

# **Thesis of Doctoral (PhD) Dissertation**

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

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(FOOD SCIENCE PROGRAMME)  
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## 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 and 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 and Aleta N 2000). Walnuts are presently cultivated for economic use in the United States, northern Africa, southern South America, 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).

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. 2015; Goulson, Lye, and Darvill 2008; Kaluza et al. 2018). This discussion aims to integrate food science and nanotechnology to address real-world challenges and support the United Nations' Sustainable Development Goals (SDGs).

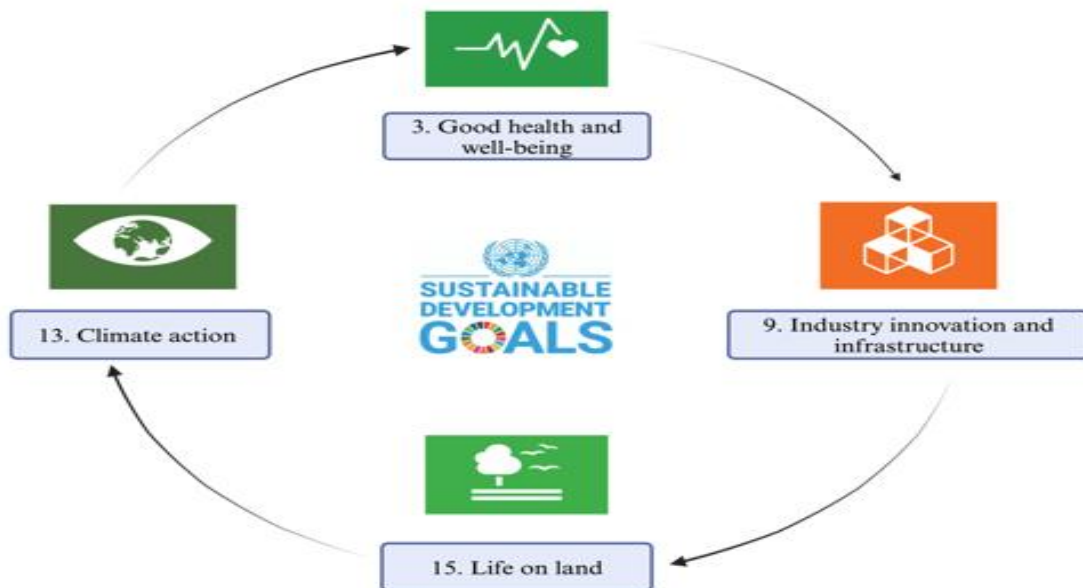


Figure 1: Sustainable development goals aimed through research (source: author, using the Biorender Design website, <https://www.biorender.com/>).

### 1.1 Background of the Study

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., Figure 2.

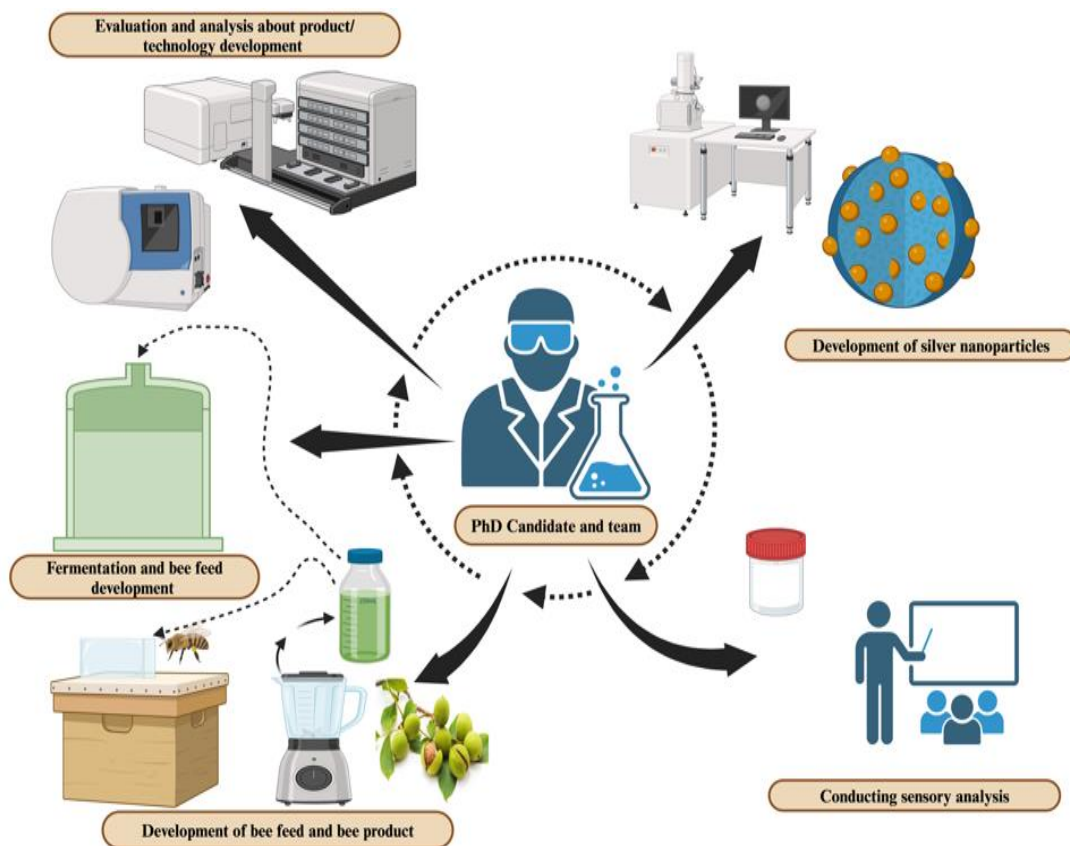
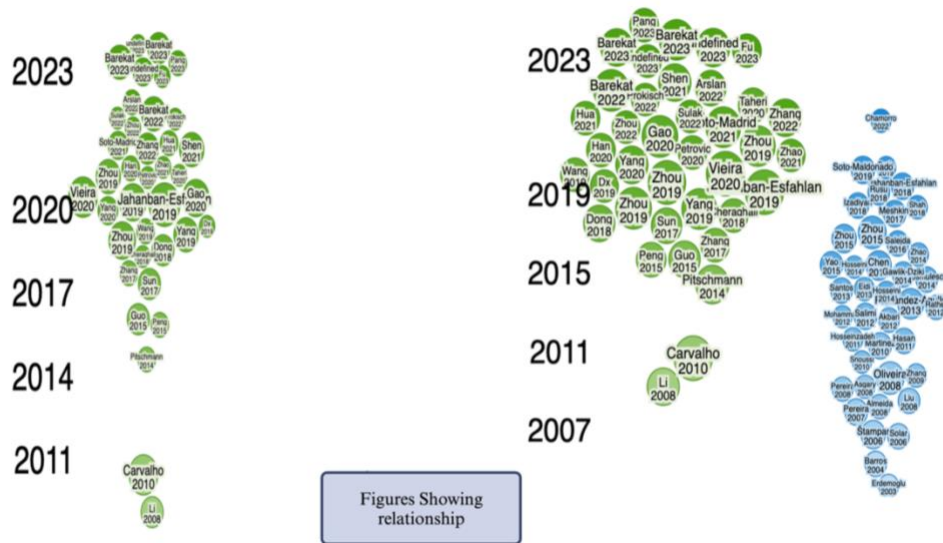


Figure 2: Graphical representation of PhD dissertation (source: author, using Biorender Design website, <https://www.biorender.com/>)

