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EVALUATION OF ELEMENT CONTENTS IN ENRICHED HONEY USING INDUCTIVE COUPLED PLASMA-MASS SPECTROSCOPY AND INDUCTIVE COUPLED PLASMA-OPTICAL EMISSION SPECTROSCOPY

Supervisor

Prof. Antonio Masi

Co-supervisor

Prof. Bèla Kovàcs (University of Debrecen)

Graduating Student

Adelabu, Bridget Omowumi

Student Number: 2016498

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ABSTRACT

One of the most intricate food sources available to humans is Honey, a viscous natural liquid made by honeybees (*Apis mellifera*) from the nectar or secretion of plants. However, essential mineral and nutritional content are often lost based on the beekeeping or processing technique. Enriching Honey with spices is a common approach to improve Honey's nutritious and beneficial effects and help make up for essential vitamin and mineral contents lost during processing. This study focuses on the evaluation of honey samples enriched with different spices from different origins (Hungary and India). It adopts the inductively coupled plasma optical emission spectrometry and mass spectrometry method to analyze the element content, which is the most appropriate for achieving the study's objective. With these chosen methods, there is a grant for comparison with other future prospective approaches. The evaluation methods offer the possibility to verify outcomes, the accuracy, and efficacy of adequate sampling, the treatment of errors, and their shortcomings. The results show that most of the element content in enhanced honey samples increased by over 50% of the blank sample. The highest concentrations of Phosphorus, Sulfur, and Calcium were found in honey samples combined with Marjoram that was obtained from Hungary; similarly, the highest concentrations of Magnesium and Sodium were obtained in honey sample mixed with Parsley from Hungary, and the honey sample mixed with Ajwain seeds from India has the highest content of Zinc and Calcium.

According to their specified origin, the rise in element contents of enriched Honey differs. These differences demonstrate how the source of spices used as additives can improve nutritional benefits differently and give them a distinctive property. Hence, making enrichment a highly recommended way of improving Honey's nutritional value domestically and industrially.

CHAPTER ONE

1.0 Introduction

Background of the study

Honey production plays a vital role in the supply of food, and it also plays a role in the local industry within the country (Encyclopedia Britannica, 2005). Honey is made by bees from the sugary parts of plants (floral nectar) or secretions of other insects (such as honeydew) by regurgitation, enzymatic activity, and water evaporation. According to (Grüter, 2020), honeybees keep Honey in wax structures called honeycombs, but stingless bees store Honey in pots made of wax and resin. The difference in Honey produced by honeybees (the genus *Apis*) is well-known, due to its nationwide commercial production and human consumption (Grüter, 2020). Honey is collected from wild bee colonies or hives of domesticated bees, a practice referred to as beekeeping or apiculture (*meliponiculture* in the case of stingless bees).

Hungary is one of the European Union's biggest suppliers of natural Honey, amounting to 19.7 thousand tonnes of supply in the year 2005. The country accounts for almost 15,000 beekeepers, which mainly sell out Honey to the more expansive continents of the world. However, 5 thousand tonnes of the country's natural Honey are locally consumed.

Hungary is notable for supplying *Robinia pseudoacacia* flower honey, whereas other flowers pollinated include the Silkweed flower (Farkas et al., 2007). Other sources of Hungarian Honey were sunflowers and fruit trees.

According to (Batista et al., 2012), the composition of Honey can be related to its plant species, climatic conditions, ecological conditions, and good beekeeping practices. Furthermore, its composition is determined by the classes of food, aroma, and flavour, as well as 200 different compounds. (Atanassova et al., 2016) stated that different kinds of Honey are produced and sold in Hungary, this includes acacia, linden, sunflower, forest, rape, silk grass, chestnut, and so forth. The type of Honey also depends on the type of flower that produced them. The most famous Honey in Hungary is Acacia honey. Moreover, factors related to the environment and Honey's geographical origin influence its chemical and physical quality.

Due to the high demand for Honey each year, there is an increase in production to meet up with the ever-increasing market demands. This statistic revealed China was the leading producer of Honey worldwide in 2020. Its production volume amounted to about 458 thousand metric tons of Honey in the year 2020. The European Union is the world's second-biggest producer of Honey after China. Every year, about 600,000 beekeepers and 17 million beehives produce about 250,000 tons of Honey (EU Analytics, 2022).

There has been an increase in the worldwide sale of Honey in recent decades by numerous and intense trade between producing and consuming countries. The EU nations that produce the most Honey (Romania, Spain, Hungary, Germany, Italy, Greece, France, and Poland) are primarily found in southern Europe, where the climate is more suited to beekeeping (EU Analytics, 2022). The most famous Honey is accredited acacia honey. Every year, Hungary produces 10 thousand tons of acacia honey. A large portion of the acacia trees in Europe is grown in Hungary because of the good soil and favourable weather conditions. Another honey, which is famous in Hungary, is silkweed honey and it has a strong spicy perfume and flavour. Hungary is also famous for silkweed honey, which is spicy and has a strong fragrance.

According to CBI (2008), the amount of Honey consumed in Hungary is below the amount of Honey produced so that Hungary can supply its domestic market. It is the largest honey producer in the EU, and it provides Honey to western European countries. In the export market, Hungary has a strong competitor in developing countries.

1.1 Elements of Honey and their health benefits

From the olden days up to recent times, Honey has been one of the most important components of the human diet. According to (Spottiswoode et al., 2016), Honey is used as a food, especially in cooking, baking, and desserts, as a spread on bread, as an addition to various beverages such as tea, and as a sweetening agent in a few commercial beverages.

Because of its energy density, hunter-gatherer cultures consume Honey as food, most importantly in warm climates, with the Hadza people ranking Honey as their favourite food (Marlowe et al., 2014). Honey hunters in Africa have a mutualistic relationship with certain species of honeyguide birds (Spottiswoode et al., 2016).

The sweetness of Honey is produced from the monosaccharide fructose and glucose and has about the same sweetness almost as sucrose (table sugar). According to National Honey Board (2012), fifteen millilitres (1 US tablespoon) of Honey make around 190 kilojoules (46 kilocalories) of food energy.

Honey's flavour is distinctive, and it has favourable chemical qualities for baking when used as a sweetening agent. (Geiling, 2013) stated that most microorganisms do not develop in Honey; with this, sealed Honey does not spoil, even after thousands of years. It is stated that Honey can be given in some traditional or custom amounts to newborn babies based on some unreliable evidence. Children and adults may benefit from honey feeding because it leads to better memory power and body growth.

Honey contains mineral substances, making it a natural product. Phosphorus, potassium, sodium, calcium, copper, Magnesium, chromium, iron, zinc, and many others are some of the main mineral substances. Honey contains most of the potassium in a proportion of one-fourth to half of the total amount of mineral substances.

According to climatic conditions and botanical sources, mineral substances are distributed in different proportions. Besides soil type, the type of plant from which the nectar was collected affects the proportion.

Honey's chemical makeup and floral source both affect its quality. Because of its nutritional benefits as well as its medicinal capabilities, Honey has always been a staple in the diet. Carbohydrates are the key nutrients and health-related components, making them a good energy source, particularly for kids and athletes. Honey is a unique, healthy, functional, and healing food. In addition to having nutritional benefits, it also inhibits several food deterioration germs. Due to its high antioxidant action, it is preferred as a food additive or preservation in addition to direct consumption.

Furthermore, Theobald (2005) identified that Honey enhances the heart, neurological, and muscular performance. It contains a calcium compound that helps in the production of teeth and bones. Several reports have shown that some specific honey contains choline (0.3-25 mg/kg) and acetylcholine (0.06-5 mg/kg). These compounds are necessary for proper brain function. It also aids in the repair and construction of the cellular membrane. Honey contains a significant amount of iron. Because iron facilitates electron transmission in cells, red blood cells must include iron. Iron aids in the transition of hydrogen peroxide into free radicals by boosting hydrogen peroxide splitting. Furthermore, it boosts the number of erythrocytes in the body as well as the level of haemoglobin in the blood.

1.3 Physical and chemical properties of Honey

The component of Honey depends on its water content. On the other hand, it relies on the type of flora used to produce it (pasturage), temperature, and the proportion of the specific sugar contents. Freshly extracted Honey is a supersaturated liquid, it contains more sugar that can be dissolved in water at ambient temperatures. However, Honey is a supercooled liquid at room temperature, in which the glucose precipitates into solid granules to form a semisolid solution of precipitated glucose crystals in fructose solution, and solution of other ingredients, having a density ranging between 1.38 and 1.45 kg/L at 20 °C

1.4 Significant effects of elements on human health

Nature contains various elements, both micro and macro elements are essential to our bodies ability to function. Frieden et al., (2018) stated that a normal human body is composed of 11 macro and micro elements, notably Magnesium, sodium, potassium, and calcium. Magnesium, sodium, potassium, and calcium constitute 1.89% of the total weight but only 8.6 g of the rest of the body. The composition of the body can be analyzed in various ways. In the first place, it can be done in terms of the chemical elements present, or by molecular type which include, water, protein, fats (or lipids), hydroxyapatite (bones), carbohydrates (such as glycogen and glucose), and DNA. In the second place, it can be analyzed in terms of tissue type, which includes, water, fat, connective tissue, muscle, bone, and so on. Thirdly, it can be done in terms of cell type.

Hundreds of different types of cells are present in the body, but notably, the dominant cells contained in a human body (though not the largest mass of cells) are not human, but bacteria residing in the normal gastrointestinal tract of humans.

Ninety-eight per cent of the human body is composed of six elements, namely carbon, oxygen, hydrogen, nitrogen, Calcium and Phosphorus. the major components include Magnesium, calcium, sodium, and potassium. Then, 1.89 per cent includes Magnesium, calcium, sodium, and potassium, but the other 0.02 per cent contains the other eleven sets of elements. According to (Khan et al., 2014), these significant parts in the functioning of the organism can be received from nature in many ways. They all play a significant role in human health, and the latest knowledge of them has been obtained from the last 100 years.

