CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Tropical AgriSciences

Chemical analysis of garlic essential oils (*Allium sativum* L.) from different countries of origin

Master's Thesis

Prague 2023

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DECLARATION

I, Kodom Prince Owusu, hereby declare that I have done this thesis entitled "**Chemical analysis of garlic essential oils (*Allium sativum* L.) from different countries of origin"** independently, all texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references and according to Citation rules of the FTA.

In Prague, 22nd April, 2023

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BSc. Kodom Prince Owusu

ACKNOWLEDGEMENTS

“To God Be The Glory”

Foremost, I would like to thank my supervisor, Ing. Klára Urbanová, Ph.D., for her selfless dedication, patience, kindness, continuous encouragement and feedback, and positive attitude. Her office door was always open to me throughout my research for consultation and advice when deeded. Without her knowledge, experience, and guidance, the successful completion of this diploma thesis would be hardly achievable.

Also, my sincere thanks go to Ing. Marketa Houdková, Ph.D. for her needful assistance, advice, and help during the practical test for antibacterial activity experiment and for making time for the consultation on the discussion part of my diploma thesis.

Lastly, I would like to express my sincere gratitude to my family, friends and classmates for providing me with unfailing support throughout my years of study. Thanks to them everything always seemed easier and achievable.

ABSTRACT

Allium sativum, likewise called garlic, is used widely around the world in both cooking and medicine. The chemical makeup of the essential oils from *Allium Sativum* was examined in the current study. The aromatic compounds extracted from five countries of origin-grown *Allium sativum* bulbs, namely; the Czech Republic, China, Spain, Slovakia and Italy, were obtained by laboratory hydro-distillation method using a Clevenger apparatus. The resulting essential oils were analyzed by gas chromatography-mass spectrometry (GC-MS). Crushing-raw fresh garlic clove were used to analyse garlic's volatiles profiles. Volatiles of headspace in the five different samples as mentioned above were extracted by a Solid-Phase Microextraction (SPME) and analyzed with GC-MS. Many volatile compounds were acknowledged in the fresh garlic using the SPME-GC-MS method. Volatile compounds found included Diallyl disulfide, Diallyl sulfide, Di-2-propenyl trisulfides, 2-Propenylmethyltrisulfide, Allyl (*E*)-1-propenyldisulfide, 3-Vinyl-1,2-dithiacyclohex-4-ene, 2-Vinyl-4H-1,3-dithiine, 4H-1,2,3-Trithiine, Methyl (*E*)-1-propenyl disulfane, and 2-Propenylmethyldisulfide.

Antibacterial activity of *Allium savitum* essential oils was determined against *Staphylococcus aureus* by oxacillin using the method of new broth microdilution volatilization. The present work's objective was to measure the variation in colour change and pH of fresh garlic. The results showed that the Hue (colour of garlic determined by the amount of carotenoids present in the garlic) was highest in the China garlic sample, followed by Slovakia, Czech Republic, Italy and Spain accordingly. Finally, in terms of total colour change, China was the best, followed by Italy, the Czech Republic, Slovakia, and Spain.

KEYWORDS: Garlic (*Allium sativum*), Volatile substance, Antibacterial activity, Colour, pH.

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LIST OF THE ABBREVIATIONS USED IN THE THESIS

AS	*Allium sativum*
CLSI	Clinical and Laboratory Standards Institute
CLH	Clevenger Laboratory Hydrodistillation
CZU-FTA	Czech University of life Science – Faculty of TropicalAgriSciences
DADS	Diallyl Disulfides
DATS	Diallyl Trisulfides
FAO	Food and Agriculture Organization
EOs	Essential Oils
EPA	Environmental Protection Agency
EVA	Ethylene Vinyl Acetate
EU	European Union
EUCAST	European Committee on Antimicrobial Susceptibility Testing
GC	Gas chromatography
GCMS	Gas Chromatography Mass-Spectrum
GI	Gastro Intestinal
HS	Headspace
HS-SPME	Headspace Solid-Phase Micro Extraction
HPLC	High-Performance Liquid Chromatography
MCSO	S-methylcysteine-sulfoxide
MIC	Minimal Inhibitory Concentration
MRSA	Methicillin-Resistant *Staphylococcus Aureus*
MSD	Mass selective detector
MTT	3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide
NAC	N-acetylcysteine

NIH	National institutes of Health
NIST	National Institute of Standard and Technology
PCSO	S-propylcyesteine-sulfoxide
PDMS	Polydimethylsiloxane
QMF	Quadrupole Mass Filter
RI	Retention Index
SAMC	S-allyl-mercapto cysteine
SD	Steam Distillation
SDE	Simultaneous Distillation and Solvent Extraction
SPME	Solid-Phase Microextraction
SPTE	Solid-phase/ Solvent Trapping Extraction

1. INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Garlic, *Allium sativum* (*A. sativum)* is a bulbous flowering plant species belonging to the Allium onion genus. The shallot, onion, chive, leek, Chinese onion and Welsh onion are all related relatives (Cun Chen et al. 2018). It is indigenous to north-eastern Iran and Central Asia and has a long history of human intake and use, spanning several thousand years (Dustin et al. 2018). It was employed as a food flavouring as a traditional remedy by the ancient Egyptians and China produces 76 % of the world's garlic (Carel et al. 2018). Garlic is a perennial flowering bulbous plant with a long, erect blooming stem that can reach a height of one metre (3 ft). Linear, flat, roughly 1.25–2.5 cm (0.5–1.0 inch) wide, and solid, with a critical apex, the leaf blade (Shuhbam Yadav et al. 2018). People have used garlic as a nutraceutical spice for thousands of years because of its polyphenolic and organosulfur content. Numerous studies have demonstrated that, garlic's antioxidant and anti-inflammatory properties and its secondary metabolites are excellent health promoters and disease preventers (as well as lipid-lowering effects) for several common diseases of humans, for example, metabolic or cardiovascular disorders and cancer (including diabetes and hypertension). The resulting chemicals give garlic its harsh or spicy taste and pungent odour. It is well known that plant-derived meals, including fruits, vegetables, spices, and grains, can influence a number of metabolic pathways to help prevent and treat chronic diseases like obesity, diabetes, cardiovascular disease, and neurodegenerative disorders. Food-based nutrients are frequently consumed as functional meals and dietary supplements (DSs). Due to their superior safety characteristics compared to pharmaceuticals, the majority of them are permitted to be taken for extended periods of time (Spyridon et al. 2018). The main classes of chemicals responsible for the antimicrobial activity of plant essential oils include oxygenated terpenoids, especially phenolic terpenes, alcohols, and phenylpropanoids (Imaël & Rodolfo 2012).

In this research, we tested essential oils of fresh garlic from five different origins, Czech Republic, China, Slovakia, Spain and Italy, with the intention of confirming the suitability for the evaluation of volatile elements produced from garlic, as representatives of distinct classes of potent phytochemical antimicrobials.

Finally, the quality of garlic is judged in terms of its aroma in particular. Aroma/Scent is one of the most important properties of garlic. However, revealing the specific effect of all these substances on the unique aroma of garlic is still in its beginning stages. Other uncertainties are associated with the presence of volatile substances in garlic in relation to the garlic's properties (such as compounds, antimicrobial activities, colour and pH).

Therefore, it is important to study the chemical compounds constituents of fresh garlic grown at different agro-ecological zones to evaluate volatile compounds, antimicrobial activities, colour change and pH.

1.2 LITERATURE REVIEW

The Alliaceae family includes garlic (*A. savitum*), which originated in Central Asia around 6,000 years ago and is today one of the most widely farmed crops on the planet. Garlic extracts have been shown to have antibacterial, antineoplastic, antithrombotic, and hypoglycemic effects in addition to their folk medicinal and spice use 13-15 (H. Takagi 2020). Garlic seedlings (GS) are a common vegetable in Chinese cuisine. In China, it is common to grow GS in open fields or greenhouses with other vegetables because of their resilience to the environment, quick growth period, and significant yield. In China, garlic seedlings treated with a blanching culture and grown in a greenhouse with low or no light are known as "blanched garlic seedlings" (BGS). Some vegetable crops, for example, hotbed chives and celery, are cultivated using the blanching culture, promoting chlorophyll degradation, and improving exterior quality and flavor (Razina Rouf et al. 2020). When BGS is blanched, it takes on a yellow tint than GS did before.

Customs authorities worldwide use the nation of origin of agricultural products to impose import tariffs and regulate commerce. Trade embargoes must be enforced, and anti-dumping and countervailing levies must be collected as part of the import process (Takagi 2020). Commodity crops are now easily transported between countries and continents because of globalisation. Because of this, consumers are more concerned about where their food comes from. To prevent the entry of agricultural products that have been tampered with, governments must have highly accurate measurement tools. Garlic's species identity can be confirmed, and its source population identified using a genomic technique (Razina Rouf et al. 2020). This has been done to identify the genetic origin of garlic while examining genetic ties among garlic clones (Chretien et al. 2020).

1.2.1 Garlic Origin and Consumption

Middle Asia is where garlic originated. Garlic is thought to have originated in West China, in the Tien Shan Mountains, and in Kazakhstan and Kyrgyzstan, according to a variety of theories (Dustin et al. 2018). The Sumerians (2600–2100 BC) actively used

the curative properties of garlic, and it is thought that they exported the garlic to China, from which it later spread to Korea and Japan. The spread of garlic most likely continued in the new world after starting in the old. Yet, certain historians continue to assert that China is where garlic first appeared (Carel et al. 2012).

Since 2700 BC, garlic has been a popular remedy in ancient China. Then, because of its warming and energizing properties, it was positioned in yang (the yin yang concept, consistent with which in the bad there is good and in the good there is bad). The use of garlic has been advocated for those with depression. Hence, the Japanese have not incorporated garlic into the tradition of Buddhist due to the stimulating qualities of the herb. Garlic is not a favorite ingredient in Japanese cuisine either (Shubham Yadav et al. 2018).

Garlic was a highly effective tonic, or roborant, in traditional Indian medicine. It was used to treat a variety of ailments, including rheumatism, haemorrhoids, common weakness, coughing, and skin conditions. Garlic was among the medicinal plants mentioned in the Vedas, the Indian holy book. The first doctors and pharmacists were Indian priests, therefore, it should come as no surprise that the healing was supplemented and accompanied by many rituals and spells, mysterious and wonderful ceremonies, and prayers (Carel et al. 2012).

Several therapeutic, fragrant, spicy, and toxic plants were known to the Egyptians. They used to be content with their medical plants from their local flora near the Nile River, when they were young and poor. The most popular ingredient was garlic. Later, as they grew in strength and commercial importance, they began looking more and more for medicinal plants with potent physiological activity as well as potent spices and smells from the East. Garlic was still used, but now it was for the poor, or slaves, as food and medicine (Carel et al. 2012). To strengthen them and enable them to perform more work, the Egyptians fed garlic to their slaves. "Inscriptions on the plates of the Egyptian pyramids remind us how much their builders utilized the garlic for this vegetable; 1600 talents of silver were spent (about 30 million dollars)," the Ancient Greek historian Herodotus (Chen et al. 2018) stated. Garlic was a vital dietary ingredient during this time (Yadav et al. 2018).

Ancient peoples like the Phoenicians, Israelites, Babylonians, Persians, and others all benefited greatly from the healing abilities, remedy preparation, and culture of ancient Egypt. All of these nomadic, basically livestock breeders, desert or semi-desert peoples, used garlic frequently. With all of the peoples residing around the Mediterranean Sea, its implications continued to be felt later during the Medieval and New Ages. As a result, the nations bordering the Mediterranean Sea, particularly those on the East coast, continue to consume garlic in significant amounts. In 1548, garlic was imported from the Mediterranean Sea shores into Great Britain, where it was widely available (Yadav et al. 2018). Lonicerus (1564) advised using external garlic to treat a variety of skin conditions, including dandruff. It was freely used in ancient Europe, especially in Italy, while the French frequently included it in their cooking.

It has been established that one of the earliest plants that man cultivated was garlic. Over time, humans have discovered how to make garlic tinctures and teas, as well as how to combine equal parts of honey and garlic, etcetera. Consequently, they discovered how to treat colds, fevers, and diarrhoea, as well as various gastrointestinal illnesses, which helped many sick individuals live longer. Due to garlic, in 1720 in Marseille, thousands of citizens were saved from the pandemic spread of plague (Yadav et al. 2018). Louis Pasteur stated that garlic murdered microorganisms in 1858. He insisted that it worked even against microorganisms that were resistant to other elements. He added that *Helicobacter pylori* was destroyed by garlic. Garlic's antiseptic capabilities were demonstrated in the suppression of cholera, typhoid fever, and diphtheria in Beirut in 1913, 1918, and 1919, respectively (Wolde et al. 2018). French phytotherapist Lekrek successfully employed garlic as a prophylactic treatment during the severe influenza epidemic, also known as "Spanish fever," in 1918. Before going out in public during the influenza epidemic in America in 1917 and 1918, many donned a necklace made of garlic. So, garlic was retitled into antibiotic natural, and almost everyone across the world is using garlic (Gia-Buu et al. 2018).

1.2.2 The Ecology, Types and Cultivation of Garlic

The optimal conditions for growing garlic are a sunny site, well-drained soil that retains moisture, and soil that contains a fair amount of organic matter. Water should be consumed in sufficient amounts for garlic. Compost or well-rotted manure are excellent soil amendments for enhancing garden soils. Between July and September in the Northern Hemisphere, the plant produces pink to purple flowers. The pungent bulb is made up of an inner sheath that encloses the clove and outer layers of thin wrapping leaves. The cloves on the bulb are typically between ten and twenty, and all but the ones closest to the center are asymmetrical in shape (Yebirzaf Yeshiwas et al. 2018). According to Tasmanian Gourmet Garlic, garlic prefers a soil pH between 6 and 7. If the pH drops below 5.8, liming is advised. Based on the findings of soil tests. Since perennial weeds hinder garlic's ability to compete with them, the land should be free of them and thoroughly tilled before planting. During the first two months of growth, once bulbs are starting to form and roots are formed, garlic needs cool temperatures air of 32 °F to 50 °F (0-10 °C). As it ages, garlic is unaffected by extreme heat (Aron 2022, Albert 2022).

The latter is best accomplished in the autumn after the temperature has cooled off. Fall planting aims to promote the development of roots but not branches. The energy expended by the plant to grow any sprouts will be lost since they will wither throughout the winter. Each clove should be buried two to three inches deep, pointy side up. Between rows, there should be a six-inch gap (Caterine et al. 2017). Before planting, the cloves shouldn't be separated from the main bulb. Fertility requirements for garlic range from moderate to high, particularly for the element nitrogen. Following the findings of a soil test, the soil should be modified before planting. A typical advice is to use three balanced fertilizer pounds (e.g., 10-10-10) per garden space of 100 square feet in the absence of the latter. In the spring, as soon as the leaves start to emerge, additional nitrogen is typically sprayed, and then again about two weeks later.

We distinguish between two primary varieties of garlic: hardneck and softneck, according to Savvy Gardening specialists. A blooming stalk (scape) surrounded by underground cloves is produced by hardneck variants. Most chefs prefer hardneck cultivars because they are thought to be tastier and simpler to peel than softneck

species. Hardneck garlic, however, does not keep well in storage. Softneck (also known as silverskin) variants frequently generate bulblets on their stems but rarely blossom or set seed. Compared to hardneck types, they are said to be more productive and simpler to grow. Softneck types can be kept for six to eight months when stored properly. The majority of the garlic you can buy in supermarkets is the softneck variety. Softneck varieties can be kept for six to eight months, whereas hardneck varieties will only keep for three to four months. German Red, Spanish Rojo, Chesnok Red, and Korean Red are some hard-neck types. California Early, Silverskin, California White, California Late, New York White, Lorz Italian, Inchelium Red, and Polish White are examples of soft-neck types (Jessica 2020).

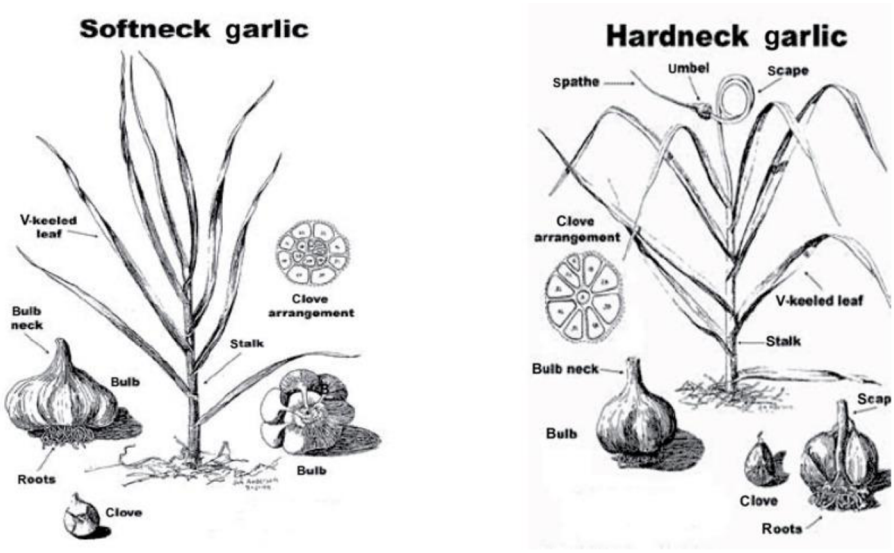


Figure 1. Types of garlic plant (Source: Jim Anderson 2017)

However, the cultivation or harvest date varies according to the type of garlic variety. By July's second week and August's first week, garlic is typically ready for harvest. Early bulb harvests don't preserve well. On the other hand, when bulbs are harvested after they reach their full maturity, individual cloves frequently "burst" out of their skin. Dig the bulb with its leaves still on when harvesting. Before brushing off extra soil from harvested plants, let them air dry; do not wash just-harvested bulbs. In a dry area with excellent air circulation, harvest bulbs (including their tops) should be allowed to cure for three to four weeks. This is frequently done by hanging the bulbs to cure while tying 10–15 of them together. After the tops have finished drying, the stems can be removed, leaving the bulb with approximately a half-inch of the stem. The optimal

conditions for storing garlic are relatively low temperatures (between 32 °F and 38 °F) and moderate humidity levels (about 60 %). While garlic can be kept at room temperature, dehydration will happen more quickly. The anticipated storage life varies by type. (David Trinklein University of Missouri Plant Science & Technology 2015)

1.2.3 Storage Conditions, Storage Life and Spoilage

Finding the appropriate curing conditions can be difficult, yet curing is necessary for optimal bulb preservation. Sunscald can occur when drying in the field, and disease can spread in poorly ventilated barns. Avoid intense sunshine and temperatures that are too hot (above 90 °F). By putting bulbs roots up on one-inch wire mesh in an open-sided, shade-cloth-covered hoop house, it is possible to speed up the curing process. It is also worked in a well-ventilated barn, nonetheless, ensure the bulbs are hung high enough off the ground or placed on open racks with ample airflow. It takes 10 to 14 days to heal. Cut the stems either before or after curing. When the chopped stem's interior is hard, the neck is constricted, and the outside skins are crispy and dry, curing is complete.

