SHERON ODMIA TITALAH

DEVELOPMENT OF A FUNCTIONAL GOAT CHEESE:

EXPLORING BIOACTIVE AND PRESERVATION EFFECTS OF *Arthrocnemum macrostachyum*

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Master of Science in Food Technology

Dissertation carried out under the co-supervision of:

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Declaration of Work Authorship

I declare to be the author of this work, which is original and unpublished. Authors and works consulted have been duly acknowledged in the text and are included in the listing of references.

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SHERON ODMIA TITALAH

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Abstract

This work explored the influence of biomass from the halophyte species *Arthrocnemum macrostachyum* as salt substitute in fresh goat cheese. Biomass was evaluated for mineral contents. The radical scavenging activity towards the 2,2-diphenyl-1-picryl-hydrazyl-hydrate free radical, and the total phenolics and flavonoids contents of ethanol extracts of the dried biomass of *Arthrocnemum macrostachyum*, and cheese, were also evaluated. Fresh and dried biomass from *Arthrocnemum macrostachyum* were evaluated for microbial quality, as well as functionalized cheese. Cheese was also analyzed in terms of microbiological, physicochemical, bioactive, and functional properties, in different preservation periods. Dried biomass (4g/L) was added to cheese, together with 8 g salt /L (B1) and 4 g salt/L (B2). Cheese with 12 g of salt was used as control (C). Cheese was analyzed at days 0(t0), 4(t4) and 8(t8) of storage at 4°C. Ethanol extracts of *Arthrocnemum macrostachyum* dried biomass were evaluated for antioxidant activity by the DPPH presented half maximal effective concentration of 4 mg/mL. Total phenolic (TPC) and flavonoid (TFC) contents, with values of 23.76 GAE/g DW and 10.35 mg QE/g DW, respectively. The minerals Na, K, Mg, Ca, and Fe were the most abundant. Ethanol extracts were prepared from dried cheeses supplemented with *Arthrocnemum macrostachyum*, and evaluated for DPPH, TPC and TFC. No antioxidant activity was detected in the cheese extract. Cheese with biomass 8 g salt/L had TPC of 15.17 (t0), 17.12 (t4) and 26.39 (t8) GAE/g DW, cheeses with 4 g salt/L increased from 19.38(t0), 18.90(t4) and 39.94 (t8) mg GAE/g DW. TFC in cheese with 8 g salt increased from 2.80 (t0) to 4.37 (t4) and decreased to 1.88(t8) mg QE/g DW. TFC increased from t4 to t8 in cheese with biomass 4 g salt. B2 was the best cheese in terms of functional and physicochemical properties. *Escherichia coli* and *Staphylococcus* coagulase positive were not present in the samples. Microorganisms at 30 °C for fresh and dry samples were 2.37 and 3.50 log CFU/g, respectively. In dry biomass, molds counts were 3.14 log CFU/g, whereas no molds were found in fresh biomass. No yeasts were found in both fresh and dry biomass. There were significant differences between L*, a*, b*, and C* of the cheeses at different storage times. Water activity and fat content were not significantly different (P>0.05) except for fats in cheese with biomass 4 g salt/L during the first week, pH and dry weight were significantly different (P<0,05) during the storage. *Escherichia coli* and *Staphylococcus* coagulase positive were absent. *Salmonella* and *Listeria monocytogene* were not detected in the cheeses. Yeast counts were higher than 5 log CFU/g with no molds.

Key words: Cheese, Halophyte, functional food, preservation, quality.

Resumo

Cada vez mais se observa uma tendência mundial para consumir alimentos considerados saudáveis acrescidos de conservantes, ingredientes naturais ou ambos. Normalmente utilizam-se produtos com os quais os consumidores já estão familiarizados, como é o caso do queijo. A utilização de plantas halófitas como fonte de novos aditivos alimentares para a conservação e produção de alimentos funcionais também tem vindo a ganhar a atenção do sector alimentar, devido à sua composição em compostos antioxidantes que os tornam interessantes para a saúde humana e indústria alimentar. Neste contexto, este trabalho visou explorar o efeito da substituição de parte do sal por biomassa seca da espécie halofita *Arthrocnemum macrostachyum*, e os eventuais efeitos bioativos e de conservação do queijo de cabra fresco durante um período de armazenamento. Os queijos foram produzidos no laboratório de Processamento de Alimentos do Instituto Superior de Engenharia, da Universidade do Algarve, utilizando leite de cabra pasteurizado e coalho de origem microbiana. Foram testadas várias condições de produção e, após prova sensorial, foram escolhidas: queijo controlo, com uma concentração de sal de 12 g/L, sem qualquer adição de biomassa; queijo B1 com adição de 4 g biomassa por litro e 8 g sal/L; e queijo B2 com adição de 4 g biomassa por litro e 4 g sal/L. Os queijos foram conservados em condições de refrigeração durante 8 dias, tendo-se feito colheitas de amostras para análise no dia da produção (t=0), no quarto dia (t=4) e no último dia (t=8). Antes da sua incorporação no queijo, a biomassa foi avaliada em termos de teor de minerais (por espectrofotometria de absorção atómica), potencial antioxidante (contra os radicais 1,1-difenil-2-picril-hidrazilo, DPPH), e composição em fenólicos e flavonoides totais (pelo método de Folin Ciocalteau e AlCl3, respetivamente). Estas duas últimas determinações foram feitas num extrato etanólico preparado a partir da biomassa seca. Os queijos aditivados com a biomassa, bem como os queijos controlo, foram avaliados quanto a cor, qualidade microbiológica, capacidade antioxidante e composição em fenólicos totais (também em extrato etanólico preparado a partir da biomassa seca de queijo).

Relativamente ao teor de minerais na biomassa seca, verificou-se que que os elementos sódio (Na), potássio (K), magnésio (Mg), cálcio (Ca) e ferro (Fe) foram os mais abundantes, especialmente o Na (24,78 ± 2,21 mg/g peso seco). O extrato de etanol da biomassa apresentou uma capacidade moderada de neutralização do radical DPPH (IC50 = 4,15 ± 0,57 mg/mL). Os teores de fenólicos e flavonoides totais foram de 23,76 ± 1,01 mg GAE/g peso seco e de 10,35 ±

0,74 mg QE/g peso seco, respetivamente. O rendimento queijeiro variou entre 28,87 % nos queijos controlo, 34,22% em queijos com adição de biomassa e 4 g de sal /L, e 36 % nos queijos

com biomassa e 8 g de sal/L. Quando testados para a atividade antioxidante (mesmo método utilizado para a biomassa), os queijos não apresentaram qualquer atividade antioxidante na máxima concentração testada (50 mg/mL). Relativamente ao teor de compostos fenólicos e de flavonoides, verificaram-se diferenças significativas com a adição de biomassa, especialmente nos primeiros dias de conservação. O teor de fenólicos totais aumentou ao longo do tempo de conservação em todos os casos, enquanto que o de flavonoides totais não apresentou variação consistente.

Relativamente à cor dos queijos, verificou-se uma diminuição do valor do parâmetro L* (luminosidade) em todos os queijos durante o período de armazenamento, enquanto que os parâmetros a* [verde (-); vermelho (+)], b* [azul(-); amarelo(+)]e C* (intensidade) aumentaram em todas as amostras testadas durante o mesmo período. Os níveis de a_W e gordura não sofreram alterações significativas ($p>0,05$) ao longo do tempo, exceto no caso da gordura da amostra B2, que aumentou durante a primeira semana.

O pH e o peso seco sofreram alterações estatisticamente significativas ($p<0,05$) ao longo do tempo. O pH do queijo de controlo aumentou na primeira semana e diminuiu durante a última semana de armazenamento. Resultados semelhantes foram obtidos nos queijos B2, enquanto o resultado contrário foi observado nos queijos B1, onde o pH aumentou ao longo do tempo em estudo.

Fazendo a comparação entre os queijos com diferentes concentrações de sal no início do período de conservação (t=0), verificou-se que o a_W e a gordura apresentaram os mesmos valores nos queijos de controlo e nos queijos B2. Os queijos B1 apresentaram um a_W ligeiramente inferior (0,96 ± 0,004) e um teor de gordura ligeiramente superior (13,00 ± 1,00) aos restantes. Relativamente ao peso seco, não foram verificadas diferenças estatisticamente significativas entre as diferentes condições. Já o pH apresentou ligeiras diferenças, variando entre 6,40 ± 0,04 para o queijo controlo e 6,57 ± 0,01 para o queijo B1.

Foi ainda realizada uma avaliação microbiológica de amostras de plantas frescas e secas de *Arthrocnemum macrostachyum* para *Escherichia coli* (ISO 16649-1:2018), *Staphylococcus* coagulase positivo (ISO 6888-1:2021), bolores e leveduras (ISO 21527-1:2008) e microrganismos a 30 ˚C (ISO 4833:2013). Os microrganismos *Escherichia coli* e *Staphylococcus* coagulase

positivos não foram encontrados em nenhuma das amostras, atestando as boas condições de higiene utilizadas durante a colheita e armazenamento desta matéria-prima. A amostra de biomassa fresca apresentou 2,73 ± 0,28 log UFC/g para microrganismos a 30 °C e a amostra de biomassa seca apresentou 3,50 ± 0,48 log UFC/g, o que são valores considerados normais para este tipo

produto. Os bolores e leveduras presentes na amostra fresca foram inferiores a 10 UFC/g, enquanto que na biomassa seca as leveduras apresentavam valores inferiores a 10 UFC/g e os bolores 3,14 ± 0,09 log UFC/g. A resistência dos bolores a ambientes com baixa humidade, pode explicar a presença destes microrganismos na amostra da planta seca, mesmo considerando as boas condições de armazenamento. A avaliação microbiológica não detetou *Escherichia coli*, nem *Staphylococcus* coagulase-positivo, durante o período em estudo, nem *Salmonella* spp ou *Listeria monocytogenes* no final desse período. As amostras de queijo com biomassa apresentaram valores para leveduras superiores a 5 log UFC/g, mas não apresentaram bolores. O valor mais elevado para os microrganismos a 30 °C foi registado aos 8 dias na amostra B1 (9,40 ± 0,07 log UFC/g).

Palavras-chave: queijo, halófitas, alimentos funcionais, conservação, qualidade

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List of abbreviations

ADI: Acceptable Daily Intake

a_W: Water Activity.

BHT: Butylated Hydroxytoluene

CCMAR: Centro de Ciências do Mar

CFU: Colony Forming Units

CPA: Cyclopiazonic Acid

CVD: Cardiovascular Disease

EFSA: European Food Safety Authority

EU: European Union

FAO: Food and Agricultural Organisation

FDA: Food and Drug Administration

FSANZ: Food Standard Australia and New Zealand

GAE: Gallic Acid Equivalent

HACCP: Hazard Analysis Critical Control Point

ICMSF: International Commission on Microbiological Specifications for Foods

IDF: International Dairy Federation

ISO: International Standard Organisation

PDO: Protected Denomination of Origin

PGI: Protected Geographical Indication

QE: Quercetin Equivalent

SCC: Somatic Cell Count.

SEs: Staphylococcal enterotoxins

SFP: Staphylococcal Food Poisoning

STC: Sterigmatocystin

TFC: Total Flavonoid Content

TPC: Total Phenolic Content

TSG: Traditional Specialty Guaranteed

1. Introduction

The human population is fast growing, thus a great challenge facing the world over the next decade is the production of sufficient food to meet the demands of the growing population. The world's population reached 7.7 billion in 2019 and the estimated medium variant projection indicates that the global population could grow to 8.5 billion in 2030, 9.7 billion in 2050, and, 10.9 billion in 2100 (UN, 2019). The increase in world population projected over the next 50 years will affect food production and supply systems.

The current growing trend toward the demand for healthy foods in developed nations has boosted the interest in goat milk and its products thereof (Park, 2017). Goat milk contains in abundance protein, fatty acids, and minerals (Dolatowska-Zebrowska et al., 2019). The demand for animal protein and energy sources particularly milk and milk products are on the rise in both the developing and developed world. The excellent digestibility and nutritional content of goat milk as well as its therapeutic potential, make it an important functional food (Ranadheera et al., 2019). These characteristics make it easier for the manufacturing of a wide range of dairy products from goat milk, including yogurt, cheese, non-fermented and fermented beverages, condensed milk, butter, ice cream, and sweets (De Oliveira et al., 2021). The production of high-quality raw goat milk is of paramount importance for the successful production, processing, and marketing of milk and its derived products. The increasing demand for milk can be met by increasing the ruminant livestock population (Devendra and McLeroy, 1982). The contribution of small ruminants and goats in meeting the demand will be very high. In Europe, the farming of small ruminants has played an important role in rural sustainability allowing the exploitation of marginal areas of otherwise low productive potential. Goat is considered valuable to local farmers for both economic and subsistence reasons, by contributing to the enhancement of the livelihood of marginal farmers (Watkins et al., 2021). The Barrocal area in the Algarve is an excellent region for the breeding of small ruminants for native consumption and goat milk cheese production.

Goat milk is mainly used in cheese making and goat milk specifically is a highly nutritious food with some interesting properties such as the smaller size of protein micelles, the smaller fat globules, and the higher levels of short-chain and medium-chain fatty acids which are responsible for its easier digestibility (Roberts, 1985), ideally suited for the growth of spoilage and pathogenic microorganisms. Hence it is highly perishable and requires careful handling. The outbreaks of milk-borne illness date back to the establishment of the dairy industry. Various bacterial infections have been linked to the consumption of raw goat milk. Raw milk is

susceptible to pathogenic microorganisms which have been found to be responsible for several food-borne diseases associated not only with raw milk but its products as well (Vasavada, 1986; Cadavez et al, 2017). Morgan et al, (2001) reported that *Listeria monocytogenes* were able to survive in soft lactic cheeses made from raw goat milk. Therefore, milk processing is an essential step to ensure its preservation and reduce milk spoilage and foodborne illness in consumers. Using traditional technologies, goat milk cheese is generally manufactured in small 'artisanal' units from raw goat milk (Lodi et al, 1996; Klinger & Rosenthal, 1997). Cheeses manufactured under these conditions may not have the minimum hygiene and sanitary guarantees necessary to obtain constant product quality (Emaldi, 1996).

Milk is processed by separation into cream and or processed into cheese, butter, or yoghurt, to increase the shelf-life quality of milk and offer the consumer a product with good flavour and high nutritive value (Niir, 2010) as well as a wide range of varieties. Moreover, cheese is a highly nutritious food with many diverse flavours and textures, which can be used as a snack or as a part of a dish or as pre-packaged convenience food, supplies abundant quantities of proteins, fat, and calcium, which are essential to growth and good health (Guinee, 2004). Goat milk is either transformed into cheese, mostly in Mediterranean countries and Latin America, or consumed raw or acidified in Africa and South Asia.

The bioactive compounds found in cheese include peptides, exopolysaccharides, fatty acids, organic acids, vitamins, γ-aminobutyric acid (GABA), and conjugated linoleic acid (CLA). Studies conducted both *in vivo* and *in vitro* have shown that these substances have antioxidant, antibacterial, and antiproliferative properties in addition to inhibiting the angiotensin-converting enzyme (ACE) (Faure et al., 2006; Sprong et al., 2010; Geurts et al., 2012). The bioactivities have health-protective effects linked to a decreased prevalence of metabolic syndrome (Bonthuis et al., 2010; Sonestedt et al., 2011), as well as cardiovascular disease risk factors such as obesity, dyslipidaemia, and type 2 diabetes (Sullivan et al., 2001).

The extraction of bioactive compounds for the sustainable valorisation of bioresources to produce value-added products has been a major topic over the last decade, and to reduce carbon footprint. The exploitation of bioactive compounds from plant sources is directly applicable in the pharmaceutical, cosmetic, agrochemical, and food industries. Bioactive compounds derived from plant extracts are an interesting substitute for synthetic additives to functionalize foods. The systematic investigation of these plants helps to evaluate their precise pharmacological properties and to determine their value as functional foods and as a source of

nutraceutical compounds, such as novel antioxidants (Miliauskas et al., 2004; Goutham Chandra et al., 2010).

Halophyte species share morphological and organoleptic characteristics presenting interesting nutritional profiles with high levels of minerals, vitamin C and β-carotene (Glenn et al., 1999; Lu et al., 2010; Redondo-Gómez et al., 2010; Ventura et al., 2011; Essaidi et al., 2013). These plants plants present significant antioxidant potential, with high radical scavenging activity (RSA), iron reducing power and total phenolic content. *Arthrocnemum macrostachyum* is rich in polyunsaturated fatty acids (PUFA) and is also a potential source of antioxidants (El- Wahab et al., 2008; Custódio et al., 2012; Rodrigues et al., 2014). A distinctive feature of halophyte is their particularly high sodium (Na) content which has been explored to reduce the salt content of goat cheese by adding *Arthrocnemum macrostachyum* biomass while obtaining a final product with bioactive, functional and/or better organoleptic and microbiological characteristics. A study suggested that consumption of *Salicornia* biomass rich in Na protected rats from hypertension and vascular diseases (Panth et al., 2016). The suitability of using these plants as food and as sources of natural antioxidants have been established by Bareira et al., (2017).

2. Objectives

2.1. General Objectives

Production of a fresh goat cheese enriched with biomass from the halophyte species *Arthrocnemum macrostachyum* to substitute part of the salt, to obtain a final product with improved bioactive properties and, eventually increased preservation time.

2.2. Specific Objectives

- To evaluate the capacity of using dried biomass from *Arthrocnemum macrostachyum* to substitute part of the salt in the composition of goat cheese.
- To determine the influence of adding dried biomass of *Arthrocnemum macrostachyum* on the physicochemical properties of the obtained goat cheeses.
- To determine the bioactive properties namely total phenolic contents, total flavonoids, and total antioxidant activity of the obtained goat cheeses.
- To evaluate the microbiological quality of the produced goat cheeses.

3. Thesis organization

This dissertation is divided into 9 parts. The organization of this work and the content of each part is presented below:

The first part is the introduction presenting the key details and elaborates the scope of this study.

The second part is the objectives made up of the general and specific objectives.

The third part is the thesis organization which describe the flow of material in this dissertation.

The fourth part is made up of the literature review which gives a background of this study, history and how it is related to the present work and other studies related to the present dissertation.

The fifth part is the materials and methods which describes the approach used to achieve the methods and objectives of this dissertation.

The sixth part is the results and discussions present the achievement and the evaluation of the success of this dissertation.

The seventh part is the conclusion which sums up the progress of the work and summarizes the dissertation.

The eighth part presents the future perspectives.

The ninth part presents the bibliographic references that have been used and presented.

Finally, the appendix is presented.

4. Literature review

4.1.The goat

A goat is any ruminant and hollow horned mammal belonging to the genus *Capra*. Related to the sheep, the goat is lighter in build and has horns that arch backward, a short tail, and straighter hair. There are a wide variety of breeds of goats existing on our planet, living on every continent except Antarctica (Figure 4.1), they thrive in an astonishing range of environments, from human settlements and tropical rain forests, to dry, hot deserts and cold, hypoxic high altitudes. The goat was previously considered a "poor man's cow," and goat milk products began gaining attention in the United States in the 1960s because of the health and nutritive values attributed to goat milk and milk products (Clark and García, 2017).

