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DETERMINATION OF VOLATILE SUBSTANCES OF NATURAL EXTRACTS INTENDED FOR COSMETIC APPLICATION

STANOVENÍ OBSAHU TĚKAVÝCH LÁTEK V PŘÍRODNÍCH EXTRAKTECH URČENÝCH PRO KOSMETICKÉ VYUŽITÍ

BACHELOR'S THESIS
BAKALÁŘSKÁ PRÁCE

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2. Design of an optimal method for the analysis of samples of natural extracts, taking into account the available analytical instrumentation.
3. Analysis of these samples with a focus on qualitative determination.
4. Evaluation, interpretation and discussion of the obtained results.

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ABSTRAKT

Tato bakalářská práce je soustředěná na zkoumaní přírodních komerčně dostupných extraktů, které se používají do přírodní kosmetiky. Literární rešerše byla zaměřena na téma obsahu těkavých látek v přírodních extraktech používaných v kosmetice, jejich charakterizace a možnosti stanovení, problematika výskytu alergenů v přírodní kosmetice. Na základě tohoto přehledu byla navržena optimální metoda pro analýzu vzorků přírodních extraktů s přihlédnutím k dostupné analytické instrumentaci. Následující výtažky byly studovány se zaměřením na kvalitativní stanovení: mandle, aloe vera, hřebíček, Etiopská káva, zázvor, cibule.

ABSTRACT

This bachelor's thesis is focused on observation of natural commercially available extracts that are used in natural cosmetics. The literature review was focused on the topic of the content of volatile substances in natural extracts used in cosmetics, their characterization and determination possibilities, and the issue of the occurrence of allergens in natural cosmetics. Based on that review was designed an optimal method for the analysis of samples of natural extracts, taking into account the available analytical instrumentation. The following extracts have been studied with a focus on qualitative determination: almond, aloe vera, clove, Ethiopian coffee, ginger, onion.

KLÍČOVÁ SLOVA

extrakt, mandle, aloe vera, hřebíček, Etiopská káva, zázvor, cibule, biokosmetika, alergen.

KEYWORDS

extract, almonds, aloe vera, cloves, Ethiopian coffee, ginger, onion, organic cosmetics, allergen.

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Paskevych Olha

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CONTENTS

1. INTRODUCTION

Cosmetics are not only decorative products, as people used to think all over the world. They are also the means of maintaining and improving the general appearance and condition of hair, nails, facial skin, and other parts of the body, e.g. mouth, hands, etc. It includes shampoos, conditioners, balms, hair masks, hair oils, shower and intimate hygiene gels, creams, lotions, massage oils, peels and scrubs, sunscreen emulsions, bath foams, tonics for the face, etc. Well-groomed, and most importantly healthy skin, nails, and hair are certainly important for a modern person. Numerous environmental factors such as chemical toxins, substances, microorganisms, prolonged wearing of masks, infections cause skin damage. Cosmetics alone are not enough for daily care; the skin needs a combination of active ingredients to maintain its tone and slow down the aging effect. The cosmetic industry pays a lot of attention to the line of so-called natural cosmetics - cosmetics that include natural extracts.

I have often come across the misconception that cosmetics with natural extracts are charlotte or homeopathic medicines. Therefore, before moving on to the theoretical part of my work, I would like to clarify what is the difference between homeopathy and herbalism.

Herbal medicine (also Herbalism) is the study of pharmacognosy and the use of medicinal plants. Plants have been the basis for medical treatments through most of human history, and such traditional medicine is still widely practiced today [1]. In fact, herbalism uses physiologically active plant preparations.

Homeopathy is a pseudoscientific system of alternative medicine [2]. Homeopathy uses highly diluted preparations, mainly plants, which are designed to exert their influence on the energy level. As a rule, in homeopathic preparations, there will be no actionable amounts of plant extracts.

I will also indicate the relevance of my work. During the pandemic, sales of the cosmetics market dropped significantly, especially in the decorative cosmetics industry [3]. This allows us to think that during the time of restrictions, consumers began to pay more attention to cosmetics of natural origin, including those containing plant extracts. While working at home, few people care about what is beautiful, but much more care about their comfort and the effect of skincare products. Moreover, naturalness is in trend now, and the further the more young people completely abandon decorative cosmetics, leaving only care products.

That is why now determination of volatile matter content in natural extracts is one of the important components of the research in this area.

2. THEORETICAL PART

2.1 Overview of extracts

For the first time, when faced with the concept of extract, it is necessary to find out what it is, and what types there are. Further, considering extracts from a chemical point of view, for initial comfort, we should answer the following questions: what exactly gets from the plant into the extract, and later into cosmetics? Should we be afraid of something, for example, the simplest allergic reactions? What active ingredients does this or that group of plants give us, how do they affect the human body and mind and, why are they generally used in cosmetology?

All these questions are answered in this section of the theoretical part of my bachelor's work.

2.1.1 Definition of extract

So, extracts are natural phytochemicals (compounds produced by plants) that have been extracted from the inert structural material of the plant that produced them. The main advantage of using extracts over raw herbs is that once extracted from the plant, phytochemicals bypass the need for digestion and are much easier to absorb. Liquid extracts are also more convenient for consuming the herb raw.

Herbal extracts come in many different forms. Extracts are usually classified by the solvent from which they are made and/or by their form. Some of the more commonly used solvents include water, alcohol, glycerin, or vinegar. The inherent properties of each of these solvents highlight various phytochemicals from the plant. For example, watery extracts prepared by infusion or decoction are used as teas, gargles, and bases for syrups and other products.

2.1.2 Classification of extracts

The following main groups of extracts are distinguished: tinctures, vinegars, oils, essential oils, powder extracts and supercritical extracts. So what's the difference?

Tinctures are liquid extracts prepared with alcohol. Alcohol extracts a wide range of phytochemicals and is an excellent preservative. It can also be diluted with water to adjust the alcohol content and glycerin added to limit the excessive settling of the finished extract. Food grade glycerin is a low glycemic index sweetener that is often used as a solvent for preparing alcohol-free liquid extracts.

Vinegars are not common. They are obtained by extracting herbs directly in vinegar. In this case, apple cider or other plant-based vinegars are most desirable.

Oils are fatty oils to which herbs are added for topical application and can be called herbal oils or infused oils. The fatty oil used as a base is usually derived from olive, sesame, or coconut oil, although many other sources can be used.

Essential oils are volatile components that have been separated from aromatic herbs. Quality essential oils are steam distilled or, in the case of herbs such as citrus peels, pressed directly from the fresh herb. Essential oils are very powerful preparations that are well diluted for internal use.

Powder extracts are prepared by drying liquid extracts, including tinctures and water extracts, often under vacuum. Powdered extracts mostly are used in capsules and tablets.

Supercritical extracts are obtained by extracting herbs with a gas, usually carbon dioxide, at low temperature and high pressure to transform them into a supercritical state. They are semi-solid extracts, which are the fat-soluble components of the herb, usually in the form of soft gels [4].

2.1.3 Chemical definition

Looking at extracts from a chemical point of view, we should pay attention to the basic terminology of the field. For example, concepts such as phytoncide, pathogen, allelopathy, and volatile organic compounds.

Allelopathy is a property of some organisms to secrete chemical compounds that inhibit or suppress the development of others [5].

Phytoncides are biologically active substances produced by plants that kill or suppress the growth and development of bacteria, microscopic fungi, protozoa and play one of the main roles in plant immunity and allelopathy [6].

Pathogens are any microorganism, including fungi, viruses, bacteria, etc., as well as a special protein - prion, which can cause a pathological condition (disease) of another living being [7].

Some plants give off very active substances (phytoncides) that help to prevent them from rotting or being eaten by some insects and animals. So some phytoncides have a detrimental effect on human and animal pathogens. Such phytoncides can be used in medicine and veterinary medicine.

Volatile organic compounds are compounds that have a high vapor pressure and low water solubility. It's a wide class of organic compounds, including aromatic hydrocarbons, aldehydes, alcohols, ketones, terpenoids, etc. VOCs include a variety of chemicals, some of which may have short- and long-term adverse health effects. The term is more often used in English-speaking countries, in the context of regulation of air pollution levels, in the environment; but also can be used for naturally produced (for example, by plants or trees) volatile substances - such as phytoncides, essential oils [8, 9].

One of the articles [10] on which my literature review is based analyzed 109 plant species (belonging to 56 different families), and it also showed that it is possible to classify species of races according to the classes of chemical compounds emitted, such as terpenes and sulfur compounds. So it can be said that plants from the same group often excrete the same or similar volatile substances.

In this study, the detection highlighted two well-defined groups of species that emit different terpenes compounds. Being released by almost any kind of tissues and type of vegetation (trees, shrubs, grass, etc.) as green leaf volatiles, nitrogen-containing compounds, and aromatic compounds, plants VOCs can be emitted constitutively, or in response to a variety of stimuli.

According to their biosynthetic origin and chemical structure, plant volatiles can be grouped into isoprenoids or terpenoids, but also oxygenated VOCs, such as methanol, acetone, acetaldehyde, methyl-ethyl-ketone, and methyl-vinyl-ketone; in few cases, sulfur compounds and furanocoumarins and their derivatives are also found.

Moreover, terpene compounds secreted by plant species are the main constituents of plant essential oils and can be used to distinguish between different species. In fact, some volatiles, especially C6 compounds and acetaldehyde, can occur in response to external stress, including wounding; this should be taken into account when using these compounds for subsequent detection analysis [10].

2.1.4 Effects on human health

Essential oils and their aromatic volatile components have a decent therapeutic potential in the prevention or treatment of diseases. Also they can be a useful part of your health or beauty regimen if you know how to properly use and dispense these substances. They are very popular as a health remedy.

Aromatic plants have been used since ancient times for their preservative and medicinal properties, as well as to impart aroma and flavor to food. By the middle of the 20th century, the role of essential oils was almost entirely concentrated in use in perfumery, cosmetics, and food flavorings, while their use in pharmaceuticals was extremely small.

They are now touted as substances that are useful for treating many conditions, from relieving anxiety and fighting infections to relieving headaches and other pains. The possible role and effects of these natural products are discussed, for example, for the prevention and treatment of cancer, cardiovascular diseases, including atherosclerosis and thrombosis, as well as their biological activity as antibacterial, antiviral, antioxidants, and antidiabetic agents. They are also used as natural skin penetration enhancers for transdermal drug delivery, therapeutic properties in aroma and massage therapies [11].

Extracts can be used both for ingestion (rinsing, tinctures, etc.) and for application directly to the skin and/or respiratory organs. Some of the popular uses are:

- Room aromatization:
    - aroma lamp;
    - diffuser;
    - aroma stones;
    - aroma pads.
- Inhalation (cold and hot);
- Aroma bath;
- Massage, rubbing;
- Compresses, bandages, wraps;
- Enrichment of cosmetics.

Interestingly, no matter how hard you try to protect yourself by choosing only something bio or organic in stores, the natural origin of the extracts does not guarantee health safety. In particular, an allergy to essential oils is not excluded. If these products are used incorrectly, in addition to other side effects, an allergy to essential oils may develop.

2.1.5 Allergens

Allergen is a substance, in fact, an antigen, that causes an allergic reaction in people who are sensitive to them. An allergen causes the body to produce antibodies [12].

Antibodies or immunoglobulins are the most important components of specific immunity. These are large globular proteins of blood plasma secreted by the plasma cells of the immune system. These compounds are designed to neutralize pathogen cells as well as protein poisons and some other foreign substances. They trigger a reaction and release chemicals to "attack" new portions of the allergen that enter the body [13].

Allergic reactions (hypersensitivity reactions) are an inadequate response of the immune system to usually harmless substances [14.]

Allergy is a condition of hypersensitivity caused by a reaction of the immune system to usually combat a perceived threat that might otherwise be harmful to the body. That causes an allergic reaction in people who are sensitive to them [15].

The overwhelming number of allergens are proteins or polypeptides. They are large molecules made up of chains of amino acids. Some polypeptides contain only a few amino acids, while proteins can contain thousands of them. Distillation, with the help of which the essential oils are obtained, excludes the presence of large molecules in the final product.

Most proteins and polypeptides are 100-1000 times larger than the largest compounds found in esters. The absence of proteins and polypeptides in essential oils that cause allergic reactions means that if a person is allergic to a plant, then there shouldn’t be an allergy to the essential oil obtained from this plant.

But does this mean that an allergy to essential oils does not exist in principle? No, because essential oils can contain phenols, haptens, hyperoxides, and epoxides (depending on the oxidation state of the essential oil). And these substances are strong allergens.

Allergic reactions are common and range from mild to life-threatening. Their symptoms usually are determined by the way the substance is used and affects such organs as the nose, lungs, throat, skin, stomach, paranasal sinuses, ears.

Considering allergens in the cosmetic environment, the main organs that can be affected are: the skin (from the direct application of the product to it), the sinuses, the throat, and the lungs (from inhalation of volatile compounds). For example, aromatherapy means that VOCs are spread through the air and inhaled. Or diluted with another oil for application to the skin [16].

Phenols in essential oils

Phenols in essential oils can cause allergic reactions. Potentially allergenic phenolic compounds present in essential oils include phenol ethers, sesquiterpenes, and diterpenes.

For example thymol [17], eugenol [18], carvacrol [19] are all phenols. These components have many useful properties: antibacterial, antiseptic, disinfectant. But essential oils rich with phenols require special care in their use: they should be used for short periods of time and in low concentration.

Haptens in essential oils

Haptens are low-molecular substances that do not possess antigenicity and acquire it with an increase in molecular weight (for example, due to attachment to a special protein carrier) [20].

Essential oils contain a high concentration of haptens. When bound, chemical compounds are formed, the so-called hapten proteins. Hapten proteins trigger an allergic response from thebody's immune system.

An example of a hapten is penicillin, which can induce anaphylaxis [21]. Even some elements, such as nickel, can trigger an immune response when they bind to proteins, even though they do not contain protein themselves [22].

Alcohols and Ethers in essential oils

Even common and well-studied essential oils such as tea tree [23] and lavender [24] can cause allergic reactions. For example allergens in lavender essential oil are geraniol [25], linalool [26], linalyl acetate [27].

Oxidation of essential oils and allergic reactions

Another problem is that essential oils oxidize over time and therefore their composition changes. For instance, geranial (also known as citral) [28], a product of geraniol oxidation, causes allergic reactions much more often than the parent compound.

The same goes for the main compounds of tea tree and lavender essential oil. Unsurprisingly, allergic reactions to aged, oxidized tea tree essential oil (epoxies are formed) were stronger than those to fresh essential oil. Likewise, when the essential oil is exposed to air, linal acetate oxidizes to hyperoxides and epoxides, the most powerful allergens. Simply put, tainted, oxidized essential oils are more likely to cause an allergic reaction.

2.1.6 Phyto-pharmacoid effects

Pharmacology is a biomedical science about medicinal substances and their effect on the body; in a broader sense - the science of physiologically active substances in general [29].

Different divisions of pharmacology may focus on specific systems that make up the body, as **neuropharmacology** (central and peripheral nervous system), **immunopharmacology** (immune system), **psychopharmacology** (psyche, mind and behavior, treatment of mental disorders). Other sections focus on cardiovascular, renal, and endocrine pharmacology [30, 31].

Earlier was defined a herbology concept that centers around **phytochemicals**. These are substances with biological activity, produced mainly by plants. They exhibit pharmacological effects useful in the treatment of bacterial and fungal infections as well as chronic degenerative diseases such as diabetes and cancer.

Phytotherapy is a method of treating various human diseases based on the use of medicinal plants and complex preparations from them. Medicinal plants can be used in fresh, dried form, as well as by isolating active substances from them using simple processing, which preserves the structure of the natural complex of components (infusion, extracts, etc.) [32].

In many cases, these substances (in particular, alkaloids) act as plant defense mechanisms against microorganisms, insects, and herbivores. Many herbs and spices used by humans to flavour food contain health-giving chemical compounds [33, 34].

It must be understood that when speaking about essential oils and other forms of extracts, first of all, we mean their active components, VOCs, and others, which can only slightly vary from the type of extract.

The most important properties of essential oils are the effect on the psyche, on the central nervous system, and a general regulating effect on the entire body. The effects can be divided into different groups, for example, one group has a tonic, stimulating effect, relieves fatigue, and increases efficiency. Others have an adaptogenic effect, allowing the body to better adapt to changing environmental conditions, for example, relieves the feeling of nausea during ship roll. The third group - relieves overexcitation, calms down, and improves sleep.