Garlic can be reserved in a decent state for 1-2 months at room temperature (68 °F to 86 °F) and relatively low humidity (i.e., <75 %) after curing. Yet, as a result of water loss in these circumstances, bulbs eventually turn soft, spongy, and shrivelled. Garlic does best when stored at low RH (60 % to 70 %) and temperatures between 30 °F and 32 °F. To avoid any moisture build-up, storage containers must have adequate airflow. Well-cured garlic can be reserved in storage for 6 to 7 months under these circumstances. In the short term, storage at higher temperatures of 60 °F might be sufficient, but it's crucial to pick a location with low relative humidity and decent airflow. Similar to onions, relative humidity requires to be under other vegetables since too much moisture encourages the formation of mildew and roots, while too little moisture causes the bulbs to dry up.

When storing garlic bulbs, keep the temperature between 50 and 70 °F and the relative humidity between 65 and 70 %. Due to the fact that garlic cloves lose their dormancy most quickly between 40 and 50 °F, continued storage in this range must be prevented. Side-shoot sprouting (witches’ brooms), rough bulbs, and initial maturity are

all effects of storing planting stock below 40 °F, whereas delayed sprouting and late maturity are effects of storing over 65 °F.

Although disease infections might spread to other fields and the harvest the following year, only the best garlic cloves should be utilized as seeds. Be out for the possible spread of the garlic blight nematode in New England on infected seed garlic. This nematode, likewise called stem nematode and a bulb, results in twisted, bloated, distorted and swollen leaves, fractured, and dark-ringed bulbs. This nematode infestation can weaken plants, making them vulnerable to secondary illnesses. Positive identification is possible thanks to the UMass Plant Disease Diagnostic Lab (Hazzard 2013).

1.2.4 Garlic Production, Trade and Economy

Eighty percent of the world's production of garlic comes from China. According to the Food and Agricultural Organization, China produced 20.7 million tons of garlic in 2020, followed by India with 2.9 million tons. South Korea, Egypt, and Russia round up the top three garlic-producing nations. The covid-19 epidemic resulted in a reduction in Chinese garlic supplies and a sharp surge in garlic prices. According to Global Market (2021) demand for garlic is increasing across a variety of businesses (such as the processing industries).

Table 1. The first ten leading production countries of garlic (Source: FAO 2019)

#	98 Countries	Metric Tons	Last	YoY
1	China	22,921,518.79	2019	+2.6 %
2	India	1,793,127.00	2019	+4.2 %
3	Bangladesh	478,284.00	2019	+3.5 %
4	South Korea	317,324.00	2019	-4.3 %
5	Egypt	291,395.00	2019	+1.8 %
6	Spain	279,589.00	2019	+2.2 %
7	Uzbekistan	258,746.00	2019	+1.5 %
8	United States	254,513.00	2019	-2.2 %
9	Myanmar	211,172.00	2019	+2.0 %
10	Russia	210,434.00	2019	-0.7 %

Global Market (2021) stated, on the Product Complexity Index (PCI), fresh or cold garlic comes in at position 4474. Fresh or cold garlic ranked as the 887th most traded goods in the world in 2020, with $3.24 billion in trade. Fresh or chilled garlic exports increased by 14.8 % during 2019 and 2020, from $2.82 billion to $3.24 billion. Fresh or cold garlic trade makes up 0.019 % of all global trade. Prices for garlic are growing, primarily in Europe. The flow of Chinese garlic to Europe is generally consistent. In the middle of 2021, the export price of garlic was approximately 10–15 % more than it had been the year before.

Global Trade (2020) stated the leading garlic exporters in 2020, chilled or fresh, were China (($2.11 B), Spain (($493 M), Argentina ($178 M), Netherlands ($84.9 M), and Egypt ($51.3 M) and prime garlic importers, chilled or fresh were Indonesia ($499 M), Vietnam ($306 M), Brazil ($281 M), United States ($220 M), and Germany ($134 M).

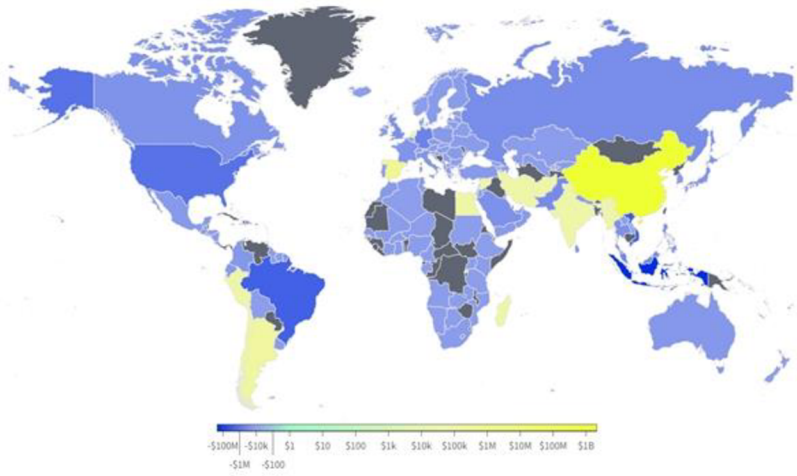


Figure 2. The top five countries of exports and imports of fresh or chilled garlic (Source: OEC, 2021).

Garlic, fresh or chilled, had an average tariff of 24.8 % in 2018, ranking it as the 198th lowest tariff according to the HS6 product classification. South Korea and Japan are the nations that charge the highest import fees for fresh or chilled garlic. Israel (101 %), Turkmenistan (100 %), Iran (100 %), South Korea (358 %), and Cyprus (85.6 %) round out the list. Mauritius (0 %), South Africa (0 %), the United Arab Emirates (0 %), Hong Kong (0 %), and Kuwait (0 %) have the lowest tariffs.

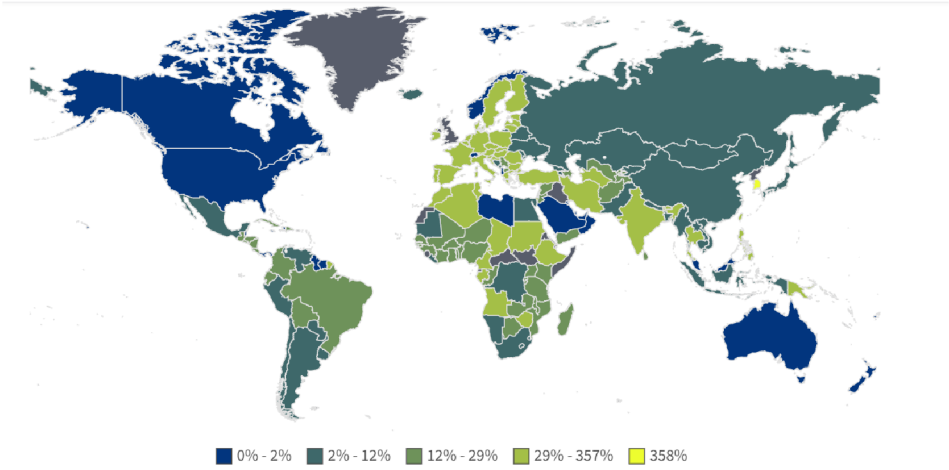


Figure 3. The nations that charge the highest and lowest average import duties for fresh or chilled garlic (Source: OEC, 2021).

Global Trading Mag (2021) stated with 89 % of the overall volume, China dominates the international export market. Chinese garlic supplies achieved historic highs of nearly $2 B, which offers a solid platform for the worldwide spike in request.

1.3 Garlic Chemical Compositions

The chemical composition of garlic is a complex combination of a large number of defined and still undefined substances. It is not possible to precisely pin down the composition of garlic in general. The individual constituents contained in clove and their amounts vary greatly. The final composition of garlic (clove) depends mainly on its genetic and physiologic aspects, such as botanical species, variety, or degree of maturation. Secondly, the chemical composition is also influenced, to a lesser extent, by extrinsic factors such as soil pH, soil composition and its fertilization, agricultural practices, drying method, processing and post-harvest techniques, and climate and storage conditions. Water evaporation is the main reason for weight loss. Its vaporization, together with the release of CO_2 and formation of volatile components. Hassan et al. (2014) One of the top twenty vegetables, garlic is used in both ancient and contemporary medicine, as well as a variety of culinary preparations around the world. Moreover, it has been scored highly for its contribution of phenolic compounds to the human diet and has been suggested as one of the richest sources of total phenolic compounds among frequently consumed vegetables (Gaber et al. 2020).

1.3.1 Major Chemical Constituents In Garlic

Garlic's pungent flavour is derived from phytochemicals released once cells of the plant are broken. Once a cell is broken down through chopping, crushing, or chewing, enzymes contained in the cell vacuoles initiate numerous sulfur-containing substances that are broken down in the cell fluids (cytosol) (Rozita Khademi et al. 2018). A wide range of pharmacological properties has emerged from garlic extracts and isolated compounds. When allicin connects with cystine and is liberated from alliin, which is generated from the protein diet, S-ally-mercapto-cysteine is formed, and garlic

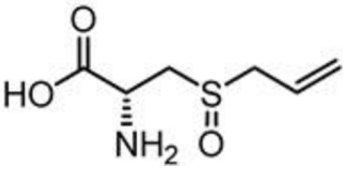
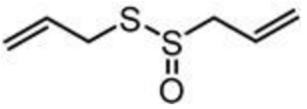
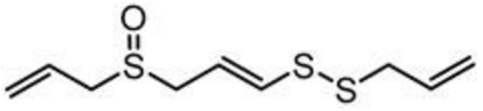
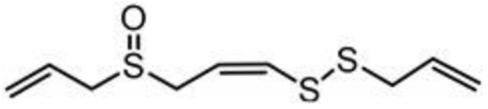
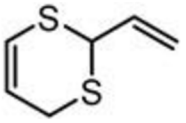
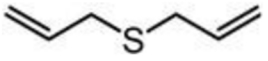
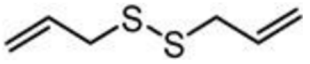
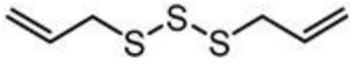
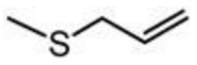
is absorbed in the GI tract (SAMC). DADS and E-ajoene are among the secondary metabolites of allicin that are found in the body's fluids and on people's faces after metabolism (Gia-Buu Tran et al. 2018). The most biologically active sulfur-containing compound in garlic, allicin [S-(2-propenyl)-2-propene-1-sulfinothioate], is what gives garlic its distinctive flavor and aroma. Approximately 70 % of the total thiosulfinates found in cloves crushed are alliin (S-allyl-L-cysteine sulfoxide). When diallyl disulfide or allicin interacts with cysteine in S-ally-mercapto-cysteine presence, the allyl mercaptan molecule is formed (Hannah Valentino et al. 2020). The four main major chemical constituents in garlic are shown in the figure (Figure 4).

Figure 4. Major chemical constituent cycle of garlic (Source: Cristian Mauricio et al. 2019).

The sulfur-containing compounds found in *Allium sativum* bulbs, such as ajoenes, thiosulfinates, vinyldithiins, and sulfides (including DADS and DATS), account for 82 % of the total sulfur content in garlic (Hannah Valentino et al. 2020) (Table 2). When the parenchyma of the garlic is cut open, the alliin enzyme is activated, transforming alliin into allicin, the main cysteine sulfoxide in garlic. Freshly milled garlic homogenates contain the main odoriferous molecules PCSO, allicin, and MCSO (S-methyl cysteine-sulfoxide) (Gülnur Ekşi et al. 2020). More than fifty metabolites of PCSO can be produced depending on water content and temperature and an enzyme called alliinase, which can

perform on the mix of MCSO, alliin, and PCSO to yield methyl methanehiosulfonate, allyl methane thiosulfonates, and added corresponding thiosulfinate (R-S-S, R') by which the allyl, propyl, and methyls groups (R and R') are present (Gaber El-Saber Batiha et al. 2020).

Table 2. List and structures of certain sulfur-containing compounds secluded from *Allium sativum* (Source: Gaber El-Saber Batiha et al. 2020)

Compounds	Molecular formula	Structure
Alliin	$C_6H_{11}NO_3S$	
Allicin	$C_6H_{10}OS_2$	
E-Ajoene	$C_9H_{14}OS_3$	
Z-Ajoene	$C_9H_{14}OS_3$	
2-Vinyl-4H-1,3-dithiin	$C_6H_8S_2$	
Diallyl sulfide (DAS)	$C_6H_{10}S$	
Diallyl disulfide (DADS)	$C_6H_{10}S_2$	
Diallyl trisulfide (DATS)	$C_6H_{10}S_3$	
Allyl methyl sulphide (AMS)	C_4H_8S	

Sulfoxides of S-alk(en)yl-l-cysteine are secondary metabolites of cysteine that accrue in Allium plants (Sanda Vladimir-Knežević et al. 2012). Garlic formulations contain various organosulfur compounds generated from alliin, including N-acetylcysteine (NAC), S-allyl-cysteine (SAC), and S-allyl-mercapto cysteine (SAMC). Notably, SAC possesses antioxidant, anti-inflammatory, redox-regulated, anti-apoptotic, pro-energetic, and signalling properties, whereas SAMC exhibits anticancer action by inhibiting cancer cell proliferation (Diogenes dos Santos Dias et al. 2020). Allyl thiosulfinate (Allicin) is a thioester of sulfenic acid whose pharmacological activity is ascribed to its activity of antioxidants and contact with thiol-containing proteins. Allicin is synthesized by converting cysteine to alliin, which is then hydrolyzed by the enzyme alliinase. Allicin is broken down by this enzyme, which is made of pyridoxal phosphate (PLP), into three extremely unstable and reactive molecules at room temperature: ammonium, allyl sulfenic acid, and pyruvate (E. Wieslander et al. 1998). When garlic is cooked, the sulfur compound known as allicin can be destroyed, and it has been linked to a variety of adverse health effects, including food intolerance and allergic reactions, as well as gastrointestinal problems (Soyeon Jo et al. 2020).

As well as adding flavor and colour to food, spices are also thought to be therapeutic due to their ability to prevent a variety of acute and chronic ailments. As a result of the different types of therapeutic properties of spices' bioactive compounds (such as alkaloids), antioxidants, ant carcinogenic, ant tumorigenic, and anti-inflammatory compounds (such as polyphenols and flavonoids), and sulfur-containing compounds, various types of therapeutic properties can be attributed to them (A. Bordia et al. 1975 & H. Amagase et al. 2001).

As a result of its numerous nutritional elements, phytochemicals, and fiber, garlic is regarded as a useful spice. While it's high in potassium and low in sodium and other vitamins and minerals (such as B-complex), it has a moderate amount of selenium (an essential trace mineral) and low amounts of calcium, magnesium, manganese, and iron. The organosulfur compound content is highlighted because it significantly contributes to garlic’s effective bioactive properties and its derived products.

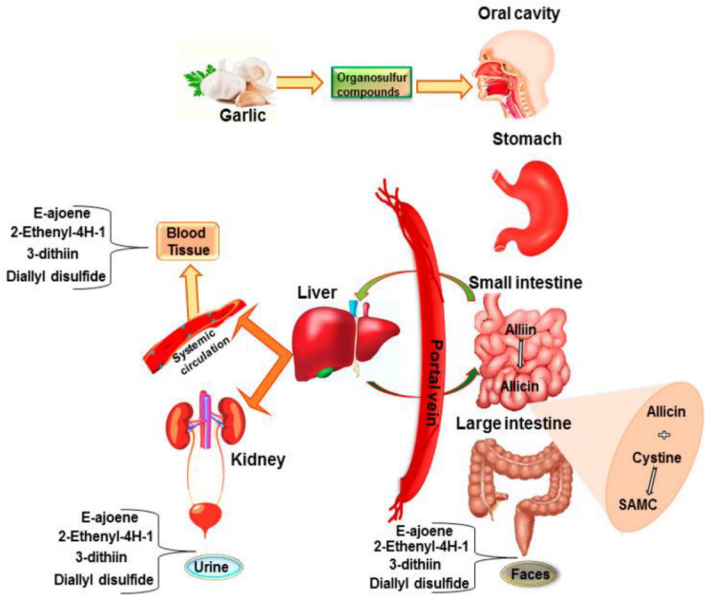


Figure 5. Garlic organosulfur components are absorbed, metabolized, and distributed in the GI tract schematically (Source: Johura Ansary et al. 2020)

1.3.2 Volatile Compounds In Garlic

Spence (2015) stated that, among the factors influencing the perception of the taste of food, the aroma is unequivocally dominating. Both academic and popular literature mention that 75 to 90 % of what we consider to be 'taste', in fact, derives from the sense of smell. Even though the number needs to be taken with caution since it can be slightly tricky to indicate the exact value of the perception of the senses, it is clear that the influence of the aroma on the taste is undeniable. The cloves contain a lot of sulfur-based chemicals, which are easily transformed into a range of volatile molecules during processing due to their reactivity.

Kazuki Abe et al. (2019) stated, processing variables like temperature, pH, and solvent have an impact on processed garlic's volatile profiles. Many forms of volatile sulfur-containing compounds have been found in both processed and fresh garlic, according to numerous investigations on volatile compound changes that take place in processing. Thiosulfinates are primarily broken down and nitrogen-containing volatile chemicals such as pyrazines and pyridines are produced when garlic is heated. Whereas

esters and phenols are the main fragrance chemicals in old garlic extract, aldehydes are the predominant compounds in black garlic.

According to Kazuki Abe, Yoji Hori, Takao Myoda (2019), in various research, 85 sulfur-containing compounds and 40 non-sulfur-containing compounds total are reported. Allicin and other reactive thiosulfinates, for example, methyl, allyl, diallyl, and diethyl mono, di, tri, tetra, penta, and hexasulfides vinyldithiins, and (*Z*) and (*E*) ajoene, can undergo a variety of transformations (Gaber El-Saber Batiha et al. 2020, Diogenes dos Santos Dias et al. 2020 & Wenxi Yang et al. 2019).

Sliced garlic's flavor composition attained by simultaneous distillation and solvent extraction (SDE), steam distillation (SD), headspace solid-phase microextraction (HS-SPME), or solid-phase trapping solvent extraction (SPTE) was compared by Lee et al. in 2003. The SDE extract's major flavorants were diallyl disulfide, allyl sulfide, and diallyl trisulfide, while only diallyl disulfide predominated in the SPTE, SD, and HS-SPME extracts. The link between the fresh garlic odour and the data was also examined, and only not diallyl disulfide; nonetheless, likewise, allicin and dithiin were taken into consideration. Commercial garlic was subjected to quantitative HPLC analysis, which revealed 20 distinct organosulfur compounds, involving minor compounds such 2-vinyl-4H-1,3-dithiin and 3-vinyl-4H-1,2-dithiin. Another garlic examination that had been macerated in oil showed that the main components in the samples were (*Z*)- and (*E*)-ajoene, 2-vinyl-4H-1, 3-dithiin, and 3- vinyl-4H-1, 2-dithiin (Cristian M. B. Pinilla et al 2019). Later, Abu-Lafi et al. used experimental data from GC-MS and HPLC chromatograms to support these findings and came to the conclusion that 2-vinyl-4H-1,3-dithiin and 3-vinyl-4H-1,2-dithiin were fresh garlic's main components (Jian Liu et al. 2019). Nevertheless, according to research, these two sulfur cyclic compounds were created accidentally during the thermal gas chromatographic (GC) examination of allicin (E. Wieslander et al. 1998). Methods involving a gas chromatograph and mass spectrometer (GC-MS) are typically employed to examine volatile substances.

