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MASTER

Thème

Caractérisation physico-chimique des jus, pulpes et huiles essentielles de différentes variétés d'espèces d'agrumes : *Citrus sinensis, Citrus paradisi* et *Citrus limonum*

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Dedication

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To all of my family and in-laws without exception.

To all those who care about my success.

When, from a long-distant past nothing subsists, after the people are dead, after the things are broken and scattered; taste and smell alone, more fragile but more enduring, more insubstantial, more persistent, more faithful, remain poised a long time, like souls, remembering, waiting, hoping, amid the ruins of all the rest; and bear unflinchingly, in the tiny and almost impalpable drop of their essence, the vast structure of recollection.
Marcel Proust
Remembrance of Things Past

Quand d'un passé ancien rien ne subsiste, après la mort des êtres, après la destruction des choses, seules, plus frêles mais plus vivaces, plus immatérielles, plus persistantes, plus fidèles, l'odeur et la saveur restent encore longtemps, comme des âmes, à se rappeler, à attendre, à espérer, sur la ruine de tout le reste, à porter sans fléchir, sur leur gouttelette presque impalpable, l'édifice immense du souvenir.
Marcel Proust
À la recherche du temps perdu

Summary

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List of abreviations

°C: degrees Celsius

C_3H_8O: Isopropanol (aldehyde free)

Cp: centpoise

g: grams

H_3NOHCl: Hydroxylamine hydrochloride

HCl: Hydrochloric acid

KOH: Potassium hydroxide

M: Molar

ml: Milliliter

mMol/l: millmolar par liter

NaOH: Sodium hydroxide

nm: nanometer

Pa s: Pascal second

Sec: second

T: transmittance

INTRODUCTION

The growing awareness of consumers concerning the relation between food and health is revolutionizing the food industry. In one hand, the application of preservatives to foods is fundamental if their safety is to be maintained, so, the ‘‘elimination” of additives used in a wide variety of foods is demanded, while ‘‘natural” additives are seen as beneficial for both quality and safety. In the face of this challenge, researchers are looking for new sources of ingredients and/or additives, among these resources, agro-food co-products are candidates (Lario *et al*., 2004).
As the use of natural additives has received importance as a trend in the replacement of synthetic preservatives (Andrade *et al*., 2014), essential oils and their main components which possess a wide spectrum of biological activities (Andrade *et al*., 2014; Raspo *et al*., 2020), may be of great importance in the field of food industry. The main advantage of essential oils is that they can be used in any foods and are considered generally recognized as safe (GRAS) (Kabara, 1991), as long as their maximum effects is attained with the minimum change in the organoleptic properties of the substrate.
The use of essential oils could represent a “natural” alternative to the synthetic antioxidants of foods, such as the butylated hydroxyanisole (BHA) or butylhydroxytoluene (BHT) which are suspected to be harmful to human health. Afterwards, consumers and food producers are increasingly interested in the essential oils application due to the antioxidant and antibacterial properties (Di RausoSimeone *et al.*, 2020). Citrus essential oils are valuable natural products, they become increasingly important in food, alternative medicines, and cosmetic industries owing to their high yields, aromas, flavors, antioxidant capacity and antimicrobial activity (Ou *et al*., 2015).
A better understanding of the composition of some citrus fruits, their by-products (after their transformation) , the extraction and characterization of their essential oils would help to better control their useful properties. The work presented in this report was carried out with the objective of analyzing the physico-chemical parameters of three species of citrus (Lemon: *Citrus limon* L., grapefruit: *Citrus paradisi* L. and orange: *Citrus sinensis* L.), juice, pulp and their essential oils, the latter in terms of their chemical composition.
The quality standards for citrus fruits for consumption and processing are mainly based on very specific physicochemical parameters. Maturity of citrus fruits is defined by parameters specified for each species including minimum juice content, minimum total soluble solids content (sugar content), sugar-acid ratio, viscosity, density and color; they are directly related to taste (Morales *et al*., 2020).
For this the present document will be divided in two parts:

1. Bibliographic study containing two different chapters (I, II) devoted on citrus fruits and essential oils, respectively;
2. Experimental part which focused on two classical chapters (III, IV) materials and methods and results and discussion, respectively.

BIBLIOGRAPHIC

STUDY

CHAPTER I

CITRUS FRUITS

I.1 General informations on citrus fruits

Citrus, which is widely cultivated in tropical and subtropical climates, is one of the most important crops in the world. Every year, approximately 130 million tons of citrus fruits are produced in more than 140 countries (Ma *et al*.,2020). Around 70% of the world's total marketable citrus are grown in the Northern Hemisphere, in particular Brazil, countries around the Mediterranean, and the United States. The greatest production in Europe is in Spain, which accounts for more than 55% of the European citrus output (Blasco *et al.*,2016).

Citrus fruits belong to the family of Rutaceae and enjoy the privilege of most traded fruit over the world (Chavan *et al*., 2018), whose name comes from the genus *Ruta* L., includes herbaceous and woody plants with essential oil glands. According to Swingle and Reece (1967), citrus are among the first fruits to be domesticated and exploited by humans. The center of its origin and diversity is southeastern Asia, particularly northeast India, Myanmar, and southern China. In those areas, citrus were exploited and consumed during ancient times (Ollitrault *et al.,* 2020).

Several species and their hybrids, considered under the more general term citrus, are of great commercial interest (Blasco *et al.*,2016).Wider range of citrus variety is present worldwide such as sweet oranges (*C. sinensis*), mandarins (*C. unshi*, *C. tangerine*), reticulate (*C. clementine*), sour/bitter oranges (Seville, *C. aurantium*), lemons (*C. limon*), limes (*C. aurantifolia* and *latifolia*), grapefruit (*C. paradisi*), and pummelos (*C. grandis*) (Chavan *et al*., 2018).

The wide diversity among such species is accompanied by an overall positive appreciation by consumers, thanks to the outstanding organoleptic characteristics of citrus fruits and, their health and nutritional value (Gentile *et al*., 2020).

I.2 The genus *Citrus*

The genus Citrus is defined by two different classification systems: Tanaka's, with 156 species, and Swingle's, with only 16 species. However, these two systems often contradict each other due to the overall sexual compatibility between the Citrus species and the frequent occurrence of apomixes (due to nucellarpolyembryony), which leads many taxonomists to consider interspecific hybrids (vegetatively propagated by apomixes) as new species. The high phenotypic and genetic variability of the *Citrus taxa* reflects a long history of cultivation, in which many mutations and natural hybridizations gave rise to the existing diversity within this mainly facultative apomictic group (Luro *et al* .,2017).The genealogy of the different citrus species indicated that they were originated through successive events of hybridization that occurred among the three “true” or “biological” ancestral species: citron (*Citrus medica*), mandarin (*Citrus reticulata*), pommelo (*Citrus maxima*), and/or their hybrids (Gentile *et al*.,2020) (See annex 1).

I.2.1Botanical classification

The citrus group belongs to the Rutaceae family, under the Aurantioideae family, the Citreae tribe and the Citrinae tribe. Citrus fruits are divided into several genera including *Poncirus*, *Fortunella* and *Citrus*, they are the three most cultivated genera around the World (Ollitrault *et al.,* 2020). The systematic position of citrus fruits is known as follows:
According to the working list of all plant species, citrus species hybridize easily and that new hybrids are continuously developed by cross pollination to obtain desired qualities such as seedless, juicy and fresh taste fruits. The working list of all plant species, the taxonomy of citrus plants follow this order; **Kingdom**: Plantae; **Subkingdom**: Tracheobionta; **Superdivision**: Spermatophyta; **Division**: Magnoliophyta; **Class**: Magnoliopsida; **Subclass**: Rosidae; **Order**: Sapindales; **Family**: Rutaceae; **Genus**: Citrus (Alexander, 2019).

I.2.2 Botanical description

The fruits of citrus are berries that are fleshy, indehiscent, many-seeded fruits containing no hard parts except the seeds. More specifically, Citrus fruits are hesperidia, in which the fleshy parts of the fruit are divided into segments and are surrounded by a separable skin. The obovoid or flattened seeds are attached adaxially near the central axis or core, have smooth or ridged seed coats, and contain one to many embryos (See annex 1). The segments are filled with stalked fusiform pulp vesicles; contain the juice, an important source of vitamin C, which plays an important role in food industry for beverage or other food product processing (Chi *et al*., 2019) and large-celled tissue (Ollitrault *et al.,* 2020). The segments are surrounded by a white endocarp, outside of which is the peel, a by-product, contains high amounts of phytochemicals including flavonoids, limonoïdes, carotenoids and pectin. Especially, essential oils, which contributes to a pleasant and refreshing aroma of citrus fruits (Chi *et al*., 2019). The peel is generally green during the early stages of fruit development and turns yellow or orange at maturity. The fruit arises from the fragrant flowers, which are borne singly or in small racemes in the axils of the leaves. The flowers of Citrus are perfect or staminate, the latter condition being due to abortion of the pistil (Ollitrault *et al.*, 2020).

I.3 Citrus and health

Citrus is one of fruits which is consumed fresh or as a juice. These fruits contain a variety of compounds: sugars, citric acid, ascorbic acid, carotenoids, minerals, essential oils, etc. and play an important role in human nutrition. Citrus fruit extracts have health benefits that are mainly attributed to the presence of bioactive compounds, such as vitamin C which is a powerful antioxidant, phenolics (e.g. flavanone glycosides, hydroxyl cinnamic acids) and carotenoids (Rita, 2018). These phytochemicals, consumed through fresh fruits or their derived products, have been suggested to have a wide variety of biological functions including antioxidant, anti inflammation, ant imutagenicity, anti carcinogenicity and anti-aging to human health (Zou *et al.,* 2016).The components responsible for these beneficial effects are not fully known, but the citrus flavonoids are one group of compounds that may be involved; such compounds are identified as methoxylated flavones, flavonones and flavonone glucosides (Goulas *et* Manganaris, 2012). Citrus fruits are also rich in dietary fibers; compared with other fruits and vegetables, the ratio of soluble to insoluble fibers is particularly high in citrus fruits. The soluble fibers in citrus fruits prevent diabetes and lower cholesterol levels. In addition to the macronutrients, citrus fruits are abundant in micronutrients, such as potassium, folate, vitamin B6, riboflavin, and calcium, which are essential for maintaining human health. In the past decades, a growing number of epidemiologic and clinical studies have demonstrated that the consumption of citrus fruits is associated with the reduction of the risks of life style-related diseases, such as cancers, cardiovascular diseases, osteoporosis, type-2 diabetes (Ma *et al*.,2020).

I.4 Description of some Citrus fruit

I.4.1 Sweet Orange (*Citrus sinensis*)

The most important of all citrus fruit is the sweet orange (*C. sinensis*), which is widely grown in those world regions adapted to citrus. Each region usually has its own characteristic varieties (Ashurst, 2016). Sweet orange (*C. sinensis* L. Osbeck) grow in warm weather countries; most of these species can be found in Africa. It is an hybrid tree originally from India, Vietnam, and south-east Asia, it is of medium size (7–10 m), erect if containing seeds and with broad crown if grafted, owns shiny leaves and white and bisexual flowers, the fruit is round of about 6–10 cm of diameter with a slightly rough peel (See annex1). According to its botany, there are four different groups within the *C. sinensis* L. Osbeck species, namely:

- Navel oranges;
- Common oranges, also called white oranges;
- Blood oranges;
- Acidless oranges, also called sugar or sugary oranges (Preedy,2016).

I.4. 2 Lemon (*Citrus limon*)

An important crop in Italy and some other Mediterranean countries, the lemon is also grown commercially in the United States and Argentina.The characteristic oval shaped, yellow fruits, apart from their culinary usage, are an importantsource of juice and flavouring for the soft drinks industry (Ashurst, 2016).

I.4.3 Grapefruit (*Citrus. paradisi*)

A large round citrus fruit with a thick yellow skin and a somewhat bitter pulp, grapefruit is generally accepted to be a hybrid between the pomelo and the orange.Today, the commercially important grapefruit is grown in many parts of the world.The most predominant cultivar to be seen in the market is the ‘Marsh Seedless’, followed by a red, pigmented version known as the ‘Star Ruby’(Ashurst, 2016) .

Grapefruits occur as clusters like grapes on the subtropical evergreen tree. The fruit is approximately spherical in shape with a diameter of four to five inches. It has a yellow- or orange-colored peel and pink or red pulp. It may or may not have seeds depending on the variety. It is juicy like oranges and its taste ranges from acidic and sour to sweet due to grapefruit mercaptan (a sulfur containing terpene). The foliage is long, slender, and dark green, the flowers are white with four petals (Preedy,2016).

I.5 Chemical composition

Citrus is one of widespread crops in the world with a production approximately of 102 million tonsper year (Di RausoSimeone *et al.*, 2020).This food and agro-foodprocessing industry yields a considerable amount of waste or by-products (peels, seeds andpulps), which represents 50% of the raw processed fruit (Geraci *et al.*, 2017).

Citrus fruits are rich sources of useful phytochemicals, such as vitamins A, C and E,mineral elements, flavonoids, coumarins, limonoids, carotenoids, pectins and essential oils (Zou *et al.*,2016).