The elements are mentioned below

I. Iron

Iron, which has the chemical symbol "Fe" and is one of the basic elements in the human body, is essential to the body's proper operation. Haemoglobin and lung tissues in the human body both contain iron. Iron can be derived from a range of foods, including fish, red meat, chicken, beans, vegetables, and so on. According to (Roger et al., 2011), unusual fatigue, a pale complexion, shortness of breath or diminished stamina, headaches and migraines, hair and skin conditions, tongue swelling, and soreness, restless legs, and migraines are all signs of iron deficiency.

II. Zinc (Zn)

Zinc is represented by the symbol "Zn". Zinc plays a significant part in metabolism as a catalyst for numerous enzymes. Inadequate zinc intake can cause serious deficiencies such as mental instability, diarrhoea, baldness, and postponed bone and sexual development (Peganova & Eder, 2004).

Lipids, carbohydrates, energy, and proteins can all be broken down with the help of zinc. It is required for skin care, takes part in metabolic processes, and helps cells develop (Osredkar & Sustar, 2011).

III. Manganese (Mn)

Although manganese is an essential mineral, it is only found in very small amounts in the human body. A vitamin that is essential to human health, it produces vital bodily tissues such as connective tissues, bones, and blood coagulation (Palacios et al., 2006). Additionally, manganese is necessary and significant for calcium absorption, blood sugar regulation, and carbohydrate digestion and metabolism. Manganese is crucial for calcium absorption, blood sugar regulation, digestion, and metabolism (Sigel et al., 2013).

IV. Copper (Cu)

All living things contain the essential metal copper. You can get copper, which has the chemical symbol (Cu), from foods like mushrooms, wheat, sunflower seeds, peanuts, beans, cashews, seafood, and poultry (Roger et al., 2011).
According to (Kodama & Fujisawa, 2009), consuming too much copper might result in several different health issues in the body. This can obstruct foot and hand procedures and comprises osteoporosis, nerve degeneration, and anaemia. Various genetic and neurological problems in humans are caused by copper.

V. Lead (Pb)

Major organs like the brain, kidney, bone marrow, liver, and different tissues are where lead is primarily found in the body. The bones and teeth of the human body contain lead (Flora et al., 2012). Additionally, it can damage practically all human organs and systems. Pesticides, water, automobile paint, and other items may contain lead, a reasonably common metal in daily life. Without any digestion, as well as through ingestion and direct contact, the circulation swiftly absorbs lead in the human body.

VI. Aluminium (Al)

The third most prevalent element on Earth is aluminium, represented chemically by the letter Al. It is non-toxic to the body and has levels in human urine and serum that vary from 3 to 9 micrograms per litre. The largest concentration of aluminium in the human body is found in the epidermis of the skin, while the lowest concentration is found in brain tissues, (Jørgensen, 2000). In healthy people, the amount of aluminium varies from 0.9 mg/kg to 4.4 mg/kg.

VII. Cadmium (Cd)

Emphysema, carcinogenicity, kidney impairment, and decreased reproductive ability are among cadmium's most prevalent and damaging effects in humans, according to (Reimann & De Caritat, 2012). Through ingestion and breathing, cadmium enters the human body.

VIII. Boron (B)

According to Li et al., (2000), the soft tissues of the kidneys and the brain have boron concentrations ranging from 0.0 to 0.6 mg/kg. Boron is represented by the scientific symbol B. An adult has a different concentration with an average of 0.03mg/kg. Additionally, because boron is a harmful gas, it can make you feel sick to your stomach and induce diarrhoea and other nauseating symptoms. On-parenteral injection could cause serious injury (Murray, 1995).

1.5 Macro Elements

The following are the important elements for human existence, I examined Phosphorous, Magnesium, Zinc, Boron, Calcium, Sulphur, and Sodium by using ICP-OES and ICP-MS during my analysis.

1. Phosphorus (P)

Phosphorus can be extracted from poultry, meat, fish, dairy products, and eggs, which are high in Phosphorus. According to Mandell et al. (2020), the right amount of Phosphorus helps in the production of energy, muscle movement, strong bone and tooth, and the production of the body's

DNA and RNA. Many deficiencies are caused by high and low quantities of Phosphorus in the human body. Diabetes results from low phosphorus levels, whereas a high level of Phosphorus promotes weakness and heart disease.

2. Calcium

Calcium is one of the most important elements found in the human body, it is symbolized by the symbol "Ca". Calcium aids in the development of bones and enhances bone health. According to (Pravina et al., 2013), excessive calcium intake increases blood pressure, increases cholesterol, and impairs renal function. It is mostly seen in dairy products, and one kilogram of hard cheese has 100 grams of calcium.

3. Sodium (Na)

Humans consume 1 to 3 grams minimum of sodium daily, which is very important for health. Sodium may be obtained from various sources of foods, including fish, meat, vegetables, and dairy products. The salt contains sodium as well, and we employ salts in the chilling process. (Strazzullo & Leclercq, 2014) link excessive salt use to a variety of diseases, including diarrhoea, perspiration, high fever, and exhaustion. It also plays an important role in the stability of human blood pressure.

4. Sulphur

The element may be obtained via dairy products and other which foods are grown using primary farming practices (Parcell, 2002). Sulfur aids in the treatment of dermatological issues such as wood healing, acne, and the effects of radioactive elements (Flagg et al., 1994). Because of its biological relevance, sulphur is an inorganic element found in amino acids, numerous compounds, vitamins, enzymes, and many other things (Komarnisky et al., 2003).

5. Potassium (K)

The chemical element Potassium plays a vital role in the electrical and cellular functioning of the human body. The electrolyte, which is made up of potassium, is one of the most important blood minerals and carries electrical potential, albeit in very minute amounts. The electrolyte contains positively charged ions and plays a crucial role in energy transmission in the bodily cell (Drobner & Tyler, 1998)

CHAPTER TWO

2.0 Research Plan and Aim

The EU as the second largest producer of Honey in the world has its products abundant in the market which includes Riga central market and the Hague market.

Many buyers consume the product raw, and others make it an important constituent of their diet, this makes me have deep pleasure in evaluating the element contents in enriched Honey using inductive coupled plasma-mass spectroscopy and inductive coupled plasma-optical emission spectroscopy. Different topics related to the study were reviewed for the dissertation. This includes food products such as spices, condiments, soybeans, cardamom, etc. The production of enriched Honey and its methods help meet consumers' daily increasing demands.

In other to achieve the aim which is to identify the element contents before and after enrichment and see which spice is best for enrichment. I have chosen the Food Technology Laboratory, University of Debrecen. Some Hungarian honey was selected and analyzed in the laboratory. The process of selection and analysis was supervised by Prof. Bèla Kovács (University of Debrecen). The most popular and largely consumed Honey is acacia, Honey amongst different other types present in Hungary. Macro and micro-elements were found in the Honey which includes K, Ca, Na, Mg, P, S, and Al, B, Mn, Fe, Cu, As, Cd, Pb, and Zn, respectively.

2.1 Planned Study Flowchart

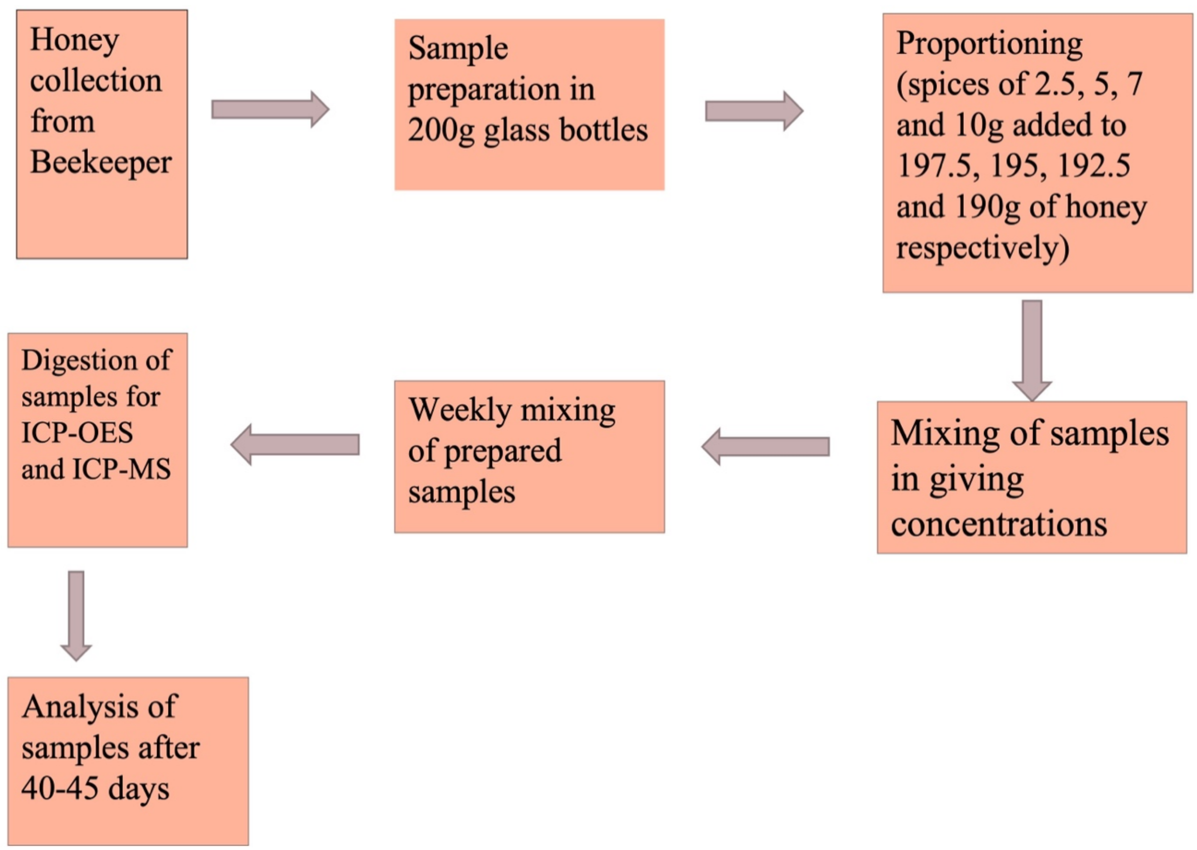

Figure 1 Research Flowchart

CHAPTER THREE

3.0 Materials and Methods

This chapter includes the research methodology that was applied in the evaluation of element contents in enriched Honey using inductive coupled plasma-optical emission spectroscopy (ICP-OES). An inductive Coupled Plasma Mass Spectrometer (ICP-MS) was used to determine the concentration of micro and macro elements in Honey. The study was conducted in the Food Technology Laboratory, University of Debrecen. Moreover, 20 samples of fortified Honey were collected and examined. Five spices were collected from Hungary and four from India.