Generally, the composition of volatile substances varies with species and cultivars. Several studies comparing samples of identical coffee originating from different countries illustrate differences in the content of some volatiles, but in general,

we cannot draw any provable conclusions from these studies as they are not considering many other relevant parameters, such as postharvest treatment, sun/shade grown, altitude and various others according to Toledo et al. (2016). The method of postharvest processing technique also substantially affects the composition of volatile compounds in garlic, which affects the quality of the beverage.

1.4 Separation and Extraction Methods of Garlic Oils

Mottaleb (2014) states that there are a number of extraction methods that differ from one another in many parameters, such as cost, efficiency, simplicity, total time consumption, length of extraction time, the necessity of solvent, selectivity, compatibility with different instruments/analytical methods and applications and many others.

One of the most physiologically active components of garlic is allicin and alliin (Figure 4). They may lessen a variety of illnesses (Yebirzaf Yeshiwas et al. 2018 & T. Wolde et al. 2018). When raw garlic is damaged or crushed, an enzymatic process results in the production of allicin. Allicin is created when the enzyme alliinase combines alliin and alliin (Rozita Khademi et al. 2018). For extracting different bioactive chemicals, numerous conventional extraction techniques, for example, reflux, maceration, percolation, soxhlet, steam distillation, etcetera, have been documented. These techniques, which have been in use for several years, take a lot of time and call for a lot of solvents. For eluting the active compounds from the animal or plant tissue, these extraction techniques typically use organic solvents or distilled water.

However, the non-traditional Solid-Phase Micro Extraction (SPME) method is well suited for the extraction of bioactive compounds from plant materials because it can increase yield in less time while using less solvent. Its benefits include low solvent consumption, high extraction efficiency, shortened extraction times, and high-purity extracts (Rozita Khademi et al. 2018). Also, just honey was used in the novel extraction, which can be considered one of the non-chemical extraction techniques.

Figueroa & Vargas (2016) looked into the suitability of three extraction methods for the isolation of volatile aromatic compounds, they compared the usefulness of simultaneous distillation and extraction (SDE), supercritical fluid extraction with carbon dioxide (SCF) and SPME with four different coatings used (PDMS/DVB, PDMS, PA and DVB/CAR/PDMS). Identification of compounds was accomplished by using GC-MS. After 24 hours of evaluating the results, Figueroa & Vargas have suggested the SPME-DVB/CAR/PDMS method as the most appropriate method for representative identification of garlic aroma compounds. Another study, published in 2008 by Viegas et al., identifies soluble garlic volatile compounds comparing even five extraction methods: dynamic headspace (purge-and-trap), static headspace, solvent extraction, simultaneous distillation-extraction (SDE) and headspace solid-phase micro extraction (HS-SPME) using four different types of coatings (DVB/CAR/PDMS with 50/30 µm thickness, PDMS with 100 µm thickness, CW/DVB with 70 µm thickness, PDMS/DVB with 65 µm thickness).

As with the previous study, after analyzing the substances by using chromatography/mass spectrometry (GC-MS), it became clear that the SPME method provides the most representative aroma profile.

1.4.1 Solid-Phase Micro Extraction (SPME) Method

Solid-phase micro extraction (SPME) is a relatively recent simple, solvent-free, rapid, easy to automate, precise and extremely sensitive solid-phase extraction sampling technique suitable for the identification of volatile and non-volatile compounds of solid, liquid and gaseous analytes according to Shirey (2012), and Merkle et al. (2015). Thanks to countless advantages over other extraction methods, SPME technique gains in popularity and finds its application in an increasing number of disciplines, according to Merkle et al. (2015). The principle of the method is based on the absorption of analytes onto an absorbent-coated fused-silica optical fibre, according to Mottaleb et al. (2014), and Sgorbini et al. (2014). Currently, the most commonly used fibre length is 1 cm. For efficiency reasons, fibres longer than 2 cm are never used. Different fibre coating durability affects the maximum number of extractions that can

be done using one single fibre. Overuse of fibre leads to its “bleeding”, which becomes evident due to the siloxane contamination. Coated fibre is attached to an inner needle or tubing that is hidden inside the outer piercing needle. An essential part of this easy to use device is the septum sealing covering the outer needle. It prevents leaking during the insertion of the needle into a pressurized injection port of a gas chromatograph (GC). The colour of the hub on the top of the tubing (plunger) indicates what kind of coating is applied to the fibre. For a detailed list of commercially available fibres together with a colour description of their hubs and technical properties (Table 3).

Table 3. List of commercially available SPME fibres and their properties (Source: Sigma-Aldrich 1999; Mottaleb 2014).

Fibre coating	Film thickness	Hub description	Polarity	Maximum operating	Compatible with	Class of compounds
Polydimethylsiloxane (PDMS)	100	Red (plain)	Nonpolar	280	GC/HPLC	Volatiles
PDMS	30	Yellow (plain)	Nonpolar	280	GC/HPLC	Nonpolar semivolatiles
PDMS	7	Green (plain)	Nonpolar	280	GC/HPLC	Medium to nonpolar semivolatiles
PDMS-divinylbenzene (DVB)	65	Blue (plain)	Bipolar	270	GC	Polar volatiles
PDMS-DVB	60	Brown (notched)	Bipolar	270	HPLC	General purposes
PDMS-DVB[a]	65	Pink (plain)	Bipolar	270	GC	Polar volatiles
Polypyrrole coated	50	—	Polar	250	HPLC–MS	Catechins and caffeine
Polyacrylate (PA)	85	White (plain)	Polar	320	GC/HPLC	Polar semivolatiles (phenols)
Carboxen–PDMS (CAR/PDMS)	75	Black (plain)	Bipolar	320	GC	Gases and volatiles
CAR/PDMS[a]	85	Light blue (plain)	Bipolar	320	GC	Gases and volatiles
Carbowax–DVB (CW/DVB)	65	Orange (plain)	Polar	265	GC	Polar analytes (alcohols)
CW/DVB[a]	70	Yellow-green (plain)	Polar	265	GC	Polar analytes (alcohols)
Carbowax-templated resin (CW/TPR)	50	Purple (notched)	Polar	240	HPLC	Surfactants
DVB-Carboxen-PDMS[a]	50/30	Grey	Bipolar	270	GC	Odours and flavours
Supel-Q-PLOT	50	—	Polar	240	HPLC-UV	Isoflavones and phenols

[a] Stableflex type is on a 2-cm fibre
(Sources: Sigma-Aldrich 1999; Mottaleb 2014)

A spring, which is a part of the manual assembly and is missing in the assembly used with auto samplers, has the task of retracting the fibre after desorption and extraction exposure, according to Shirey (2012). The schematic diagram of the SPME manual fiber assembly is shown in figure below (Figure 6).

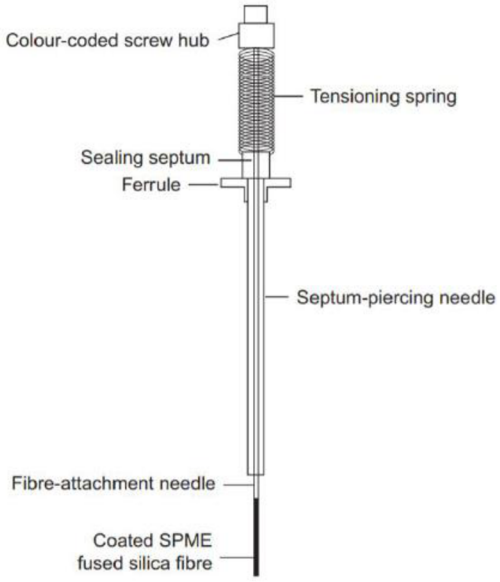


Figure 6. Schematic diagram of the SPME manual fiber assembly (Source: Shirey 2012)

For better manipulation, the assembly is inserted into the manual holder (Figure 7). Because the needle is relatively brittle and can be quite easily damaged, the holder is provided with a needle guide depth gauge that determines how far the needle goes either into the vial or in the injection port by being screwed down or up. Fibre can be locked in the exposed position by a z-slot attached to the manual holder, according to Shirey (2012).

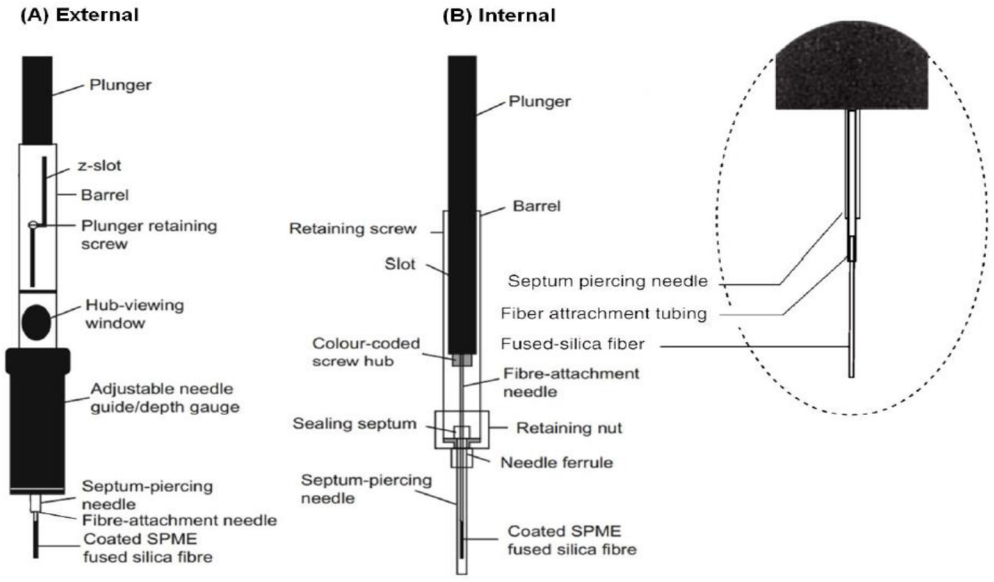


Figure 7. Schematic diagram of external (A) and internal (B) view of SPME manual fibre assembly holder. (Source: Zhang & Yang (1994), and Shirey (2012))

During the transfer of the device, the plunger is located in the uppermost position of the z-slot, which indicates that the fibre is securely hidden in the hollow needle. The downward movement of the plunger along the z-slot drives the fused-silica fibre out of a hollow needle during extraction and subsequent injection into the chromatograph (GC). The whole process is illustrated in detail in Figure 8 below;

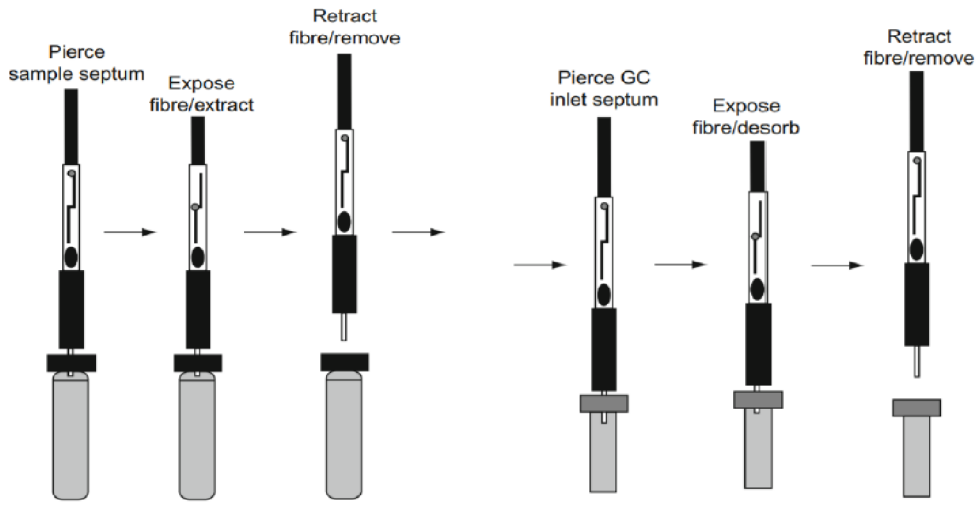


Figure 8. Typical steps of SPME extraction (direct immersion) and subsequent thermal desorption (Source: Shirey 2012).

Three basic modes of SPME operation are distinguished. The first of them is a direct extraction (illustrated in Figure 9 A), during which the needle is directly immersed into the sample. In case the fibre does not come into contact with the sample at all and is inserted only into the headspace slightly above the sample, we talk about headspace SPME (Figure 9 B). In practice, this method is widespread in the analysis of samples with high-molecular-weight interferences. Heating the vial facilitate release of volatile substances and thus reduce extraction time. Enhanced sensitivity of extraction can be achieved by minimizing the headspace volume. The last method, membrane-protected SPME (Figure 9 C), is suitable for the accurate identification of samples, including both high-molecular weight interfering compounds and non-volatile target analysts at the same time. The time extraction is dependent on the time length that is required for the analyst concentration to achieve equilibrium between the sample matrix and the fibre coating, according to Pawliszyn (2012b). This time is, among other parameters, for example, significantly influenced by the thickness of the coating. For the measurement

of volatile substances, a thick coating is used, whereas semi-volatile compounds require thin coatings to be absorbed/desorbed with the highest effectiveness possible, according to Mottaleb et al. (2014).

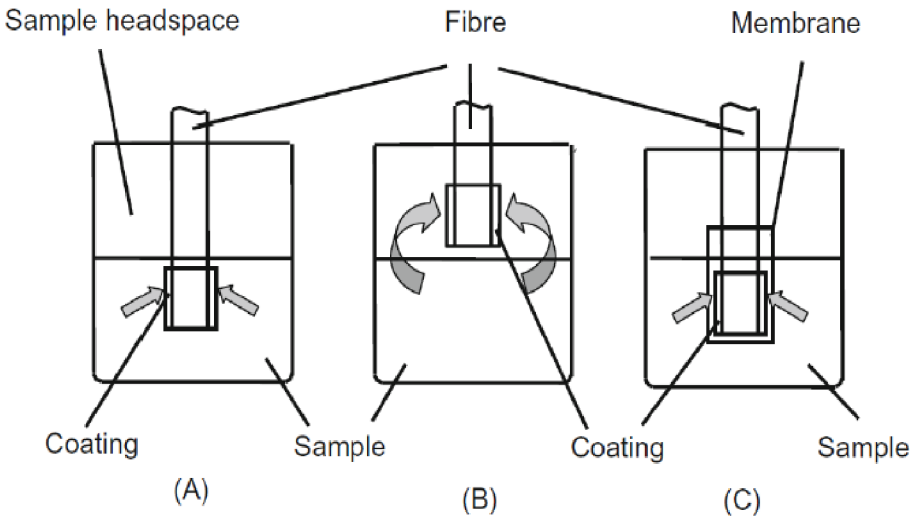


Figure 9. Three basic SPME operation modes: (B) headspace SPME; (A) direct extraction; and (C) membrane-protected SPME (Pawliszyn (2012b)

1.4.2 Hydro-Distillation Methods

Traditional techniques for obtaining bioactive components from plants include hydro distillation. This process involves packing plant materials into a compartment that is motionless, adding just enough water, and then bringing it to a boil. Steam direct can likewise be injected into the plant sample as an alternative. Water is indirectly used to chill the water and oil vapor mixture in order to condense it. Bioactive compounds and oil are separated automatically from the condensed mixture of water as it goes from the condenser to the separator, according to Azmir et al. (2013).

Its use is restricted due to limitations on high applications of temperature for phenolic heat-sensitive compounds, despite the method's benefits, which include the absence of organic solvents in the process, the need elimination to dehydrate materials of plant, and quicker extraction times according to Ouzzar et al. (2015), and Azmir et al. (2013).

Researches have shown that the three different commonly used distillation methods used are Laboratory Hydro-distillation, Industrial Hydro-distillation, and Industrial Steam Distillation. These methods use a Clevenger apparatus for 3 hours, 4 hours and 5 hours, respectively, according to Prabodh Satyal et al. 2017.

Yahya and Yunus (2013) discovered that the amount of time spent extracting the essential patchouli oil had an impact on its quality. The contents of various components may change when the extraction time is lengthened or shortened (Yahya and Yunus (2013), Strati et al. (2015), Zhang et al. (1994), and Su (2002)).

According to Robert Tisserand, Rodney Young PhD, in Essential Oil Safety (Second Edition) (2014), the majority of essential oils are now extracted using a newer adaptation of this technique that involves passing steam through the plant material. The use of water or steam is chosen because it entails a lower risk of decomposition and exposes plant constituents to lower temperatures than would be required for simple distillation. Early Persian distillers occasionally employed simple heating, or "dry distillation." Volatile plant components are vaporized during steam distillation and then condensed after cooling to create an immiscible mixture of an oil phase and an aqueous phase. Essential oil is a complex blend of mostly odoriferous, occasionally colored, and frequently physiologically active chemicals. Aromatic chemicals are also present in the aqueous layer, which is often referred to as a hydrosol, aromatic water, or hydrolyte, but they are at much lower concentrations and in different ratios to the essential oil.

Robert Tisserand, Rodney Young PhD (2014) A plant component needs to exert a significant vapour pressure at 100 °C in order to volatilize and go through steam distillation. As a result, some solid substances and liquids that are less volatile than water may co-distil with water. Furanocoumarin derivatives, notably psoralen and bergamottin, are notable examples of such solids. They are referred to as "non-volatile chemicals" in the Essential Oil Profiles when they are present in essential oils in considerable levels.

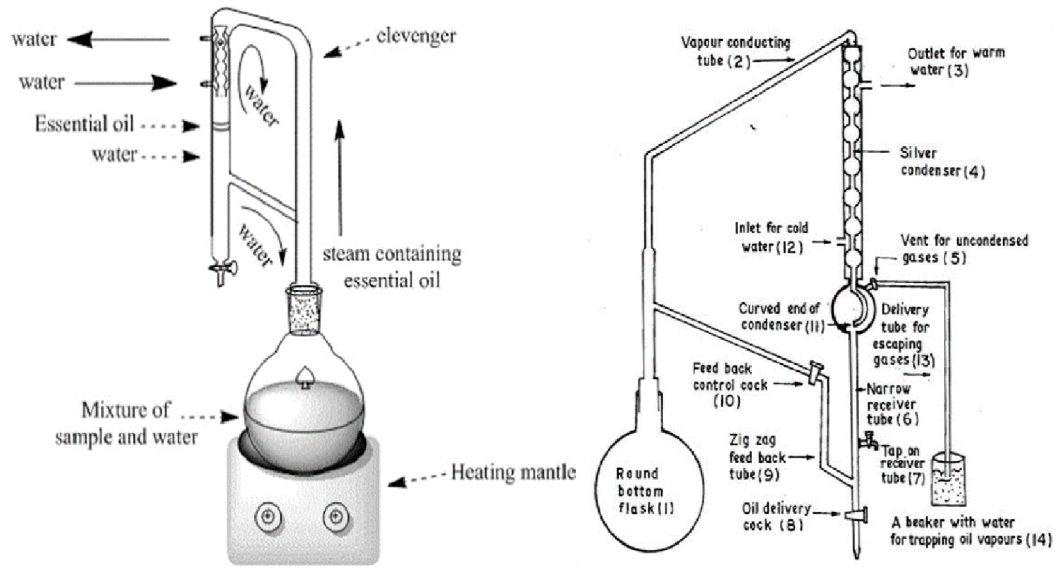


Figure 10. A Schematic diagram of Clevenger apparatus (Mahtab Samadi et al. (2016) and Mahesh Dissanayake et al. (2010)

Traditionally, the essential oil is produced by hydro-distillation using apparatus based on the circulatory distillation device Clevenger invented in 1928. Apparatus and techniques of operation are now well-established.