CHAPTER II

ESSENTIAL OILS

II.1 General informations on essential oils

II.1.1 Definition

Essential oils (EOs), also known as essences, volatile oils, etheric oils, are natural products formed by aromatic compounds that are widely used all over the world (Safitri *et al*., 2020). Their production is constantly increasing because of the strong demand for pure natural ingredients in many fields: cosmetics, flavors, fragrances, agriculture, food and health industries (aromatherapy and phytomedicine) (Gentile *et al*., 2020). Currently, about 300 aromatic plants are commercially available (Kumar *et al*., 2020). Essential oils are mixtures of more than 200 different compounds (Kamaliroosta *et al*., 2016). These compounds are mainly formed of monoterpene and sesquiterpene hydrocarbons and their oxygenated derivatives such as esters, alcohols and aliphatic aldehydes and ketones (Gentile *et al*., 2020) (see Appendix 4).

According to the International Standard Organization on Essential Oils (ISO 9235: 2013) and the European Pharmacopoeia (Council of Europe , 2004), an essential oil is defined as the product obtained from plant raw material by hydrodistillation, steam distillation or dry distillation or by a suitable mechanical process (*e.g*. Citrus fruits). The term 'oil' denotes the lipophilic and viscous nature of these substances, while the term 'essential' signifies the preciousness and typical fragrance of the plants (Gentile *et al*., 2020).

The essential oils are typically complex liquids, clear and unusually colored, and their constituents are volatile, characterized by a strong odor. They are synthesized by aromatic plants as secondary metabolites, which protect them against microorganisms and insects. They can be synthesized in several plant organs such as buds, flowers, leaves, stems, branches, seeds, berries, roots, wood or bark, being stored in secretory cells, cavities, channels, epidermal cells or trichomes (Safitri *et al*., 2020).

Essential oils of citrus are the most popular natural essential oils and account for the largest proportion of commercial natural flavors and fragrances. Depending on the plant source, citrus essential oils are extracted from pericarp, flower, fruit juice, crushed fruits, leaf and twigs and sometimes from little green fruits (Gentile *et al*., 2020).

II.1.2 Essential oils components

Essential oils are aromatic and volatile liquids obtained from plant material, including flowers, roots, bark, leaves, seeds, peels, fruits, wood, and whole plants (Adelakun *et al*., 2016). There are two main types of component in essential oils: hydrocarbons (carbon and hydrogen only) and oxygenated hydrocarbons, which also contain oxygen (Clarke,2009).

Limonene is the major chemical component of citrus Eos; amount ranging from 32 to 98%. It belongs to the class of monoterpenes and the specific aroma of these compounds is characterized especially by the length of unsaturated straight chain aldehydes C8 –C14, acetate and a-selinenone (Mahato *et al.*, 2017).

II.2 Essential oil extraction methods

The extraction is known as separation of dissolvable materials from insoluble residues, which might be solid or liquid, by utilizing different solvents (Bagade et Patil, 2019). Table I summarizes the advantages and disadvantages of various extraction processes of essential oils.

Table I: Advantages and disadvantages of various extraction processes (Buckle, 2015).

Extraction Process	Advantages	Disadvantages
Distillation	Economical Large quantities can be processed Little labor needed	Changing constituents Depending on time/temp
Expression	No heat required Simple apparatus	Some flavoring left Only citrus peel oils Oxidize quickly
Enfleurage	Low temperature needed	Time consuming Labor intensive Expensive
CO2 extraction	Constant product No heat used	Expensive Different chemistry to essential oil
Solvent extraction	Constant product	Solvent residues Different chemistry to essential oil

II.3 Citrus essential oils

Citrus essential oils are widely used to the production of natural fruity perfumes and as flavoring ingredients in food, pharmaceutical and cosmetic products. They are obtained mainly from the fruit rind (flavedo), although flowers or leaves have also been used (González-Mas *et al.*, 2019).

Citrus essential oils (CEOs) are volatile aromatic compounds usually present in the peels of the citrus fruits at a concentration between 0.6% and 3.8% dry basis (Chen *et al.*, 2019). Bizzo (2009) has reported that maximum of 0.4 g essential oil can be extracted per 100 g of pulp. Extraction of essential oil from the citrus residue is very popular field of research among many scientists now days. Economy of the extraction of essential oils can be justified by its high value in wider pharmaceutical industry, as flavoring agent in food and beverage industry and cleaning products. The main constituents of this oil is a mixture of volatile compounds like terpenes and oxygenated derivatives such as aldehydes (citral), alcohols and esters. Virot *et al*. (2008) had demonstrated its application in oleochemical, wax, resin, paint, and glue industries as a nontoxic solvent as alternative to hazardous petroleum solvent (Chavan *et al*., 2018).

II.3.1 Chemical composition

The chemical composition and biological efficacy can be dramatically different in oils extracted from different varieties of citrus, specific citrus cultivar or from identical citrus materials using different extraction and separation methods (See annex 3).

Citrus essential oils are complex mixtures of approximately 400 compounds (Ou *et al*., 2015). They contain 85-99% volatile and 1-15% non-volatile components. The volatile constituents are a mixture of monoterpene (such as limonene) and sesquiterpene hydrocarbons and their oxygenated derivatives, including aldehydes (such as citral), ketones, acids, alcohols (such as linalool) and esters. Limonene being the major compound of citrus essential oils (Raspo *et al* ., 2020), ranging from 32 to 98% (Espina *et al*.,2011; Mahato *et al*.,2017)(See annex 4).

The USA Food and Drug Administration considered limonene as a GRAS (Generally Recognized as Safe) material. Aissou *et al*. (2017) have used limonene from agro-industrial waste streams as a primary chemical to obtain different oxidized and high added-value compounds, such as α-terpinolene, 3-methyl cyclopentanone and cis-Linalool oxide. Several researchers have used limonene as a polymer precursor using catalytic reactions. Linalool and β-pinene are other important compounds present in citrus essential oils, which have shown antidepressant and sedative activities when used in alternative medicines. Haselton *et al*. (2015) have shown that α- pinene had repellent properties against the house fly (*Musca domestica*) in laboratory conditions. Myrcene and linalool have been shown to have anesthetic properties (Raspo *et al*., 2020).

II.3.2 Citrus oil extraction

Citrus flavor products can be listed as peel oil, obtained by mechanical rupture of fruit oil glands either prior to, or during juice extraction; essence oil and aqueous essence (sometimes called aqueous aroma), consisting of volatile juice compounds both formed from the condensate of the evaporation process of citrus juices; petigrain oil, a product from steam distillation of citrus leaves and twigs; and oil of neroli, a floral oil produced from steam distillation of orange blossoms. Citrus juice volatile compounds are mainly represented by monoterpene hydrocarbons, sesquiterpene hydrocarbons, and oxygenated compounds, mainly represented by aldehydes, monoterpene alcohols, and esters (Wang *et al*., 2020) (See annex 3).

The essential oils extraction from citrus vegetal material (peel, flowers, and leaves) is based on steam distillation mainly in Clevenger-type hydrodistillation. Recently, an improved Clevenger-type apparatus with a second condenser preventing thermal reactions and reducing the oxidation of some monoterpene compounds has been described. Moreover, essential oils obtained from citrus peel such as sweet oranges (*Citrus sinensis* L.) and bitter oranges (*Citrus aurantium* L.), lemons (*Citrus limon* L.), bergamots (*Citrus bergamia*), mandarins (*Citrus deliciosa*), grapefruits (*Citrus paradise*), etc, can also be performed by cold press extraction, whereas this technique should not be applied for citrus flowers and leaves (González-Mas *et al*., 2019).

The extraction process can be summarized in three fundamental steps, namely:

(1) A mechanical action enables the rupture of the peel utricles and oil release;

(2) A stream of water is exploited to transport the essential oil;

(3) The oil and water are separated via centrifugation.

From the residues of the cold-extraction process it is possible to recover oils of lower grade by distillation. The flowers and leaves of bitter oranges are exploited for the production of distilled oils, defined as neroli and petigrain oils, respectively (Tranchida *et al*.,2012).

The essential oils should only be obtained either by distillation (water steam distillation or Clevenger hydrodistillation) or cold pressing. To prevent confusion, an organic extract from citrus peel should not be named EO, although it could simply be named oil. In fact, medium polarity solvents (diethyl ether, dichloromethane or ethyl acetate) extract more polar and higher molecular weight compounds such as hexadecanal, squalene, linoleic acid, heptadecanoic acid or neophytadiene, than distillation or cold press extraction, and it fails to extract many monoterpene and sesquiterpene compounds which are characteristic of citrus essential oils (González-Mas *et al*.,2019).

The extraction method used has an effect upon the physical properties of citrus oils. The qualitative characteristics of an essential oil are almost always closely related to the yield obtained. An exhaustive extraction procedure produces a larger quantity of high-boiling components, with high molecular weight. As a result, the oil has high specific gravity, non-volatile residue and refractive index values, while the optical rotation value is lower because of the lower relative percentage of d-limonene.

In ideal conditions, the extraction of essential oils would not involve the use of water. However, almost all the industrial procedures required the use of water to wash away the oil from the surface of the whole fruit or peel. Since the fragrance of essential oils is directly related to the content of aldehydes and esters, the amount of water which circulates during processing is very important. A relatively simpler extraction method is stream stripping and distillation method. This is an effective method for removing oil components from oil-milled sludge.

Distillation is sometimes considered as an economical way to recover the oils (with better yield of 0.21%) compared to cold pressing (yield-0.05%). During distillation, the citrus peels are exposed to boiling water or steam. The oils are released into water and then collected through distillation. The steam and EO vapors are condensed and collected in specialized vessel called “Florentine flask” (Mahato *et al*., 2017).

The conventional methods for the extraction of citrus essential oils have some disadvantages. When using cold pressing, citrus essential oil is agitated vigorously with water and a gradual diminution in citral and terpene alcohols contents will be observed. Furthermore, during agitation, air is thrashed into the liquid, thereby creating favorable conditions for hydrolysis, oxidation and resinification. For steam distillation and hydrodistillation, the elevated temperatures and prolonged extraction time can cause chemical modifications of the oil components and often a loss of the most volatile molecules (Boukhatem *et al*, 2016). These shortcoming have led to the consideration of the use of new “green” technique in essential oil extraction, such as simultaneous distillation and extraction, microwave-assisted extraction, and ultrasonic-assisted extraction that use solvent (González-Mas *et al*.,2019).

EXPERIMENTAL

STUDY

CHAPTER III

MATERIALS AND METHODS

The objective of the present study is the extraction of juice from three citrus fruits collected in Bejaia city, and the characterization of their respective essential oils provided by Cosmos aromatica (Barcelona, Spain). Indeed, the extraction of essential oils from our samples was not possible because of the COVID 19 pandemic. For this, we tried to interpret the results of the physicochemical and phytochemical analyzes of essential oils supplied by a Spanish company.

III.1 Plant material and essential oils samples

Sweet orange (*Citrus sinensis*), grapefruit (*Citrus paridisi*) and lemon (*Citrus limon*) fruits were collected by hand in the beginning of August from Bejaia precisely in the surrounding of Toudja in 500 m of altitude; the average of temperature was 28°C and humidity was 62% (data were provided by national institute of metrology of Algeria).

After that, the fruit were prewashed to get rid of immediate dirt and pesticide residue. The leaves and stems still attached to the fruit are removed. Then the manual inspection was done to remove any unsuitable fruit. Sound fruit is conveyed to the transformation.

Concerning the essentials oils provided by the Spanish company they are presented in the table below.

Table II: Citrus essential oils obtained from peel using different industrial extraction methods

					
SAMPLE	I *Citrus Limon* essential oil	II *Citrus Limon* concentrated essential oil (5fold)	III *Citrus paradisi* essential oil	IV *Citrus sinensis* essential oil	V *Citrus sinensis* concentrated essential oil (5 fold)
Extraction	Cold press	Distillation	Cold press	Cold press	Distillation
Origin	Spain	Spain	Spain	Brasil	Brasil

III.2 Extraction of juice

Extraction involves squeezing or reaming juice out of either whole or halved citrus fruits by means of mechanical pressure. The citrus fruits are directed to domestic juice extractor in order to achieve optimum juice yield.

After extraction, the pulpy juice (about 50% of the fruit) is clarified by primary finishers which separate juice from pulp (method based on sieving). The juice stream is further clarified by centrifugation.

