POLITÉCNICO DE LEIRIA | ESCOLA SUPERIOR DE TURISMO E TECNOLOGIA DO MAR

Dissertation

Masters degree in Food Quality and Safety Management

Physicochemical and bioactive characterization of a wild natural resource of the West Coast of Portugal: *Corema album L (D.) Don*

Hugo Sá

Peniche, December 2021

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Dissertation submitted to obtain the master's degree in Food Quality and Safety Management

Master's dissertation conducted under the supervision of Doctor Maria Joaquina da Cunha Pinheiro and Doctor Rui Manuel Maneta Ganhão of the Escola Superior de Turismo e Tecnologia do Mar do Instituto Politécnico de Leiria

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Escola Superior de Turismo e Tecnologia do Mar
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Resumo

A *Corema album* L (D.) don é uma baga selvagem nativa da Península Ibérica e atualmente é subutilizada mas possui alto potencial antioxidante. Esta baga, devido à competição por espécies invasoras e à destruição do seu habitat esta em risco de ser perdida. Este estudo contribui para a caracterização dos atributos físico-químicos com foco na sua capacidade antioxidante. O estudo foi realizado durante o período de maturação entre Setembro e Outubro, tendo sido obtidas as amostras de duas áreas diferentes (Serra d'El Rey (CAM-SR) e Peniche (CAM-CV)). Foi também realizado um estudo durante o armazenamento posterior à apanha, a duas temperaturas diferentes. (20 ° C e 4 ° C). Para além disso, a adição da baga a um iogurte natural adoçado também foi feita. O estudo de maturação revelou que os tamanhos das bagas tendem a diminuir nas fases posteriores do período de maturação, diminuindo 11% e 19% para as bagas de CAM-SR e CAM-CV a partir do valor inicial de 0.461 ± 0.043 e 0.467 ± 0.033. Observou-se também uma diminuição de 20% e 40% para o peso. Para os parâmetros de cor os locais de colheita comportaram-se de forma diferente, com as bagas de CAM-SR apresentando um valor inicial de 53.03 ± 2.64 e um valor final de 55.65 ± 2.97, enquanto as bagas de CAM-CV apresentaram um valor inicial de 50.82±5.42 e um valor final de 52.07±1.91 no entanto o valor máximo de luminosidade foi no dia 21 de setembro (56.27 ± 3.19), com uma diminuição posterior. Os valores de vermelhidão não mostraram alteração estatisticamente significativa com os valores pairando em torno do lado positivo de 0. A propriedade de textura (dureza) aumentou em ambos os locais de colheita, sem alteração na coesão das amostras. O pH diminuiu em ambos os locais de colheita com o valor inicial de 2.67 ± 0.01 para CAM-CV e 2.54 ± 0.01 para CAM-SR e um valor final de 2.53 ± 0.01 para CAM-CV 2.44 ± 0.01 para CAM-SR, O teor de sólidos solúveis também diminuiu a partir do valor inicial de 10.44 ± 0.02 e 8.78 ± 0.01 para 7.37 ± 0.02 e 8.59 ± 0.02 para CAM-SR e CAM-CV respetivamente. A capacidade antioxidante quando medida por FRAP mostrou um aumento nas bagas de CAM-CV a partir do valor inicial de 9.85 ± 0.90 para o valor final de 19.24 ± 6.13. Ao medir usando a metodologia DPPH, a capacidade antioxidante aumentou 128% e 136% a partir dos valores iniciais de 8.51 ± 1.97 e 11.85 ± 0.98 para CAM-SR e CAM-CV respetivamente. O conteúdo fenólico total não apresentou alteração para as bagas de CAM-SR, mas apresentou um aumento temporário nas bagas de CAM-CV. Os efeitos das condições de armazenamento

nas propriedades da baga mostraram que o frio induziu uma mudança mais lenta na cor (os valores de luminosidade diminuíram e os valores de vermelhidão aumentaram, mais lento do que à temperatura ambiente), maturação mais lenta, menor percentagem de perda de peso. A capacidade antioxidante medida com DPPH diminuiu para a temperatura de 4° C, sem alteração em comparação com as bagas armazenadas a 20 ° C. Quando medido usando FRAP, nenhuma mudança foi observada em ambas as condições. O conteúdo fenólico total não apresentou alterações em ambas as condições de temperatura. Finalmente, a aplicação da baga como extrato proporcionou uma influência mais forte na capacidade antioxidante geral e no conteúdo fenólico quando comparada com a adição da baga liofilizada. Os resultados do questionário hedônico mostraram que a adição da baga não foi percetível.

Palavras-chave: Camarinha; *Corema Album L.* (D) Don; Antioxidantes; Fruto Silvestre; Iogurte

Abstract

The wild coastal berry native to the Iberian Peninsula is an underused berry with high antioxidant potential. This berry due to manly competition from invasive species and habitat destruction this berry might become lost. This study contributes to the characterization of the physicochemical attributes with a focus on their antioxidant capacity. This was done during their maturation period between September and October, obtaining the samples from two different areas ("Serra d'El Rey" (CAM-SR) and "Peniche" (CAM-CV)), and during storage at two different temperatures (20°C and 4°C). Additionally, the addition of the berry to a sweetened natural yogurt was also made. The maturation study revealed that the berries sizes tend to reduce on later stages of the maturation period dropping 11% and 19% for the berries from CAM-SR and CAM-CV from the initial value of 0.461±0.043 and 0.467±0.033. A decline of 20% and 40% for their weight was also observed. For the colour parameters the harvesting sites behaved differently with the berries from CAM-SR showing an initial value of 53.03±2.64 and a final value of 55.65±2.97, whilst CAM-CV showed the highest L* value on the 21st of September (56.27±3.19), with a posterior decrease. The a* values showed no statistically significant change with the values hovering around the positive side of 0. The texture property (hardness) increased on both harvesting sites, with no change in the cohesiveness of the samples. The pH decreased on both harvesting sites with the initial value of 2.67 ± 0.01 for CAM-CV and 2.54 ± 0.01 for CAM-SR and a final value of 2.53 ± 0.01 for CAM-CV 2.44 ± 0.01 for CAM-SR, SSC also decreased from the initial value of 10.44±0.02 and 8.78 ± 0.01 to 7.37±0.02 and 8.59 ± 0.02 for CAM-SR and CAM-CV respectively. The antioxidant capacity when measured using FRAP showed an increase in the berries from CAM-CV from the initial value 9.85±0.90 of to the final value of 19.24±6.13. When measuring using DPPH methodology the antioxidant capacity increased 128% and 136% from the initial values of 8.51 ± 1.97 and 11.85 ± 0.98 for CAM-SR and CAM-CV. Total phenolic content showed no change for the berries form CAM-SR and a temporary increase on the berries from CAM-CV. The effects of storage conditions on the berry properties showed that cold temperatures induced slower change in colour (L* values dropped and a* values increased, slower than ambient temperature), slower maturation, lower weight loss %. The antioxidant capacity when measured using

DPPH showed a decrease while under 4°C, with no change in comparison with the berries stored at 20°C. When measured using FRAP, no change was observed under both conditions. The total phenolic content showed no changes under both temperature conditions. Finally the application of the berry as an extract provided a stronger influence in the overall antioxidant capacity and phenolic content when compared with the addition of the freeze dried berry. The hedonic questionnaire results showed that the addition was not perceived.

Keywords: “Camarinha”; *Corema Album L.* (D) Don; antioxidants;Wild berry; Yogurt

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1. Introduction

Currently the global berry market is projected to achieve US$ 70,000 Mn by 2027, with €40.39 Mn belonging to the food and beverage industry, the rest distributed between the pharmaceutical, cosmetics and dietary supplements [1]. Recently there has been an increase in the consumption of fruits and vegetables in Europe, in 2020 Freshfel Europe reported an increase of 4% in relation to 2017 data, this new value of 363.76g, however in still below the recommended intake by the World Health Organization of 400g. In Portugal the average from 2016 to 2020 in daily fruit intake was 266.1g per habitant [2]. Therefore, there is a need to further increase this average value. In 2019 the Organization for economic co-operation and development reported that 66.4% of the population above 15 years old consumed fruit daily [3].

The Statistics Portugal reported that the fruits consumed by the Portuguese people were oranges, apples, fresh peach, pear, and table grapes. These five fruits contribute 75.1% of the total fruit consumption [4].

In the 24.9% that remains includes the category of small fruits, of these several can be foraged in various parts of Europe. Berries such as blackberries (*Rubus fruticosus agg*), raspberries (*Rubus idaeus*), red currants (*Ribes rubrum*), bilberries (*Vaccinium myrtillus*), cowberries (*Vaccinium vitis-idaea*), elderberries (*Sambucus nigra*), rowan (*Sorbus aucuparia*), hawthorn (*Crataegus monogyna*), cherry plum,[5] as well as Portuguese crowberries (*Corema album L (D.) Don*). Some of these wild berries are currently being cultivated for mass production, blueberries, raspberries, and blackberries are readily available is various supermarkets, as fresh fruits as processed products, such as jams, compotes, as highly processed products such has sausages developed by Osipova M.V. (2021) [6], or the hamburgers developed by Ganhão [7]. Other uses include the application in dairy products such as yogurts [8].

1.1 Nutraceutical properties

The application of the berries in foods stuffs mainly focus on using the highly antioxidant capacity of these fruits. These compounds reduce the oxidative stress of the

environment they are present [9]. Besides the effect ROS, there is also some evidences that wild berries might exhibit anticarcinogenic properties[10].

Oxidative stress is related to reactive oxygen species (ROS), these molecules are free radicals that happen naturally in living organism during chemical reactions. [11] Free radicals are not stable and therefore seek stability. If the radical reach DNA molecules it could cause damage, via unwanted oxidation, continuous damage to the DNA causes premature aging and could lead to the early manifestation of known age associated diseases such as Parkinson's, and Alzheimer's [12]–[15] On the other hand, if the radicals are captured by molecules that can stabilize these ROS the oxidation is minimized. Therefore, it has been known that consumption of antioxidants is recommended to help reduce oxidative stress [12], [13], [16]–[18]. *Corema album L* (D.) Don has shown to have positive results in a yeast Parkinson's Disease model, this results are not entirely related to the capture of free radicals. [19] There are various sources of high amounts of known antioxidants in plants and algae[20].

Products that have high levels of fatty acids, especially those with a high fraction of unsaturated fatty acids, are prone to lipid oxidation. The compounds released in the reactions are more volatile and give the unpleasant smell of rancidity[21]. Artificial preservatives can be used to delay this process. Compounds such as Propyl gallate (E-310), Tertiary-butyl hydroquinone (E319) and Butylated hydroxyanisole (E-320) are some of the authorized artificial preservatives that are used as antioxidants. Natural alternatives are also available and some of the authorized compounds are rosemary extract (E-392) and ascorbic acid (E-300)[22].

1.1.1 Phytochemical composition

The compounds that have nutraceutical properties can be related to secondary metabolites these have various functions, from defensive mechanisms to pigments[23]. The largest group of phytochemicals is the alkaloids, these are secondary metabolites consisted of nitrogen containing, cyclical compounds. Although common not all alkaloids are beneficial, such as the caffeine and berberine have beneficial uses, whilst others are toxic (sanguinarine, scopolamine) or highly addictive (cocaine, morphine). These compounds are often exploited for pharmaceutical uses, poisons, or narcotics[24].

Another group of phytochemicals are the glycosides, and these molecules consisted of condensed sugar molecules, which have a strong bitter taste, and various uses depending on the structure, some can act as flavouring, others aid digestion, and other if not handled properly can be poisonous. Some examples of plants known to have beneficial compounds are, *Juniperus phoenicea*, this is a plant that has been extensively studied, and the compounds extracted from the leaves and berries were shown to have antimicrobial properties [25]–[27], hepaprotective effect due to high levels of antioxidants[28]. The ethyl acetate extract of the leaves of *Corema album L* (D.) Don was shown to have a cytotoxic activity against HT-29 human colon adenocarcinoma cell line [29]. The cytotoxic response was related to the presence of two dihydrochalcones, 2',4'-dihydroxydihydrochalcone and 2'-methoxy-4'-hydroxydihydrochalcone. The activity of these compounds was decreased when they were administered in the presence of higher levels of antioxidants, this interaction was used to determine the reactions mechanisms of the compounds, in this case this behaviour helped determine that the reaction mechanism involves the presence of free radicals[29].

1.1.2 Plant phenolics

A large variety of fruits and phenolics extracts have been reported to be effective enhancers of the oxidative stability of foods [30] with berries being among the best sources of phenolic compounds[31].

These compounds are synthesised in plants and have various functions related to them. These compounds contain multiple aromatic rings with the most basic phenolic compound being the C6 phenol (Figure 1). Their classification differs depending on their carbon number and functional groups. One pathway the produces phenolic compounds is the shikimic acid pathway, this synthesizes the aromatic amino acids phenylalanine, tyrosine and tryptophan, these compounds themselves are the starter units in the phenylpropanoid pathway [32], that leads to the flavonoid biosynthesis pathway [33], which give the starter compounds in the anthocyanin biosynthesis[34].

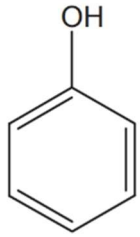

Figure 1 - Phenol[35]

Anthocyanins (Figure 2) are C_{15} phenolic compound [35] that play a role as a powerful antioxidant, and are responsible for the colour of multiple plants and fruits. The colours vary depending on the pH. Under acidic conditions they appear red and under basic conditions the appear blue. Anthocyanins are not very stable by themselves and therefore usually are coupled to glycosides, turning the compound into an anthocyanidin, the most common anthocyanin in plants is cyanidin-3-glucoside[36]. Although common in plants not all anthocyanins are found in the same amounts in the various types of plants. In red berries the unmethylated anthocyanins and their corresponding glycosides are most abundant.

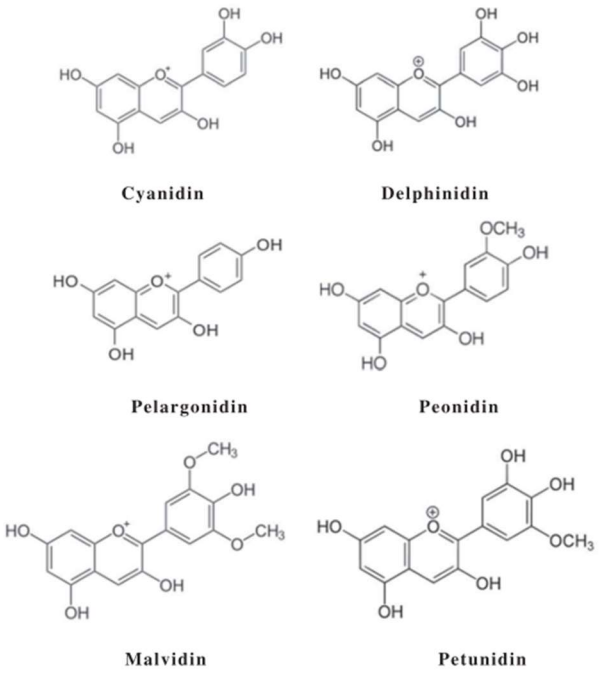

Figure 2 - Struture of the major Anthocyanins [45]

A mutual exclusive compound with anthocyanins are the Betalains, these are present in the order of *caryophyllales*, this order includes plants such as beetroot, amaranth, cacti[37], [38]. Another C_{15} phenolic compounds are the flavanols, the functional groups are different from anthocyanins. They are present in high amounts in berries as well, and the main function is as pigments[39].

