FACULTY OF PHARMACY OF THE UNIVERSITY OF PORTO

Stability study of flavoring agents in different oral pharmaceutical vehicles

MASTER IN QUALITY CONTROL

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ABSTRACT

Flavoring agents are added substances that make it possible to give an original flavor and/or smell to a food, drink, or medicine, improving or modifying it, and that is felt mainly by the senses of taste and smell. The various flavoring agents are classified as natural, artificial, or their combinations, and have different chemical structures, including esters, acids, alcohols, aldehydes, and volatile complexes, among others. The useof flavoring agents is very important for the food, veterinary, cosmetic, and pharmaceutical industries to enhance or mask certain sensory characteristics.

The incorporation of flavoring agents into pharmaceutical formulations is challenging due to stability issues, but it is an essential strategy to improve adherence to oral pharmacological treatments. Based on this, the present dissertation aimed to study the stability of flavored vehicles at room temperature, under refrigeratedand after forced degradation, aided by rheological parameters (viscosity, amplitude, frequency, and thixotropy), pH, and the released volatiles evaluated by SPME-GC-MS. After the previous characterization of diverse vehicles and flavoring agents, the selected vehicles were simple syrup, Syrspend®, and Suspendit®. As to the flavoring agents, andbased on the richness in their composition, banana and mint were selected from a groupthat also included orange and marshmallow.

During the 90 days of analysis, the flavored vehicles remained stable with respect to rheological parameters, pH, and organoleptic characteristics. for both conditions (room temperature and refrigerated) and for both flavoring agents, The only relevant modification was a decrease of consistency index (from Herschel-Bulkley model) after the incorporation of both flavoring agents in Suspendit®. Regarding the relative abundance of the aroma compounds released from the flavored vehicles, Suspendit® showed more stable results at room temperature. Under refrigeration, both Syrspend® and Suspendit® behaved well with banana flavor but mint volatiles were more stable with Syrspend®. As for the forced degradation banana flavoring showed higher stability with Suspendit® while mint flavor was more stable with Syrspend®.

The present work allowed us to prove that the Syrspend® and Suspendit® vehicles when incorporating flavoring agents, are more stable in comparison to the simple syrup vehicle. Additionally, it presents the SPME-GC-MS technique as an interesting green analytical approach to understanding the potential variations in aroma perceptions when applied to different pharmaceutical vehicles.

Key-words: Flavoring agent; Oral vehicles; Mint; Banana; GC-MS/SPME, Rheology

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Abbreviations and acronyms

APIs	Active Pharmaceutical Ingredients
FDA	Food And Drug Administration
GC-MS	Gas Chromatography-Mass Spectrometry
HPLC	High-Pressure Liquid Chromatography
ICH	International Conference on Harmonization

Introduction

1.1 Flavoring agents

Several definitions of flavor have been proposed. According to the Food and Drug Administration (FDA) and the European Council (EU), flavoring agents are substances added with the purpose to give an original taste and/or smell to foods, drinks or medicine, enhancing or modifying them (1, 2). The Society of Flavor Chemists identifies a flavoring agent as a substance that can be a single chemical entity or a mixture of chemicals of natural or synthetic origin whose primary purpose is to provide all or part of the taste/smell to any food or other product ingested through mouth (3, 4).

The mixed sensation of taste(s), touch (texture, hardness, viscosity, temperature, density, consistency, etc.), smell (smell or odor), vision (shape, color, appearance, size, contrast, aspect, etc.) and hearing (crunchy sounds, softness, hardness, etc.), involves a combination of physicochemical and physiological actions, which combine to produce an infinite number of graduations in the perception of a product (5, 6).

Flavoring agents are very diverse regarding their chemical complexity. In this class of additives, many substances can be found, *e.g.,* some products can naturally have more than a thousand substances that, together, give a characteristic aroma. The odor of fruits, flowers and other natural products frequently used with flavoring purposes is the combined result of hundreds of volatile substances (7). Coffee is a good example: roasted coffee has such a complex aroma that it has been possible to identify more than a thousand components in its constitution. Other examples that can be mentioned are honey, apples and strawberries. Honey has an aroma made up of more than 200 individual aromas. Apple has more than 130 individual, volatile components responsible for its aroma, and strawberries about 100 substances (7).

The perception of flavoring agents is possible due to the joint action of the senses of flavor, which is the result of a combination of taste, smell and trigeminal perception. The sense of vision has also a contribution. The gustatory and olfactory perceptions are the only senses in the human body that present chemical receptor cells (chemoreceptors), and for this reason, they are considered special senses (8). The receptor cells for the sense of smell are located in the nasal cavity in the region of the olfactory bulb (8) and the receptor cells for the sense of taste are distributed in the oral cavity and spread mainly on the tongue(9).

There are two fundamental elements to the perception of flavor. The tongue and the saliva. The tongue, has 4 large groups of taste buds, or taste corpuscles, responsible for its rough texture, that can be fungiform (mushroom-shaped), filiform (small pointed lumps

disseminated in large numbers throughout almost the entire upper extension of the tongue), foliate and calciform/circumvallate (located behind, on the upper surface of the tongue (Figure 1). These taste buds have receptors which can be classified as surface proteins or ion channels, capable of sending electrical stimuli which are transformed into neurotransmission to the brain, translating into salty, sweet, bitter, sour and umami tastes. The saliva is also relevant for the perception of flavor, and during the chewing process, odorant particles are released in the retronasal region (olfactory sensation) (Figure 2), which the stimuli will also be sent to the brain, where it will distinguish thousands of different odors, even if they are in low concentrations (8, 10).

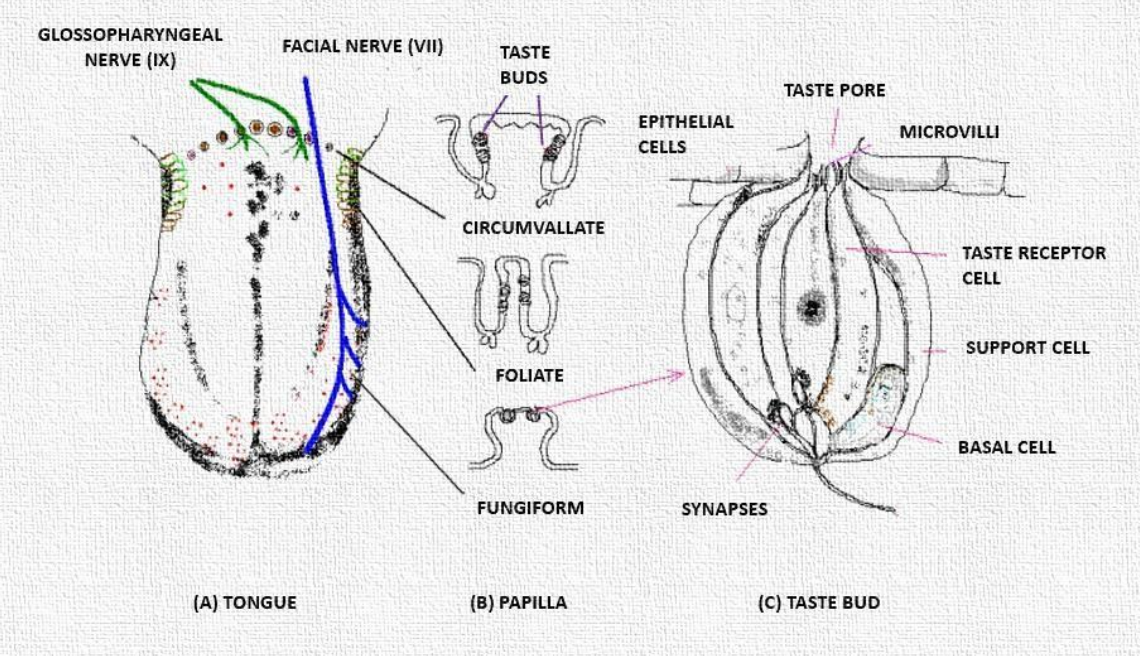


Figure 1. *Peripheral gustatory system (11).*

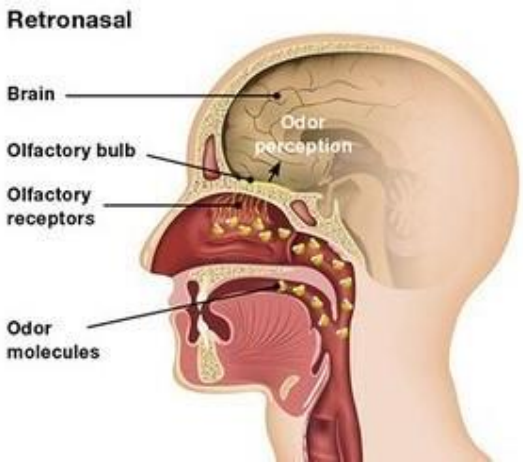


Figure 2. *Retronasal Olfaction (12)*

Sweet and salty flavors are considered nutritious flavors, as they reflect the identification of potential nutrients, while bitter and sour flavors are considered antagonistic flavors and reflect the rejection of toxic components (13).

1.1.1 Historical perspective and classification of flavoring agents

The history of flavor and fragrances started in ancient times, when people discovered that characteristic components of the aroma of natural products, such as the presence of spices and resins of animal and vegetable origin, could be obtained from simple processes to enrich the taste/smell of a product. Some methods used for oil extraction and distillation have endured for more than two thousand years. There is a considerable range of variations when it comes to fragrance and flavor materials, which can vary from simple chemicals to much more convoluted mixtures (14).

The Arabs, in the 9th century AD, improved distillation techniques. The production and application of blends were unchanged for centuries. Systematic progress began in the 13th century, when the preparation of medicinal oils by pharmacies began. Further on, the properties and effects of these oils were recorded in the pharmacopoeias. Many of the essential oils used today by perfumers and flavorists were originally used and developed by distillation in the 16th and 17th centuries (14).

The middle of the 19th century was another important stage in the history of flavor and fragrances. The production of essential oils started to be industrialized due to the increase in demand. This progress resulted in the isolation of cinnamaldehyde from cinnamon oil by Dumas and Peligot in 1834, and the isolation of benzaldehyde from bitter almond oil by Liebig and Wohler in 1837. Between 1845 and 1850 the first synthetic aromatic oils were introduced and these consisted of low molecular weight fatty acid esters of various alcohols that were synthesized by the chemical industry for presenting a fruity odor, such as methyl salicylate, extracted from wintergreen oil, which was obtained in 1859. Development of vanillin from coniferin in 1874 and coumarin in 1878 by Haarmann and Reimer was responsible for the foundation of the first factory in the world where synthetic flavors were produced (14, 15).

In the 1950s, the flavors were employed predominantly in the liquid form. They were made of natural extracts and essential oils. The first major paradigm change took place in the 1960s, as a result of a sum of simultaneous developments. The first synthetic components began to be used, at the same time that instrumental analysis and information technology started to influence the flavor and fragrance industry (15).

Today, new flavor chemicals continue to enter the market annually through biotechnological processes by biotransformation, which allows the generation of compounds recognized as natural by European and United States America legislation,

making this alternative even more attractive to industries. Besides the advantage of obtaining a product called 'natural', this methodology overcome limitations, such as the dependence on agriculture for the production of raw materials and the climatic and social setbacks added to this sector. It is also considered a more sustainable method with the possibility of translation on an industrial scale, obtaining more selective products (16, 17).

Based on the discoveries made from ancient times to the present day, it is possible to classify flavoring agents based on their production, as natural or synthetic.

According to, the Code of Federal Regulations 21CFR101.22 FDA flavors can be classified in four different categories, contingent on their constitution, as described below (1).

1.1.1.1 Natural flavors

A natural flavor is described by the FDA as "essential oil, oleoresin, essence or extractive, protein hydrolysate, distillate, or any roasting, heating or enzyme product, that is, obtained exclusively by physical, microbiological or enzymatic methods, which contains the constituents flavorings derived from a seasoning, such as those of floral origin (saffron and cloves), fruit or fruit juice (strawberry, vanilla, citrus), vegetable or vegetable juice, edible yeast, herb, seeds (mustard, nutmeg , anise), root (ginger, turmeric), leaf or similar plant material (oregano, thyme, bay leaf), bark (cinnamon), meat, seafood, poultry, eggs, dairy products or fermentation products, whose significant role in food is flavoring instead of nutrition." (1).

When it comes to natural flavors, it is necessary to obtain the aroma chemicals from the source material. For example, if a natural strawberry flavor is desired, it must be obtained from a fruit, leaf or stem of a strawberry plant. Fruit flavors can be obtained through vacuum concentration, extraction methods that may involve extrusion, solvent extraction, distillation or fermentation (16,18).

The flavors of highest quality are usually natural, but their design is troublesome due to the natural origin. They are not commonly employed in drug products and their low stability is another hindrance to their wide application. (1, 18).

1.1.1.2. Natural with Other Natural Flavors

The flavors can be extracted from natural sources and mixed with other natural flavors. Because of these blends, they can also have a more unique flavor profile and can be cheaper to produce than their natural counterparts. As an example, the case of strawberries being mixed with other flavors (1, 18).

1.1.1.3. Artificial flavors

Artificial flavors result from the production of flavors similar to natural ones. What sets them apart from natural flavors is that they feel the same, but are obtained through synthetic processes; and present high purity (1,18) and thus they have consistent quality during processing, which leads to greater durability. For this reason, they are commonly used in pharmaceuticals drug products.

1.1.1.4. Natural and Artificial Flavors

Natural and artificial flavors when combined, with a greater proportion of artificial flavors than natural ones, ensure a higher flavor quality in comparison with artificial flavors used alone. The cost of these flavors is lower than of the natural ones (WONF). As they guarantee greater stability, they are commonly used in pharmaceutical preparations (1, 18).

1.1.2 Importance and use of flavoring agents

Flavoring agents are widely used by food, veterinary, cosmetic and pharmaceutical industries.

Palatability influences food/beverage choice. The food and beverage industry has sought to add better nutritional quality to its products, as well as a broad exploration of their sensory and organoleptic properties (19 – 21). Thus, researchers have increasingly sought to understand the mechanisms involved with the multisensory perception of flavor since this relationship affects both the personal choice of an individual and the ingestion of a particular food (21, 22).

The use of flavoring agents in the veterinary industry is very similar to the use of medicines for pediatric use with respect to the purpose of improving the medication adherence. Typically, oral dosage forms developed for animals are composed of a flavor enhancing agent. Yeast-based or meat-based flavors are more common for developing the drug product. Adding just one flavor may not always be suitable to all animal species due to different flavor perception. But in the case of dogs, the simple addition of flavor has been shown to lead to an 80% acceptance rate (23).

In the cosmetic industry, organoleptic agents such as flavoring agents constitute an important category of ingredients. The main purpose of adding a flavoring agent is to make the cosmetic product more attractive to consumers. Their use is essential for the manufacture of cosmetics that come into contact with the oral cavity such as lipsticks and toothpastes. The flavor used will depend on the age of the consumer among other variables. For example, children have a greater preference for fruity and sweet flavors than adult consumers (24).

For the pharmaceutical industry, the main objective of adding an organoleptic agent in oral/buccal formulations, such as tablets, orodispersible tablets, solutions or suspensions, is to make the pharmaceutical product more attractive to patients and, thus, increase adherence to dosage regimens.

The pharmacological treatment often relies on the administration of the drug in dosage forms suitable for specific groups of patients, such as the elderly and children, for whom medications in liquid pharmaceutical form or in the form of reconstitution powder are more suited. However, oral liquid forms present three major difficulties: the drug solubility, stability of the formulation and taste masking effectiveness. Solubilization enhances the taste of the drugs, which can make the patient resistant to ingesting the drug product. Therefore, it is important to use flavoring agents in the liquid formulations so that the flavor becomes more pleasant and adherence to the treatment is improved (25). Typically, flavoring agents are fruity and sweet for children and based on mint for the elderly.

For example, the pediatric population has sensitive sensory systems, as they have a greater amount of taste buds compared to adults and therefore can detect tastes and smells more sensitively, and if tastes and smells do not suit their preference, they immediately reject it. Therefore, the use of flavoring agents is generally recommended in pediatric formulations (24). For the selection and proposal of a formulation for children´s use, parameters such as children´s palatability, food restriction, suitability of sweeteners and flavorings, and pharmacokinetic compatibility have to be considered.

With the expansion of technology in the flavor industry, many artificial flavors were created. Cough syrups, laxatives, sedatives, antihistamines, antibiotics, vitamins, and pediatric and geriatric formulations in general are now available in a variety of flavors that mask unpleasant tastes without affecting physical and chemical stability (5).

As an example of the diversity of flavoring agents, a study carried out in Brazil analyzed flavorings that are used in the sensory correction of cannabis extract for pediatric use (26). Cannabis oil extract has an unattractive taste since it is bitter and oily. The study showed that the preferable flavorings to mask the bitter taste were chocolate, chocolate + mint, mint, orange, lemon, cherry and raspberry, while mint and cinnamon could be used to mask the oily taste.

1.1.3 Flavoring agents used in pharmaceutical formulations

Medicines entering the oral cavity, whether administered orally, sublingually, or inhaled, must have an acceptable flavor. One of the main barriers preventing patients from complying with treatment is the unpleasant taste of the active pharmaceutical ingredients in these formulations. Therefore, it is necessary to flavor the pharmaceutical formulation to ensure good adherence to drug treatment by the patient, especially if the formulation is

intended for pediatric or geriatric use, since for these patients, oral medications in solid dosage form, for example tablets, present greater swallowing difficulty.

Several criteria are used to select flavors for a pharmaceutical preparation. Different flavor concentrations produce highly subjective sensations. Specific balance requirements depend, in part, on the drug substance and the physical form of the product, as there are several variables such as product texture (e.g., formulation viscosity), water content or vehicle/excipients. In addition, the patient's preference regarding aspects such as color and odor must be taken into account, as they also influence the acceptability of a pharmaceutical preparation by the patient. Also, it is important to verify a possible allergic sensitivity to a certain flavoring agent (27). The bitter taste is the most common since the number of bitter compounds exceeds the compounds that exhibit a sweet taste (28). The main flavorings used for masking the bitter taste include chocolate, mint (menthol), lemon (citral aldehyde and limonene), orange (d-limonene), cherry and raspberry. For the sweet taste, vanilla, tutti-frutti, grape, strawberry and raspberry are often used. For the sour taste preference is given to lemon, orange, cherry, raspberry while to mask salty taste raspberry, cherry syrup and chocolate syrup are prefered. When the taste is a mixture of saline and bitter, orange syrup is usually used. Mint is widely used for the oily taste while for metallic strawberry, raspberry, cherry and grape are preferred (27).

Some of the most common flavoring agents for pharmaceutical formulations are described below in more detail.

Mint essential oil - is used as a flavoring in antacids, tablets and chewable gums. It has a desensitizing effect on the taste, making it useful for masking bitterness. It is obtained from *Mentha piperita*. Its composition includes menthol, menthyl acetate and other volatile compounds. It is usually used in concentrations between 0.1% to 0.3% (27, 29).

Lemon oil – is a volatile oil, obtained from the bark of the hole *Citrus limon* (Linnaeus) *Burmann filius* (Fam. Rutaceae). It is composed of citral aldehyde (4%), d-limonene (more than 70%), among other compounds such as α-pinene (1–2%), β-pinene (6–13%), sabinene (1–2%) and γ -terpinene (8–10%) (15).

Orange essential oil – it's mainly used in elixirs and other preparations. It is a volatile oil obtained from the rind of the fruit *Citrus sinensis* (Linnaeus) Osbeck (Fam. Ruteaceae) and has in its composition d-limonene (90%) and 5% to 10% of other volatile constituents. It is usually used in concentrations between 0.1% to 0.3% (27, 29).

Cherry essence - There are two types of cherry. Sweet (*Prunus avium*) and acidic (*Prunus cerasus*). Sweet cherry contains many volatile compounds such as benzaldehyde, (E) – 2 – hexanal and hexanal and sour cherry also has benzaldehyde, being the most important benzyl alcohol, eugenol and vanillin (15).

Strawberry essence – It is related to the group called red fruits, as is the raspberry. It is predominantly composed of esters (25 to 90%), alcohols, ketones and aldehydes. The most important aromas in strawberry are methyl butanoate, methyl hexanoate, ethyl hexanoate, furaneol, linalool, among others (15).

Vanilla – Produced from Andrews *Vanilla Planifolia* (Orchidaceae). They are base flavors for masking unpleasant flavors and odors of some active ingredients such as caffeine and some vitamins. Widely used in food, beverage and cosmetics. And they can be used in formulations of tablets, syrups, solutions and powders (15, 29).

In addition to these compounds, it is possible to classify as flavoring agents, for example:

Aniseed Oil - Often used for the correction of bitter and oily preparations. It is a volatile distilled oil obtained from *Pimpinella anisum*, Linnaeus. It has in its composition anethole, about 80 to 90%, methyl chavicol, and anisic acetone.

Cinnamon essential oil - for oily, salty, bitter and dental preparations. It is a volatile distilled oil obtained from *Cinnamonum cassia* (Linnaeus). It has in its composition 80% of total aldehydes, mainly cinnamaldehyde.

Clove essential oil - for dental preparations. It is an essential oil distilled from *Eugenia caryoohyllus* flower buds. Its composition is composed of 85 to 90% eugenol.

Methyl salicylate - used as flavoring in small concentrations and local analgesic. It is an oil obtained synthetically or from the leaves of *Gaultheria procumbens* and from the bark of *Betula slow*.

Maltol - its application and use are intended for flavoring agents and flavor enhancers in pharmaceutical and food products. It enhances the sweet notes of the preparation. And in diluted solutions, it gives the preparation a flavor and odor related to pineapple and strawberry. In addition to these, there are several other flavor correctors (29).

Recommended flavoring agents can also vary with the drug therapeutic classes (Table 1).

Table 1. *Recommended flavors according to the therapeutic class (25, 27)*

DRUG CLASSES	FLAVORS
ANTIBIOTICS	Cherry, pineapple, orange, raspberry, strawberry with vanilla, tutti-frutti, banana with pineapple, banana with vanilla, caramel, coconut cream, etc.
ANTIHISTAMINE	Cherry, orange with peach, raspberry, vanilla, chocolate, apricot, cinnamon, grape, honey, lemon.
ANALGESIC/ANTIPYRETICS	Blackberry, lemon, orange, menthol, strawberry, etc.
DECONGESTANTS AND EXPECTORANTS	Cherry, mint with strawberry, strawberry, orange, pineapple, raspberry, fennel, apricot, caramel, coconut cream, blackcurrant, cilantro, etc.
ANTI-INFLAMMATORY	Cherry, grape, raspberry, orange, lemon, etc.

A pool of oral medicines that are present in the Portuguese market and respective flavors were analyzed based on data described in INFOMED (database of medicinal products for human use) as detailed in Table 2. (30).

Table 2. *Examples of oral medicines present in the market and their respective flavors (30)*

THERAPEUTIC CLASS	ACTIVE SUBSTANCE	DRUG PRODUCT COMMERCIAL NAME	PHARMACEUTICAL FORM	FLAVOR
Antibiotic	Amoxicillin	Amoxicilina Sandoz 250mg/5ml	Powder for oral suspension	Lemon, Peach, Apricot, Orange
		Amoxicilina Sandoz 500mg/5ml	Powder for oral suspension	
		Amoxicilina Generis 250mg/5ml	Powder for oral suspension	Peach
		Amoxicilina Generis 500mg/5ml	Powder for oral suspension	Strawberry
		Amoxicilina Labesfal 250ml/5ml	Powder for oral suspension	Tangerine
		Amoxicilina Aurobindo 375/500/750mg	Dispersible tablet	peach and orange
		Amoxicilina toLife 1000mg	Dispersible tablet	Strawberry
Antihistamine	Hydroxyzine	Atarax 2mg/ml	Syrup	Hazelnut
	Desloratadine	Desloratadina Abdi 0,5mg/ml	Syrup	Tutti Frutti
		Desloratadina Aristo 0,5mg/ml	Oral solution	Chewing gum
		Desloratadina Azevedos 0,5mg/ml	Oral solution	Strawberry
		Desloratadina Alter^{MG} 5mg	Orodispersible tablet	Strawberry

Table 2 (cont). *Examples of medicines present in the market and their respective flavors (30)*

THERAPEUTIC CLASS	ACTIVE SUBSTANCE	DRUG PRODUCT NAME	PHARMACEUTICAL FORM	FLAVOR
Antihistamine	Desloratadine	Desloratadina Azevedos 2,5/5mg	Orodispersible tablet	Tutti Frutti
Analgesic / Antipyretic	Ibuprofen	Brufen 200-400-600mg	Effervescent granules	Orange
		Brufen no sugar 20-40mg/ml	Oral suspension	Orange
		Ib-u-ron 20-40mg/ml	Oral suspension	Strawberry
		Ibuprofeno Algik 20mg/ml	Oral suspension	Strawberry
		Ibuprofeno Farmoz 20-40mg/ml	Oral suspension	Orange
		Ibuprofeno Generis 20mg/ml	Oral suspension	Tutti Frutti
		Danofen 200-400mg/10ml	Oral suspension in sachet	Orange
		Danofen 600mg/15ml	Oral suspension in sachet	Strawberry
		Dolomate 200mg	Powder for oral suspension	Lemon
		Ibuprofeno Codramol 200mg	Granules for oral solution	mint
		Neurofen 100mg	Soft capsule to chew	Orange
		Kifen 200mg	Dispersible tablet	Orange
		Trifene dispersible 200mg	Dispersible tablet	Orange
		Trifene® effervescent 200mg	Effervescent tablet	Orange

Table 2 (cont). *Examples of medicines present in the market and their respective flavors (30)*

THERAPEUTIC CLASS	ACTIVE SUBSTANCE	DRUG PRODUCT NAME	PHARMACEUTICAL FORM	FLAVOR
Decongestants	Xylometazoline	Vibrocil Actilong menthol 1mg/ml	Nose drops and nebulized inhalation solution	Eucalyptol
Expectorant	Ambroxol	Broncosil 3-6mg/ml	Syrup	Forest fruits and vanilla
		Ambroxol Generis 3mg/ml	Syrup	Strawberry
		Muscosolvan 6mg/ml	Syrup	Strawberry and vanilla
		Broncoliber 3-6mg/ml	Syrup	Pineapple
		Drenoxol 30mg/10ml e 3mg/ml	Syrup	Wild strawberry
		Drenoxol 6mg/ml	Syrup	Pineapple
		Benflux 3mg/ml	Syrup	Lemon and gooseberry
	Bromhexine	Bromexina Tissulene 1,6mg/ml	Syrup	Tangerine
		Bromexina Farmoz 0,8mg/ml	Syrup	Cherry
		Lisomucin	Solution drops (2mg/ml) and syrup (1.6mg/ml)	Raspberry
Anti-inflammatory	Nimesulide	Aulin 100mg	Granules for oral suspension	Orange
		Nimed 100mg	Granules for oral solution	Orange
		Nimesulida Basi 100mg	Granules for oral suspension	Orange
	Acetylsalicylic acid	Aspirina Direkt 500mg	Granulate	Orange and Cola

According to the analyzed medicines (n=48), that the flavoring agent with the highest usage frequency was orange (Figure 7), with 14 formulations which represents 29% of the total sample, followed by strawberry (9), which represents 19%, tutti-frutti and peach, both with 3 (6%), lemon, tangerine, vanilla and pineapple with 2 (4%). The remaining flavoring agents were used in 1 of the oral formulations studied, representing 2% of each in the total sample.

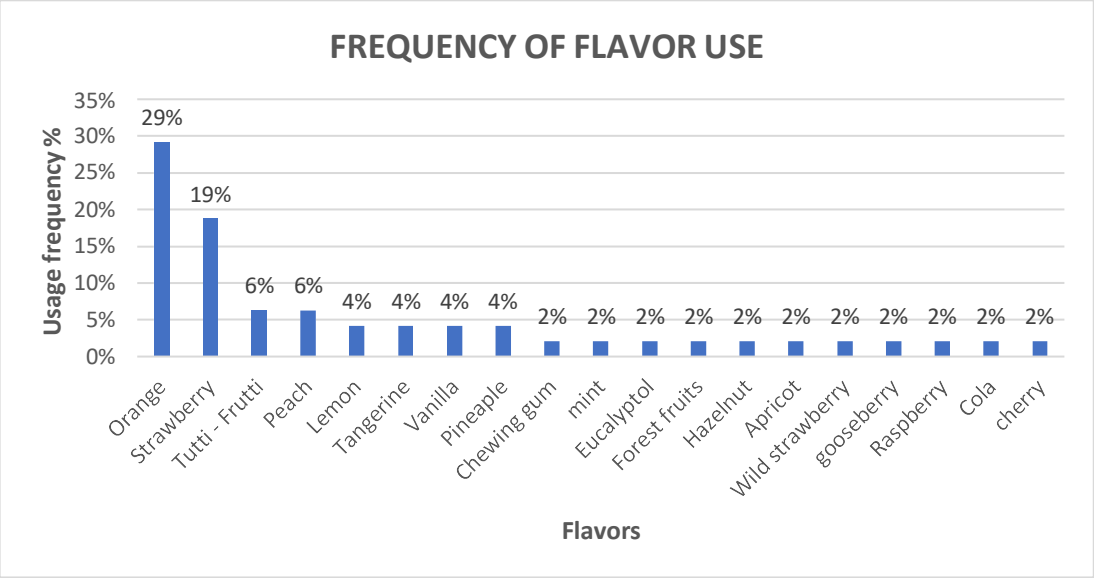


Figure 3. *Relative frequency of use of flavors in marketed oral pharmaceutical formulations (30).*

1.2 Vehicles for oral compounding formulations

A pharmaceutical dosage form is the final state drugs are presented in order to facilitate their administration, and to maximize the therapeutic effect (31). When it comes to liquid preparations, the carrier of the active substances is known as the vehicle and consists of one or more excipients (32).

Liquid pharmaceutical forms for oral use expand treatment options for patients who have difficulty swallowing tablets or capsules. Specific populations that may benefit from the composition of these dosage forms include pediatric patients, geriatric patients, and patients with enteral feeding tubes (33).

Several oral vehicles are available and these products differ in their physicochemical and organoleptic properties. These vehicles can be used to prepare a variety of oral liquid dosage forms, and the commonly used forms are solutions, suspensions, emulsions (34 - 37).

Some preparations for oral use are prepared by dilution of concentrated liquid preparations, or of powders or granules for the preparation of oral solutions or suspensions, for oral drops or for syrups, using a suitable vehicle (38).

The vehicle for any preparations for oral use is chosen taking into account the nature of the active substance(s) and to provide organoleptic characteristics suitable for the intended use of the preparation, since when ingested these pharmaceutical forms for oral use arouse a range of olfactory-gustatory, texture and visual sensations.

Several categories of preparations can be distinguished (38):

- Oral solutions, emulsions and suspensions
- Powders and granules for oral solutions and suspensions;
- Oral drops;
- Powders for oral drops;
- Syrups;
- Powders and granules for syrups.

Solutions are liquid preparations that contain one or more chemical substances dissolved, that is, molecularly dispersed, in a suitable solvent or in a miscible mixture of solvents. They can be intended for oral, topical, or parenteral use. One of their advantages is that the active components conveyed in this form are more rapidly absorbed by the gastrointestinal tract and there is homogeneity in dosage regardless of agitation. On the other hand, one of its disadvantages is its lower physicochemical and microbiological stability (39).

Emulsions are liquid preparations that contain small particles of one liquid dispersed in another liquid. Generally, emulsions involve the dispersion of water in oil, and whenever this pharmaceutical form is to be administered there is a prior need for agitation. This pharmaceutical form is indicated for drugs that are unstable in aqueous formulations. Physical stability challenges of emulsions include creaming (weak associations between drops of the internal phase) and cracking (irreversible coalescence of drops of the internal phase). And to improve the stability of emulsions it is necessary to add viscosity regulators and thickening agents (40).

Suspensions are preparations in which the drug is not completely dissolved in the liquid medium. They usually settle to the bottom of the container. But they should have an easy resuspension after slight agitation. The ideal for suspensions is to obtain a uniform average particle size, since larger diameter particles tend to sediment faster and smaller particles tend to aggregate and form agglomerates that are difficult to resuspend, this phenomenon is called "caking" (41).

Powders and granules for the preparation of oral solution or suspensions generally conform to the definitions in the monographs on oral powder <1165> or granules <499>, as appropriate. They may contain excipients, in particular to facilitate dispersion or dissolution and to prevent caking (38).

Oral drops are solutions, emulsions or suspensions that are administered in small volumes such as drops by means of a suitable device. If powders are added for the preparation of oral drops, generally, these formulations may contain excipients to facilitate dissolution or suspension in the prescribed liquid or to prevent caking. After dissolution or suspension, they meet the requirements for oral drops (38).

Syrups are aqueous pharmaceutical forms characterized by high viscosity. They present in their composition about 64% to 66,7% (w/w) of sucrose or other sugars, and may contain one or more chemical substances. They are well preserved because they are hypertonic solutions, thus ensuring the dehydration of microorganisms. They are generally used for substances with a very unpleasant taste. If the syrup makes use in its formulation of powders and granules it is generally necessary to contain excipients to facilitate dispersion or dissolution and to avoid caking (38).

In relation to the organoleptic properties of liquid formulations for oral use, it is essential that they have a pleasant taste. For, as cited by some authors: "The fulfillment of a prescription is directly related to the pleasant taste or not" (34, 36). When there are unpleasant tasting formulations, generally, they force the patient to mix them with other products, especially food, in order to ingest them, which may decrease the absorption or cancel the effect of the drug. Thus, to improve the taste of many drugs, correctives are used, such as sweeteners and flavorings, alone or in combination with each other, for example (42).

There are a number of commercially available oral liquid vehicles, that are used to prepare oral compounded formulations. They usually provide greater drug stability since are buffered to a slightly acidic pH (pH ~ 4.2). This slightly acidic pH provides a reduction in the degradation of the drugs through oxidation. In addition, these vehicles contain effective preservatives, (40,43). A summary of the features of commonly used commercial oral vehicles is presented in table 3 (44,45):

Table 3 *Summary of the features of commonly used commercial oral vehicles*

Vehicle	Manufacturer	pH	Taste	Appearance	Further information
OraPlus®	Perrigo	4.0 to 4.5	Unflavored	Translucid, milky-white	Oral suspension that can be diluted up to 50% or more with other ingredients and still retain appropriate properties as a suspending agent.
Ora Sweet syrup®	Perrigo	~4.3	Sweet citrus-berry flavor	Clear liquid with a slight tint	Solution for extemporaneous oral preparations.
Ora Sweet SF®	Perrigo	4.0 to 4.4	Sweet citrus-berry flavor	Clear liquid with a slight tint	Sugar-free version
Syrspend®	Fagron	4.0 to 5	Unflavored	Hazy, white, translucid syrup	Provide adequate sedimentation rates, same pouring capacity and good resuspension.
Suspendit®	PCCA	NA	Unflavored	Viscous white opaque liquid	Provide adequate sedimentation rates, same pouring capacity and good resuspension.
Simple syrup	Portuguese galenical form	NA	Unflavored	clear, viscous, colorless or pale-yellow solution	-
Vehicle for preparation of oral suspension - sugar free (FGP B.9)	Portuguese galenical formulary	5.0 to 7	Unflavored	Opaque liquid, white in color and homogenous appearance	-
Vehicle for preparing oral solutions and suspensions (FGP B.12.)	Portuguese galenical form	5 to 6.0	Unflavored	Colorless and translucent liquid; homogeneous appearance	-
Oral suspend®	Medisca	4.0 to 5.0	Unflavored	Viscous off-white aqueous liquid	-

1.3 Quality control of pharmaceutical excipients and formulations

According to ISO 9000:2015, quality control is the part of quality management focused on meeting quality requirements, in other words. quality control has the responsibility to verify that the quality of products/services is in accordance with technical specifications, the analysis is carried out from raw materials to the finished product (46)

In the flavor industry, the main objectives of quality control are to determine the main flavor impact compounds, in order to maintain a consistent flavor profile, as well as to identify unwanted flavors that occasionally appear, and to determine purity, adulteration, spoilage, authenticity, among others.

The quality control of aromas, as well as their raw materials, is a very complex area and the quality control laboratories in the aroma industry encompasses chemical, instrumental, microbiological and biological areas, and can use more than 500 analytical procedures. All raw materials used, all intermediate products, as well as all flavors that are delivered to customers, must be controlled by physicochemical, biotechnological (enzymatic or immunological), sensory and, if necessary, microbiological methods (15, 47).

Classical methods prioritized basic attributes such as coloring and clarity. Other quality parameters include specific gravity, optical rotation, refractive index, melting point, among others. These techniques are used to determine the authenticity of flavoring materials. Currently, there are a range of instrumental techniques available that are employed to identify and quantify individual chemical substances. Especially for quick identification verification purposes, spectroscopic methods such as infrared and near infrared spectroscopy, nuclear magnetic resonance (NMR), ultraviolet spectroscopy, mass spectroscopy and atomic absorption spectroscopy are widely used. In addition, chromatographic procedures, such as gas chromatography, high-pressure liquid chromatography and capillary electrophoresis can be used. Modern tandem techniques such as gas chromatography-mass spectrometry (GC-MS), high-pressure liquid chromatography-mass spectrometry (HPLC-MS) and capillary electrophoresis- mass spectrometry (CE-MS) can be also used (48).

Sensory tests have also become extremely important tools in quality control as sensory characteristics (sight, taste, texture, smell and hearing) must be controlled to ensure product quality. In addition to quality control, sensory testing is also used for product development, product matching, shelf-life study, product reformulation and product acceptability. In addition to smell and taste, sensory assessment covers trigeminal impressions, e.g., hot, burning, and visual impressions such as coloration, opacity and particle size (48). In addition to sensory tests (descriptive tests), there are also instrumental analyses such as gas chromatography/olfactometry (GC-O) and electronic tongue that combine the information provided by chemical characterization and odor and taste perception respectively.

Comparatively, microbiological tests play a less significant role than physico-chemical methods when it comes to quality control in the flavor industry. Most liquid flavors contain solvents such as ethanol, propylene glycol or edible oils in a concentration of between 70 to 90% and thus have bacteriostatic or bactericidal properties (49). In the case of herbs and spices, one of the main sources for the aroma industry, it is necessary to carry out microbiological tests (total count, yeast and mold count, detection of coliform bacteria and E. coli, for example) during the entire preparation phase, from harvesting to storage

and transport of the raw material because the composition of microflora varies according to the part of the plant used (leaves, seeds, flowers, among others) (15).

The technique used will depend mainly on the information needed and also on the techniques available in the work environment. Physical state, volatility, solubility, and sample size are additional characteristics that may rule out some techniques (48).

1. 4 Stability evaluation

1.4.1 Stability of flavoring agents

As mentioned in the previous topic, stability represents the physical, chemical and sensory quality of a final product, throughout its life cycle under appropriate and defined conditions.

When using flavoring agents in pharmaceutical and cosmetic formulations, it is important to evaluate their stability profile, as they may interfere with the physical, chemical and microbial integrity of the formulation (50). The interference may be caused by storage conditions such as temperature, pH, oxygen, humidity, physical and interaction with the ingredients (51).

