INSTITUTO POLITÉCNICO DE BRAGANÇA

AMBROSIA
sustainable reuse of prickly pear seeds to extract oil with compound of high interest

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Acknowledgement

To my professors and department head Pr. Ana Pereira and Pr. Vera Ferro Lebres ...

To my supervisors Pr. Paula Cabo and Pr. Maria João Sousa

To my family and my friends

To you dear reader

Thanks for your contribution …

ABSTRACT

The prickly pear or *Opuntia ficus indica*, is a robust plant abundant in the Portuguese territory from south to north, but remains poorly exploited and integrated in the culture and consumption habits of the Portuguese.

This report presents a study of the pure oil of prickly pear seeds extracted by cold pressing and without additives. This study has involved 3 plans, physical, chemical and toxicological.

We obtained acceptable results and in accordance with the standards as the density, pH, refractive index. Also an interesting and competitive profile of the fatty acids of *opuntia ficus indica* was detected by a gas chromatography. The main fatty acid was linoleic acid with a value of 70.277%, followed by palmitic and oleic acids with respective values equal to 15.401% and 8.349%. According to these found results, our oil is considered polyunsaturated which presents multiple benefits for our health.

It was also shown that the median lethal dose LD50 is equal to 10%, a result to be reinforced.

Key words: Opuntia ficus indica, prickly pear seed oil, fatty acid, toxicity, entrepreneurship

RESUMO

A pêra espinhosa ou *Opuntia ficus indica*, é uma planta robusta e abundante no território português de sul a norte, mas permanece pouco explorada e integrada na cultura e hábitos de consumo dos portugueses.
Este relatório apresenta um estudo do óleo puro de sementes de figo da Índia extraído por prensagem a frio e sem aditivos. Este estudo envolveu 3 planos, fisícos, químicos e toxicológicos.
Obtivemos resultados aceitáveis e de acordo com as normas como densidade, pH, índice de refração. Também um perfil interessante e competitivo dos ácidos gordos de *Opuntia ficus indica* foi detectado por cromatografia gasosa. O ácido gordo principal é o ácido linoleico com um valor de 70,277%, seguido pelos ácidos palmítico e oléico com valores respectivamente de 15,401% e 8,349%. De acordo com estes resultados elaborados, o nosso óleo é considerado poliinsaturado, o que pode apresentar múltiplos benefícios para a saúde.
Foi também verificado em estudos preliminares que a dose mínima letal LD50 é igual a 10%, um resultado a ser comprovado.

Palavras-chave: Opuntia ficus indica, óleo de semente de figo da Índia, ácido gordo, toxicidade, empreendedorismo

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Introduction

In a society increasingly sensitive to the depletion of natural resources and the planet, some industries are struggling to keep up with the ecological wave and the need for the population to respect this ideology and to avoid continuing to increase environmental stress. The cosmetics and body care industry is facing the challenge of adapting their production process and contributing to a positive impact on the environment. The marine, terrestrial, forest and air ecosystems are suffering unlimited damage from industrial waste. This damage is also suffered by the consumer without him being aware of it.

Also farmers today force production by using pesticides and chemical fertilizers to meet the needs of the cosmetic industry such as for almonds and avocados and this can contribute to soil deterioration and loss of fertility. The question that arises is the following: What if natural beauty products that can be recycled without any effect on the environment were more highlighted?

Cosmetic product means any substance with an action that is rubbed or sprinkled on the various parts of the human body for the sole purpose of cleaning, beautifying, increasing the attractiveness, protecting and maintaining them in good condition.

Ambrosia project adopts the vision of respecting and protecting the planet as much as the well-being and natural beauty, through a production strategy which is based on a quantitative and not qualitative organic agriculture, knowing that the raw material comes from a robust plant that requires little, recyclable packaging to reduce the accumulation of plastic and most importantly is that we will develop partnerships with food industries to collect their waste of prickly pear which is the seeds and give it a second life.

This business is based on a robust and little exploited plant despite its unlimited benefits. The prickly pear tree, also called "*Opuntia Ficus Indica*", has its origin in Central America, and more precisely in Mexico. This plant was imported for the 1st time on the European continent between the end of the 15th and the beginning of the 16th century. Indeed, it appeared in Europe following the discovery of the Americas.

Originally, this plant was used as food for animals and men living on the earth thanks to its fruits and snowshoes, through time man discovered many other functions and benefits such as medicinal, maintenance of the environment, economic and industrial.

Seeing that this plant has contributed to the economic progress of some countries in Africa and Europe such as Morocco, Italy and Spain, we decided in this study and project to focus on this plant poorly exploited and known in Portugal, while the land of this latter cultivates it perfectly and in several regions from north to south, where it is enough present.

Knowing that not only the private sector has started to set up commercial plantations of prickly pears in south Portugal (Alentejo and Algarve) but also the north present a significant cultivation (Gimonde, Tras os Montes), the purpose of our work is the valorisation of the derivatives of the plant (seed) in the form of products with high added value.

The present study will thus include two main parts. First, a bibliography that will focus on the knowledge of some aspects of the prickly pear, then a study of the method of extraction, physicochemical tests, toxicity test and a second part devoted to a first marketing step.

Chapter 1. Phytotherapy

The term "phytotherapy" is etymologically composed of two Greek Roots : phuton and therapeia which respectively mean "plant" and "treatment".

Phytotherapy is defined above all as an allopathic discipline, based on the therapeutic use of medicinal plants, which is based on knowledge from the tradition. It is intended to prevent, repair or treat certain minor functional disorders or pathological conditions by using plants, parts of plants or plant preparations, whether consumed or used externally.(CARILLON, 2009)

This method presents essentially an advantage of multiplicity of the complementary active principles permitting a use in pharmacologically weak doses even physiological, it also presents a benefit of the effects of synergy and potentiation of the therapeutic action of the plant, an excellent tolerance of the medicinal plant which allows to minimize the side effects, the problems of rebound, negative feedbacks and dependence so frequently met with the medicines of medicinal plant which allows to minimize the side effects, the problems of rebound, negative feedbacks and dependence so frequently met with the synthetic medicines, and without forgetting the undeniable economic aspect (ratio cost/efficiency).

These characteristics are accompanied by another major aspect which is the existence of physiological harmony between the constituents of the plant and the human organism. The constituents of plant origin present a certain analogy of molecular structure spatial structure with those of the human being (Institut Européen des Substances Végétales, 2017).

Medicinal plants are practically the only therapeutic arsenal available to traditional healers in some third world countries (more than 80% of the population). In the industrialized countries of Europe, the consumption of medicinal plants has doubled in the last decades. (Hostettmann, 1997).

Used in the formulation of food supplements or medicines, phytotherapy products are the result of various processes and are found in many galenic forms, being mainly intended for the oral route, the inhaled route or the topical route.

Among the different existing forms, the active principle can be presented in different aspects. It is initially in the form of a powder, extract or dye and constitutes what is initially known as a galenic form.

1.1. Liquid forms

1.1.1. Aqueous extracts

1.1.1.1. Herbal water preparation

These are aqueous preparations obtained from suitably divided vegetable drugs, the quantity to be used of which will vary according to the plant, which are placed in contact with water for a variable time and at a high temperature. They can be prepared by: infusion, decoction, maceration or digestion.

1.1.1.2 Hydrolats:

These are aqueous preparations containing most of the volatile principles, and can make emulsion in water. They are obtained by distillation of a fresh plant using a still, they are in fact the secondary products collected after hydrodistillation during the preparation of essential oils.

1.1.2. Alcoholic extracts

1.1.2.1. Alcoholatures

These are liquid preparations obtained by maceration of fresh vegetable drugs in alcohol. They generally correspond to the fifth of the dehydrated plant. Their alcoholic strength varies between 75 and 95°.

1.1.2.2. Alcoholates

They are obtained first of all by a maceration of fresh or dry drugs in alcohol varying from 60 to 80°.

1.1.3. Hydroalcoholic extracts

1.1.3.1. Stains

They are defined as liquid preparations generally obtained by hydroalcoholic extraction of the fresh dried drug. The alcoholic strength is between 60 and 90° depending on the nature of the substance to be dissolved.

1.1.3.2. Tinctures

These are liquid preparations obtained by hydroalcoholic extraction of fresh plant drugs. Their alcoholic strength is between 60 and 70°. They are generally prepared to the tenth by maceration of a fresh plant.

1.1.3.3. Integral suspensions of fresh plants (SIPF)

They are obtained from fresh vegetable drugs. These are crushed in the presence of liquid nitrogen (-196°C), allowing to obtain grinds at a temperature below -50°C. Consisting of extremely fine particles, these will be suspended in ethanol to obtain a concentration of 30% (w/v).

1.1.4. Glycerine extracts

1.1.4.1. Glycerine macerates

The buds and young organs are macerated in a water-alcohol-glycerine mixture or in an alcohol-glycerine mixture to obtain a mother macerate.

1.1.4.2. Standardized fresh plant glycerine fluid extracts

Fluid extracts are liquid preparations, whose final fluid extract weight should be equal to that of the dried drug.

1.1.5. Oily extracts

These preparations result from a digestion by maceration of the vegetable drug in oil. The oil used can be of different nature, like sunflower oil, sweet almond, or olive. One gram of dry plant at the beginning will be used to obtain one gram of finished product. The first half of the plant is placed in a water bath with vegetable oil for two hours, or cold for several weeks. In this case they stirred daily. After filtration, the rest of the plants are treated with the infused oil.

Finally, we can conclude by specifying that all these liquid forms of use of medicinal plants can be used to prepare other galenic forms such as potions, solutions, solutes, mellitus, drops, drinkable ampoules, gargles, etc.

1.2. Solid forms Capsules and dry tablets to be swallowed

Capsules are preparations of solid consistency consisting of a hard shell based on gelatin or cellulose derivatives, each containing a dosage unit of one or more active ingredients (Jean Raynaud, 2006).

1.3. Forms used topical application

1.3.1. Ointments

These are semi-solid preparations composed of a single-phase excipient in which liquid or solid substances can be dissolved or dispersed. They are intended to be applied to the skin or mucous membranes (Jean Raynaud, 2006).

1.3.2. Liniment

A liniment is a semi-solid preparation for skin application only, belonging to the category of lipophilic creams. It is composed of oil or fat, as well as one or more active ingredients such as plant extracts or essential oils (Jean Raynaud, 2006).

1.3.3. Other forms

Many other forms are reserved for external use. We can find gels, ointments, with a harder consistency than ointments, balms, creams, pastes, lotions (Jean Raynaud, 2006).