The antioxidant activity of phenolic compounds in fruits, vegetables and spices is mainly due to their redox properties and chemical structures, which can act as reducing agents, free radical scavengers, metal chelators and singlet oxygen quenchers (Rice-Evans et al., 1997)[40].

1.2 Methods for antioxidant detection

Various methodologies exist to determine the concentration of the antioxidant's species present in a sample, below some are described briefly.

1.2.1 Folin-ciocalteu method

The Folin-Ciocalteu methodology can determine the total phenolic content (TPC) of a liquid substrate. It is based on the reduction of the folin-ciocalteu reagent in alkaline conditions, this reduction causes a change in the colour of the solution from yellow to blue. The change in colour increases the absorbance values at λ765 nm. Using a standard curve of gallic acid it is possible to establish a correlation between the absorbance and the concentration of phenolic compounds in the sample.

1.2.2 DPPH radical scavenging

This method exploits the physical changes of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) to determine the antioxidant capacity of solutions. This molecule has a free radical and in proximity with other molecules it loses its radical and swaps from purple

to yellow (Figure 3), the purple molecule has an maximum absorbance at 517 nm and it decreases proportionally with the radical concentration[41].

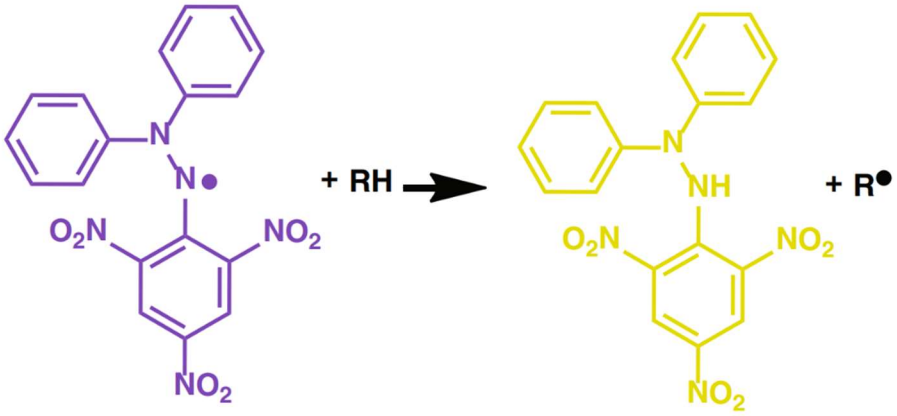

Figure 3 Structure and reaction of 2,2-Diphenyl-1-picrylhydrazyl [58]

1.2.3 FRAP

This methodology described by Benzie & Strain (1996)[42] measures the reductive potential of antioxidants by reacting a complex of ferric ions (Fe^{3+}) with the antioxidants. The reaction occurs when the antioxidant molecule gives an electron to the complex converting it into a ferrous (Fe^{2+}) complex. Depending on the complex used the resulting ferrous complex has different absorption values, the original complex, ferrous tripyridyltriazine has an intense blue colour with a maximum absorption 593 nm, alternatively potassium ferricyanide can also be used, and the resulting compound is called prussian blue which has a maximum absorption at 735nm. There is, however, a disadvantage in using Prussian blue as the reactant, this complex tends to form a suspension and stain the measurement vial. A tensioactive compound may be used to stabilize. Other variations of the FRAP method have been developed with some using electrochemical processes to determine the reductive power of antioxidants. Since this method is a non-radical single electron transfer method it can be used in conjunction with other methods to help determine the characteristics of the antioxidants in a sample[43].

1.2.4 ORAC

This methodology measures the capacity of an antioxidant to donate a hydrogen atom to stop the chain reaction caused by free radicals. The radical scavenging capacity can be measured for different radicals by using different radical generators, for the peroxyl radical ($ROO^{\bullet}$) the various options can be sorted into two main categories, hydrophilic and hydrophobic, α,α,-azobisizobutyronytril (AIBN), 2,2-azobis (2-amidinopropane) chlorohydrate (ABAP), 2,20-azobis (2,4-dimethylvaleronytril) (AMVN) are examples of hydrophobic and 2,20-azobis(2-amidinopropane) dihydrochloride (AAPH) are examples of hydrophilic compounds. As for hydroxyl radical ($^{\bullet}OH$) generators the system of H_2O_2-$CuSO_4$ can be chosen to generate the radical. The results are measured using fluorescence; therefore, a fluorescent probe is needed, this probe can be proteins such has β-phycoerythrin or synthetic dyes such has fluorescein and nile blue. These compounds lose their fluorescence in the presence of free radicals. This change is used to measure the presence and capacity of radical scavengers[43].

Due to the differences between each analysis method and their chemical pathways, the benefits and drawbacks of each method should be considered when choosing the ones that will be applied.

1.3 Corema album L (D.) Don

The berry with particular interest in this study is the Portuguese crowberry (*Corema album L* (D.) Don), also known as "camarinha" this evergreen shrub is endemic to the Atlantic coast of the Iberian Peninsula. There are two species in the genus *Corema*, the species *Corema conradii* which is native to the north American east coast, and the *Corema album L* (D.) Don, which is native to the western coast of the Iberian Peninsula. There is as well an subspecies in the Azores archipelago promptly named *Corema album* subsp. *Azoricum*, present in 66% of the islands and under 200m of altitude[19]. The differences between the two species seems to be the type and leaf density as well as fruit density [44].

The shrub grows mostly in sand dunes and cliffs systems [45] sharing its habitat with other plants, namely *Calluna vulgaris* (Figure 4)the invasive species *Carpobrotus edulis (*Figure *5), Ulex sp (*Figure 6*)* and *Juniperus phoenicea* (Figure 7) [46]. The soil

in which the berries are found is part of the coastal dunes with a slightly acidic to neutral pH[47], [48]. In the study developed by Jose M. Fedriani (2009) it was shown that the shrub growth changes if it is ingested by animals, with some specimens having a more significant growth under a mother plant if ingested by foxes [49]. Also, in another study, an increase of growth in open sites if ingested by seagulls, blackbirds, and rabbits, was showed [45].

The shrubs are dioecious, which means there are male and female organisms. It is a small shrub growing to less than a meter high with small thin leaves with curled edges. The blooming season is between March and April, both male and female flowers grow at the extremity of the branch. The male specimens have stamen whilst the female flowers do not. During the months of June to October the distinction between male and female becomes easier, as only the female produces the white, pearl shaped, edible berries. [50] The flowering branch keeps growing from the end even after pollination, therefore the fruits can be in the end or at the middle of the branch, in clusters of up to 4 berries [51]. The growth and maturation period of the plant was studied by Tomás Magalhães (2015) [52] and 8 stages were suggested, as described in Table 1.

Figure 4 *Calluna vulgaris [53]*

Figure 5 *Carpobrotus edulis [54]*

Figure 6 *Ulex sp. L.* picture by J. Antúnez Glez - CC BY-NC *[55]*

Figure 7 *Juniperus turbinata* subsp. turbinata*[56]*

Table 1 Growth stages of *Corema album L* (D.) Don. [adapted from Tomás Magalhães (2015) *[52]*

STAGE	DESCRIPTION
0	Bud development
1.1	Leaf development (main branch) winter
1.2	Leaf development (main branch) spring
3	Elongation of the vegetative branch / shoot development
5.1	Emergence of male inflorescence
5.2	Emergence of the female inflorescence
6.1	Male flowering
6.2	Female flowering
7	Fruit development
8	Fruit and seed maturation

The berries at the end of stage 7 are fully white with some of them develop a slight green discoloration which is associated with chlorophylls, afterwards in stage 8 they start to become translucent, meaning that the seed is visible through the skin and some turn pink. [19], [52]. The shrubs shown in Figure 8 represent the plants under different conditions, Figure 8 A shows a shrubs in the midst of other plants whilst Figure 8 B Shows an isolated specimen.

Figure 8 *Corema album L* (D.) Don shurb in harvesting site (A) Cabo Carvoeiro (CAM-CV) and (B) Serra d'El Rey (CAM-SR)

1.3.1 Quality attributes

The quality attributes of fruits are varied [9], sugar content can be measured using soluble solid content (SSC) measured in °brix and in *Corema album L. (D) Don* there are some studies that represent their results. In a study developed by Sonia C. Andrade (2015)[57] the berries from *Corema album* L (D.) Don collected in Mira beach, Coimbra found an average value of 6.1 °Brix for the soluble solids content (SSC), whilst Pimpão et al. (2013) [58] found a higher value of 6.8° Brix in *Corema album L (D.) Don* berries from the Comporta region. Other studies but in this time made with commercially available berries, such has blueberries, Julie A. Mennella et al. (2017) [59] found values of SSC from 8.0 to 12.0 °Brix in 3 different cultivars from Florida, USA. The cultivars were, Arcadia, Kestrel and the one with the highest SSC value Keepcrisp, Molina et al (2008) [60] reported highest SSC values between 9.8-12.7 °Brix, in blueberries grown in Andalusia, Spain. In another type of commercially available berries, the raspberries analysed by Lee et al. (2014) [61] showed sugar contents between 10.4 to 16.2 °Brix in a study in south Korean grown berries. Palonen and Weber (2019) [62] in a study on raspberries from different genotypes grown in multi-bay high tunnels in New York, USA found values ranging from 9.5 to 11.4 ºBrix.

Although the sugar values of fruits are an important quality in berries and berry products there is also the relation between acidity and sweetness. The sweetness of a product is counteracted with the acidity and when acidity increases perceived sweetness decreases. In berries the acidity is related to the presence of organic acids such has malic and citric[63]. Berries acidity values can be represented in pH values or using total titratable acidity, these two values cannot be correlated [64] and only the total titratable acidity can be related with the sugar content[65]. In *Corema album L (D.) Don* berries Sonia C. Andrade (2015) [57] found the values for total acidity of 10.7 % (citric acid). Pimpão et al. (2013) [58] found the values of 7.4 [g(citric acid)/L]. In another berries such as blueberries the values found were between 2.6-5.6 TAc [g(tartaric acid)/L], raspberries values between 9.1-14.6. The acidity of the berries seems to vary highly between different types.

Another aspect that is of importance to the acceptability of the berries is their colour due to being part of the visual appearance and therefore associated with the perceived quality. The values of the colour parameters can be quantified for better understand the relationship between colour and quality.

Another factor in the acceptability is the texture of the fruit, as this component changes during maturation, the analysis of how these changes occur is of interest. The changes in texture can be attributed to the behaviour of the compounds that make the cell walls, as these change with the maturation time, increasing of their solubility, therefore softening the berry. Compounds such has pectin, cellulose, hemicellulose and glycoproteins are involved in these changes.[66] Understanding the expected values of the particular berry in a given analysis can be used to predict the maturation state of berries and certain relationships between quality and texture can be drawn.[67] Due to these changes when ripe berries are susceptible to damage and therefore proper precautions should be taken to increase their shelf-life. Proper storage conditions and harvesting time are factors involved in these precautions. As far as methodologies goes several methods can be used to analyse these proprieties, Changying Li (2011) [68] used a laser air-puff firmness test to evaluate the quality of blueberries. With this technic they were able to differentiate from two cultivars. Other

techniques can be used to analyse the texture of foodstuffs, texture profile analysis (TPA) simulates the experience that would be felt during mastication by using two sequential plunges. This technique has been used for solid and liquid food analysis, however the interpretation of the results is quite different, whilst in solid food analysis the force values can be associated with the hardness in liquids the behaviour of compression is different, and therefore the interpretations cannot be the same [69].

1.3.2 Post-Harvest Handling

After harvest the berries start to decay, during this time there is an increase in the softness of the fruit which by the time it reaches the consumer, the change might be too high and therefore reducing the value of the product. These changes are the result of enzymatic action within the fruit; therefore, several techniques can be used to slowdown the decay. Storing the fruits in at a lower temperature slows the activity of enzymes that leads to softening. As a benefit to this storage lower temperatures (0ºC) reduce the metabolic rates of the organisms involved, and therefore storage at these temperatures suppress fungal decay [70], with the reduction of storage temperatures from 22ºC to 5ºC achieving an increase of 290% to 360% with regards to post harvest life in blue berries[71]. The container that the berries are kept is also important, rigid containers protect from physical damage, plastic clamshell boxes can be used, wooden boxes or larger crates. Each with their own benefits and drawbacks [66].

The storage of *Corema album L* (D.) Don berries at ambient temperatures can be used for limited amounts of time, in an open container the berries remain in good condition after two weeks, with only some presenting pink coloration. After three weeks the berries deteriorate fast, however some berries remain unchanged. During a study made by Luís Simões (2018) [51] the berries of the Portuguese crowberry remained edible after four weeks at 4°C. Studies found that under these conditions the rates of respiration and water loss are reduced, the stability of phenolic antioxidants

is increased. Anthocyanins productions seems to be lower at reduced temperatures [70].

The storage at below 0°C berries is also a good way of preserving foodstuffs, however freezing creates ice crystals, and that can cause damage to the cells. The slower the freezing time the bigger the crystals and the more damage it causes to the fruits, faster freezing such has using liquid nitrogen can create smaller crystals and therefore longer shelf-life [72]. These changes however can be adequate if the intention is to have the berries further processed.

1.4 Objectives

During the last years, numerous plant materials have been investigated as sources of substances with antioxidant properties. However, there is a lack of knowledge about the composition and antioxidant potential of wild fruits from the West Coast of Portugal. The primary objective of the present work was to investigate the composition and antioxidant potential of *Corema Album L (D.) Don*. To achieve this a study is going to be conducted during the maturation period of the berry and their behaviour during storage at two different temperatures. An additional objective is to evaluate how the berries affect the properties of a sweetened natural yogurt when added as an extract or whole.

2. Materials and methods

The present work is divided in three phases, phase (1) maturation evolution, stage (2) storage temperature study and finally the stage 3 with the application of the berry extract in a manufacturing yogurt, as shown in Figure 9. Also, it can be seen in the flowchart, the physico-chemical methodologies performed in each one stage.

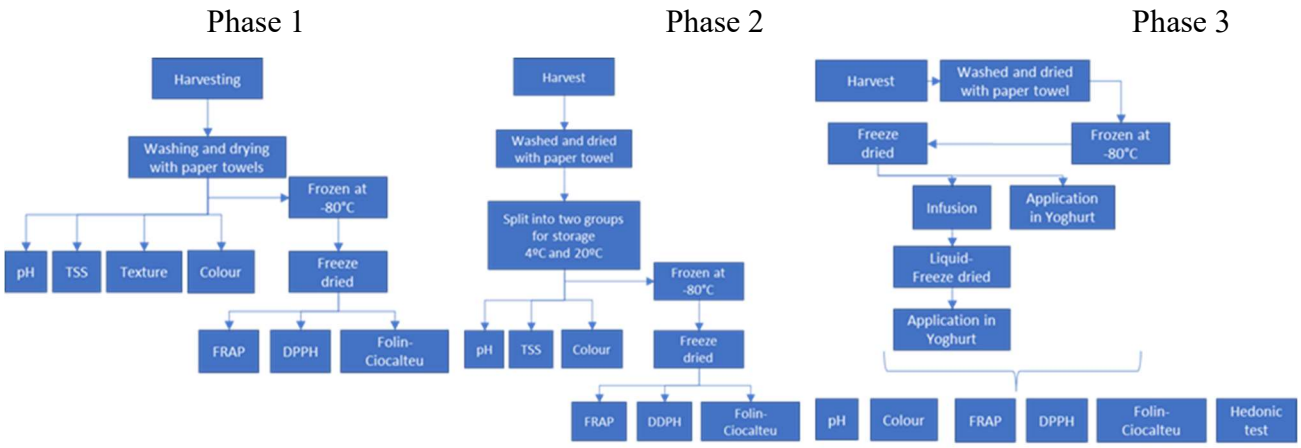

Figure 9 – Flowchart of the processes and analysis realized in "camarinhas" used in the three phases of the present study.