Some organoleptic agents are known to potentiate Active Pharmaceutical Ingredients (API) degradation, leading to the instability of the pharmaceutical product. For example, ethyl vanillin reacts with neomycin sulfate (antibiotic) or succinyl sulfate (antibacterial sulfonamide), resulting in a color change in the formulation, yellow discoloration. Spearmint oils (active flavoring agent: menthol) are incompatible with chloral hydrate (anxiolytic) formulations along with other flavoring agents containing thymol as the main flavoring component, as it degrades API and potentially causes formulation instability (52).

There is a great occurrence of API and flavoring agent interactions leading to instability since flavors are complex substances that are composed of many chemicals. For example, the natural cherry flavor contains more than 70 components and the artificial flavor has more than 20; natural banana flavor has more than 150 components and the artificial ones have more than 17; natural grape bristles have around 225 components and artificial ones have more than 18 (53).

Some flavoring agents are also unstable. For example, citrus flavors (orange, lemon, lemongrass) contain citral, that may show instabilities due to the α, β-unsaturated aldehyde that becomes quite susceptible to acid-catalyzed cyclization and oxidative degradation, particularly in the presence of light and heat. Vanilla flavor has an active component, vanillin, that is unstable due to three functional groups (aldehyde, phenolic hydroxyl and aromatic nucleus) and may undergo oxidative degradation at high

temperatures. Caramel, strawberry, raspberry, grape and pineapple flavors, contain furanone derivatives that may present instability due to the presence of air and in aqueous solutions. Their stability depends on pH and the occurrence of tautomerization. They are also very susceptible to elevated temperatures, leading to thermal degradation, and can undergo photo-oxidation due to ring opening after a free radical attack. For coriander, sweet basil and lavender, the main flavoring component is linolool and it may undergo auto-oxidation to form hydroxides, which are allergenic. Another example of widely used flavoring is the mint flavor, whose main components are menthol, terpenoids and menthofuran and instability may be due to the polymerization of these constituents leading to an increase in flavor viscosity (54).

Stability tests of organoleptic agents used in pharmaceutical formulations are performed in accordance with the excipient stability guidelines of the International Council of Pharmaceutical Excipients (IPEC). The IPEC Excipient Stability Guide is intended to provide excipient manufacturers with strategies to assess the stability of their excipients. This guideline recognizes that the stipulations in ICH Q1A (R2) are not suitable for excipients because ICH Q1A (R2) is intended for drugs, however, it is necessary recommended to use the conditions and recommendations of ICH Q1A (R2) for analysis of stability (55).

The guideline is primarily intended to provide evidence that the excipient will continue to meet specifications from the point at which manufacturing is completed (usually after packaging) to the point at which the packaging is opened. Stability of excipients ranges from very stable (60 months), stable (24 to <60 months) or with limited stability (<24 months) (55).

Manufacturers must be responsible for the quality of the products, ensuring that they are suitable for the purposes for which they are intended, complying with established safety, quality and efficacy requirements, based on analytical validation methodologies. Ensuring that the product meets the exact, adequate, reproducible and robust standards required under the Good Manufacturing Practices

1.4.2 Stability of pharmaceutical compounding formulations

The stability of a liquid formulation for oral use is based on the principle of the time over which the preparation retains, within specified limits, and during the period of storage and use, the same properties and characteristics as it had at the time of its preparation (42).

The stability of a pharmaceutical formulation is essential to define a period of time over which its quality, efficacy and safety are guaranteed (41).

The stability study provides data on how the physical, chemical and microbiological characteristics of the formulation vary over time under the influence of various

environmental factors, such as temperature, humidity and light. At the same time, it allows establishing the expiry or use by date and defining the storage conditions (41).

Chemical stability requires integrity and stated potency of the active ingredient. Hydrolysis and oxidation are the most common degradation reactions in oral formulations (56). However, it can be corrected with pH adjustment, elimination of oxygen from the pharmaceutical form, use of chelating agents, antioxidants and prevention of light exposure. Physical stability refers to the retention of the original physical properties, including appearance, palatability, uniformity, dissolution and viscosity (56). The microbiological stability of a liquid formulation is the result of resisting bacterial growth. The most common signs of instability are discoloration, turbidity or gas formation.

According to the Portuguese Galenic Form, the determination of the shelf life should be done in real time, under the conditions recommended for the conservation of each product after opening the package. For this purpose, there are general rules for the attribution of use-by dates to manipulated drugs. For these attributions, bibliographic research concerning their stability must be carried out beforehand and take into consideration the nature of the different raw materials, their degradation mechanism, the packaging used, the storage conditions and the expected duration of the treatment (57).

In the absence of data on the stability of a given medicinal product, the following shelf-life is recommended for manipulated non-sterile drugs (table 4), kept in tightly closed packaging, protected from light and at room temperature (57):

Table 4 *Expiry date evaluation for non-sterile manipulated drugs*

Non-aqueous liquid preparations and solid preparations	Where the origin of the active substance is an industrialized product, the period of use of the manipulated medicinal product shall be equal to 25% of the time remaining before the expiry of the shelf life of the industrialized product. If the period calculated in this way is longer than 6 months, a period of use of 6 months should be adopted. If it is an individualized raw material, the period of use shall not exceed 6 months.
Liquid preparations containing water a (prepared with active substances in solid state)	The period of use of the manipulated drug should not exceed 14 days, and it should be kept in the refrigerator.
Remaining of the preparations	The period of use of the manipulated medicinal product should correspond to the duration of treatment. If the treatment lasts longer than 30 days, a maximum period of use of 30 days should be adopted.

However, there are studies, such as those by Belayneh and Tessema on the systematic review of the stability of extemporaneous pediatric oral formulations using 28

articles as a basis says that most of the extemporaneous formulations were stored at temperatures of 4°C, 25°C and 40°C with and without light and that the duration of storage ranged from 24 hours to 150 days (58).

With this, it is possible to say that the storage conditions of pharmaceutical products, whether industrial or extemporaneous, for stability studies are planned according to the guidelines of the International Conference on Harmonization (ICH) and the Committee for Medicinal Products for Human Use (CHMP). According to these guidelines, stability studies must be conducted at least as long as the pharmaceutical product is in use. They also define the conditions under which stability studies must be conducted. Thus, in general cases, long-term stability studies, according to the ICH Q1A guideline in climatic area II where Portugal is included, must be conducted at a temperature of 25ºC±2ºC and 60%±5% relative humidity. The accelerated trial, on the other hand, is a method used to estimate the storage period of the product under conditions that accelerate degradation and the data is based on the relationship between the stock temperature and the degradation observed at high temperatures. For formulations being stored in the refrigerator, the study should be performed at 4°C ± 2°C (59).

1.4.3 Forced degradation studies

The forced degradation test or stress test is used to test the stability formulations, drugs and excipients, under extreme conditions, even more than those used for the accelerated stability study (60, 61). Forced degradation studies are also used to facilitate the development of an analytical methodology, providing conditions to gain a better understanding of formulation components and product stability during the development phase (62, 63). Also, they can provide information about the possible degradation reactions and products formed (64 – 66).

In summary, forced degradation studies are performed to be able to differentiate the degradation products that are related to the drug from those that are generated from excipients in a formulation (64, 67, 68). In addition, it is also possible to elucidate the structure of degradation products (64,68), determine the intrinsic stability of the formulation (68), elucidate the mechanisms of degradation, develop suitable analytical method to indicate stability, among others.

Forced degradation studies are described in several international guidelines. Guidelines published by the International Conference on Harmonization (ICH), have been discussed and approved by North American, European and Japanese regulatory authorities (69). These guidelines become essential for organizing forced degradation studies of new pharmaceutical products.

Forced degradation testing is usually performed early in the formulationdevelopment process where the formulation is subjected to a series of conditions, such ashydrolysis (acid-base), photostability, peroxide oxidation, and temperature (above the temperature for accelerated testing, i.e., greater than 50°C), in order to evaluate the resulting by-products and their possible degradation pathways, according to ICH guidelineQ1A(R2) (61, 65, 66).

FDA and ICH guidelines determine the forced degradation requirement to recognize how the quality of a drug substance and drug product varies with time and different environmental factors (70, 71).

Experimental part

2.1 Aims

This dissertation has the following aims:

- To characterize the rheological behavior of commercially available vehicles for oral compounding formulations;
- To characterize four commercial flavoring agents used in oral pharmaceutical formulations (orange, banana, mint and marshmallow);
- To study the stability of the oral flavored formulations prepared with Suspendit®, Syrspend and Simple syrup over a period of 90 days at room temperature and refrigerated conditions.
- To study the stability of the oral flavored formulations prepared with Suspendit®, Syrspend® and Simple syrup against forced degradation by high temperature

2.2 Materials and Methods

2.2.1 Materials

2.2.1.1 Flavorings agents

The banana flavoring (mixture of natural and synthetic flavoring) and orange flavoring, lot 215091-J-1 and 214456-J-1, respectively, was offered by Acofarma Distribuición, S. A. The mint cream and Marshmallow flavoring, lot C200300 and C200134, respectively, was supplied by PCCA. Regarding the physicochemical aspects, the banana essence presents a clear, oily liquid, with a color that can be colorless or yellowish, and has a characteristic odor (banana fruit); the orange flavoring has a yellow liquid with a characteristic citrus, orange odour; the mint cream has a clear liquid with greenish coloration and has a characteristic odor of mint and the marshmallow flavoring agent, which has an off-white to pale yellow liquid with a sweet, characteristic odor and taste.

2.2.1.2 Vehicles for oral compounding formulations

The commercial vehicles used for the preliminary study of vehicle choice were the following: Syrspend® vehicle (Fagron Ibérica, Barcelona - Spain), Suspendit® vehicle (PCCA, Houston - Texas), simple syrup vehicle, prepared according to the Portuguese Galenic Form (FGP), Ora plus® vehicle (Perrigo, Minneapolis – Minnesota - EUA), and oral suspension (Guinama, La Pobla de Vallbona - Spain). Their composition is indicated in the table below (Table 5).

Table 5. *Vehicles composition*

Vehicle Composition	**Simple syrup**	**Oral suspension (Guinama)**	**Ora Plus (Guinama)**	**Syrspend® SF PH4 (Fragon)**	**Suspendit® (PCCA)**	**Function**
Purified water	x	x	x	x	x	Solvent
Natural sweetener (monk fruit)					x	Sweetener
Sucrose	x					Sweetener
Sucralose				x		Sweetener
Microcrystalline cellulose		x	x			Thickener
Cellulose gum		x				Thickener
Sodium carboxymethyl cellulose			x			Thickener
Modified food starch				x		Thickener
Carrageenan		x	x			Thickener
Amorphophallus Konjac Root powder					x	Thickener
Citric acid		x	x	x	x	Acidifier
Malic acid				x		Acidifier
Sodium citrate				x		Buffer and chelating agent
Sodium Phosphate		x	x			Buffer and chelating agent
Trisodium Phosphate			x			Buffer and chelating agent
EDTA disodium					x	Chelating agent
Sodium benzoate < 0.1%				x	x	Preservative
Sodium Methylparaben		x				Preservative
Potassium sorbate		x	x		x	Preservative
Propylparaben		x				Preservative
Methylparaben			x			Preservative
Simethicone		x		x		Anti-foaming agent
Dimethicone Emulsion			x			Anti-foaming agent

2.2.2 Methods

2.2.2.1 Characterization of the flavorings

The characterization of the flavoring agents was done based on their technical specifications, on the literature and through analysis by gas chromatography coupled to mass spectrometer.

2.2.2.1.1 Gas Chromatography - Mass spectrometry (GC-MS)

Chromatography is first and foremost a physicochemical method of analysis that is widely employed both in the separation of chemical compounds and in the identification (qualitative analysis) and quantification (quantitative analysis) of the separated species. Chromatography is a separation technique that, when coupled with hyphenated techniques, becomes a very important tool in the investigation of chemical compounds. It is a physical method of separation in which the components to be separated are distributed between two phases: a stationary phase and a mobile phase, which moves in a defined direction.

Gas chromatography is a chromatographic separation technique based on the difference in species distribution between two non-miscible phases in which the mobile phase is a carrier gas (e.g., helium) that moves with or past the stationary phase (e.g., a silica capillary column) contained in a column. It is applicable to substances or their derivatives that are volatilized under the temperatures employed (38,72).

The injection can be performed at the top of the column, using a syringe or an injection valve, or in a vaporization chamber that can be equipped with a flow divider (splitter). Compounds introduced into the column that do not have much interaction with the stationary phase are first eluted by the carrier gas into the detector, and then those that have more affinity for the stationary phase are eluted out.

Flame ionization detectors are generally employed, but additional detectors such as electron capture, mass spectrometry (MS), thermal conductivity, Fourier transform infrared spectrophotometry, and others can be used depending on the purpose of the analysis.

In the case of ionization detectors with additional mass spectrometry detectors, they are based on the direct measurement of the ratio between the mass and the number of positive or negative elementary ion charges (m/z) in the gas phase obtained from the analyte. This ratio is expressed in units of atomic mass (1 a.m.u. = one-twelfth the mass of 12C) or in daltons (1 Da = the mass of the hydrogen atom) (38, 72).

The mass spectrometer is capable of providing valuable information about unknown compounds. Therefore, it has a wide field of application in industry and research, in several areas such as: Biotechnology (analysis of proteins, peptides, nucleotides, polymers); Pharmaceutical (quality control of drugs, drug metabolism); Clinical (drugs, compounds in

blood and urine); Environmental (degradation by-products, pesticides, volatile organics for water and air quality control); Food (volatiles in beverages, contaminants in food), among many other areas of application (38, 72).

The main components of the instrumentation system for this combination are: the carrier gas introduction system, flow regulator, injector, column, oven, ionization source, mass analyzer, detector and the data acquisition system (Figure 4)

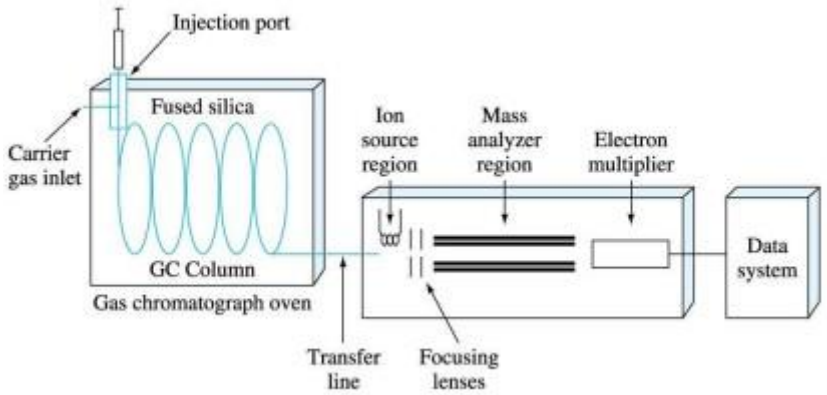


Figure 4. *components parts of instrumentation of a gas chromatography (73).*

Gas chromatography in conjunction with mass spectrometry combines fast analysis, high resolution, easy operation, excellent quantitative and qualitative results, and moderate cost.

For the GC-MS analysis of the flavorings, an Agilent Technologies 7890A equipment with an 5977B MS detector was used. The injection was performed in a classical and automated manner. The flavoring (less than 1µl) was introduced directly into the inlet liner, being injected into the column with a ten-fold dilution (split 10:1). The separation of the compounds was achieved using a DB5-MS column (60 m x 0.25 mm, 0.5µm film thickness, J&W Scientific, Agilent Technique, Foster City, CA). The column flow rate was 1.5 mL/min (helium). The oven temperature was set at 40ºC for 5 min, and then to 100ºC ate 5 ºC/min, and to 250ºC at 10 ºC/min with a 3 min hold at final temperature.

Injection, MS transfer line, and ion source temperatures were 220, 240, and 180°C, respectively. Electron ionization mass spectrometric data from m/z 25 to 300 were collected in scan mode, with an ionization voltage of 70eV. Compound identifications were made by mass spectral data from the Wiley 275.L database (G1035).

Figure 5. *Gas chromatograph with mass spectrometry from Agilent Technologies used in the assays.*

2.2.2.2 Characterization of the vehicles

2.2.2.2.1 Mechanical properties

Rheology is defined as the branch of physics that studies how materials deform and flow when they are subjected to a stress, under certain thermodynamic conditions, and over an interval of time (31,74). At rest, a material has a thickness, length, and width. For a deformation flow to occur, a certain force (F) must be applied, over a certain area (A). It is assumed that the fluid is divided into several layers. The upper layer has a higher velocity than the lower layer, and is considered stationary. When the force is applied to the area, the upper layer is displaced by dv and the thickness by dx (Figure 6). The force acting on the area (top layer) is called shear stress (τ), expressed in the International System of Units(SI) unit Pascal. The shear ratio or shear rate ($\dot{\gamma}$), expressed by the unit s-1, will be definedby the velocity gradient, which evaluates the displacement as a function of time. Then, the viscosity (η) will be the coefficient of proportionality between shear stress and shear ratio,and is expressed by the unit Pascal.second (Pa.s) (75,76).

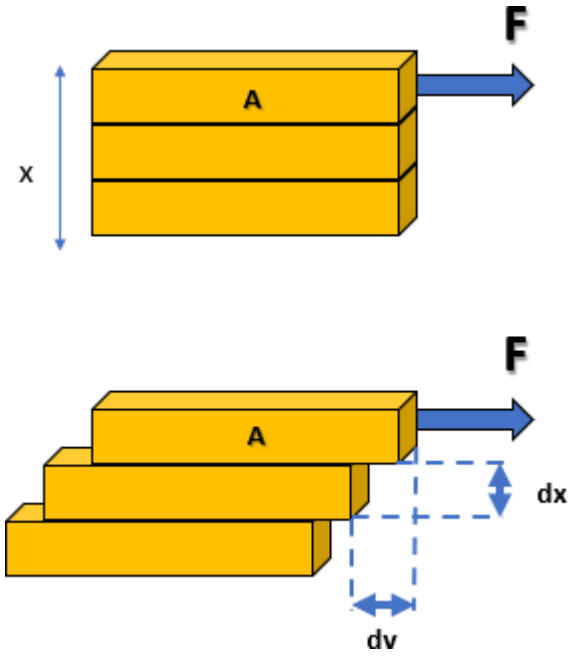


Figure 6. *Representation of a material subjected to shear stress. A - area; F - force; x – thickness.*

Through the analysis of the mechanical behavior arising from the relationship between shear rate and shear stress, fluids can be classified as Newtonian or non-Newtonian.

The Newtonian system, according to Newton's law, for ideal fluids, the shear stress (τ) is directly proportional to the strain rate (Eq. 1), i.e., it has a constant viscosity, independent of the applied force for a given temperature. Thus, all fluids that follow this behavior are called Newtonian fluids, such as all gases and non-polymeric and homogeneous liquids like water, glycerin and vegetable oils (77, 78, 79).

$$\eta = \frac{\tau}{\dot{\gamma}} \qquad (1)$$

However, a non-Newtonian fluid is characterized by a change in viscosity with increasing applied shear force, i.e., the relationship between strain rate and shear stress is not constant (77, 78). The causes of this behavior are the formation of an organized structure of the system through the orientation of asymmetric particles in the direction of flow (79). Materials that exhibit a non-Newtonian type flow can be classified into time independent, time dependent and viscoelastic (78) (Figure 7).

When a preparation shows yielding only after a certain stress value, it is said to have plastic behavior. At rest, these materials behave like solids due to inter-particle association.

The external force must overcome these internal forces and destroy the structure of the material. The minimum shear stress required to produce yielding is called the yield strength. Above this value, the material begins to exhibit liquid-like behavior, flowing freely (76).

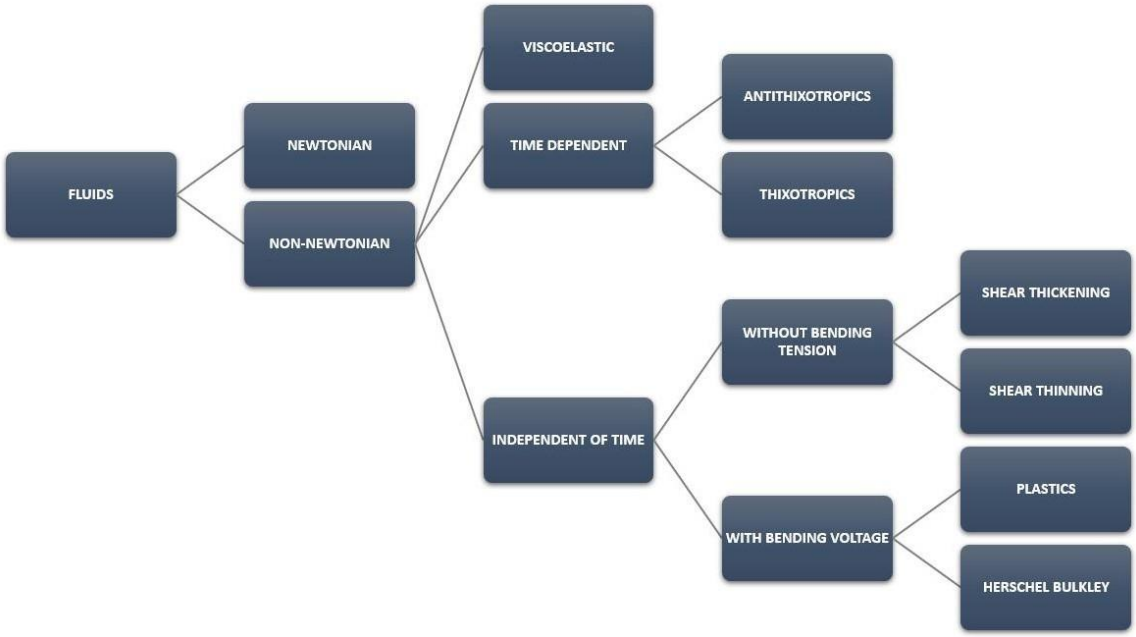


Figure 7. *Classification of fluids according to their rheological behavior. Adapted from (80).*

The flow curves that translate different types of rheological behavior can exhibit the relationships viscosity "versus" shear rate or shear stress "versus" shear rate, as depicted in figure 8.

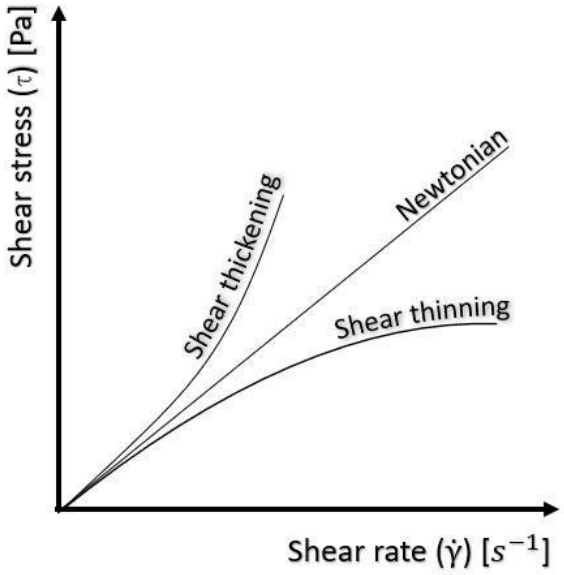


Figure 8. *Rheograms typical of liquid formulations (81).*

Shear thinning behavior often presents itself in semisolid systems containing polymeric components, due to the existence of intermolecular interactions between the polymer chains, as can be seen in Figure 9. When a stress is applied to these materials, deformation initially occurs with difficulty, but becomes progressively easier as the applied stress increases. The shear thinning behavior, presents a constant zone for lower strain rates, called the first Newtonian region, where microscopic rearrangements in the structure of the material may be at the beginning of maintaining equilibrium and hence viscosity. When the shear ratio is high, that is, when the materials are at rest, they exhibit a reticular structure that may consist of clusters of molecules that attract each other or a network of entangled polymer chains, a second Newtonian zone is reached, where the viscosity becomes constant. When the material reaches the maximum rearrangement, the intermolecular bonds break down, and the viscosity decreases. Emulsions and suspensions are examples of systems that exhibit this type of behavior (75, 76).

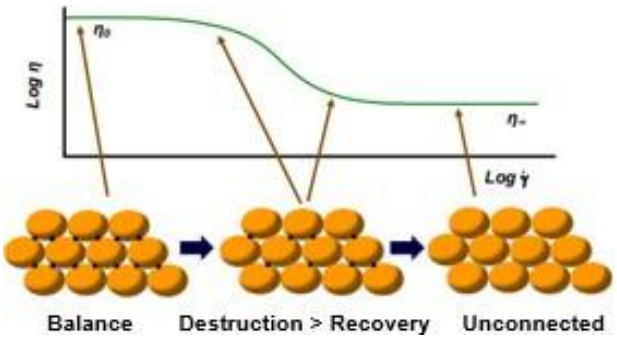


Figure 9. *Intermolecular interactions of the components in shear thinning materials (76).*

To study the rheological behavior, a rotational rheometer - Malvern Kinexus, lab + (Figure 10), was used, with the use of the rSpace software. This device allows viscosity analysis (flow tests and dynamic properties) and viscoelasticity analysis (oscillatory tests).

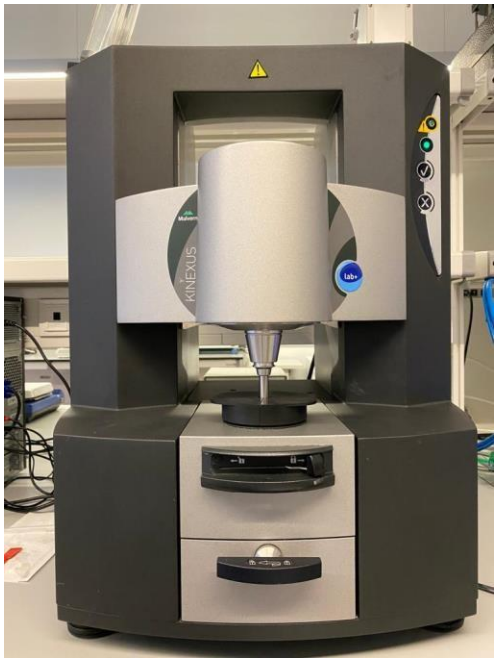


Figure 10. *Malvern Kinexus lab+ rotational rheometer.*

The geometry used will depend on the consistency of the sample to be analyzed and can be of the type parallel plates and dish cone. In the present work the dish cone geometry was used (reference - CP/40 SR 4321 SS:PL61 ST S2974SS). First the geometry is fitted into the instrument and the height calibration (zero gap) is performed. A small amount of the sample (approx. 1.5ml) is added to the lower plate (figure 11-a). The upper plate descends to meet the lower plate, compressing the sample (Figure 11-b); it is necessary toremove the excess that overflows (Figure 11-c).

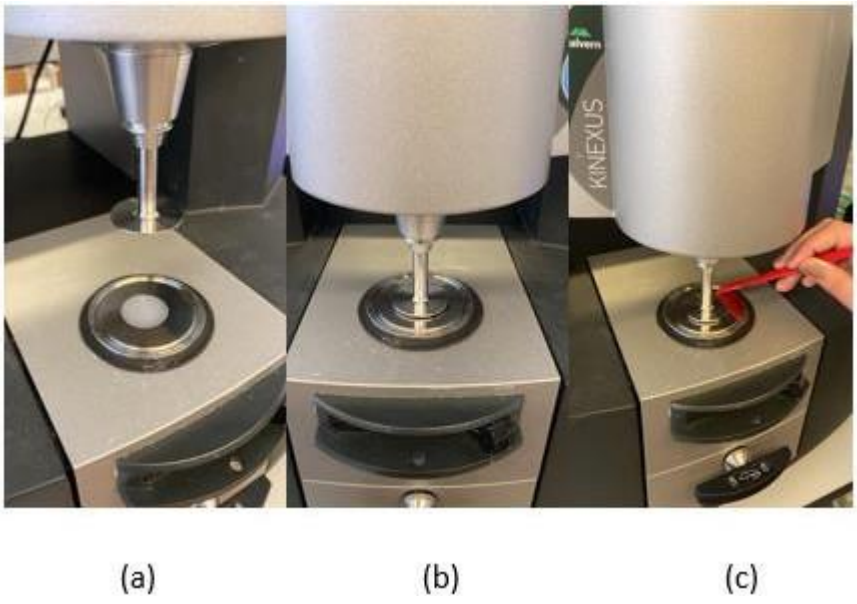


Figure 11. *Sample addition scheme.*

Then the type of test must be chosen, which can be either rotational or oscillatory, as shown in figure 12. The vehicles in question were previously characterized using rotational and oscillatory tests such as viscosity as a function of shear rate, thixotropy, amplitude, and frequency.

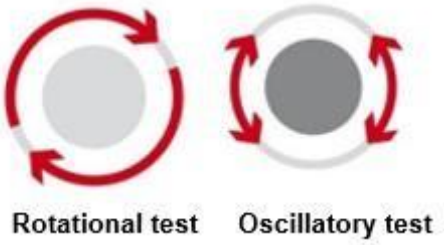


Figure 12. *Types of tests for evaluating rheological behavior.*

Rotational tests can be performed defining the shear ratio as constant, which allows the characterization of materials essentially dependent on the flow velocity, or those that define the shear stress as constant. Oscillatory tests, on the other hand, are applied to materials with viscoelastic behavior (elastic and viscous), allowing measurements of components such as elastic modulus, viscous modulus, and viscosity as a function of the shear ratio (75).

The stress exerted on the sample can be of the normal type, where perpendicular stress is applied, or shear stress, where tangential stress is applied (75).

For characterization of the vehicles Regular Syrup, Oral Suspension - Guinama®, OraPlus®, Syrspend® and Suspendit® the following analyses were performed:

2.2.2.2.1.1 Flow curve

To perform this test, a shear ratio is applied with varying intensity over a specified period of time, and we can observe whether there are any changes in the viscosity of the system. The test was performed with the following test conditions (Table 6):

Table 6*. Viscosity test conditions as a function of the shear rate.*

Initial shear rate (s-1)	0.1
Final shear rate (s-1)	500
Samples per decade	10

2.2.2.2.1.2 Degree of thixotropy

The property of a hard material that becomes less viscous over the time it is flowing is defined as thixotropy (31,74). This term, comes from the Greek word thixis (agitation) and trepo (change), i.e., more clearly this word expresses a meaning of "change by torque" and is designated for materials that exhibit the property of modifying their internal structure by agitation and after a certain time, resumes rest. In general, all materials that exhibit shear thinning flow usually exhibit, to a greater or lesser extent, a certain degree of thixotropy (31,74).

One way to evaluate the thixotropic degree is to perform a 3-stage deformation test (Figure 13):

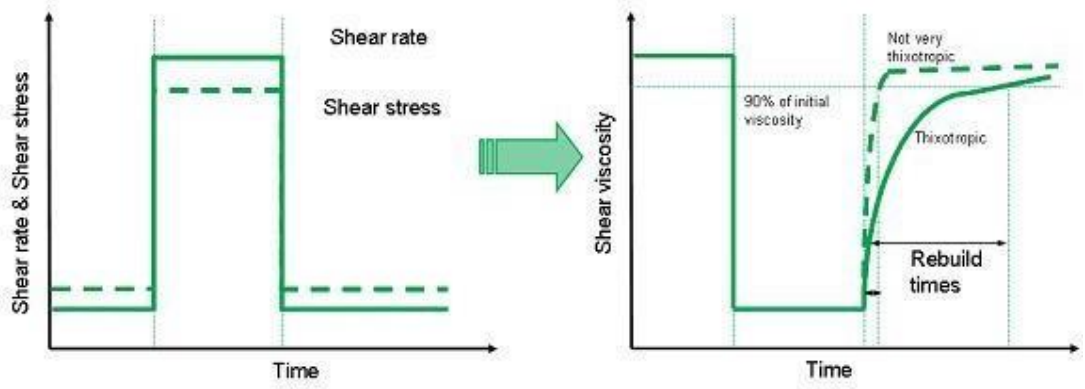


Figure 13 *Schematic of the 3-step thixotropy test (82).*

- First stage: low strain rate, similar to the resting state.
- Second stage: application of a high strain rate over a specified period of time to destroy the structure of the matter.
- Third stage: low strain rate equal to the first stage so that viscosity recovery can be evaluated.

The degree of thixotropy is then evaluated by the time to 90% viscosity recovery, and a system is more thixotropic if it takes longer to recover its initial viscosity (75). The test conditions are shown in Table 7.

Table 7. *Test conditions for the analysis of the degree of Thixotropy.*

Step 1	
Shear rate	$0.1s^{-1}$
Time 1º fase	1min
Sampling interval	0.5s

Step 2	
Shear rate	100^{s-1}
Time 2º fase	30s
Sampling interval	0.5s

Step 3	
Shear rate	0.1^{s-1}
Time 3º fase	10min
Sampling interval	0.5s

2.2.2.2.1.3 Amplitude sweep

The amplitude sweep test is an oscillatory test with continuous cycling movements applied to determine the linear viscoelastic region limit (LVER). The linear viscoelastic region is one in which the sample responds to the applied force or stress in a homogeneous manner, i.e., the stress and strain are linearly related and the fluid behavior can be described as a simple function of time. This test applies an increasing amplitude of deformation, then determines the maximum deformation that the sample is capable of withstanding, as shown in Figure 14, while fully recovering its initial characteristics. This test serves as a parameter to be adopted in other oscillatory rheological tests, since it defines the maximum limit of the deformation region. The test was conducted only once, under the conditions shown in Table 8.

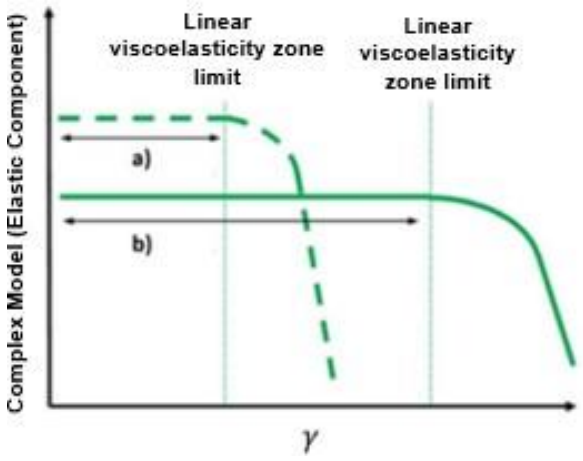


Figure 14. *Linear viscoelasticity region (82).*

Table 8. *Test conditions for amplitude sweep test.*

Start shear strain	0.1%
End shear strain	100%
Frequency	1.0 Hz
Sample per decade	10

2.2.2.2.1.4 Frequency sweep

The evaluation of shear stress as a function of frequency should be performed through the frequency sweep test in the region of linear viscoelasticity (determined previously), where the proportionality between stress and strain is guaranteed, applying a strain in which the microstructure of the material is not compromised. An oscillatory frequency sweep test uses a certain shear strain at different frequencies and allows obtaining several important rheological parameters for the classification of materials, namely: viscous (or loss modulus, G''), elastic (or storage modulus, G'), phase angle (δ) and complex viscosity (η*). The complex modulus parameter (G *) is a measure of the strength of the material and its value is given by slope of the shear stress versus shear ratio curve in the elastic region and is expressed in N/m2 (3,85). The relationships between these parameters are as follows: G'=G* x cos δ and G''=G* x sen δ. For apparent liquids, at high frequencies the elastic modulus (G') predominates, i.e., it exhibits solid properties (δ < 45º), whereas at lower frequencies the viscous modulus (G'') predominates, i.e., liquid properties (δ > 45º). For apparent solids, the elastic modulus (G') is independent of frequency and the viscous modulus (G'') increases with frequency (31,74). The test was performed under the conditions shown in Table 9.

Table 9. *Test conditions for frequency sweep test.*

Start frequency	10Hz
End frequency	0.1Hz
Shear strain	0.3%

2.2.2.3 Preparation of oral flavored formulations

After characterizing the flavorings and the vehicles, it was possible to define, from chromatographic and rheological analyses, which ones would be used for the stability study. The flavorings were banana and mint and the vehicles, simple syrup, Syrspend® and Suspendit®.

According to the literature it was possible to establish the concentration of the banana flavoring (1%) that should be added to each vehicle and its different batches (92). For the mint cream flavoring, its ideal concentration (0.5%) was established through a blind test. According to the percentage of flavoring agent, each vehicle was added to a final volume of 230 ml.

Three batches of the oral flavored formulations were prepared and stored at room temperature and in the fridge (2-8 ºC).

The preparation for the vehicles containing banana flavours was carried out as follows: 227.7g of the vehicle (Syrup, Syrspend® and Suspendit®) and 2.3g of the flavouring were added to the amber bottle. For the mint flavored vehicles, 228.85g of the vehicles and 1.15g of the mint flavoring were added. The preparation was done individually for each bottle and after the addition of the vehicle and the flavouring agent, manual agitation (40 times) was performed and followed the same procedure for the different storage conditions.

Figure 15*. Image of the prepared oral flavored formulations.*

2.2.2.4 Evaluation of flavoring stability

The stability tests of the commercial flavoring agents included a chromatographic analysis by GC-MS, their organoleptic characteristics, and their refractive index through refractometer equipment.

2.2.2.4.1 GC-MS

The GC-MS technique and methodology was described in section 2.2.2.1.1 when referring to the characterization of the flavoring agents.

The assays were performed at time zero and time 90. During this period, the flavoring agents are stored in a small transparent bottle, as shown in the figure below (Figure 16) at room temperature and protected from light.

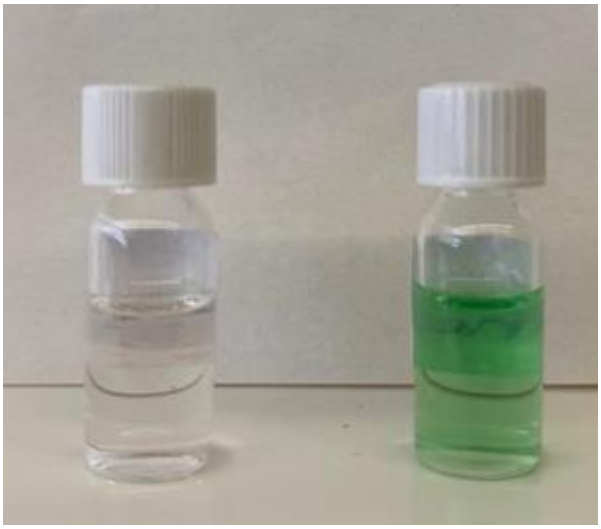


Figure 16. *Appearance of tested flavoring agents (Banana - left and mint - right)*

2.2.2.4.2 Organoleptic characteristics

The organoleptic characteristics of the flavoring agents evaluated during the stability tests were the appearance, color, and aroma.

Coloration was analyzed through macroscopic observation and photo recording over a period of 90 days. The aroma was evaluated by olfaction.

