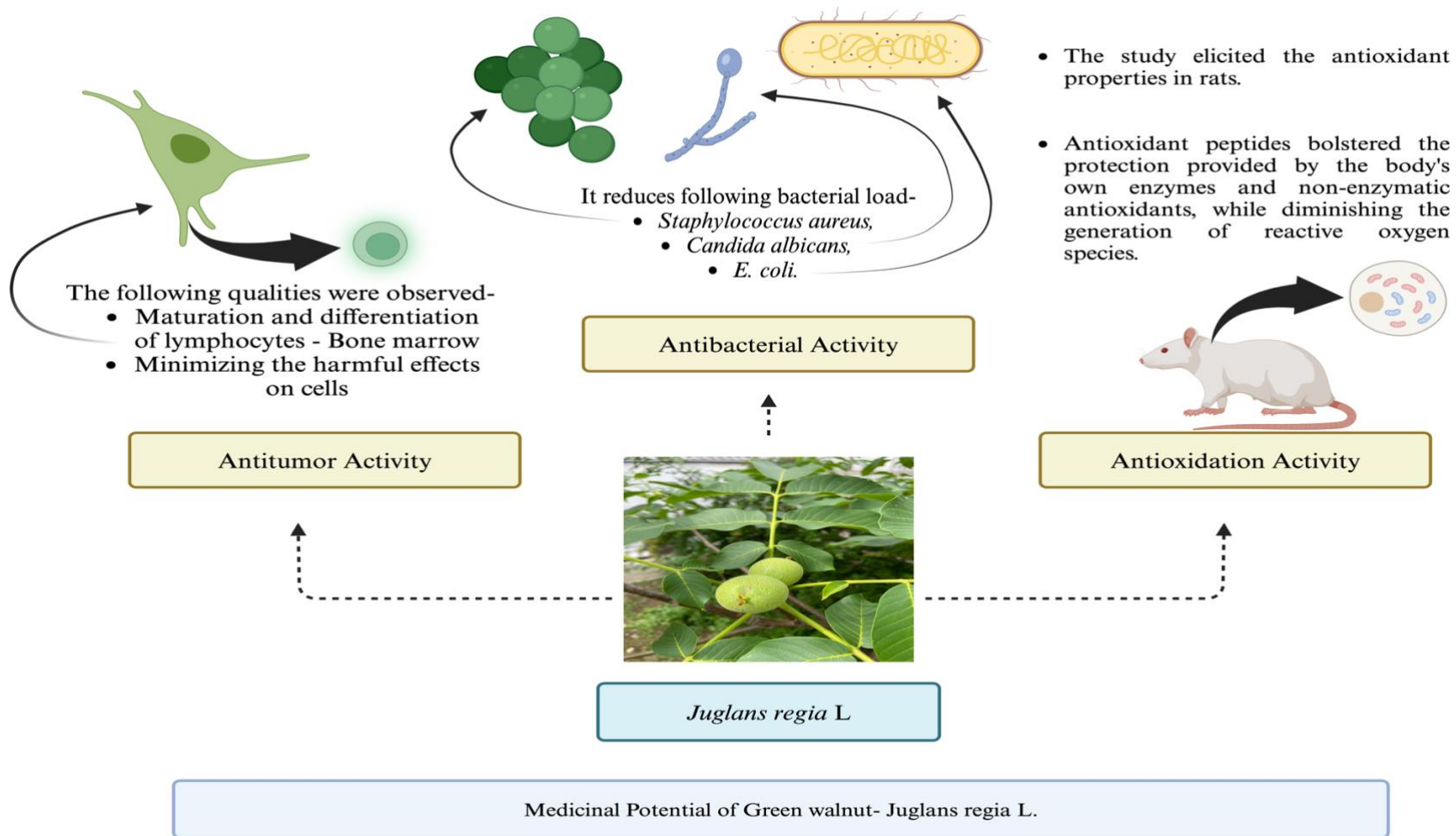


Figure 13. Different therapeutic benefits of green walnut (*Juglans Regia L.*) globally (source: author, Created using Biorender Design website, <https://www.biorender.com/>) (Alshatwi, 2011; Chaleshtori et al., 2011; Cosmulescu & Trandafir, 2012; Dzidziguri et al., 2016; Izadiyan et al., 2018; Raja et al., 2017; Soto-Madrid et al., 2021; Zhang et al., 2022; Zhang et al., 2015) (Carey et al. 2013; Barekat et al. 2022; Croitoru et al. 2019)

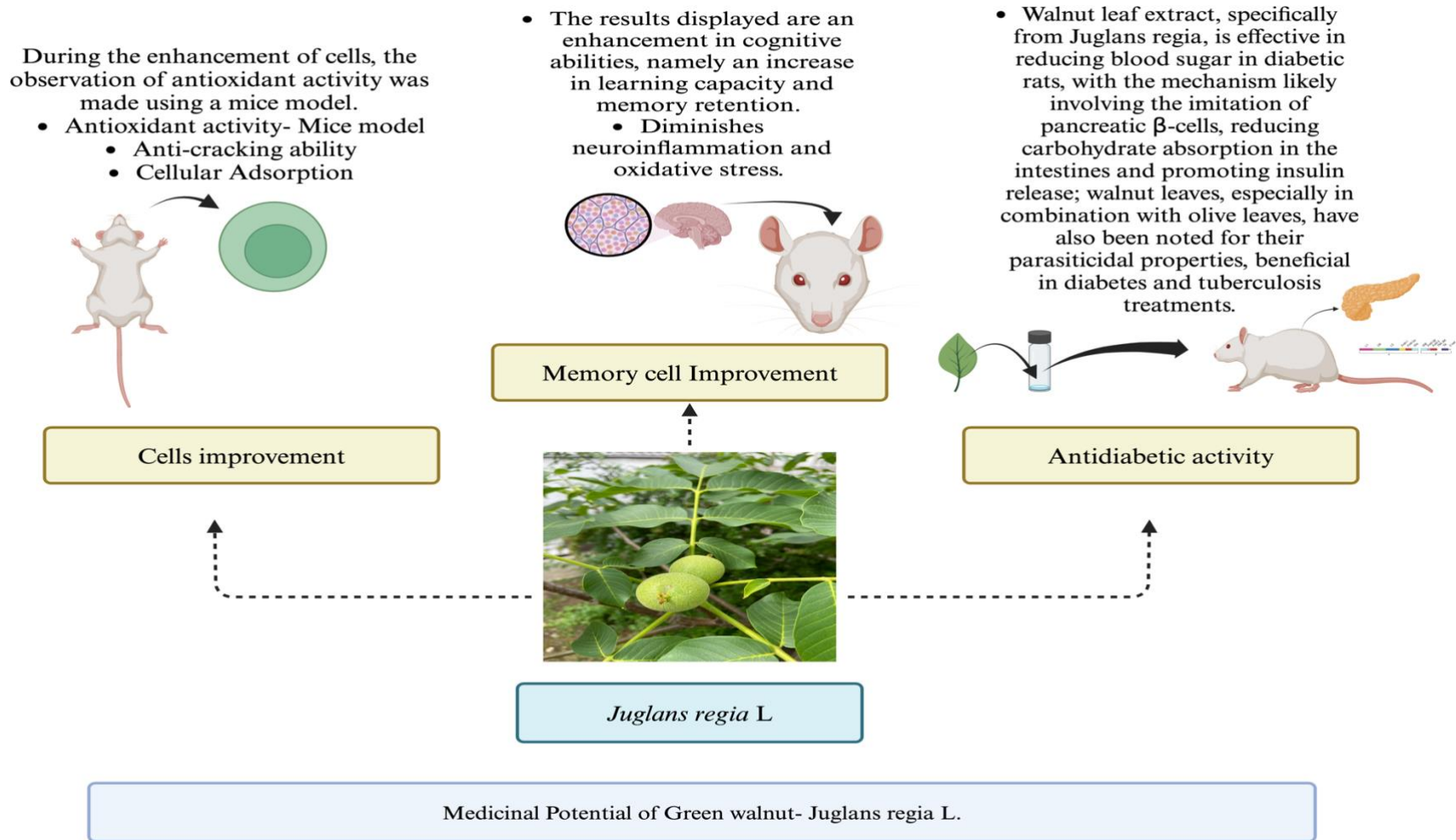


Figure 14. Different therapeutic benefits of green walnut (*Juglans Regia L.*) globally (source: author, Created using Biorender)(Feng et al., 2018; Mohammadi et al., 2011; Moravej et al., 2016; Ramishvili et al., 2016; Tan B. et al., 2022; Wang et al., 2021; Wang et al., 2018).

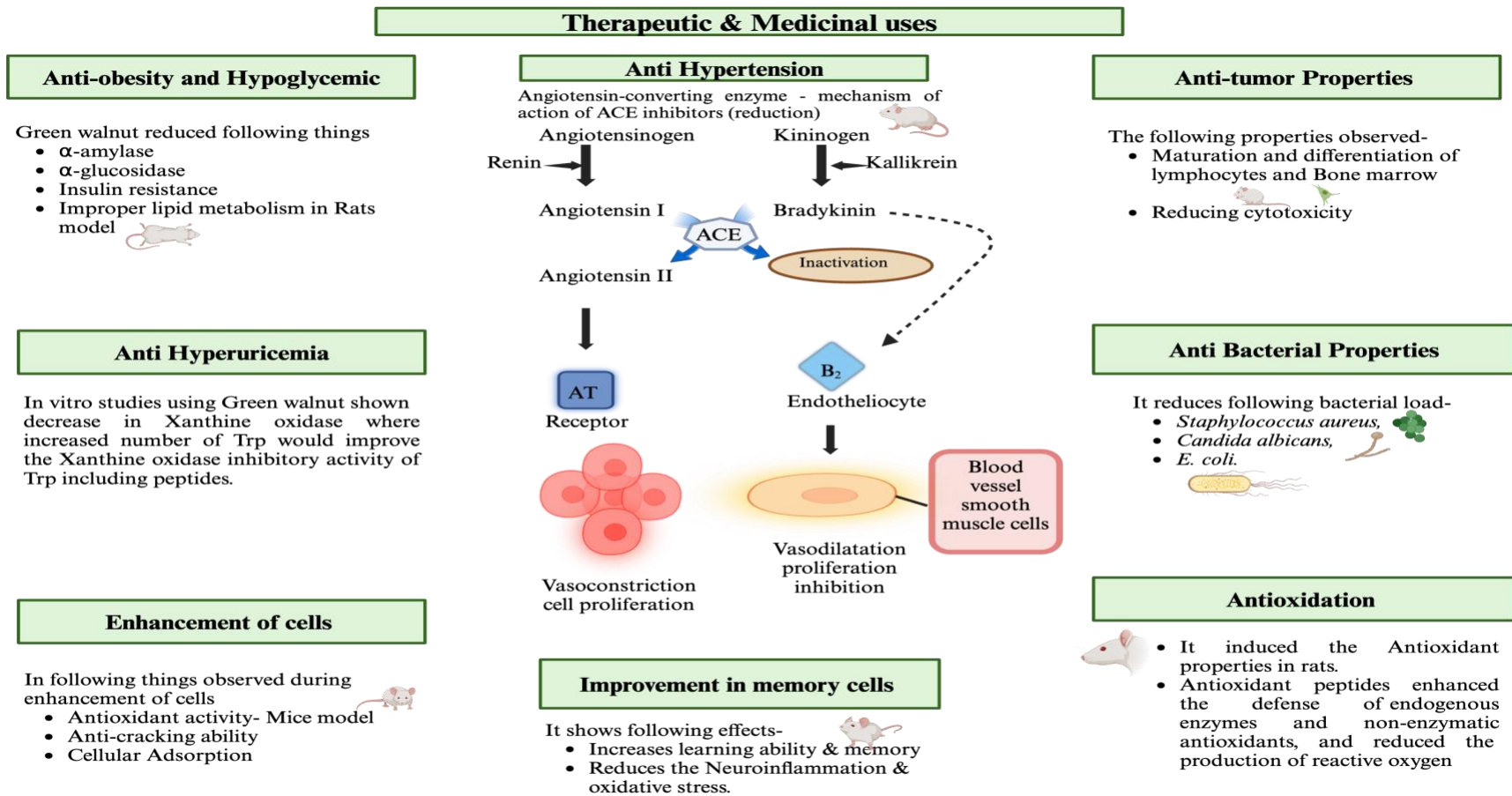


Figure 15. Different therapeutic benefits of ree walnut (*Jugals Regia L.*) globally (source: author, Created using Biorender)(Feng et al., 2018; Mohammadi et al., 2011; Moravej et al., 2016; Ramishvili et al., 2016; Tan B. et al., 2022; Wang et al., 2021; Wang et al., 2018). (Alshatwi, 2011; Chaleshtori et al., 2011; Cosmulescu & Trandafir, 2012; Dzidziguri et al., 2016; Izadiyan et al., 2018; Raja et al., 2017; Soto-Madrid et al., 2021; Zhang et al., 2022; Zhang et al., 2015) (Carey et al. 2013; Barekat et al. 2022; Croitoru et al. 2019) (Ademiluyi et al., 2019; Hosseinzadeh et al., 2011; Meshkini & Tahmasbi, 2017; Salem et al., 2022; Simopoulos, 2002; Verma et al., 2013).

2.8 Green walnuts as a feed for bees

Qualitative and quantitative studies of the application of walnuts in feed technology have garnered global attention. Walnut is regarded as the "King of Nuts" due to its nutritious importance in human diets. Nonetheless, the significance of walnuts extends beyond human-related endeavours, as they have demonstrated economic viability as an alternative to feed technologies (Popescu et al., 2020). Walnuts can be incorporated into a diverse array of gourmet creations owing to their remarkable adaptability. They complement other nutrient-dense foods and can serve as plant-based alternatives to meat products. Walnut (*Juglans regia L.*) is a premium feed ingredient that has been effectively utilized in animal diets for many years. It is regarded as a substantial source of energy and high-quality protein, rendering it an optimal component of monogastric diets. The advantageous fatty acid composition and presence of many bioactive substances with antioxidant characteristics have enhanced their significance in animal nutrition (Yang et al., 2021; Zhao et al., 2023). Herbal extracts have been shown to have beneficial effects on the growth of bee colonies. The application of IMMUNOSTART HERB, a natural formulation derived from herbal extracts, markedly enhanced the vitality of bee colonies and the quantity of sealed worker bee broods in comparison to control groups that were solely provided with a sugar solution (Shumkova & Balkanska, 2021; Shumkova et al., 2022).

Herbal extracts bolster the immune system of bees. Hemp extract enhances the natural immunity of bees by elevating protein concentrations and diminishing protease activity, which are essential for combating pathogens (Skowronek et al., 2021). The application of NOZEMAT HERB and NOZEMAT HERB PLUS supplements correlated with increased overwintering survival rates of bee colonies, presumably attributable to improved immunological function and antioxidant capacity (Shumkova & Balkanska, 2021). Different plant extracts (s (i.e., acacia, Chlorella alga, green walnut, and sea buckthorn) have also been utilized for the development of bee feeds (Prokisch et al., 2022).

2.9 Green walnut for making Silver Nanoparticles by using green synthesis.

Silver nanoparticles (AgNPs) produced from herbal extracts have garnered considerable interest because of their environmentally sustainable and economical fabrication techniques. These nanoparticles demonstrate many biological activities, such as antibacterial, anticancer, and wound-healing characteristics, making them potential candidates for numerous medicinal applications. Herbal extracts function as reducing and capping agents in the environmentally

friendly manufacturing of silver nanoparticles. This method is superior to conventional chemical and physical techniques owing to its biocompatibility and eco-friendliness. Numerous herbs, including *Matricaria chamomilla*, *Scutellaria barbata*, and *Iresine herbstii*, have been utilized to synthesize AgNPs, which are characterized using techniques such as UV-visible spectroscopy, X-ray diffraction (XRD), and Fourier transform infrared spectroscopy (FTIR) to verify their formation and stability (Dadashpour et al., 2018; Dipankar & Murugan, 2012; Nguyen et al., 2020; Veeraraghavan et al., 2021). In the food service industry, where microbial contamination may result in spoiling and foodborne diseases, AgNPs provide an effective means of food preservation owing to their strong antibacterial properties against a wide range of pathogens. They can be integrated into packaging materials, food contact surfaces, and food additives to suppress microbial proliferation and prolong the shelf life of perishable items (Ahmed et al., 2016). Silver nanoparticles (AgNPs) are often classified as Generally Recognised as Safe (GRAS) by regulatory bodies, including the US Food and Drug Administration (FDA), when utilized within designated parameters. In contrast to traditional antibacterial drugs, AgNPs are less prone to inducing microbial resistance owing to their diverse targets and modes of action. Furthermore, progress in nanotechnology has resulted in the creation of nanocomposite materials with regulated release characteristics, guaranteeing the safe and efficient application of AgNPs in the food context. Silver nanoparticles (AgNPs) are used in nanotechnology-driven detection techniques for food safety and quality assurance. Functionalized AgNPs act as sensitive probes for detecting pollutants, poisons, and allergens in food matrices. They provide swift and precise identification of pathogens and chemical pollutants (e.g., pesticides, antibiotics, heavy metals) in food samples, assuring adherence to regulatory standards and improving consumer safety (Ivanišević et al., 2021; Mustafa & Andreescu, 2020).

Numerous techniques exist for nanoparticle synthesis, each with distinct advantages and limits. A variety of physical processes are commonly employed, including radiation, electro-deposition, evaporation-condensation, vapor and gas phases, ball milling, lithography, spray pyrolysis, sonication, arc discharge, pulse wire discharge, and pulsed laser ablation. Chemical approaches include microwave, photochemical, electrochemical, sonochemical, hydrothermal, co-precipitation, pyrolysis, redox, and microemulsion methods. Biological approaches include green synthesis techniques that utilize plants, fungi, yeasts, viruses, bacteria, and biomolecules or biopolymers (Ahmed et al., 2022; Ealia & Saravanakumar, 2017). Physical and chemical techniques have effectively generated nanoparticles; however, traditional synthesis methods

are costly and often require toxic substances and harsh temperatures, resulting in environmental damage and health hazards. Green synthesis entails the environmentally sustainable fabrication of nanomaterials utilizing biomolecules derived from plants or microorganisms such as fungi, bacteria, yeast, viruses, and algae as substrates. This entails three essential steps: selecting a solvent medium, selecting environmentally benign reducing agents, and selecting non-toxic chemicals to ensure the stability of AgNPs. It is secure, cost-effective, ecologically sustainable, and hygienic (Huston et al., 2021; Saratale et al., 2018).