Chapter 2. Generality about prickly pear and its seed oil

2.1. Generality about Prickly pear

The prickly pear is native to Mexico, it is well adapted to arid and semi-arid areas (Reynolds et al., 2003). It can occupy an important part in human nutrition by its fruits and it is also used as fodder for animals and plants. It is an advantageous plant because of the environmental conditions in which it grows and its resistance to extreme climatic conditions.

2.1.1. Botanical description

Prickly pear is a sturdy arborescent plant 3-5 m tall with a thick, woody trunk (Neffar S, 2012).

Cladodes (snowshoes): organized in flattened articles, with an elliptical or ovoid shape and a green-mat color, the length can vary between 30 and 50 cm, width between 15 and 30 cm and thickness between 1.5 and 3 cm. The cladodes provide chlorophyll function and are covered with a waxy cuticle (the cutin) that limits transpiration and protects against predators (Neffar S, 2012). They are covered with small areoles, spines, and white glochids.

Figure 1: *Opuntia ficus indica L* (Mill) species

(Source: Schweizer M., 1997, https://antropocene.it)

Flowers: The flowers, marginal on the top of the cladodes, are hermaphroditic, 4 to 10 cm wide and yellowish, becoming reddish as the plant approaches senescence (Schweizer M., 1997, Mulas M. et Mulas G., 2004). A fertile cladode can bear up to 30 flowers (Reyes-aguero J et al., 2006).

Fruits: The flowers give birth to fruits, in large quantities (100 to 150g), oval or elongated and fleshy, with a juicy pulp. The most remarkable feature of these fruits is that they contain many seeds (polysemic). The color and the shape of the fruit are variable according to the varieties: yellow, red, white (Schweizer M., 1997, Piga A., 2004, Feugang M.J. and Coll, Reyes aguero J. and Coll, 2006). The red compounds are betacyanins and the yellow ones are betaxanthins (Gibson A.C et Nobel P., 1986).

Seeds: are hard, indigestible, but rich in vitamins. We obtain after processing, a highly valued oil and a nourishing flour (Schweizer M, 1997).

2.1.2. Geographical distribution

Its geographical extension is located mainly in: Mexico, Sicily, Chile, Brazil, Turkey, Korea, Argentina and North Africa (Felker P. et al, 2005, Kabas O. and Coll, 2006, Snyman H.A., 2006).

It was first introduced in Spain and later, in the 16th century, in North and South Africa. It spread rapidly in the Mediterranean basin (Barbera G. et al, 1992, Habibi Y., 2004). It is developed in the western part of the Mediterranean as in southern Spain, Portugal and North Africa, Tunisia, Algeria and Morocco (Bensalem H. et al, 2002, Arba M., 2009).

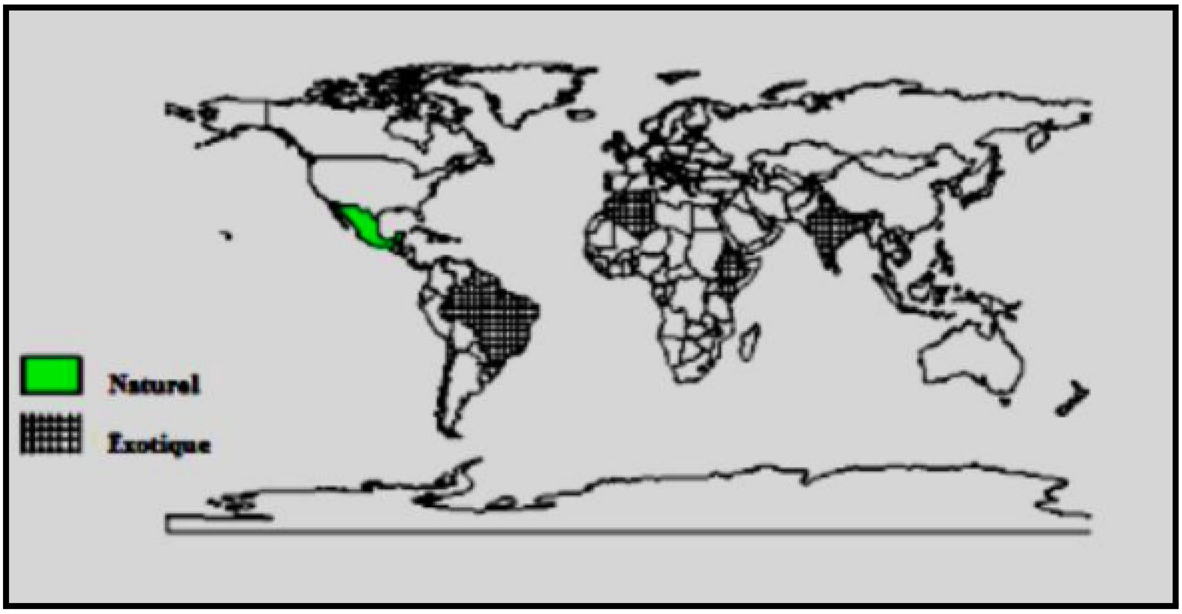


***Figure 2* :** Geographical distribution of the prickly pear
(Source: Neffar, 2012)

2.1.3. Taxonomy

Kingdom: Plantes.

Ordre: Caryophyllalles.

Subclass: Caryophyllidae.

Family: Cactaceae.

Groupe: Opuntia Eae.

Genre : Opuntia.

Sub-genre: Platyopuntia

Species : *Opuntia ficus-indica*.

The genus *Opuntia* belongs to the family Cactaceas, order Caryophyllales and the subclass Caryophyllidaes. The family Cactaceaes includes about 130 genus and 1500 species, of which 300 belong to the genus *Opuntia* (Mulas et al., 2004).

The group Opuntiaeaes includes the genus Opuntia, which is in turn divided into four subgenera: Platyopuntia, Cylindropuntia, Tephrocactus and Brasiliopuntia. The subgenus Platyopuntia includes 150 to 300 described species, we have the species *Opuntia megacantha* and the series of *ficus-indicae*, which include *Opuntia ficus-indica* that are known as prickly pear (Mulas et al., 2004).

The two species *Opuntia ficus-indica* and *Opuntia megacantha* are among the most important cacti in terms of agronomy, both for their eatable fruit and for the raquettes which can be used as fodder or vegetables (Mulas et al., 2004).

2.1.4. Nomenclature and terminology of the prickly pear

The prickly pear is known by several names in the world.

In Mexico: Nopal comes from the word nochtli in Nahuatl, the classical language of the Aztecs (Schweizer, 1997).

In Spain: Besides Nopal, Nopallito, it is familiarly called Nopalcito, Tuna, Ensada, Higos de Pala, Higos de Mauro (Benattia, 2017).

In Brazil: palma de gado (FAO and ICARDA, 2018).

In Portuguese: palma forrageira, figo da Índia, figo de piteira, figueira da Índia, palmatória sem espinhos, tabaido

In France: Figue de barbarie

In Italy: Fichi d'India

In German: frucht des feigenkactus, Indianische feige (FAO, 2001).

In Arabic: El-tin-el-Choki, El-tin-el-Hindi, El-Kemtheri-el-Chaik (Schweizer, 1997).

2.1.5. Habitat

Cacti have evolved and adapted to all kinds of biotopes and environments. The vast majority of species live in biotopes where they receive at least 500 minutes of average annual rainfall.

Some types of cactus appreciate even more humid habitats, in particular epiphytic species of tropical evergreen forests where the atmospheric humidity is constantly high. Cacti occupy biotopes from the coast to the interior coastline to inland regions, from sea level to altitudes approaching 4000 m. Some cacti have adapted to cope with intense cold and snow as well as those growing in the sand a few meters above sea level (Lamb, 1991).

2.1.6. Ecological conditions of the prickly pear

The ecological characteristics of the prickly pear, depend mainly on the climatic factors that control its growth and distribution, notably the rainfall, the atmospheric humidity, the winter temperature, the nature of the soil and drainage atmospheric humidity, winter temperature, the nature of the soil and drainage (Report of the Ministry of (Report of the Ministry of Agriculture and Maritime Fisheries, 2010).

2.1.6.1. Temperature

The prickly pear is resistant to cold and can withstand about -5 ° C. Its heat needs are important and especially during the growth phase of the fruit, it's between 15 and 25 °C.

It is resistant to drought and the average annual temperatures of 15 to 18 °C are perfectly suitable for it.

It can withstand high temperatures of over 50 °C. In addition, the lowest level of where the development is possible is the isotherm of 1,5 to 2 °C of the daily average of the coldest month (usually January) which corresponds to the monthly average of 7 to 9 °C, but with a daily maximum greater than or equal to 12 °C.

2.1.6.2. Precipitation

Precipitation requirements are variable depending on the nature of the soils. Indeed, for sandy and deep soils, the minimum precipitation required is around 300 to 400 mm/year, while on muddy and silty soils, the average minimum precipitation required is 200 mm/year. Average minimum rainfall required is 200 mm/year; but the cactus can be cultivated as long as the additional water from erosion allows the soil to store 300 to 400 mm.

2.1.6.3. Humidity

Atmospheric humidity, in case of great deficit of saturation of the atmosphere (low relative humidity) also affects Opuntia species. Empirical observations have shown that the prickly pear is eliminated from regions where the average relative humidity is below 40% for more than thirty consecutive days.

2.1.6.4. Soil

The species is highly adaptable with a preference for very permeable, sandy or stony soils with low clay content.

2.1.6.5. Drainage

This is also one of the most important ecological requirements. Indeed, the prickly pear is very sensitive to the lack of oxygen at the root level; for this reason, it is recommended to avoid clay soils which can be temporarily saturated and poorly drained.

2.1.7. Use

2.1.7.1. Human food

Fruit: There are nearly 200 species of Opuntia, but only the fruits of about 20 species are consumed.

The fruits are known for their high content of sugar glucose and fructose, minerals, vitamins and an acid pH=5.6. They are produced and marketed in summer and autumn, depending on the maturity of the variety. In Mexico, for example, these edible fruits, called "tuna", are marketed fresh or transformed: dried, frozen, in the form of confit, juice and alcohol (Report of the Ministry of Agriculture and Maritime Fishing 2010).

Cladodes: Young cladodes are eaten as a vegetable because they are tender and full of fibre. Their nutritional value is similar to that of many vegetables and leaves. They are rich in water, carbohydrates, protein, vitamin C and β-carotene which is a precursor of vitamin A. These young cladodes are called "Napolitos" in Mexico where they have been considered as a traditional vegetable for centuries. They are eaten fresh or cooked like a green and fresh vegetable (Arba, 2009).