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

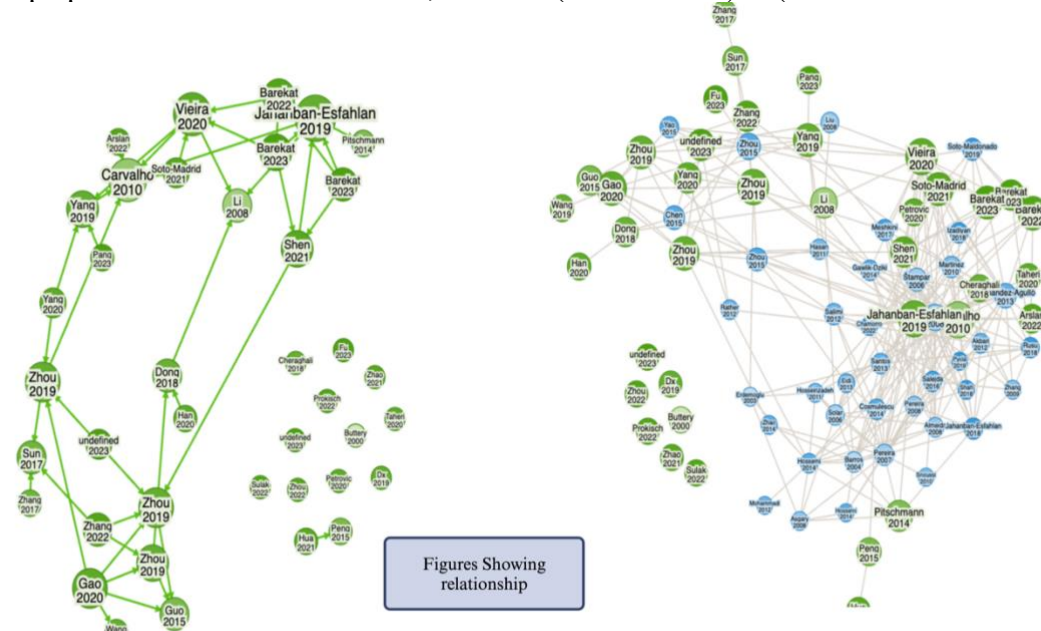
### 2.4.1 Literature review using AI Tool- Research Rabbit

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. Research Rabbit, 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 3–6). Approximately 40 papers pertained to its therapeutic applications and other uses, whereas approximately 40 studies focused on its morphology and characterization.



**Figure 3 & 4.** 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. 4: Similarities within work conducted on *Juglans regia* L. and different scientific papers matched to its own properties and characteristics, etc. (blue colour) (from 2000 to 2023).



**Figure 5-6:** 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 VOS viewer. The authors used the following terms in WoS: ("green walnut" OR "unripe walnut" OR "*Juglans regia*" OR "walnut extract" OR "walnut oil") AND (medicinal OR pharmaceutical OR therapeutic OR "health benefits") AND (antioxidant\* OR anti-inflammatory OR nutraceutical\* OR "natural products"). Subsequently, the search results were examined using VOS Viewer software (Version 1.5.5) produced by Leiden University, Netherlands (Eck and Waltman 2017).

Subsequent outcomes were obtained for the primary keywords. Refer to figure 7. The primary keywords associated with green walnut, walnut and its applications, bioactive substances, their properties and characterization, and walnut husk were analyzed using Vos Viewer software.

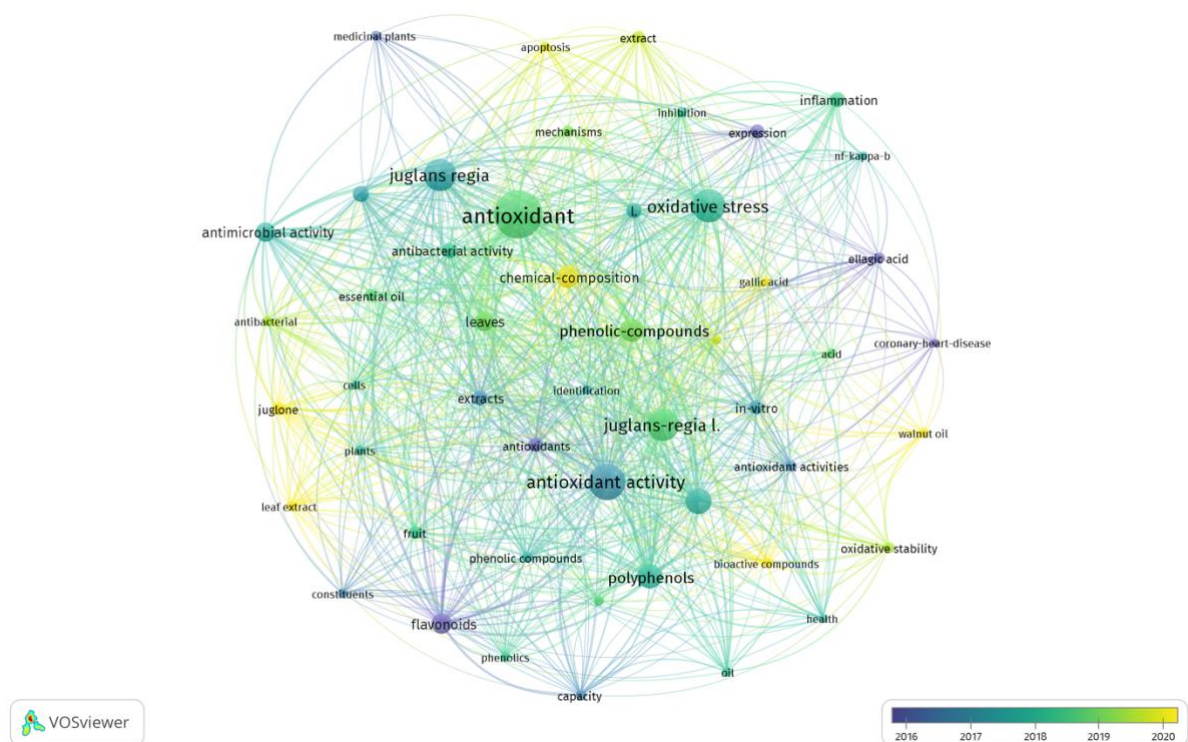


Figure 8. Widely searched/used keywords for *Juglans regia L.* globally.

## 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  $1.23 \text{ g cm}^{-3}$ . 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 (Figure 9 - 10.).

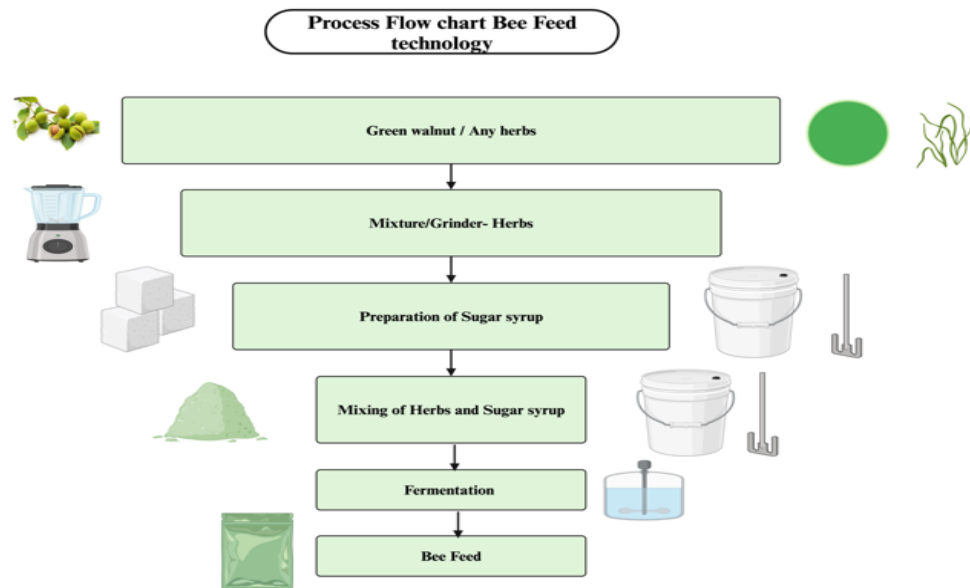


Figure 9. Process flow chart for Bee Feed technology development (Source: own photo, created using Biorender)