3.1 Samples

3.1.1 Honey Sample

Honey made from acacia trees is called acacia honey. It is a common honey because of its mild fragrance. In comparison to wide other varieties of nectar, acacia honey tastes gentler and doesn't crystallize. There are two hues of acacia honey: pale yellow and dark yellow. Famous and consumed for its nutritional value and therapeutic benefits (Revathi et al., 2017), from bright yellow to practically transparent as crystal.

Rape honey easily solidifies and has a light amber colour. It smells like a rape flower and is sweet with a distinctive flavour. Rape honey shares the same hue as acacia honey in terms of colour. Acacia honey generally has a lower calorific production than rape or forest honey, and its market price is significantly higher than rape honey. Furthermore, acacia honey is more popular with consumers than forest, linden, or rape honey. As a result, rape honey is mostly used to substitute for acacia honey in the market (*Wang, 2013*, n.d.). The acacia honey has a distinctive flavour and is used to increase the quality of blended Honey, which is a popular commerce item in Hungary.

Acacia honey obtained from various explorations is of excellent quality and contains a variety of beneficial compounds, including minerals, phenolics, flavonoids, and unsaturated fats. It is very nutritional and has potent neurologic, immunomodulatory, antiproliferative, and cell-reinforcing properties, making it a strong candidate for both cancer prevention and treatment. From a neurological perspective, it might function as a plausible restorative agent in the treatment of Alzheimer's disease (Muhammad et al., 2016).

Acacia honey significantly reduced the amount of acetylcholinesterase (AChE) activity in the brain, which was amplified in the presence of sodium arsenate. Additionally, a strong correlation between sodium and calcium particle levels and acetylcholinesterase activity in the brain was observed. These findings suggest that acacia nectar regulates acetylcholinesterase activities, which may be investigated in the treatment of Alzheimer's infections. (Muhammad et al., 2015). Other effects of acacia honey include hepatoprotection by lowering serum aminotransferase levels and maintaining the integrity of hepatocytes, nephroprotection by lowering blood urea and creatinine levels, and raising of haematological parameters (white platelets, red platelets).

The Acacia Honey sample (*Robinia pseudoacacia*) used in this study was collected from a Beekeeper from the Miskolc region. Honey was collected from the mountains of Bükki National Park in December 2021. The samples were brought in sterile jars, the samples were kept away from sunlight at room temperature.

Macro and microelement analysis was done in June 2022, but mixing spices was done at the end of February 2022 and early March 2022.

Figure 1 Control Sample 200g of Acacia honey

3.1.2 SPICES

- Ajwain

Trachyspermum ammi is an annual herb in the Apiaceae family that is also known as ajowan caraway, thymol seeds, Bishop's weed, or carom. (Setiawan et al., 2020). It is also called Bishop's weed, and the plant can also be eaten by humans. (Naquvi et al., 2022), Ajwain possesses anti-disease qualities in humans. However, there is little high-quality clinical evidence available. It is also used as a dietary supplement in medicines (Capsules, liquids, and powders) (Bishop's weed: *Trachyspermum ammi L*. Ajwain is utilized in herbal blends in traditional medicine systems such as Ayurveda on the belief that it can treat a variety of ailments (Duke, 2002).

Figure 2 Ajwain seeds

- Fenugreek

(*Trigonella foenum-graecum* L.) also called methi (in Hindi), has been used as a culinary spice, flavouring agent, and medicinal plant since antiquity. Fenugreek is a spice that is used to increase the flavour and colour of food and make it tastier. It is also used to change the texture of foods (Ghosh et al., 2015).

Mature seeds are high in amino acids, fatty acids, vitamins, saponins, and acids (84mg/100g). Fenugreek also contains the following compounds: diosgenin, gitogenin, neogitogenin, homorientin saponaretin, neogigogenin, and trigogenin (Mohammad et al., 2006). Fenugreek use has been shown safe for human life and may be easily applied for health benefits due to its high fibre content and other bioactive components. With its high concentration of phytochemicals, fenugreek seed not only helps to lower low-density cholesterol and triacylglycerol but is also utilized to lower blood sugar levels (Ghosh et al., 2015).

Figure 3 Fenugreek seeds

- Cumin

Cumin is derived from the herb *Cuminum cyminum*, which is a member of the parsley family. Cumin is hand-harvested when it reaches a height of 30-50 cm (12–20 in). It's a slender, glabrous, branched annual herbaceous plant that grows to a height of 20-30 cm and has a diameter of 3-5 cm (1+14-2 in) (8–12 in) (Divakara Sastry & Anandaraj, 2013).

In a 100-g reference number, cumin seeds provide a large percentage of the Daily Value for fat (especially monounsaturated fat), protein, and dietary fibre. Vitamin B, vitamin E, and a range of dietary minerals, including iron, Magnesium, and manganese, are all abundant. Cumin seeds contain petroselinic acid. (Bettaieb et al., 2011) (Hewlings & Kalman, 2017).

Figure 4 Cumin seeds

- Marjoram

Marjoram (*Origanum majorana*) is indigenous to Cyprus and Southern Turkey. It is mostly cultivated in Hungary, Greece, France, the United States, and the Mediterranean. Marjoram has its classification as a medicinal herb that has been in use for thousands of years. Essential oils have been extracted from this herb all over the world (Greathead, 2003).

Due to the presence of volatile aromatic compounds such as eugenol, citral, geranyl acetate, cadinene, ocimene, linalyl acetate, carvacrol, terpineol, linalool, and various other terpenes, the essential oil extracted from various species of Marjoram has a high nutritional value and extreme therapeutic potential. This oil has been used to treat rheumatism, muscle pains, and flatulence because of its anti-bacterial and anti-inflammatory properties. Terpineol, terpinene-4-ol, p-cymene, and myrcene are some of the key chemical elements of this plant (Liu et al., 2021).

Figure 5 Marjoram leaves

- Oregano

Oregano (Origanum vulgare) is a strong, potent herbaceous herb. Although commonly used as a fragrant cooking herb, oregano has been employed in traditional practices for generations because of its camphorous and herbal fragrance. Carvacrol is the major component of oregano: a phenol with antioxidant capabilities. Oregano generates essential oil, although it is not often utilized in aromatherapy due to its potent chemical component.

Figure 6 Oregano flowers

- Parsley

Parsley (*Petroselinum crispum*) is a biennial aromatic plant from the *Apiaceae* family that has an unbranched root, pinnately split leaves, umbels, and schizocarp. It includes essential oil in every portion, with the primary components being phenylpropane and terpene chemicals. It is high in flavonoids and other polyphenolic chemicals, including furanocoumarins, carotenoids, and polyacetylenes, and its leaves are high in vitamins and minerals (Punoševac et al., 2021). As a result, parsley has powerful diuretic and disinfectant qualities, and frequent use of parsley as food or supplement can help to prevent the effects of free radical-induced carcinogenesis and cancer (Danciu et al., 2018).

In addition to its medical benefits, parsley is widely used as a culinary additive around the world, mostly in salads, sauces, and soups, and as an ingredient in herb butter and cheese, but also a variety of foods and beverages (Burlando et al., 2010).

Figure 7 Parsley Leaves

- Cardamom

E. cardamomum is a woody, fragrant, strong, and distinctive flavour-characterized spice with two varieties which are utilized in food and beverages as flavourings, cooking spices, and medical purposes. Smoked *E. cardamomum*, often known as green cardamom, is used as a spice and masticator (The Uses of Cardamom, 2018)

Cardamom is the major spice that people liken to mint because of its potent, slightly sweet flavour. It is used in both sweet and savoury cuisines and has its origins in India. It is currently available everywhere. Cardamom is a spice whose seeds, oils, and extracts have long been employed in traditional medicine due to their reputedly potent therapeutic effects.

Figure 8 Green Cardamom seeds

- Rosemary

An aromatic evergreen shrub known as Rosemary, *Salvia Rosmarinus*, has needle-like leaves and white, pink, purple, or blue flowers. After cultivation, the leaves, twigs, and flowering apices are gathered for use (Burlando and others, 2010). A variety of cuisines, including stuffing and roasting meats, use the leaves (Rosemary: Information and Uses, 2021).

It has been discovered that rosemary oil can increase the thermal stability and shelf life of rancid omega-3-rich oils. (Oregano and rosemary extracts promise preservation of omega-3s, 2017) Additionally, Rosemary has antimicrobial properties (Nieto et al., 2018).

Figure 9 Rosemary leaves

Table 1 Spice and their source

SPICES	SOURCE
Ajwain	India
Cardamom	India
Cumin	Hungary and India
Fenugreek	India
Marjoram	Hungary
Oregano	Hungary
Parsley	Hungary
Rosemary	Hungary

3.2 Sample preparation

The samples to be evaluated were prepared in different concentrations of A (2.5%), B(5%), C (7.5%) and D (10%) to make a total of 200g, respectively.