1.5 Gas Chromatogram-Mass Spectrometry (GC-MS)

Hites (1997) Gas chromatography-mass spectrometry (GC-MS) is an analytical method combining the features of two highly compatible techniques to quantify and identify semi-volatile and volatile organic compounds in complex mixtures with a great resolution. The gas chromatograph, developed in the mid-1950s, is used to precisely physically separate the compounds in the sample, but unfortunately, the device is not able to further reliably selectively detect separated particles. For this reason, shortly after its development, it has been connected with the mass spectrometer, that has the opposite problem – it provides detailed information about the structure of the compounds, directly leading to their exact identification, but the device is not able to readily separate the mixture according to Hites (1997), and Sneddon et al. (2007).

Aniszewski (2007) The common gas chromatograph consists of the separation column, injector port, high-pressure cylinder with a supply of carrier gas, flow control

meters, attendant pressure regulators, detector, electrometer, and data-processing unit.

The sample, in the form of a liquid solution or a collection of molecules absorbed on the surface of the fiber (SPME method), is introduced via an injection port into the inlet (called injector). Injectors can be divided into two main categories: on-column injectors and vaporization, according to Forgács & Cserháti (2003). Injectors of vaporization use rapid exposure of the sample to high temperatures (200 - 300 °C). The sample volatilizes immediately and mixes with a continuous flow of carrier gas. The carrier gas acts only as a background gas facilitating the detection, and thus must be inert or non-reactive. The most commonly used gases are helium, hydrogen, nitrogen, and argon, according to Stauffer et al. (2008), and Stashenko & Martinéz (2014). The second category, represented by on-column injectors, omits vaporization. Without using any heat, the sample is deposited directly into the column, according to Forgács & Cserháti (2003).

The separation is based on the selective distribution of the compounds between the two phases, the mobile phase (gas) and liquid in the case or gas–liquid chromatography (GLC) or the stationary phase (solid in the case of gas–solid chromatography (GSC). The gaseous mobile phase enters the separation section, where the long capillary tubing, called the column, is located, according to Forgács & Cserháti (2003), and Stashenko & Martinéz (2014). The column is enclosed in a temperature-controlled oven, according to Stauffer et al. (2008). The columns can be generally classified into two distinct categories: capillary and packed columns, also known as open tubular columns. Packed columns, made of a rigid metal or glass tubing, are, as their name indicates, densely packed with a solid support (like fluorocarbons, graphitized carbon black, diatomaceous earth or glass beads) coated with a stationary stage, represented by a thin layer of high molecular weight polymer according to Forgács & Cserháti (2003). The second group, capillary columns, is further divided into two types. The first type is a wall-coated open tubular (WCOT) column with a microscopic film of the stationary stage coated directly along the inner wall columns. The column of support-coated open tubular (SCOT), the second type, has the inner walls of the capillary

coated with an adsorbant solid or a thin layer that is preserved with the phase of liquid stationary, according to Poole (2002).

As the sample mixed with the stream of the carrier gas is swept through the column, the gaseous compounds interact with the phase of stationary. The column retain molecules and gradually elute from the column at dissimilar times, known as the retention times. Individual components of the gasified sample are separated by the column according to their physical characteristics, such as boiling point, polarity differences, or molecular size (molecular sieve columns). The most volatile compounds leave the column first. The quantity (concentration) of each compound exiting the column is measured by detectors according to Emerson Process Management (2012). A plot of the detector signal as a function of time generated by the GC system is referred to as a chromatogram and it is the main output of the technique. Each peak on a chromatogram corresponds to a different compound of the mixture. By integrating the chromatogram, peak areas are obtained and so the mole fraction of each compound can be quantified. , An example of a chromatogram obtained from a sample of garlic is shown in Figure 11.

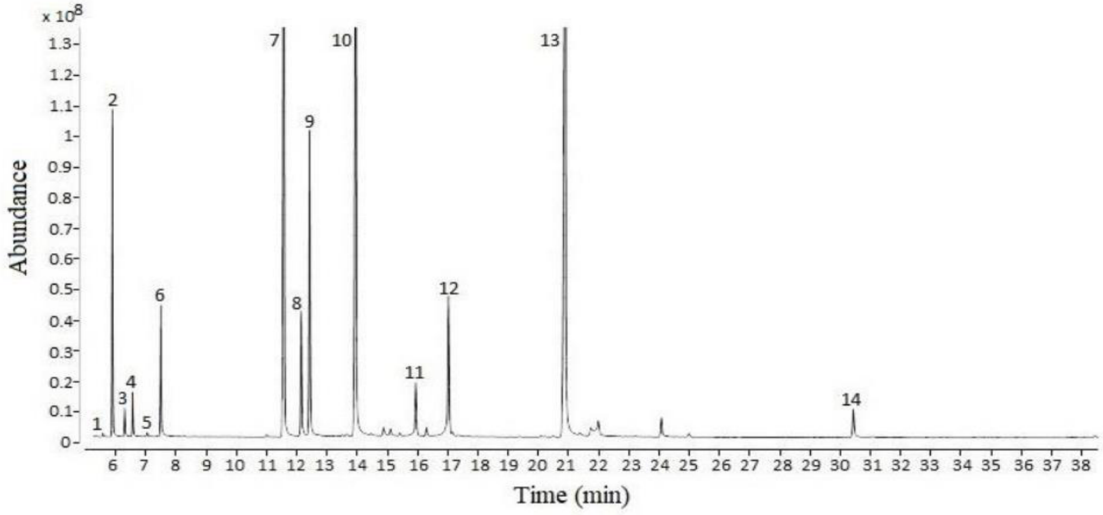


Figure 11. Chromatogram obtained from a sample of garlic by using HS-SPME–GC–MS method of compound identification (Source: Özge Süfer & Fuat Bozok 2019).

Degradation products eluted from the stationary phase may cause the normal background signal recognized as a column bleed. Column bleeding occurs to some extent in all cases, regardless of the quality or source, and does not necessarily mean damage of the column. With increasing column length, column diameter, and film thickness, the bleed levels will also increase. A slightly higher rate of bleeding is exhibited by polar phases compared to nonpolar ones, according to MSP Kofel (2005). Discrete peaks that appeared during the blank runs always indicate contamination of the front portion or inlet. As, a peak generation, a one-time, isolated event, can never be caused by a continuous process, such as stationary phase degradation. Mass spectrometry is a highly sensitive and accurate tool for the identification of molecular structure. The major components of the mass spectrometer are inlet, such as for example, a gas chromatograph, an ionization source, one or more mass analysers, a detector, and a data processing system. The first step after the introduction of the sample into the mass spectrometer is its ionization. The most commonly used ionization methods are electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI). The ionized sample continues into the mass analyser, where the ions are parted consistent with their ratio of mass-to-charge (m/z). Several types of mass analysers are available, including ion trap, quadrupole, orbitrap, and time-of-flight (TOF), according to Pan et al. (2014), and Vandell & Limbach (2017)). Eventually, selected ions are fragmented, and the fragments are further analysed in the second mass analyser. After the ions emerge the last analyser, they are detected, measured in terms of their abundance, and converted into electrical signals. The electrical signals are processed, transmitted into the computer and displayed as a mass spectrum, according to Hoffmann & Stroobant (2007). The mass spectrum represents a molecular fingerprint presented usually as a vertical bar graph. Each bar stands for an ion with a specific mass-to-charge ratio (m/z). The relative abundance of individual ions is indicated by the length of the bars. In case the abundance is expressed in absolute form, the most intense ion (the highest bar) is assigned an abundance of 100 % and the others are normalized to this value according to Chudoba (2016). For illustration, Figure 12 shows the mass spectrum of (-)-limonene.

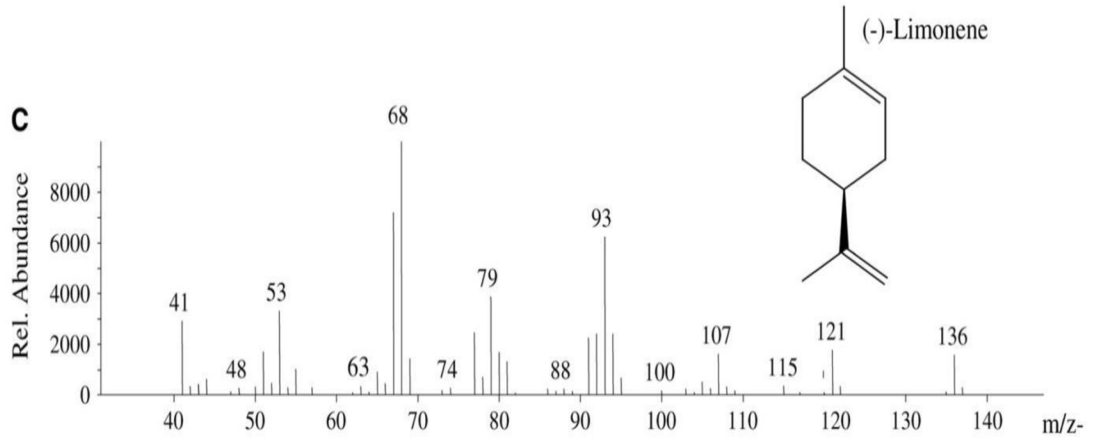


Figure 12. Mass spectrum of (-)-limonene library standard (Source: Byun-McKay et al. 2006).

For the final identification of sample compounds, the mass spectrum together with the retention time of the molecule is the most important because these two attributes are compared and matched to the standard reference compounds analyzed under the same conditions.

Almazov & Konenkov (2017) In modern spectrometry, one of the most commonly used mass analysers is a quadrupole mass filter (QMF). Quadrupole Mass Filter (QMF) is formed by a parallel array of four metal electrodes with a hyperbolic or circular profile and length of between 15 and 25 cm. Electrodes are arranged in the shape of the block, forming a space between them. Opposing pairs of electrodes are electrically connected together. Tanna & Lawson (2016) Alternate current (AC) and direct current (DC) voltages are applied using the rule that opposite electrodes have the same voltage. Clarke (2017) The oscillating electrical fields created around the rods are able to selectively destabilize or stabilize the trajectories of ions passing through a radio frequency (RF) quadrupole field between the rods and thus filter the ions according to their mass-to-charge ratio values (m/z). Somogyi (2008) Main disadvantages of this relatively fast and simple operation, with no requirement for very high vacuum (> 10^{-7} Torr), are especially low (generally unit) resolution, a low *m/z* cutoff and low transmittance**.** A simplified schematic diagram of the GC-MS system with a quadrupole mass analyser is shown in Figure 13.

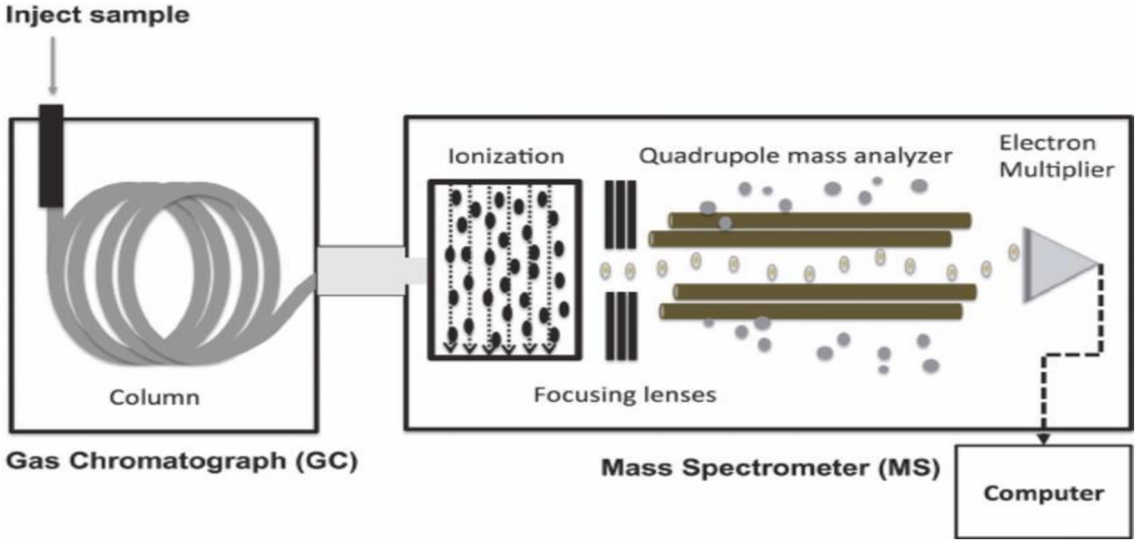


Figure 13. Simplified schematic diagram of GC-MS system with quadrupole mass analyzer (Source: Il-Young et al. 2016).

1.6 New Microdilution Volatalization and Modified MTT Assay

In order to examine their volatile ingredient's potential for novel inhalation treatments' development, a number of techniques for assessing essential oils' antimicrobial effects have been developed in recent years (Lívia C. Galvão et al. 2012). The vapour phase tests are the most suited in vitro method for demonstrating essential oils' antibacterial activity and their constituents because it has been established that their vapours are more potent antimicrobials than their liquid stages (Laird & Phillips 2011). Guo et al. (2012) examined the effect of garlic supplement meals on development and illness, as well as garlic's in-vitro antibacterial efficacy against *Streptococcus iniae* (*S. iniae*). In order to identify the amounts effective against some of swine intestinal microbiota's most important populations, Ruiz et al. (2010) examined in-vitro two of these garlic-derived chemicals' effects (PTS-O and PTS). Moreover, activity against *Salmonella typhimurium* and *Escherichia coli,* two popular pigs' pathogens, was also tested. M. Houdkova et al. (2017) designed and evaluated a novel screening approach (method of new broth microdilution volatilization) for simple and rapid simultaneous willpower of antibacterial possibility of plant volatiles in the vapour phase and liquid at dissimilar concentrations. Additionally, cinnamaldehyde, antibacterial activity of carvacrol, 8-hydroxyquinoline, eugenol, thymoquinone and thymol was determined

against *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Haemophilus influenzae* using a novel method of broth microdilution volatilization. These compounds' cytotoxicity was assessed using the test of MTT in fibroblast lung cells MRC-5. Lenka Nedorostova el al. (2011) evaluated and identified antimicrobial properties of designated essential oils in the phase of vapour against diverse strains of *Staphylococcus aureus*, involving resistant clinical isolates and MRSA. The resistant strains of *Staphylococcus aureus* were checked by the method of disc diffusion volatilization against three antibiotics Vancomycin, Erythromycin and Oxacillin.

In order to comprehend the essential oils' capacity to fight germs in the respiratory tract, it may be helpful to understand the findings of these experiments (Györgyi Horváth & Kamilla Ács 2015). There are zero assays standardized for determining the sensitivity of microbial to volatile compounds in the phase of vapour, such as those suitable for the European Committee on Antimicrobial Susceptibility Testing (EUCAST) or Clinical and Laboratory Standards Institute (CLSI) (CLSI 2015 & E. Matuschek et al. 2014). This is in contradiction to well-established approaches for susceptibility testing of antimicrobial in liquid media. The most popular technique for assessing plant volatiles' antibacterial properties in the vapour stage is the disc volatilization test (Juan Bueno et al. 2015). It has also been developed in many variations employing Petri dishes with a dressing model (V. Edwards-Jones et al. 2004), sterile adhesive tape (P. Lopez et al. 2005), four section Petri dishes (Pavel Kloucek et al. 2012), and agar sealing on the lid (L. Nedorostova et al. 2009). In a different investigation, (Hyun-Sun Seo et al. 2015) employed a unique apparatus created for the evaluation of essential oils' gaseous phase. Despite the fact that all of these techniques are frequently employed to evaluate the antibacterial qualities of volatile substances in the vapour stage. These are not intended for screening of high-throughput; some only analyse samples at one concentration, while others require specialized equipment that is not frequently available. Additionally, they assess whether volatile plant-derived chemicals are antibacterial in the gaseous or liquid phases.

While essential oils are typically regarded as harmless substances with positive effects on human health (Claudia & Florian 2013), there is a chance that they could cause pulmonary toxicity when used as an inhalation therapy (Laird & Phillips 2011).

Determining the therapeutic preparations' safety for the respiratory system should therefore be a part of the development process (Lívia Câmara de Carvalho Galvão et al. 2012). One of the most popular techniques for assessing in vitro cytotoxicity employing design of microtiter plate is the thiazolyl blue tetrazolium bromide (MTT) colorimetric test. This technique can be used to evaluate the cytotoxicity of natural compounds, including volatiles (Claudia & Florian 2013 and A. Khadir et al. 2016). Yet, as various writers have previously stated (Novy et al. 2014, Thellen et al. 1989) volatile chemicals can affect the outcomes of biological tests utilizing microtiter plates. It is required to alter testing procedures in order to stop the spread of vapours. While investigating the combinatorial effects of volatile substances and antibiotics, (Rondevaldova et al. 2017) demonstrated the need to utilize ethylene vinyl acetate (EVA) Capmat™ as a reliable barrier vapour to stop volatiles from scattering into nearby wells.

1.7 Measurements of Colour and pH In Garlic

Allicin is in charge of the distinctive smell, as well as the antibacterial, anti-inflammatory, anti-cancer, anti-atherosclerosis, anti-thrombosis, and antioxidant effects (Rabinkov et al. 1998). In other to measure the colour change and pH in garlic, respectively, the Minolta Colorimeter (Chrome Meter CR410) and the Thermo Scientific™ Orion Star™ A211 Benchtop pH Meter instruments are thoroughly needed.

1.7.1 Minolta Colorimeter (Chrome Meter CR 410)

The CR-410 Chroma Meter is a hand-held, portable measurement tool made to assess the colour of items, especially those with texture, uneven surface conditions, or significant colour variation. This highly accurate, dependable colour meter assists users in maintaining the colour quality, consistency, and look of their samples in a more streamlined, effective manner both internally and throughout the supply chain. It precisely identifies colour traits in things, establishes colour disparities across items, and offers pass/fail assessments to quickly ascertain whether the sample complies with the predefined criteria. Because of this, the CR-410 excels in quality control, quality

assurance, and research and development fields for colour inspections of food, building materials, and textile applications. The CR-410 Chroma Meter is compatible with SpectraMagic NX software, which records measurements and offers a more thorough colour analysis, as well as an optional data processor for on-site printing of results (Konica Minolta Measuring Instruments).