2.1 Reagents

The reagents used in the physical-chemical methodologies have the required degree of purity so as not to interfere in the analysis (P.A.) and are listed below.

- ✓ 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich, Germany)
- ✓ 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) (≥ 98%Sigma-Aldrich, Switzerland)
- ✓ 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (TROLOX) (97% Sigma-Aldrich, Russian federation)
- ✓ Acetic acid glacial (VWR Chemicals, France)
- ✓ Ethanol (Absolut Food grade, AGA – Álcool e géneros Alimentares S.A, Portugal)
- ✓ Folin-Ciocalteu's phenol reagent (Merck, Germany)

- ✓ Gallic Acid (anhydrous Merck, Germany)
- ✓ Hydrochloric Acid (36.2%, VWR Chemicals, France)
- ✓ Iron (III) chloride hexahydrate (extra pure, Scharlau, Spain)
- ✓ Methanol (100% VWR Chemicals, France)
- ✓ Phenolphthalein (VWR Chemicals, EC)
- ✓ Sodium Acetate trihydrate (100% VWR Chemicals, Belgium)
- ✓ Sodium carbonate (BIOCHEM Chemopharma, France)
- ✓ Sodium hydroxide (Labchem)

2.2 Equipment

The equipment's used were in good condition as to not interfere with the quality of the analysis, and are listed below:

- ✓ Analytical Scale (max 120g, d=0,1mg)
- ✓ Centrifuge (Eppendorf, 5810R, Germany)
- ✓ Cooking robot (Bimby – Vorwerk, Thermomixer 31-1)
- ✓ Digital calliper (Traceable, VWR, país)
- ✓ Digital scale (max 3500g d=0,01g, Kern)
- ✓ Microplate reader (Epoch 2, Biotech Instruments Inc, USA)
- ✓ Oven (Memmert, IF110er)
- ✓ Paper filter
- ✓ Cylindrical probe of 2 mm diameter)
- ✓ Potentiometer (WTW, Inolab pH/ION)
- ✓ Refractometer (RFM340+, Bellingham-stanley)
- ✓ Texturometer (Stable Micro Systems, Surrey, England)
- ✓ Tristimulus colorimeter (Minolta chromameter, CR-400, Osaka, Japan))
- ✓ Ultrapure water filter (Milli-Q Advantage A10, Merck)

2.3 Fruits sampling

White "camarinhas" (*Corema album* L. (D.) don) that were not visually damaged nor showed signs of translucence were collected during the months of September and

October of 2020, from two locations, one in Serra d'El Rey, near to "praia de Covões" in Óbidos and another in *Cabo Carvoeiro*, at Peniche. (Figure 10). After the berries harvest, they were kept in a food grade plastic bag and transported in a cold environment. Afterwards the berries were washed to remove any leaves and debris and patted dry using paper towels. Any berries that showed signs of discoloration or damage were discarded at this point.

(A) **(B)**

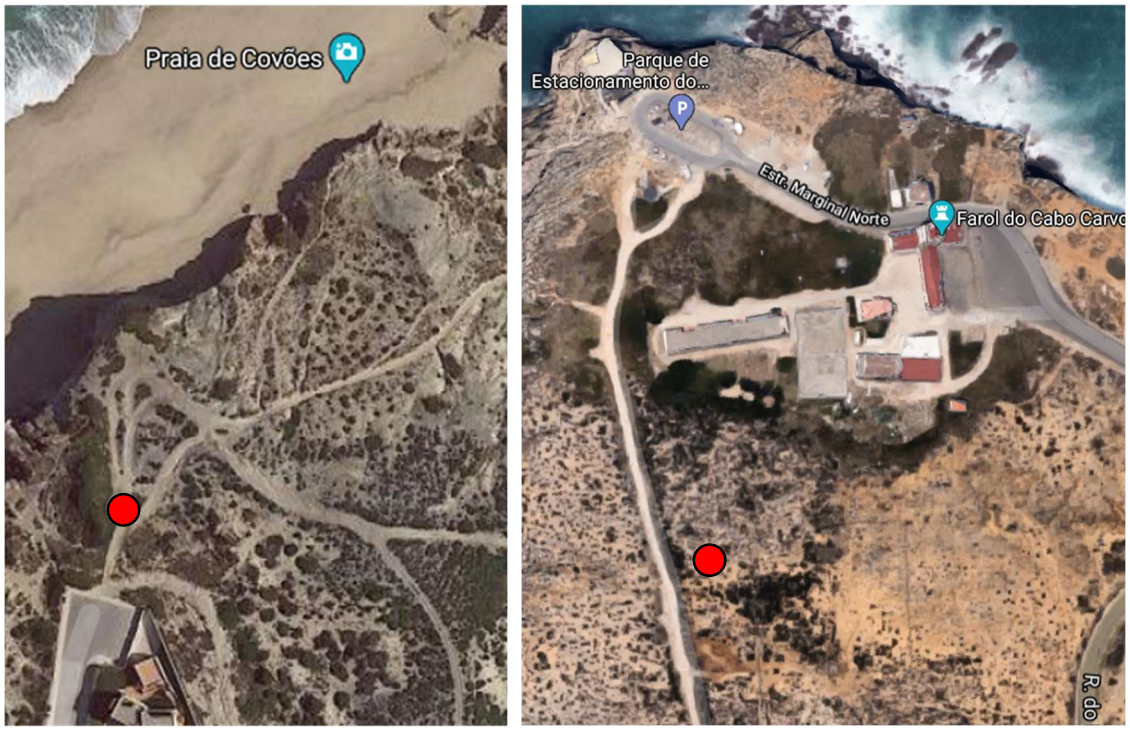

Figure 10 – Harvesting location in *Serra d'El Rey* (*Óbidos*, *Portugal*, 39°24'03.9"N 9°16'25.7"W) (A) and *Cabo Carvoeiro* (*Peniche*, *Portugal*, 39°21'35.2"N 9°24'20.9"W) (B). The red dot indicates the precisely location that berries were harvested.

2.4 Evaluation of maturation stage of berries harvested in "*Cabo Carvoeiro*" ("*Peniche*") and "*Serra d'El-Rei*" ("*Óbidos*")

The study of evaluation of berries maturation stage was performed during the September (17th, 21st and 30th) and October (12th and 30th) of 2020. In each day analysis, about 300 berries were collected in "*Serra d'El Rey*" and "*Cabo Carvoeiro*" and were

identified as CAM-SR and CAM-CV, respectively. To reduce the variance of the sample, the collection was taken from the various shrubs in the studied area.

2.5 Effect of postharvest storage at room and refrigerated temperature on berry quality

The changes of quality berries during the postharvest storage at room and refrigerated temperature (4±1°C), were evaluated with 700 berries collected in *Cabo Carvoeiro* at different days analysis: 0, 1, 4, 8, 12, 15 and 28 and 0, 1, 4, 8, 12, 15, 19, 22, 25, 28, 32, 39, 55 for room (RT) and refrigerated temperature (REF), respectively.

For both samples' groups, the berries were left in a single layer and in a large petri dish uncovered and untouched.

2.6 Impact of berry extract on yogurt quality

The enrichment of yogurt was performed after freeze-dried of berries which was prepared as follows. After, the freeze-dried berries were crushed in small piece, and mixed with recently boiled water in a proportion ratio of 1:10 (m:v) and left for 5 minutes. Then, the mixture was filtered through a sieve to collect the liquid phase and proceed to freeze-dried for used in yogurt manufacturing. In this, two types of yogurts were performed. Firstly, the CAM yogurt sample was prepared using 5g of the freeze-dried extract and the LIO yogurt sample was prepared using 10g of freeze-dried berries. For both yogurt samples, the next ingredients were mixed: 500 mL of pasteurized milk ("Pingo Doce"), 60 g of natural yogurt ("Pingo Doce"), 20 g of dried milk powder ("Nido") and 40g of white granulated Sugar ("Pingo Doce"). After the mixture, the heat treatment at 50 °C for 4 minutes in the cooking robot was performed. A control sample (CTR) was made using 50g of sugar. The mixture was then transfer into sterilized jars (around 90 g per jar) and left in a pre-heated oven at 45 °C. pH measurements were taken every half hour until the value reached the value of pH 4.5. The yogurts samples were then removed from the oven, cooled at room temperature, and stored at 4°C.

2.7 Analytical procedures

The physical and chemical methodologies used in each phase of the present work are described below.

2.7.1 Visual evaluation and weight loss

Regarding the importance of visual appearance of the berries during the maturation development, in the study of impact of storage temperature, about 30 berries (in triplicate) was placed in a petri dish. These berries were monitored periodically for evaluation in appearance and weight loss. One group of these berries was kept at room temperature (20 ± 1°C) and another under the refrigerated temperature (4 ±1 °C). In order to register the visual fruit changing in appearance, a photographic record was taken, in each day of storage analysis: 0, 1, 4, 8,12, 15, 19, 22, 25, 28, 32, 35, 39.

For the weight loss evaluation, the methodology described by Pinheiro et al. (2019)[73] was followed. Briefly, after the weight measurements were taken, the berries were placed back in their original conditions, and the Equation 1 was used, where the W_0 is the average of the initial weight (three samples) and W_t is the average weight at storage day t (the same three samples at t days in storage).

$$(\%)Weight\ loss = \frac{W_0 - W_t}{W_0} \times 100 \qquad \text{(Equation 1)}$$

2.7.2 Biometric parameter

The biometric parameters like size, and shape measurements of 30 berries picked randomly, were realized using a digital calliper. The height of berries was defined as the distance between the stem and the end of the berry, and the diameter (Figure 11) was defined as the perpendicular distance to the height (Figure 11). The results were expressed as the average of 30 berries.

(A) **(B)**

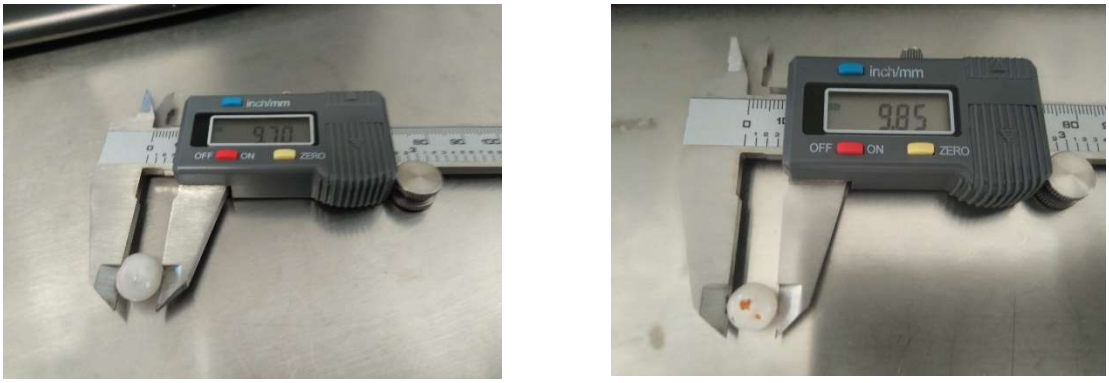

Figure 11 - Height (A) and diameter (B) of a single *Corema album* L (D.) Don berry.

2.7.3 Colour

Colour analysis was determined following the methodology described by Pinheiro et al (2019) [73]. Briefly, the colour parameters used in the CIELab L*a*b* system were obtained using a colorimeter, after calibration using a white standard tile (L*=97.10, a*=0.19, b*=1.95). The illuminate and observer used were D65 and 2°, respectively. Thirty measurements were realized in a group of 20 berries randomly selected. The obtained colour parameters were the L* (from 0 to 100), represents the black to white, a* (from -60 to +60) represents the greenness to redness and b* (from -60 to + 60) that represents the blueness to yellowness.

2.7.4 pH and SSC

The pH of the pulp and juice of the *Corema album L* (D.) Don berries were determined in a potentiometer. Before the determination, the equipment was calibrated using the manufactured recommended procedure. To obtain the pulp and juice of berries, twenty berries were pierced with tweezers and then seeds were removed, this was done to minimize the damage to berry prior to being crushed in a mortar and pestle. The

resulting pulp and juice were then measured in triplicate and the results were expressed as the mean value ± standard deviation.

The soluble solids content (SSC) of berries was measured in a refractometer, where a drop of the berries juice was placed in the equipment. The results were expressed as ºBrix and represents the average of three measurements ± standard deviation.

2.7.5 Texture

The texture evaluation was made using a texture profile analysis (TPA) following the methodology described by Pinheiro et. al. (2019) [73]. Briefly, the berries were placed sideways in the plate of a texturometer equipped with a load cell of 5kg, a cylinder probe of 2.00 mm and using a speed of 1.00 mm/s with 5.00 seconds waiting time between the two bites. The berry was in place loosely while the piston moved. From this analysis the values of cohesiveness (A2/A1) (Figure 12) and hardness were obtained and expressed as the average of 30 berries ± standard deviation.

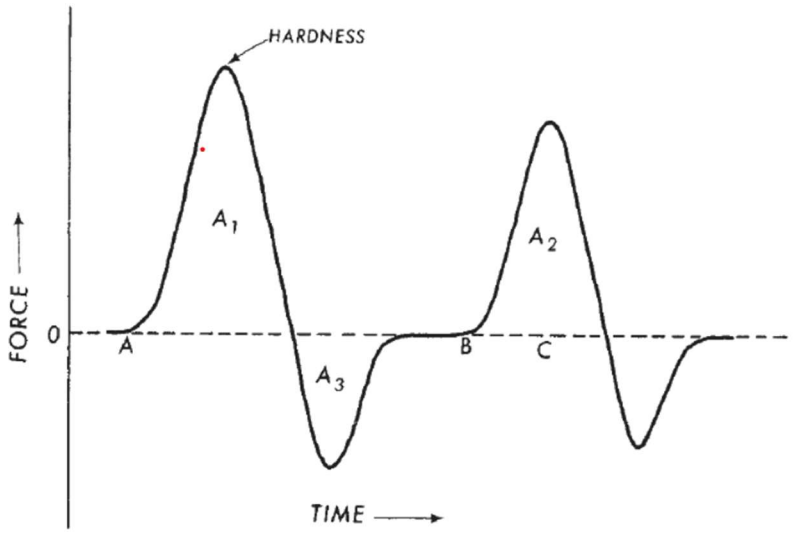

Figure 12 – An example of the texture profile analysis (TPA) graph [69]

2.7.6 Extraction optimization of antioxidant activity and phenolic content

The extraction procedure of antioxidant capacity and phenolic content was adapted from the methodologies used by João Jacinto and colleagues [74]. Briefly, the freeze-dried berries were cut into small pieces, and 0.2g was weighed into the extraction vessel and 3.6 mL of ethanol concentration (0%, 25%, 50%, 75%, 100%- Ethanol/Water) was added and were kept overnight at 4°C. The next day the samples were centrifuged at 4°C and the supernatant was decanted into paper filter, and the final extract was kept at 4°C until use After the obtained results and discussion of them, the solution of ethanol at 50%, was taken account as better extraction solution and was used in the present study. In this sense, the extraction procedure was as follows: The freeze dried berries were mixed with ethanol at 50% at a ratio of 1:18 (m:v) and kept at 4°C overnight. Then, the mixture was centrifuged, filtered and the clear supernatant was analysed. The extraction was made in triplicates.