Green synthesis techniques employ natural resources, including flora, microbes, and eco-friendly chemicals as reducing and stabilizing agents. Green synthesis methods promote environmental sustainability by minimizing the use of toxic chemicals and solvents, decreasing hazardous waste production, and preventing the emission of harmful pollutants (Kharissova et al., 2013).

3. Materials and Methods

3.1.1 Preparation of Plant Extracts (Bee Feed)

First, we selected the Raw Green walnut fruit crushed (whole fruit) for using as a main source of plant extract. An important part of the entire process is the development of a plant extraction method using sugar syrup with fermentation. The entire process begins with an understanding of the idea of making different plant extracts in different proportions. It varied from 1:5, 1:8, and 1:2 depending on the plant extract and sugar syrup. The conventional honey production process begins with bees foraging for nectar from flowering plants. After extracting nectar from flowers, bees metabolize it using enzymes, transforming it into simpler chemicals, primarily fructose and glucose, which are stored in their abdomens. The fundamental premise of syrup production involves combining basic plant material with five times its weight in sugar. As a result, water was included in the sugar-plant mixture. The final sugar content in the syrup was approximately 50%, with the precise quantity ascertained through the density of the syrup. Density and pH play crucial roles in syrup production. Based on extensive experience, beekeepers recommend a density value of 1.23 g cm^{-3} . If the concentration is elevated, the bees encounter challenges in syrup absorption; conversely, if it is diminished, fermentation may occur excessively, altering the pH, which would likely deter the bees. The pH may be adjusted using partially fermented syrup if required. The inventors evaluated, manufactured, and marketed over ten distinct honey varieties derived from plant extracts. The three chosen items were selected based on consumer satisfaction and beekeeper experience. If these syrup varieties are favoured by bees, it would be feasible to enhance their production values.

A specialized feeding approach was employed to administer the syrup. A sealed thin polyethylene (PE) bag containing syrup was positioned beneath the top of the beehive. The proboscis can perforate the polyethylene bag, allowing the bee to extract syrup. The bag was hermetically sealed, and its mass was readily quantified. This procedure prevents infection or contamination of syrup.

The process flow chart is shown below which has been developed specially for the green walnut. We also used other plant extracts to prepare feed extracts to check how bees behave and consume, so we used (1) green algae or *Chlorella* (*Chlorella sp.*) which were procured from Albitech Kft (Budapest, Hungary), (2) sea buckthorn (*Hippophae rhamnoides L.*) collected from the organic garden of Orbán Fruzsina (Debrecen, Hungary), and (3) green walnut (*Juglans regia L.*) gathered from the organic garden of beekeeper János Sáfián

(Debrecen, Hungary). We also collected samples from our campus, where there are trees grown close to the Laboratory of Dr. Prokisch József. - Faculty of Agriculture, Food Science and Environmental Management, University of Debrecen. This information was used for further internal testing. The extracts were utilized to prepare feeding syrups, which were created by the subsequent technique and subsequently employed to produce honey products from each feeding syrup as the initial stage (Figure16. - 17.).

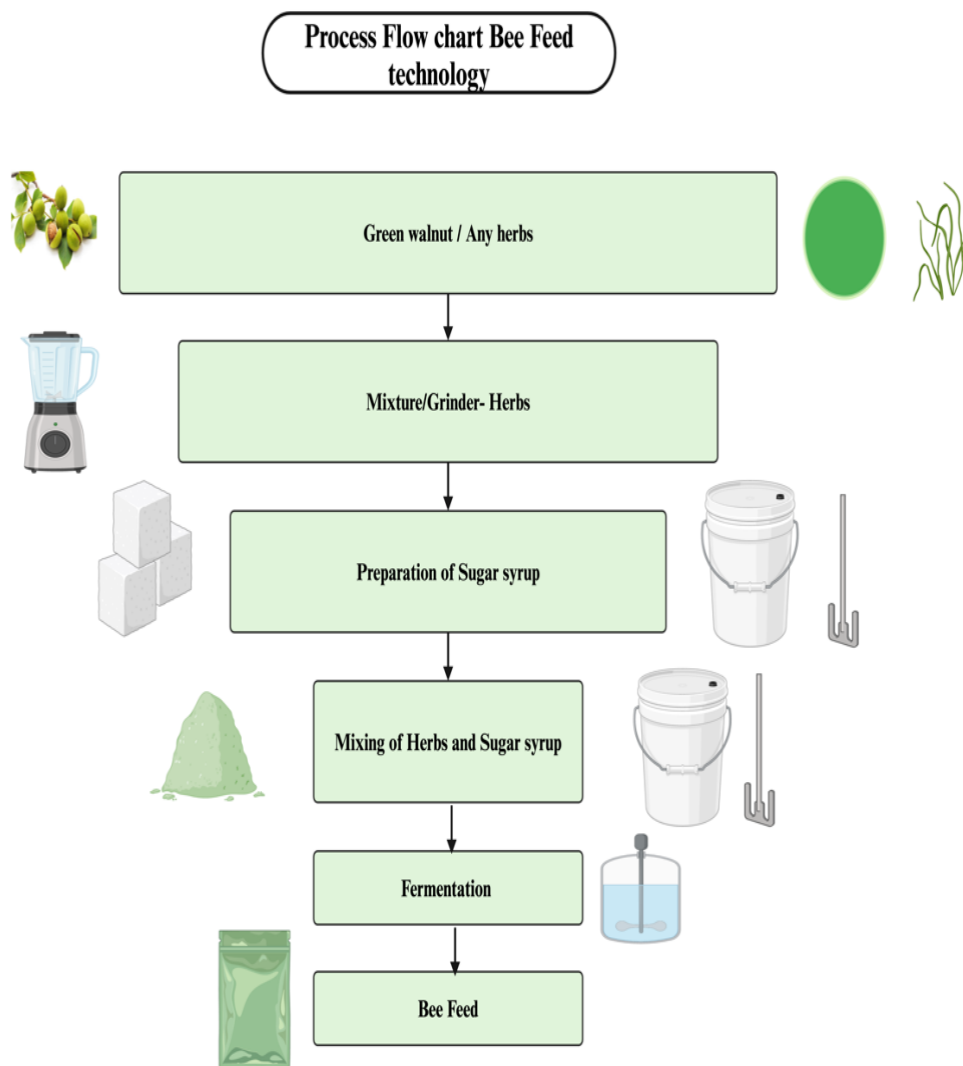


Figure 16. Process flow chart for bee feed technology development (Source: author, created using Biorender)

This above process flow chart shows that how the process conducted for preparing the Bee feed. First the process starts with the selection of raw green walnut which are uniped (entire fruit) is crushed into mixture. The quantity depends on the batch of the feed. The above process

shows generalise process but for small batch we can use Water and sugar syrup like if you are preparing total volume 400L the extract of Walnut should be 40 Kg, So basically general principle with sugar syrup preparation is ratio should be 5 times than that of water like 1:5 (water plant extract: sugar syrup) The concentration of sugar is 50% in syrup (final) where it can be examined using density of syrup which is 1.23 g cm⁻³ and also pH is also important (starting pH was 4.6 and end was 3.9). Secondly if the Density is higher than in this case the bee has difficulties in the consumption of syrup and if it is lower than the fermentation is very intensive and can change pH easily and Bee's will not consume it at all. The pH should be maintained within the range (Daróczy, 2020) Table 3 showing different bee feeds properties post fermentation.

Table 3. Types of Bee Feeds and its properties

Item in Detail	Group of Green Algae	Group of Sea Buckthorn	Group of Green Walnut
Syrup preparing	Water and sugar	Water and sugar	Water and sugar
- Total volume †	50 L	24 L	400 L
- Extract, kg or L	15 L	8 kg	40 kg
Feeding rate	5 L per day	2 L per day	5 L per day
Feeding period	8 days	12 days	20 days
Syrup density	1.20 kg L ⁻¹	1.25 kg L ⁻¹	1.20 kg L ⁻¹
Syrup pH (start)	5.8	4.3	4.6
Syrup pH (end)	5.2	3.9	3.9

†Notes Feeding rate /bee colony

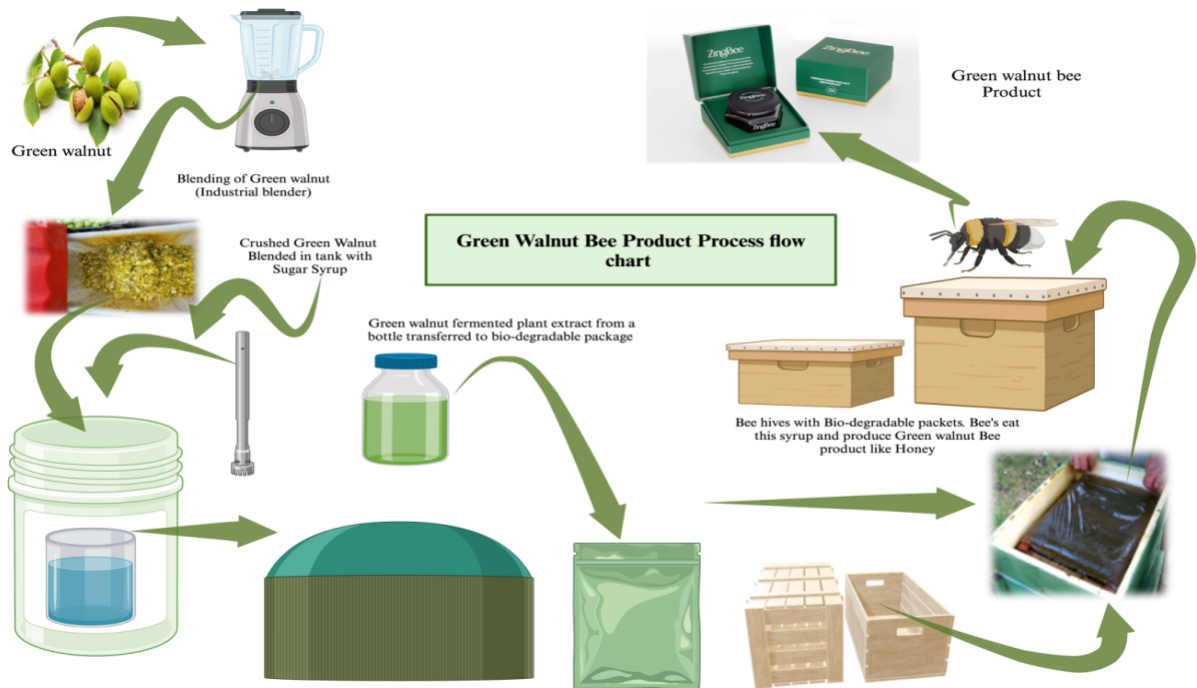


Figure 17. Process flow chart for the bee feed development (Green walnut- *Juglans Regia L.*) (Source: own photo, created using Biorender)

3.1.3 Preparation of Samples Yoghurt Samples fortified with Bee Products

Yoghurt inoculated using the heat-treated technology was transferred from the aging tank to a plastic bucket. After ascertaining the quantity of the raw material, quantified honey and apiarian products were incorporated. After homogenization, the blended semi-finished product was transferred to the ballast tank of a beaker. The product was then dispensed into a plastic cup within a circular dosing machine, sealed with welded aluminium foil, and gathered in a collection tray holding 20 units. The samples were stored in this manner in a refrigerator at 4–6 °C. in Figure 18. as an illustration of the preparation of the green walnut honey). The freezing chain of the polystyrene refrigerator was extended to the site of human clinical trials at the Borsod-Abaúj-Zemplén County Central Hospital and University Teaching Hospital in Miskolc, Hungary. The chemical makeup of the honey utilized before administering treatments from various groups was quantified, as presented in Table 3. The methodology for the parameters in Table .5 involved determining basic parameters, such as water content, fructose + glucose, fructose, glucose, free acid content, HMF content, and diastase activity, in accordance with the standard methods established by the International Honey Commission ("Harmonized Methods of the International Honey Commission," 2009). Nutrient concentrations, including boron, calcium, copper, iron, potassium, magnesium, sodium, phosphorus, sulphur, and zinc, were

quantified using ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometer) (ICP-OES; Thermo Scientific iCAP 6300, Cambridge, UK), as per Czipa et al.(Czipa et al., 2019)

150 g of Yogurt is infused with different bee products like Green algae, sea buckthorn and Green walnut along with acacia honey as an control samples, whereas the quantity of them was 30 g. 45 samples were prepared for giving it for clinical trials.

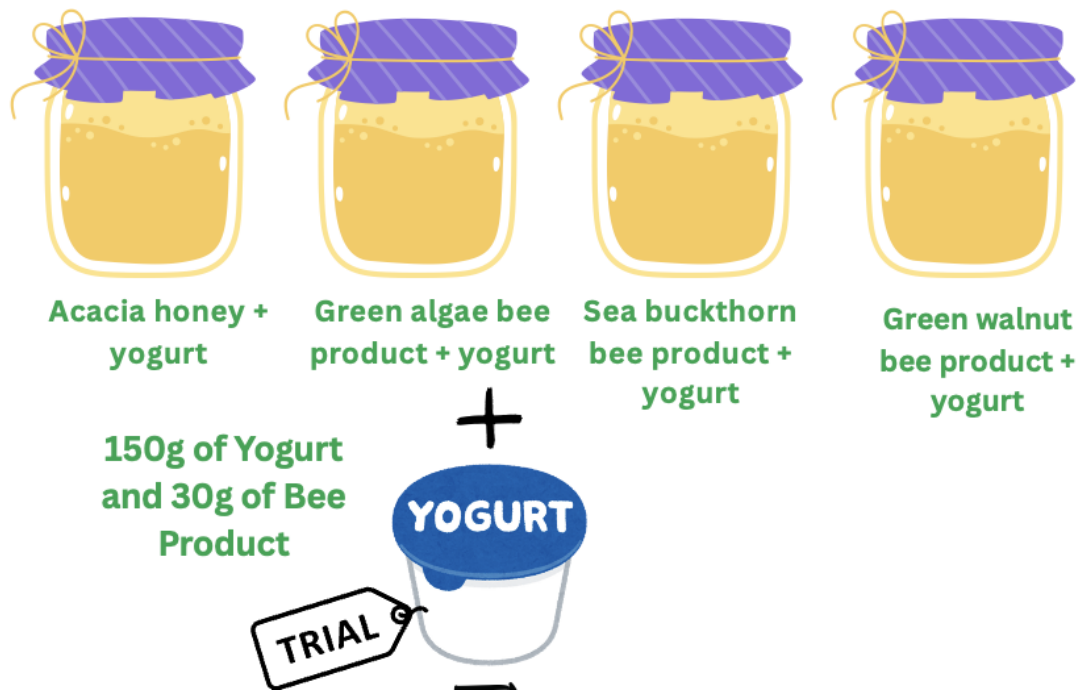


Figure 18. Fortified Yogurt Preparation by adding different bee products like green algae bee product, sea buckthorn and green walnut along with Acacia honey as a control sample.



Figure 19. The tested honey in the clinical products belonging to each tested group includes (1) Acacia, (2) Chlorella alga, (3) Sea buckthorn, and (4) Green walnut. (Source: Own pictures)

3.1.4 The Protocol for Human studies

Sixty participants aged 24 to 55 years participated in the clinical investigation, comprising 30% males and 70% females, and were allocated into four distinct groups, each consisting of 15 unique persons. Participants were selected according to certain criteria and were consistently monitored using a flow chart (Table 4.). Selection criteria were established based on the following criteria: a signature on a consent form and healthy male or female participants aged 18 or older. The exclusion criteria were as follows: (1) a history of stroke or severe cerebrovascular accident; (2) a history of acute myocardial infarction; (3) surgery within the past six months, (4) pregnancy or lactation; (5) malignancy; (6) severe acute immunological or pulmonological conditions; (7) history of multiple drug sensitivities; (8) known milk protein allergy or lactose intolerance; (9) coeliac disease, gluten-sensitive enteropathy, or during disease; (10) Crohn's disease or ulcerative colitis; (11) allergy to flower powder; (12) thyroid disorders; and (13) diabetes or elevated blood glucose levels. Figure 19 illustrates the primary steps of this study.

Table 4. The main criteria used in the current clinical study for selecting the participants

	Baseline	3 Weeks	3 Months
Signing a statement of consent	X		
Recording of anamnesis, somatic status	X	X	
Blood pressure, heart rate, weight control	X	X	
SF 36 questionnaire	X	X	
EuroQol EQ-5D Quality of Life Questionnaire	X	X	
EORTC QLQ-C30 Questionnaire	X	X	
Gastronomic Questionnaires		X	
Inspection about sleeping pattern issue	X	X	
Inspection about viral disease	X	X	X

Note: Participants received functional yoghurt daily for 21 days, incorporating extracts from the following honey products: (1) green walnut, (2) Chlorella algae, (3) sea buckthorn, and (4) acacia, with each extract comprising 20 g in 150 mL of natural yoghurt. Throughout the trial, the participants maintained their usual lifestyle habits. We did not request any modifications to their diets or lifestyles. General parameters and medical condition records were analysed. Upon the conclusion of the trial, participants filled out a straightforward culinary questionnaire regarding the product's flavour.

The process flow chart is given below about the human studies (Figure 19?).