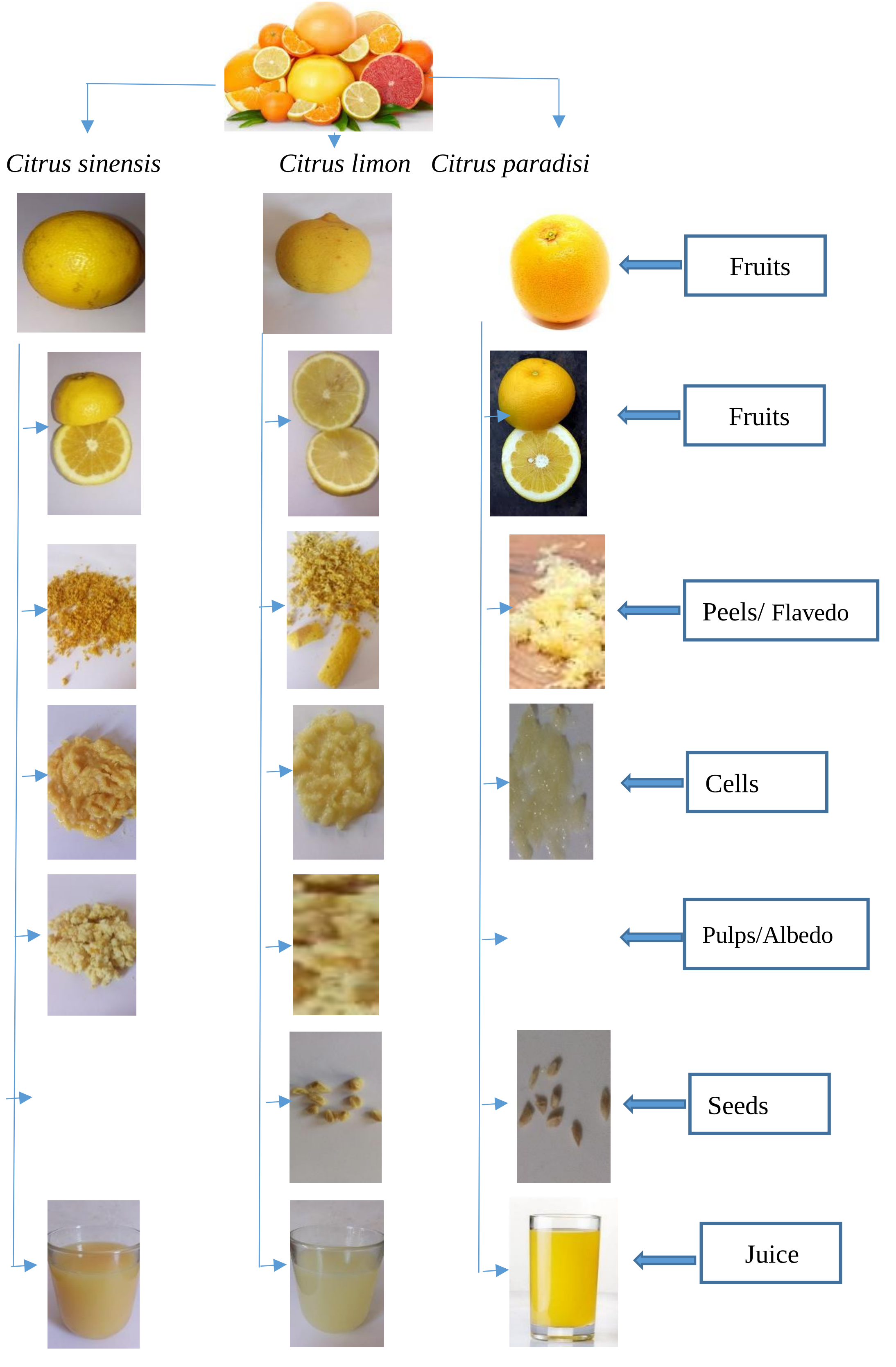

Figure 1: Three samples of citrus fruits along with their by products

The extraction yield obtained is defined as follows:

$$\text{Rate of the extracted fraction } \mathbf{(\%) = [(W_1 – W_0) /WS].100}$$

With:

W0: Weight of empty narrow neck Erlenmeyer flask (mg);

W1: Weight of the Erlenmeyer flask with narrow neck plus weight of the extracted fraction (mg);

WS: Sample weight (fruit) (mg);

The extracted products are stored at 4 °C and protected from light, they will be the subject to physicochemical analyzes in the following 5 minutes.

III.3 Physicochemical analyzes of citrus fruits

III.3.1 Refractometer ºBrix

- **Principle**

Measurement of light refraction: Light travels at different speeds in different media such as air, water or sugar solutions. When light passes from one medium to another it is refracted, that is, it changes direction slightly. This property of a medium can be quantified as its refractive index. The refractive index depends on its total soluble solids. The °Brix scale is based on standard measurements at 20 °C. Brix can be measured by either density measurements or by measuring the refractive index. Both are then related to a 100 % sucrose solution (Ringblom, 2004).

Brix values properties that give an indication of the density of sugar in a solution at a specific temperature. Brix values are frequently used to express the sugar levels in fruits, beverages and sugar juices. Named by the German inventor A. F. W. Brix (IFIS, 2009).

- **Procedure**

The °Brix (degree Brix) is determined by measuring the refraction of light, digital refractometers was used (Arenas-Arenas *et al*., 2017)

- **Corrected ºBrix or Soluble Solids**

In order to obtain the real percentage of soluble solids, known as corrected ºBrix, the refractometer Brix should be corrected for the presence of acids and temperature (temperature per definition 20ºC) (Ringblom, 2004).

III.3.2 Acid content

- **Principle**

Juice acidity is measured using a chemical titration method. Orange juice contains acids which release hydrogen ions (H+) in solution. When a base which releases hydroxyl ions (OH^-) is add to an acid media, a chemical reaction takes place which gradually turns the solution neutral.

$$H^+ + OH^- \rightarrow H_2O$$

Acid content (% w/w) is determined by direct titration against a standardized alkali solution (e.g. 0.1 Molar sodium hydroxide), using a pH meter, to an end point at pH 8.1. Where the juice is naturally clear, or has been clarified, and is of low colour intensity, the end-point may be accurately found using phenolphthalein as indicator.

Although there are other acids present in fruit juices (e.g. oxalic, iso-citric, tartaric, etc.), it is usual to record acidity in terms of citric acid, both for citrus fruit juices and for the majority of soft fruit juice (Ashurst, 2016).

- **Procedure**

10 ml of single-strength juice of juice are poured into a beaker and a volume of sodium hydroxide (NaOH) at 0.1562 N in a burette. The pH can be indicated by adding 5 drops of phenolphthalein or by using a pH meter. Titrate until a slight darkening in the juice persist or until pH 8.20. Read the amount of NaOH used from the burette (Ringblom, 2004).

- **Calculation**

Single-strength juice: % acid = ml titration solution/10.4;

Concentrate: % acid = ml titration solution/g concentrate.

III.3.3 Ratio

The percentage soluble solids, known as corrected ºBrix is divided by the percentage of titratable acidity (as % citric acid anhydrous) to obtain the ºBrix/Acid ratio. The Brix: acid ratio is very important for taste as it is a measure of the balance between the sweet and sour sensation. The ratio decides the maturity of the fruit before harvesting (Arenas-Arenas *et al*., 2017).

The ºBrix and ratio are criteria used in determination of fruit maturity and juice quality. ºBrix is also used to determine the degree of concentration in the production of juice concentrates. The higher the ratio the sweeter the products (Arenas-Arenas *et al*., 2017).

Ratio =°Brix / % (w/w) citric acid

III.3.4 Determination of pH

- **Principle**

pH measure of the degree of acidity or alkalinity of a substance. pH (an abbreviation for potential of hydrogen) is defined as the negative logarithm of the hydrogen ion concentration. The scale ranges from 0 (very strongly acid) to 14 (very strong alkaline). A neutral solution, such as pure water, at 25°C has a pH of 7 (Rodrigues *et al.*, 2019).

- **Procedure**

Set pH meter with a standard buffer solution as per instructions on the meter using pH 4.0 and 7.0 buffer. Place sample in a 50 – 100 ml beaker and immerse electrodes. Use sufficient sample so that the tips of the electrodes are covered. Adjust for temperature and read the pH (Arenas-Arenas *et al.*, 2017).

III.3.5 Viscosity

- **Principle**

Viscosity measure of the ease with which a fluid can flow when subjected to shear stress, measured in Newton seconds per square meter or Pascal seconds. Low viscosity, e.g. that of a gas, allows flow through a fine tube to be quite rapid, whereas high viscosity (as with thick oils) makes motion sluggish. Viscosity arises from the intermolecular forces in a fluid (internal friction); the stronger these forces, the greater the viscosity. With a rise in temperature, attraction between the molecules is reduced, enabling them to move more freely (IFIS, 2009). Viscosity (η, Pa.s) is defined as$\eta = \tau/\gamma$ Rheological behavior of fluids is characterized by measurement of viscosity. If a plot of shear stress (τ) vs shears rate (γ.) results in a straight line, viscosity (η) is constant and that material is classified as Newtonian. Fluid that does not obey this relationship ($\tau = \eta.\gamma.$), is non-Newtonian, which includes most of the food materials (Rui *et al.*, 2014).

- **Procedure**

Viscometer is used for measure viscosity. The apparent viscosity is generally expressed as centipoise (100 g/sec cm) and shear rate (sec-I). For non-Newtonian fluids, the method that is most applicable and most commonly used is the rotation of a spindle or cylinder within a coaxial cylindrical tube. With non-Newtonian solutions, such as citrus fruits juice and concentrate, the varying shear rates can lead to misleading results (Kimball, 1999).

III.3.6 Density

- **Principle**

Density one of the physical properties of a substance, defined as the mass contained in a given volume. This parameter is routinely determined for a wide range of foods, including fruits and vegetables (some times related to ripeness and composition), fats and oils, foods produced by extrusion, and cereals. Density determination can also be used as process control steps in food processing (IFIS, 2009).

- **Procedure**

Densimeter is used for measure the density.

III.3.7 Sinking pulp

- **Principle**

Different procedures for measuring the pulp and concentration of suspended solids of orange juice were used by the industry.

The juice sample is centrifuged in graduated tubes for a known time and speed. Solid material above a certain particle size will settle in the tubes according to the time and centrifugal force of the lab centrifuge (Ringblom, 2004).

- **Procedure**

The recommended procedure in the juice industry is to spin the juice sample at 370 g for 10 minutes at 26 °C (USDA method).

The sedimented quantity is expressed as volume %. Not only may the time-speed combination vary greatly from method to method, but the results may be referred to suspended solids, suspended pulp, and centrifuged pulp or sinking pulp.

III.3.8 Floating pulp

Floating pulp is often measured by a sieving method.

- **Procedure**

Sieves with different whole sizes are used to determine the amount and size of pulp in juice (Ringblom, 2004).

III.3.9Cloud stability

- **Principle**

This analysis method is based on the fact that both soluble and insoluble solids absorb light, with the result that only a certain amount of total light entering a sample will pass through (Ringblom, 2004).

- **Procedure**

Cloud stability is detected by measuring the transmittance of orange juice in a spectrophotometer.

- **Lector**

The citrus juice sample is centrifuged to take away larger suspended particles (sinking pulp).
The light transmittance of the sample serum is measured at 650 nm wavelength. The denser the cloud in orange juice, the lower the transmittance, % T. Citrus juice cloud is not considered stable if the % T at 650 nm is greater than 36 (Ringblom, 2004). .

III.4 Physicochemical analyzes of Citrus essential oils

III.4.1 Optical Rotation

- **Principle**

Molecules within essential oils have the ability to rotate a plane of polarized light. Molecules that rotate counterclockwise are called levorotatory, or l for short. Those that rotate clockwise are called dextrorotatory, or d. This ability is indicated in the name of the molecule, for example, d-limonene. Almost all essential oils show optical activity. Optical rotation can reveal synthetic compounds that alter the optical rotation. Synthetic menthol rotates in a different way to menthol from the mint plant (Buckle, 2015).

- **Procedure**

• Polarimeter with a standard 100 mm tube and a sodium vapor lamp Procedure

1. Adjust oil sample and instrument temperature to 20°C (68°F);
2. Fill the polarimeter tube with oil and wipe off excess oil on the exterior;
3. Place the tube in the polarimeter;
4. Slowly turn the analyzer until both halves of the field, viewed through the telescope, show equal intensities of illumination, better perform in dark;
5. Read rotation degree together with the direction of rotation from the zero position {counter-clockwise, (-) and clockwise, (+)}.

- **Calculations**

The reading is presented as result. If measurement is performed at prism temperatures other than the recommended 20°C, the observed optical rotation is corrected by adding or subtracting 0.22° for orange and grapefruit oils and 0.14° for lemon oil for each degree centigrade above or below 20°C.

III.4.2 Refractive Index

- **Procedure**

This was determined as reported by Adepoju and Eyibio (2016).

• Refractometer is use for this mesure

1. Calibrate the refractometer against a standard provided by the manufacturer. For daily use, check with distilled water (RI of 1.3330 at 20°C/68°F and 1.3325 at 25°C/76°F);
2. Condition the oil sample and refractometer to 20°C (68°F);
3. The temperature should be carefully adjusted and maintained since the refractive index varies significantly with temperature;
4. Clean prism by wiping with cotton pad moistened with a solvent (e.g., isopropanol) and let air dry;
5. Apply a couple drops of sample and allow time for temperature equilibrium between instrument and sample;
6. Adjust board line so that it falls on point of intersection of cross hairs;
7. Read RI.

- **Calculations**

The reading is presented as the result. If measurement is performed at prism temperatures other than the recommended 20°C, the observed refractive index is corrected by adding or subtracting 0.00045 for orange oil and 0.00046 for lemon oil for each degree centigrade above or below 20°C (Fakayod et abobi, 2018).

III.4.3 Total aldehyde determined by AOAC method (JBT, 2018)

- **Apparatus**

Analytic balance with resolution of 1 mg;

pH Meter with resolution of 0.01;

10 ml Buret with 0.05 ml graduations;

Magnetic stirrer and Teflon;

Coated stirring bar;

50 ml Glass-stoppered graduate;

1000 ml Graduated cylinder;

Potassium hydroxide (KOH);

Isopropanol (aldehyde free) (C_3H_8O);

Bromophenol blue;

Hydroxylamine hydrochloride (H_3NOHCl);

Hydrochloric acid (HCl).

- **Reagents**

A. Isopropanol (60%): Dilute 632 ml of 95% isopropanol with distilled water to 1000 ml;

B. Alcoholic potassium hydroxide (0.5 M): Dissolve 28.06 g of KOH in 60% ethanol and make up to 1000 ml with the same solvent;

C. Bromophenol blue solution (0.01%): Triturate and dissolve 0.1 g of bromophenol blue in 5 ml of 0.05 N NaOH and make up to 100 ml with 60% isopropanol;

D. Hydroxylamine hydrochloride solution (0.5 N): Dissolve 34.745 g of H3NO.HCl in 875 ml of 60% isopropanol, add 1.5 ml of bromophenol blue solution and enough 0.5 M alcoholic KOH solution to give permanent blue solution, and make to 1000 ml with 60% isopropanol.

