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INFLUENCE OF HIGH PRESSURE TREATMENTS ON GOAT'S MILK CHEESE FOR THE IMPROVEMENT OF ITS SENSORY AND COMMERCIAL CHARACTERISTICS

Memòria presentada per a optar al grau de Doctora
en Ciència i Tecnologia dels Aliments

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FAN CONSTAR: que NATÀLIA NICOLAU I VILLELLAS ha realitzat sota la seva direcció, a l'Àrea de Ciència i Tecnologia dels Aliments de la Universitat Autònoma de Barcelona, el treball titulat **'I*nfluence of High Pressure treatments on goat's milk cheese for the improvement of its sensory and commercial characteristics*'** que presenta per a optar al grau de Doctora.

I per a que així consti, signem el present document a:

Bellaterra, Cerdanyola del Vallés, a dia 10 d'Abril de 2015

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A tú, Pare.
A tí, Madre.
A tú, Nena.
A tí, Asier.

He denunciado que la educación formal tradicional es un desperdicio por demás destructivo en tiempos donde nuestra mayor necesidad no es otra cosa que la de una auténtica cultura, entendimiento y un buen corazón. Creo que la educación es nuestra mayor esperanza, porque ya ha sentado las bases institucionales para lo que solamente tenía contemplado llevar a cabo, ayudar en el desarrollo personal. Ahora bien, debido a que el problema más grave y más básico que tenemos en común es el subdesarrollo de la consciencia, es necesario que hagamos hincapié en la prevención. Solamente deberíamos percatarnos de qué tan destructivo ha sido el querer educar a la juventud para que sean un reflejo de lo que nosotros somos, y de cómo, al creer que les estamos transmitiendo nuestros valores, lo que hacemos es mostrar una arrogante ceguera respecto a la forma en que les transmitimos nuestras plagas, y hasta qué grado lo hacemos.

Si la gran esperanza de cambiar la educación ha de realizarse - y más vale pronto que nunca - habrá de basarse en la transformación de los educandos, puesto que resultaría ridículo pensar que ello pudiera lograrse mediante una reforma curricular solamente. Y es así que surge el interrogante: ¿contamos con un método efectivo y factible a través del cual pudiéramos educar a los docentes ofreciéndoles las experiencias y entrenamiento que el mundo académico nunca les pudo brindar, y que sin embargo resultan indispensables para una educación orientada hacia la evolución personal y social?

Claudio Naranjo

Agraïments

Primer de tot m'agradaria anomenar a les persones que van confiar en mi des del primer momento i van creure que la meva historia d'amor amb els formatges anava en sèrio. A en Ventura Guamis per tastar els meus formatges cassolans des del primer dia i per acollir-me en el seu equip durant tots aquests anys brindant-me infinites oportunitats. A en Toni Trujillo per fer de trampolí i pensar en mi per al projecte PETRI que ha acabat donant lloc a aquesta tesi. Seguidament vull dedicar una part important d'aquesta plana al Martín Buffa, per la seva paciència, i per la seva paciència altra vegada amb mi, per la seva mà esquerra i les ganes de formar-me que finalment han donat fruit i m'han fet qui sóc avui en dia professional i personalment. Veurem quantes ampolles de Fernet em costa la Tesi…

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SUMMARY

The overall goal of the present PhD thesis was to study some factors related to the choice of pressure intensity (100, 200 or 300 MPa) and the moment of high-pressure (HP) treatments application (before and after brining; BS and AS respectively) that could influence water binding, textural characteristics and sensory profile of pressurized goat's milk cheeses compared with the control cheeses.

First of all several cheese making productions were carried out in order to tune up the manufacture conditions, especially pursuing the goal of major water binding by conventional methods. Different pressure and time conditions were tested during pressing, two different brining times and two different relative humidity values in the ripening room were studied. From these works, the best conditions retaining higher moisture content were chosen and applied to cheese manufacturing. Results indicated that pressure intensity was the variable which most affected goat's milk cheeses, and in a lesser extent the moment of application.

