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MESTRADO EM CONTROLO DA QUALIDADE COM ESPECIALIZAÇÃO EM ÁGUA E ALIMENTOS

Opuntia ficus-indica (L.): Chemical Composition and Nutritional Valorization towards functional food ingredients

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Resumo:

Opuntia ficus-indica (Figo-da-Índia) é um cato frutífero, nativo do México, atualmente considerado uma espécie invasora no território Europeu. Cresce em regiões áridas e semiáridas. As recentes mudanças climáticas têm vindo a aumentar o território desta planta.

Amostras de frutos e de cladódio (caule do cato) foram recolhidas numa colónia de *Opuntia* no Noroeste de Portugal. Foram avaliadas amostras colhidas no verão e no inverno, inclusive uma amostra com rega diária. Uma amostra do subproduto de uma empresa produtora de sumos foi também alvo de estudo. As amostras foram avaliadas através de diversos ensaios, incluindo a determinação de macronutrientes, ensaios de poder antioxidante, determinação de ácidos gordos e vitamina E.

A semente tem uma elevada quantidade de Vitamina E, principalmente α-tocoferol, que contrasta com os habituais altos teores de δ-tocoferol encontrados na *Opuntia*. Os ensaios de atividade antioxidante, destacaram a possibilidade de a rega diária causar stress na planta, apresentando esta os maiores valores nos ensaios. A fruta de inverno demonstrou uma quantidade incomum de frutose, que foi atribuída a um mecanismo de adaptação ao frio. A parte da planta que apresentou maior poder antioxidante foi a casca do figo-da-índia, devido ao seu funcionamento como camada protetora. O óleo é altamente insaturado, mas a planta regada apresentou os níveis mais altos de saturação.

A comparação do cladódio e o respetivo sumo com a planta de Aloé Vera permitiu planear a sua utilização como base para um produto alimentar. Os cladódios de *Opuntia* têm uma quantidade superior de açúcar, mas em geral são composicionalmente semelhantes ao Aloé. Desenvolveu-se um iogurte enriquecido com cladódio, substituindo parte do leite de vaca por sumo de cladódio. Os melhores resultados a nível da textura foram obtidos com uma concentração de sumo de 10%. No entanto, concentrações até 25% mostraram uma produção de ácido lático estatisticamente semelhante ao controlo.

Apesar das grandes taxas de crescimento na Europa, esta plante continua sobre explorada. As suas adaptações a climas áridos e baixa necessidade de manutenção são promissoras. Os autores consideram assim a *Opuntia como* uma oportunidade para utilização em vários produtos alimentares e cosméticos.

Palavras-chave: *Opuntia ficus-indica*, figo-da-índia, desenvolvimento de produto alimentar, antioxidantes, análise de ácidos gordo

Abstract:

Opuntia ficus-indica (Prickly-pear cactus) is a fruit-bearing cactus, native to Mexico, currently an invasive species throughout Europe and Africa. Flourishing in arid and semi-arid regions, recent climate changes are increasing this plants territory further north.

Four fruit samples and one cladode (cactus stem) sample were collected in an Opuntia colony in Northeast Portugal. These samples varied in collection year, collection season (Summer or Winter) and watering. A sixth sample of a juicing company leftovers of the same fruit was also collected. Various laboratory assays were made, including macronutrient determination, antioxidant assays, fatty acid and vitamin E determination.

The seed shows a high amount of Vitamin E, mainly α-tocopherol, which contrasts with the usual high amounts of δ-tocopherol usually found in *Opuntia*. The watered plant, despite showing a more nutritionally poor profile, also displayed the highest results in the antioxidant activity, showing the possibility of t the watering actually causing stress on the plant. The winter fruit shows an unusual high amount of fructose, which was attributed to a cold-adaptation mechanism. The plant part that shows the highest antioxidant score was the peel as it functions as a protective layer. The oil is highly unsaturated, but the watered plant shows higher levels of saturation.

Comparing the cladode and cladode juice to Aloe Vera allowed us to plan on using it as a basis for a product. *Opuntia* stems show a higher amount of sugar, but is overall similar to aloe. Furtthermore, we developed a cladode enriched yogurt by replacing part of the milk with cladode juice. The best results in texture were obtained when the juice concentration was 10%. However concentrations until 25% showed a lactic acid production that was statistically the same as the control.

Opuntia ficus-indica's potential is underexplored, especially in the western civilization, where we now find it growing in adverse conditions. We consider this plant a big opportunity for elaboration of various products, including food, beverages, cosmetic and even textiles. Its lack of watering need makes it a plant adapted to climate change and a low-maintenance crop. *Opuntia's* future is, for a fact, very promising.

Keywords: *Opuntia ficus-indica,* Prickly-pear, Food product Development, Antioxidants, Fatty Acid Analysis

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List of Abbreviations

SD	Standard deviation
Wat	Prickly-pear fruit sample from the daily watered plant from summer 2020
S2019	Prickly-pear fruit sample from the wild plant from summer 2019
W2019	Prickly-pear fruit sample from the wild plant from winter 2019
S2020	Prickly-pear fruit sample from the wild plant from summer 2020
Cla	Sample of cladodes (cactus stems) from summer 2020
Jui	Sample of juiced cladodes from summer 2020
DPPH	Compound 2,2-diphenyl-1-picrylhydrazyl, used in a common antioxidant assay where the compound is inhibited (DPPH radical inhibition antioxidant assay)
FRAP	Ferric Reducing Antioxidant Power Assay
GAE	Gallic acid equivalents
CE	Catechin equivalents
TE	Trolox equivalents

Introduction

"Una mitología de sangre que entretejen los hondos dioses muertos, los cladodees que dan horror a los desiertos y el amor de una sombra que es anterior al día. ¡ Cuántas cosas eternas!"
*-**México**, by Jorge Luís Borges, Mexican Poet*

1. What is *Opuntia-ficus indica*?

Opuntia ficus-indica (Prickly-pear cactus) is a fruit-bearing cactus, native to Mexico, currently spread on all continents due to its outstanding resistance and propagation. *Opuntia* is an invasive species throughout Europe and Africa. Recent changes in water availability further increased this plant's territory as it flourishes in semi-arid and arid regions. Due to its habitat preference and low water need, this plant is a suitable alternative to improve commercial income in these areas.

Regarding the plant's usage for human consumption, all the plant parts are consumable, including the fruits, cladodes (*Opuntia*'s flattened stems) and the flowers, which are used in infusions. This ensures a low-waste crop cultivation as all parts of the plant are used as food. The cladodes have also been shown to be a good source of food for cattle [1]. Traditionally it has also been used to treat various conditions.

The consumption of this plant has been associated with several benefic properties. This is due to its high content of digestible carbohydrates and antioxidant dietary fiber [2], the capacity of some of its components to behave as radical scavengers [3], its high content in minerals, antioxidants, and vitamin C [4], and its seed oil being particularly rich in PUFAs which bring benefits to conditions such as coronary heart disease and rheumatoid arthritis, as well as having a high concentration in linoleic acid (5). An example of a direct action of the consumption of the prickly pear fruit is the protective ability against ethanol-induced stomach ulcers [5].

Fresh consumption is not the only use of this cactus. The fruit can be sundried and consumed, made into liquor, or transformed into syrup to name a few uses. Young cladodes are traditionally consumed as a vegetable and the seeds are made into seed oil. The vast

majority of the plant is edible, which only expands its beneficial horizons in regard to its environmental benefits and human health benefits.

Figure 1 and 2: Prickly-pear plant from Northeast Portugal. The winter fruit (on the left) is a darker color than the Summer fruit (on the right).

i. <u>The prophesized plant – A tale of the genesis of a civilization</u>

A nomadic tribe in South America, known as the *Nahua* wandered the desert in search of a divine sign that would guide them to the place where they would settle to build their capital. By word of their god Huitzilopochtli, the precise spot would be marked by an eagle devouring a snake, perched on top of a cactus (*Opuntia ficus-indica)*. After two hundred years of wandering, they found the promised sign on a small island in the swampy Lake Texcoco. It was there they founded their new capital, Tenochtitlan, and their civilization became known as the Aztecs. By 1428 the Aztec state had emerged, and Tenochtitlan was the most important city in Mesoamerica. At its height, it was one of the largest cities in the world, with over 200,000 inhabitants. [6]

Currently, an *Opuntia* full of fruits, growing on a rock, with an eagle holding a snake on its beak is the coat of arms of Mexico, having a central spot on the national flag.

Figure 3. Teocalli of the Sacred War sculpted in 1325, Museo Nacional de Antropología, México [7]

ii. Botany

Although this review focuses on *Opuntia ficus-indica* it is important to mention that there are almost 300 species of the genus *Opuntia* [8].

The taxonomy of the plant presents various challenges, among them the phenotypes which vary within regions, the polyploidy, having both sexual and asexual reproduction, and the existence of numerous hybrids. Most species also blossom during the same time of the year having little to no biological barriers separating them [9]. Different subgenus have developed adaptive anatomical, morphological, and physiological traits and particular plant structures [10]. Among these adaptations are asynchronous reproduction, the Crassulacean Acid Metabolism (CAM), and structural adaptations such as succulence.

Most varieties follow the same development throughout the year. Flowering occurs in Spring, usually in May, and is followed by fruit development which is harvested in Summer. Some plants also present a second out-of-season fruit production in winter. This is found to be dependent on abiotic factors such as nitrogen presence in the soil [11], and the removal of cladodes and flowers during the main season [12].

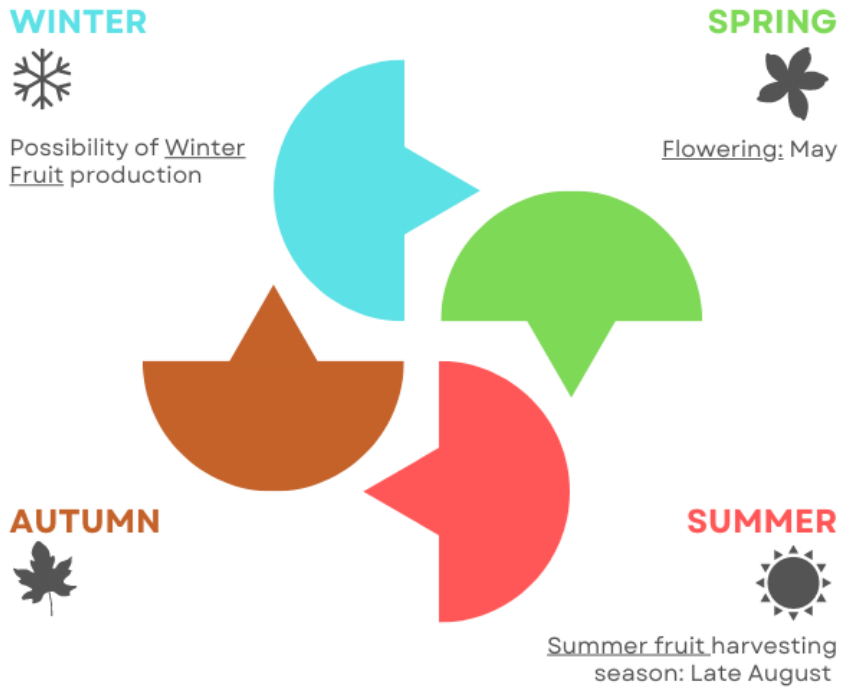

Figure 4. Yearly timeline of *Opuntia* development.

iii. Tradition and history

The plant *Opuntia ficus-indica L.* is native to Mexico and is currently distributed throughout all the Americas as well as the Mediterranean and Africa. Traditionally, it has been used to treat several diseases and conditions due to its anti-inflammatory potential, and its hypoglycemic and neuroprotective properties. Prickly-pear cacti were brought to Europe by the first Spanish conquerors between the end of the 15th century and the beginning of the 16th century. Earlier on, the plant was already of great importance to the Aztecs in their economic, social and religious life [13]. The main reason the species was spread to Europe was that the plant was a reliable host to a cochineal insect (*Dactylopius coccus* Costa), whose body was used to produce an expensive red clothes and food dye [14].

In the milder Mediterranean areas, at first in the territories controlled by the Spanish Empire, namely Sicily and southern Italy, then in northern Africa, prickly pear cacti proved to be so well-suited to the environmental conditions that they quickly became naturalized. They spread by vegetative propagation near the cultivated fields and, through birds feeding on the fruits, spread by seed to more distant areas [13].

Opuntia is now part of the natural landscape in most world regions. Its intensive commercial plantation is common in a lot of countries as well as family orchards. They are well adapted to poor soils that are dry and subject to erosion being perfectly adapted to arid regions. Their consumption during drought times may be lifesaving for both humans and animals. Common usage of the cactus includes feeding livestock with cladodes diminishing their need for supplementation with hay or barley and water contributing to sustainable agriculture in arid areas. *Opuntia* started as a wild plant but is now becoming an endless source of products and functions for both humans and cattle contributing to the food security of populations in agriculturally marginalized areas.

Figure 5. Depiction of a native American harvesting the cochineal insect for red dye production [15].

iv. Prickly-pear fruit

Opuntia ficus-indica is of high economical value in various countries, being the most significant Mexico. Some European and North African countries have also been growing this crop commercially at a much smaller scale. Many studies have been made on the fruit composition and antioxidant activity, most from commercial sources [5, 28, 50-52].

In the northeast region of Portugal, a colony of wild *Opuntia* has been growing over the past few decades and probably came from Spain. Due to the lack of studies regarding wild varieties of the plant, we decided to conduct a nutritional study into this variety both summer and winter fruit as well as the same variety subjected to watering.

In this study we will analyze the wild variety of *Opuntia*'s fruits found in the northeast of Portugal, from the orange variety. We will proceed in studying the peel, the pulp, the juice, and the seed separately

v. Cladodes

The cladodes are an often-leftover part of *Opuntia ficus-indica* as the main purpose of the plant is the fruit.

The cactus needs to be trimmed frequently due to its rapid growth making it hard to reach for the economically important fruit. The cladodes that are trimmed constitute themselves a problem. *Opuntia* can propagate and reproduce itself from these trimmed cladodes, which rapidly develop roots and grow a new plant in very little time. This characteristic is one of the reasons why the cactus is considered a highly problematic invasive species.

In the light of this, it is necessary to find a solution to this waste, since *Opuntia* cladodes are edible and can be introduced into the food chain. Cactus such as Aloe Vera have been processed into electrolyte drinks, gelatins and yogurts, which have had a great success in the market. The modern consumer looks for plant-based, organic, and natural drinks, making these products very sought after. In fact, according to Grand View Research, in 2019 the Aloe Vera drink market was valued at USD 77.8 million and expected to grow at a compound annual rate of 11.3% from 2020 to 2027 [46]. The *Opuntia* cladodes are an opportunity to make a drink with the same nutritional and general properties as the popular Aloe drinks.

In this study, a chemical characterization of the cladode and the respective juice was made, the juice being obtained through a conventional juicer machine. If the aim is valorize cladodes and create a new product using it, it is of big importance to analyze and compare these two samples.

vi. Opuntia seed

After extraction of the pulp, seeds are usually discarded as a waste product. The prickly-pear fruit contains a large amount of hard seeds in its interior, which amount can vary from

30-40% on a dry weight basis [16]. Proper utilization of these waste products could lead to an important new source of oil and meal [38].

Seeds are mainly composed by insoluble fiber and fatty acids, as these act as an important carbon storage for the new embryo. We gathered seed samples from a wild *Opuntia* colony, growing in the Northeast region of Portugal, as well as a juicing industrial byproduct, mainly composed of seeds. We conducted a nutritional study into these samples, as well as an analysis of antioxidant and bioactive compounds.

2. Work Objective

This dissertation focuses on *Opuntia Ficus-indica* and it is divided into 2 parts: Nutritional analysis and practical usage of the plant.

In the first part, we proceeded with the physicochemical characterization of never-before studied wild Prickly-pear from northeast Portugal. We also determined the composition of the rarely studied winter fruit from the same plant. Additionally, we had the chance to compare the composition of the regular plant with a regularly watered plant from the same area.