Almost all essential oils have anti-inflammatory, antiseptic, bactericidal, and antiviral effects. Some essential oils have an analgesic, antipyretic, drainage effect, and are useful for dysfunctions of the cardiovascular system and gastrointestinal tract.

The effect of essential oils on the skin is very apparent - they have the ability to deeply penetrate the skin, directly affect the cells of its various layers, blood and lymphatic capillaries, and metabolism. That is why essential oils help to eliminate functional disorders ("stale" complexion), help with local disorders (striae, bruises, edema), and general disorders (eczema, neurodermatitis) [35].

2.1.7 Organic cosmetics

Today the cosmetic line is full of products, and it is not always easy to tell which cosmetics are natural and which are organic. Different countries have different certifications for certain products. Consumers believe that the more certificates a product has, the better. However, you cannot blame them for this, because getting even one certificate is associated with meticulous quality control, a large amount of bureaucracy, and paperwork.

Since the extracts for the practical part of the bachelor's work were provided by a country that is a member of the European Union, the norms for EU will be further analyzed.

To describe in more detail one of the European certifications, we will take as an example the ECOCERT-COSMOS® standard. The COSMOS-standard association consists of five founding members (whose labels can be seen on Figure 1) who now authorize and oversee the certification, including the Soil Association (UK), Ecocert (France), Cosmebio (France), BDIH (Germany), and ICEA (Italy) [36].

Figure 1 Labels of the COSMOS (Cosmetic Organic Standard)

The COSMOS-standard does not only evaluate the ingredients, but also the entire manufacturing process and packaging on the basis of sustainability criteria. The certification process inspects a range of criteria in the following categories:

- Origin and processing of ingredients;
- Composition of the total product;
- Storage, manufacturing, and packaging;
- Environmental management;
- Labeling and communication;
- Inspection, certification, and control.

A cosmetics product is COSMOS ORGANIC certified only if:

- 99% of all ingredients are of natural origin;
- For leave-on products, 95% of the plant-based ingredients must be organic and at least 20% of the total formula must be organic ingredients;
- For rinse-off products, again 95% of the plant-based ingredients must be organic, but at least 10% of the total formula must be organic ingredients.

There are also many other certificates for cosmetic products in Europe:

SOIL ASSOCIATION - Association for the certification of eco-farms and their products in the UK (Figure 2) [37].

Figure 2 Soil Association label

In order to be considered organic according to the organization's standards, it must contain at least 95% of agricultural substances marked "organic" (excluding water).

Despite the difficulties, various certification bodies are still trying to bring their standards to a common denominator and come up with a single logo that would be understandable to everyone, everywhere.

The result of such agreements was the NaTrue mark - a new European standard.

NATRUE-Label is a generally accepted standard developed on the recommendation of the councils of Europe (Figure 3) [38].

Figure 3 The NATRUE label

To use the NATRUE-Label, it is necessary that at least 75% of all individual products (in terms of formulation) of one limited edition of the same product have been certified as “organic / BIO cosmetics”. This requirement does not apply to cosmetic raw materials and preservatives. The use of preservatives identical to natural ones is allowed.

Also, the **French Cosmebio** standard separates cosmetics into “eco” and “bio”. Its ECO mark means that at least 95% of all ingredients in this product are of natural origin and at least 5% are organic. To achieve the BIO mark, the percentage of organic components rises to 10.

In addition to certification stamps, you can find a lot of useful information on the packaging. For example, a leaping bunny icon (Figure 4) indicates that the product has not been tested on animals. In general, in organic matter, all dermatological tests are carried out in vivo, that is, on volunteers with sensitive skin types, because allergies can be caused not only by chemistry but also by essential oils [39].

Figure 4 The cruelty-free label

2.2 Analyzed substances

A literature search was made for all analyzed extracts. This section is based on at least six previous studies. It describes (for each extract separately) which extract was extracted from what, which analysis methods were used, and which substances were found. For each extract, I selected a work that would be as close as possible to my method of analysis and to the types of extracts provided.

This section will show the main volatiles detected by different methods in the analyzed liquid extracts. Based on which we can compare our results with the expected ones and draw appropriate conclusions.

2.2.1 Almond

For almond extract, I chose the article called «Classification of almond cultivars using oil volatile compound determination by HS-SPME – GC – MS». As it is clear from the title the headspace solid-phase microextraction–gas chromatography–mass spectrometry (HS-SPME–GC–MS) analytical method for isolation and determination of the volatile compounds in almond oils was used. Main HS-SPME variables optimized were extraction temperature, extraction time, and stirring speed. Several volatile compounds including aldehydes, alkanes, alcohols, and aromatic hydrocarbons were identified. Twenty-four samples from three different almond cultivars were used [40].

Based on this article, I have compiled a table (Table 1) with the major volatiles usually identified in almond essential oils.

Table 1 Expected major volatile compounds identified for almond oils

Peak No.	Volatile compound	Retention time [min]
1	3-Methyl-3-pentanol	3.1
2	Octane	4.1
3	1,3-Dimethylbenzene	6.2
4	Nonane	7.8
5	5-Hexen-2-ol	8.4
6	2-Methylpentane	9.8
7	Decane	14.3
8	Octanal	14.5
9	Undecane	19.5
10	Nonanal	19.6
11	Dodecane	23.7
12	Decanal	23.8
13	2-Decenal	25.8
14	Nonanoic Acid	26.3
15	Tridecane	27.3

16	Undecanal	27.5
17	2-Undecenal	29.4
18	Tetradecane	30.7
19	Dodecanal	30.9
20	Tetradecanal	37.1
21	Pentadecanal[b]	42.5
22	n-Hexadecanoic acid	46.1

2.2.2 Aloe vera

For aloe vera extract, I chose the article called «Antimicrobial activity and volatile constituent analysis of three commercial herbal toothpastes containing Aloe vera L. and Fragaria vesca L. extracts». The chemical characterization was performed using solid-phase microextraction/gas chromatography-mass spectrometry-flame ionization detection (SPME/GC-MS-FID) in three herbal gel toothpastes in the amount of 100 μL. Oxygenated monoterpenes were the main class of organic compounds in all samples that were studied. Also were identified the next chemical compound classes: monoterpene hydrocarbons, sesquiterpene hydrocarbons, alcohols, esters, and aliphatic hydrocarbons. According to the VOCs results, the major compounds identified in the SPME of the three herbal toothpastes were anethole, menthol, eucalyptol, and menthone [41].

Based on this article, I have compiled a table (Table 2) with the volatiles usually identified in aloe vera essential oils.

Table 2 Expected major volatile compounds identified for aloe vera oils

Peak No.	Volatile compound	Retention time [min]
1	Isobutyl acetate	08.036
2	Ethyl butyrate	08.647
3	Ethyl isovalerate	10.144
4	(3Z)-Hexenol	10.344
5	(2Z)-Hexen-1-ol	10.646
6	α-Pinene	13.153
7	Camphene	13.865
8	β-Pinene	14.982
9	β-Myrecene	15.313
10	Octan-3-ol	15.512
11	Ethyl caproate	15.581
12	Hexyl acetate	16.060
13	o-Cymene	16.849
14	Limonene	17.039
15	Eucalyptol	17.246

16	Isopentyl butyrate	17.878
17	Menthone	22.476
18	γ-Terpinene	18.240
19	Octanol	18.766
20	α-Terpinolene	19.490
21	2-Nonanone	19.543
22	Linalool	19.952
23	Isopentyl isovalerate	20.096
24	Isopulegol	22.181
25	İsomenthone	22.420
26	Menthone	22.471
27	Menthol	23.244
28	Isomenthol	23.504
29	α-Terpineol	24.075
30	Trans-dihydrocarvone	24.333
31	Phenoxethol	25.405
32	Pulegone	26.161
33	Carvone	26.326
34	Piperitone	26.812
35	Citral	27.270
36	Neo-menthyl acetate	27.496
37	Anethole	28.187
38	Menthyl acetate	28.252
39	Bicycloelemene	30.160
40	(E)-Methyl cinnamate	31.958
41	β-Bourbonene	32.184
42	Tetradecane	32.337
43	(E)-Caryophyllene	33.575
44	(E)-α-Ionone	33.711
45	α-Humulene	34.889
46	Dodecanol	35.331
47	Germacrene D	35.903
48	Ledene	36.590
49	Hexadecane	39.885

2.2.3 Clove

For clove extract, I chose the article called «GC-MS Analysis of Clove (Syzygium aromaticum) Bud Essential Oil from Java and Manado». The essential oils were isolated from cut clove bud samples by steam distillation method. The chemical constituents of clove bud oil were analyzed by using gas chromatography-mass spectrometry (GC-MS). Constituents were then identified by comparing the results of the chromatogram and reference retention time using Wiley mass spectra library (Wiley W9N11). Major classes of compounds are sesquiterpenes, phenyl propanoid, oxygenated sesquiterpenes, and esters. In total, 44 constituents were identified based on the GC-MS analysis. Cloves buds were obtained from the clove warehouse of one of the largest tobacco companies in Indonesia - PT H. M. Sampoerna Tbk [42].

Since there are very few works in this area with clove, I had to turn to several sources and make a combined table. Since the main substances found in the extracts of this plant are identical, the table was only slightly supplemented (only 2 compounds – №44 and №45) from the following works: «Rapid quantification of clove (Syzygium aromaticum) and spearmint (Mentha spicata) essential oils encapsulated in a complex organic matrix using an ATR-FTIR spectroscopic method» [43] and «Chemical composition and phytotoxic activity of clove (Syzygium aromaticum) essential oil obtained with supercritical CO2» [44], where the clove oils were characterized by gas chromatography (GC) coupled to a mass spectrometer (MS).

Based on those articles, I have compiled a table (Table 3) with the volatiles usually identified in clove essential oils.

Table 3 Expected major volatile compounds identified for clove oils

Peak No.	Volatile compound	Retention time [min]
1	2-Heptanol acetate	9.22
2	2-Nonanone	10.46
3	(E)-4,8-Dimethyl-1,3,7-nonatriene	11.05
4	Acetic acid, phenylmethyl ester	12.29
5	Methyl salicylate	13.06
6	Bicyclobutylidiene	13.98
7	Chavicol	14.49
8	α-Cubebene	16.62
9	Eugenol	16.96
10	α-Copaene	17.25
11	Cis-isoeugenol	17.40
12	β-Elemene	17.52
13	Caryophyllene	18.23
14	β-Gurjunene	18.32
15	α –Ylangene	18.76
16	α-Humulene	18.86
17	Alloaromadendrene	19.07

18	δ-Cadinene	19.23
19	γ-Muurolene	19.31
20	α-Amorphene	19.36
21	α-Muurolene	19.43
22	β-Selinene	19.55
23	α-Selinene	19.71
24	β –Cadinene	19.80
25	α-Farnese	19.91
26	γ-Cadinene	20.08
27	Eugenyl acetate	20.41
28	Cadina-1,4-diene	20.51
29	α-Cadinene	20.60
30	α-Calacorene	20.73
31	1-Vinyl-2,6,6-Trimethylcyclohex-1-Ene	20.93
32	1,Z-5,E-7-Dodecatriene	21.04
33	Caryophyllenyl alcohol	21.25
34	(-)-Caryophyllene oxide	21.49
35	Junipene	21.84
36	Ledol	21.93
37	Humulene Oxide	21.99
38	β-Guaiene	22.59
39	Tau-Muurolol	22.64
40	Caryophylla-4(12),8(13)-dien-5.beta.-ol	22.94
41	Vulgarol B	23.19
42	2,3,4-Trimethoxyacetophenone	23.52
43	Benzyl benzoate	24.85
44	2-Heptanone	-
45	Tridecane	-

2.2.4 Ethiopian coffee

For Ethiopian coffee extract, I chose the article called «Detection and Differentiation of Volatile Compound Profiles in Roasted Coffee Arabica Beans from Different Countries Using an Electronic Nose and GC-MS». The results of the volatile compound profile analysis provided by the Agrinose device were verified with the GC-MS technique. Chemometric tests demonstrated a dominant role of alcohols, acids, aldehydes, azines, and hydrazides in the coffee volatile compound profile. The differences in their content had an impact on the odor profile of the coffees originating from the different countries. The results of the Agrinose analysis of volatile substances were consistent and correlated with the GC-MS results. The material used in the study was roasted Arabica coffee beans from Brazil, Ethiopia, Guatemala, Costa Rica, and Peru [45].

Based on this article, I have compiled a table (Table 4) with the volatiles usually identified in Ethiopian coffee essential oils.

Table 4 Expected major volatile compounds identified for Ethiopian coffee cultivars oils

Peak No.	Volatile compound	Retention time [min]
1	2-Buten-1-ol	1.10
2	2-oxopropanal	1.17
3	Methyl-D3 1-diderterio-2-propenyl ether	1.35
4	Acetaldehyde	1.45
5	Pyridine	1.76
6	Butan-2-one	2.31
7	2-methylpyrimidine	2.54
8	2-furanccarboxaldehyde	2.64
9	2-furanmethanol	2.92
10	Acetic acid ethenyl ester	3.16
11	4.6-dimethylpyrimidine	4.25
12	2-pethylpyrazine	4.35
13	Cis-ocimene	4.87
14	5-methylfuran-2-carbaldehyde	5.78
15	2-furylmethyl acetale	6.91
16	N.N-dimethylpyridyn-4-amine	7.06
17	1-methyl-2-cyano-2-piperidine	7.23
18	2.5-dimethyl-3-ethylpyrazine	9.73
19	Heptasiloxan	34.36

2.2.5 Ginger

For ginger extract, I chose the article «Volatile phytochemical composition of rhizome of ginger after extraction by headspace solid-phase microextraction, petroleum ether extraction, and steam distillation extraction». The following most common compounds were found in ginger essential oil: about 98% of the compounds were authenticated with α-zingiberene, β-sesquiphellanderen, α-farnesen, β-phellandrene, β-bisabolene, γ-curcumene and curcumene. They make up about 74% of the essential oil. Samples of rhizome of ginger were obtained commercially from Shuicheng of Guizhou Province, China [46].

Based on this article, I have compiled a table (Table 5) with the volatiles usually identified in ginger essential oils.

Table 5 Expected major volatile compounds identified for ginger oils

Peak No.	Volatile compound	Retention time [min]
1	Camphene	13.2
2	6-Methyl-5-hepten-2-one	15.1
3	Phellandrene	17.3
4	Borneol	24.5
5	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-	24.8
6	Aromadendrene	37.4
7	Curcumene	38.4
8	Zingiberene	39.2
9	Farnesen	39.5
10	β- bisabolene	39.6
11	β- Sesquiphellandrene	40.3
12	β- Eudesmol	45.3

2.2.6 Onion

For onion extract, I chose the article «Emission of volatile organic compounds from yellow onion (Allium cepa L.) bulbs during storage». VOCs were extracted from the headspace of bulbs by solid-phase microextraction (SPME) up to 5 times during storage and analyzed by gas chromatography–mass spectrometry (GC–MS). A total of twenty-nine compounds were measured and twenty-seven of these were identified while thirteen were reported for the first time from yellow onion bulbs. These compounds included an acid, alkanes, alkenes, alcohols, aldehydes, an ester, furans, ketones, sulfides, and thiols. The raw material was taken from commercial storage house facilities in March 2015 after approximately six months in storage [47].

Based on this article, I have compiled a table (Table 6) with the volatiles usually identified in onion essential oils.

Table 6 Expected major volatile compounds identified for onion oils

Peak No.	Volatile compound	Retention time [min]
1	Propene	5.89
2	Acetaldehyde	7.29
3	Methanethiol	7.59
4	Methyl formate	7.95
5	Propanal	9.31
6	Acetone	9.42
7	Carbon disulfide	9.45
8	Isopropyl alcohol	9.87
9	Isoprene	9.98
10	Pentane	10.04
11	1-propanol	10.40
12	2-methyl-2-propanol	10.70
13	2-methylfuran	10.83
14	3-methylfuran	10.96
15	2-butanone	11.02
16	1-propanethiol	11.15
17	Hexane	11.53
18	Acetic acid	11.63
19	2,5-dimethylfuran	12.20
20	Pentanal	12.46
21	2-pentanone	12.35
22	Methyl propyl sulfide	12.56
23	(Z)-methyl 1-propenyl sulfide	12.60
24	Dimethyl disulfide	12.73
25	Heptane	12.91
26	Hexanal	14.17
27	3,4-dimethylthiophene	15.77
28	Methyl 1-propenyl disulfide	17.04
29	Methyl propyl disulfide	17.20

2.3 Observational Methods of extracts

This section is devoted to the theory of the used analytical methods and devices. In total, three methods were used: solid-phase microextraction, gas chromatography, and mass spectrometry.