There are other products derived from cladodes such as jam, gherkins and candied cladodes. While cladodes have been used as a meat substitute traditionally, they are now served with a meal to replace green beans (Stintzing and Carle, 2005).

2.1.7.2. Production of fodder for animals

It represents the second most important economic use of cactus in the world.

Cacti have long been used in dryland livestock feed and are more profitable than other forage species such as maize and sorghum. It is grown as a forage species in several countries to provide a food supply for livestock during the transition periods in summer and autumn and in drought years, like for example Mexico, USA, Brazil, Peru, Morocco. Cladodes are appreciated by livestock because they are rich in water, fibre, protein and mineral elements, which improves the flavour of milk and the colour of butter (Arba, 2009).

Cladodes are either burnt directly on the field as in Mexico and the USA or harvested and cut as is the case in some north african countries such as Morocco and Tunisia.

Compared to other fodder elements, the energy value of the raquettes is close to that of alfalfa with 0.12 fodder unit/kg. A (Arba, 2009).

2.1.7.3. Beekeeping

The cactus is an abundant flowering plant and its flowering cycle can extend from 3 to 6 months depending on the region and the variety.

Its flowering attracts bees in masse with their large yellow flowers, abundant pollen and nectar (Arba, 2009).

2.1.7.4. Therapeutic indications

Modern medical research shows the considerable properties of prickly pear, due to the active molecules that compose it. This species can effectively fight against the most severe diseases, like anxiety, arteriosclerosis, cholesterol, diabetes, obesity, stress (Schweizer M., 1997).

In Australia and South Africa, "Nopalitos" are used in the treatment of non-insulin-dependent diabetes (Araba A. et al, 2000).

The capsules of the dried flower corollas are used as a remedy against prostate dysfunction (benign prostatic hypertrophy), and also as a diuretic regulator (Arba M., 2009).

In Sicily, the tea prepared with the flowers of this plant is used as treatment against kidney pain (Park E.H., 2001, Araba A. and Coll, 2000).

In Morocco the fruits are known to stop colic and diarrhea (Schweizer M., 1997, Araba A. et Coll, 2000).

The mucilage also allows to reduce the cholesterol level in the blood. The dried powder of cladodes also has an effect on the control of sugar and cholesterol in the blood (Fernandez M.L. and Coll, 1990).

Cladode juice contains a large variety of antioxidant compounds such as polyphenols (especially some flavonoids), vitamin C and E, β-carotene (provitamin A), glutathione (El kharrassi Y, 2015).

An interesting proportion of polyphenols reduces the risk of cancer, cardiovascular diseases and neurodegenerative diseases such as Alzheimer's disease (Feugang M.J. et al., 2006).

The extracts of cladodes could have anti-ulcer and anti-inflammatory effects. The aqueous extract remarkably improves wound healing (El-Mostafa K. et al., 2014).

The nopal is used in the cleaning of the colon because it contains soluble dietary fiber soluble dietary fiber facilitating intestinal transit but it also contains fiber non soluble fibers that is to say unassimilable which accelerate the transit while regulating its movements (Schweizer M, 1997). It also has a galactogenic and fortifying property, it favors and activates the production of milk in women who breastfeed (Schweizer M, 1997).

2.1.7.5. Production of oils

The oil extracted from the seeds of the prickly pear fruit belongs to the family of polyunsaturated oils like most vegetable oils.

The commercial value of this oil is interesting because of its particular cosmetic properties. It is rich in unsaturated fatty acids such as linoleic acid (64.43%) and oleic acid (18.46%). Among the saturated fatty acids, the most important is palmitic acid (12.60%) and stearic acid (2.82%). On the other hand, its particularity is its richness in unsaponifiable matter (sterols and tocopherols). This characteristic could be a lever for its exploitation in the field of cosmetology, given the beneficial effects of these substances skin elasticity, cell metabolism and restoration of the skin structure. It has cosmetic properties, as it prevents skin aging and wrinkles. The seeds are also used for the preparation of creams for dermal use (Report of the Ministry of Agriculture and Maritime Fishing, 2010).

2.2. Generality about prickly pear seeds

2.2.1. Study of the seed of prickly pear

The seeds of prickly pear have generated considerable interest in recent years, interest has like other seeds in particular those of grapes and studies have multiplied to characterize their constituents in order to evaluate their interesting nutritional value (Sawaya et al, 1983).

According to (Uchoa et al, 1998), the protein reserves of the seed are albumins. However, most attention has been focused on the oils contained in these seed coats. The extraction of these oils generates a cake that constitutes up to 90% of the weight of the raw material.

This residue is very rich in cellulosic fibers. The other constituent polysaccharides are very rare, even non existent. (El Kossori et al, 1998).

Table 1. Chemical composition of the seeds of the prickly pear (Habibi, 2004)

components	percentage
water	5-6%
oil	7-8.5%
minerals	1.3%
lignin cluster	18%
protein	11-12%
cellulose	30%
other polysaccharides	27%

The mineral composition is: P 1.628, K 533, Ca 471, Mg 117, Na 71 and Fe 290 12 mg / kg dry weight. Moreover, the seed oil contains linoleic acid as the main fatty acid, followed by oleic and palmitic acids.

Similarly, myristic, stearic and arachidonic acids were detected in small quantities. The fatty acid composition of prickly pear oil was close to that of sunflower and grape seed (Angulo-Bejarano et al, 2014).

2.2.2. Morphological study of the seed

A morphological study was carried out in order to identify the composition and organization of the cellular tissues that form the seed.

The study was carried out on semi-finished and ultrafine sections made with an MTX RMC ultramicrotome.

Figure 3: Cross-section of the seed of the prickly pear (Habibi, 2004).

Observation of a cross-section of the seed under a light microscope shows that it consists mainly of two distinct parts: an envelope (pericarp) and a core (endosperm). The morphological analysis was performed by scanning and transmission electron microscopy.

2.2.2.1. The endosperm

It represents 5 to 10% of the total weight of the seeds of prickly pear. It is constituted by cells of parenchyma of reserve with very fine walls containing numerous leucoplasts which form small grains of starch.

Between the tissues rich in starch is interposed a layer of gluten (layer a aleurone) which gives the nucleus a viscous aspect. The whole of these cells is covered with a thick cell wall in the shape of an inverted tile.

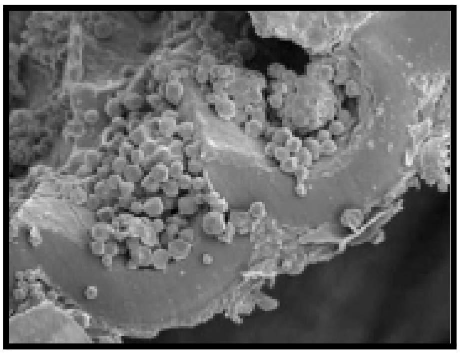


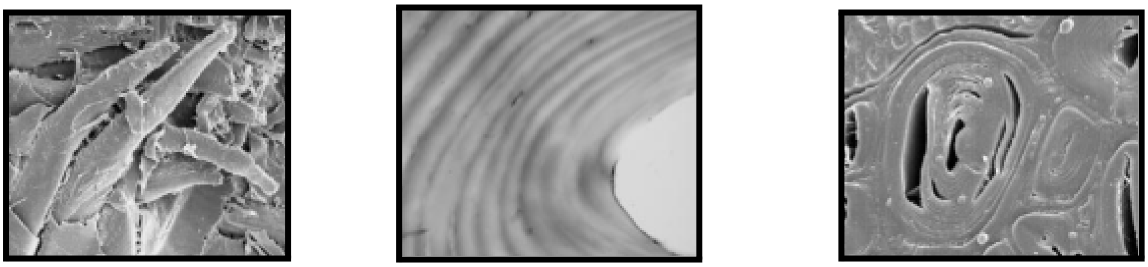
***Figure 4*: Endosperm of prickly pear seed**
(Habibi, 2004)

2.2.2.2. The pericarp

The pericarp of the prickly pear seed represents 90 to 95% of the total weight of two seeds.

We can distinguish two types of cells: the majority of very compact long cells in the form of spindle-shaped fibers and some spiral vessels.

The fibers are commonly called sclerenchyma fibers. This support tissue is widely distributed in seed coats, fruit cores, stone cells, spines and prickles of stems and leaves. The regular layers of cells, which line their thick walls, have a helical arrangement.



(a) (b) (c)

Figure 5: (a) Spindle-shaped sclerenchyma fibers (spindle), (b) organized in helical strata of cellulose; (c) external faces of helices (Habibi, 2004)

2.2.3. The importance of the prickly pear seed

There has been an intensive orientation and progressive interest in the use of prickly pear seed in developed countries due to the enormous advantages of the active substances of this plant, fruit and seed. Indeed, the seed is a part rich in fatty acid, it can be exploited for the extraction of oils for food, pharmaceutical, medical and cosmetic use (Fadili, 2000).

They are characterized by their richness in xylans, which is a principal component of hemicelluloses, and the second most abundant natural polysaccharide, it is endowed with very diverse applications, which can go from the plastic industry, the paper industry to medical applications. The amphiphilic alkyl derivatives of xylans have excellent emulsifying properties, and are widely used in the food industry (Habibi, 2004). Prickly pear seeds can be used as a source of edible oils due to their richness in essential fatty acids (Ramadan et Morsel, 2003).

Recently, it has been shown that the addition of prickly pear seed powder to the diet decreases serum glucose concentration, increases glycogen in liver and skeletal muscle and significantly increases LDL cholesterol levels, suggesting a potential application for diabetes and atherosclerosis (Ennouri et al., 2006). Also increase in their intake reduces the risk of cardiovascular diseases and coronary diseases (Ennouri et al., 2006).

The richness of these seeds in non-saponifiable matter (sterols and tocopherols) makes them a asset for their exploitation in cosmetology, given the beneficial effects of these substances on skin elasticity, cellular metabolism and restoration of skin structure (Habibi, 2004)

2.3. Prickly pear seed oil

2.3.1. Definition of the oil of prickly pear seeds

The oil of prickly pear seeds extracted from prickly pear seeds using different extraction techniques, from conventional to advanced, is a precious product. This oil consists mainly of acids oleic (22%), palmitic (12%) and linoleic (60%) acids that give it very nourishing and emollient nourishing and emollient properties very interesting in cosmetics. The oil of prickly pear is 100% natural. Repairing, nourishing and moisturizing the skin, it also fights the vagaries of time thanks to its high level of antioxidants and essential fatty acids (Dali, 2017).