In regards to physic-chemical characteristics, 300 MPa HP-treated cheeses showed highest pH values and moisture content. Additionally, these cheeses appeared as much more ripened than others, probably due to color enhanced characteristics and higher proteolysis values. In relation to textural parameters, pressurized cheeses at 300 MPa were less firm and account for higher strain values compared with the control and the rest of pressurized cheeses. Significant differences referring to high-pressure treatments at different moments of brining appeared in 100 and 200 MPa cheeses, resulting AS samples in lower fracture stress (σ_t) values at day 30 of ripening. Micrographs were quantified and the microstructure of cheese analyzed obtaining a different microstructure determined by low levels in porosity, especially in 300 MPa cheeses. At the end of ripening, higher lipidic area was another characteristic attributed to 300 MPa cheeses compared to the rest of cheeses. Cheeses high-pressure treated at 300 MPa received a significantly higher overall grade than did other samples, mostly with respect to textural parameters, leading to better mouthfeel cheeses as punctuated by panelists.

Internal moisture profiles of goat's milk cheeses were affected in a great manner by pressure intensity applied and in a lesser extent, by the moment of HP application. 300 MPa cheeses showed greater amounts of W1 (free water) released at all sampling points during ripening. Although little oscillations of W3 were found at first stages of ripening, 300 MPa samples finished their ripening with major amounts of W3, together with the control cheese, showing both samples a better binding of water at the optimum ripening point compared with 100 and 200 MPa cheeses. Most of samples reached the equilibrium in regards to the salt content between inner and outer parts of cheese at the determinate optimum ripening point for cheeses in this study (day 30). Regarding the pressure effect, HP samples, especially 300 MPa cheeses revealed a greater penetration of salt at day 1, showing higher values than control and the rest of pressurized cheeses in the inner part of cheese. While control or other pressurized cheeses did not reach salt uptake equilibrium before day 30, 300 MPa cheeses revealed a faster diffusion of salt during ripening obtaining similar values between both cheese parts studied (inner and outer) at day 7 of ripening. No large effect can be observed in high-pressure treated-samples respect to the moment of brining and the salt content.

In this study, HP-treatments caused several changes on the overall amount of volatile compounds found in goat's milk cheeses. While pressures of 300 MPa seemed to increase total amount of volatile compounds, cheeses treated at 100 and 200 MPa revealed lower levels compared with the control cheese leading to an impoverished volatile profile. The absence or presence of several compounds in 300 MPa treated-cheeses modified its volatile profile enhancing it by minimizing the mouldy or sharpness and goaty notes of 300 MPa.

It seems that novel textures and flavors, certainly due to the better water binding could be developed by HP processing applying 300 MPa HP-treatment. This technology may provide new textures to traditional cheeses or even the possibility to create novel types of cheese enhancing their commercial characteristics being more appealing to consumers and providing beneficial factors, economically speaking.

RESUM

L'objectiu principal d'aquesta tesi va ser estudiar l'efecte de la intensitat de pressió (100, 200 o 300 MPa) i el moment (abans o després del salat; BS i AS, respectivament) de l'aplicació dels tractaments d'Alta Pressió (AP) sobre la capacitat de retenció d'aigua, les característiques de textura i el perfil sensorial dels formatges de cabra pressuritzats comparats amb els formatges control.

En un inici, es van posar a punt les condicions d'elaboració del formatge amb l'objectiu d'aconseguir una máxima retenció de l'aigua en els formatges mitjançant mètodes convencionals. Es van estudiar diferents combinacions de temps i pressions durant el premssat, dos temps durant el salat i dos valors d'humitat relativa a la cambra de maduració. Dels resultats obtinguts, es van escollir les millors condicions per a conformar el diagrama d'elaboració òptim. La variable que va afectar els formatges de cabra en major grau va ser la intensitat de la pressió, i en menor grau el moment d'aplicació de l'alta pressió.