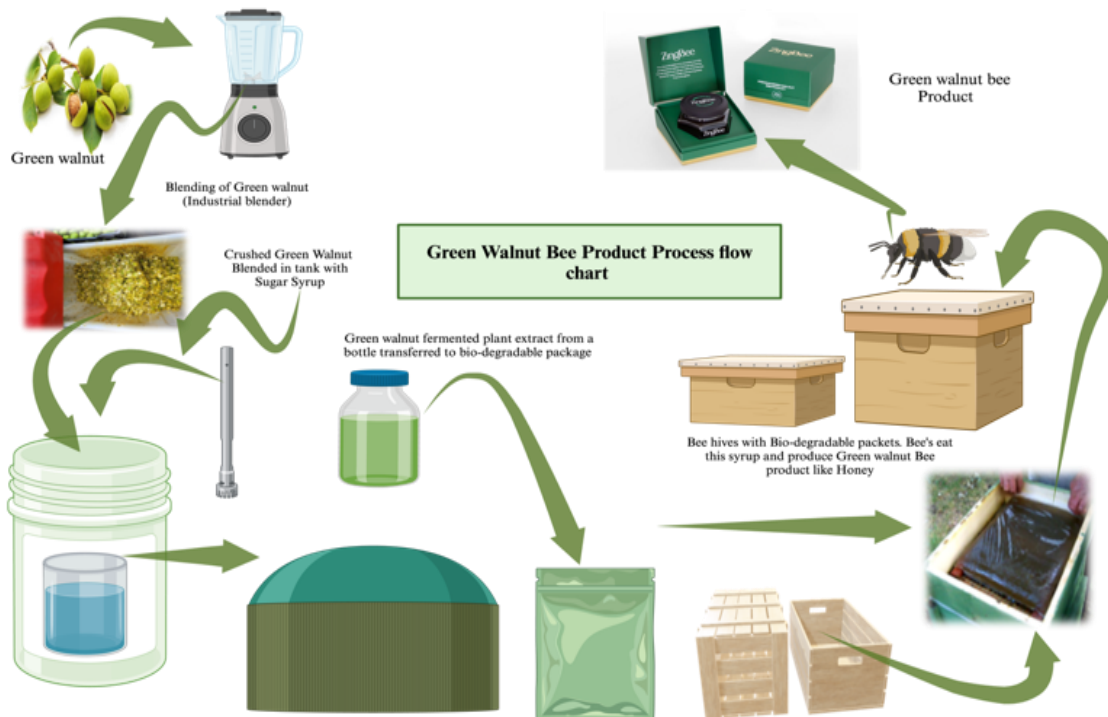


Figure 10. Showing the process flow chart for the Bee feed development (Green walnut- *Juglans Regia L.*) (Source: own photo, created using Biorender)

### 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 1. 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 a control samples, whereas the quantity of them was 30 g. 45 samples were prepared for giving it for clinical trials.

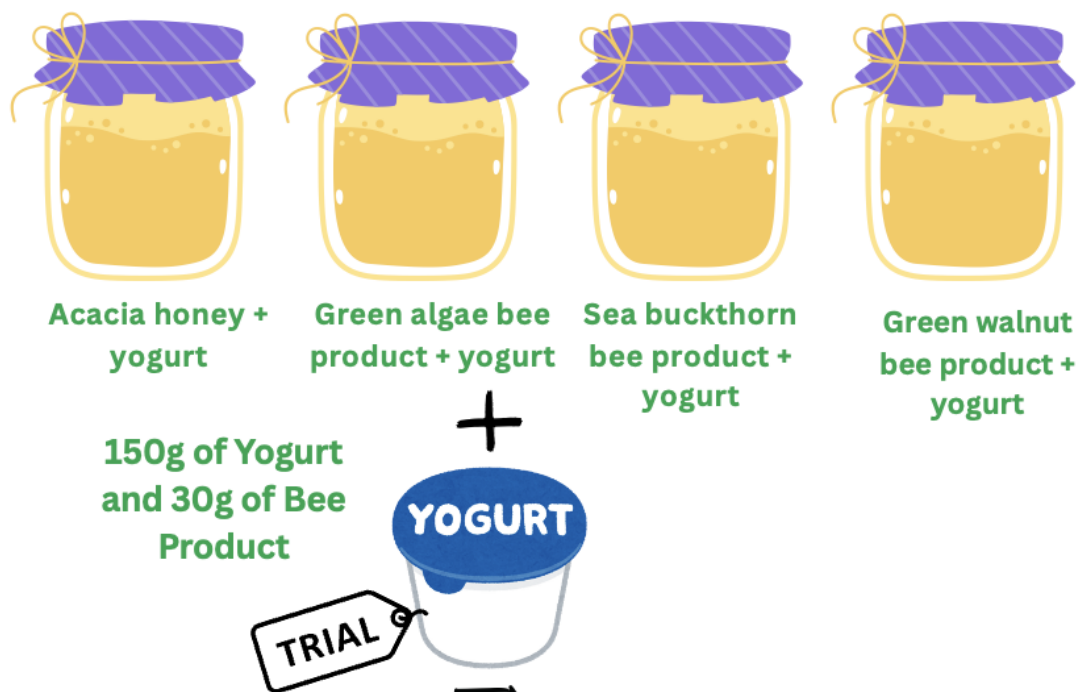


Figure 11. Fortified Yogurt Preparation by adding different bee products like green algae bee product, sea buckthorn and green walnut along with Acacia honey as a control sample.

### 3.1.2 Preparation of Samples with Yoghurt

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 apianian 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 aluminum 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 9. 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 1. The methodology for the parameters in Table 3 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, sulfur, 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, Phillips, and Kovács 2019)

**Table 1 The detailed information about development of syrup for honey and bee product for each group**

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 <sup>-1</sup>	1.25 kg L <sup>-1</sup>	1.20 kg L <sup>-1</sup>
Syrup pH (start)	5.8	4.3	4.6
Syrup pH (end)	5.2	3.9	3.9

Notes- †Feeding rate /bee colony



Figure 12. 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)

### 3.1.3 The process/ Protocol for Human based 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 flow chart 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 11 illustrates the primary steps of this study.

**Table 2 The main criteria used in the current study for selecting the participants.**

	<b>Baseline</b>	<b>3 Weeks</b>	<b>3 Months</b>
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 analyzed. Upon the conclusion of the trial, participants filled out a straightforward culinary questionnaire regarding the product's flavor.

The process flow chart is given below about the Human case study,

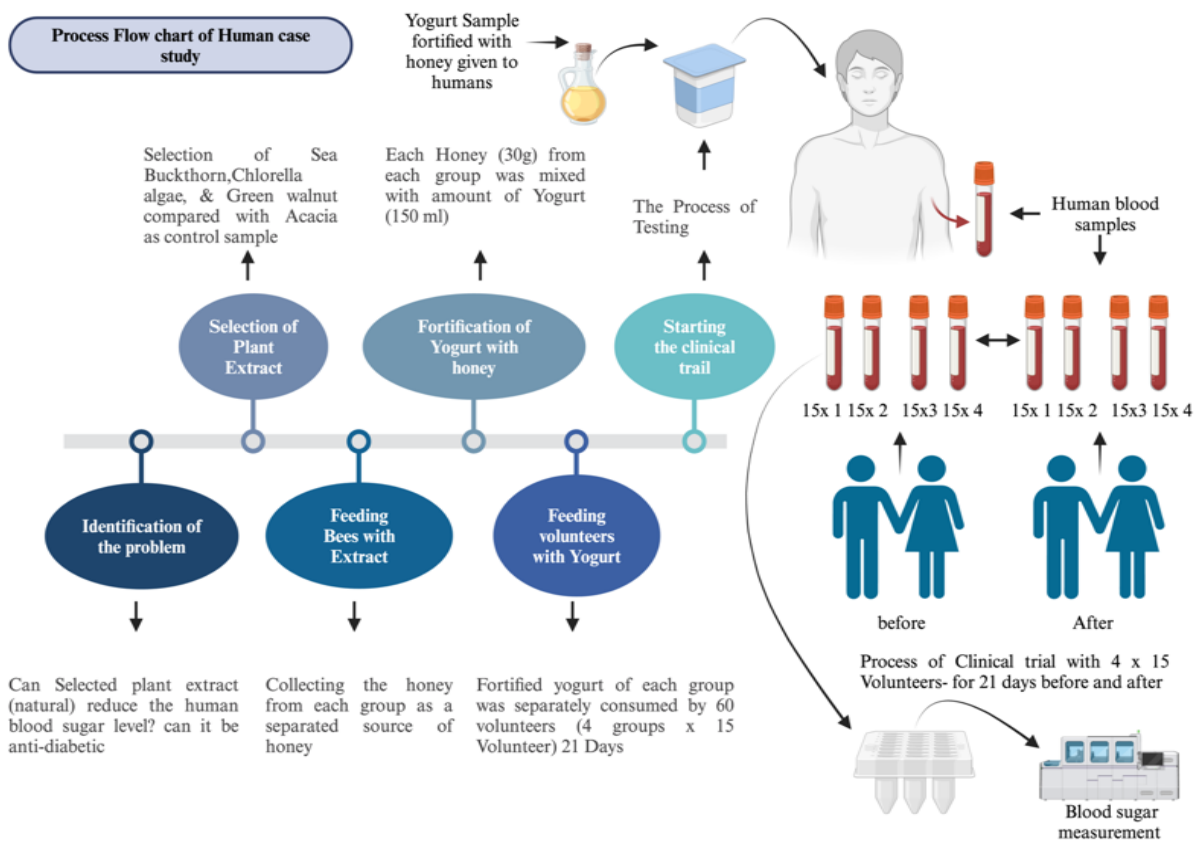


Figure 13. The primary steps undertaken in this study encompass the clinical trial. The evaluated honey products were ingested with yoghurt. (Source; Own, Created using Bio-render).

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.

### 3.1.4 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 analyzed, 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.