2.3.1 Solid Phase Microextraction

Solid-phase microextraction, or SPME, is a solid-phase extraction sampling technique that involves the use of a fiber coated with an extracting phase, that can be a liquid (polymer) or a solid (sorbent), which extracts different kinds of analytes (including both volatile and non-volatile) from different kinds of media, that can be in liquid or gas phase. The quantity of analyte extracted by the fiber is proportional to its concentration in the sample as long as equilibrium is reached or, in case of short-time pre-equilibrium, with help of convection or agitation [48].

Microextraction technology consists of the extraction of analytes from samples by their adsorption onto a SPME fiber and subsequent desorption in a GC inlet or an HPLC injector. SPME is considered a new approach in sample preparation and was first described in scientific papers in the 1990s by Pawliszyn J.

The analytes are extracted onto a special fiber (a solid fused silica substrate coated with a polymer phase, for example, polyacrylate or PDMS) which are located in a special holder (Figure 5). The length of the fiber is 1 cm. Extraction takes place when the fiber is placed in the analyzed sample, and due to diffusion, the analytes are sorbed. This method is usually used for volatile, medium-volatile, non-polar organic compounds [50]. It usually takes quite a long time to reach equilibrium - up to 30 minutes.

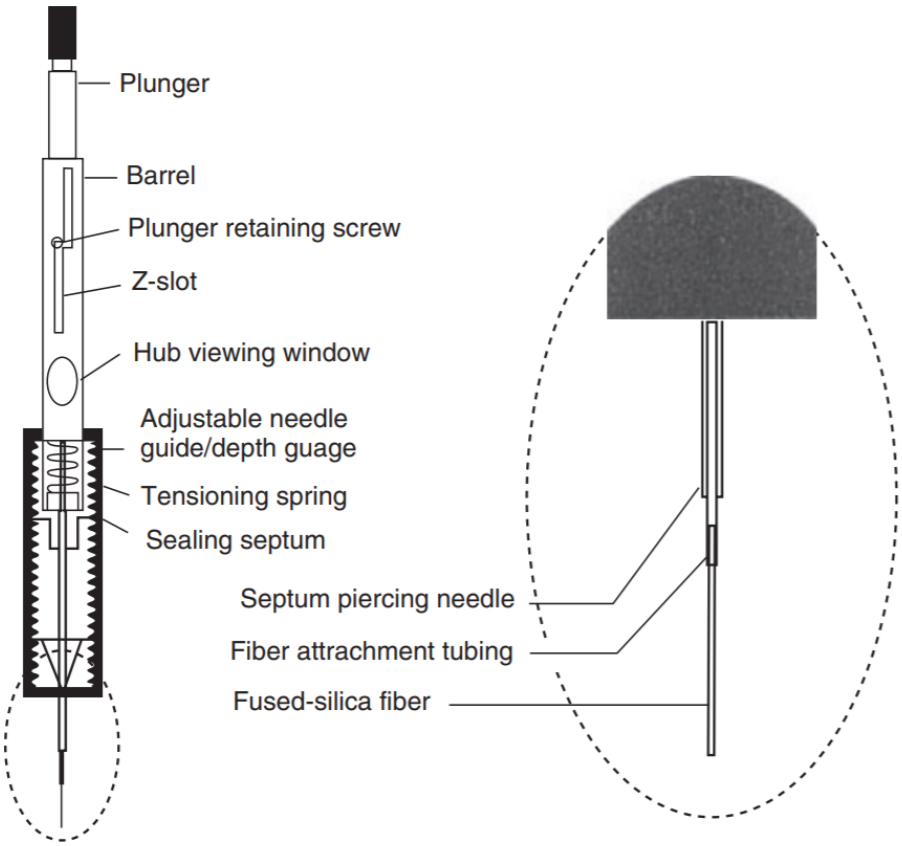

Figure 5: A commercial SPME device [49]

When working with volatile substances, the fiber is transferred to the inlet of a gas chromatograph (Figure 6). Chromatographic separation and analysis are then performed by thermal desorption of the analytes. The use of SPME in combination with GC turned out to be quite promising and is now used to solve environmental problems and analyze food products.

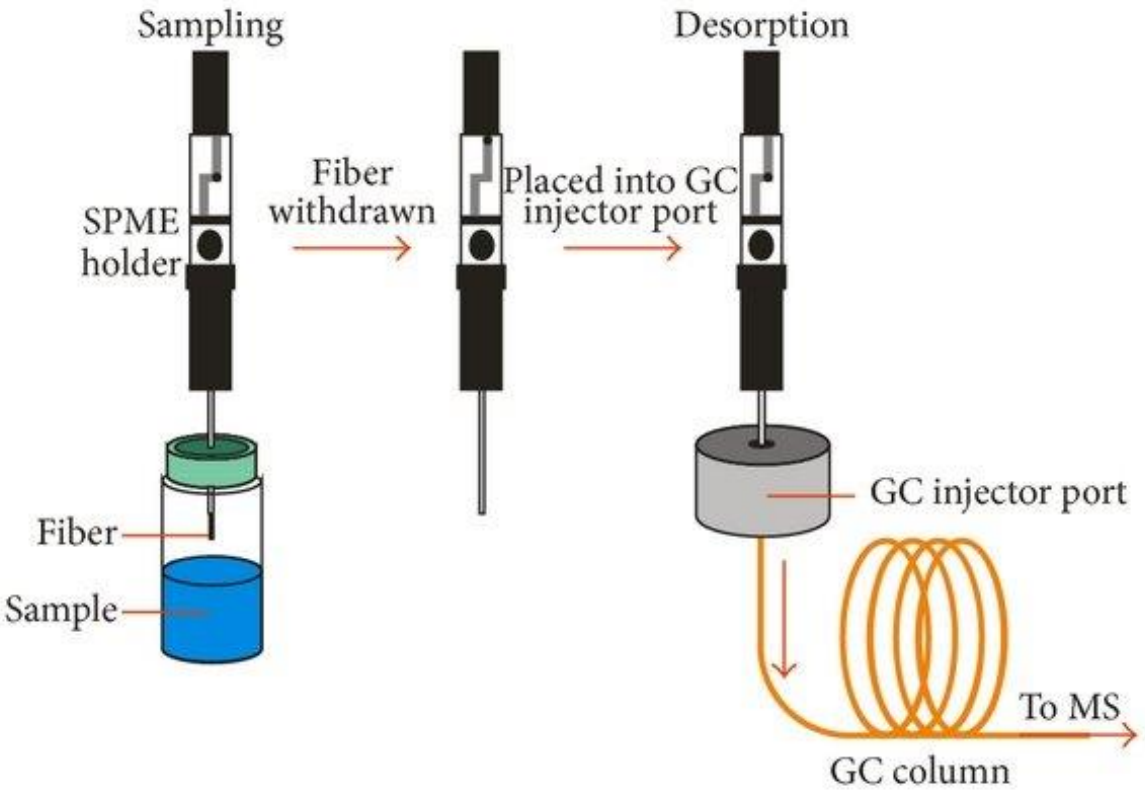

Figure 6 Transferring the fiber from the sample to the GC inlet [51]

For non-volatile substances, the desorption of the analytes is carried out by elution (solvent) using HPLC analysis. However, this approach has proven to be less efficient than GC analysis and has found limited application [52].

The selection of fiber type for SPME depends on the nature of the analyte (Table 7).

Table 7 Guide to selection of fiber type [52]

Analyte and matrix	**Fiber type and coating**
Low molecular weight or volatile compounds with a low boiling point	PDMS, 100 μm
Medium volatility compounds	PDMS, 30 or 7 μm
Polar analytes in polar samples	Polyacrylate, 85 μm
Volatile polar analytes (alcohols, amines)	PDMS / Divinylbenzene, 65 μm
Trace Volatile Compounds	PDMS / Carboxene, 75 μm
A wide range of analytes (C3 - C20)	Divinylbenzene / PDMS / Carboxene
Non-volatile	PDMS / Divinylbenzene, 60 μm

In SPME, two main types of analyte extraction by a sorbent are considered (Figure 7): a) concentration on the sorbent through the vapor phase; b) concentration on a sorbent immersed in a sample [48, 49].

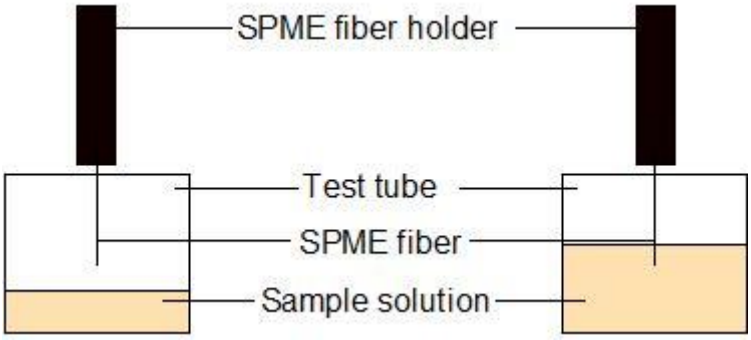

Figure 7 Two main types of analyte extraction by SPME: to the left - through the vapor phase; to the right - immersed in a sample [50]

If we talk about the advantages of the SPME method, then first it is its simplicity. The procedure for using the SPME device for sample analysis usually contains up to 10 actions. You begin with placing the sample in a vial and seal with a suitable septum. Then (if needed) heat the vial to the wanted extraction temperature and wait for equilibration. Insert the fiber holder into the vial and position it in the gas phase above the sample (for volatile analytes) or directly in the sample (for non-volatile analytes). Strip fiber and cure for needed extraction time. Hide the fiber in the safety needle and remove the holder from the vial. Insert the fiber holder into the heated gas chromatograph injector port. Strip the fiber and cure for the desired desorption time. Hide the fiber in the safety needle and remove the holder from the gas chromatograph injector.

Other advantages of the SPME method are its speed, inexpensiveness (one fiber> 500 analyzes, only a vial and septum are needed for analysis). It does not require toxic solvents. Only volatile compounds are extracted from the gas phase, which simplifies the maintenance of the GC sample injector. As well the process can be easily automated using autosamplers. Finally, SPME is a "green" method (very low environmental impact) which is very much appreciated in the modern world [53].

Also, the SPME method is very flexible. Many measurement parameters are optimizable. For example, the ratio of the volumes of the sample and the gas phase above it, the composition and size of the extraction coating, the extraction temperature, the extraction time, the amount of salt and other substances added, pH, desorption temperature, desorption time, etc.

Even though the fiber SPME device has a disadvantage (its fragility), the application of the SPME method is very diverse. It is used both for the analysis of environmental objects (air, water, soil), food quality control, medicine, and for forensic examination and any scientific research [50].

2.3.2 Gas chromatography

Gas chromatography, or GC, is a common type of chromatography used in analytical chemistry for separating and analyzing compounds that can be vaporized without decomposition. Typical uses of GC include testing the purity of a particular substance or separating the different components of a mixture (the relative amounts of such components can also be determined). In some situations, GC may help in identifying a compound [54].

A typical gas chromatograph is mainly composed of the following parts (Figure 8): carrier gas in a high-pressure cylinder with appropriate pressure regulators and flow meters, sample injection system, separation column, liquid phases, supports, detector, recorder.

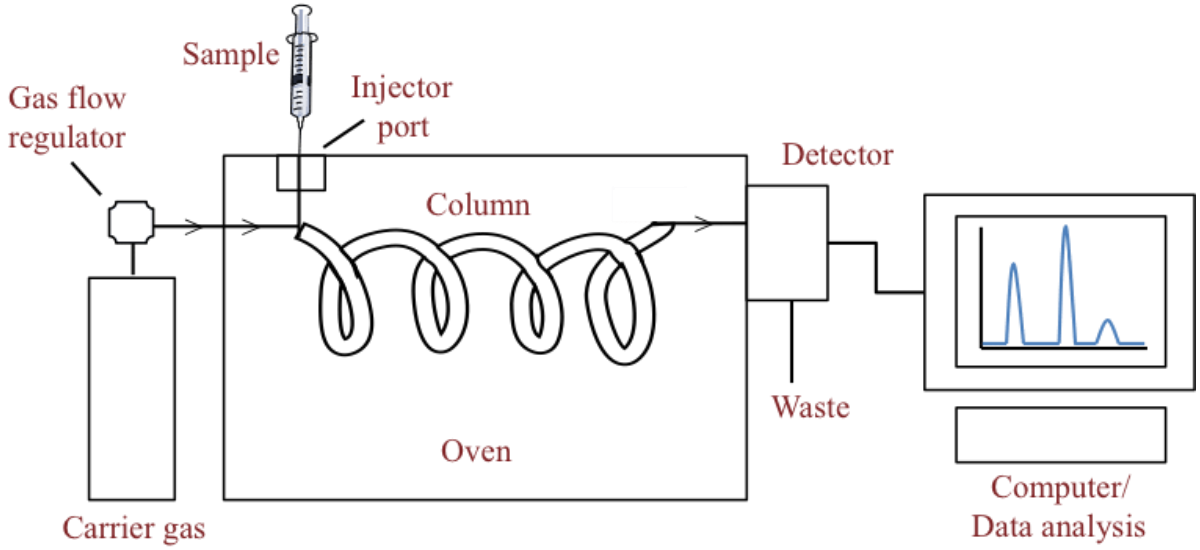

Figure 8: A gas chromatography system [55]

To separate the compounds in gas chromatography, a sample that contains organic compounds of interest is injected into the injector port. Since it is in the oven, as soon as the sample is injected, it will immediately evaporate. The gaseous molecules are then mixed with the mobile phase, which carries the sample through the column. The sample components will be distributed between two phases: the stationary phase and the mobile phase.

The mobile phase is a carrier gas, usually an inert gas such as helium or an unreactive gas such as nitrogen. The stationary phase is a microscopic layer of liquid or polymer on an inert solid support, inside a piece of glass or metal tubing called a column.

The gaseous compounds being analyzed interact with the walls of the column, which is coated with a stationary phase. In the column, the compounds are separated on the basis of different abilities to bind with different strengths to the stationary phase. Those, that spend more time in the column and elute later have a longer retention time (Rt). The comparison of retention times is what gives GC its analytical usefulness.

Separated components and carrier gas are getting into the detector, which provides a quantitative measurement of the components of the mixture as they elute in combination with the carrier gas. Here, substances that differ in their physicochemical properties from the carrier gas are registered and converted into an electrical signal. This is followed by amplifying this signal and converting it to an analog voltage. At this stage, the data are digitized.

The recorder (usually a PC) builds a graph where the x-axis is the retention time, and the y-axis is the abundance or the absorbance. This graph is commonly named as a chromatogram [56,57].

Gas chromatography can be used in many different fields such as pharmaceuticals, cosmetics, and even environmental toxins. Since the samples must be volatile, human breath, blood, saliva, and other secretions containing large amounts of organic volatiles can be easily analyzed using GC [58].

GC/MS is also another useful method which can determine the components of a given mixture using the retention times and the abundance of the samples. This method is applied to many pharmaceutical applications such as identifying the number of chemicals in drugs. Moreover, cosmetic manufacturers also use this method to effectively measure how much of each chemical is used for their products [59].

2.3.3 Mass spectrometry

Mass spectrometry or MS is an analytical technique that is used to measure the mass-to-charge ratio of ions (m/z). The results are typically presented as a mass spectrum, a plot of the intensity of the ion signal as a function of the mass-to-charge ratio.

These spectra are used to identify a substance, to determine the concentration of various components in it (isotopic, elemental, or chemical composition), as well as to find out the chemical identity or structure of molecules and other chemical compounds.

A mass spectrometer consists of three components: an ion source, a mass analyzer, and a detector.

Conventionally, the methods of ionization of organic substances can be classified according to the phases in which the substances are located before ionization. For the gaseous phase, there are two main methods - electron ionization (EI) and chemical ionization (CI). In this work, electron ionization was used.

Electronic ionization takes place in a vacuum to prevent the massive formation of atmospheric gas ions that can recombine with analyte ions and destroy them. Since the energy of electrons significantly exceeds the energy of a chemical bond, fragmentation of ions occurs. The chemistry of ion fragmentation during electron fragmentation is well studied, therefore, knowing the masses of the fragments and their intensity, one can predict the initial structure of the substance [60]. Mass spectra obtained using the electron ionization method are well reproducible; therefore, today there are libraries containing hundreds of thousands of spectra of various substances, which greatly facilitate qualitative analysis.