2.7.7 DPPH radical scavenging activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity of the optimized extracted of berries was determined according to the method reported by Brand-Williams et al. (1995) [75] and adapted to 96 well-plate assay. The DPPH solution was prepared by dilution with methanol at 80% until reaching the absorbance of 1.1 at microplate reader at wavelength of 517 nm. Briefly, 50µL of sample/standard was mixed with 150 µL of DPPH solution and kept in the dark for 30 minutes. After, the reaction was realized in a microplate at λ517 nm and used the Trolox standard for calibration curve, at concentration level between 0.1 and 0.01mg/mL. This analysis was made in triplicate and the results were expressed as the mean of 9 determination in Trolox Equivalent Antioxidant Capacity per gram of freeze-dried extract (mg TEAC/ g))[75]–[77].

2.7.8 FRAP

The antioxidant methodology by FRAP (Ferric reducing antioxidant power) was performed in the same berry extract, as before mentioned, with a dilution of 1:4, and using the Ethanol solution at 50%. The procedure realized was according the Benzie and Strain (1996) [42], with alterations by Santos et al. (2019) [78]. Briefly, the solutions required to prepare the FRAP reagent were made freshly: 10mM TPTZ dissolved in a 40 mM HCL solution (A), 20mM $FeCL_3.6H_2O$ (B), and a 300 mmol of acetate buffer pH 3. 6(C). The FRAP reagent was prepared using 2.5mL A + 2.5 mL B and 25mL C. The reaction was realized with FRAP solution (2.7 mL), 270 µL nanopure water with the extract samples (90 µL), and afterwards, warmed in a water bath at 37 °C for 30 min. Then, the final product (ferrous tripyridyltriazine complex) was read at 595 nm. The results were obtained using a Trolox calibration curve and then expressed as the average of 9 determination, as Trolox Equivalent Antioxidant Capacity per gram of freeze-dried extract (mg TEAC/ g).

2.7.9 Total phenolic content

The total phenolic content of the optimized and previously described extracted of berries was determined using the Folin-Ciocalteu methodology [79]. Briefly, 20 µL of sample/standard was mixed with 100 µL of Folin-Ciocalteu diluted with ultrapure water at proportion of 1:10 (v:v). The mixture was left to react for 4 minutes and then 80 µL of a Na_2CO_3 (7.5%, w/v) solution and left in the dark for 120 min. Analysis of the results was made in a microplate and read at λ750 nm. Results were expressed using the average of 9 determination, in mg of gallic acid equivalents per gram of freeze-dried berry (mg GAE/g), using a curve of gallic acid with concentration between 0.25 to 0.050 $mg.mL^{-1}$.

2.7.10 Titratable acidity

The titratable acidity of yogurt samples was determined by titration methodology according to the NP 701 (1982). Briefly, 10 g of yogurt samples was mixed with 10 mL of water and 5 drops of phenolphthalein at 1%. The neutralisation of acid part of yogurt samples was performed by addition of NaOH solution (0.1 N) until the pink colour, as

endpoint was reached. The results of titratable acidity were performed in triplicate and are expressed as %lactic acid.

2.7.11 Sensorial analysis

Sensorial analysis of the yogurts enriched with extracts of berries was performed by the hedonic test [80], to evaluate the acceptability of the three yogurts samples produced (CTR – control; CAM – enriched with freeze-dried infusion of berry, LIO – enriched with freeze-dried berry and COM – commercial yogurt). A panel of 15 semi-trained-panellists (member of SensoMarES panel from MARE-Polytechnic of Leiria), performed the sensorial analysis of yogurts samples identifying the sensorial attributes related to appearance (visual aspect, colour), flavour (global flavour, berry flavour), taste (sweetness, texture) rating the numeric scale of 9-point. Also, the global appreciation and intention of purchase of yogurt enriched with berry were evaluated in a 5-point scale (from low to high intensity).

2.8 Statistical analysis

The analysis of variance (ANOVA) of data was carried out using the Statistica™v.8.0 Software from Statsoft. The obtained results were considered statistically significant at level of significance 5% (p-value < 0.05), following the Tukey HSD (Honestly Significant Difference) test. All the data were presented as mean values and standard deviation (SD).

3. Results and discussion

3.1 Evaluation of maturation stage of berries harvested in *Cabo Carvoeiro* (*Peniche*) and *Serra d'El-Rei* (*Óbidos*)

This study hopes to find of there are differences in the berries that could be attributed to the harvesting time of the berry. Knowing these possible changes can help the producer harvest the berry in the optimal time, so that a particular quality is maximized. For this, the following parameters were analysed, height, diameter, colour, texture, pH, SSC, antioxidant levels, and total phenolic content. The results are presented below.

3.2 Biometric parameter

The physical characteristics of berries is of importance because this qualification allowing the separation of the berries during the maturation/development stage and can help the choice of the best moment for picked to guarantee the best quality during the fruits postharvest. The biometric characterization of berries (table 2), harvested in both selected localizations, was performed by the height and diameter.

At the beginning of the maturation study, both berries harvested in *Óbidos* and *Peniche*, denoted a similar radius (Tukey test, p-value > 0.05), 0.46±0.04 and 0.47±0.03, respectively. There is however, a statistically significant (Tukey test, p-value < 0.05) trend after 43 days, where a decrease on radius value was observed in both berries' samples. The berries from CAM-CV having a radius decrease of 19% and the berries from CAM-SR a decrease of 11%. Similar values were found in two studies reported by Jacinto et al., (2021) [74] and Sónia C. Andrade (2015) [57]. The study reported by Jacinto et al. (2021) [74] the berries were cultivated in greenhouses from the starting plants that were collected from various points along the coast and in the study by Sonic C. Andrade (2015) [57] the studied berries where from *Mira* and *Coimbra*. According to the obtained results in the present study, the confirmation that the *Corema album L.* (D.) don is within the value of 8-10 mm in diameter, although some achieve values above 10 mm, most do not. The plants that are able to yield this larger fruits should be further studied since by comparison with other commercially available berries, such as

blueberries, these are considered small when their diameter is around 11,5 mm and large when its diameter reaches 15 mm [81], and by comparison our largest berries are below the small threshold.

Comparing the weight of berries picked at the first day, the samples from *Cabo Carvoeiro* showed a higher value when compared to the ones picked in *Serra D´El Rey*, 0.460 and 0.407, respectively. At the last day, on 30 October 2020, the berries weight from *Cabo Carvoeiro* were lower than those found in *Serra d'El Rey*, 0.239 and 0.327 respectively. These difference reveals a decline of 48% and 20%, for *Cabo Carvoeiro* and *Serra D'el Rey*. The decline of weight is not a surprise since it has been shown in previous studies that berries harvested later can have smaller sizes. Mallik and Hamilton (2017)[82], showed a decrease of ≈30% in blueberries harvested 20 days apart. These decrease may be associated to the dehydration of the berry [83]. Bras de Oliveira (2012) [19] found that the weight of *Corema album L* (D.) Don berries from several cultivars across the Portuguese coast, giving the results between 0.31 ± 0.08 g and 0.49 ± 0.06 g. Regarding the studied berries are within their measured interval and therefore consistent. However, these values are considerable lighter than blueberries and raspberries, these berries vary in weight from 0.75 - 1.94 g and 4 g per berry for blueberries and raspberries, respectively[81], [84]. This comparison shows that in the berries of *Corema album L* (D.) Don a reduction in size and weight during the maturation period, and later harvest result in lower rewards.

Table 2 Biometric results from the berries collected in "*Cabo Carvoeiro*" (CV) and "*Serra D'EL Rey"* (SR) in the months of September and October in 2020.

		Biometric parameter			
Identification of berry samples	**Harvest (date)**	**Height (mm)**	**Diameter (mm)**	**Radius (mm)**	**Weight (g)**
CAM-SR	17-Oct	8.75 ± 0.92^{d}	9.69 ± 0.89^{de}	0.46 ± 0.04^{d}	0.407
CAM-SR	21-Sep	7.91 ± 0.77^{bc}	8.69 ± 0.88^{abc}	0.42 ± 0.04^{bcd}	0.387
CAM-SR	30-Sep	8.89 ± 0.71^{d}	9.38 ± 0.82^{cde}	0.46 ± 0.04^{d}	0.341
CAM-SR	12-Oct	7.17 ± 1.15^{a}	8.04 ± 0.98^{a}	0.38 ± 0.05^{a}	0.309
CAM-SR	30-Oct	7.93 ± 1.13^{abce}	8.57 ± 0.97^{abc}	0.41 ± 0.05^{abc}	0.327
CAM-CV	17-Sep	8.66 ± 0.67^{de}	10.02 ± 0.77^{e}	0.47 ± 0.03^{d}	0.460
CAM-CV	21-Sep	7.68 ± 0.79^{abc}	8.58 ± 0.76^{ab}	0.41 ± 0.04^{abc}	0.296
CAM-CV	30-Sep	8.25 ± 0.69^{cde}	9.11 ± 0.65^{bcd}	0.43 ± 0.01^{cd}	0.368
CAM-CV	12-Oct	7.30 ± 0.73^{ab}	8.10 ± 0.68^{a}	0.39 ± 0.03^{ab}	0.272
CAM-CV	30-Oct	7.16 ± 0.80^{ab}	8.01 ± 0.94^{a}	0.380 ± 0.04^{a}	0.239

Results are presented as average ± standard deviation.

In the same column, the different lowercase letters represent significant differences at p-value < 0.05

3.3 Colour

The appearance of the berries is the first thing that a potential consumer sees when want to buy the products and for berries a consistent and predictable colour is important. The L* values of the berries can be related to the whiteness of this fruit. Since our berries (Table 3), at the first day, was white and opaque, higher values would be expected. On the other hand, in the later stages of maturity, the berries start to lower whiteness and the seeds become more visible. These seeds are darker and therefore lower the L* values. Lower L* values would indicate a berry at a later maturation stage. In our measurements the values that were found at the beginning were 53.03±2.64 for CAM-SR and 50.82±5.42 for CAM-CV (Table 3), with statistically differences between the sites (Tukey test p-value <0.05).

During the harvesting period, the value of L* of both berries' samples increased after one week, following a decrease in both sample group. The maximum value of L* of CAM-CV berries was achieved at the end of September month, afterwards the values decreased slightly, with the following values having no statistically differences (Tukey test p-value >0.05). The values obtained in CAM-SR berries, also achieved a maximum at the same day, however the decrease afterwards was not observed.

Our values were consistent with the findings of Alegria et al. [85], in which they found L* value of 53.51±3.93. In the study, the values were measured in berries that contained a mixture of white berries and translucent ones, although the berries used in this study were all opaque and the values are closer to those of a mixture of white and translucent. Jacinto et al (2021) [74] obtained values of L* of 72.50 and Sonia C. Andrade (2015) [57], 79.82, in berries classified as white. The difference between the studies could be related to the time of harvest or seasonal changes causing a changing of maturation berries. The changes measured during the study could be related to the higher translucence of the berries, this might not be easily detected by eye, but the equipment is able to discern this low translucence change.

The redness of berries is expressed by the behaviour of colour parameter a*, reflects the progression in the maturation of berries, and can be observed in Table 3. During the study, the a* values were around the positive side of 0, with minimal changes. Some of these berries have a tendency of developing a pink coloration when they become

translucent therefore the conjunction of the a* with the L* can be used to predict the current stage of the berries. At the last day, 30 October 2020, the values achieved a maximum of 1.32 ± 0.85 in CAM-CV and the lowest value was obtained at 21st of September of 2020 with a value of 0.12±0.61, in the CAM-SR. Overall, the CAM-CV berries achieved the higher value than CAM-SR. These highest values combined with increase in the last day is one indicator of a faster maturation rate from this harvesting site. The obtained values were lower than those found by Sonia C. Andrade [57], but within those found by Jacinto et al. [74], 1.27 and 0.24–1.68, respectively. The differences found could be attributed to harvesting sites, weather conditions and berry manipulation during the measurement.

Table 3 - Colour changes of berries of *Corema album L* (D.) Don, expressed by luminosity (L* value), redness (a* value), yellowness (b*) during the study of maturation evaluation.

Identification of berry sample	**Harvest day**	**L***	**a***	**b***
CAM-SR	17/set	53.03±2.64^{cde}	0.52±0.73^{ab}	4.71±0.82^{ac}
CAM-SR	21/set	56.01±3.50^{ab}	0.12±0.61^{b}	3.91±0.49^{cd}
CAM-SR	30/set	53.78±2.11^{abcd}	0.50±0.70^{ab}	4.96±0.83^{ab}
CAM-SR	12/out	54.94±2.58^{abc}	0.61±0.81^{ab}	5.63±1.66^{b}
CAM-SR	30/out	55.65±2.97^{ab}	0.72±0.70^{ac}	4.65±0.93^{ac}
CAM-CV	17/set	50.82±5.42^{e}	0.74±0.88^{ac}	4.96±1.05^{ab}
CAM-CV	21/set	56.27±3.19^{b}	0.35±0.68^{ab}	3.8±0.73^{d}
CAM-CV	30/set	55.21±2.25^{abc}	0.23±0.45^{ab}	5.46±0.85^{ab}
CAM-CV	12/out	53.69±2.87^{acd}	0.45±0.79^{ab}	4.76±0.89^{a}
CAM-CV	30/out	52.07±1.91^{de}	1.32±0.85^{c}	5.07±1.09^{ab}

Results are presented as average ± standard deviation. In the same column, the different lowercase letters represent significant differences at p-value < 0.05

Regarding the yellowness of berries, expressed by colour parameter b* (Table 3) no distinct trend in both sites, was obtained. Based on the characteristic colour of berries, this behaviour has been expected with little changes occurring during maturation.

Since high L* values and low redness indicate a white berry with at the optimal stage it seems that the last week of September was the optimal harvest for both sites during this year, however this optimal time of harvest could change depending on the yearly conditions.

3.4 Texture

The determination of texture changes in berries was performed in order to determine the resilience of berries against external physical pressures, especially in two moments, during packaging and consumption. During the TPA test, the berries had two distinct behaviours, the whiter berries showed a minimal deformation in their shape before being pierced, slightly more translucent berries bulged under the pressure before being pierced, some burst from the side instead of being pierced.

Those berries that compressed changed from white to translucent during the compression. These changes remained after compression stopped. Some results from this study were discarded because the piston sometimes would compress the seeds of the berries and given the high strength of this seeds. The values show a very significant increase, with an instance that cause the equipment to stop the analysis halfway because the maximum value was exceeded the maximum load (5kg), whilst the berries themselves did not go above 390g. The presence of the seeds in a food product needs to be considered due to their high strength.