### 3.1.8 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.

### 3.1.9 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).

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

A standardized consumer acceptance test was employed for sensory evaluation, during which panelists (International Students) evaluated several sensory aspects of the samples. Forty panelists (International Students) engaged in the evaluation. Each panelist received coded samples in a controlled environment to reduce external variables. The assessment was performed in a controlled environment with a scope to guarantee impartial evaluation. Panelists (International Students) were directed to evaluate the samples according to their appearance, aroma, flavor, texture, consistency, complexity, quality, overall preference, and purchase intent. The evaluations were assigned using a 9-point hedonic scale ranging from 1 (very dislike) to 9 (highly like). All panelists refreshed their palates with water and plain crackers between samples to prevent sensory tiredness. The protocol shown in figure 14. Below,

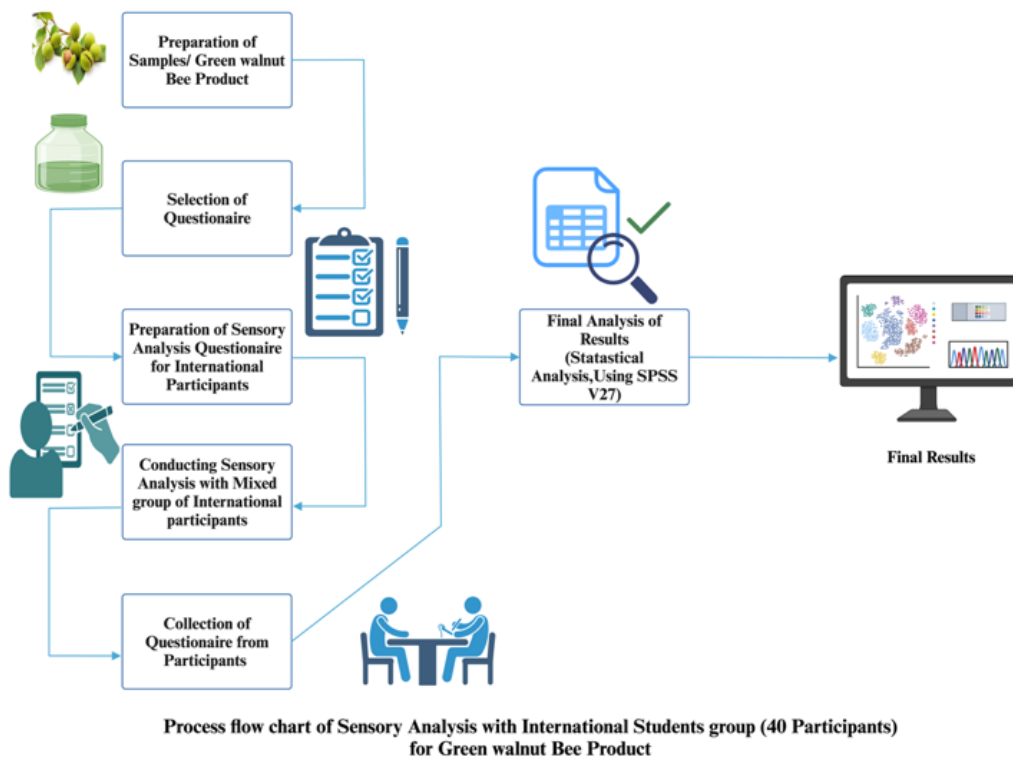


Figure 14. Protocol conducted to carry out sensory analysis with a group of international students at Faculty of Agriculture, Food Science and Environmental Engineering, University of Debrecen, from 01/02/2024 to

01/01/2025, with Ethical Approval ELMTI/2/2025. With 40 Participants (Source: Author, Created using Biorender).

### **3.2.2 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 Analysis of Correlation 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.

### **Objective 3**

#### **3.4.1 Materials and Methods for Silver nano particles synthesis**

Silver nitrate ( $\text{AgNO}_3$ ) 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- $\mu\text{m}$  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  $\text{AgNO}_3$  solution under ambient temperature and pressure conditions. The herbal extract facilitates the reduction of  $\text{Ag}^+$  ions to  $\text{Ag}^0$ , 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 analyzed 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 analyzed 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  $20^\circ/\text{min}$  in the  $2\theta$  range of  $20^\circ$ - $80^\circ$ . (All experiments were carried out in collaboration with the University of Pannonia, Veszprem, Hungary- Especially TEM, XRD) Figure 15. A and B: Preparation of Silver Nanoparticles using green walnut powder and its various applications.

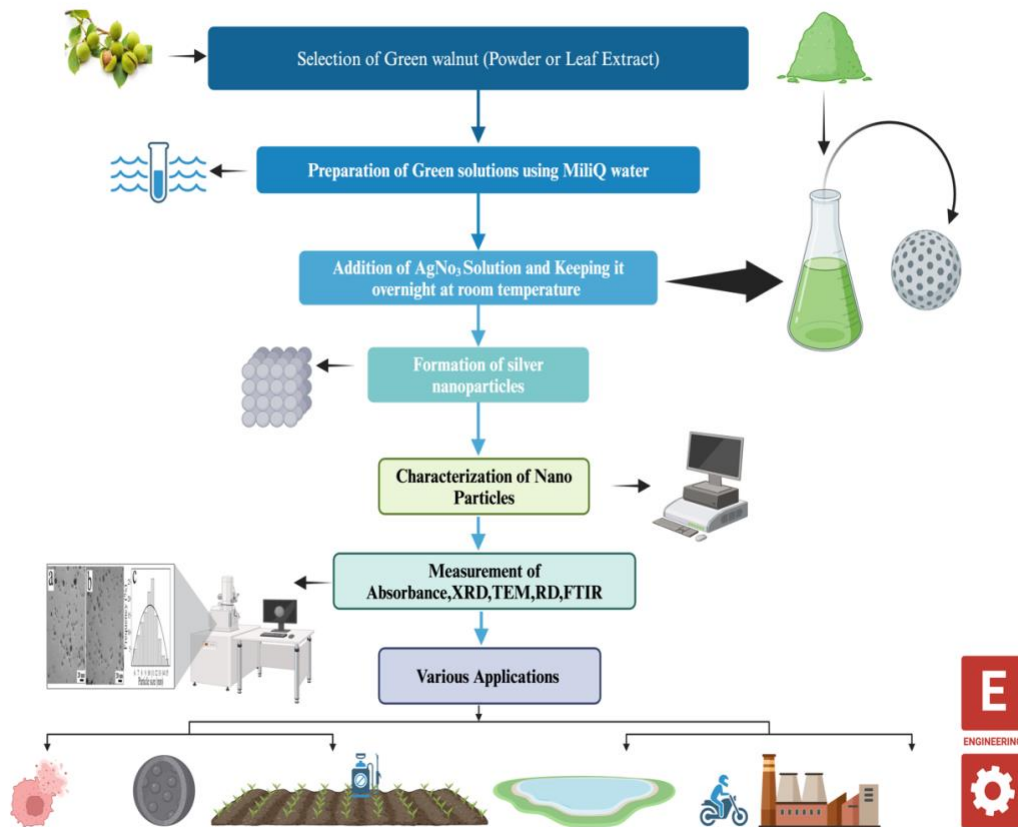


Figure 15 A. Process of Preparation of silver nanoparticles using Green walnut whole powder, its synthesis and its application along with measurement process. (Source: Author, Created using Biorender).