The ions obtained during ionization are transferred to the mass analyzer using an electric field. There, the second stage of mass spectrometric analysis begins - sorting ions by mass (more precisely, in relation to mass-to-charge, or m/z). The following types of mass analyzers are available: continuous and pulse mass analyzers. The most popular of the continuous ones are quadrupole mass analyzers (divided into single quadrupole (Q) and triple quadrupole (QQQ)). Mass spectrometers with a quadrupole mass analyzer are widely used in combination with liquid and gas chromatography in analytical solutions to problems of chemistry, biotechnology, medicine, ecology, etc. But in this work is used a pulse mass analyzer, specifically the simplest Time-of-flight mass spectrometry (TOFMS).

TOFMS is a method of mass spectrometry in which an ion's mass-to-charge ratio is determined by a time of flight measurement. Ions are accelerated by an electric field of known strength [61]. In it, ions come from the source during the flight tube in portions where there is no electric field (no field gap) at certain time intervals. Having flown a certain distance, the ions are registered by an ion detector with a flat or almost flat recording surface (Figure 9). The advantages of time-of-flight mass analyzers include a high upper threshold for the detectable ion mass, limited only by the fact of a sharp decrease in the sensitivity of the ion detector when slowly (velocity <20000 m/s) flying ions are detected [62].

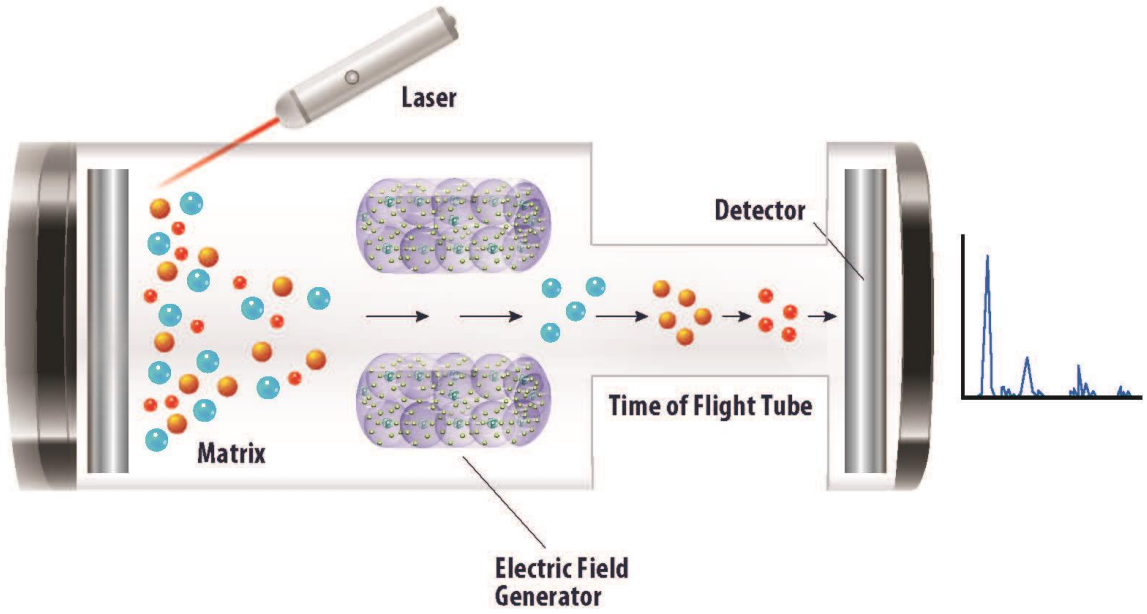

Figure 9 Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) [64]

So, the last element of the mass spectrometer we are describing is a charged particle detector. Dynode secondary electron multipliers, or photomultipliers, are now used. The most important technical characteristics of mass spectrometers are sensitivity, dynamic range, resolution, scanning speed. Because the number of ions leaving the mass analyzer at a particular instant is typically quite small, considerable amplification is often necessary to get a signal. The detector measures the value of an indicator quantity and thus provides data for calculating the abundances of each ion present [63].

3. EXPERIMENTAL PART

3.1 Materials and Instruments

3.1.1 Extracts

All 6 analyzed extracts were provided by GREENTECH. All information about the extracts (Tables 8, 9, 10, 11, 12, 13) was taken from the safety data sheets provided by the manufacturer. The raw material is certified by ECOCERT Greenlife according to the COSMOS standard. The contact information of the company is indicated on the safety data sheets, which can be found in the attachments.

3.1.1.1 Almond

Table 8 Basic information from safety data sheets about the Rostalmond extract

Name	ROSTALMOND HYDROGLYCOLIC EXTRACT ECO
Shelf life	24 months
Stockage	+15 / +25 °C Keep in the original packaging, without opening, in the recommended storage conditions

Composition	**Raw materials**	**Solvents**
Sweet almond	Seed	Water
Vanilla	Pod	Natural propylene glycol

	Proceedings	**Specifications**
Organoleptic characteristics		
Sight	GT001	Liquid
Color	GT002	Amber to dark brown
Odor	GT003	Characteristic
Physical & chemical characteristics		
Solubility 10% in water	GT004	Soluble
Solubility 10% in ethanol	GT004	Partially soluble
pH (direct)	GT005	4.2 - 6.2
Dry matter	GT006	1.0 - 2.0 %
Refraction index	GT007	1.370 - 1.410
Specific gravity	GT008	1.010 - 1.060
Microbiological analysis		
Enumeration of mesophilic aerobic bacteria	ISO 21149	≤ 100 ufc/mL - cfu/mL
Enumeration of yeasts and moulds	ISO 16212	≤ 100 ufc/mL - cfu/mL
Detection of enterobacteria	ISO 18415	Absence

3.1.1.2 Aloe vera

Table 9 Basic information from safety data sheets about the Aloe vera extract

Name	ALOE VERA ORGANIC JUICE 1:1 (SB) FAIR FOR LIFE
Shelf life	24 months
Stockage	+15 / +25 °C Keep in the original packaging, without opening, in the recommended storage conditions

Composition	**Raw materials**	**Solvents**
Organic aloe vera	Leaf	Water
Sodium benzoate	-	-
Potassium sorbate	-	-
Citric acid	-	-

	Proceedings	**Specifications**
Organoleptic characteristics		
Sight	GT001	Liquid
Color	GT002	Colorless to yellow
Odor	GT003	Characteristic
Physical & chemical characteristics		
Solubility 10% in water	GT004	Soluble
Solubility 10% in ethanol	GT004	Partially soluble
pH (direct)	GT005	3.5 - 4.7
Dry matter	GT006	0.4 - 1.0
Refraction index	GT007	1.330 - 1.380
Specific gravity	GT008	1.000 - 1.050
Microbiological analysis		
Enumeration of mesophilic aerobic bacteria	ISO 21149	≤ 50 ufc/mL - cfu/mL
Enumeration of yeasts and moulds	ISO 16212	≤ 10 ufc/mL - cfu/mL
Detection of enterobacteria	ISO 18415	Absence

3.1.1.3 Clove

Table 10 Basic information from safety data sheets about the Clove extract

Name	CLOVE BIOGREEN (SB)
Shelf life	24 months
Stockage	+15 / +25 ℃ Keep in the original packaging, without opening, in the recommended storage conditions

Composition	**Raw materials**	**Solvents**
Clover	Flower	Water
Sodium benzoate	-	Glycerin
Potassium sorbate	-	-
Citric acid	-	-

	Proceedings	**Specifications**
Organoleptic characteristics		
Sight	GT001	Liquid
Color	GT002	Light brown to dark brown
Odor	GT003	Characteristic
Physical & chemical characteristics		
Solubility 10% in water	GT004	Soluble
Solubility 10% in ethanol	GT004	Partially soluble
pH (direct)	GT005	3.4 - 4.0
Dry matter	GT006	0.4 - 1.0 %
Refraction index	GT007	1.350 - 1.380
Specific gravity	GT008	1.020 - 1.100
Microbiological analysis		
Enumeration of mesophilic aerobic bacteria	ISO 21149	≤ 100 ufc/mL - cfu/mL
Enumeration of yeasts and moulds	ISO 16212	≤ 100 ufc/mL - cfu/mL
Detection of enterobacteria	ISO 18415	Absence

3.1.1.4 Ethiopian coffee

Table 11 Basic information from safety data sheets about the Ethiopian coffee extract

Name	ETHIOPIAN COFFEE ORGANIC BIOGREEN
Shelf life	24 months
Stockage	+15 / +25 °C Keep in the original packaging, without opening, in the recommended storage conditions

Composition	**Raw materials**	**Solvents**
Organic Ethiopian coffee	Seed	Water
Sodium benzoate	-	Glycerin
Potassium sorbate	-	-
Citric acid	-	-

	Proceedings	**Specifications**
Organoleptic characteristics		
Sight	GT001	Liquid
Color	GT002	Yellow to amber
Odor	GT003	Characteristic
Physical & chemical characteristics		
Solubility 10% in water	GT004	Soluble
Solubility 10% in ethanol	GT004	Partially soluble
pH (direct)	GT005	3.4 - 4.0
Refraction index	GT007	1.350 - 1.380
Specific gravity	GT008	1.020 - 1.100
Quantifications		
Caffein (HPLC)	GT243	0,50 - 0,85 g/L
Microbiological analysis		
Enumeration of mesophilic aerobic bacteria	ISO 21149	≤ 100 ufc/mL - cfu/mL
Enumeration of yeasts and moulds	ISO 16212	≤ 100 ufc/mL - cfu/mL
Detection of enterobacteria	ISO 18415	Absence

3.1.1.5 Ginger

Table 12 Basic information from safety data sheets about the Ginger extract

Name	GINGER ORGANIC BIOGREEN
Shelf life	24 months
Stockage	+15 / +25 °C Keep in the original packaging, without opening, in the recommended storage conditions

Composition	**Raw materials**	**Solvents**
Organic ginger	Root	Water
Sodium benzoate	-	Glycerin
Potassium sorbate	-	-
Citric acid	-	-

	Proceedings	**Specifications**
Organoleptic characteristics		
Sight	GT001	Limpid to opalescent liquid
Color	GT002	Pale yellow to amber
Odor	GT003	Characteristic
Physical & chemical characteristics		
Solubility 10% in water	GT004	Soluble
Solubility 10% in ethanol	GT004	Partially soluble
pH (direct)	GT005	3.4 - 4.0
Refraction index	GT007	1.360 - 1.380
Specific gravity	GT008	1.020 - 1.080
Microbiological analysis		
Enumeration of mesophilic aerobic bacteria	ISO 21149	≤ 100 ufc/mL - cfu/mL
Enumeration of yeasts and moulds	ISO 16212	≤ 100 ufc/mL - cfu/mL
Detection of enterobacteria	ISO 18415	Absence

3.1.1.6 Onion

Table 13 Basic information from safety data sheets about the Onion extract

Name	ONION BIOGREEN (SB)
Shelf life	24 months
Stockage	+15 / +25 ℃ Keep in the original packaging, without opening, in the recommended storage conditions

Composition	**Raw materials**	**Solvents**
Onion	Bulb	Water
Sodium benzoate	-	Glycerin
Potassium sorbate	-	-
Citric acid	-	-

	Proceedings	**Specifications**
Organoleptic characteristics		
Sight	GT001	Liquid
Color	GT002	Colorless to yellow
Odor	GT003	Characteristic
Physical & chemical characteristics		
Solubility 10% in water	GT004	Soluble
pH (direct)	GT005	3.5 - 4.0
Refraction index	GT007	1.330 - 1.380
Specific gravity	GT008	1.020 - 1.100
Microbiological analysis		
Enumeration of mesophilic aerobic bacteria	ISO 21149	≤ 100 ufc/mL - cfu/mL
Enumeration of yeasts and moulds	ISO 16212	≤ 100 ufc/mL - cfu/mL
Detection of enterobacteria	ISO 18415	Absence

3.1.2 Chemicals

In addition to the directly analyzed extracts were used only highly purified water - Milli-Q and common salt - sodium chloride.

3.1.3 Apparatus and Instruments

The following tables list used apparatus (Table 14) and instruments (Table 15).

Table 14 Utilized apparatus

Specification	Apparatus
Analytical balance	Kern Serie 770-14
Apparatus for the production of Mili-Q water	Millipore QGARD, Academic
Magnetic stirrer with heating	IKA® RCT basic, safety control
SPME fiber marked in gray, 50/30 μm	DVB / CAR / PDMS, SUPELCO
SPME fiber marked in blue, 50/30 μm	PDMS / DVB, SUPELCO
Thermometer	–

Table 15 Utilized instruments

Specification	Instruments
Manual Solid Phase Microextraction device	SUPELCO
Gas chromatograph	Agilent 6890N, Agilent, USA
Mass Spectrometer	LECO Pegasus IVD, USA

3.2 Methods

3.2.1 Solid Phase Microextraction

The analysis of the samples was carried out with the following parameters. PDMS/DVB fiber, which was cleaned every day as recommended by the manufacturer. A water bath was heated to 60 ℃ and stirred with a magnetic stirrer at a speed of 200 rpm, in which a test tube with a sample and a magnetic stirrer was placed for 20 minutes. The concentration of the sample solution was 10%. After these 20 minutes of heating, the purified SPME fiber was placed in a test tube for the next 20 minutes, after which, it was immediately transferred to the inlet of the gas chromatograph.

3.2.2 Gas chromatography – Mass spectrometry

The GC-MS analysis was performed by using the LECO Pegasus IVD Agilent 6890N gas chromatograph, sample injection was splitless manual (SPME). A SLB-5MS column (30 m × 0.25 mm, the film thickness 0.25 μm) (USA). A mass detector is equipped with a Time of Flight spectrometer (TOF). The oven temperature was increased from 50 °C (hold for 1 minute) then increased by 15 °C/min up to 300 °C and hold for 5 minutes. The carrier gas was Helium with a flow rate of 1 mL/min. Injector and detector temperatures were 250 °C, transfer line temperature was 270 °C.

For mass spectrometer the electron ionization energy was 70 eV. The ion source temperature was 250 °C. The range of monitored molecular weights: 30–500 u. The scanning speed was 15 spectra/s. And detector voltage was 1,620 V.

4. RESULTS AND DISCUSSION

One of the goals of the work was to develop an optimal method for analyzing samples of natural extracts, taking into account the available analytical equipment. Starting with the optimization in the order of using the analytical equipment, first of all, the solid-phase microextraction part was optimized.

The usual main parameters of SPME optimization are: the chemical composition of the adsorbent, the thickness of the adsorption coating, pH, the nature and concentration of the salting-out agent, the extraction time, the intensity of stirring, temperature, desorption conditions, derivatization of the analyte [50].

Optimization was carried out in the following points:

Fiber selection

The first 10-12 measurements (until the desired temperature and concentration of the sample were established) were made on both fibers – gray (DVB/CAR/PDMS) and blue (DVB/PDMS). Many substances were detected, and the blue fiber had a higher intensity for the target substances. This can be seen on the chromatogram attached below (Figure 10).

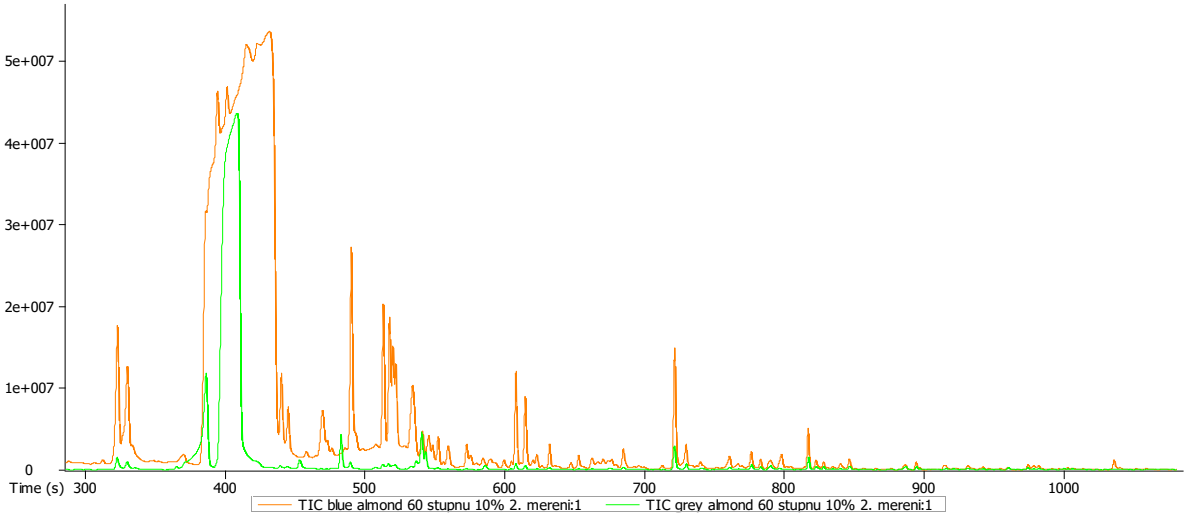

Figure 10 Chromatogram for fiber selection: orange line is for blue (DVB/PDMS) fiber and green line is for grey (DVB/CAR/PDMS) fiber

Solution concentration

To begin with, we tried 2 extremes - 1% solution and concentrate. The chromatogram below (Figure 11) clearly shows that the concentrate lacks accuracy due to too large peak areas, and the 1% sample, in turn, lacks the intensity of the peaks. we tried a 10% solution and it turned out to be quite legible, with more peaks appearing both quite clear and well separated from others.