The results obtained from the TPA analysis are represented in Figure 13. In relation of hardness of berries from the Cabo Carvoeiro (CAM-CV), no significant differences (Tukey test, p-value < 0.05) was observed during the two months of maturation/development stage. The values from CAM-SR showed an increase in their hardness with no significant changes (Tukey test, p-value < 0.05) when compared with CAM-CV berries, except for the first analysis day. Our results were consistent with those

found by Jacinto et al. [74] and although the values in their studied are only represented graphically they are within the same order of magnitude of obtained in our study.

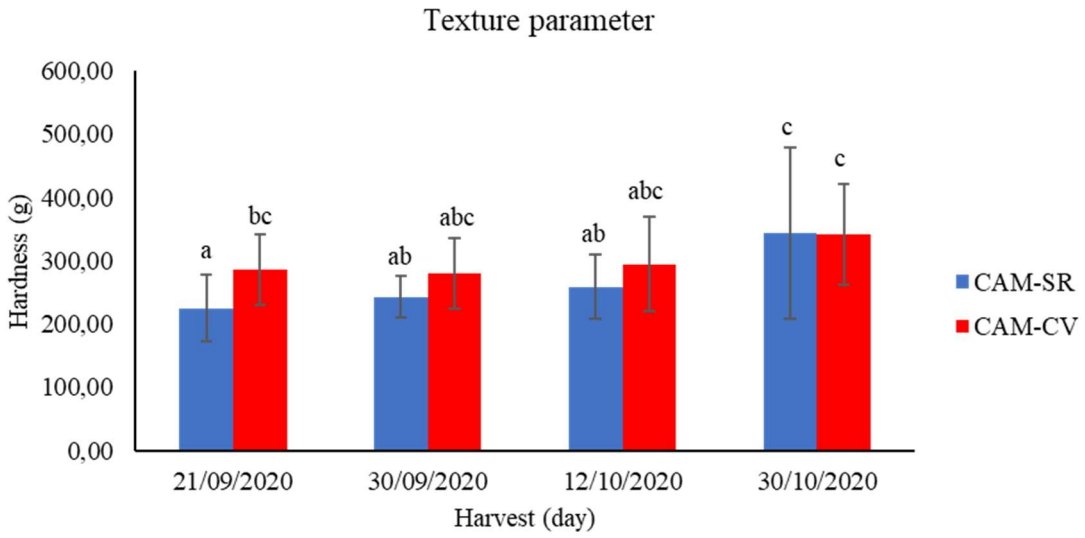

Figure 13 Texture attributes (Hardness)of berries of *Corema album L* (D.) Don during the study of maturation evaluation. Error bars denote standard deviation. [a,b,c] Different letters denote statistically significant differences (Tukey test p-value<0.05)

The cohesiveness values (Figure 14) obtained in both samples, varied between the 0.08 and 0.24 with no significant differences (Tukey test, p-value < 0.05). These results are lower than those found for raspberries, 0.284 ± 0.047 measured by Mierzwa et al. (2019) [86] in a study where the effects of drying on texture of berries, was evaluated and with the blueberries studied by Chiabrando et al 2009 [87] with the value 0.41. This seems to indicate that the berries after physical deformation do not hold their shape, this is backed by the permanent changes that occur after physical damage to the berry.

The higher average compression force of the berries could be related to the smaller size of the berries, as the berries measured were the same as the used for texture analysis and there is an inverse correlation between size and compression forces. However, larger berries seem to be desired, in the market of small fruits and berries, focusing this quality as fruits preference[88].

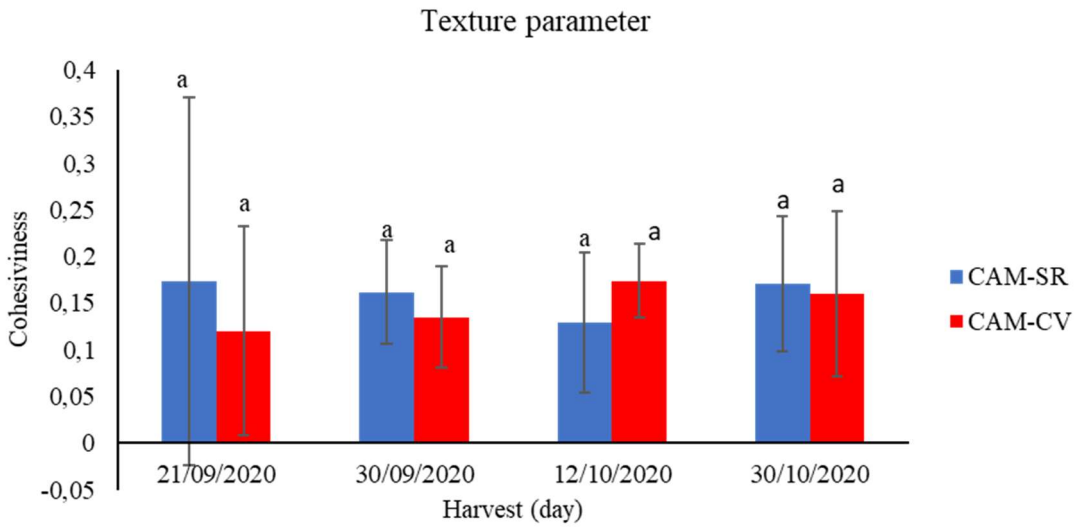

Figure 14 Texture attributes (Cohesiveness) of berries of *Corema album L* (D.) Don during the study of maturation evaluation. Error bars denote standard deviation. [a,b,c] Different letters denote statistically significant differences (Tukey test p-value<0.05)

3.5 pH and SSC

The pH levels and the soluble solid content, which is an indirect measurement of the sugar content, are two quality attributes that are associated with the acceptability of the berries by consumers, being decisive in the purchase consideration. In the difference in terms of pH in both harvested berries, was observed. The initial value of pH, as shown in Figure 15 was 2.67 ± 0.01 for CAM-CV and 2.54 for CAM-SR. After this initial value a decrease in the values to the final value of 2.53 for CAM-CV and 2.44 for CAM-SR, was statistically different (Tukey test, p-value < 0.05). During the study, the berries from CAM-SR registering values below those found in CAM-CV with these differences being statistically different (Tukey test p-value < 0.05) in all days of analysis, with exception of the 12th of October.

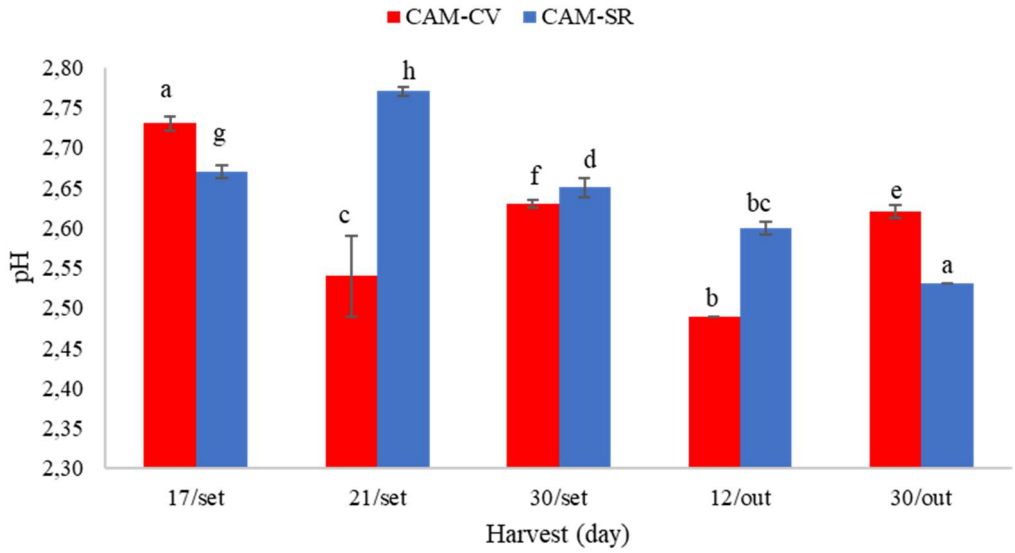

Figure 15 Comparison between harvesting sites for the values of SSC, error bars denote standard deviation, different letters denote statistically significant differences (Tukey test p-value<0.05)

The sweetness of the berries is another decisive factor that influence the acceptability of berries by the consumer. This factor is partially associated with the sugar concentration of fruits. The results of the solid soluble content (SSC) of berries are represented in Figure 16.

Along the study, the values of SSC of the CAM-CV berries, was decreased from 10.44±0.02 to 7.37±0.02, revealing the decrease of total sugars in the berries. The CAM-SR started with value of 8.78 and ended with 8.59. Following the statistical analysis, the values of SSC between the both harvesting location, a statistically difference (Tukey test, p-value < 0.05) were denoted.

The detected values were consistent with those reported by Jacinto et al. (2021) [74]. Although consistent there are some different notably, our lowest values were below 8, whilst the ones found in their study didn't drop below 8.4. This fact could be associated with the different climate or topological conditions. Zhang et al (2020) [65] found that in blueberries the sugar content varies between 9.90 and 19.50. Lee et al (2014) [61] found that in raspberries the sugar content was contained between 10.40 and 16.20. These values are higher than those of our berries, with these differences being firstly attribute to the difference species of plants and secondly to the difference between growing methods,

since these berries are grown commercially their environment is more controlled, in contrast with the wild environment of our berries[89].

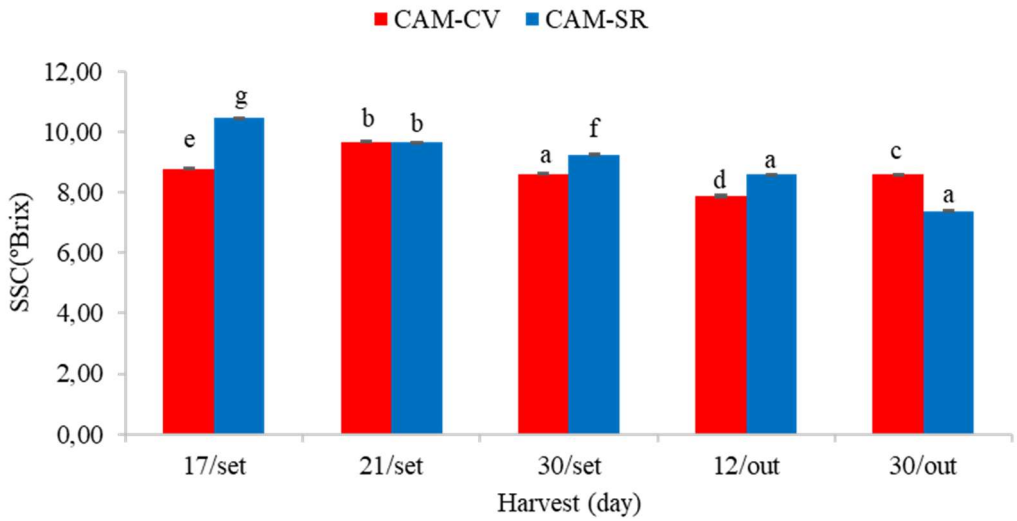

Figure 16 Comparison between harvesting sites for the values of SSC, error bars denote standard deviation, different letters denote statistically significant differences (Tukey test p-value<0.05)

3.6 Extraction optimization procedure

The optimization of the extraction procedure for antioxidant activity is essential to evaluate and understand the impact of different condition on the extractions. The various solvent has extraction rates regarding the different compounds. In the procedure, various fractions of solvents were used to detect the optimal solution and proportion for antioxidant and phenolic content extraction.

The results of antioxidant activity obtained after the extraction procedure can be observed in Figure 17. The maximum value of antioxidant capacity was achieved using the ethanol fraction of 0%, 25% and 50%, with no statistical differences between them (Tukey test, p-value > 0.05). The fractions of 75% and 100% achieved the lowest value of antioxidant activity.

The TPC analysis showed that there were no statistically significant differences between the sites (Figure 18). The results show that for both DPPH and TPC analysis the highest response was obtained with a solution of ethanol and water at 50%, although statistically the first three solutions were similar (Tukey test, p-value > 0.05). (Figure 18) Therefore the %50 solution was chosen for the extraction procedure.

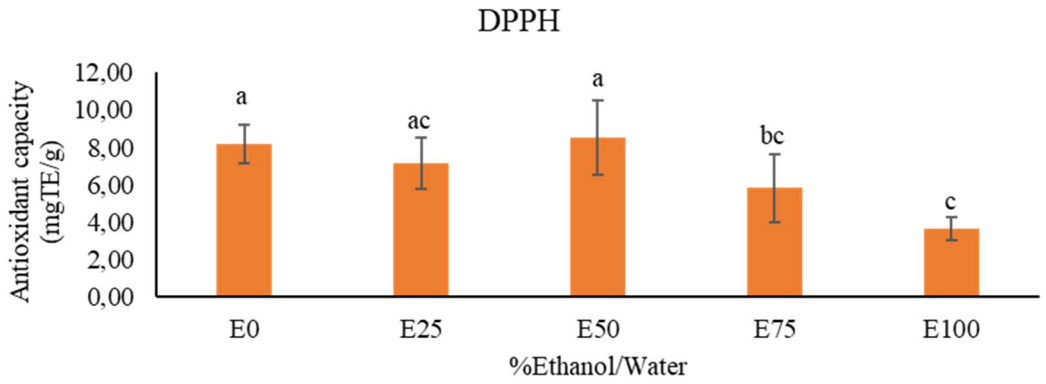

Figure 17 Antioxidant capacity (mgTE/g) obtained after the optimization extraction procedure in berries. Error bars denote standard deviation and different letter denote statistically significant differences (Tukey test, p-value < 0.05).

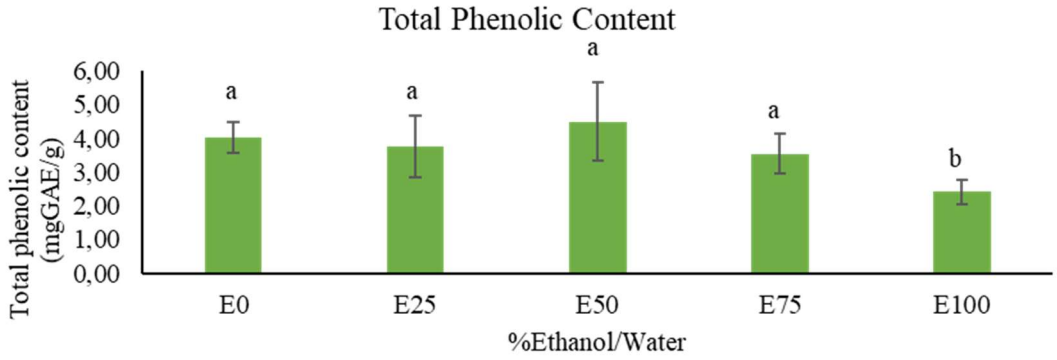

Figure 18 - Total phenolic content (mgGAE/g) obtained after the optimization extraction procedure in berries. Error bars denote standard deviation and different letter denote statistically significant differences (Tukey test, p-value < 0.05).

3.7 Antioxidant

The analysis of antioxidant compounds is an important evaluation because of their known contribution to the overall well-being and consumer health. The benefits which are related with the regular consumption of high antioxidant containing foods. Fruit and in particular the berries are known for their high concentration of antioxidants.