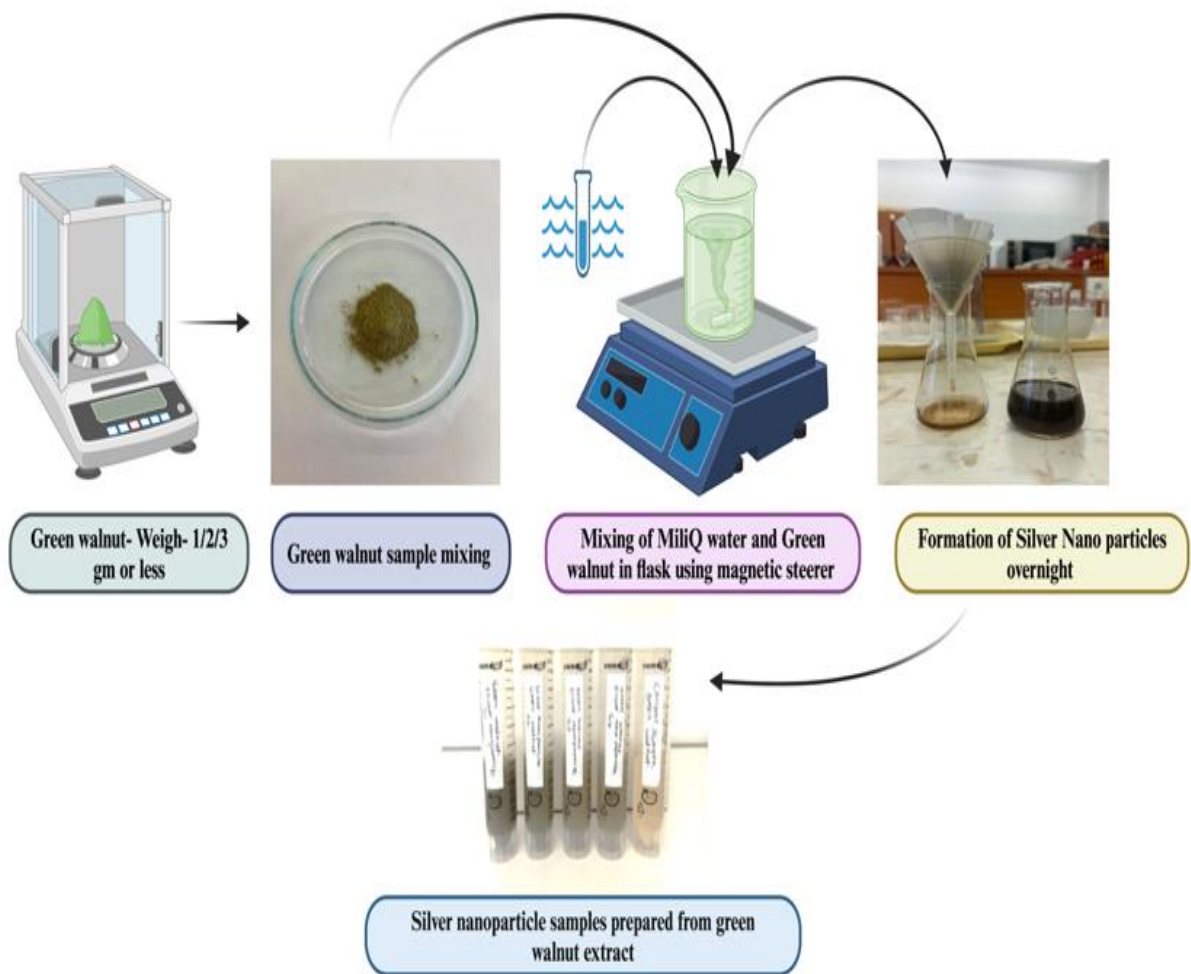


Figure 15 B. Flow chart for silver nano Particles synthesis. (Source: Author, Created using Biorender).

### 3.4.2 Characterizations of Silver nano particles

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.

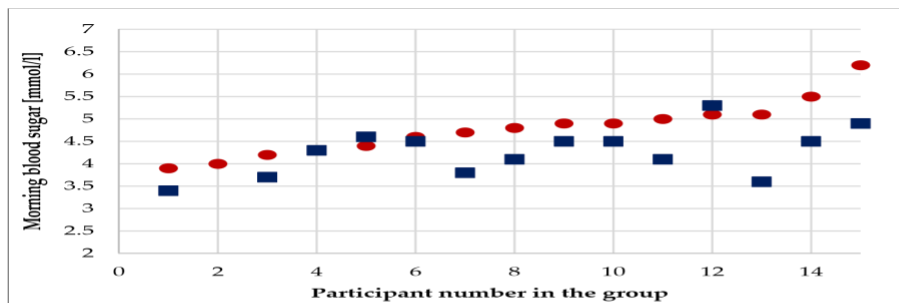
## **Results & Discussions**

### **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/ Personal data protection; 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. 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 26). In the second and third groups, the correlation between blood glucose levels and the intake of yoghurt enriched with honey was ambiguous and statistically insignificant. Conversely, in the fourth group (walnut), all participants' blood glucose levels following the consumption of honey-fortified yoghurt were lower than those recorded after treatment

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. Figure 16.

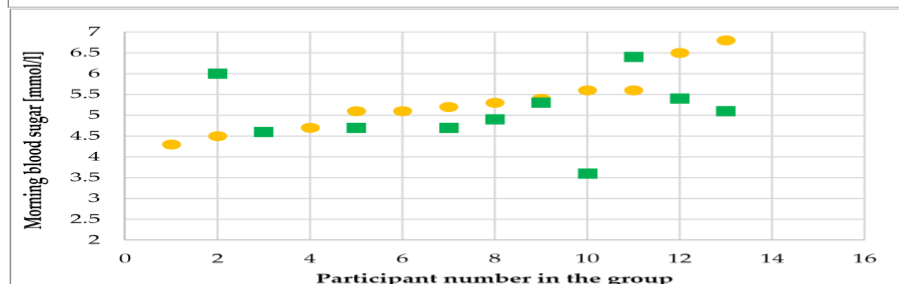
Fundamental analyses of honey in the four groups are presented in Table 4. The data indicate that the chemical composition of honey across all groups is generally comparable, with no significant disparities, including sugar content (fructose + glucose was elevated in *Chlorella alga*) and various measured 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.



**Group 1.**  
Acacia honey +  
yogurt

before: "●"

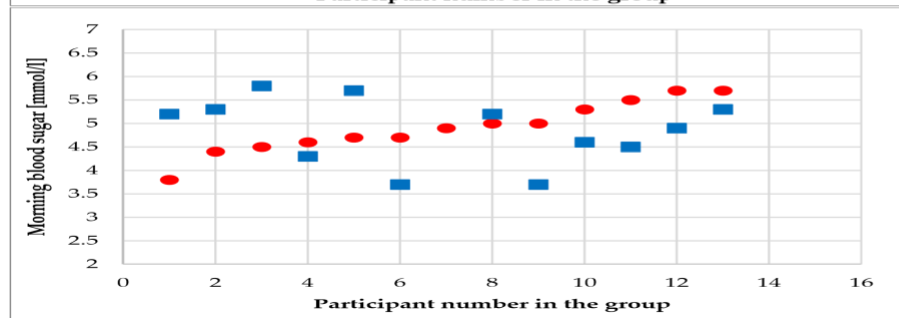
after: "■"



**Group 2.**  
Green algae honey  
+ yogurt

before: "●"

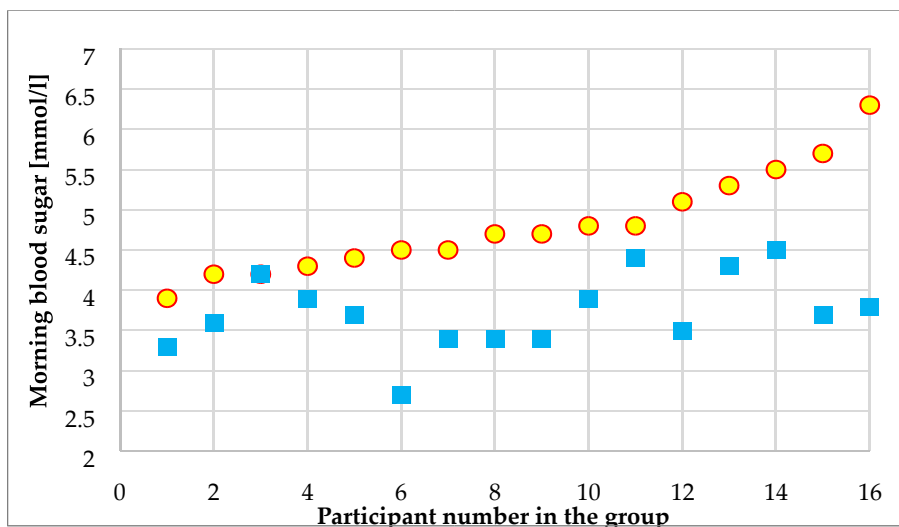
after: "■"



**Group 3.**  
Sea buckthorn  
honey + yogurt

before: "●"

after: "■"



**Group 4.**  
Walnut honey +  
yogurt

before: "●"

after: "■"

Figure 16. 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.

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

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 16), 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). For group 4, the pre-treatment morning blood sugar levels in the volunteers were 4.8 mmol L<sup>-1</sup>, while post-treatment levels were 3.7 mmol L<sup>-1</sup>, as illustrated in Figure 26, group 1, 2 The data in Figure 15, group 3 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<sup>-1</sup>, with a high of 2.5 mmol L<sup>-1</sup> (ranging from 6.3 to 3.8 mmol L<sup>-1</sup>).