2.2.2.4.3 Refractive Index

The refractive index is a dimensionless number that describes how light propagates through a medium. It is defined as:

$$n = \frac{c}{v} \qquad (2)$$

where c is the speed of light in vacuum (299 792 458 m/s), which is usually approximated to 3.0 × 108 m/s, and v is the speed of light in the material medium. Since v is usually smaller than c, it follows that the index of refraction is greater than 1 for all material media found in nature. For example, the index of refraction of water is 1.33 at 20ºC, which means that light propagates 1.33 times slower in water than under vacuum. We say that the higher the index of refraction of a material, the more refractive it is, or the higher its optical density (38).

Refractometers usually determine the critical angle. In such an apparatus the essential part is a prism of known refractive index in contact with the liquid to be examined. Certified reference materials are used for calibrating the instrument. When white light is used, the refractometer is provided with a compensation system. The instrument provides accurate readings to at least the third decimal place and is supplied with a means of operating at the prescribed temperature. The thermometer is graduated in intervals of 0.5 °C or less (38).

Figure 17*. Refractometer*

The refractive index analysis for the stability study of the flavoring agents (banana and mint cream) is performed at T0 and T90.

2.2.2.5 Stability evaluation of oral flavored vehicles

The stability tests of the vehicle plus flavoring were performed at time zero and then at different storage periods (T30 and T90) for the formulations at room temperature. For formulations of the vehicle plus flavoring agent stored at refrigerated temperature, the analysis was performed at T0 and T90. To evaluate the stability of the formulations the tests described below are performed.

2.2.2.5.1 Organoleptic characteristics

Organoleptic characteristics are the most accessible indicator to assess the quality of a semisolid preparation and to detect changes. The simple visual examination can function as an indication of the homogeneity of the preparation. The organoleptic analysis performed on the formulations took place after production (T0), at T30 and T90 for the samples at room temperature, and after production (T0) and T90 for the samples at refrigerated temperature. From the visual analysis it was possible to evaluate the color, appearance and odor.

2.2.2.5.2 pH Assessment

The pH value is related to the compatibility of the formulation components, with the efficacy and safety of use, constituting an important parameter to be evaluated in stability studies. The pH analysis over time occurred on predetermined days (T0, T30 and T90) for samples at room temperature and (T0 and T90) for samples at refrigerated temperature. Approximately 7ml of the mixture (vehicle plus flavoring) was placed in a 15ml falcon and the pH value of each batch of the different vehicles and flavorings was read with the help of a pH meter Hanna® HI5221, Germany.

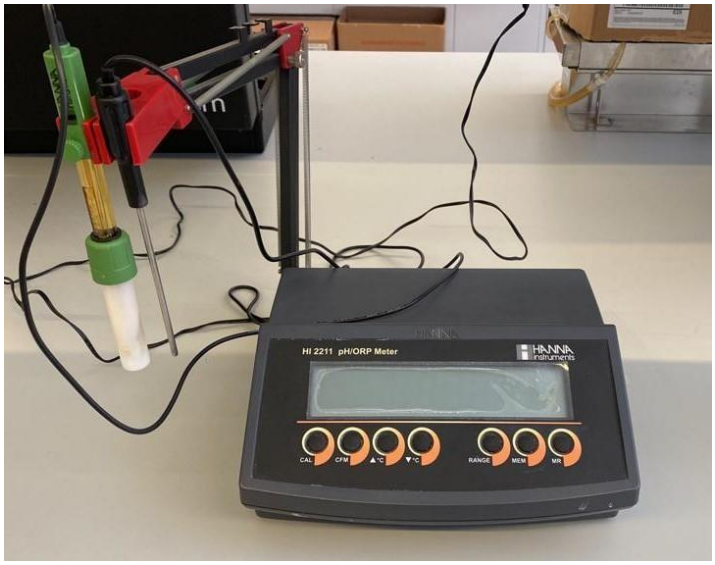


***Figure 18**. pH meter Hanna® HI5221*

2.2.2.5.3 Mechanical properties

For the samples at room temperature, the rheological analysis of viscosity, thixotropy, amplitude and frequency were performed at T0. At T30, only the viscosity analysis was performed, and at T90 all the analyses were performed, just as at time zero. For the cooled samples, analyses were performed at T0 and T90, both times the complete analysis (viscosity, thixotropy, amplitude, and frequency) was performed. The test conditions are

described in sections (2.2.2.2.1.1 to 2.2.2.2.1.4) when the characterizations of the vehicles were discussed.

2.2.2.5.4 GC-MS/SPME

The general description of GC-MS was covered in section 2.2.2.1.1. However, the methodology used for evaluating the characterization and stability of flavorings is different from the stability of the mixtures containing vehicle with the flavoring agent. The extraction method used in this case was solid phase microextraction (SPME). It is an extraction technique that integrates extraction and pre-concentration, introducing the sample into the chromatographic system in a single step and without the use of solvents (83, 84).

SPME is based on the equilibrium of analytes between a fused silica fiber or a fiber coated with a non-volatile polymer (adsorption and partition equilibrium, respectively) and the sample matrix. As no solvents are used in this technique, there is a large reduction in waste generation and occupational exposure. Moreover, it is a simple technique, easy to handle in relation to other techniques, and has the advantage of using a small amount of sample, and can be applied to various areas, such as environmental, pharmaceutical, food, forensic aromas and toxicology (85, 86, 87).

A weakness found in this technique is that it has a very low amount of adsorbent available for volatiles or has a variable extraction of volatiles due to competitive reactions between the SPME fiber, the aqueous phase, glass walls of the vessel, and the stir bar used (88). However, the speed and simplicity of this method and refinements on SPME make it very attractive.

In the SPME technique a syringe is used where its needle is retractable. The retractability is to provide protection against physical damage and contamination to the inert fiber that the syringe has (Figure 19). This fiber is coated with an adsorbent and this is placed in the sample headspace and allowed to adsorb volatiles (Figure 20). The "charged" fiber is then thermally desorbed in a GC carrier gas stream and the released volatiles are analyzed (89).

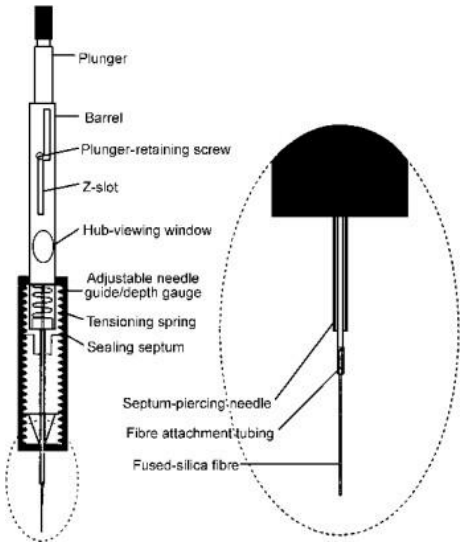


Figure 19. *Schematic of an SPME device (89).*

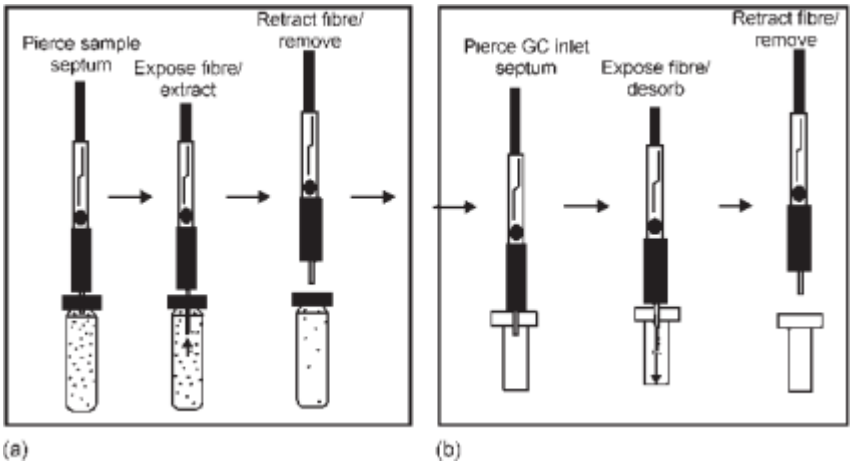


Figure 20. *Schematic showing the steps involved in the use of an SPME device: (a) extraction procedure; (b) desorption procedure (89).*

As each analyte has particular physical-chemical characteristics, behaving in a specific way in relation to the existing equilibrium of partition or adsorption between the fiber and the sample matrix, it is necessary to obtain extraction conditions whose balance favors the concentration of analytes in the polymeric coating. Therefore, the first step to obtain good results is the appropriate choice of the fiber stationary phase according to its volatile profile, that is, affinity for the analyte or for most of the analytes to be investigated. The second step is to optimize the extraction conditions such as medium salinity, pH, temperature and extraction time (42, 90, 91).

For the stability analyses of the vehicle plus flavoring, a glass container with a septum aluminum screw cap, stir bar, samples to be analyzed (approximately 60mg), refrigerated ultrapure water (1,9 mL), NaCl (100 mg), and nonanal solution (internal standard – 50 mg/L; 100 µl) were added.

The container was properly sealed with the screw cap and exposed for 25 minutes under stirring at 45°C. After the 25 minutes the syringe is introduced and the 2 cm fused silica fiber coated with divinylbenzene/carboxen/polidimethylsiloxane 50/30 µm is exposedto the headspace environment for a further 20 minutes at 45 ºC.

The volatiles adsorbed on the SPME fiber were desorbed in split mode for 5 min in the GC injector at 250 °C. All the remaining chromatographic characteristics were describer in 2.2.2.1.1.

2.2.2.6 Thermal degradation analysis

The samples, stored in amber glass vials containing the vehicles with the flavoring agents, were placed in an oven at a temperature of 60ºC for a period of 24 hours. After period, the vials containing the preparations were removed and allowed to cool to room temperature for further analysis in GC-MS.

Results and discussion

3.1 Preliminary study for the selection of flavorings and vehicles

3.1.1 Flavoring agents

3.1.1.1 Dosing of volatile compounds

There is an extensive range of flavoring agents available on the market for use as a flavoring agent. In the laboratory, we had available for preliminary study the banana, orange, mint, and marshmallow flavoring agents. For the children's palate, the banana and orange flavors were selected, which are commonly used flavors in pediatric formulations (92), since there is a preference for fruity flavors. And for an adult palate, the mint and marshmallow flavorings were selected, which present a softer and refreshing taste. Thereafter, the flavoring agents were analyzed by direct dosing of volatile compounds by GC-MS in an automated way, and later, after analyzing the areas of each compound and the total area of each compound, the relative abundance of each compound was estimated.

Figure 21 shows the banana chromatogram. The identifications of the peaks corresponding to the analyzed compounds are detailed in Table 10. Of a total of 13 compounds detected, allyl heptanoate stands out, being the major one, followed by benzyl alcohol and 1-butanol, 3-methyl-, acetate, typical of banana flavoring. Salmon et al. (1996), conducted a study of commercial banana flavorings (93), where the prevalent chemical compounds were pentan-2-one, isobutyl acetate, 2-methylpropan-1-ol, isoamyl acetate, pentan-2-ol, isobutyl butyrate, isoamyl alcohol, isoamyl butyrate, isoamyl isovalerate, and

eugenol. Many of the components present in the article are the same or similar in structure to the components found in the analyses performed, as shown in table 10. There is also a similarity in that most of the compounds belong to the ester functional group. This class is related to fruity flavor notes, and most flavorings belong to this family (94).

Propylene glycol is the vehicle of the flavorings, reason why it appears with more relevance. Note that the relative percentage of chromatographic areas does not necessarily correspond to the relative concentration of the compounds, because each compound has a distinct fragmentation pattern and intensity. For quantification, it would be necessary to have analytical standards for each compound, which was not available and is not necessaryfor the context of this exploratory analysis.

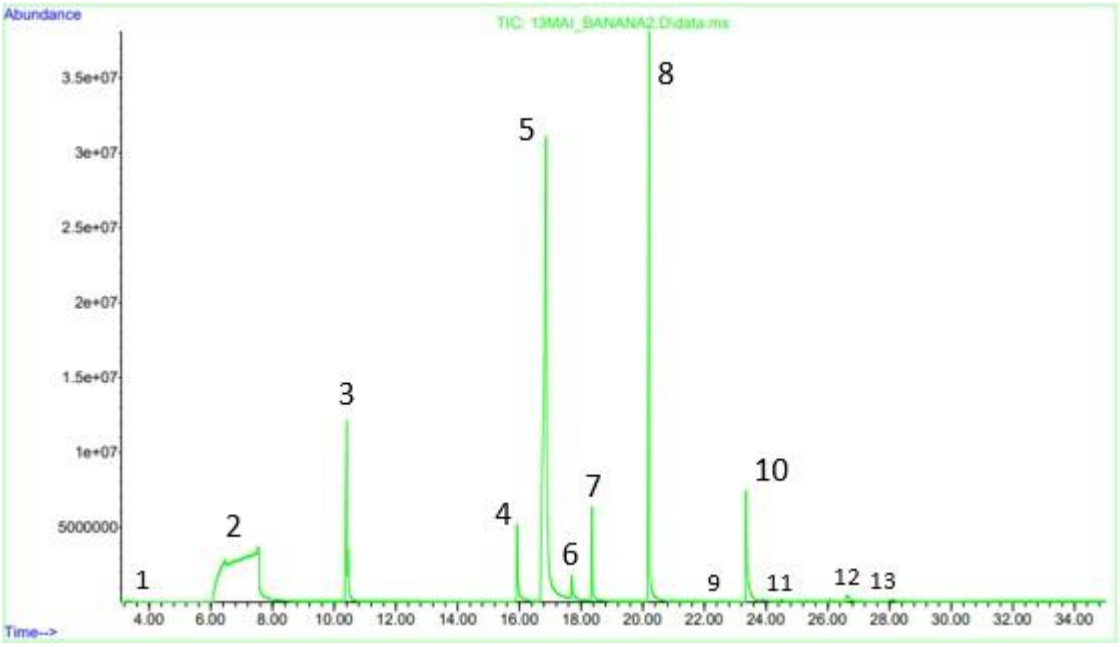
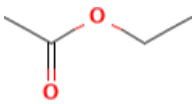
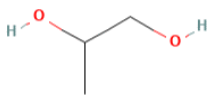
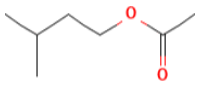
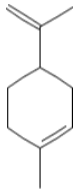
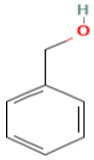
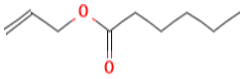
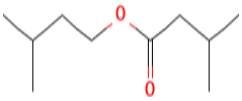
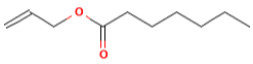
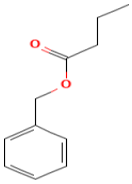
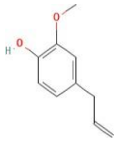
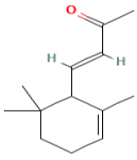


Figure 21 *Chromatographic representation of the banana flavoring agent (for numbering please consult Table 10).*

Table 10 *Compounds present in banana flavoring agent, after direct GC-MS analysis*

Characterizations of flavoring agents						
Banana						
Compound name	MM g/mol	Formula	Chemical structure	Retention rate	Main ions	Relative %
Ethyl Acetate **(1)**	88	C4H8O2		612	43 | 29 | 45 | 61	0.1
Propylene Glycol **(2)**	76	C3H8O2		740	45 | 43 | 29 | 31	35.0

Isoamyl acetate (**3**)	130	C7H1402		880	43 | 70 | 55 | 41 |	6.2
Limonene **(4)**	136	C10H16		1030	68 | 67 | 93 | 39 |	2.6
Benzyl alcohol **(5)**	108	C7H8O		1036	79 | 108 | 77 | 107 |	32.9
Allyl hexanoate **(6**)	156	C9H16O2		1076	43 | 41 | 99 | 71 |	0.8
Isoamyl isovalerate **(7)**	172	C10H20O2		1104	70 | 43 | 85 | 57 |	2.3
Allyl heptanoate **(8)**	170	C10H18O2		1180	43 | 41 | 113 | 55 |	14.7
Benzyl butyrate **(9)**	178	C11H14O2		1345	108 | 91 | 43 | 71 |	4.4
Eugenol **(10)**	164	C10H12O2		1357	164 | 103 | 77 | 149	0.1
α-Ionone **(11)**	192	C13H20O		1426	121 | 93 | 43 | 136 |	0.1
γ-Undecanolactone **(12)**	184	C11H20O2		1574	85 | 29 | 55 | 41	0.5

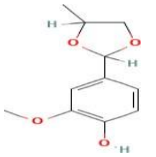
Vanillin propylene glycol acetal **(13)**	210	C11H14O4		1686	151 | 87 | 209 | 124	0.3

Figure 22 shows a chromatogram of the mint flavoring solutions. The peaks corresponding to the compounds are detailed in table 11. In this case, menthone, and levomenthol, together they represent more than 25% of the flavoring agents.

To our knowledge, there are no reports on the GC-MS spectra of the mint-based flavoring agent. This is why we expanded our analysis to volatile compounds found in the family mint. It was observed that there are no significant differences in the volatile compounds between the different mint species (95, 96, 97).

Comparing the compounds obtained through the analysis, of the synthetic flavoring agent, and with those present in the literature, even if it is compounded from plant species, it is possible to say that most of the compounds obtained in the laboratory are present in the literature, such as limonene, eucalyptol, menthone, levomenthol, pulegone, caryophyllene, β-copaene, that is, out of 15 compounds, 7 of them are present in the literature. It can be said that the range of compounds is very rich and diverse, possessing considerable compositional diversities, including alcohols, aldehyde, ketones, and terpene derivatives such as monoterpenes.

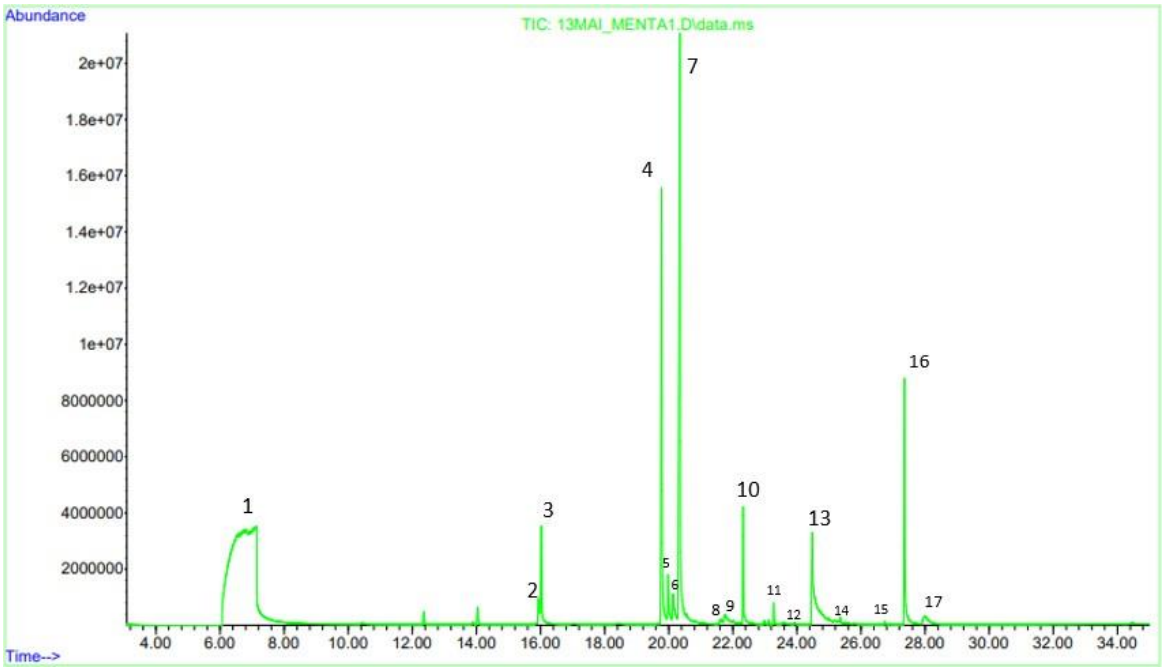
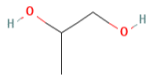
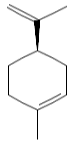
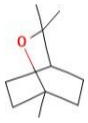
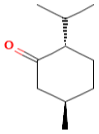
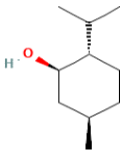
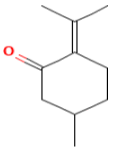
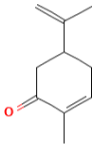
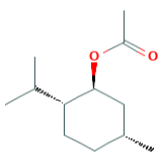
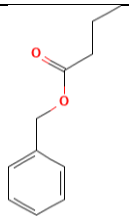
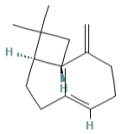
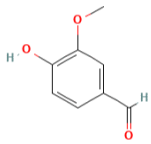
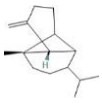
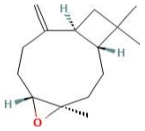
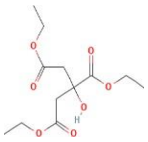
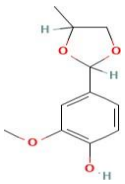


Figure 22 Chromatogram representation of the mint flavoring agent.

Table 11 *Compounds present in the mint flavoring agent, after direct GC-MS analysis.*

Characterizations of flavoring agents						
Mint						
Compound name	MM g/mol	Formula	Chemical structure	Retention rate	Main ions	Relative %
Propylene Glycol **(1)**	76	C3H8O2		740	45 | 43 | 29 | 31	50.7
Limonene **(2)**	136	C10H16		1030	68 | 93 | 67 | 79 |	0.7
Eucalyptol **(3)**	154	C10H18O		1032	43 | 81 | 108 | 71 |	2.5
Menthone **(4 and 5)**	154	C10H18O		1164	112 | 69 | 41 | 55 |	11.0
Levomenthol **(6 and 7)**	156	C10H20O		1175	71 | 81 | 95 | 41 |	17.8
Pulegone **(8)**	152	C10H16O		1237	81 | 152 | 67 | 109 |	0.2
Carvone **(9)**	150	C10H14O		1242	82 | 39 | 54 | 27 |	0.7
Isomenthol acetate **(10)**	198	C12H22O2		1305	43 | 95 | 81 | 138 |	2.1

Benzyl butyrate **(11)**	178	C11H14O2		1345	91 | 108 | 43 | 71	0.1
Caryophyllene **(12)**	204	C15H24		1419	93 | 133 | 91 | 41	0.9
Vanillin **(13)**	152	C8H8O3		1404	151 | 152 | 81 | 109	6.0
β-copaene **(14)**	204	C15H24		1443	161 | 105 | 91 | 41	0.1
Caryophyllene oxide **(15)**	220	C15H24O		1581	43 | 41 | 79 | 93	0.1
Triethyl citrate **(16)**	276	C12H20O7		1658	157 | 29 | 115 | 43	5.8
Vanillin propylene glycol acetal **(17)**	210	C11H14O4		1686	151 | 87 | 209 | 124	0.9

The mass spectra for the orange and marshmallow flavoring agents are depicted in Figures S1 and S2, respectively.

After analyzing the chromatograms and their compositions it was decided to select the two flavoring agents based on their richness of compounds, that is, those that from the chemical point of view presented more diverse compounds. The flavoring agent that was selected to cater to children's tastes was the banana flavoring agent. This flavoring agent presents a very diverse range of compounds compared to the orange flavoring agent. For the young/adult palate, the mint flavoring agent was selected. Even though both the mint and marshmallow flavoring agents had a diverse range of compounds, when adding the

marshmallow flavoring agent to one of the vehicles under study, it was observed that the abundances of its compounds were relatively low compared to the mint flavoring agent.

3.1.2 Vehicles for Oral compounding formulation

3.1.2.1 Rheological behavior

Analyzing Figure 23 and 24 and table 12 and 13, we can conclude that all the studied vehicles, except the simple syrup, are considered non-Newtonian fluids with a shear-thinning behavior since the apparent viscosity decreases with the increase in the shear rate. The simple syrup, on the contrary, presents a Newtonian rheological behavior.

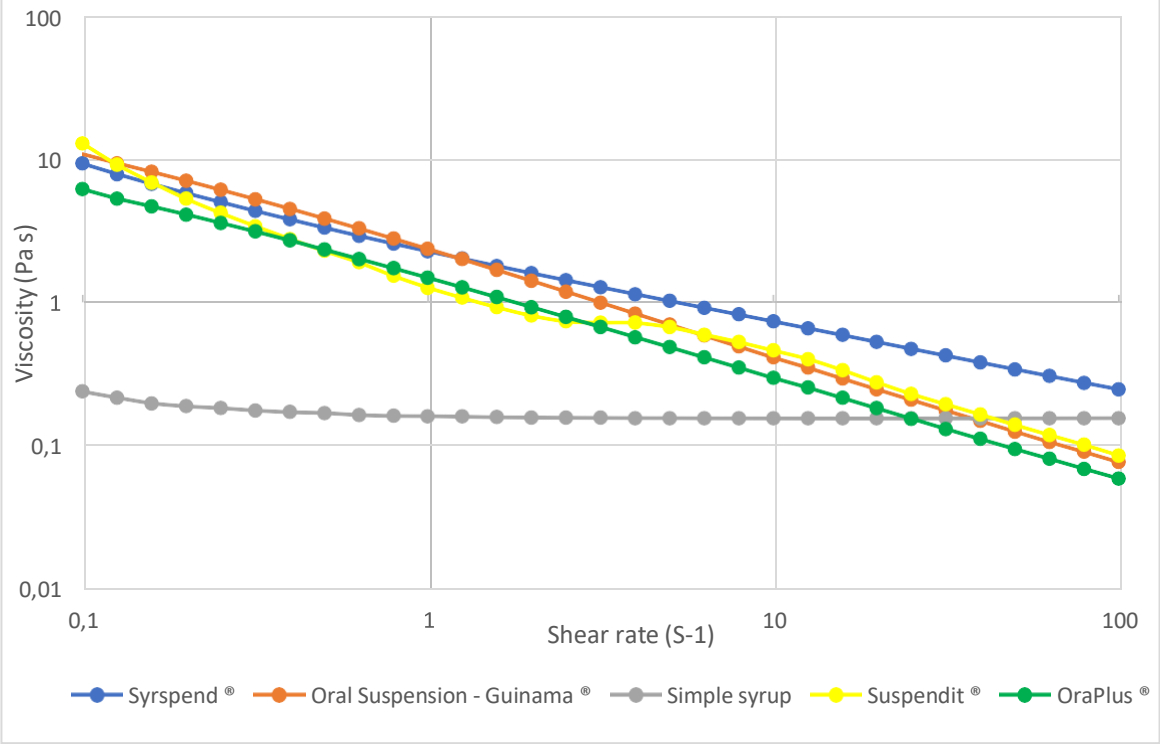


***Figure 23**. Flow curves of tested oral vehicles.*

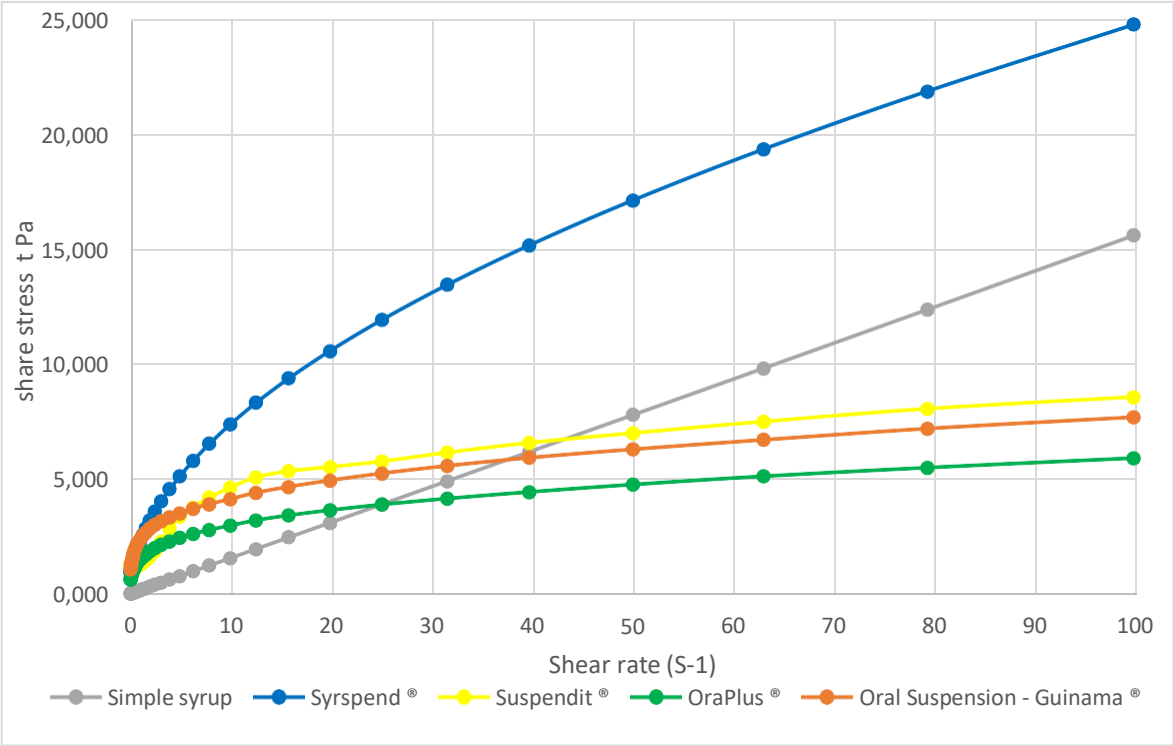


Figure 24 *Rheogram of the oral vehicles tested at room temperature.*

Table 12 *Parameters of the Herschel-bulkley model for the oral vehicles tested*

Vehicle	Herschel-bulkley model			
	K (Pa. s)	**n**	**τ_0 (Pa)**	**R^2**
Oral suspension – Guinama®	1.80	0.31	0.27	0.9950
OraPlus®	0.90	0.40	0.27	0.9889
Suspendit®	2.00	0.31	0.10	0.9751
Syrspend®	1.53	0.60	0.90	0.9983

Table 13 *Parameters of the Power law model for syrup*

Vehicle	Power Law		
	K (Pa. s)	**n**	**R^2**
Simple syrup	0.15	1.00	1.000

In addition, oscillatory tests were performed (strain sweep and frequency sweep) and thixotropic behaviour was characterized for Syrspend®, oral suspension-Guinama, Suspendit® and OraPlus®.

Figure 25 shows the viscous modulus (G"), the elastic modulus (G') and the phase angle as a function of strain. As can be seen, up to a strain value of 1%, in both vehicles,

there is no change with G' and G" modulus. It was established in this work the strain limit value of 0.3% (linear viscoelastic region) for all the vehicles.

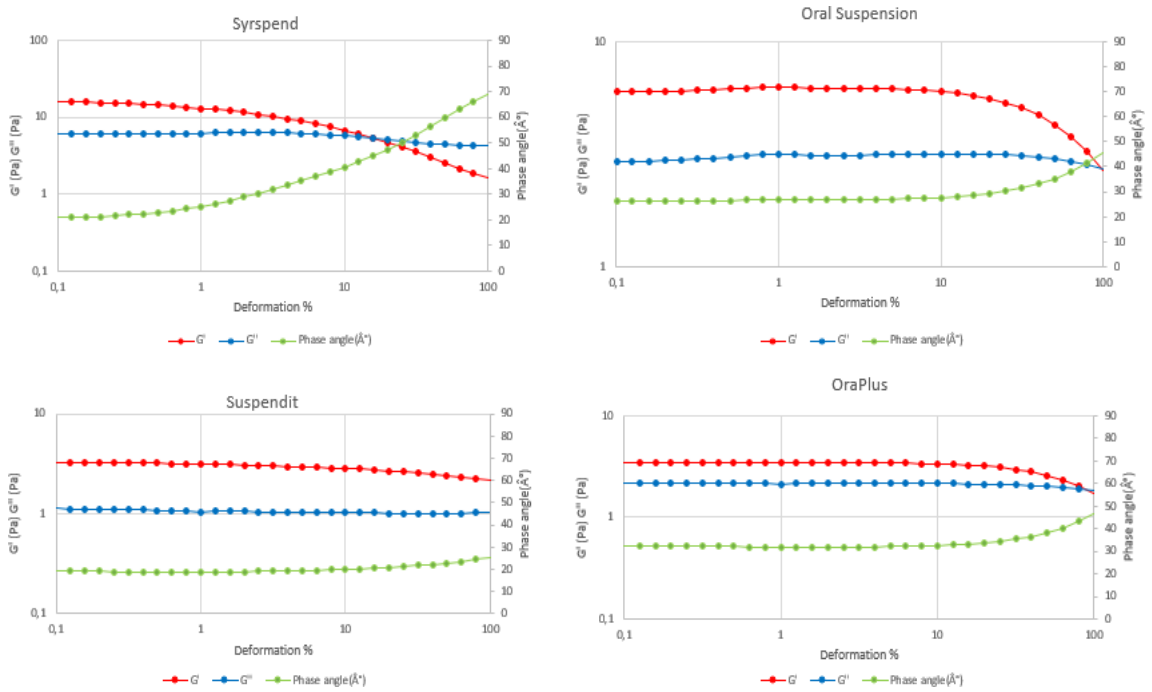


***Figure 25**. Strain sweeps of tested oral vehicles.*

After the LVER (Linear Visco-Elastic Region) was determined, a frequency sweep was performed. For all vehicles there is a predominance of G' (elastic modulus) and a phase angle of less than 45° (Figure 26) and thus, they present a solid-like behavior.

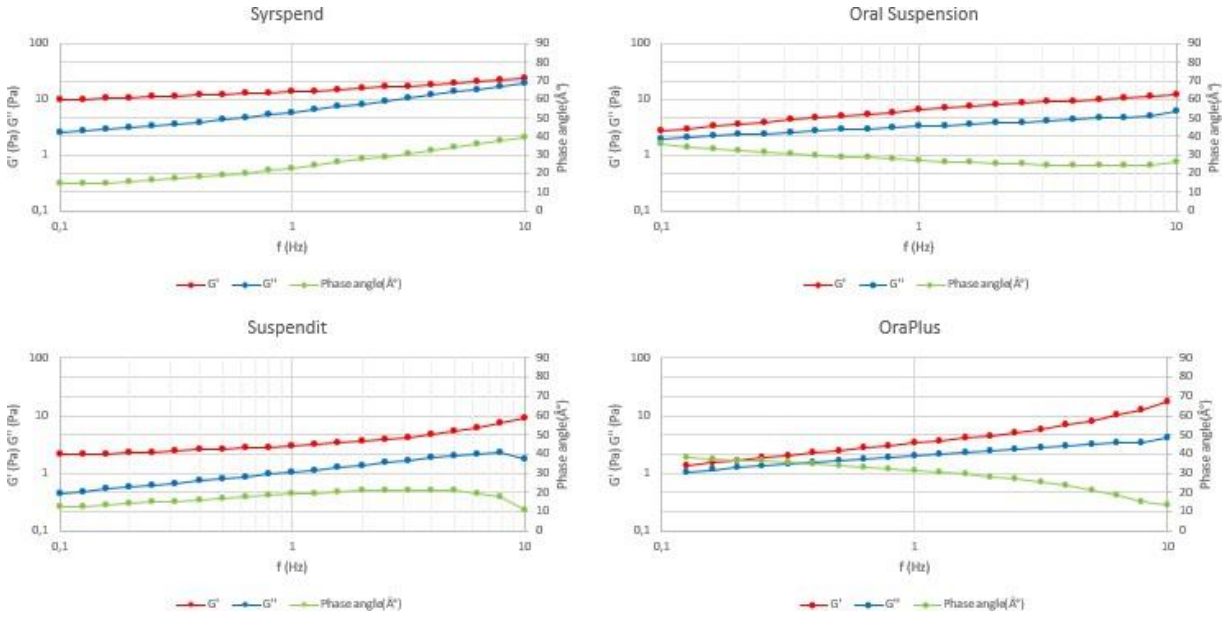


***Figure 26.** Mechanical spectrums of tested oral vehicles.*

In Figure 27, it is possible to differentiate the 3 distinct phases of the thixotropy analysis of the different vehicles under study.

The Syrspend® vehicle is characterized as non-thixotropic, with a recovery rate of 102%. In the first phase it is possible to observe a very low strain stress and the viscosity remains constant; in the second phase, with high shear rate, there is a sharp drop in viscosity; in the third phase, where the strain conditions of the first phase are restored, there is an almost immediate and total reconstitution of the vehicle's structure.

Oral suspension – Guinama®, Suspendit®, OraPlus® vehicles are characterized as thixotropic, showing a recovery rate of 87%, 68%, 89% respectively. The recovery period, i.e. the time where there was the highest viscosity value in the third phase, for the Suspendit® vehicle was 165s and for the Suspendit® Oral-Guinama® and OraPlus® vehicles it was not possible to determine a recovery period as the viscosity continued to increase after 300 seconds.

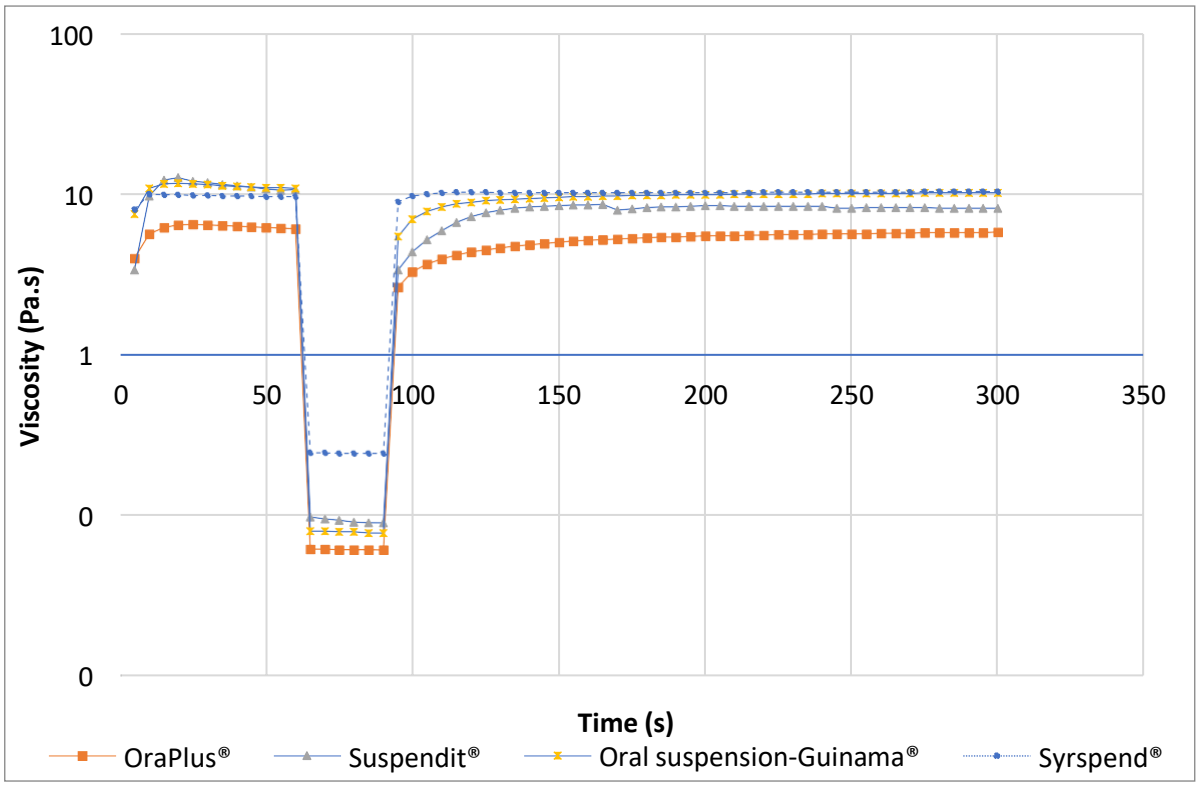


Figure 27 *Degree of thixotropy of the different vehicles under study.*

Based on the characterization of the vehicles, the simple syrup was selected because it is a Newtonian vehicle and because it is widely used in pharmaceutical formulations. The Suspendit®, characterized as non-Newtonian shear thinning and thixotropic fluid and Syrspend®, which is also characterized as non-Newtonian shear thinning, but does not present thixotropy, were also selected. Suspendit® and Syrspend®

were the most studied vehicles in the studies related with compounding oral formulations reported in the literature [46].