3.7.1 DPPH scavenging activity

This method can be used to detect the presence of hydrophobic antioxidants by either receiving an electron or an hydrogen[43]. The results of DPPH scavenging activity in both berries are shown in Figure 17. Our data shows a steady increase for both harvesting sites with a clear pivot point after the last day of September. After this date, the values of antioxidant activity started to show statistically differences (Tukey test, p-value < 0.05), with an increase from the initial value of 8.51 ± 1.97 to the final value of 38.99 ± 8.75 for CAM-SR and 11.85 ± 0.98, to the final value of 44.56 ± 1.05, for CAM-CV berries. This shows an increase of 128% and 136% of antioxidant capacity during the maturation stage of both berries samples. This is a quite substantial increase in both samples berries, about CAM-SR and CAM-CV, respectively. Despite the augment of antioxidant capacity expressed by DPPH scavenging, no significant difference (Tukey test, p-value > 0.05) was denote between them. This increase could be attributed to the earlier maturation of berries, since the data also shows that during the maturation period the antioxidant value increase[82]. This higher values is still lower that those reported by Andrade et al (2017) [90], where they found higher concentrations. This difference could be attributed to different edaphoclimatic conditions of the area and time of harvest.

Regardless the increase it is considerable it is still between ten to one-hundred times lower than blueberries as reported by Okan et al (2018) [91]. In comparison with the raspberries studied by Lee et al. (2014) [61], our values are shown to be half, of those reported by them. This significant difference, revealed the impact of wild and cultivated berries in their antioxidant capacity as constated by Guerrero et al. (2010) [89]. Their

study showed that there were differences between wild and cultivated berries with no positive or negative correlation between them.

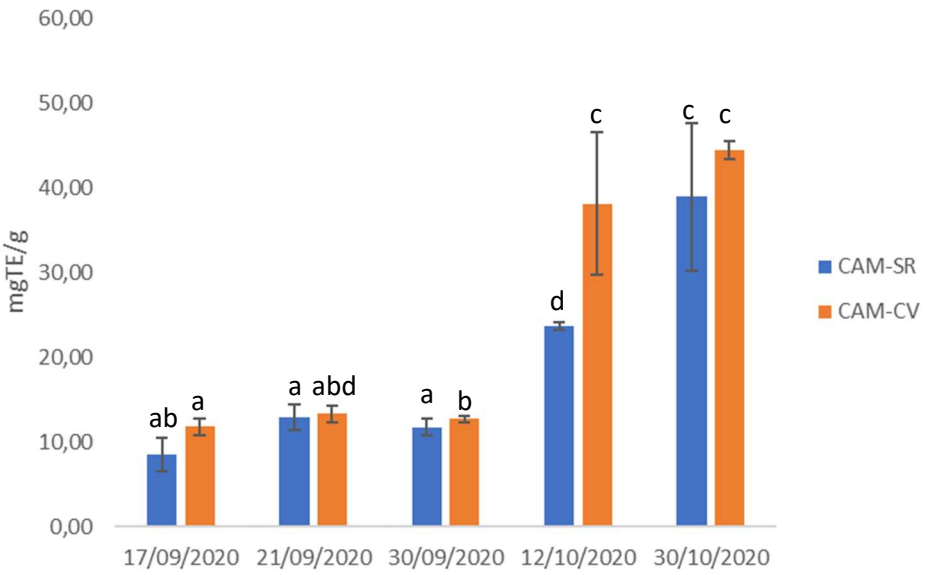

Figure 19 – Antioxidant capacity expressed as DPPH scavenging activity (mgTE/g) from both harvest locations. Error bars denote standard deviation and different letters denote a statistical and significant differences (Tukey test, p-value < 0.05).

3.7.2 Ferric Reducing Antioxidant Power (FRAP)

This methodology detects the hydrophilic antioxidants that are able to donate a single electron [43]. The use of ferric reducing antioxidant power (FRAP) analysis contributes to the range of methods to help understand the changes of the antioxidant content of the berries during the present studies Our results show that the berries from CAM-SR showed no significant changes throughout the study whilst the berries from CAM-CV showed an increase from the initial value. Our highest measured vale was 19.24±6.13 at the last measured day for CAM-CV and 15.69±2.03 for CAM-SR.

In comparison with blueberries 454.93 - 36832.96 µmol TE/100 g this is several orders of magnitude higher than those found in our berries. In raspberries, the values for

this type of analysis found by Lee et al. (2014)[61] were between around 70 to 95.04 µmol TE/100g, which are still higher than those found in our berries. This these differences could be attributed with the different species of berries.

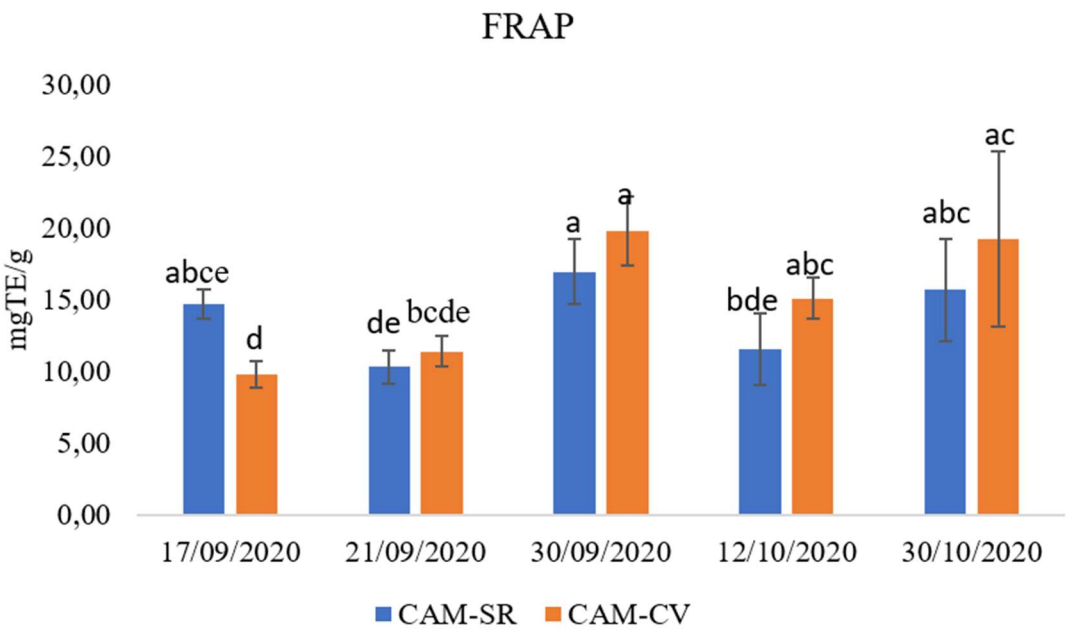

Figure 20 Comparison between Harvesting sites, error bars denote standard deviation, different lowercase letters signify statistically significant differences

3.7.3 Total phenolic compounds (TPC)

The analysis of the total phenol content is important to the relationship between these group of compounds and their antioxidant capacity as well as their association with fruits[92]. These compounds are also involved in the colour change of fruits. The results from the Folin-ciocalteu method showed a strong relation with the colour of the extract[23], [93]. The extracts with a pink colour showed a higher response, meaning that there is a possibility that the colour is created by the presence of phenolic antioxidants. The total phenolic content of berries samples is shown in Figure 21. In the CAM-SR berries there wasn't statistically significant changes (Tukey test p-value <0.05), showing that this type of antioxidants remains constant during the harvesting period. On average, the CAM-CV berries showed higher values that CAM-SR, this can be associated with a

faster ripening. The extracts from the last day showed a brownish colour which is associated with the degradation of polyphenols[94]. In this study, the highest value, 8.05± 1.52 mg GAE/g was detected on the 21st of September in the CAM-CV. This result is within the range of values of 463–1614 mg GAE/100g reported by Andrade et al (2017) [90]. This similarity could be attributed to the selective harvesting of the berries and the influence of the higher phenol content on colour. Due to their ability to change colour berries with higher phenol content will tend to have a colour different than the pretended opaque white. In comparison with raspberries the amounts are higher than those reported by Lee et al (2014) [61], in which it is found that the values are within 122.91 and 182.97 mg GAE/100g, this difference could be attributed to the difference species of berries. In blueberries Okan et al (2018) [91] found values between 77.26-215.12 mg GAE/100g.

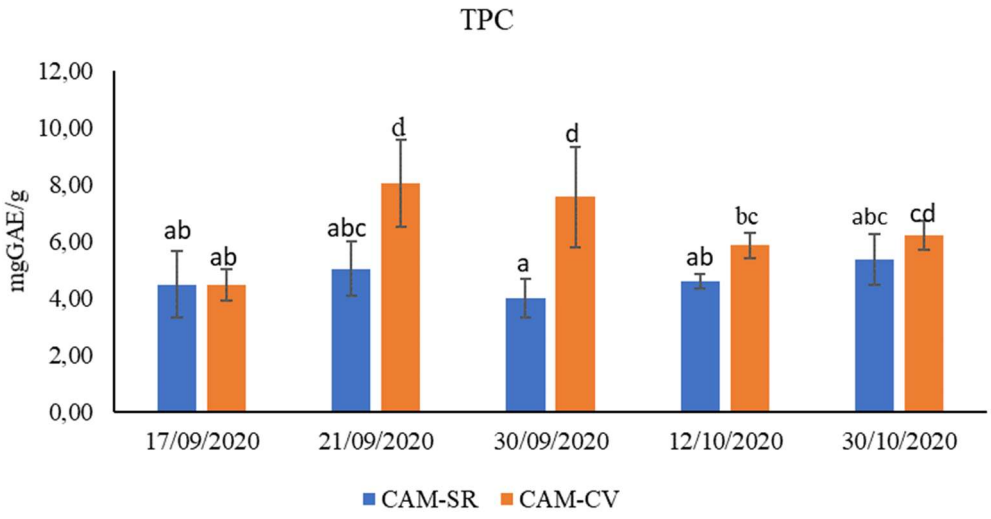

Figure 21 Total phenolic content (mg GAE/g) of berries harvested in both locations. Error bars denote standard deviation and different letters denote a statistical and significant differences (Tukey test, p-value < 0.05).

3.8 Effect of postharvest storage at room and refrigerated temperature on berry quality

There are several factors that occur in the storage of berries that can affect the parameters of berry quality. The analysis of the results obtained from the visual analysis

of the berries is explained below. It is fundamental to understand the impact of two storage temperature, 20 ºC and 4 ºC in the berries quality. Along the storage period the visual appearance of berries was evaluated by eye and with a photographic record, as can be observed in Figure 7, which consists of 6 stages. From the sample of each stored berries constituted by 30*3 fruits, the ones stored at refrigerated temperature showed a lower quantity of fruits colour change with the highest berries count of 11 whilst berries stored at ambient temperature achieved a maximum of 27 berries out the 30.

Besides the total amount of changed berries, the quantification of their state was also determined. For this a guide was developed based on the different observed states. Figure 22 shows a visual representation of each state: stage 1 in a white non translucent state, stage 2 represents the berries that are starting to become translucent, fully translucent berries are in stage 3. The first 3 stages represent the berries that are visually edible, the last 3 stages show an unappealing coloration and texture. Berries in stage 4 are brown, whilst berry in stage 5 are a dark brown with black tinge. The last stage represents a berry that has shrivelled and lost most of its pulp.

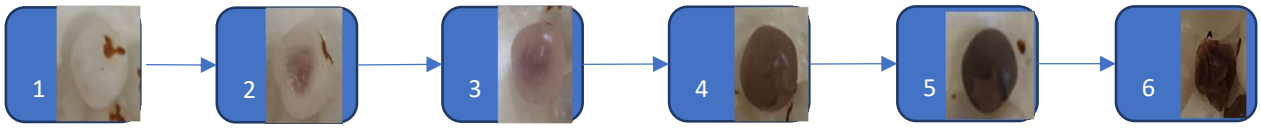

Figure 22 Proposed scheme of berries stages based on colour behaviour during storage .

3.8.1 Biometric parameter and visual analysis

From the analysis and separation of the berries in the various stages it was revealed that the berries do not follow the same speed of decay. Whilst under room temperature the decay was accelerated and the total amount of berries in stage 1 were low there were still berries in that group. This results are similar with reported by Miguel da Silva (2018) [51]. After the study was over it was observed that some berries did not show signs of decay. In the present study a similar effect was also observed where some berries that were kept in refrigeration (4±1°C) undisturbed, showed no signs of decay even after several months.

Until the 8th day of storage at both temperatures, a similar behaviour in the number of berries each stage, afterwards the berries stated to ripen at different speeds, was observed. The number of berries in stage 1 decreased the minimum of 3.2 ± 1.7 in room temperature and 18 ± 0.47 for the refrigerated samples at the last day of the study. The berries in the second stage were the most prevalent in both studies in a maximum of 21±0.60 and 8.0±1.6 for room temperature and refrigerated stored samples, respectively. Only the berries stored at room temperatures showed fruits at the 6th stage. The first fruit that was classified at this stage as at the 22nd day at storage.

Weight loss was increased in the berries stored at room temperature. Berries stored under refrigerated temperature did not suffer significant weight loss during storage (Table 4). The weight loss was higher in the berries stored at room temperature than those stored under refrigeration, these did not suffer significant difference (Tukey test, p-value > 0.05) This results are backed by the findings made by Miguel da Silva (2018)[51], in which it is said that the berries under refrigeration appeared to be edible even after five weeks in storage.

Table 4 Weight loss comparison between storage temperature 4°C and 20°C

Weight loss		
Storage (day)	**T (4°C) %**	**T (20°C) %**
0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
1	0.33 ± 0.00^{ba}	0.97 ± 0.00^{cba}
4	0.79 ± 0.00^{cba}	3.17 ± 0.00^{fedcb}
8	1.61 ± 0.00^{dcba}	5.97 ± 0.00^{hgf}
12	2.01 ± 0.00^{edcba}	8.92 ± 0.01^{ji}
15	2.60 ± 0.01^{edcba}	11.33 ± 0.01^{j}
19	3.45 ± 0.01^{gfedc}	15.91 ± 0.01^{k}
22	4.06 ± 0.01^{hgfed}	18.80 ± 0.02^{k}
25	4.23 ± 0.01^{hgfed}	21.89 ± 0.01^{l}
28	4.82 ± 0.01^{hgfe}	24.32 ± 0.02^{l}
32	5.93 ± 0.01^{hgf}	27.73 ± 0.01^{m}
35	6.31 ± 0.01^{ihg}	31.01 ± 0.00^{n}
39	6.65 ± 0.01^{ih}	33.22 ± 0.00^{n}

Results are presented as average ± standard deviation. The different lowercase letters represent significant differences at p-value < 0.05

3.8.2 pH & SSC

The analysis of the pH and the soluble solids content (SSC) of berries during storage is important to understand what the appropriated storage conditions is, so that the characteristic of the berry is not lost. The values for pH under both refrigerated and room temperatures showed the same tendency to drop but only slightly Figure 23 with the

starting pH value being 2.67±0.01. The berries stored at refrigerated temperature maintained the pH with only the 28th day being below, the starting value. The berries stored at room temperatures showed a similar behaviour. However, the drop below the initial value was measured at the 4th day and then again at the 15th day in storage. These results seem to indicate that under lower temperatures the pH remains more stable.