- **Procedure**

1. Accurately weigh, to the nearest 10 mg, about 10 g of oil sample into glassstoppered 50 ml graduate;
2. Add 7 ml of hydroxylamine solution;
3. Add 0.1 ml of bromophenol blue solution;
4. Mix thoroughly;
5. Titrate with 0.5 M alcoholic KOH to a permanent full alkaline color of the bromophenol blue solution (in the lower layer separated after shaking vigorously for 2 minutes). Reaction time is complete in about 15 min and KOH solution used should be less than 5 ml.

- **Calculations**

The major aldehyde component present in orange oil and lemon oil are decanal and citral, respectively. Accordingly, each ml of 0.5 M alcoholic KOH is equivalent to 0.07813 g of decanal or 0.07612 g of citral.

Percent Aldehyde (W/W) = ((ml titrant/1000 ml/l)(M titrant)(MW of aldehyde)) /(Sample Weight)*100

Or

% Aldehyde (Citral, W/W) = ((ml KOH/1000ml/l)(0.5MKOH)(152.23 g/mole)) /(g Sample)*100

= (ml KOH)/(g Sample)*7.612

And

% Aldehyde (Decanal, W/W) = ((ml KOH/1000ml/l)(0.5MKOH)(156.27 g/mole)) /(g Sample)*100

=(ml KOH)/(g Sample)*7.813

The % Aldehyde for accurately weighed oil samples is calculated as :

- 10 g of cold pressed lemon oil

  %Aldehyde (Citral,w/w)=(ml KOH)*0.7612
- 10g of cold pressed orange oil

  %Aldehyde (Decanal, w/w)=(ml KOH)*0.7813

III.4.4 Total aldehyde determined by USP method (JBT, 2018)

- **Apparatus**

Analytic balance with resolution of 1 mg;

pH Meter with resolution of 0.01;

10 ml Buret with 0.05 ml graduations;

Magnetic stirrer and Teflon coated stirring bar;

150 ml Flasks with ground glass stoppers;

Hydroxylamine hydrochloride ($H_3NO.HCl$);

Potassium hydroxide (KOH);

Tertiary butyl alcohol;

Isopropanol (C_3H_8O).

- **Reagents**

A. Alcoholic potassium hydroxide solution (0.5 M): Dissolve 28.055 g of KOH in 1000 ml of 95% isopropanol;

B. Hydroxylamine hydrochloride solution: Dissolve 45 g of H_3NO. HCl in 130 ml of distilled water, add 850 ml of tertiary butyl alcohol. Mix and adjust pH to 3.4 with

0.5 N alcoholic KOH solution.

- **Procedure**

1. Weigh accurately 5 g of oil into a 250-ml flask with stopper;
2. Add 50 ml of hydroxylamine hydrochloride solution into the flask;
3. Stopper the flask right away and swirl to mix the solution;
4. Incubation during 30 min at ambient temperature with occasional

Swirling;

5. Titrate samples, drop-wise and slowly, with 0.5 M alcoholic KOH to pH 3.4;
6. Read volume of titrant used.

- **Calculations**

The aldehyde content in citrus oils is expressed in equivalent of the major aldehyde component present in the tested oil. For orange and lemon oils, they are decanal and citral, respectively. One mole of KOH reacts with one mole of aldehyde and therefore, each ml of 0.5 M alcoholic KOH is equivalent to 0.07813 g of decanal or 0.07612 g of citral.

Percent Aldehyde (W/W) = ((ml titrant/1000ml/l)(M titrant)(MW of aldehyde)) /(Sample Weight)*100

Or

% Aldehyde (Citral, W/W) = ((ml KOH/1000 ml/l)(0.5M KOH)(152.23 g/mole)) /(g Sample)*100

= (ml KOH)/(g Sample)*7.612

And

% Aldehyde (Decanal, W/W) = ((ml KOH/1000ml/l)(0.5MKOH)(156.27 g/mole)) /(g Sample)*100

=(ml KOH)/(g Sample)*7.813

The % Aldehyde for accurately weighed oil samples is calculated as:

- 5 g of cold pressed lemon oil

  % Aldehyde (Citral, w/w)=(ml KOH)*1.522

- 5g of cold pressed orange oil

  % Aldehyde (Decanal, w/w)=(ml KOH)*1.563

III.4.5 Determination of peroxide value of orange essential oil

This parameter was determined by the procedure described by Njoku and Evbuomwan (2014). Thirty milliliters of acetic acid chloroform solution was measured into a flask containing 2 g of the oil sample. A 0.5 ml saturated solution of potassium iodide was then added, followed closely by the addition of 30 ml of distilled water. The flask content was then titrated against 0.1 M sodium thiosulfate until the yellow color almost disappeared; 0.5 ml starch indicator was added and the titration continued until the end point (where the blue-black color just disappeared). A blank titration was equally performed. The peroxide value was calculated using Eq (Fakayod et abobi, 2018).

$$PV = \frac{100 \times (B - S)}{W_o}$$

PV= peroxide value of orange peel essential oil (mEq O_2/kg)

III.4.6 Determination of volatile compounds by gas chromatography

In chromatographic methods, such as gas or liquid chromatography coupled with mass spectrometry detectors, the goal is to identify compounds and compare their concentrations across and within samples. To achieve this goal, data processing must fulfil two criteria:

- it must correctly determine the mass spectrum of the individual compounds for identification and;

- it must accurately calculate the abundance of chromatographic peaks corresponding to those compounds in each sample.

These two tasks are often challenging and time consuming mainly due to the co-elution of chromatographic peaks within a single chromatogram, as well as retention time (RT) shift of peaks across samples. These two challenges lead to mixed mass spectra and complicate compound identification and quantification. For these reasons processing of GC-MS data is challenging using currently available techniques that may perform inadequately both with respect to identification and quantification leading to compounds being wrongly interpreted or simply left undetected (Johnsen *et al.*, 2017).

Injection conditions are not given because they are specific to the Spanish company.

Chapter IV

RESULTS
AND
DISCUSSION

IV.1 Extraction yield of juice from citrus fruits

On a small scale, the juice is usually extracted by hand reaming where the fruits are halved in two parts and juice is extracted by reaming the halves on a suitable rosette. This extraction method of juice is the best one as it does not break the oil cells of the peel nor crush the seeds. On the contrary, mechanical juice extractors are also available for juice extraction where sometimes, the peel is removed manually and peeled citrus fruit is fed into the domestic juice extractor to extract the juice. The average yield of juice obtained by manual method is around 39% for *Citrus sinensis*, 33% for *Citrus limon* and 34% for *Citrus paradise* (Figure 2); while that of clean, healthy and ripe fruit, obtained by mechanical extraction method can reach 60% (Sharma, 2009).

This variation in yield can be attributed not only to the origin of the fruit, variety, genome diversity, climate, cultural practices and the technique of juice extraction but also to the time of fruit picking (Boutakiout *et al*., 2018). In fact, in this study, the fruits were picked in August before reaching the stage of maturity that’s why the extraction yields are less than 50%.

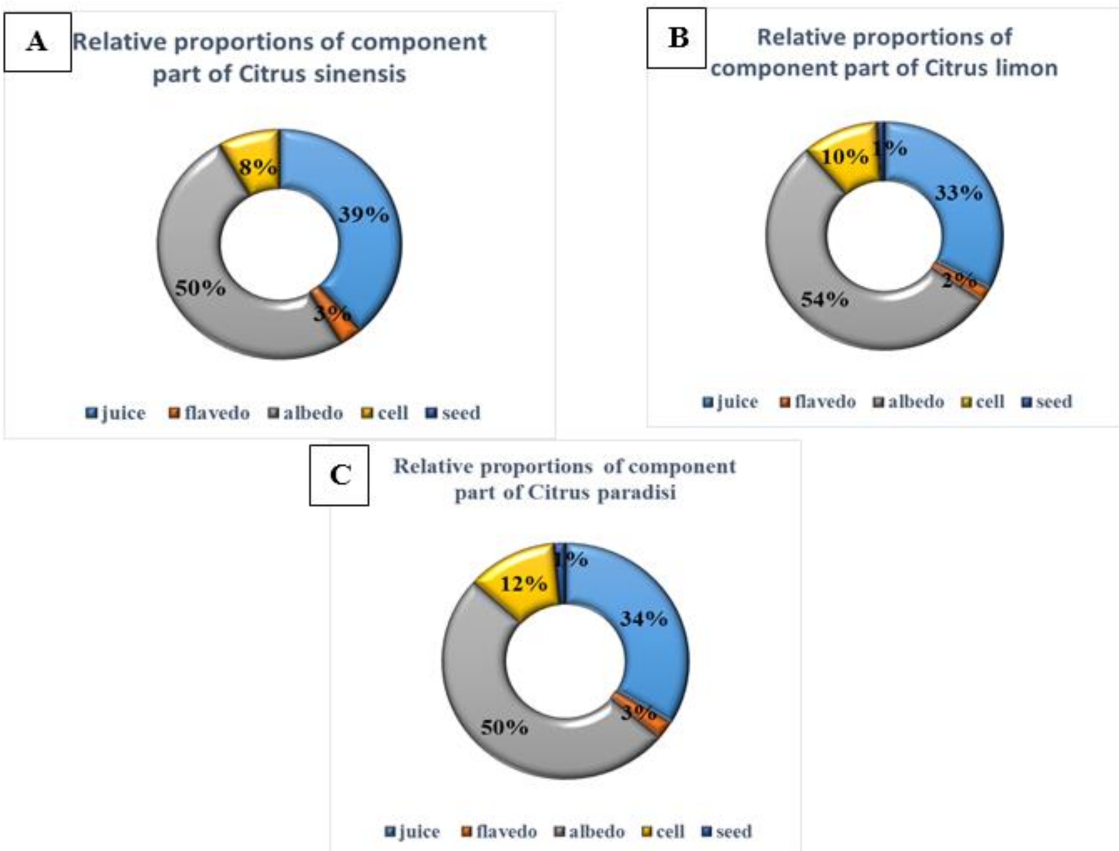

Figure 2: Relative proportions of component part of some citrus fruits:

A) *Citrus sinensis*, B) *Citrus limon*, C) *Citrus paradisi*

IV.2 Physicochemical characteristics

IV.2.1 Juice and pulp

Figures 3,4,5,6 and 7 provide the physicochemical characteristics presented by the three selected samples of citrus fruits analyzed in the present work.

- **Refractometer °Brix**

°Brix is commonly used for analysing juice quality (Rizzon and Miele, 2012). The results given in Figure 3 shows that there is no specific trend observed in quantity of various constituents in the juice of different species of citrus.

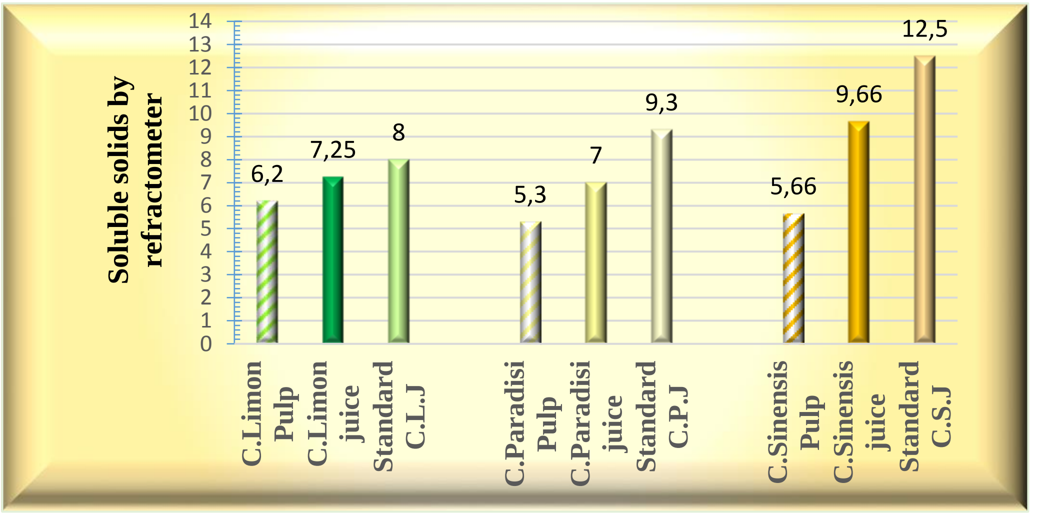

Figure 3 : Graphic representation of the soluble solids content expressed in ° Brix.

The juice of *Citrus sinensis* contains the highest amount (9.66 g/100 ml) of total soluble sugars as compared to other species of citrus, whereas *Citrus paradisi* contains the lowest amount of the same compounds i.e 7 g/100 ml, *Citrus limon* contain 7.25g /100 ml. Indeed, these findings are similar than those already reported in the litterature (Chavan, 2018). In Brazil, the legislation for juice quality establishes a minimum value of 14 °Brix measured under a controlled temperature of 20°C. For integral juice, this value represents only sugars naturally present in the fruit (Brasil, 2000). The composition of fruit nutrients may vary largely due to climatic conditions, topographical variation nature of soil and maturity of fruit (Kumar, 2013).

The pulps obtained from the different citrus fruits have a lower Brix than the brix of the juice of the same fruit, it varies from 5.3% for the pulp of *Citrus paradisi* up to 6.2% in the case of the pulp of *Citrus limon*.