In the second part, taking into account the need for new processed products based on the prickly-pear, we explored the making of a yogurt enriched with the cactus cladode juice, showing promising results.

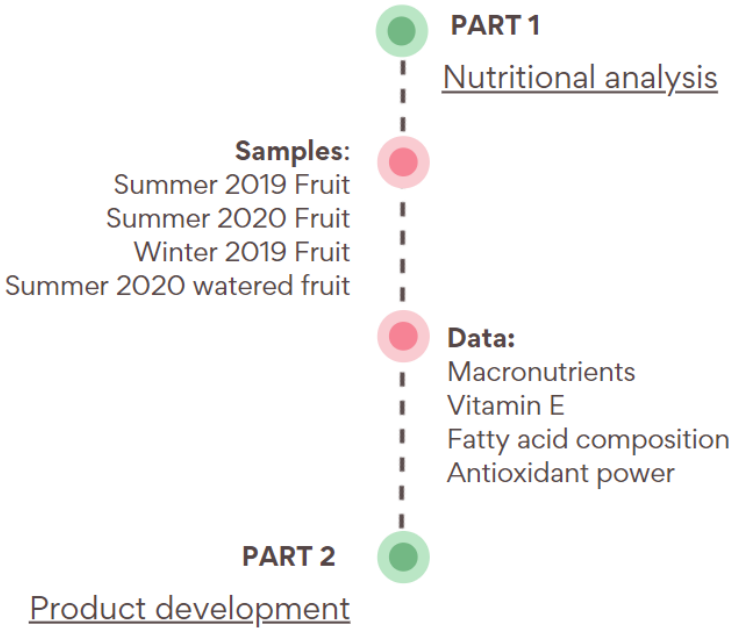

Figure 6. Scheme of the dissertation and research organization.

i. Specific work objectives

- Characterize the nutritional composition of wild fruit from *Opuntia ficus-indica* from northeast Portugal.
- Characterize the nutritional composition of wild cladodes from *Opuntia ficus-indica* from northeast Portugal and secondarily characterize the obtained cladode juice.
- Characterize the fatty acid composition of the oil obtained from *Opuntia* fruit juice byproduct, comparing it to regular oils.
- Compare the physicochemical composition of:
    - Prickly-pear from 2 consecutive years (Summer 2019 and 2020);
    - Summer and Winter fruit from the same year (2019);
    - Watered and non-watered fruit from the same year (2020).
- Create an easily produced appellative product from leftover cladodes.

Methodology

Scientific laboratory techniques were carried out at Faculdade de Farmácia da Universidade do Porto (FFUP) REQUIMTE/LAQV research group (Rede de Química e Tecnologia/Laboratório Associado para a Química Verde, Tecnologias e Processos Limpos). The work was done under the responsibility of Prof. Dr. Beatriz Oliveira, who provided all the necessary conditions and equipment, as well as a wide experience in the chosen methodologies.

All determinations were done according to methodologies already in use in the research group laboratory.

1. Sample gathering

Fruit samples

All fruit samples (Summer 2019 and 2020, Winter 2019 and Watered) were collected on the same area located on the arid slopes near the Ferradosa Dam in the municipality of Freixo de Espada à Cinta, Bragança and by the same time of day (late morning). Samples were transported to the laboratory within less than 24 hours, where they were kept in a refrigerated unit. The samples were all from well-developed plants that did not show any signs of disease or were occulted by other vegetation, having direct sunlight exposure. To later fruit deterioration we cut along with the fruit a piece of the cladode. This ensures that the fruit's moisture content won't vary in the time it takes to reach the lab and start the analysis.

Figures 7 and 8. The tree where the samples were collected. On the left is the tree in the Summer (5th September 2019) and on the right is the same tree in the Winter (27th December 2019).

This Opuntia wild variety seems to prefer the arid steep slopes as its growing place. This ground is characterized by its lack of growth of other flora, being most of it seasonal and only present in winter, when the humidity increases, this fact is visible in the Summer/Winter pictures above. This gives Opuntia a lack of competition, easing its permanence in the region. The plant seems to prefer slopes directed to the west, perhaps due to increased sun exposure.

Figure 9. View from the sampling place to the Douro River (27th December 2019)

It is to note the proximity of the trees to the Douro River (figure 7). Although being a specialized arid weather plant, the plant prevalence in this region is higher closer to the river's margins. The present sample is located about 150 meters from the water and about 400-600 meters from the average sea level [17]. The zone experienced a moderate drought in the Summer of 2019 and 2020 when the sample was collected, and moderate rain during the colder months when the Winter 2019 sample was collected [18]. The drought experienced in the region incentives the prickly pear's growth, giving it advantages to the local flora as this plant already has drought resistance mechanisms.

Cladode samples

The cladode samples were collected from the same plant aggregate as the fruit samples on the 2nd of October 2021. In the Summer of 2021, mirroring what happened during previous years, the territory went through a Moderate drought [18]. Samples were transported to the laboratory within less than 24 hours, where they were kept in a refrigerated unit.

Opuntia Commercial Juicing Leftover By-product

This sample was obtained from a juicing industry that takes *Opuntia* fruits from commercial orchards in the Alentejo region of Portugal. Samples were transported to the laboratory within less than 24 hours, where they were kept in a refrigerated unit.

2. Sample preparation

Fruit samples were manually separated into different components (Pulp, seed, juice, and peel) and homogenized (using the laboratory blender GRINDOMIX GM 200, Retch, Germany).

Cladode samples were separated into whole cladode and cladode juice. The whole cladode sample was directly homogenized (using the laboratory blender GRINDOMIX GM 200 (Retch, Germany). The juice sample was prepared by peeling the cladodes manually followed by juicing using a regular kitchen juicing machine.

Opuntia Commercial Juicing Leftover By-product was simply homogenized (using the laboratory blender GRINDOMIX GM 200 (Retch, Germany).

Following this step, some sample was separated to determine the moisture content, while the rest proceeded to a lyophilization process (Lyophilizer Freeze Dryer, Telstar, Spain). Lyophilized samples were kept in a dry place, away from sunlight exposure.

3. Reagents and standards

All reagents used were of analytical standard.

Protein Kjeldahl determination

Kjeldahl tablets without the addition of Se and Hg, ($Na_2S_2O_8/CuSO_4$) 5g/tablet, (Merck, Darmstadt, Germany); Sulfuric acid ACS reagent (H_2SO_4) 95-97% (Honeywell, NC, USA); Sodium hydroxide (NaOH) 98.5% pellets, (VWR chemicals, Leuven, Belgium); Boric Acid (H_3BO_3) (Panreac, Barcelona, Spain); Sulfuric acid (H_2SO_4) 0.5 M (Carlo Erba, Val de Reuil, France)

Total Lipid Soxhlet Extraction Determination

Anhydrous sodium sulfate (Na_2SO_4) (Merck, Darmstadt, Germany); Petroleum Ether (Sigma Chemical Co., St. Louis, USA); Sand (VWR International, Leuven, Belgium).

Vitamin E Composition Determination

n-Hexane HPLC grade (Merck, Darmstadt, Germany); 1,4-Dioxane 94:6 v/v (Sigma Chemical Co., St. Louis, USA); Tocopherol and tocotrienol standard solution: α, β, γ, δ-tocopherol and α, β, γ, δ-tocotrienol (Calbiochem, La Jolla, CA, USA); tocol internal standard solution: 3,4-dihydro-2-methyl-2-(4,8,12-trimethyltridecyl) (Matreya Inc ; PA, USA).

Fatty Acid Composition Determination

Dichloromethane (VWR International, Leuven, Belgium); Sodium Hydroxide (Panreac, Barcelona, Spain); n-Hexane HPLC grade (Merck, Darmstadt, Germany); Boron Trifluoride (BF_3, 14% Methanol) (Sigma Chemical Co., St. Louis, USA); Standard mixture of Fatty Acids (FAME 37), (Supelco, Bellefonte, PA, USA).

Total Fiber Determination

Enzyme kit (α-amylase, protease, and amyloglucosidase) (Sigma Chemical Co., St. Louis, USA); Anhydrous Disodium hydrogen phosphate and sodium dihydrogen phosphate (Merck, Darmstadt, Germany); Sodium hydroxide and chloric acid 1.0 M (Carlo Erba, Val de Reuil, France); Ethanol 96% (Aga, Prior Velho, Portugal); Acetone (VWR international, Leuven, Belgium).

Free sugar Composition Determination

Acetonitrile HPLC Grade (Riedel-de Haën, Honeywell, NC, USA); Fructose, glucose, and sucrose (Sigma Chemical Co., St. Louis, USA).

Extraction and Determination of Bioactive compounds and Antioxidant Activity

Absolute Ethanol, (Fischer Chemical, Loughborough, England); Sodium carbonate (Na_2CO3), Gallic acid, catechin, DPPH• (1,1-Diphenyl-2-picrylhydrazyl, Free Radical), Trolox, TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine), sodium nitrite ($NaNO_2$), aluminum chloride ($AlCl_3$), Ferrous sulfate ($FeSO_4$), Sodium acetate, Glacial Acetic Acid and Ferric Chloride ($FeCl_3$), (Sigma Chemical Co.,St. Louis, USA); Folin-Ciocalteau reagent (Merck, Darmstadt, Germany); Sodium hydroxide (NaOH) 1.0 M (Carlo Erba, Val de Reuil, France).

4. Equipment

- Laboratory Blender GRINDOMIX GM 200 (Retch, Haan, Germany);
- Freezer;
- Freeze dryer CRYODOS -50, (Telstar, Barcelona, Spain);
- Automatic Moisture Measuring Oven KERN® DBS 60-3 (KERN & SOHN GmbH, Balingen, Germany);
- Muffle Oven Thermolyne 48000 (Electrothermal Engineering Ltd, Essex, England);
- Automatic Sample Digestor Kjeldahl K-438 (Büchi®, Büchi Labortechnik AG, Switzerland);
- Gaseous Vapor Neutralizer B-414 (Büchi®, Büchi Labortechnik AG, Switzerland);
- Automatic Destilation Unit K-360 (Büchi®, Büchi Labortechnik AG, Switzerland);
- Heating mantles for extraction Electromantle® EME (VWR International, Darmstadt, Germany);
- Refrigerator Huber Minichiller 300 (HUBERTM, Huber Kältemaschinenbau AG, Germany);
- Filtration Unit CSF6 (VELP®SCIENTIFICA, Usmate Velate, Italy);
- Enzymatic Digestor GDE (VELP®SCIENTIFICA, Usmate Velate, Italy);
- Incubator oven (WTB binder 78532, Tuttlingen, Germany);
- Centrifuge (Heraeus Sepatech Labofuge Ae, Heraeus Instruments, Hanau, Germany);
- Microcentrifuge (Heraeus Sepatech Biofuge Pico, Heraeus Instruments, Hanau, Germany);
- Centrifuge (Heraeus Megafuge 16, Thermo Scientific, Massachusetts, USA);
- Vortex Mixer (Reax top, Heidolph, Schwabach, Germany);
- Block heater (Block Heater SBH130D/3, Stuart, Staffordshire, England);
- Microplate reader (BioTek Synergy HT, Biotek HT, Winooski, USA);
- Spectrophotometer UV-1800 (Shimadzu – UV spectrophotometer, Kyoto, Japan);
- GC-FID (GC- 2010 Plus, Shimadzu, Kyoto, Japan) equipped with an automatic sampler and injector (AOC-20i Shimadzu) with a 50:1 split at 250ºC; a silica capillary column CP-Sil 88, 50.0m x 0.25 mm of internal diameter and 0.20 μm film thickness (Varian, Middelburg, Netherlands) and a flame ionization detector (Shimadzu, Tokyo, Japan) at 270 ºC.

- HPLC system for Vitamin E determination (Jasco, Tokyo, Japan) equipped with a DAD MD-2015 multiwavelength diode array detector (Jasco, Tokyo, Japan), a fluorescence detector (FP-2020) (Jasco, Tokyo, Japan) programmed for excitation at 290 nm, and emission at 330 nm. An auto sampler AS-4050 autosampler (Jasco, Tokyo, Japan) and a pump PU-4180 (Jasco, Tokyo, Japan) were used. The capillary column used was Supelcosil™ LC-Si HPLC, 3 µm particle size, L x I.D. 15 cm x 4.6 mm (Merck, Darmstadt, Germany).
- HPLC system for sugar content determination (Jasco, Tokyo, Japan) equipped with an automatic sample injector (AS-4050), a pump (PU-4180), an oven (CO-4061), and an evaporative detector with light scattering (ELSD- evaporative light scattering detector) Sedex 80 (SEDERE, Alfortville - France).
- TA-Texture Analyzer (Stable Micro System Ltd., Godalming, U. K.) equipped with a 2 kg load. The samples were compressed with a 25mm diameters cylindrical probe.
- Thermo Scientific HAAKE Viscotester 550 (Thermo Scientific, Massachusetts, USA).

5. Methodology description

Moisture determination

Samples are loaded on an automatic moisture analyzer in an aluminum tray. The sample is heated at 105 ºC and the weight is successively measured until no further weight oscillation is detected by the device. The device then compares the weight before and after the heating process and calculates the moisture percentage.

Ash content determination (AOAC 920.153)

Samples were loaded on crucibles that were previously left overnight at 450 ºC in a laboratory furnace to ensure that impurities on the surface of the crucibles were burned off. The crucible with the sample is put into the furnace at 120 ºC and then the temperature is increased gradually until it reaches 500 ºC. The samples were cooled down on a desiccator and weighed. The ash content is determined based on the difference between weights.

Protein determination – Kjeldahl Method (AOAC 982.08)

For the determination of protein content in the fruit and fruit parts, a Jones factor of 4.05 according to *Izhaki et al.* [19] was used. This academic study was chosen as reference because it focused on 26 different fleshy fruits of Mediterranean habitat, and the prickly pear is a fleshy fruit and in this case, grown in Mediterranean habitat.

The protocol followed was the one on standard analytical methods published by AOAC (AOAC 982.08).

After sample homogenization, it was weight to roughly 0.5 grams and placed in a digestion tube. Two Kjeldahl catalyst tablets (10 g) were added as well as 20 mL of concentrated sulfuric acid. The sample was heated to 400 ºC while connected to a scrubber to absorb the resulting exhaust fumes during digestion. The following distillation step was done using an automatic distiller with the following parameters: 50 mL of H_2O; 90 mL of NaOH; reaction time 5 s; distillation time 240 s; Aspiration enabled; steam power 100%. The titration was manual and used sulfuric acid 0.2 M.

Fat Determination - Soxhlet extraction Method (AOAC 991.36)

Flat-bottom boiling flasks were placed in the incubator at 105 ºC overnight to ensure the stability of the weight of the flask. About 5 g of fresh sample were transferred into a Soxhlet extraction thimble and put into a Soxhlet extractor. The bottle was then filled with petroleum ether 40º-60º C and put on a heating mantle. The Sample was heated for 8 hours to ensure an efficient fat extraction. The solvent was recovered for further usage. The flask was then incubated until the solvent was completely evaporated, placed on a desiccator to cool, and weighted. This process was repeated until the weight was stable. The flask and its dried content were weighted. The comparison of the extracted fat and the sample's original weight allows us to calculate the fat percentage of the sample.

Fiber determination – Enzymatic gravimetric method (AOAC 985.29)

A lyophilized sample in a buffer solution goes through several steps of enzymatic digestion including starch gelatinization and hydrolysis to dextrin (Thermostable α-amylase, 30 min 95 ºC pH 6.0); Protein hydrolysis (Protease, 30 min 60 ºC pH 7.5) and starch dextrin hydrolysis (Amyloglucosidase, 30 min 60 ºC pH 4.5). Samples are then left to precipitate with ethanol overnight followed by vacuum filtration. One of the samples then follows through with protein determination (following the protocol described above for protein determination) while another follows ash determination (following the protocol described above for ash determination with some modifications).