3.2 Study of the stability of flavoring agents

3.2.1 Dosing of volatile compounds

The stability analysis of the flavoring agents was performed simultaneously with the characterization of the flavoring agents, that is, after the analysis performed at the initial time (T0), an analysis was performed at T90 days to verify the stability of the flavoring agents selected for the study.

3.2.1.1 Banana flavoring

Below are the chromatograms of the banana flavoring agent at T0 and T90. By observing the chromatograms (Figure 28) of the banana flavoring it is possible to say that after 90 days the same compounds.

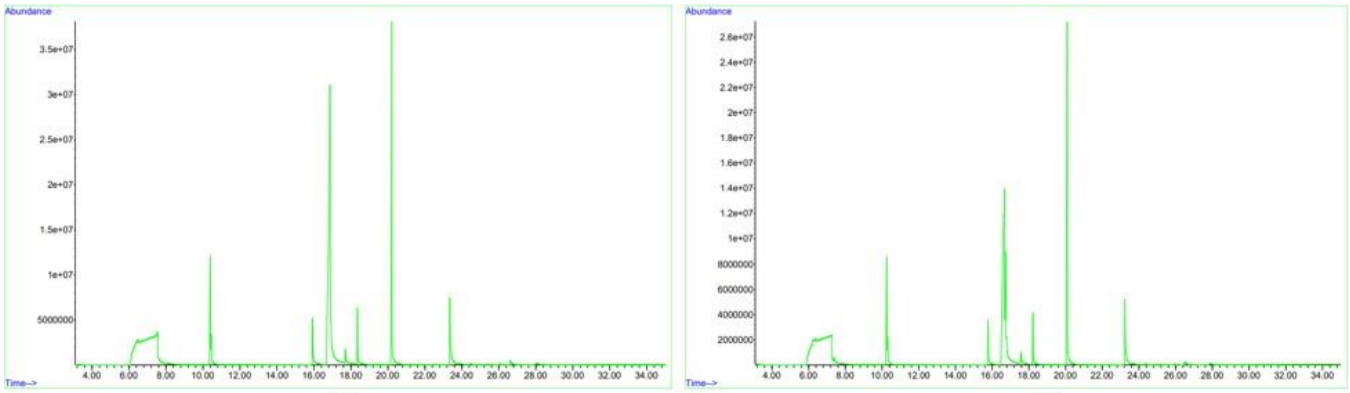


Figure 28 *Representative chromatograms of time 0 (a) and time 90 (b) of the banana flavoring agent*

To make a more detailed comparison, the relative abundance of the 0 and 90 days was averaged and an ANOVA statistical analysis was performed. Table 14 presents the results of the mean relative abundance and standard deviation of the banana flavoring compounds.

Table 14 *Results of the mean relative abundance and standard deviation of the compounds of the banana flavoring agent*

Compounds	T0 (% ± SD)	T90 (% ± SD)	*p*-value
Ethyl Acetate	0.08 ± 0.02	0.08 ± 0.01	0.870
Propylene Glycol	34.95 ± 0.27	34.37 ± 0.21	0.226
Isoamyl acetate	6.22 ± 0.36	7.30 ± 0.20	0.120
Limonene	2.60 ± 0.26	2.49 ± 0.08	0.731
Benzyl alcohol	32.89 ± 0.16	31.85 ± 0.26	0.077
Allyl hexanoate	0.85 ± 0.04	0.69 ± 0.02	0.081
Iso-amyl isovalerate	2.26 ± 0.13	2.32 ± 0.02	0.706
Allyl heptanoate	14.74 ± 0.11	15.72 ± 0.05	0.160
Benzyl butyrate	4.43 ± 0.19	3.96 ± 0.15	0.184
Eugenol	0.07 ± 0.07	0.41 ± 0.05	0.066
α-Ionone	0.12 ± 0.04	0.10 ± 0.01	0.563
γ-Undecanolactone	0.54 ± 0.01	0.38 ± 0.06	0.124
Vanillin propylene glycol acetal	0.26 ± 0.07	0.21 ± 0.04	0.598

*p>0.05 - normal distribution

Regarding the data treatment, it can be observed that only non-significant (p>0,05) variations were observed, without appearance of new compounds at T90 compared to T0.

Thus, the evidence points to the stability of the flavoring agent over the analytical period studied here.

3.2.1.2 Mint flavoring

By observing the T0 and T90 chromatograms of the mint flavoring agent (Figure 29), it is possible to see a decrease in peak abundance and the appearance of new peaks near retention times 33 and 34 min. However, these compounds are related to these peaks are from the solid-phase of the column (degradation peaks), and therefore not used for calculation purposes.

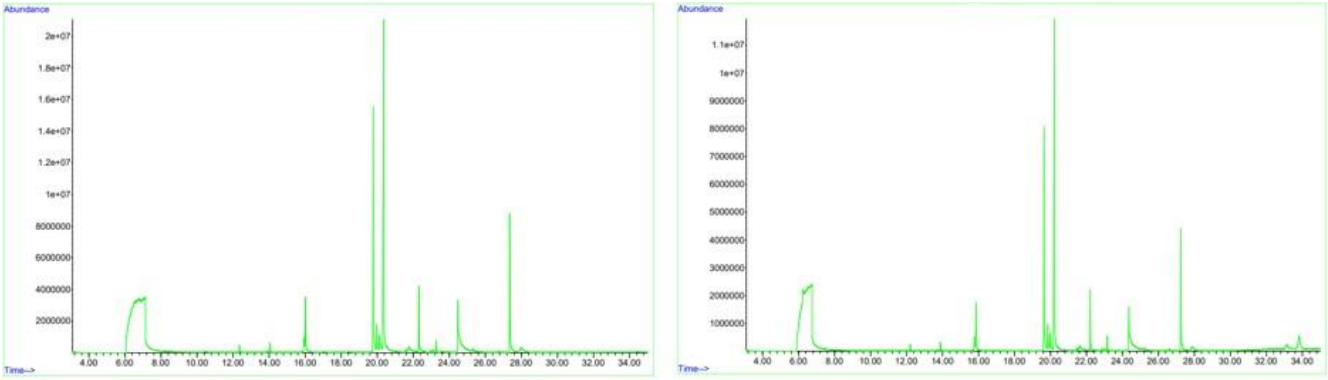


Figure 29 *Representative chromatograms of time 0 (a) and time 90 (b) of the mint flavoring agent.*

Table 15 *Results of the mean relative abundance and standard deviation of the compounds of the mint flavoring agents.*

Compounds	T0 (% ± SD)	T90 (% ± SD)	*p*-value
Propylene Glycol	50.73 ± 1.52	53.84 ± 0.01	0.179
Limonene	0.73 ± 0.07	0,96 ± 0.01	0.095
Eucalyptol	2.50 ± 0.07	2.47 ± 0.06	0.776
Menthone	11.07± 0.14	11.95 ± 0.08	0.032
Levomenthol	17.84 ± 0.25	18.12 ± 0.05	0.401
Pulegone	0.25 ± 0.01	0.22 ± 0.01	**0.020**
Carvone	0.68 ± 0.21	0.57 ± 0.01	0.645
Isomenthol acetate	2.14 ± 0.01	2.26 ± 0.05	0.162
Caryophyllene	0,95 ± 0.93	3.78 ± 0.14	0.096
Vanillin	6.06± 1.69	0.11 ± 0.02	0.072
β-copaene	0.13 ± 0.02	0.08 ± 0.01	0.130
Caryophyllene oxide	0.08 ± 0.01	0.10 ± 0.02	0.349
Triethyl citrate	5.84 ± 0.87	4.87 ± 0.00	0.381
Vanillin propylene glycol acetal	0.95 ± 0.18	0.67 ± 0.02	0.269

*p>0.05 - normal distribution

With respect to the percentage of relative abundance, no significant differences were observed between T0 and T90, indicative of the formula stability over the 90 days.

3.2.2 Organoleptic characteristics

The organoleptic characteristics were studied for both selected flavoring agents. Banana and the mint cream flavoring agents presented as a liquid with transparent and greenish coloration, respectively, with homogeneous aspect and characteristic banana and mint odors.

During 90 days, the flavoring agents kept their original organoleptic characteristics under the storage conditions (room temperature and protected from light), with no perceived changes in color or odor.

3.2.3 Refractive Index

The refractive index analysis performed showed that after the 90 days, the refractive index of the flavoring agents remained stable (Table 16).

Table 16 *Refractive Index of flavoring agents*

	Index of refraction	
	Banana flavoring	Mint flavoring
Reference Value	1.4470	1.4070
T0	1.4480	1.4060
T90	1.4445	1.4060

3.3 Stability study of the flavored oral vehicles

3.3.1 Rheological analysis

The stability study was carried out over a period of 90 days for the different preparations containing the vehicle with the flavoring agent.

3.3.1.1 Room temperature

3.3.1.1.1 Flow curve

Analyses were performed at T0, T30 and T90 for preparations obtained with the different vehicles and flavoring agents. The data were obtained through the rheological model analyses: power law model was used for the simple syrup vehicle and the Herschel-Bulkley model was used for the Syrspend® and Suspendit® vehicles.

The preparations containing the Simple syrup vehicle with the banana and mint flavoring agents (Figure 30) did not change significantly during the 90 days of analysis. Both times of analysis show similarity with the vehicle without the addition of the flavoring agent.

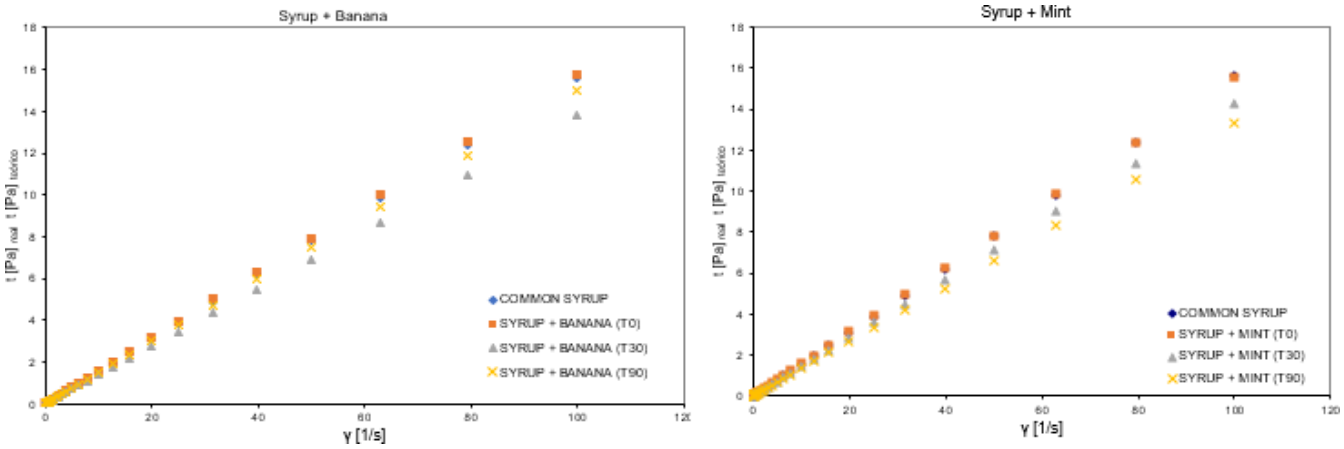


Figure 30. *Flow curve of simple syrup vehicle preparations flavored with banana (left) and mint (right) flavoring agent.*

Through the rheological power law model (Tables 17 and 18) it is also possible to state that there were no significant changes between the analysis times.

Table 17 *Power law model of simple syrup vehicle preparations flavored with banana*

	Simple syrup + Banana - Power Law			
	Simple syrup (mean ± SD)	T0 (mean ± SD)	T30 (mean ± SD)	T90 (mean ± SD)
K (Pa.s^n)	0.150 ± 0.000	0.152 ± 0.005	0.138 ± 0.002	0.148 ± 0.002
n	1.005 ± 0.000	1.005 ± 0.000	1.000 ± 0.000	1.000 ± 0.000
R^2	1.000 ± 0.000	1.000 ± 0.000	1.000 ± 0.000	1.000 ± 0.000

Table 18 *Power law model of simple syrup vehicle preparations flavored with mint*

	Simple syrup + Mint - Power Law			
	Simple syrup (mean ± SD)	T0 (mean ± SD)	T30 (mean ± SD)	T90 (mean ± SD)
K (Pa.s^n)	0.150 ± 0.000	0.150 ± 0.004	0.141 ± 0.004	0.130 ± 0.008
n	1.005 ± 0.000	1.005 ± 0.000	1.000 ± 0.000	1.000 ± 0.000
R^2	1.000 ± 0.000	1.000 ± 0.000	1.000 ± 0.000	1.000 ± 0.000

For the preparations containing the Syrspend® vehicle and both flavoring agents (Figure 31), there were also no significant changes during the time of analysis and there was also a similarity with the vehicle without added flavoring agent.

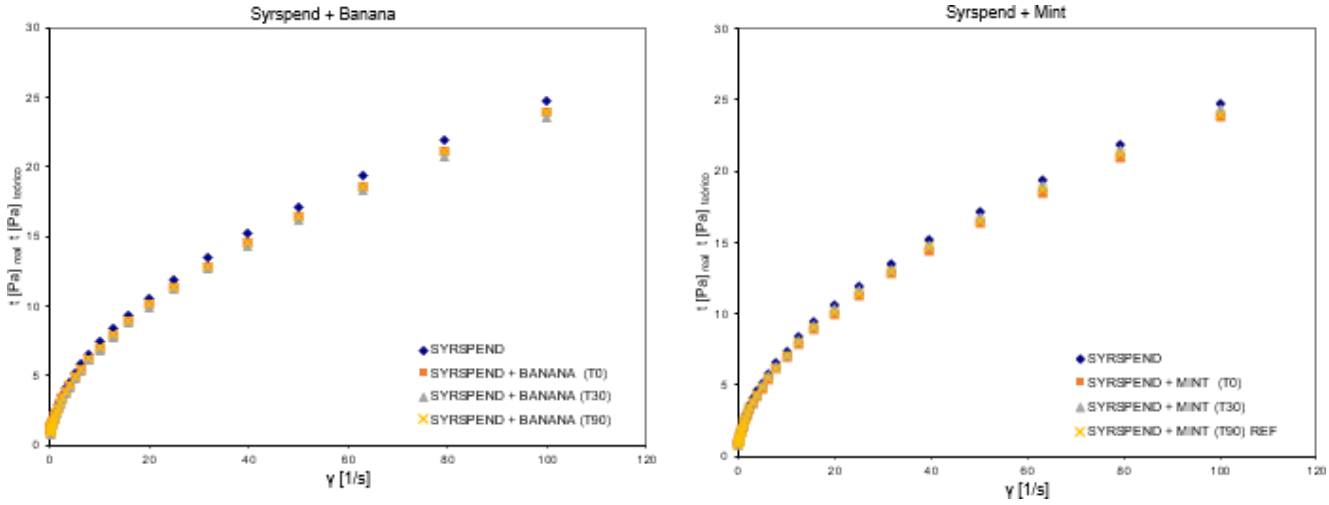


Figure 31. *Shear stress versus shear rate graph of the Syrspend® vehicle preparations plus banana (left) and mint (right) flavoring agent.*

As for the syrup vehicle, it is possible to state from the analysis of the Herschel-Bulkley rheological model (Tables 19 and 20) that there were also no significant changes between the analysis times.

Table 19 *Herschel-Bulkley model of Syrspend® vehicle preparations flavored with banana*

	Syrspend® + Banana - Herschel-Bulkley			
	Syrspend® (mean ± SD)	T0 (mean ± SD)	T30 (mean ± SD)	T90 (mean ± SD)
K (Pa.s^n)	1.583 ± 0.062	2.100 ± 0.000	2.100 ± 0.000	2.133 ± 0.047
n	0.600 ± 0.000	0.520 ± 0.000	0.520 ± 0.000	0.520 ± 0.000
t_o (Pa)	1.033 ± 0,189	0.500 ± 0.000	0.150 ± 0.000	0.150 ± 0.000
R^2	0.998 ± 0.000	1.000 ± 0.000	1.000 ± 0.000	1.000 ± 0.000

Table 20 *Herschel-Bulkley model of Syrspend® vehicle preparations flavored with Mint*

	Syrspend® + Mint - Herschel-Bulkley			
	Syrspend® (mean ± SD)	T0 (mean ± SD)	T30 (mean ± SD)	T90 (mean ± SD)
K (Pa.s^n)	1.583 ± 0.062	1.933 ± 0.047	2.200 ± 0.000	2.100 ± 0.000
n	0.600 ± 0.000	0.530 ± 0.000	0.520 ± 0.000	0.520 ± 0.000
t_o (Pa)	1.033 ± 0.189	0.500 ± 0.000	0.150 ± 0.000	0.150 ± 0.000
R^2	0.998 ± 0.000	1.000 ± 0.000	1.000 ± 0.000	1.000 ± 0.000

As for the preparations with the Suspendit® vehicle with the flavoring agents (Figure 32) during the time of stability analysis, the preparations did not change significantly. However, it is possible to observe that after the addition of the flavoring agent to the vehicle there was a decrease in viscosity.

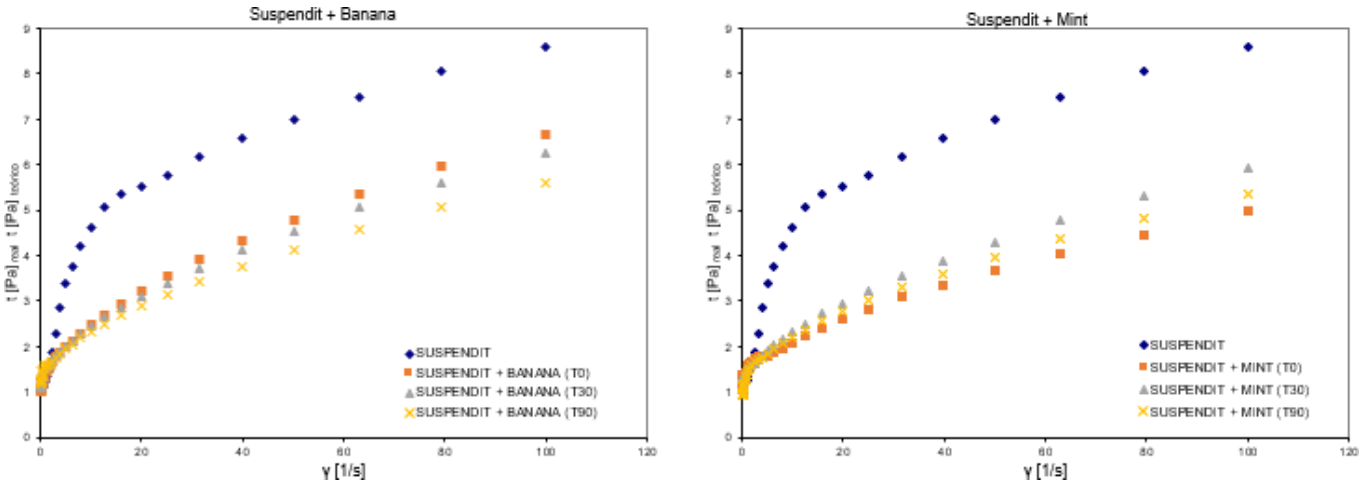


Figure 32. *Rheograms of Suspendit® vehicle preparations plus banana (left) and mint (right) flavoring agent.*

Using the Herschel-Bulkley model (Table 21 and 22), it is possible to confirm a difference when the banana and mint flavoring agent is added to the Suspendit® vehicle. There is a significant decrease in consistency index (K) values and a slight increase in behavioural index (n) values, leading to a decrease in viscosity.

Table 21 *Herschel-Bulkley model of Suspendit® vehicle preparations flavored with Banana*

	Suspendit® + Banana - Herschel-Bulkley			
	Suspendit® (mean ± SD)	T0 (mean ± SD)	T30 (mean ± SD)	T90 (mean ± SD)
K ($Pa.s^n$)	2.000 ± 0.000	0.455 ± 0.000	0.455 ± 0.000	0.350 ± 0.000
n	0.312 ±0.000	0.553 ± 0.002	0.540 ± 0.008	0.545 ± 0.000
t_o (Pa)	0.100 ± 0.000	0.833 ± 0.047	0.800 ± 0.000	1.067 ± 0.047
R^2	0.974 ± 0.005	0.999 ± 0.000	0.998 ± 0.000	0.996 ± 0.002

Table 22 *Herschel-Bulkley model of Suspendit® vehicle preparations flavored with mint*

	Suspendit® + Mint - Herschel-Bulkley			
	Suspendit® (mean ± SD)	T0 (mean ± SD)	T30 (mean ± SD)	T90 (mean ± SD)
K ($Pa.s^n$)	2.000 ± 0.000	0.420 ± 0.021	0.455 ± 0.000	0.373 ± 0.083
n	0.312 ± 0.000	0.494 ± 0.008	0.523 ±0.009	0.550 ± 0.035
t_o (Pa)	0.100 ± 0.000	0.833 ± 0.124	0.800 ± 0.000	0.800 ± 0.282
R^2	0.974 ± 0.005	0.978 ± 0.019	0.996 ± 0.001	0.991 ± 0.009

The amplitude and frequency sweep, and thixotropy were analyzed only at time 0 and time 90 for the Syrspend® and Suspendit® vehicles, in order to characterize the formulation at the initial and final time of the study.

3.3.1.1.2 Amplitude sweep

Through the figures (33 to 36) it is possible to observe that in the amplitude test no relevant changes were observed for both preparations containing the Syrspend® and Suspendit® vehicle and the banana and mint flavoring agents. The linear viscoelastic region remained the same and the strain of 0.3% used throughout the oscillatory testes remains within in the linear region.

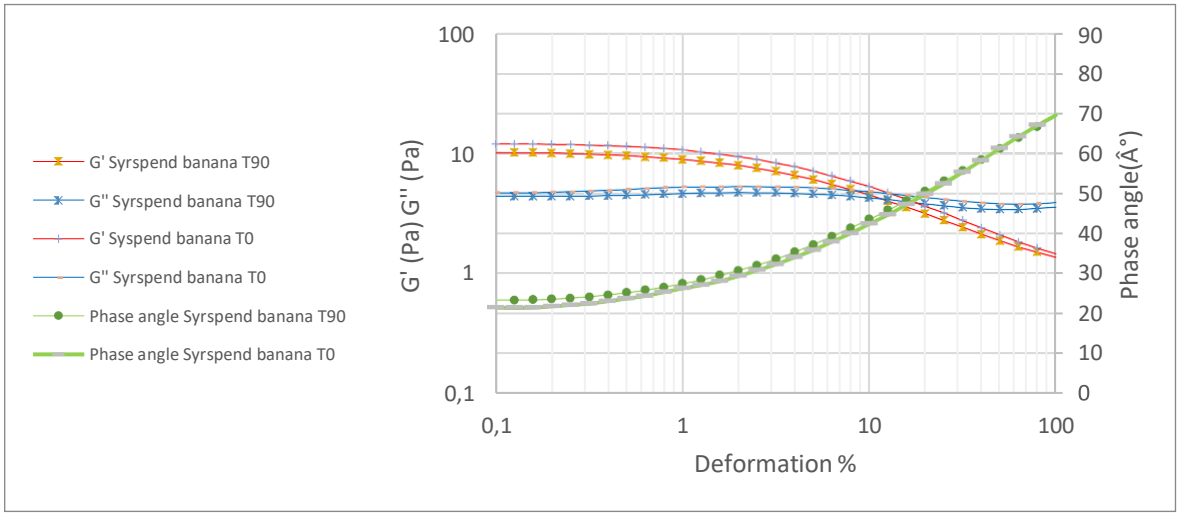


Figure 33. *Amplitude sweep, at times 0 and 90 days, relating the mixture of the Syrspend® vehicle with the banana flavoring agent.*

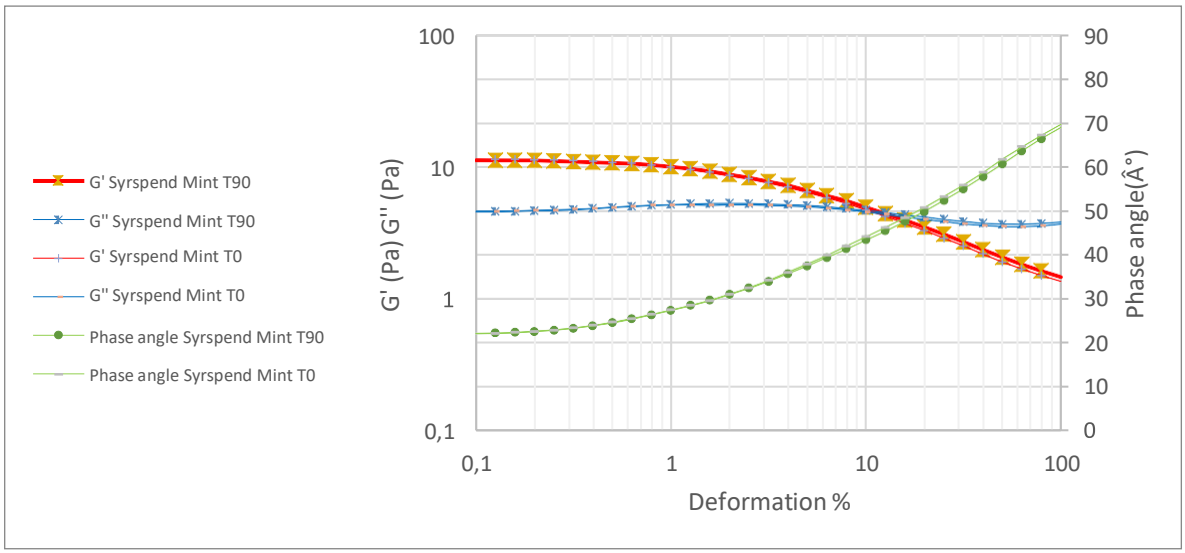


Figure 34. *Amplitude sweep, at times 0 and 90 days, relating the mixture of the Syrspend® vehicle with the mint flavoring agent.*

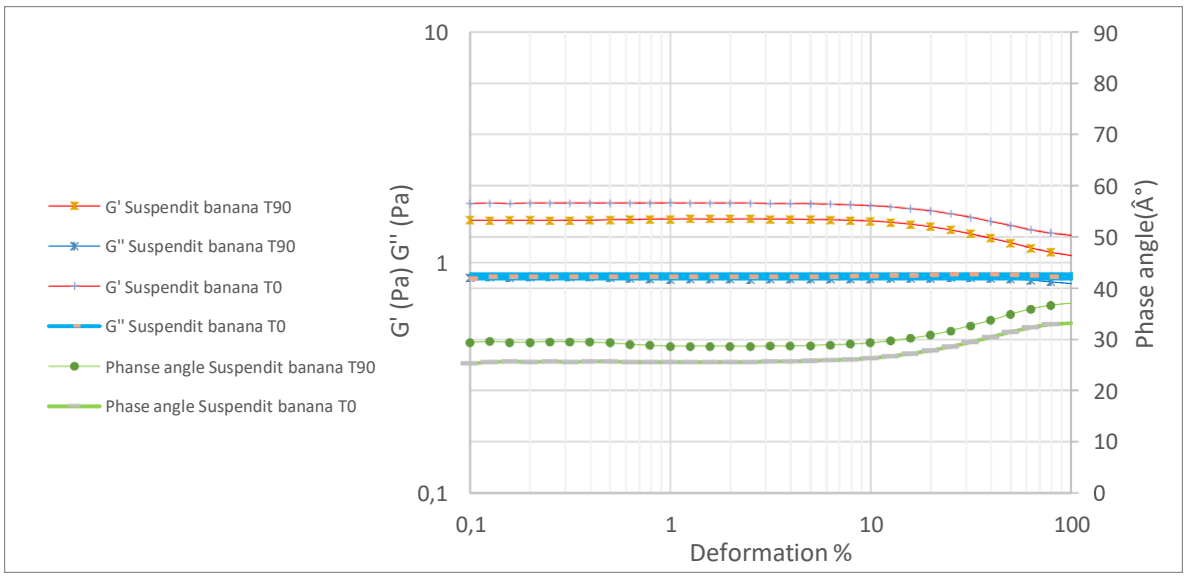


Figure 35. *Amplitude sweep, at times 0 and 90 days, for the mixture of the Suspendit® vehicle with the banana flavoring agent.*

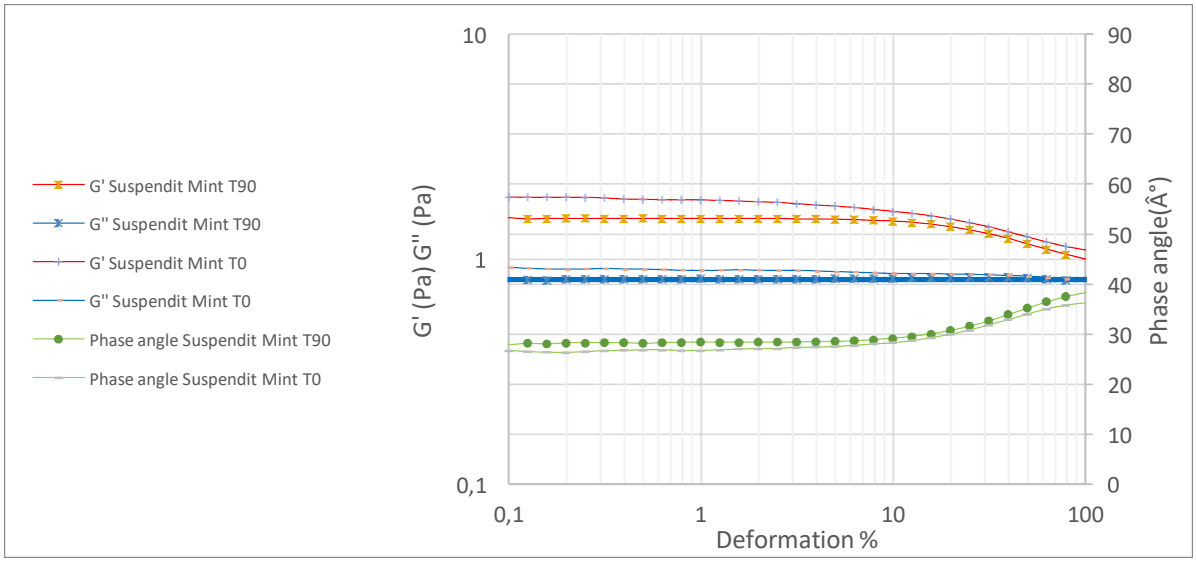


Figure 36. *Amplitude sweep, at times 0 and 90 days for mixing the Suspendit® vehicle with the mint flavoring agent.*

3.3.1.1.3 Mechanical spectrum

In Figures 37 to 40, the mechanical spectrums of the preparations with the respective vehicles and flavoring agents, obtained from the frequency sweep test are presented. During the 90 days, G' remained higher than G'' and the phase angle less than 45º, representing a solid-type behavior.

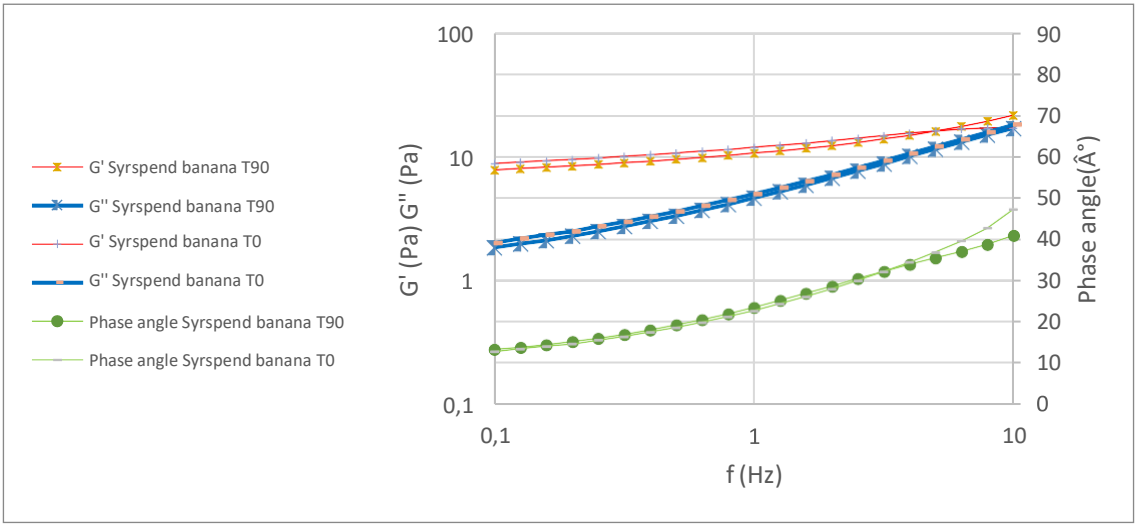


Figure 37 *Mechanical spectrum, at times 0 and 90 days, of the mixture of the Syrspend® vehicle with the banana flavoring agent.*

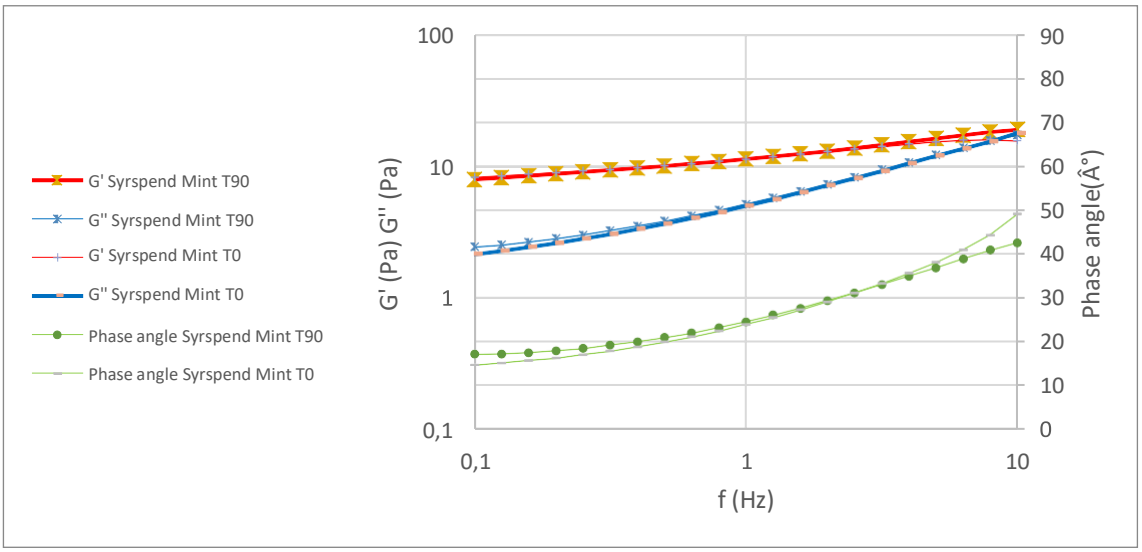


Figure 38 *Mechanical spectrum, at time 0 and 90 days, of the mixture of the Syrspend® vehicle with the mint flavoring agent*

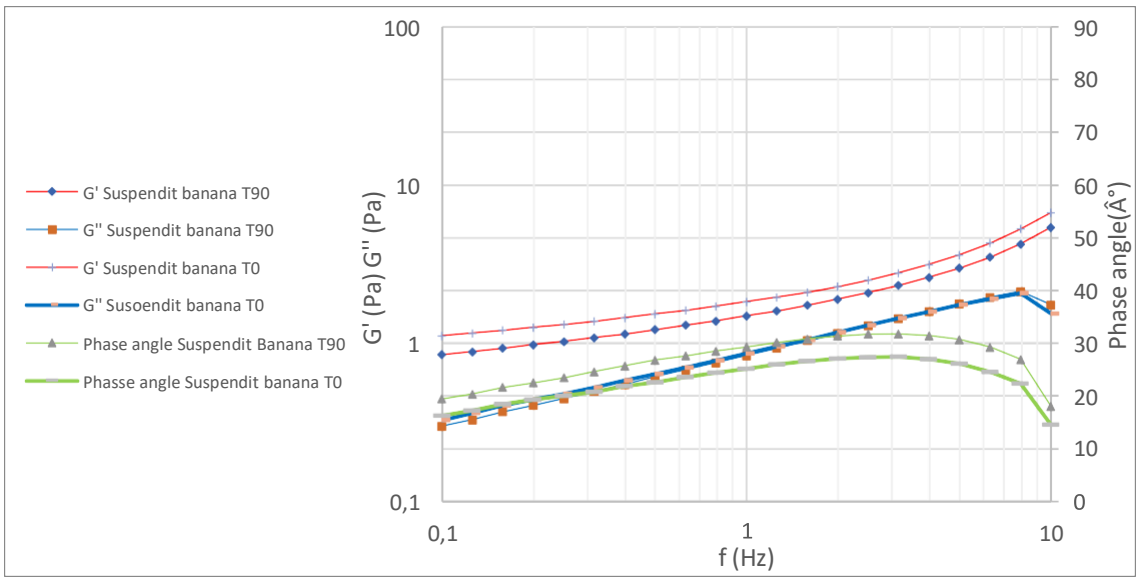


Figure 39 *Mechanical spectrum, at times 0 and 90 days, of the mixture of the Suspendit® vehicle with the banana flavoring agent.*

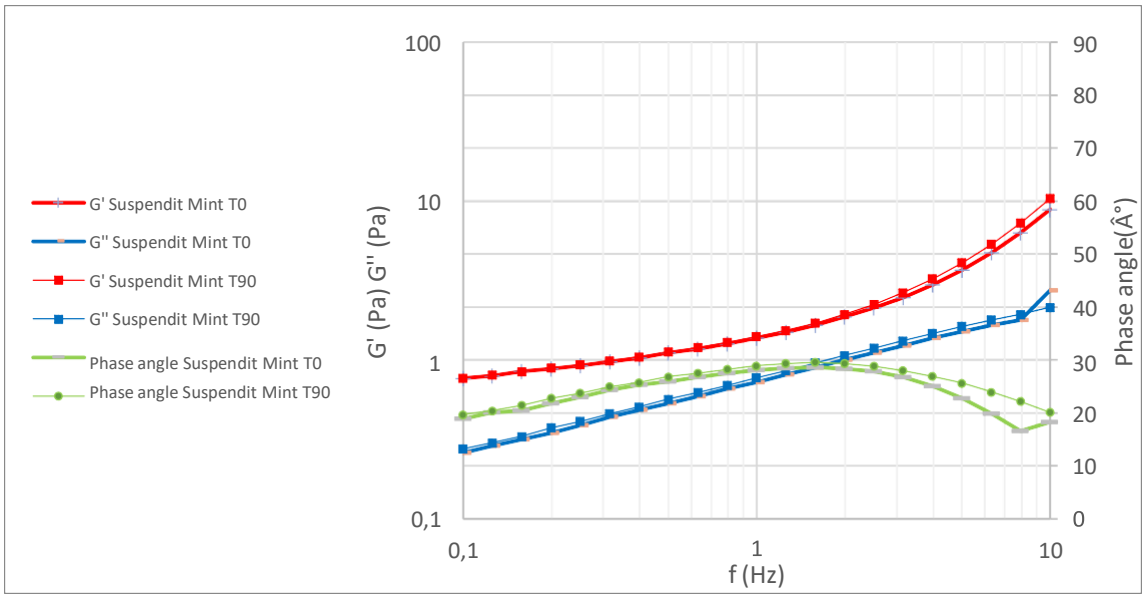


Figure 40 *Mechanical spectrum, at times 0 and 90 days, of the mixture of the Suspendit® vehicle with the mint flavoring agent.*

3.3.1.1.4 Thixotropy

There was no relevant change in the thixotropic behavior during the 90 days (Figure 41 to 44). There was only a small decrease in the recovery rate, for the Suspendit® vehicle mixed with the banana flavoring agent. The Syrspend® vehicle mixtures with the flavoring agents remained non thixotropic, with a recovery rate above 90%. The Suspendit® vehicle mixtures with flavoring agents remained thixotropic because the recovery rate is below 90% and also because its recovery period reaches the end point (300s) and there was no stability in the recovery period.