- **Titratable acidity**

The acidic flavor is linked to the perception of the H $^+$ ion. The measurement of acidity is defined by three parameters: pH, titratable acidity, and organic acid composition. The very high acidity of the pulp is the main characteristic of citrus fruits. It is linked to the composition of the juice and pulp in organic acids and more particularly in citric acid. Citric acid, in general, represents from 60% in so-called "intermediate" fruits (orange, clementine and mandarin) to more than 90% in so-called "acid" fruits (lemon and lime) of total organic acids (Khififi, 2015).

For the studied fruit juice, the percentage of acids is 3.4% for *Citrus limon* juice, 1.1% for *Citrus paradisi* juice and 0.86% for *Citrus sinensis* (Figure 4). Citrus fruit pulps acidities are lower than that of the same species. They are 1.085%, 0.9% and 0.443% for *Citrus limon*, *Citrus paradisi* and *Citrus sinensis*, respectively.

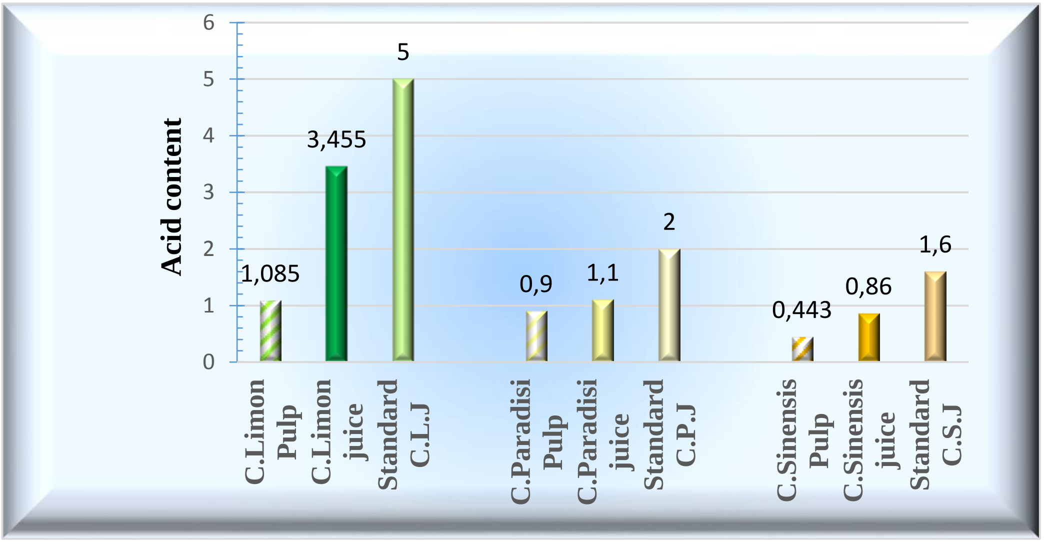

Figure 4 : Graphical representation of the acidity expressed in % (m/m citric acid).

The acidity of mature citrus juices are 6% for *Citrus limon*, 1.3% for *Citrus paradisi* and 2.1% for *Citrus sinensis* (Karadeniz, 2004; Marsh *et al*., 2003; Pailly *et al*., 2004), these values are higher than those of our samples, this can be explained by the immaturity of the analyzed fruits.

- **Ratio**

The ° Brix / titratable acidity ratio is an indicator of the maturity of a fruit, as well as its flavor, the more the sugar level increases (° Brix), the more the concentration of organic acids decreases (titratable acidity). Brix: acid ratio is important for describing taste as a measure of the balance between the sweet and sensations of the consumers which prefer a ratio of around 15:1 (tetrapak). The more the fruit becomes mature for orange juice this value is 9° and 14° (Sadler *et* Murphy, 2010).

The average of the ratios obtained during the analysis of the different citrus juices are presented in the figure 5. *Citrus sinensis* juice has a ratio of 11.2 which is higher than that of *Citrus limon* juice (2.1) and *Citrus paradisi* juice (6.4), this is due to the high acidity of these last two fruits and their low Brix.

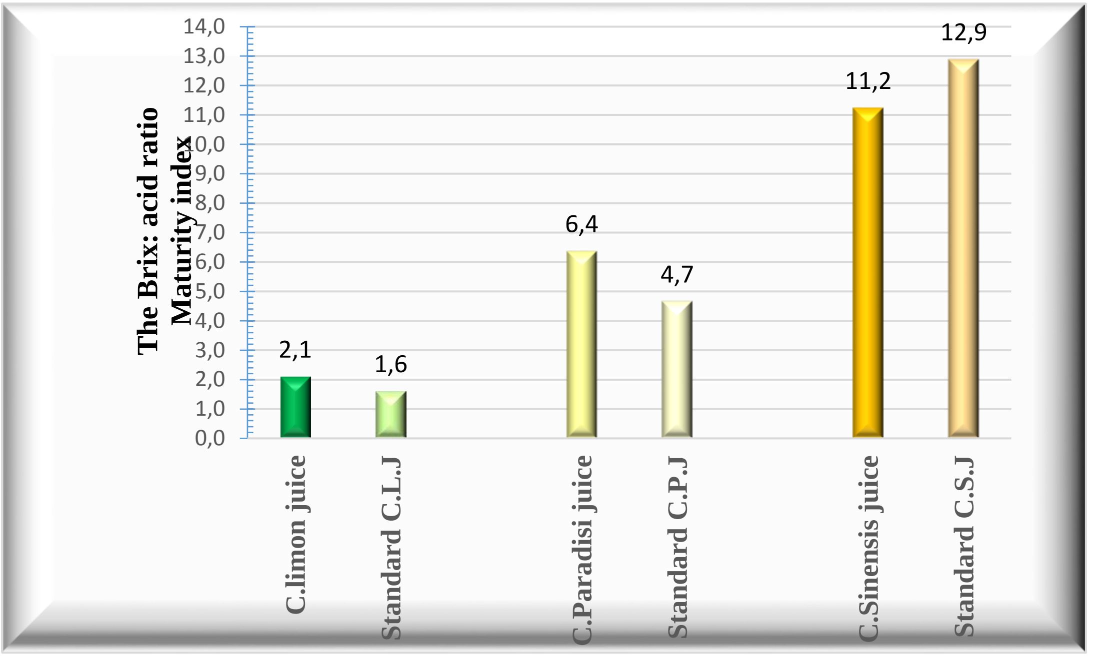

Figure 5 : Graphical representation of Ratio Brix/Acidity

The values obtained are higher than the standard values (1.6 and 4.7) in the case of *Citrus limon* and *Citrus paradisi* juices, respectively. So that the *Citrus sinensis* juice presents a ratio lower than the value of the standard which is about 12.9. This can be explained by the stage of maturity, ie the maximum acidity and brix not reached.

During the ripening of the fruits, the percentage of sugars, especially that of sucrose, increases gradually and steadily in most species citrus fruits with the exception of species whose fruits are acidic such as limes and lemons (Lado *et al*., 2018).

It should be noted that the accumulation of sugars does not occur not only at the level of the juice vesicles but also at the level of the flavédo. Citrus fruits also contain starch which is present in small amounts. The concentration of this polysaccharide continues to increase during growth and ripening of the fruit (Sinha *et al*., 2012).

- **Viscosity**

Viscosity is a measure of the"thickness" of a fluid. It affects the "body" of the juice created primarily by pectin-related stabilisation of the cloud or colloids in the juice. The presence of insoluble materials also contributes to the increases of juice body or viscosity (Ringblom. 2004).

The results presented in the figure 6 shows that the viscosity of the juicefrom *Citrus limon* (450cp) is lower than that of *Citrus paradisi* and *Citrus sinensis* which (790cp and 1176cp, respectively), this is probably due to the level of citric acid contained in the juice which destabilizes the pectin network and the low amount of soluble matter which contributes to the decrease in the body which lead to the decrease of the viscosity of the juice. The viscosity of the pulp is much higher than that of the juices because of the high quantity of pectin present in the albedo and its richness in fibers (Enkuahone, 2018).

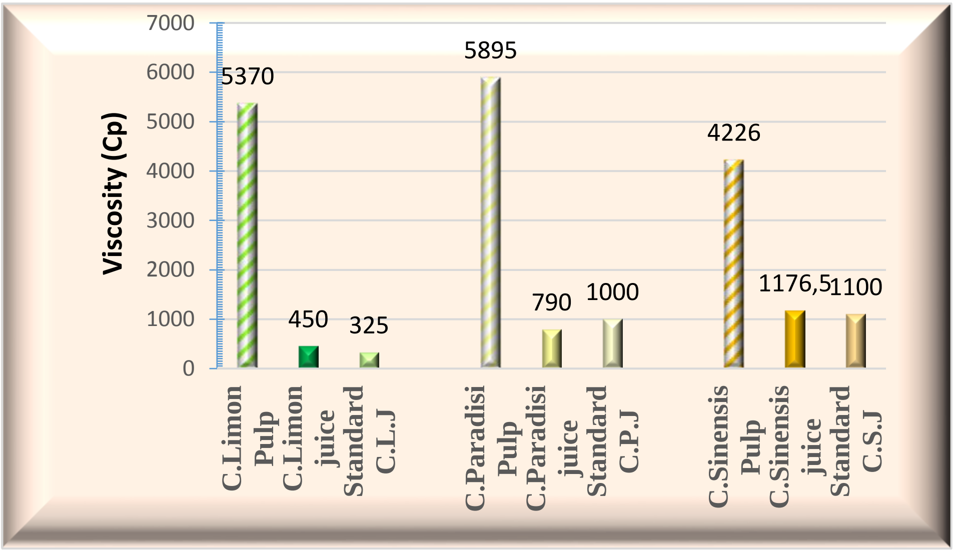

Figure 6 : Graphical representation of some citrus juice and pulp viscosity

- **Density**

Figure 8 shows the experimental density values obtained for the juice and pulp of the three analyzed citrus varieties.

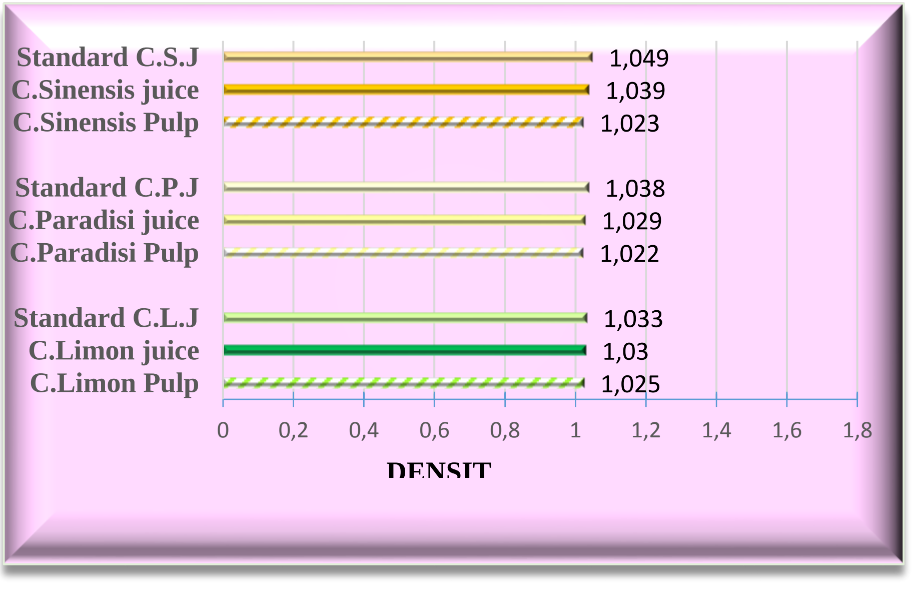

Figure 7 : Graphical representation of some citrus juice and pulp density .

The densities of the juices of *Citrus sinensis*, *Citrus paradisi* and *Citrus limon* are 1.039, 1.029 and 1.03, respectively, these values are very close because of the similarity of the composition of different juices (slightly cloudy liquid solution having a low brix and containing small particles of fiber). The obtained values are lower than those of the references (1.049, 1.038 and 1.033) because of the brix and the degree of maturity of the fruit which is not reached.

The variability in composition between and within each kind of studied citrus fruit in this work is influenced by many factors such as genetic factors, rootstock, maturity, position of the fruit on the tree, field factors, and orchard practices. Moreover, there is even variability in the composition within individual fruits. All of these factors which influence variability should be taken into account when a population of citrus fruits is sampled for analysis; however, the validity of the results should be assessed in the light of the sampling adopted procedures (kefford, 1960).

IV.2-2 Essential oils of the three citrus fruits

The physicochemical properties of the sample from citrus essential oil are presented in Table III.

Tableau III: Physicochemical analysis of some citrus essential oils.