As an illustration, four different samples were obtained: we added 2.5g of spices to 197.5g of Honey in a, 5g of spices to 195g of Honey, 192.5g of Honey and 7.5 g of spices, and 190g of Honey to 10g of spices.

We created 109 samples in all, including the replicates and the blank sample, which we maintained in a dark room for 40 days, mixing each sample once a week to ensure homogeneity.

Sensory analysis was performed every week to track the changes continuously. Each week, the Honey's flavour has been intensely enhanced with spices.

Table 2 Examined Acacia honey with enriched spices, all the honey samples were prepared from the same type of Acacia honey

Sample	Concentrations
Acacia Honey- Cumin India (AC-1- CUI)	
1.00	A
2.00	B
3.00	C
4.00	D
Acacia Honey- Cumin Hungary (AC-1- CUH)	
5.00	A
6.00	B
7.00	C
8.00	D
Acacia Honey- Cardamom (AC-1- CRI)	
9.00	A
10.00	B
11.00	C
12.00	D
Acacia Honey- RosemaryHungary (AC-1- RSH)	

13.00	A
14.00	B
15.00	C
16.00	D
Acacia Honey- Marjoram Hungary (AC-1- MJH)	
17.00	A
18.00	B
19.00	C
20.00	D
Acacia Honey- Oregano (AC-1-ORH)	
21.00	A
22.00	B
23.00	C
24.00	D
Acacia Honey- Fenugreek India (AC-1- FEI)	
25.00	A
26.00	B
27.00	C
28.00	D
Acacia Honey-Parsley Hungary (AC-1- PRH)	
29.00	A
30.00	B
31.00	C
32.00	D
Acacia Honey- Ajwain India (AC-1- AJI)	
33.00	A
34.00	B
35.00	C
36.00	D

3.3 Mixing

3.3.1 Acacia Honey (Hungary) - Cumin Spice (India) and
3.3.2 Acacia Honey (Hungary) - Cumin (Hungary)

The Honey was collected from Bükki national park near Miskolc through Beekeeper, and the spice (Cumin) samples were collected from India and Hungary. A total of 4*4 concentrations were prepared: 2.5, 5, 7.5 and 10 % of Cumin which were further added in 197.5, 195, 192.5 and 190 g of Honey inglass bottles.

The Following pictures (Figure 10 & 11) shows the cumins from India and Hungary bought from the Asian market and the SPAR store in Hungary.

The Cumin (Figures 10 & 11) was mixed in honey samples in different concentrations and kept for 45 days in a dark room and at maintained room temperature. Weekly mixing has been done for increasing its flavour and sensitivity.

Figure 11 Cumin (Jeera) India

Figure 10 Cumin Hungary

3.3.3 Acacia Honey (Hungary)- Cardamom (India)

Honey was obtained from a beekeeper in the Bükki National Park near Miskolc, while spice (cardamom) samples were obtained from India. A total of four concentrations of Cardamom 2.5, 5, 7.5, and 10% were p 192.5, and 190 g of Honey in glass bottles respectively.

Figure 12 Green Cardamom India

Various Cardamom spice co ples of Honey, which were then stored for 45 days at a constant room temperature in a dark environment. For improving its flavour and sensitivity, mixing has been done weekly.

3.3.4 Acacia Honey (Hungary) - Rosemary (Hungary)

The spice (Rosemary) samples were obtained from the SPAR store in Debrecen (Hungary), and the Honey was obtained from Bükki National Park near Miskolc through a beekeeper. A total of four Rosemary concentrations 2.5, 5, 7.5, and 10%—were prepared and then added to 197.5, 195, 192.5, and 190g of Honey in glass bottles.

Figure 13 Rosemary Hungary

3.3.5 Acacia Honey (Hungary)- Fenugreek (India)

The Honey was obtained through a beekeeper from Bükki National Park in Miskolc and the spice (fenugreek) samples were purchased from the Asian market in Debrecen, Hungary. Glass bottles containing 197.5, 195, 192.5, and 190g of Honey were filled with respective amounts of four different fenugreek concentrations: 2.5, 5, 7.5, and 10%.

Figure 14 Fenugreek India

Varying amounts of the spice (fenugreek) were added to samples of Honey, which were then stored for 45 days at a constant room temperature in a dark space. To increase its flavour and sensitivity, weekly mixing has been done.

3.3.6 Acacia Honey (Hungary)- Parsley (Hungary)

The samples of parsley spice were acquired from the SPAR supermarket in Debrecen, Hungary, while the Honey was obtained from a beekeeper in Miskolc's Bükki National Park. Glass bottles containing 2.5, 5, 7.5, and 10% of four different parsley concentrations were each filled with 197.5, 195, 192.5, and 190g of Honey, respectively.

Different amounts of the parsley spice were added to samples of Honey, which were then stored for 45 days at a constant room temperature in a dark space. To increase its flavour and sensitivity, weekly mixing has been done.

Figure 15 Parsley Leaves (Hungary)

3.3.7 Acacia Honey (Hungary)- Marjoram (Hungary)

The marjoram spice samples were from a SPAR store in Debrecen, Hungary, and the Honey came from a beekeeper in Miskolc's Bükki National Park. Each glass container held 197.5, 195, 192.5, and 190g of Honey, respectively, and included 2.5, 5, 7.5, 10%, and 10% of four different marjoram concentrations.

Figure 16 Marjoram (Hungary)

Samples of Honey were mixed with various amounts of Marjoram before being kept for 45 days in a dark, constant room-temperature environment. Mixing has been done weekly to improve the product's flavour and sensitivity.

3.3.8 Acacia Honey (Hungary) - Ajwain (India)

The Honey was collected from Bukk national park near Miskolc through Beekeeper, and the spice (Ajwain) samples were collected from India; and Total four concentrations were prepared 2.5, 5, 7.5 and 10% of Cumin which was further added in 198.5, 195, 192.5 and 190 g of Honey in glass bottles.

Figure 17 Ajwain Seeds (India)

Ajwain was added to honey samples in varying amounts, and the mixture was then held for 45 days at a constant room temperature and in a dark environment. Its flavour and sensitivity have been increased through weekly mixing.

3.3.9 Acacia Honey (Hungary) – Oregano

Honey was procured from a beekeeper in the Bukk National Park near Miskolc, and samples of the spice (Ajwain) were procured from India. A total of four concentrations of the spice 2.5, 5, 7.5, and 10% were prepared before they were mixed with 197.5, 195, 192.5, and 190 g of Honey, respectively, and placed in glass bottles. Honey samples were mixed with oregano in varied proportions, and the mixture was then kept for 45 days in a dark, constant room-temperature environment. Through weekly mixing, its flavour and sensitivity have improved.

Figure 18 Oregano leavees

3.4 Pre- digestion and Digestion of Samples

The sample preparations were carried out by the procedures suggested by Kovács et al., (1996). 10 ml of nitric acid (69% v/v) (VWR International Ltd, Radnor, USA) was added to 2.5 to 3 ml of enriched honey samples in digestion tubes, and the samples were then left overnight to undergo additional reaction.

During the pre-digestion stage, the honey samples were heated in the digestion unit at 60 °C for 30 minutes (Figures 19, 20 & 21). 3 mL of hydrogen peroxide (30% v/v) (VWR International Ltd, Radnor, USA) was added after the honey samples had cooled. During the initial digestion phase, the materials were heated at 120°C for 90 minutes. The final volume of the sample, 50 ml, was created by chilling ultrapure water (Millipore S.A.S. Molshein France; Millipore Q water purification equipment). The ingredients were then homogenized and filtered through subjective filter paper into sterile centrifuge tubes (Sartorius Stedim Biotech SA, Gottingen, Germany). The samples were stored in these tubes until they were examined using ICP-OES and ICP-MS.

Figure 19 Digestion unit with Nitric acid and Hydrogen peroxide

Figure 20 Samples after pre-digestion with Nitric acid

Figure 21 Samples after final digestion with Hydrogen Peroxide

Figure 22 Filtration of digested samples into centrifuge tubes

3.5 Determination of Element Content

Using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) (Thermo Scientific iCAP 6300, Cambridge, UK) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS, Thermo Scientific X-Series II) the amounts of Phosphorus, boron, Magnesium, potassium, zinc, calcium, sulfur, iron and sodium were determined. The following picture is taken from the laboratory, which shows the ICP-OES and ICP-MS process

Figure 24 Inductively Coupled Plasma Optical Emission Spectrometer

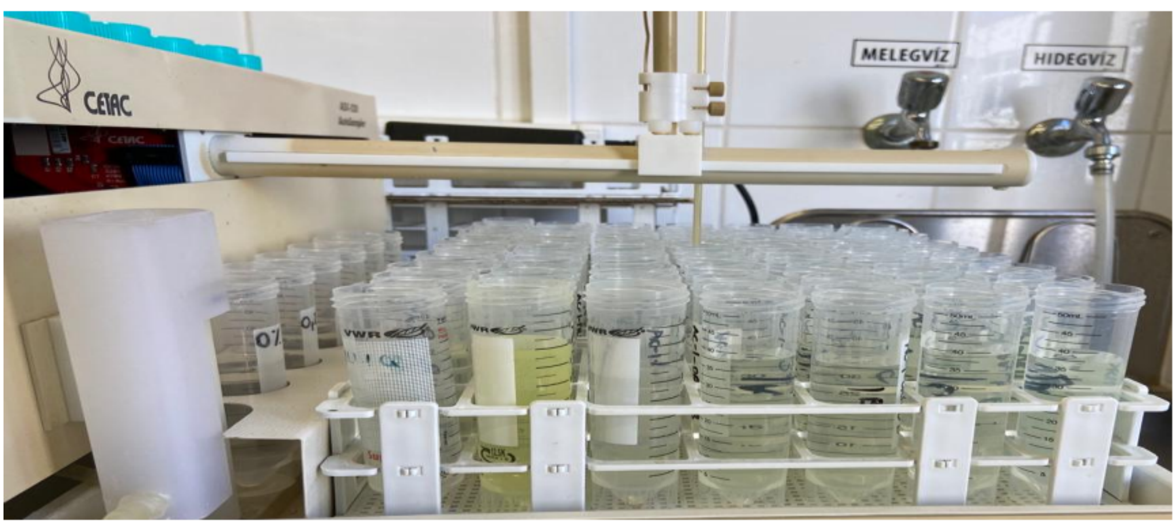

Figure 23 Samples injected through ICP-OES

Figure 25 Inductively Coupled Plasma Mass spectrometer

Figure 26 Samples injected through ICP-MS

CHAPTER FOUR

4.0 Results & Discussions

The table below shows the result obtained from nine different spices samples mixture with Acacia Honey with four different concentrations in each. Here A, B, C, and D stands for four different concentration of spices which are respectively 2.5%, 5%, 7.5% and 10%.