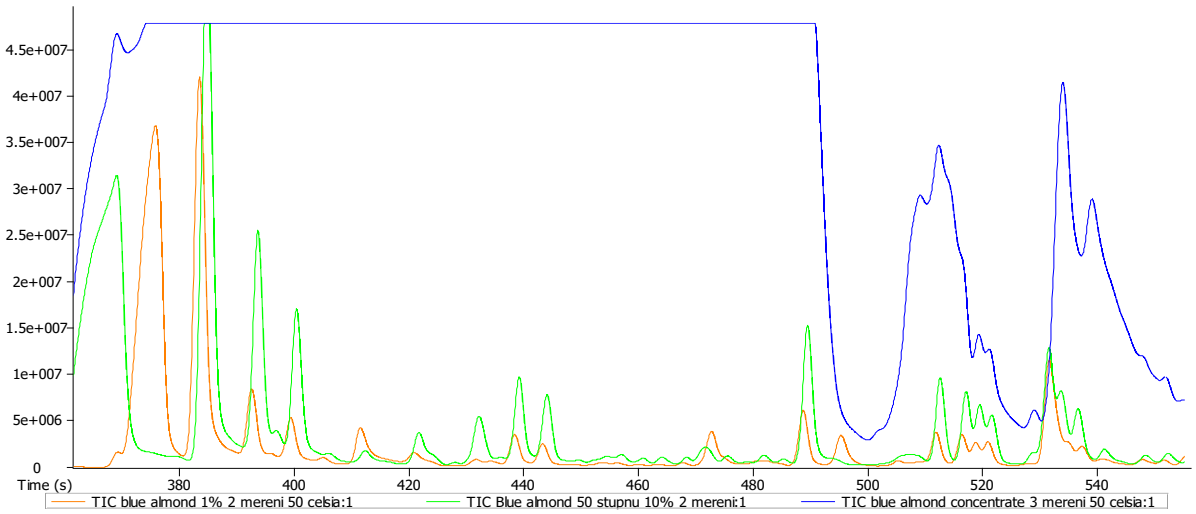

Figure 11 Chromatogram for solution concentration: orange line is for 1% solution, green line is for 10% solution, and the blue line is for concentrate

Temperature

We tried temperatures from the laboratory (20-22 °C) to 60 °C with a step of 10 °C. The chromatogram below (Figure 12) shows that the higher is the temperature the clearer are results, so we decided to stuck with 60 °C, because higher temperatures (with the probability of the best result) are harder to keep constant in a water bath.

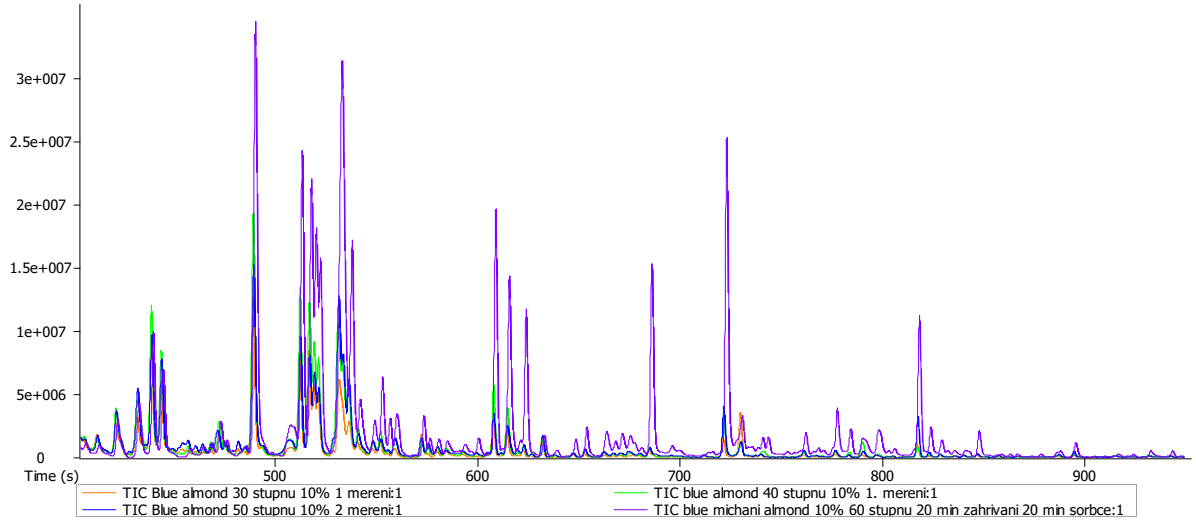

Figure 12 Chromatogram for temperatures: orange line is for 30 °C, green line is for 40 °C, the blue line is for 50 °C, and the violet line is for 50 °C

Heating time

We tried 10, 20, and 30 minutes of heating the sample before sorption, and the average time of 20 minutes worked best for the target substances, which is shown on the chromatogram below (Figure 13).

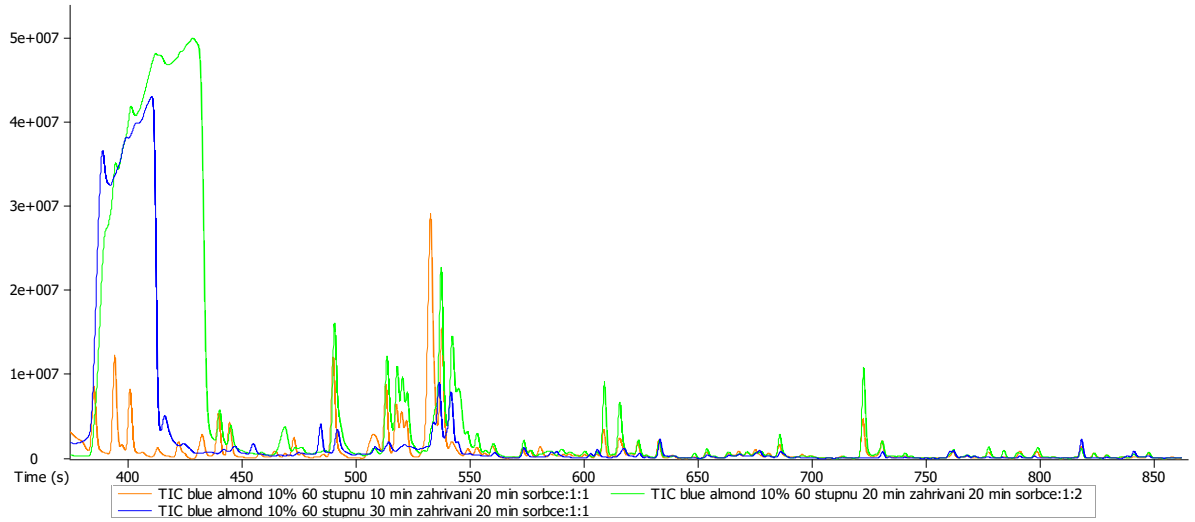

Figure 13 Chromatogram for heating time: orange line is for 10 minutes, green line is for 20 minutes, and the blue line is for 30 minutes

Stirring speed

As we can see on the chromatogram below (Figure 14) the peaks are a bit clearer with stirring of the sample solution, than without it. Since it was not difficult and the result was quite visible, we decided to use stirring.

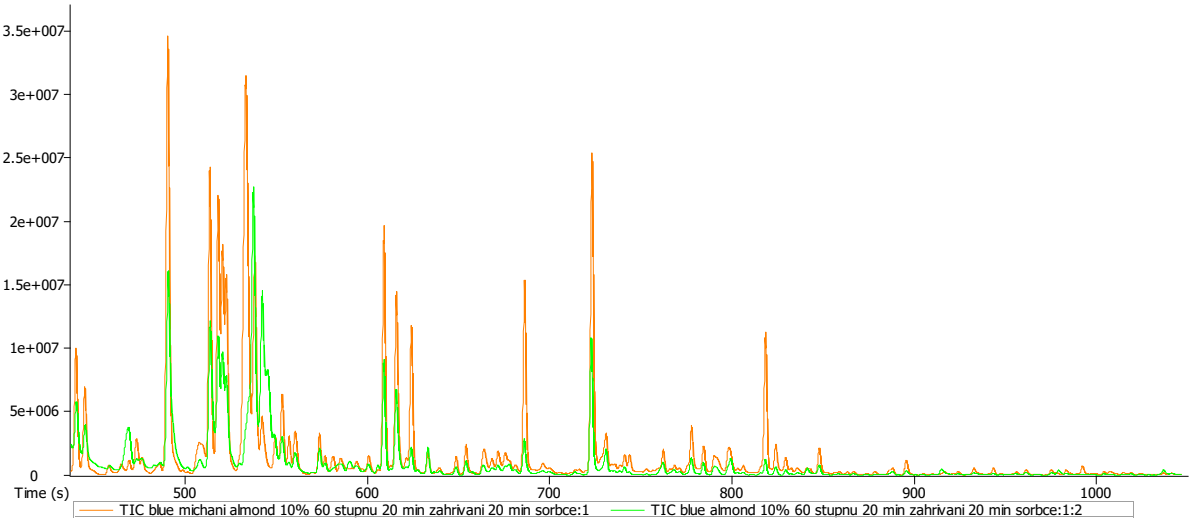

Figure 14 Chromatogram for **s**tirring speed: orange line is for stirred sample, and the green line is for still sample

Sorption time

We tried 10, 20, and 30 minutes of sorption of the VOCs from the sample with SPME fiber. The average time worked best, despite the fact that peaks weren't always higher, but they were certainly clearer, which is shown on the chromatogram below (Figure 15).

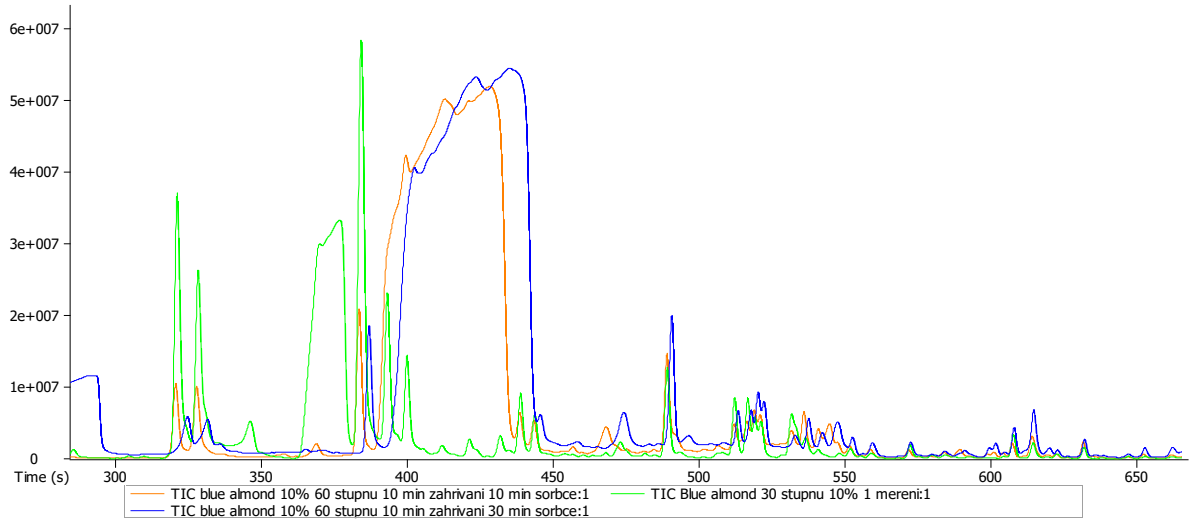

Figure 15 Chromatogram for sorption time: orange line is for 10 minutes, green line is for 20 minutes, and the blue line is for 30 minutes

Salting out

To increase the efficiency of extraction of polar and weakly polar analytes, they are often salted out. Therefore, an additional test was carried out with the addition of 2.5 grams of sodium chloride, which, however, did not give any significant improvement (Figure 16). Therefore, it was decided not to do salting out.

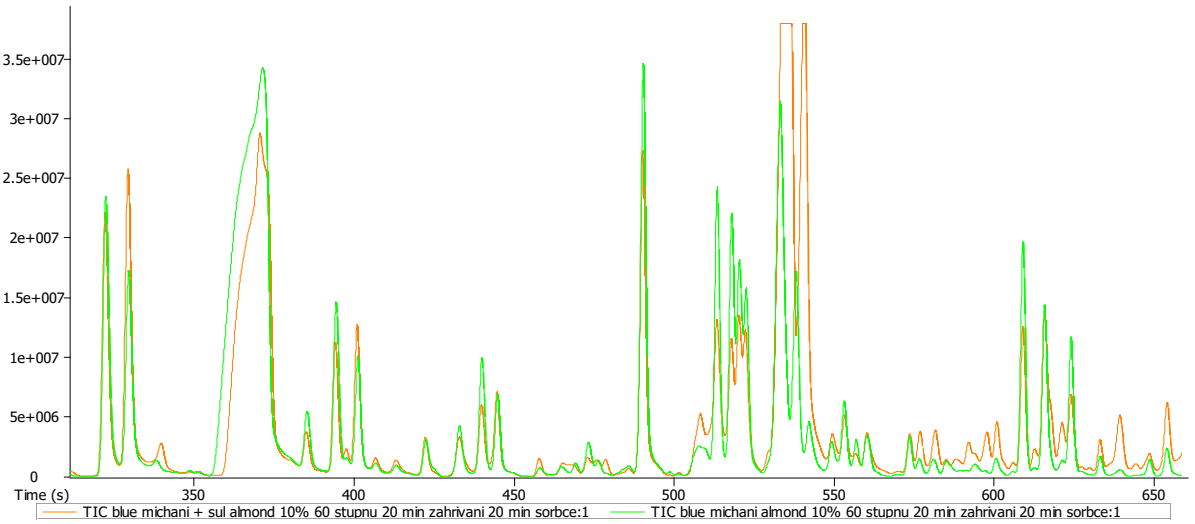

Figure 16 Chromatogram for salting out: orange line is for salted out sample, and the green line is for regular sample

Based on these experiments carried out, the SPME part of the analysis method was optimized as follows: PDMS/DVB (blue) fiber, which was cleaned every day as recommended by the manufacturer. A water bath was heated to 60 °C and stirred with a magnetic stirrer at a speed of 200 rpm, in which a test tube with a sample and a magnetic stirrer was placed for 20 minutes. The concentration of the sample solution was 10%. After these 20 minutes of heating, the purified SPME fiber was placed in a test tube for the next 20 minutes, after which, it was immediately transferred to the inlet of the gas chromatograph.

With the above indicated optimized method, the provided extracts were analyzed with a focus on the qualitative determination.

Comparing the found compounds with the assumed ones, several matches were found. They are listed in the tables below (Tables 16, 17, 18, 19, 20, 21).and denoted by the index "a". Compounds found with our method that have been added by the manufacturer and are indicated in the safety data sheets are designated by the index "b". An index "c" denotes those compounds that the manufacturer indicated as contained in the extracts, but our method could not determine them. Compounds not indicated by any index are compounds found using the selected optimized method, that were identified according to the NIST 2.0 library, and our chosen criterion was that they had a spectrum similarity greater than 70%.

The tables include only those compounds that, according to the PubChem® library [64] could be of interest in the use of natural extracts, such as a food flavoring or aroma agent, antioxidant, etc. A chromatogram with measurements will be placed under each table (Figures 17, 18, 19, 20, 21, 22).

Some potential allergens that have also been found are also included in tables. For simplicity, here's a list: thymol, eugenol, isoeugenol, lilial, propylene glycol, cinnamaldehyde, farnesol, linalool. And in general, compounds belonging to the classes listed in the allergens section (phenols, ethers, haptens, alcohols, aldehydes, sesquiterpenes, and diterpenes) are potential allergens. It just depends on their concentration, so for the future if someone wants to quantify those compounds these tables (Tables 16, 17, 18, 19, 20, 21, 22) could be a good fulcrum.