CEO	Extraction process/ part of the fruit	**Density (20ºC,g/ml)**	**Refractive Index Nd 20**	**Optical Rotation (º)**	**Acid mgKOH /g**	**Indice peroxide mmol/l**	**Pont d inflamation** °C	**Aldehyde** (%)
I Lemon Essential oil	cold press, Peel	0.8535 ±0.0045	1.4735 ±0.0035	62 ±7.5	/	/	/	As citral 2.5
II Lemon 5Fold essential oil	Distillation Peel	0.867 ±0.005	1.474 ±0.004	/	/	/	/	As citral 6.5
III Citrus paradisi oil	Cold press peel	0.845 ±0.0015	1.475 ±0.01	95 ±5	/	/	46	/
IV Citrus sinensis oil	Cold press peel	0.846 ±0.004	1.473 ±0.003	96.5 ±2.5	2	20	48	As decanal 1

V Citrus sinensis 10 absatract	Distillation Peel	0.875 ±0.005	1.4825 ±0.0075	/	/	20	/	As decanal 8

- **Density**

The extracted essential oil has a specific density less than 1 which implies that it is lighter than water and consequently will be insoluble in water. The density values of essential oils are practically identical, they vary from 0.846 to 0.865 for *Citrus limon*, 0.842 to 0.88 for *Citrus sinensis* and from 0.83 to 0.86 for *Citrus paradisi*, noting that the densities obtained after cold extraction are lower than that obtained after distillation, which is explained by the high concentration of oils extracted by the latter method and indicates that the physical properties of these three oils are quite similar.

- **Refractive Index**

The refractive indices are commonly used for testing how the speed of light is altered when passing through the oil. Values obtained in the present study are low and varies from 1.465 to 1.49, noting that the RIs obtained after extraction by distillation are slightly higher than those obtained by cold extraction. This can be explained by the high concentration of essential oil resulting from the distillation. Kumar (2014) categorized a refractive index of 1.47 as highly pure (Fakayod et abobi 2018), which confirm that the extracted essential oil of the studied samples are pure.

- **Indice peroxide**

The essential oil extracted from the peel of *Citrus sinensis* exhibits a peroxide value equal to 20 mEqO2/kg, a value similar to the upper limit described by Mailer and Beckingham (2006) from which the oil becomes less stable requiring a shorter shelf life. Peroxide value gives a measure of the extent to which the oil has undergone primary oxidation. The double bonds found in essential oils play a role in the autoxidation; a free radical reaction involving oxygen that leads to the deterioration of oils which form off-flavors and off-odors; it shows an initial evidence of rancidity in unsaturated fats and oils. Peroxides are intermediates in the autoxidation reaction and the peroxide number is useful in assessing the progression of spoilage (Fakayod et abobi, 2018).

- **Acidity**

The acid value of the essential oil was 2 mg KOH/g, oils with low acidity are considered as neutralized and safe for making skin care products as high acidity of oils may be harmful for skin. Essential oils are concentrated and contain several volatile aroma compounds which are majorly free fatty acids. Free fatty acids present in essential oil are considered as degrading in oils because they are responsible for oil rancidity (Kumar, 2014).

- **Aldehyde**

The major aldehyde is citral for the essential oil of *Citrus limon* with 2.5% (cold extraction) and 6.5% (distillation). For *Citrus sinensis* the decanal represents 1% (cold extraction) and 8% (distillation). During distillation the majority of volatile molecules are recovered while during cold extraction a loss of volatile substance is observed. The content of both aldehydes, defined as “citral,” is an important parameter in the commercial transactions of essential oil as these substances determine its olfactory peculiarities (Sahraoui *et al*., 2011).

For citrus oils, the terpenes represent the main composite class, constituted mainly of D - limonene. The oxygenated terpenoids, which are the lesser constituents, contribute a higher intensity to the oil aroma.

In general, the components present in sweet orange EO can be grouped into five classes: monoterpenes, oxygenated monoterpenes, sesquiterpenes, oxygenated sesquiterpenes, and other oxygenated compounds (Espina *et al*., 2011). Monoterpenes hydrocarbons ($C_{10}H_{16}$) represented the main coumpounds of *Citrus sinensis* essential oil. Among which, limonene is the main compound present (71–95.1%) in sweet orange EO, this was in consistent with most citrus species (Espina *et al*., 2011, Sahraoui *et al*., 2011). The chemical compositions of essential oils are reported in Table IV.

In the present study, the results depicted that the major compounds of different *Citrus sinensis* samples are:

Citrus sinensis peel obtained by cold press: limonene (93%), alpha pinene (0.4%), Beta Mycene (1.5%), Sabinene (0.2%), Linalool (0.15%) and Decanal (0.1%);

Citrus sinensis peel obtained by distillation: limonene (85%), Octanal (1.5%), Linalool (6%) and Decanal (5%).

The presences of terpenes with traces of oxygenated components give the characteristic aroma of the orange and peel essential oils. The volatile components present in *C. sinensis* peel are largely terpenes: Mono- and sesquiterpene hydrocarbons, together with several oxygenated derivatives comprising alcohols, aldehydes, ketones, esters, and epoxides, are also found as main compounds (Qiao *et al*., 2008).

Table IV: Chemical composition of different citrus essential oils

Citrus essential oils	***IV Citrus sinensis oil***	***V Citrus sinensis 10 abstract***	***Peel oil of Citrus sinensis (Singh et al.,2010)***	***Peel oil of Citrus sinensis (Njoroge et al.,1995)***	***Leaf oil of Citrus sinensis (Guridfakim et Demarne, 1995)***
From	**Brasil**	**Brasil**	**Hindu**	**Kenya**	**Mauritius**
Monoterpene					
Alpha pinene	0.4		0.36	0.3	0.94
Beta pinene	0.02		0.03		0.32
Beta myrcene	1.5		0		0
Limonene	93	85	90.66	92.5	0
Sabinene	0.2		0.37	0.2	0
OxygenatedMonoterpenes					
Linalool	0.15	6	0	0.5	0
Sesquiterpene					
Valencene	0.01				
OxygenatedSesquiterpenes					
Beta sinensal	0.01		0.37		0
OtherOxygenated Compounds					
Neral(Citral B)	0.03				2.2
Geranial(Citral A)	0.05				1.04
Octanal	0.1	0.2	0.43		0
Nonanal	0.01		0.05		0
Decanal	0.1	5	0.02	0.2	0

IV.2-3 Characterization of the three Spanish citrus essential oils varieties by GC/ MSD

The GC/MSD screening compounds in different varieties were illustrated in the chromatograms (Figure 8), which exhibited numerous compounds. Monoterpene hydrocarbons (limonene, α-pinene, β-myrcene, α-terpinene, β-ocimene, γ-terpinene, and terpinolene) constitute the main volatile groups of citrus essential oils. Sesquiterpene hydrocarbons are mainly represented by, α-bergamotene and β-bisabolene, while oxygenated compounds are mainly aldehydes (nonanal, decanal, undecanal, dodecanal, neral, and geranial), monoterpene alcohols (linalool, α-terpineol, nerol, and geraniol), and esters (octylacetate, nonyl acetate, citronellyl acetate, neryl acetate) and geranyl acetate (Wang *et al*., 2020).

All samples contained d-limonene (51.98 -86.38%), myrcene (2.65-4.34%) and α-pinene (1.6-2.93%) as the main molecules.

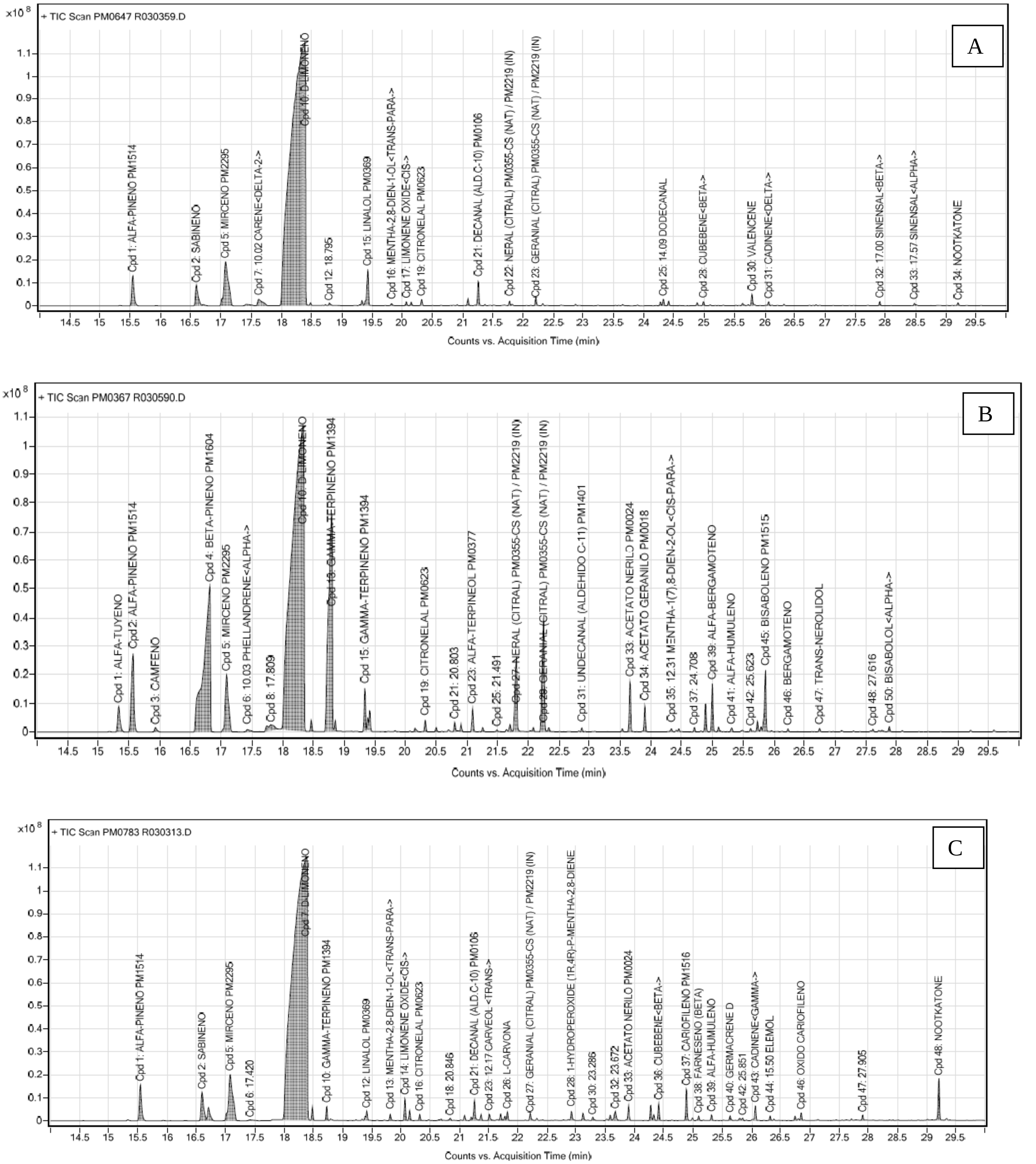

Figure 8: Typical analysis of Spanish citrus peel essential oil using GC/MSD (Industrial sample obtained by distilled, Production date: August 2020): A: ***Citrus sinensis*, B: *Citrus Limon*** and **C: *Citrus paradisi.*** The detailed composition table is in annex 6 .

The chemical composition profiles were simplified, the majors 10 components (based on the ratio of content) of each EO were screen out and ranged in descending order (Table V). Every "dominant components" were marked by different colors shown as below.

Table V: Major components in citrus essential oils from three Spanish citrus varieties

Essential oil / Number order of dominant components	*Citrus Sinensis*	*Citrus Paradisi*	*Citrus Limon*
1	D limonene (86.38%)	D limonene (80.17%)	D limonene (51.98%)
2	Myrceno (4.34%)	Myrceno (4.12%)	Beta pinene (13.75%)
3	Alpha pinene (1.6%)	Alpha pinene (1.74%)	Gama terpenene (11.88%)
4	Linalool (1.35%)	Sabineno (1.66%)	Geranial (3.62%)
5	Sabineno (1.31%)	Nootkatone (1.3%)	Alpha pinene (2.93%)
6	Decanal (0.8%)	Cariofileno (0.92%)	Myrceno (2.65%)
7	Carene (0.73%)	Beta pinene (0.74%)	Neral (2.21%)
8	Valencene (0.36%)	Decanal (0.7%)	Bisableno (1.43%)
9	Geranial (0.25%)	Limonene oxid (0.65%)	Neral acetate (1.05%)
10	Alpha terpineol (0.21%)	Cubebene (0.47%)	Alpha bergamote (0.97%)

The chemical composition of the three citrus essential oils showed great variability (Table VI). Indeed, the composition of essential oils may be influenced by many factors as genetic differences, soil type, maturity stages, weather types and culturing conditions (Deng *et al*., 2020), also environmental factors (climate, season of harvesting, geographical regions), and the extraction methods (Guo *et al*., 2019).

Table VI: Relative composition of *Citrus sinensis*, *Citrus limon* and *Citrus paradisi* essential oils from different geographical regions using GC-MS

	(Raspo *et al* .,2020)	(Raspo *et al* .,2020)	(Raspo et al .,2020)	(Di Roseau someone et *al.*,2020)	(Deng et al .,2020)	Sample
Region	United state	Argentine	Commercial	Italy	/	Spain
	Citrus sinensis					
Monoterpenehydrocarbons						
Alpha pinene			0.5			1.6
Myrcene	1.3	1.3	1.1			4.34
Limonene	91.5	96.1	97.3			86.38
Alpha terpene		0.3				0.3
Oxygenatedmonoterpene						
Geraniol						0.3
Nerol						0.3
Sequiterpenehydrocarbons						
Trans bergamotene						
Bisabolene						0.9
	Citrus limon					
Monoterpenehydrocarbons						
Alpha pinene	1.5	0.9		1.24		11.88
Myrcene	0.7	1	0.9	0.54		13.75
Limonene	60	72	69.7	71.76		51.98
Alpha terpene	0.3	0.3		8.59		
Oxygenatedmonoterpene						
Geraniol	0.3	0.1		1.16		
Nerol	0.3	0.1		0.85		
Sequiterpenehydrocarbons						
Trans bergamotene				0.12		
Bisabolene	0.9	1.1		0.12		
	Citrus paradisi					
Monoterpenehydrocarbons						
Alpha pinene					0.75	1.74
Myrcene	1.1		0.9		2.16	4.12
Limonene	95		98.2		93.3	80.17
Alpha terpene						
Oxygenatedmonoterpene						
Geraniol						
Nerol						
Sequiterpenehydrocarbons						
Trans bergamotene						
Bisabolene	0.9					

CONCLUSION

In term of the present work we can concluded that:

- The yield of the extracted juice from *Citrus sinensis*, *Citrus paradisi* and *Citrus limon* differs from one species to another and within each citrus species;

- The physico-chemical characterization of juices, pulps and essential oils of different varieties of citrus species shows also variability in different parameters and different part of the fruits;

- The chemical composition of the essential oils from peels of the three fruits of citrus verities was determined by GC-MS. Major compounds are monoterpenes hydrocarbon: d-limonene, β-myrcene, α-pinene, decanal and linalool, sabinene, β-pinen, and d-limonene was the major component (51.98-86.38%).