Referent als resultats físico-químics, els foramtges tractats a 300 MPa van mostrar els valors més alts de pH i un major contingut d'humitat. Adicionalment, aquests formatges semblaven més madurats que la resta, probablement degut a la millora de les característiques de color i als valors més elevats de proteolysis. En relació als paràmetres de textura, els formatges tractats a 300 MPa van obtenir valors més baixos de fermesa i més elevats de deformació en el punt de fractura en comparació amb el control i la resta de formatges pressuritzats. Observant les mostres tractades a 100 i 200 MPa, són remarcables algunes diferències significatives pel que fa al moment, indicant que els formatges AS van obtenir valors més baixos de fermesa en el punt de fractura al dia 30 de maduració. Els micròsgrafs es van quantificar per a analitzar la microstructura, els resultats de la qual van apuntar als formatges tractats a 300 MPa com els de valors de porositat més baixos i en canvi, mostrant valors d'àrea lipídica més elevats que la resta del formatges. Les puntuacions sensorials més elevades van ser per als formatges tractats a 300 MPa, majorment pel que fa als atributs de textura, donant lloc a formatges amb millor palatibilitat i sensació en boca.

Els perfils de retenció d'aigua dels formatges de cabra es van veure afectats en major grau per la intensitat de la pressió aplicada i només lleugerament pel moment d'aplicació dels tractaments. Els formatges tractats a 300 MPa van alliberar una quantitat més elevada de W1 (aigua lliure) en tots els punts de mostreig. Encara que es van observar petites oscil·lacions en els valors de W3 (aigua lligada) durant els primers estadis de maduració, els formatges tractats a 300 MPa van finalitzar la seva mauració amb valors més elevats de W3, juntament amb els formatges control, els quals van donar lloc a formatges amb una elevada capacitat de retenció d'aigua comparat amb els formatges tractats a 100 o 200 MPa. La majoria de les mostres van assolir l'equilibri pel que fa al contingut de sal entre les parts externes i internes del formatge al punt òptim de maduració establert per a aquests formatges (dia 30). Pel que fa als efectes de la pressió, els formatges pressuritzats, especialment els tractats a 300 MPa van destacar per mostrar una elevada penetració de la sal a dia 1, obtenint valors més elevats en la part interior del formatge comparat amb el control i la resta de mostres pressuritzades. Mentres que el altres formatges pressuritzats i el control no van aconseguir l'equilibri de l'homogeneització de sal en el formatge fins al dia 30, els formatges tractats a 300 MPa van mostrar una ràpida difusió de la sal durant la maduració assolint valors similars a les dues parts analitzades del formatge (interior i exterior) a dia 7.

En aquest estudi, els tractaments d'alta pressió van afectar al perfil volàtil dels formatges, augmentant l'abundància de diversos compostos en el cas de 300 MPa, i per altra banda disminuint els valors d'alguns compostos en el cas de 100 i 200 MPa.

Tenint en compte els resultats obtinguts, sembla ser que applicant pressions de 300 MPa als formatges de quallada enzimàtica de cabra es podrien aconseguir formatges de noves textures i aromes. Aquesta tecnología podria proporcionar la possibilitat de crear nous tipus de formatge de caracterísitques sensorials i comercials millorades, éssent més atractius pels consumidors i aportant factors beneficiosos com ara un estalvi econòmic important.

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List of abbreviations

Al	alia (others)
ANOVA	Analysis of variance
AS	After brining cheeses
Au	Area unit
Aw	Activity of water
α-LA	α – Lactoalbumine
α_s-cn	α_s caseins
β-LG	β – Lactoglobuline
β-cn	β caseins
BS	Before brining cheeses
C	Control cheese
CAR	Carboxen
Cd Cl_2	Cadmium Chloride
CLSM	Confocal Laser Scanning Microscopy
DVB	Divinyl benzene
EU	European Union
FAA	Free Amino Acids
FAO	Food and Agriculture Organization from the United Nations
FFA	Free Fatty Acids
Fig	Figure
GC	Gas Chromatography
HP	High Pressure
IDF	International Dairy Federation

KPa	Kilo Pascal
LAB	Lactic cid bacteria
Leu	Leucine
LOD	Limit of detection
L	Litre
Min	Minute
MPa	Mega Pascals
MS	Mass spectra
Na^+ Cl^-	Sodium Chloride
P	Pressure
PCA	Principal Component Analysis
PDMS	Polydimethylsiloxane
PDO	Protected denomination of origin
PGI	Protected geographical indication
SD	Standard deviation
SEM	Scanning Electron Microscopy
SN	Soluble Nitrogen
SPME	Solid Phase Micro Extraction
Tª	Temperature
t	Time
TGA	Thermogravimetrical analysis
TN	Total Nitrogen
UAB	Universitat Autònoma de Barcelona
WSN	Water Soluble Nitrogen
ΔE	Color difference