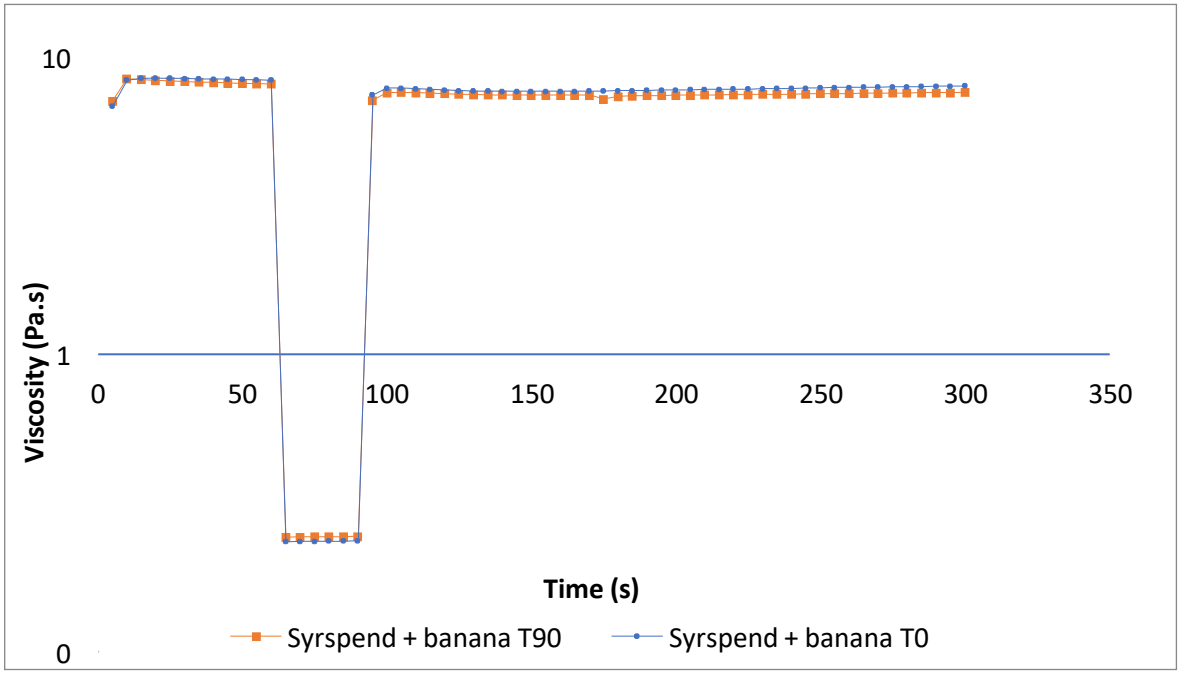


Figure 41 *Thixotropy graph of Syrspend® preparations with banana at time 0 and 90 days.*

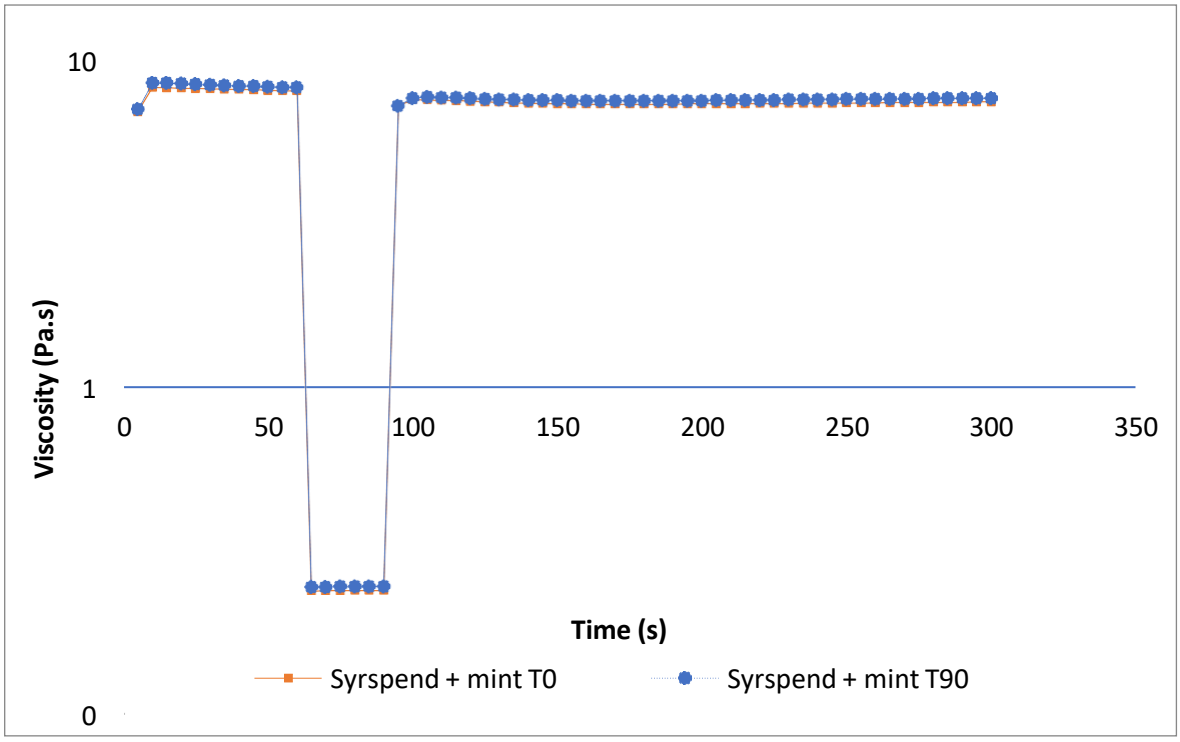


Figure 42 *Thixotropy graph of Syrspend® preparations with mint at time 0 and 90 days.*

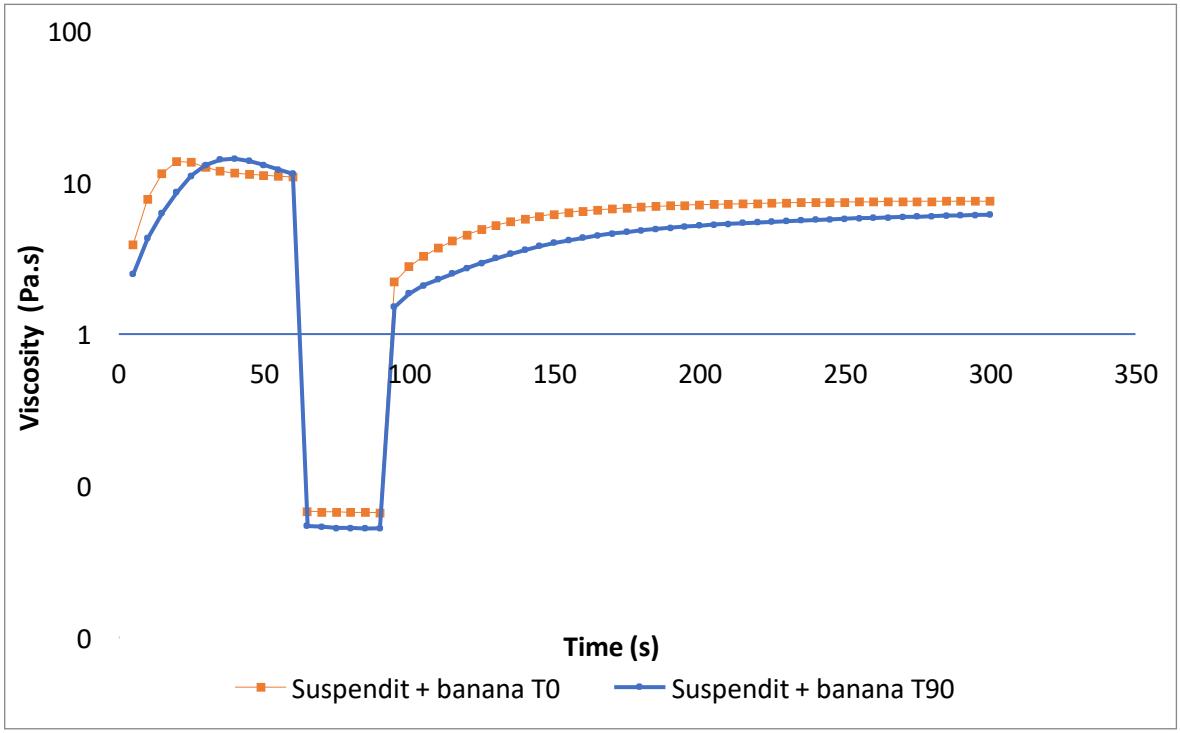


Figure 43 *Thixotropy graph of Suspendit® preparations with banana at time 0 and 90 days.*

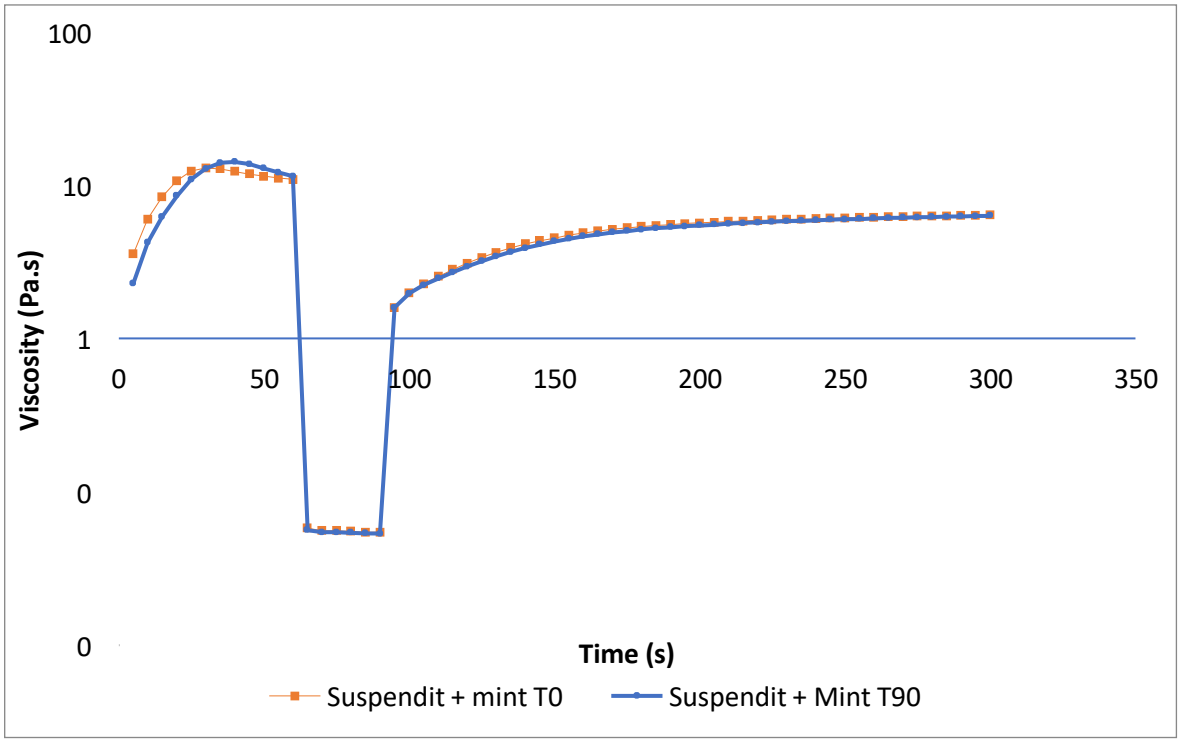


Figure 44 *Thixotropy graph of Suspendit® preparations with mint at time 0 and 90 days.*

3.3.1.2 Refrigerated temperature

The tests were performed at the initial time (T0) and after 90 days, for the preparations stored in the amber glass inside the refrigerator at a temperature of approximately 4°C.

3.3.1.2.1 Flow curve

Flow analysis was performed at T0 and T90 for preparations obtained with the different vehicles and flavoring agents. Rheological models were fitted to the results. Power law for the regular syrup vehicle and the Herschel-Bulkley model for the Syrspend® and Suspendit® vehicles provided the best fit.

The preparations containing common syrup vehicle with banana and mint flavoring agents (Figure 45), did not undergo significant changes during the 90 days of analysis. Results were similar to the vehicle without the addition of the flavoring agent.

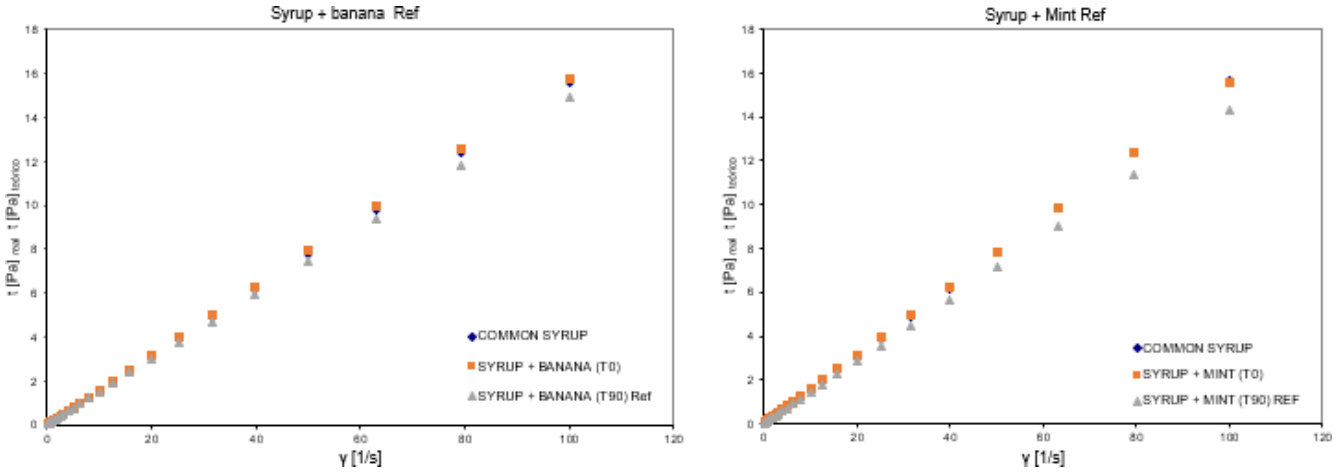


Figure 45 *Flow curve of simple syrup vehicle preparations plus banana (left) and mint (right) flavoring agent.*

Through the rheological model of the power law (Table 23 and 24) it is possible to confirm that the analyses of time 0 and time 90 did not obtain significant changes. As well as, there were no changes between the pure syrup and after the addition of banana and mint flavoring agents.

Table 23 *Power law model of simple syrup vehicle preparations flavored with banana at refrigerated temperature*

	Simple syrup + Banana - Power Law		
	Simple syrup (mean ± SD)	T0 (mean ± SD)	T90 (mean ± SD)
K (Pa.s^n)	0.150 ± 0.000	0.152 ± 0.005	0.147 ± 0.000
n	1.005 ± 0.000	1.005 ± 0.000	1.005 ± 0.000
R^2	1.000 ± 0.000	1.000 ± 0.000	1.000 ± 0.000

Table 24 *Power law model of simple syrup vehicle preparations flavored with mint at refrigerated temperature*

	Simple syrup + Mint - Power Law		
	Simple syrup (mean ± SD)	T0 (mean ± SD)	T30 (mean ± SD)
K (Pa.s^n)	0.150 ±0.000	0.150 ± 0.004	0.143 ± 0.000
n	1.005 ± 0.000	1.005 ± 0.000	1.000 ± 0.000
R^2	1.000 ± 0.000	1.000 ± 0.000	1.000 ± 0.000

For the preparations containing the Syrspend® vehicle and both flavouring agents (Figure 46), there was a slight change in the viscosity of the mixture containing the vehicle and banana flavouring agent between times 0 and 90 and there was a closer proximity between the pure Syrspend® vehicle and time 0. As for the mixture of the vehicle with the mint flavouring agent, it seemed more stable when compared to the banana one, both the pure vehicle and the mixtures at time 0 and 90 seemed to be closer to each other.

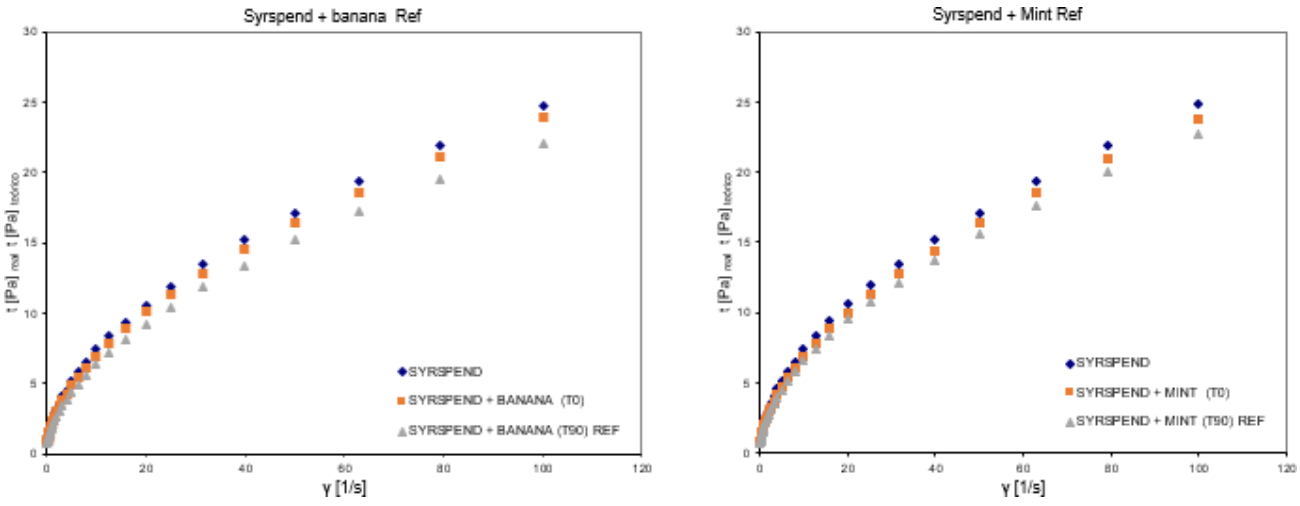


Figure 46 *Flow curve of Syrspend® vehicle preparations plus the flavoring agent banana (left) and mint (right).*

As for the analysis through the rheological models (table 25 and 26), it is possible to numerically evaluate the difference that was observed in Figure 46. The consistency index (K) had a small variation between the analysis times and between the vehicle withoutand with the banana flavoring. The behavior index (n) also had a small variation, but n is still less than 1, ensuring a shear thinning behavior. Where there was the greatest variation,was in the yield stress between the pure vehicle and the mixture containing the vehicle withthe banana flavouring. For the mixture of the Syrspend® vehicle with the mint flavouring, the only one that also showed greater variation was in the yield stress between the pure vehicle and the mixture of Syrspend® with the mint flavouring. However, even though these variations exist, it is possible to consider that both vehicles remained stable over the time of analysis and after the addition of the flavouring agent.

Table 25 *Herschel-Bulkley model of Syrspend® vehicle preparations flavored with banana at refrigerated temperature*

	Syrspend® + Banana - Herschel-Bulkley		
	Syrspend® (mean ± SD)	T0 (mean ± SD)	T90 (mean ± SD)
K ($Pa.s^n$)	1.583 ± 0.062	2.100 ± 0.000	1.700 ±0.000
n	0.600 ± 0.000	0.520 ± 0.000	0.552 ± 0.001
t_o (Pa)	1.033 ± 0.189	0.500 ± 0.000	0.500 ± 0.000
R^2	0.998 ± 0.000	1.000 ± 0.000	1.000 ± 0.000

Table 26 *Herschel-Bulkley model of Syrspend® vehicle preparation flavored with mint at refrigerated temperature*

	Syrspend® + Mint - Herschel-Bulkley		
	Syrspend® (mean ± SD)	T0 (mean ± SD)	T90 (mean ± SD)
K ($Pa.s^n$)	1.583 ± 0.062	1.933 ± 0.047	1.783 ±0.062
n	0.600 ± 0.000	0.530 ± 0.000	0.547 ±0.002
t_o (Pa)	1.033 ± 0.189	0.530 ± 0.000	0.400 ± 0.000
R^2	0.998 ± 0.000	1.000 ± 0.000	1.000 ± 0.000

For the Suspendit® vehicle, during the stability analysis period, the sample the banana flavoring agent (Figure 47), had significant changes. There was a decrease in the apparent viscosity of the vehicle itself when the flavoring agent was added, and from the initial time to the final time of the analysis there was also a decrease in viscosity. However, in the sample containing Suspendit® and the mint flavoring agent, there was a decrease in viscosity when adding the mint flavoring agent, but there the preparation remained stable from the initial time to the final period of the analysis.

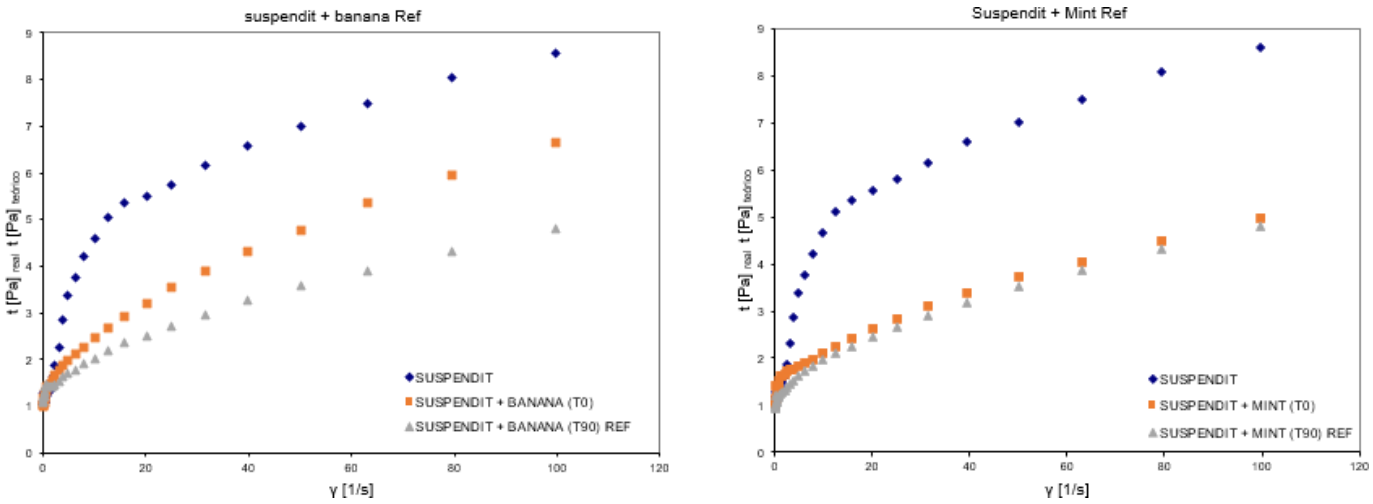


Figure 47 *Flow curve of Suspendit® vehicle preparations plus banana (left) and mint (right) flavoring agent.*

Using the Herschel-Bulkley model (Table 27 and 28), it is possible to confirm through the values of K, n and $t_{0,}$ that for Suspendit® with banana, besides the difference when the flavoring agent is added to the vehicle, there are also differences over the time of the analyses. For Suspendit® with mint flavoring, there is a difference in viscosity only when the flavoring is added to the vehicle. After addition, the mixture remains with stable values.

Table 27 *Herschel-Bulkley model of Suspendit® vehicle preparation flavored with banana at refrigerated temperature*

	Suspendit® + Banana - Herschel-Bulkley		
	Suspendit® (mean ± SD)	T0 (mean ± SD)	T90 (mean ± SD)
K (Pa.s^n)	2.000 ± 0.000	0.455 ± 0.000	0.200 ± 0.000
n	0.312 ± 0.000	0.553 ± 0.002	0.633 ±0.005
t_o (Pa)	0.100 ± 0.000	0.833 ± 0.047	1.033 ± 0.094
R^2	0.974 ± 0.005	1.000 ± 0.000	0.994 ±0.001

Table 28 *Herschel-Bulkley model of Suspendit® vehicle preparation flavored with mint at refrigerated temperature*

	Suspendit® + Mint - Herschel-Bulkley		
	Suspendit® (mean ± SD)	T0 (mean ± SD)	T90 (mean ± SD)
K (Pa.s^n)	2.000 ± 0.000	0.420 ±0.022	0.420 ± 0.000
N	0.312 ± 0.000	0.494 ± 0.008	0.483 ±0.005
t_o (Pa)	0.100 ± 0.000	0.833 ± 0.125	0.733 ± 0.047
R^2	0.974 ± 0.005	0.979 ± 0.020	0.994 ± 0.002

3.3.1.2.2 Amplitude sweep

Through the figures 48 to 51, it is possible to observe that in the amplitude test no relevant changes were observed for both preparations containing the Syrspend® and Suspendit® vehicle and the strain of 0,3% used throughout the oscillatory testes remains within the linear region.

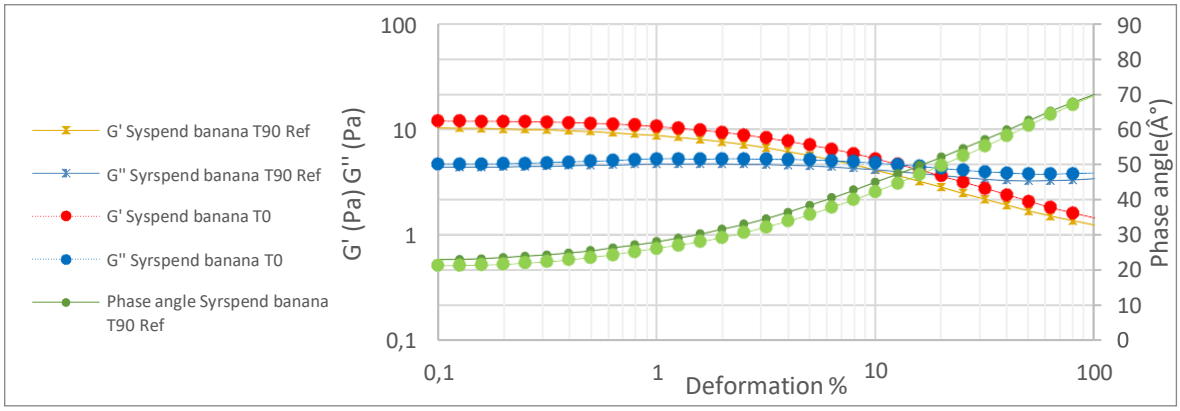


Figure 48 *Amplitude sweep, at times 0 and 90 (refrigerated) days, relative to the mixture of the Syrspend® vehicle with the banana flavoring agent.*

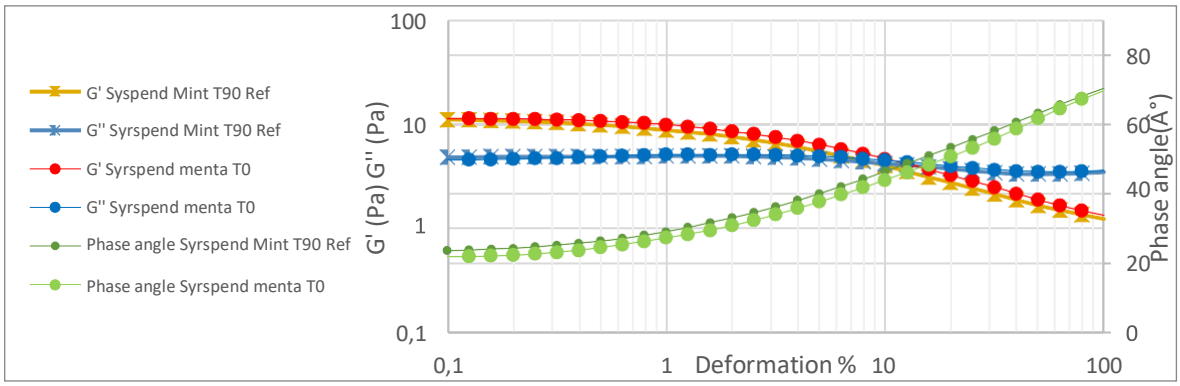


Figure 49 *Amplitude sweep, at times 0 and 90 (refrigerated) days, relative to the mixture of the Syrspend® vehicle with the Mint flavoring agent*

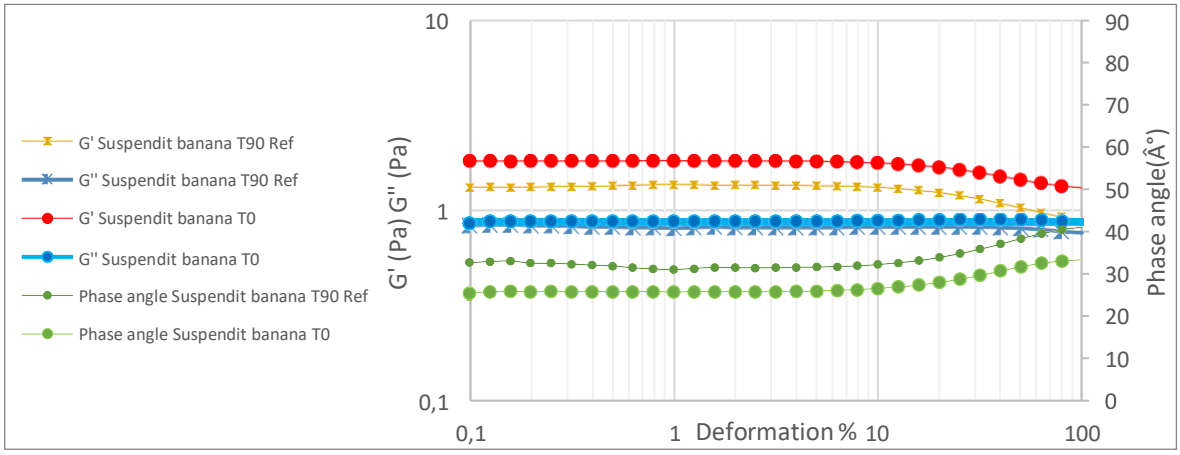


Figure 50 *Amplitude sweep, at times 0 and 90 (refrigerated) days, relative to the mixture of the Suspendit® vehicle with the banana flavoring agent.*

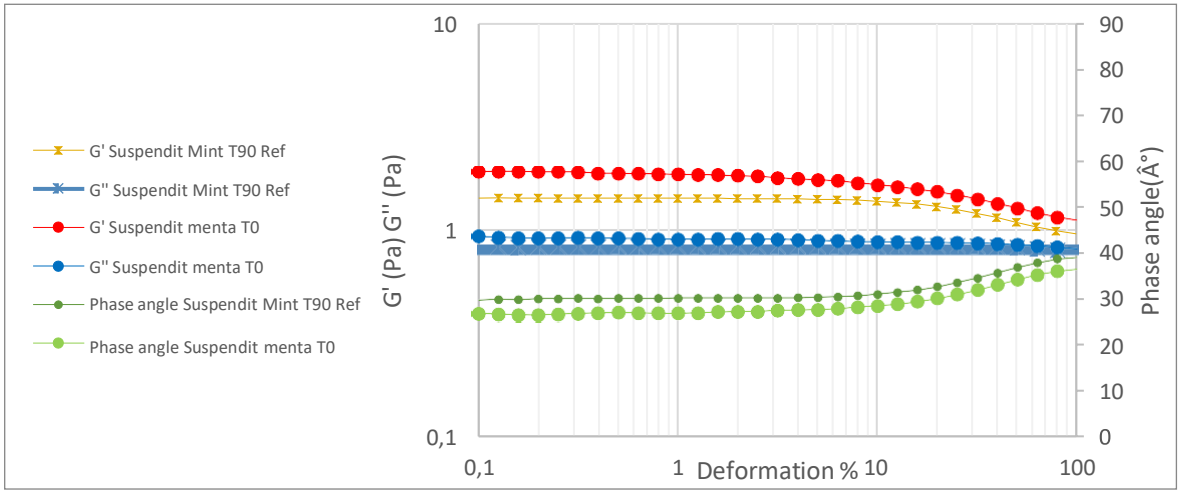


Figure 51 *Amplitude sweep, at times 0 and 90 (refrigerated) days, for mixing the Suspendit® vehicle with the mint flavoring agent.*

3.3.1.2.3 Mechanical spectrum

In Figures 52 to 55, the mechanical spectrums of the preparations with the respective vehicles and flavoring agents, obtained from the frequency sweep test are presented. During the 90 days, G' remained higher than G'' and the phase angle less than 45º, representing a solid-type behavior

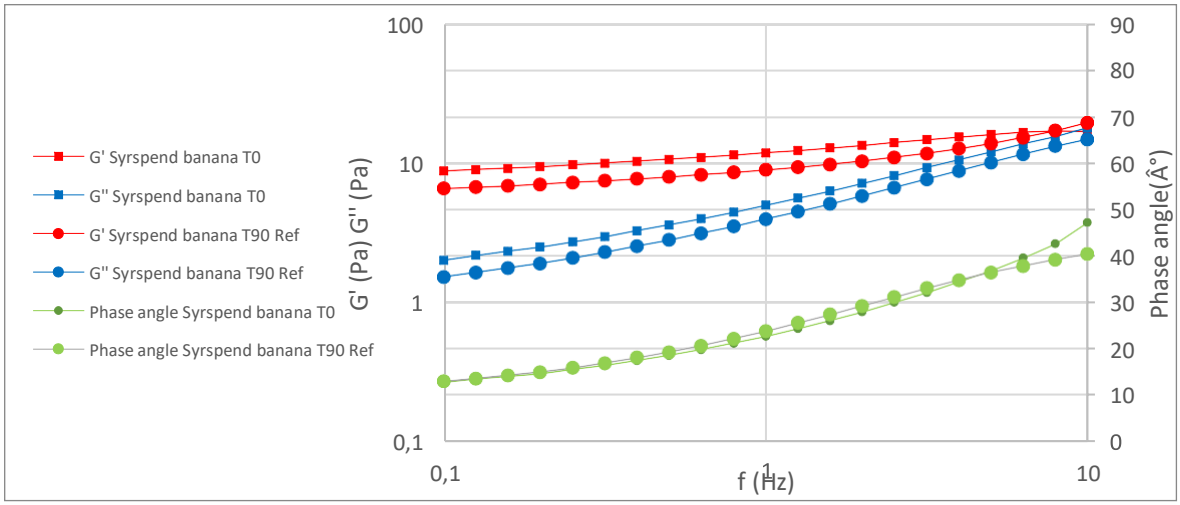


Figure 52 *Mechanical spectrum, at times 0 and 90 days, of the mixture of the Syrspend® vehicle with the banana flavoring agent.*

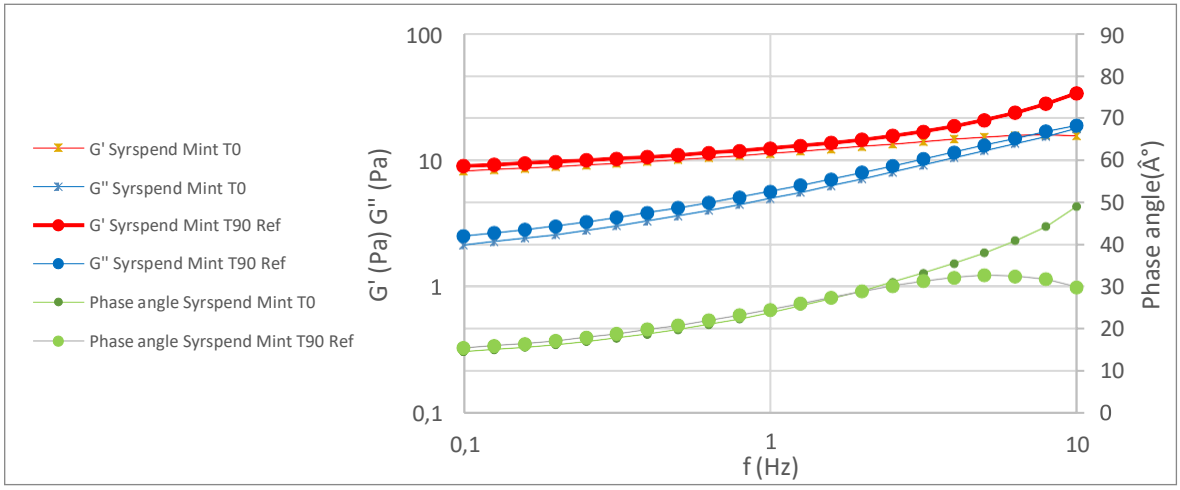


Figure 53 *Mechanical spectrum, at times 0 and 90 days, for the mixture of the Syrspend® vehicle with the mint flavoring agent.*

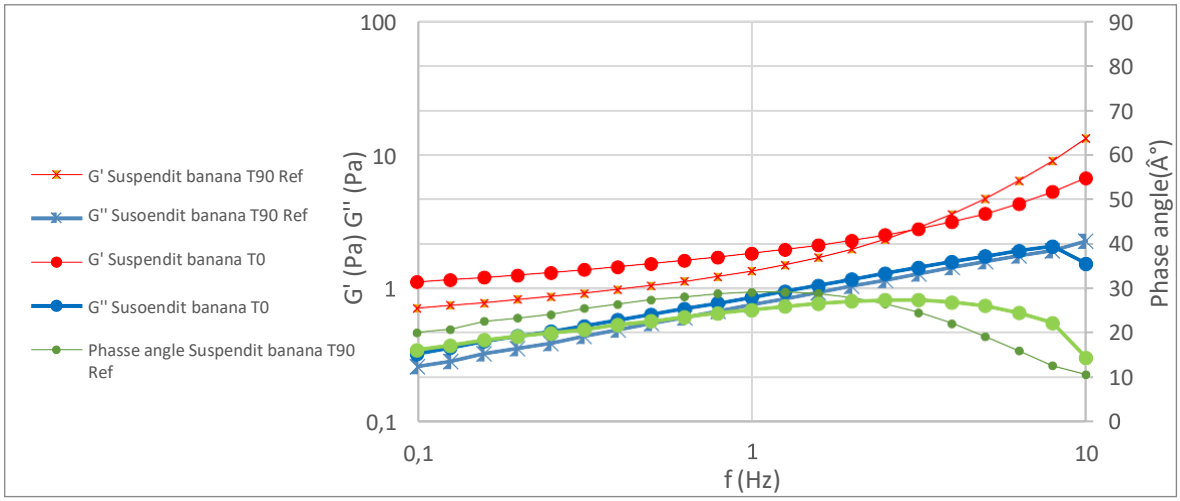


Figure 54 *Mechanical spectrum, at times 0 and 90 days, for the Suspendit® vehicle mixed with the banana flavoring agent.*

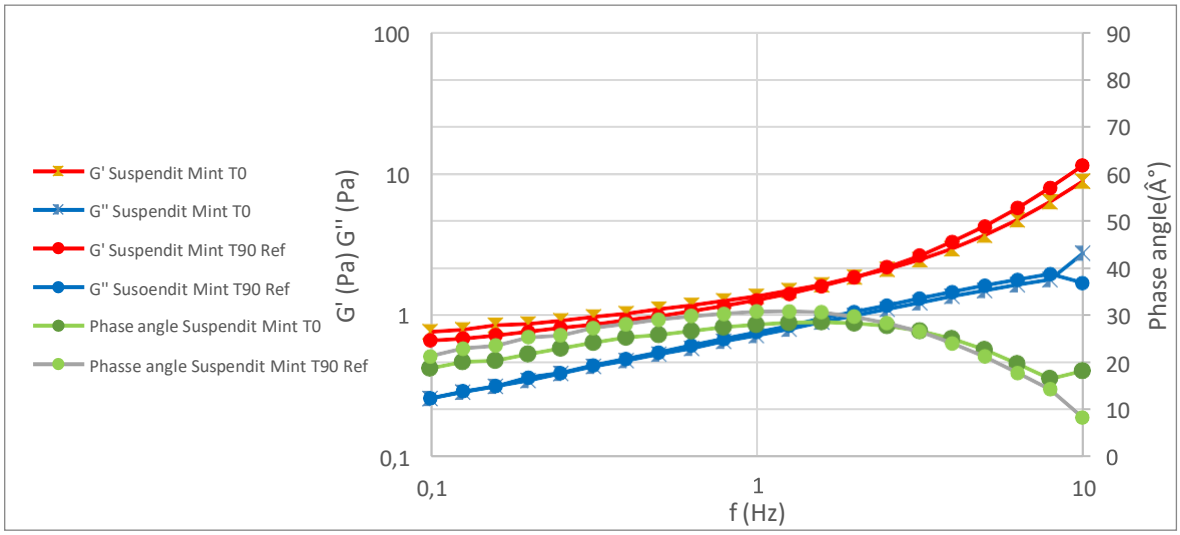


Figure 55 *Mechanical spectrum, at times 0 and 90 days, for the mixture of the Suspendit® vehicle with the mint flavoring agent.*

3.3.1.2.4 Thixotropy

Through the graphs below Figure 56 to 59, it is possible to observe that there was a slight decrease in the apparent viscosity after 90 days for the mixture of Syrspend® with banana. For the mixture of Suspendit® with banana there was also a decrease in viscosity and recovery rate: 55% (T0) and 41% (T90). Both vehicles with mint flavoring agent showed to be more stable in relation to the mixture with banana. However, the Syrspend® vehicle mixtures with the flavoring agents, remained with the non-thixotropic effect with a recovery rate above 90% As for the Suspendit® vehicle mixtures with flavoring agents remained with the thixotropic effect with a recovery rate below 90%.