As a perspective, it would be interesting to:

1. Use efficient, affordable and "green" methods for extraction of essential oils from citrus fruit (juice, pulp and peel);
2. Study the physico-chemical properties, biological activities (antioxidant and antimicrobial) and the safety of citrus by-products for their better exploitation in the various industries (agrifood, cosmetics and pharmaceuticals).

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ANNEX

Annex 1

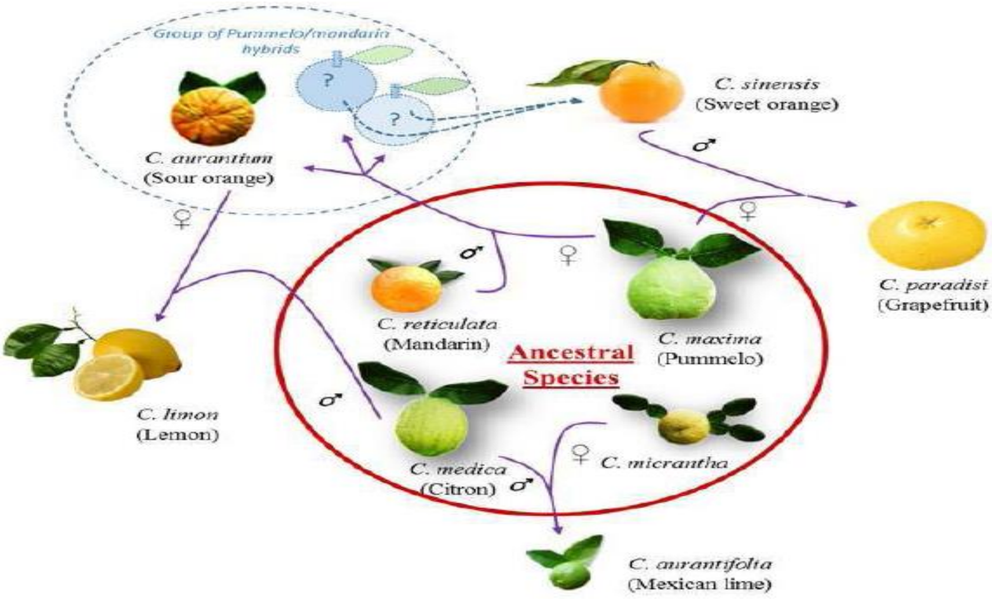

Figure 1 :phylogenetic origins of major secondary citrus species with the maternal and paternal ancestors (dotted lines are hypothetical cross) (Luro *et al* .,2017).



Figure N ° 2: Citrus sinensisFigure N ° 3: Citrus limonumFigure N ° 1: Citrus paradisi

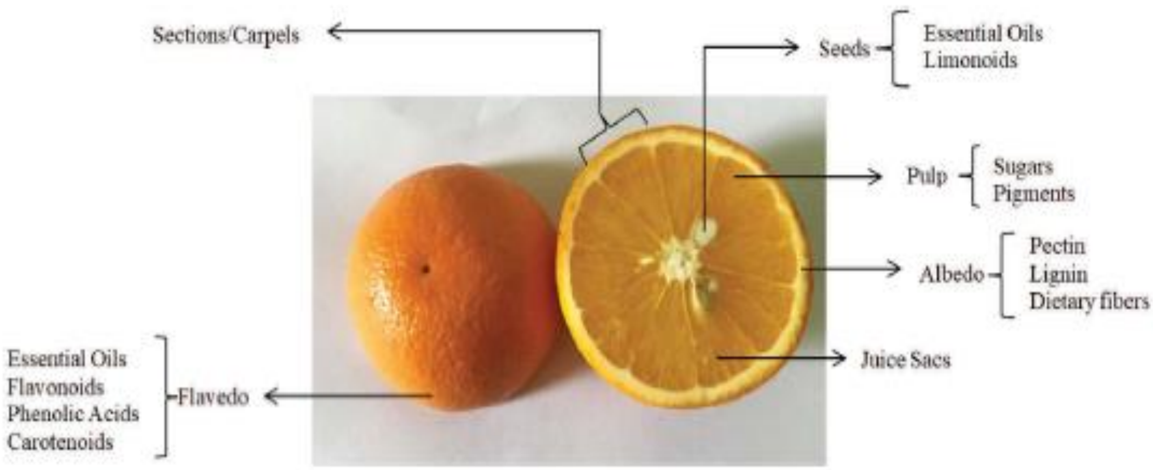

Figure 3 : Component parts of a typical citrus fruit (Panwar D *et al.*,2019).

Annex 2

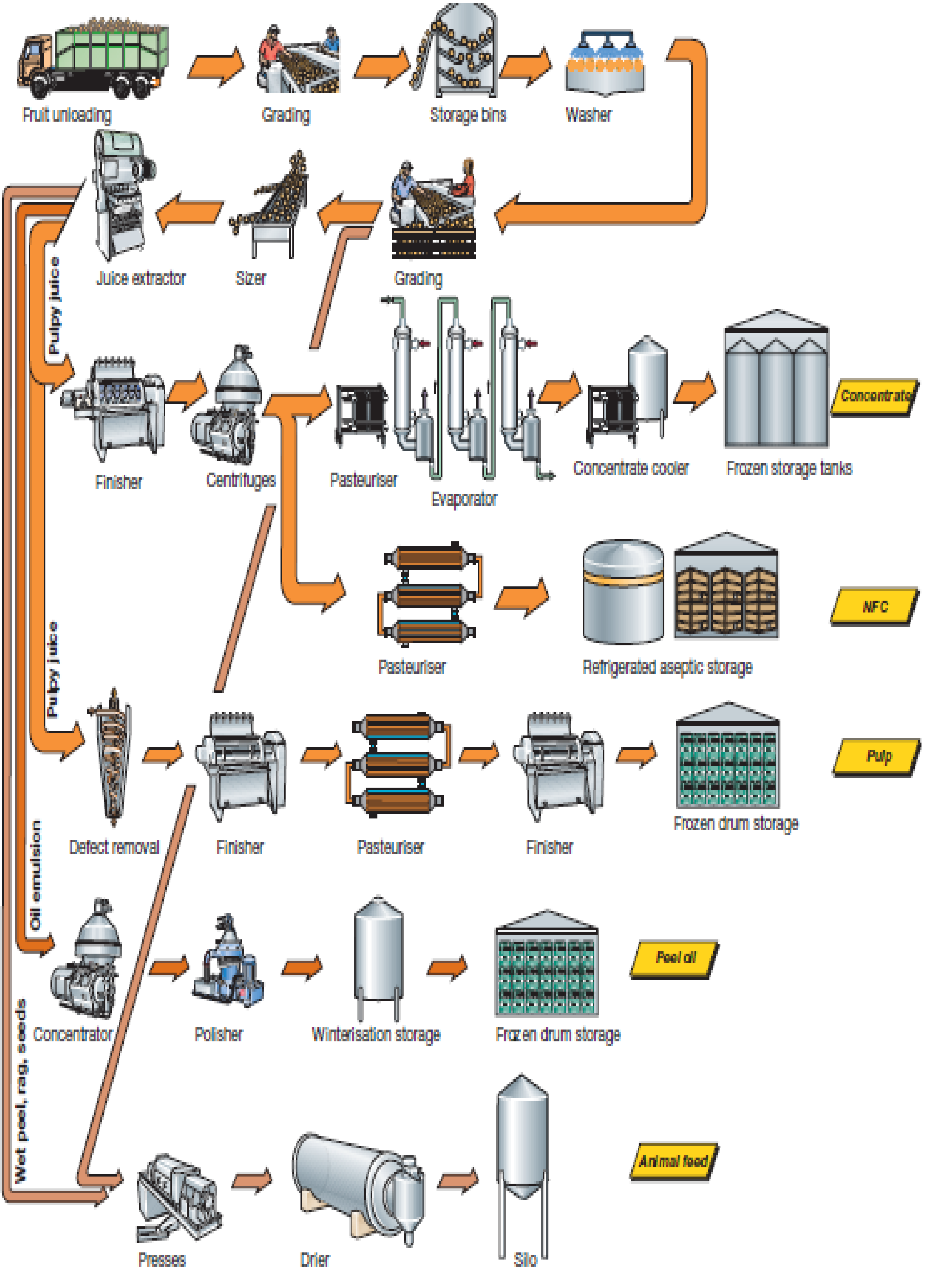

Figure 4 : Flow chart showing typical processing steps found in orange processing plant

Annex 3

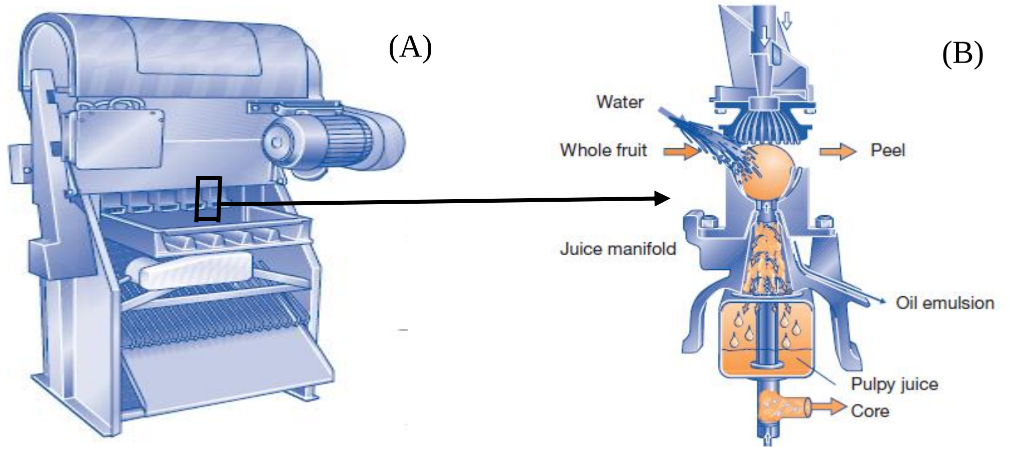

Figure 5 :(A) A squeezer type orange juice extractor , (B) Operation of the squeezer type orangejuice extractor (Ringblom.2004).

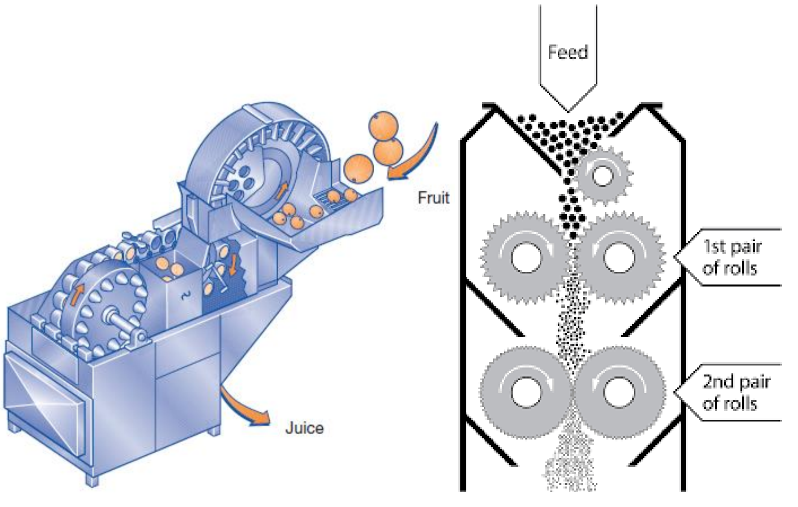

Figure 6 : A reamer type orange juice extractor (Ringblom .2004).

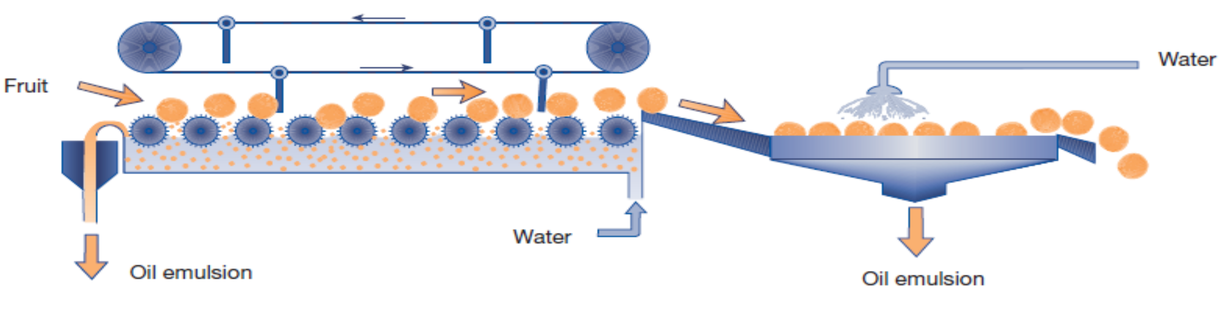

Figure 7 : An oil extraction system (Ringblom .2004).