Table 3 Quantity of Chemical elements (mg/kg) in examined samples (Cumin (India, Hungary), (Cardamom India)

Sample Name	P	Zn	Mg	Ca	S	B	Na
Blank Sample	79,783±1.49	1.29 ± 0.28	2.87±0.01	23.09±0.43	13.30±0.25	3.59±0.34	11.96±0.19
AC-Cumin India A	71.97±4.22	1.36±0.15	7.49±0.73	34.37±1.21	23.30±0.10	3.74±0.51	26.37±2.99
AC-Cumin India B	73.50±2.72	1.08±0.12	11.77±0.71	52.63±0.46	36.97±0.68	3.54±0.40	36.57±2.15
AC-Cumin India C	75.97±0.65	0.94±0.07	14.90±0.56	77.30±1.13	43.17±0.64	3.39±0.26	47,70±1.80
AC-Cumin India D	76.10±3.93	0.92±0.11	20.77±1.66	88.37±0.45	55.37±0.98	3.83±0.01	63.63±1.51
AC-Cumin Hungary A	77.33±1.69	0.97±0.10	5.05±0.39	23.30±1.85	20.03±0.81	3.61±0.28	9.26±0.36
AC-Cumin Hungary B	72.43±4.82	0.88±0.08	4.37±0.12	38.97±0.67	18.93±0.55	3.31±0.13	7.52±0.43
AC-Cumin Hungary C	78.63±2.84	0.98±0.12	4,83±0.29	29.33±0.31	21.43±1.21	3.71±0.11	8.29±0.12
AC-Cumin Hungary D	74.33±1.17	0.93±0.03	4.90±0.23	26.33±0.31	20.90±0.70	3.45±0.18	9.56±0.22

AC-Cardamon India A	56.30±1.15	0.66±0.00	6.64±0.45	23.40±2.21	19.23±0.38	3.19±0.19	24.07±2.36
AC-Cardamon India B	57.33±0.67	0.79±0.08	10.57±0.29	24.77±1.74	21.17±1.03	3.20±0.17	38.90±0.72
AC-Cardamon India C	64.73±0.40	0.75±0.03	15.10±0.62	25.73±1.80	22.80±0.66	3.41±0.37	57.47±1.55
AC-Cardamon India D	64.47±1.29	0.75±0.04	17.53±0.12	25.00±2.09	23.07±0.64	3.46±0.27	77.20±1.57

Table 4 Quantity of Chemical elements (mg/kg) in examined samples Fenugreek (India), Oregano (Hungary), Parsley (Hungary)

Sample Name	P	Zn	Mg	Ca	S	B	Na
Blank Sample	79.78±1.49	1.29±0.28	2.87±0.01	23.09±0.43	13.30±0.25	3.52±0.40	12.03±0.14
AC-Fenugreek India A	52.7±2.12	0.63±0.00	6.60±0.46	46.67±3.33	25.43±0.15	3.30±0.34	19.70±1.65
AC-Fenugreek India B	54.33±1.72	0.74±0.05	11.53±0.35	74.90±1.54	32.40±1.32	3.22±0.23	31.33±1.21
AC-Fenugreek India C	55.33±0.57	0.68±0.06	15.33±0.25	90.80±0.70	38.83±1.10	3.32±0.32	40.47±0.57
AC-Fenugreek India D	58.33±1.36	0.84±0.02	21.37±1.05	115.67±1.49	47.00±1.15	3.74±0.03	57.23±1.76
AC-Oregano Hungary A	83.97±2.35	1.36±0.23	7.61±0.59	77.40±5.40	23.63±0.45	3.15±0.19	14.73±1.86
AC-Oregano Hungary B	91.13±1.31	1.04±0.03	8.05±1.35	59.23±4.81	27.30±0.61	3.11±0.48	13.90±1.44
AC-Oregano Hungary C	105.0±4.58	1.11±0.03	12.87±0.21	103.67±3.79	34.23±1.44	3.23±0.32	11.67±0.29

AC-Oregano Hungary D	100.33±0.58	0.84±0.05	14.17±0.23	99.70±5.47	34.80±3.33	3.23±0.19	17.00±2.09
AC-Parsley Hungary A	93.93±11.00	0.89±0.07	16.03±1.76	76.03±8.79	24.70±2.88	2.58±0.36	42.23±2.75
AC- Parsley Hungary B	121.33±7.64	1.16±0.11	34.37±4.27	139.67±2.52	35.87±1.85	3.47±0.37	83.43±5.99
AC- Parsley Hungary C	118.0±5.57	1.28±0.33	35.90±1.48	136.33±24.83	44.87±3.71	3.57±0.62	100.20±7.23
AC- Parsley Hungary D	121.67±5.51	1.14±0.12	34.40±0.17	117.0±4.36	41.63±0.64	3.27±0.10	107.33±3.21

Table 5 Quantity of Chemical elements (mg/kg) in examined samples Ajwain (India), Marjoram (Hungary), Rosemary (Hungary)

Sample Name	P	Zn	Mg	Ca	S	B	Na
Blank Sample	79.91±1.52	1.31±0.26	2.85±0.04	23.13±0.42	13.27±0.31	3.52±0.40	12.03±0.14
AC-Ajwain India A	101.30±12.82	1.34±0.23	5.51±0.38	57.13±3.33	23.43±3.01	3.60±0.66	17.87±1.17
AC-Ajwain India B	89.40±3.50	1.16±0.25	9.03±0.20	103.23±6.19	26.77±2.04	3.32±0.03	14.71±0.77
AC-Ajwain India C	96.13±8.02	2.25±0.12	11.47±0.50	121.67±14.01	30.60±2.10	3.60±0.37	15.93±1.55
AC-Ajwain India D	88.80±7.73	1.14±0.09	14.27±2.12	155.33±10.02	33.50±2.91	3.61±0.37	10.91±2.71
AC-Marjoram Hungary A	96.20±6.29	1.19±0.25	9.62±0.12	87.30±13.08	31.13±2.51	2.99±0.28	19.13±1.22
AC-Marjoram Hungary B	108.67±2.08	0.99±0.10	15.37±0.67	115.33±4.04	42.23±1.01	3.45±0.31	20.07±1.45

AC-Marjoram Hungary C	120.33±12.90	1.29±0.44	15.80±10.10	152.33±4.04	52.80±5.59	3.33±0.55	20.00±2.54
AC-Marjoram Hungary D	129.67±8.02	1.36±0.35	26.47±3.92	159.00±23.64	61.07±4.27	3.52±0.28	24.40±1.18
AC-Rosemary Hungary A	89.20±4.28	1.07±0.04	3.74±0.36	39.67±9.45	19.90±1.15	3.38±0.31	7.92±2.26
AC-Rosemary Hungary B	91.47±4.56	1.19±0.14	4.41±0.56	28.93±0.25	21.67±0.80	2.84±0.12	10.42±1.17
AC-Rosemary Hungary C	96.53±4.27	1.36±0.16	4.52±0.42	32.70±1.13	21.87±0.51	3.57±0.07	10.37±0.76
AC-Rosemary Hungary D	95.23±4.53	1.16±0.15	5.00±0.14	59.57±4.89	21.73±1.18	3.15±0.19	16.67±1.55

4.1 Phosphorus Content

Phosphorus is utilized by the body to maintain bone strength and health. Phosphorus is also important in the removal of waste and the restoration of damaged tissues. Most people obtain sufficient Phosphorus from their diet. Herbs and spices are good sources of Phosphorus.

When we carried out this experiment (Figure 27), we observed that the amount of P was greater than 70 milligrams in all the concentrations tested. In the blank sample, the amount of Phosphorus found was 79.78±1.48 mg/kg. This revealed Acacia honey as a rich source of Phosphorus. The samples found to be less quantity of Phosphorus than the blank sample are AC-Cumin India and AC-Cumin Hungary at all levels of concentration. It was found that the highest amount of Phosphorus was found in AC- Marjoram Hungary 10% concentration where the quantity was 129.67±8.02 mg/kg, while the lowest amount was discovered in AC- Fenugreek India 2.5 % concentration, where the amount was 52.7±2.12 mg/kg. Phosphorus was identified as the second most abundant chemical element in the Acacia Honey and mixed spices, following Potassium, after carefully examining all the samples.