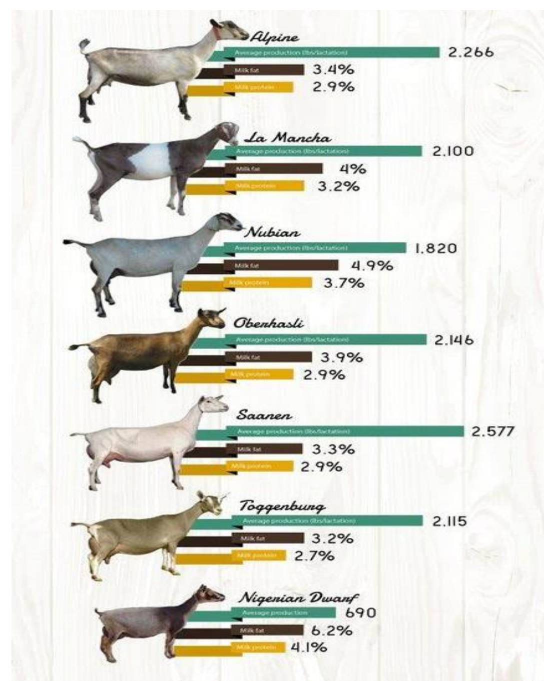

*Figure 4 1.*Goat distribution worldwide (Chaffhaye, 2018).

4.1.1. Origin and History

Goats were the first animals to be domesticated over 10,000 years ago. According to Hirst (2019) goats were among the first domesticated animals, adapted from wild bezoar ibex *(Capra aegagrus*) in western Asia which played an important role in the advancement of Neolithic agricultural technology, providing meat, milk, hides, and bones. Other authors argue that three different groups were involved in the origin: *Capra aegarus* belongs to the European group (*Ovis capra european*), *Capra falconeri* and *Capra prisca* belonging to the Asian group (*Ovis capra asiatica*) and *Capra nubiana* or *sinaiantica* to the African group (Almendra, 1996; Kukovics, 2018). Solaiman (2010) considered the existence of five wild ancestors: *Capra hircus* (the true

goat including the bezoar), *Capra ibex* (the ibexes), *Capra caucasica* (the Caucasian tur), *Capra pyrenaica* (Spanish ibex), and *Capra falconeri* (the markhor). Goats contribute to about 2% production supply of the world's total milk. Their significance in the economic upliftment and nutritional wellbeing of the human population is crucial in several regions around the world, particularly in the Middle East and Mediterranean countries (Park, 2017). Goats have been categorized into varieties of breeds and are raised in different environments. Goat breeds meant for dairy products and milk production are receiving more attention in studies regarding milk quality and milk yield (Zobel and Nawroth, 2020).

4.1.2. Goat breed in Portugal

In Portugal, goat production is extremely important, not only for the produced meat and milk, but also because they provide an income in impoverished and highly depopulated regions in mountainous regions of the interior and where alternative economic activities are scarce (Silva et al., 2007). There are six autochthonous goat breeds officially recognized: *Bravia, Serrana, Preta de Montesinho, Charnequeira, Serpentina,* and *Algarvia* (Figure 4.2). These breeds represent 12.5% of the total of the national goat inventory, whereas exotic breeds represent only 5% of such inventory, and the remaining percentage are a result of crosses between several breeds (Carolino et al., 2017). Even though their origin is not fully clear, Portuguese goat breeds have been indicated in previous studies to have descended from three groups of goats of the Quaternary. In the Iberian Peninsula, with animal evolution and because of migrations in mountain ranges, the Pyrenees goat (*Capra pyrenaica*) replaced the original wild species. *Capra pyrenaica* was therefore the direct ancestor of Portuguese and Spanish breeds (Almendra, 1996). According to Bruno-de-Sousa et al. (2011), the *Bravia* and the *Algarvia* breeds descend from independent ancestral populations whereas other breeds descend from various ancestral populations present in the Iberian Peninsula for a long time. Autochthonous breeds closely located show a weak differentiation although present high levels of genetic diversity. Lopes et al. (2016) clearly established the *Algarvia* and *Serrana* breeds as being the sources of the *Crespa* breed.

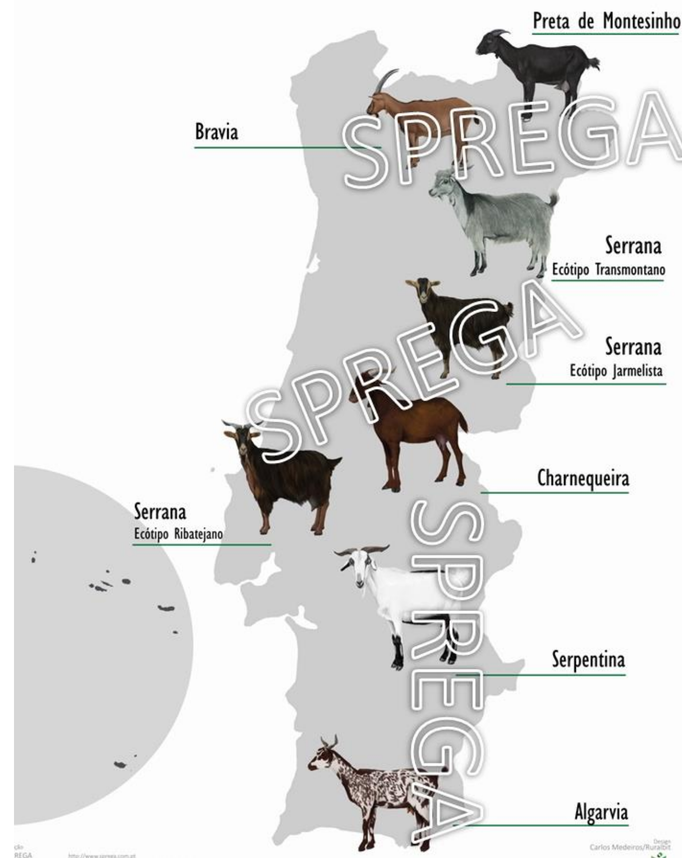

Figure 4 2. Six goats breed distribution in Portugal *(*SPREGA, 2020a)

4.1.3. Goat breed in Algarve

The *Cabra Algarvia* (Figure 4.3) is known as a hardy breed of goat in the Algarve region. It is said to be indigenous to the Algarve since the 19th century and it is part Charnequeira and part Moroccan with a little bit of Spanish goat thrown into the mix in the early 20th century. They are short-haired multi-coloured goats, with the majority being mostly white with brown spots usually seen grazing in the Algarvian hills. Some stay on farms while others are being managed in the traditional way with a goatherd moving them throughout the day (Becky, 2016). This autochthonous goat breed has great relevance in goat milk production, and it is well adapted to the difficult environment. In 2020, according to the Portuguese Society of Animal Genetic Resources (SPREGA), the Algarvian goat's workforce was 2813 heads, which produces about 166 liters of milk per lactation (SPREGA, 2020b).

Figure 4 3. Algarvian goat breed (Becky, 2016).

4.1.4. Goat milk

Goat milk is a cream lacteal secretion free from colostrum, acquired by the complete milking of healthy goats. Council Directive 92/46/EEC of 16 June 1992 define raw milk as "milk produced by secretion of the mammary glands of one or more cows, sheep, goats, or buffaloes from a single holding that has not been heated beyond 40 °C or undergone any treatment having a similar effect".

For decades goat milk has been considered nutraceutical touted for its easy digestibility and lower allergic properties as compared to cow milk. The importance of goats for human nutrition has likely been recognized since the beginning of domestication (Clark and García, 2017). Goat milk is a product of high nutritional value. In microbiological terms, raw milk is a rich medium for the development of pathogenic microorganisms responsible for several foodborne diseases (Ombarak and Elbagory, 2017). Goat milk secreted by a healthy udder can be considered non-infected and contain natural antibacterial inhibitors, such as lactoferrin and lactoperoxidase, which prevents the growth of bacteria, at ambient temperatures, during the first three or four hours immediately after milking (Ay and Bostan, 2017). For this reason, during this initial period, the milk ought to be cooled to 4 °C to 6 °C to preserve its original quality. In regions with poor hygienic standards, the use of a lactoperoxidase system is recommended to control the microbiological quality of raw milk (Ay and Bostan, 2017; *Codex Alimentarius,* 2007). This method makes use of a naturally occurring antibacterial system in milk known as the lactoperoxidase system. The lactoperoxidase system has been recommended to be used for the preservation of raw milk in areas where there is no possibility to use mechanical refrigeration for technical and or economic reasons (IDF, 1988; FAO, 1999). The enzyme lactoperoxidase

catalyses the oxidation of thiocyanide (SCN^-) by hydrogen peroxide (H_2O_2) and generates the hypothiocyanite ($OSCN^-$) ion, which has proven to have antibacterial activity (Reiter, 1985).

Goat milk production is a historical practice in southern Europe with an intensive specialization in milk production. Spain is one of the main producers of goat milk in the European Union. Particularly, the Canary Islands have a long tradition of raising native goats, and foreign breeds with higher production yields that have not been allowed to be exploited to favor local breeds whose dairy production has a highly distinguishable characteristic (Torres et al., 2013). The production of goat milk (caprine) is of major importance in several countries where climatic conditions are unfavorable for cattle rearing (Juarez and Ramos, 1984). According to Eurostat 2016, milk production in the European Union member states was approximately 168.2 million tonnes, 96.8 % represented the production of cow milk, 1.7 % ewe´s milk, 1.3 % goat milk, and 0.18 % buffalo´ milk in 2015. In 2019, the nonbovine annual milk production stands at 133 million tons, representing more than 17 % of the total milk output worldwide with goat milk representing 13.5 % of the total output making it the most significant contributor (Ranadheera et al, 2019). Goats raised in pasture-based feeding systems are shown to have a better milk nutritional composition than its counterpart. Goat milk cheese contains potential bioactive components and micronutrients, which aid in the maintenance of the proper metabolism and functioning of the human body (Korhonen and Pihlanto, 2006).

4.1.4.1. Physical chemical characteristics

The physical and chemical composition of the milk is directly influenced by the diets given to the animals, the forage concentrate ratio directly interferes with the volume of milk produced as well as the concentrations of the components, especially the fat content (Chilliard et al., 2014). The composition of milk depends on different factors such as breed, diet, stage of lactation, and environmental and management conditions (Park, 1990). The breed is the main genetic aspect affecting milk quality and, consequently, milk coagulation properties and cheese characteristics. Among them, cheese yield, physicochemical characteristics, and sensorial properties are affected by several genetic factors (Coulon et al., 2004).

The composition and physical characteristics of goat milk vary from species to species. Being a complex oil-in-water emulsion, goat milk contains fat, protein, lactose, minerals as well as enzymes, cells, hormones, and immunoglobulins. There are two major categories of milk proteins insoluble proteins (the casein family) and soluble proteins (whey proteins) found in lactoserum. The caseins include αs1, αs2, β, and K-caseins, while the whey proteins are α-lactalbumin and β-lactoglobulin (Jenness, 1980; Park et al., 2007). Milk also comprises important

minor proteins, such as serum albumin, immunoglobulins, lactoferrin, transferrin, a calcium-binding protein, prolactin, folate-binding protein, and proteose-peptone.

Goat milk has high biological value and nutritional qualities due to its higher digestibility and its dietary characteristics with smaller diameter fat globules. It presents a chemical composition composed of proteins of high biological value and essential fatty acids, besides its mineral and vitamin content (Haenlein, 2004; Park et al., 2007). Goat milk is said to have a buffering capacity which might be useful in the treatment of stomach ulcers. Some of the physicochemical characteristics of goat milk, such as shorter fat globules, softer curd creation, and a greater percentage of medium and short-chain fatty acids, are beneficial for higher digestion and better metabolism of lipid when compared to cow milk (Turkmen, 2017). The buffering capacity of goat milk is due to its relatively higher content of protein, phosphate content, and non-protein nitrogen (Turkmen, 2017). The total content of non-protein nitrogen is around 5-8 % of total nitrogen (Prosser et al., 2008). The main components of the non-protein nitrogen fraction are urea (30 %), free amino acids (with taurine, L-glycine, L-glutamic acid, and L-glutamine being the most abundant), nucleosides, nucleotides, and polyamines (Park et al., 2007; Prosser et al., 2008). A special characteristic of goat milk is the higher level of polymorphism of αS1-CN, where clear differences in the levels of protein synthesized between alleles exist which correlates to the composition of milk and with some milk processing parameters. This relationship between the occurrence of polymorphic forms of αS1 and physicochemical properties of milk is emphasized; this relation affects caprine milk processing quality (Mahaut and Korolezuk, 1994; Tziboula, 1997; Mahmood and Usman, 2010).

4.1.4.2. Microbiological characteristics

Milk has a high nutritional profile, from the smaller size of protein micelles, fat globules, higher levels of short and medium-chain fatty acids, and therefore a desirable medium suitable for microbiological development. Raw goat milk has a mixed microbiome; bacteria, fungi, yeast, and parasites derived from several sources including direct transfer from the blood of an animal and exterior environments such as the surface of goat, udder, faeces, milk handling equipment, and physical contact by personnel (FSANZ, 2009; Verraes et al., 2015). Fresh goat milk collected from a healthy udder under good hygienic (sanitary) conditions contains relatively fewer microorganisms (Burgess et al., 1994) due to the naturally enclosed and intact delivery system. Common routes of raw goat milk contamination can be classified into animal factors: animal health and husbandry; environmental factors: housing, faeces, feed, soil, and water; and milking

practices: milking methods, personnel, equipment, storage, packaging, and delivery (FSANZ, 2009).

According to European Regulation (EC) No 853/2004 bacterial count and Somatic cell count (SCC) in raw cow milk should not exceed 10^5 and $4x10^5$ CFU per millilitre, respectively as hygiene standards of raw milk from cows and other animals. The main microorganisms associated with the consumption of raw milk from cows, goats, and sheep are *Bacillus cereus, Campylobacter spp*., *Staphylococcus aureus, Helicobacter pylori*, Shiga toxin-producing *Escherichia coli* (STEC), *Listeria monocytogenes*, *Salmonella spp*., *Clostridium botulinum, Brucella spp., Mycobacterium bovis, Leptospira, Cryptosporidium parvum* and *Toxoplasma gondii* (Claeys et al., 2013; Verraes et al., 2015; EFSA, 2015). *Staphylococcus aureus, Escherichia coli,* moulds and fungi are microorganisms used as hygienic indicators in the food industry. Pasteurization is considered an effective treatment against foodborne pathogens in milk and milk products, but this is not completely applicable in the case of *Staphylococcus aureus* since they produce *Staphylococc*i Enterotoxins that are thermostable which could cause foodborne intoxication in milk consumers (Oliveira et al., 2011).

4.1.4.3. Organoleptic characteristics

The combination of tastes and aromas associated with caprine milk leads to a range of unique flavors and sensory properties that are critical to the overall quality of caprine milk and its processed products (García et al., 2014). The goaty flavors perceived from goat milk are a result of the high content of free octanoic acids present, which may reduce its acceptance by certain consumers (Young et al., 2012). Cheese flavor arises from a series of complex reactions involving microbial metabolism and enzymatic reactions, which include proteolysis of proteins, lipolysis of fats, and fermentation of carbohydrates (Park, 2001). The products of these microbiological and enzymatic activities result in a vast array of flavor compounds (Yvon and Rijnen, 2001; Marilley and Casey, 2004). These reactions occur throughout the cheese production phase and are concentrated in the ripening phase (Park, 2005). Most of these reactions occur due to both endogenous and microbial-produced enzymes (Smit et al., 2000; Collins et al., 2003). Proteases and lipases mediate many of the most important flavor-generating reactions in goat cheese by the processes of lipolysis and proteolysis (Park, 2001; Jin and Park, 1995). The less firmness described for goat milk products is associated with lower amounts of αS1 casein, greater micellar dispersion, and larger amounts of colloidal calcium among others (Park et al., 2007). Goat milk has unique alkalinity, greater buffering capacity, and therapeutic potential in human nutrition and medicine, which varies from human milk or cow milk (Verruck et al., 2019). Goat milk has a higher proportion of fatty acids including capric, caprylic, and caproic acids. The composition of

these short-chain fatty acids gives goat milk and its cheeses their unique tangy flavor (Kosikowoski and Mistry, 1999).

When goat milk cheese is aged, the tangy flavor may cause a creamy and earthy cheese taste. The color of milk and cheeses depends on the composition of the forage fed to the animals. Milk contains variable amounts of pigments including carotene. The type of diet fed to goats has a direct effect on carotene levels in milk, and on the color of cheese (Coulon et al., 2004). Carotene is present in significant amounts in green forage and contributes to the yellow coloration of dairy products (Park et al., 2017). It has been discovered that cheeses made with spring milk are more yellowish in color than those made with winter milk. Cheeses made with winter milk from dairy goats fed with grass silage are more yellowish than those made with milk from animals fed with hay. Maize silage, containing very little carotene, produces very whitish cheeses (Verdier-Metz et al., 2002). When forage is well conserved, the conservation method has little influence on the sensory characteristics of cheeses, except on the color.

4.1.4.4. Nutritional characteristics

There are certain specie specific differences between goat milk and cow milk, the basic nutrient composition of goat milk is like that of cow milk (Park, 2006). Goat milk as a great dietary source provides ample benefits for health maintenance, physiological process, in the nutrition of the young and the elderly population and studies have reported that goat milk may be consumed by susceptible populations allergic to cow milk (Song et al., 2020). Goat milk is considered superior in terms of numerous health benefits and lower risk of allergy when compared to cow milk and therefore, has been used as a hypoallergenic in infant foods or milk to substitute for an infant's allergy to cow milk (Park, 2006). In a study carried out by Park (2009), the bioactive components of goat milk treatment cured a large population of children suffering from cow milk allergies and in another allergy case study, 49 of 55 treated children benefited from goat milk treatment. Goat milk contains a higher mineral content; calcium, iron, zinc, and magnesium, a higher vitamin content; A and B complexes, and has fewer atherogenic fatty acids, which characterize it as a highly nutritious food for consumers (Haenlein, 2004; Park et al., 2007; Slacanac et al., 2010).

The physiological and biochemical facts of the unique qualities of goat milk are barely known and little exploited, especially the high levels of short and medium-chain fatty acids in goat milk, which have recognized medical values for many disorders and diseases of people (Haenlein, 2004). Goat milk exceeds cow and sheep milk in monounsaturated, polyunsaturated fatty acids, and medium-chain triglycerides, which all are known to be beneficial for human health,

especially for cardiovascular conditions. Capric, caprylic acids, and medium-chain triglycerides have become established medical treatments for an array of clinical disorders, including malabsorption syndromes, chyluria, steatorrhea, hyperlipoproteinemia, intestinal resection, premature infant feeding, non-thriftiness of children, infant malnutrition, epilepsy, cystic fibrosis, coronary by-pass, and gallstones, because of their unique metabolic ability to provide direct energy instead of being deposited in adipose tissues, and also because of their actions of lowering serum cholesterol, inhibiting and limiting cholesterol deposition (Alferez et al., 2001; Greenberger and Skillman, 1969; Kalser, 1971; Schwabe et al., 1964; Tantibhedhyanangkul and Hashim, 1978).

The nitrogen content in goat milk varies according to breed, genetics, season, stage of lactation, and type of feed (Park, 2007; Park et al., 2007). Crude protein extract derived from goat milk whey has the potential to act as a bacteriostatic, cytotoxic compound for the destruction of tumour cells, and a potent antioxidant (Medeiros et al., 2018).

According to USDA (1976) and, Jenness (1980) as cited by Park et al. (2017), caprine milk contains 12.2 % total solids, consisting of 3.8% fat, 3.5% protein, 4.1% lactose, and 0.8% ash, indicating that it has more fat, protein and ash, and less lactose than cow milk (Table 4.1).

Table 4-1. The basic composition of goat and cow milk (mean values per 100 g). Data from USDA (1976), Larson and Smith 1974, Posati and Orr 1976, Jenness (1980), and Haenlein and Caccese (1984).