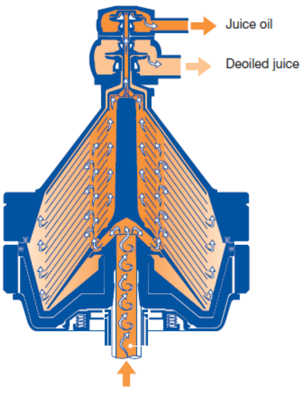

Figure 8 : Operational principle of hermitic centrifuge for deoiling juice (Ringblom .2004).

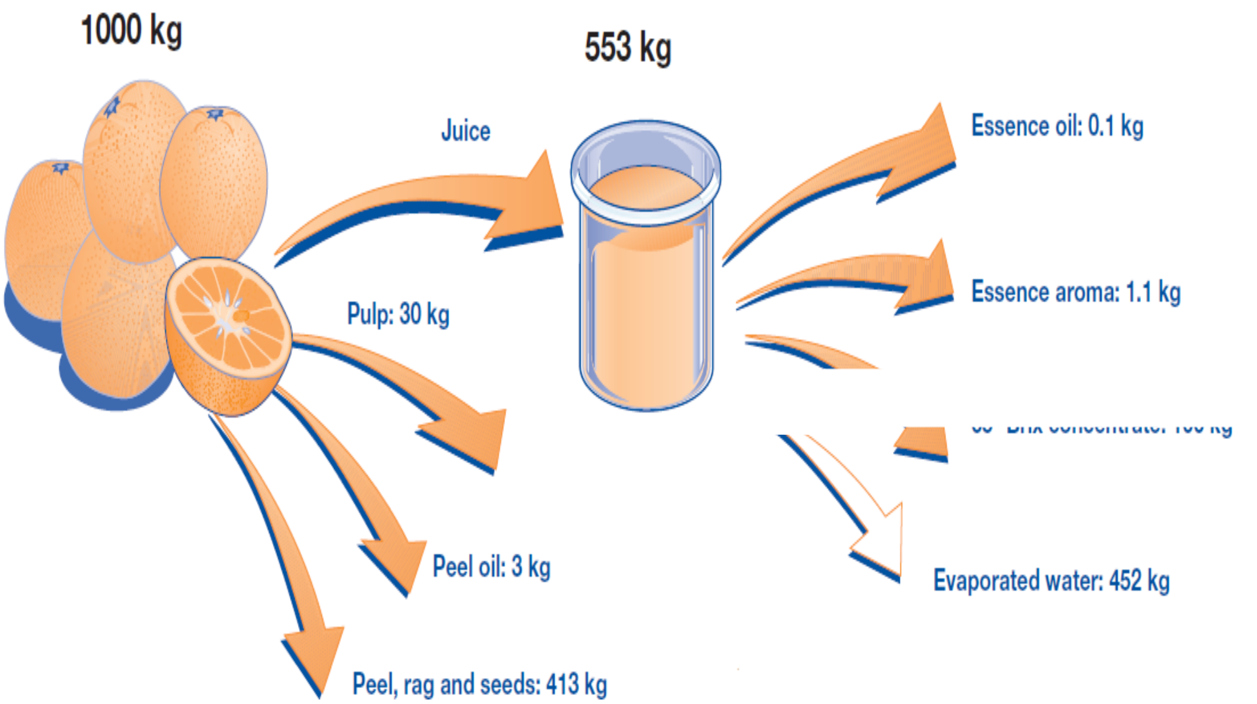

Figure 9 : Products derived from 1000kg of Oranges fruits (Ringblom .2004).

Annex 4

Table 1 : Two major classes of compounds found in essential oils

Hydrocarbons	**Oxygenated compounds**
- Also called aliphatic hydrocarbons - Names end in-ene as they are unsaturated (have double bonds);Terpenes based on the isoprene unit (5 carbon atoms) Monoterpenes: 2 isoprene units (up to 10 carbon atoms) Sesquiterpenes: 3 isoprene units (up to 15 carbon atoms) Diterpenes: 4 isoprene units (up to 20 carbon atoms)	- If they are derived from terpenes - they are called terpenoids Alcohols Phenols Aldehydes Ketones Esters Lactones

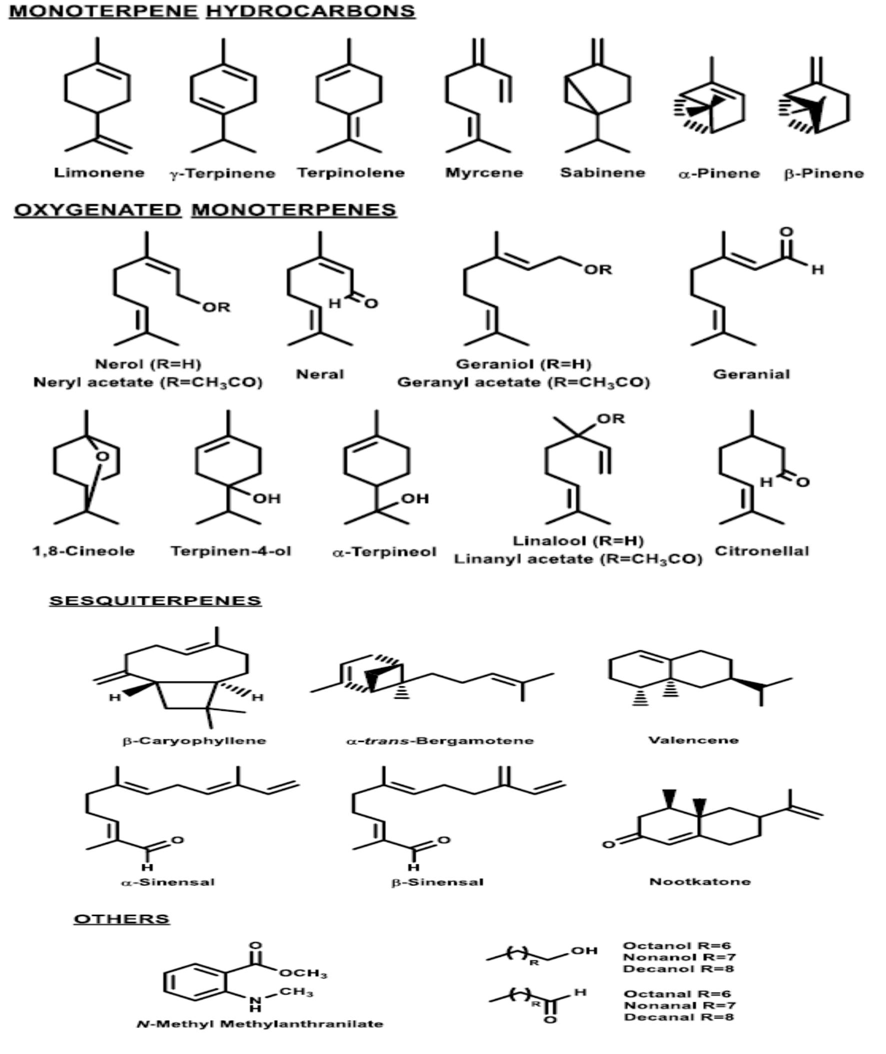

Figure 10 : Selection of the most representative volatile components of Citrus essential oils.

Annex 5

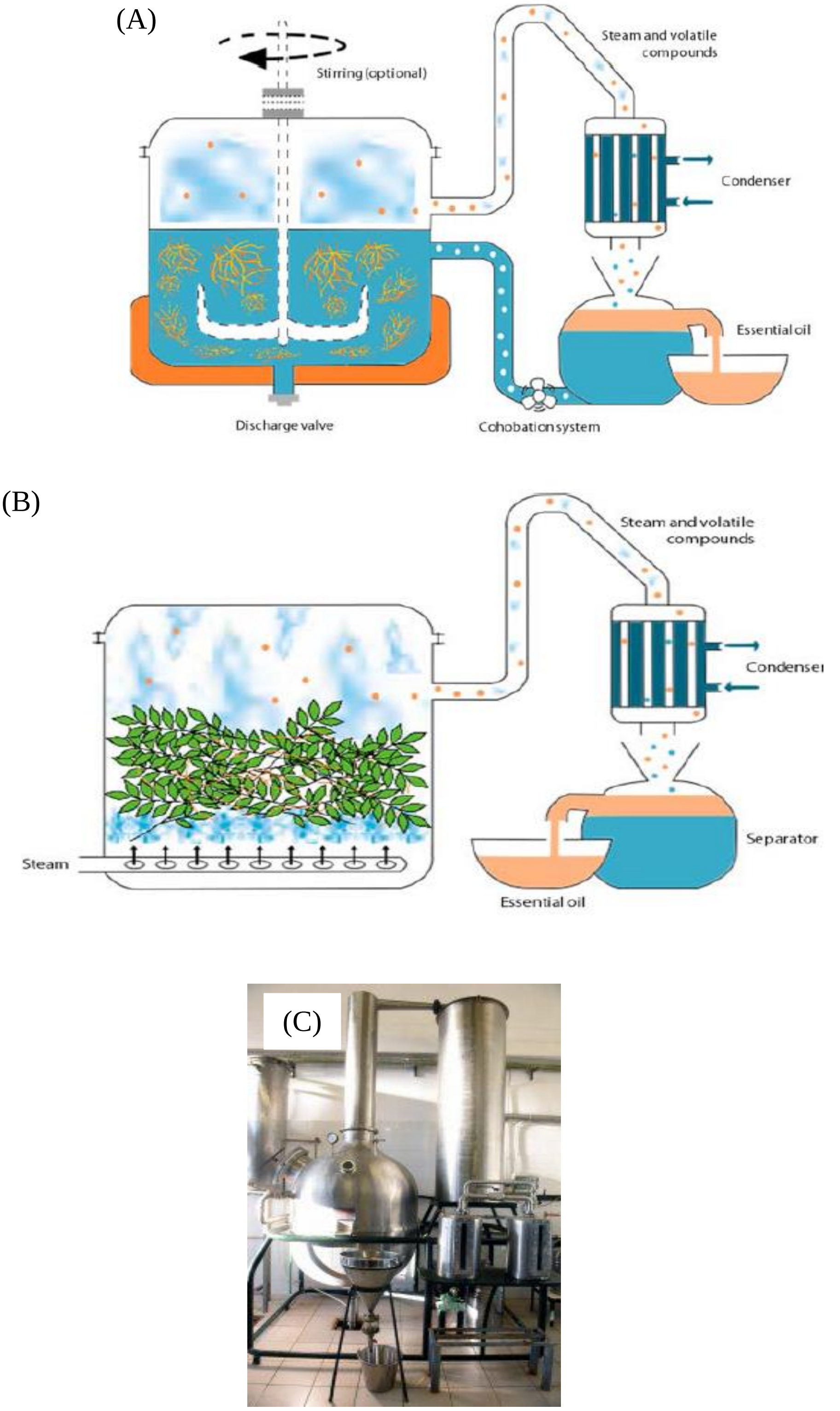

Figure 11 : (A) Hydrodistillation of essential oils (Burger P *et al*.,2019)
(B) Direct steam distillation of essential oils (Burger P *et a*l.,2019)
(C) Industrial steam distillation setup (Clarke .2009)

Abstract

Three species of the genus Citrus, namely *Citrus sinensi*, *Citrus paradisi* and *Citrus limon* from locality of Bejaia were studied. Their juice was extracted using a domestic juice extractor and their respective yields are: 39%, 34% and 33%. The relative component proportions of both varieties were established (juice and pulp) and analyze for their physicochemical properties which varied from one species to another. The results shows that the maturity stage is not reached for the three studied species. Physicochemical analyzes were carried out on five essential oils from Spanish citrus fruit peels obtained by distillation and cold pressing, then their chemical composition was determined by gas chromatography-mass spectrometry (GC-MS). The main components of these oils are: d-limonene, β-myrcene, α-pinene, decanal and linalool. According to the results, d-limonene was the major compound (51.98-86.38%).

Keywords: Citrus essential oil; Citrus fruits; GC/ MSD; D-limonene.

Résumé

Trois espèces du genre Citrus, à savoir Citrus sinensi, Citrus paradisi et Citrus limon de la localité de Bejaia ont été étudiées. Leur jus a été extrait à l'aide d'un extracteur de jus domestique et leurs rendements respectifs sont de: 39%, 34% et 33%. Les proportions relatives des composants des deux variétés ont été établies (jus et pulpe) et analysées pour leurs propriétés physico-chimiques qui variaient d'une espèce à l'autre. Les résultats montrent que le stade de maturité n'est pas atteint pour les trois espèces étudiées. Des analyses physico-chimiques ont été effectuées sur cinq huiles essentielles issues d'écorces d'agrumes espagnoles obtenues par distillation et pression à froid, puis leur composition chimique a été déterminée par chromatographie en phase gazeuse-spectrométrie de masse (GC-MS). Les principaux composants de ces huiles sont: le d-limonène, le β-myrcène, l'α-pinène, le décanal et le linalol. Selon les résultats, le d-limonène était le composé principal (51,98-86,38%).