Vitamin E determination

Knowing the fat percentage of each sample, they were weighted to have about 20 mg of fat present and put in a falcon tube. A BHT solution (75 µL) to avoid fat oxidation as well as an internal standard (190 µg/mL, 50µL) and absolute ethanol (1 mL) were added to each tube. The tubes were then mechanically homogenized for 30 min in an orbital vortex mixer, followed by the addition of 4 mL of n-hexane and another cycle of 30 minutes of mixing. Following this mixing period, 2 mL of NaCl (m/v) was added. The mixture was briefly vortexed and centrifuged (2 min at 5000 RPM, or 2795 g). The organic phase was collected, and the residue was reextracted twice again with 2 mL of n-hexane. The organic phases were combined, and anhydrous Na_2SO_4 was added to make sure no water was left in the sample as this can damage the HPLC column. The extract was then evaporated under a nitrogen stream and resuspended with 500 mL of n-hexane. This was finally injected into the HPLC system. The vitamin E vitamers were identified by chromatographic comparison with authentic standards and their UV spectrum. Quantification was performed based on the internal standard method, using the UV spectrum as well.

The chromatographic separation of the compounds was achieved on a normal phase Supelcosil™ LC-Si HPLC column, 3 µm particle size, 15 cm x 4.6 mm, operating at constant room temperature (21.8 ºC). A mixture of n-hexane (HPLC grade) and 1,4-dioxane (94: 6 V/V) was used as eluent, at 0.7 mL/min. The detection was performed by both the DAD connected in series with the fluorescence detector (MD-2015 multiwavelength diode array detector, programmed for excitation at 290 and emission at 330 nm (gain 10).

Fatty acid profile determination

The same lipid extraction protocol was used as in the Vitamin E determination mentioned above. The collected fatty acids were then derivatized into methyl esters, according to ISO 12966-2: 2011 (ISO & STANDART, 2010). The lipid saponification process was made in a basic medium and its methylation was catalyzed by boron trifluoride (BF_3 14% in methanol solution) and heat.

First, the sample was dissolved in 2 mL of dichloromethane, followed by the addition of 1.5 mL potassium hydroxide methanolic solution 0.5 M. The sample was heated to 100 ºC for 10 minutes. After cooling it in ice for 5 minutes, 2 mL of boron trifluoride were added, and the solution subjected to another 30 minutes at 100 ºC. After cooling down in ice again, 2 mL of water and 4 mL of n-hexane were added. The samples were then agitated in a vortex mixer and centrifuged (5 min, 3000 RPM or 1006 g) for the separation of organic and aqueous phases.

The organic phase was transferred to a vial and added some anhydrous Na_2SO_4 to get rid of any water that may still be present. The organic phase was injected into the GC-FID system.

The equipment used was a GC-FID equipped with an automatic sampler and injector with a 50:1 split at 250 ºC; a silica capillary column CP-Sil 88, 50.0 m x 0.25 mm of internal diameter and 0.20 μm film thickness and a flame ionization detector, at 270 ºC. The drag gas was helium (3.0 mL/min) and the temperature program used was: 120 ºC for 5 min, 2 ºC/min at 160 ºC for 2 minutes, and 2 ºC/min at 220 ºC for 10 minutes. The injection volume was 1.0 μL. The fatty acid methyl esters were identified by comparing them with a standard mixture (FAME 37). The data was analyzed based on the relative peak areas of each component.

Free sugar content determination

A trial run was made using several sample weights to make sure that the detection method was not saturated.

Samples were weighted and added 30 mL of water followed by agitation in a vortex mixer for 20 minutes. The samples were then centrifuged, and 1 mL of the supernatant was filtered and transferred to an HPLC injection vial. All analyses were made in triplicate.

The chromatographic analysis was carried out in a HPLC equipment, integrated with an evaporative light scattering detector (ELSD), two high-pressure pumps, and an automatic injector. The compounds were separated in a Shodex column (Asahipak NH2P-50 4E) (4.6 mm I.D. x 250 mm). An isocratic system with 2 eluents (water/acetonitrile 1:3) and the samples were eluted with a 1 mL/min flux for 20 minutes at a temperature of 30 ºC.

Antioxidant and bioactive compounds determination

a. Preparing the extracts

   The solvent used was water/ethanol (1:1), as with similar samples it was shown to be the most effective solvent for extracting the target compounds [20].

   Various sample weights were tested to make sure the methods were not saturated or non-detectable, therefore contributing to the method's optimization.

   The extractions were made for 1 hour at 40 ºC with constant agitation, followed by cooling and filtration. The obtained extracts were stored at -20 ºC for the following analysis.

b. Total Phenolic content

   The total phenolic content was determined by a spectrophotometric method using the Folin-Ciocalteu reagent [21]. In a microplate, 30 μL of the extract were placed in the

well, to which 150 µL of Folin-Ciocalteu reagent (diluted 1:10) and 120 µL of $Na_2CO_3.10H_2O$ (7.5% m/v) were added. The microplate was then incubated at 45 °C for 15 minutes, followed by 30 minutes at room temperature. Absorbance was determined at 765 nm, using a microplate reader. A calibration curve was elaborated using gallic acid.

c. Total flavonoids content

The total flavonoid content was determined by a spectrophotometric method [22]. In a tube, 1 mL of extract was mixed with 4 mL of water and 300 µL sodium nitrite ($NaNO_2$ 25%). After 5 minutes, 300 µL of Aluminum chloride was added ($AlCl_3$ 10%). After 1 minute, 2 mL of sodium hydroxide (NaOH 1M) and 2.5 mL of water were added. The tube was then mixed in a vortex mixer. The absorbance was read at 510 nm in a microplate reader. A calibration curve was elaborated with catechin.

d. Antioxidant activity determination by DPPH radical inhibition

The DPPH radical inhibition was determined using a spectrophotometric method described by Costa *et al.*[22]. In a microplate, 30 µL of the extract was mixed with 270 µL of a DPPH solution (6.10^{-5} mol/L in ethanol). This solution was prepared shortly before as it is very sensitive to deterioration. The absorbance was read at 525 nm in a microplate reader. A calibration curve was elaborated with Trolox.

e. Antioxidant activity determination by Ferric ion reduction (FRAP)

The Ferric ion reduction capacity was determined by a spectrophotometric method, using the method described by Benzie *et al.* [23]. In a microplate, 30 µL of the extract was mixed with 270 µL of FRAP reagent (acetate buffer solution 0.3M with 10 mM TPTZ and 20 mM iron chloride). The plate was then kept at 40° C for 30 minutes. After cooling, absorbance was read at 595 nm in a microplate reader. A calibration curve was elaborated with iron sulfate.

Large Deformation Analysis

Large deformation analysis was carried out with a single compression test, using a TA-XT2 Texture Analyzer (Stable Micro System Ltd., Godalming, U. K.) equipped with a 2 kg load. The samples were compressed with a 25mm diameters cylindrical probe to a depth of 5 mm at a constant speed of 3 mm/s. The gel firmness was determined as the maximum force (N) on the compression force-time curve. 3 separate determinations were made in each batch.

Thixotropy tests

Thixotropy tests were applied to characterize the flow behavior of the yogurt samples. The tests were carried out at 10 ºC and allowed 900 s to equilibrate. Shear stress was then recorded at increasing shear rates from 1 to 100 s^{-1} (upward flow curve), followed by decreasing shear rates from 100 to 1 s^{-1} (downward flow curve). To model the flow behavior, the upward flow curve was fitted to the Ostwald model ($\sigma=k\ \gamma n$), where σ represents shear stress (Pa), K is the consistency index (Pa.sn), n is the flow behavior index (dimensionless) and ɣ is the shear rate (s-1).

6. Statistical analysis

Mean values, standard deviations (SD), medians, and mean deviation (Mean Dev.) were calculated with Microsoft Excel®, Version 2202. For multiple comparisons, analysis of variance (one-way ANOVA) was used. The statistical significance of the differences between the two samples was examined using a post-hoc Tuckey HSD (honestly significant difference) test. The test was performed with the online web calculator Astatsa (2016, Navendu Vasavada).

Part 1.

Results and Discussion

1. A Note on Variations

Nutritional and compositional variations are a natural occurrence as abiotic and biotic factors change in the environment. A lot of studies have taken these into account and tried to come up with a correlation between composition and fruit maturity, plant age, and plant variety among other factors. Here we explore some of those correlations as they should be considered in the sections that follow.

Small variations exhibited in the chemical composition of *Opuntia ficus-indica*'s parts can be explained by differences in the growth environment and characteristics. Variation in moisture values may result from the difference in seasons, whether wet or dry. The ash content depends on both the age of the fruit and the season (the older the fruit the higher the ash content). The sugar values depend on the season (the drier the season, the lower the sugar value) [24]. Accompanying the maturation of both the cladodes, its fiber content gets higher. In its interior part, a network of hard cellulose forms which in turn increases the fiber content as well as the ash and mineral content of the plant, as described by Guzmán Loayza *et al.* [25]. Other favorable conditions include irrigation, and low temperatures during harvest and storage [24].

Various studies have described the changes in the plant's physicochemical composition throughout its maturation period. Hernández-Urbiola *et al.* [26] described the fruit composition at different stages of maturity. The fiber content increased with age with a decrease in soluble fiber but a higher increase in insoluble fiber, as seen in Figure 8. Fat content also tended to decrease while mineral ash composition showed a tendency to increase with cladode age.

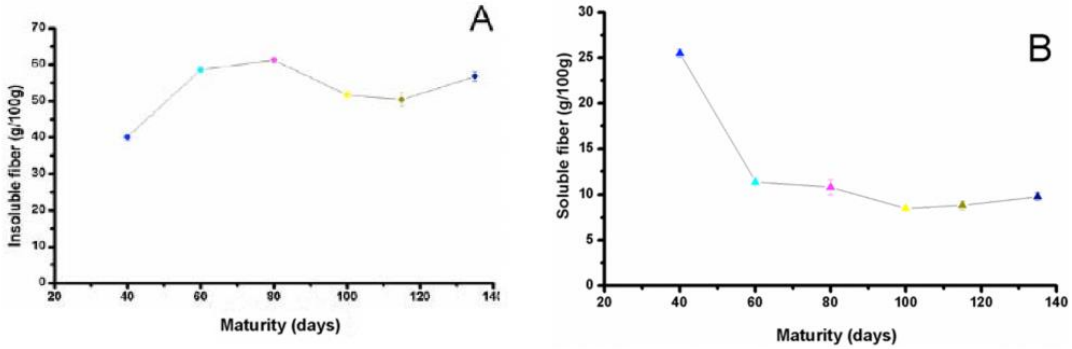

Figure 10. Insoluble fiber (A) and soluble fiber (B) contents of the fruit throughout the maturity stage [26].

Coskuner *et al.* [27] studied the fat composition and maturation of the seed 15 weeks after flowering (figure 9).

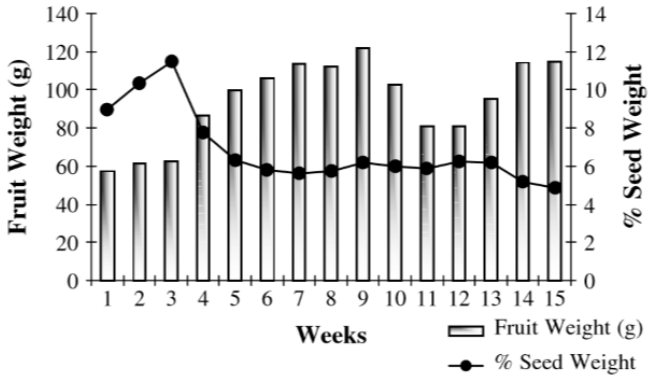

Figure 11. Fruit weight vs. seed weight over the fruit maturation period [27].

Seed weight percentage is largest in the first 3 weeks as the fruit has just started to develop. During the fruit maturation that follows, there is a significant amount of weight gain on the fruit while the seed weight percentage remains relatively constant.

Regarding oil quality, during maturation, it appeared that linolenic and linoleic acids were saturated and converted to oleic and stearic acids. A small increase in palmitic acid content was also observed [27]. Generally, the oil from more mature fruit is richer in saturated fatty acids while the oil from a younger one is more unsaturated.

The same study reported a higher oil content by the 4th to 6th week after flowering, meaning that economically this might be the period where seeds should be collected.

2. Macronutrient Composition, sugar composition and antioxidant activity of Prickly-pear fruit divided into various parts

Table 1. Sample names, descriptions, collection dates, and area of origin.

Sample		Collection date	Origin
Wat	Fruit from daily watered plant*	28th August 2020	Northeastern Portugal (Bragança)
S2019	Summer fruit from wild plant	5th September 2019	Northeastern Portugal (Bragança)
W2019	Winter fruit from wild plant	27th December 2019	Northeastern Portugal (Bragança)
S2020	Summer fruit from wild plant	9th September 2020	Northeastern Portugal (Bragança)

*_Opuntia_ cultivars are conventionally not watered, as the plant is adapted to arid conditions
**from an industrial juicing method

Table 2. Macronutrient composition of various fruit parts (Juice, Pulp, and Peel) of the collected samples (Watered, Summer 2019, Winter 2019, and Summer 2020) (dry weight % ± SD).

Sample		Moisture (fresh weight %)	Fat	Total Fiber	Protein	Ash	Sugar	Carbohydrates*
Juice	Wat	87.48 ± 0.06 [a]	0.20 ± 0.02 [a]	1.86 ± 0.17 [a]	4.56 ± 0.20 [a]	0.35 ± 0.02 [a]	88.17 ± 0.38 [a]	93.03 ± 0.41
	S2019	88.44 ± 0.06 [a]	0.36 ± 0.02 [b]	2.50 ± 0.11 [b]	3.26 ± 0.06 [b]	1.48 ± 0.12 [b]	91.66 ± 0.22 [b]	94.88 ± 0.20
	W2019	88.80 ± 0.16 [a]	0.40 ± 0.02 [b]	3.74 ± 0.06 [c]	0.72 ± 0.06 [c]	0.37 ± 0.01 [a]	82.20 ± 7.02 [c]	98.51 ± 0.09
	S2020	85.06 ± 1.27 [b]	0.16 ± 0.00 [a]	2.21 ± 0.14 [b]	1.62 ± 0.12 [d]	0.87 ± 0.01 [b]	96.89 ± 0.35 [d]	97.35 ± 0.13
Pulp	Wat	81.39 ± 1.28 [a]	0.53 ± 0.04 [a]	13.39 ± 1.37 [b]	4.24 ± 0.36 [a]	0.40 ± 0.03 [a]	64.63 ± 0.17 [a]	94.83 ± 0.43
	S2019	82.26 ± 0.63 [a]	0.66 ± 0.05 [b]	32.19 ± 2.02 [c]	3.48 ± 0.04 [b]	1.53 ± 0.16 [b]	55.35 ± 5.39 [b]	92.37 ± 0.25
	W2019	84.95 ± 0.91 [b]	1.96 ± 0.15 [c]	32.68 ± 1.06 [c]	4.24 ± 0.08 [a]	0.43 ± 0.03 [a]	65.71 ± 4.17 [c]	93.37 ± 0.26
	S2020	79.79 ± 0.84 [a]	0.24 ± 0.01 [d]	13.01 ± 0.62 [b]	2.11 ± 0.09 [c]	0.48 ± 0.02 [a]	62.98 ± 0.42 [d]	97.52 ± 0.12
Peel	Wat	83.31 ± 0.26 [a]	0.56 ± 0.04 [a]	24.42 ± 0.33 [a]	3.11 ± 0.16 [a]	1.66 ± 0.00 [a]	34.88 ± 0.25 [a]	94.67 ± 0.20
	S2019	84.56 ± 0.43 [a]	0.94 ± 0.01 [b]	26.18 ± 0.48 [b]	1.43 ± 0.13 [b]	1.51 ± 0.07 [a]	56.81 ± 0.12 [b]	96.12 ± 0.21
	W2019	86.48 ± 0.29 [b]	0.81 ± 0.01 [c]	18.31 ± 0.24 [c]	2.96 ± 0.20 [a]	1.76 ± 0.11 [a]	58.99 ± 2.09 [c]	94.47 ± 0.32
	S2020	79.68 ± 0.22 [c]	0.58 ± 0.02 [a]	31.44 ± 0.45 [d]	1.65 ± 0.11 [b]	1.13 ± 0.08 [b]	61.03 ± 0.25 [d]	96.64 ± 0.21

* Carbohydrates calculated by subtracting the fat, protein, and ash from 100%

Within each column, for each part of the fruit (juice, pulp or peel, separately), different letters represent significant differences between collected samples (Wat, S2019, W2019, S2020), at $p<0.05$. The same letter represents no significant differences ($p>0.05$), determined by One-way ANOVA with post-hoc Tukey HSD Test. Wat – Watered plant; S2019 – Fruit Summer 2019; W2019 – Fruit winter 2019; S2020 – Fruit summer 2020.