The chemical characterization of the oil has been reported, and it is sufficiently understood that this oil has high nutritive value.

2.3.2. The interest of prickly pear seed oil

The existence of oils in plants or seed, even if their function is not always precisely known, would meet the needs of a specific protection of species according to their environment (Thielmann et al., 2019).

The composition of prickly pear seed oil gives it many significant properties because it contains a large amount of polyunsaturated fatty acids (Sawaya et al., 1982), and contains a much higher percentage of tocopherol than to other oils as it can reach up to 850mg/kg (Zine et al., 2013).

Various researchers have reported the fatty acid composition of this oil. Gas chromatography of the prickly pear seed oil methyl ester was the main method for fatty acid composition. It is characterized by a high level of unsaturated fatty acids (80–88%), among which are linoleic acid (49.3–78.8%), oleic acid (12.8–25.3%), vaccenic acid (4.3–6.3%) and linolenic acid (0.23–1.1%). The main saturated fatty acids are palmitic (9.3–14.3%) and stearic (2.2–4.3%) acid. Various factors have an influence on fatty acid content, including prickly pear variety, geographical location, methods and solvents used for oil extraction, cultivar, degree of maturity, and harvesting season (Shahidi et al., 2016).

Prickly pear seed oil contains other valuable compounds, such as sterols and tocopherols, that have been known and approved scientifically to decrease or alleviate human diseases, such as atherosclerosis, diabetes and cancer. The content and composition of the phytosterol is considered as a fingerprint of the oil, and it can be used for authenticity or detecting adulterations. β-sitosterol and campesterol were found to be the major phytosterols compounds (Shahidi et al., 2016).

Other than sterols and tocopherols, the antioxidant activity may be partly due to some compounds. Few studies have reported the polyphenol content in prickly pear seed oil, but recently, total polyphenol content (TPC) in Algerian oil extracted by the cold press method was reported to be 55.82 ± 3.84 mg of gallic acid equivalents/100 g of oil (Brahmi et al., 2020).

In vitro antioxidant activity of the oil was determined using different methods, the authors (Brahmi et al., 2020) indicated that prickly pear seeds oil extracted by the cold press method showed antioxidant activity of 0.56 ± 0.01 AU, an other study was performed by (Berraaouan et al., 2014), who showed that the Moroccan oil extracted by cold press exhibited a good antioxidant effect in DPPH scavenging assay (0.001%; *w*/*v*) with an IC50 value of 0.96 mg/mL.

In addition, this seed oil was shown to have wound healing potential in vivo. The findings from this study have shown that the topical application of it at 0.6 µL/mm2 accelerated skin closure and improved the healing process significantly, The authors explained that the wound healing effect of the prickly pear seed oil is due to the active components that are present in the oil, including unsaturated fatty acids, triacylglycerols, phytosterols, and tocopherols; these compounds work to enhance the speed of wound contraction, complete reepithelialization, and improve the external scar’s aspect (Khemiri et al., 2019)

This oil has curative effects against chronic diseases, it is very useful in the management of diabetes mellitus (Chougui et al., 2013) and can significantly reduce blood triglyceride levels and total lipids in the liver. It has been reported that prickly pear seed oil lowers cholesterol and lowers blood sugar (Ennouri et al., 2006).

Deeply repairing, nourishing and moisturizing the skin, it fights the signs of aging or what is called wrinkles thanks to its high level of antioxidants and essential fatty acids (Dali, 2017).

2.3.3. The techniques of extractions of the prickly pear oil

To extract oils from a natural plant material can be proceeded according to different techniques of extraction which consist in removing one or more chemical species from a solid or liquid medium.

The extraction process is based on the differences in solubility of compounds in a mixture in a solvent, there are several extraction methods, among these we can mention the following methods:

2.3.3.1. Soxhlet extraction

Soxhlet extraction is a classical method for solid-liquid extraction, simple and convenient, allowing to repeat unlimitedly the extraction cycle with a fresh solvent until total exhaustion of the solute in the raw material (Wang et al., 2006).

Evidently, every procedure has its limiting inconveniences for the extraction, and for this reason the choice of technique is an essential part of oil production. For Soxhlet extraction, the inconvenience is the heat that can degrade certain chemical components (Wang et al., 2006).

2.3.3.2. Extraction by cold pressing

Cold pressure extraction is used to pass the seeds through a screw oil press that causes an increasing pressure at about 60°C; the recovered oil is decanted, weighed and then stored at -20°C (Nitiema et al ,. 2012).

This technique allows the preservation of the content of essential fatty acids and natural antioxidants, and consequently avoids an alteration of the properties of the oil (Nitiema et al ,. 2012).

2.3.3.3. Extraction by supercritical fluid

Extraction by supercritical fluid and more specifically by supercritical CO_2 has been introduced as an alternative to these solvent extraction processes (Danielski et al., 2006).

2.4. Chemical characteristics in other countries

Successive studies made in the countries of North Africa (Morocco, Tunisia and Algeria), indicate that the cactus with its different parts fruits, pulp, seed and the ecorce by different extraction methods were rich in linolenic, oleic and palmitic acids (Ennouri M. et Coll, 2005, Ramadan M.F. et Coll, 2003). A high level of omega-6 fatty acids (linoleic acid) has been detected in cactus seed oil (53.5% to 70.29%).

The oil of the seed of the prickly pear belongs to the category of oils "polyunsaturated" as most vegetable oils (Habibi Y, 2004).

This extract presents a fluid oil, relatively odorless and color that varies from one variety to another, ranging from light yellow to greenish (El hachimi F. et al., 2015). It presents a high content of unsaturated fatty acids and linoleic acid (Inglese P. et Coll, 2018).

In Tunisia, a study was carried out by (Tlili N. et al., 2011) on the composition of *Opuntia ficus indica* seeds coming from the region of Kairouan, collected during three different years.

The crushed seeds were submitted to an oil extraction using the Soxhlet equipment and then fatty acid analysis. Gas chromatographic analysis (GC) of the seed oil revealed the following data: Unsaturated fatty acids represent the majority of fatty acids (83.2%). Linoleic acid was the main fatty acid (56.6%), followed by oleic acid (20.1%).

In Algeria, (Chougui N. et al., 2013) studied the composition of the seeds of four varieties of *Opuntia ficus-indica* from the Bejaïa region. The result of the chromatographic analysis of the seed oil revealed high percentages of linoleic acid in the four varieties ranging from 58% to 63%.

On the other hand, the works carried out in Morocco, precisely in Oudja, show that the majority acid in the seed oil, extracted by the solvent hexane, is linoleic acid with a percentage of 58.79%. Then comes the palmitic acid (11.18%). Stearic acid was present in small amounts (1.50%) (Ghazi Z. et al, 2013).

In continuity, in this report this work is going to characterize the prickly pear seed oil coming from Aldeia do Vale do Pereiro located in the sunny region of Portugal, Alentejo. The fruits are harvested at the end of August and cold extracted.

Chaptre 3. Material and methods

The experimental work of this project was carried out in the research laboratory at Polytechnic Institute of Bragança - Agricultural School, during the period between September and June of the year 2021-2022. The objective of the study is to evaluate the physicochemical characteristics of the seed oil of the Portuguese prickly pear.

Also a ecorce try of the composition of the anti-aging cream, was carried out during the year 2020-2021 in the Laboratory CIMO Centro de Investigação de Montanha.

3.1. Physicochemical study of *Opuntia ficus-indica* seed oil

3.1.1. Harvest site

The samples of *Opuntia ficus-indica* fruits, come from São Bento do Mato, also called Azaruja , is a Portuguese parish of the municipality of Évora, in the region of Alentejo, characterized by an average altitude of 240 meters. The climate of the area is Mediterranean, with rainfall unevenly distributed throughout the year. The warm Mediterranean climate has dry summers. Over the year, the average temperature in Alentejo is 9.6°C and the average rainfall is 987.9 mm.

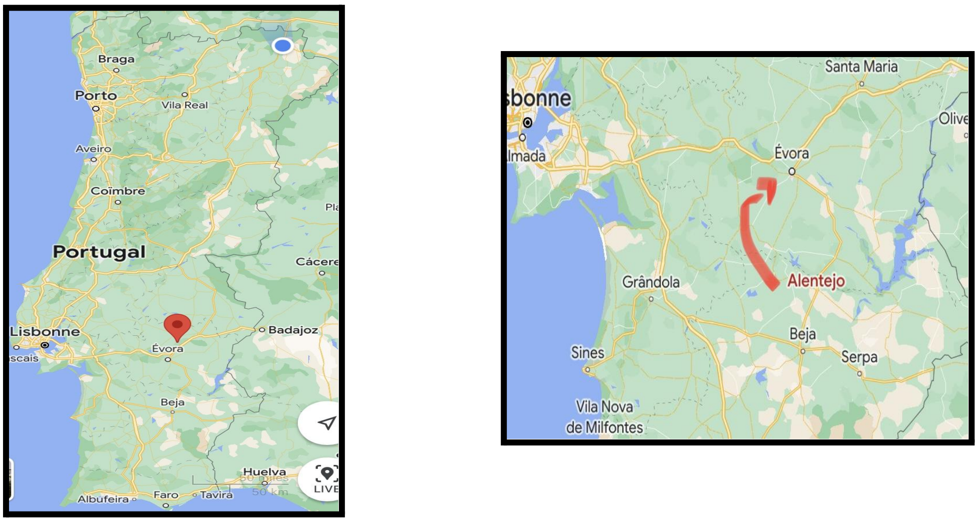


Figure 6: harvesting site

3.1.2. Preparation of plant material

The fruits, of yellowish green and orange color, were picked in the period of maturity (from the end of July to the beginning of September 2021), from the area mentioned above. These fruits were carefully picked by the family and employees of this plantation, the fruits must be picked from the plant as if it falls to the ground it means that it is more mature than desired.

The harvest is done by hand using gloves or traditional tools since they are very thorny.

Having the same commitment, to obtain the prickly pear seeds, a separation of materials and a total management of the fruits will be done.

The fruits have been washed with running water to eliminate glochids and impurities. Then, they were peeled in order to the pulp (which will be used to derive other products such as jam). This pulp is strewn with several small seeds (seeds that we will need) that are stacked fairly evenly. The cohesion between the seeds is ensured by the mucilage and the fibers contained in the pulp.

After washing, the seeds are separated from the pulp by using water and sieves with increasingly small pores, rinsing thoroughly with running water to be sure to eliminate all the mucilage. These seeds are then dried in a well-ventilated place away from the light.