Constituents	Goat milk	Cow milk	Sheep milk	Human milk
Fat (g)	3.8	3.6	7.9	4
Protein (g)	3.5	3.3	6.2	1.2
Lactose (g)	4.1	4.6	4.9	6.9
Ash (g)	0.8	0.7	0.9	0.2
Total Solids (g)	12.2	12.3	12.0	12.3
Calories (cal)	70	69	105	68

4.2.Halophytic Plants

Halophytes are flowering plants highly salt-tolerant that grow in soil or water of high salinity, sand dunes, rocky coasts, saline depressions, or inland salt flats, and in marine environments such as coastal marshes (Redondo-Gómez et al., 2010; Ksouri et al., 2012). To withstand the unfavourable abiotic conditions that characterize their habitats, such as high salinity and high UV radiation levels, halophytes species have evolved several physiological traits that allow them

to retain and acquire water, protect cells from the damage caused by the accumulation of reactive oxygen species (ROS), and maintain ion homeostasis (Ksouri et al., 2012; Flowers et al., 2010). Some of these traits include the biosynthesis of different primary and secondary metabolites, such as vitamins, terpenoids, phenolics, polysaccharides, and glycosides, which display several biological activities, including antioxidant, antimicrobial, anti-inflammatory, and antitumoral, and thus they can be crucial for the prevention of a variety of diseases as, for instance, cancer, chronic inflammation, and cardiovascular disorders (Rodrigues et al., 2014). Such compounds especially antioxidants can also be very useful in the food industry as additives.

4.2.1. *Arthrocnemum macrostachyum*

Arthrocnemum macrostachyum (Figure 4.4) is found on the Mediterranean coasts of North Africa, Europe, the Orient, and the Red Sea coasts, extending north to Jordan Valley. It is also present in the Middle East, including Iran and Pakistan. It also grows in eastern Africa and at an Altitude range of 0 to 400 meters above sea level having a flowering season from April to September. *Arthrocnemum macrostachyum* is a Carbon 3 (C3) perennial shrub belonging to the family *Amaranthaceae* a coastal halophyte that can tolerate hypersaline concentrations reaching up to 1.02 M (Khan et al., 2005) and can survive in arid desert climates where temperatures exceed 60 ^{o}C in mid-summer days (Redondo-Gomez et al., 2010). This species grows in clumps to a height between 30 and 100 cm, with erect branches with terminal cylindrical obtuse spikes measuring between 3 to 4 mm. The stems are green, with red-colored pigmentation seen on the stems in the hot dry season attributed to the presence of pigments such as anthocyanin and betacyanin (Mora-Ruiz et al., 2016). *Arthrocnemum macrostachyum* has been cultivated for oils and contains a remarkable quantity of edible oils. The seeds contain between 22 and 25% oil per weight of which unsaturated fatty acids account for 60% to 80% of total oil content (Weber et al., 2007; Cybulska et al., 2014). Samples collected from southern Portugal were reported to contain a high amount of polyunsaturated fatty acids up to 75.0% of total fatty acids content, and of which α-linolenic acid and linoleic acid were the most predominant (Barreira et al., 2017) α-linolenic acid is bioactive and was reported by several studies to exhibit anti-inflammatory effects (Ren and Chung, 2007) and have the capacity to decrease coronary disease (Connor, 2000), exhibit neuroprotection effects (Lauritzen, 2000), and exhibit antifungal activity (Walters et al., 2004). *Arthrocnemum macrostachyum* is a potential biological source of biochemicals counteracting free radical-induced oxidative damage and degenerative diseases associated with metabolic stress such as cancer and neurological disorders (Custodio et al., 2012). Moreover, *Arthrocnemum macrostachyum* contains phenols, flavonoids, tannins, and alkaloids which are secondary metabolites responsible for different

bioactivities, such as antioxidant and anti-inflammatory. Therefore, this plant has been used as traditional medicine in the treatment of microbial infections, reduction of blood pressure, treatment of cancerous tumours, and many other cases *in vitro* studies have affirmed these ethnopharmacological uses. It also plays a prominent role as an antibiotic (Zabka et al., 2011) and as an alexipharmic to remedy snake bites and scorpion stings in Tunisia (Agoramoorthy et al 2008; Boulaaba et al., 2013) and plant extracts used as hypoglycaemic agents in India (Sekii et al., 2015; Al-Tohamy et al., 2018).

Arthrocnemum macrostachyum have been traditionally consumed for their organoleptic and/or medicinal properties (Davy et al., 2001; Ventura and Sagi, 2013), and used in gourmet cuisine due to their salty taste (Ventura et al., 2011). Due to their tolerance to salt and resistance to pests and diseases, they can be grown in brackish or saline water rather than freshwater in marginal or salinized soils (Ventura and Sagi, 2013; Diáz et al., 2013; Glenn et al., 1999).

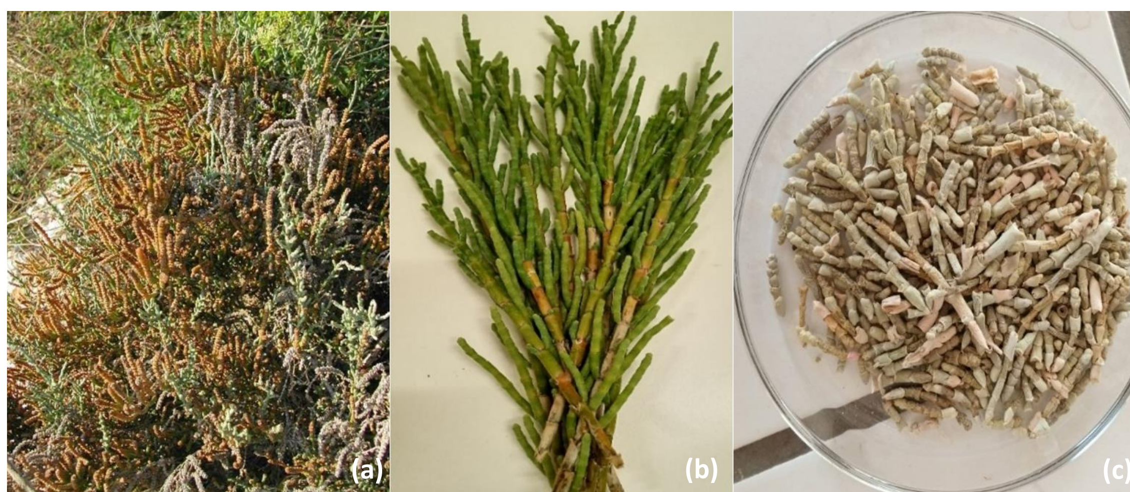

*Figure 4 4. Arthrocnemum macrostachyum (a) Plant in nature, (b) fresh plant, (c) dry edible part of plant (*Titalah Sheron, 2022*).*

4.2.1.1. Antioxidant Activity of *Arthrocnemum macrostachyum*

Oxidative stress and free radicals are a concern with regards to the homeostasis of the body since when the process is not properly regulated, it can contribute to the emergence of various chronic and degenerative diseases such as heart disease, cancer, arthritis, stroke, respiratory diseases, immune deficiency, emphysema, Parkinson’s disease, and other inflammatory or ischemic conditions. Antioxidants can stabilize or disable free radicals, therefore protecting the human body against cell damage caused by oxidative stress and contribute to the maintenance

of health (Valko et al., 2006; Rodrigues et al., 2020). Antioxidants can also be added to food products to prevent or delay food oxidation (Halliwell et al., 1995).

Halophyte plants are adapted to the most varied abiotic stresses, such as high salinity, UV intensity, and drought (Barreira et al., 2017) in part due to the synthesis and accumulation of bioactive primary and secondary molecules, which have an important nutritional value and relevant biological properties, such as antioxidant and antiparasitic activity. *Arthrocnemum macrostachyum* is an important source of antioxidant molecules. According to Rodrigues et al. (2014), while working on different extracts, it was identified that *Arthrocnemum macrostachyum* is a good source of phenolic and flavonoid compounds. *Arthrocnemum macrostachyum* has high commercial importance exploited not only for its traditional medicinal properties as antibiotics (Zabka et al., 2011), alexipharmic (Agoramoorthy et al., 2008; Boulaaba et al., 2013), antifungal activity (Walters et al., 2004), anti-inflammatory effects (Ren and Chung, 2007), exhibit neuroprotection effects (Lauritzen, 2000), and hypoglycaemic agents in India (Sekii et al., 2015; Al-Tohamy et al., 2018) but also used in gourmet cuisines (Barreira et al., 2017).

4.3. Cheese

4.3.1. Origin, history, and evolution

The earliest root of cheese production dates to ancient times during the domestication of goats about 10,000 years ago in the mountain regions of Iran (Haenlein, 2007). Formally, goats were considered a marginalised species for subsistence farming of poor populations underestimating their role in economic and other potentialities. As of now, goats are no more synonymous to underdevelopment and poverty, truth be told, goat milk assumes an imperative part in human nourishment in the area acknowledged as the cradle of modern civilization (Hatziminaoglou and Boyazoglu, 2004; Selvaggi et al., 2014). Their importance in the economic upliftment and nutritional well-being of the human population is significant in several regions around the world, particularly in the Middle East and the Mediterranean countries (Park, 2017). What makes goats so famous is their ability to provide high quality food under diverse climatic conditions, as well as their resilience to extreme environments (Silanikove, 2000).

There are hundreds of different types of cheeses produced all over the world. Different styles and cheese flavours are the results of using different species of starter bacteria and ripening moulds; different concentrations of milk fat; differing coagulation methods and processing treatments including cheddaring, pulling, brining, mould wash; variations in the length of ageing; and using milk from different breeds of cows, sheep, or other mammals. Other factors include

variations in animal diet and the addition of flavouring agents such as herbs, spices, or wood smoke. Cheesemaking has remained an art rather than science until relatively recently. Although the names of many varieties of cheeses have existed for hundreds of years (Table 4.2), cheeses were not standardised and there may have existed great variations within any one cheese type (Fox, 2004).

Table 4-2. First recorded dates for some major cheese varieties (Adapted from Scott, 1986 *in* Fox et al., 2004).

Variety	Year	Variety	Year
Gorgonzola	897	Gouda	1697
Roquefort	1070	Gloucester	1783
Grana	1200	Stilton	1785
Cheddar	1500	Camembert	1791
Parmesan	1579		

The contribution of goat milk production to the economic and nutritional wellbeing of humanity is undeniable in many developing countries, especially in the Mediterranean, Middle East, Eastern Europe, and South American countries. In developed countries belonging to Europe, Oceania, and North and South America, goat milk production is assuming an increasingly economic relevance, especially due to the production of goat cheeses which are suitably selected as gourmet food and receive the highest prices among cheeses on the market in France and Italy. In addition, dairy goat and dairy sheep farming are a traditional and fundamental part of the national economy in many Mediterranean countries, including Spain, Greece, Turkey, and Morocco (Park et al., 2007).

In 2014, the world production of goat cheeses counted totally was 523,000 tons, showing a rapid increase from 2011 to 2013, just over 15%, as compared to the world cow dairy production in the same period which was 5.4% (FAOSAT, 2018). A large proportion of goat milk in the world was produced in Asia (10.55 million tons, that is 56% in 2014, and 57% in 2017), the highest production of goat cheese came from Africa, particularly from South Sudan (110,750 tons). Europe produced 35% of world goat cheese with only 15% of goat heads. The world regions top producers of goat milk cheese were Eastern Africa particularly South Sudan, and Western Europe with 87,407 tons, from France, Germany, and Austria, followed by Northern Africa with 85,105 tons, from Sudan, Morocco, and Tunisia, and Southern Europe with 81,854 tons, from Greece, Spain, Italy, Portugal, Albania, and Malta (Sepe and Argüello, 2019).

Goat milk cheese is the most important and highly consumed manufactured product of goat milk. According to the FAO data, goat milk is mainly processed into cheese, and the world's goat cheese production is 564,075 tons (FAO, 2021). The production of high-quality goat cheeses is

associated with Mediterranean countries such as France, Italy, and Spain, while in most countries, goat milk is consumed more locally (Miller and Lu, 2019). In Portugal goat production is more meat than dairy oriented. However, goat farms reveal a greater milk tendency than sheep farms although goat farms are very small (Tibério and Diniz, 2014). The average size of goat herds (13 animals) is considerably lower than that of sheep and increased by only 3 heads in 1999 (IVE, 2011). The dairy herd of about 150,000 animals, represents about 35% of the goat population and is distributed over approximately 12,000 farms, which represents approximately 36% of the total number of goat farms (Tibério and Diniz, 2014).

In Europe, the traditional products are protected and valorised by the acknowledgment of a labelling system where the production is strictly regulated. Goat products are differentiated in Portugal between high quality and current products using specific denominations and standards. Products bearing the Protected Denomination of Origin (PDO) label are those whose production, transformation, and elaboration occur within a well delimited, geographical area. The PDO follows the narrowest rules. There is also the Protected Geographical Indication (PGI), indicating that only a stage of the whole process of production, transformation, or elaboration needs to be done in a well delimited geographical area (Tibério and Diniz, 2014). The last differentiation is the Traditional Specialty Guaranteed (TSG). Products bearing this label do not have to be manufactured in a specific zone at any stage of the process but must be produced according to a traditional method.

Since April 2019, the European Union had 216 PDO and 59 PGI types of cheese (European Commission, 2019). Among them, 41 were from goat milk. In Portugal, "Queijo de Cabra Transmontano/ Transmontano Velho", "Queijo Rabaçal", "Queijo Amarelo da Beira Baixa/ Queijo Picante da Beira Baixa" are DPO; while "Queijo mestiço de Tolosa" is PGI. Food consumers appreciate the opportunity to purchase products made directly on the farm, since they identify the products with their place of origin and are confident of their quality. This quality is often associated with the region of production, the climate, and the vegetation present in each region, which distinguishes it from efficiently manufactured food (Medeiros et al., 2014). It must be noticed that specific character and difficult environmental conditions of the mountain areas, where the food products are produced have been recognised by the European Commission for a long time by their presentation of the quality term "mountain product" (European Regulation No. 1215/2012).

The dairy industry has also beheld the chance to produce functional dairy products from goat milk, which may potentially benefit human health and contribute to attenuating issues related

to CVD, overweight, obesity, and diabetes (Sepe and Argüello, 2019). One of the greatest challenges for mankind is to combat the changing climatic conditions, which propels us to develop sustainable strategies to adapt to the changes in water availability temperature, soil system, vegetable, and animal biodiversity, both preserving the environment and satisfying the increasing food demand. Therefore, Novel functional foods with health-promoting natural ingredients instead of synthetic additives have been intensively developed and commercialized by the food industry (Caleja et al., 2015; Carocho et al., 2015). In the current work, the main goal was to substitute the added salt to the goat cheese with Na present in the biomass of *Arthrocnemum macrostachyum*, while exploring the bioactive properties and preservation effects on the goat cheese. Substituting part of the salt in the cheese with *Arthrocnemum macrostachyum* biomass can have several benefits. Firstly, to reduce the sodium content of the cheese, which can be beneficial for people who are sensitive to or need to limit their sodium intake. Secondly, the biomass may have functional properties that can improve the texture and shelf life of the cheese. Additionally, the use of a natural ingredient like *Arthrocnemum macrostachyum* biomass can be appealing to consumers who are looking for healthier and more natural food options.

In the current work, fresh goat cheese enriched with *Arthrocnemum macrostachyum* biomass was produced, substituting the added salt in the cheese with dietary Sodium (Na) present in the biomass to obtain a final product with improved bioactive properties and eventually increased preservation time.

4.3.2. Definition and composition

Cheese is a concentrated food with a selective concentration of components which differs from milk. In addition, the microbial fermentation adds a new dimension of nutrition to both cheese and cultured milk foods. According to Fox et al. (2004), cottage cheese contains 79-82.5% moisture, 12.5-17.5% protein, 0.7-1.4% ash 2.6- 3.6% carbohydrate and 72-103 Kcal/100g energy when cheese contain 0.4-4.5% fat. Cottage cheese is a soft unripen white cheese with 80 % maximum moisture and 4 % minimum fat in dry matter (Scott, 1998). Cream and salt can be added. The cheese has been commonly produced using skim milk with the addition of herbs or fruits to give the product flavour, but full fat or whole milk cottage cheeses are also available (Robinson, 1995).

Cheese can be grouped according to their composition and manufacturing technique, consistency, or rheology (softness or hardness), country of origin, general appearance (size, shape, colour, surface ripening), source of milk, and chemical analysis, and as acid or rennet

(enzyme), and as natural or processed cheeses. Natural cheese is an industrial term used to describe cheeses made directly from milk. Processed cheeses are made using natural cheese plus other ingredients that are cooked together to change the textural and/or melting properties and increase shelf life.

4.3.3. Manufacturing technology

Cheese making likely began as a way of preserving soured and curdled milk through pressing and salting, when it was noticed that cheese made in an animal stomach produced more solid and better-textured curds with rennet later introduced. Asian nomads were the first people to discover the coagulation properties of milk when they stored it in bags made from the stomach of herbivorous animals that contain rennet (Braga, 2003). The main ingredient used in cheese making is milk. Cheese is made using cow, goat, sheep, water buffalo or a blend of these milks. The cow is the most significant of every species whose milk is used, but sheep, goat, and buffalo are commercially important in certain areas (IDF, 2007). Each cheese is a biologically dynamic material, and its production represents a series of successive biochemical stages prompting to a final product with exceptionally desirable aromas and flavours. Other flavourings may be added depending on the cheese type. Some common ingredients include herbs, spices, hot and sweet peppers, horseradish, and Port wine.

The fundamental processing techniques involved in the processing goat milk are milk collection, filtration, standardisation, pasteurisation, chilling, packaging, storing, and distribution of goat milk (Moatsou and Park, 2017). A typical farmhouse goat milk cheese making process consists of the nine fundamental stages as listed (Paschino et al., 2020); milk filtering, renneting, milk coagulation, putting the curds into appropriate cheese molds, draining, unmoulding, salting, drying, ripening (Medina and Nuñez, 2017). The major steps and conditions in the making of 4 textured cheese varieties adapted from Scott et al. (1998) and Fox et al. (2000) (Figure 4.5).

Dairy farming provides one of the most cost-effective methods of converting crude animal feed resources into high-quality protein-rich food for human consumption. However, since milk is a very perishable foodstuff special measures and considerations are necessary to ensure that it reaches the market in an acceptable condition. The collection of milk from the farmers and transportation to the dairy is the most critical link in the total handling chain of milk. This problem is recognized worldwide (Barron del Castillo, 1990; Claesson, 1992). After the milk is collected from an individual animal, while in a bulk tank, or a milk transportation truck, it is filtered to remove impurities like sediments, udder body cells, and certain bacteria (Moatsou and Park, 2017). Clarification is used in the removal of excess impurities through the distribution

of the milk into thin layers over conical disks, revolving at a relatively high speed. Before cheese making the first step is to is obtain high quality goat milk that is free from visible impurities, abnormal odour or taste, foreign substances, pathogenic microorganisms, and should possess desirable acidity between pH 6.2 to 6.55 (Park and Guo, 2006).