Table 3. Sugar composition of various fruit parts (Juice, Pulp, and Peel) of the collected samples (Watered, Summer 2019, Winter 2019, and Summer 2020) (% (m/m) of dry weight ± SD).

		% (m/m) of dry weight			
Samples		Fructose	Glucose	Sucrose	Total
Juice	Wat	29.43 ± 0.12 [a]	58.74 ± 0.26 [a]	n.d.	88.17 ± 0.38 [a]
	S2019	24.24 ± 0.10 [b]	67.43 ± 0.13 [b]	n.d.	91.66 ± 0.22 [b]
	W2019	26.24 ± 5.57 [c]	55.96 ± 1.45 [c]	n.d.	82.20 ± 7.02 [c]
	S2020	30.32 ± 0.11 [d]	66.57 ± 0.23 [d]	n.d.	96.89 ± 0.35 [d]
Pulp	Wat	21.54 ± 0.06 [a]	43.09 ± 0.11 [a]	n.d.	64.63 ± 0.17 [a]
	S2019	14.77 ± 1.58 [b]	40.58 ± 3.80 [b]	n.d.	55.35 ± 5.39 [b]
	W2019	24.73 ± 1.57 [c]	40.98 ± 2.59 [c]	n.d.	65.71 ± 4.17 [c]
	S2020	19.67 ± 0.13 [d]	43.30 ± 0.29 [d]	n.d.	62.98 ± 0.42 [d]
Peel	Wat	15.61 ± 0.10 [a]	19.28 ± 0.15 [a]	n.d.	34.88 ± 0.25 [a]
	S2019	17.19 ± 0.10 [b]	38.51 ± 0.02 [b]	1.11 ± 0.04 [a]	56.81 ± 0.12 [b]
	W2019	23.62 ± 0.85 [c]	34.10 ± 1.09 [c]	1.27 ± 0.15 [b]	58.99 ± 2.09 [c]
	S2020	21.91 ± 0.10 [d]	39.12 ± 0.15 [d]	n.d.	61.03 ± 0.25 [d]

n.d. not detected

Within each column, for each part of the fruit (juice, pulp or peel, separately), different letters represent significant differences between collected samples (Wat, S2019, W2019, S2020), at $p<0.05$. The same letter represents no significant differences ($p>0.05$), determined by One-way ANOVA with post-hoc Tukey HSD Test. Wat – Watered plant; S2019 – Fruit Summer 2019; W2019 – Fruit winter 2019; S2020 – Fruit summer 2020

Table 4. Antioxidant power and bioactive compounds in dry weight (Ethanol/water extract) of various fruit parts (Juice, Pulp, and Peel) of the collected samples (Watered, Summer 2019, Winter 2019, and Summer 2020).

		DPPH	FRAP	Total Phenols	Flavonoids
Samples		mg.g^{-1} of TE	µmol.g^{-1} of Fe^{2+} E	mg.g^{-1} of GAE	mg.g^{-1} of CE
Juice	Wat	2.66 ± 0.29 [a]	27.79 ± 1.27 [a]	4.12 ± 0.49 [a]	n.d.
	S2019	2.15 ± 0.12 [b]	30.77 ± 1.68 [b]	5.85 ± 0.33 [b]	0.43 ± 0.01 [a]
	W2019	1.84 ± 0.14 [b]	39.49 ± 1.36 [c]	5.88 ± 0.60 [b]	n.d.
	S2020	2.07 ± 0.17 [b]	22.28 ± 1.07 [d]	3.35 ± 0.41 [c]	n.d.
Pulp	Wat	2.16 ± 0.21 [a]	20.84 ± 1.04 [a]	3.60 ± 0.24 [a]	n.d.
	S2019	1.34 ± 0.06 [a]	19.88 ± 0.81 [a]	3.49 ± 0.39 [a]	0.36 ± 0.03 [a]
	W2019	2.12 ± 0.15 [a]	23.66 ± 0.99 [b]	2.56 ± 0.49 [b]	n.d.
	S2020	1.74 ± 0.17 [b]	18.22 ± 0.93 [c]	2.64 ± 0.35 [b]	0.35 ± 0.00 [a]
Peel	Wat	3.25 ± 0.24 [a, b]	62.11 ± 1.45 [a]	11.57 ± 0.79 [a, b]	n.d.
	S2019	3.08 ± 0.10 [a]	93.72 ± 4.47 [b]	16.31 ± 0.96 [c]	0.95 ± 0.11 [a]
	W2019	3.14 ± 0.24 [a]	74.96 ± 2.20 [c]	11.10 ± 0.95 [a]	0.95 ± 0.05 [a]
	S2020	3.48 ± 0.22 [b]	53.13 ± 4.71 [d]	12.78 ± 0.91 [b]	1.18 ± 0.09 [b]

n.d. not detected

Within each column, for each part of the fruit (juice, pulp or peel, separately), different letters represent significant differences between collected samples (Wat, S2019, W2019, S2020), at $p<0.05$. The same letter

represents no significant differences (p>0.05), determined by One-way ANOVA with post-hoc Tukey HSD Test. Wat – Watered plant; S2019 – Fruit Summer 2019; W2019 – Fruit winter 2019; S2020 – Fruit summer 2020.

The juice is the part that shows the least fiber, as all the fibrous parts have been removed from it in the juicing process. The pulp still contains the seeds, showing a variable amount of fiber depending mainly on the number of seeds it contains. The peel's fiber works mainly as a physical barrier to protect the fruit, as well as give it structure. The peel, therefore, shows a lower amount of protein due to its high fiber concentration and ash, which is usually associated with fiber.

Sugar content is highest in the juice, as the juicing process removes the fibrous components, therefore increasing the amount of sugar. The pulp shows the second-highest concentration, and the lowest concentration is found on the peel. The summer fruits tend to show a higher amount of sugar in the juice, probably due to the increased production of this metabolite due to more sun exposure. Glucose is always found in higher amounts compared to fructose and sucrose.

The part that shows the highest antioxidant power and bioactive compounds is consistently the Peel. This is to be expected as the peel is the outer protection of the fruit, therefore being responsible for keeping threats out and is in direct contact with stress sources such as sunlight.

CONCLUSION

The Prickly pear shows a typical macronutrient composition of a fruit. A high amount of water is observed, followed by sugar.

Juicing removed most fiber from the fruit, but it also concentrated the sugar amount. The peel has a higher fiber concentration than all other fruit parts (except the seed) and shows the highest antioxidant power due to its protective role.

Summer fruits produced higher amount of sugar as the increased sun exposure heightens the plants metabolism. Mainly sugar is found in the form of glucose, but fructose and sucrose were also detected, always in relative same amounts.

3. Macronutrient composition, sugar composition and antioxidant power of cladodes and cladode juice

The cladodes were divided into 2 samples: a juice sample (Jui) and a whole cladode sample (Cla). The whole cladode sample (Cla) is composed of the homogenized cladode. The juice sample (Jui) was obtained by removing the cactus skin with a conventional kitchen potato skin peeler and then processing the cladode through a household juicer. The samples were then lyophilized before analysis.

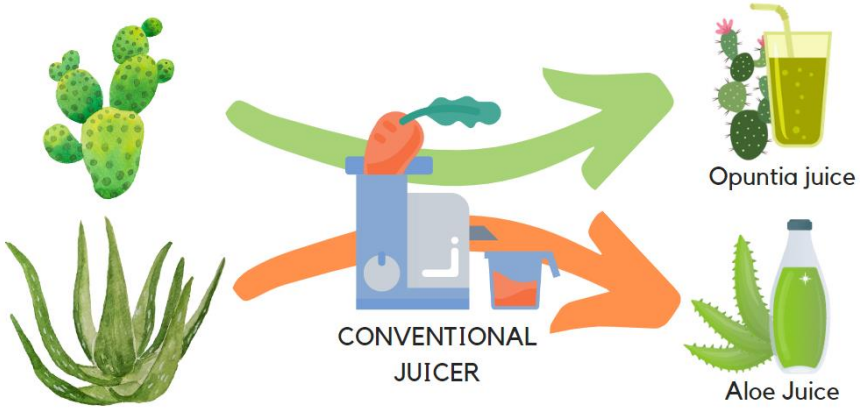

Figure 12. Scheme of sample treatment before testing.

Table 5. Macronutrient composition of the cladode and comparison to bibliography data (dry weight %).

Nutritional Parameter	Cladode	**Cladodes bibliography data**	
Moisture (%)	93.61 ± 0.16	94.81	Hernández-Urbiola et al. [26]
		92.57	Guzmán Loayza *et al.* [25]
		94.33	Guzmán Loayza *et al.* [25]
		91.03-94.33	L. Espírito Santo [20]
Fat	1.03 ± 0.05	1.50	Hernández-Urbiola et al. [26]
		1.17-1.73	L. Espírito Santo [20]
		1.42 ± 0.01	Bensadon *et al.* [2]
		1.94	Guzmán Loayza *et al.* [25]
Total Fiber	31.83 ± 0.61	23.33	Hernández-Urbiola et al. [26]
		22.95-40.00	L. Espírito Santo [20]
		62.05 ± 3.99	Bensadon *et al.* [2]
		18.70	Guzmán Loayza *et al.* [25]
Protein	5.40 ± 0.02	7.7	Hernández-Urbiola et al. [26]
		3.48-7.62	L. Espírito Santo [20]
		1.14 ± 0.31	Bensadon *et al.* [2]
		8.47	Guzmán Loayza *et al.* [25]
Ash	16.39 ± 0.06	21.92	Hernández-Urbiola et al. [26]
		16.54 ± 0.15	Bensadon *et al.* [2]
		17.21-26.99	L. Espírito Santo [20]
		28.22	Guzmán Loayza *et al.* [25]
Free Sugars	19.45 ± 0.16	11.39-19.48	L. Espírito Santo [20]

Table 6. Macronutrient composition of the cladode (Cla) and juice (Jui) samples and comparison to aloe vera (dry weight %).

Nutritional Parameter	Samples		Aloe Vera leaf [28]	Aloe Juice [28]
	Cla	Jui		
Moisture (%)	93.61 ± 0.16 [a]	95.36 ± 0.17 [b]	---	---
Fat	1.03 ± 0.05 [a]	0.37 ± 0.02 [b]	2.2	4.3
Total Fiber	31.83 ± 0.61 [a]	10.43 ± 0.20 [b]	23.8	1.2
Protein	5.40 ± 0.02 [a]	2.67 ± 0.00 [b]	7.7	4.5
Ash	16.39 ± 0.06 [a]	19.89 ± 0.05 [b]	14.9	28.6
Free Sugars	19.45 ± 0.16 [a]	35.74 ± 0.23 [b]	11.3	21.8

Within each line, different letters represent significant differences between collected samples at $p<0.05$. The same letter represents no significant differences ($p>0.05$), determined by One-way ANOVA followed by a t-student test.

The cladode is mostly composed of water being 93.61% of its fresh weight. This value corresponds with bibliography values (91.03-94.81%) [2, 20, 25, 26, 29, 30]. Moisture variation can be explained by abiotic factors (as explored in section 3.1. A Note on Variations). We theorize that the plant by growing in Portugal, a country with colder and wetter conditions (*versus* the countries in the bibliography Mexico and Tunisia) there might be a tendency to accumulate more water, therefore having more moisture.

Both fiber, fat, and ash values in the cladodes are highly dependent on the cladodes' age, increasing with age. Hernández-Urbiola *et al.* [26] described the cladodes composition at different stages of maturity. The fiber content increases with age, with a decrease in soluble fiber but a higher increase in insoluble fiber. Fat content also tended to decrease while mineral ash composition showed a tendency to increase with age. Values found in other articles are sometimes quite distinct because of this. For example, Bensadon *et al.* [2] report 64.24% of dry weight of fiber while Guzmán Loayza *et al.* [25] report 18.70%. Our value was 31.33% of dry weight, staying in between these values.

From comparing the macronutrient composition of the juice and cladode we can also conclude that juicing decreases the amount of fiber and the nutrients associated with it (lipids are hydrophobic compounds and tends to remain with the fiber, the same thing happens with some proteins, and some ash) while consequentially increasing the more hydrophilic components like sugar. Since we completely removed the cladodes skin before juicing, some of these nutrients might have also been associated with it. Being the outside protection of the

cladodes, this peel is expected to have a higher antioxidant capacity and protective molecules. Therefore, removing it may result in a decrease in the accompanying macromolecule groups.

The main difference between Aloe vera and *Opuntia ficus-indica* is that the latest has a much higher amount of sugar. This difference is more significant when we compare the juice composition, as it shows almost 14 g more sugar per 100 g of dry weight. Juicing generally increases the amount of sugar present as it removes the second main component, fiber.

Table 7. Sugar composition of the cladode (Cla) and juice (Jui) samples (% m/m of dry weight).

	% (m/m) of dry weight			
	Fructose	Glucose	Sucrose	Total
Cla	6.61 ± 0.11 [a]	10.59 ± 0.02 [a]	2.25 ± 0.03	19.45 ± 0.16 [a]
Jui	14.04 ± 0.16 [b]	21.70 ± 0.07 [b]	n.d.	35.74 ± 0.23 [b]
L. Espírito Santo [20]	6.17-9.02	3.14-7.41	2.09-3.05	11.39-19.48
Aloe Vera leaf **[28]**	3.3	8.0	n.d.	11.3
Aloe Vera Juice **[28]**	5.0	16.6	0.16	21.8

n.d. not detected

Within each column, different letters represent significant differences between collected samples at $p<0.05$. The same letter represents no significant differences ($p>0.05$), determined by One-way ANOVA followed by a t-student test.

The sugar profile in the cladodes is mainly composed of glucose (10.59 g/g of dry weight) with also a considerable amount of fructose (6.61 g/100 g of dry weight). We can also find sucrose in lower amounts (2.25 g/100 g of dry weight). This amounts to 1.24 g of sugar per 100 g of fresh cladode.

The juice shows a higher amount of sugar at 1.66 g/100 g of fresh weight. However, no sucrose was detected. We can assume that the juicing process removed the sucrose and decreased the dilution of fructose and glucose, therefore increasing the total sugar amount.

Opuntia ficus-indica contains more glucose, fructose, and sucrose compared to Aloe Vera.

Table 8. Antioxidant power and bioactive compounds in dry weight (Ethanol/water extract).

	DPPH mg.g^{-1} of Trolox equivalents	FRAP µmol.g^{-1} of Fe^{2+} equivalents	Total Phenols mg.g^{-1} of gallic acid equivalents	Flavonoids mg.g^{-1} of catechin equivalents
Cla	3.32 ± 0.32 ^{a}	56.24 ± 1.79 ^{a}	13.91 ± 1.20 ^{a}	1.18 ± 0.05 ^{a}
Jui	4.26 ± 0.41 ^{b}	58.00 ± 1.67 ^{a}	23.11 ± 1.68 ^{b}	1.39 ± 0.03 ^{b}
L. Espírito Santo [20]	1.09-3.46	38.07-48.19	10.53-18.24	0.86-1.26

Within each column, different letters represent significant differences between collected samples (Wat, S2019, W2019, S2020), at $p<0.05$. The same letter represents no significant differences ($p>0.05$), determined by One-way ANOVA followed by a t-student test.