Figure 7: Prickly pear seed

The extraction method chosen for our work is the extraction of vegetable oil by cold press. It is a type of mechanical pressing that does not require any chemical product. It is the passage of the seeds in a screw press (Benattia k.F., 2014).

This method is used mainly to generate extra-virgin edible oils or for small capacity units, allowing the extraction of oil by simple or successive pressing at a temperature below 60°C (Sylvain P et al., 2012). The recovered oil was decanted, weighed and then stored at -20°C (Mouden M et al.,2012).

It is important to know that the yield of this method is the lowest, as the fat content of the press residue (the cake) remains typically between 6 and 18% depending on the type of press used (screw press, bar press) and most importantly that the oil is of good quality, can be used directly after its filtration and contains few phospholipids. (Sylvain P et al.,2012)

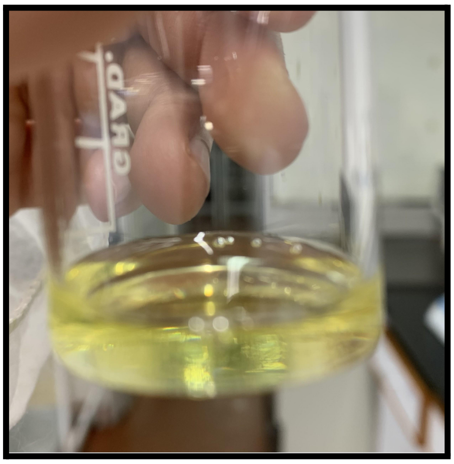


Figure 8: Prickly pear seeds oil

Despite the low yield of this extraction technique, there are major advantages such as simple use, no need for labor, low cost, environmental friendliness, lack of harmful organic solvents and production possibilities.

The environment, the lack of harmful organic solvents and its high quality production possibilities. In addition, the product is not usually applied to heat treatment (cold press). Therefore, high quality oils are obtained. These oils are generally suitable for direct consumption and do not require refining or special treatment. (Çakaloğlu B et al., 2018)

Figure 9: Raw material preparation steps

3.1.3. Organoleptic characteristics

The organoleptic examination is one of the criteria for evaluating the quality of oils. It is based on the odor, the color and the aspect at 20°C.

3.1.4. physicochemical characteristics

3.1.4.1. Density

The relative density at 20°C of an essential oil is the ratio of the mass of a sample of essential oil at 20°C of mass (m1), to a mass (m2) of water distilled at 20°C of the same volume.

The relative mass of the empty pycnometre (m0) is determined, then the pycnometre is filled with distilled water (m2), the pycnometre is brought to a temperature of 20°C with the help of a precision balance. The density is given by the following formula.

$$d = m2 - m0/ m1 - m0$$

3.1.4.2. Determination of the acid number

The acid number is the number of milligrams of potassium hydroxide required to neutralize the free fatty acids present in one gram of fat. This index is determined experimentally according to the standard (Benattia k.F, 2014).

The test sample is dissolved in a solvent mixture. The solution thus obtained is titrated with an ethanolic solution of potassium hydroxide in the presence of an indicator, so the principle consists in an acid-base dosage corresponding to the neutralization of which the reaction scheme is as follows (Benattia k.F, 2014).

R-COOH + KOH RCOOK + H2O

The acid number (AI) expressed in mg KOH /g oil is given by the following formula:

IA = (56.1 x N x V) / m

IA: acid index

N: normality of the ethanolic KOH solution

v: volume of the ethanolic KOH solution expressed in ml

m: mass of the oil test sample in g

Weigh 10 g of oil in an Erlenmeyer flask. Pour 150 ml of mixture (ethanol 96% - diethyl ether/ 1:1). Add a few drops of phenolphthalein. Shake very vigorously until dissolved. Then titrate with a solution of ethanolic potash at 0.1 N potassium hydroxide solution until a persistent pink color appears (Paquot C, 1979).

3.1.4.3. Determination of the pH

The pH is measured using a potentiometric method. We measure the potential difference between a glass electrode and a reference electrode (MartinandLurin E., Gruber R., 2012).

Weigh 2g of the oil in a 25 ml Erlenmeyer flask. Add 13 ml of hot water while agitating. Refresh the Erlenmeyer in a cold water bath at ambient temperature. After calibrating the pH meter with buffer solutions and immersing the electrode in the Erlenmeyer flask, note the pH value read on the pH meter (Saeed-Ul-Hassan S et al., 2013).

3.1.4.4. Determination of refraction index

The refractive index of a material, is a number that characterizes the power that this material has, to slow down and to deflect the light. The refractive index is characteristic of the group to which the material belongs. It is a physical quantity related to the unsaturation but which does not really determine it. It is influenced by many factors such as: free acidity, oxidation, polymerization.

The principle consists of a measurement with an appropriate refractometer of the refractive index of the liquid sample at a temperature of 20±0.5C (Paquot C, 1979).

The measurement made by the Abbe type refractometer, the latter must be adjusted so as to give, at a temperature of 20°C for distilled water, an index of 1.3330.

Carry out the measurements according to the operating instructions of the instrument used. Read the refractive index in absolute value and note the temperature of the prism of the machine (Journal officiel N° 65, 2012).

3.1.5. Gas chromatography

The separation and detection of fatty acid methyl esters was carried out by gas chromatography (GPC). This technique of separation of the components of a specific mixture is based on the difference of affinity of the substances to be analyzed towards a current mobile phase called carrier gas carrier gas and a non-volatile stationary phase.

In the case of fatty acids, the separation depends on the length of the carbon chain and the number of double bonds (El hachimi F et al., 2005).

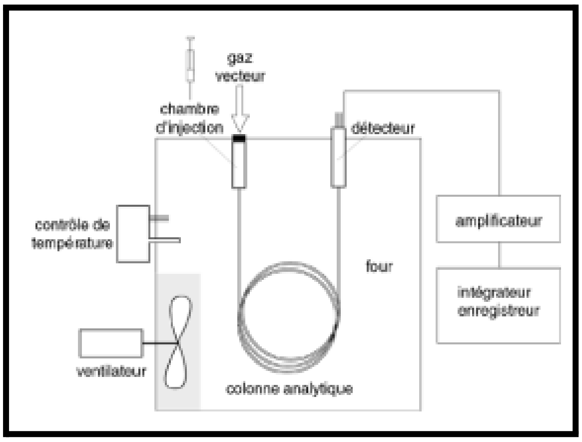


Figure 10: scheme of gas chromatograph
(Source: Jiri Urban, 2009)

Essentially, in gas chromatography, a sample is injected into the hot inlet of a gas chromatogram, which volatilizes the sample components. Then an inert gas ("carrier gas") transports the volatile compounds through a coated capillary column. The capillary coating or "stationary phase" is housed inside the capillary column. The flame ionization detector (FID) passes the sample and the "carrier gas" in the column through a hydrogen-air flame.

The hydrogen-air flame alone creates few ions, but when an organic compound is burned, there is an increase in the ions produced. A polarizing voltage draws these ions to a collector near the flame. The current produced is proportional to the amount of sample burned. This current is detected by an electrometer, converted into digital form and sent to a data system where a chromatogram is constructed electronically. The time it takes for a specific compound to pass through the capillary column and reach a detector is called its "retention time". Retention time, which is inherently related to the affinity of a compound to the stationary phase, can be used to identify the compound in question against a reference standard.

For this manipulation it is necessary to prepare the sample of oil of prickly pear and methyl esters.

Weigh approximately 0.1g of the oil sample into a 5ml screw-capped test tube , add 2ml of heptane and shake. Add 200ul of 2 M KOH methanol solution then close tightly and shake vigorously for 30 seconds. allow it to stand until the upper phase of the solution clears.

Transfer about 1ml of the organic phase into a beaker suitable for the automatic injector of the gas chromatograph. it's possible to keep this solution in the refrigerator until the chromatographic analysis.

3.1.6-.Study of the toxicity of prickly pear seed oil

3.1.6.1. Brine shrimp toxicity testing

The choice of the model organism takes into consideration its geographical distribution, adaptive capacity, life cycle, ecological and socio-economic relevance, and also the ease of manipulation and maintenance.

Artemia salina. (crustacea, anostraca), known as the saltwater, is characteristic of saline biotopes, and can be found in more than 500 ecosystems distributed throughout the tropics, subtropics, and temperate zones of the zones of the globe. Furthermore, the relative low cost and the absence of ethical constraints, encourages the use of this organism to develop work in the field of ecotoxicology.

The methodology for the toxicity test using Artemia sp. as a bioindicator will be described in detail. After testing, *Artemia salina*. will be exposed for 24 h to the prickly pear seed oil in order to determine the potential lethal effects of the expressed by the LD 50 value (Lethal Dose 50), which is a measure of acute toxicity of the compound/substance under study, and it makes possible to compare toxicity levels between two or more compounds. (Artoxkit M., n.d)

3.1.6.2. Toxicity testing

3.1.6.2.1. Decapsulation

- In a beaker, the cysts are placed for 1-2h in freshwater; it's necessary to check the package recommendations to use the good amount of water. Hydration should be done under aeration, at 25 ºC. When hydrated, the cysts should have a spherical shape.
- Water passed through the filter and washed abundantly with tap water.

- In order to facilitate the decapsulation of the cysts, these should be immersed in a 3% hypochlorite solution (common lixivia) for 10-15 minutes. Beaker keeped in an ice bath and with agitation/ stirring. When decapsulated, the cysts should be orange in color.

NOTE: The commercial bleach has 5g hypochlorite per L. To calculate the volume of bleach to be pipetted, use the formula CiVi=CfVf.

- Washing thoroughly with tap water. If there is an odor of bleach, efficiently wash the cysts with a 3% thiosulfate solution and again rinse thoroughly with water.

3.1.6.2.2. Incubation

- Preparation of incubation medium

$NaCl$ (26.4 g - dissolved in 1 l. = 26.4 g/l)

KCl (840 mg - dissolved in 1 l. = 840 mg/l)

$CaCl_2.2H_2O$ (1670 mg - dissolved in 1 l. = 1670 mg/l)

$MgCl_2.6H_2O$ (4600 mg - dissolved in 1 l. = 4600 mg/l)

$MgSO_4.7H_2O$ (5580 mg - dissolved in 1 l. = 5580 mg/l)

$NaHCO_3$ (170 mg - dissolved in 1 l. = 170 mg/l)

H_3BO_3 (30 mg - dissolved in 1 l. 3 = 30 mg/l)

- The cysts are placed in the previously prepared incubation medium.
- The container with the cysts placed under constant illumination (photoperiod 16h/8h) and insulated with parafilm.
- Incubate for 48h.