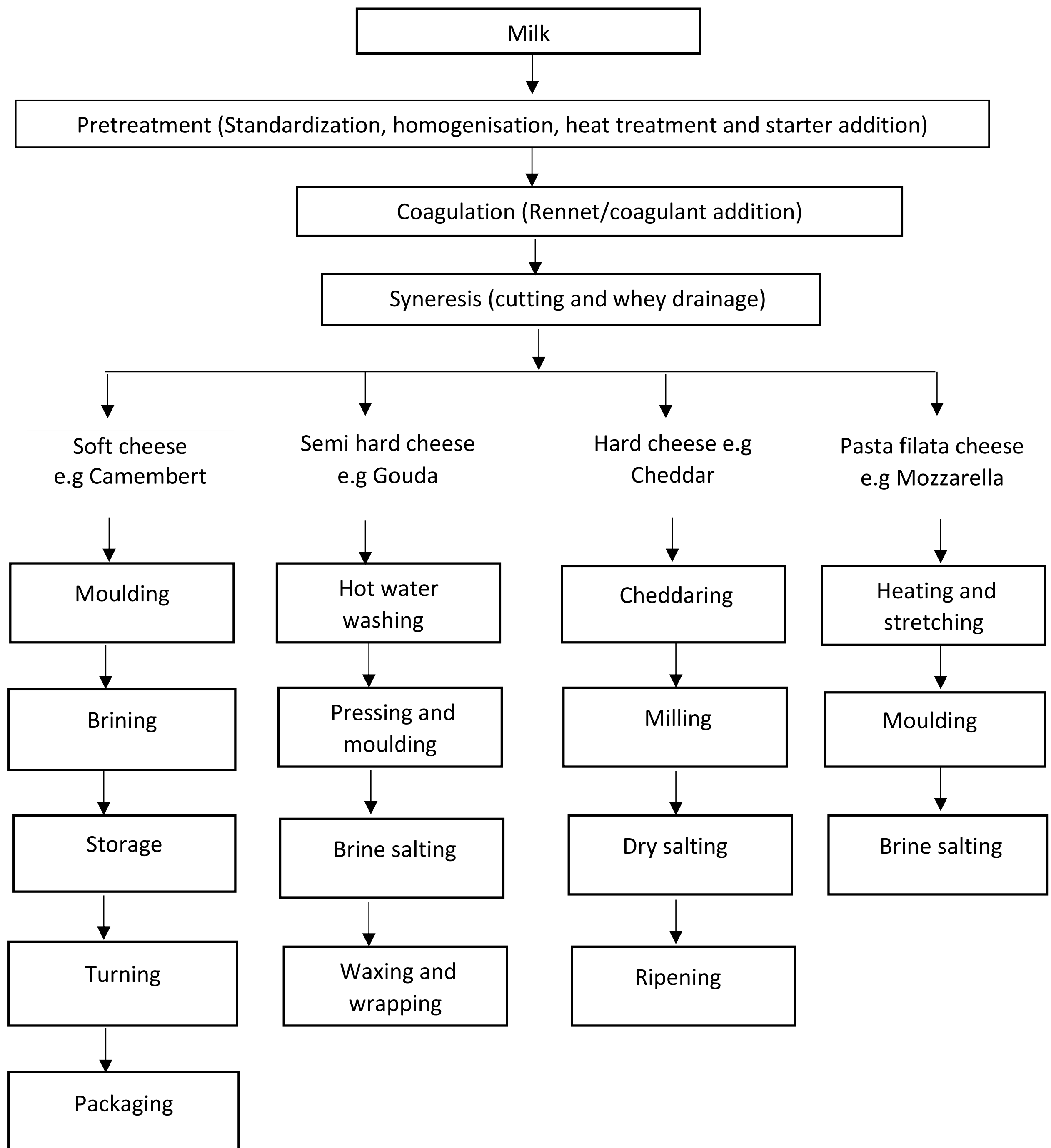

Figure 4 5. Major steps and conditions in the making of 4 textured cheese varieties (adapted from Scott et al., 1998 and Fox et al., 2000).

Pasteurization or pre-treatment of cheese milk

After milking the milk for cheese production is immediately cooled to 4 °C and may be held at this temperature for several days on the farm or at the processing facility (Fox, 1987). At this temperature, the development of total bacteria counts, and some pathogenic bacteria growth is limited but increases the growth of psychotropic microorganisms such as Pseudomonas. These bacteria, contribute to the rapid and progressive degradation of milk proteins and lipids through their enzymes that are heat resistant which can occur not only during storage, but also after heat treatment. Cold storage can provoke the destabilisation of the balance of the minerals and proteins in milk. Under refrigeration conditions, the solubility of micellar calcium and β-casein increases which cause degradation of technological properties like an increase in the renneting time. However, this effect seems to be less marked in goat milk than in cow milk (Raynal and Remeuf, 2000). Heat treatment depending on time and temperature relation. Pasteurization can be performed as low temperature long time (65 °C, 30 min); high temperature short time (72-75 °C for 25 seconds) or ultra-high temperature (125 ± 5 °C for 4s or 135 ± 5 °C, 4s) (Chen et al., 2019; Cole et al., 2020; Deeth, 2020). The purpose of pasteurization is to increase milk safety for the consumer by destroying vegetative pathogenic microorganisms that may be present in milk and reduces the natural occurring fermentation. Spore formers and thermoduric gram-positive bacteria may survive pasteurisation. Spore formers can either be unaffected by pasteurisation or activated (Jay, 2000; Giffel et al., 1995). Pasteurization increases the shelf life and standardizes quality of milk products by destroying spoilage microorganisms and enzymes. The aim is to cause minimum change in composition and flavour acceptability of the milk. The effective heat treatment does not necessarily entail the destruction of all the microorganism originally present, but it must destroy any pathogens, which are mostly sensitive to heat (Kay et al, 1962). Contamination with pathogens and spoilage bacteria can happen during the addition of starter culture, addition coagulant, and salting (Marler, 2009).

Addition of Starter Culture

The application of starter culture in the production of cheese encourages whey separation, inhibits the growth of pathogenic bacteria, generates some aromatic compounds, and increases the degree of ripening. In the dairy industry, various starter types used for cheese making include *Lactococcus lactis* sub sp. *lactis* or *cremoris, Streptococcus salivarius* sub sp. *thermophilus, Lactobacillus delbruckii sub sp. Bulgaricus, and Lactobacillus helveticus*. The lactic acid bacteria that are used to induce lactic fermentation are also essential in the manufacture

of cheese and fermented milk products (Harrigan and MacCance, 1976). After pasteurization, the milk is then inoculated with starter cultures usually *Lactococcus lactis* sub-spp *lactis* and *cremoris* (Robinson and Wilbey, 1998).

Coagulation

During coagulation milk undergoes a profound physical and rheological change known as gelation. Milk gel is achieved by aggregation of milk protein casein, by the action of proteolytic enzymes, lowering the pH below the isoelectric point of protein (~4.6), heating to about 90 °C at a pH of about 5.2, higher than the isoelectric point (Kosikowski and Mistry, 1997). The type of coagulant used depends on the type of cheese desired. For acid cheeses, an acid source such as acetic acid (the acid in vinegar) or gluconodelta-lactone (a mild food acid) is used. For Rennet cheeses, calf Rennet or more commonly, a Rennet produced through microbial bioprocessing is used. Calcium chloride is sometimes added to the cheese to improve the coagulation properties of the milk. The cheeses from enzymatic coagulation with animal (calf Rennet, porcine pepsin) or plant Rennet (*Cynara cardunculus* from cardom), represents most of the world production for both farmstead and industrial applications.

Salting

During cheese making, salt can be applied at two stages: before coagulation as done in cheeses from the Algarvian goat breed and salting of curd before pressing or immersion in brine after pressing. The salt level of cheese significantly affects microbial growth, enzymatic activity and biochemical changes that determine the flavor, aroma, texture, and overall quality during ripening (Guinee and Fox, 2004; Ardö et al., 2014). Consequently, precise control of this factor is essential to cheesemaking to ensure consistent and optimal quality (Guinee and Fox, 2004). With temperature, pH, water activity, redox potential, and microbiota, salt helps conserve cheese, minimizing deterioration and prevent the growth of pathogens. All these factors interact with each other and, in many cases, the distinction between them is not clear (Tabla et al., 2015).

Ripening

The ageing of cheese allows the development of specific flavor, structure, and textural qualities by the action/activity of specific enzymes and microorganisms maintained under the conditions favorable to the desired activity and growth. The ageing conditions can also result in objectionable changes if the original milk is contaminated with undesirable microorganisms or if improper manufacturing procedures are used. Therefore, knowledge of the main

physicochemical, biochemical, and microbiological characteristics at various stages of ripening is required for the development of an acceptable product (Guizani et al., 2006). The biochemical processes of cheese ripening include proteolysis, lipolysis, and glycolysis. Cheeses, depending on the varieties, undergo ripening from 4 weeks to more than two years before consumption. Proteolysis is the principal and most complex biochemical event occurring during the ripening process (Grappin and Beuvier, 1982) which directly contributes to the cheese flavor through the formation of peptides and amino acids, and textural characteristics attributable to the breakdown of the protein network (Fox, 1989). Textural properties have significant effect on consumers acceptance (Bugaud et al., 2001). Lipolysis also plays an important role in cheese flavor by generating free fatty acids (FFA) that are precursors of volatile compounds, like methyl ketones, alkanones, and lactones (Nájera et al., 1993).

Cheeses made from raw milk tends to develop stronger flavors and ripen quickly than cheeses from pasteurized milk. Yet, most commercial cheeses are produced from pasteurized milk, rather than raw milk, to eliminate pathogens.

4.3.4. Quality control

4.3.4.1. Physical chemical Control

Cheese yield

The increasing demand for goat cheese, coupled with an increase in the price of milk, has stimulated new interest in the cheesemaking ability of goat milk formulae predicting cheese yield based on milk components (Zeng et al., 2007). The main problem with those formulae regarding goat milk is the wide range of variation of its composition in relation to the different breeds and dairy systems. Cheese yield relies solely on the fat and protein particularly the casein content of milk and on the technological properties of processed milk (Law and Tamine, 2010). Milk fat and protein contents are considered formulas to predict cheese yield (Emmons and Modler, 2010). One of the most important attributes of milk affecting the profitability of dairy farmers is the percentage ratio between milk processed (% CY) and cheese manufactured (Emmons et al., 1993). Cheese yield is defined as the amount of cheese, expressed in grams, obtained from 100 kg of milk (Mona et al., 2011). This means that the higher the percentage of milk solids recovered, the greater the amount of cheese obtained and therefore the profit gained in economic terms.

Color

Colour is a visual attribute of food quality that determines consumers' choices and preferences. A physical stimulus is captured by the vision and encoded in the brain, producing a sensation in those who observe (Pedrosa, 2003). Color is simpler, faster, and correlates well with other physicochemical qualities, therefore a color measurement of food products has been employed as an indirect indicator of other quality attributes such as flavor and pigment content (Pathare et al., 2012). In 1948, CIELAB L*, a*, and b*, was created for photoelectric measurements. In 1976, CIE L*a*b* color space was established to provide consistent color differences regarding human perception. The presence of color necessitates the presence of an object, a light source (illuminant), and an observer. A light source can be used to view an object and can be turned on and off. An illuminant, on the other hand, is a mathematical description of a light source. The parameter a* is positive for reddish colors and negative for greenish colors, whereas the parameter b* is positive for yellowish colors and negative for bluish colors. L* is a rough measure of luminosity, which is the quality that allows each color to be considered comparable to a member of the greyscale, which ranges from black to white (Granato and Masson, 2010). Chroma (C*) is the qualitative feature of colorfulness used to assess the difference between a hue and a grey color of the same luminance. The color intensity of samples perceived by humans is proportional to their chroma values (Pathare et al., 2012).

The color of the cheese results from the penetration of light into the surface layers captured by fat globules and serum bags (Lemay et al., 1994; Paulson et al., 1998). Homogenization increases the number of fat globules, increasing the capture centers and thus increasing the luminosity (Everett and Auty, 2008). This increased luminosity can contribute to increased perception of white color (Fife et al., 1996). The trend towards green is characteristic of low-fat cheeses, where the small size of the blood cells of fat allows the color of the serum to be revealed (Fife et al., 1996). The yellowish color of dairy products originates mainly from pigments like carotenoids present in the animal diet. Goat milk is poorer in these elements, which makes the cheese clearer than that of cow, a factor that is often a determinant of the greater acceptability by certain consumers (Fox and McSweeney, 1998; Fox et al., 2000; Park, et al., 2006; Fuquay et al., 2011).

Potential Hydrogen (pH)

Potential hydrogen (pH) is a physical-chemical parameter used to indicate whether a solution is acidic, neutral, or basic (Magri, 2015) was introduced by the biochemist Soren Sorensen in 1909. The determination of pH represents an important factor in the evaluation of the quality various

foods such as cheeses, since it is considered to have an important role in relation to microbiological activity, texture, and maturation. These chemical reactions catalyzed by the enzyme rennet and microorganisms depend directly on the pH (Sousa et al., 2014).

Water Activity (a_W)

In the food industry, food scientists, employ a_W in foods for product development, quality control and food safety. It is also a criterion for assessing and monitoring food safety and quality. Water activity (a_W) is the relationship between the water vapor pressure exerted by water in a food system (P) and that of pure water (P0) at the same temperature: a_W=P/P0. Together with temperature and pH, and food storage conditions (temperature, atmosphere, and pressure), a_W is one of the main parameters for evaluating chemical, biochemical and microbiological changes that occur in food, since a high a_W favors the development of most microorganisms (Aung and Chang, 2014; Gram et al., 2002).

Dry weight

Dry matter, dry weight, or dry extract refers to the residue that remains after evaporation of water from the sample and subsequent drying at the temperature of 101±1 °C (NP 3544:1987). The dry matter residue of food contains carbohydrates, fats, proteins, vitamins, minerals, and antioxidants. The energy in foods is provided by carbohydrates, fats, and proteins (measured in kilocalories or kilojoules), which make up 90 % of the dry weight of a diet. For both milk and its products, the dry weight is a fraction of the importance of its quality and nutritional profile.

Ash Content

Ash content is the inorganic residue that remains after ignition or complete combustion of organic matter in a foodstuff (Marshall, 2010). The ash constituents' residues include potassium, sodium, calcium, and magnesium, which are present in larger amounts as well as smaller quantities of aluminum, iron, copper, manganese or zinc, arsenic, iodine, fluorine, and other elements present in traces. The determination of ash content involves the removal of water and other volatile constituents as vapors and the organic constituents are burnt off in the presence of oxygen to carbon dioxide and oxides of nitrogen and eliminated together with hydrogen from water. Ash content is important in the food industry as it is a base for nutritional evaluation. Ash content is the first step in preparing a food sample for specific elemental analysis. Ash content analysis are usually of significant importance when working with foods with high mineral content (Marshall, 2010).

Fat

Fat content is one of the most important components of the technological, nutritional, or dietetic quality of goat milk. After parturition goat milk fat content is high and decreases during a major part of lactation. This is associated to the dilution effect due to the increase in milk volume until the lactation peak, and a decrease in fat mobilization that decreases the availability of plasma non-esterified fatty acids, especially C18:0 and C18:1, for mammary lipid synthesis (Chilliard et al., 2003). Goat milk fat is composed primarily of 98% triglycerides or triacyl glycerides and a small part made of phospholipids and sterols. Three medium chain fatty acids caproic (C6:0), caprylic (C8:0), and capric (C10:0) are predominant in goat milk. They contribute to about 15% of the total fatty acid content in goat milk in comparison to 5% in cow milk (Haelein, 1993). The presence of relatively high levels of medium chain fatty acids (C6:0 to C10:0) in goat milk fat could be responsible for its inferior flavor (Skjevdal, 1979).

Protein

Cheese is a solid milk concentrate that consists mainly of proteins. Curd formation and whey draining result from the coagulation of milk proteins that takes place by means of the addition of rennet or other coagulating agents (*Codex Alimentarius* Commission, 2013). The main protein fraction in all milk is casein which represents approximately 80 % of the total protein. The most significant aspect of the structure of milk casein is the fact that α-s1, β, and calcium phosphate are in the interior of the micelles, whereas K-caseins is predominantly located on the surface of the spherical casein micelle to form a protective layer at the surface of each spherical micelle (Christrian, 1996). The size of casein micelles varies considerably within and between species. They seem to be smaller in goats' milk than in cows' milk. According to Le-Jaouen (1981), the peak frequency of micelle diameters of cows' and goats' milk was 75 and 50 nm respectively.

Antioxidant activity of Cheese

The chemical and nutritional composition of pasture plants changes seasonally, which gives cheeses made from milk of grazing animals different nutritive properties and flavors during year-round seasons, being a significant source of bioactive compounds (Sanz Sampelayo et al. 2007), metabolites that are thought to be an important part of both human and animal diets (Galina et al., 2007; Ruiz-Teran et al., 2008). Milk and by-products provide a wide range of these compounds with potentially functional properties which are transferred from the animal diet (O'Connell and Fox, 2001; Cuchillo et al., 2009; Puga et al., 2009). Soft goat's cheese antioxidant activity can be modified by the animal's feeding system that is grazing management represents a better option than indoor feeding to produce a healthy profile of bioactive compounds,

providing an increase in total polyphenol, hydroxycinnamic acids, and flavonoid concentrations. A tenuous relationship has been established between forage intakes and antioxidant compounds in goat milk, particularly in flavonoids like rutin and quercetin (De Feo et al., 2006). Several compounds exhibit antioxidant activity, protecting lipids and other molecules against the oxidation or the production of free radicals, which have been regarded as a dangerous forerunner of cardiovascular diseases (CVD).

4.3.4.2. Microbiological Control

Escherichia coli

Escherichia coli belongs to the coliform group of microorganisms that ferment lactose with both gas and acid production within 48 h at 32 to 35°C (Davidson et al., 2004). Coliform is a general term used for Gram-negative, anaerobic, facultative bacteria that have a rod shape. The members of the coliform group include *Enterobacter, Citrobacter, Escherichia,* and *Klebsiella. Escherichia coli* bacterium is commonly found in intestinal microbiota of humans and warm-blooded animals, and it is the primary indicator of hygiene deficiency during food production (Cadavez et al., 2017). *Escherichia coli* is a Gram-negative bacterium, with no endospores which among coliform bacteria is considered the species that most effectively indicates fecal contamination and the possible presence of enteric pathogens.

The ingestion of food or liquids with certain types of *Escherichia coli* can lead to mild or severe gastrointestinal diseases. Some types of pathogenic *Escherichia coli* can be fatal (FDA, 2019). Patients generally have an abrupt onset of diarrhea that does not contain blood, pus, or mucus, and is usually mild to moderate in severity. Some patients may have symptoms like severe fluid loss, in addition to low fever, nausea, vomiting, and abdominal pain or stomach cramps. Dehydration can become severe or fatal in newborns and children. Contamination occurs mainly by the fecal-oral route and is more common in developing countries, which lack adequate sanitation facilities and treatment of drinking water. According to the European Regulation No. 1441/2007 (Table 4.3), the criterion for *Escherichia coli* in cheeses is n=5, c=2, m=100, M=1000 cfu/g (n = number of samples or units analyzed; c = maximum allowable number of sample units yielding marginal results, i.e. results between m and M; m = microbiological level that separates good quality from defective, or in a three-class plan good from marginally acceptable quality; M = microbiological level in a three class plan that separates marginally acceptable from unacceptable (defective) quality).

Staphylococcus coagulase positive

Staphylococcus species is facultative anaerobic Gram-positive, non-spore-forming spherical-shaped bacteria commonly found in the environment, humans (nose and skin), and animals. Although several *Staphylococcus* species can produce *Staphylococcal Enterotoxins* (SEs), including both coagulase-negative and coagulase-positive isolates, most Staphylococcal Food Poisoning (SFP) is attributed to SE produced by coagulase-positive *Staphylococcus aureus* (FDA, 2012; FSANZ, 2013). *Staphylococcus aureus* growth can occur at temperatures between 7 to 48°C, pH of 4.0 to 10.0, and a minimum a_W of 0.83 when other conditions are near optimum. SEs are resistant to heat inactivation and cannot be destroyed by cooking. SEs remain stable under frozen storage conditions (FSANZ, 2013). Generally, SE is a moderate hazard that causes illness of short duration and usually no sequelae (ICMSF, 2002). People of all ages are susceptible to SFP. However, the severity of symptoms may vary depending on the quantity of SEs consumed and the general health status of individuals. The young and elderly are more likely to develop more serious symptoms (FSANZ, 2013). SFP is characterized by rapid onset gastroenteritis that appears around three hours after ingestion (normal range of 1 to 6 hours). Common symptoms of SFP include nausea, vomiting, abdominal cramps, and diarrhoea. Recovery is usually between 1 to 3 days (FSANZ, 2013). People become ill after exposure to very small quantities of SEs (less than 1 µg). These levels of toxin are generally observed when *Staphylococcus aureus* populations exceed 10^5 CFU/g of food (FDA, 2012). Milk could be contaminated by *Staphylococcus aureus* by the infected herd. The risk of contamination can be negated through chain controls by effective heat treatment during cheese processing. Post-processing contamination of cheese with *Staphylococcus aureus* can occur, although several processing factors and or product physical-chemical characteristics influence the potential growth of *Staphylococcus aureus* in cheese including pH, salt concentration, water activity, and maturation or ripening conditions (FSANZ, 2006). Although pasteurisation will inactivate *Staphylococcus aureus*, SE is thermostable and will not be affected by the pasteurisation process. The European Union has microbiological criteria for *Staphylococcus aureus* and *Staphylococcus* coagulase-positive in cheese made from pasteurised milk (European Regulation 1441/2007). According to this European Regulation criterion for *staphylococcus* coagulase-positive in cheeses is n=5, c=2, m=100, M=1000 cfu/g (n = number of samples or units analyzed; c = maximum allowable number of sample units yielding marginal results, i.e. results between m and M; m = microbiological level that separates good quality from defective, or in a three-class plan good from marginally acceptable quality; M = microbiological level in a three class plan that separates marginally acceptable from unacceptable quality (Table 4.3).