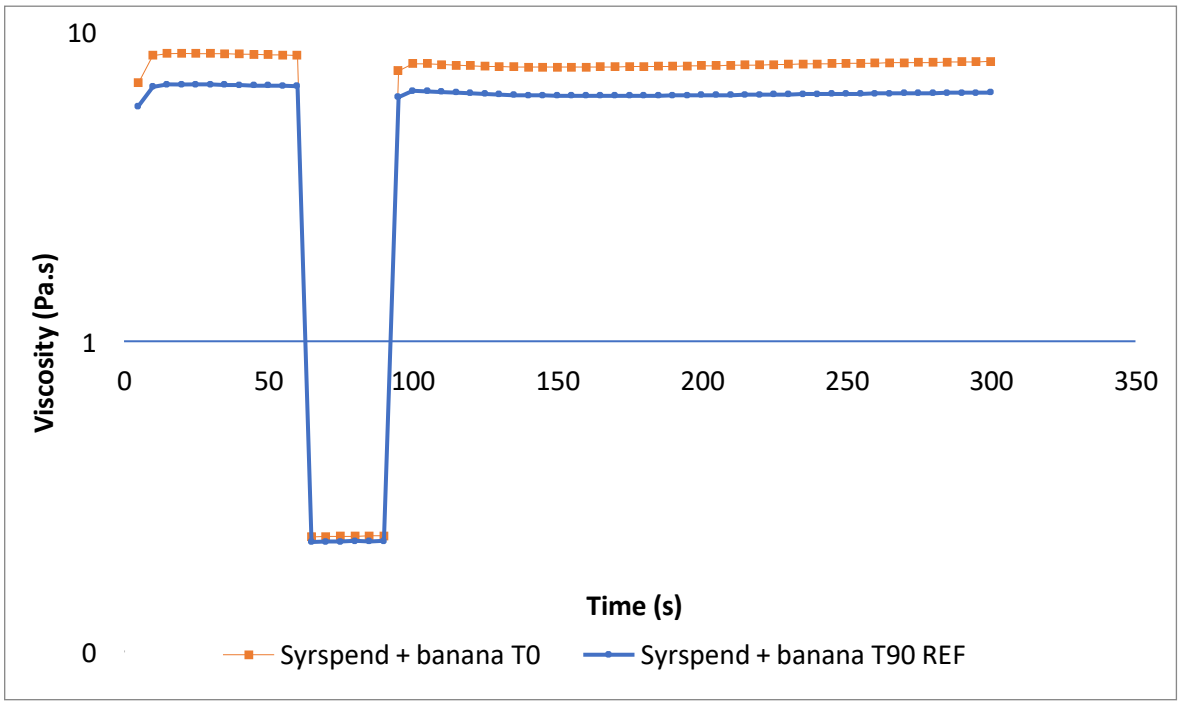


Figure 56 *Thixotropy graph of Syrspend® preparations with banana at time 0 and 90 Ref.*

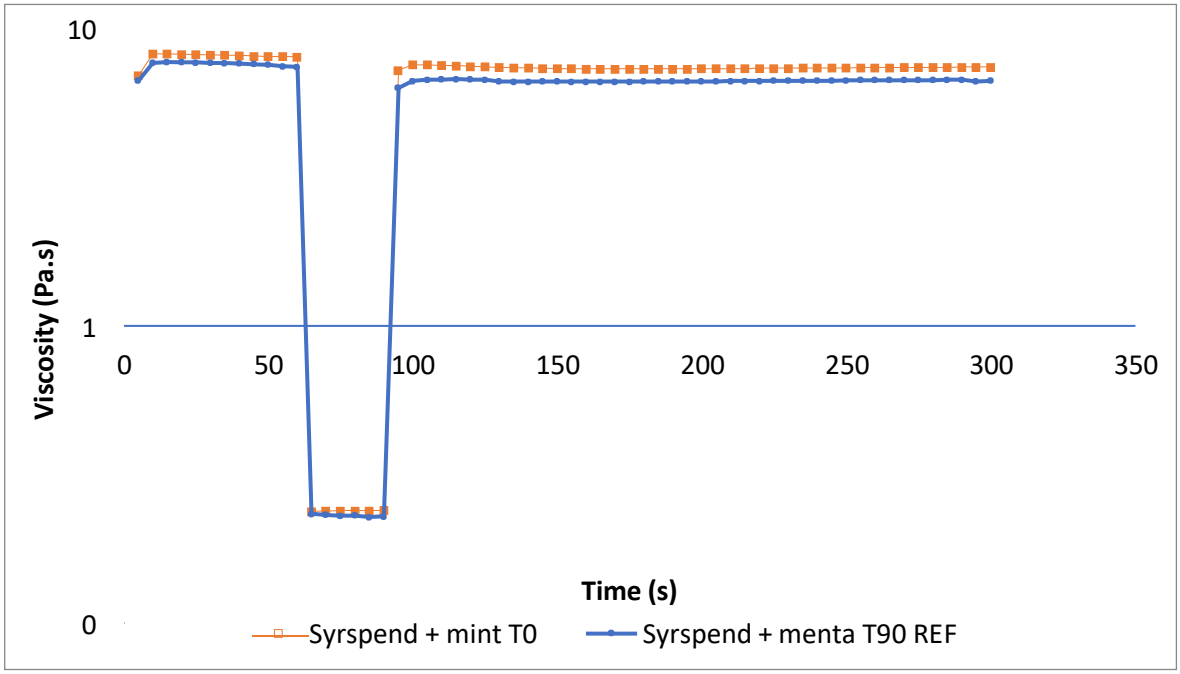


Figure 57 *Thixotropy graph of Syrspend® preparations with mint at time 0 and 90 Ref.*

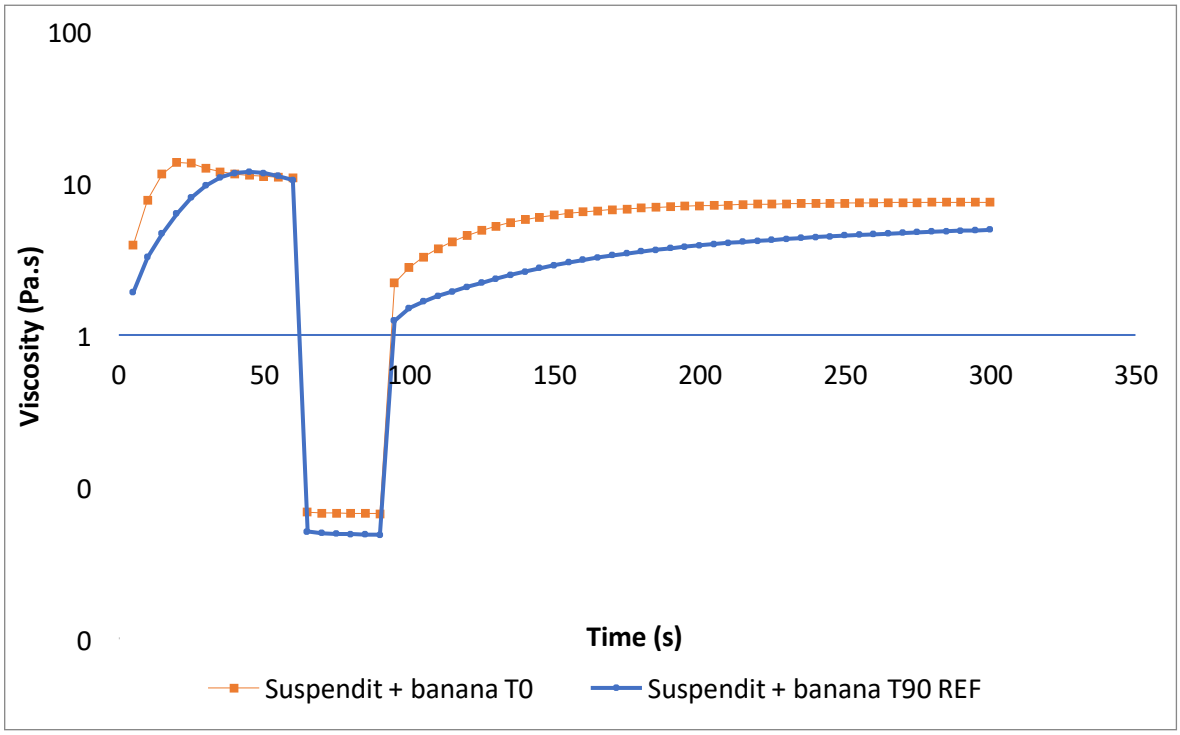


Figure 58 *Thixotropy graph of Suspendit® preparations with banana at time 0 and 90 Ref.*

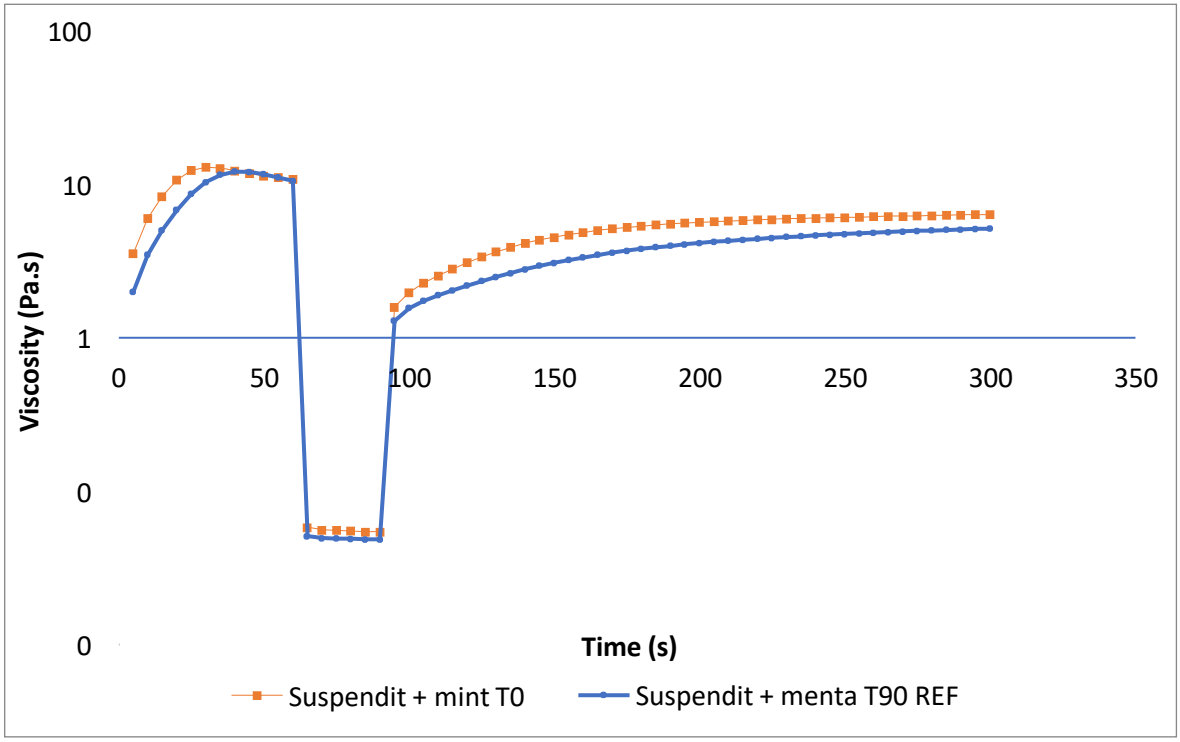


Figure 59 *Thixotropy graph of Suspendit® preparations with mint at time 0 (left) and 90 Ref (right).*

3.3.2 Dosing of volatile compounds in the flavored vehicles

In addition to the stability studies of the flavoring agents in their pure form, the stability studies of the flavored vehicles in the times 0, 30, and 90 were also performed.

3.3.2.1 Optimization of the SPME analysis method

The extraction of the volatile compounds in the mixture of vehicle and flavoring agent was done by the SPME method since direct injection of such a complex mixture was not feasible, nor was the selective extraction of only the flavoring compounds. The solid-phase microextraction technique allows to analyze only the volatile compounds that under optimized conditions of temperature, agitation, ionic strength, and time pass into the headspace of the extraction vial and are adsorbed onto the SPME fiber. This fiber can also have specific characteristics, and there are fibers more adapted to lower or higher molecular weight compounds. In our case, we used a CAR-DVB-PDMS fiber, described as being ideal for volatile and semi-volatile compounds, and therefore adapted to the object of this study. To perform this assay, it was necessary to perform preliminary tests to optimize the method, i.e., to obtain an optimal amount of sample, water, salt, internal standard, temperature, and time to achieve stable results.

For the optimization of the internal standard, a selection was made to know which internal standard would be used for the semi-quantification of the volatile compounds. Two candidates were tested to be chosen as internal standard for the experiments, which were cyclohexanone and nonanal. When the test was performed with cyclohexanone and the sample, it was found that it overlapped with some compounds present in the sample, making its use as an internal standard unfeasible. Then, the chosen standard was nonanal, because it is volatile and does not interact with any other volatile compound present in the sample, that is, it does not overlap with other compounds in the chromatogram. However, it is not highly soluble in water, and therefore it was necessary to use it in a very low concentration and after careful homogenization of the solution when in use.

In addition, the amount of flavored vehicle needed to give enough chromatographic signal for analysis was tested while ensuring that no saturation of the SPME fiber occurred. These tests were performed using the syrup vehicle as a model and then transposed to the other vehicles.

The initial test was performed with sample dilution in water, containing the syrup vehicle and the banana flavoring agent, for the fiber saturation test. The following compounds were selected: isoamyl acetate; limonene; isoamyl isobutanoate; isoamyl isovalerate, and allyl heptanoate. The chromatographic results were converted to equivalents of the internal standard (IS), which is presented in the formula below.

$$Eq.IS = \frac{(Sample\ area\ x\ IS\ mass)}{IS\ area} \quad (2)$$

The figure below (figure 60) is an example of the assays, representing the graph for the compound allyl heptanoate, in relation to the parameters: sample mass and internal standard equivalent for the syrup vehicle and the banana flavoring agent.

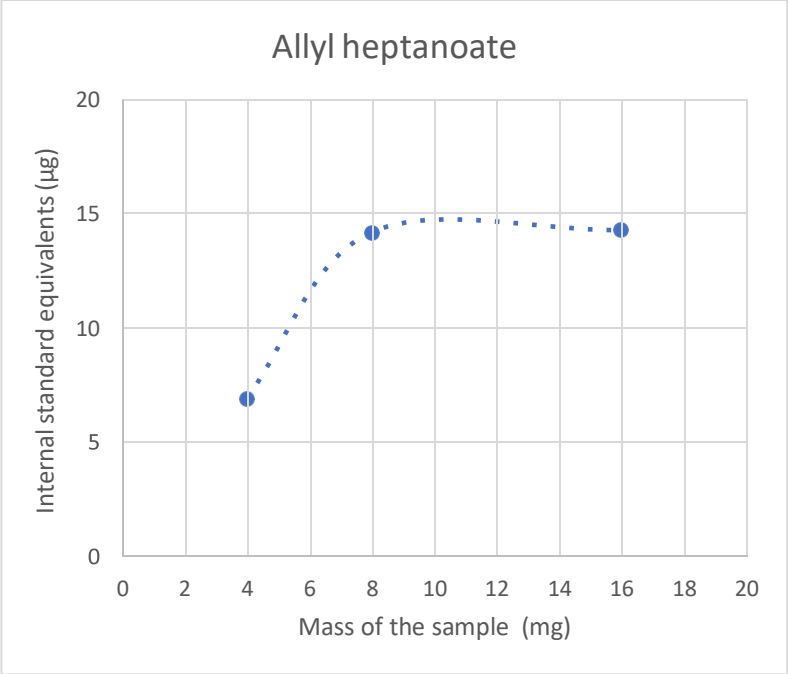


Figure 60 *Graph of Allyl heptanoate acetate compound against sample volume and internal standard equivalents parameters for syrup vehicle and banana flavoring*

As can be seen in the graph for allyl heptanoate, for the mass of 4 to 8 mg there was an increase in the equivalence value of the internal standard. This suggests that the fiber (CAV/DVB/PDMS) used in the SPME is still adsorbing the analyte in question. However, this was not observed in the 16 mg volume. According to Pawliszyn et al., (2012)the adsorption mechanism is of the adsorbent, pore type. Therefore, at a certain concentration of the compounds, all the pores will be saturated no longer having the linearincrease in the signal (98). Thus, it is suggested that for the volume of 16 mg the fiber is already saturated giving a similar signal to that of 8 mg. To evaluate with total certainty, thesaturation point of the fiber, it would be necessary to perform the test by adding more volumes between the 8 and 16 mg volumes. For all the other compounds evaluated for thesaturation test, similar profiles were observed, corroborating that the fiber at the 16 mg point was already saturated.

After this evaluation of the banana flavor, the test was performed for the mint flavor. The volumes for this test were: 1, 2, 4, and 6 mg. The following compounds were selected: eucalyptol, menthol, and levomenthol. The response chosen was the same as for the banana flavouring (equation 2).

The figure below represents the graph relating to the compound levomenthol, in relation to the parameters: sample mass and equivalent of the internal standard of the syrup vehicle and the mint flavouring agent.

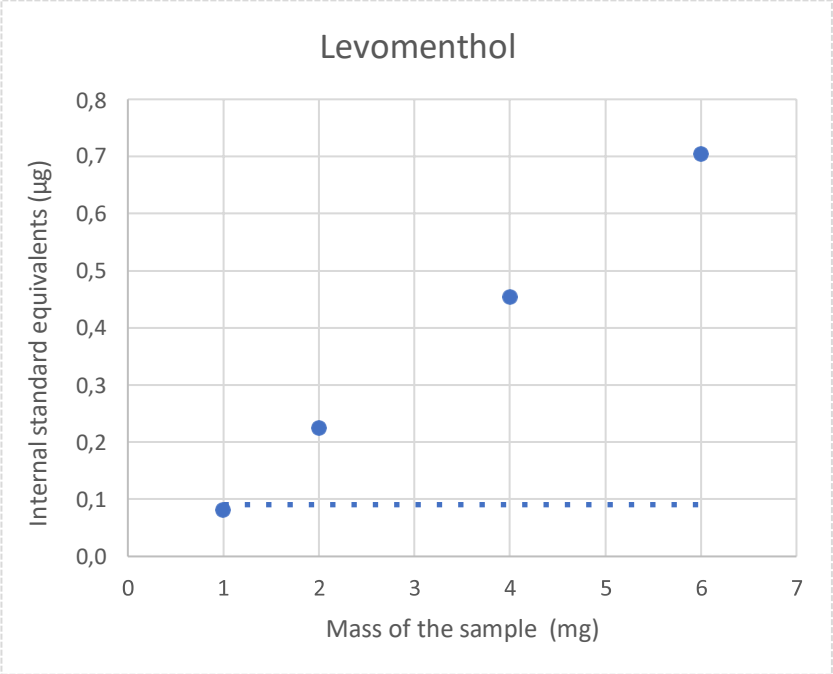


Figure 61 *Graph of Levomenthol compound against sample volume and internal standard equivalents parameters for syrup vehicle and mint flavoring*

For all the sample masses used in the test, there was a proportional increase in the response in equivalent to the internal standard.

As can be seen in the graph, for the compound Levomenthol, after the analysis of the samples presented in the graph (Figure 61), it is verified that the saturation of the fibers was not reached, because there was a linear increase in the response (equivalence of the internal standard). In order to evaluate the fiber saturation point, for this compound, it would be necessary to perform more tests by adding other masses greater than 6 mg, until the fiber saturation level was reached.

As for the banana flavouring, it was observed that saturation was reached at the mass of 8 mg, and as there was no fiber saturation for the mixture containing the mint flavouring, it was decided to increase the volume of sample addition for analysis in order to standardize the same amount of sample for the two types of flavorings.

To help in the choice of the sample mass to be used, some tests were performed on the sample injection mode in the gas chromatograph. The two modes evaluated were: split mode (10:1), with the intention of diluting the sample in the equipment itself instead of injecting it totally, and splitless mode (total injection). After obtaining the chromatograms for the two modes of injection, it was verified that the split mode presented better peak resolution, increased linearity range (data nor shown) and that the homogeneity of the sample was not compromised, and was chosen to proceed with the experiments.

After all these experiments, including checking the peak intensities of the compounds in the chromatograms, it was decided that the sample mass could be increased

to 60 mg, with a split 10:1. This decision was made to standardize a single sample mass for both the banana flavouring mixture and the mint flavouring mixture, as the added valueof the mixture would ensure that for both, this value would not result in saturation of the fiberand peaks of the compounds in the chromatogram had good resolution.

After all optimizations, the final analysis parameters were: 60 mg of sample, 100 µl of internal standard solution (nonanal at 50 mg/L), 1.9 ml of cold ultrapure water, 100 mg of NaCl (salting out effect), and the magnet (to mix the components present in the bottle and help to release the compounds). All these components were packed in a glass vial with a septum screw stopper. The flask was exposed for 25 minutes in a magnetic stirrer with heating at a temperature of 45ºC (equilibrium of the compounds in the headspace), followed by exposure of the fiber for further 20 minutes. And finally, the fiber was placed in the GC-MS injector, where it was exposed for 5 minutes at 250ºC to release the compounds.

After defining the conditions, "blanks" were performed with the syrup vehicle, Syrspend®, and Suspendit® in order to observe whether there would be interference of the matrix in the analysis. The blank was performed in the same way as the vehicle samples plus the addition of the flavoring agent, that is, by SPME and with the addition of the internal standard (corresponding to the peak in the chromatogram - Figure 62). The internal standard was added to be able to make a comparison between the samples analyzed.

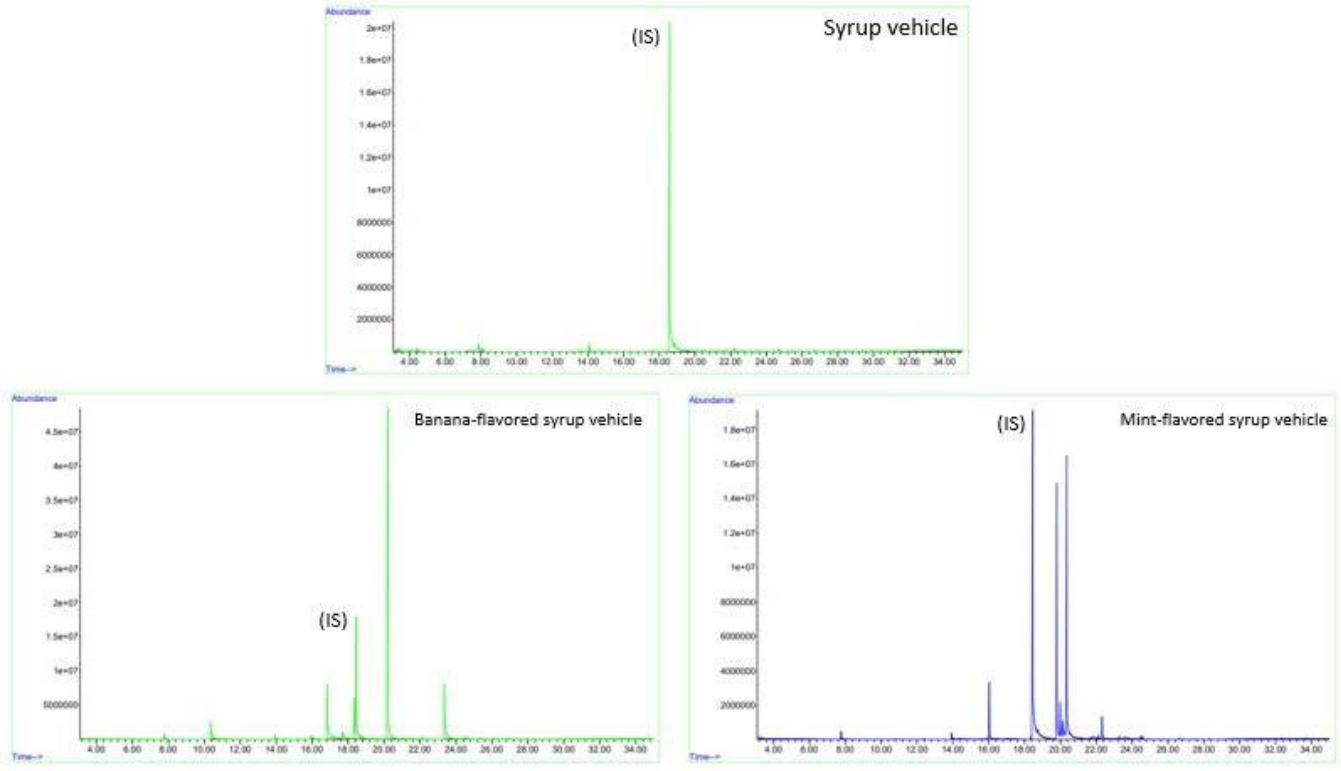


Figure 62 *Representative chromatograms of the syrup vehicle and samples.*

Comparing the chromatograms of the blank for the syrup vehicle, the syrup plus banana flavoring agent and the syrup plus mint flavoring agent, it is possible to confirm that there was no interference of the matrix in the volatile profile. The same was done for the other tested vehicles that were Syrspend® and Suspendit® confirming that there was no matrix interference.

To analyze the figure of merit repeatability, the equivalence of the internal standard was evaluated for each sample in triplicate, with the coefficient of variation (CV%) as the response. Table 29 shows the results of the coefficient of variation of the mean internal standard equivalent of the triplicates of each sample flavored with the banana flavouring agent, comparing the three vehicles analyzed.

Table 29 *Results of the coefficient of variation of the internal standard equivalents of the triplicates of each banana-flavored sample, comparing the three vehicles analyzed*

	CV% of internal standard equivalents
Syrup	17.6%
Syrspend®	2.0%
Suspendit®	6.5%

As acceptance criteria, values below 20% of coefficient of variation are within the statistical error and are considered valid. Observing Table 29, it can be seen that all the values found are within the acceptance criteria. Analyzing the data for each vehicle, it can be seen that for the syrup there was greater instability in the repeatability of the samples, followed by the Suspendit® vehicle. As for the Syrspend® vehicle, it is observed that it was the vehicle which obtained most similarity among the triplicate, that is, they were more repeatable.

It was also decided to evaluate the repeatability of the individual compounds of each sample which were performed in triplicate, with the coefficient of variation as the response. Table 30, presents the CV% results of the mean of the relative abundance values of each compound present in the mixture of the vehicles with the banana flavoring agent.

Table 30 *Results of coefficient of variation of the mean relative abundance of triplicates of each mixture containing banana syrup and flavoring.*

	Simple syrup	Syrspend®	Suspendit®
Isoamyl acetate	9%	3%	6%
Limonene	19%	2%	2%
Isoamyl isobutanoate	15%	2%	1%
Allyl hexanoate	2%	1%	2%
Isoamyl isovalerate	3%	1%	1%
Allyl heptanoate	3%	1%	1%
Benzyl butyrate	2%	3%	2%
α-Ionone	2%	5%	7%
average	**7%**	**2% 2%3%**	**3%2%**

2%

Through table 30, it is possible to observe that the syrup, as well as in the semiquantitative analysis with the internal standard, presented the greater variation, with higher CV% when compared to the Syrspend® and Suspendit® vehicles.

For Syrspend® and Suspendit®, it was observed that the CVs% were low, indicating that the analyses were more repetitive. Thus, with respect to repeatability under this analytical technique, the vehicle that showed greater stability with the banana flavoring was Syrspend®.

For the mint flavouring, the coefficient of variation was also evaluated for both the total volatile amounts in internal standard equivalents and the relative abundance of each volatile for each sample. Table 31 shows the results of the coefficient of variation of the total internal standard equivalents of the triplicates of each sample of the vehicles containing the mint flavouring, comparing the vehicles analyzed.

Table 31 *Results of the coefficient of variation of the internal standard equivalence of the triplicates of each mint-flavored sample, comparing the three vehicles analyzed*

	CV% of internal standard equivalents
Syrup	7,9%
Syrspend®	2,3%
Suspendit®	8,1%

With the same acceptance criteria (CV <20%), it is possible to observe that the methodology is adequate for the analysis of the three vehicles. Analyzing the data for each vehicle it is possible to say that the one that presented the lowest CV%, that is, presented

more repeatable results was, once again, the Syrspend vehicle containing mint flavor. However, it is possible to conclude that for both vehicles the analyses were repeatable.

It was also decided to evaluate the repeatability of the individual compounds in the mint flavored mixtures, performed in triplicate. Table 32, presents the CV% results of the mean of the relative abundance values of each compound present in the mixture of the vehicles with the mint flavoring agent.

Table 32 *Results of coefficient of variation of the mean relative abundance of triplicates of each mixture containing banana syrup and flavouring.*

	Simple syrup	Syrspend®	Suspendit®
Limonene	20%	4%	14%
Eucalyptol	9%	6%	4%
Menthone	1%	1%	2%
Levomenthol	2%	1%	2%
Pulegone	11%	3%	15%
Carvone	30%	7%	12%
Isomenthol acetate	20%	2%	3%
Caryophyllene	26%	3%	2%
average	**15%**	**3%**	**7%**

Through Table 32, it is possible to observe that the simple syrup, once again, presented a greater variation. Regarding the vehicles Syrspend® and Suspendit®, both showed acceptable repeatability (CV%<20%) for most compounds. Thus, indicating that they are vehicles with a higher repeatability rate. But, as in banana, the Syrspend® vehicle remained more stable in the repeatability level and showed a lower CV% compared to the Suspendit® vehicle.

3.3.2.2 Vehicle with flavoring agent at room temperature

3.3.2.2.1 Evaluation of the stability of compounds released from banana flavoring in the mixture with Syrup, Syrspend® and Suspendit® vehicles

After analyzing the interference of the matrix, it was possible to carry out stability studies of the formulations. The stability was performed at after preparation and then at 30, and 90 days for the mixtures of vehicles: syrup, Syrspend®, and Suspendit®. The stability test was performed for all the vehicles mentioned above together with the banana and mint flavorings.

During the analyses, it was possible to verify that the internal standard had an irregular behavior over the sampling time. Thus, it did not prove to be the best option to

ensure the comparability of results over time, allowing only intra-day comparisons within the same set of samples. Therefore, comparisons over time were made with the percentage of relative abundance of the volatile compounds, i.e., by the ratio of the area of the analyzed compound and the total area of the compounds excluding the area of the IP. For intra-day quantification purposes, the PI area was used, allowing the repeatability of the analyses to be compared from the coefficient of variation (CV%), as discussed earlier.

At first, it is possible to analyze by observing the graphs how the mixtures of the vehicles with the flavoring agents behaved. The chromatograms of figure 66 correspond to the three vehicles (Syrup - left; Syrspend® - center; Suspendit® - right) containing the banana flavoring agent, for the times: T0 (upper), T30 (center), and T90 (down).

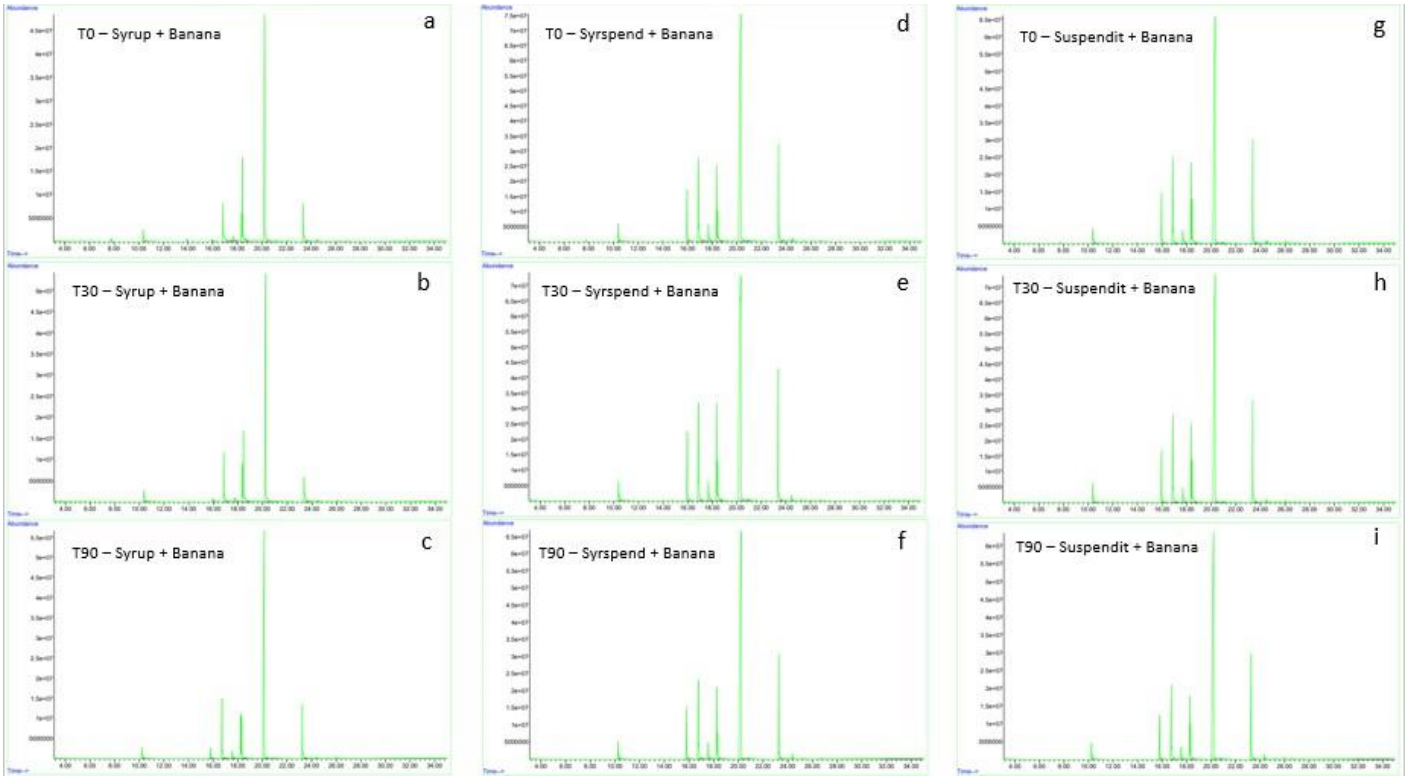


Figure 63 *representative chromatograms of banana flavoring agent with the carriers: syrup, Syrspend® and Suspendit®*

In the chromatograms represented in Figure 63 it can be seen that for the syrup vehicle (Figure 63a, b, c, all left) there was the detection of fewer compounds when compared to the Syrspend® (Figure 63d, e, f) and Suspendit® (Figure 63g, h, i) vehicles. Between Syrspend® and Suspendit® there is a similarity in the diversity of compounds.