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Chapter I

Hypothesis, objectives and working plan

Hypothesis, objectives and working plan

1.1 Hypothesis

Spain is an important producer of goat and sheep milk (Martínez, Franco, & Carballo, 2011). Being a country belonging to the Mediterranean area, it has a high population of these animals and numerous traditional products involving their milk. In this area almost all goats (and 60% of the sheep) are totally or partially milked, and about the 90% of the milk is transformed into good quality products (Scintu & Piredda, 2007), such as cheese. Even natural characteristics of goats, allows them adapting to different farming systems and environments, pastoralism in goat has been a very important part of most of the Mediterranean agricultural systems for centuries (Dubeuf, de A. Ruiz Morales, & Castel Genis, 2010). Goat milk cheese is part of the historic cultural heritage of Spain as the breeding of dairy goats is characteristic of marginal and less favorite areas, where local breeds must adapt to local resources. Therefore, in these areas, typical products have been developed according to local resources available. Thus, goat's milk cheese keeps preserving traditional cheese making processes resulting in conservation of old crafts and valuable skills related to rural areas, raw materials and disadvantaged regions.
However, not always these issues cause positive perception on the consumer. In Spain the situation of goat milk cheeses is very different than other countries in Europe, i.e. compared to France. Small-scale and farm-made sectors develop their activity by producing and selling local cheeses. Until several years ago, goat milk has been less appreciated than sheep milk for cheese production, probably due to its physicochemical composition and its poor aptitude to be curdled. An inherited negative image of dairy goat products has been a handicap for organizing and appreciating an effective dairy goat sector, and there is no defined market for goat milk in contrast with other neighbor countries in Europe. In addition, the sensory aspects of goat products, such as more intense flavor and aroma, also contribute to the low acceptance of these products by some consumers (Dubeuf et al., 2010). Due to the goat underdevelopment market, and a lack of technology and research characterizing dairy products made with goat milk, there is a limitation on its expansion in the consumer's level. However, goat cheeses are consumed worldwide and have been associated in recent years with increased goat milk production and demand in numerous countries (Dubeuf et al., 2010; Facó et al., 2011; Gürsoy, 2006; Hayaloglu & Karagul-Yuceer, 2011; Medeiros et al., 2013; Queiroga et al., 2013).

Milk composition, and especially proteins and fat, may vary according to genetic diversity of the animals and different feeding systems, giving peculiar features to the milk utilized to make typical cheeses (Scintu & Piredda, 2007). Several authors have pointed out specific differences in composition between goat and cow's milk, giving different behaviors during milk coagulation and cheese ripening (Attaie & Richter, 2000; Attaie, 2005; Mora-Gutierrez, Farrell, Attaie, McWhinney, & Wang, 2007; A J Trujillo, Guamis, & Carretero, 1997; A.J. Trujillo, Guamis, & Carretero, 1997). It is well known that casein in milk from goats does not react with rennet the same as caseins from bovine milk resulting in a gel not as firm as that formed from bovine milk. It is more fragile and the

yield of the cheese is less than that obtained from bovine milk (Remeuf, Verdaletguzman, & Lenoir, 1995).

Because of the differences in goat's milk composition the ripening of goat milk cheeses involves a higher water loss and the subsequent weight loss, a bit much firming of the paste and an overmuch drying with a possible lack of flavor and an inappropriate texture at the supposed optimum maturation point. These final sensory characteristics of goat milk cheese could lead to a less acceptance of these products by the consumers and thus, the decrease of retail marketing.

Among the emerging technologies, high pressure (HP) has been used targeting several aims on foodstuff and specifically in cheeses. Nowadays consumers give high importance to natural food products, low-processed and no added in additives nourishment. In this line, high-pressure is a non-invasive technology which meets all requirements of consumers and is one of the most promising methods for preservation of food at room temperature (Cheftel, 1995). Besides, high-pressure do not include thermal treatment, what is more, could be a substitute of this conventional technology, being this fact very advantageous, as food treated by high pressure keeps all nutrients and do not lead to a degradation of essentials compounds in food.