Microorganisms that grow at 30 °C

The large microbial population responsible for food contamination could be assessed by the counts of mesophilic aerobic bacteria in colonies growing at 30 °C. Although there is no distinction between pathogenic microorganisms and microorganisms causing changes in food, the determination of mesophilic aerobic bacteria gives a general indication of the contamination of food or utensils used during processing or storage (Garayoa et al., 2017). These groups of microorganisms develop in a wide range of intermediate temperatures, with their optimum growth between 30 °C and 45 °C. Minimum temperatures are between 5 °C and 15 °C and the highest between 35 and 47 °C (FDA, 2009). The counting of mesophilic microorganisms can be used to evaluate hygienic quality, verify the application of good manufacturing practices (Silliker, 1963).

Yeasts and moulds

Fungi, also known as molds and yeasts, are heterotrophic eukaryotes, which have a rigid cell wall and can be unicellular or multicellular. The fungi responsible for cheese contamination during production are diverse and belong to several genera as *Acremonium, Alternaria, Aspergillus, Aureobasidium, Botrytis, Cladosporium, Epicoccum, Eurotium, Exophiala, Fusarium, Gliocladium, Lecanicillium, Mucor, Penicillium, Rhizopus,* and *Wallemia* (Garnier et al., 2017). The genera responsible for cheese spoilage are *Penicillium* and *Aspergillus* (Garnier et al., 2017 and Marin et al., 2015). Under favorable conditions, spores germinate and produce hyphae that can invade seeds, grains, and other substrates (Borges, 2013). Some species of fungi like *Penicillium* are used in the production of cheeses. However, there are species that are responsible for the deterioration of materials and cause some diseases in living beings (Adams et al., 2016). The contamination of cheeses by mold species may produce mycotoxins and some of the toxins such as cyclopiazonic acid (CPA) and sterigmatocystin (STC) have been shown to be stable under normal processing conditions. CPA is produced by *Penicillium* and *Aspergillus* species and typically contaminates cereals (rice and maize), peanuts, figs, tomatoes products and cheese (Burdock and Flamm, 2000; Ostry et al., 2018). STC is a polyketide secondary metabolite and *Aspergillus* specie contaminating grains, coffee bean, cheese, spices, and soybean (Veršilovskis and De Saeger, 2010).

Table 4-3. Microbiological criteria for food stuff- milk and dairy products (adapted from Regulation No 1441/2007).

Food category	microorganisms	Sample plan		limits		Analytical reference method	Stage where the criterion applies
		n	c	m	M		
2.2.2 Cheese made from milk or whey that has undergone heat treatment	*Escherichia coli* ([1])	5	2	100 cfu/g	1000 cfu/g	ISO 16649-1 or 2	At the time during the manufacture process when the *Escherichia coli* count is expected to be highest ([2])
2.2.5 Unripen soft cheeses (fresh cheeses) made from milk or whey that has undergone pasteurisation or a stronger heat treatment ([3])	Coagulase positive *staphylococci*	5	2	10 cfu/g	100 cfu/g	EN/ISO 6888-1 or 2	End of the manufacture process.

Notes: ([1]) *Escherichia coli* is used here as an indicator for the level of hygiene. ([2]) For cheeses which are not able to support the growth of *Escherichia coli, Escherichia coli* count is usually the highest at the beginning of the ripening period, and for cheeses which are able to support the growth of *Escherichia coli* it is normally at the end of the ripening period. ([3]) Excluding cheeses where the manufacturers can demonstrate to the satisfaction of the competent authorities, that the product does not pose a risk of *Staphylococci enterotoxins*.

The main mold contamination source is the environment in the production facilities. Air is generally an effective medium for distribution of mold, therefore air filtration and even practice of cleanroom technique have been introduced in some places.

Yeasts differ from molds because they are unicellular, presenting in spherical, ovoid, and ellipsoid forms (Adams et al., 2016). Fermented milks and yogurts are commonly deteriorated by the action of yeasts. Yeast is present in cheese because of its ability to survive low pH and low water activity with high nutritional profile. When packed in vacuum or modified atmospheres, packages may swell due to the large amount of CO_2 produced by yeasts (Ledenbach and Marshall, 2009).

Listeria monocytogenes

Listeria monocytogenes is a pathogenic microorganism, and an aetiological agent of human and animal listeriosis, a highly fatal infection associated with the ingestion of contaminated food. Several outbreaks of foodborne listeriosis and sporadic cases of varying extent reported in North America and Europe have been associated with the consumption of contaminated milk and milk products particularly cheeses. It is recognized as a major issue to public health authorities due to its high hospitalization rate (94%) and a high case-fatality rate (12.8 to 17 % of cases) affecting susceptible groups, such as pregnant women, neonates, children, elderly people, and immunocompromised patients (European Centre for Disease Prevention and Control, 2013). In pregnant women invasive listeriosis can cause spontaneous abortion, stillbirth, or neonatal infection. Influenza-like symptoms, fever, and gastrointestinal symptoms can also occur in the mother. In immunocompromised individuals and the elderly invasive listeriosis can cause potentially fatal bacterial meningitis with symptoms of fever, malaise, ataxia and altered mental status. The onset of illness of invasive listeriosis generally ranges from 3 days to 3 months after infection. Invasive listeriosis has a fatality rate of 15-30 % (FDA, 2012; FSANZ, 2013).

Listeria monocytogenes is a Gram-positive, non-spore-forming rod-shaped bacterium that can grow in both aerobic and anaerobic conditions. They are found in abundance on farms, where it lives in the soil, plant matter, water, and manure. Throughout the environment it has been isolated from domestic and wild animals, birds, soil, vegetation, fodder, and wet areas of food processing environments (FSANZ, 2013). This means that cheesemakers who work on farms, as many artisan producers do, must be extremely cautious. Through chain controls, including effective heat treatment during cheese production, will inactivate *Listeria monocytogenes* present in this category of cheese. However, re-contamination can occur after this processing step as *Listeria monocytogenes* is a ubiquitous organism and can become established in

processing environments. A distinguishing feature of *Listeria monocytogenes* is its ability to grow at refrigeration temperatures. Growth can occur at temperatures between 1.5 – 45.0 °C, pH of 4.0 – 9.6 and a minimum water activity of 0.90 when other conditions are near optimum. Temperatures above 50 °c, are lethal to *Listeria monocytogenes*, however, it can survive frozen storage at -18 °C (ICMSF, 1996; FSANZ, 2013). Due to the inherent characteristics of cheese relatively high moisture content and low acidity growth of *Listeria monocytogenes* can occur, even when stored at <4°c. Good hygienic practices in food manufacturing and food handling minimize *Listeria monocytogenes* contamination of food (FSANZ 2006; 2014). The implementation of control measures (for example temperature control) so that high levels of growth of *Listeria monocytogenes* will not occur in the food and are expected to have the greatest impact on reducing rates of listeriosis, as nearly all cases of listeriosis result from the consumption of high numbers of the pathogen.

According to this European Regulation No. 1441/2007, the criterion for *Listeria monocytogenes* in cheeses is n=10, c=0, m= absence in 25 g, M=absence in 25 g (n = number of samples or units analyzed; c = maximum allowable number of sample units yielding marginal results, i.e. results between m and M; m = microbiological level that separates good quality from defective, or in a three-class plan good from marginally acceptable quality; M = microbiological level in a three class plan that separates marginally acceptable from unacceptable quality. In a two-class plan M is assimilated to m (Table 4.4).

Salmonella

Salmonella species are facultative anaerobic Gram-negative, non-spore-forming rod-shaped bacteria which growth can occur at temperatures between 5.2 – 46.2 °C, pH of 3.8 – 9.5, and a minimum a_W of 0.93 when other conditions are near optimum. They are ubiquitous bacterium present in the intestinal tract of warm and cold-blooded vertebrates and in the surrounding environment (FSANZ, 2013). *Salmonella* is sensitive to normal cooking conditions, however, foods that are high in fat and low in moisture may have a protective effect against heat inactivation (FSANZ, 2013; Li et al., 2013).

Salmonella can survive for months or even years in low moisture foods and are able to survive frozen storage at -20 °C *Salmonella* is a serious hazard as they cause incapacitating but not usually life-threatening illness of moderate duration, and sequelae are rare (ICMSF, 2002). People of all ages are susceptible to salmonellosis. However, the elderly, infants and immunocompromised individuals are at a greater risk of infection and generally have more severe symptoms (FSANZ, 2013) The symptom of *Salmonella* includes abdominal cramps,

nausea, diarrhea, mild fever, vomiting, dehydration, prostration, and headache. The onset of illness of salmonellosis is typically 24 to 48 hours after infection, ranging from 8 to 72 hours and symptoms usually last for 2 to 7 days. Severe diseases such as septicemia (sepsis) sometimes develop, predominantly in immunocompromised individuals. The fatality rate for salmonellosis is generally less than 1% (FDA,2012; FSANZ, 2013).

Salmonella is one of the four key global microbes causing diarrheal diseases, and it causes annually 93.8 million cases of foodborne illness and 155,000 deaths (Eng et al., 2015).

The food matrix and strains of *Salmonella* influence the level of *Salmonella* required for illness to occur. It has been reported that as low as one or 100 cells caused illness, however, in other cases significantly more cells are required for illness to occur (ICMSF, 1996b; FDA, 2012).

Salmonella Enteritidis is one of the five most reported serovars from human salmonellosis cases acquired in the EU. In total, 926 salmonellosis food-borne outbreaks were reported by 23 EU Medical Specialists in (2019), causing 9169 illnesses, 1915 hospitalizations (50.5% of all outbreak-related hospitalizations), and 7 deaths. *Salmonella* caused 17.9% of all food-borne outbreaks during 2019. The vast majority (72.4%) of the salmonellosis food-borne outbreaks were caused by *Salmonella Enteritidis* (EFSA, 2021).

Many salmonellosis outbreaks are linked to the consumption of cheeses and have been reported worldwide in recent years, including cheeses made from raw milk, which is evident that *Salmonella* is also a pathogen of concern in these food products (FDA, 2012; FSANZ, 2013).

Milk can be contaminated by *Salmonella* by the infected herd. The risk can be nullified through chain controls, including effective heat treatment during cheese production. In the finished product, contamination can occur because of inadequate heat treatment during processing or cross-contamination with raw milk during processing. Post-processing contamination can occur, although several processing factors and product physical-chemical characteristics influence the potential for growth of *Salmonella* in cheese including pH, salt concentration, water activity, maturation, and ripening conditions (FSANZ, 2006). According to this European Regulation No. 1441/2007, the criterion for *Salmonella* in cheeses is n=10, c=0, m= absence in 25 g, M=absence in 25 g (n = number of samples or units analyzed; c = maximum allowable number of sample units yielding marginal results, i.e. results between m and M; m = microbiological level that separates good quality from defective, or in a three-class plan good from marginally acceptable quality; M = microbiological level in a three class plan that separates marginally acceptable from unacceptable quality. In a two-class plan M is assimilated to m (Table 4.4).

Table 4-4. Microbiological criteria for food stuff- milk and dairy products (adapted from Regulation No 1441/2007).

Food category	Microorganisms	Sample plan		limits		Analytical reference method	Stage where the criterion applies
		n	C	m	M		
1.2 Ready to eat foods ready the support the growth of *Listeria monocytogenes* other than those intended for infants and for special medical purposes.	*Listeria*	10	0	Absence in 25g ([1])		ISO 11290-1	Before the food has left their immediate control of the food business operator who has produced it.
1.11 Cheese butter and cream made from raw or milk that has undergone a lower heat treatment than pasteurization.	*Salmonella*	5	0	Absence in 25g		EN/ISO 6579	Products placed on the market during their shelf life.

Notes: ([1]) This criterion shall apply to products before they have left the immediate control of the producing food business operator, when he is not able to demonstrate, to the satisfaction of the competent authority that the product will not exceed a limit of 100cfu/g throughout the shelf life.

4.3.5. Shelf Life

Food additives and preservatives are substances used to prolong the shelf-life of foods by protecting them against deterioration caused by microorganisms and/or protect against the growth of pathogenic microorganisms (European Regulation 1333/2008). The European Regulation (EU) No. 1129/2011, amending Annex II to Regulation (EC) No 1333/2008 establishes a list of food additives, including the use of preservatives such as sorbic acid and sorbates (E 200–203) for unripen and ripened cheeses. Although these preservatives are safe for human health in specific dosages, extensively applicable in the food industry, the excessive consumption of these additives and preservatives may give rise to health problems and complications (Abdulmumeen et al., 2012). Therefore, safety concerns about chemical preservatives and consumers' negative concerns about additives have led to a growing interest in more natural alternatives, among which are plant-based compounds (Carocho et al., 2015). The use of herbs and spices in cheese making is a widespread practice since ancient times, but it generally involved physically rubbing the cheese with certain herbs or spices, or their oils (Vazquez et al., 2001), and is often related to local traditions.

The main advantage regarding the use of *Arthrocnemum macrostachyum* in this cheese making is due it´s potential biological source of biochemicals (total phenols, flavonoids, tannins, and alkaloids) counteracting free radical-induced oxidative damage (Custodio et al., 2012). High mineral content of the biomass particularly Sodium (Na) used to substitute part of the salt. Excessive salt intake is linked to high blood pressure in some people, which can lead to serious health issues, such as cardiovascular disease and stroke. High antioxidant activity, extending shelf life by direct scavenging of reactive oxygen species (ROS), because oxidation processes in cheese often result in strong off-flavors and deterioration of its nutritional quality (Dimick and Kilara, 1983).

5. Material and methods

5.1. Harvesting plant material and biomass treatment

Biomass from *Arthrocnemum macrostachyum* was supplied by the XtremeBio laboratory of the Algarve Center of Marine Sciences (CCMAR) of the University of Algarve. The fresh biomass was harvested from adult plants grown in greenhouse located in the Portuguese Institute for the Sea and Atmosphere (IPMA), Division of Aquaculture and upgrading (DivAV), Olhão (EPPO) in October 2021. The plants were frozen, dehydrated, and lyophilized for 3 days. Dry biomass was then grounded to powder and stored in Falcon tubes, out of light at room temperature.

The fresh biomass for microbiology analysis was collected in the above-mentioned places and transported to the laboratory under refrigeration conditions and immediately used for microbiological analysis.

5.2. Determination of Bioactive Properties of *Arthrocnemum macrostachyum.*

5.2.1. Preparation of the extract

The dried biomass was mixed with absolute ethanol (1:10, w/v) and then extracted in an ultrasonic water bath for 30 minutes at room temperature (RT, about 20 °C). The extract was filtered using a Whatman No. 4 filter before being dried under reduced pressure at 40 °C. The dried extract was weighed and dissolved in ethanol at the concentration of 50 mg/mL and stored at a temperature of -20 °C in dark conditions until needed.

5.2.2. Determination of Antioxidant Activity of *Arthrocnemum macrostachyum*

The radical scavenging activity (RSA) of *Arthrocnemum macrostachyum* extract was assessed by the DPPH (2,2-diphenyl-1- picrylhydrazyl) solution (120 µL) assay (Moreno et al., 2006). For that, 22 µL of extract at concentrations ranging from 20 mg/mL to 50 mg/mL was mixed with 200 µL of 120 µM DPPH (0,0024 g + 50 mL of ethanol) in a 96 well plates and incubated for 30 minutes at room RT in the dark. A positive control, 1 mg/mL butylated hydroxytoluene (BHT) was utilized. RSA was determined as a percentage of inhibition in relation to a negative control that included ethanol and DPPH**.** The color control was 22 µL of extract plus 200 µL of the solvent used (ethanol). The absorbance was measured at 517 nm (Biotek Synergy 4, BioTek Instruments, Winooski, VT, US microplate reader). The results were expressed as antioxidant activity (%) in

relation to a negative control containing water, and the medium inhibitory concentrations (IC_{50}), that is, the antioxidant concentration necessary to neutralize 50% of DPPH radicals.

5.2.3. Determination of Total Phenolic (TPC) and Flavonoid Contents (TFC)

The TPC was determined by the Folin-Ciocalteu assay (Velioglu et al, 1998). In brief, 5 µL of sample (10 mg/mL) was mixed with 100 µL of F-C reagent (10x diluted) and incubated for 10 minutes at room temperature (RT, 20°C). Then, 100 µL of sodium carbonate (75 g/L in water) were added and the plates were Incubated for 90 minutes at RT. The absorbance was measured at 725 nm on a microplate reader (Biotek Synergy 4, BioTek Instruments, Winooski, VT, US). A calibration curve was built by placing 10 µL gallic acid (1 mg/mL) in a microplate from which 5 µL was pipetted out and mixed in 5 µL of water. Then were added, 100 µL of Folin-Ciocalteu solution and incubated for 10 minutes after which, 100 mL of sodium carbonate was added and incubated for 90 minutes. The absorbance was read 725 nm. Results were expressed as milligrams of gallic acids equivalents (GAE) per g of dry weight (mg GAE/g DW).

TFC was estimated by the $AlCl_3$ colorimetric assay modified to 96 well microplates (Pirbalouti et al., 2013)**.** 50 µL of the sample (50 mg/mL) were placed in 96-well microplates and mixed with 50 µL of 2% $AlCl_3$-ethanol solution and incubated for 10 minutes at room temperature (RT). The absorbance was read at 420 nm in a microplate reader (Biotek Synergy 4, BioTek Instruments, Winooski, VT, US). A calibration curve was prepared using 100 µL of quercetin (1 mg/mL) as standard. The results were expressed as milligrams (mg) of quercetin equivalents (QE) per gram of dry weight (mg QE/g DW).

5.2.4. Determination of mineral content of *Arthrocnemum macrostachyum*

Three replicates of the biomass of about 250 mg each were added 6ml of supra-pure HNO_3 (65%; Fluka, Sigma-Aldrich Co. LLC, St. Louis, MO, US), a blank was produced and included in the batch of samples and microwave digested (Ethos Touch, Milestone Srl, Sorisole, Italy) in high-pressure Teflon vessels and high temperature for 15minutes. Then were added, 1 mL of $HClO_4$ (p.a. 70 %; Riedel-de Haën, Honeywell, Morris Plains, NJ, US), and 1 mL of supra-pure H_2O_2 (30%, Merck KGaA, Darmstadt, Germany). A clear liquid of HNO_3 and minerals was obtained and diluted in pure water by serial dilutions (1:10, 1:100, 1:1000 v/v). A calibration curve was prepared using stock solution of 100 mg/L with concentration 10 mL for macro-minerals Ca, Fe, K, Mg, Na with dilutions at concentrations (20 mg/L, 10 mg/L, 7,5 mg/L, 5 mg/L, 2.5 mg/L) and from the dilutions

(1 mg/L, 0.5 mg/L, 0.25 mg/L). A similar procedure was repeated for micro minerals, and a calibration curve was prepared with dilutions (1000 ppb, 750 ppb, 500 ppb, and 250 ppb) and from these dilutions were repeated (100 ppb, 50 ppb, and 25 ppb). Procedural blanks always accounted for less than 1% of the metal concentrations in samples.