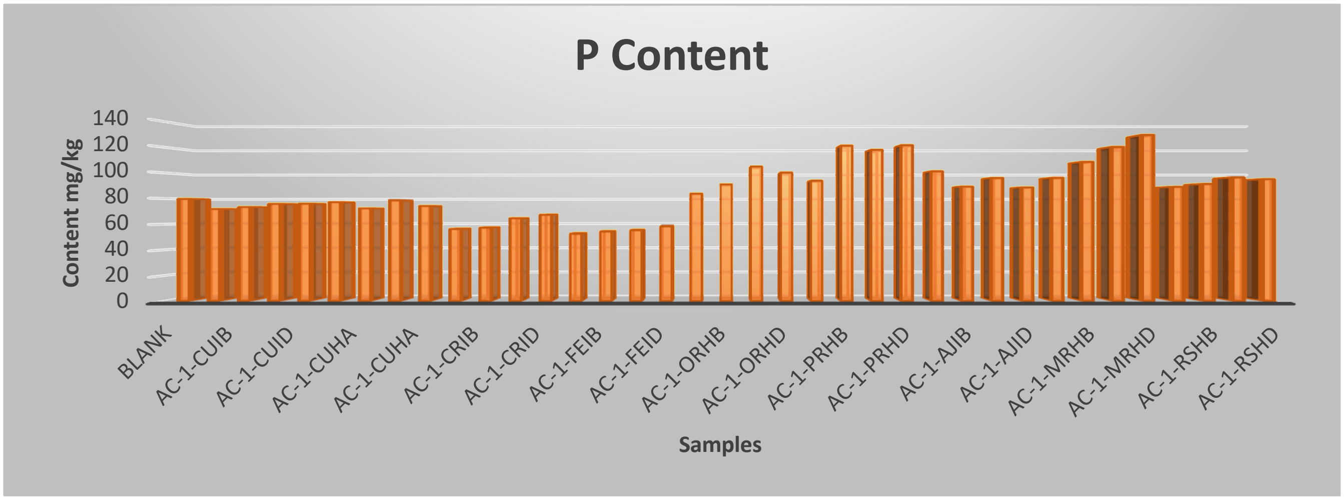

Figure 27 Phosphorus Content in Acacia Honey and mixed spices

4.2 Zinc Content

Zinc is essential for the proper functioning of our immune system and metabolism. Zinc is also necessary for wound healing and the senses of taste and smell. Our bodies typically obtain enough zinc through a well-balanced diet. Chicken, red meat, and fortified morning cereals are all good sources of zinc, however spices and Honey have a lower zinc concentration than these foods.

Similar results were seen in our experiment (Figure 28), except that the amount of zinc was the smallest compared to the other chemical components present. Approximately 1 mg of zinc may be detected in each of the examined mixed samples. The zinc level in AC-Ajwain India at 7.5% concentration was the highest with a total content of 2.25±0.12 mg/kg, which is over 50% higher than the blank sample. Mixed honey sample groups which display a good quantity at all four concentrations, are AC- Oregano, AC- Marjoram, AC- Ajwain, and AC- Rosemary. Other samples show low content and the lowest group being AC-Fenugreek at all concentration levels.

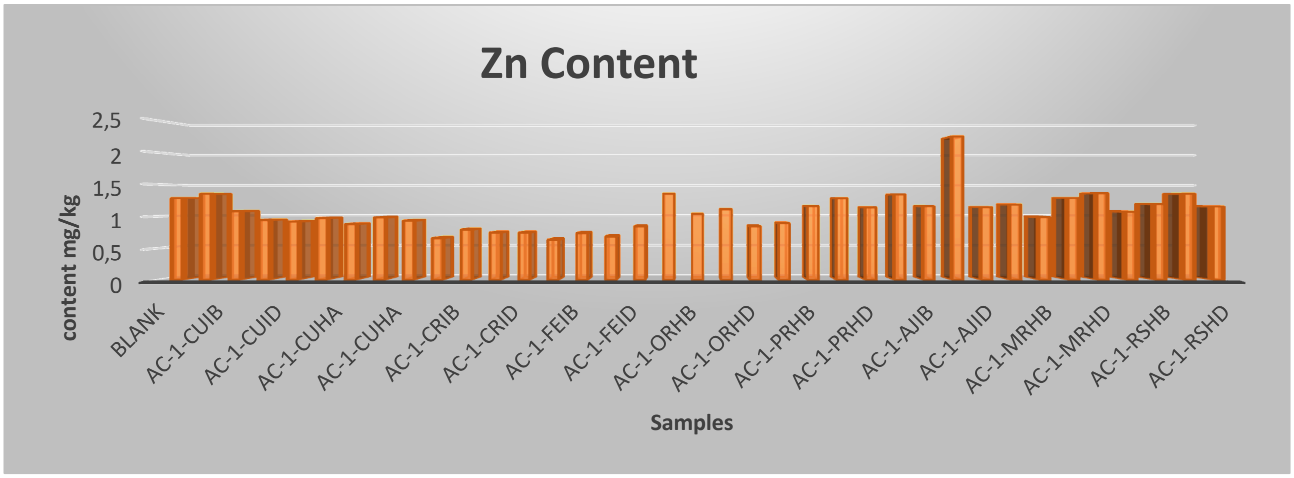

Figure 28 Zinc Content in Acacia Honey and Mixed spices.

4.3 Magnesium Content

Herbs and Spices are rich in Magnesium while the amount is found moderate in Honey in many studies. All samples show higher content than the blank sample (Figure 29) contains, which is only 2.87±0.01 mg/kg, this indicates the less Mg contained in Honey in comparison with the spices. The highest Magnesium content was found in AC-Parsley Hungary mixed samples at a concentration of 7.5%, and the lowest concentration was found in AC-Rosemary Hungary at a concentration of 2.5 % with a quantity of 3.74±0.36 mg/kg. The sample groups with low and similar Mg content are AC-Cumin Hungary and AC-Rosemary Hungary at all levels of concentrations 2.5%, 5 %, 7.5 %, and 10 % with a quantity of 5.05±0.39 mg/kg, 4.37±0.12 mg/kg, and 4.83±0.29 mg/kg, and 4.90±0.23 mg/kg in the Cumin mixed samples and 3.74±0.36 mg/kg, 4.41±0.56 mg/kg, 4.52 ±0.42mg and 5.00±0.14 mg/kg in Rosemary mixed samples. In the AC-Marjoram samples of Hungary, we noticed an increase in Mg content with increasing concentration, with values of 9.62±0.12mg/kg, 15.37±0.67 mg/kg, 15.80±10.10 mg/kg, and 26.47±3.92 mg/kg, respectively. In this evaluation, there was a good increase in Mg content all through the samples.

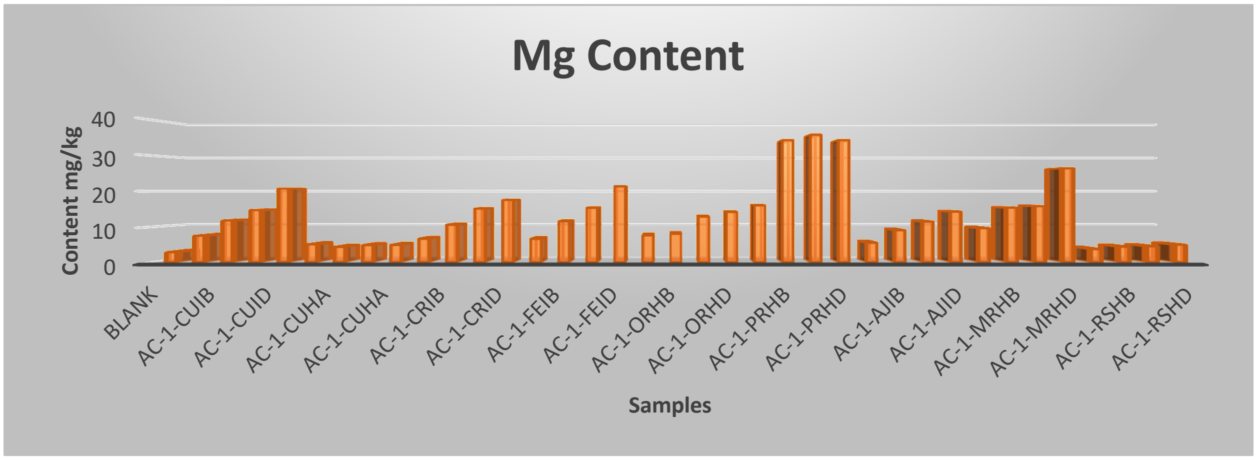

Figure 29 Magnesium Content in Acacia Honey and Mixed spices

4.4 Calcium content

Calcium is essential for the function of other nutrients, including Vitamins D and K, as well as hormones. AC- Ajwain India sample and Ac- Marjoram sample at 10% concentration were found to contain the highest quantity of calcium in this experiment (Figure 30). The amounts found were 155.33±10.02 mg/kg and 159.00±23.64 mg/kg respectively. All mixed samples showed a content increase of Calcium across all levels of concentrations. Ca levels in the AC-Cumin Hungary and AC-Cardamom samples are practically identical, and they are both in the lowest group on the calcium scale. AC-Cumin Hungary had the lowest content of Ca with a value of 23.30±1.85 mg/kg, it was noted that in this sample category, at the highest concentration of 10%, the amount of Ca present was higher than in the blank. In AC-Marjoram Hungary and AC-Ajwain India, the amount of calcium increased with the increase in spice concentration; the results were: (87.30 ±13.08 mg/kg, 115.33±4.04mg/kg, 152.33±4.04 mg/kg, and 159.00±23.64 mg/kg) and (57.13±3.33 103.23±6.19 mg/kg, 121.67±14.01 mg/kg, mg/kg, and 155.33±10.02 mg/kg respectively. This examination has shown a significant increase in the calcium content of mixed samples.