Regarding the HP processing of cheese, most of the studies have been focused on cheese making from HP-treated milk (Buffa, Trujillo, Pavia, & Guamis, 2001; Guamis, Trujillo, Sendra, Buffa, & Saldo, 2000; A J Trujillo et al., 2000; Zamora, Ferragut, Juan, Guamis, & Trujillo, 2011), or when HP treatments were directly applied on cheese, to acceleration of ripening (Johnston & Darcy, 2000; Kolakowski, Reps, & Babuchowski, 1998; O'Reilly, O'Connor, Murphy, Kelly, & Beresford, 2000; J. Saldo, Sendra, & Guamis, 2000; Yokohama, H., Sawamura, N. and Motobayashi, 1992), deceleration or stop of ripening (Reps, Wiśniewska, Dajnowiec, & Iwańczak, 2000), decrease of spoilage microorganisms and increase microbiologiocal safety or increase of the shell life of cheese (Capellas, Mor-Mur, Gervilla, Yuste, & Guamis, 2000; Evert-Arriagada, Hernández-Herrero, Guamis, & Trujillo, 2014; Lopez-Pedemonte, Roig-Sagues, De Lamo, Hernandez-Herrero, & Guamis, 2007) or accelerating or improving salting of cheeses (W Messens, Dewettinck, Camp, & Huyghebaert, 1998; Winy Messens, Dewettinck, & Huyghebaert, 1999; Pavia, Jose Trujillo, Guamis, & Ferragut, 2000).

Although all these applications were well conducted to specific purposes, there was a lack of conscience of side effects due to the HP treatments, such as the firming of the paste because of the drop of water content, or the decrease of weight and therefore of money (Bibiana Juan, Ferragut, Guamis, & Trujillo, n.d.; Pavia et al., 2000; Jordi Saldo et al., 2003). When any of these goals are pursued, it is required that high pressure treatments do not interfere with interesting physic and biochemical changes like the development of specific flavor, aroma, and texture (Delgado, González-Crespo, Cava, & Ramírez, 2011).

Another aspect regarding to previous studies on high-pressure and cheese is that most of authors have applied the high-pressure treatments at the same step of cheese-making process. Several authors have applied high-pressure treatments on cheese at different days of ripening or with

different goals like Pavia et al. (2000) that elucidated the salt uptake and the rate of salt diffusion in Manchego-type cheese brined while applying 50 and 200 MPa on cheese. Other studies were conducted by Messens (W Messens et al., 1998; Winy Messens, Estepar-Garcia, Dewettinck, & Huyghebaert, 1999) to assess the effect of high pressure at the brining stage of Gouda and its serum to accelerate the brining by high-pressure in Gouda cheese, respectively.

In processing, salt has an important role during the manufacture process largely affecting water content of cheeses which in turn modifies the enzyme activity of cheese, its proteolysis and hence, its texture and microstructure. In regards to sensory characteristics, salt confers its own specific flavor and modifies the flavor of other ingredients contributing to the overall aroma of cheese. Additionally, salt has a direct effect on water activity diminishing availability of water for microorganisms to growth. Consequently, it regulates the activity of starter culture microorganisms, retarding the growth of most bacteria and acting as a preservation agent on cheese (Arboatti et al., 2014; Guinee & Sutherland, 2011; Man, 2007; Paulson, McMahon, & Oberg, 1998).

From a technological point of view, being the salt such determining ingredient of cheese, seems interesting to find out more about its behavior under high-pressure conditions or its possible synergistic effects. However, to date no authors have compared the effect of HP treatments applied before and after salting of cheese.

The experimental design of many of these studies carried out with high pressure and cheese included different conditions, especially modifying variables such as pressure, temperature, duration of treatment and the time point of ripening when it was applied.

Low temperatures are more expensive and difficult to achieve regarding to financial and energetic terms. Since the warming of fluid water due to high pressure is 3 º C per each 100 MPa reached and the warming of fat is 8-9 º C per each 100 MPa reached (Patazca, Koutchma, & Balasubramaniam, 2007; Rasanayagam et al., 2003), treating cheese by HP at 14 º C seems to be an energetically and experimentally interesting temperature as fat cheese may not reach upper temperatures than 41 º C during the high pressure treatments.