A general increase was observed in the juices of both bioactive compounds and antioxidant power. This shows that these antioxidants are not generally bound to fiber and are hydrophilic. The cladode sample also constantly showed a higher antioxidant score and bioactive compounds than the bibliography.

CONCLUSION

Registered moisture variations can be attributed to abiotic factors such as precipitation and temperature registered when the fruit was growing, as our samples display a higher value than bibliographic registered. We attribute this to a colder and wetter weather conditions in Portugal compared to countries where the plant was studied (Africa).

Other macronutrients such as ash and fiber are highly dependent on the cladodes age, showing a considerable range in bibliography. The low amount of some macronutrients in the juiced sample can be attributed by the juicing process removal of fiber and its associated nutrients (some lipids and ash). As expected, the juicing process increased the amount of sugar in the sample, as it got more concentrated. No sucrose was detected in the Juice.

Comparing *Opuntia* with Aloe Vera, it contains a higher amount of sugar, but overall, the macronutrient amount is relatively the same. This highlight the potential of *Opuntia* to be used in the same products as Aloe, perhaps with a lower amount of added sugar even.

Regarding the antioxidant assays, a general increase was observed from the juicing process. We can conclude that juicing concentrates the plant´s bioactive compounds.

4. Analysis of *Opuntia* seed

Four *Opuntia ficus-indica* seed samples were collected from different growing conditions and growing years (summarized in table 3). A fifth sample was obtained from the leftover by-product of juice extraction, constituted by ground seeds and insoluble fiber present on the fruit.

Table 9. Sample names, description, collection dates and area of origin.

Sample		Collection date	Origin
Wat	Fruit from daily watered plant*	28th August 2020	Northeastern Portugal (Bragança)
Byp	Juice byproduct ** from conventional cultivar plant	October 2020	Central Portugal (Alentejo)
S2019	Summer fruit from wild plant	5th September 2019	Northeastern Portugal (Bragança)
W2019	Winter fruit from wild plant	27th December 2019	Northeastern Portugal (Bragança)
S2020	Summer fruit from wild plant	9th September 2020	Northeastern Portugal (Bragança)

*_Opuntia_ cultivars are conventionally not watered, as the plant is adapted to arid conditions
**from an industrial juicing method

i. Macronutrient composition, bioactive compounds and antioxidant activity

Table 10. Macronutrient composition of the various seed samples (Watered, Summer 2019, Winter 2019, and Summer 2020) (dry weight % ± SD).

Sample	Moisture (fresh weight %)	Fat	Total Fiber	Protein	Ash	Sugar
Wat	56.72 ± 0.42 [a]	2.41 ± 0.36 [a]	74.69 ± 0.37 [a]	5.03 ± 0.41[a]	2.11 ± 0.03 [a]	4.31 ± 0.03 [a]
S2019	49.52 ± 1.41 [b]	6.14 ± 0.34 [b, c]	80.20 ± 0.16 [b]	6.83 ± 0.25 [b]	1.77 ± 0.11 [b]	5.44 ± 0.21 [b]
W2019	54.96 ± 1.01 [c, a]	7.07 ± 0.10 [c, d]	73.98 ± 1.56 [a]	6.72 ± 0.00 [b]	1.00 ± 0.00 [c]	9.82 ± 0.14 [c]
S2020	53.25 ± 0.27 [c]	7.85 ± 0.44 [d]	81.93 ± 0.09 [b]	10.07 ± 0.13 [c]	1.96 ± 0.06 [d]	3.43 ± 0.00 [d]
Byp	44.06 ± 0.88 [d]	5.69 ± 0.16 [b]	80.28 ± 0.30 [b]	3.76 ± 0.23 [d]	1.96 ± 0.190 [d]	3.33 ± 0.05 [e]

Within each column, different letters represent significant differences between collected samples (Wat, S2019, W2019, S2020), at $p<0.05$. The same letter represents no significant differences ($p>0.05$), determined by One-way ANOVA with post-hoc Tukey HSD Test.
Wat – Watered plant; Byp – Fruit Juice Byproduct; S2019 – Fruit Summer 2019; W2019 – Fruit winter 2019; S2020 – Fruit summer 2020.

Table 11. Sugar composition of the various seed samples (Watered, Summer 2019, Winter 2019, and Summer 2020) (% (m/m) of dry weight ± SD).

Samples	Fructose	Glucose	Sucrose	Total
Wat	1.05 ± 0.00 [a]	2.74 ± 0.02 [a]	0.52 ± 0.01 [a]	4.31 ± 0.03 [a]
S2019	1.43 ± 0.06 [b]	2.96 ± 0.15 [b]	1.05 ± 0.02 [b]	5.44 ± 0.21 [b]
W2019	3.45 ± 0.05 [c]	5.32 ± 0.07 [c]	1.05 ± 0.02 [b]	9.82 ± 0.14 [c]
S2020	1.05 ± 0.00 [a]	1.79 ± 0.00 [d]	0.58 ± 0.00 [a]	3.43 ± 0.00 [d]
Byp	1.03 ± 0.00 [a]	1.75 ± 0.01 [d]	0.55 ± 0.04 [a]	3.33 ± 0.05 [d]

Within each column different letters represent significant differences between collected samples (Wat, S2019, W2019, S2020), at $p<0.05$. The same letter represents no significant differences ($p>0.05$), determined by One-way ANOVA with post-hoc Tukey HSD Test.

Wat – Watered plant; Byp – Fruit Juice Byproduct; S2019 – Fruit Summer 2019; W2019 – Fruit winter 2019; S2020 – Fruit summer 2020.

Table 12. Antioxidant activity and bioactive compounds in dry weight (Ethanol/water extract) of the various seed samples (Watered, Summer 2019, Winter 2019, and Summer 2020).

Samples	DPPH mg.g^{-1} of Trolox equivalents	FRAP µmol.g^{-1} of Fe^{2+} equivalents	Total Phenols mg.g^{-1} of gallic acid equivalents	Flavonoids mg.g^{-1} of catechin equivalents
Wat	3.79 ± 0.19 [a]	30.89 ± 1.19 [a]	1.63 ± 0.11 [a]	1.03 ± 0.07 [a]
S2019	2.98 ± 0.10 [b]	25.64 ± 1.13 [b]	1.55 ± 0.16 [a]	0.77 ± 0.07 [b]
W2019	2.92 ± 0.23 [b]	24.10 ± 0.99 [b]	1.58 ± 0.19 [a]	0.75 ± 0.07 [b]
S2020	2.64 ± 0.19 [c]	20.75 ± 1.97 [c]	1.02 ± 0.13 [b]	0.75 ± 0.04 [b]
Byp	1.26 ± 0.11 [d]	21.56 ± 0.98 [c]	1.34 ± 0.06 [b]	0.85 ± 0.01 [b]

Within each column different letters represent significant differences between collected samples (Wat, S2019, W2019, S2020), at $p<0.05$. The same letter represents no significant differences ($p>0.05$), determined by One-way ANOVA with post-hoc Tukey HSD Test.

Wat – Watered plant; Byp – Fruit Juice Byproduct; S2019 – Fruit Summer 2019; W2019 – Fruit winter 2019; S2020 – Fruit summer 2020.

The watered sample was the one that showed the least fat in its composition, accounting for 2.41% of its dry weight. Additionally, it showed the lowest protein content of the wild samples (5.03% of dry weight). It showed the highest water content as well as the highest ash percentage. With these results, we can say that by watering the plant we lower its metabolism rate; therefore, it produces less fat and protein. However, we may also associate this to a later fruit development caused by the watering, as the amount of ash tends to get lower and the amount of fat tends to get higher during the development of the fruit according to Coşkuner *et al.* [27].

Surprisingly, the sample that shows the highest antioxidant power and bioactive compounds amount was the watered sample. Antioxidant *status* rises when the organism is in a stressful situation, such as in lack of water, too much sunlight exposure, soil salt concentration being too high, etc. Drought is a major environmental stress factor that affects the growth and development of plants [31]. Therefore, it was to be expected that the watered plant would be exposed to the least stress, yet the results hint at the opposite situation to be true.

Sugar composition was consistent throughout the samples, except for the winter sample (W2019) which shows a much higher amount of total sugar, 9.82% of dry weight, while the rest of the samples show 3.33-5.44% of dry weight. The winter sample shows an increased amount of fructose, which is on average 3 times higher than the other samples. The glucose amount is double the other samples, while the sucrose amount seems to be unvarying. The role of fructose in a plant organism is not only as a source of energy but as a regulatory metabolite. Increased fructose amounts in plants have been linked to cold resistance. Fructose and its metabolites, mainly fructose-6-phosphate (F6P) and fructose-1,6-biphosphate (FBP) have been shown to contribute to the preservation of homeostasis in plants under cold temperatures [32]. This could explain the accumulation of fructose seen in the winter fruit when compared to the others.

CONCLUSION

The watered sample showed the worst nutritional composition, showing the least fat and protein. We can attribute these results to a metabolism modification caused by the watering. Despite this, this same sample showed the highest antioxidant power. As bioactive compounds arise from stress, we can surprisingly conclude that the act of watering this specific plant species causes it stress.

The winter fruit showed an inconsistent amount of sugar when compared to the other samples. This high sugar amount was mainly caused by a 3 times higher amount of fructose, which was attributed to a plant adaptation to a colder climate.

ii. Vitamin E

Vitamin E is an essential nutrient in the human diet. The term refers to a group of tocopherols and tocotrienols, from which α-tocopherol is considered to bear the highest biological activity [33]. This family of compounds also shows high antioxidant activity and is associated with the prevention of chronic diseases related to oxidative stress. It is widely used in cosmetic products to prevent oxidation of the product, enhance shelf life, as well as protect

the skin against oxidative agents. It is advertised as reducing redness and inflammation [34] and as a moisturizing ingredient, all while being completely safe as it is already naturally present in the skin's sebum.

Vitamin E is liposoluble, being found mainly in plant oils including *Opuntia ficus-indica* seed oil. Since the fruit juice by-product was mostly seeds, it also showed a high-fat percentage being relevant for this analysis as well. The vitamin E profile was expected to be different in this case as the by-product also consists of insoluble fibers from the peel and pulp. This oil is used in the cosmetic industry, being advertised as rich in Vitamin E and unsaturated fatty acids. The fat content in the seeds is reported to be 6.1-7.0% of fresh weight [27, 35, 36]. It comes as no surprise that the oil has a very high market value as the low number of seeds per fruit means that tons of fruit are needed to extract a substantial amount of oil.

The results are summarized in the following table (Table 13).

Table 13. Vitamin E content of the seeds of the Watered, Summer 2019, Winter 2019, and Summer 2020 samples; and of the By-product of fruit juice sample (mg.kg^{-1} of dry weight ± SD).

Vitamin E (mg.kg^{-1} of dry weight)	Samples				
	Wat	Byp	S2019	W2019	S2020
α-tocopherol	3.50 ± 0.22 ^{a}	3.91 ± 0.34 ^{a}	5.17 ± 0.59 ^{b}	11.69 ± 1.16 ^{c}	4.90 ± 0.64 ^{b}
β-tocopherol	0.46 ± 0.02 ^{a}	1.01 ± 0.08 ^{b}	1.16 ± 0.08 ^{b}	1.43 ± 0.03 ^{b}	1.13 ± 0.01 ^{b}
γ-tocopherol	22.82 ± 0.52 ^{a}	0.91 ± 0.02 ^{b}	39.02 ± 2.82 ^{c}	33.15 ± 3.29 ^{d}	3.96 ± 0.15 ^{b}
δ-tocopherol	0.34 ± 0.01^{a}	n.d.	0.82 ± 0.03	0.82 ± 0.02^{a}	n.d.
Total	27.13 ± 0.58 ^{a}	5.83 ± 0.40 ^{b}	46.17 ± 3.04 ^{c}	47.09 ± 4.26 ^{c}	9.99 ± 0.61 ^{b}

Wat – Watered plant; Byp – Fruit Juice Byproduct; S2019 – Fruit Summer 2019; W2019 – Fruit winter 2019; S2020 – Fruit summer 2020.
n.d. Not detected
Within each line different letters represent significant differences between collected samples (Wat, S2019, W2019, S2020), at $p<0.05$. The same letter represents no significant differences ($p>0.05$), determined by One-way ANOVA with post-hoc Tukey HSD Test.

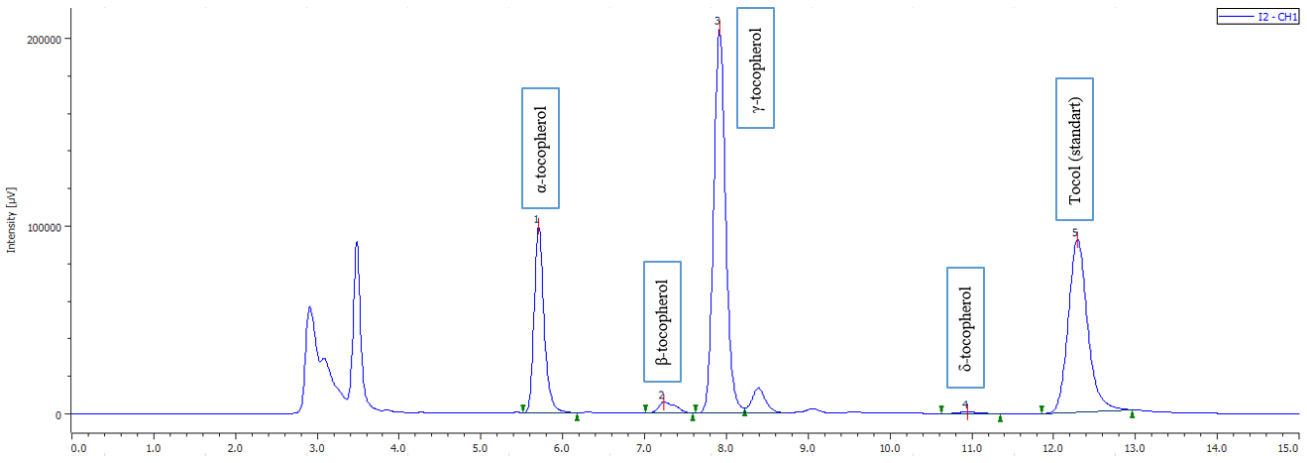

Figure 13. Chromatogram with identified peaks for the sample W2019.

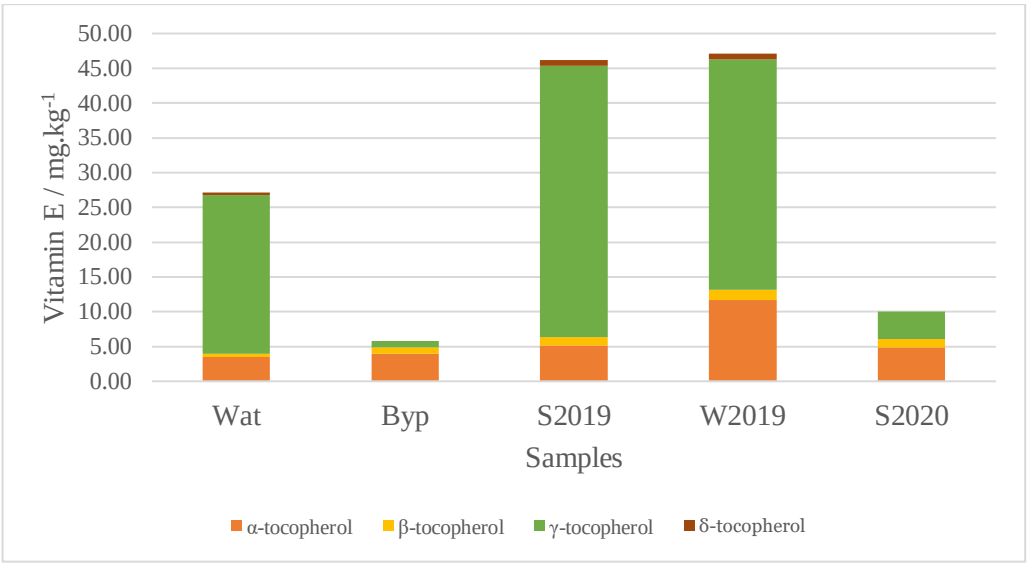

Figure 14. Vitamin E content of the seeds of the Watered, Summer 2019, Winter 2019, and Summer 2020 samples; and the Byproduct of fruit juice sample.