At the end of the incubation period (total 48h), discard the cysts that are floating cysts.

3.1.6.2.3. Toxicity tests

The tricky point for our test was the solvent to be used to mix the oil as much as possible in the aqueous medium of the nauplius, knowing that the solvent should be at a well studied concentration so as not to be toxic itself and to allow to measure just the toxicity of the oil.

After multiple tests of different concentrations made in the aquaculture lab at IPB, the choice is taken on TWEEN 20 with an optimal non-toxic concentration equal to 8%.

Tween 20 is a soft, non-irritating, non-toxic solubilizer. It is a perfect dispersing agent that allows with manual agitation the dissolution of the prickly pear oil in the nauplius medium.

The beginning was with the TWEEN 8% solution which consists of 8ml of TWEEN 20 diluted in 82ml of incubation media.

From this solution we will make the mother solution of 10% of our oil, so the starting concentration is 10%, then make the dilution to obtain in total 5 different concentrations, 5%, 2.5%, 1.25% and 0.62%.

For each concentration, we make 4 replicates which make 4 wells with 4 nauplius in each one, here is an explanatory shema.

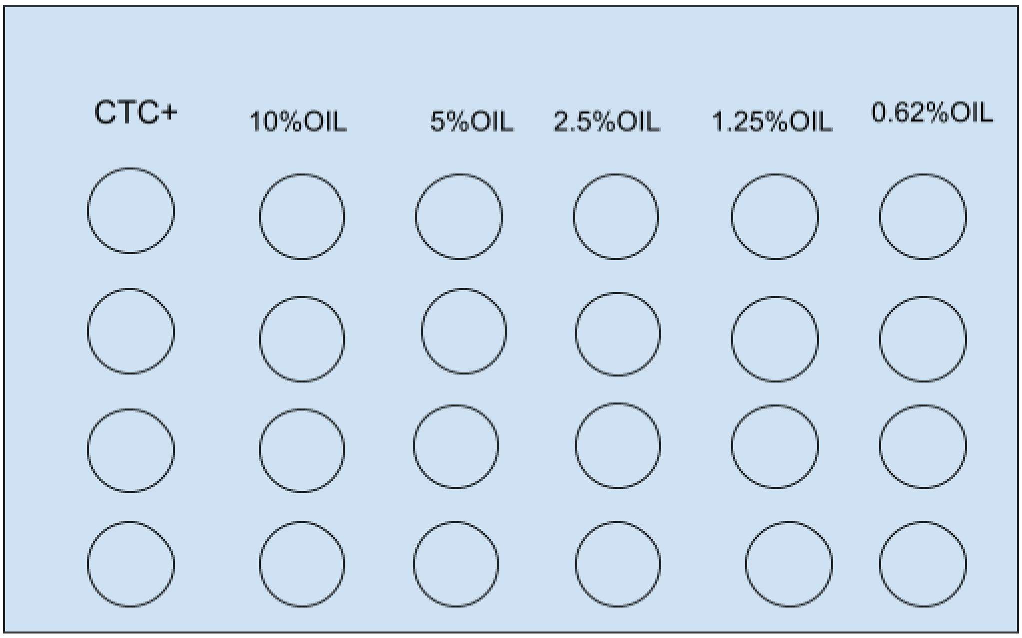


Figure 11: organization of wells at different concentrations

A positive control is an experimental control that gives a positive result at the end of the toxicity experiment. This type of test always gives the result as an "yes". This is a good indication of whether the test works. Therefore, positive controls are used to assess the validity of a test.

After having estimated the volume of solution in the wells, we put 4 young nauplius and we kept them in a dark place.

We Count the number of live and dead nauplii after 6, 12 and 24h of exposure (t0h, t6h, t12h, t24h, respectively). Between counts, the plate has to be preserved (sealed with parafilm) in a dark environment and with a temperature equal to 25ºC.

This manipulation will allow the determination of the LD50 value. This indicator measures the dose of substance causing the death of 50% of a given animal population under specific experimental conditions using the method of Kabba and Berhens (Adjoungoua et al, 2008).

$$DL\,50 \;=\; DL\,100 \;-\; \Sigma\, a \times b \,/\, n$$

LD50: dose giving 50% of deaths.

DL100: dose giving 100% of deaths.

a: average deaths between two successive doses.

b: difference between two successive doses.

n: Average of animals used.

Chapter 4. Results and discussion

4.1. Organoleptic results

From a cold extraction of prickly pear seeds without any additive addition, the oil sample represente 100% pure product based on a natural ingredient, the appearance is limpid light yellow, a slight variation in color may take place, the smell is fruity, strong and powerful enough, not soluble in water.

This prickly pear seed oil is soft and light. It does not leave a greasy film on the skin.

4.2. Physicochemical parameters

Several physicochemical parameters have been determined for a Portuguese species of prickly pear seed oil. The results obtained are described below.

- **Density**

The value of the density corresponds to 0.901 and the relative density corresponds to 0.920.

For comparison with Mediterranean species, this found relative value is a little bit higher than the one reported by (Gharby S. et al, 2015) and (Musa Ozcan M.and Al Juhaimi F.Y., 2011), their studies of the latter give the following respective values 0.906 and 0.907. On the other hand the value found by (Benattia F.k, 2017) is 0.925 which is very close to ours.

The absolute density at room temperature is 0.901, which is comparable with the value reported by (Ennouri M. et al, 2005) which corresponds to 0.903. We can conclude that our oil is of acceptable purity.

- **Acid number**

Apart from the fact that it is a chemical criterion of freshness and purity of oil, the knowledge of acid index is considered necessary and efficient to study the degree of alteration of an oil (belarbi F, 2010).

The acid index is a quality parameter, knowing that a very high free acidity makes the oil very fragile to oxidation.

The sample of prickly pear seed oil studied represents an acidity index equal to 1.7mg KOH/g of fat. First of all, it is a result that perfectly meets the required standard for virgin fats and oils, which validates 4mg KOH/g maximum (Codex Alimentarius,1981).

Then, comparing to other medical works, this value is lower than 2.66mg of KOH/g, given by (Benattia F.k ., 2017), but also higher than 0.56mg of KOH/g found by (gharby S. et al, 2015, Mouden M. et al, 2016, El Mannoubi I. et al, 2009).

It can be concluded that the acidity value detected for prickly pear seed oil from Portuguese lands remains comparable and similar to those recorded for the same extract from the mediterranean area.

- **pH**

The pH gives an indication of the acidity or alkalinity of the medium, it is determined from the number of free hydrogen ions contained in the oil (Addou S, 2017).

As for the pH value obtained, it is 6. The higher the pH, the lower the rate of acidity is low.

- **Refractive index**

This parameter is also an important criterion to confirm the purity of the oil. It is proportional to the molecular weight of the fatty acids of the oil (Ollë M, 2002). The refractive index determined at a temperature of 20 ° is 1.478.

The researchers (El Mannoubi I. et al, 2009) show a value of 1.471 and (Ennouri M. et al, 2005) a value of 1.4750. The results are clearly close.

- **Results of the chromatographic analysis**

Prickly pear seed oil was analyzed by gas chromatography to decipher the chemical composition

Table 2 Fatty acid composition of prickly pear seed oil

N°	Fatty acid	Retention time (min)	Area (%)	Concentration (%)
1	Capric acid (C 10:0)	5.11	0.021	0.033
2	Lauric acid (C 12:0)	6.940	0.140	0.206
3	Myristic acid (C 14:0)	9.320	0.165	0.215
4	Palmitic acid (C 16:0)	12.452	13.085	15.401
5	Palmitoleic acid (C 16:1)	13.040	0.938	1.131
6	Margaric acid (C 17:0)	14.196	0.042	0.051
7	Stearic acid (C 18:0)	16.128	2.984	3.348
8	Oleic acid (C 18:1)	16.640	15.707	8.349
9	Linoleic acid (C 18:2)	17.824	66.000	70.277
10	Linolenic acid (C 18:3)	19.152	0.177	0.196
11	Arachidic acid (C 20:0)	19.992	0.256	0.278
12	Gadoleic acid (C 20:1)	20.412	0.140	0.157
13	Behenic acid (C 22:0)	23.492	0.152	0.159
14	brassidic acid (C 22:1)	23.664	0.041	0.042
15	Tricosanoic acid (C 23:0)	24.220	0.049	0.051
16	Lignoceric acid (C 24:0)	24.732	0.103	0.106

A separation of 16 different fatty acids was observed. The oil of prickly pear seeds has a high level of unsaturated fatty acids.

The main fatty acid detected is linoleic acid with a concentration of 70.277%, followed by palmitic acid with a percentage of 15.401% then oleic and stearic acid with percentages of 8.349% and 3.348% respectively.

The figure above shows the chromatogram obtained during the chemical characterization of prickly pear seed oil by gas chromatography (GC).

From this chromatogram, we observe four major and significant peaks with retention times of 12.452, 16.128, 16.640 and 17.824 minutes respectively, corresponding to the major fatty acids (stearic acid, oleic acid, palmitic acid and linoleic acid, respectively).

In general, the high level of unsaturation particularly of linoleic acid, combined with a low level of linolenic acid (C 18:3) which adversely affects the stability of the oil, indicates that the seeds of Opuntia ficus-indica could be an excellent potential source of edible oil for human consumption, which corresponds to our results, a high level of linoleic acid associated with a low concentration of (C 18:3) equal to 0.19% (Sawaya W.N.et Khan P., 1982).

To have a more extensive vision, a comparative study of the chemical composition of the oil (some fatty acid) of *Opuntia ficus-indica* collected from different regions of the world using the same extraction method, has been made.

The comparison is based on several reports, the researchers (Mouden M. et al, 2016) studied the oil from the Moroccan region, (El Mannoubi I. et al, 2009) characterized the oil coming from the north of Tunisia, (Ramadan M.F. et Mörsel J.T., 2003) established the data for Germany and finally (Ciriminna R. et al, 2017) studied the oil coming from the Sicily region in Italy.

Table 3 Comparing 4 major fatty acids of *Opuntia ficus indica* oil from different regions of the world (%)

Fatty acid	Maroc	Tunisie	Allemagne	Italie	Portugal
Linoleic acid	57,47	60,69	53,5	57,98	70,277
Palmitic acid	10,92	12,76	20,1	12,29	15,401
Oleic acid	15,37	16,41	18,3	17,61	8,349
stearic acid	4,70	3,20	2,72	3,92	3,348

- **results of toxicity test**

The oil of prickly pear seed was tested by the vivasite of nauplius or the so-called young artemia. The table below presents the number of deaths after a specific time interval.