1.7.2 Thermo – Scientific Orion Star A211 Benchtop Meter

Thermo Scientific™ Orion Star™ A211 benchtop pH meter (Spectrophotometer) is perfect for a variety of uses and sophisticated pH lab studies. With the help of the free, downloadable Orion Star™ Com software, you can quickly do up to a 5-point pH calibration, log up to 2000 data sets of points with a date/time stamp, and send data logs and calibration by RS232 or USB to a computer or printer. Use the supplied stand electrode to make it simple to store sensors in samples and reduce damage. It records accurate and reliable pH, mV, ORP and temperature measurements. The Orion Star A211 Benchtop pH Meters from Thermo Scientific are dependable laboratory meters with simple operation and cutting-edge features. You have flexibility and control over your testing thanks to the stability indication and several read modes. The date, temperature, time, measurement stability, read type, electrode status icon, and active calibration information are all displayed along with your measurements. Instructions for calibration and setup on screens can be seen in a number of regional languages, and more languages can be added through software upgrades.

The extensive keypad's menu-specific function buttons, dual-purpose scroll and shortcut keys, with measure, power, stirrer keys, and log view that guarantees uncomplicated operation, provide for quick and easy navigation. You may easily access, assess, and modify measurement and instrument settings since all setup options are conveniently located in one place and are directly accessible with a single shortcut key, according to Spektro Tec (2023).

2. AIMS OF THE THESIS

The diploma thesis focused on garlic's chemical composition analysis with a special emphasis on the description of the volatile compounds found in fresh garlic from five different countries of origin, namely; the Czech Republic, China, Slovakia, Spain and Italy.

The main objective of the diploma thesis was to analyze the volatile compounds of fresh garlic (*A. sativum*).

The specific objective was to analyze the essential oils' antimicrobial activity present in the fresh garlic (*A. sativum*), originating from the five geographical locations.

The secondary subject of interest of this research was to compare, whether fresh garlic originating from different geographical locations responds dissimilarly to colour change and pH.

Overall changes were considered to include differences in the volume quantity of individual volatile compounds present in the samples, the presence of antimicrobial activity in the essential oils, as well as differences related to the colour change and pH.

3. MATERIALS AND METHODS

3.1 Clevenger Laboratory Hydro-Distillation (CLH)

3.1.1 Plant Materials

Five samples of *Allium sativum* were subjected to the research. Sampling was based on the country of origin and the availability in the market (supermarket) at the time of purchasing, specific countries of origin were the Czech Republic, China, Spain, Slovakia and Italy.

Fresh bulbs of *Allium sativum* from the selected countries were purchased from the local supermarkets in Prague, Czech Republic, in October 2021. Clevenger apparatus, knife, blender, weighing machine, distilled water, and chemical (Hexane, Sigma-Aldrich) were provided in the school laboratory.

3.1.2 Sample Preparation

All five samples were brought to the laboratory and grouped according to their origins. One garlic bulb comprises numerous wedge-shaped cloves that are coated with inner layers, and garlic bulbs have numerous white layers, papery coatings. Garlic bulbs were finely peeled with a knife by hand. All the cloves which seemed to have gone bad were removed. Each country of the garlic was weighed in order to know the amounts of the weight of the various garlic. 200 grams of crushed garlic was blended since they were subjected to boiling and the addition of 500 ml of distilled water. The Clevenger apparatus was nicely set up and all the parts were carefully checked before powering on. Boiling stone was then added to the garlic mixture. The laboratory hydro-distillation method using the Clevenger apparatus takes 4 hours in order to get the essential oils. However, the boiling temperature was carefully monitored and regulated to avoid spilling.

3.1.3 Extraction of Garlic Essential Oils

The steam containing essential oils began to evaporate through the vapour conducting tube during the boiling of the garlic mixture. The essential oils together with the steam went through the condenser, where the oils floated on the steam. The oil delivery cock was then carefully opened to get the oil separately into a beaker. The steam water went back to the round bottom flask, through the feedback control cock. Hexane was added to the extract of essential oils in order to separate water and the essential oils. The essential oils were stored in a refrigerator at a temperature of 4 °C. The essential oils can be stored in the refrigerator until GC-MS analysis.

3.1.4 GC-MS Analysis

The essential oils from each garlic sample dissolved in hexane (5 mg/ml) were injected for analyses, it was conditioned at 250 °C, and the blank measurement was performed for 1 hour total. The GC-MS analysis was performed on an Agilent 7890B/5977A GC/MSD System (Agilent Technologies, USA) equipped with the HP-5 column ((5 %-phenyl)-methylpolysiloxane, 30 m length, 250 µm internal diameter, 0.25 µm film thickness) was employed. Thermal desorption was done directly into the injection GC port at 250 °C and maintained during the whole chromatography run, which was set to 1 hour. The GC injector port was operated in the splitless mode with a 0.75 mm i.d. liner. The enhanced GC oven program temperature was 70 °C (2 min) to 280 °C at 10 °C/min (final temperature held for 5 min). As the carrier gas Helium, with a flow of 1 ml/min, was used. Values of the Retention Index (RI) for the volatile substances were calculated by running *n*-Alkanes (Sigma-Aldrich, Czech Republic, Prague) under the same conditions. The MSD transfer line temperature was kept at 250 °C with the electron energy of 70 eV. Spectra mass was attained in the range mass to 600 from *m/z* 30, using 1 s scan time. Data were processed using Mass Hunter Workshop software for qualitative analysis version B.o7.00 using the electronic integration peak areas. The identification was confirmed by comparing the measured of RI with the database of the National Institute of Standards and Technology (NIST, USA). Not all substances could be verified by comparison of RI, because some retention indexes were not available.

3.2 Solid-Phase Micro Extraction (SPME)

3.2.1 Plant Materials

The garlic samples were the same as those used for Hydro-distillation method.

3.2.2 Sample Preparation

The garlic samples were prepared the same way as those used for Hydro-distillation method.

3.2.3 Extraction of Garlic Compounds

1 g of the grinded garlic was placed in a clear 4 ml vial hole cap and PTFE-faced silicone septa. The hermetic sealing of the packaging protects garlic against light, oxygen, moisture, and evaporation. A silica fibre coated with 100 µm thick polydimethylsiloxane (PDMS) film set in the manual SPME device was inserted and exposed to the garlic sample without touching it for 5 minutes.

3.2.4 GC-MS Conditions/Analysis

Before using the fibre for analyses, it was reconditioned at 250 °C each day, and the blank measurement was performed for 1 hour in total. The GC-MS analysis was performed on an Agilent 7890B/5977A GC/MSD System (Agilent Technologies, USA) equipped with a HP-5 column ((5 %-phenyl)-methylpolysiloxane, 30 m length, 250 µm internal diameter, 0.25 µm film thickness) was employed. Needle was inserted into the injection port immediately after extraction. Thermal desorption was performed directly into the GC injection port at 250 °C and maintained during the whole chromatography run, which was set to 1 hour. The GC injector port was operated in the splitless mode with a 0.75 mm i.d. liner. Helium was utilized as the carrier gas, and the temperature oven was raised to 160 °C from 40 °C at 6 °C per minute rate, then to 220 °C from 160 °C at 10 °C per minute rate. Retention Index (RI) values for the volatile substances were

calculated by running *n*-Alkanes (Sigma-Aldrich, Czech Republic, Prague) under the same conditions. The MSD transfer line temperature was maintained at 250 °C with the electron energy of 70 eV. Mass spectra were acquired in the mass range from *m/z* 30 to 600, using a scan time of 1 s scam time. Data were processed using Mass Hunter Workshop software for qualitative analysis version B.o7.00 using the electronic integration of peak areas. Identification of volatile compounds was done by comparison of their mass spectra against mass spectra covered by the NIST/EPA/NIH library version 2.2. The identifications were comfirmed by comparing the measured RI with the database of the National Institute of Standards and Technology (NIST, USA). Not all substances could be verified by comparison of RI, because some retention indexes were not available.

3.2.5 Statistical Recording of Analysed Data for CLH and SPME

The results of the overall areas of peak and the chosen key volatiles. All data were sorted and saved in Microsoft Excel 2016.

3.3 Antimicrobial Activity

3.3.1 Chemicals

The essential oils of garlic from the Czech Republic, China, Slovakia, Spain, Italy and oxacillin (7240-38-2, 86.3 %); and other chemicals: dimethyl sulfoxide (DMSO), blue dye thiazolyl tetrazolium bromide, and Tween 20 % were bought from Sigma-Aldrich (Czech Republic, Prague).

3.3.2 Bacteria Strains and Culture Media

Staphylococcus aureus ATCC 29213, the standard strain from the American Type Culture Collection (ATCC), was utilized. Yeast extract was added as a Mueller-Hinton (MH) complement to assay media and the cultivation (broth/agar) (*S. aureus*). Using

Trizma® base, the broths' pH was adjusted to its final 7.6 value (Sigma-Aldrich, Czech Republic, Prague). Oxoid was used to purchase the microbial strains and growing media (Basingstoke, UK). Prior to testing, stock cultures of several strains of bacteria were grown in the proper medium at 37 °C for 24 hours. Then, using a Densi-La-Meter II (Lachema, Brno, CZ), the turbidity of the bacteria suspension was adjusted to 0.5 McFarland standard to obtain 107 CFU/mL's final concentration. As effective antibiotic controls, the susceptibilities of *S. aureus* and oxacillin were examined, respectively.

3.3.3 Antimicrobial Assay

Using a newly created broth microdilution volatilization method, plant volatile components' antibacterial potential in the vapour and liquid phases was assessed. Standard Nunclon 96-well microtiter plates with a well capacity of 800 µL were used for the tests, which were then covered with tight-fitting lids with evaporation-reducing flanges (Thermo Scientific, Roskilde, Denmark).

The initial concentration of oxacillin was 16 µg/mL and stock solution concentration of oxacillin (100x higher) so we had 1600 µg/mL. However, we needed 600 µL of concentration (c) was 16 µg/mL but an amount of 800 µL will be needed for the starting concentration. An amount of 8 µL will be taken from the stock solution. An amount of 792 µL was added to the buffer broth. Finally, the efficiency or assay was ɧ = 86,3 % (863 µg/mg), the minimal weight was 12.8 µg, the real weight was 550 µg, the efficiency was 0.863 and the solvent (oxacillin in distilled water) was 296.6563 µL.

The initial concentration of essential oil for analysis was 1024 µg/mL and stock solution concentration (100x higher) that's 102400 µg/mL. An amount of 800 microliters was prepared instead of 600 microliters of concentration 1024 µg/mL (3*200 microliters). Also, the stock solution concentration (100x higher) so we had 102400 µg/mL. An amount of 792 µL was added to the buffer broth. Finally, the efficiency or assay was ɧ = 100 % (µg/mg), the volume of essential oil used was 2 µL, the minimal weight was 819.2 µg, the real weight was 2170 µg, the efficiency was 1, the solvent (DMSO) was 19.1914 µL.

Oxacillin against *Staphylococcus aureus* ATCC 29213 (CLSI, 2009)

MIC 0,12 - 0,5.

Table 4. Oxacillin against *Staphylococcus aureus* ATCC 29213, Minimal Inhalatory Concentration (MIC).

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B	EO IT	PURITY CONTROL	GROWTH CONTROL	1024	512	256	128	64	32	16	8	
C	EO SL			1024	512	256	128	64	32	16	8	
D	EO CH			1024	512	256	128	64	32	16	8	
E	EO SP			1024	512	256	128	64	32	16	8	
F	EO CZ			1024	512	256	128	64	32	16	8	
G	OXACILLIN			16	8	4	2	1	0.5	0.25	0.125	
H												

First, 30 µL of agar was pipetted into each flange of the plate and lid, and then, after the agar had solidified, 5 µL of bacterial suspension was added. The second step of this approach involved dissolving each sample's volatile components in DMSO at 1 % maximum concentration before diluting them in the suitable broth medium. For all substances, seven twofold diluted serially concentrations of samples beginning at 1024 µg/mL were made. In the case of *S. aureus* was assayed within a concentration range 0.125–16 µg/mL. The last volume in the individual well was 19.1914 µL. Bacterial suspensions were then added to the plates as an inoculant. As growth and purity controls, wells with inoculated and non-inoculated broth were simultaneously created. In order to avoid the edge effect, the outermost wells were left unfilled.

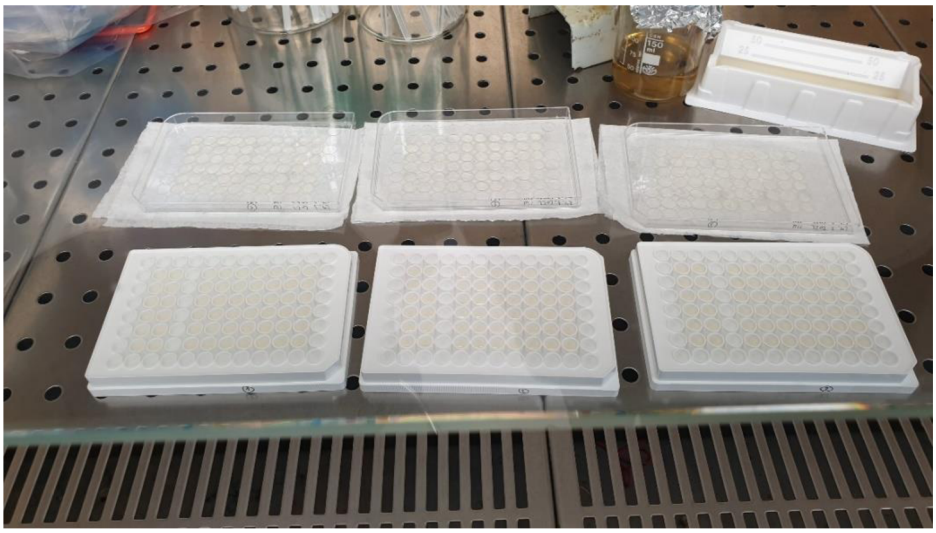


Figure 14. agar was pipetted into all flanges on the plate and lid

Plates of microtiter were then incubated for 24 hours at 37 °C before the clamps (Lux Tool, Prague, CZ) and handcrafted hardwood pads (size 8.5 × 13 × 2 mm) were used to secure the plate and lid together (Figure 15). Every experiment was performed in triplicate in each independent experiment.

Figure 15. The clamps (Prague, Lux Tool, CZ) were utilized for fastening lid and plate together.

However, the schematic design of the experiment is shown in Figure 16 below;

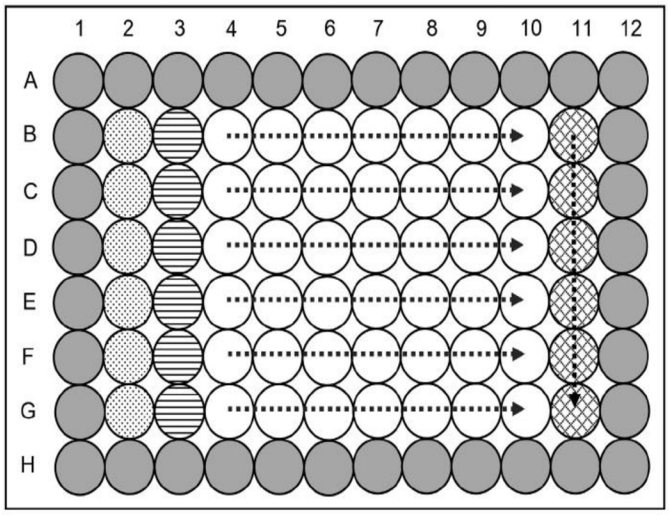


Fig. 3. Schematic design of experiments demonstrating: (a) template for 6 compounds with 7 serial dilutions (two-fold dilution series); ○ white coloured wells: serial two-fold dilution of tested volatile compounds; ◍ grey coloured wells: empty wells, not used in data calculation (problem of evaporation); ◉ dotted wells: purity control (non-infected medium control; 0% growth of bacteria); ◌ striped wells: growth control (100% growth of bacteria); ◎ gridded wells: serial two-fold dilution of positive antibiotic control.

Figure 16. Schematic design of experiment (Source: M. Houdkova et al. 2017.)

Figure 17 below provides a detail of one microtiter plate's cross-sectional view well with a flange on the lid that is filled with agar and broth;

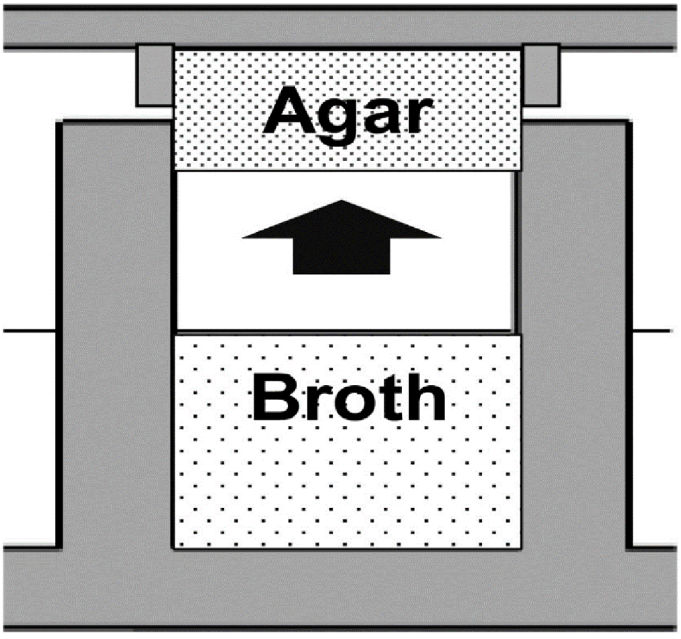


Figure 17. Detail of a cross-sectional image of a plate microtiter well with a flange on the lid that is filled with agar or broth, as appropriate (Source: Houdkova et al. 2017)

3.4 Colour Measurement

3.4.1 Materials

Garlic samples were selected and stored in a refrigerator at (5 ± 1 °C) until use. The CR-410 Chroma Meter, white ceramic plate and Petri dishes were all provided by the CZU-FTA laboratory.

3.4.2 Sample Preparation

A laboratory knife was used to peel, clean, and slice the garlic cloves, which had diameters and thicknesses of 15 ± 2.40 and 1 ± 0.35 mm, respectively. The Minolta colorimeter (Chroma Meter CR 410) is well checked and make sure the lens is cleaned as well as the Petri dishes. The colour parameters to be measured are L_o*, a_o* and b_o*, respectively.

3.4.3 Colour Measurements

The garlic sample is prepared and placed in dishes of Petri (height = 1 cm and diameter = 5 cm) to be measured. The garlic sample is then placed on white filter paper, and a direct reading of the garlic sample was measured the colour in a colorimeter Minolta (Chroma Meter CR 410) using a system of tridimensional *CIE*L*a*b*. The colour measurement of each of the samples was repeated five times, however, there was calibration in-between each sample. A schematic diagram of the colour measurement of the garlic sample is shown in Figure 18 below.