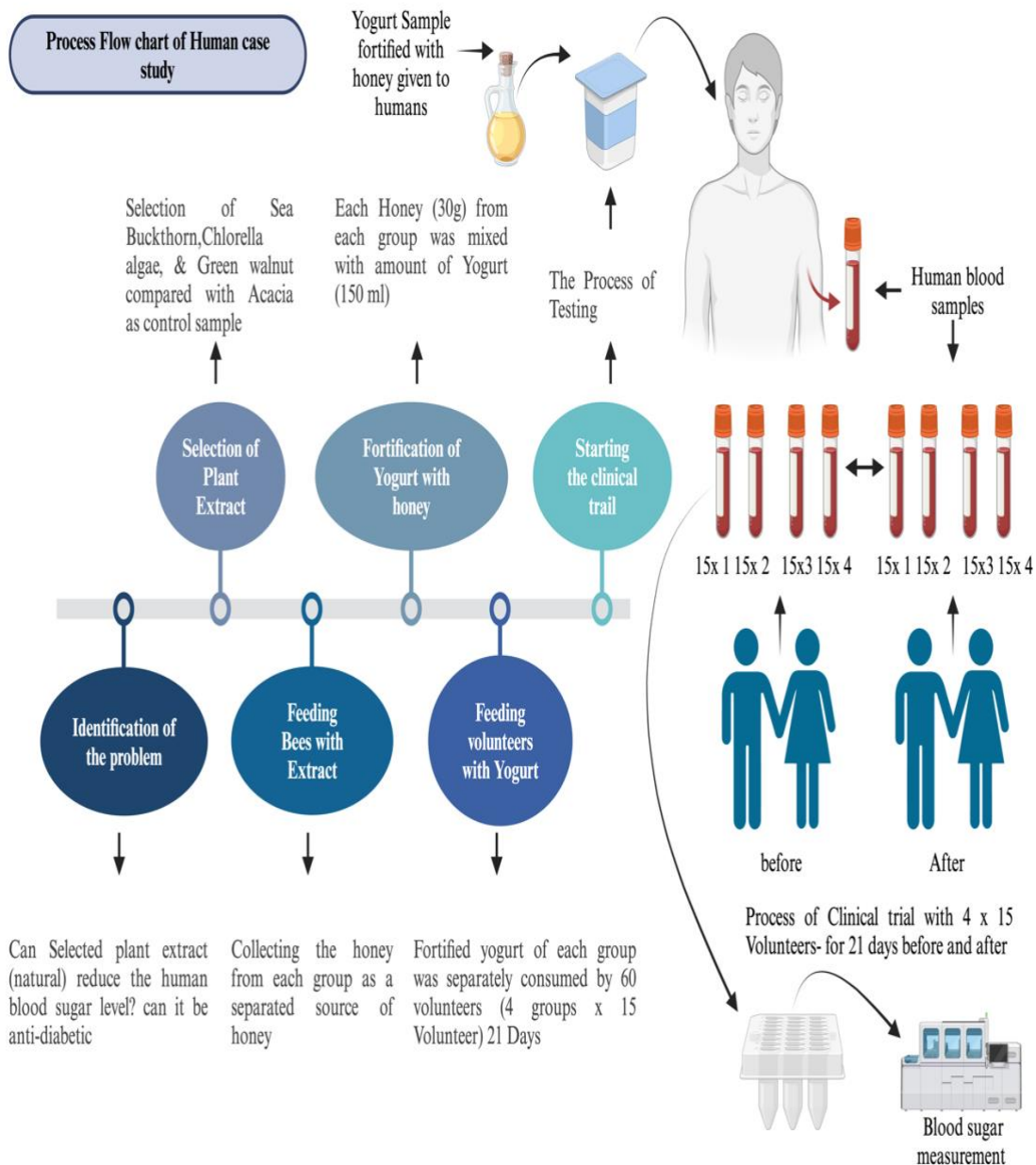


Figure 20. The primary steps undertaken in this study encompass the clinical trial. The evaluated honey products were ingested with yoghurt. (Source; author, Created using Bio-render).

The treatments included: 1) green walnut bee product, (2) Chlorella algae, (3) sea buckthorn, and (4) Acacia, A 30 g of honey was added to 150 mL of yoghurt.

The subjects ingested a single yogurt-honey box daily for 21 days, with blood samples collected prior to and following yoghurt ingestion during the clinical trial. It is crucial to highlight that certain volunteers were omitted from the study: one from the first group, three from the second group, and one from the third group. One participant exhibited elevated early blood sugar

levels, while others withdrew from the trial. Conversely, all individuals in the fourth group remained engaged from commencement to conclusion.

3.1.5 Sampling

Blood samples were collected by qualified personnel strictly adhering to sterility protocols, on the 1st and 21st days of the study, and biochemical and hematological parameters were assessed using the ADVIA Chemistry XPT System (Siemens Medical Solutions Inc., Malvern, PA, USA). Blood samples were analysed, and the following chemical assays were conducted: IgGAM and IgE, Sysmex 1000 (Sysmex, Kobe City, Japan) for complete blood count, Adams A1c HA-8180V (Arkray, Kyoto City, Japan) for HgbA1c, and Hydrasys (Sebia, Lisse, France) for agarose gel electrophoresis. The findings of this study did not include any repetition during the meaning process.

3.1.6 Determination of Total Sugars and Moisture content of the samples

A digital refractometer (ATAGO Master-Honey/BX, ATAGO Co., Ltd., Japan) was used to ascertain moisture and total sugar concentrations. It is seen in Figure 20. This apparatus directly quantifies the moisture and total sugar content of honey.



Figure 21. Refractometer (Source: From Laboratory, Institute of Food Science, University of Debrecen, Manufacturer- Atago)

3.1.7 Determination of pH value of the samples

The pH was determined in accordance with the MSZ 6943-3:1980 standard. Prior to the study, we calibrated the pH meter, as it must be calibrated at pH 2 and 7 before measurements, given

that the pH values of the honeys ranged from 2 to 5. as shown in Figure21. Samples were taken primarily from green walnut samples.



Figure 22. Digital pH meter (Source: From Laboratory, Institute of Food Science, University of Debrecen, Manufacturer- METTLER TOLEDO)

Sample preparation. It was dissolved 10 g of homogenized honey samples in 25 ml of distilled water. The beaker containing the sample was positioned on a magnetic stirrer and the pH electrode was immersed in the solution. The mixture was stirred, and the pH of the sample was recorded using a pH meter. The digital pH meter instantaneously measured the pH value of the solution; hence, no correction was required. Figure 21. illustrates the digital pH meter.

3.1.8 Determination of Diastase activity of samples

Diastase activity was assessed using a spectroscopic approach established by Bogdanov (2004). Initially, we formulated a starch solution by dissolving 2 g of anhydrous starch in 100 ml of distilled water and subsequently assessed its concentration. Preparation of samples: 10 g of homogenized honey was measured in a 100 ml beaker, to which 5.0 ml of acetate buffer (pH 5.3), 20 ml of distilled water, and 3.0 ml of sodium chloride solution (0.5 M) were added. We aliquoted it into a 50 ml volumetric flask and adjusted the volume with distilled water. We transferred 10 ml of the solution into a flask and immersed it in a water bath at 40 °C. Upon reaching a temperature of 40°C, we introduced 5 ml of starch solution and initiated the timer. Every five minutes, 1 ml of the solution was pipetted into 35 ml of iodine solution (0.0035 M)

and distilled water, and the quantity of distilled water was calculated during the concentration assessment of the starch solution and the absorbance was assessed at 660 nm against a blank solution. The technique was continued until the absorbance was below 0.235 nm. We constructed a regression line and determined the time (t) at which absorbance reached 0.235. Diastase activity was determined using the following equation:

$$\text{Diastase activity (DN)} = \frac{300}{t}$$

3.1.9 Ethical Permission

This clinical trial was a double-blind, randomized, controlled pilot study for follow-up purposes. The test commenced with the authorization of the RKEB Ethics Committee. The ethical approval number is IG-50-102/2019.

3.1.10 Statistic Analysis

Laboratory data were assessed using a t-test and one-way ANOVA using SPSS statistical software (SPSS V22.0, New York, United States).

3.1.11 Solutions and Reagents for samples

All the compounds were of analytical grade or superior quality. Nitric acid (69% v/v) and hydrogen peroxide (30% v/v) were obtained from VWR International Ltd. (Radnor, USA). Ultrapure water generated by a Milli-Q water purification system (Millipore SAS, Molsheim, France) was used to prepare solutions and dilutions. Elemental standard solutions were prepared from a mono-elemental standard solution (1000 mg/l; Scharlab S.L., Barcelona, Spain).

3.1.12 Analysis of Element content

The digestion of samples was conducted following the method developed by (Kovács et al., 1996) which was validated with animal and plant materials at an ISO/IEC 17025:2005 recognized laboratory at the Institute of Food Science, University of Debrecen. Three grams of honey was added to 10 ml of nitric acid (69% v/v), and the samples were allowed to rest overnight. Samples were pre-digested at 60°C for 30 min. After cooling, 3 ml of hydrogen peroxide (30% v/v) was added, and the samples were heated at 120 °C for 90 min. (Shown below in Figure 22. A and B)



Figure 23. Digestion unit and tubes (Left Fig A. Digestion unit with Nitric acid and Hydrogen peroxide, Right Fig B. Digestion tubes with samples for green walnut and other samples). (Source: Picture from Laboratory, Institute of Food Science, University of Debrecen).

Following digestion, ultrapure water was added to a final volume of 50 ml. The samples were homogenized and subsequently filtered using qualitative filter sheets (Sartorius Stedim Biotech S.A., Göttingen, Germany). Elemental analysis was conducted using ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) Fig. 23.). (Thermo Scientific iCAP 6300, Cambridge, UK) or Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Thermo Scientific XSeries 2, Bremen, Germany) according to the expected concentration.



Figure 24. Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) (Source: Picture from Laboratory, Institute of Food Science, University of Debrecen).

3.2.1 Conducting the Sensory Analysis with International students by using green walnut Bee product.

The sensory analysis of green walnut bee product along with control sample as acacia honey, where participants were informed about the honey samples and their responses were recorded on one questionnaire sheet, which included the following details: .

1. Age of the participants
2. Smoking habits
3. Drinking habits
4. Physical activity-frequency (times per day/week/month)
5. Honey eating habits-frequency (times per day/week/month)
6. Purchasing capacity
7. Education
8. Country
9. Sex
10. Marital Status
11. Birth year
12. Health habits (junk food consumptions habits)

All the concerns related to GDPR (General Data Protection Regulation policy were strictly followed by EU norms, the names and personal details were not recorded).

3.2.2 Sensory Evaluation of Green Walnut Bee Product

Participants evaluated the green walnut bee product based on key sensory attributes, including as follows.

1. Appearance
2. Aroma
3. Flavour
4. Texture
5. Consistency
6. Complexity
7. Quality
8. Overall Liking
9. Purchase Intent

A standardized consumer acceptance test was employed for sensory evaluation, during which panellists (international students) evaluated several sensory aspects of the samples.

Forty panellists engaged in the evaluation. Each panellist received coded samples in a controlled environment to reduce external variables and to guarantee impartial evaluation. The evaluations were assigned using a 9-point hedonic scale ranging from 1 (very dislike) to 9 (highly like). All panellists' refreshed their palates with water and plain crackers between samples to prevent sensory tiredness. Figure 24. shows the protocol for sensory analysis.

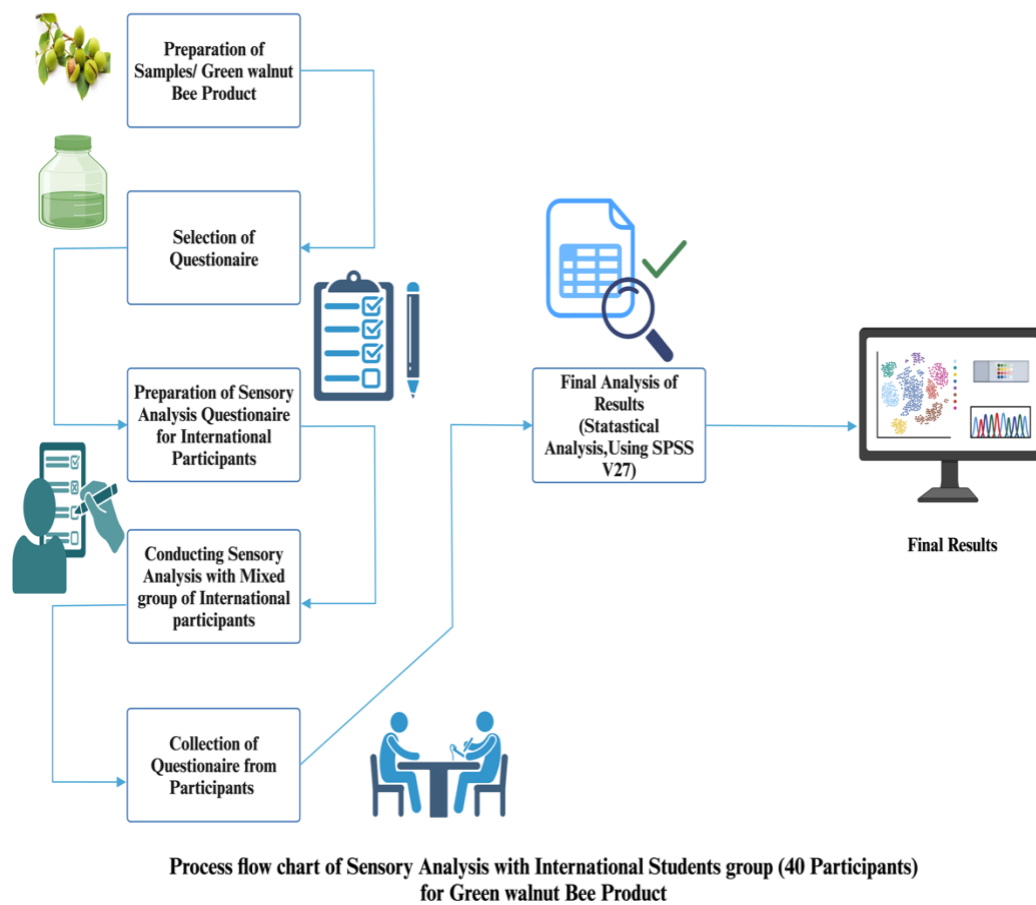


Figure 25. Protocol conducted to carry out sensory analysis with a group of international students at Faculty of Agriculture, Food Science and Environmental Engineering, University of Debrecen, from 01/02/2024 to 01/01/2025, with Ethical Approval ELMTI/2/2025. With 40 Participants (Source: Author, Created using Biorender).

3.3.3 Statistical analysis for the sensory attributes

The gathered data were examined using Principal Component Analysis (PCA) and Multiple Factor Analysis (MFA) in XLSTAT (Version 2023.2.1414) to determine significant sensory aspects affecting customer perception. The mean and standard deviation were computed for each attribute to ascertain central tendency, and variability. Pearson's

correlation coefficient was calculated to evaluate the correlations among various sensory qualities. Bar charts were added to show the average sensory scores. A heat map was designed to explain the correlation matrix among the attributes. Statistical significance was set at $p < 0.05$, and all analyses were conducted using Microsoft Excel with the XLSTAT plugin.

3.4.1 Materials and Methods for Silver nano particles synthesis & Characterizations of silver nano particles

Silver nitrate (AgNO_3) was obtained from Sisco Research Lab (SRL). All the compounds were of analytical grade and excellent purity. All other reagents and solvents were procured from commercial providers and were utilized as received. All aqueous solutions were formulated using ultrapure water sourced from a Mili-Q water system. To prepare the herbal extract, 2 g of dried walnut powder was heated with 100 mL of sterile distilled water and 100% ethanol for 20 minutes in 500 mL Erlenmeyer flasks. The boiled herbal extract was filtered using 0.2 μm filter paper, and the resulting broth was stored at 4 °C for future use. Silver nanoparticles were synthesized by combining 2 mL of plant extract broth with 10 mL of 1 mM aqueous AgNO_3 solution under ambient temperature and pressure conditions. The herbal extract facilitates the reduction of Ag^+ ions to Ag^0 , caps the nanoparticles, and subsequently inhibits their aggregation. The reaction was conducted for 72 h and the herbal extract was isolated using filter paper under sterile conditions. The synthesized Ag nanoparticles were analysed using UV–Vis absorption spectroscopy, transmission electron microscopy (TEM), and X-ray diffraction (XRD). UV–Vis spectroscopy measurements of Ag nanoparticles were conducted using a Jasco spectrophotometer (Thermo Scientific, USA) with a resolution of 1 nm across a wavelength range of 200-800 nm. The morphology of the Ag nanoparticles was analysed via TEM using a FEI Talos F200X G2 scanning transmission electron microscope (STEM) at an accelerating voltage of 200 kV. To do this, we prepared samples by drop-coating particles suspended in an aqueous medium onto carbon-coated copper grids. To verify the crystallinity of the synthesized Ag nanoparticles, powder XRD patterns were recorded using a Rigaku Miniflex-II X-ray diffractometer, which was equipped with high-intensity $\text{Cu K}\alpha$ radiation ($\lambda = 1.5406 \text{ \AA}$) and operated at a voltage of 30 kV and current of 15 mA, with a scan rate of 20°/min in the 2θ range of 20°-80°. (All experiments were carried out in collaboration with the University of Pannonia, Veszprem, Hungary-Especially TEM, XRD) Figure 25. A and B illustrate preparation of

silver nanoparticles using green walnut powder and its various applications.