Table 16 Compounds identified for the Rostalmond extract

№	Volatile compound	Chemical group	Applying
1	Octanal[a]	Aldehyde	Flavoring agent
2	Nonanal[a]	Aldehyde	Flavoring agent
3	Decanal[a]	Aldehyde	Flavoring agent
4	Undecanal[a]	Aldehyde	Flavoring agent
5	Dodecanal[a]	Aldehyde	Flavoring agent
6	Tetradecanal[a]	Aldehyde	Flavoring agent
7	Tetradecane[a]	Alkane	Flavoring agent
8	Nonanoic acid[a]	Acid	Flavoring agent
9	n-Hexadecanoic acid[a]	Acid	Flavoring agent
10	Vanillin[b]	Benzaldehyde	Flavoring agent
11	Caramel[c]	Ketone	Flavoring agent, Food additive (colorant)
12	Propylene glycol[c]	Alcohol	Food additive (solvent)
13	α-Terpineol	Alcohol	Flavoring agent
14	α-Bisabolol	Sesquiterpenoid	Flavoring agent, Food additive
15	Supraene	Triterpene	Cosmetics (Antistatic, Emollient, Hair conditioning, Refatting)
16	Benzyl Benzoate	Ester	Flavoring agent, Food additive (solvent)

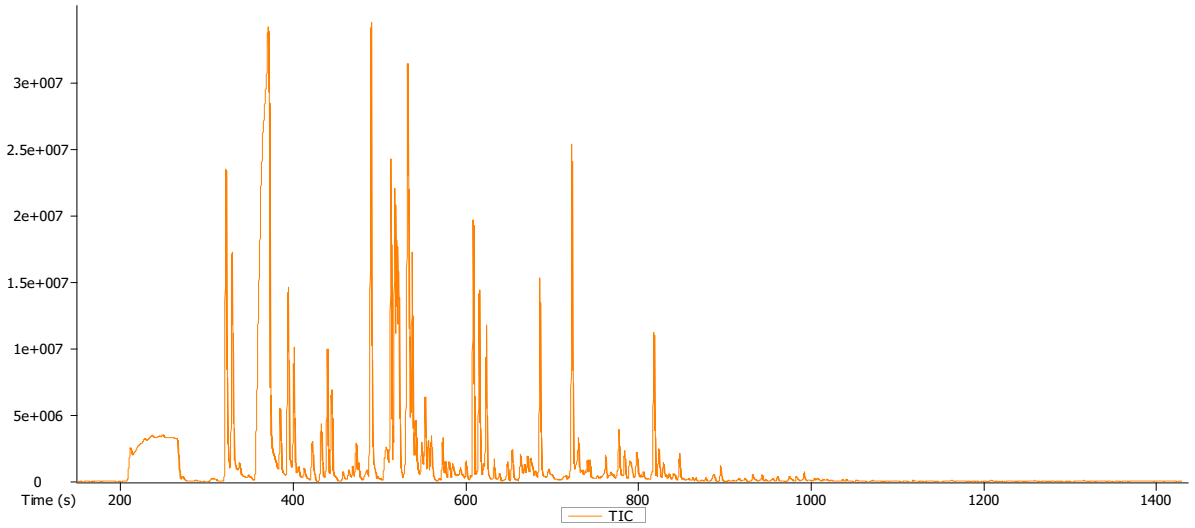

Figure 17 A chromatogram for compounds identified for the Rostalmond extract

Table 17 Compounds identified for the Aloe vera extract

№	Volatile compound	Chemical group	Applying
1	Octan-3-ol[a]	Alcohol	Flavoring agent
2	Dodecanol[a]	Alcohol	Flavoring agent
3	Tetradecane[a]	Alkane	Flavoring agent
4	Hexadecane[a]	Alkane	Fragrance
5	Eucalyptol[a]	Monoterpenoid	Flavoring agent
6	α-Pinene[a]	Monoterpene	Flavoring agent
7	Sodium benzoate[c]	Benzoic acid	Preservative
8	Potassium sorbate[c]	Fatty acid	Preservative
9	Citric acid[c]	Acid	Flavoring agent
10	Benzoic acid	Acid	Flavoring agent, Preservative
11	Sorbic acid	Acid	Flavoring agent, Preservative
12	Furfural	Aldehyde	Flavoring agent (solvent)

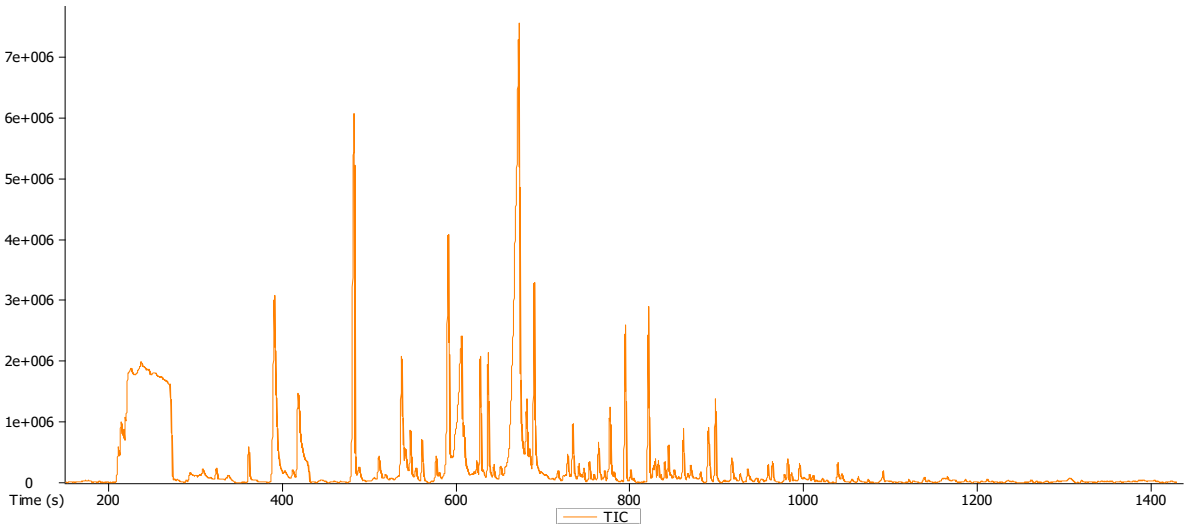

Figure 18 A chromatogram for compounds identified for the Aloe vera extract

Table 18 Compounds identified for the Clove extract

№	Volatile compound	Chemical group	Applying
1	Methyl salicylate [a]	Ester	Flavoring agent
2	Tridecane[a]	Alkane	Flavoring agent, Food additive
3	2-Heptanone[a]	Keton	Flavoring agent
4	Eugenol[a]	Phenol	Flavoring agent
5	Propylene glycol[b]	Alcohol	Food additive (solvent)
6	Glycerin[b]	Alcohol	Flavoring agent, Food additive (solvent, emulsifier, humectant, thickener)
7	Sodium benzoate[c]	Benzoic acid	Preservative
8	Potassium sorbate[c]	Fatty acid	Preservative
9	Citric acid[c]	Acid	Flavoring agent
10	Vanillin	Benzaldehyde	Flavoring agent
11	Furfural	Aldehyde	Flavoring agent (solvent)
12	Squalene	Triterpene	Cosmetics (Antistatic, Emollient, Hair conditioning, Refatting)
13	Thymol	Phenol	Flavoring agent

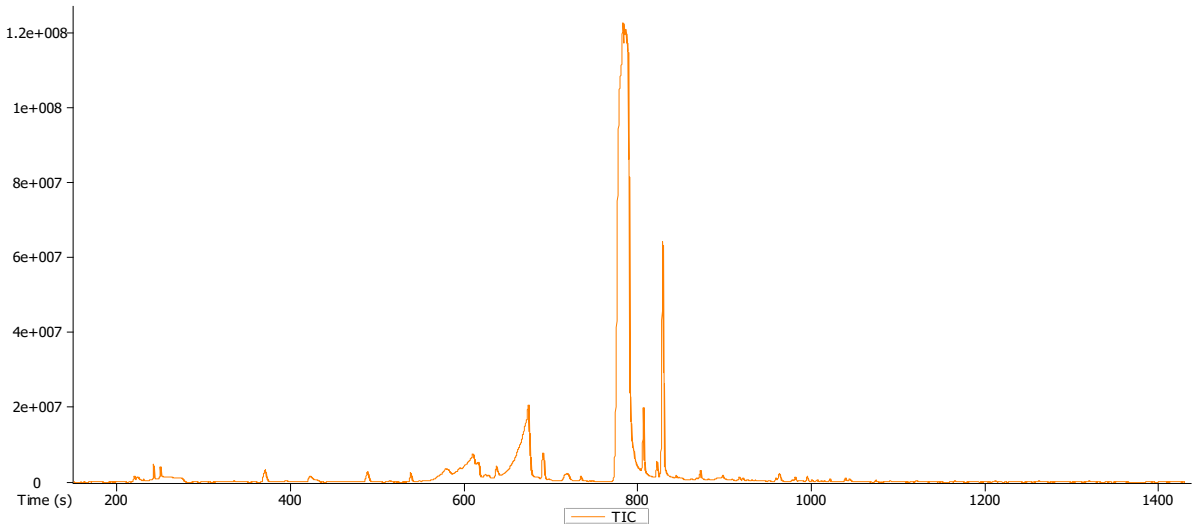

Figure 19 A chromatogram for compounds identified for the Clove extract

Table 19 Compounds identified for the Ethiopian coffee extract

№	Volatile compound	Chemical group	Applying
1	5-methylfuran-2-carbaldehyde[a]	Aldehyde	Flavoring agent
2	Pyridine[a]	Aromatic heterocycle	Flavoring agent, Adjuvant
3	Glycerin[b]	Alcohol	Flavoring agent, Food additive (solvent, emulsifier, humectant, thickener)
4	Sodium benzoate[c]	Benzoic acid	Preservative
5	Potassium sorbate[c]	Fatty acid	Preservative
6	Citric acid[c]	Acid	Flavoring agent
7	Caffeine	Alkaloid	Flavoring agent, Adjuvant
8	α-Ionone	Ketone	Flavoring agent
9	α-Terpineol	Alcohol	Flavoring agent
10	Benzyl alcohol	Alcohol	Flavoring agent, Food additive (solvent)
11	α-Myrcene	Monoterpene	Flavoring agent
12	D-Carvone	Monoterpene	Flavoring agent
13	Limonene	Monoterpene	Flavoring agent

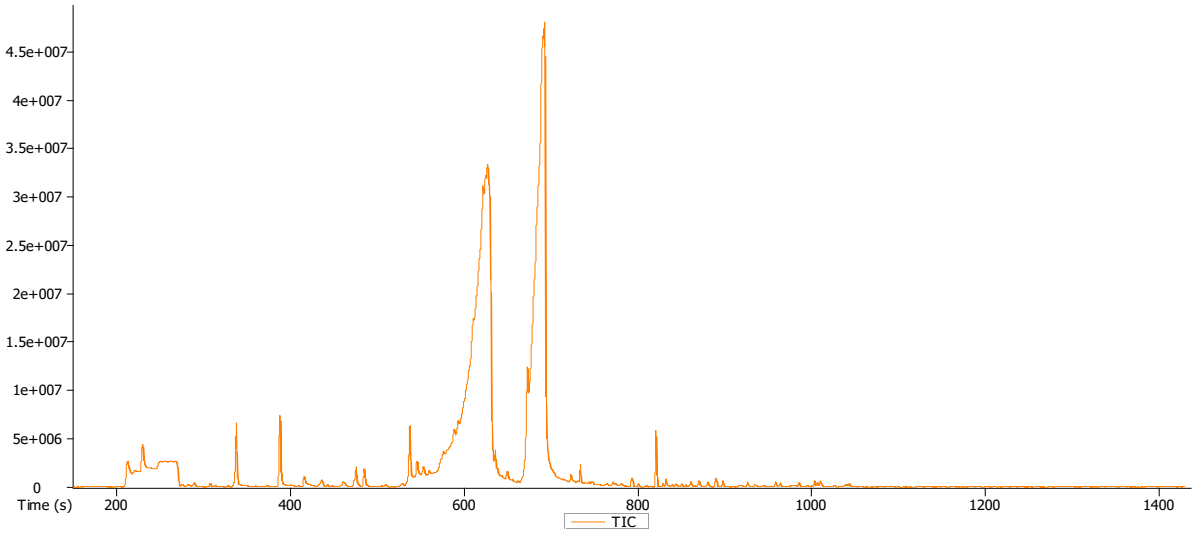

Figure 20 A chromatogram for compounds identified for the Ethiopian coffee extract

Table 20 Compounds identified for the Ginger extract

№	Volatile compound	Chemical group	Applying
1	6-Methyl-5-hepten-2-one[a]	Keton	Flavoring agent
2	Borneol[a]	Terpene derivative	Flavoring agent, Fragrance
3	Aromadendrene[a]	Sesquiterpenoid	Fragrance
4	β-Eudesmol[a]	Sesquiterpenoid	Food additive, Fragrance
5	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)- [a]	Alcohol	Flavoring agent
6	Glycerin[b]	Alcohol	Flavoring agent, Food additive (solvent, emulsifier, humectant, thickener)
7	Sodium benzoate[c]	Benzoic acid	Preservative
8	Potassium sorbate[c]	Fatty acid	Preservative
9	Citric acid[c]	Acid	Flavoring agent
10	Mandelic acid	Acid	Cosmetics (Antimicrobial)
11	α-Terpineol	Alcohol	Flavoring agent
12	α-Cubebene	Sesquiterpenoid	Emulsifier
13	Mequinol	Ether	Flavoring agent, Depigmenting agent

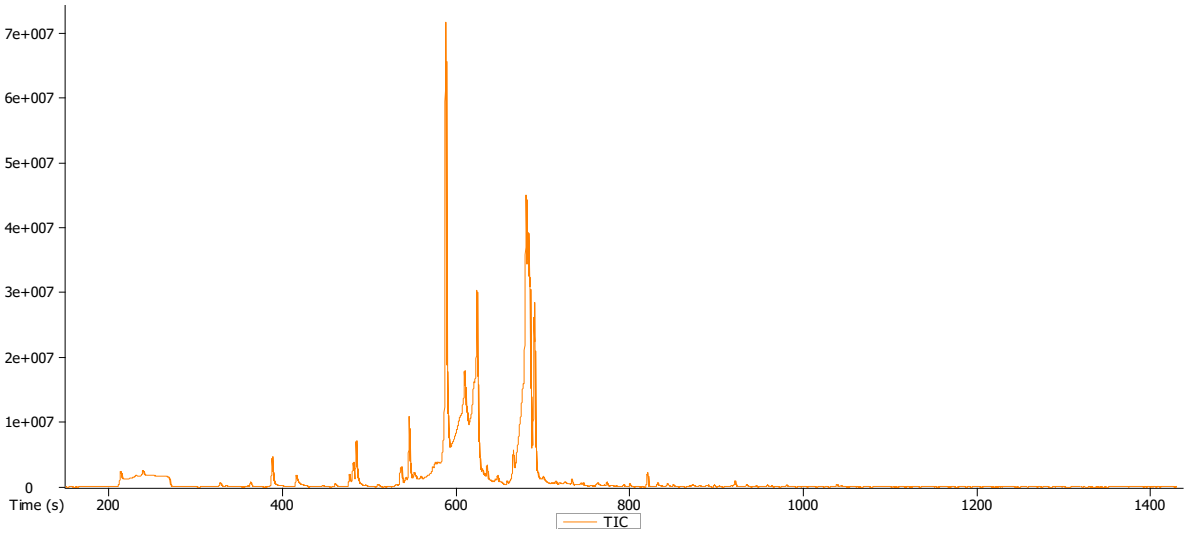

Figure 21 A chromatogram for compounds identified for the Ginger extract

Table 21 Compounds identified for the Onion extract

№	Volatile compound	Chemical group	Applying
1	2-methylfuran[a]	Furan	Flavoring agent
2	Hexane[a]	Alkane	Food additive (solvent)
3	Heptane[a]	Alkane	Food additive (extraction solvent)
4	Hexanal[a]	Aldehyde	Flavoring agent
5	Acetic acid[a]	Acid	Flavoring agent, Food additive (acidity regulator, preservative)
6	Glycerin[b]	Alcohol	Flavoring agent, Food additive (solvent, emulsifier, humectant, thickener)
7	Sodium benzoate[c]	Benzoic acid	Preservative
8	Potassium sorbate[c]	Fatty acid	Preservative
9	Citric acid[c]	Acid	Flavoring agent
10	α-Ionone	Ketone	Flavoring agent
11	α-Bisabolol	Sesquiterpenoid	Flavoring agent
12	α-Farnese	Alkene	Flavoring agent
13	Cinnamaldehyde	Aldehyde	Flavoring agent, Food additive
14	Benzyl alcohol	Alcohol	Flavoring agent, Food additive (solvent)