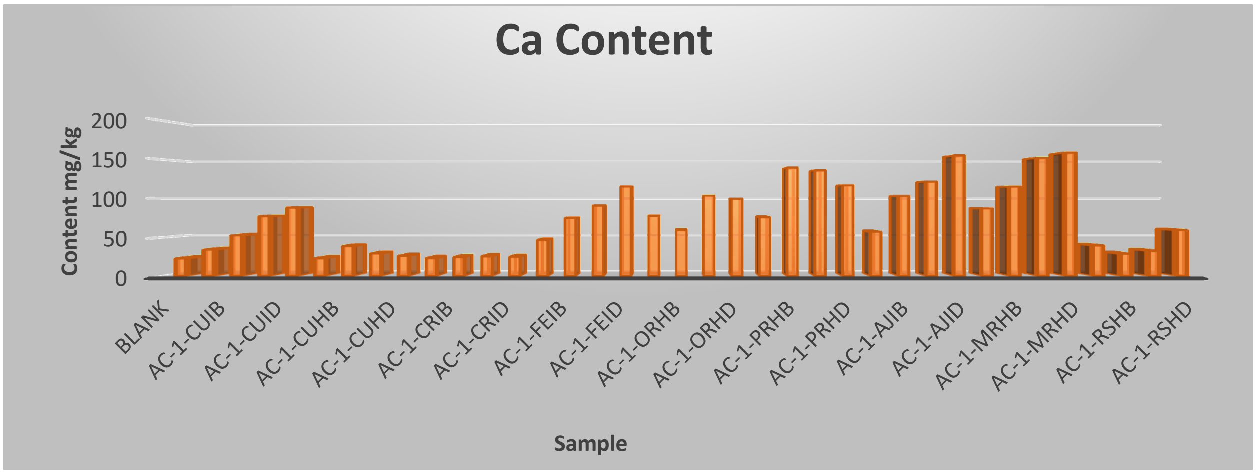

Figure 30 Calcium Content in Acacia Honey and Mixed Spices

4.5 Sulfur Content

The amount of sulfur detected (Figure 31) in the Acacia honey blank sample was 13.30±0.25 mg/kg, but the amount of sulfur found in mixed samples of the Honey and spices samples was higher. The sulfur content of AC-Marjoram Hungary increases with the concentration of the compound, and it is the sulfur-containing group with the highest content. In each case, the values obtained were 31.13±2.51 mg/kg, 42.23±1.01 mg/kg, 52.80±5.5 mg/kg, and 61.07±4.27 mg/kg respectively. When we examined the honey mixed samples, we found that there was not much quantity decrease as the concentration increased except in a few samples, and this content was still higher than the amount in the blank. It was observed that the lowest amount of sulfur was found in AC Cumin Hungary at 5 % concentration, with a value of 18.93±0.55 mg/kg. This was also the group with the least amount of sulfur. In the AC Rosemary mixed sample from Hungary, we detected an increase in the amount of sulfur present was low from 5% to 10%.

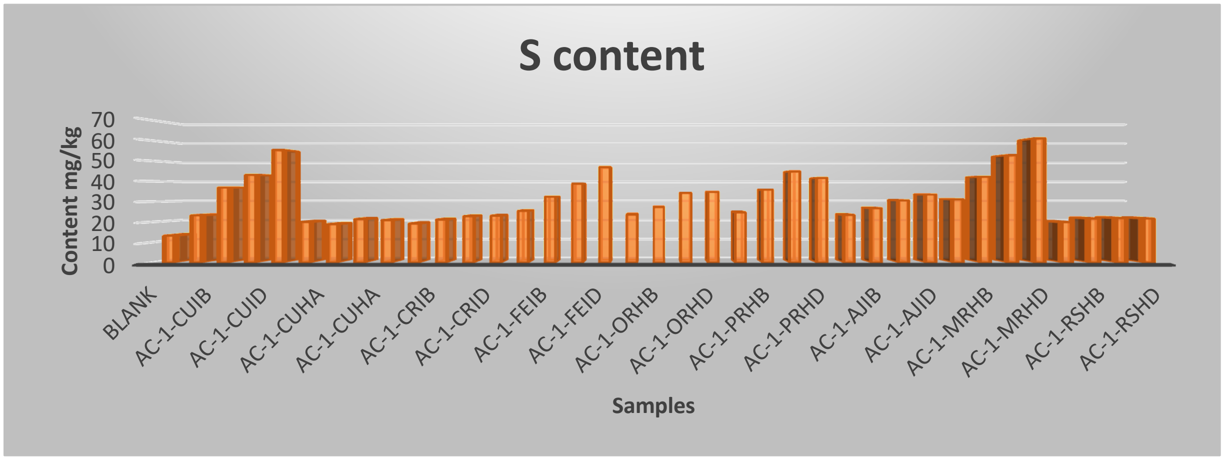

Figure 31 Sulfur content in Acacia Honey and Mixed spices

4.6 Boron content

The amount of Boron present (Figure 32) in Honey was depicted. The highest concentration of Boron was found in a 2.5% concentration sample of AC-Parsley Hungary, with a concentration of 2.58±0.36 mg/kg. The AC- Marjoram and AC-Rosemary Hungary samples produced results that were similar to the blank sample. In AC-Rosemary mixed honey, the amount of Boron decreased as the spice concentration increased from 3.89±0.33 mg/kg to 3.87±0.49 mg/kg, 3.40±0.14 mg/kg, and 3.30±0.16 mg/kg.

In summary, this experiment found that Boron was the second least amount of chemical element present in the Acacia Honey and mixed Spice samples.

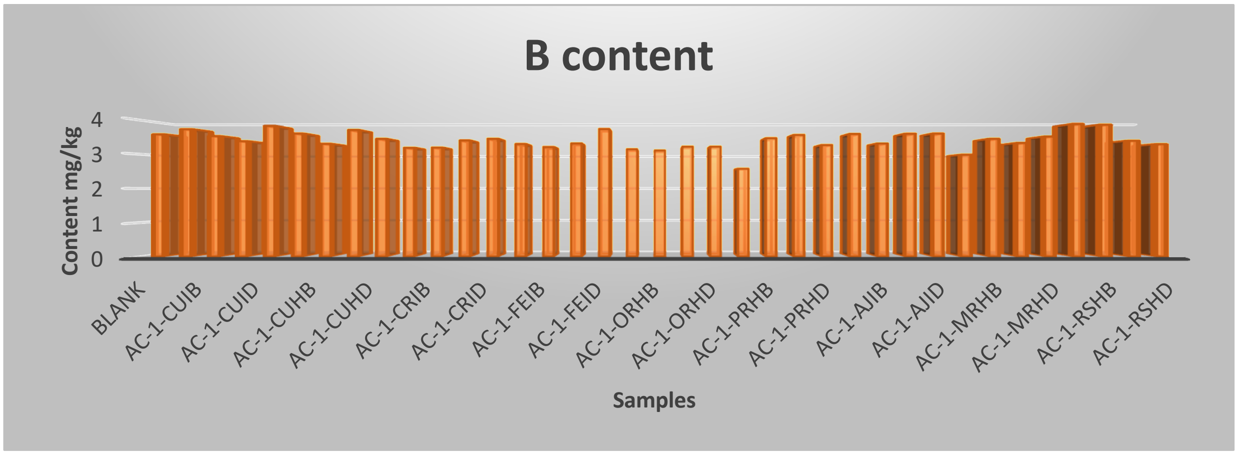

Figure 32 Boron Content in Acacia Honey and Mixed spices.

4.7 Sodium content

The sodium in the body is used to regulate blood pressure and blood volume. The human body requires sodium for the proper functioning of the muscles and neurons.

According to our findings (Figure 33) from our analysis of the Honey and mixed spices, the sample with the highest concentration is AC- Parsley at 10% concentration, having a quantity of 107.33±3.21 mg/kg. The AC-Cumin India mixed samples at concentration levels of 2.5%, 5%, 7.5%, and 10%, with Na quantity of 26.7 ± 2.99 mg/kg, 36.57 ± 2.15 mg/kg, 47.70 ± 1.80 mg/kg, and 63.63±1.51 mg/kg. Another mixed sample which shows a high content of Na in a proportional form of an increase in concentration determines the amount of Na present is the AC- Cardamom India; the increase is so significant and can be described as having almost a 50% increase as the concentration increases. AC-Rosemary Hungary samples have Na content like the blank sample. This indicates that this spice has a lower sodium concentration than other spices. The other sample groups with low sodium content are AC Cumin Hungary, where the amount of Na present is lower than the blank at 2.5 %, 5 %, 7.5 %, and 10 % concentrations; the amount was 9.26±0.36 mg/kg, 7.52±0.43 mg/kg, 8.29±0.12 mg/kg, and 9.56±0.22 mg/kg respectively.

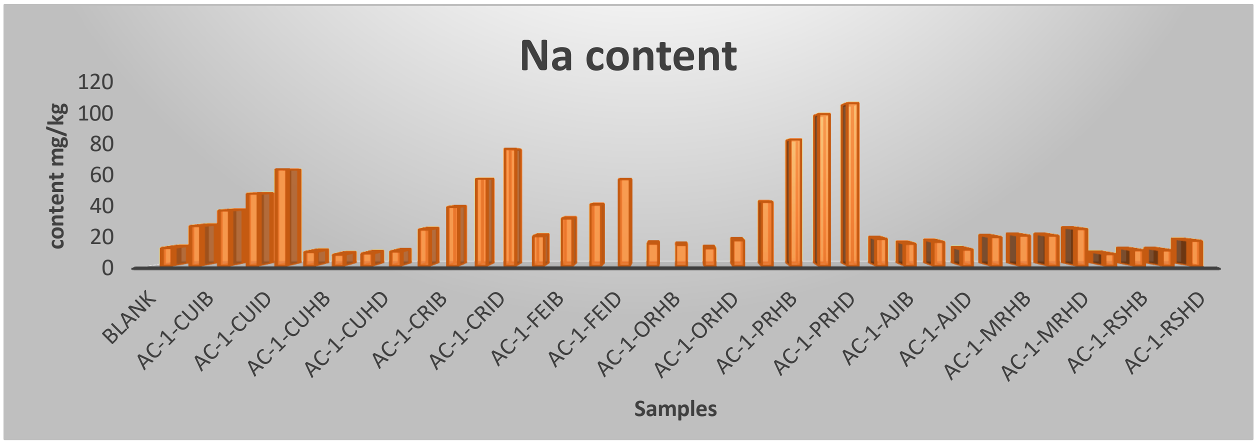

Figure 33 Sodium Content in Acacia Honey and Mixed spices.

CHAPTER FIVE

5.0 SUMMARY AND CONCLUSIONS

The main purpose of this research was to analyze enriched Honey with different spices. We intended to use six spices but going through some other research works; we discovered that some other spices, such as Parsley, Cardamom, and Ajwain, were interesting; therefore, we decided to add them to the spices to be used in the enrichment process. We were able to survey by reading articles on spices that may contribute to a healthier life.