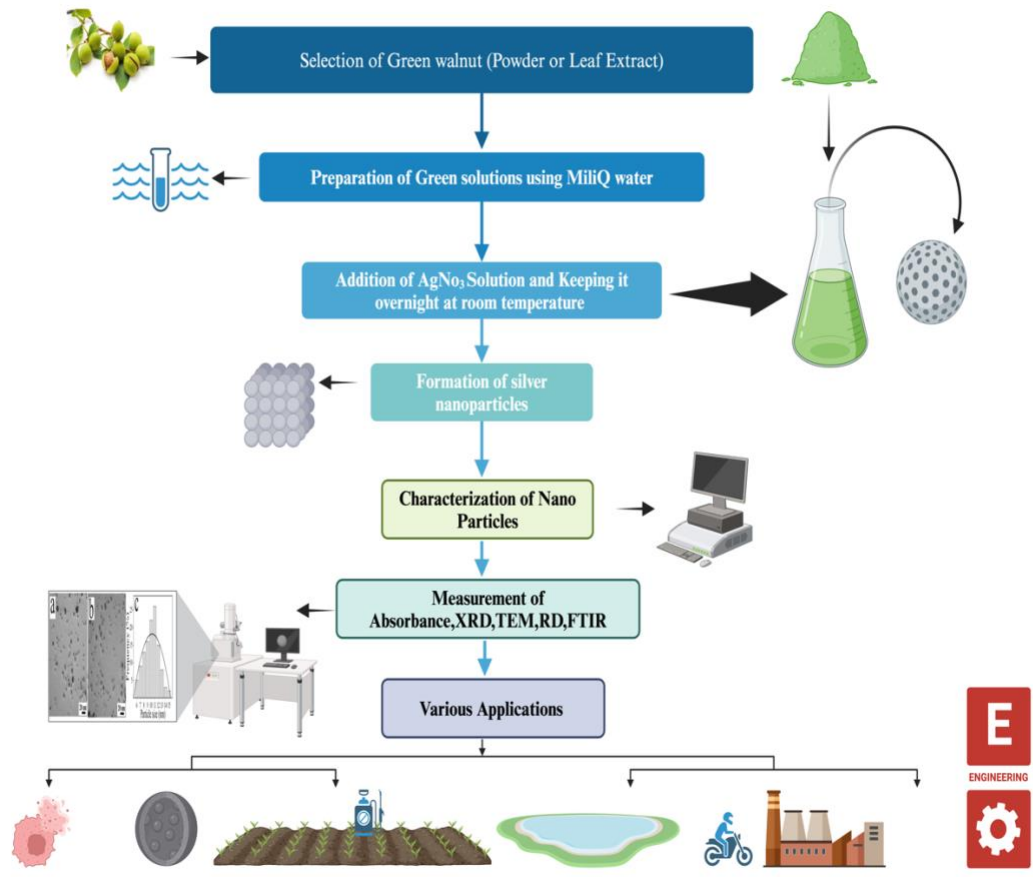


Figure 26 A. Process of preparation of silver nanoparticles using green walnut whole powder, its synthesis and its application along with measurement process. (Source: author, Created using Biorender).

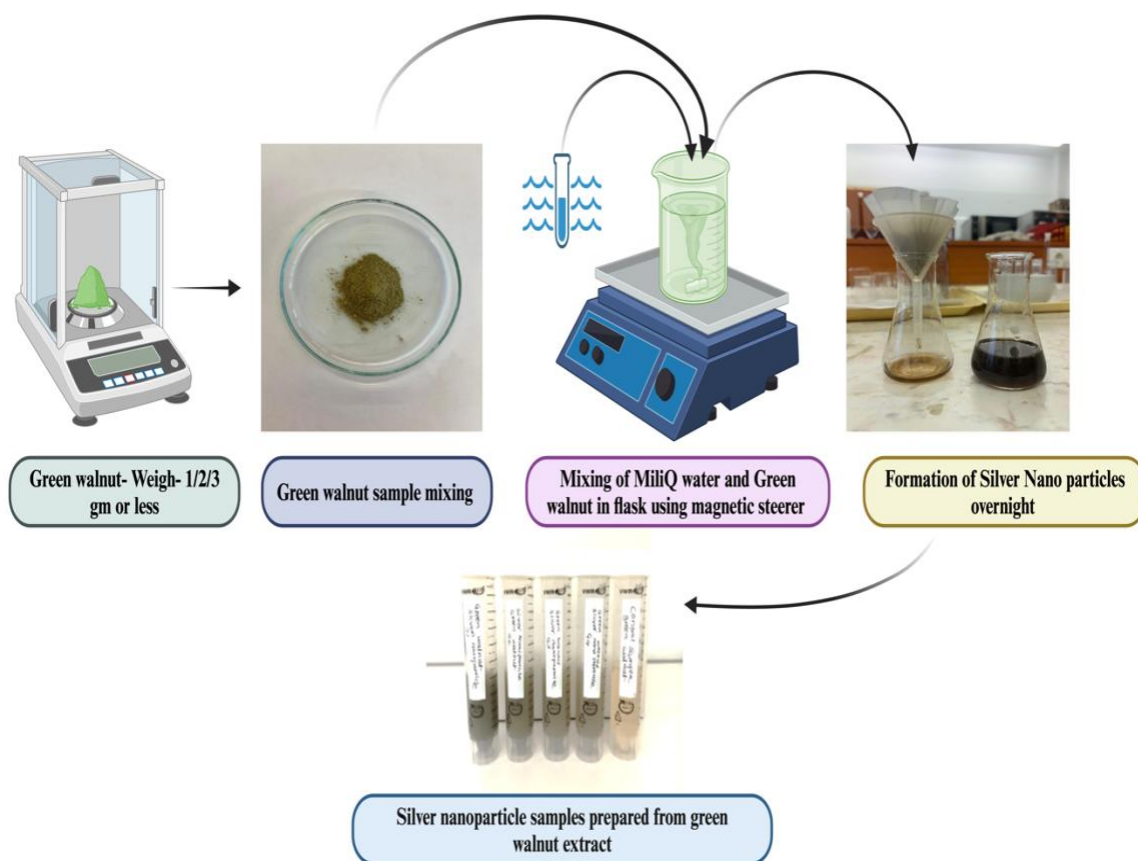


Figure 26 B. Flow chart for silver nano particles synthesis. (Source: author, Created using Biorender).

The synthesized silver nanoparticles were characterized using UV–visible spectroscopy (Thermo Scientific, USA) within the wavelength range of 200-800 nm, transmission electron microscopy (TEM) analysis (FEI Talos F200X G2 scanning electron microscope (STEM) operating at an accelerating voltage of 200 kV), and X-ray diffraction analysis (XRD). These are some of the very expensive equipment, and they are very sensitive in nature; therefore, we cannot add their pictures.

2 Results and Discussions

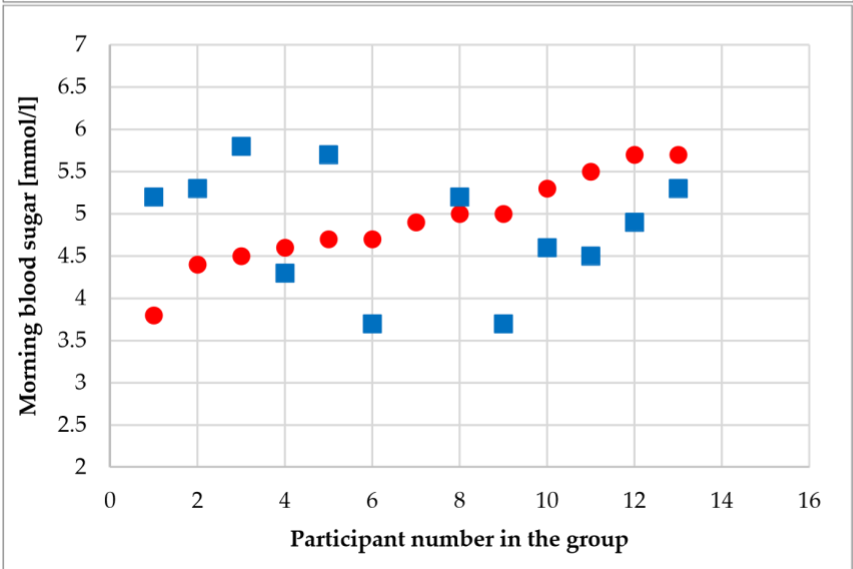
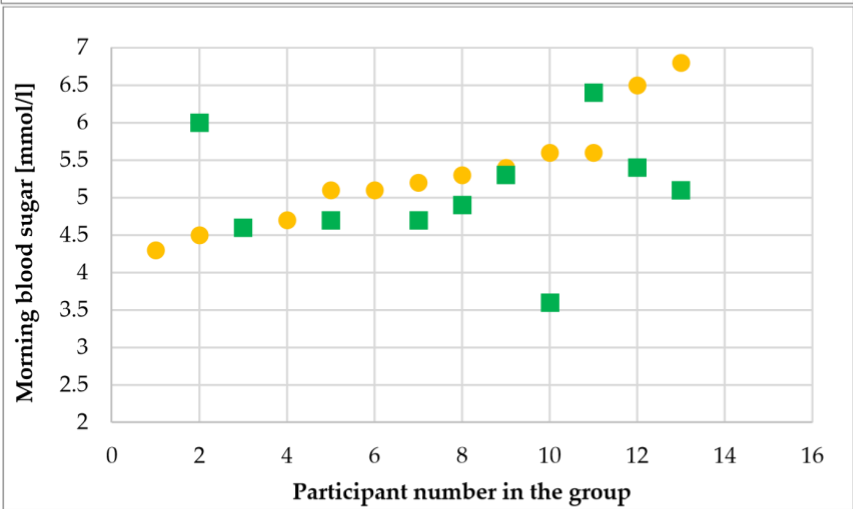
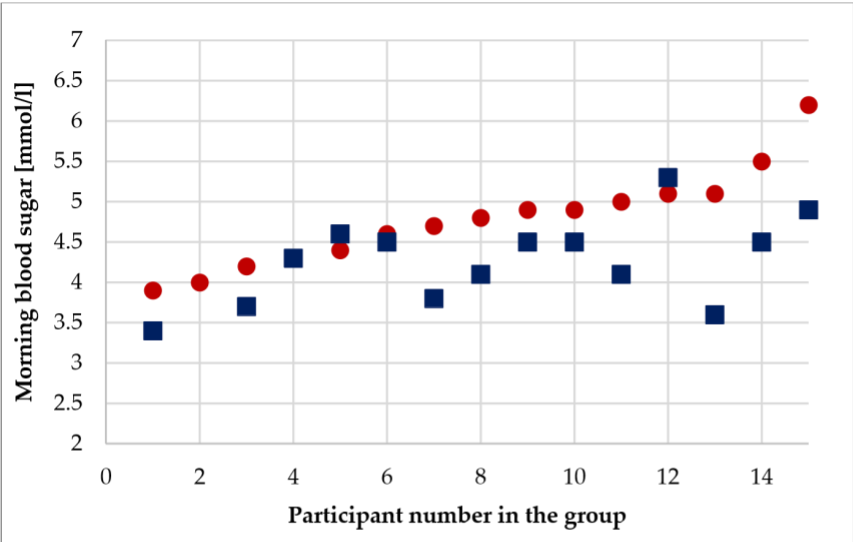
4.1 Results related to green walnut and its fortified yogurt with clinical trials

4.1.1 Results about green walnut bee product with fortified yogurt along with other samples developed using bee feed method

This study aimed to identify which plant extracts from the investigated options could reduce morning blood sugar levels in a clinical trial. Blood samples obtained throughout the clinical trial were refrigerated at 4°C until the assessment of their basic chemical makeup before and after ingestion. The assessed parameters included glucose, triglycerides, cholesterol, creatinine, immunoglobulin, uric acid, ferritin, albumin, transferrin, and blood serum constituents including calcium, magnesium, and iron, which we did not plan to include because of GDPR.; We only obtained consent for blood sugar levels. Blood glucose levels were assessed in all individuals across each group, and in each honey therapy group.

The following groups show the difference between morning blood sugar levels before and after giving samples such as acacia honey+yogurt, green algae honey+ yogurt, sea buckthorn+ yogurt, and green walnut bee product+ yogurt. There are four groups for this, which indicate control + samples with honey and bee products (Table 5)

Fundamental analyses of honey in the four groups are presented in Table 5. The data indicate that the chemical composition of honey across all groups is generally comparable, with no significant disparities, including sugar content (fructose + glucose was elevated in Chlorella alga) and various measured nutrients in the honey. Eleven nutrients were quantified in the honey before the application of treatments, including Ca, Mg, K, B, I, Cu, and Zn.



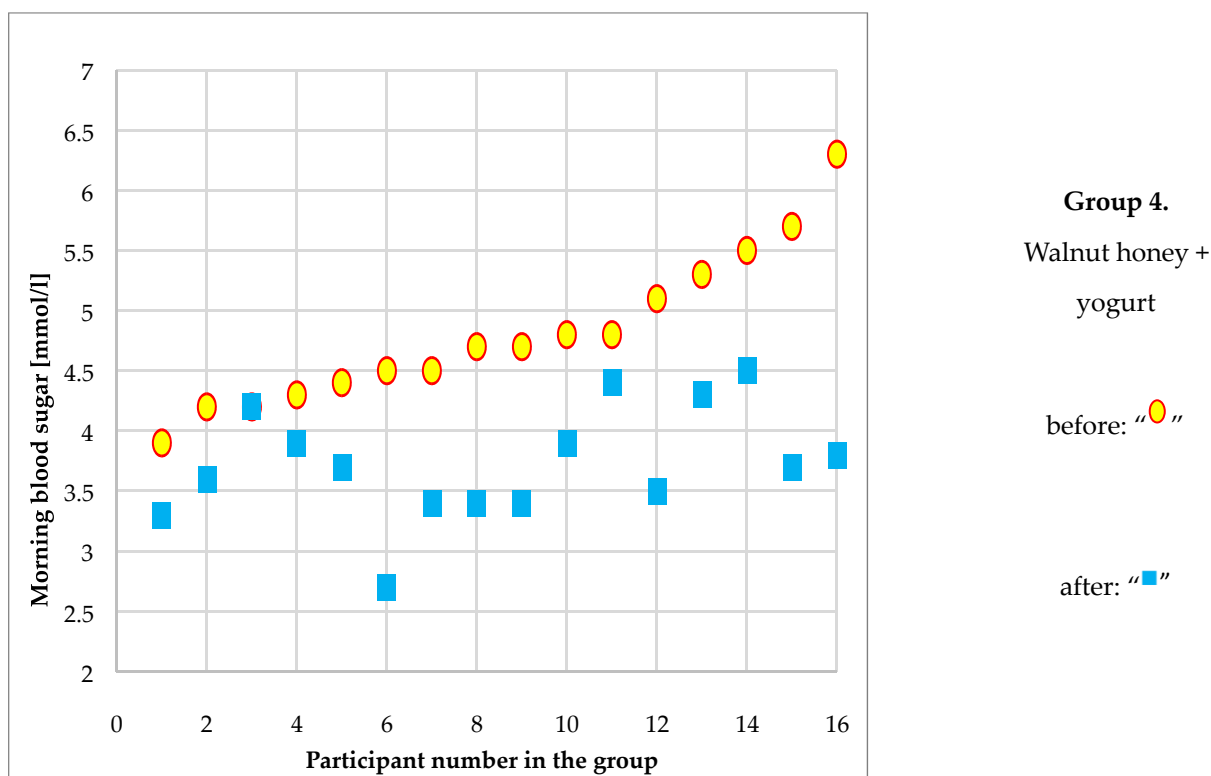


Figure 27. The blood glucose levels of each participant in the four treatment groups of the clinical trial, measured before (day 0) and after (day 21) the trial.

Participants were administered 30 g of four distinct honey products mixed with 150 g of yoghurt over 21 days. Certain volunteers were eliminated from the study across the first, second, and third groups because one individual had an elevated initial blood sugar level, whereas the others withdrew from participation in the trial. In the fourth group, all participants engaged from start to finish. Regarding the initial group (acacia honey as a control), the blood glucose levels of participants ingesting acacia honey-fortified yoghurt exhibited a decline, albeit not in a pronounced manner (Figure 27.). In the second (Seabuckthron) and third groups (Green algae), the correlation between blood glucose levels and the intake of yoghurt enriched with honey was ambiguous and statistically insignificant. Conversely, in the fourth group (Green walnut), all participants' blood glucose levels following the consumption of honey-fortified yoghurt were lower than those recorded after treatment (Figure 27).

Table 5. Composition of the analysed honey from selected plant extracts (i.e., chlorella algae honey, sea buckthorn honey, and green walnut honey) in comparison to the acacia honey utilized prior to treatments

Measured Parameter/Nutrient	Unit	Acacia Honey (Control)	Chlorella Algae Honey	Sea Buckthorn Honey	Green Walnut Bee Product
Water content	% (m/m)	19.2 ± 0.1 ^a	18.5 ± 0.1 ^b	18.6 ± 0.1 ^b	19.0 ± 0.1 ^c
Fructose + glucose	% (m/m)	64.7 ± 0.20 ^a	69.1 ± 0.20 ^b	65.9 ± 0.6 ^c	65.0 ± 0.6 ^{ac}
Fructose	% (m/m)	34.9 ± 0.4 ^a	35.4 ± 0.5 ^a	41.0 ± 0.4 ^b	36.2 ± 0.2 ^c
Glucose	% (m/m)	29.8 ± 0.3 ^a	33.7 ± 0.4 ^b	24.9 ± 0.3 ^c	28.8 ± 0.3 ^d
Free acid content	mmol L ⁻¹	23.5 ± 0.4 ^a	37.5 ± 0.6 ^b	75.6 ± 0.4 ^c	45.3 ± 0.2 ^d
HMF content	mg kg ⁻¹	1.73 ± 0.20 ^a	55.6 ± 2.0 ^b	42.3 ± 2.0 ^c	4.88 ± 0.3 ^d
Diastase activity	Goethe number	6.07 ± 0.10 ^a	4.17 ± 0.12 ^b	<4.0 ^c	8.68 ± 0.1 ^d
Boron (B)	mg kg ⁻¹	3.12 ± 0.20 ^a	2.14 ± 0.23 ^b	0.71 ± 0.12 ^c	2.09 ± 0.19 ^d
Calcium (Ca)	mg kg ⁻¹	62.8 ± 2.9 ^a	125 ± 13 ^b	131 ± 13 ^b	120 ± 26 ^b
Copper (Cu)	mg kg ⁻¹	0.45 ± 0.11 ^{ab}	0.46 ± 0.03 ^a	0.36 ± 0.02 ^b	0.56 ± 0.09 ^a
Iron (Fe)	mg kg ⁻¹	<0.10 ^a	0.68 ± 0.11 ^b	1.60 ± 0.15 ^c	1.28 ± 0.04 ^d
Iodine (I)	mg kg ⁻¹	<0.10 ^a	<0.10 ^a	310 ± 10 ^b	<0.10 ^a
Potassium (K)	mg kg ⁻¹	142 ± 9.0 ^a	415 ± 8.0 ^b	428 ± 41 ^b	321 ± 8.0 ^c
Magnesium (Mg)	mg kg ⁻¹	2.75 ± 0.05 ^a	15.7 ± 0.1 ^b	27.0 ± 1.7 ^c	15.5 ± 0.7 ^b
Sodium (Na)	mg kg ⁻¹	14.0 ± 1.6 ^a	55.4 ± 2.1 ^b	49.6 ± 3.1 ^c	51.9 ± 4.6 ^{bc}
Phosphorus (P)	mg kg ⁻¹	68.5 ± 4.9 ^a	113 ± 3 ^b	79.9 ± 6.4 ^a	89.6 ± 0.7 ^c
Sulfur (S)	mg kg ⁻¹	19.0 ± 1.5 ^a	38.2 ± 0.4 ^b	36.8 ± 1.9 ^b	38.7 ± 0.5 ^b
Zinc (Zn)	mg kg ⁻¹	0.86 ± 0.04 ^a	1.53 ± 0.12 ^b	3.55 ± 0.25 ^c	2.39 ± 0.25 ^d

The letters a–d indicate the results of Duncan’s test. Identical letters indicate that there is no substantial variation between the values in the row.