The minerals were analysed by atomic absorption spectrometry-AAS (GBC Avanta Sigma, GBC Scientific Equipment PTY Ltd., Dandenong, Vic., Australia) provided with a deuterium background correction. The accuracy of the analytical procedure was assessed by the analysis of certified reference material BCR60 (Lagarosiphon major). The Results were expressed per g of dry weight (DW).

5.3. Microbiological characterization of *Arthrocnemum macrostachyum*

Biomass (fresh and dry) of *Arthrocnemum macrostachyum* was analysed for enumeration of *Escherichia coli*, *Staphylococcus* coagulase positive, microorganisms at 30 °C, and yeast and moulds.

5.3.1. Sample Preparation (ISO 6887-1:2017)

Fresh biomass was chopped into small pieces and mixed with Ringer solution in the stomacher (VWR Star blender Ib 400) to obtain the initial suspension. From these, serial dilutions were performed for the different microbiological analyses.

Dry biomass of *Arthrocnemum macrostachyum* was mixed with Ringer solution to obtain the initial suspension and from this, serial dilutions were made.

The two samples (fresh and dry biomass) of *Arthrocnemum macrostachyum* were prepared according to ISO 6887-1:2017.

5.3.2. *Escherichia coli* (ISO 16649-2:2001)

Escherichia coli was enumerated on Tryptone Bile Glucuronic Agar (TBX, Scharlau 01-619-500) and incubated at 44 °C and 5-bromo-4-chloro-3-indolyl Beta-D-glucuronide, according to ISO 16649-1:2018. Dilutions 10^{-1} and 10^{-2} were used.

5.3.3. *Staphylococcus* coagulase positive (ISO 6888-1:2021)

Staphylococcus coagulase-positive was enumerated by inoculation on Baird Parker Agar Base (BP, Scharlau 01-030-500), according to ISO 6888-1:2021. Dilutions 10^{-1} and 10^{-2} were used.

5.3.4. Microorganisms that grow at 30 °C (ISO 4833-1:2013)

Total mesophilic bacteria were enumerated on Plate count agar (PCA, HIMEDIA M091-500G) according to ISO 4833-1:2013. Dilutions 10^{-2} to 10^{-5} were used. The number of colonies was counted considering plates presenting from 30 to 300 colony forming units per millilitre (CFU/ml).

5.3.5. Yeasts and molds (ISO 21527-1:2008)

The enumeration of yeast and molds was performed on Rose Bengal Chloramphenicol Agar (RB, HIMEDIA M640-500G) according to ISO 21527-1:2008. Dilutions 10^{-1} to 10^{-5} were used. The number of colonies was counted considering plates presenting as from 15 to 150 colony forming units per millilitre (CFU/ml).

5.4. Milk acquisition

The pasteurized milk was purchased from Portal dos Queijo, Sao Bras de Alportel and transported to the processing laboratory under refrigeration**.**

5.5. Cheese processing

The fresh goat cheeses were produced under laboratory conditions (Food Processing Laboratory, Instituto Superior de Engenharia, University of Algarve) in three separate vats. The processed conditions were 12 g salt /L milk without biomass (control), 8 g salt /L milk with 4 g biomass/L milk (B1), and 4 g salt /L milk with 4 g biomass /L milk (B2). The milk was inoculated with coagulant (Liquid Britex Rennet) following the technological scheme (Figure 5.1) of the production of cheeses. The curd cutting is shown in figure 5.2. The whey was separated from the curds by using plastic cheese moulds (figure 5.3) which were manually filled with the curds and pressed lightly with continual drainage of whey. The cheeses inside the cheese moulds were stored at refrigeration condition (4 °C to 6 °C). Before and during the process of goat cheese production, the equipment and utensils used were washed properly and disinfected with a hydrated ethyl alcohol solution of 70 % to avoid cross-contamination. Gloves, masks, and disposable caps were worn for the same intention.

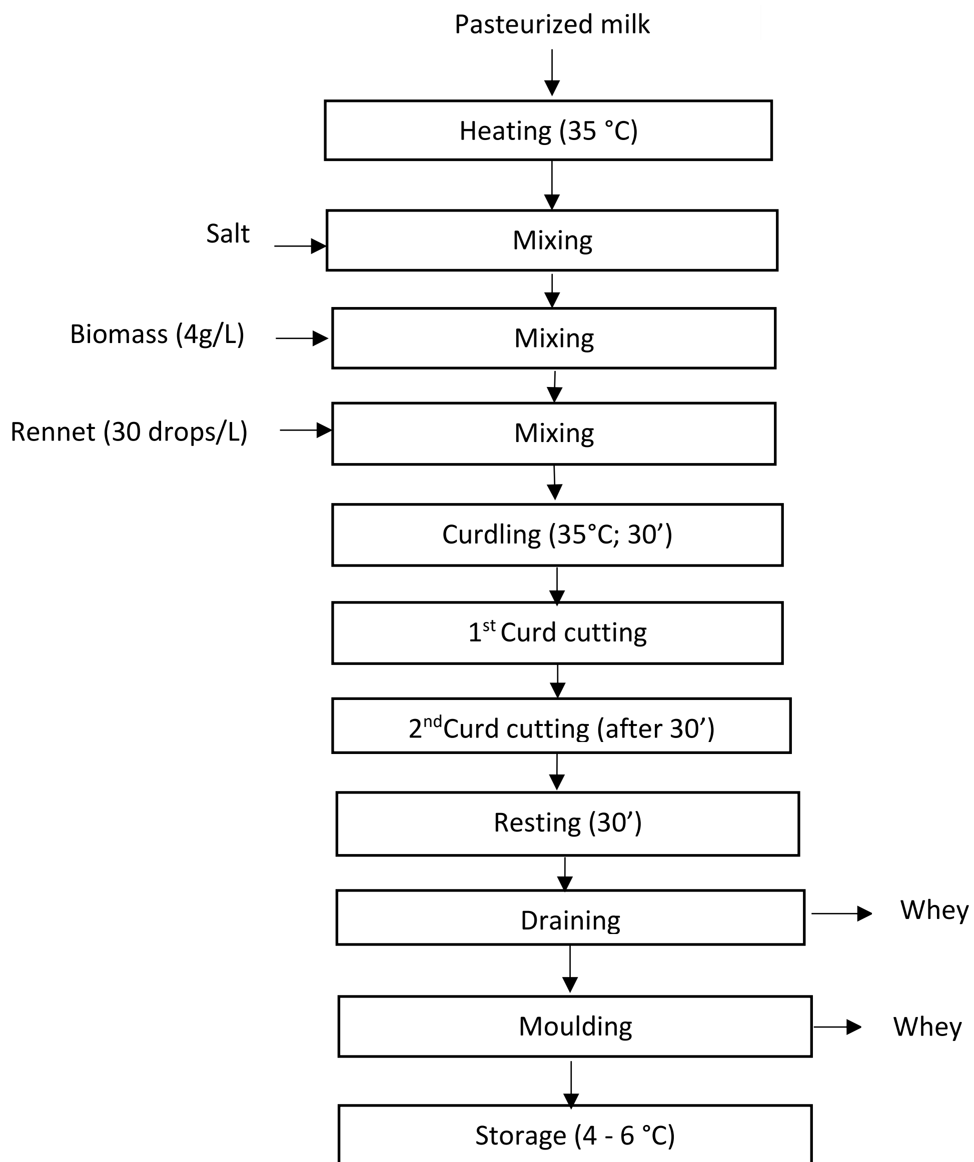

Figure 5 1. Technological scheme of goat cheese processing. The quantity of salt added depends on the condition.

Figure 5 2. After cutting coagulum containing biomass of *Arthrocnemum macrostachyum* (Titalah Sheron).

Figure 5 3. Cheese curds in perforated plastic moulds (Titalah Sheron).

5.6. Test for optimal concentration of biomass and salt

To determine the ideal concentrations of biomass and salt to be used for cheese production and further analysis, several essays were performed (Table 5.1). These tests were performed on 1 L of milk per essay.

Table 5-1. Test for optimal concentration of biomass before coagulation.

Essay	Plant biomass quantity (g/L)	Salt quantity (g/L)
1	0	12,5
2	0	12,04
3	148,8	3,12
4	148,8	0
5	99,2	6,24
6	99,2	0
7	49,6	0
8	24,8	0
9	12,4	0
10	11,64	0
11	6,2	0
12	6,2	5,48
13	6,2	8,2
14	4,16	8,16
15	4,16	4,16
16	4,04	4,08
17	4	2
18	3,04	5,48
19	2	2

5.7. Physical chemical control

5.7.1. Cheese yield

According to Raimundo *et al*. (2015) the cheese yield is the relationship between the quantity of milk in liters (L) used and the quantity of cheese obtained in kilograms (kg) and it is expressed in percentage. Thus, the determination of cheese yield was carried out based on the following formula:

$$\eta = 100 \times m_Q / m_L$$

Where η is the processing yield (%), m_Q is cheese mass (kg), and m_L is milk mass (L).

5.7.2. Cheese Color

The determination of colour was performed using the CIEL coordinate system defined by the Commission Internationale de L'Éclairage CIE 1976. A PSE-CSM 10 colorimeter (Instruments LDA, UK) was used, using illuminant D65.

In the colorimetric space, defined by L*, a*, b*, the **L*** coordinate (luminosity) corresponds to the ratio between the reflected and absorbed light, which makes it defined as the product of the black colour (0 - total absorbed light) and white (100 - total reflected light) (Granato and Masson, 2010); **a*** varies between -60 (green) and +60 (red) and the **b*** component varies between -60 (blue) and +60 (yellow) (Buffa et al., 2001).

The chromaticity or saturation (**C**) was also measured, which indicates the intensity or purity of the tone, regardless of how light or dark the colour is. The higher its value, the more intense the colour, appearing luminous or concentrated, while low values (achromatic) indicate greyish, weak, or diluted colour (Granato and Masson, 2010).

Thirty measurements were performed on each sample, with the device previously calibrated, using different parts of the cheese. Readings were taken on days t=0, t=4 and t=8. The cheeses were kept in refrigerated storage at 4 °C to 6 °C.

5.7.3. pH, a_W, Dry weight, and Fat content

The **pH** was measured by the direct method using a potentiometer (Crison, Instruments, S.A., Spain). 15 measurements were performed per sampling time, using 3 different cheeses.

The **water activity** (a_W) was determined on an a_W meter (Rotronic Hydrolab – Rotronic AG, Bassersdorf, Switzerland), thermally stable at 25 °C. A sample of the cheese was placed in a polystyrene cell, which was introduced into the measuring station for 25 minutes. Measurements were performed in triplicate.

Gravimetric analysis was used to obtain the total **dry weight** content, using a conventional oven. 3 g of cheese sample were placed in a Petri dish, previously dried and tared. After homogenization, the sample was placed in an oven at 100 °C for 24 h. Then, the plates were transferred to desiccators for cooling and weighing followed. The analysis was performed in triplicate (NP 3544-1987).

To determine the total **fat** content, the Gerber method, modified by Egan et al. (1981) was used. Samples of 3 g were homogenized and weighed directly in a Van Gulik butyrometer (for cheese). Then, 10 mL of sulphuric acid solution, about 6 mL of water or until the sample was covered,

and 1 mL of isoamyl alcohol were added. The butyrometer was made up to volume with hot water and the butyrometer was transferred to a water bath at 65 °C, shaking until all the sample was dissolved. Once dissolved, it was centrifuged for 5 minutes at 1100 rpm and the fat was read directly on the butyrometer scale. All assays were performed in triplicate.

5.8. Determination of Bioactive Properties of the Cheese

5.8.1. Determination of Antioxidant activity of the cheese

To evaluate the antioxidant activity, the fresh cheeses were lyophilized, and the resulting dried biomass was extracted with ethanol (Abderrezak, 2018), at a ratio 1:10, w/v, in an ultrasound water bath for 30 minutes at RT (about 20 °C). According to Directive 2009/ 32/EC of the European Parliament and of the Council of April 23, ethanol may be used during the processing of raw materials, foodstuffs, food components, or food ingredients. The extracts were filtered using a Whatman No. 4 filter before being dried under reduced pressure at 40 °C. The dried extracts were weighed and dissolved in ethanol at the concentration 50 mg/mL and stored at a temperature of -20 °C away from light until needed.

The antioxidant activity of the cheese's biomass was analyzed using DPPH as described in 5.2.2.

5.8.2. Determination of Total Phenolic (TPC) and Flavonoid Content (TFC) of the Cheese

Total phenolic and Flavonoids contents were determined from the cheese extract 50 mg/mL prepared above following the same method of determination of TPC and TFC described in point 5.2.3.

5.9. Microbiological control of the Cheese

5.9.1. Sample preparation (ISO 6887-1-2017)

A composite sample was prepared by weighing 25 g from 3 cheeses. The sample was mixed in Ringer solution of 225 mL and placed in a stomacher (VWR Star Ib 400) to obtain the initial suspension. From these, serial dilutions were performed for the enumeration of the different microorganisms.

5.9.2. *Escherichia coli* (ISO 16649-2:2001)

The enumeration of *Escherichia coli* was done on Tryptone Bile Glucuronic Agar (TBX, Scharlau 01-619-500) according to ISO 16649-2:2001. (Microbiology of food and animal feeding stuff - Horizontal method for the enumeration of β-glucuronidase-positive *Escherichia coli* - Part 2: the colony count technique at 44 °C using 5-bromo-4-chloro-3-indolyl β-D-glucuronide).

Sampling was performed at the beginning (t=0), in the middle (t=4) and at the end (t=8) of the study period.

5.9.3. Staphylococcus coagulase positive *(ISO 6888-1:2021)*

The enumeration of *Staphylococcus* coagulase positive was performed in Baird Parker Medium following the ISO 6888-1:2021. (Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) -Part 1: Method using Baird-Parker agar medium. Genève, Switzerland: International Organization for Standardization, standardization). During the study period, sampling techniques were used at the beginning (t=0), in the middle (t=4), and at the end as (t=8 days).

5.9.4. Microorganisms that grow at 30 ˚C (ISO 4833-1:2013)

Standard 4833-1:2013 Part 1 "Microbiology of food and animal feeding stuffs- Horizontal method for the enumeration of microorganisms - Colony-count technique at 30 °C" was used for the enumeration of microorganisms at 30 °C.

From the batch, sampling was carried out at the beginning (t=0), in the middle (t=4) and at the end (t=8 days) of the period under study.

5.9.5. Yeasts and molds (ISO 21527-1:2008)

For Yeast and Molds enumeration, ISO 21527-1:2008 Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of yeasts and molds. Part 1: Colony count technique in products with water activity greater than 0.95 was used.

From the batch, sampling was carried out at the beginning (t=0), in the middle (t=4), and at the end (t=8 days) of the period under study.

5.9.6. *Listeria monocytogenes* (ISO 11290-1:2017)

For the detection of *Listeria monocytogenes*, Microbiology of the food chain — Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. — Part 1: Detection method.

From the batch produced, sampling was carried out at the end of the period under study (t=8 days).

5.9.7. *Salmonella (*ISO 6579-1:2017*)*

For the detection of *Salmonella* spp. Microbiology of the food chain - Horizontal method for detection, enumeration, and serotyping of *Salmonella*- Part 1: Detection of *Salmonella spp.* was used.

From the batch produced, sampling was carried out at the end of the period under study (t=8 days).

5.10. Experimental plan and data analysis

For each test, 12 litters of milk were used, which allowed the manufacture of 27 cheeses, 9 cheeses for each condition (12g/L salt without biomass, 8 g salt /L milk with 4 g biomass /L, and 4 g salt/L with 4 g biomass/L). 3 sets from 3 cheeses were analysed each time. Quality parameters including color, pH, water activity, dry matter, fats, and microbiological analysis were carried out on fresh cheeses on day t=0. These physicochemical and microbiological analysis were repeated on days t=4 and t=8, to determine the effects of the addition of biomass on the preservation time of the cheeses.

Statistical analyses were performed with the SPSS software (IBM SPSS Statistics 26 Software). The descriptive and inferential statistical outputs are presented in the appendix. To assess whether preservation time (independent variable) significantly affected the dependent variables of color (L, a, b, and C), pH, a_W, dry extract, fat, total phenolic (TPC) and flavonoid (TFC) content of the cheeses, One-way ANOVA and Post-hoc HSD multiple comparison test (Tukey) were used to compare the mean at t=0, t=4 and t=8 using a significant level of 0,05 ($p<0,05$). The assumption of the Normal distribution of these dependent variables was evaluated by the Kolmogorov-Smirnov and Shapiro-Wilk tests. It was considered that the variables showed a normal distribution when the p-value was greater than 0.05 ($p>0,05$). The assumption of homogeneity of variance (homoscedasticity) of the dependent variables was evaluated using the Levene test. It was considered that the variables showed homogeneous variances when the p-value was greater than 0.05. in cases where heteroscedasticity was verified, the complementary Welch test was performed, recommended by Lix et al. (1996). When there is a deviation to normality, no corrective mathematical transformation was carried out since there were small deviation and ANOVA is robust to mild violations of this assumption (Maroco, 2010). In a case where there is no homogeneity of variances, the ratio between the smallest and the largest variance is less than 1:4 and the size of the groups is greater than 5, thus the Parametric Test ANOVA remains robust (Maroco, 2010).

The same statistical treatment was used to study the influence of the salt concentration on the pH, a_W, dry weight, fat, TPC, and TFC.

Bioactive analysis including antioxidant, total flavonoids, and phenolic compounds were also determined. The antioxidant activity of *Arthrocnemum macrostachyum* biomass and cheeses was calculated as the percentage of reduction (uptake) of the radical, in relation to the negative control (sample solvent), according to the formula:

$$DOr=DOa-DOcc$$

$$\% \, RSA=(DOcn-DOr) / DOcn \times 100$$

Where: % RSA - % Radical Reducing Activity; DOr - Real absorbance; DOa -Sample absorbance; DOcc -Absorbance of color control; DOcn - Absorbance of negative control. The Values of IC50 were calculated by sigmoidal fitting of the data in GraphPad Prism version® 6.0c.

The total phenolic and flavonoid content of *Arthrocnemum macrostachyum* were expressed as TPC ± SD mg GAE/g biomass DW, and TFC ± SD mg QE/g biomass DW. The TPC, TFC and standard deviation (SD), n=6.

6. Results and discussion

6.1. Bioactive Properties of *Arthrocnemum macrostachyum*

6.1.1. Antioxidant Activity of *Arthrocnemum macrostachyum*

The RSA of the ethanol extract was determined by the DPPH assay, which displayed an IC_{50} value of 4,15 ± 0,57 mg/mL. The extracts´ antioxidant activity was higher than that reported by Barreira et al. (2017). El-Naker et al. (2020) noted the presence of a variety of phytochemical compounds in *Arthrocnemum macrostachyum* and discovered sixteen that were potentially bioactive, some of which have antioxidant (quercetin, 4 hydroxybenzoic, and caffeic acids), antiviral, antibacterial, and/or anti-tumor properties (hesperidin, salicylic, chlorogenic, and coumaric acids), including substances for the treatment of diabetes (rhamnetin).