Table 4 number of dead nauplius at different concentrations

		Duplicates				%mortes	Valor Probit
CTC +	t0	4	4	4	4		
	t1	0	0	1	0		
	t2	0	0	0	0		
total deaths		0	0	1	0	6,25	
10%	t0	4	4	4	4		
	t1	0	0	1	1		
	t2	3	2	1	0		
total deaths		3	2	2	1	50	5,0
5%	t0	4	4	4	4		
	t1	0	0	0	0		
	t2	1	1	2	1		
total deaths		1	1	2	1	31,25	4,50
2,50% t0	4	4	4	4			
	t1	1	0	0	0		
	t2	0	1	0	1		
total deaths		1	1		1	18,75	4,45
1,25% t0	4	4	4	4			
	t1	1	0	0	0		
	t2	0	0	1	0		
total deaths		1		1		12,50	3,87
0,62 t0	t1	t2	t3	t4			
	t1	0	0	0	0		
	t2	0	0	0	0		
total deaths		0	1	0	0	6,25	3,45

To obtain the LD50 value, the log dose-probit graph is required.

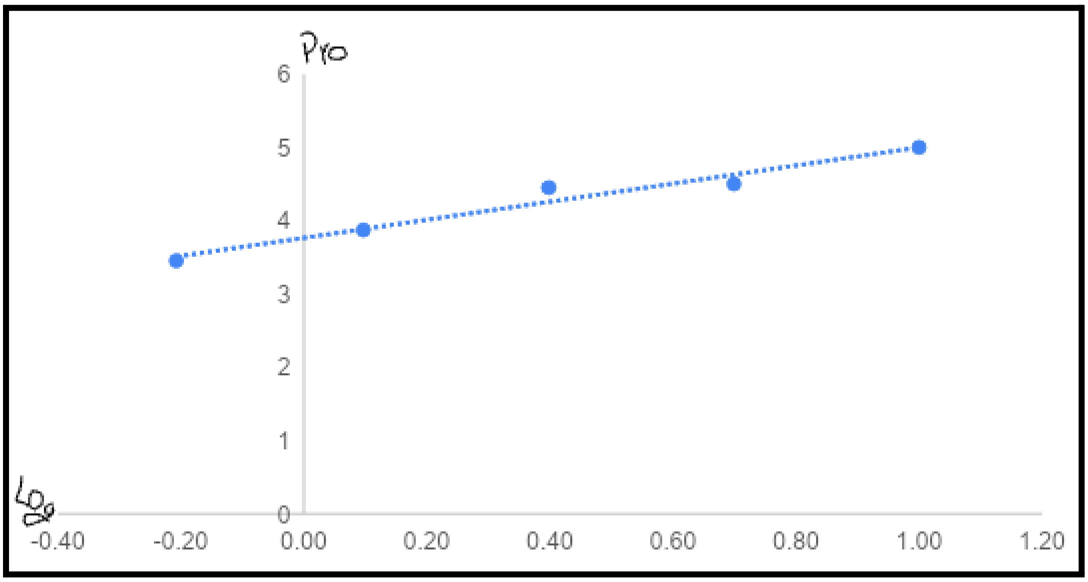


Figure 12: The log dose-probit graph

Therefore the oil presents a LC50 value of 10% (in tween), as we could see directly from the results (50% deaths at 10% concentration). This result alone, does not allow us to conclude if the oil of prickly pear is toxic at what dose exactly.

So this remains our research idea in order to deepen our knowledge.

Chapter 5. Entrepreneurship project

AMBROSIA is a project idea which is being developed from 2021 to produce the oil of prickly pear seeds, for direct use or in industry, using a cold press extractor.

The products are unique since the oil and the raw materials will be 100% Portuguese from local agriculture at first, and from the waste of the agri food industry in the future.

Also, the products will not be tested on animals since it is one of the most important values of the company, the respect of nature, animals and environment.

AMBROSIA will have an e-commerce website in order to promote and sell the products and the process of making them. Furthermore, the company will develop a solid marketing campaign and plan in order to expand the business and fulfill the financial objectives.

5.1. Company summary

5.1.1. Mission

A mission is a statement of why the company operates. It outlines what the firm does and its overriding purpose. The mission is used to support the vision and to share the purpose and focus with the employees, clients, suppliers and other partners.

AMBROSIA wants to: "Offer to everyone, fresh, organic, respectful, efficient and high quality products for the best of their skin and life routine." Create, produce, design and market natural skin care products, of the highest quality, without chemical ingredients and according to international and European standards in order to find solutions to the specific needs of our customers and contribute to their self success and awareness while respecting their values and the planet.

5.1.2. Vision

A vision is a description of the organization as it would be seen in a future successful situation. An efficient vision statement is both inspiring and empowering. It provides a visual image of the future status that the company would like to achieve. A vision is meant to challenge and invite employees to be inspired.

This points represents the vision of AMBROSIA:

- Valorization of agri food industry waste, prickly pear seed.
- Achieve full integration of our production chain, our culture and distribution.
- Ensure a high quality of products.
- Offer products free of chemical ingredients, that are not tested on animals, respecting the environment and 100% portuguese.
- Assure a contact dynamic of sales through different ranges of products.

5.1.3. Values

A values statement describes what the company believes in and shows the way it will behave. Values are a guideline for the enterprise and its employees. They define the core philosophies and beliefs of the corporate culture and identity.

The values of AMBROSIA constitue her heart. AMBROSIA represents transparency with her customers and puts it as a must to be. The company has the respect of the environment and animals as an important subject that she wants to reflect through all the proces.

This flowing point represent the main values of the company:

- Passion: Faithful to its goals and visions.
- Sustainability: Driven by environmental responsibility and integrity.
- Innovation: Delivering new and innovative products and solutions to its customers.
- Excellence: Delivering superior quality on its own products.

5.2. SWOT analysis

SWOT analysis is a framework used to evaluate a company's competitive position and to develop strategic planning. SWOT analysis assesses internal and external factors, as well as current and future potential.

This analysis is designed to facilitate a realistic, fact-based, data-driven look at the strengths and weaknesses of an organization, its initiatives, or an industry. The organization needs to keep the analysis accurate by avoiding preconceived beliefs or gray areas and instead focusing on real-life contexts. Companies should use it as a guide and not necessarily as a prescription.

5.2.1. Strength

We were able to highlight four important points representing the strengths that our company possesses.

First, the quality of our products and future products. We are going to develop and produce 100% natural products without any chemical or solvent additions. Therefore, our products will be pure and of high quality as they have more active ingredients due to their purity.

Our second point is the use of organic products. Our raw material will be bought from Portuguese farmers who do not use insecticides and who have an organic production that respects the environment or what is even more sustainable and preferable is that we will use the waste from compal industry after using the fruit for their juice.

This in order to always promote our values of naturalness and respect for the environment and the skin of our customers, whether in B2C or B2B.

Third, our ability to innovate. Certainly our first products will be prickly pear seed oil and skin care products based on this oil. However, We will have the ability and opportunity to develop new products. We are already studying these probabilities. Such as for example an oil based on chestnut, oil mixes, care products based on our different oils that we were going to develop.

And finally, one of our strengths is our diverse team. Currently we are two people for the beginning.

TAAMALLAH Marwa, who has the scientific skills for the development of our products and the technological knowledge for the extraction for our basic product which is oil. She also has the biological knowledge to know the components of our products and the most favorable mixtures for our products. She has been able to practice and develop this knowledge through her university career, the different internships and work she has done during the last 6 years.

ZAIBI Feryel, who has gained the necessary skills in marketing, communication, finance, accounting and entrepreneurship through her academic background but also through her internships and practical work in these different fields.

The mix of these two skills represents a development power for our company since it allows us to have a balance but also will spare us some expenses with different contributors such as an accountant or a laboratory technician.

5.2.2. Weakness

Like any company, we also have weaknesses that we need to work on. We were able to identify three essential points.

Since we are a new company on the Portuguese market but also on the international market, we have little or no notoriety. Only a small circle of people know our company and our brand, but this can be explained by the fact that we have not yet started marketing our products, but also by the lack of communication on social networks. These are important points to develop but we plan to start these actions once our products are well developed and tested to ensure the good quality and efficiency of our offers.

We are two Tunisian entrepreneurs and newly arrived in Portugal, which means that our knowledge of Portuguese law in terms of business creation is very limited as well as in terms of administrative procedures. For this reason, we need to carry out further research, either on the internet or with people who have already developed a company or are about to do so, in order to acquire the necessary and exact notions for a good start and development.

And last but not least, one of our weaknesses that we consider to be among the most important is our financial capacity, which is weak and limited at this stage. This factor is generally one of the most problematic points for the majority of new companies.

5.2.3. Opportunities

Opportunities are the advantages that the market and the environment possesses and that can benefit us for the development, growth and prosperity of our project.

The market for skin care products is constantly growing. Articles and sources affirm this point such as the world census site Statista.com which is a leading provider of market statistics and consumer behavior. Or Vividata.ca , which is a leading Canadian multimedia and consumer research site, offering essential information on global consumption.

In the same continuity, if a market is growing, it also means that the demand is more and more present. If the demand is growing, it also means that the potential customers or consumers are expanding and their number is growing. For us this is a positive point and an opportunity to reach more people.

In parallel with our strengths, there is a strong possibility of product diversification. Certainly this is a strength because we have the possibility to do so, but it is also an opportunity offered by the natural environment as well as the competition. To avoid marketing myopia, we must always be on the lookout for innovations and technologies offered or developed by competitors. This may be perceived as a threat by some industries, but it is also an opportunity for us to create and market new products.

Furthermore, the natural environment offers a multitude of exploitable raw materials, either fruits or wild herbs with substances that can bring a lot to the skin or to the health of the human being and this while respecting our ecological and biological values.

To continue in the same perspective of nature's offer, one of the opportunities we can mention is the availability of the primary fruit in Portugal, the Prickly Pear. In fact, this fruit is cultivated and is present enormously in the south of the country and more precisely in the Algarve. But also, one of the largest cactus cultivation and consequently the fruit can be found on the island of Madeira. This point allows us to use and import this fruit always within the Portuguese legal framework and without the need of other countries exporting this fruit. This allows us to develop the Portuguese agricultural market while maintaining a constant production.

In addition, one of the significant opportunities is the establishment of 500 hectares of prickly pears in southern Portugal by the private sector to develop the market. This is a not negligible point for us because it allows us to have a constant and above all local supply.