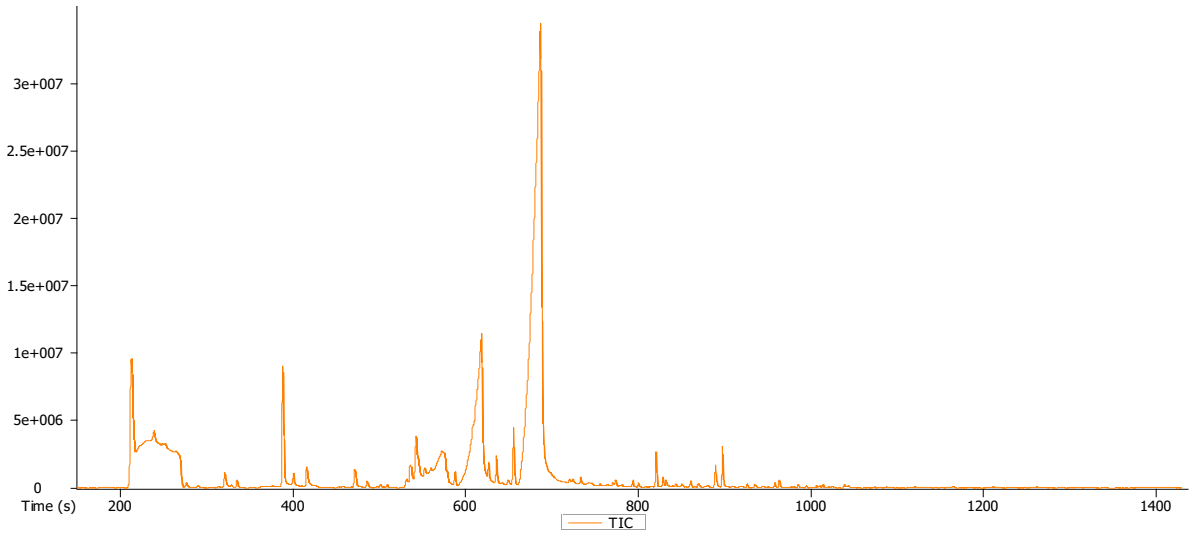

Figure 22 A chromatogram for compounds identified for the Onion extract

The following compounds listed in Table 22 were found in all or most of the extracts, and their similarity was at least 80%. Each extract is marked with a symbol: «A» is for Almond, «B» is for Aloe Vera, «C» is for Clove, «D» is for Ethiopian Coffee, «E» is for Ginger and «F» is for Onion.

Table 22 Compounds found in two or more extracts

№	Volatile compound	Chemical group	Applying	A	B	C	D	E	F
1	Tetradecanoic acid	Acid	Flavoring agent	X		X	X		X
2	Sorbic Acid	Acid	Flavoring agent, Preservative		X	X		X	X
3	Valeric acid	Acid	Flavoring agent, Food additive			X	X		X
4	Nonanoic acid	Acid	Flavoring agent	X	X				X
5	2-Ethylhexanoic acid	Acid	Flavoring agent		X			X	
6	4-Pentenoic acid	Acid	Flavoring agent				X	X	
7	Formic acid	Acid	Flavoring agent, Preservative		X	X	X	X	
8	Lauric acid	Acid	Flavoring agent	X			X		X
9	Undecylenic acid	Acid	Flavoring agent				X	X	
10	Carbamic acid, monoammonium salt	Acid	Cosmetics Buffering	X			X		X
11	Benzoic acid	Acid	Flavoring agent, Preservative		X	X	X	X	X
12	Acetic acid	Acid	Flavoring agent, Preservative	X	X	X		X	X
13	Glycerin	Alcohol	Flavoring agent, Food additive (solvent, emulsifier, humectant, thickener)			X	X	X	X
14	3-Buten-2-ol, 2-methyl-	Alcohol	Flavoring agent				X	X	
15	2-Tridecen-1-ol, (E)-	Alcohol	Flavoring agent	X	X				
16	2-Heptanol	Alcohol	Flavoring agent				X	X	
17	1-Undecanol	Alcohol	Flavoring agent (Mandarin)	X	X	X	X	X	X
18	1-Octen-3-ol	Alcohol	Flavoring agent				X	X	
19	1-Octanol	Alcohol	Flavoring agent (bitter almond)	X	X				

20	1-Nonanol	Alcohol	Flavoring agent	X			X		X
21	1-Hexanol, 2-ethyl-	Alcohol	Flavoring agent, Adjuvant		X		X		X
22	1-Hexanol	Alcohol	Flavoring agent	X			X	X	
23	1-Hexadecanol	Alcohol	Flavoring agent		X	X			X
24	Propylene glycol	Alcohol	Food additive (solvent)			X	X		X
25	1-Dodecanol	Alcohol	Flavoring agent	X	X		X	X	
26	Isoamyl alcohol	Alcohol	Flavoring agent				X	X	
27	Tridecanol	Alcohol	Emollient, Emulsion stabilizing, Refatting, Viscosity controlling	X		X	X	X	
28	Ethanol	Alcohol	Flavoring agent (solvent)	X	X	X	X	X	X
29	5-Methylfurfural	Aldehyde	Flavoring agent	X	X	X	X	X	X
30	Nonanal	Aldehyde	Flavoring agent	X	X	X			X
31	Tetradecanal	Aldehyde	Flavoring agent	X			X		X
32	Octanal, 2-(phenylmethylene)-	Aldehyde	Flavoring agent	X	X		X		X
33	Octanal	Aldehyde	Flavoring agent	X	X		X	X	X
34	Undecanal	Aldehyde	Flavoring agent	X	X	X	X	X	X
35	Lilial	Aldehyde	Fragrance	X				X	
36	Hexanal	Aldehyde	Flavoring agent	X	X		X	X	X
37	Heptanal	Aldehyde	Flavoring agent	X	X		X		X
38	Furfural	Aldehyde	Flavoring agent (solvent)			X	X		
39	Decanal	Aldehyde	Flavoring agent	X	X	X	X	X	X
40	Butanal, methyl-	Aldehyde	Flavoring agent	X			X		X
41	Dodecanal	Aldehyde	Flavoring agent	X	X	X	X	X	X
42	Vanillin	Benzaldehyde	Flavoring agent	X		X			
43	Chloroform	Alkane	Extraction solvent	X	X			X	X
44	Tetradecane	Alkane	Flavoring agent	X	X	X	X	X	X
45	Pentadecane	Alkane	Flavoring agent		X	X		X	

46	Nonadecane	Alkane	Additive to paraffin and vaseline	X	X	X	X	X	
47	n-Hexane	Alkane	Oil and fat extraction	X	X		X	X	X
48	Dichloromethane	Alkane	Extraction solvent	X	X		X	X	X
49	Hexadecane	Alkane	Fragrance		X	X	X	X	X
50	Heptane	Alkane	Food additive (extraction solvent)		X		X	X	X
51	Tridecane	Alkane	Flavoring agent, Food additive	X			X		X
52	4-Methyl-2-pentadecyl-1,3-dioxolane	Alkane	Ficus VOC	X			X		
53	Phenanthrene	Aromatic hydrocarbon	Fragrance	X	X	X	X	X	
54	Dioctyl ether	Ether	Cosmetic solvent	X	X		X	X	X
55	Diphenyl ether	Ether	Flavoring agent		X		X		X
56	Isopropyl myristate	Ester	Flavoring agent, Moisturizer	X	X	X	X	X	X
57	Homosalate	Ester	Skin conditioning, UV absorber, UV filter	X				X	
58	Ethyl Acetate	Ester	Flavoring agent				X	X	
59	Glutaric acid, di(isobutyl) ester	Ester	Solvent				X	X	X
60	Dodecyl acrylate	Ester	Colorant			X			X
61	Dodecanoic acid, methyl ester	Ester	Flavoring agent	X	X				
62	Vinyl acetate	Ester	Cosmetics (film forming)	X					X
63	4-Pentenoic acid ethyl ester	Ester	Flavoring agent				X	X	
64	Benzoic acid, different esters*	Ester	x	X	X	X	X	X	X
65	Butyrolactone	Furan	Flavoring agent (caramel)		X	X			
66	Furan, 2-methyl-	Furan	Flavoring agent				X		X
67	Furan, 2-ethyl-5-methyl-	Furan	Flavoring agent					X	X
68	Furan, 2,5-dimethyl-	Furan	Flavoring agent	X				X	
69	2-Acetyl-5-methylfuran	Furan	Flavoring agent		X				X

70	2,4-Heptadien-6-one	Keton	Black tea, Ficus VOC	X			X		X
71	4-Hydroxy-3-methylacetophenone	Keton	Fragrance		X			X	X
72	3-Penten-2-one, (E)-	Keton	Flavoring agent		X				X
73	2-Buten-1-one, 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-	Keton	Flavoring agent, Food additive, Cosmetics, Fragrance				X	X	
74	2-Butanone	Keton	Flavoring agent, Fragrance	X				X	
75	2-Propanone, 1-hydroxy-	Keton	Flavoring agent	X	X	X	X		
76	5-Hepten-2-one, 6-methyl-	Keton	Volatile oil component of citronella oil, lemon-grass oil and palmarosa oil	X	X	X	X	X	X
77	2-Heptanone	Keton	Flavoring agent			X		X	X
78	Geranylacetone	Keton	Flavoring agent	X	X	X	X	X	X
79	Camphor	Keton	Flavoring agent	X				X	
80	Benzophenone	Keton	Flavoring agent	X	X				X
81	Eucalyptol	Monoterpenoid	Flavoring agent	X				X	
82	2-Methoxy-4-vinylphenol	Phenol	Clove flavoring agent			X	X		
83	cis-Isoeugenol	Phenol	Fragrance (cacao)		X	X			
84	Isoeugenol	Phenol	Vanilla composition, producing vanillin and eugenol		X	X			
85	2,4-Di-tert-butylphenol	Phenol	Antioxidant	X	X	X	X	X	X
86	Pyridine	Pyridine	Flavoring agent, Adjuvant				X		X

For number 64 in Table 22, different variants of Benzoic acid esters were found. Each extract is marked with a symbol: «A» is for Almond, «B» is for Aloe Vera, «C» is for Clove, «D» is for Ethiopian Coffee, «E» is for Ginger and «F» is for Onion.

Benzoic acid, methyl ester	Flavoring agent	C, F
Benzoic acid, ethyl ester	Flavoring agent	D
Benzoic acid, 2-hydroxy-, pentyl ester	Flavoring agent	A, B, D
Benzoic acid, 2-hydroxy-, 2-methylbutyl ester	Flavoring agent	A, B, C, D
Benzoic acid, 2-ethylhexyl ester	Flavoring agent	A, B, D, E

5. CONCLUSION

This bachelor's thesis was focused on the observation of natural commercially available extracts that are used in natural cosmetics. The literature review was focused on the topic of the content of volatile substances in natural extracts used in cosmetics, their characterization and determination possibilities, and the issue of the occurrence of allergens in natural cosmetics.

In the theoretical part, these basic concepts were explained: an extract, its classification, an allergen, and which classes of chemical substances could cause an allergic reaction. There was also described what exactly gets from the plant into the extract, and then into cosmetics, what active substances this or that group of plants gives us, how they affect the human body and mind, and why they are generally used in cosmetology.

Based on that review was designed an optimal method for the analysis of samples of natural extracts, taking into account the available analytical instrumentation.

The SPME part of the analysis method was optimized as follows: PDMS/DVB (blue) fiber, which was cleaned every day as recommended by the manufacturer. A water bath was heated to 60 °C and stirred with a magnetic stirrer at a speed of 200 rpm, in which a test tube with a sample and a magnetic stirrer was placed for 20 minutes. The concentration of the sample solution was 10%. After these 20 minutes of heating, the purified SPME fiber was placed in a test tube for the next 20 minutes, after which, it was immediately transferred to the inlet of the gas chromatograph.

With the above indicated optimized method, the provided extracts (almond, aloe vera, clove, Ethiopian coffee, ginger, onion) were analyzed with a focus on the qualitative determination. Both some of the expected compounds and some of the compounds added by the manufacturer were found. Some potential allergens have also been found, such as: thymol, eugenol, isoeugenol, lilial, propylene glycol, cinnamaldehyde, farnesol, linalool. All the discovered compounds were identified according to the NIST 2.0 library, and our chosen criterion was that they had a spectrum similarity greater than 70%.

And in general, compounds belonging to the classes listed in the allergens section (phenols, ethers, haptens, alcohols, aldehydes, sesquiterpenes, and diterpenes) are potential allergens. It just depends on their concentration.

Based on the above, we can conclude that the goals set for this work have been achieved. The optimized method has shown itself well and can be applied in the future for works based on this. Further research with a focus on quantitative determination might be a good follow-up to this work for those interested.

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7. ATTACHMENTS

BIOTECHNOLOGIES
GREENTECH

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BULLETIN D'ANALYSES / CERTIFICATE OF ANALYSIS

MELANGE ACV EXTRAIT HYDROGLYCOLIQUE ECO
ROSTALMOND HYDROGLYCOLIC EXTRACT ECO

Réf. : **3001165**
N° de lot / Batch number : **ACV1903L1-ECO**
Production : **03/2019**

DLUO / Shelf life : 24 mois / months : 03/2021
Stockage : +15 / +25°C
Conservation dans le contenant d'origine, sans ouverture, dans les conditions de stockage préconisées.
Keep in the original packaging, without opening, in the recommended storage conditions.
Conseil avant utilisation / Advice before use : Agitation

COMPOSITION	Matières premières / Raw materials	Solvants
	Amande douce / Sweet almond *(Prunus amygdalus dulcis)*.....Graine / Seed Caramel Vanille / Vanilla *(Vanilla planifolia)*..... Gousse / Pod	Propylène glycol végétal / Natural propylen glycol Eau / Water

	Proceedings	Specifications	Analyse / Analysis	Result.
CARACTÉRISTIQUES ORGANOLEPTIQUES / ORGANOLEPTIC CHARACTERISTICS				
Aspect Sight	GT001	Liquide Liquid	Liquide Liquid	Conforme *OK*
Couleur Color	GT002	Ambré à brun foncé Amber to dark brown	Ambré à brun foncé Amber to dark brown	Conforme *OK*
Odeur Odor	GT003	Caractéristique Characteristic	Caractéristique Characteristic	Conforme *OK*
CARACTÉRISTIQUES PHYSICO-CHIMIQUES / PHYSICAL & CHEMICAL CHARACTERISTICS				
Solubilité 10% dans l'eau Solubility 10% in water	GT004	Soluble	Soluble	Conforme *OK*
Solubilité 10% dans l'éthanol Solubility 10% in ethanol	GT004	Partiellement soluble Partially soluble	Partiellement soluble Partially soluble	Conforme *OK*
pH (direct)	GT005	4.2 - 6.2	5.2	Conforme *OK*
Matière sèche Dry matter	GT006	1.0 - 2.0 %	1.5 %	Conforme *OK*
Indice de réfraction Refraction index	GT007	1.370 - 1.410	1.390	Conforme *OK*
Densité Specific gravity	GT008	1.010 - 1.060	1.037	Conforme *OK*
ANALYSES MICROBIOLOGIQUES / MICROBIOLOGICAL ANALYSIS				
Dénombrement des bactéries aérobies mésophiles Enumeration of mesophilic aerobic bacteria	ISO 21149	≤ 100 ufc/mL - cfu/mL	ND	—
Dénombrement des levures et moisissures Enumeration of yeasts and moulds	ISO 16212	≤ 100 ufc/mL - cfu/mL	ND	—
Détection des enterobactéries Detection of enterobacteria	ISO 18415	Absence	ND	—

Commentaires / Comments :

Accepté
Accepted

Visa contrôle qualité
D. GORACY

Visa resp. labo. analyses
A. SEVESTRE

Version 1 du 22/11/2016

Date de libération / Delivred date : 01/04/2019

1/1

Attachment 1 GREENTECH Certificate of analysis for a Rostalmond extract

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BULLETIN D'ANALYSES / CERTIFICATE OF ANALYSIS

ALOE VERA JUS BIO 1:1 (SB) EQUITABLE
ALOE VERA ORGANIC JUICE 1:1 (SB) FAIR FOR LIFE

Réf. : **341121**
N° de lot / Batch number : **ALV1907L1-AQBIO**
Production : **07/2019**