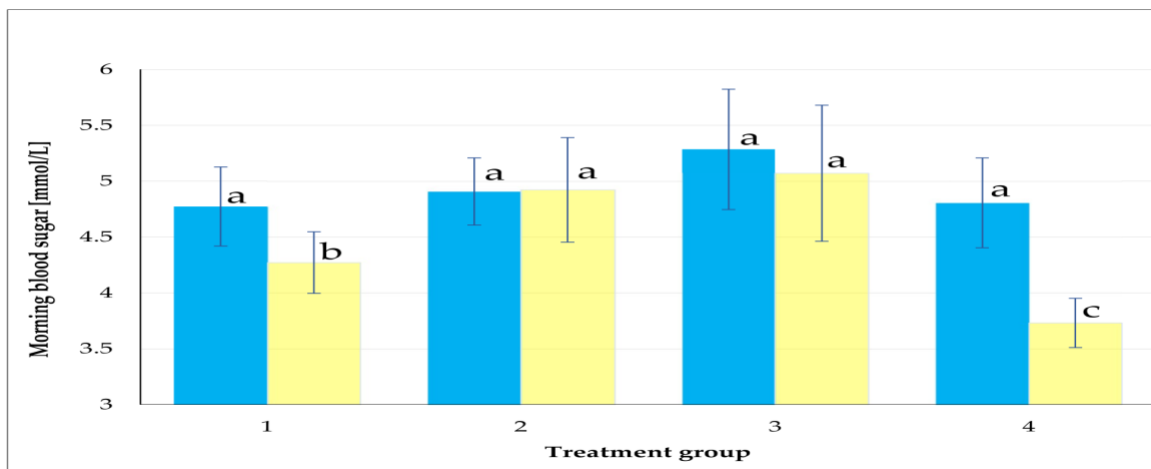


Figure 17. Average morning blood glucose levels of participants prior to (blue column) and following (yellow column) the human clinical trial. The treatment groups consisted of group (1) acacia honey combined with yoghurt; group (2) green algae honey combined with yoghurt; group (3) sea buckthorn honey 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<sup>-1</sup>, with a high of 2.5 mmol L<sup>-1</sup> (ranging from 6.3 to 3.8 mmol L<sup>-1</sup>).

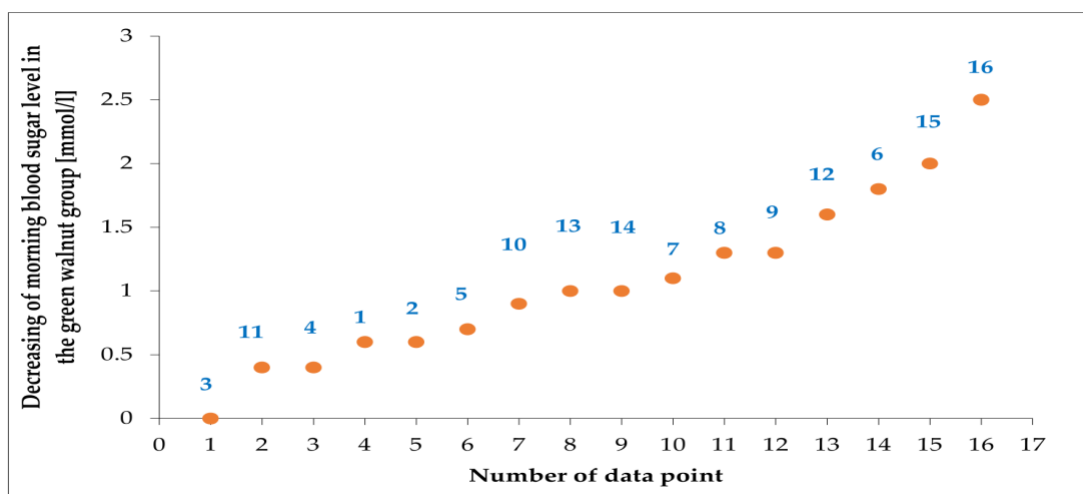


Figure 18. The reduction of morning blood glucose levels in the blood of participants in treatment group 4. The data were arranged in ascending order and computed by subtracting the morning blood sugar measurement recorded on the 21st day of the trial from that of day 0.

#### **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. 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, Abou-Shaara, and Ansari 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; Sowa et al. 2019; Tlak Gajger et al. 2020; Shumkova and Balkanska 2021).

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 and 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 analyzed 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 hydroxy-methyl-furfural (HMF) at 1.73 mg kg<sup>-1</sup> and fructose plus glucose content of 64.7%. Additionally, it recorded the lowest concentrations of several nutrients (in mg kg<sup>-1</sup>): 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  $\beta$ -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 (Sharma et al. 2020; Samarghandian, Farkhondeh, and Samini 2017).

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 Phyto additive and/or bioactive phytonutrient, lipid, carotenoid, and protein content (Bazarnova et al. 2021; Caporgno and 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 50 g 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 (Tkacz et al. 2021; Ren 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 and 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  $6.3 \text{ mmol L}^{-1}$ , which reduced to  $3.8 \text{ mmol L}^{-1}$  after 21 days of treatment. The reduction in persons was  $2.5 \text{ mmol L}^{-1}$  (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 ( $4.88 \text{ mg kg}^{-1}$ ) 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).

## **Objective 2**

### **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 19. 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  $\text{Ag}^+$ , resulting in the creation of silver nanoparticles. The addition of herbal extract to the  $\text{AgNO}_3$  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.

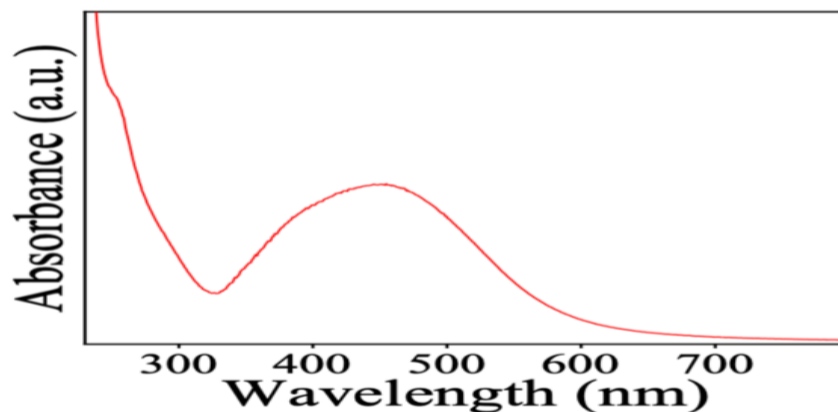


Figure 19. UV–Visible spectra of Silver Nanoparticles after 72 hours keeping in lab.

The crystallinity of the biosynthesized silver nanoparticles produced using walnut extract was assessed using X-ray diffraction. The sample was examined from drop-cast films of as-synthesized Ag nanoparticles on a glass substrate and exhibited well-defined X-ray diffraction patterns corresponding to the (111), (200), (220), (311), and (222) planes. Figure 20. distinctly shows the contribution of the amorphous material resulting from the biomolecules in the herbal extract, which provide stability and capping of the nanoparticles.

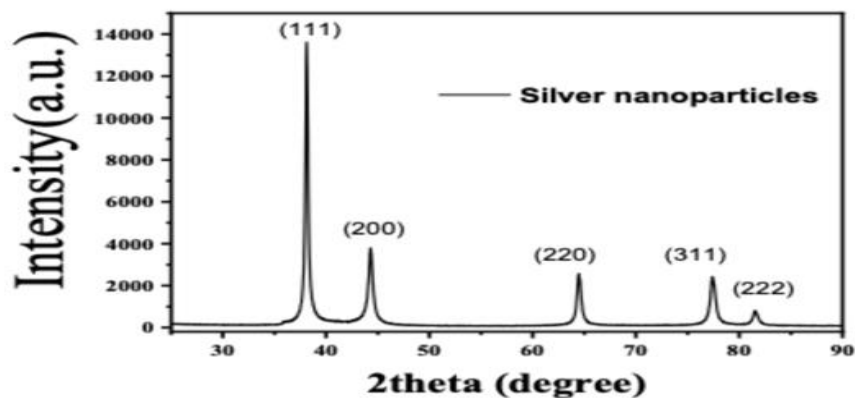


Figure 20. X ray Diffraction (XRD) pattern of a drop-cast film of AgNo<sub>3</sub> on a glass substrate following reaction with the herbal extract of green walnut.

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 21 (A, B, C, and D) Shows the Transmission electron micrograph of silver nanoparticles AgNo<sub>3</sub>, characterized by uneven shapes and an overall quasispheroidal morphology. Figure 21 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.