Statistical analysis of the examined honey groups revealed a notable reduction in morning blood sugar levels in the walnut group 4 (Figure 27.), while groups 2 (green algae) and 3 (sea buckthorn) exhibited no significant differences; however, all four groups maintained a comparable lower level of morning blood sugar (below 6 mmol L⁻¹). For group 4, the pre-treatment morning blood sugar levels in the volunteers were 4.8 mmol L⁻¹, while post-treatment levels were 3.7 mmol L⁻¹, as illustrated in Figure 28., group 4.

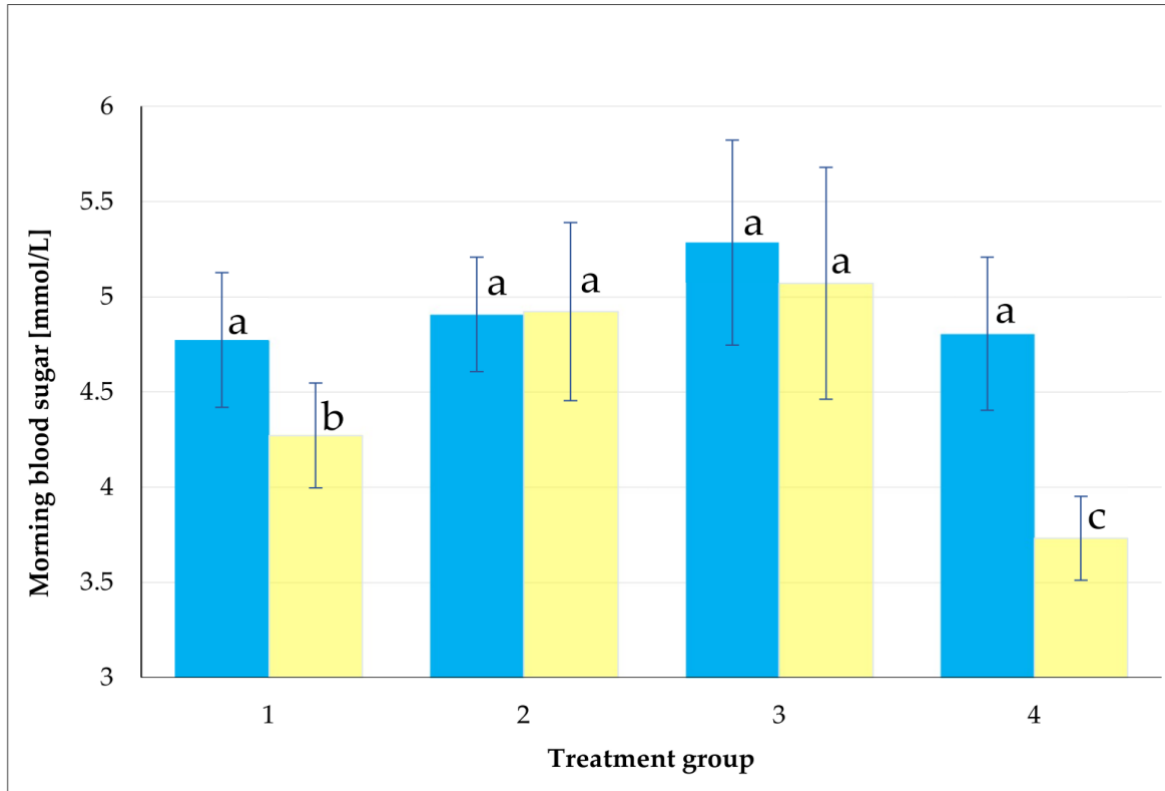


Figure 28. Average morning blood glucose levels of participants prior to (blue column) and following (yellow column) the human clinical trial. The treatment groups consisted of group (1) acacia honey combined with yoghurt; group (2) green algae honey combined with yoghurt; group (3) sea buckthorn honey combined with yoghurt; and group (4) walnut honey combined with yoghurt.

The participants were administered 30 g of honeybee product mixed with 150 g yoghurt for 21 days. Blood samples were collected before and after the 21 day study period. Identical letters indicate that the values are not statistically significant at the 5% level.

The data in Figure 28. different groups were arranged in ascending order, with the values determined by subtracting the morning blood sugar measurement on the 21st day of the trial from the measurement on day 0. The majority of subjects exhibited reduced blood

sugar levels. The average reduction rate for the walnut group was 22.45%, specifically 1.07 mmol L⁻¹, with a high of 2.5 mmol L⁻¹ (ranging from 6.3 to 3.8 mmol L⁻¹).

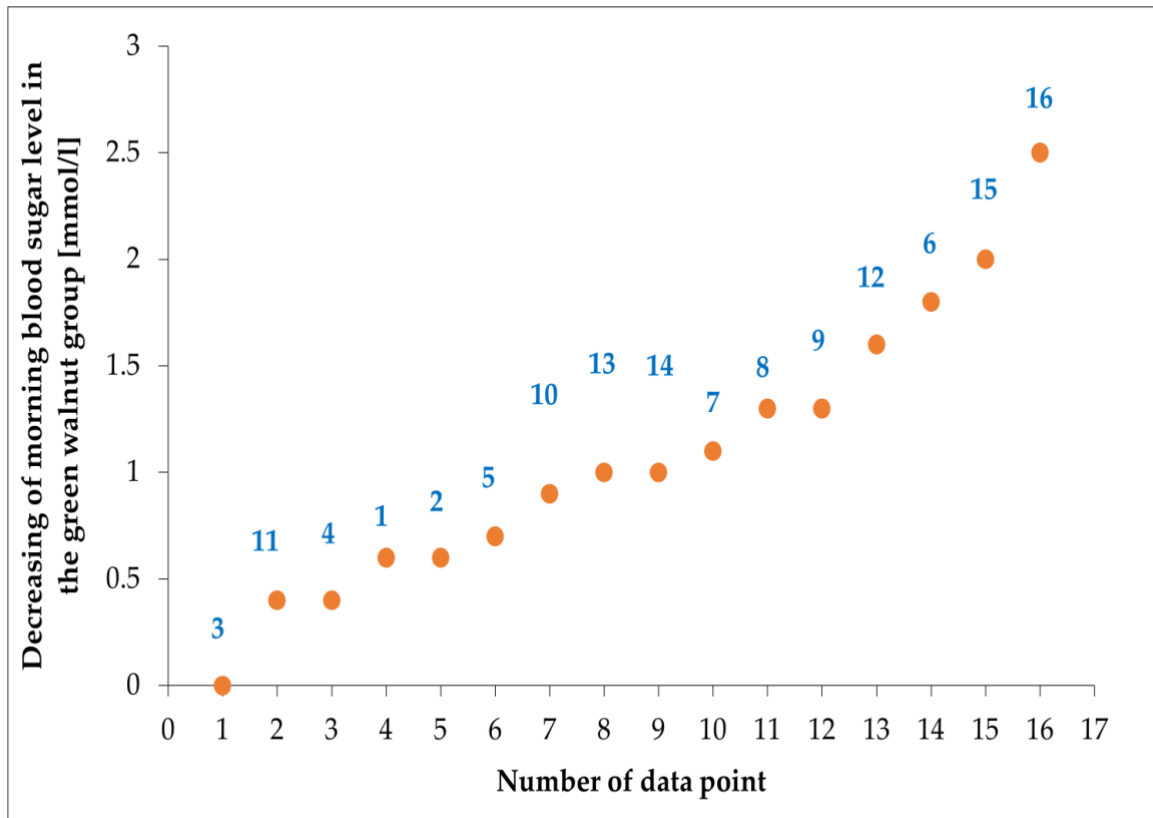


Figure 29. The reduction of morning blood glucose levels in the blood of participants in treatment group 4. The data were arranged in ascending order and computed by subtracting the morning blood sugar measurement recorded on the 21st day of the trial from that of day 0.

4.1.2 Discussions on development of the process of feed and food development using green herbs/ plants

This study employed a proprietary approach that does not include supplying bee colonies with sugar or sugar syrup during the dearth period, instead of nourishing bees with specific herbs or immune-enhancing natural active constituents derived from plants or minerals. The process of Bee feed has been patented (Daróczi, 2020). The Beekeeper Mr. Lajos Daroczi has provided us the necessary support to utilise this process and work further to find feasible possible solutions. Natural, active chemicals exert a positive physiological impact on bees and are present in honey, which in turn benefits individuals who consume it, perhaps by enhancing immunity and providing other advantageous physiological effects. This study revealed that honey can be ingested with yoghurt as a functional yoghurt

enriched with honey. These functional preventive honey products may be created as functional foods derived from bees, with the quantity of active components potentially surpassing the beneficial compounds found in the natural varieties of honey. The "orientated production" of honey can be regulated to enhance its composition with specific active compounds by administering these substances to the bees, such as by the provision of certain herbs (Džugan et al., 2017).

Plant extracts from mint, cinnamon, and chamomile (Al-Ghamdi et al., 2021), or the administration of *Spirulina platensis* extract enhances the antioxidant and phenolic contents of honey (Guldás et al., 2022).

In this study, three natural plant extracts were selected to generate honey enriched with the active compounds present in these extracts, thereby promoting the health of both bees and humans. This renders honey products economically competitive relative to other conventional technologies that extract active compounds from natural sources. Consequently, analytical investigations have confirmed that approximately 80% of the physiologically advantageous groups of active chemicals present in the herb are also transmitted to sugar syrup and subsequently converted by bees into honey-like products. Consequently, it is necessary to regulate the feeding process of bees as a viable approach to enhance honey with beneficial bioactive plant constituents achievable through the use of natural herbs. This method is regarded as a creative and new approach for the production of bee products that are abundant in bioactive chemicals, as indicated in recent studies (Durazzo et al., 2021; Shumkova & Balkanska, 2021; Sowa et al., 2019; Tlak Gajger et al., 2020).

The presence of these bioactive chemicals is primarily contingent upon the plant species, such as coumarin found in the flowers of *Melilotus officinalis* (Sowa et al., 2019), or in trademarked herbal extracts (Shumkova & Balkanska, 2021). This study presents a novel and patented technique that is essential as an innovative option for assisting beekeepers in providing bees with a preservative substance, in contrast to existing beekeeping methods.

The creation of yoghurt enriched with bioactive components for human consumption is a significant strategy in food production, as evidenced by numerous studies utilizing extracts from *Nyctanthes arbor-tristis* L. flowers (Amadarshanie et al., 2022), fennel seeds (Atwaa et al., 2022), and nutmeg (Shori, 2022), as well as white and black peppers. This research utilized a fortification strategy to create yoghurt enriched with bioactive compounds

derived from natural herbs, thereby enhancing its nutritional and health advantages. The present study involved fortifying yoghurt with honey derived from bees that were underfed with extracts from three natural plants (green algae, sea buckthorn, and green walnut), in contrast to acacia, which served as a control for the treatment of diabetes. The honey-fortified yoghurt prepared for each group was analysed individually for each natural plant extract. Acacia honey, along with oilseed, rapeseed, sunflower, and woodland honey, is prevalent and of superior quality in Hungary. Hungary is regarded as one of the leading honey producers in the European Union (Sajtos et al., 2022; Varga et al., 2020). The present study revealed that acacia honey exhibited the highest water content (19.2%) among honey samples from various plant sources. However, it demonstrated the lowest levels of hydroxymethyl-furfural (HMF) at 1.73 mg kg^{-1} and fructose plus glucose content of 64.7%. Additionally, it recorded the lowest concentrations of several nutrients (in mg kg^{-1}): phosphorus (68.5), sulphur (19), and zinc (0.86), calcium (62.8), potassium (142), sodium (14), in comparison to other treatments.

Numerous studies have been conducted, including those by researchers (Sharma et al., 2020) substantiated the association between honey and its anti-diabetic properties. Their findings elucidated the mechanism of honey's proposed antibiotic effects, which encompass the modulation of pancreatic β -cell activity and associated hormones, as well as the kidney, liver, ocular system, intestine, nervous system, gastrointestinal tract, musculature, and vasculature, through its antioxidant, antimicrobial, antihypertensive, anti-inflammatory, immunomodulatory, wound healing, hypolipidemic, hypoglycemic, and nutritional properties. Honey possesses a low glycemic index attributed to its primary sugars, fructose, and glucose, along with substantial levels of amino acids, minerals, enzymes, phenolic compounds, and vitamins, which may influence diabetes management based on its source, composition, and dosage (Samarghandian et al., 2017; Sharma et al., 2020).

The significant potential of *Chlorella algae* as microalgae has been established as a sustainable food source to fulfil the population's requirements because of their substantial phytoadditive and/or bioactive phytonutrient, lipid, carotenoid, and protein content (Bazarnova et al., 2021; Caporgno & Mathys, 2018; Udayan et al., 2023). Numerous studies have documented the medicinal potential (Khavari et al., 2021) and applications of microalgae, including their relevance to diabetes (Nigam et al., 2022). *Chlorella*, a unicellular microalga, is widely consumed in several nations, especially in East Asia (e.g., China, Korea, Indonesia, Japan, and Taiwan), owing to its nutritional significance as a

reasonably comprehensive food source (Hosseini et al., 2021). The current investigation indicated that the function of green algae in lowering blood sugar levels is not substantial.

Sea buckthorn is a member of the *Elaeagnaceae* family, and its berries are associated with a decreased risk of various human ailments, including cardiovascular diseases (Tkacz et al., 2021). This plant possesses significant potential for lowering blood glucose levels as an antidiabetic medication via glycemic regulation (Hameed et al., 2020; Ren et al., 2021). Human experiments indicate that the application of 40 g of dried sea buckthorn to 200 g of yoghurt and 50g of glucose may mitigate the peak insulin response and stabilize postprandial hyperglycemia (Ren et al., 2021). This plant is abundant in many beneficial components, including flavonoids and fat-soluble vitamins A, E, and K (Ren et al., 2021). Although numerous studies have validated the role of sea buckthorn in lowering blood sugar levels (Ren et al., 2021; Tkacz et al., 2021), the present investigation revealed no significant difference before and after the administration of yoghurt supplemented with honey derived from bees nourished with sea buckthorn extract.

Green walnuts were chosen as juvenile specimens because of their non-allergenic properties; elevated levels of vitamin C (exceeding those of oranges), iron, and tannins; and their exceptional richness in phenolic compounds. Walnut fruits offer an extensive array of nutrients, including vitamins (B9, B6, E, B3, etc.), minerals (K, Ca, Mg, etc.), and other bioactive components such as antioxidants, phytosterols, and phenolic compounds (Verde et al., 2022). This plant is produced for use in the cosmetic, medicinal, and agricultural sectors, encompassing green seeds, husks, kernels, shells, bark, and leaves, as it is an abundant source of ascorbic acid, phenolics, and tocopherols (Liu et al., 2020). Walnuts exhibit hypoglycemic effects, reducing blood glucose levels in diabetes mellitus, as evidenced by studies on walnut seeds (Alsuhaibani & Al-Kuraieef, 2019) and leaves (Liu et al., 2020), attributed to the influence of phenolic components and fatty acid content.

The present investigation demonstrated a pronounced and significant effect of green walnut honey on lowering human blood glucose levels, as shown in Figure 16. and 17.. The initial average blood sugar level in the walnut group was 6.3 mmol L⁻¹, which reduced to 3.8 mmol L⁻¹ after 21 days of treatment. The reduction in persons was 2.5 mmol L⁻¹ (a rate of 39.7%), representing the most significant decrease in blood glucose levels among all treatments (Acacia, Sea Buckthorn, and Chlorella algae honey groups). The most notable characteristic of green walnut honey is the HMF concentration, which recorded the lowest

value (4.88 mg kg^{-1}) compared to the other treatments (second and third groups). The HMF value is regarded as a significant indicator of honey quality, as noted by a researcher (Shapla et al., 2018).

4.2.1 Determination of Silver Nano particles made using green walnut using TEM (Transmission Electron Microscope), Powder XRD (Xray diffraction), UV-Visible spectra and its characterization

Figure 30. shows the UV-Vis absorption spectrum, with absorbance peaks at approximately 265 nm and a wide absorption feature around 465 nm. The presence of these absorption peaks may result from active chemical components such as biomolecules. These bioactive chemicals actively engage with Ag^+ , resulting in the creation of silver nanoparticles. The addition of herbal extract to the AgNO_3 solution evidently alters the absorption spectrum, with the emergence of a new absorption band at 465 nm signifying the creation of Ag nanoparticles. The bioorganic ingredient in the herbal extract facilitates charge transfer between bioactive chemicals and Ag nanoparticles, which are encapsulated on the nanoparticle surface, enhancing their stability and preventing aggregation.