DLUO / Shelf life : 24 mois / months : 07/2021
Stockage : +15 / +25°C
Conservation dans le contenant d'origine, sans ouverture, dans les conditions de stockage préconisées.
Keep in the original packaging, without opening, in the recommended storage conditions.
Consell avant utilisation / Advice before use : Agitation

COMPOSITION	Matières premières / Raw materials	Solvants
Aloe vera bio / Organic aloe vera *(Aloe barbadensis)*	Feuille / Leaf	Eau / Water
Benzoate de sodium / Sodium benzoate		
Sorbate de potassium / Potassium sorbate		
Acide citrique / Citric acid		

	Proceedings	Specifications	Analyse / Analysis	Result.
CARACTÉRISTIQUES ORGANOLEPTIQUES / ORGANOLEPTIC CHARACTERISTICS				
Aspect Sight	GT001	Liquide Liquid	Liquide Liquid	Conforme *OK*
Couleur Color	GT002	Incolore à jaune Colorless to yellow	Incolore à jaune Colorless to yellow	Conforme *OK*
Odeur Odor	GT003	Caractéristique Characteristic	Caractéristique Characteristic	Conforme *OK*
CARACTÉRISTIQUES PHYSICO-CHIMIQUES / PHYSICAL & CHEMICAL CHARACTERISTICS				
Solubilité 10% dans l'eau Solubility 10% in water	GT004	Soluble	Soluble	Conforme *OK*
Solubilité 10% dans l'éthanol Solubility 10% in ethanol	GT004	Partiellement soluble Partially soluble	Partiellement soluble Partially soluble	Conforme *OK*
pH (direct)	GT005	3.5 - 4.7	4.1	Conforme *OK*
Matière sèche Dry matter	GT006	0.4 - 1.0 %	0.7 %	Conforme *OK*
Indice de réfraction Refraction index	GT007	1.330 - 1.380	1.334	Conforme *OK*
Densité Specific gravity	GT008	1.000 - 1.050	1.003	Conforme *OK*
ANALYSES MICROBIOLOGIQUES / MICROBIOLOGICAL ANALYSIS				
Dénombrement des bactéries aérobies mésophiles Enumeration of mesophilic aerobic bacteria	ISO 21149	≤ 50 ufc/mL - cfu/mL	ND	—
Dénombrement des levures et moisissures Enumeration of yeasts and moulds	ISO 16212	≤ 10 ufc/mL - cfu/mL	ND	—
Détection des enterobactéries Detection of enterobacteria	ISO 18415	Absence	ND	—

Commentaires / Comments :
Commerce équitable contrôlé par le référentiel Fair for life
Matière certifiée par ECOCERT Greenlife selon le référentiel COSMOS
Raw material certified by ECOCERT Greenlife according to COSMOS standard

Accepté
Accepted

Visa contrôle qualité D. GORACY

Visa resp. labo. analyses A. SEVESTRE

Version 3 du 23/07/2018

Date de libération / Delivred date :

Attachment 2 GREENTECH Certificate of analysis for an Aloe vera extract

BIOTECHNOLOGIES
GREENTECH

Biopôle Clermont-Limagne
63360 Saint-Beauzire – FRANCE
Tél.: +33 4 73 33 99 00
Fax.: +33 4 73 33 91 32
email : greentech@greentech.fr

BULLETIN D'ANALYSES / CERTIFICATE OF ANALYSIS

CLOU DE GIROFLE BIOGREEN (SB)
CLOVE BIOGREEN (SB)

Réf. : 400054
N° de lot / Batch number : **SYA180701-BG**
Production : **07/2018**

DLUO / Shelf life : 24 mois / months : 07/2020
Stockage : +15 / +25°C
Conservation dans le contenant d'origine, sans ouverture, dans les conditions de stockage préconisées.
Keep in the original packaging, without opening, in the recommended storage conditions.
Conseil avant utilisation / Advice before use : Agitation

COMPOSITION	Matières premières / Raw materials	Solvants
	Clou de girofle / Clover *(Eugenia caryophyllus)*.....Fleur / Flower	Glycérine / Glycerin
	Benzoate de sodium / Sodium benzoate	Eau / Water
	Sorbate de potassium / Potassium sorbate	
	Acide citrique / Citric acid	

	Proceedings	Specifications	Analyse / Analysis	Result.
CARACTÉRISTIQUES ORGANOLEPTIQUES / ORGANOLEPTIC CHARACTERISTICS				
Aspect Sight	GT001	Liquide Liquid	Liquide Liquid	Conforme *OK*
Couleur Color	GT002	Brun clair à brun foncé Light brown to dark brown	Brun clair à brun foncé Light brown to dark brown	Conforme *OK*
Odeur Odor	GT003	Caractéristique Characteristic	Caractéristique Characteristic	Conforme *OK*
CARACTÉRISTIQUES PHYSICO-CHIMIQUES / PHYSICAL & CHEMICAL CHARACTERISTICS				
Solubilité 10% dans l'eau Solubility 10% in water	GT004	Soluble	Soluble	Conforme *OK*
Solubilité 10% dans l'éthanol Solubility 10% in ethanol	GT004	Partiellement soluble Partially soluble	Partiellement soluble Partially soluble	Conforme *OK*
pH (direct)	GT005	3.4 – 4.0	3.7	Conforme *OK*
Indice de réfraction Refraction index	GT007	1.350 – 1.380	1.366	Conforme *OK*
Densité Specific gravity	GT008	1.020 – 1.100	1.064	Conforme *OK*
ANALYSES MICROBIOLOGIQUES / MICROBIOLOGICAL ANALYSIS				
Dénombrement des bactéries aérobies mésophiles Enumeration of mesophilic aerobic bacteria	ISO 21149	≤ 100 ufc/mL – cfu/mL	<10 ufc/mL – cfu/mL	Conforme *OK*
Dénombrement des levures et moisissures Enumeration of yeasts and moulds	ISO 16212	≤ 100 ufc/mL – cfu/mL	<10 ufc/mL – cfu/mL	Conforme *OK*
Détection des enterobactéries Detection of enterobacteria	ISO 18415	Absence	Absence	Conforme *OK*

Commentaires / Comments :

Accepté
Accepted

Visa contrôle qualité D. GORACY

Visa resp. labo. analyses M. CARRÉ

Version 4 du 09/07/2018

Date de libération / Delivred date : 18/07/2018

Attachment 3 GREENTECH Certificate of analysis for a Clove extract

BIOTECHNOLOGIES
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BULLETIN D'ANALYSES / CERTIFICATE OF ANALYSIS

CAFÉ D'ÉTHIOPIE BIOGREEN BIO
ETHIOPIAN COFFEE ORGANIC BIOGREEN

Réf. : 410019
N° de lot / Batch number : COAE190401-BGBIO
Production : 04/2019

DLUO / Shelf life : 24 mois / months : 04/2021
Stockage : +15 / +25°C
Conservation dans le contenant d'origine, sans ouverture, dans les conditions de stockage préconisées.
Keep in the original packaging, without opening, in the recommended storage conditions.
Conseil avant utilisation / Advice before use : Agitation

COMPOSITION	Matières premières / Raw materials	Solvants
	Café d'Ethiopie bio / Organic Ethiopian coffee *(Coffea arabica)*..... Graine / Seed	Glycérine / Glycerin
	Benzoate de sodium / Sodium benzoate	Eau / Water
	Sorbate de potassium / Potassium sorbate	
	Acide citrique / Citric acid	

	Proceedings	Specifications	Analyse / Analysis	Result.
CARACTÉRISTIQUES ORGANOLEPTIQUES / ORGANOLEPTIC CHARACTERISTICS				
Aspect Sight	GT001	Liquide Liquid	Liquide Liquid	Conforme *OK*
Couleur Color	GT002	Jaune à ambré Yellow to amber	Jaune à ambré Yellow to amber	Conforme *OK*
Odeur Odor	GT003	Caractéristique Characteristic	Caractéristique Characteristic	Conforme *OK*
CARACTÉRISTIQUES PHYSICO-CHIMIQUES / PHYSICAL & CHEMICAL CHARACTERISTICS				
Solubilité 10% dans l'eau Solubility 10% in water	GT004	Soluble	Soluble	Conforme *OK*
Solubilité 10% dans l'éthanol Solubility 10% in ethanol	GT004	Partiellement soluble Partially soluble	Partiellement soluble Partially soluble	Conforme *OK*
pH (direct)	GT005	3.4 - 4.0	3.7	Conforme *OK*
Indice de réfraction Refraction index	GT007	1.350 - 1.380	1.369	Conforme *OK*
Densité Specific gravity	GT008	1.020 - 1.100	1.068	Conforme *OK*
QUANTIFICATIONS / QUANTIFICATIONS				
Caféine (CLHP) Caffein (HPLC)	GT243	0.50 - 0.85 g/L	0.82 g/L	Conforme *OK*
ANALYSES MICROBIOLOGIQUES / MICROBIOLOGICAL ANALYSIS				
Dénombrement des bactéries aérobies mésophiles Enumeration of mesophilic aerobic bacteria	ISO 21149	≤ 100 ufc/mL - cfu/mL	<10 ufc/mL - cfu/mL	Conforme *OK*
Dénombrement des levures et moisissures Enumeration of yeasts and moulds	ISO 16212	≤ 100 ufc/mL - cfu/mL	<10 ufc/mL - cfu/mL	Conforme *OK*
Détection des enterobactéries Detection of enterobacteria	ISO 18415	Absence	Absence	Conforme *OK*

Commentaires / Comments :
Matière première certifiée par ECOCERT Greenlife selon le référentiel Ecocert des Cosmétiques Ecologiques et Biologiques disponible sur http://cosmetiques.ecocert.com/
Matière certifiée par ECOCERT Greenlife selon le référentiel COSMOS
Raw material certified by ECOCERT Greenlife according to Natural & Organic Cosmetics Ecocert's standards available on http://cosmetiques.ecocert.com/
Raw material certified by ECOCERT Greenlife according to COSMOS standard

Accepté
Accepted

Visa contrôle qualité — D. GORACY
Visa resp. labo. analyses — M. CARRÉ

Version 9 du 06/12/2017
Date de libération / Delivred date : 15/04/2019

Attachment 4 GREENTECH Certificate of analysis for an Ethiopian coffee extract

BIOTECHNOLOGIES
GREENTECH

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BULLETIN D'ANALYSES / CERTIFICATE OF ANALYSIS

GINGEMBRE BIOGREEN BIO
GINGER ORGANIC BIOGREEN

Réf. : **410045**
N° de lot / Batch number : **ZIO1906L1-BGBIO**
Production : **06/2019**

DLUO / Shelf life : 24 mois / months : 06/2021
Stockage : +15 / +25°C
Conservation dans le contenant d'origine, sans ouverture, dans les conditions de stockage préconisées.
Keep in the original packaging, without opening, in the recommended storage conditions.
Conseil avant utilisation / Advice before use : Agitation

COMPOSITION	Matières premières / Raw materials	Solvants
	Gingembre bio / Organic ginger *(Zingiber officinale)*.....Racine / Root	Glycérine / Glycerin
	Benzoate de sodium / Sodium benzoate	Eau / Water
	Sorbate de potassium / Potassium sorbate	
	Acide citrique / Citric acid	

	Proceedings	Specifications	Analyse / Analysis	Result.
CARACTÉRISTIQUES ORGANOLEPTIQUES / ORGANOLEPTIC CHARACTERISTICS				
Aspect Sight	GT001	Liquide limpide à opalescent Limpid to opalescent liquid	Liquide limpide à opalescent Limpid to opalescent liquid	Conforme *OK*
Couleur Color	GT002	Jaune pâle à ambré Pale yellow to amber	Jaune pâle à ambré Pale yellow to amber	Conforme *OK*
Odeur Odor	GT003	Caractéristique Characteristic	Caractéristique Characteristic	Conforme *OK*
CARACTÉRISTIQUES PHYSICO-CHIMIQUES / PHYSICAL & CHEMICAL CHARACTERISTICS				
Solubilité 10% dans l'eau Solubility 10% in water	GT004	Soluble	Soluble	Conforme *OK*
Solubilité 10% dans l'éthanol Solubility 10% in ethanol	GT004	Partiellement soluble Partially soluble	Partiellement soluble Partially soluble	Conforme *OK*
pH (direct)	GT005	3.4 - 4.0	4.0	Conforme *OK*
Indice de réfraction Refraction index	GT007	1.360 - 1.380	1.366	Conforme *OK*
Densité Specific gravity	GT008	1.020 - 1.080	1.043	Conforme *OK*
ANALYSES MICROBIOLOGIQUES / MICROBIOLOGICAL ANALYSIS				
Dénombrement des bactéries aérobies mésophiles Enumeration of mesophilic aerobic bacteria	ISO 21149	≤ 100 ufc/mL - cfu/mL	ND	—
Dénombrement des levures et moisissures Enumeration of yeasts and moulds	ISO 16212	≤ 100 ufc/mL - cfu/mL	ND	—
Détection des enterobactéries Detection of enterobacteria	ISO 18415	Absence	ND	—

Commentaires / Comments :
Matière première certifiée par ECOCERT Greenlife selon le référentiel Ecocert des Cosmétiques Ecologiques et Biologiques disponible sur http://cosmetiques.ecocert.com/
Raw material certified by ECOCERT Greenlife according to Natural & Organic Cosmetics Ecocert's standards available on http://cosmetiques.ecocert.com/

Accepté
Accepted

Visa contrôle qualité — D. GORACY
Visa resp. labo. analyses — A. SEVESTRE

Version 9 du 12/03/2018
Date de libération / Delivred date : 19/06/2019

Attachment 5 GREENTECH Certificate of analysis for a Ginger extract

BIOTECHNOLOGIES
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FICHE DE SPÉCIFICATIONS / SPECIFICATIONS DATA SHEET

OIGNON BIOGREEN (SB)
ONION BIOGREEN (SB)

Réf. : **400833**

DLUO / Shelf life : 24 mois / months
Stockage : +15 / +25°C
Conservation dans le contenant d'origine, sans ouverture, dans les conditions de stockage préconisées.
Keep in the original packaging, without opening, in the recommended storage conditions.
Conseil avant utilisation / Advice before use : Agitation

Taux de plante mis en œuvre à l'extraction / Plant rate implementation extraction : ~10 %

COMPOSITION	Matières premières / Raw materials	Solvants
Oignon / Onion *(Allium cepa)*	Bulbe / Bulb	Glycérine / Glycerin
Benzoate de sodium / Sodium benzoate		Eau / Water
Sorbate de potassium / Potassium sorbate		
Acide citrique / Citric acid		

	Proceedings	Specifications
CARACTÉRISTIQUES ORGANOLEPTIQUES / ORGANOLEPTIC CHARACTERISTICS		
Aspect Sight	GT001	Liquide Liquid
Couleur Color	GT002	Incolore à jaune Colorless to yellow
Odeur Odor	GT003	Caractéristique Characteristic
CARACTÉRISTIQUES PHYSICO-CHIMIQUES / PHYSICAL & CHEMICAL CHARACTERISTICS		
Solubilité 10% dans l'eau Solubility 10% in water	GT004	Soluble
pH (direct)	GT005	3.4 - 4.0
Indice de réfraction Refraction index	GT007	1.350 - 1.380
Densité Specific gravity	GT008	1.020 - 1.100
ANALYSES MICROBIOLOGIQUES / MICROBIOLOGICAL ANALYSIS		
Dénombrement des bactéries aérobies mésophiles Enumeration of mesophilic aerobic bacteria	ISO 21149	≤ 100 ufc/mL - cfu/mL
Dénombrement des levures et moisissures Enumeration of yeasts and moulds	ISO 16212	≤ 100 ufc/mL - cfu/mL
Détection des enterobactéries Detection of enterobacteria	ISO 18415	Absence

Version provisoire n°1 du 02/03/2018 1/1

Attachment 6 GREENTECH Certificate of analysis for an Onion extract

8. LIST OF SYMBOLS AND ABBREVIATIONS

1. VOCs – volatile organic compounds
2. SPME – solid-phase microextraction
3. GC – gas chromatography
4. TOF – time of flight
5. MS – mass spectrometry
6. e.g. – for example
7. etc. – and so forth
8. ℃ – the degree Celsius
9. DVB – divinylbenzene
10. CAR – carboxen
11. PDMS – polydimethylsiloxane
12. Rt – retention time