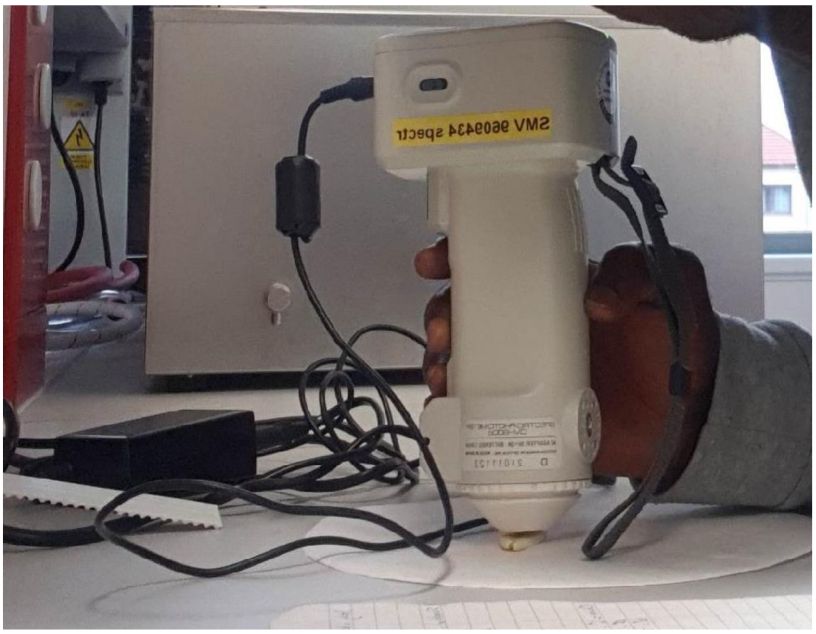


Figure 18. Colour measurement of a garlic sample in CZU-FTA Lab

3.4.4 Data Analysis Recording

Results of each of the colour measurements of each garlic sample by each country of origin were recorded and saved in Microsoft Excel 2016 as shown in (Table 10) respectively.

3.5 pH Measurements

3.5.1 Materials

Garlic samples were selected and stored in a refrigerator at (5 ± 1 °C) until use. Thermo Scientific™ Orion Star™ A211 Benchtop pH Meter (Denver, model A211), distilled water, mortar and pestle, and Petri dishes were all provided by the CZU-FTA laboratory.

3.5.2 Sample Preparations

The cloves of each of the garlic samples were peeled and cleaned thoroughly. 5 grams of each sample were crushed using the laboratory mortar and pestle and the addition of 40 ml of distilled water to each garlic sample. The electronic pH meter (Denver, model A211) is well cleaned and all parts are checked before use.

3.5.3 Measurements of Garlic pH

The pH measure electrode is placed in the sample, the automatic calibration enables the machine to read or measure and record the pH of the garlic sample as soon as placed in. The machine beep after recording the value and it will be displayed on the screen. The pH of each of the samples was repeated five times at one-minute time intervals. The value on the pH meter is always set to zero before another test is performed. A schematic diagram below shows the pH testing of the garlic sample.

Figure 19. A schematic diagram showing pH measurement of a garlic sample in CZU-FTA lab.

3.5.4 Data Analysis Recording

Results of each of the pH of each garlic sample were recorded and saved in Microsoft Excel 2016 as shown in Table 4.

4. RESULTS

4.1 Chemical Analysis of Garlic Essential Oils

The garlic (*A. sativum*) essential oils from the Czech Republic, China, Italy, Slovakia and Spain, were obtained using the Clevenger laboratory hydro-distillation method and were characterized by GC-MS (Table 5). The amount of essential oils obtained after the Clevenger laboratory hydro-distillation method show that the Czech Republic had the highest volume in grams, the second highest in Spain, followed by China as the third highest, Italy was the fourth highest in volume, and least was recorded by Slovakia. These essential oils were later subjected to the GC-MS method and a total of twenty-four (24) volatile compounds combined were identified using the peak heads. The number of volatile compounds found in each of the garlic samples by each country the Czech Republic is nineteen (19), China and Spain had the same number with eighteen (18), Slovakia had seventeen (17) and Italy had sixteen (16).

These essential volatile compounds, Diallyl sulphide, 2-Propenyl methyl disulfide, 3H-1, 2-Dithiole, Diallyl disulphide, Allyl (*Z*)-1-propenyl disulfane, Allyl (*E*)-1-propenyl disulfane, Methyl 2-propenyl trisulfide, 3-Vinyl-1,2-dithiacyclohex-4-ene, 4H-1,2,3-Trithiine, 2-Vinyl-4H-1,3-dithiine, and 5-Methyl-1,2,3,4-tetrathiane were found in all the garlic samples of all countries.

Methyl propyl sulfoxide, (1*E*)-1-propenyl methyl trisulfide, Butyl propenyl sulfide, and 3-Isopropyl-4-methyl-dec-1-en-4-ol were only found in the Czech Republic sample. Methyl (*E*)-3-propenyl trisulfane was only found in Slovakia sample. Butyl propenyl sulfide, Methyl propyl sulfoxide, (1*E*)-1-propenyl methyl trisulfide, and 3-Isopropyl-4-methyl-dec-1-en-4-ol were only found in the Czech Republic.

Methyl (*Z*)-1-propenyl disulfane was identified in the Czech Republic, China, Slovakia except in Italy and Spain. Methyl (*E*)-1-propenyl disulfane, and Dimethyl trisulfide were identified in all the countries except Italy. Di-2-propenyl trisulfide, and Di-2-propenyl tetrasulfide were not found in Slovakia but in all the other samples. 1, 2-Dithiolane was found in all the other samples except the Czech Republic. 4-Methyl-1, 2,

3-trithiolane cannot be found in the Czech Republic and Slovakia samples but in other samples.

A detailed table of garlic (*Allium Savitum*) compounds obtained by Clevenger Laboratory Hydro-distillation using GC-MS is shown below.

Table 5. Evolution of the area (× 10^4) of selected volatile compounds identified in the garlic samples obtained by Clevenger Laboratory Hydro-distillation using GC-MS.

			Czech Republic	China	Slovakia	Spain	Italy
Volatile Compounds	***RI ^{cal}***	***RI ^{lit}***	***Average Area***	***Average Area***	***Average Area***	***Average Area***	***Average Area***
Methyl propyl sulfoxide	840	*	47.86	ND	ND	ND	ND
1, 2-Dithiolane	842	986	ND	51.57	24.28	37.27	45.07
Diallyl sulphide	851	859	37.73	127.04	141.47	135.43	26.45
2-Propenyl methyl disulfide	916	918	264.47	475.97	767.94	421.60	94.02
Methyl (*Z*)-1-propenyl disulfane	931	932	27.30	25.48	54.30	ND	ND
Methyl (*E*)-1-propenyl disulfane	940	940	45.96	34.95	78.71	29.93	ND
3H-1, 2-Dithiole	966	959	188.31	216.66	96.26	82.23	249.00
Dimethyl trisulfide	976	977	71.06	84.60	163.06	124.02	ND
Butyl propenyl sulfide	1072	*	28.83	ND	ND	ND	ND
Diallyl disulphide	1088	1088	2301.78	3458.67	4278.26	1307.49	1886.55
Allyl (*Z*)-1-propenyl disulfane	1103	1097	342.07	204.21	402.23	2628.46	282.73
Allyl (*E*)-1-propenyl disulfane	1109	1103	842.84	535.09	787.41	499.92	769.12
Methyl 2-propenyl trisulfide	1151	1144	1195.45	1489.41	1433.31	911.54	585.39

Table 6. Evolution of the area (× 10^4) of selected volatile compounds identified in the garlic samples obtained by Clevenger Laboratory Hydro-distillation using GC-MS. (Continue)

			Czech Republic	China	Slovakia	Spain	Italy
Volatile Compounds	***RI ^{cal}***	***RI ^{lit}***	***Average Area***	***Average Area***	***Average Area***	***Average Area***	***Average Area***
(1*E*)-1-propenyl methyl trisulfide	1174	1169	47.25	ND	ND	ND	ND
4-Methyl-1, 2, 3-trithiolane	1153	1157	ND	29.26	ND	811.59	47.48
Methyl (*E*)-1-propenyl trisulfane	1164	1169	ND	ND	31.61	ND	ND
3-Vinyl-1,2-dithiacyclohex-4-ene	1204	1191	65.21	70.99	39.2	50.95	70.15
4H-1,2,3-Trithiine	1220	1202	37.7	67.41	43.65	61.73	66.42
2-Vinyl-4H-1,3-dithiine	1230	1217	152.93	216.69	122.28	113.06	195.91
Di-2-propenyl trisulfide	1319	1304	3864.28	4929.64	ND	1369.7	3963.49
Allyl (*E*)-1-propenyl trisulfane	1342	1333	229.71	ND	67.46	2453.64	311.37
5-Methyl-1,2,3,4-tetrathiane	1393	1369	192.2	189.3	119.41	120.28	210.82
Di-2-propenyl tetrasulfide	1557	1555	109.74	88.33	ND	154.23	87.92
3-Isopropyl-4-methyl-dec-1-en-4-ol	1789	*	50.13	ND	ND	ND	ND

* indicates that the retention index of the volatile compounds is not available in the literature used for comparison.

Chromatogram of *A. sativum* essential oil from Clevenger laboratory hydro-distillation using GC-MS indicating volatile compounds of the various countries of origin (Figures 20 - 24).

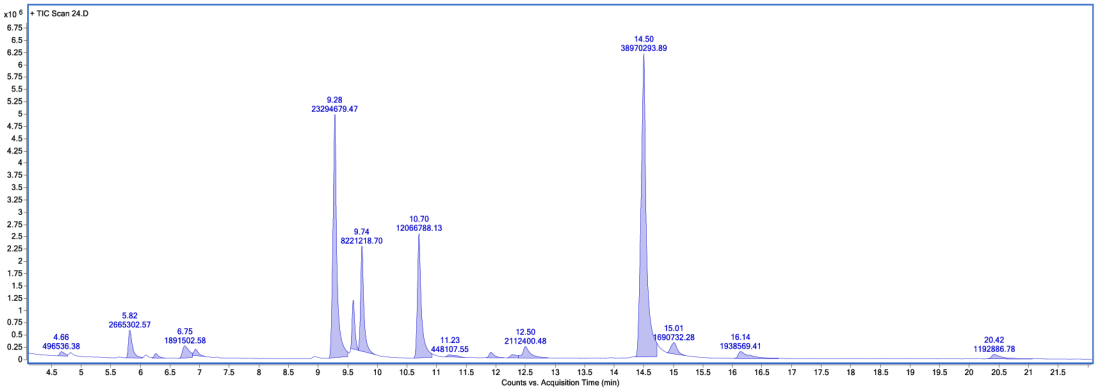


Figure 20. Volatile compounds of Czech Republic essential oil from Clevenger laboratory hydro-distillation using GC-MS

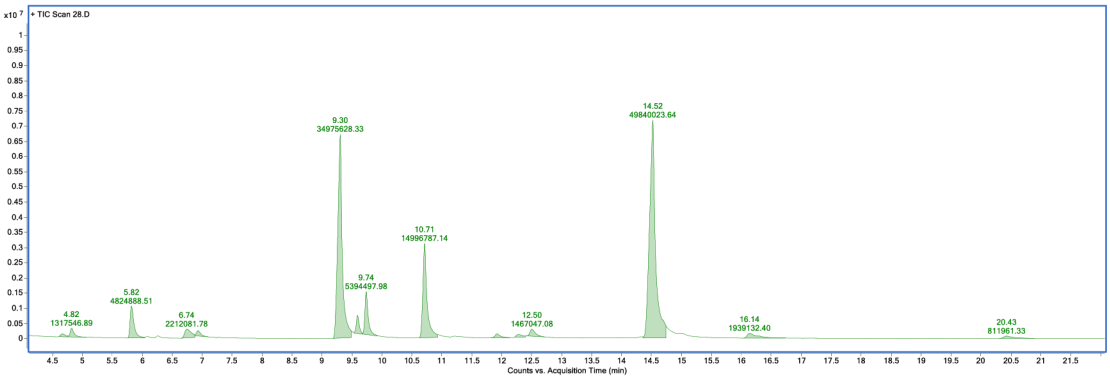


Figure 21. Volatile compounds of China essential oil from Clevenger laboratory hydro-distillation using GC-MS

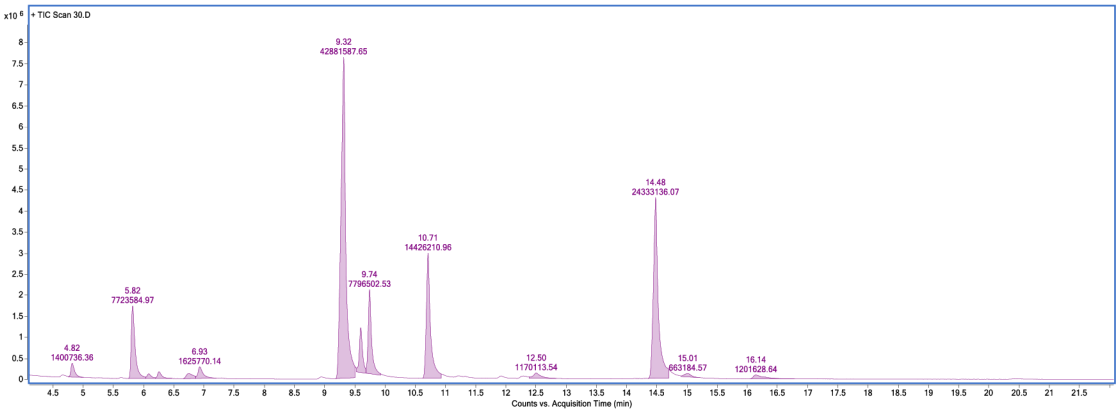


Figure 22. Volatile compounds of Slovakia essential oil from Clevenger laboratory hydro-distillation using GC-MS

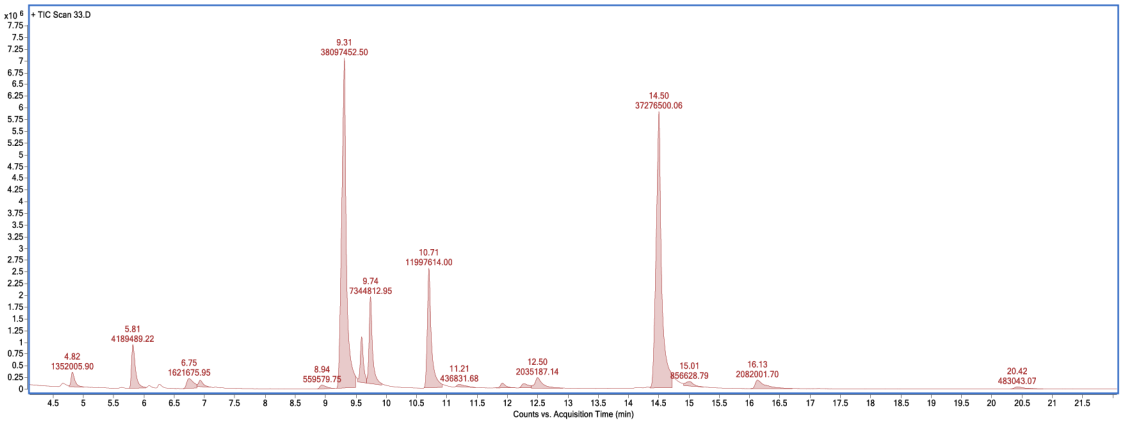


Figure 23. Volatile compounds of Spain essential oil from Clevenger laboratory hydro-distillation using GC-MS

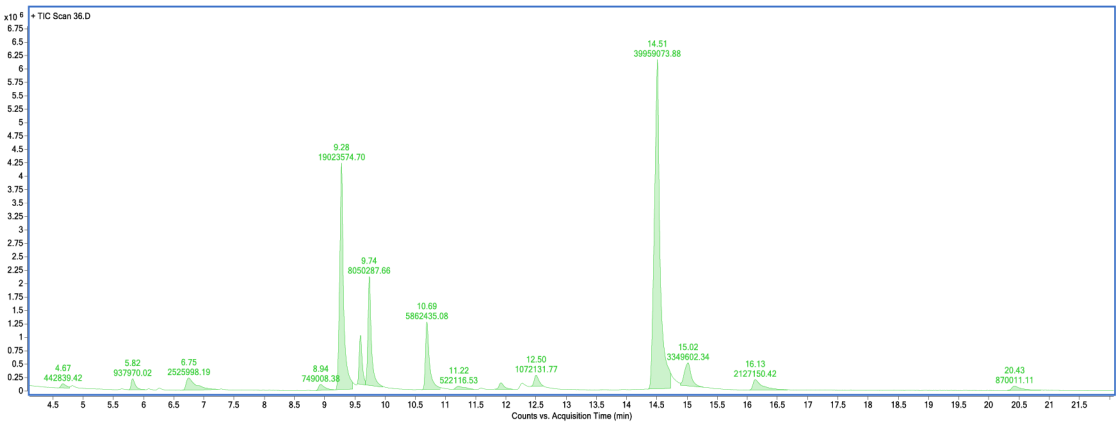


Figure 24. Volatile compounds of Italy essential oil from Clevenger laboratory hydro-distillation using GC-MS

Chromatogram of *A. sativum* essential oil from Clevenger laboratory hydro-distillation using GC-MS indicating volatile compounds of the various countries of origin, including major sulfur-containing compounds, are shown below in Figures 23 and 24, respectively.

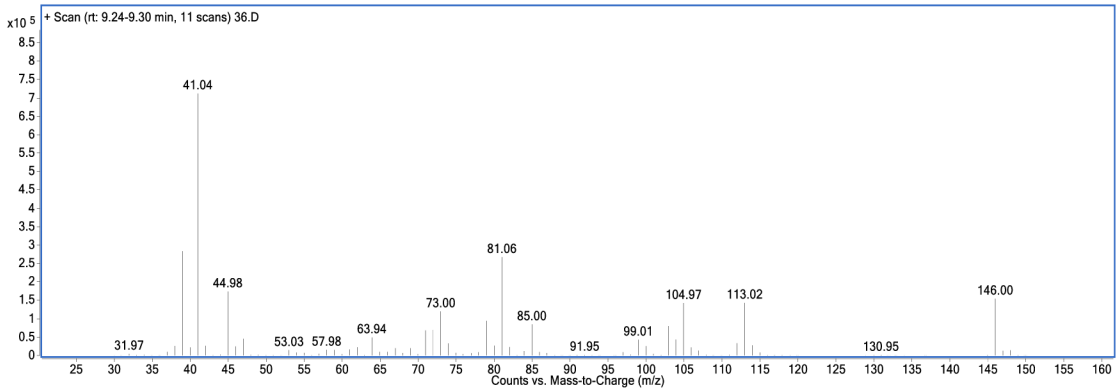


Figure 25. Diallyl disulphide

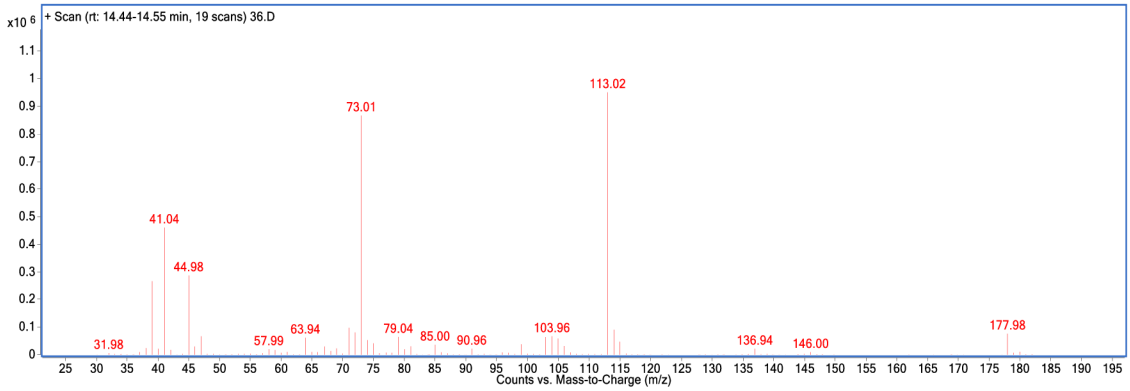


Figure 26. 2-Propenyl methyl trisulfide

However, in SPME, a total, twenty-six (26) compounds were identified. Of these, thirteen (13) compounds detected in the garlic samples can be found in all five different geographical origins, which includes; Methyl thiirane, Allyl methyl sulfide, Dimethyl disulfide, Diallyl sulfide, Allyl (*E*)-1-propenyl sulfane, 3,4-Dimethylthiophene, 2-Propenyl methyl disulfide, 3H-1,2-Dithiole, Diallyl disulphide, Allyl (*E*)-1-propenyl disulfane, 2-Propenyl methyl trisulfide, Di-2-propenyl trisulfide, and Allyl (*E*)-1-propenyl trisulfane.