6.1.2. Total Phenolic (TPC) and Flavonoid Content (TFC) of *Arthrocnemum macrostachyum*

TPC and TFC values obtained for *Arthrocnemum macrostachyum* extract were 23.76 ± 1.01 mg GAE/g DW and 10.35 ± 0.74 mg QE/g DW respectively, indicating its significance as a source of antioxidant compounds. The highest TPC (49 mg G.A.E./g DW) was reported in ethanolic extracts of *Arthrocnemum macrostachyum* collected from Faro, south of Portugal which explains it's higher antioxidant potential (Barreira et al., 2017). *Arthrocnemum macrostachyum* was also identified by Rodrigues et al. (2014) as a beneficial source of phenolics and flavonoids despite working with different extracts. Since they can reduce the negative consequences of oxidative stress, phenolic compounds also known as excellent radical scavengers play a significant role in maintaining human health. The phenolic molecules known as flavonoids are thought to be dietary antioxidants with anti-inflammatory, antiviral, antibacterial, and metal chelating properties (Gargouri et al., 2013). This result is consistent with other publications showing a relationship between halophyte extracts' phenolic content and their ability to scavenge free radicals (Falleh et al., 2011; Trabelsi et al., 2013). Flavonoids are secondary metabolites that have a variety of roles in the physiology and cellular mechanisms of plants, including pigmentation and resistance to pathogens, predators, and oxidative stress.

6.1.3. Mineral Content of *Arthrocnemum macrostachyum*

Table 6.1 displays the mineral composition of *Arthrocnemum macrostachyum* biomass. The minerals Na, K, Mg, Ca, and Fe were the most abundant similarly to that of Ventura et al. (2011).

Arthrocnemum macrostachyum is particularly high in Na (24, 78 ± 2, 21 mg/g DW). Some halophytes need NaCl for optimum growth and development, whereas others may tolerate a high concentration of Na in the root zone (Yuan et al., 2019). Na is a necessary nutrient, but it's over consumption has been related to several illnesses, such as hypertension and cardiovascular diseases (Kotchen et al., 2013). As a result, consuming halophytes that were grown in saline environments increases the risk of consuming too much sodium. Therefore, the recommended ADI of Na for adults is 2 g per day (WHO, 2012) as higher intakes raise the risk of hypertension and cardiovascular disorders. This halophyte can be regarded as a good nutritional source of minerals like K (4, 62 ± 0, 49 mg/g DW), Mg (2, 01 ± 0,12 mg/g DW) and Ca (1,34 ± 0,07mg/g DW) because the amounts of other minerals are comparable to those found in several edible wild plants. Halophytes have the capacity to accumulate metals like Zn, Cr, Pb, Ni, and Cd when found in contaminated salt marshes (Caetano et al., 2008). Cd was below the limit of quantification and therefore it was not detected. Most harmful metal concentrations (Cr, Pb, Ni, and Cd) fell below the legal limits (0.3 mg/kg ww for Pb and 0.2 mg/kg ww for Cd) according to European Regulation 1881/2006.

Table 6 1. Mineral amounts (per gram of sample dry weight) of *Arthrocnemum macrostachyum* (mean ± standard deviation).

Macro minerals (mg/g DW)	**Mean ± standard deviation**	**Micro minerals (µg/g DW)**	**Mean ± standard deviation**
Ca	1,34 ± 0,07	Zn	7,57 ± 2,05
K	4,62 ± 0,49	Cd	nd
Mg	2,01 ± 0,12	Cu	4,08 ± 0,04
Na	24,78 ± 2,21	Ni	1,18 ± 0,93
Fe	0.02 ± 0.00	Pb	3,25 ± 1,95
		Mn	64,74 ± 4,38
		Cr	1,48 ± 0,78

Note: nd: not detected.

6.2. Microbiological Characterisation of *Arthrocnemum macrostachyum*

6.2.1. *Escherichia coli* (ISO 16649-2:2001)

The enumeration of *Escherichia coli* for both fresh and dry samples of *Arthrocnemum macrostachyum* were < 10 CFU/g, which indicates that this plant can be consumed if proper hygienic practices are maintained throughout production. The maximum permissible limit of *Escherichia coli* for vegetables (ready-to-eat) is 100 CFU/g according to Regulation 1441/2007. As far as we know, no studies have been conducted on the microbial contamination of halophytes meant for human consumption. However, we can assume that halophyte-based food

preparation contamination by bacteria can be evaluated according to the general guidelines used for other vegetables (Lombardi et al., 2022).

6.2.2. *Staphylococcus* coagulase positive (ISO 6888-1:2021)

The enumeration of *Staphylococcus* coagulase positive for both fresh and dry samples of *Arthrocnemum macrostachyum* were < 10 CFU/g, which indicates that, the plant is suitable for consumption. As far as we know, no studies have been conducted on the microbial contamination of halophytes meant for human consumption. However, we can assume that halophyte-based food preparation contamination by bacteria can be evaluated according to the general guidelines used for other vegetables (Lombardi et al., 2022).

6.2.3. Microorganisms that grow at 30 ˚C (ISO 4833-1:2013)

The enumeration of microorganisms at 30 °C for *Arthrocnemum macrostachyum* fresh sample was 2,73 ± 0,28 log CFU/g and dry sample was 3,50 ± 0,48 log CFU/g. The dry sample which presented higher contamination than the fresh sample, could give general indication of the contamination of plant sample or equipment used during processing and/or storage (Garayoa et al., 2017). Since the differences is so low, the process and storage conditions does not seem to contribute to the contamination of the material.

6.2.4. Yeasts and Molds (ISO 21527-1:2008)

The enumeration of yeast and molds for *Arthrocnemum macrostachyum* fresh samples were <10 CFU/g. Meanwhile, in the dry samples, Yeasts were <10 CFU/g and molds were 3,14 ± 0,09 log CFU/g. Due to their adapted xerotolerance and halotolerance, numerous mycotoxin-producing fungal species have been identified from low water activity conditions ($a_W \leq 0.8$) (Cantrell et al., 2006; Biango-Daniels and Hodge, 2018).

6.3. Test For Optimal Concentration of Biomass and Salt to be added to goat cheese

The results for optimal concentration of biomass and salt to be added were discussed based on the coagulation and salt taste of the cheeses from each essay. No coagulation was observed in essays 3,4,5,6,7,8 and 9 (Table 6.2) probably due to the high concentration of biomass hindering the formation of curds during enzymatic coagulation by rennet. These samples were also slightly salty. Different degrees of coagulation were observed in essays 10 to 13 and 16 to 19 with low and medium salt taste. Yet not all of that were the best samples as the curds were not firm enough. The different quantities of biomass used for this work are meant to produce a functional

food, with halophyte substituting the quantity of salt to be added with Na in the biomass, maintaining the acceptability and good quality. In the preliminary, organoleptic test was performed using 5 tasters (with no significant statistical results). The most acceptable essays that were used for the final determination of this work were essays 14 and 15, with 2 being the control. Essay 15 with equal quantity of biomass and salt was well coagulated with firmer curds and excellent salt taste. Essay 18 with half quantity of biomass to salt was equally appreciated and was accepted for formation of firm curds and less salt taste (Table 6.2) but not used for the final determination of this work because it was similar in salt taste, and texture like essay 14.

Table 6 2. Results of optimal concentration of biomass and salt (conditions on table 5.1).

Essay	Results/observation
1	Coagulated and slightly salty
2	Coagulated with excellent salt
3	No coagulation and too salty
4	No coagulation and salty
5	No coagulation and too salty
6	No coagulation and salty
7	No coagulation and slightly salty
8	No coagulation and slightly salty
9	Almost coagulated with low salt
10	Coagulated with low salt
11	Coagulated with medium salt, likable
12	Coagulated with medium salt, not likable
13	Coagulated with low salt
14	Coagulated with excellent salt
15	Coagulated with excellent salt
16	Coagulated with less salt
17	Coagulated with medium salt
18	Coagulated with less salt
19	Coagulated with medium salt

6.4. Cheese Yield

The yield of cheeses without biomass (control) with 12 g salt /L was 28,87%, with 4 g biomass and 8 g salt /L was 36% and with 4 g biomass and 4 g salt /L was 34,22%. It was observed that cheeses prepared with biomass have high yield as compared to the cheese sample without biomass (control). which is possible to understand the yield of the two batches (8 g salt /L and 4 g salt /L) with same quantities of biomass having different yields. Salt directly influences the properties of cheese by controlling syneresis, and texture of curd (Fox, 1993).

6.5. Physicochemical Control of the Cheese During the Storage Period

6.5.1. Cheese color

The parameters of color L*, a*, b*, and C* were seen to have a statistically significant difference during storage for the control cheeses (Table 6.3). For the control cheeses (12 g salt/L), L* diminished, a* increased but remained greenish (-0,62 ± 0,07 to -0,50 ± 0,15), b* was more yellowish from 3,90 ± 0,21 to 4,22 ± 0,10 and C* also increased during storage. Based on the analysis of variance (ANOVA) $P<0.05$. For L*, a*, b*, and C*. $F(2, 84) = 17.411$; $P<0.001$ (Table 10.1), $F(2, 87) = 21.81$; $P<0.001$ (Table 10.2), $F(2,82) = 70.139$; $P<0.001$ (Table 10.1), $F(2, 82) = 69.82$; $P<0.001$ (Table 10.1); respectively.

For the cheeses with biomass (B1) 8 g salt/L, the parameters L*, a*, b*, and C* were significantly different during preservation (Table 6.3). There was significant difference between each parameter at different study times (t=0, t=4, and t=8) L*(lightness) diminished during storage from 76,57 ± 0,60 to 75,22 ± 0,72. The value a* increased from -0,42 ± 0,13 (greenish) to 0,30 ± 0,10 (reddish). The values b* was yellowish and increased during the study period from 7,13 ± 0,35 to 8,67 ± 0,29. C* also increased. Based on the analysis of variance ANOVA the $P<0.05$ for all the parameters of color L*, a*, b*, and C*. For L*, $F= (2, 79) = 33.561$; $P=<0.001$ (Table 10.4). For a*, $(2, 86) = 222.51$; $P=<0.001$ (Table 10.3). For b*, $F(2, 87) = 180.334$; $P=<0.001$. For C*, $F= (2,87) = 191.688$; $P=<0.001$ (Table 10.2).

The cheese with biomass (B2) 4 g salt/L milk, the parameters L*, a*, b*, and C* were significantly different at preservation time (t=0, t=4 and t=8) (Table 6.3). L* (lightness) diminished during the weeks of storage, a* increased from greenish (-0,47 ± 0,08) to reddish (0,14 ± 0,24), b* remained yellowish and increased during the weeks of storage from 6,93 ± 0,38 to 8,43 ± 0,25. As there was a significant difference between all the parameters at different preservation times the analysis of variance ANOVA had $P<0.05$ for all the parameters studied. L*, $F(2, 87) = 28.497$; $P<0.001$ (Table 10.2). a*, $F= (2, 83) = 77.70$; $P<0.001$ (Table 10.1). b*, $F= (2, 85) = 187.14$; $P=<0.001$. C*, $F(2, 85) = 186.003$; $P=<0.001$ (Table 10.3).

Generally, there were significant differences between L*, a*, b*, and C* at different preservation times. According to Buffa et al., (2001) studies on the characteristics of colour during cheese ripening, the a* value was bluish during the last week in samples containing biomass. The highest values for b* (reddish) were obtained in the samples with biomass (B1) in the last week (t=8) of preservation time. Color was significantly affected by preservation time.

Table 6 3. Color results of cheeses without biomass (control 12 g salt/L) and with biomass (B1 8 g salt/L and B2 4 g salt/L) carried out within a duration of 8 days.

	Control (12 g salt /L)			**B1 (8 g salt/L)**			**B2 (4 g salt /L)**		
	t=0	t=4	t=8	t=0	t=4	t=8	t=0	t=4	t=8
L*	$79,25±1,2^b$	$79,87±0,4^a$	$78,64±0,2^c$	$76,57±0,6^a$	$75,55±0,5^b$	$75,22±0,7^b$	$76,96±1,5^a$	$74,71±1,2^b$	$74,63±1,3^b$
a*	$-0,62±0,07^b$	$-0,60±0,08^b$	$-0,50±0,08^a$	$-0,42±0,13^c$	$-0,05±0,15^b$	$0,30±0,10^a$	$-0,46±0,08^c$	$-0,31±0,25^b$	$0,19±0,24^a$
b*	$3,95±0,21^b$	$4,28±0,05^a$	$4,21±0,10^a$	$7,13±0,35^c$	$8,18±0,32^b$	$8,67±0,29^a$	$6,93±0,38^c$	$7,82±0,26^b$	$8,43±0,25^a$
C*	$3,91±0,20^b$	$4,32±0,05^a$	$4,24±0,11^a$	$7,12±0,32^c$	$8,18±0,32^b$	$8,68±0,30^a$	$6,95±0,38^c$	$7,83±0,25^b$	$8,43±0,25^a$

L*: luminosity/Lightness; **a***: varies green-red component; **b***: varies between blue-yellow component; **C***: chromaticity. Values with different letters (a, b, and c) in the same row, within the conditions “12 g salt/L”, “8 g salt /L”, or “4 g salt /L”, indicate statistically significant differences (Test Tukey's HSD, $p<0.05$).

The samples underwent some changes in terms of their appearance during the 8 days of the study (Figure 6.1).

Figure 6 1. Cheese samples appearance throughout the study (duration of 8 days). (C0) Control 12g/L salt, t=0 day ; (C4) Control 12g/L salt, t=4 days; (C8) Control 12g/L salt, t=8 days; ($B_1$0) Biomass 8g/L salt, t=0 day; ($B_1$4) Biomass 8g/L salt , t=4 days; ($B_1$8) Biomass 8g/L salt, t=8 days; ($B_2$0) Biomass 4g/L salt, t=0 day; ($B_2$4) Biomass 4g/L salt , t=4 days; (B_2 8) Biomass 4g/L salt, t=8 days. (Titalah Sheron).

6.5.2. pH, a_W, Dry weight, and Fat content

The pH of the cheeses without biomass control (12g/L salt) and with biomass (8 g salt /L and 4 g salt /L) showed a significant difference during the preservation time (Table 6.4). From the analysis of variances (ANOVA), the $p<0.05$ for all the cheeses. For control $F(2, 42) = 28,17$; $p=0.00$. For (B1) $F(2, 42) = 5.418$; $p=0.01$. For (B2) $F(2, 42) = 133,960$; $p=0.00$ (Table 10.5).

The a_W of the cheeses did not show any significant difference with preservation time (Table 6.4). For the analysis of variance (ANOVA) the $p>0.05$ for all the cheeses. For control $p=0.185$; $F(2, 6) = 2.267$. B1, $F(2, 6) = 3.561$; $p=0.96$. B2, $F(2, 6) = 3.296$; $p=0.108$ (Table 10.5).

The results for dry weight were significantly different between the preservation time of control (12 g salt/L) cheeses and cheeses with biomass B1 (8 g salt/L). The cheeses with biomass (4 g salt /L) were not significantly different with preservation time (Table 6.4). For the analysis of variance (ANOVA) the $p<0.05$ for control, $F(2, 6) = 15.624$; $p= 0.004$, and cheeses with biomass B1, $F(2, 6) = 25.149$; $p=0.001$. The $P>0.05$ for cheeses with biomass B2 (4 g salt /L), $F(2, 6) = 1.465$; $p=0.303$ (Table 10.6).

The results for fat content of the cheeses were not significantly different during time for control cheeses and the cheeses with biomass B1 (8 g salt /L), while cheeses with biomass B2 (4 g salt /L) were significantly different between days t=0 and t=4 (Table 6.4). For the analysis of variance (ANOVA) the $p>0.05$, for control cheeses $F(2, 6) = 1.909$; $p= 0.228$ and cheeses with biomass B1, $F(2, 6) = 4.333$; $p=0.068$. The $P<0.05$ for cheeses with biomass B2, $F(2, 6) = 27.000$; $p=0.001$ (Table 10.6).

Table 6 4. Physical chemical results of pH, a_W, dry weight, fat, and protein (duration of 8 days).

	Treatment	t=0	t=4	t=8
pH	Control 12 g salt /L	6,40 ± 0,04^{c}	6,51 ± 0,01^{a}	6,47 ± 0,06^{b}
	B1 (8 g salt /L)	6,57 ± 0,01^{a}	6,58 ± 0,01^{a}	6,46 ± 0,15^{b}
	B2 (4 g salt /L)	6,54 ± 0,01^{b}	6,66 ± 0,02^{a}	6,67 ± 0,04^{a}
a_W	Control (12 g salt /L)	0,97 ± 0,01^{a}	0,96 ± 0,01^{a}	0,97 ± 0,001^{a}
	B1 (8 g salt /L)	0,96 ± 0,005^{a}	0,95 ± 0,001^{a}	0,95 ± 0,004^{a}
	B2 (4 g salt /L)	0,97 ± 0,003^{a}	0,96 ± 0,003^{a}	0,97 ± 0,005^{a}
Dry weight (%)	Control (12g salt/L)	22,17 ± 1,07^{b}	26,80 ± 1,31^{a}	27,63 ± 1,46^{a}
	B1 (8 g salt /L)	23,57 ± 2,05^{b}	29,30 ± 1,40^{a}	32,13 ± 0,80^{a}
	B2 (4 g salt /L)	21,90 ± 0,62^{a}	24,50 ± 10,91^{a}	30,70 ± 2,46^{a}
Fat (%)	Control (12 g salt /L)	12,00 ± 1,00^{a}	12,33 ± 0,58^{a}	13,63 ± 1,53^{a}
	B1 (8 g salt /L)	13,00 ± 1,00^{a}	13,50 ± 0,50^{a}	15,00 ± 1,00^{a}
	B2 (4 g salt /L)	12,00 ± 0,00^{b}	15,00 ± 0,00^{a}	15,00 ± 1,00^{a}

Note: Values with different letters (a, b, and c) in the same row, within the conditions “12 g salt /L”, “8 g salt /L”, or “4 g salt /L”, indicate statistically significant differences (Test Tukey's HSD, $p<0.05$).

6.6. Bioactive Properties of the Cheese

6.6.1. Antioxidant activity of cheese

Ethanol extracts from cheese with biomass added displayed no radical scavenging activity at the concentration tested. One reason maybe that the quantity of bioactive compounds in the cheese were not enough to be detected at the concentration 50 mg/mL tested. However, when trying to increase the concentration to be tested, a white solution was obtained, hindering the feasibility of performing the assay.

Some possible reasons could be that the concentration of the biomass was not high enough to have an effect. The processing or storage conditions may have caused degradation of the active components in the biomass, or the radical scavenging activity of the biomass may have been inhibited by other components in the cheese matrix.

6.6.2. Total Phenolic (TPC) and Flavonoid Content (TFC) of the Cheese

TPC increased in all the cheeses with preservation time from t=0 to t=8 $P<0.05$ (Table 6.5). Similar results were reported about total phenolic content in cheeses that increased during the storage period, mainly in those made with the higher content of catechin. The antioxidant activity values generally increased with storage and/or ripening time in both control and treated batches, but the cheeses made with catechin showed higher antioxidant activity than the control cheeses, suggesting that catechin remained in the treated cheeses during storage (Rashidinejad et al., 2013).

For control cheese $F (2, 15) =5{,}626$, $P=0.015$. For B1 $F (2, 15) =6{,}982$, $P=0.007$. For B2 $F (2,15) =11.469$, $P=0.001$ (Table 10.7). The highest TPC were observed in cheeses with biomass, B2 (4 g/L salt) from 19.38 to 39,94 mg GAE/g DW compared to the control 9,94 to 15.66 mg GAE/g DW, which is correlated to radical scavenging activity.

Arthrocnemum macrostachyum contains phenols, flavonoids, tannins and alkaloids, the contents of which have been reported by several studies (Rodrigues et al., 2014; Lopes et al., 2016; Barreira et al., 2017; Zengin et al., 2018; Chekroun-Bechlaghem et al., 2019). During the cheese-making process, these phenolic compounds may have been extracted from the biomass and transferred to the cheese, resulting in higher total phenolic contents.

TFC was not detected (nd) in control cheeses at t0 and t4, and in B2 at t0. There was a significant difference in the TFC between the cheeses (Table 6.5). The analysis of variance ANOVA, $P<0.05$. For the control cheeses $F (2, 15) =58.725$, $P<0.001$. For B1 $F (2,15) =3.364$ $P= 0.062$. For B2 $F (2,15) =34.352$, $P<0.001$ (Table 10.7).