Saldo et al. (2002) applied moderate high pressure treatments on goat's milk cheese (50 MPa at 25 º C for 72h) and did not observed higher values of proteolysis and neither any modification in the texture of HP cheeses compared to the control cheeses. However, Juan et al. (2008) studied the effects of 300 MPa treatments on sheep's milk cheese and highlighted that conformational changes in cheese matrix and cellular lyses were produced.

Other studies (B. Juan, Trujillo, Guamis, Buffa, & Ferragut, 2007; J. Saldo et al., 2000) including HP treatments from 200 to 500MPa, times of treatment from 5 to 10min and treatments at days 1, 15, 21 and 60 of ripening, showed a decrease in free fatty acids (FFA) and ripening index in HP cheeses treated at 400 MPa. What is more, at the same pressure it seemed to be a blockage of both lipolysis and residual rennet activity. Cheeses treated at other dates after day 1 during de ripening did not show large differences compared to the control cheeses in proteolysis or texture

modification due to the lack of water content in the cheese matrix in the moment of the HP treatment. Water is a good pressure conductor material, and it is known that lower is the water content in a food product, lower is the effect of high pressure treatment. It is important to note that even 400 and 500 MPa pressures were the cause of several detentions in biochemical reactions that take place in cheese during ripening, these samples showed higher pH and free amino-acids values which suggest that many changes in proteolysis take place.

Delgado et al. (2011) studied the effect of high pressure on goat's milk PDO Queso de los Ibores. Referring to the sensory characteristics, no large differences in flavor and taste were detected; however appearance, odor and texture were significantly different between HP cheeses and control cheeses. Saldo et al. (2002) also noted that HP cheeses showed lower values of hardness and friability and higher springiness compared to the control cheese.

Delgado et al. (2011) and Kolakovsky et al. (1998) stated that high pressure could be a good option to develop novel (new) textures preserving the appearance and keeping intact the sensory profile of cheese.

These results suggest that HP treatments could be really beneficial commercially speaking, as they shorten the proteolysis and improve the texture but without modification of other sensory parameters like flavor and taste which contribute substantially to the typicality of cheese. Moderate HP-treatments seems to be appropriate as they are not very energetically expensive, they are commercially easy to achieve with the equipments nowadays manufactured and will allow reaching the changes in the matrix that we search for obtaining a better water binding and an improvement of the sensory and commercial characteristics of goat's milk cheese. For all that reasons cited above, pressures of 100, 200 and 300 MPa, time of 5 min and temperature of 14 º C were chosen to be applied before and after brining of goat's milk cheeses as the main conditions of the experimental design in this study.

1.2 Objectives

General Aim

The general objective of this study was to improve commercial characteristics of goat's milk cheese applying high-pressure treatments before and after brining during the cheese-making process.

Specific aims

In order to achieve the general objective HP cheeses were compared to control cheeses which were non pressurized chasing the following specific objectives.

- Study the sensory and physico-chemical characteristics of goat's milk cheese induced by high pressure treatments before and after salting.

- Determine the microstructural changes and final texture of the goat's milk cheese high pressure treated before and after salting.

- Evaluate the water holding capacity and the water activity in the goat's milk enzymatic cheese treated by high pressure before and after salting.

- Determine the effect of high-pressure treatments on the salt penetration and salt final content in goat's milk cheese treated before and after salting.

- Evaluate the effect of high-pressure treatments before and after salting on the volatile profile of goat's milk cheese.

1.3 Working plan

According to the objectives, Figures 1.1 and 1.2 schematically represent the experimental design of all the assays performed in the framework of this thesis.

Preliminary cheese-making productions were carried out in order to tune up the cheese making process to specific pressing, brining and ripening conditions to carry out this study (Annex I).

Additionally, the next step included the application of the best conditions obtained to perform three different HP treatments (100, 200 or 300 MPa) at different moments during the cheese making process (before and after brining) on goat's milk cheeses. The goal of this study was to evaluate the changes caused on the resultant goat's milk cheeses referring to its physic-chemical properties, water holding capacity, salt content and salt uptake, their texture and microstructure, their sensory characteristics and their volatile profile. This phase includes the cheese production, HP treatment and the subsequent analyses as compositional, instrumental, proteolysis, microstructural and sensory analyses.