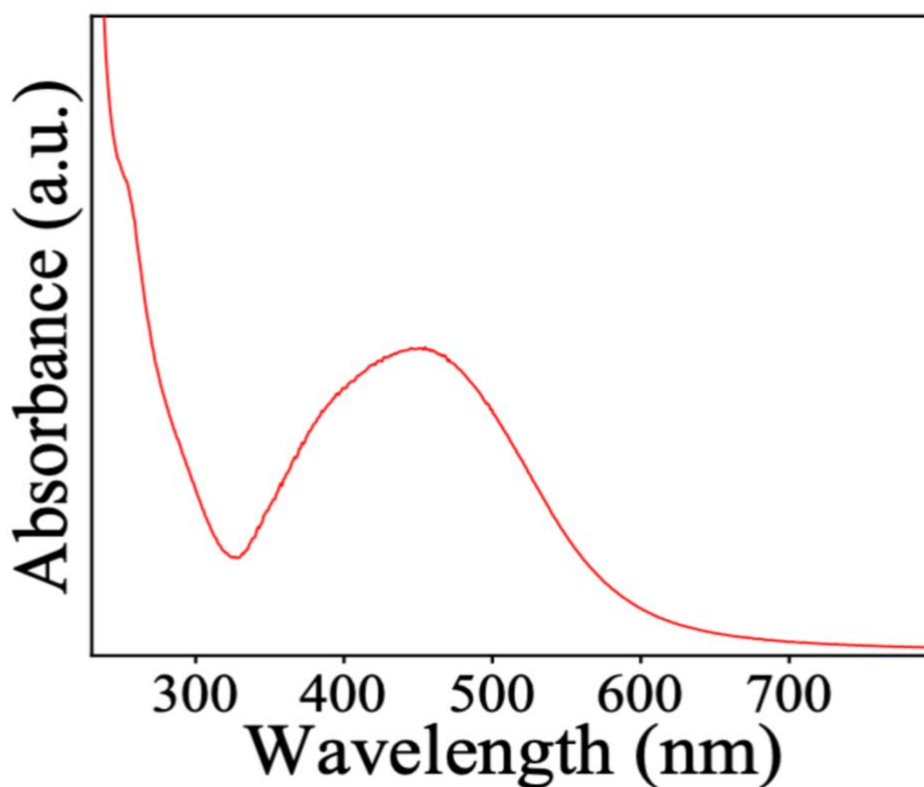


Figure 30. UV–Visible spectra of silver nanoparticles after 72 hours keeping in laboratory. The crystallinity of the biosynthesized silver nanoparticles produced using green walnut extract was assessed using X-ray diffraction method. The sample was examined from drop-cast films of as-synthesized Ag nanoparticles on a glass substrate which exhibited well-defined X-ray diffraction patterns corresponding to the (111), (200), (220), (311), and (222) planes. Figure 31. distinctly shows the contribution of the amorphous material resulting from the biomolecules in the herbal extract, which provide stability and capping of the nanoparticles.

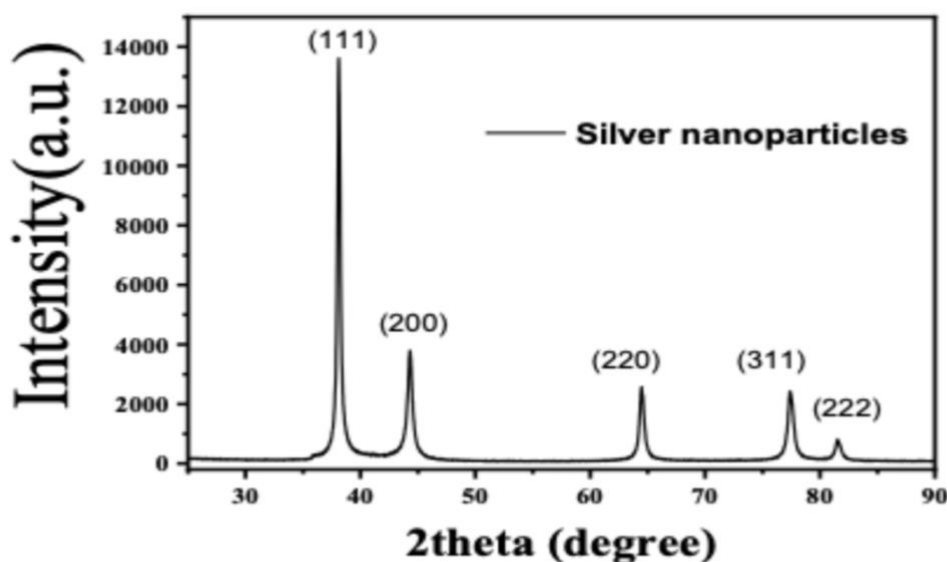


Figure 31. X ray diffraction (XRD) pattern of a drop-cast film of Ag Nanoparticles on a glass substrate following reaction with the herbal extract of green walnut.

The morphology and dimensions of the synthesized Ag nanoparticles were analyzed via transmission electron microscopy. An aliquot was obtained at 72 h after the process. Figure 32. (A, B, C, and D) shows the transmission electron micrograph of silver nanoparticles, characterized by uneven shapes and an overall quasispheroidal morphology. Figure 32 E illustrates the dimensions of the nanoparticles, with the histogram indicating that the mean size of the nanoparticles was between 10 and 11 nm. The size distribution graph indicates that the particles were homogeneous and adequately spaced apart. Biosynthesized

nanoparticles have enhanced stability and comparatively smaller dimensions than those of nanoparticles produced by alternative processes. This results from the capping and functionalization of nanoparticles by biomolecules during synthesis, which inhibits nanoparticle development and adheres to their surfaces.

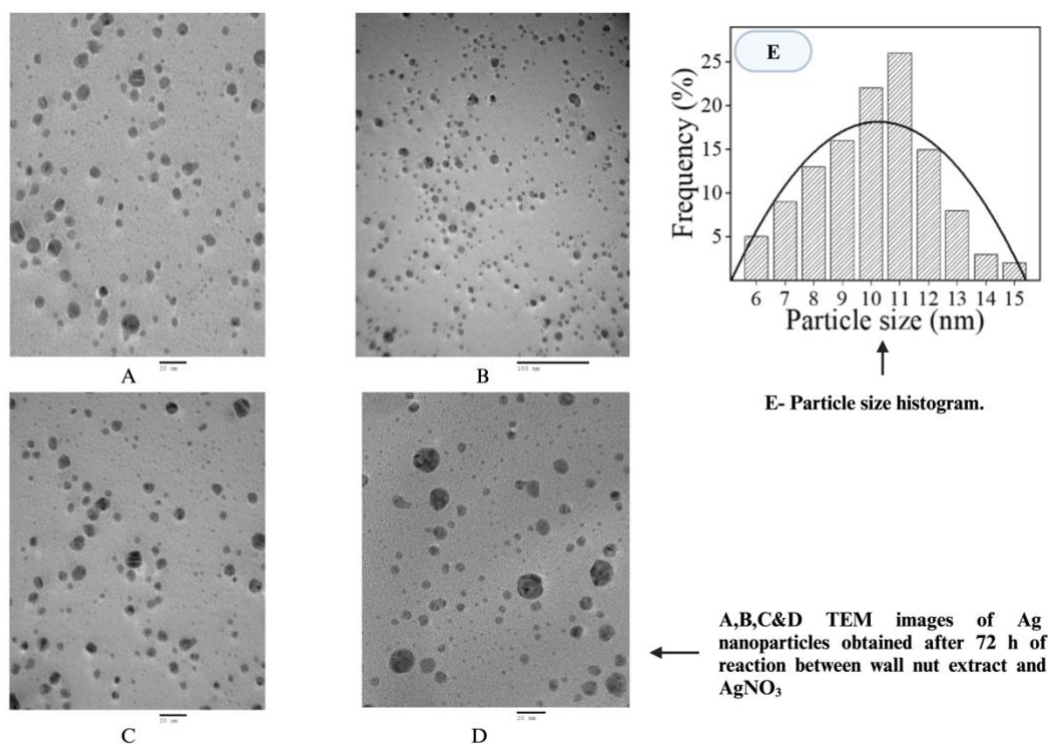


Figure 32. A, B, C and D Showing the Transmission Electron Microgram produced using green walnut silver nanoparticles, while Figure 33.E Shows the Particle size histogram.

The characterization part is wide, and it can be continued in the connection of postdoctoral dissertations since it has wide results. All its Antibacterial, Antimicrobial properties should be studied further. Transmission Electron Microscopy (TEM) conducted at 72 hours after synthesis (using green walnut extract + AgNO₃)

Figure shows from A–D

1. Shows Ag nanoparticles with varied morphology
2. Mostly quasi-spherical shapes with a well-dispersed structure

Figure 32E (Histogram)

1. Mean particle size: between 10–11 nm
2. Homogeneous size distribution and spacing observed

Key Insights

1. Biosynthesized AgNPs display
2. Higher stability
3. Smaller and more uniform dimensions

Due to capping and surface functionalization by walnut-derived biomolecules

4.3.1 Principal Component Analysis (PCA) of Sensory Attributes

Sensory evaluation of the samples (Acaica Honey as a control and green walnut bee product as main sample) was conducted based on various attributes, including appearance, Aroma, flavour, texture, consistency, complexity, quality, and overall Liking. The dataset consisted of 80 observations, and statistical summaries of these sensory attributes are presented in Table 6. below,

Table 6. Statistics of sensory attributes

	Observations	Min.	Max.	Mean \pm Std Dev
Appearance	80	4	9	7.17 \pm 1.51
Aroma	80	2	9	6.5 \pm 1.63
Flavour	80	2	9	6.92 \pm 1.6
Texture	80	3	9	7.11 \pm 1.67
Consistency	80	2	9	7.04 \pm 1.54
Complexity	80	2	9	6.51 \pm 1.54
Quality	80	3	9	7.25 \pm 1.61
Overall Liking	80	3	9	7.06 \pm 1.44
Purchase Intent	80	1	9	6.69 \pm 1.77

The mean scores for the attributes ranged between 6.51 (Complexity) and 7.25 (Quality), indicating that panellists generally had a positive perception of the product. The Standard deviations ranged from 1.43 to 1.66, suggesting moderate variability in responses.

4.3.2 Variance Explained by Principal Components

PCA was performed to reduce the dimensionality of the dataset and to identify the most influential sensory attributes. The Scree plot (Figure.33) represents the variance explained by each principal component.

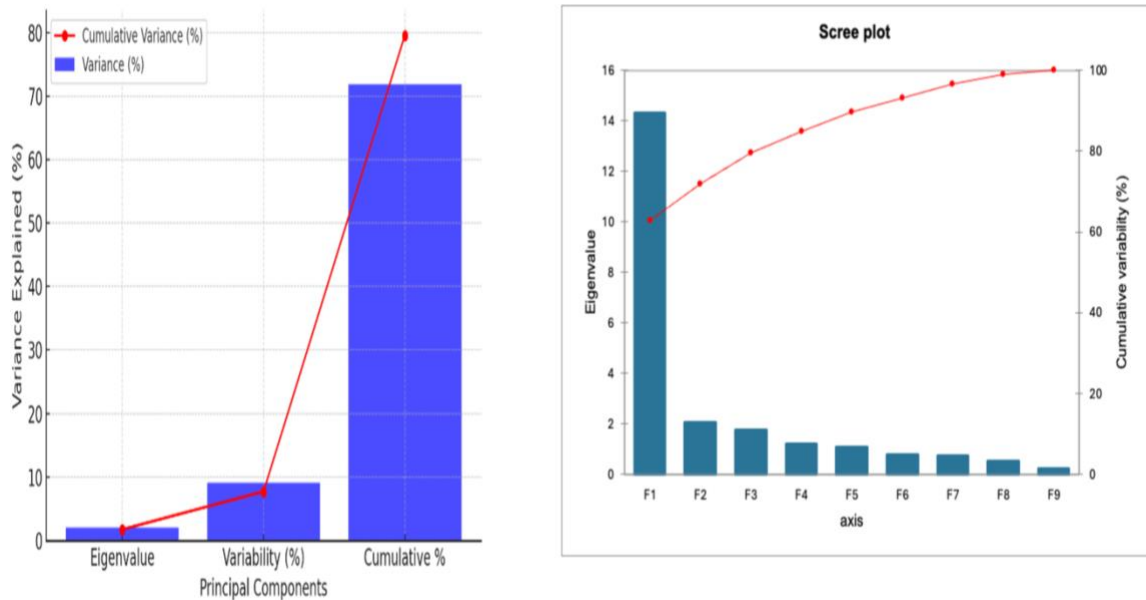


Figure 33. Variance explained by principal components by using Scree plot (Source: XLSTAT Output)

The first principal component (PC1) had an eigenvalue of 14.31, explaining 62.72% of the total variance and making it the most significant factor influencing sample differentiation. The second component (PC2) explained an additional 9.09% of the variance, while the third component (PC3) accounted for 7.73%. Together, the first three components explained 79.55% of the total variance, indicating that most of the variation in the sensory attributes could be captured using these three components as shown in Table 7. below.

Table 7. Variance explained by principal components

	F1	F2	F3	F4	F5	F6	F7	F8	F9
Eigenvalue	14.314	2.076	1.765	1.224	1.097	0.794	0.748	0.546	0.257
Variability (%)	62.722	9.096	7.735	5.366	4.806	3.481	3.276	2.391	1.126

Cumulative %	62.722	71.818	79.553	84.919	89.725	93.206	96.483	98.874	100.00
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4.3.3 Factor Loadings and Sensory Attribute Contributions

The factor loading matrix (Table 8.) represents the correlations between the original sensory attributes and the extracted principal components. A higher absolute value of the factor loading indicates a stronger relationship with the respective principal components.

Table 8: Factor loadings of sensory attributes

	F1	F2	F3	F4	F5	F6	F7	F8	F9
Appearance	1.118	0.158	-0.206	-0.539	0.769	0.069	0.258	-0.048	-0.006
Aroma	0.941	1.238	-0.333	-0.079	-0.307	-0.042	-0.099	-0.129	0.007
Flavour	1.223	-0.522	-0.472	-0.467	-0.220	-0.441	-0.342	0.075	-0.033
Texture	1.424	0.149	0.565	-0.195	-0.235	0.112	0.166	0.524	-0.088
Consistency	1.149	-0.045	0.859	-0.108	0.081	0.075	-0.379	-0.360	-0.097
Complexity	1.193	0.164	0.025	0.720	0.450	-0.394	-0.152	0.170	0.004
Quality	1.295	-0.257	-0.551	0.271	0.014	0.632	-0.257	0.056	-0.076
Overall Liking	1.330	-0.182	0.133	0.024	-0.125	0.076	0.068	-0.055	0.458
Purchase Intent	1.570	-0.310	-0.103	0.260	-0.286	-0.122	0.538	-0.287	-0.151

Additionally, the heatmap (Figure 34.) visually represents the relationship between the sensory attributes and principal components.

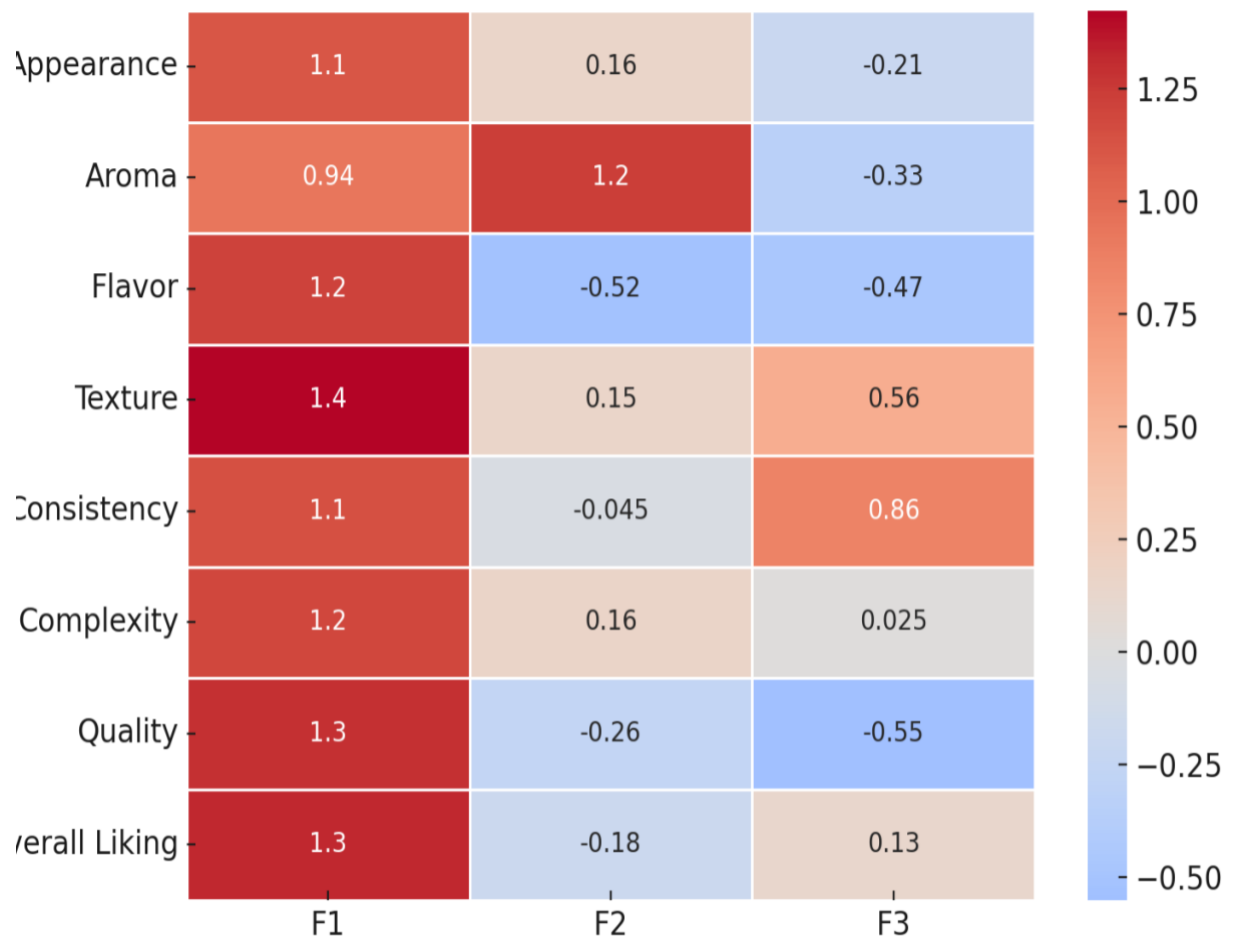


Figure 34: Factor loadings heatmap (Source: XLSTAT Output)

- PC1 (62.72%): The first principal component was highly correlated with flavour (1.22), texture (1.42), and appearance (1.11). This suggests that flavour, texture, and appearance are the most influential attributes for differentiating the samples.
- PC2 (9.09%): The second principal component had the highest loading for aroma (1.23), suggesting that olfactory perception plays a key role in product variation.
- PC3 (7.73%): The third principal component showed a moderate influence from texture (0.56) but had lower associations with other attributes.