Table 6 5. Total phenolic (TPC) mg GAE/g DW and flavonoid (TFC) mg QE/g DW content of the cheese (duration 8 of days).

	Treatment	t0	t4	t8
TPC (mg GAE/g DW)	Control (12 g salt/L)	9,94 ± 2,44^{b}	11,38 ± 2,32^{ab}	15,66 ± 4,13^{a}
	B1 (8 g salt/L)	15,17 ± 5,35^{b}	17,12 ± 3,95^{b}	26,39 ± 6,96^{a}
	B2 (4 g salt/L)	19,38 ± 3,41^{b}	18,90 ± 2,23^{b}	39,94 ± 14,49^{a}
TFC (mg QE/g DW)	Control (12 g salt/L)	nd	nd	10,16 ± 3,95^{a}
	B1 (8 g salt/L)	2,80 ±0,58^{a}	4,37 ± 2,76^{a}	1,88 ± 0,68^{a}
	B2 (4 g salt/L)	nd	0,53 ± 0.91^{b}	1,94 ± 0,90^{a}

Note: nd – not detected. Values with different letters (a, b, and c) in the same row, within the conditions “12 g salt/L”, “8 g salt/L”, or “4 g salt/L”, indicate statistically significant differences (Test Tukey's HSD, $P<0.05$).

6.7. Microbiological control of the cheese during the storage period

6.7.1. *Escherichia coli* (ISO 16649-2:2001)

The enumeration of *Escherichia coli* in the cheeses was <10 CFU/g. This means that the results follow the legislation in force, which indicates a minimum limit of <10 CFU/g (Regulation 1441/2007). Similar results were found by Lemos et al (2019) where no *Escherichia coli* was detected. This suggest that the cheeses are microbiologically safe making it a relevant outcome.

6.7.2. S*taphylococcus* coagulase positive (ISO 6888-1:2021)

The enumeration of *Staphylococcus* coagulase positive in the cheeses was <10 CFU/g. This means that the results follow the legislation in force, which indicates a minimum limit of <10 CFU/g (European Regulation 1441/2007). Different results were found by Lemos et al. (2019) where *Staphylococcus* coagulase positive was 1.0 x 10^2 CFU/g.

6.7.3. Microorganisms that grow at 30 ˚C (ISO 4833-1:2013)

The enumeration of microorganisms at 30 °C was determined. The number of CFU/g increased during preservation from t=0, t=4 and t=8 in the cheeses of all the conditions (Table 6.6). Similar results were recorded by Lemos et al. (2019) where total microorganisms at 30 °C were 5.5x10^5 CFU/g.

Table 6 6. Results of microorganisms at 30 °C (duration of 8 days)

Time	Control (12 g Salt /L) (log CFU/g)	B1 8 g Salt /L (log CFU/g)	B2 (4 g Salt /L) (log CFU/g)
t=0	5,02 ± 0,05	4,62 ± 0,43	3,74 ± 0,02
t=4	6,13 ± 0,06	6,76 ± 0,07	5,90 ± 0,08
t=8	7,74 ± 0,05	9,40 ± 0,07	8,38 ±0,11

6.7.4. Yeasts and moulds (ISO 21527-1:2008)

In the enumeration of yeast and moulds, no moulds were found in the cheeses. Yeasts were present from days t=4 to t=8 of the storage period. On the last week (t=8) the number of yeast colonies were >5 log CFU/g for conditions B1 and B2 (Table 6.7). Lemos et al (2019) recorded the number of yeast as 2.5x10^3 CFU/g.

Table 6 7. Results for detection of fungi yeast on cheeses (duration of 8 days).

	Time	Control (12 g Salt/L) (log CFU/g)	B1 (8 g Salt/L) (log CFU/g)	B2 (4 g Salt /L) (log CFU/g)
Yeast	t=0	<1	<1	<1
	t=4	2,95 ± 0,04	4,82 ± 0,09	2,92 ±0,08
	t=8	4,46 ± 0,22	>5	>5

6.7.5. *Listeria monocytogenes* (ISO 11290-1:2017)

Listeria monocytogenes was not detected in the cheeses. The standard is absence in 25 g before the food has left the immediate control of the producer (European Regulation No. 1441/2007). The cheeses were safe for consumption at any given time within the days of the analysis. Similar results were recorded by Lemos et al. (2019).

6.7.6. *Salmonella (ISO 6579-1:2017)*

Salmonella was not detected in the cheeses. As stipulated by European Regulation EC No. 1441/2007 is complete absence in 25 g. This therefore means that the cheeses were safe for consumption at any given time within the days of the analysis. Similar results were recorded by Lemos et al. (2019).

6.8. Effect of different salt concentrations on pH, a_W, dry weight and fat content

The pH of the cheeses with different salt concentration were significantly different (P<0.05). Highest pH of 6,57 was observed in the cheeses containing 4 g biomass with 8 g salt/L, followed by pH of 5,54 in the cheese with 4 g biomass and 4 g salt/L and the lowest pH of 6,40 in the control cheese with 12 g salt/L (Table 6.8) (ANOVA, F (2, 42) =232,036; P<0.001. (Table 10.8). Tukey HSD, P<0,001 (Table 10.9). The addition of biomass seems to contribute to the increase in pH.

The a_W, dry weight and fat contents of the cheeses with different salt concentrations were not statistically significantly different, P>0.05 (Table 6.8) (ANOVA, a_W F (2,6) =2,611; P=0.153; Dry weight F (2,6) =1,255; P=0.350; Fat F (2, 6) =1,500; P=0.296 (Table 10.10).

Table 6 8. Effect of different salt concentrations on pH, a_W, dry weight, and fat content before storage at time (t=0).

	Control (12 g salt /L)	B1 (8 g salt/L)	B2 (4 g salt/L)
pH	$6,40 \pm 0,04^{c}$	$6,57 \pm 0,01^{a}$	$6,54 \pm ,01^{b}$
a_W	$0,97 \pm 0,005^{a}$	$0,96 \pm 0,004^{a}$	$0,97 \pm 0,003^{a}$

Dry weight (%)	22,17 ± 1,07^{a}	23,57 ± 2,05^{a}	21,90 ± 0,62^{a}
Fat (%)	12,00 ± 1,00^{a}	13,00 ± 1,00^{a}	12,00 ± 0,00^{a}

Note: Values with different letters (a, b, and c) in the same row, within the conditions "12 g salt /L", "8 g salt /L", or "4 g salt /L", indicate statistically significant differences in the salt concentration (Test Tukey's HSD, P<0.05).

6.9. Effect of different salt concentrations on Total Phenolic (TPC) and Flavonoid Contents (TFC).

Total phenolic and flavonoid content of the cheeses with different salt concentration were significantly different (P<0.05) (Table 6.9). (ANOVA F (2,15) =8,719; P<0,003; F (2,15) =194,513; P<0,0001) (Table 10.11). TPC was had highest values in B2 followed by B1 and lowest in the control cheeses. Tukey HSD, P<0,185 (Table 10.12). TFC was not detected in the control and B2 cheeses. Tukey HSD, P<0,001 (Table 10.12).

Table 6 9. Effect of different salt concentrations on Total Phenolic (TPC) and Flavonoid (TFC) Contents before storage at time (t=o).

	Control (12 g salt/L)	**B1 (8 g salt/L)**	**B2 (4 g salt/L)**
TPC (mg GAE/g DW)	9,33 ± 2,43^{b}	15,17 ± 5,35^{ab}	19,38 ± 3,41^{a}
TFC (mg QE/g DW)	Nd	2,81 ± 0.58^{a}	nd

Note: nd – not detected. Values with different letters (a, b, and c) in the same row, indicate statistically significant differences (Test Tukey's HSD, P<0.05).

7. Conclusion

Arthrocnemum macrostachyum is endowed with antioxidants and phenolic compounds which can be related to their radical scavenging activity. *Arthrocnemum macrostachyum* presented antioxidant activity with an IC_{50} value approximately 4 mg/mL.

Total phenolic (TPC) and flavonoid (TFC) contents of *Arthrocnemum macrostachyum* were 23.76 GAE/g DW and 10.35 mg QE/g DW, respectively.

Macrominerals were the most abundant with Na 24,78 mg/g DW being the highest. Mn (64,74 µg/g DW) was the most accumulated and Cd not detected.

The microbiological evaluation of the biomass, both fresh and dry showed excellent results free from the contamination by hygiene indicator bacteria's *Escherichia coli* and *Staphylococcu*s coagulase positive and no *Salmonella* and *Listeria monocytogenes* were detected indicating microbiological safety of the biomass. The results for microorganisms at 30 °C and yeast and molds leads us to the conclusion that biomass is in conditions, from the hygienic point of view, to be added to cheese manufacture.

The influence of the addition *Arthrocnemum macrostachyum* biomass on the physicochemical properties of the cheeses was evaluated. The cheese yield ranged from 28,87 % in the control to 36 % in the cheese with biomass, showing that not only the added salt, but also the biomass directly influences the properties of cheese by controlling syneresis, and the texture of the curd.

The colour parameters L* (lightness) values decreased in the cheeses with biomass relatively to the control cheeses. a* increased in all the samples tested and remained green during storage. The values of b* and C* (chroma) increased with the addition of biomass indicating yellowness and more colorfulness of the cheeses. The highest values for L* were observed in the control cheeses and decreased during the last week of storage. The highest value for a* (-0,05 ± 0,15) was observed in the cheeses containing biomass with 8 g salt/L during the second week of storage. The highest values for b* and C* were observed in the cheeses containing biomass with 8 g salt/L during the second and last week of storage.

The pH and dry weight content were highest in the cheeses containing biomass with 8 g salt/L followed by the control and the lowest in the cheeses containing biomass with 4 g salt/L.

a_W remained virtually unchanged throughout the storage period. Fat content slightly increased during the storage period. The antioxidant activity of the cheeses was not detected.

TPC increased in all the cheeses during preservation, being the highest in the cheese containing biomass and 4 g salt/L. TFC was not detected in the control cheeses and cheeses containing biomass with 4 g salt/L.

There was no effect of the added biomass in the extension of cheese preservation time, since the microbial load was higher on cheeses produced with added biomass. Nevertheless, the microbiological quality remains acceptable during the first four days of storage in all studied cheeses. Thus, since part of the added salt can be replaced by *Arthrocnumum macrostachyum* without quality and safety problems, the cheese with 4 g of biomass and 4 g salt/L milk was considered the best within the studied ones.

8. Future perspectives

Due to the known functional advantages of *Arthrocnemum macrostachyum*, it is recommended that extracts of this plant should be incorporated into the cheeses and studied for better antioxidant activity and other nutrients like proteins.

Sensory analysis should be carried out with a panel of judges to obtain significant results that allows a more definitive conclusion about the acceptability of the cheese studied.

Similar studies on other plants could be performed to test whether they provide radical scavenging ability to this type of cheese.

9. References

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10. Appendix

1.1. Data analysis output tables from IBM SPSS

Table 10 1. One-way ANOVA with variables of colour (L-control, b-control, C-control, and a-4g/L).

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
L-control	Between Groups	21,627	2	10,814	17,411	,000
	Within Groups	52,172	84	,621		
	Total	73,799	86			
b-control	Between Groups	2,931	2	1,465	70,139	,000
	Within Groups	1,713	82	,021		
	Total	4,644	84			
C-control	Between Groups	2,784	2	1,392	69,819	,000
	Within Groups	1,635	82	,020		
	Total	4,419	84			
a-4g/L	Between Groups	6,675	2	3,338	77,703	,000
	Within Groups	3,565	83	,043		
	Total	10,240	85			

Table 10 2. One-way ANOVA with variables of color (a-control, b-8g/L, C- 8g/L and L-4g/L).

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
a-control	Between Groups	,259	2	,130	21,807	,000
	Within Groups	,517	87	,006		
	Total	,777	89			
b-8g/L	Between Groups	37,225	2	18,612	180,334	,000
	Within groups	8,979	87	,103		
	Total	46,204	89			
C-8g/L	Between Groups	37,640	2	18,820	191,688	,000
	Within Groups	8,542	87	,098		
	Total	46,182	89			
L-4g/L	Between Groups	105,030	2	52,515	28,479	,000
	Within Groups	160,426	87	1,844		
	Total	265,455	89			

Table 10 3. One-way ANOVA with variables of color (a-8g/L, b-4g/L, and C- 4g/L).

ANOVA

		Sum of squares	df	Mean Square	F	Sig.
a-8g/L	Between Groups	7,570	2	3,785	222,517	,000
	Within Groups	1,463	86	,017		
	Total	9,033	88			
b-4g/L	Between Groups	36,595	2	18,298	187,136	,000
	Within Groups	8,311	85	,098		
	Total	44,906	87			
C-4g/L	Between Groups	35,929	2	17,964	186,003	,000
	Within Groups	8,209	85	,097		
	Total	44,138	87			

Table 10 4. One-way ANOVA with variables of color L – 8 g/L

ANOVA

L-8 g/L

	Sum of squares	Df	Mean square	F	Sig.
Between Groups	25,622	2	12,811	33,561	,000
Within Groups	30,157	79	,382		
Total	55,779	81			

Table 10 5. One-way ANOVA with variables (pH control, 8g/L, and 4g/L).

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
pH-control	Between Groups	,096	2	,048	28,167	,000
	Within Groups	,072	42	,002		
	Total	,168	44			
pH-8g/L	Between Groups	,089	2	,045	5,418	,008
	Within Groups	,347	42	,008		
	Total	,436	44			
pH-4g/L	Between Groups	,170	2	,085	133,960	,000
	Within Groups	,027	42	,001		
	Total	,196	44			

Table 10 6. One-way ANOVA a_W, dry weight (ES), and Fats.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
aw-Control	Between Groups	,000	2	,000	2,267	,185
	Within Groups	,000	6	,000		
	Total	,000	8			
aw-8g/L	Between Groups	,000	2	,000	3,561	,096
	Within Groups	,000	6	,000		
	Total	,000	8			
aw-4g/L	Between Groups	,000	2	,000	3,296	,108
	Within Groups	,000	6	,000		
	Total	,000	8			
ES-control	Between Groups	52,047	2	26,023	15,624	,004
	Within Groups	9,993	6	1,666		
	Total	62,040	8			
ES-8g/L	Between Groups	114,287	2	57,143	25,149	,001
	Within Groups	13,633	6	2,272		
	Total	127,920	8			
ES-4g/L	Between Groups	122,640	2	61,320	1,465	,303
	Within Groups	251,080	6	41,847		
	Total	373,720	8			
Fat-control	Between Groups	4,667	2	2,333	1,909	,228
	Within Groups	7,333	6	1,222		
	Total	12,000	8			
Fat-8g/L	Between Groups	6,500	2	3,250	4,333	,068
	Within Groups	4,500	6	,750		
	Total	11,000	8			
Fat-4g/L	Between Groups	18,000	2	9,000	27,000	,001
	Within Groups	2,000	6	,333		
	Total	20,000	8			

Table 10 7. One-way ANOVA of Total flavonoid (TFC) and phenolic contents (TPC) of the cheese

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
TFC-Control	Between Groups	616,218	2	308,109	58,725	,000
	Within Groups	78,700	15	5,247		
	Total	694,918	17			
TFC-8g/L	Between Groups	18,956	2	9,478	3,364	,062
	Within Groups	42,265	15	2,818		
	Total	61,220	17			
TFC-4g/L	Between Groups	43,284	2	21,642	34,352	,000
	Within Groups	9,450	15	,630		
	Total	52,734	17			
TPC-control	Between Groups	106,402	2	53,201	5,626	,015
	Within Groups	141,834	15	9,456		
	Total	248,236	17			
TPC-8g/L	Between Groups	431,335	2	215,668	6,982	,007
	Within Groups	463,344	15	30,890		
	Total	894,679	17			
TPC-4g/L	Between Groups	1731,787	2	865,893	11,469	,001
	Within Groups	1132,492	15	75,499		
	Total	2864,279	17			

Table 10 8. One-way ANOVA of Effect of different salt concentrations on pH before storage at time (t=0).

ANOVA

pH Conc

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	,243	2	,121	232,036	,000
Within Groups	,022	42	,001		
Total	,265	44			

Table 10 9. Tukey HSD of Effect of different salt concentrations on pH before storage at time (t=0).

Multiple Comparison

Dependent variable: pH Conc

Tukey HSD

(I) Salt Concentration	(J) Salt Concentration	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
4 g/L	8 g/L	-,03600*	,00835	,000	-,0563	-,0157
	12 g/L	,13467*	,00835	,000	,1144	,1550
8 g/L	4 g/L	,03600*	,00835	,000	,0157	,0563
	12 g/L	,17067*	,00835	,000	,1504	,1910
12 g/L	4 g/L	-,13467*	,00835	,000	-,1550	-,1144
	8 g/L	-,17067*	,00835	,000	-,1910	-,1504

*.The mean difference is significant at the 0.05 level.

pH Conc

Tukey HSD[a]

Salt Concentration	N	Subset for alpha = 0.05		
		1	2	3
12 g/L	15	6,4013		
4 g/L	15		6,5360	
8 g/L	15			6,5720
Sig.		1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.

a.Uses Harmonic Mean Sample Size = 15,000.

Table 10 10. One-way ANOVA of Effect of different salt concentrations on a_W, dry weight, and fat content before storage at time (t=0).

ANOVA

		Sum of Square	Df	Mean Square	F	Sig.
a_W Conc	Between Groups	,000	2	,000	2,611	,153
	Within Groups	,000	6	,000		
	Total	,000	8			
DW Conc	Between Groups	4,809	2	2,404	1,255	,350
	Within Groups	11,493	6	1,916		
	Total	16,302	8			
Fat Conc	Between Groups	2,000	2	1,000	1,500	,296
	Within Groups	4,000	6	,667		
	Total	6,000	8			

Table 10 11. One-way ANOVA of Effect of different salt concentrations on TPC and TFC before storage at time (t=0).

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
TFC	Between Groups	89,159	2	44,579	194,513	,000
	Within Groups	3,438	15	,229		
	Total	92,597	17			
TPC	Between Groups	268,290	2	134,145	8,719	,003
	Within Groups	230,787	15	15,386		
	Total	499,078	17			

Table 10 12. Tukey HSD of Effect of different salt concentrations on TPC and TFC before storage at time (t=0).

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Salt Concentration	(J) Salt Concentration	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
TFC	4	8	-4,62333*	,27640	,000	-5,3413	-3,9054
		12	,19000	,27640	,774	-,5279	,9079
	8	4	4,62333*	,27640	,000	3,9054	5,3413
		12	4,81333*	,27640	,000	4,0954	5,5313
	12	4	-,19000	,27640	,774	-,9079	,5279
		8	-4,81333*	,27640	,000	-5,5313	-4,0954
TPC	4	8	4,20833	2,26464	,185	-1,6740	10,0907
		12	9,43833*	2,26464	,002	3,5560	15,3207
	8	4	-4,20833	2,26464	,185	-10,0907	1,6740
		12	5,23000	2,26464	,085	-,6523	11,1123
	12	4	-9,43833*	2,26464	,002	-15,3207	-3,5560
		8	-5,23000	2,26464	,085	-11,1123	,6523

*. The mean difference is significant at the 0.05 level.

TPC

Tukey HSD[a]

Salt Concentration	N	Subset for alpha = 0.05: 1	Subset for alpha = 0.05: 2
12	6	9,9383	
8	6	15,1683	15,1683
4	6		19,3767
Sig.		,085	,185

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6,000.

TFC

Tukey HSD[a]

Salt Concentration	N	Subset for alpha = 0.05	
		1	2
12	6	-2,0050	
4	6	-1,8150	
8	6		2,8083
Sig.		,774	1,000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6,000.