To make a more detailed comparison, an average of the relative abundance of the times 0, 30 and 90 and a statistical ANOVA analysis were performed. Tables 34, 35 and 36

present the results of the mean relative abundance and standard deviation of the syrup, Syrspend® and Suspendit® vehicles with the banana flavoring, respectively.

Table 33 *Compounds detected in Syrup, Syrspend®, Suspendit® vehicles flavored with banana compared to pure banana flavor*

	Syrup	Syrspend®	Suspendit®
Ethyl Acetate	-	-	-
Propylene Glycol	-	-	-
Isoamyl acetate	x	x	x
Limonene	x	x	x
Benzyl alcohol	x	-	-
Allyl hexanoate	x	x	x
Isoamyl Isovalerate	x	x	x
Allyl heptanoate	x	x	x
Benzyl Butyrate	x	x	x
Eugenol	-	-	-
α-Ionone	x	x	x
γ-Undecanolactone	-	-	-
Vanillin propylene glycol acetal	-	-	-

Based on the tables presented below it is possible to observe that even for some compounds that presented relatively similar averages, there were statistically relevant differences between times 0, 30, and 90 for some of the compounds analyzed.

Table 34 *Results of the mean relative abundance and standard deviation of the banana-flavored syrup vehicles*

Syrup + Banana				
Compounds	T0 (% ± SD)	T30 (% ± SD)	T90 (% ± SD)	*p*-value
Isoamyl acetate	4.11 ± 0.37	5.22 ± 0.79	2.73 ± 0.33	**0.010**
Limonene	1.30 ± 0.25	2.34 ± 0.06	2.79 ± 0.24	**0.001**
Isoamyl Isobutanoate	12.06 ± 1.81	11.62 ± 0.44	11.84 ± 0.1	0.919
Benzyl alcohol	0.09 ± 0.05	0.05 ± 0.04	n.d.	0.325
Allyl hexanoate	2.33 ± 0.05	2.64 ± 0.12	2.12± 0.11	**0.006**
Isoamyl Isovalerate	5.79 ± 0.18	6.88 ± 0.17	6.65± 0.10	**0.001**
Allyl heptanoate	61.27 ± 1.89	59.87 ± 1.11	64.40 ± 0.42	**0.031**
2-Octynoic acid, methyl ester	n.d.	n.d.	0.06± 0.01	**0.000**
Menthyl acetate	n.d.	n.d.	0.02 ± 0.02	0.422
Benzyl Butyrate	12.74 ± 0.25	9.76 ± 0.65	8.82 ± 0.26	**0.000**
α-Ionone	0.34 ± 0.01	0.36 ± 0.01	0.57 ± 0.08	**0.006**

Table 35 *Results of the mean relative abundance and standard deviation of the Syrspend® vehicles with banana flavoring*

Syrspend® + Banana				
Compounds	T0 (% ± SD)	T30 (% ± SD)	T90 (% ± SD)	*p*-value
Isoamyl acetate	2.33 ± 0.28	2.18 ± 0.14	2.30 ± 0.07	0.711
Limonene	6.20 ± 0.25	6.69 ± 0.27	5.43 ± 0.14	**0.004**
Isoamyl Isobutanoate	13.24 ± 0.66	12.46 ± 0.32	11.65 ± 0.18	**0.031**
Allyl hexanoate	2.08 ± 0.06	2.14 ±0.02	1.84 ± 0.02	**0.001**
Isoamyl Isovalerate	7.26 ± 0.3	7.61 ± 0.26	6.78 ± 0.08	**0.032**
Allyl heptanoate	58.04 ± 0.7	57.84 ± 0.48	61.98 ± 0.23	**0.000**
2-Octynoic acid, methyl ester	0.43 ± 0.04	0.39 ± 0.04	0.22 ± 0.04	**0.007**
Benzyl Butyrate	9.92 ± 0.51	10.16 ± 0.56	9.27 ± 0.31	0.231
α-Ionone	0.49 ± 0.02	0.54 ± 0.03	0.54 ± 0.03	0.170

Table 36 *Results of the mean relative abundance and standard deviation of the Suspendit® vehicles with banana flavoring*

Suspendit® + Banana				
Compounds	T0 (% ± SD)	T30 (% ± SD)	T90 (% ± SD)	*p*-value
Isoamyl acetate	2.23 ± 0.28	2.70 ± 0.17	2.63 ± 0.46	0.352
Limonene	5.97 ± 0.09	6.08 ± 0.12	5.39 ± 0.31	**0.030**
Isoamyl Isobutanoate	12.07 ± 0.44	12.51 ± 0.14	12.06 ± 0.55	0.504
Allyl hexanoate	2.18 ± 0.02	2.26 ± 0.04	2.07 ± 0.05	**0.008**
Isoamyl Isovalerate	7.28 ± 0.27	7.23 ± 0.05	6.90 ± 0.48	0.474
Allyl heptanoate	59.17 ± 0.34	58.85 ± 0.17	59.07 ± 1.68	0.947
2-Octynoic acid, methyl ester	0.37 ± 0.04	0.33 ± 0.03	0.25 ± 0.07	0.120
Benzyl Butyrate	10.24 ± 0.88	9.61 ± 0.24	11.02 ± 0.69	0.181
α-Ionone	0.48 ± 0.33	0.43 ± 0.03	0.61 ± 0.04	**0.003**

Of the 13 compounds found in the GC-MS analysis for the banana flavoring in its pure form, 8 of these were found in the mixture of syrup and banana. The compounds that were not detected included ethyl acetate, propylene glycol, eugenol, γ-undecanolactone, and Vanillin propylene glycol acetal. In addition, in this same mixture, for the 90 days time point two more compounds that had not been detected in the banana flavoring agent, 2-Octynoic acid, methyl ester, and menthyl acetate were present with a low relative abundance ratio (0.1± 0.01 and 0.02 ± 0.02, respectively). This may indicate that the values may be below the detection limit of the analytical technique analyzed or that they are in fact degradation products that could increase their concentrations with times longer than T90.

To validate the first hypothesis, a quantitative analysis using analytical standards of the product in question would be necessary. It is important to note that the compounds analyzed by SPME correspond to those that, under the physicochemical conditions of the sample (vehicle with flavoring agent), temperature and agitation, have the ability to be volatilized and have an affinity with the SPME fiber to allow their concentration. Thus, the results of a direct flavoring analysis, as performed in the initial phase of flavoring characterization, cannot be compared to the results obtained by SPME. However, the SPME results obtained from optimized conditions, such as those previously tested, can be compared with each other, allowing for verifying not only the stability of the compounds over time, but also their relative potency in each vehicle (the amount that the vehicle allows to be released under the test conditions). Regarding the possibility that they are degradation products, it would be important to continue the test for a longer time, in order to verify if it is a degradation of the flavoring agent itself, probably present in all three formulations, or the interaction of the flavoring agent with the chemical environment of the vehicle.

For the Syrspend®, 7 compounds out of the 13 presents in the banana flavoring in its pure form were detected, as presented in table 33. Moreover, out of the two compounds that appeared at 90 days in the syrup, one of them (2-Octynoic acid, methyl ester) appeared in the Syrspend® throughout all the analysis times also with a low relative abundance rate. It is hypothesized that these compounds were not detected in the pure flavoring because their concentrations were too low and the injection of the flavoring agent in its pure form was done directly, without pre-concentration of these compounds by SPME.

Suspendit® vehicle behaved similarly to Syrspend® base, showing the samepattern and proportions of compounds.

Analyzing the data of the three vehicles by the results of ANOVA, the mixture of syrup with banana presented 8 out of 11 compounds with statistically significant differences ($p<0.05$). In relation to the Syrspend® vehicle, 6 of the 9 compounds showed statistical differences. However, for the Suspendit® vehicle, only 3 of the 9 compounds showed statistical differences. This leads us to understand that the Suspendit® vehicle with the addition of banana flavoring agent presents greater stability of volatile compounds compared to the other two vehicles. However, all the variation are minor, without a clear identification of signs of degradation of the aroma compounds.

On the other hand, it is interesting to observe that, although using the same proportion of vehicle and aromatizing agent, the relative proportion of the peaks are difference between the vehicles. This occurs because of the interaction of the vehicles with the aroma compounds, and the “release” occurring at the sampling temperature (45ºC). Limonene, for instance, represents around 6% of the chromatographic areas in Suspendit

and Syrspend, while in syrup it represents just 1%. On the opposite way, isoamyl acetate was higher in syrup against the commercial vehicles. Although not representing a true perception of the aroma when being ingested by the patient, it can be an indication that the true perception of the aroma can differ based on the vehicle being used, an issue that could be explored in the future.

3.3.2.2.2 Evaluation of the stability of compounds released from mint flavoring in the mixture with the following vehicles: syrup, Syrspend® and Suspendit®

All the statistical tests for the mixture containing mint flavoring were done according to the banana analysis.

The chromatograms in Figure 64 correspond to the three vehicles (Syrup - left; Syrspend® - center; Suspendit® - right) containing mint flavoring, for the times: T0 (upper), T30 (center) and T90 (down).

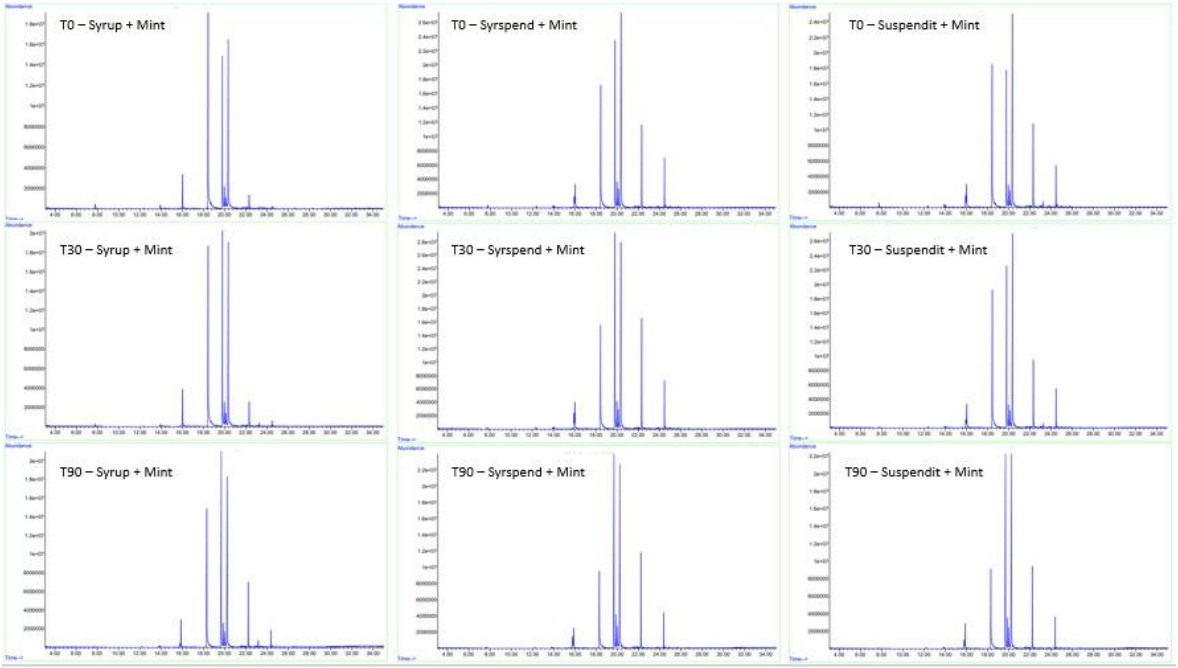


Figure 64 *representative chromatograms of mint flavoring agent with the carriers: syrup, Syrspend® and Suspendit®*

In the chromatograms represented in figure 64 it was observed that in relation to the detection of the compounds, the same happened to the banana, i.e., the syrup presented fewer compounds in relation to the Syrspend® and Suspendit® vehicles. And between Syrspend® and Suspendit®, the chromatogram profiles are similar, i.e., a high homogeneity between these vehicles is perceptible over time, thus demonstrating the stability of the preparations.

The table below represents a comparison between the pure flavoring agent and when added to the vehicles under study.

Table 37 *Compounds detected in the vehicles Syrup, Syrspend®, Suspendit® compared to pure mint flavoring.*

	Syrup	Syrspend®	Suspendit®
Propylene Glycol	-	-	-
Limonene	X	X	X
Eucalyptol	X	X	X
Menthone	X	X	X
Levomenthol	X	X	X
Pulegone	X	X	X
Carvone	X	X	X
Isomenthol acetate	x	X	X
Benzyl Butyrate	-	-	-
Caryophyllene	X	X	X
Vanillin	-	-	-
β-copaene	-	-	-
Caryophyllene oxide	-	-	-
Triethyl citrate	-	-	-
Vanillin propylene glycol acetal	-	-	-

For a more detailed comparative analysis a relative rate average of the times 0, 30, and 90 and a statistical ANOVA analysis were performed for better understanding. Tables 38, 39, and 40 present the results of the average relative abundance and standard deviation of the syrup, Syrspend® and Suspendit® vehicles with mint flavoring, respectively.

Table 38 *Results of the mean relative abundance and standard deviation of the mint-flavored syrup vehicles*

Syrup + Mint				
Compounds	T0 (% ± SD)	T30 (% ± SD)	T90 (% ± SD)	*p*-value
Limonene	n.d.	n.d.	0.89 ± 0.18	**0.000**
Eucalyptol	7.92 ± 0.36	8.21 ± 0.17	5.62 ± 0.52	**0.001**
Isoamyl isovalerate	0.09 ± 0.12	n.d.	0.04 ± 0.05	0.569
Menthone	38.70 ± 1.42	42.82 ± 0.41	41.37 ± 0.63	**0.012**
Levomenthol	50.30 ± 1.84	44.02 ± 0.85	42.51 ± 1.08	**0.002**
Pulegone	n.d.	n.d.	0.43 ± 0.05	**0.000**
Carvone	n.d.	n.d.	0.65 ± 0.20	**0.002**
Isomenthol acetate	n.d.	n.d.	6.86 ± 1.41	**0.008**
Caryophyllene	0.36 ± 0.01	0.71 ± 0.08	1.62 ± 0.42	**0.006**

Table 39 *Results of the mean relative abundance and standard deviation of the Syrspend® vehicles with mint flavoring*

Syrspend® + Mint				
Compounds	T0 (% ± SD)	T30 (% ± SD)	T90 (% ± SD)	*p*-value
β-Pinene	0.38 ± 0.03	0.41 ± 0.02	0.19 ± 0.01	**0.000**
o-Cymene	n.d.	0.19 ± 0.02	0.20 ± 0.01	**0.000**
Limonene	2.47 ± 0.25	3.47 ± 0.27	3.27 ± 0.14	**0.01**
Eucalyptol	4.438 ± 0.03	5.02 ± 0.03	3.93 ± 0.22	**0.000**
γ-Terpinene	n.d.	0.12	0.21 ± 0.1	**0.038**
Isoamyl Isovalerate	0.13 ± 0.01	n.d.	0.06 ± 0.05	0.216
Menthone	29.91 ± 0.59	37,59 ± 0,41	37.33 ± 0.53	**0.000**
Levomenthol	45.56 ± 1.13	38.95 ± 1.37	37.79 ± 0.39	**0.001**
Pulegone	0.38 ± 0.04	0.24 ± 0.003	0.28 ± 0.01	**0.005**
Carvone	0.75 ± 0.17	0.37 ± 0.02	0.67 ± 0.05	**0.022**
Isomenthol acetate	10.48 ± 0.65	13.72 ± 0.77	11.87 ± 0.24	**0.005**
Caryophyllene	5.60 ± 0.25	n.d.	3.79 ± 0.1	**0.000**
Humulene	n.d.	n.d.	0.15 ± 0.01	**0.000**

Table 40 *Results of the mean relative abundance and standard deviation of the Suspendit® vehicles with mint flavoring.*

Suspendit® + Mint				
Compounds	T0 (% ± SD)	T30 (% ± SD)	T90 (% ± SD)	*p*-value
β-Pinene	0.85 ± 0.1	0.68 ± 0.07	0.40 ± 0.04	**0.030**
o-Cymene	n.d.	n.d.	0.08 ± 0.004	0.080
Limonene	2.81 ± 0.47	1.82 ± 0.25	1.65 ± 0.29	**0.032**
Eucalyptol	4.12 ± 0.62	4.97 ± 0.19	3.99 ± 0.76	0.262
Menthone	25.57 ± 1.71	32.79 ± 0.54	36.29 ± 1.90	**0.001**
Levomenthol	48.79 ± 2.11	42.47 ± 0.85	43.20 ± 0.42	**0,006**
Pulegone	0.34 ± 0.03	0.32 ± 0.05	0.36 ± 0.004	0.570
Carvone	0.57 ± 0.08	0.39 ± 0.05	0.78 ± 0.18	**0,045**
Isomenthol acetate	10.92 ± 0.81	8.93 ± 0.28	8.75 ± 1.31	0.096
Caryophyllene	6.03 ± 0.85	4.71 ± 0.11	4.18 ± 0.85	0,088
Humulene	n.d.	n.d.	0.33 ± 0.21	0,054

A similar analysis with the banana flavoring can be performed with the vehicle mixtures with the mint flavoring. Of the 15 compounds found in the GC-MS analysis of the pure mint flavoring, 8 were found in the mint syrup mixture. Propylene glycol, benzyl butyrate, Vanillin, β-copaene, caryophyllene oxide, triethyl citrate, and Vanillin propylene glycol acetal were absent. One compound was not present in the mint flavoring agent, isoamyl isovalerate, however, the compound only appeared at time 0 and time 90 and with a very low relative abundance ratio (0.09 ± 0.12 and 0.04 ± 0.05 respectively).

Regarding Syrspend®, 8 of the 15 compounds present in mint flavoring in its pure form were detected, as presented in table 37. Compared to the Syrspend®, the detection of 4 new compounds was observed. The compounds were β-Pinene (T0, T30, and T90), o-Cymene (T30 and T90), γ-Terpinene (T90), and Humulene (T90). However, they also show a low relative abundance ratio.

The Suspendit® vehicle with the mint flavoring behaved similarly to the Syrspend® mixture. For the compounds that did not appear in the syrup, 3 of that appeared in the Suspendit® were β-Pinene (T0, T30, and T90), o-Cymene (T90), and Humulene (T90). Again, these three compounds show low relative abundance cues.

Overall, in the syrup-mint mixture, they showed 8 out of 9 compounds with statistically significant differences ($p<0.05$). As for the Syrspend® vehicle 12 out of 13 compounds showed statistical differences. However, for the Suspendit® vehicle, only 5 out of 11 compounds showed statistical differences. As was observed for the Suspendit® with banana flavoring, the Suspendit® with mint flavoring was the one that presented fewer compounds with significant statistical differences between T0, T30, and T90 among the vehicles studied, indicating greater stability of the flavor.

3.3.2.3 Vehicle with flavoring agent at refrigerated temperature

In order to understand if any variations could be seen with time and if it was dependent on storage temperature, replicates kept under refrigeration during the entire trial period were analyzed at the end of the trial.

Figure 65 represents the chromatograms and their analytical replicates, of the vehicles with the banana flavoring agents.

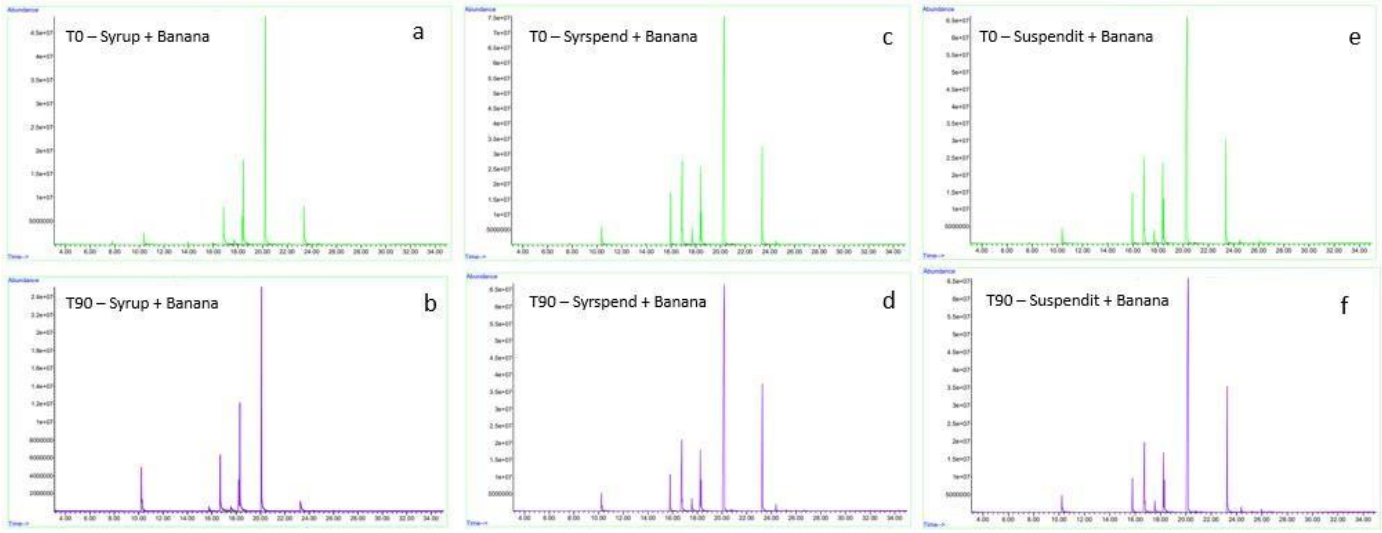


Figure 65 *Chromatogram representation of the vehicles: Syrup, Syrspend® and Suspendit® with banana flavoring at refrigerated temperature*

Regarding the chromatograms, it is possible to observe that there were no significant changes between the time 0 and 90 days with the Syrspend® and Suspendit® vehicles. For the syrup vehicle, it is seen that there is a decrease in the intensity of some peaks and an increase in others. In addition, the syrup remains to show fewer compounds compared to Syrspend® and Suspendit®. It is suggested that there is no stability of the compounds in a refrigerated environment for the syrup vehicle with the banana flavoring.

To make a more detailed comparison, an average of the relative abundance of the times 0 and 90 and a statistical ANOVA analysis were performed. Tables 41, 42, and 43 present the results of the mean relative abundance and standard deviation of the syrup, Syrspend®, and Suspendit® vehicles with banana flavoring, respectively, for refrigerated temperature.

Table 41 *Results of the mean relative abundance and standard deviation of the banana-flavored syrup vehicles at refrigerated temperature*

Syrup + Banana			
Compounds	T0 (% ± SD)	T90 (% ± SD)	*p*-value
Isoamyl acetate	4.11 ± 0.37	15.67 ± 0.84	**0.000**
Limonene	1.30 ± 0.25	2.15 ± 0.24	**0.026**
Isoamyl Isobutanoate	12.06 ± 1.81	16.17 ± 0.61	**0.038**
Benzyl alcohol	0.06 ± 0.06	0.09 ± 0.13	0.752
Allyl hexanoate	2.33 ± 0.05	2.32 ± 0.15	0.888
Isoamyl Isovalerate	5.79 ± 0.18	6.78 ± 0.15	**0.004**
Allyl heptanoate	61.27 ± 1.89	50.35 ± 0.70	**0.002**
Benzyl Butyrate	12.74 ± 0.25	6.25 ± 0.16	**0.000**
α-Ionone	0.34 ± 0.01	0.22 ± 0.02	**0.002**

Table 42 *Results of the mean relative abundance and standard deviation of the Syrspend® vehicles with banana flavoring at refrigerated temperature*

Syrspend® + Banana			
Compounds	T0 (% ± SD)	T90 (% ± SD)	*p*-value
Isoamyl acetate	2.33 ± 0.28	2.63 ± 0.13	0.237
Limonene	6.20 ± 0.25	4.50 ± 0.29	**0.003**
Isoamyl Isobutanoate	13.24 ± 0.66	11.28 ± 0.36	**0.021**
Allyl hexanoate	2.08 ± 0.06	1.92 ± 0.02	**0.029**
Isoamyl Isovalerate	7.26 ± 0.30	6.51 ± 0.35	0.080
Allyl heptanoate	58.04 ± 0.70	6.96 ± 0.25	**0.022**
2-Octynoic acid, methyl ester	0.43 ± 0.04	0.35 ± 0.12	0.053
Benzyl Butyrate	9.92 ± 0.51	12.15 ± 1.05	**0.014**
α-Ionone	0.49 ± 0.02	0.70 ± 0.07	0.418

Table 43 *Results of the mean relative abundance and standard deviation of the Suspendit® vehicles with banana flavoring at refrigerated temperature.*

Suspendit® + Banana			
Compounds	T0 (% ± SD)	T90 (% ± SD)	*p*-value
Isoamyl acetate	2.23 ± 0.28	2.54 ± 0.08	0.203
Limonene	5.97 ± 0.09	3.97 ± 0.19	**0.000**
Isoamyl Isobutanoate	12.07 ± 0.44	11.12 ± 0.01	**0.037**
Allyl hexanoate	2.18 ± 0.02	1.90 ± 0.03	**0.000**
Isoamyl Isovalerate	7.28 ± 0.27	6.24 ± 0.22	**0.013**
Allyl heptanoate	59.17 ± 0.34	60.20 ± 0.41	0.052
2-Octynoic acid, methyl ester	0.37 ± 0.04	0.29 ± 0.02	0.055
Benzyl Butyrate	10.24 ± 0.88	13.01 ± 0.38	**0,015**
α-Ionone	0.48 ± 0.03	0.69 ± 0.03	**0.002**

Regarding the statistical analysis, there are some statistically relevant differences. These data were similar to the analyses at room temperature. The same compounds that appeared for the samples containing the Syrspend® and Suspendit® vehicles at room temperature also appeared at refrigerated temperature. Regarding the samples containing the syrup vehicle, the two compounds that appeared at time 90 in the room temperature analyses (2-Octynoic acid, methyl ester, and menthyl acetate), did not appear, for both times, for the samples at refrigerated temperature.

About the mean relative abundance, standard deviation, and pValue values, it can be seen that the greatest variations once again occurred in the syrup vehicle, being most significant for isoamyl acetate, which nearly tripled, as opposed to benzyl butyrate which was reduced to half the total area. The majority compound, Allyl heptanoate, reduced its relative ambiguity by about 10%, from 61% to 50%. Overall, the variations were higher in the replicates stored under refrigeration compared to room temperature across the storage times.

Comparing the Syrspend® and Suspendit® samples at refrigerated temperature with at room temperature, it can be seen that there were greater statistical differences, but this does not compromise the assessment of stability at refrigerated temperature for these two vehicles, as the variations in relative abundance were not high between the compounds, showing stability in a refrigerated environment.

These data reinforce that the refrigeration of the syrup vehicle changes the characteristics of the mixture since after returning to room temperature for analysis and sampling under the conditions of the tests (release of the flavors at 45ºC), there is a loss of compounds in the Simple syrup. The other vehicles proved to be stable under refrigeration

and also, after returning to room temperature, not changing the release profile of the flavoring agent.

Figure 66 represents the chromatograms and their analytical replicates, of the vehicles with the mint flavoring agents.

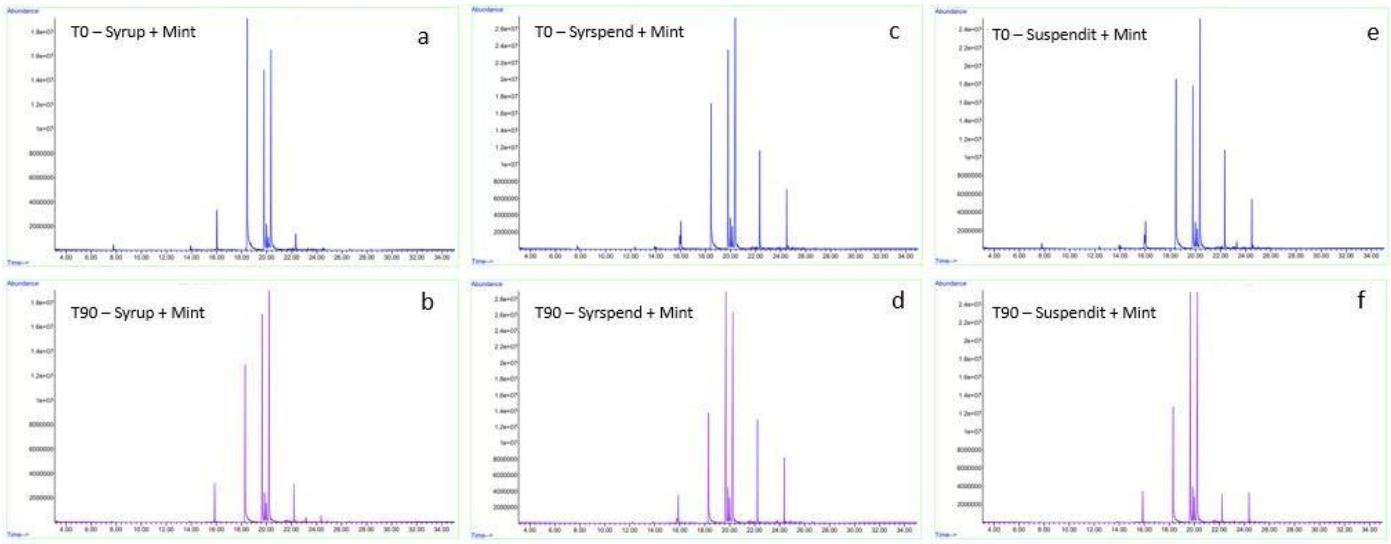


Figure 66 *Chromatogram representation of the vehicles: Syrup, Syrspend® and Suspendit® with mint flavoring at refrigerated temperature*

In the chromatograms represented in Figure 66 it can be seen that for the syrup vehicle (Figure 66 a and b) there was the detection of fewer compounds when compared to the Syrspend® (Figure 66d and e) and Suspendit® (Figure 69g and h) vehicles. Between Syrspend® and Suspendit® there is a similarity in the diversity of compounds. For Syrspend® and Suspendit®, there was an increase in some compounds and decrease in others. Regarding Syrspend®, both times (0 and 90), showed similarity among the compounds. For the two vehicles, syrup and Suspendit®, it is suggested that there is not a great stability in the compounds present in the chromatograms when compared to Syrspend®, which presents greater stability of the compounds under refrigeration.

As performed in the previous analyses, to make a more detailed comparison, an average of the relative abundance of the times 0 and 90 and a statistical ANOVA analysis were performed. Tables 44, 45, and 46 present the results of the mean relative abundance and standard deviation of the syrup, Syrspend®, and Suspendit® vehicles with mint flavoring, respectively.

Table 44 *Results of the mean relative abundance and standard deviation of the Syrup vehicles with mint flavoring at refrigerated temperature*

Syrup + Mint			
Compounds	T0 (% ± SD)	T90 (% ± SD)	*p*-value
Limonene	n.d.	2.54 ± 3.40	0.350
Eucalyptol	7.92 ± 0.36	6.67 ± 0.49	0.046
Isoamyl Isovalerate	0.09 ± 0.12	n.d.	0.374
Menthone	38.70 ± 1.42	41.93 ± 0.32	0.035
Levomenthol	50.30 ± 1.84	45.48 ± 0.77	0.027
Pulegone	n.d.	0.43 ± 0.07	0.001
Carvone	n.d.	0.52 ± 0.22	0.029
Isomenthol acetate	2.63 ± 0.15	4.23 ± 0.99	0.087
Caryophyllene	0.36 ± 0.01	0.63 ± 0.19	0.120

Table 45 *Results of the mean relative abundance and standard deviation of the Syrspend® vehicles with mint flavoring at refrigerated temperature*

Syrspend® + Mint			
Compounds	T0 (% ± SD)	T90 (% ± SD)	*p*-value
β-Pinene	0.38 ± 0.03	n.d.	**0.000**
Limonene	2.47 ± 0.25	0.77 ± 0.08	**0.001**
Eucalyptol	4.38 ± 0.03	3.67 ± 0.14	**0.002**
Isoamyl Isovalerate	0.09 ± 0.06	0.04 ± 0.02	0.365
Menthone	29.91 ± 0.59	37.42 ± 1.79	**0.005**
Levomenthol	45.56 ± 1.13	42.08 ± 0.58	**0.018**
Pulegone	0.38 ± 0.04	0.45 ± 0.04	0.172
Carvone	0.75 ± 0.17	0.76 ± 0.13	0.932
Isomenthol acetate	10.48 ± 0.65	9.58 ± 0.52	0.203
β-Elemene	n.d.	0.10 ± 0.01	**0.000**
Caryophyllene	5.60 ± 0.25	5.13 ± 0.78	0.453

Table 46 *Results of the mean relative abundance and standard deviation of the Suspendit® vehicles with mint flavoring at refrigerated temperature*

Suspendit® + Mint			
Compounds	T0 (% ± SD)	T90 (% ± SD)	*p*-value
β-Pinene	0.85 ± 0.10	n.d.	**0.000**
Limonene	2.81 ± 0.47	0.02 ± 0.02	**0.001**
Eucalyptol	4.12 ± 0.62	4.10 ± 0.34	0.970
Pentanoic acid, pentyl ester	n.d.	0.05 ± 0.04	0.124
Menthone	25.57 ± 1.71	40.00 ± 0.31	**0.000**
Levomenthol	48.79 ± 2.11	48.25 ± 2.31	0.820
Pulegone	0.34 ± 0.03	0.47 ± 0.01	**0.009**
Carvone	0.57 ± 0.08	0.68 ± 0.08	0.245
Isomenthol acetate	10.92 ± 0.81	3.70 ± 0.80	**0.001**
Caryophyllene	6.03 ± 0.85	2.69 ± 1.76	0.073
Caryophyllene oxide	n.d.	0.07 ± 0.01	**0.001**

Corroborating with the chromatogram profiles, it was possible to observe that the compounds present in the Syrspend® mixture with mint, between T0 and T90 showed better stability at refrigerated temperature compared to the syrup and Suspendit® vehicles. The profile of the compounds in relation to relative abundance for Syrspend® at room temperature and refrigerated temperature is similar, verifying that refrigeration does not compromise the compounds. Some compounds that were detected in the Syrspend® containing the mint flavoring at room temperature were not detected in the analysis under refrigeration, such as o-cymene, γ-Terpinene, for example, since they already had lower relative abundances. For mint flavoring, the vehicle that behaved best was Syrspend® in a refrigerated environment.

3.3.2.3 Flavoring agent vehicle after forced temperature degradation

Forced degradation by temperature tests was also performed, submitting the sample to a temperature of 60ºC for 24 hours.

At first, it is possible to analyze by observing the graphs how the mixtures of the vehicles with the flavoring agents behaved. The chromatograms in Figure 67 correspond to the three vehicles (Syrup - left; Syrspend® - center; Suspendit® - right) containing the banana flavoring agent, for the times: Initial time (upper) and after the forced degradation by temperature (down).

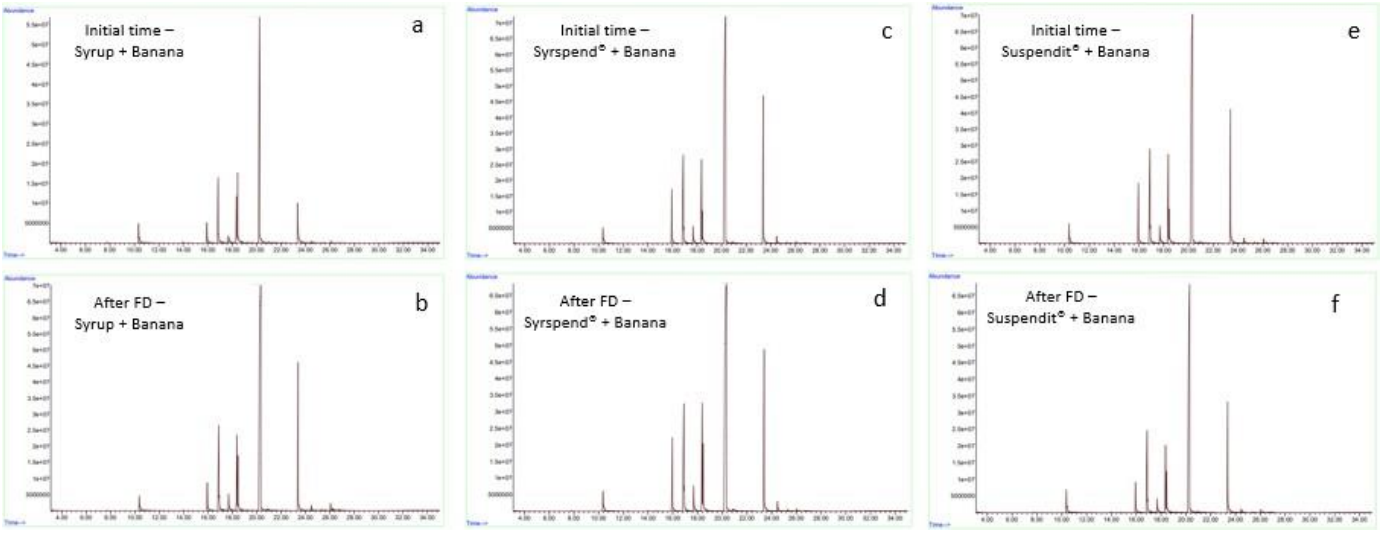


Figure 67 *Chromatogram representation of the vehicles: Syrup, Syrspend® and Suspendit® with banana flavoring at forced degradation*

As observed in all other tests, the banana flavor does not show good stability in syrup vehicle compared to Syrspend® and Suspendit®. Between Syrspend® and Suspendit® there is also a similarity between the compounds detected. Both in the initial period and after the forced degradation, there was similarity in the profile of the compounds.