4.3.4 Discussions

These findings indicate that flavour, texture, and appearance are the primary drivers of consumer perception, followed by aroma, respectively. This suggests that any modifications or innovations in product formulation should prioritize these attributes to enhance the sensory appeal.

The high variance explained by principal component 1 (62.72%) shows that most variations in the sensory data are due to differences in Flavor, Texture, and Appearance. This is consistent with previous sensory evaluation studies, in which these attributes have been reported as key determinants of overall liking and consumer acceptance.

The relatively lower contribution of Principal Component 2 and Principal Component 3 shows that while Aroma and Texture have some influence, they are secondary factors in differentiating the samples. In conclusion, our Principal Component Analysis (PCA) of sensory attributes identified Flavor, Texture, and Appearance as the primary key drivers of consumer perception in the examined food products. These findings match those of recent studies that emphasize the critical role of sensory properties and parameters in deciding consumer choices. For instance, research has shown (Haider et al., 2022; Jaeger, 2006; Steptoe et al., 1995) that taste is a key factor influencing consumer perception, purchase intention, and consumption of convenience foods as per their intent.

Moreover, sensory perception is influenced by a combination of sensory properties, personal factors, such as age and health conditions, and environmental factors, such as regional food habits and choices. Understanding these multifaceted influences is essential for developing products that meet consumers' expectations and choices. Our analysis underscores the importance of focusing on Flavor, Texture, and Appearance in product development to enhance consumer satisfaction and acceptance. By prioritizing these attributes, beekeepers can better align their products with consumer preferences, potentially increasing their market potential.

Future research should continue to explore the complex interplay between sensory attributes and consumer perception (in our terms, international students), considering factors such as cultural differences and individual variability in sensory experiences. This comprehensive approach will contribute to a deeper understanding of consumer behaviour and support the development of products that cater to diverse consumer needs after covid 19 especially in the Global South.

4.4.1 Multiple Factor Analysis (MFA) for sensory attributes

The following Table 9 and Figure 35 shows the MFA conducted with data collected from the panelists (international students) from the given questionnaire. It shows their overall liking for two different types of samples like acacia honey and green walnut bee products.

Table 9. Variable for Acacia Honey and Green walnut bee product

Variable	Observations	Minimum	Maximum	Mean \pm Std
Acacia				
Appearance	40	4	9	6.97 \pm 1.46
Aroma_Acaica	40	2	9	5.95 \pm 1.45
Flavor_Acaica	40	2	9	6.95 \pm 1.68
Texture_Acaica	40	3	9	6.9 \pm 1.71
Consistency_Acaica	40	2	9	6.78 \pm 1.67
Complexity_Acaica	40	2	9	6.22 \pm 1.75
Quality_Acaica	40	3	9	7.2 \pm 1.57
Overall Liking_Acaica	40	3	9	6.97 \pm 1.48
Purchase Intent_Acaica	40	1	9	6.58 \pm 1.72
GWBP				
Appearance	40	4	9	7.38 \pm 1.55
Aroma-GWBP	40	3	9	7.05 \pm 1.63
Flavor-GWBP	40	4	9	6.9 \pm 1.55
Texture-GWBP	40	3	9	7.33 \pm 1.62
Consistency-GWBP	40	4	9	7.3 \pm 1.36
Complexity-GWBP	40	4	9	6.8 \pm 1.26
Quality-GWBP	40	3	9	7.3 \pm 1.67
Overall Liking-GWBP	40	4	9	7.15 \pm 1.41
Purchase Intent-GWBP	40	2	9	6.8 \pm 1.83

The sensory evaluation results are summarized in Table 10. The highest-rated attributes for the acacia sample were quality (7.2 \pm 1.57) and overall liking (6.9 \pm 1.48), suggesting a generally favourable reception. However, aroma (5.9 \pm 1.45) had the lowest score, indicating possible improvements in the formulation.

In contrast, the GWBP sample had slightly higher mean values across most attributes, with appearance (7.38 \pm 1.55) and overall liking (7.15 \pm 1.41) being the most preferred attributes. Notably, purchase intent for GWBP (6.8 \pm 1.83) was higher than acacia's ones, suggesting a stronger market potential, which shows that it has the potential to be launched in the market. Moreover, it also shows the choice of panelists (international students), although their food

habits are different because of different cultures, food preparation methods, and taste differences.

The following tables (Table 10.-11.) show eigenvalues for acacia honey and green walnut bee product.

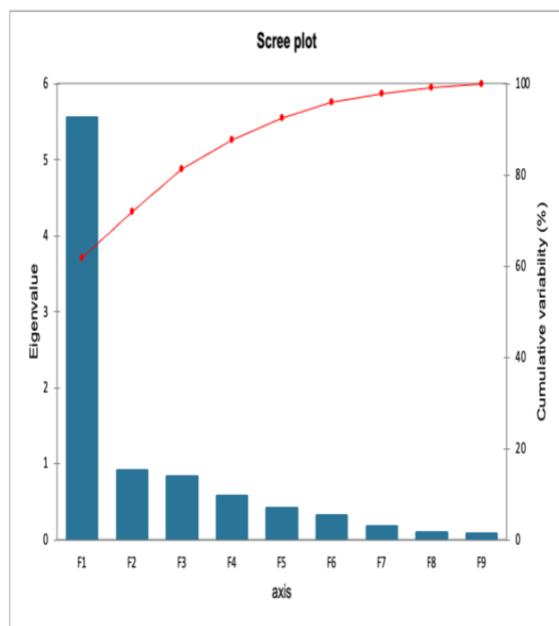
Table10. Eigenvalues for Acacia Honey

	F1	F2	F3	F4	F5	F6	F7	F8	F9
Eigenvalue	5.563	0.914	0.838	0.575	0.426	0.322	0.171	0.105	0.085
Variability (%)	61.813	10.155	9.309	6.393	4.732	3.578	1.902	1.171	0.947
Cumulative %	61.813	71.968	81.277	87.670	92.402	95.980	97.882	99.053	100.000

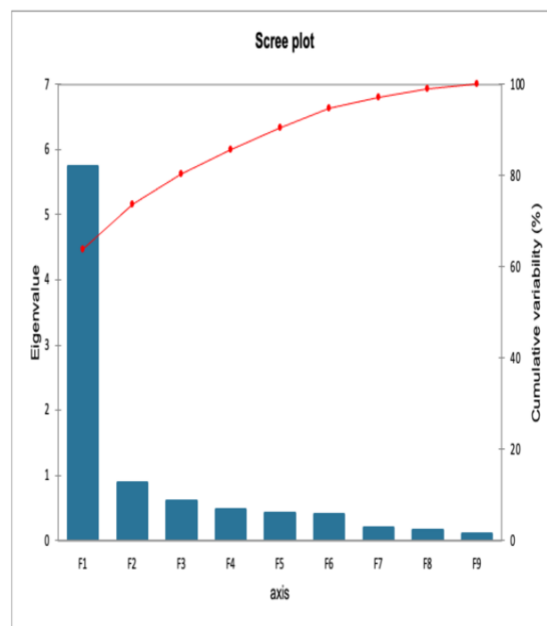
Table11. Eigenvalues for Green walnut bee product

	F1	F2	F3	F4	F5	F6	F7	F8	F9
Eigenvalue	5.740	0.894	0.603	0.473	0.428	0.400	0.205	0.157	0.099
Variability (%)	63.780	9.937	6.699	5.257	4.757	4.446	2.280	1.746	1.098
Cumulative %	63.780	73.716	80.416	85.672	90.429	94.876	97.155	98.902	100.000

Based on Tables 10. - 11., the scree plots are presented below.



Scree Plot for Acacia Honey



Scree Plot for Green walnut bee product

Figure 35. Scree plots for the acacia honey and reen walnut bee product (Source: XLSTAT output)

The scree plots for acacia honey and green walnut bee products demonstrated the outcomes of Principal Component Analysis (PCA). These graphs assist in identifying the ideal quantity of primary components that account for variability within the dataset.

Each scree plot comprises bars (eigenvalues) which indicates the extent of variance elucidated by each primary component and red line (Cumulative Variability, %) which represents the cumulative percentage of variation obtained by incorporating consecutive components. The X-axis (Factors F1 to F9) represents the main components (PCs). The Y-axis (Eigenvalues and Variance, %) assesses the significance and impact of each component.

The first principal component (F1) possessed the highest eigenvalue (~5.8), accounting for the greatest share of variation in the sample. F2 contributed substantially, albeit to a lesser extent than F1 did. From F3 onwards, the eigenvalues declined sharply, indicating that the succeeding factors provided negligible extra variance. The cumulative variance curve (red line) indicates that the initial three to four components accounted for most of the variability in the dataset.

The initial two to three main components adequately explained most of the information in the acacia honey dataset. Elements beyond F3 provide minimal new insight and may be excluded from subsequent analysis, whereas F1 possesses a superior eigenvalue (~6.2) compared to acacia honey, indicating that the first principal component accounts for a greater variation in this dataset. F2 and F3 provided a moderate contribution; however, the eigenvalues beyond F3

were significantly diminished. The cumulative variability curve (red line) indicates that the initial three components accounted for almost all the variability in the dataset.

The green walnut bee product exhibited a robust first principal component, suggesting that most of the variability can be accounted for by one or two causes. This indicated greater consistency or more potent influencing characteristics in the dataset relative to acacia honey.

The green walnut bee product exhibited a greater structure, and the predominance of variance accounted for by F1 indicated a significant dominant factor affecting sensory characteristics, likely related to flavour, texture, or appearance. Acacia honey exhibits greater variability, and a larger number of components are required to account for the variance, indicating a broader range of influencing elements (e.g., colour, taste complexity, and scent changes). The principal component analysis results suggest that concentrating on the top two principal components may suffice for capturing essential quality indicators in product optimization for both items. Correlations between variables and factors for acacia honey are shown below in Table 12. -13.

Table .12 Correlations between variables and factors

	F1	F2	F3	F4	F5	F6	F7	F8	F9
Appearance_Acaica	0.726	-0.052	-0.326	0.456	-0.365	-0.120	0.076	-0.048	-0.001
Aroma_Acaica	0.468	-0.379	0.777	0.144	-0.052	-0.096	0.027	-0.029	-0.002
Flavor_Acaica	0.758	-0.373	-0.215	0.210	0.412	0.004	0.098	0.129	-0.002
Texture_Acaica	0.849	0.366	0.174	0.111	-0.119	0.144	-0.153	0.196	0.075
Consistency_Acaica	0.743	0.572	0.158	0.125	0.184	0.085	0.100	-0.091	-0.145
Complexity_Acaica	0.827	-0.022	-0.007	-0.453	-0.201	0.011	0.253	0.074	-0.002
Quality_Acaica	0.806	-0.387	-0.114	-0.104	-0.076	0.375	-0.132	-0.095	-0.063
Overall Liking_Acaica	0.936	0.128	-0.038	-0.099	0.160	-0.063	-0.015	-0.155	0.207

Purchase Intent_Acaica	0.872	-0.031	-0.109	-0.221	0.023	-0.355	-0.200	0.014	-0.107
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Table 13. Correlations between variables and factors

	F1	F2	F3	F4	F5	F6	F7	F8	F9
Appearance-GWBP	0.768	0.369	0.075	0.051	0.268	0.410	-0.144	0.050	-0.050
Aroma-GWBP	0.659	0.625	-0.310	-0.002	-0.151	-0.042	0.230	0.021	0.029
Flavor-GWBP	0.785	-0.300	-0.084	-0.187	0.451	-0.060	0.202	-0.048	0.029
Texture -GWBP	0.845	-0.093	-0.261	-0.342	-0.167	-0.030	-0.152	-0.144	-0.138
Consistency-GWBP	0.758	-0.187	0.455	-0.158	-0.281	0.216	0.179	0.029	-0.012
Complexity-GWBP	0.719	0.353	0.433	-0.047	0.084	-0.385	-0.104	-0.041	0.019
Quality-GWBP	0.807	-0.170	0.001	0.531	-0.034	-0.030	0.054	-0.157	-0.091
Overall Liking-GWBP	0.924	-0.163	-0.122	0.050	-0.114	0.069	-0.137	-0.046	0.252
Purchase Intent-GWBP	0.888	-0.231	-0.126	0.087	-0.038	-0.158	-0.054	0.319	-0.057

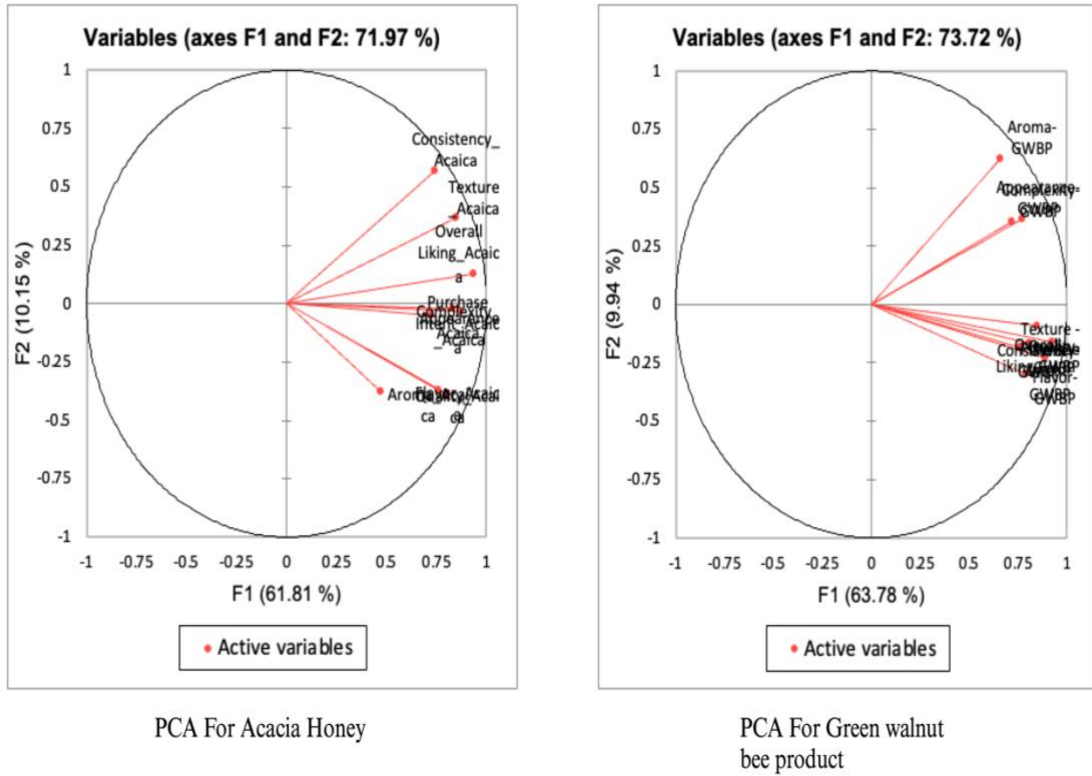


Figure 36. Principal component analysis done for both of samples, acacia honey and GWBP. (Source: XLSTAT Output)

The Principal Component Analysis (PCA) biplots for acacia honey and green walnut bee products demonstrate the correlations among several sensory qualities and their impact on the overall variation within the dataset. PCA is effective for dimensionality reduction and identifying the principal sensory qualities that account for most of the variability in consumer perception. In both instances, the first main component (F1) accounted for the greatest variation (61.81% for acacia honey and 63.78% for green walnut bee product), but the second principal component (F2) explained the supplementary variance (10.15% and 9.94%, respectively). The red vectors in the biplots denote sensory attributes, with longer arrows signifying a stronger influence on the principal components and attributes aligned in comparable directions exhibiting a positive correlation.

PCA results for acacia honey indicate a strong correlation among consistency, texture, and overall liking, suggesting that items with a uniform texture are typically favoured by consumers. The aroma is distinctly positioned, resulting in a diminished influence on the overall preference relative to texture-related qualities. Purchase Intent was significantly correlated with texture and overall liking, indicating that these attributes predominantly

affect consumers' purchasing decisions. Improving texture and consistency can increase product acceptability, whereas alterations in scent complexity may further help consumers' product acceptance.

Research on green walnut bee products reveals a substantial correlation between aroma and appearance, which significantly impacts F1, suggesting that both aspects are crucial in shaping consumer acceptance and behaviour. Moreover, texture, consistency, and overall Liking constitute distinct clusters, emphasizing that structural features substantially influence product liking. purchase intent and impressions correlate with consistency and texture, indicating that consumers are more inclined to buy the product due to its physical attributes, because they are first visuals that attract their attention to the product (in our case, its honey and bee samples). In contrast to acacia honey, aroma significantly influences the perception of green walnut bee products, but visual appeal (appearance) exerts a more substantial effect on preference because of its innovation in nature, which might be a substantially different aspect of looking at it.

These data indicate that although texture and consistency are essential for both goods, aroma and appearance exert a greater influence on green walnut bee products. This underscores the necessity for texture optimization in acacia honey and an emphasis on enhancing aroma and looking in green walnut bee products to boost consumer acceptance and purchasing choices, which helps us with the business perspective to take the further step in launching the product commercially.

Summary

This research investigates the integration of food science and nanotechnology to address real-life challenges in food and agriculture. This study primarily focuses on the medicinal properties of green walnut (*Juglans regia L.*), its potential as an innovative bee feed, and the development of silver nanoparticles (AgNPs) using green walnut extracts, which are getting popular post covid 19. This research aims to enhance sustainable agricultural practices while contributing to the United Nations Sustainable Development Goals (SDGs), it supports, 3, 9, 13 and 15.

This study explored the nutritional and pharmacological significance of green walnut, which contains bioactive compounds, such as polyphenols, and essential fatty acids. It also examined its use as a functional food ingredient, particularly in fortified bee feed, which contributes to honey production with enhanced bioactive properties. This study also highlights the use of Artificial Intelligence (AI) in conducting literature reviews using ResearchRabbit, Litmaps applications, and VOSviewer software for constructing and visualizing bibliometric networks.

. This research integrates a human clinical trial to evaluate the effect of green walnut-fortified honey on blood sugar levels and demonstrates a significant reduction in fasting blood glucose.