In the samples, we identified α-, β-, γ-, and δ-tocopherol. The samples Wat and S2020 showed a much lower amount of Vitamin E compared to the rest and we can observe an absence of δ-tocopherol in the samples Byp and S2020. However, δ-tocopherol might have been present but in amounts lower than the method's detection limit, since it is present in all other samples. The lower amount of Vitamin E in the case of Byp may be attributed to a lower amount of overall fat in the sample, as it contains not only seeds but also plant fiber leftover from juicing.

In plants, α-tocopherol seems to be similarly distributed throughout the plant, while γ-tocopherol predominates in the seeds [37]. The obtained results seem to follow this trend as γ-tocopherol is predominant in most samples. Little γ-tocopherol is found in Byp, which can be justified by this sample being constituted not only by seeds but a mixture of seeds and leftover fiber from juice extraction. α-tocopherol has been linked to cold resistance during winter in the seagrass *Cymodocea nodosa* [38]. A similar result can be witnessed here as the winter sample (W2019) shows a significantly higher amount of α-tocopherol compared to the rest of the samples.

Samples S2019 and S2020 were expected to be the most similar as they represent the same type of samples but from different years. However, S2020 shows a much lower amount of total Vitamin E. The difference may then be attributed to either, 1. the fruit being in different developmental stages due to external abiotic factors or 2. differential degradation of the vitamin E during storage. Vitamin E is known to be sensitive to light [39]. Although samples were kept in the same conditions as best as possible, the more recent samples would be on the top of the storage box being more exposed to light.

In a review, Al-Naqeb *et al.* [40] report a γ-tocopherol content of 67.5-98.5% of total vitamin E followed by δ-tocopherol 0.46-8.26% and α-tocopherol 0.11-1.95%. These results are similar to the ones obtained except for α-tocopherol which is found in much higher quantities in any of our samples. However, it is noted that different extraction methods have an influence on Vitamin E composition, for example, using a Soxhlet extraction method here the sample achieves a higher temperature that may degrade the vitamin E. Ennouri *et al.* [41] reported a γ-tocopherol content of 94.12% of total vitamin E while Khémir *et al. [42]* reported a γ-tocopherol content of 92% of total vitamin E, δ-tocopherol 6.24% and α-tocopherol 1.33%. All these studies reported on plants from Tunisia, Morocco, Algeria, and Turkey where average temperatures are higher than in Portugal. If we consider the possibility of α-tocopherol being higher in plants exposed to colder weather, it makes sense for plants grown in Portugal to show a much higher amount of this Vitamin E form. This actually represents a nutritional advantage to the Portuguese plant, as α-tocopherol is the most biological active form.

CONCLUSION

The seed of Opuntia contains the following Vitamin E tocopherols: α-, β-, γ-, and δ-tocopherol.

The juicing by-product samples showed a much lower amount of Vitamin E and we can observe an absence of δ-tocopherol in the by-product and Summer 2020 samples. The by-product sample has a low amount of fat as it includes some peel leftovers mixed in, which can justify the lower amount of Vitamin E found.

High amounts of α-tocopherol have been linked with cold resistance in plants [37], justifying the Winter 2019 showing the highest amount of this tocopherol. In fact, when compared to bibliography, our samples reveal a significantly higher level of α-tocopherol. We attribute this to the plants we collected growing in a colder climate in Portugal, when compared to bibliography's plants origins (Africa). Since α-tocopherol is the most biological active form of Vitamin E, the higher amount of this tocopherol can be seen as a nutritional advantage.

iii. Fatty acids

Fat makes up a reduced part of *Opuntia ficus-indica,* having the highest content on the seeds. Fatty acids are important carbon storages on seeds, being the main lipid-containing part of the plant. The edible part of the prickly pear contains a relatively large number of seeds, whose amount can vary from 30% to 40% on a dry weight basis [16]. Seeds are usually discarded as a waste product, after extraction of the pulp. Millions of kilograms of fruit seeds are discarded yearly, resulting in disposal problems, while proper utilization of these waste products could lead to an important new source of oil and meal [43].

It is important therefore to study the oil composition to clarify the possible uses and benefits.

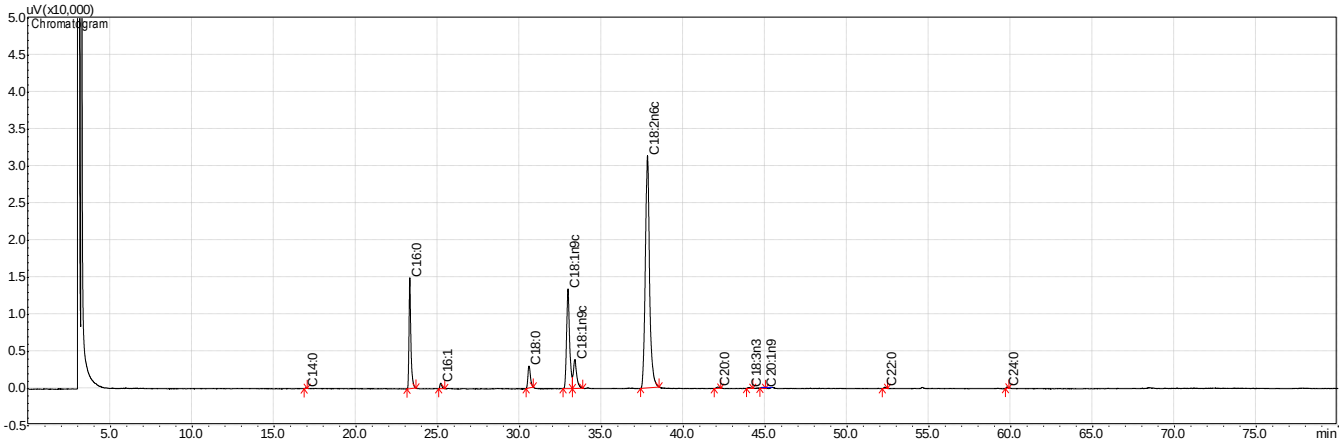

Figure 15. Chromatogram with identified peaks for the sample S2019.

Table 14. Fatty acid content of the seeds of the Watered, Summer 2019, Winter 2019, and Summer 2020 samples; and of the Byproduct of fruit juice sample (Total fatty acid % ± SD).

Compound Name	Wat	Byp	S2019	W2019	S2020
Myristic Acid (C14:0)	0.09 ± 0.01 [a]	0.13 ± 0.00 [b]	0.06 ± 0.01 [a]	0.10 ± 0.00 [a]	0.20 ± 0.01 [c]
Palmitic Acid (C16:0)	14.76 ± 0.80 [a]	15.66 ± 0.05 [a]	12.24 ± 0.06 [b]	12.86 ± 0.34 [b]	13.15 ± 0.25 [b]
Palmitoleic Acid (C16:1)	0.74 ± 0.02 [a]	0.84 ± 0.02 [a, b]	0.67 ± 0.03 [a, c]	0.81 ± 0.02 [a, b]	0.58 ± 0.05 [c]
Stearic Acid (C18:0)	3.85 ± 0.19 [a]	3.75 ± 0.03 [a]	3.59 ± 0.19 [a]	3.77 ± 0.23 [a]	3.90 ± 0.09 [a]
Oleic Acid (C18:1n9c)	14.19 ± 0.26 [a]	18.16 ± 0.54 [b]	17.61 ± 0.40 [b]	18.09 ± 1.80 [b]	19.55 ± 1.00 [b]
Vaccenic Acid (C18:1n7)*	6.25 ± 0.05 [a]	6.87 ± 0.06 [b]	5.71 ± 0.13 [c]	5.90 ± 0.23 [a, c]	6.22 ± 0.17 [a]
Linoleic acid (C18:2n6c)	58.80 ± 1.25 [a]	52.38 ± 0.52 [b]	59.26 ± 0.21 [a]	57.39 ± 1.73 [a, c]	54.93 ± 1.12 [b, c]
Arachidic Acid (C20:0)	0.31 ± 0.01 [a]	0.30 ± 0.03 [a, b]	0.23 ± 0.01 [b, c]	0.25 ± 0.02 [a, c]	0.31 ± 0.02 [a]
α-Linolenic Acid (C18:3n3)	0.24 ± 0.02 [a]	0.19 ± 0.01 [b]	0.17 ± 0.01 [b]	0.24 ± 0.02 [a]	0.17 ± 0.00 [b]
Eicosanoic Acid (C20:1n9)	0.10 ± 0.01 [a]	0.09 ± 0.00 [a]	0.14 ± 0.01 [b]	0.17 ± 0.00 [c]	0.19 ± 0.01 [c]
Behenic Acid (C22:0)	0.23 ± 0.01 [a]	0.26 ± 0.02 [a]	0.18 ± 0.01 [a]	0.18 ± 0.01 [a]	0.23 ± 0.01 [a]
Lignoceric Acid (C24:0)	0.45 ± 0.04 [a]	1.37 ± 0.06 [b]	0.11 ± 0.01 [c]	0.25 ± 0.02 [c]	0.59 ± 0.06 [a]
Total SFA	19.68 ± 1.07	21.47 ± 0.18	16.43 ± 0.28	17.41 ± 0.63	18.37 ± 0.45
Total MUFA	21.28 ± 0.33	25.96 ± 0.63	24.14 ± 0.57	24.97 ± 2.06	26.54 ± 1.23
Total PUFA	59.04 ± 1.27	52.57 ± 0.53	59.44 ± 0.22	57.62 ± 1.75	55.09 ± 1.12
Total UFA	80.32 ± 1.60	78.53 ± 1.16	83.58 ± 0.79	82.59 ± 3.81	81.63 ± 2.35

*Identified as Vaccenic acid according to bibliography, as the standard (FAME) used didn't include this fatty acid
Within each line different letters represent significant differences between collected samples (Wat, S2019, W2019, S2020), at $p<0.05$. The same letter represents no significant differences ($p>0.05$), determined by One-way ANOVA with post-hoc Tukey HSD Test.
Wat – Watered plant; Byp – Fruit Juice Byproduct; S2019 – Fruit Summer 2019; W2019 – Fruit winter 2019; S2020 – Fruit summer 2020.

According to literature, dry weight fat percentage varies from 4.47 to 12.52% [27, 35, 36, 44]. Most of our samples fall in this interval except for the watered plant (Wat) which showed a reduced-fat percentage of 2.41% of dry weight. None of the samples in the bibliography were watered, so we can conclude that watering causes the fat percentage on the seed to lessen. Compared to S2020, which was harvested in the same year and in the same area, this

difference in fat composition becomes more apparent as it registers a 7.85% dry weight. The juice by-product sample (Byp) also showed a low value of fat. This is because, as the seed has the most fat percentage on the plant and the sample is constituted by not only the seed, this lowers the overall fat percentage on the sample.

The main fatty acid found in all the samples was Linoleic acid (C18:2n6c), constituting 52.38-59.26%. This is according to the bibliography where it is also registered as the main fatty acid component, making up 53.84-70.3% of total fatty acids [16, 27, 35, 36, 43, 45-47]. Linoleic acid is a polyunsaturated omega-6 fatty acid, and it is one of the two (the other being linolenic acid) that humans must obtain through diet. Research has also shown that this component has beneficial properties on the skin, including anti-inflammatory properties, acne reduction, skin-lightening, and moisture retention [48, 49]. The application of *Opuntia* oil in cosmetic products has been increasing and its high amount of linoleic acid can justify some of its reported benefits.

Oleic acid (C18:1n9c) is the second most predominant acid accounting for 14.19-19.55% of fatty acids followed by Palmitic acid (C16:0) at 12.24-15.66%. Bibliography reports 15.2-25.52% of oleic acid content and 9.32-14.06% of palmitic acid [16, 27, 35, 36, 43, 45-47].

Vaccenic acid is commonly reported in *Opuntia ficus-indica* oil (5.0-5.5% of total fatty acids). The FAME standard used in the determination of the fatty acids did not include Vaccenic acid. However, a peak presented itself where vaccenic acid would be expected (reported in figure 12 as the second peak at y=34 min, labeled as C18:1n9c), being detected right after Oleic Acid. Taking the bibliography into account, and the fact that the amounts registered were very close to those reported (5.71-6.87% of total fatty acid), we safely concluded that this peak corresponded to vaccenic acid. Furthermore, Chougi *et al.* [16] studied the seed oil composition of different prickly pear varieties (Green, yellow, and orange), finding that vaccenic acid was only present in the green and orange varieties. This comes in sync with our findings as the variety studied is the orange variety.

Other fatty acids found in low amounts also coincide with those commonly reported. However, we must highlight the amount of Arachidic acid (C20:0) as it is only reported by Coşkuner *et al.* [27] varying from 0.42 to 0.66 % of total fatty acids throughout the fruits maturation period and by El Mannoubi *et al.* [36] at 0.30% of total fatty acids. In our sample, the amount was 0.23-0.31% of total fatty acids agreeing with the mentioned authors.

The sample obtained from the juice by-product (Byp) shows the most saturated fat compared to the other samples. The saturated fat percentage is also relatively higher than the one commonly reported (21.47% vs. 12.43-19.34%) [16, 27, 35, 36, 43, 45-47]. The sample

shows a minor amount of linoleic acid (C18:2n6c) an unsaturated acid and a higher amount of palmitic acid (C16:0), a saturated one, making the oil overall more saturated. Again, as this sample is not only constituted by the seeds, this plays a role in the oil composition.

The watered sample shows the second-highest saturation, having a lower amount of oleic acid (C18:1n9c), an unsaturated acid. We can conclude that watering the plant highers the seed oil saturation. The seed oil from the same year is more saturated in winter but the following year's summer sample shows even higher amounts of saturation, suggesting that there must be another factor influencing oil saturation.

CONCLUSION

The main Fatty acid found in all the sample was Linoleic acid (C18:2n6c), being an essential omega-6 fatty acid. Oleic acid (C18:1n9c) is the second most predominant followed by palmitic acid (C16:0).

The FAME standard used in the determination of the fatty acids did not include vaccenic acid, however, a peak presented itself where vaccenic acid would be expected. Because Vaccenic acid is commonly reported in the oil of *Opuntia*, and in amounts that were close to our reported amount, we safely concluded that this peak corresponded to vaccenic acid. Therefore, Vaccenic acid is the 4th most common fatty acid present in the oil.

The watered plant sample shows an unusual low fat percentage level 2.41% of dry weight which can be attributed to its daily watering treatment. Compared to S2020, which was harvested in the same year and in the same area, this difference in fat composition becomes more apparent as it registers a 7.85% dry weight of fat. The watered sample shows the second-highest saturation. We can conclude that watering the plant highers the seed oil saturation.

The lower values of fat in the juice by-product can be attributed to the peel leftover in its composition, which naturally has a lower lipidic composition than seeds. The by-product also shows the most saturated fat percentage, being justified as well by its different composition.

Part 2.