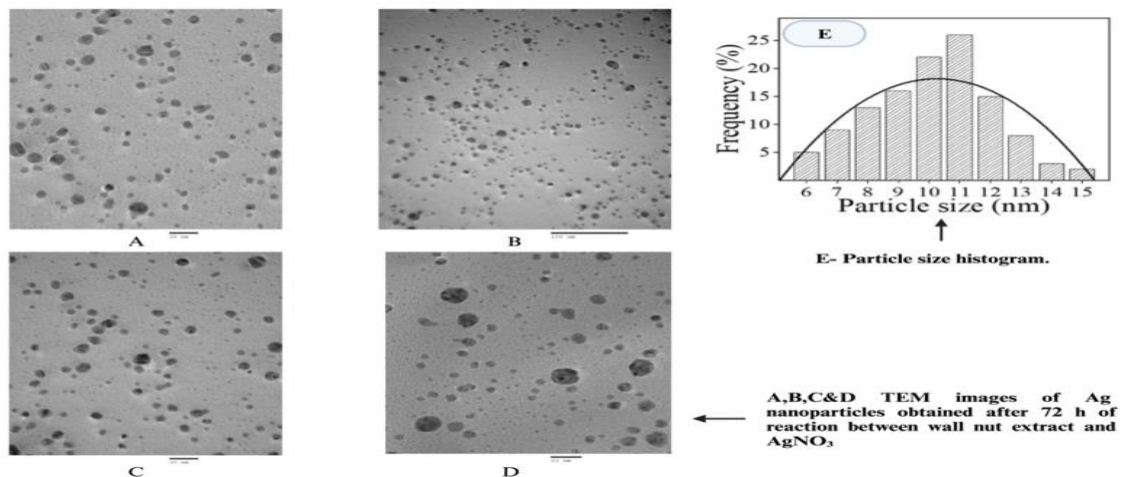


Figure 21. A, B, C and D Showing the Transmission Electron Microgram produced using green walnut silver nanoparticles, while Figure 33.E Shows the Particle size histogram.

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 + AgNO<sub>3</sub>)

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 was conducted based on various attributes, including Appearance, Aroma, Flavor, Texture, Consistency, Complexity, Quality, and Overall Liking. The

dataset consisted of 80 observations, and statistical summaries of these sensory attributes are presented in Table .4 below,

**Table 4: Statistics of Sensory Attributes**

	Observations	Min	Max	Mean $\pm$ Std Dev
<b>Appearance</b>	80	4	9	7.175 $\pm$ 1.508
<b>Aroma</b>	80	2	9	6.500 $\pm$ 1.630
<b>Flavor</b>	80	2	9	6.925 $\pm$ 1.605
<b>Texture</b>	80	3	9	7.113 $\pm$ 1.669
<b>Consistency</b>	80	2	9	7.038 $\pm$ 1.538
<b>Complexity</b>	80	2	9	6.513 $\pm$ 1.543
<b>Quality</b>	80	3	9	7.250 $\pm$ 1.611
<b>Overall Liking</b>	80	3	9	7.063 $\pm$ 1.435
<b>Purchase Intent</b>	80	1	9	6.688 $\pm$ 1.769

The mean scores for the attributes ranged between 6.51 (Complexity) and 7.25 (Quality), indicating that panelists generally had a positive perception of the product. The Stannard 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) represents the variance explained by each principal component.

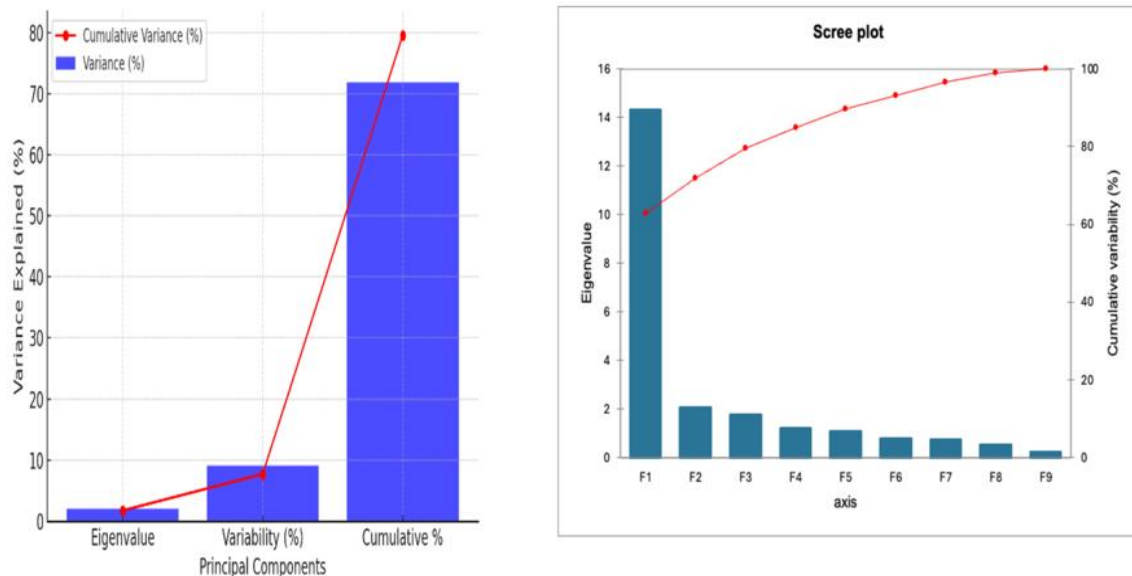


Figure 22. Variance Explained by Principal Components by using Scree plot (Source: XLSTAT Output)

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. Shown in table 5 below,

**Table 5: Variance Explained by Principal Components**

	<b>F1</b>	<b>F2</b>	<b>F3</b>	<b>F4</b>	<b>F5</b>	<b>F6</b>	<b>F7</b>	<b>F8</b>	<b>F9</b>
<b>Eigenvalue</b>	14.314	2.076	1.765	1.224	1.097	0.794	0.748	0.546	0.257
<b>Variability (%)</b>	62.722	9.096	7.735	5.366	4.806	3.481	3.276	2.391	1.126
<b>Cumulative %</b>	62.722	71.818	79.553	84.919	89.725	93.206	96.483	98.874	100.000

#### 4.3.3 Factor Loadings and Sensory Attribute Contributions

The factor loading matrix (Table 6) 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 6: Factor Loadings of Sensory Attributes**

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

Additionally, the heatmap (Figure.21) visually represents the relationship between the sensory attributes and principal components.

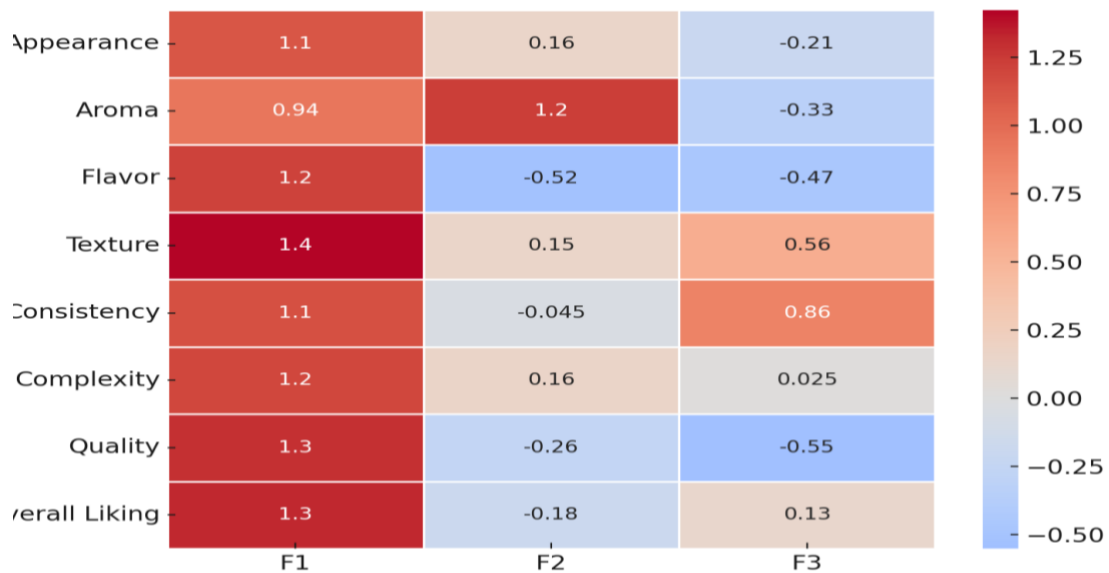


Figure 23: Factor Loadings Heatmap, (Source: XLSTAT Output)