Additionally, this study developed an eco-friendly method for synthesizing silver nanoparticles using green walnut extracts. The research highlights a novel approach to sustainable food innovation, demonstrating how natural plant-based ingredients can be utilized in bee product development and nanotechnology for health and environmental benefits. It also opens the door for new product development and conducting sensory analysis in a completely international environment that shows the choices can vary depending upon different factors such as food habits, taste choices, purchasing intent, cultural differences, etc. It also helps beekeepers take this outcome to transform into value-added, ecofriendly, and profit-making sustainable business ventures or start-ups.

New Scientific results

1. I proved the benefit in application to be used by researchers of AI-based tools such as ResearchRabbit, Litmaps and VOSviewer in compiling literature reviews, constructing and visualizing bibliometric networks by conducting a systematic assessment of ecological, pharmaceutical, and culinary properties of green walnut, it is new insights for the researchers.
2. I demonstrated that feeding the bee colony on medical herbs like *Juglans regia L.* (green walnut extract-based sugar syrup) in its natural and usual life cycle, and because of the application of a special feeding method, a Green-colored new functional Bee Product was produced by bees containing green walnut (green walnut bee product). The contribution of green walnut as natural enrichment to the increased nutritional and health promoting value of bee Product was proved by its increased fructose/glucose ratio, diastase activity, free acid and mineral content in comparison to acacia honey used as control. (Sensory analysis) (Prokisch et al., 2022) , furthermore in bee feed process-the concentration of sugar is 50% in syrup with water , where it can be examined using density of syrup which is 1.23 g cm^{-3} and also pH is also important (starting pH was 4.6 and end was 3.9) for green walnut type of group. The main process has been patented (Daróczy, 2020).
3. I found that the consumption of green walnut-fortified bee product with yogurt significantly reduced fasting blood glucose levels in human clinical trials. The initial average blood sugar level in the treatment group was 4.8 mmol L^{-1} , which decreased to 3.7 mmol L^{-1} after 21 days, reflecting a reduction of 1.1 mmol L^{-1} (a 22.9% decrease). This effect was more pronounced than in groups consuming acacia, sea buckthorn, and chlorella algae honey.
4. I developed a green synthesis method for producing silver nanoparticles using green walnut extracts. This environmentally friendly approach reduces dependence on toxic chemicals traditionally used in nanoparticle synthesis, while maintaining desirable nanoparticle properties. This finding highlights the potential for sustainable nanotechnology applications in food science and medicine. The formation of green walnut silver nanoparticles is possible after 72 hours with nanoparticles size ranged between 10 and 11 nm by using green walnut samples at room temperature.

Practical New Scientific Results

1. The AI-based tools (Research Rabbit, VOS viewer, LiteMaps) developed and utilized in this study can be practically applied by researchers for efficient literature analysis, saving time and reducing mental workload which is need of current PhD students and researchers globally.
2. The specialized bee feed technology, incorporating medicinal herbs, offers a practical solution for beekeepers to enhance bee health and productivity. This feed can be customized with various medicinal plants to address specific health needs in bee populations and helps in combating climate change issues.
3. Green walnut-fortified Bee Product can be developed as a functional food product targeting diabetes management. Its significant blood glucose-lowering effect, combined with superior honey quality (low HMF content), makes it a valuable addition to the functional food market in EU and globally.
4. The green synthesis of silver nanoparticles from green walnut extracts provides a sustainable method for producing nanoparticles for use in food packaging, medical applications, and environmental remediation, reducing the environmental impact of conventional synthesis methods which opens the new possible ways of Nanotechnology research.
5. The integration of plant-based bioactive compounds with food technologies can be commercialized into health-promoting product ranges. This research (study) offers a pathway for transforming scientific findings into viable business opportunities for beekeepers, food technologists, and entrepreneurs focused on sustainable agriculture and food.

Publications



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Candidate: Ayaz Mukarram Shaikh
Doctoral School: Doctoral School of Nutrition and Food Sciences
MTMT ID: 10083294

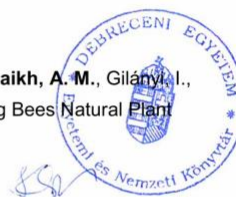
List of publications related to the dissertation

Foreign language international book chapters (1)

1. **Shaikh, A. M.**, Panda, L. R., Suthar, T., Wandhekar, S. S., Kovács, B., Boruah, T., Uddin, J., Nayik, G. A., Ramniwas, S., Singh, R.: Raman spectroscopy methods in honey characterization, authentication and adulteration. In: Advanced techniques of honey analysis: Characterization, Authentication, and Adulteration. Ed.: Gulzar Ahmad Nayik, Jalal Uddin, Vikas Nanda, Academic Press Inc Elsevier Science, San Diego, 243-257, 2024. ISBN: 9780443131752

Foreign language scientific articles in international journals (3)

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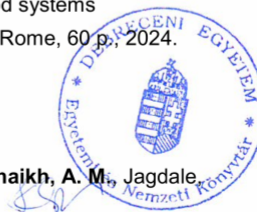
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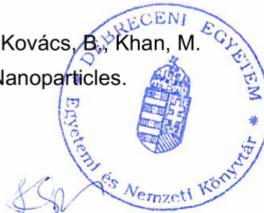


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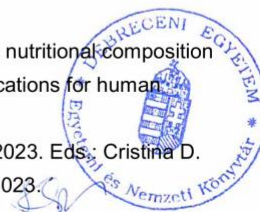




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Annexure I

Sensory Analysis questionnaires- green walnut Bee Product / Acacia Honey

- Age group- 15-20 20-25 26-30 30-35 35-40
- Smoking? Yes/No..... (Prefer not to disclose- Mark here.....)
- Drinking Alcohol? Yes/No..... (Prefer not to disclose- Mark here.....)
- Physical activity/Daily Exercise? Yes/No..... (Prefer not to disclose- Mark here.....)
- If yes, please write time per day/week per day
- Do you eat honey on a daily basis? Frequency? (Low/Moderate/High) (Per day/Per week/ Per month)
.....
.....
- How much do you spend on Honey Purchase (Approximate price)
.....
- Education
(Bachelors/Master/PhD).....
- Country.....
.....
- Sex (M/F/ Not disclose**)
.....
.....
- Status (Single/Married/ Widowed/Not to disclose)
.....
- Birth Year.....
- Health Habits (Junk food consumption Per day/ Per Week/ Per month)
.....

Annexure II

Honey characteristics

1-9 Scale (Please tick mark in appropriate section (1-9 Points boxes in each column))

Points	Appearance	Aroma	Flavour	Texture	Consistency	Complexity	Quality	Overall Liking	Purchase intent
1									
2									
3									
4									
5									
6									
7									
8									
9									

- 1 - Extremely Dislike: The sample is highly unappealing and is not acceptable.
- 2 - Dislike Very Much: The sample is very unappealing and is barely acceptable.
- 3 - Dislike Moderately: The sample is somewhat unappealing and is marginally acceptable.
- 4 - Dislike Slightly: The sample is slightly unappealing and is somewhat acceptable.
- 5 - Neither Like nor Dislike: The sample is neither appealing nor unappealing and is moderately acceptable.
- 6 - Like Slightly: The sample is slightly appealing and is somewhat acceptable.
- 7 - Like Moderately: The sample is somewhat appealing and is marginally acceptable.
- 8 - Like Very Much: The sample is very appealing and is barely acceptable.
- 9 - Extremely Like: The sample is highly appealing and is extremely acceptable.

Annexure III

Collected Data (With International students) as panelists. (green walnut bee products).

Participant Number	Appearance	Aroma	Flavor	Texture	Consistency	Complexity	Quality	Overall Liking	Purchase Intent	Average total
1	8	6	7	8	7	7	7	7	7	7
2	7	7	7	8	8	7	7	7	7	7
3	4	5	8	8	6	4	8	7	7	6
4	8	6	8	7	8	7	7	7	7	7
5	9	9	8	9	9	8	9	9	9	9
6	6	6	8	8	7	7	8	7	8	7
7	9	9	9	9	7	8	9	9	9	9
8	7	5	9	8	8	8	9	9	8	8
9	6	3	7	4	6	6	5	5	5	5
10	5	6	5	6	7	4	6	6	5	6
11	8	4	6	5	7	5	3	5	3	5
12	8	8	7	7	8	6	7	8	7	7
13	4	5	4	6	6	6	5	6	4	5
14	4	5	6	7	7	6	5	6	7	6
15	4	7	4	5	4	6	4	4	2	4
16	8	8	5	8	8	8	6	7	5	7
17	9	9	8	9	9	8	8	7	8	8
18	9	8	6	8	7	7	9	8	8	8
19	7	8	4	4	4	5	8	6	5	6
20	8	7	8	9	8	8	9	9	9	8

21	9	9	9	9	8	8	9	9	9	9
22	8	9	9	8	9	7	8	9	8	8
23	8	7	6	5	5	7	7	6	5	6
24	6	5	5	3	8	7	8	5	4	6
25	8	9	7	9	9	8	9	8	9	8
26	6	7	6	5	7	6	7	5	6	6
27	7	6	8	7	6	7	7	6	6	7
28	9	9	8	9	9	9	9	9	9	9
29	7	9	6	7	7	8	6	7	8	7
30	9	8	9	8	7	8	9	8	9	8
31	9	9	9	9	9	9	9	9	9	9
32	7	5	4	6	7	6	7	7	7	6
33	8	8	7	9	8	7	7	8	5	7
34	9	8	8	8	9	8	8	8	8	8
35	9	8	9	9	8	5	9	9	8	8
36	7	8	6	7	4	5	3	5	5	6
37	7	5	6	8	8	5	8	8	8	7
38	9	7	7	8	8	7	9	7	6	8
39	8	8	6	8	7	7	7	7	6	7
40	7	7	7	8	8	7	7	7	7	7

Annexure IV

Collected Data (With International students) as a Panelists. (Acacia Honey)

Participant Number	Appearance	Aroma	Flavor	Texture	Consistency	Complexity	Quality	Overall Liking	Purchase Intent	Average total
1	6	5	5	6	6	5	5	6	6	6
2	8	6	7	8	8	6	7	7	7	7
3	8	5	7	5	6	4	5	6	6	6
4	8	7	8	8	8	7	8	8	8	8
5	7	5	7	7	6	6	8	7	7	7
6	5	7	9	9	9	8	9	9	7	8
7	9	6	5	7	7	7	7	7	6	7
8	6	2	6	3	3	2	5	3	3	4
9	7	5	8	6	7	5	6	6	6	6
10	5	7	6	3	2	5	7	4	4	5
11	5	7	6	3	2	5	7	4	4	5
12	5	3	4	5	6	7	5	5	5	5
13	5	5	4	5	5	5	6	6	5	5
14	4	5	2	7	8	2	3	4	1	4
15	8	6	9	7	8	7	8	8	8	8
16	9	5	8	9	8	8	9	9	8	8

17	8	6	6	8	9	8	7	7	7	7
18	8	8	7	8	6	6	7	7	7	7
19	5	5	6	7	6	5	6	7	8	6
20	7	6	7	5	7	5	8	8	8	7
21	7	9	9	9	9	9	8	9	9	9
22	7	8	7	7	6	8	7	7	7	7
23	5	6	6	5	7	4	3	7	5	5
24	9	5	9	9	8	9	9	9	9	8
25	7	5	4	8	6	8	8	7	7	7
26	6	5	6	6	5	6	6	7	6	6
27	6	5	7	6	6	5	7	6	7	6
28	9	5	9	8	8	9	9	9	9	8
29	8	5	9	8	7	6	9	8	6	7
30	4	6	7	5	7	7	8	7	6	6
31	7	9	6	9	8	7	9	7	8	8
32	7	5	7	8	8	6	7	7	7	7
33	8	5	9	8	8	9	9	9	9	8
34	8	7	7	7	6	6	7	7	7	7
35	9	8	9	9	9	9	9	9	8	9
36	8	6	8	7	7	5	9	7	4	7
37	8	7	8	9	8	6	8	8	8	8

38	8	7	8	7	7	5	8	7	6	7
39	7	6	7	7	7	6	7	7	7	7
40	8	8	9	8	7	6	8	7	7	8

Annexure V

Demographic Data (With International students) as a Panelists.

Participant Number	Age Group	Smoking	Drinking Alcohol	Physical Activity	If Yes - Per Day	If Yes - Per week	Do you eat honey on daily basis?	Frequency	How much do you spend on Honey Purchase? (in HUF)	Education	Country	Sex	Status	Birth Year	Health Habits (junk food consumption)
1	26-30	No	Yes	Yes	1hr	N/A	Low	Per month	900	Master	Philippines	Female	Single	1997	2 Per month
2	20-25	No	Yes	NO			Low	Per month	1000	Master	Philippines	Female	Single	1998	3 Per week
3	30-35	No	Yes	NO			Low	PER DAY	2000	PHD	Philippines	MLE	Single	1989	2 Per month
4	26-30	No	Yes	Yes	1 HR		Low	Per month	0	Master	Philippines	Female	Single	1997	2 Per month
5	20-25	No	NO	Yes		2 HR	MODERTE	PER WEEK	2000	BACHELORS	UGANDA	MLE	Single	1999	
6	20-25	No	NO	Yes		3 DAYS	MODERTE	PER WEEK	2000	BACHELORS	BANGLADESH	MLE	Single	2004	1 PER WEEK
7	20-25	Yes	Yes	Yes		5 DAYS	Low		N/A	Master	KENYA	Female	Single	1998	PER WEEK
8	15-20	No	NO	NO			Low		0	BACHELORS	PAKISTAN	Female	Single	2005	PER MONTH
9	20-25	No	NO	Yes		2 HR	Low		0	BACHELORS	CHINA	MLE	Single	2002	1 PER WEEK
10	20-25	No	Yes	NO			Low	Per month	0	BACHELORS	CHINA	MLE	Single	2003	2 PER WEEK

11	20-25	Yes	Yes	Yes	1 HR		Low	PER DAY	1000	Master	CHINA	MLE	Single	1999	2 PER WEEK
12	20-25	No	NO	Yes	1 HR		Low	Per month	0	Master	CHINA	MN	Single	2000	2 PER WEEK
13	26-30	No	Yes	Yes		1 DAY	Low	Per month	0	Master	KENYA	M	Single	1995	NO
14	15-20	No	Yes	Yes		5 DAY	Low	Per month	0	BACHE LORS	BOTSWANA	M	Single	2005	PER WEEK
15	26-30	No	NO	NO			Low	2 DY PER WEEK	500	Master	KENYA	M	Single	1994	2 PER WEEK
16	20-25	No	NO	Yes		1 DAY	MODERTE	Per month	1500	BACHE LORS	GHANA	Female	Single	2004	1 PER MONTH
17	26-30	No	NO	Yes		1 DAY	Low		0	Master	GHANA	Female	Single	1997	1 PER WEEK
18	20-25	No	NO	NO			HIGH	EVERY DAY	2000	BACHE LORS	NIGERIA	Female	Single	2002	1 PER MONTH
19	20-25	No	NO	NO			Low	2 PER MONTH	N/A	BACHE LORS	GHANA	Female	Single	2003	1 PER MONTH
20	20-25	No	NO	Yes		3 DAYS	MODERTE	Per month	3000	BACHE LORS	NIGERIA	Female	Single	1998	2 Per month
21	20-25	No	NO	NO			Low	Per month	1500	BACHE LORS	GHANA	Female	Single	2003	1 PER MONTH
22	20-25	No	NO	NO			Low		2000	BACHE LORS	GHANA	M	Single	2001	1 PER MONTH
23	26-30	No	NO	Yes		4 DAYS	Low	3 PER MONTH	2000	Master	HUNGARY	M	Single	1998	1 PER WEEK
24	20-25	No	NO	Yes		3 DAYS	Low		N/A	Master	GAHNA	M	Single	2000	NO
25	20-25	No	NO	NO			Low	Per month	1000	BACHE LORS	GAHNA	Female	Single	2002	1 PER WEEK
26	15-20	No	NO	N/A			Low	Per month	3000	BACHE LORS	CHINA	Female	Single	2005	2 PER WEEK

27	20-25	No	Yes	NO			Low		1000	BACHELORS	CHINA	Female	Single	2003	2 PER WEEK
28	15-20	No	NO	Yes		1 DAY	MODERTE	2 PER WEEK	2000	BACHELORS	INDIA	Female	Single	2003	1 PER WEEK
29	25-20	No	NO	Yes		2 DAYS	MODERTE	PER WEEK	2000	BACHELORS	INDIA	Female	Single	2005	1 PER WEEK
30	26-30	No	Yes	Yes		3 DAYS	Low	1 PER MONTH	2000	Master	HUNGARY	M	Single	1996	3 PER WEEK
31	26-30	No	NO	Yes			Low		N/A	PHD	INDIA	Female	Single	N/	1 PER MONTH
32	26-30	NO	Yes	Yes		4 DAYS	HIGH	20 DY PER MONTH	4000	Master	MALAYSIA	M	Single	1993	2 PER WEEK
33	20-25	No	Yes	Yes		2 DAYS	Low	1 PER MONTH	0	Master	EUADOR	Female	Single	1997	3 PER WEEK
34	20-25	No	NO	Yes		2 DAYS	Low	1 PER MONTH	1500	Master	KOSOVO	Female	Single	1998	1 PER MONTH
35	20-25	No	Yes	Yes		2 DAYS	Low	1 PER WEEK	3000	Master	SYRIA	Female	Single	1998	2 PER WEEK
36	20-25	No	NO	Yes		3 DAYS	Low	PER MONTH	N/A	Master	KOSOVO	M	Single	2000	1 PER MONTH
37	20-25	No	NO	Yes		3 DAYS	Low		2000	Master	INDONESIA	M	Single	1998	2 PER WEEK
38	20-25	No	N/A	Yes		4DAYS	HIGH	PER DAY	N/A	Master	KOSOVO	Female	Single	2000	1 PER MONTH
39	26-30	No	NO	Yes		3 DAYS	Low	Per month	1000	Master	INDIA	m	Single	1995	1 per week
40	26-30	No	Yes	Yes		4 DAYS	NO		N/A	Master	PARAGUAY	Female	Single	1992	2 PER WEEK

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