Product development – Yogurt enriched with cladode

1.1. Developments in enriched yogurts – examples and research

Fortified food is commonly targeted towards infants, pregnant and lactating women, as specific nutrient deficiencies in these stages can be detrimental towards the development of the child and to the mother's well-being [50]. However, in the last decade, we have seen the rise of another type of fortified food: fiber fortified food. Dietary fiber deficiency is widespread in developed countries, here processed and refined foods are very common. A diet rich in this type of foods leads to a prevalence of obesity and cardiometabolic disease [51]. The benefits of the consumption of these fortified foods were described by Lee et al. [51] and include reduced body weight, fat mass, total cholesterol, insulin, among others. This type of fortified food is therefore targeted to all consumers, as recent surveys have shown that only about 5% of the North American population[52] and 10% of the European population [53] meet the recommended intake.

Yogurts are a very common food to be fortified. We can find 2 types of enriched yogurts: specific nutrient enriched yogurts (e.g. Calcium enriched yogurt, Vitamin D fortified yogurt) and food byproduct enriched yogurt (e.g. pea fiber enriched yogurt, olive fruit polyphenols enriched yogurt, flaxseed enriched yogurt).

A recent trend in byproduct enriched yogurt is to use food waste. Examples include carrot waste [54], grape seed extract [55], olive pomace [56], bioactive compounds from red pepper waste [57], hazelnut skin [58] among many others. Plant-based food processing waste contains high amounts of phytonutrients and fiber. Using this waste product in yogurts allows us to re-introduce these beneficial nutrients in our diet. At the same time, the waste products are made use of.

1.2. The idea and how it came to be

One of the problems in prickly-pear intensive cultivation is its pruning. Pruning a prickly-pear plant is necessary due to overgrown cladodes that block the paths workers walk or that block reaching the economically important fruit. The leftover from pruning is cut cladodes. A substantial problem arises from this type of waste: if simply left on the soil to compost, the cladodes will develop their root system creating a new prickly-pear plant. To maintain order in

an orchard and to avoid the trouble of having to eliminate new prickly-pear plants, the main solution farmers came up with is to grind the cladodes using an industrial grinding machine.

Highlighting the potential of the cladodes, some ways to repurpose this waste have been developed:

1. Production of a plant-based leather alternative (Opuntia MX® https://opuntiamx.com)
2. Production of biofuel (Elqui Global Energy® https://www.elquiglobalenergy.com)

As discussed previously, the cladode is an edible part of the prickly-pear plant, and it is comparable to *aloe vera* in its composition. Hence, a lot of products that have been developed from the aloe plant, need only minimal modifications to be applied to the prickly pear. Products starring this low-impact by-product will be an environmentally friendly alternative.

When thinking of product formulation, we decided to create a product that used already common transformation techniques in the food industry, and that was familiar to the consumer, adding only a twist to an already existing product. Therefore, we decided to use yogurt as our base to create a cladode enriched yogurt.

1.3 Potential health benefits – what are the advantages of enriching a yogurt with cladode juice?

- Calorie lowering

Milk's main macronutrient is fat, which is also the most calorie-dense one (9 kcal/gram). The cladode is mainly composed of sugar which is less calorie dense (4 kcal). Another way to look at this aspect is that yogurts usually have added sugar, and by adding cladode juice, the need for added sugar later is reduced.

- Fiber

As an animal product, milk is devoid of dietary fiber. Adding cladode juice, which has 10.43 % of dry weight fiber, would increase the amount of fiber in the final product and therefore increase its nutritional benefits.

- Antioxidant properties

Cladode juice is rich in bioactive substances with antioxidant power. Adding it to the yogurt would make it a source of these compounds.

2. Methodology

To obtain the best flavor with the most percentage of cladode juice, we studied various mixes of cow's milk and cladode juice. A commercial lyophilized yogurt culture was used, consisting of powdered milk, *Streptococcus thermophilus,* and *Lactobacillus bulgaricus* (Yaourt Brassé, Nat all®). Instructions provided in the package were followed for the amount of culture to add to a certain amount of liquid (milk and cladode juice), temperatures, and time.

The cladode juice was prepared by passing raw cladodes through a conventional kitchen juicer, using the same method as in section 3.4. After this process, the juice was pasteurized, heated at 80 ºC for 30 minutes, and then cooled in a closed recipient. We opted to use this method to avoid higher temperatures, where certain bioactive compounds would certainly be degraded.

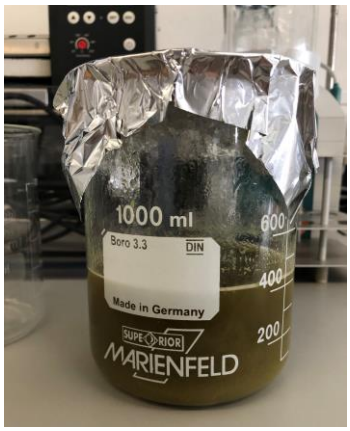

Figure 16. Cactus stem juice after being sterilized (80 ºC for 30 min).

The different cladode juice/milk mixes were heated until they reached 45 ºC when 1.2 grams of lyophilized culture was added. After a 5-minute homogenization period at 45 ºC, the mixed were placed in a laboratory oven at 40 ºC for 8 hours. After these 8 hours, the yogurts were removed from the oven and allowed to settle for a few hours in a fridge.

The following mixes were tested (cladode juice%/milk%): 75/25; 50/50; 25/75; 20/80 and 10/90.

The yogurts were then subjected to testing. All the tests were performed in the first (1) day and again 7 days later following their preparation. Titratable acidity (lactic acid %) was measured using the AOAC titration method 947.05, with NaOH 0.1 M. Using this value, we can estimate how active the yogurt cultures were, correlating to how successful the fermentation was.

Large deformation analysis was carried out with a single compression test, using a TA-XT2 Texture Analyzer (Stable Micro System Ltd., Godalming, U. K.) equipped with a 2 kg load. The samples were compressed with a 25mm diameters cylindrical probe to a depth of 5 mm at a constant speed of 3 mm/s. The gel firmness was determined as the maximum force (N) on the compression force-time curve. Three separate determinations were made in each batch.

Thixotropy tests were applied to characterize the flow behavior of the yogurt samples. The tests were carried out at 10 ºC and allowed 900 s to equilibrate. Shear stress was then recorded at increasing shear rates from 1 to 100 s^{-1} (upward flow curve), followed by decreasing shear rates from 100 to 1 s^{-1} (downward flow curve). To model the flow behavior, the upward flow curve was fitted to the Ostwald model ($\sigma=k\,\gamma^n$), where σ represents shear stress (Pa), k is the consistency index ($Pa.s^n$), n is the flow behavior index (dimensionless) and ɣ is the shear rate (s^{-1}). Another parameter that was considered was the difference between the upward flow curve and the downward flow curve areas. A higher area difference represents a higher hysteresis. In rheological systems, hysteresis is associated with thixotropy, classically defined as the time-dependent reduction of apparent viscosity under a constant shear rate" [59].

3. Results and discussion

The concentrations of 75% and 50% juice were discarded in the first trials due to various factors. The main factor was the low fermentation rate compared to the control sample and the 25% juice sample, which can be assessed by the low quantity of lactic acid present in these samples (see table 15). Adding to this, the sample did not have the characteristics of yogurt being too liquid and with green coloration, not being appellative (see figure 15).

Figure 17. Aspect of the tested samples (from right to left: 75/25 – yogurt of 75% cladode juice and 25% milk; 50/50 – yogurt of 50% cladode juice and 50% milk; 25/75 – yogurt of 25% cladode juice and 75% milk; 20/80 – yogurt of 20% cladode juice and 80% milk; 10/90 – yogurt of 10% cladode juice and 90% milk; Control – milk yogurt).

Table 15. Lactic acid percentage for the first trial of cladode juice enriched yogurt (Control – milk yogurt; Blank – milk without yogurt ferments; 75/25 – yogurt of 75% cladode juice and 25% milk; 50/50 – yogurt of 50% cladode juice and 50% milk; 25/75 – yogurt of 25% cladode juice and 75% milk; 20/80 – yogurt of 20% cladode juice and 80% milk; 10/90 – yogurt of 10% cladode juice and 90% milk).

	% of lactic acid		
Control	8.25	±	0.62 [a]
Blank	1.38	±	0.08 [b]
75/25	5.18	±	0.05 [c]
50/50	7.07	±	0.16 [d]
25/75	8.25	±	0.70 [a]
20/80	7.52	±	0.08 [a]
10/90	8.08	±	0.70 [a]

Different letters represent significant differences between samples at $p<0.05$. The same letter represents no significant differences ($p>0.05$), determined by One-way ANOVA with post-hoc Tukey HSD Test.

The control values were statistically the same as the samples 25/75, 20/80, and 10/90, meaning that the production of lactic acid by the yogurt ferments was the same in all these samples. The presence of cactus juice in these concentrations (25%, 20%, and 10%) did not hinder the bacteria's ability to produce a fermented product.

Table 16. Firmness results of cladode juice enriched yogurt (Control – milk yogurt; 25/75 – yogurt of 25% cladode juice and 75% milk; 20/80 – yogurt of 20% cladode juice and 80% milk; 10/90 – yogurt of 10% cladode juice and 90% milk).

	Sample age	Firmness (N)		
Control	1 day	0.55	±	0.01
	7 days	0.48	±	0.01
25/75	1 day	0.18	±	0.01
	7 days	0.17	±	0.01
20/80	1 day	0.26	±	0.00

	7 days	0.23	±	0.01
	1 day	0.45	±	0.03
10/90	7 days	0.36	±	0.00

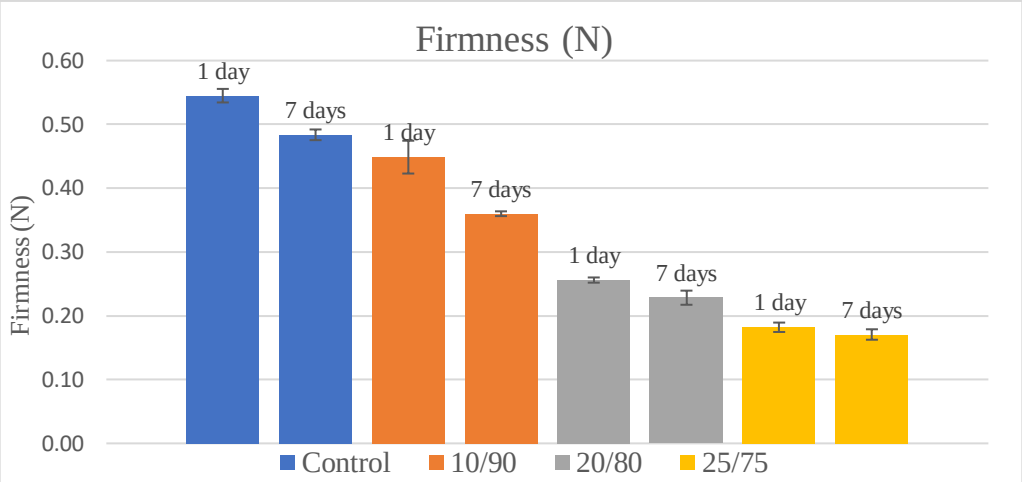

Figure 18. Firmness of the enriched yogurt samples (Control – milk yogurt; 25/75 – yogurt of 25% cladode juice and 75% milk; 20/80 – yogurt of 20% cladode juice and 80% milk; 10/90 – yogurt of 10% cladode juice and 90% milk).

As we can see from figure 16, no sample behaved the same as the control sample, being all statistically different. The firmness in yogurt is caused by the low pH (due to the lactic acid production) causing the agglutination of the milk. By adding cactus juice to the milk, even though the production of lactic acid was the same (as seen in Table 15), the amount of agglutinated milk fat globules is low due to the decreased amount of milk in the product. Therefore, a reduced firmness was observed, which decreased with the addition of cactus juice. This reduced firmness does not necessarily make the product unviable. Adding thickeners to yogurts is a very common practice in the yogurt industry, especially with low-fat and plant-based yogurts that also have a decreased amount of milk fat globules or no milk at all. Some widely used thickeners are modified starch, tapioca starch, Agar agar, guar gum, arrowroot starch, etc.

The samples were tested on the first and seventh days, as to see if this firmness was maintained during storage. A relatively small decrease was recorded in all samples, including in the control. We can conclude that this decrease is to be expected in this product.

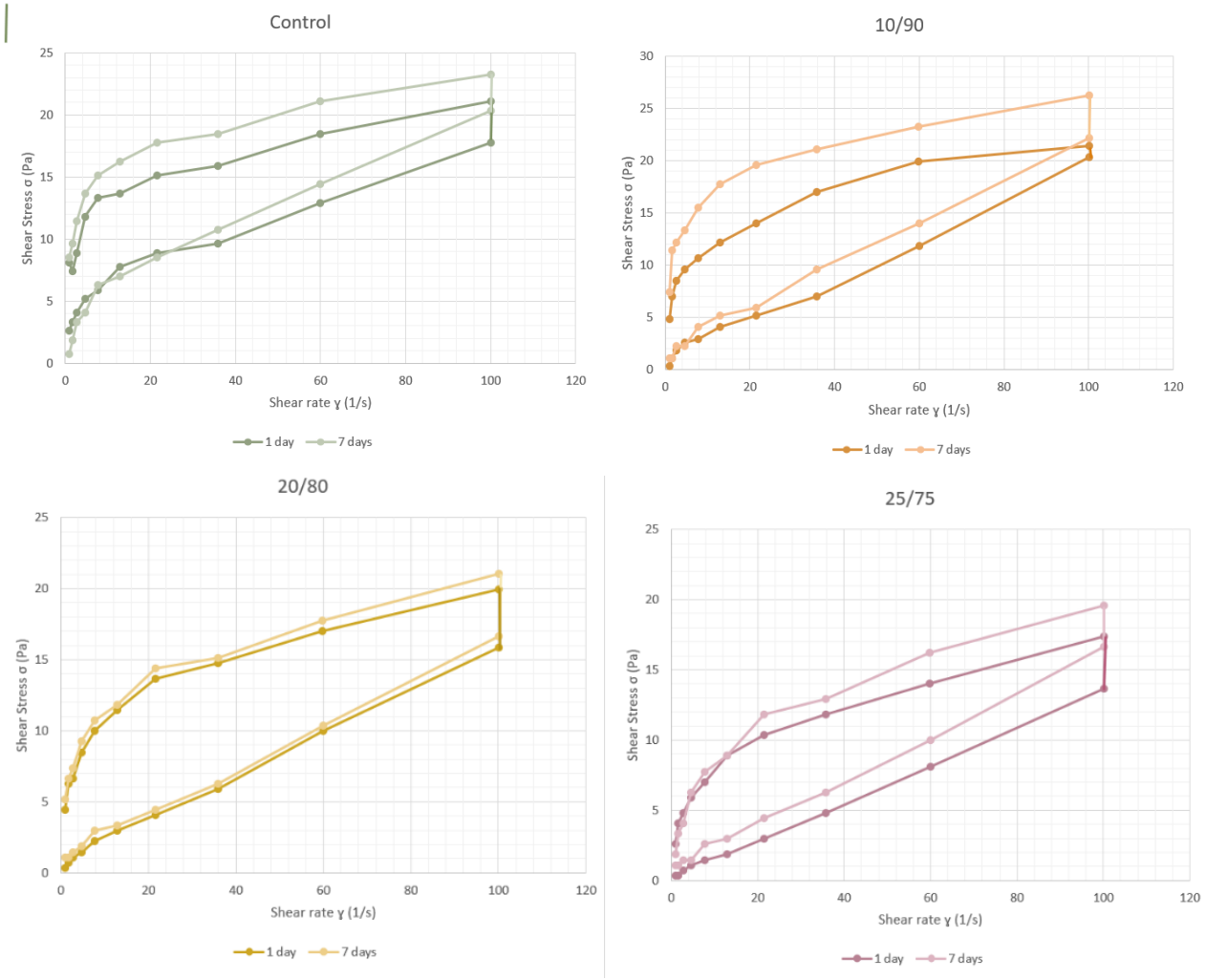

Figure 19. Yield stress and hysteresis determinations for the first and seventh days (Control – milk yogurt; 25/75 – yogurt of 25% cladode juice and 75% milk; 20/80 – yogurt of 20% cladode juice and 80% milk; 10/90 – yogurt of 10% cladode juice and 90% milk).