Table 47 *Results of the mean relative abundance and standard deviation of the Syrup vehicles with banana flavoring at forced degradation.*

Syrup + Banana			
Compounds	Initial time (% ± SD)	After FD (% ± SD)	*p*-value
Isoamyl acetate	5.11 ± 1.76	2.25 ± 0.08	0.083
Limonene	4.61 ± 0.08	3.14 ± 0.09	**0.000**
Isoamyl Isobutanoate	13.87 ± 0.25	10.95 ± 0.44	**0.001**
Allyl hexanoate	2.56 ± 0.04	2.18 ± 0.09	**0.006**
Isoamyl Isovalerate	6.85 ± 0.31	6.96 ± 0.14	0.669
Allyl heptanoate	58.25 ± 1.71	61.07 ± 1.64	0.167
2-Octynoic acid, methyl ester	0.05 ± 0.05	0.24 ± 0.06	0.250
Benzyl Butyrate	8.46 ± 0.97	12.68 ± 1.27	**0.020**
α-Ionone	0.23 ± 0.03	0.53 ± 0.08	**0.006**

Table 48 *Results of the mean relative abundance and standard deviation of the Syrspend® vehicles with banana flavoring at forced degradation*

Syrspend® + Banana			
Compounds	Initial time (% ± SD)	After FD (% ± SD)	*p*-value
Isoamyl acetate	2.38 ± 0.21	2.33 ± 0.15	0.788
Limonene	5.42 ± 0.21	5.98 ± 0.17	**0.041**
Isoamyl Isobutanoate	11.77 ± 0.17	12.80 ± 0.09	**0.002**
Allyl hexanoate	2.15 ± 0.06	2.26 ± 0.07	0.176
Isoamyl Isovalerate	6.71 ± 0.16	7.75 ± 0.38	**0.024**
Allyl heptanoate	56.27 ± 0.80	55.91 ± 0.80	0.677
2-Octynoic acid, methyl ester	0.31 ± 0.07	0.31 ± 0.01	1.000
Benzyl Butyrate	14.24 ± 0.52	11.97 ± 0.25	**0.005**
α-Ionone	0.76 ± 0.02	0.70 ± 0.08	0.322

Table 49 *Results of the mean relative abundance and standard deviation of the Suspendit® vehicles with banana flavoring at forced degradation*

Suspendit® + Banana			
Compounds	Initial time (% ± SD)	After FD (% ± SD)	*p*-value
Isoamyl acetate	2..64 ± 0.15	3.75 ± 0.38	**0.019**
Limonene	6.07 ± 0.33	4.02 ± 0.28	**0.003**
Isoamyl Isobutanoate	12.33 ± 0.91	12.61 ± 0.3	0.693
Allyl hexanoate	2.15 ± 0.12	2.29 ± 0.07	0.219
Isoamyl Isovalerate	7.22 ± 0.45	6.74 ± 0.24	0.258
Allyl heptanoate	57.17 ± 0.73	59.25 ± 0.55	0.320
2-Octynoic acid, methyl ester	0.29 ± 0.08	0.17 ± 0.06	0.173
Benzyl Butyrate	11.58 ± 1.05	10.68 ± 0.69	0.370
α-Ionone	0.55 ± 0.04	0.47 ± 0.02	0.067

In relation to the analysis of relative abundance presented in the tables above, it was found that although there are statistical differences ($p<0.05$), the relative abundance does not show large variations for each compound in the mixtures of the vehicles with banana flavoring. This indicates that the forced degradation did not influence the release of compounds or the appearance of new compounds, showing a good stability of the vehicles with banana flavoring in this test of forced degradation.

The chromatograms of figure 68 correspond to the forced degradation test for the three vehicles (Syrup - left; Syrspend® - center; Suspendit® - right) containing the mint flavoring, for the initial time (upper) and after the forced degradation at 60ºC for 24 hours (down).

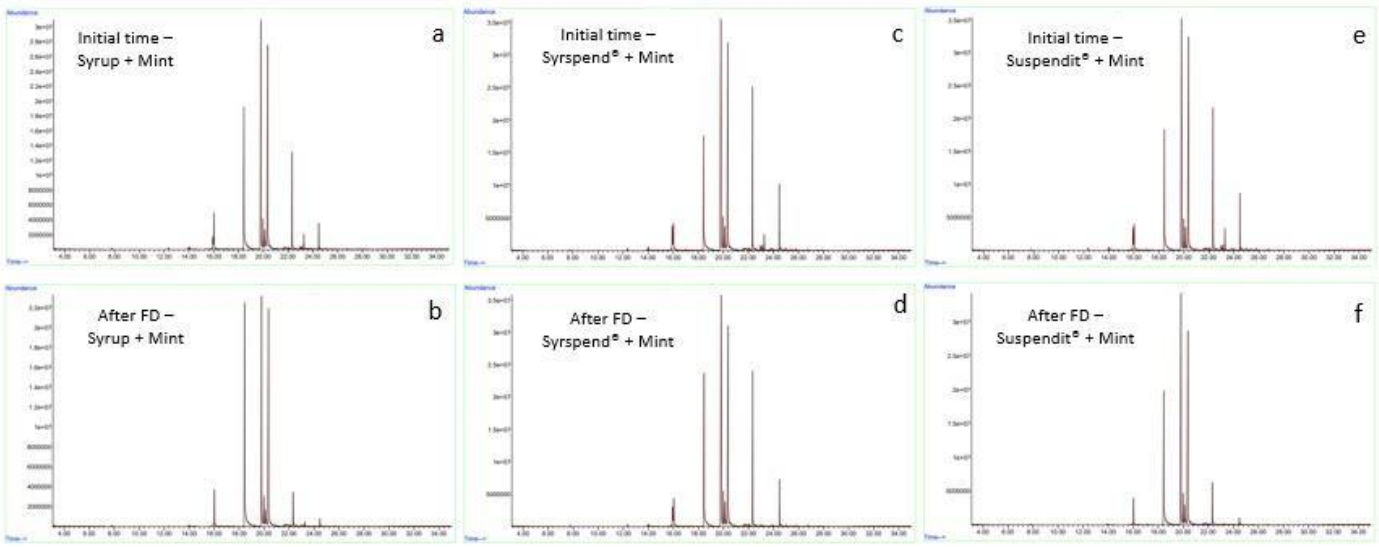


Figure 68 *Chromatogram representation of the vehicles: Syrup, Syrspend® and Suspendit® with mint flavoring at forced degradation*

Observing the chromatograms and the results on Table 50, 51 and 52, it can be seen that the only vehicle with a similar profile of compounds for the initial and final time of the degradation test is Syrspend®. For syrup and Suspendit®, some compounds disappeared after the forced temperature degradation test, indicating that mint flavoring was not stable under these test conditions.

Table 50 *Results of the mean relative abundance and standard deviation of the Syrup vehicles with mint flavoring at forced degradation.*

Syrup + Mint			
Compounds	Initial time (% ± SD)	After FD (% ± SD)	*p*-value
β-Pinene	0.36 ± 0.04	0.13 ± 0.09	**0.033**
o-Cymene	0.04 ± 0.06	n.d.	0.374
Limonene	1.64 ± 0.45	0.40 ± 0.14	**0.021**
Eucalyptol	5.60 ± 0.67	6.08 ± 0.30	0.410
Isoamyl Isovalerate	0.07 ± 0.05	n.d.	0.124
Menthone	42.00 ± 1.79	43.15 ± 0.14	0.417
Levomenthol	40.42± 0.79	45.13 ± 1.03	**0.007**
Pulegone	0.43 ± 0.06	0.34 ± 0.17	0.492
Carvone	0.78 ± 0.30	0.61 ± 0.42	0.659
Isomenthol acetate	8.93 ± 1.16	4.50 ± 0.81	**0.011**
Humulene	0.06 ± 0.04	n.d.	0.116
Caryophyllene oxide	0.10 ± 0.07	n.d.	0.117

Table 51 *Results of the mean relative abundance and standard deviation of the Syrspend® vehicles with mint flavoring at forced degradation*

Syrspend® + Mint			
Compounds	Initial time (% ± SD)	After FD (% ± SD)	*p*-value
o-Cymene	0.23 ± 0.01	0.19 ± 0.02	**0.038**
Limonene	3.28 ± 0.10	2.71 ± 0.17	**0.015**
Eucalyptol	3.59 ± 0.17	3.82 ± 0.17	0.237
γ-Terpinene	0.11 ± 0.09	n.d.	0.142
Isoamyl Isovalerate	0.07 ± 0.01	0.07 ± 0.10	0.965
Menthone	36.82 ±1.20	39.71 ± 0.89	0.052
Levomenthol	35.28 ± 0.97	35.47 ± 0.52	0.812
Pulegone	0.45 ± 0.01	0.35 ± 0.01	**0.003**
Carvone	0.73 ± 0.14	0.65 ± 0.01	0.545
Isomenthol acetate	14.71 ± 0.36	14.27 ± 0.43	0.337
Humulene	4.95 ± 0.32	2.85 ± 1.73	0.168
Caryophyllene oxide	0.13 ± 0.01	0.16 ± 0.01	0.065

Table 52 *Results of the mean relative abundance and standard deviation of the Suspendit® vehicles with mint flavoring at forced degradation*

Suspendit® + Mint			
Compounds	Initial time (% ± SD)	After FD (% ± SD)	*p*-value
o-Cymene	0.20 ± 0.02	n.d.	**0.000**
Limonene	3.34 ± 0.25	0.20 ± 0.19	**0.000**
Eucalyptol	3.81 ± 0.26	4.33 ± 0.13	0.059
Isoamyl Isovalerate	0.02 ± 0.03	0.04 ± 0.03	0.638
Menthone	34.83 ± 0.83	45.10 ± 0.73	**0.000**
Levomenthol	37.66 ± 97	42.84 ± 1.32	**0.011**
Pulegone	0.42 ± 0.01	0.42 ± 0.04	1.000
Carvone	0.55 ± 0.03	0.66 ± 0.15	0.381
Isomenthol acetate	13.84 ± 0.61	5.12 ± 0.89	**0.000**
Humulene	4.96 ± 0.17	1.26 ± 0.62	**0.001**
Caryophyllene oxide	0.15 ± 0.02	0.02 ± 0.03	**0.011**

For the forced degradation test, confirming with the chromatograms presented in figure 68, it is verified that syrup and Suspendit® vehicles for the mint flavoring, presented greater statistical differences than the Syrspend® vehicle. Comparing the initial and final time of the degradation test, it is observed that some compounds are absent after the final time of the forced degradation analysis.

Comparing the forced degradation test for the best candidate vehicle with the mint flavoring that is Syrspend® with the room temperature data, it was found that the relative

abundances are very similar indicating stability of the compounds even after the forced degradation test. It is concluded that the best vehicle for the mint flavoring is Syrspend®.

3.3.3 pH evaluation

The pH was analyzed both for the preparations at room and chilled temperature and for the vehicle without the addition of a flavoring agent.

The data analysis was divided by the different types of vehicles. In Figure 69, it is possible to observe the evolution of the pH stability test for simple Syrup vehicle. This vehicle presented the highest instability when compared to the other two vehicles. For the mixture of syrup with banana flavor at room temperature, it is observed that between time zero and after 30 days of storage, the pH remained stable, but there was a slight increase at time 90. As for the syrup mixture with mint flavor at room temperature, there was a slight increase at time 30 in relation to the initial analysis and remained stable between time 30 and time 90. For the syrup mixtures with the two flavorings that remained at refrigerated temperature, it was observed that the mixture containing the mint flavoring remained stable and a slight decrease in pH was observed with the mixture containing the banana flavoring.

In relation to the vehicle without flavoring and with the flavoring, it is possible to note the increase in pH from 4.5 to 5.5/6.0 when the flavoring was added to the simple syrup.

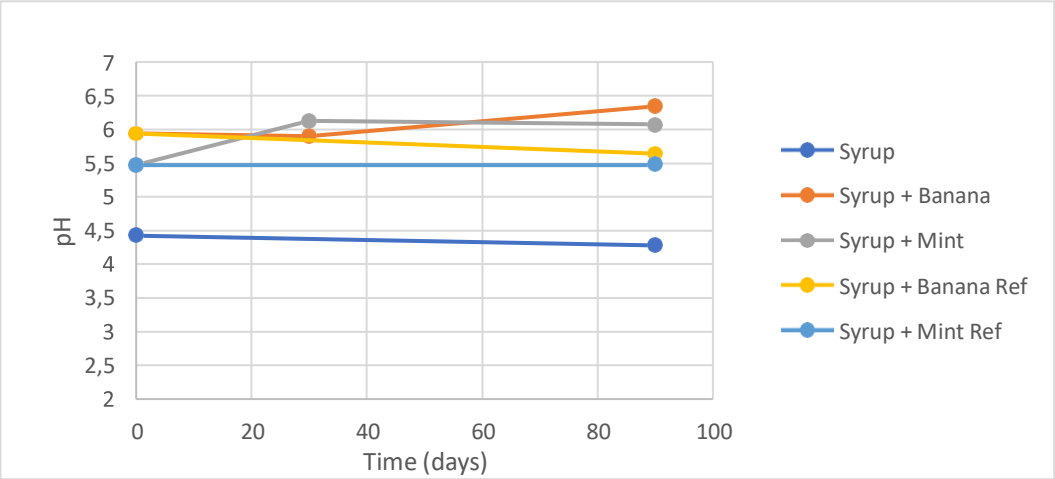


Figure 69 *Evaluation of pH versus time of the syrup vehicle.*

For the Syrspend® and Suspendit® vehicles it is shown in Figures 70 and 71 respectively, that there were no significant differences, i.e., they remained stable throughout the study.

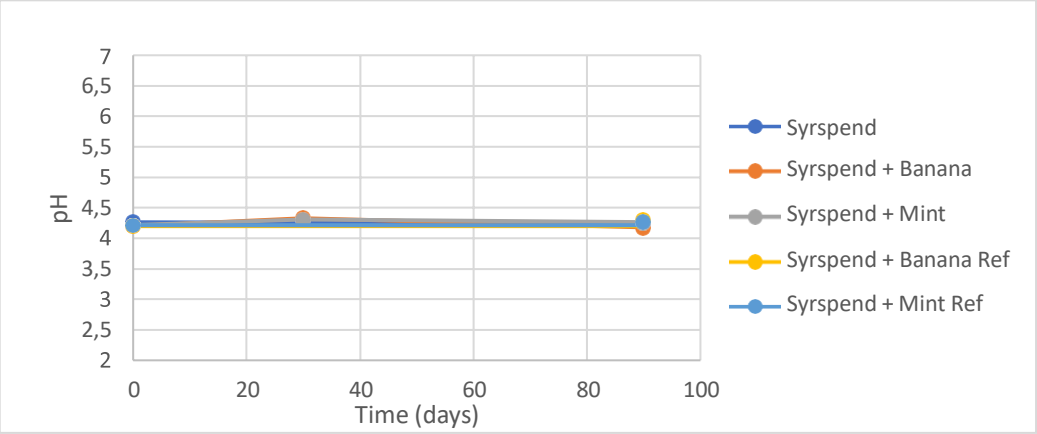


Figure 70 Evaluation of pH versus time of the Syrspend® vehicle.

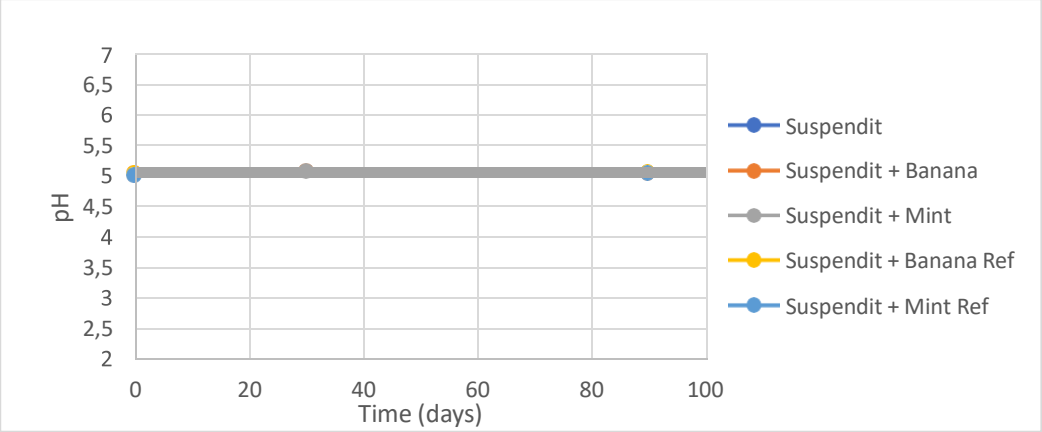


***Figure 71** Evaluation of pH versus time of the Suspendit® vehicle.*

3.3.4 Organoleptic characteristics

The organoleptic characteristics of the mixtures containing the vehicle and the flavouring agent were analyzed according to the time of analysis and the storage temperature.

For the samples stored at room temperature it was possible to observe that both the vehicle and the flavouring agent had no alteration in their coloring. The smell was analyzed whenever the sample was used for the rheological analysis according to the analysis times, and also, did not present any alteration. For samples stored at refrigerated temperature and for samples after forced degradation (temperature at 60ºC) there was also no change in coloration and smell. The figures of coloration analysis are present in the supplementary material (S3 to S8).

Conclusions

This work aimed to evaluate the stability of flavoring agents in diverse oral compounding vehicles. After an initial rheological characterization of simple syrup, Syrspend®, Suspendit®, OraPlus®, and oral suspension – Guinama®; and the chemical characterization of diverse flavoring agents (banana, orange, mint, and marshmallow) by GC-MS; the stability study was performed for 90 days with two flavoring agents in three vehicles, under three different storage conditions: room temperature, refrigeration (2 to 8ºC), and under forced temperature degradation (60ºC for 24 hours).

The vehicles selected included simple syrup, a Newtonian vehicle widely used as a vehicle for pharmaceutical formulations, and Syrspend® and Suspendit® commercial vehicles, representing non-Newtonian fluids commercially available. Syrspend® is non-thixotropic, while Suspendit® is a thixotropic fluid. Concerning the flavoring agents, banana and mint flavorings were selected for the stability study due to their richness of compounds, that is, they presented a higher chemical diversity of volatile compounds.

The addition of flavoring agents did not impact the flow behavior of simple syrup and Syrspend® while for the Suspendit® vehicle a decrease of consistency index (from Herschel-Bulkley model) was observed, with both flavorings. However, after storage for 30 and 90 days at room temperature the flow parameters remained unchanged, except for a slight decrease of the consistency index at room temperature observed again with Suspendit® vehicle, as well as under refrigerated conditions with banana flavor. For the Syrspend® and Suspendit® vehicles, besides the flow analysis, the amplitude and frequency sweep tests were performed as well as the evaluation of thixotropy. The moduli G' was higher than G" and phase angle lower than 45°, indicating solid-like properties, for both commercial vehicles and this behaviour remained unchanged throughout the stability test. The linear viscoelastic region also remained similar after 90 days storage supporting a good mechanical stability. The thixotropic behavior of each commercial vehicles was unchanged after the storage period.

After analysis of the volatiles released from the preparation by SPME-GC-MS, it is possible to state that the syrup vehicle presented a worse stability than the commercial counterparts, under all the temperature conditions andfor both flavoring agents, with higher variations in the individual compounds proportion and total volatile amounts. The Syrspend® and Suspendit® vehicles presented the best results, both chromatographic and statistical, with increased stability. In detaill, Suspendit presented a more stable release of volatile compounds for both flavoring agents when preserved at room temperature, while both behave similarly under refrigeration, except with mint agent, with increased stability

with Syrspend®. In the analysis of forced degradation, although both vehicles presented good results, Suspendit® preserved better the banana flavor, while Syrspend® gave more constant results with mint.

Overall, all the vehicles with both flavoring agents remained fairly stable with respect to mechanical properties during storage for 90 days (at both temperatures) being Suspendit® vehicle the least stable, showing a viscosity decrease with the addition of both flavorings, which should be taken in to account in the design of the medicines to ensure the target flow properties. With respect to flavoring stability, the results could have a putative impact in treatment adherence if the ability to mask unpleasant flavors is diminished. The SPME-GC-MS method developed represents a valuable tool to potentially address undetectable aroma changes.

The chemical stability of flavorings incorporated into oral vehicles can also potentially influence the chemical stability of drugs and thus it its recommended to select the flavoring and vehicle considering stability results, besides taste masking effectiveness and patient preferences. In-use stability studies, simulating real use could provide additional information on flavoring degradation. Further studies with drugs commonly used in oral compounded formulations could also be performed, with the methodologies and stability protocol developed in this work, to reveal the impact of the stability of the flavoring in a given vehicle on the drug and flavoring agents stability.

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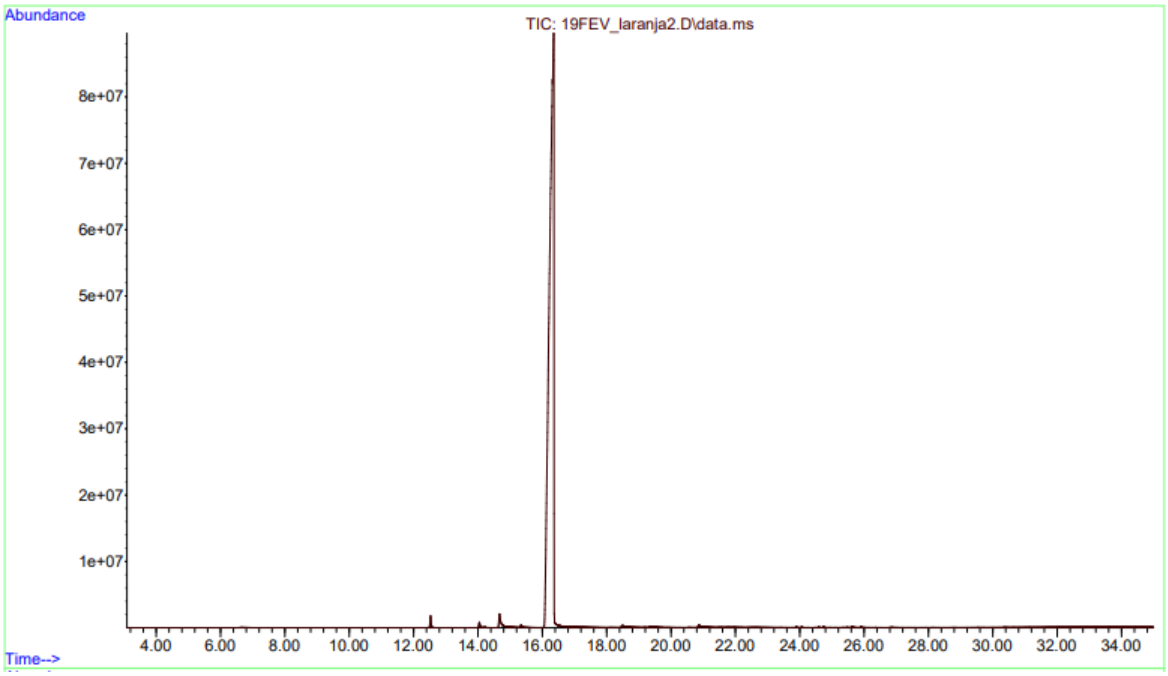
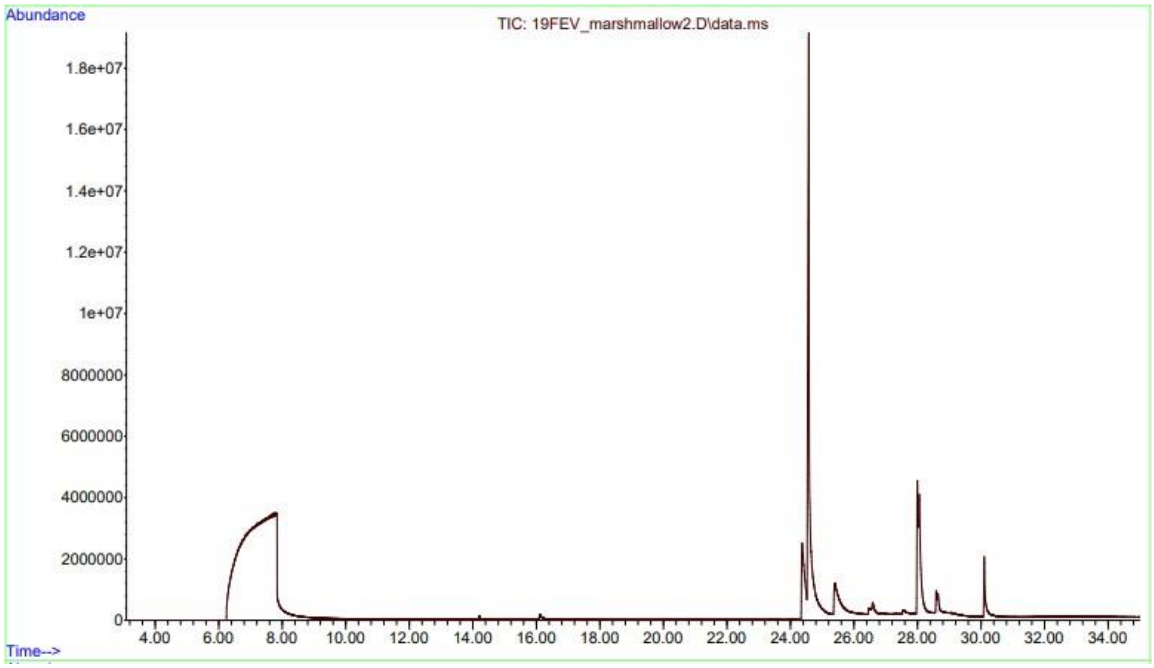
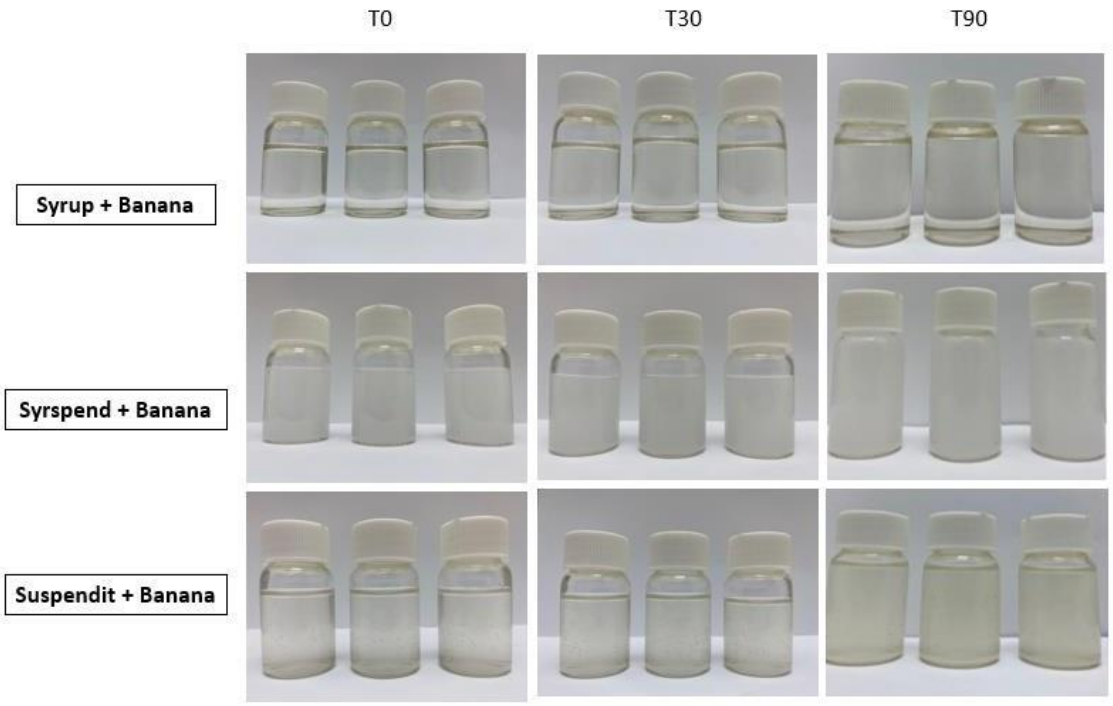
Supplementary Material



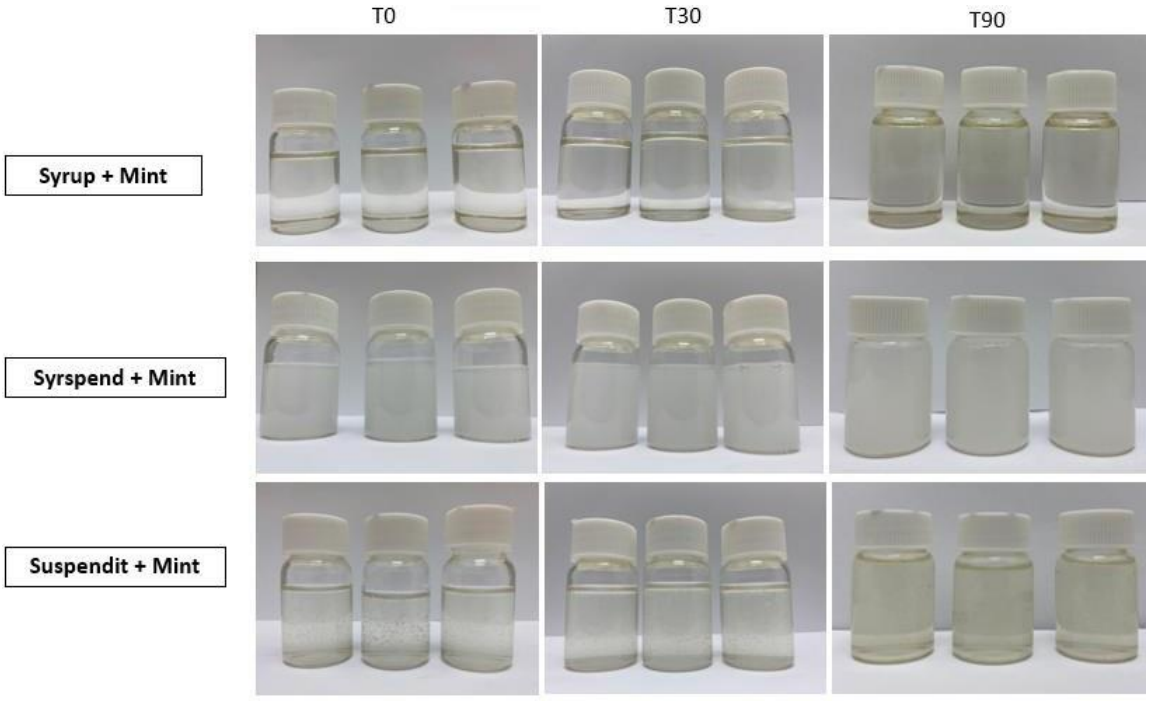
Supplementary Figure 1 – GC-MS Chromatogram of the orange flavouring agent



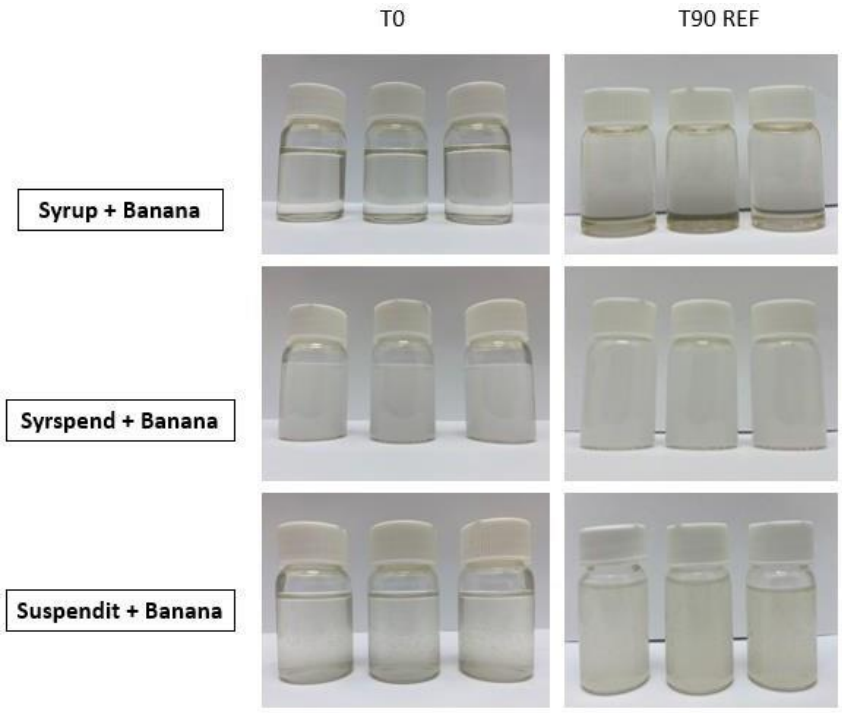
Supplementary Figure 2 - GC-MS Chromatogram of the marshmallow flavouring agent



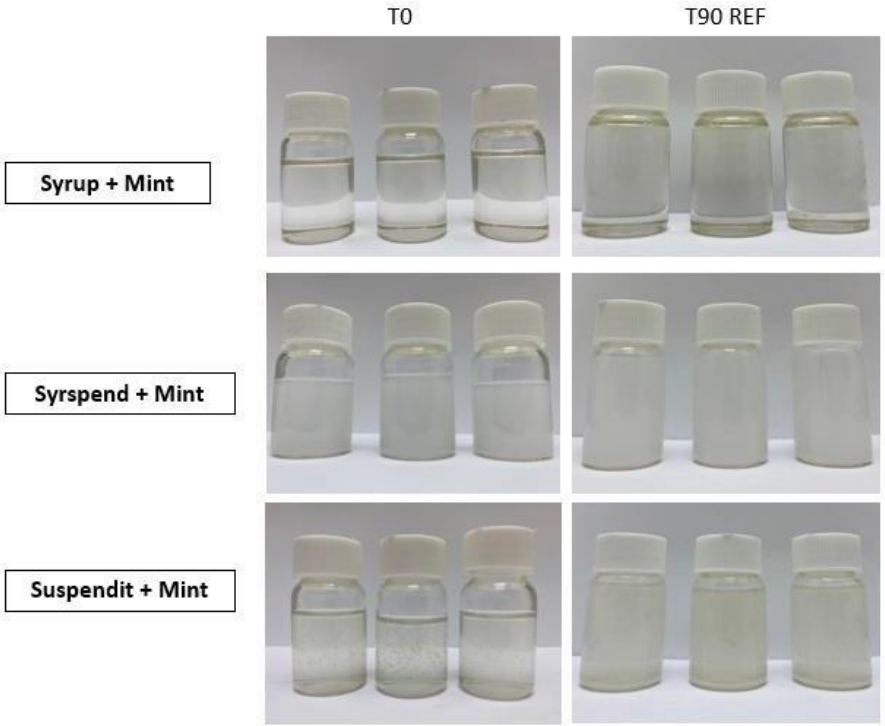
Supplementary Figure 3 Organoleptic characteristics (coloring) of vehicles with banana flavoring agent at room temperature



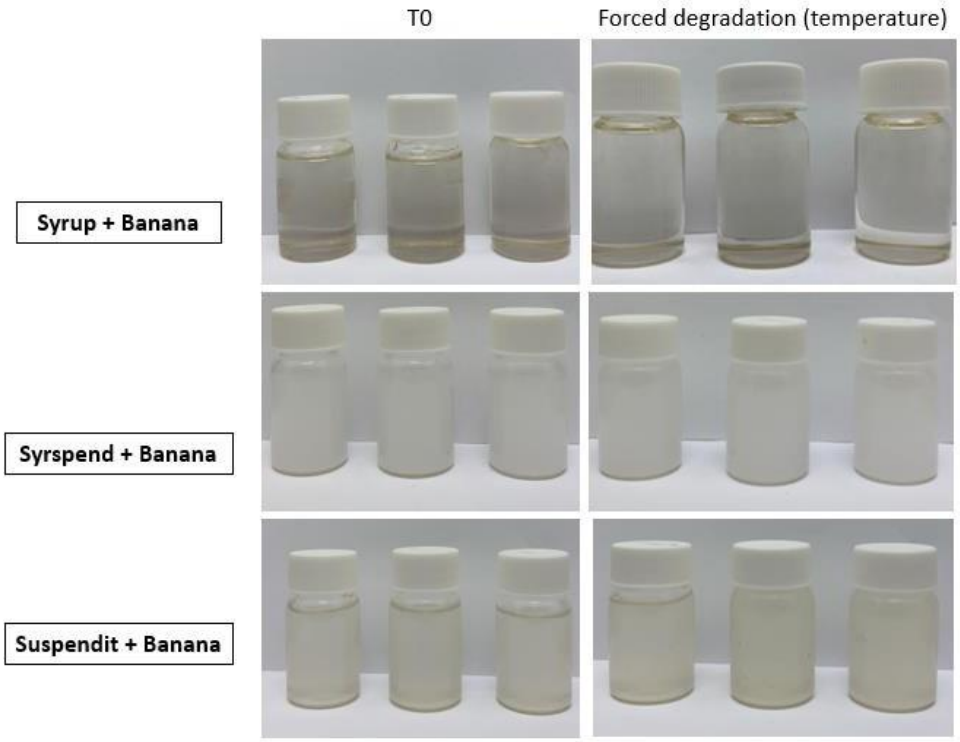
Supplementary Figure 4 Organoleptic characteristics (coloring) of vehicles with mint flavoring agent at room temperature



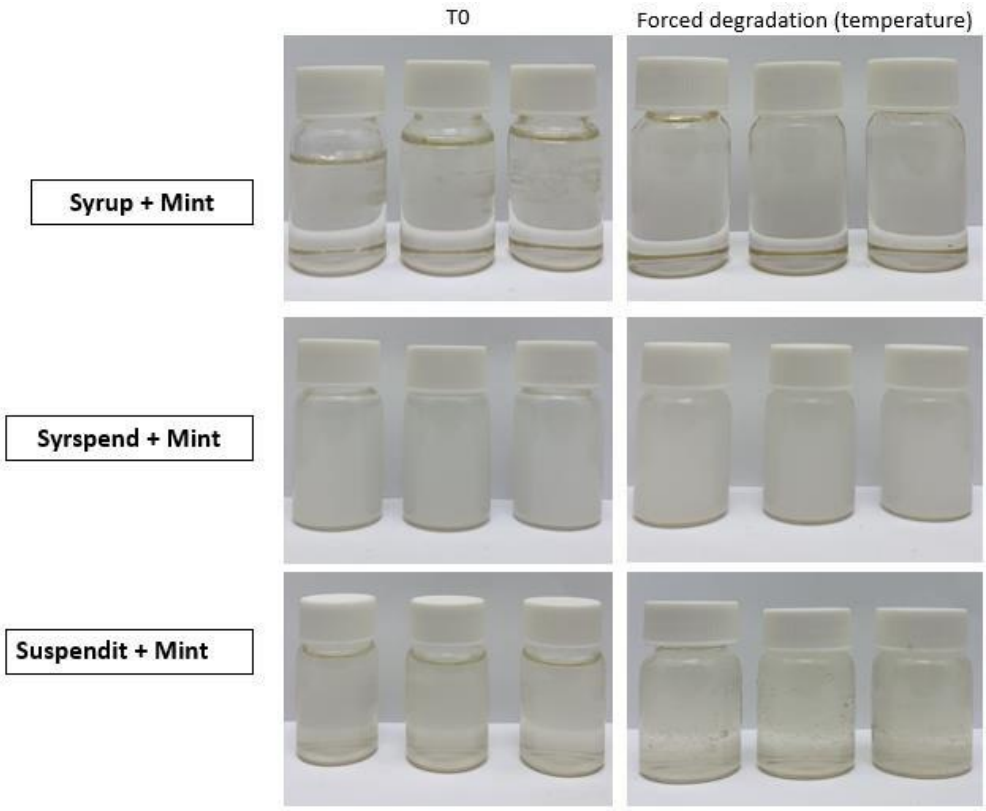
Supplementary Figure 5 Organoleptic characteristics (coloring) of vehicles with banana flavoring agent at refrigerated temperature



Supplementary Figure 6 Organoleptic characteristics (coloring) of vehicles with mint flavoring agent at refrigerated temperature



Supplementary Figure 7 Organoleptic characteristics (coloring) of vehicles with banana flavoring agent at forced degradation (temperature)



Supplementary Figure 8 Organoleptic characteristics (coloring) of vehicles with mint flavoring agent at forced degradation (temperature)