- PC1 (62.72%): The first principal component was highly correlated with flavor (1.22), texture (1.42), and appearance (1.11). This suggests that Flavor, 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 Flavor, 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 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 behavior 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. Figure 7 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. 7 Showing the Variable for Acacia Honey and Green walnut bee product**

Variable	Observations	Minimum	Maximum	Mean $\pm$ Std Dev
Appearance_Acaica	40	4	9	6.975 $\pm$ 1.459
Aroma_Acaica	40	2	9	5.950 $\pm$ 1.449
Flavor_Acaica	40	2	9	6.950 $\pm$ 1.679
Texture_Acaica	40	3	9	6.900 $\pm$ 1.707
Consistency_Acaica	40	2	9	6.775 $\pm$ 1.672
Complexity_Acaica	40	2	9	6.225 $\pm$ 1.747
Quality_Acaica	40	3	9	7.200 $\pm$ 1.572
Overall Liking_Acaica	40	3	9	6.975 $\pm$ 1.476
Purchase Intent_Acaica	40	1	9	6.575 $\pm$ 1.723
Appearance-GWBP	40	4	9	7.375 $\pm$ 1.547
Aroma-GWBP	40	3	9	7.050 $\pm$ 1.632
Flavor-GWBP	40	4	9	6.900 $\pm$ 1.549
Texture-GWBP	40	3	9	7.325 $\pm$ 1.623
Consistency-GWBP	40	4	9	7.300 $\pm$ 1.363
Complexity-GWBP	40	4	9	6.800 $\pm$ 1.265
Quality-GWBP	40	3	9	7.300 $\pm$ 1.667
Overall Liking-GWBP	40	4	9	7.150 $\pm$ 1.406
Purchase Intent-GWBP	40	2	9	6.800 $\pm$ 1.829

The sensory evaluation results are summarized in Table X. The highest-rated attributes for the acacia sample were quality (7.200  $\pm$  1.572) and Overall Liking (6.975  $\pm$  1.476), suggesting a

generally favorable reception. However, Aroma ( $5.950 \pm 1.449$ ) 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 ( $7.375 \pm 1.547$ ) and Overall Liking ( $7.150 \pm 1.406$ ) being the most preferred attributes. Notably, Purchase Intent for GWBP ( $6.800 \pm 1.829$ ) was higher than Acacia, 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 shows, Eigenvalues for Acacia Honey and Green walnut Bee Product

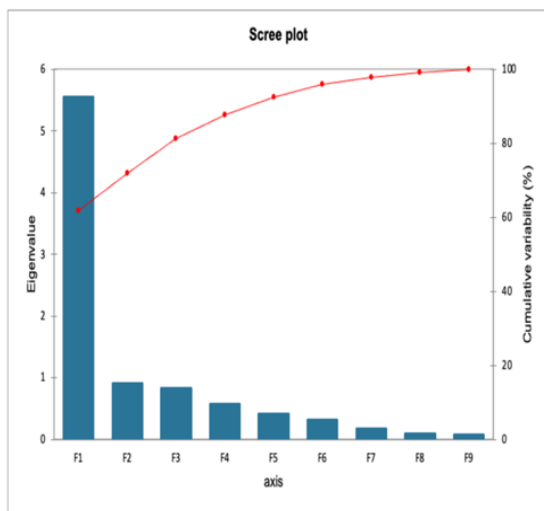
**Table.8 Showing the Eigen values for Acacia Honey**

	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

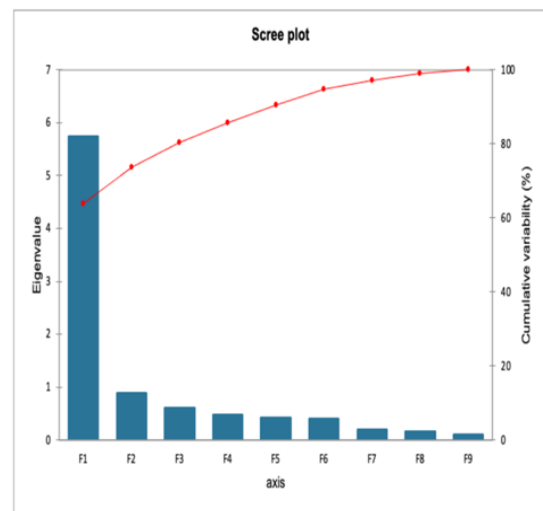
**Table.9 Showing the Eigen values for green walnut bee product**

	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 8 and 9, the scree plots are presented below.



Scree Plot for Acacia Honey



Scree Plot for Green walnut bee product

Figure. 24: Showing the Scree plots for the Acacia Honey and Green 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): indicates the extent of variance elucidated by each primary component Red Line (Cumulative Variability %) and 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 & 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 flavor, 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., color, 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

**Table. 10 Showing Correlations between variables and factors for Acacia Honey.**

	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

**Table 11. Showing Correlations between variables and factors for green walnut bee product**

	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

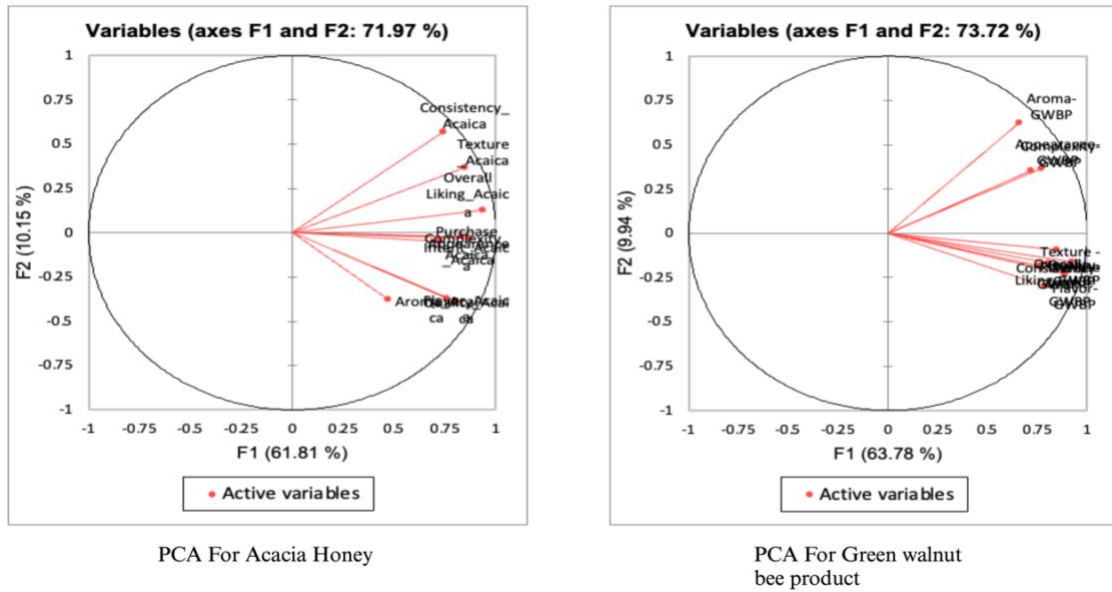


Figure 25. Showing the Principal component analysis done for both of samples (Acacia Honey and GWBP, Source: XLSTAT Output)

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 favored 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 behavior. 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.

## 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  $1.23 \text{ g cm}^{-3}$  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 (Daróczi, 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  $4.8 \text{ mmol L}^{-1}$ , which decreased to  $3.7 \text{ mmol L}^{-1}$  after 21 days, reflecting a reduction of  $1.1 \text{ mmol L}^{-1}$  (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.

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## Publications



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### 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.  
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#### Foreign language scientific articles in international journals (3)

2. **Shaikh, A. M.**, Wandhekar, S. S., Ahmed, A. E. M., Pandey, V. K., Oláh, C., Daróczy, L., Prokisch, J., Harsányi, E., Kovács, B.: Exploring the Ecological Implications, Gastronomic Applications, and Nutritional and Therapeutic Potential of *Juglans regia* L. (Green Walnut): A Comprehensive Review.  
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5. **Shaikh, A. M.**, Ahmed, A. E. M., Kovács, B., Daróczy, L., Oláh, C., Prokisch, J.: "Walnut wonders: a dive into bee product development with green extract".  
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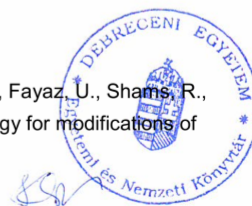


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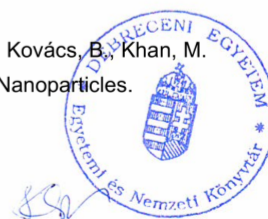


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