However, there are six compounds which were detected in four of the garlic samples, namely; Methyl (*Z*)-1-propenyl disulfane, Methyl (*E*)-1-propenyl disulfane, 4-Methyl-1,2,3-trithiolane, 3-Vinyl-1,2-dithiacyclohex-4-ene, 2-Vinyl-4H-1,3-dithiine, and 5-Methyl-1,2,3,4-tetrathiane.

Also, Allyl (*Z*)-1-propenyl sulfane, 1,2-Di((E)-propenyl) disulfane, and Tridecane were the compounds detected in three of the garlic samples in the countries of origin.

(*E*)-2-Butenal, Allyl (*Z*)-1-propenyl disulfane, 2-Mercapto-3,4-dimethyl-2,3-dihydrothiophene, and 2-Methyl-octahydro-thiochromen-4-ol were the compounds detected in two of the garlic samples in the countries of origin.

The number of volatile compounds found in each of the garlic sample by each country the Slovakia twenty-six (26), Czech Republic and Italy had the same number twenty-two (22), China was twenty (20), and Spain was sixteen (16).

A detailed table of garlic (*A. Savitum*) compounds obtained by SPME are shown in the Table 8 below.

Table 7. Evolution of the area (× 10^4) of selected volatile compounds identified in the garlic samples obtained by SPME – GC-MS.

			Czech Republic	China	Slovakia	Spain	Italy
Volatile Compounds	***RI ^{Cal}***	***RI ^{Lit}***	***Average Area***	***Average Area***	***Average Area***	***Average Area***	***Average Area***
Methyl thiirane	**	606	1057.12	455.5	778.97	1059.39	1110.93
(*E*)-2-Butenal	**	657	ND	265.46	361.29	ND	ND
Allyl methyl sulfide	**	698	1715.82	542.23	1739.85	2570.09	971.76
Dimethyl disulfide	735	740	426.8	778.56	1249.48	1083.54	392.27
Diallyl sulfide	851	859	7792.85	2432.48	10751.93	15196.7	10453.96
Allyl (*Z*)-1-propenyl sulfane	880	888	ND	123.55	775.44	ND	622.63
Allyl (*E*)-1-propenyl sulfane	887	890	169.67	164.23	904.37	545.93	722.34
3,4-Dimethylthiophene	902	908	984.24	111.6	1935.6	724.29	1358.14
2-Propenyl methyl disulfide	916	919	6475.24	1948.43	13231.01	23617.25	8985.81
Methyl (*Z*)-1-propenyl disulfane	931	940	261.63	930.6	757.18	ND	324.29
Methyl (*E*)-1-propenyl disulfane	940	940	934.27	ND	2538.67	2228.7	1622.93
3H-1,2-Dithiole	966	959	602.63	330.68	990.29	1044.59	941.44
Diallyl disulphide	1088	1088	13034.79	97652.08	37513.08	86985.03	48235.63

Table 8. Evolution of the area (× 10^4) of selected volatile compounds identified in the garlic samples obtained by SPME – GC-MS. (Continue)

			Czech Republic	China	Slovakia	Spain	Italy
Volatile Compounds	***RI ^{Cal}***	***RI ^{Lit}***	***Average Area***	***Average Area***	***Average Area***	***Average Area***	***Average Area***
Allyl (*Z*)-1-propenyl disulfane	1103	1103	1199.94	ND	4406.6	ND	ND
Allyl (*E*)-1-propenyl disulfane	1109	1103	4825.82	21077.36	19247.5	24505.3	22290.76
1,2-Di((E)-propenyl) disulfane	1120	1129.1	232.12	419.39	283.97	ND	ND
2-Propenyl methyl trisulfide	1151	1144	395.75	593.52	986.1	1507.62	592.15
4-Methyl-1,2,3-trithiolane	1153	1157.3	391.11	ND	535.44	562.54	381.13
2-Mercapto-3,4-dimethyl-2,3-dihydrothiophene	1160	*	ND	ND	463.37	ND	271.2
3-Vinyl-1,2-dithiacyclohex-4-ene	1204	1191	497.17	701.27	496.62	ND	276.04
2-Vinyl-4H-1,3-dithiine	1230	1217	317.07	634.81	324.47	ND	252.24
Tridecane	1300	1300	173.17	ND	1092.62	578.13	ND
Di-2-propenyl trisulfide	1319	1304	198.05	2139.77	137.33	2754.48	1646.98
Allyl (*E*)-1-propenyl trisulfane	1342	1332.5	200.35	399.35	822.37	553.59	737.41
5-Methyl-1,2,3,4-tetrathiane	1393	1369	141.17	312.83	324.18	ND	284.6
2-Methyl-octahydro-thiochromen-4-ol	1400	*	ND	ND	380.57	ND	551.09

* indicates that the retention index of the volatile compounds is not available in the literature used for comparison.

** indicates that the retention index is out of calibration

4.2 Antimicrobial Activity

First, the resistance pattern of each strain was examined using the disc diffusion method. The *S. aureus* was sensitive to every tested antibiotic and showed resistance to oxacillin. The results of EOs' anti-*S. aureus* actions in the liquid and vapour phases, expressed in µg/mL. At the measured concentration range (MICs 8–1024 µg/mL), every EO was not effective against every bacterial strain.

Once colour interface changes from brown and yellow (relative to control wells colours) were documented in agar and broth, the minimum inhibitory concentrations (MICs) were determined by visual bacterial growth assessment after coloration of the metabolically bacterial active colony after coloration with MTT dye (Figures 25 and 26). The median/modal MIC values were calculated as the bottom concentrations that prevented the growth of bacterial as likened to control of compound-free.

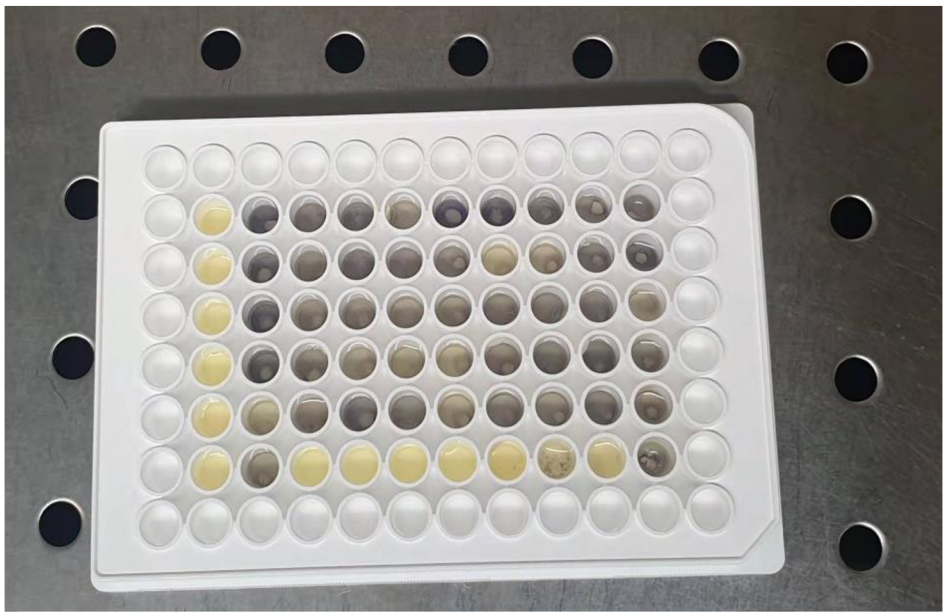


Figure 27. Visual assessment of bacterial growth on the plate

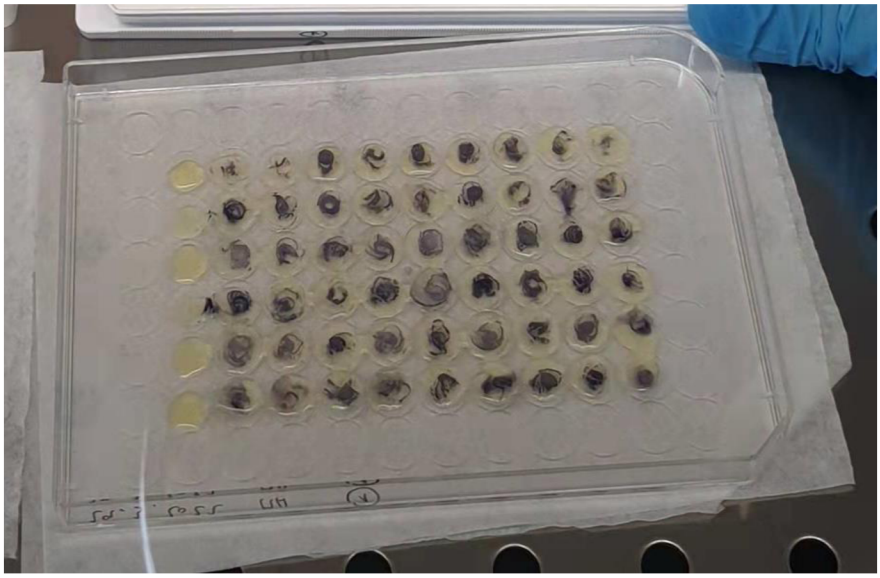


Figure 28. Visual bacterial growth assessment on the lid

Second, Table 7 summarizes the findings of an in-vitro study employing the volatilization method of broth microdilution to examine garlic volatiles' growth-inhibitory activity against bacteria linked to infections of the respiratory system in both vapor and liquid phases. In all medium, all chemicals examined had a similar level of antibacterial activity, although their potency varied greatly, ranging to 1024 µg/mL from 8 in agar and to 1024 µg/mL from 8 in broth.

In the phase of liquid, the lowest values of MIC were observed 0.125 and 16 for *S. aureus* (µg/mL). A very weak inhibitory antibacterial activity effect was shown by EO Italy, EO Slovakia, EO China, EO Spain, and EO Czech Republic, with MICs ranging from 8 to 1024 µg/mL, respectively.

Table 9: Vapour and liquid phase using the method of broth microdilution volatilization

	Bacteria/Grown Medium/MIC (µg/mL)		
	Staphylococcus aureus		
Essential Oils	**broth (µg/mL)**	**agar (µg/mL)**	**(µg/cm³)**
EO Italy	>1024.0	>1024.0	>256
EO Slovakia	>1024.0	>1024.0	>256
EO China	>1024.0	>1024.0	>256
EO Spain	>1024.0	>1024.0	>256
EO Czech Republic	>1024.0	>1024.0	>256
Positive antibiotic control			
Oxacillin	0.125	>16	ND

MIC: minimum inhalatory concentartion; >: determined; ND: not determined

As can be seen for *S. aureus* ATCC 29213, the results of our assay generally differ from those other authors obtained using the conventional method of broth microdilution. This suggests that different strains of *S. aureus* may respond differently to antibacterial agents, which may account for the disparate outcomes seen in our study and the literature.

4.3 Colour Measurement

It was decided to use a computerized, practical method for colour analysis. Similar-sized cuttings of garlic bulbs were digitally photographed using the Minolta colorimeter (Chroma Meter CR 410) under identical settings with natural light and the same ISO sensitivity in order to compare the colour density of fresh garlic. The RGB colour scheme was used to measure the concentration of red, blue, and green hues as well as the luminance of 10,000 pixels from the center of each sample. The average results of each garlic sample are shown in the Table 10 below using a system of tridimensional *CIE*L*a*b*, where a* is a measurement changing to red (+60) from green

(-60), L* specifies the luminosity (changing to 100 = white from 0 = black) and b* varies to yellow (+60) from blue (-60). Using a white plate, the instrument was calibrated.

Table 10. Results of colour measurement of various garlic samples

Parameters	*Czech Republic*	*China*	*Slovakia*	*Spain*	*Italy*
*L**	84.478	78.746	83.604	87.202	84.834
*a**	-1.234	-0.28	-0.824	-1.778	-0.68
*b**	18.908	32.198	24.106	17.088	15.906

The parameters $L_o^*a_o^*b_o^*$ were utilized to define the Hue (Eq. (2)), Chroma (Eq. (1)), a total colour difference (△E) (Eq. (3))

Chroma = $(a_o^{*2} + b_o^{*2})^{1/2}$ ..... (1)

Hue = $\tan^{-1}(b_o^*/a_o^*)$ .....(2)

$\triangle E = \sqrt{(L_o^* - L^*)^2 + (a_o^* - a^*)^2 + (b_o^* - b^*)^2}$ .....(3)

L*, a* and b* refer to the reading of the colour of garlic which are not fresh. How their values are zeros since we focused only on fresh garlic.

However, inputting our values of L_o^*, a_o^* and b_o^*, the results of our equations (1), (2) and (3) are shown below in Table 9.

Table 11. Results of Chroma, Hue and Total Colour Difference of various garlic samples

Origin (Countries)	Chroma	Hue	△E
Czech Republic	18.86768952	-1.505625369	86.57694672
China	32.19678251	-1.562100354	85.07480309
Slovakia	24.09191273	-1.53662727	87.01387239
Spain	16.99524816	-1.467119773	88.87828662
Italy	15.89145796	-1.52807118	86.31495115

4.4 pH Measurement

After a series of repetitions of the tests from the five samples of fresh *A. sativum*, the results have been tabulated below (Table 10). We repeated the tests of each garlic five times with a thirty (30) seconds time interval for the same garlic sample and two (2) minutes time interval between each garlic sample from a different origin. The average of our results of all the samples was also calculated.

Table 12. Results of pH in all various garlic samples

	Number of Tests					
Countries of Origins	**1st**	**2nd**	**3rd**	**4th**	**5th**	**Average**
CHINA	6.7	6.67	6.65	6.65	6.64	6.662
CZECH REPUBLIC	6.42	6.41	6.39	6.37	6.37	6.392
ITALY	6.47	6.44	6.43	6.42	6.41	6.434
SLOVAKIA	6.53	6.42	6.42	6.41	6.37	6.430
SPAIN	6.53	6.47	6.46	6.42	6.42	6.460

5. DISCUSSION

The garlic (*A. sativum*) essential oils from the Czech Republic, China, Slovakia, Spain and Italy, gained using two dissimilar extraction approaches (Clevenger laboratory hydro-distillation, CLH and Solid Phase Micro Extraction, SPME) were categorized by GC-MS (Table 5 and 6). The essential oils were conquered by polysulfides allyl, comprising Diallyl disulfide (11.38 – 63.42 %), Diallyl sulfide (8.59 – 16.59 %), Di-2-propenyl trisulfides (11.93 – 43.39 %), 2-Propenylmethyltrisulfide (6.41 – 12.90 %), Allyl (*E*)-1-propenyldisulfide (4.35 – 19.19 %), and 2-Propenylmethyldisulfide (1.03 – 13.78 %).

Our research results show that *A. sativum* essential oil's major components extracted by distillation of Clevenger-type laboratory were Diallyl disulfide (11.38 – 38.51 %), Di-2-propenyl trisulfide, (11.93 – 43.39 %), 2-Propenylmethyl trisulfide (6.41 – 12.90 %), Allyl (*E*)-1-propenyl disulfide (4.35 – 8.42 %) and 2-Propenylmethyl disulfide (1.03 - 6.91 %) (see Table 5).

However, the oil compositions from this study compare and contrast quantitatively with those from earlier studies on garlic oil. Diallyl disulfide (20.8 %), Diallyl trisulfide (allitridin) (33.4 %), Allyl (*E*)-1-propenyl disulfide (5.2 %), Allyl methyl trisulfide (19.2 %), and Allyl methyl disulfide (4.4 %) were the main components of *A. sativum* essential oil extracted by laboratory distillation of Clevenger-type, which was similar to our result.

The main components of the hydro-distilled Egyptian garlic essential oil were Diallyl disulfide (25.2 %), Allyl methyl trisulfide (23.8 %), and Diallyl trisulfide (21.1 %) (Hye Young et al. 2012). Through hydrodistillation, Diallyl trisulfide (33.6 %), Allyl methyl trisulfide (17.8 %), and Diallyl disulfide (28.1 %) were Serbian essential garlic oil's main components (Sunčica et al. 2012). Diallyl trisulfide (30.4 %) and Diallyl disulfide (49.1 %) were the two primary ingredients of the hydrodistilled Tunisian garlic essential oil (Zhao et al. 2013). The profile discovered in this investigation differed from the French garlic oil published by Mnayer et al. (2014) were Diallyl trisulfide (28.1 %), Diallyl disulfide (37.9 %), Diallyl sulfide (6.6 %), Allyl methyl trisulfide (7.3 %), Allyl methyl disulfide (3.7 %) and Diallyl tetrasulfide (4.1 %). According to Douiri et al. (2013), Diallyl disulfide (16.0 %),

Diallyl trisulfide (46.5 %), Diallyl disulfide (7.2 %), and Allyl methyl trisulfide (10.9 %) were the main sulfur compounds in the essential oil of *A. sativum* produced by Clevenger hydrodistillation. Similar to this, six geographical variants of essential oils produced by steam-distilling garlic fresh grown in India have been examined by Rao and colleagues. These researchers discovered that the primary components were Diallyl trisulfide (19.9–34.1 %) and Diallyl disulfide (27.1–46.8 %), which were then followed by Allyl methyl disulfide (4.4–12.0 %) and Allyl methyl trisulfide (8.3–18.2 %) (Rao et al. 2007). Diallyl trisulfide (18.5-23.4 %), Diallyl disulfide (45.1-63.2 %), Diallyl tetrasulfide (6.3-10.5 %), and Diallyl sulfide (4.5-11.4 %) are all present in large amounts in commercial Chinese garlic oil (Wei-Chuan Chen et al. 2011, Wei-Wen Kuo et al. 2013 & Kuang-Chi Lai et al. 2012). Using the Likens-Nickerson hydro-distillation-extraction method, Kimbaris and colleagues obtained garlic oil from Greece and discovered Diallyl trisulfide (18.2-22.1 %), Diallyl disulfide (23.1-28.4 %), Allyl methyl disulfide (8.5-11.2 %), and Allyl methyl trisulfide (16.3-17.5 %) (Park et al. 2012, Pyun & Shin 2006).