Table 17. Fluid hysteresis loop test measurements based on the graphical area of the graph Shear rate ɣ (1/s) / Shear Stress σ (Pa) (Hysteresis loop). Calculated by subtracting the area under the Downward flow curve (decreasing shear stress) from the area under the Upward flow curve (increasing shear stress).

	Sample age	Graphical area
Control	1 day	1070
	7 days	1341
10/90	1 day	1391
	7 days	1877
20/80	1 day	1414
	7 days	1464
25/75	1 day	1149
	7 days	1102

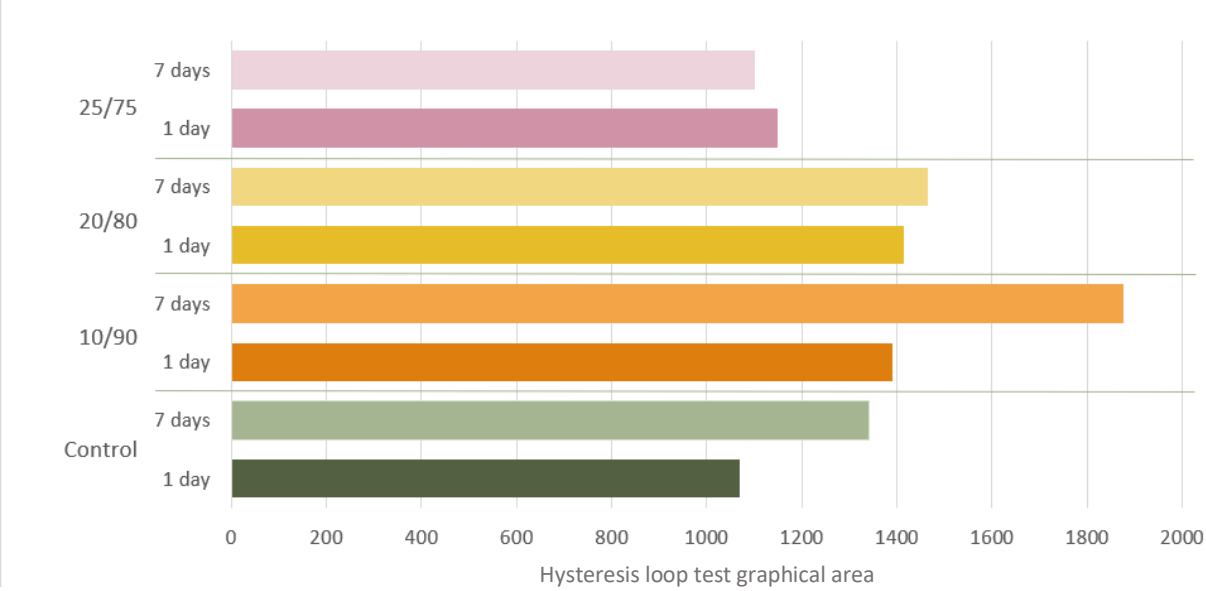

Figure 20. Hysteresis loop test graphical area. Based on the graph Shear rate ɣ (1/s) / Shear Stress σ (Pa) (Hysteresis loop). Calculated by subtracting the area under the Downward flow curve (decreasing shear stress) from the area under the Upward flow curve (increasing shear stress).

Table 18. Ostwald model determinations – " $\eta = K\gamma^n$ " (η is the shear stress in Pa, γ is the shear rate in 1/s, K is the flow consistency index in Pa.s and n is the flow behavior index which is dimensionless) (Control – milk yogurt; 25/75 – yogurt of 25% cladode juice and 75% milk; 20/80 – yogurt of 20% cladode juice and 80% milk; 10/90 – yogurt of 10% cladode juice and 90% milk).

	Ostwald Model			
	Sample age	K (Pa.s)	n	R^2
Control	1 day	7.38	0.23	0.96
	7 days	9.47	0.20	0.98
10/90	1 day	5.69	0.30	0.99
	7 days	9.06	0.24	0.97
20/80	1 day	5.03	0.31	0.99
	7 days	5.61	0.29	0.99
25/75	1 day	3.12	0.38	0.99
	7 days	2.50	0.48	0.98

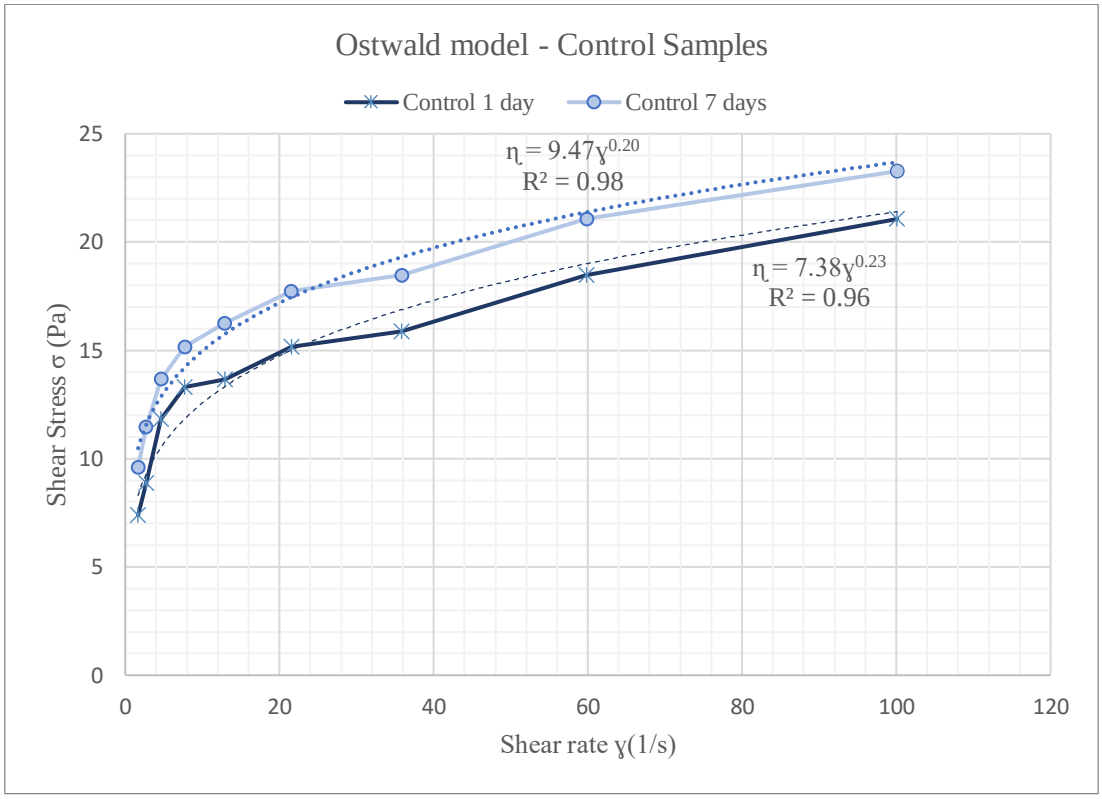

Figure 21. Ostwald model applied to the control sample (Control – milk yogurt). The plots only include the upward flow curve

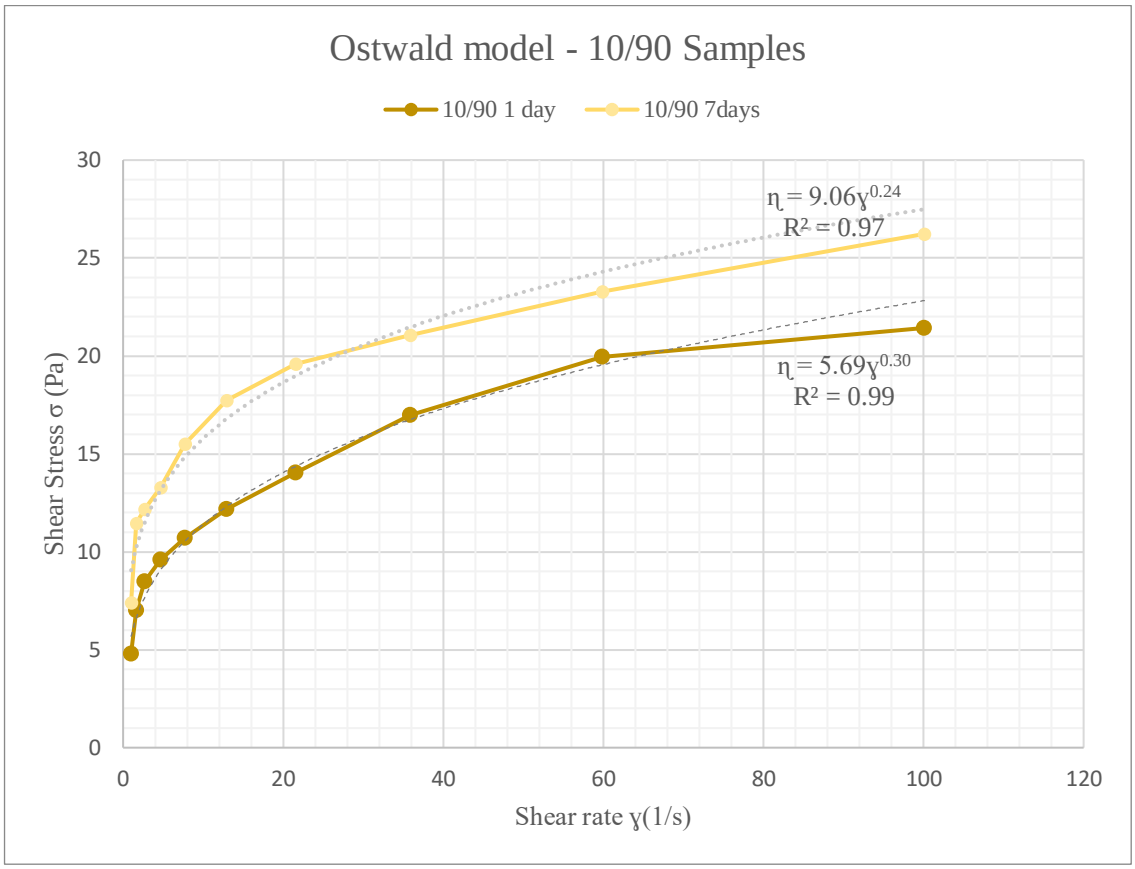

Figure 22. Ostwald model applied to the 10/90 sample (10/90 – yogurt of 10% cladode juice and 90% milk). The plots only include the upward flow curve.

A note on the fluid model used: The most complete non-Newtonian fluid model is the Herchlel-Bulkley model " $\eta = \sigma_0 + K\gamma^n$ " (η is the shear stress in Pa, γ is the shear rate in 1/s, σ_0 is the yield stress in Pa, K is the flow consistency index in Pa.s and n is the flow behavior index which is dimensionless). However, the apparent viscosity associated with the Herschel-Buckley stress diverges to infinity as the strain rate approaches zero. This divergence makes the model difficult to implement in numerical situations. Despite various attempts, no satisfactory fit was obtained for the Herschel-Buckley model. As a result, we opted to use the more easily applied Ostwald model.

Yogurt and the cactus juice-enriched yogurt are both non-Newtonian fluids, which means the apparent viscosity changes with the stress applied to the fluid (Figure 17). This is very visible in the traced plots where we never observe constant shear stress. We can also conclude that the behavior of the sample is Pseudoplastic as its flow behavior index is <1 (Table 18 and Figures 19 and 20). This translates to a decreased apparent viscosity with a higher

stress-strain. We can as well observe a phenomenon known as thixotropy, which is a time-dependent thinning property that non-Newtonian fluids can have. The longer the fluid is stirred, the less viscous it becomes (Less shear stress). In a plot, this is observable by an area formed between the downward flow curve and the upward flow curve (table 19 and figure 18).

All the samples showed a higher apparent viscosity on the 7th day. We can observe this in the plots as the 7th day is constantly represented on top of the 1st day. This is, with the same shear rate applied to the sample, the 7th day sample shows a higher stress value. This is expected, as with time the yogurt "settles" becoming more viscous. Since we also observed this behavior in the control sample, we can conclude that the sample enriched with the cactus juice, behaved the same way.

From the area obtained in the hysteresis loop test (see table 19), we can conclude the level of thixotropy of each sample. The higher the area, the higher the thixotropy phenomenon. From the 1st to 7th day, we see a significant increase in the thixotropy in the control and the 10/90 samples. In the rest of the samples, the change in thixotropy is not significant. Therefore, in this parameter, the sample that shows the most yogurt-like behavior is 10/90.

Regarding the Ostwald model, all samples had a very good fit to the model, showing an R^2 always higher than 0.95. The flow behavior index (n) tended to decrease from the 1st to the 7th day, translating to an increase in the pseudoplastic behavior of the fluid. This index also shows an increase in samples higher in cactus juice percentage, lowering their pseudoplastic behavior. The flow consistency index (K) tends to increase from the 1st to the 7th day, the difference being more significant in the Control sample. Overall, the sample that behaved the most like the control group was the 10 % cactus juice yogurt.

4. Conclusion

The overall similarities between the regular yogurt and the cactus juice enriched version show high potential for this product. The sample with 10% cactus juice displays the behavior that more closely resembled the yogurt in rheological characteristics. The samples with 10%, 20%, and 25% produced statistically the same amount of lactic acid as the regular yogurt. This shows that the fermentation was successful and that the modified environment was able to support the bacteria's growth and metabolism.

The loss of firmness for higher cactus juice concentration is due to the lesser amount of milk fat that agglutinates as the fermentation proceeds. This lack of firmness does not mean that the high amounts of cactus juice are not viable for yogurt production. The addition of thickeners is a usual practice when producing yogurt, especially yogurt with added non-conventional ingredients or transformations. Another modification that will probably be needed is the amount of yogurt culture added to each batch, as this may improve the firmness of the higher concentrations.

The next step is to conduct a sensorial analysis with subjects so that we can be provided with the opinion and satisfaction of real consumers. The addition of cactus juice gives the yogurt a certain hint of freshness, but as the concentrations get higher this shift into a "grass-like" taste which is not appellative. There is also a slight color change as the cactus juice is added as we can see in figure 15. Overall, the product was sweeter than the regular yogurt as the cactus juice contains sugar.

The results of these tests were satisfactory, but more tests are needed to go forward with the product, mainly ingredient modifications, and sensorial acceptance testing.

General Conclusions

In European countries with warm climate, such as Portugal, *Opuntia* has an excellent adaptation to the driest and hottest parts of the country, being found in Trás-os-Montes and Alentejo regions. Yet, it has the *status* of an invasive plant. In this paper we proposed a new vision of this plant, as an opportunity.

Therefore, we explored *Opuntia ficus-indica's* potential as a food source. The fruit is already widely used in Central America where its native. The cladodes are less used, however edible, due to difficulties in preparation and usage.

Studying and concluding on the Portuguese wild *Opuntia* composition opens doors to its usage in the food or cosmetics industries. Our conclusions on various fruit and plant parts highlight the plants multifaceted potential, such as, preferential exploring the fruits peel for cosmetics production due to its higher antioxidant power; watering the plant to increase its antioxidant potential; or using the juicing by-product to produce oil of known general composition.

A spotlight of our work was showing in a practical way an application of the cladodes in a simple, yet appellative product. The cladode enriched yogurt showed excellent results, having a preparation very simple to the regular yogurt, and using techniques and machines already widely used in the food processing industry.

Opuntia ficus-indica's potential is underexplored, especially in the western civilization, where we now find it growing in adverse conditions. We consider this plant a big opportunity for elaboration of various products, including food, beverages, cosmetic and even textiles. Its lack of watering need makes it a plant adapted to climate change and a low-maintenance crop. *Opuntia's* future is, for a fact, very promising.

The authors hope to keep exploring the elaboration of the cladode enriched yogurt, including the addition of thickeners and a sensorial analysis.

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