Finally, after a series of discussions, we agreed to use nine spices, five from Hungary and four from India. I followed the instructions given by Prof. Dr Béla Kovács and his PhD student regarding this research.

After carefully preparing the samples, we did the analyses and observed the changes in enriched honey samples with spices. We found that some of the samples have shown increased element content. More so, after performing the analysis through ICP-OES and ICP-MS, we decided to focus on and discuss more important elements that have a distinctive increase in element content in the mixed honey samples.

There are significant changes observed in Honey samples mixed with Marjoram obtained from Hungary, which showed high content of Phosphorus, Sulphur, Zinc, and Calcium. Also, Honey mixed with Parsley obtained from Hungary showed a high increase in Phosphorus, Sodium, and Magnesium, while Honey samples mixed with Ajwain obtained from India have shown an increase in Phosphorus, Zinc, and Calcium.

There was no significant increase in Boron content in any of the honey mixed samples, as seen in the results, the Boron element content remained at almost the same level as in the blank sample.

This shows that there is huge scope for this research to conduct detailed studies with different spices infused with different types of honey samples and check how it increases its micro and macro element concentration without adding any chemicals.

There is a huge perspective to develop functional food kind of innovative honey type by using these spices which can be presented in this growing market.

Nowadays, people want healthier food products and are ready to spend some extra funds to become healthier.

This research has inspired my knowledge and given me more prospects for food innovation while putting in mind that nutrition for healthy living is the most important aspect of food development.

If I get the opportunity to study further, I would like to conduct further research on the antimicrobial properties and shelf-life of these honey samples and carry out a sensory analysis through a survey.

As a future prospect, it would be interesting to compare enriched honey types from different geographical origins.

REFERENCES

Atanassova, J., Pavlova, D., Lazarova, M., & Yurukova, L. (2016). Characteristics of Honey from serpentine area in the Eastern Rhodopes Mt., Bulgaria. *Biological Trace Element Research*, *173*(1), 247–258.

Batista, B. L., da Silva, L. R. S., Rocha, B. A., Rodrigues, J. L., Berretta-Silva, A. A., Bonates, T. O., Gomes, V. S. D., Barbosa, R. M., & Barbosa, F. (2012). Multi-element determination in Brazilian honey samples by inductively coupled plasma mass spectrometry and estimation of geographic origin with data mining techniques. *Food Research International*, *49*(1), 209–215. https://doi.org/10.1016/j.foodres.2012.07.015

Bettaieb, I., Bourgou, S., Sriti, J., Msaada, K., Limam, F., & Marzouk, B. (2011). Essential oils and fatty acids composition of Tunisian and Indian Cumin (Cuminum cyminum L.) seeds: a comparative study. *Journal of the Science of Food and Agriculture*, *91*(11), 2100–2107.

Burlando, B., Verotta, L., Cornara, L., & Bottini-Massa, E. (2010). *Herbal principles in cosmetics: Properties and mechanisms of action*. CrC Press.

Danciu, C., Zupko, I., Bor, A., Schwiebs, A., Radeke, H., Hancianu, M., Cioanca, O., Alexa, E., Oprean, C., Bojin, F., & others. (2018). Botanical therapeutics: Phytochemical screening and biological assessment of chamomile, parsley and celery extracts against A375 human melanoma and dendritic cells. *International Journal of Molecular Sciences*, *19*(11), 3624.

Divakara Sastry, E. V, & Anandaraj, M. (2013). Cumin, fennel and fenugreek. *Soils, Plant Growth and Crop Production. Encyclopedia of Life Support Systems (EOLSS)*, 1–10.

Drobner, U., & Tyler, G. (1998). Conditions controlling relative uptake of potassium and rubidium by plants from soils. *Plant and Soil*, *201*(2), 285–293.

Duke, J. A. (2002). *Handbook of medicinal herbs*. CRC press.

Farkas, Á., Zajácz, E., Spring, E., Pasture, B. E. E., & Summer, E. (2007). Nectar Production for the Hungarian Honey Industry. *Plant Science*, *January 2007*.

Flora, G., Gupta, D., & Tiwari, A. (2012). Toxicity of lead: a review with recent updates. *Interdisciplinary Toxicology*, *5*(2), 47.

Geiling, N. (2013). The Science behind Honey's Eternal Shelf Life. *Smithsonian Mag. Com*.

Ghosh, B., Chandra, I., & Chatterjee, S. (2015). Fenugreek (Trigonella foenum-graecum L.) and its necessity. *Fire J. Eng. Technol*, *1*(1), 66–67.

Grüter, C. (2020). Stingless bees. *Cham, Switzerland: Springer International Publishing*.

Hewlings, S. J., & Kalman, D. S. (2017). Curcumin: A review of its effects on human health. *Foods*, *6*(10), 92.

Jørgensen, S. (2000). *Principles of pollution abatement*. Elsevier.

Khan, N., Choi, J. Y., Nho, E. Y., Jamila, N., Ho, J., Ryu, K. Y., Park, K. S., & Kim, K. S. (2014). *Analytical Letters*. *June*, 37–41.

Kodama, H., & Fujisawa, C. (2009). Copper metabolism and inherited copper transport disorders: molecular mechanisms, screening, and treatment. *Metallomics*, *1*(1), 42–52.

Komarnisky, L. A., Christopherson, R. J., & Basu, T. K. (2003). Sulfur: its clinical and toxicologic aspects. *Nutrition*, *19*(1), 54–61.

Liu, T., Ming, K., Wang, W., Qiao, N., Qiu, S., Yi, S., Huang, X., & Luo, L. (2021). Discrimination of Honey and syrup-based adulteration by mineral element chemometrics profiling. *Food Chemistry*, *343*(October 2020), 128455. https://doi.org/10.1016/j.foodchem.2020.128455

Muhammad, A., Odunola, O. A., Gbadegesin, M. A., Sallau, A. B., Ndidi, U. S., & Ibrahim, M. A. (2015). Inhibitory effects of sodium arsenite and acacia honey on acetylcholinesterase in rats. *International Journal of Alzheimer's Disease*, *2015*.

Muhammad, A., Odunola, O. A., Ibrahim, M. A., Sallau, A. B., Erukainure, O. L., Aimola, I. A., & Malami, I. (2016). Potential biological activity of acacia honey. *Frontiers in Bioscience-Elite*, *8*(2), 351–357.

Murray, F. J. (1995). A human health risk assessment of boron (boric acid and borax) in drinking water. *Regulatory Toxicology and Pharmacology*, *22*(3), 221–230.

Naquvi, K. J., Ansari, S. H., Salma, A., Ahamad, J., & Najib, S. (2022). A Review on Phytochemical Investigations and Biological Activities of Trachyspermum ammi (L.) Sprague. *Research Journal of Pharmacy and Technology*, *15*(5), 2364–2370.

Nieto, G., Ros, G., & Castillo, J. (2018). Antioxidant and antimicrobial properties of Rosemary (Rosmarinus officinalis, L.): A review. *Medicines*, *5*(3), 98.

Osredkar, J., & Sustar, N. (2011). Copper and zinc, biological role and significance of copper/zinc imbalance. *J Clinic Toxicol S*, *3*(2161), 495.

Parcell, S. (2002). Sulfur in human nutrition and applications in medicine. *Alternative Medicine Review*, *7*(1), 22–44.

Peganova, S., & Eder, K. (2004). Elements and their Compounds in the Environment. *MERIAN, E., ANKE, M. e M, S.(Ed.): Wiley-VCH2*, 1203–1239.

Pravina, P., Sayaji, D., Avinash, M., & others. (2013). Calcium and its role in the human body. *International Journal of Research in Pharmaceutical and Biomedical Sciences*, *4*(2), 659–668.

Punoševac, M., Radović, J., Leković, A., & Kundaković-Vasović, T. (2021). A review of botanical characteristics, chemical composition, pharmacological activity and use of parsley. *Archives of Pharmacy*, *71*(Notebook 3), 177–196.

Reimann, C., & De Caritat, P. (2012). *Chemical elements in the environment: factsheets for the geochemist and environmental scientist*. Springer Science \& Business Media.

Revathi, S., Govindarajan, R. K., Rameshkumar, N., Hakkim, F. L., Mohammed, A.-B., Krishnan, M., & Kayalvizhi, N. (2017). Anti-cancer, antimicrobial and antioxidant properties of Acacia nilotica and their chemical profiling. *Biocatalysis and Agricultural Biotechnology*, *11*, 322–329.

Roger, V. L., Go, A. S., Lloyd-Jones, D. M., Adams, R. J., Berry, J. D., Brown, T. M., Carnethon, M. R., Dai, S., De Simone, G., Ford, E. S., & others. (2011). Heart disease and stroke statistics—2011 update: a report from the American Heart Association. *Circulation*, *123*(4), e18--e209.

Setiawan, E., Darnaedi, D., Rachman, I., Triono, T., & Webb, C. O. (2020). The Digital Herbarium: Solutions for Data Collection and Identification of Indonesian Plant Diversity. *Biogenesis: Jurnal Ilmiah Biologi*, *8*(2), 203–209.

Sigel, A., Sigel, H., & Sigel, R. K. O. (2013). *Interrelations between essential metal ions and human diseases* (Vol. 13). Springer.

Spottiswoode, C. N., Begg, K. S., & Begg, C. M. (2016). Reciprocal signaling in honeyguide-human mutualism. *Science*, *353*(6297), 387–389.

Strazzullo, P., & Leclercq, C. (2014). Sodium. *Advances in Nutrition*, *5*(2), 188–190.

THE CHEMICAL ELEMENTS OF LIFE Author(s): Earl Frieden Source: Scientific. (2018). http://www.jstor.org/stable/24927384

Wang, 2013. (n.d.).