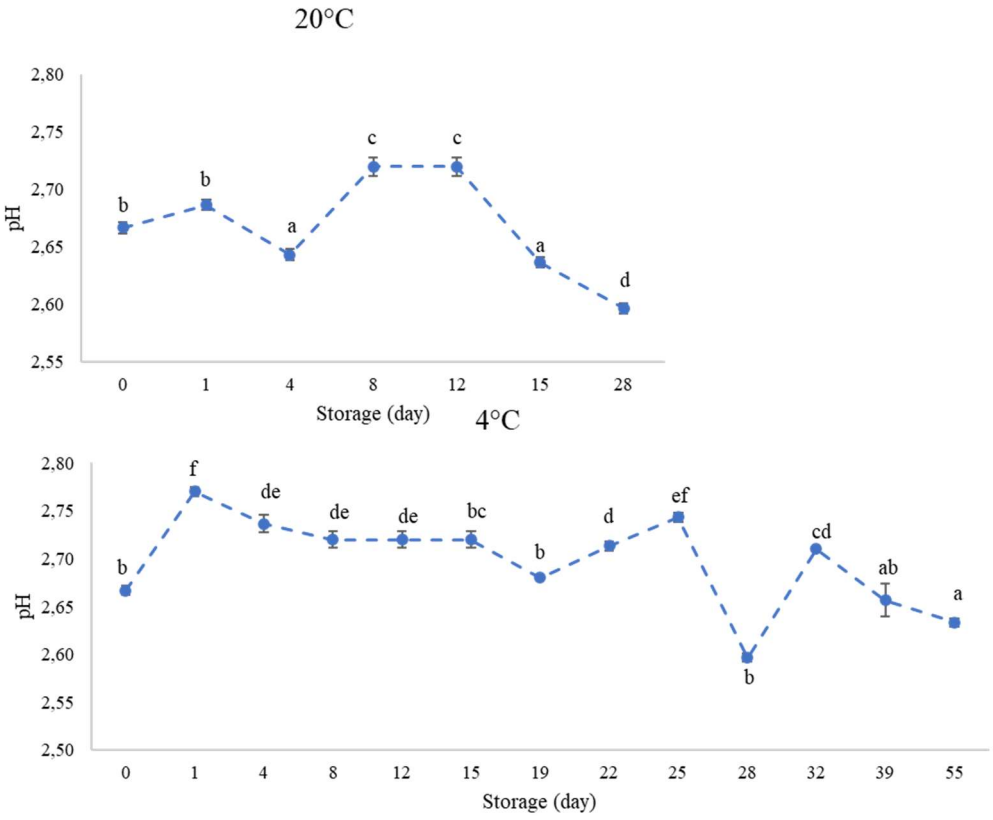

Figure 23 pH measurements during storage for both temperatures (20°C and 4°C) Error bars denote standard deviation and different letters denote a statistical and significant differences (Tukey test, p-value < 0.05).

As for the SSC, these values remained statistically different (Tukey test, p-value <0.05) from each other with the maximum value at the 15th day (9.17±0.01 °brix) for the room temperature and berries with the 8th and 12th day showing no statistically difference. However, the berries under refrigeration, achieved the highest value after 39 days in storage (9.62±0.01 °brix). These values are higher than reported by Miguel da silva (2018) [51], 7.0 °brix, for room temperature (20°C) and 8.0 °brix for refrigerated temperature (4°C). The difference, however can be attributes to the different storage time, harvesting location, as well as the edaphoclimatic conditions of the area during the harvest

season. This change might also be correlated with the weight loss of the berries during storage since this weight is mostly due to dehydration, and the loss of water would concentrate the sugar and therefore show a higher result of SSC.

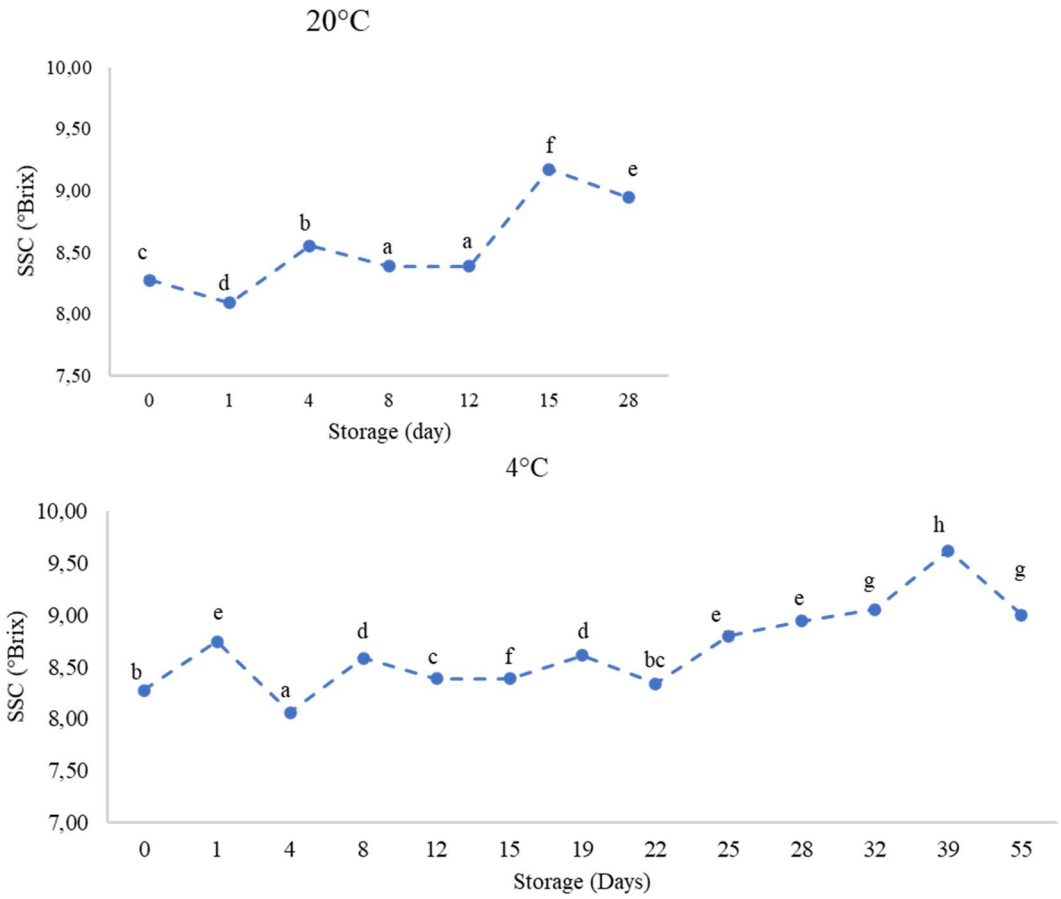

Figure 24 Soluble solids content (SSC) measurements during storage for both temperatures (20°C and 4°C) Error bars denote standard deviation and different letters denote a statistical and significant differences (Tukey test, p-value < 0.05).

3.8.3 Colour Analysis

The visual appearance of the berries and the associated changes during storage are important due to their correlation with the acceptability of the product. The colour parameter L*, a*, b* of berries stored at both temperatures can be observed in Table 5 and 6. Regarding the L* of berries the value remained constant under both stored

conditions with an increasing variability in later stages. This can be attributed to the high heterogeneity of the behaviour of berries. The values for the berries under refrigeration achieved the highest at the 12th day in storage. However, there no significant change from the majority of this study, the lowest value was measured after 55 days in storage with a value of 46.46±6.23. This value is consistent with the results expected of berries that are translucent as shown by the results obtained by Alegria et al. (2020) [85].

The redness values of berries stored at both temperature conditions increased, however the increase is more consistent (lower standard deviation) in the berries stored at lower temperatures. The time to achieve the same values was also extended in the berries stored at lower temperatures having a slower increase in this parameter. The changes in the redness are associated with the development of the red colour that accompany the maturations of the berries. The changes in the coloration of the berries is not one of the quality attribute desirable, since the uniformity and the lack of changed is recognizable by the consumer[95].

Table 5 – Colour values associated with the storage temperature (20°C)

Storage Temperature (ºC)	Storage Time (Days)	L*	a*	b*
20°C	0	52.31±3.55	0.26±0.80	4.51±1.03
20°C	1	52.86±4.02	0.62±1.57	5.46±0.86
20°C	4	51.63±6.20	1.22±2.75	5.51±0.95
20°C	8	50.85±4.06	1.60±1.28	6.20±1.86
20°C	12	50.05±5.12	1.28±1.41	6.22±0.97
20°C	15	50.34±5.12	1.57±1.11	5.60±0.81
20°C	28	52.46±4.20	1.29±1.17	5.21±1.32

Values expressed as mean value ± standard deviation

Table 6 – Colour values associated with the storage temperature (4°C)

Storage Temperature (ºC)	Storage Time (Days)	L*	a*	b*
4°C	0	52.31±3.55	0.26±0.80	4.51±1.03
4°C	1	52.86±3.21	0.73±0.89	4.37±0.88
4°C	4	51.96±3.83	0.97±0.81	4.72±0.86
4°C	8	52.66±3.13	1.03±0.81	4.98±0.96
4°C	12	53.15±2.45	1.15±0.73	4.74±0.78
4°C	15	51.84±3.72	1.33±1.01	4.48±1.07
4°C	19	52.69±3.25	1.09±0.87	4.85±0.92
4°C	22	50.13±4.79	1.47±1.32	4.57±1.01
4°C	25	50.96±6.17	1.63±1.50	4.30±0.87
4°C	28	53.01±2.78	1.36±0.73	4.06±0.75
4°C	32	50.69±4.15	1.88±1.32	4.27±0.75
4°C	39	51.93±2.83	1.19±0.96	5.52±1.43
4°C	55	46.46±6.23	2.74±1.95	6.07±1.24

Values expressed as mean value ± standard deviation

3.8.4 Antioxidant capacity (DPPH & FRAP)

The monitoring of the quality changes that occur during the storage of berries, is useful to determine if there are significant changes that might influence the qualities of

the product that is presented. Therefore, the measurement of the progression of the antioxidant capacity in the berries is shown below with the data relating to the DPPH analysis in Figure 25 The berries stored under refrigeration followed a similar behaviour with an increase in the values after the first day in storage, this increase of 3 mg/TEg remained until the last day of measure, in which the value returned to within statistical insignificance from the start of the study.

A similar behaviour was observed with the berries stored under ambient temperatures although higher values were measured in all samples, these higher values (an increase of 8 mg) could be related to the different temperature condition, or to this harvest having higher antioxidant content, regardless there was a measurable increase in the antioxidant capacity for 2 out of the 5 days, with the others having a stable value. The results from this methodology are that the lower temperature does not seem to affect the hydrophobic antioxidants.

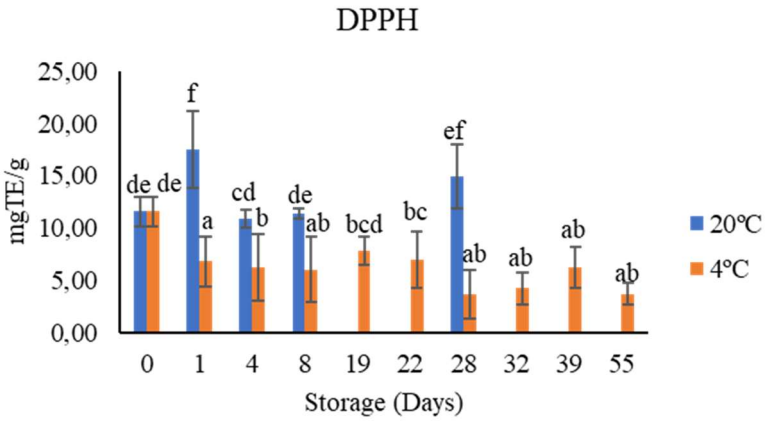

Figure 25 Results from the antioxidant analysis (DPPH) of the berries that were stored under refrigeration and ambient temperature., error bars denote standard deviation. Lower case letters denote statistically significant results (Tukey test p-value < 0.05)

The measurements using FRAP methodology represented in Figure 26, these values show a slight decrease from the initial value, there aren't however significant differences between the harvesting sites, this would seem to indicate that the temperatures do not have an effect in the storage behaviour of the hydrophilic antioxidants that contribute to the total antioxidant capacity. From the starting value of 19.13±2.15 to a

value of 14,36±1,71 and 9.80±1.32 for the 20°C and 4°C respectively. This corresponded with a drop of ≈25% and ≈49%. This drop remained with no significant changes for both conditions until the 12th day in storage. At the 15th day in storage the value showed an increase of ≈162% to a value of 17.46±1.55, this increased brough the values of the antioxidant capacity back to values similar to the start of the study (Tukey test p-value >0.05). After this day the values dropped again to values that have statistical significance to the ones prior to the 15th

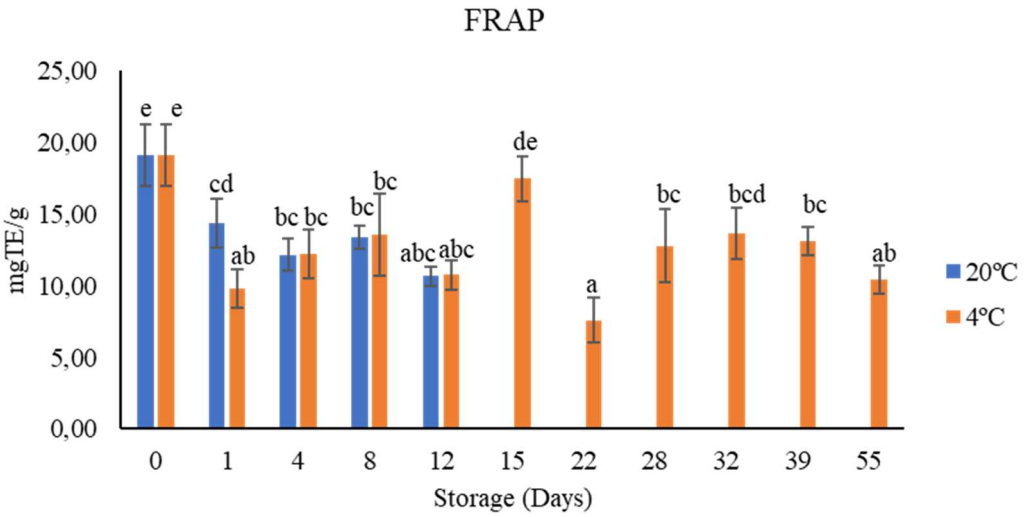

Figure 26 Results from the antioxidant analysis (FRAP) of the berries that were stored under refrigeration and ambient temperature., error bars denote standard deviation. Lower case letters denote statistically significant results (Tukey test p-value < 0.05)

3.8.5 Total phenolic Content

The performance of phenolic content of berries stored under refrigerated and room temperatures are presented in Figure 27. During ambient temperature storage temperature there wasn't statistically significant differences with a exception of the first day after storage. The refrigerated temperature however showed an increase of the phenolic content with statistically significant changes.

Our values were within those measured previously. Research shows conflicting results in regards to the behaviour of fruits under storage, Connor et al (2002) [96] showed that in blue berries there wasn't a significant change in the total phenolic content. Shin et

al (2008) [97] showed that strawberries when harvested earlier showed no change and when harvested later showed a decrease in phenolic content reaching to. However in study developed Sayyari et al [98], demonstrated that pomegranates when stored under cold temperatures have lower phenolic content.