In contrast to SPME, the major components of *A. Sativum* essential oil extracted were Diallyl disulfide (27.74 – 63.42 %), Diallyl sulfide (8.59 – 16.59 %), Allyl (*E*)-1-propenyl disulfide (10.27 – 19.19 %), 2-Propenyl methyl disulphide (7.74 – 13.78 %) and Allyl methyl sulfide (0.35 – 3.63 %) (See Table 6).

Taylor & Francis (2014), garlic's essential oil extracted by SPME and its major components was conquered by Diallyl disulfide (17.5–35.6 %), Diallyl trisulfide (37.3–45.9 %), 2-vinyl-1,3-dithiane (3.9–5.9 %) and Methyl allyl trisulfide (7.7–10.4 %) and the sulfur group accounts for above 84 % total oils which are similar to our research results. However, most of these components are reported already as garlic essential oil's main volatile components (Young Kim et al. 2011 & Abu-Lafi et al. 2004). Amr E. Edris & Hoda M. Fadel (2002) results also show the chemical make-up of molecules that contain sulfur and are present in the essential oil extracted from green garlic leaves. It is clear that the main components of the oil are Methyl allyl trisulfide (11.40 %), Diallyl disulfide (31.35 %), and Diallyl trisulfide (32.32 %). Their findings indicated that garlic bulb oil also contained other sulfur-containing compounds, such as Methyl allyl trisulfide (1.81 %), Isobutyl isothiocyanate (2.28 %), and Propenyl allyl trisulfide (2.33

%) (Rao et al. 2007 & Abu-Lafi et al. 2004). Moreover, there are a few minor components found in garlic leaf oil that are thought to be powerful garlic taste substances, such as Diallyl sulfide (0.40 %) and Dimethyl trisulfide (0.41 %) (Bordia et al. 1975). Mugao et al. (2020) results showed that some of the major components found were Allyl (*Z*)-propenyl trisulfide (4.30 %), Diallyl sulphide (2.94 %) and Methyl (*E*)-1-propenyl trisulfide (3.40 %) which is found in our results in small quantity (Taylor & Francis 2014, Mugao et al. 2020). The major organosulfur that were acknowledged in this research were comparable to those other researchers identified (Benkeblia 2004, El-Zemity et al. 2006, Zenner et al. 2003, Ayaz et al. 2008, Rui et al. 2010, & Fukaya et al. 2020).

However, according to our results we observed that the major compounds found in various garlic origins were similar to above mention articles. In CLH-GCMS, the amount of area volume of volatile compounds for each garlic sample for Diallyl disulfide was Czech Republic (22.64 %), China (28.09 %), Slovakia (38.51 %), Spain (11.38 %) and Italy (20.66 %), 2-Propenylmethyltrisulfide was Czech Republic (11.76 %), China (12.10 %), Slovakia (12.90 %), Spain (7.79 %) and Italy (6.41 %), Allyl (*Z*)-1-propenyldisulfane was Czech Republic (3.36 %), China (1.66 %), Slovakia (3.62 %), Spain (23.08 %) and Italy (3.10 %); Allyl (*E*)-1-propenyl disulfane was Czech Republic (8.29 %), China (4.35 %), Slovakia (7.09 %), Spain (4.37 %) and Italy (8.42 %), 2-Propenylmethyldisulfide was Czech Republic (2.60 %), China (3.87 %), Slovakia (6.91 %), Spain (3.69 %) and Italy (1.03 %); and Di-2-propenyl trisulfide was Czech Republic (38.01 %), China (40.03 %), Slovakia (0.00 %), Spain (11.93 %) and Italy (43.39 %).

In SPME-GC-MS, the amount of area volume of volatile compounds for each garlic sample for Diallyl sulfide was Czech Republic (16.59 %), China (1.58 %), Slovakia (9.90 %), Spain (8.59 %) and Italy (9.00 %), Diallyl disulphide was Czech Republic (27.74 %), China (63.42 %), Slovakia (34.52 %), Spain (49.20 %) and Italy (41.52 %), Allyl (*E*)-1-propenyldisulfane was Czech Republic (10.27 %), China (13.69 %), Slovakia (17.71 %), Spain (13.86 %) and Italy (19.19 %), 2-Propenylmethyldisulfide was Czech Republic (13.78 %), China (12.65 %), Slovakia (12.18 %), Spain (13.36 %) and Italy (7.78 %), and

Allyl methyl sulfide, was Czech Republic (3.65 %), China (0.35 %), Slovakia (1.60 %), Spain (1.45 %) and Italy (0.84 %).

In conclusion, In CLH, of the twenty-four (24) volatile compounds, only eleven (11) of them can be identified in all the samples and in SPME, only thirteen (13) out of the twenty-six (26) volatile compounds can be identified with GC-MS in all the samples of all the five (5) geographical origins. In total, fifty (50) volatile compounds were identified for both CLH and SPME by GC-MS, but only five major volatile compounds can be identified in all samples. However, the five major volatile compounds that were found in all garlic samples in both CLH and SPME with GC-MS were Diallyl sulphide, 2-Propenyl methyl disulfide, 3H-1, 2-Dithiole, Diallyl disulphide, and Allyl (*E*)-1-propenyl disulfane.

Recent epidemiological investigations have shown that sulfur compounds, such as Diallyl disulfide and Diallyl trisulfide, were the cause of the antifungal (Motsei et al. 2003 & Eliades et al. 2006), antibacterial (Ross et al. 2001 & Benkeblia 2004), acaricidal (El-Zemity et al. 2006), antiparasitic (Zenner et al. 2003), nematicidal (Ayaz et al. 2008), antiviral (Douiri et al. 2013), and insecticidal (Zouari et al. 2014) properties. Both diallyl disulfide and dipropyl disulfide exhibit hypolipidemic and hypoglycemic effects, respectively (Young et al. 2011 & Fukaya et al. 2020). It has been demonstrated that the allicin derivatives Diallyl trisulfide and Diallyl disulfide activate antioxidant enzymes and have antibacterial properties (Inouye et al. 2001).

However, as is shown in Table 7, every compound tested produced the same antibacterial level effect in both media, ranging substantially to 1024 µg/mL from 8 on agar and in broth. This is growth-inhibitory in vitro effect of garlic volatile compounds against bacteria related to infections of the respiratory system in the vapour and liquid phase.

The MIC values for oxacillin against *S. aureus* in the liquid phase did not change (µg/mL). Moreover, only a very modest inhibitory impact (8 to 1024 µg/mL) and extremely weak antibacterial activity were demonstrated. As can be seen for *S. aureus* ATCC 29213, the results of our assay do not generally agree with those of other authors that obtained using the conventional method of broth microdilution.

Likewise, in the broth microdilution assay, oxacillin was not effective antibacterial agent against *S. aureus* in the phase of vapour with MIC values µg/mL. Additionally, EOs of all the garlic withstood effective inhibition growth of oxacillin against *S. aureus* and possessed no activity of antibacterial in the phase of vapour with MICs ranging 8 – 1024 µg/mL. Contrary to numerous investigations on volatile chemicals' antibacterial action in liquid media, there are solely a few reports on their impact in the phase of vapour. Standardized methodologies have not yet been utilized to determine plant volatile chemicals' antibacterial efficacy in the phase of vapour.

Oxacillin inhibitory concentrations against *S. aureus* ATCC 29213 were obtained by Nedorostova et al. (2009) using the vapour diffusion method, and they were 8 and 17 µl/L, respectively. The MICs in µl/l of atmosphere volume were used to express EOs effects in the phase of vapour against different *S. aureus* strains. According to EOs effects in the phase of vapour on *S. aureus* strains, all of the tested EOs were effective against all of the tested strains bacterial in the 8.3 to 530 µl/l concentration range. *A. rusticana (8.3-17* µl/l*), followed by O. syriacum (8.3-130* µl/l*), S. hortensis (17-130* µl/l*), A. sativum (8.8-530* µl/l*), S. montana (33-260* µl/l*), , T. serpyllum (33-530* µl/l*) and T. vulgaris (33-260* µl/l*)* demonstrated the MICs that were the lowest and most reliable. Additionally, the antibiotics were tested by disc diffusion method which shows that the first clinical isolates of oxacillin were not effective but however, from the second to sixth clinical isolates of oxacillin were all effective against *S. aureus*.

Ahmed et al. (2018) determined concentrations (*Penicillin, Amikacin, Tetracycline, Clavulanate/Amoxicillin, Erthrocin, Ciprocin, Tazobactam/Piperacillin,* and *Chloramphenicol*) against *S. aureus ATCC 25923*, for the antibacterial effect of Australian (red garlic) and Chinese plants of garlic likened to that of the antibiotics at definite minimum inhibitory concentrations. There were no growth effects on concentrations of *Penicillin*, *Tetracycline*, *Erthrocin* and *Chloramphenicol* all in 30 µg while other concentrations *Amikacin*, *Amoxicillin/Clavulanate*, *Ciprocin* and *Piperacillin/Tazobactam* have growth effects against *S. aureus* ATCC 25923. Additionally, the experiment has done dry garlic of both origins.

The values of MIC found in our investigation, in contrast to the results given above, are typically lower (8–1024 μg/mL). The variance in results between our study and others that have been published before (Novy et al. 2014, Lopez et al. 2005, Inouye et al. 2001 & Krumal et al. 2015) may be attributable to the use of various techniques for examining the antibacterial impact in the vapour phase. The bacterial strains utilized as well as different essential oil compositions' differing antibacterial efficacies (Inouye et al. 2001 & Krumal et al. 2015) may potentially contribute to the variable findings.

The results indicated above show the viability of a unique method of broth microdilution volatilization, which joins the principles of the disc volatilization test with the classic broth microdilution assay. The volatile chemicals' antimicrobial efficacy generated from garlic varies under these circumstances, despite the ability of previously developed methodologies to test the activity of antibacterial in vapour and liquid phases separately, according to Juan Bueno (2015).

Hence, the primary benefit of our method is its applicability for quick comparison of MIC values in solid media and liquid. It can be adjusted to work with microtiter plates with fewer wells (for example, 48) and is appropriate for testing a variety of concentrations in a 96-well microtiter plate. Our approach, which is based on a 96-well plate microtiter design, uses less material and labor than modified disc volatilization experiments utilizing Petri dishes (Novy et al. 2014, Lopez et al. 2005). Antimicrobial agents' final concentrations can be impacted by the transition between gaseous and liquid systems on top of by losses that their evaporation throughout the processing of the assay caused. Despite this novel antibacterial assay's clear advantages, it does not address specific shortcomings of earlier methods developed caused by volatile compounds' physico-chemical properties tested.

The concentrations in the vapour phase should only be regarded as indicative values because of this. The concentrations can be stated as the weight of volatile agent per unit volume of a well if volatiles' distribution in the gaseous and liquid phases is uniform; however, their real values were 128, 256, 32, 64, 8, 16, 2 and 4 μg/cm^3 for 512; 1024; 128; 256; 32; 64; 8 and 16 μg/mL, respectively. Unfortunately, the volatile substances are typically not dispersed equally throughout the well. As a result,

depending on how much of an evaporated component from the broth, the concentrations employed in our experiment might range to 341.3 µg/mL of air from traces (for 1024 µg/mL). If necessary, the precise concentrations can be determined, for instance, by combining mass spectrometry/gas chromatography analysis with solid phase micro extraction (Lopez et al. 2005). However, the broth microdilution test with repeated doses can be utilized to express the results of screening trials in the vapour phase.

Moreover, in this research, it was regarded as significant to measure L_o*, a_o* and b_o* in all the garlic samples of different origins. The biometric parameters (L_o*, a_o* and b_o* per bulb are revealed in Table 10). These parameters were utilized to define and investigate the variances among diversities using the tridimensional *CIE*L*a*b* system to describe Chroma, Hue and the total colour difference.

Our results of L_o* from the five origins were 84.478, 78.746, 83.604, 87.202 and 84.834, respectively, which is very similar to Selen Akan et al. (2019). whose results were 73.54, 88.15, 87.24, and 84.85 when he conducted a same test on fresh garlic from four countries of origins (China, Turkey, Spain and French).

However, our results of a_o* after calibration were -1.234, -0.28, -0.824, -1.778 and -0.68, which shows a big difference as compared to Selen Akan's results -3.01, -2.93, -3.14, and -2.21 respectively. The highest value of a_o* after calibration of all our samples was -1.778, and the least value was -0.28 as compared to -3.14 and -2.21, respectively.

And the b_o* results of our research showed, from the least 15.906, 17.088, 18.908, 24.106 and 32.198, which is significantly different from Selen Akan et al. (2019) whose results were 17.89, 20.32, 21.33 and 26.38 respectively. The difference between our least results and Selen Akan et al. (2019) results are very small. However, the difference between our highest results are quite bigger as compared to his (Selen Akan et al. 2001) results. Moreover, for fresh garlic, the rectangular coordinates L_0^*, b_0^* and a_0^* were 62.55 ± 0.81, 21.61 ± 0.46 and−3.45 ± 0.06, respectively, Luciane & Caciano (2012) and Ahmed and Shivhare (2001) found values of 69.37, −3.25 and 15.95 for L_o^*, a_o^* and b_o^*, respectively.

According to our results, arranging from the highest to the least value of L_o*, a_o*, and b_o*, Spain (87.202), Italy (84.834), Czech Republic (84.478), Slovakia (83.603), China (78.746), and Spain (-1.778), Czech Republic (-1.234), Slovakia (-0.824), Italy (-0.68), China (-0.28) and China (32.198), Slovakia (24.106), Czech Republic (18.908), Spain (17.088), Italy (15.906) respectively. The differences between L^*_o, a_o*, and b_o* of our results are 8.456, -1.498, both between Spain and China, and 16.292, which was between China and Italy. The b_o* results of our research show a big difference between the highest and the lowest. The results of our research show that Spain has the best luminosity (brightness) and greenish to reddish colour as compared to the other origins, but Chinese garlic has the best bluish to yellowish colour. Also, Chinese garlic has the least luminosity and greenish to reddish colour. It was also shown that the luminosity and greenish to reddish colour between Italy and the Czech Republic garlic were very small (Table 8).

Our results show that in determining the Chroma, China (32.20) was the highest value, followed by Slovakia (24.09), the Czech Republic (18.87), Spain (16.20), and Italy (15.89), respectively. Our results also show that from the highest to the lowest value of Hue, China (-1.56), followed by Slovakia (-1.54), Italy (-1.53), Czech Republic (-1.51), and the lowest was Spain (-1.48) accordingly. Lastly, the total colour change was determined by each of the various garlic origins researched in our paper. The results show that Spain (88.88) was the highest, followed by Slovakia (87.01), the Czech Republic (86.58), Italy (86.32), and the least was China (85.08)

In conclusion, our results show that the Hue (colour of garlic determined by the amount of carotenoids present in the garlic) was highest in the China garlic sample, followed by Slovakia, Czech Republic, Italy and Spain accordingly. Finally, in terms of total colour change, China is the best, followed by Italy, the Czech Republic, Slovakia, and Spain.

Depending on the method employed for extraction and quantification, the amount of allicin in fresh garlic ranged from 1.7 to 4.6 mg/g (Bocchini et al. 2001). In contrast to alliin, which an acidic methanol-water solution can be used to extract (Nasim

et al. 2010 & Arnault et al. 2003), allicin may be recovered from the plant crushed using water or ethanol as the solvent extraction (Wang et al. 2015 & Bocchini et al. 2001).

Therefore, the results of pH of fresh garlic from the five samples were higher at the first test of all the samples, with China was the highest figure of 6.66, followed by Spain 6.46, Italy 6.43, Slovakia has 6.43 and Czech Republic is lowest of 6.39. Selen Akan et al. 2019 results on fresh garlic were 5.50, 6.16, 6.33, 6.33 from China, Spain, Turkey and France, respectively. UC Davis et al. 2016 (Athena Hessong), the pH level of garlic ranges between 5.3 and 6.3, making it a low-acid vegetable, consistent with California University at Davis.

Also, the difference between the first test sample and the last sample test of each garlic sample was China 0.03, Czech Republic 0.05, Italy 0.06, Slovakia 0.06 and Spain 0.11. We realized that Spain has the highest difference with Slovakia and Spain having the same value, the Czech Republic was fourth, and China is the sample with the lowest value from the above. Our findings also confirmed that the average values for each samples show that China has the highest value of pH (6.62), Spain (6.460), Italy (6.434), Slovakia (6.430) and the Czech Republic (6.392).

Despite the fact that the allicin in s aqueous extracts is light- and temperature-sensitive (greatest stability in pH = 5-6), according to Wang et al. (2015). Moreover, it was discovered that the extraction solvent affected the first order breakdown reaction, which reduced the activity against the pylori Helicobacter bacterium (Caizares et al. 2008). Wang also noted that the allicin concentration decline rate at 40 °C was quicker than at 4 °C or 27 °C. This was most likely due to the fact that there were two different methods through which allicin may decompose: self-decomposition and thermolysis (Wang et al. 2015 & Dongsheng et al. 2014). Our results were able to meet our research aim, so we can say that our findings were correct since the best stability in garlic pH is 5-6, according to Wang et al. 2015.

6. CONCLUSION

Clevenger Laboratory Hydro-distillation (CLH) and Solid Phase Micro-Extraction (SPME) are clean, sensitive and effective methods for extraction of volatile compounds from the headspace of fresh garlic samples. Combining CLH and SPME with Gas Chromatography-Mass Spectrometry (GC-MS) provided detailed and precise information on the chemical composition of the volatile profile of *Allium Sativum* samples.

According to our results and literature review, it is clear that the essential oils of garlic are subjugated by sulfur-containing compounds, mainly garlic oils and allyl polysulfide from many geographical sites have revealed qualitative similarities, nonetheless differences of quantitative in organosulfur compounds' concentrations, and are probable to affect the organoleptic and medicinal properties of the garlic. Also, regardless of the approach, sulfur compounds, which have a variety of biological functions, dominated the volatile profile of garlic essential oils.

The antibacterial activity of *Allium savitum* essential oils was determined by oxacillin against *Staphylococcus aureus* using the new broth microdilution volatilization method. Our results demonstrated that EOs of fresh garlic (*A. sativum*) were not effective for the development of antibacterial growth.

The use of the Chroma Meter CR410 for measuring fresh garlic colour is easy, clean and very sensitive. The results of our findings using the tridimensional *CIE*L*a*b* system significantly shows that all the fresh garlic samples from the five different origins, Czech Republic, China, Slovakia, Spain, and Italy, are all in the range of luminosity (*L**), thus all our garlic samples are whitish. Also, our results show that all our garlic samples are likely to be in greenish and yellowish colour rather than reddish and bluish colour as determined by *a** and *b**, respectively. Hence, garlic growers and consumers over the world favor all these qualities. Researchers who are interested in garlic may find some of these findings to be appealing. Also, comparing several kinds gives the opportunity to choose the best breeding stock for upcoming research.

According to our result, we observed that the value of pH in all samples was decreased slowly in each stage of the tests. However, we had some samples having the same figures. According to the results of our research, the pH of garlic confirmed decreasing trend of allicin in water with time during the measurements. However, further research focused on the pH of garlic using different methodologies can be used in future studies and researchers that are interested in fresh garlic may find some of these findings to be appealing. Although, the garlics were from different countries, the results did not show significant differences. Also, comparing several kinds give the opportunity to choose the best breeding stock for upcoming research.

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