We also have the opportunity to make partnerships with food industries in order to supply us with their prickly pear waste. Knowing that the food industries do not use the seeds of the fruits because they can give a bitter taste to juices or other food products, we wish to make partnerships with industries to recover the seeds so that we can use them for the extraction of the oil.

The last point of opportunity that we consider essential for our business is the Government. The latter has put and continues to put in place measures to encourage the launch and prosperity of new startups and companies in order to improve the economic factor of the country and encourage young entrepreneurs, which can also help reduce the unemployment rate through the employment of skilled workers or even laborers.

5.2.4. Threat

While the external environment offers opportunities, it does not prevent the presence of threats that can slow business growth.

Three major threats have been identified.

The competition at the national level is certainly weak with the presence of only one company manufacturing prickly pear oil of Portuguese origin. However, at the international level a large number of industries market this oil and its derivatives, as well as skin care products based on this oil, despite the fact that the origin of the fruit used is often imported from North Africa or Latin America. This may hinder our expansion on a broader level.

Despite the statistics cited above regarding the growth of the cosmetics market and more specifically the skin care market, we cannot overlook the possible threat of slow global purchasing power as a result of the health crisis facing the world. This threat may cause the shutdown of several industries and consequently the dismissal of a large number of employees. And this leads to a decrease in purchasing power and the prioritization of products to be purchased from certain households. As the Maslow Pyramid tool can demonstrate, some individuals or households have a different purchasing priority to others, and therefore skin care products or essential oils may have a low priority for some.

Finally, the last threat that we have identified is the demand of local consumers, which may be more difficult to convince than some other international segments.

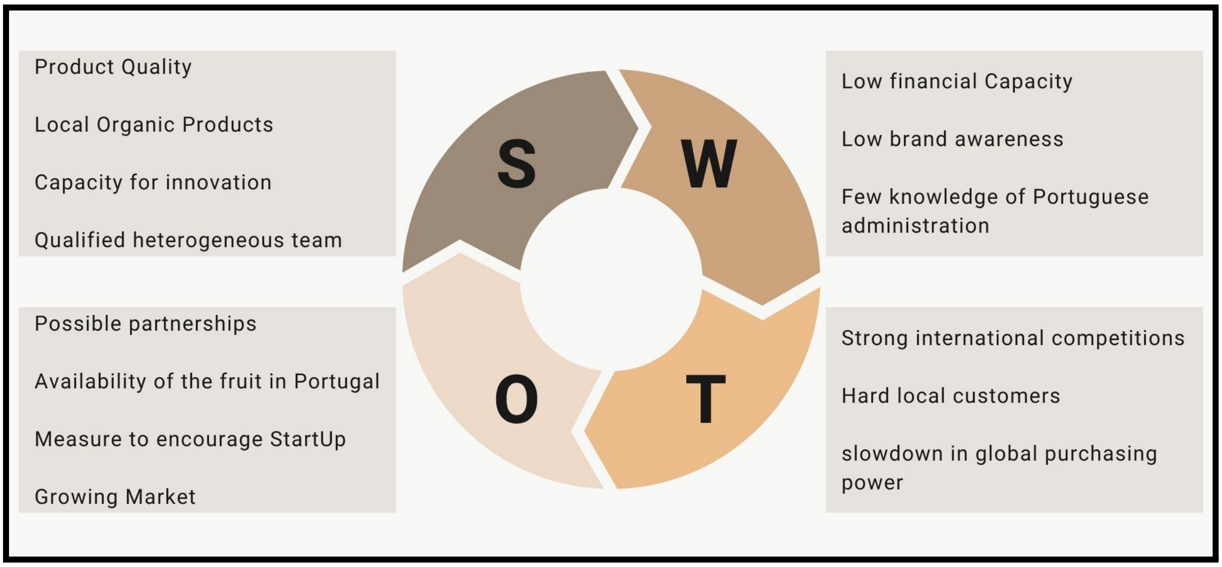


Figure 13: Swot analyse

5.3. Future Enterprise

A wide range of traditional preservation technologies can be applied to prickly pears (FAO, 2018). Nothing is thrown away, everything has a real value added that can constitute a niche investment in its own right: Prickly pears, mucilage, fruit and/or its seeds as well as the petals of its flowers (Agroligne, 2016). This will allow the company to evolve by manufacturing other products such as: juice, jam, flour from cladodes or seeds, vinegar and colorants.

Chapter 6. Conclusion

The cactus has been largely ignored by scientists until the beginning of 1980, this renewed interest is partly attributed to the multifunctionality of prickly pear fruit and the numerous benefits present in the different parts of the plant. Recent studies have revealed their high levels of certain chemical compounds, which can make this fruit an added nutritional value, such as taurine, calcium, magnesium, phenolic compounds and betalains (Piga, 2004). Cactus also has a renewed interest in several countries due to its ecological role, environmental and socio-economic: the fight against erosion and desertification, and fruit production fodder (Bouzoubaa et al., 2014). The prickly pear is also the origin of products and by-products to various industrial uses, medicinal, pharmaceutical and cosmetics (Barbera, 1995).

In a general context of valorization of waste or use of industrial by-products, the AMBROSIA project targets prickly pear seeds as a starting point, a study of the *Opuntica ficus indica* prickly pear tree species is necessary. This plant is present on the Portuguese territory, more frequent in the south.

The objective of this work is to determine the physicochemical indices, the composition of fatty acids of the cold extracted oil, which is the most ecological technique.

The results obtained confirm the nutritional quality of the oil of prickly pear seeds, which strongly confirms our project ideology, knowing that this oil is not sufficiently highlighted in Portugal.

These qualitative results not only validate the process of project creation but also open perspectives and research tracks both for innovation in the market and the possibility of being more sustainable. Additional studies to be considered:

-An evaluation of the biological activities (antioxidants, antibacterial)

- Exploitation of the different parts of the plant (racket, flowers..) to enlarge the range of organic products.

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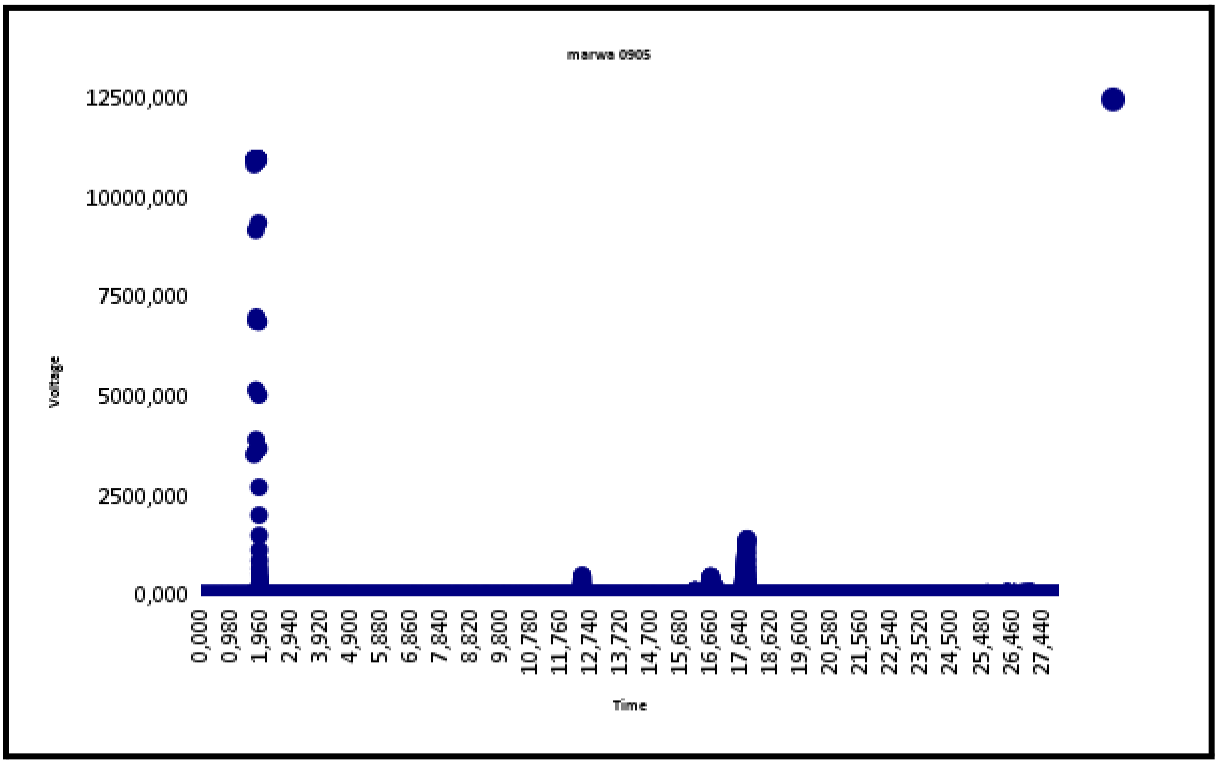
Annex A



Chromatogram of the oil of prickly pear seeds

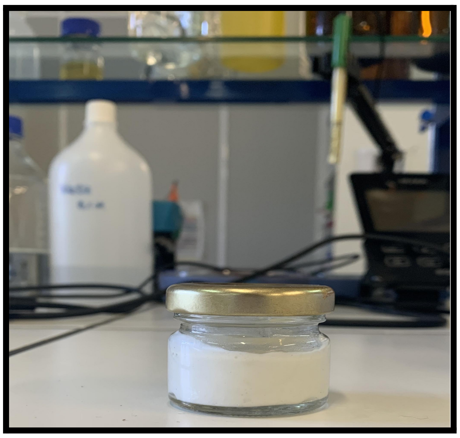
Annex B

First ry of anti aging cream:

The anti-aging cream is one of the basic products for the AMBROSIA project, the composition will be more developed in the coming days.

The current formulation is composed of two phases, an aqueous phase which contains 15ml of water and 0,2g of xanthan gum and an oily phase which contains 0.5g of olivem 1000, 0.2g of olivem 300, 0.25g of oliwax and 2 ml of pure prickly pear seed oil.

- Weigh into 2 separate tubes the FA and FO ingredients, respectively;
- Secure the tubes inside the bath with the help of the holder and the two clamps;
- Heat both phases (FA and FO) to 70-75 oC;
- Remove the tubes from the heating and then add the FO phase to the FA phase;
- Shake by hand and then on the vortex mixer;
- Add the desired additives and homogenize gently with the help of a spatula.
- Transfer to a container for storage.



First try of the anti aging cream