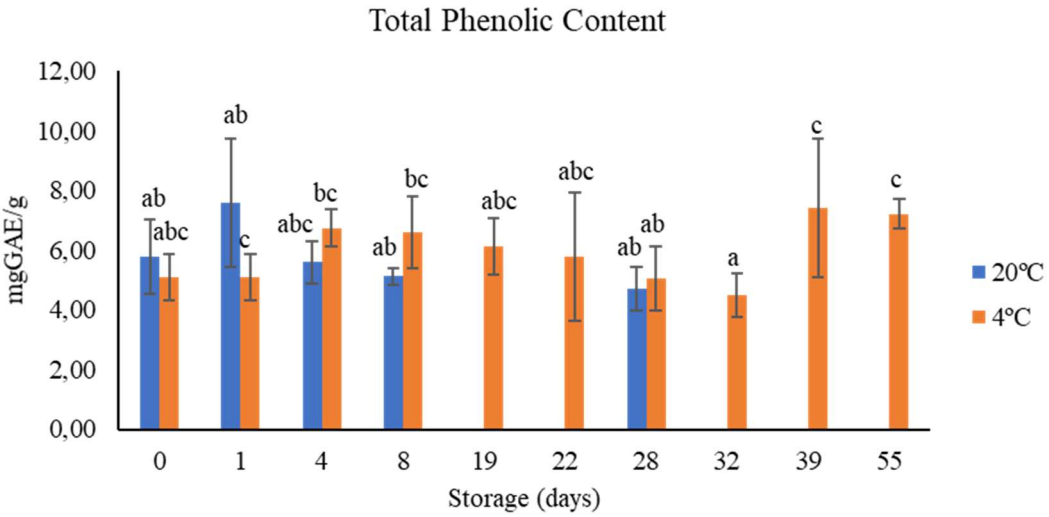

Figure 27 Total phenolic content measurements from the berries stored at refrigeration temperatures and at ambient temperatures, error bars denote standard deviation. Lower case letters denote statistically significant results (Tukey test p-value < 0.05)

3.9 Enrichment of natural yogurt with berry extract

Since the berries in this study are rich in antioxidants, we attempted to verify what would change if the berries, or an extract made from them were added to a yogurt. To test how this enrichment would proceed the addition of the freeze-dried crushed berry (identified as LIO), or with the addition of the infusion made from these berries (identified as CAM).

3.9.1 Manufacturing of yogurt with freeze-dried *Corema album L.* (D.) Don berry

The additions of the berries as an extract or has a whole was made by adding to a control mixture of ingredients, afterwards the solution was added to the sterilized flasks and left to ferment in the oven. The mixture had the pH measured every thirty minutes until the pH of 4.5 was achieved as per Lee and Lucey (2010) [99]. The progression of these results is shown in Figure 29. The three samples started at different pH, with the addition of the berries lowering from pH. The control samples (CTR) started with the value of 6.35, the samples with the added freeze-dried berry (LIO) started with 5.83, and finally the extract with the added extract starting with ta pH of 5.45. The CTR sample and the LIO sample achieved the same pH value after 90 minutes at the value of 5.44±0.02 and after 120 minutes all three samples achieved the same values at 5.15±0.02. The CTR sample achieved the target pH value after 240 minutes, and the LIO and CAM samples achieved the target pH after 300 minutes. The resulting yogurt is shown in Figure 28.

Figure 28 Control yogurt after fermentation

The yogurt that had the extract added to it (CAM) upon reaching the target pH of 4.5 wasn't completely solid, this result could be attributed to the disruption of the symbiotic relationship between the two main bacteria involved. This yogurt starting pH was lower than the control. This could replace the initial growth of Streptococcus thermophilus, which could be the reason behind the slower pH drop, and non-complete solidification[99].

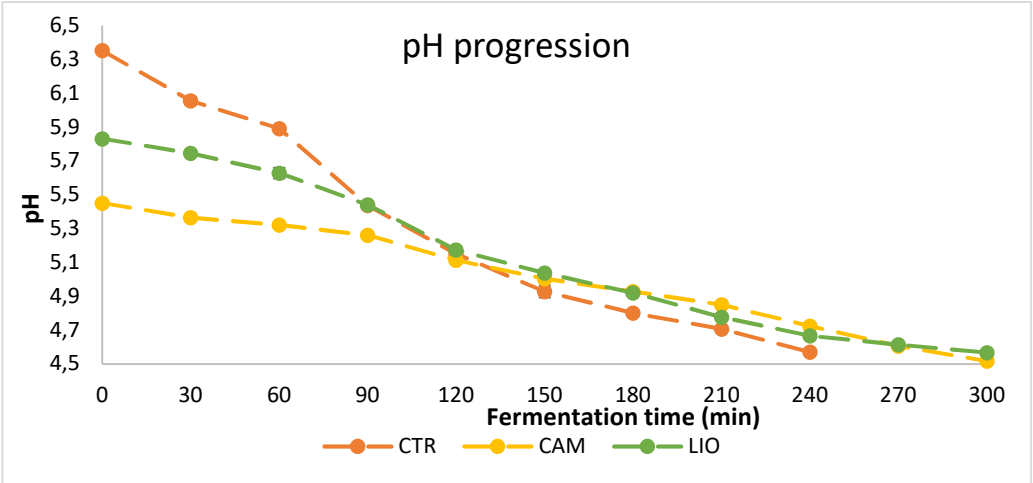

Figure 29 pH measurements during the yogurt production

3.9.2 Physical properties of the yogurt

As part of the parameters measured the titratable acidity is one of the required [100], as this as an important effect on the safety of the product. These values are represented in Table 7. We found that the yogurt that had the extract added (CAM) showed the lowest results, with 9.8% lower than the CTR, LIO and COM. These values in similar to those found by Izadi et al (2015) [101] 0.79±0.01%, in their studies about fortified yogurts.

Table 7 - Titratable acidity of yogurt samples (CTR-control, CAM-enriched with berry infusion: LIO-berry freeze-dried, COM-commercial yogurt) expressed as % lactic acid.

Yogurt sample identification	**Titratable acidity (% lactic acid)**
CTR	0.82±0.02[a]
CAM	0.74±0.01[b]
LIO	0.81±0.02[a]
COM	0.80±0.02[a]

Results are presented as mean ± standard deviation.

Different lowercase letters represent significant differences at p-value < 0.05 (Tukey test)

As part of this study and with the addition of an extract that is of the colour pink (Figure 30), the colour of yogurt samples was analysed and can be observed Table 8. The L* values showed that CTR sample was between LIO and CAM samples, with statistically significant differences (Tukey test, p-value < 0.05) between them. The redness (a*) parameter showed statistically significant differences for the CAM sample, the value however still remained within the negative range of the parameter, this means that the addition of the extract added some redness to the yogurt so that it was detectable with the equipment, but it wasn't enough to change the colour of the yogurt as a whole.

The yellowness (b*) parameter showed similar behaviour has the L*, with the CTR sample being in between the LIO and CAM.

The change in colour of the berries from white to pink was observed to be triggered with cell damage, at all temperatures, freezing, ambient, and near boiling. Further work is required to better understand the changes, and what it implies in the quality of the fruit, and its phytochemicals. However, controlling the changes under low temperatures is advised due to the already understood best practices [70] and the possible presence of compounds of interest that might be highly sensitive to higher temperatures.

Figure 30 - *Corema album L.* (D) Don extract post freeze-drying process.

Table 8 - Colour analysis (L*, a* and b* colour parameters) of the yogurt samples (CTR-control, CAM-enriched with berry infusion: LIO-berry freeze-dried)

Yogurt sample identification	L*	a* colour parameter	b* colour parameter
CTR	87.70±0.60[a]	-3.91±0.06[a]	5.89±0.62[a]
CAM	82.23±0.94[b]	-3.61±0.28[b]	6.13±1.33[a]
LIO	89.08±0.41[c]	-3.95±0.08[a]	8.33±0.49[b]

Results are presented as mean ± standard deviation.

Different lowercase letters represent significant differences at p-value < 0.05 (Tukey test)

The humidity values in the yogurt samples showed no significant differences (Tukey´s test, p-value > 0.05) between them (Table 9) and in comparison, with the commercial used as a starter for the fermentation. By comparison with the available literature, our yogurts have higher humidity results compared with reported by Zubairi et al [102] measured a value of 73%. This difference could be related to the different methods of manufacture.

Table 9 Relative humidity (%) of the yogurt samples (CTR-control, CAM-enriched with berry infusion: LIO-berry freeze-dried, COM-commercial yogurt and COM-commercial yogurt)

Yogurt sample identification	Relative humidity (%)
CTR	92.99±0.22[a]
LIO	79.43±0.01[a]
CAM	86.98±0.09[a]
COM	81.39±0.00[a]

Results are presented as mean ± standard deviation.

Different lowercase letters represent significant differences at p-value < 0.05 (Tukey test)

3.9.3 Hedonic test

The acceptability of a new food product from the consumer point of view is an important and major factor in the success of the product and based on this goal, the sensory analysis of the enriched yogurts was performed achieved the results shown in Figure 31. These results showed equal response in most evaluated attributes with the control and the commercial sample. The CAM sample achieve an overall lower rating with the only similar value being the sweetness. These lower values could be associated with contrasting aspect of this yogurt since this was the only one that was not completely

solid. Our control achieved similar values with a commercially available yogurt, the yogurt with added solid berries (LIO) achieved good results with high global appreciation. The flavour and smell of the berry wasn't detected, and the colour wasn't perceived to be different. These results are promising as the yogurt that achieved total solidification were as well accepted as the commercial one used for the starter; however this results alone do not imply that the addition of the berry was a successful one.

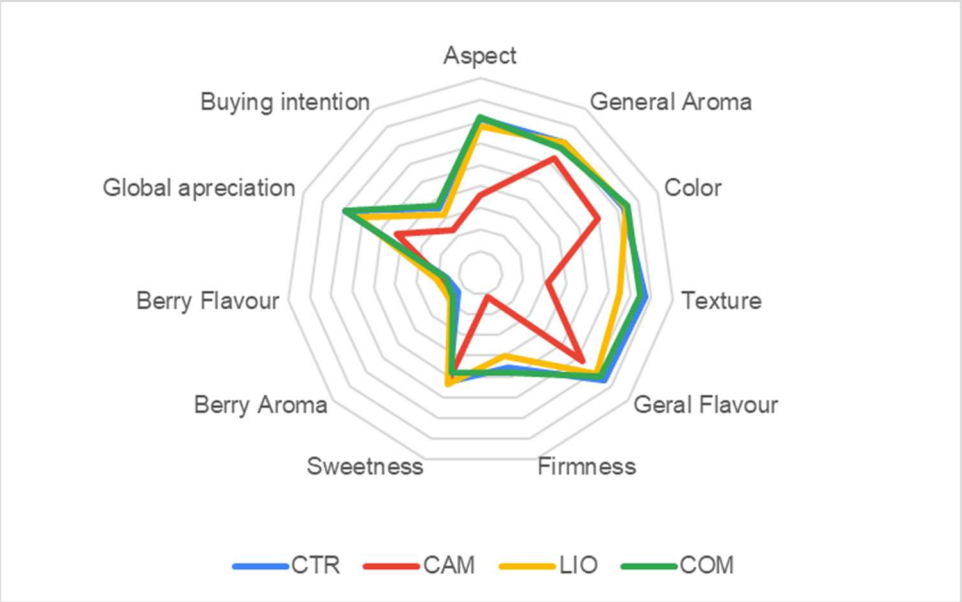

Figure 31 Results from the hedonic test realized on the yogurts samples (CTR - control, CAM – yogurt with added extract, LIO-yogurt with added freeze-dried berry, COM- commercial yogurt used as a started culture)

3.9.4 Antioxidants

To verify if the addition of the berry carried the benefit if their high antioxidant potential the of the final yogurt samples was made the results are shown in Table 10 The CAM yogurt achieved the highest antioxidant value in all methodologies with LIO achieving a lower but measurable increase. These results show that the addition of the berry extract transfers more antioxidants than the addition of the freeze-dried berries alone.

Table 10 Antioxidant's capacity of (DPPH, FRAP) and total phenolic content (TPC) of the yogurt samples (CTR-control, CAM-enriched with berry infusion: LIO-berry freeze-dried)

Yogurt sample identification	DPPH (mg TE/g)	FRAP (mg TE/g)	TPC (mg GAE/g)
CTR	0.043 ± 0.113^{a}	0.199 ± 0.024^{d}	0.705 ± 0.310^{g}
CAM	0.390 ± 0.102^{b}	0.408 ± 0.061^{e}	1.353 ± 0.059^{h}
LIO	0.083 ± 0.122^{c}	0.282 ± 0.027^{f}	1.266 ± 0.152^{g}

Results are presented as mean ± standard deviation.

Different lowercase letters represent significant differences at p-value < 0.05 (Tukey test)

3.10 Future work

Due to the interaction between the extract and the yogurt product, it is advised that the extract after being made be first dissolved in a basic solution, to increase its pH to that of the milk used, a strong base is recommended such as KOH, or NaOH. Since this change adds additional minerals to the yogurt it is recommended that the final product be analysed for their mineral content to verify if there is a measurable change. Another suggestion is to increase the amount of extract used to attempt to achieve a perceivable change in colour and flavour, however it is also noted that the amounts of berries required to do such studies is high and therefore the sustainability of the source of the berries needs to be taken into consideration.

4. Conclusion

The results from the study of evaluation of berry maturation, harvested in two locations in the centre coastal region of Portugal (Peniche and Óbidos) was that the harvest time influences the berries qualities. Depending on the final product different harvesting times should be considered. The average of berries size tended to reduce. the pH values of the berries remained stable throughout the study. However, the SSC decreased. The L* values changed during the study with an increased on both samples followed by a decreased on the CAM-CV sample, redness showed an increase for the berries from CAM-CV whilst berries from CAM-SR showed no change. During storage the berries stored under ambient temperatures showed a faster decay, with higher colour change and weight loss %. Whilst the berries under refrigeration showed little transformations during the study, with a lower weight loss %. The pH values of the berries under both conditions showing an initial increased followed by a slow decrease, the SSC values showed an increase on both condition with the berries under ambient temperature showing a faster increase. The L* values showed no significant changes, however there was an increase in the variability for the later measurements, for both conditions. The redness showed a faster increase for the berries stored at ambient temperature. The antioxidant potential showed a decrease after the initial value with no statistically significant changes after the first measurements when using the frap methodology. Using the DPPH methodology shows a decrease in the berries stored at 4°C. The berries stored at 20°C showed an initial change with the berries from the 4°C showing a decrease whilst the berries at 20°C showed an increase afterwards both samples remained within statistical significance within the sample. The total phenolic content of the berries stored at ambient temperature showed no significant changes with the berries stored at refrigeration temperatures showing a decrease under storage. From the results obtain, lower temperatures seem to prolong the berries shelf life without reducing their qualities.

The enrichment of the berry yogurt with the extract showed that the addition of the berry as an extract provoked the largest changes with this yogurt (CAM) showing statistically significant changes in the titratable acidity, the highest antioxidant capacity of both samples but it also gathered the worse results in the sensory evaluation, this might

be due to the contrast between this yogurt and the others evaluated, as this yogurt didn't solidify completely. The addition of the whole berries wasn't as effective, but it also increased the antioxidant capacity. The panel couldn't detect the berries flavour or aroma in the yogurt.

To conclude the wild berry provides high antioxidant potential, with the potential of being a new source of antioxidants for the consumer.

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