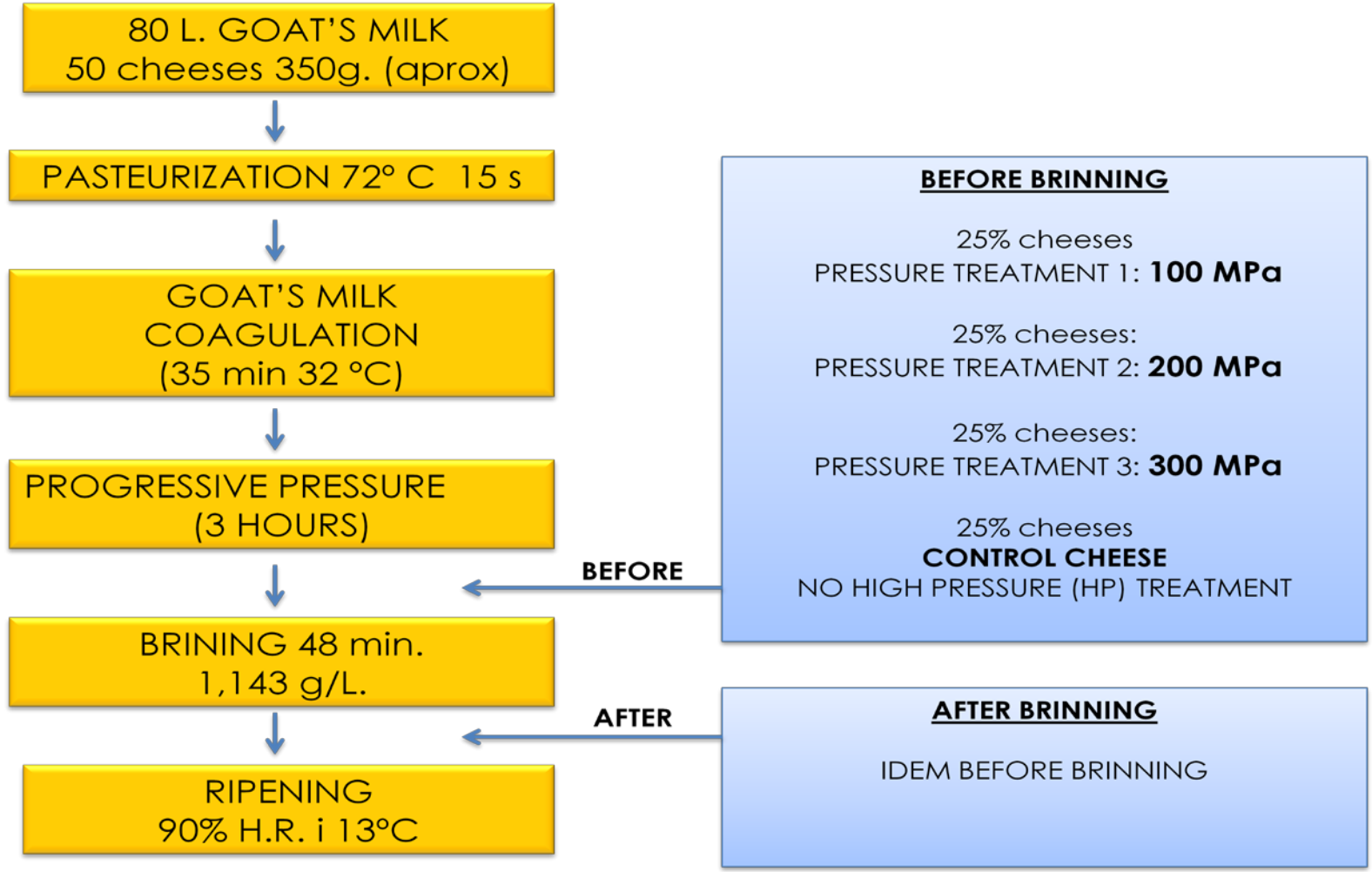

Figure 1.1 Working plan followed in experiments and HP treatment of cheeses

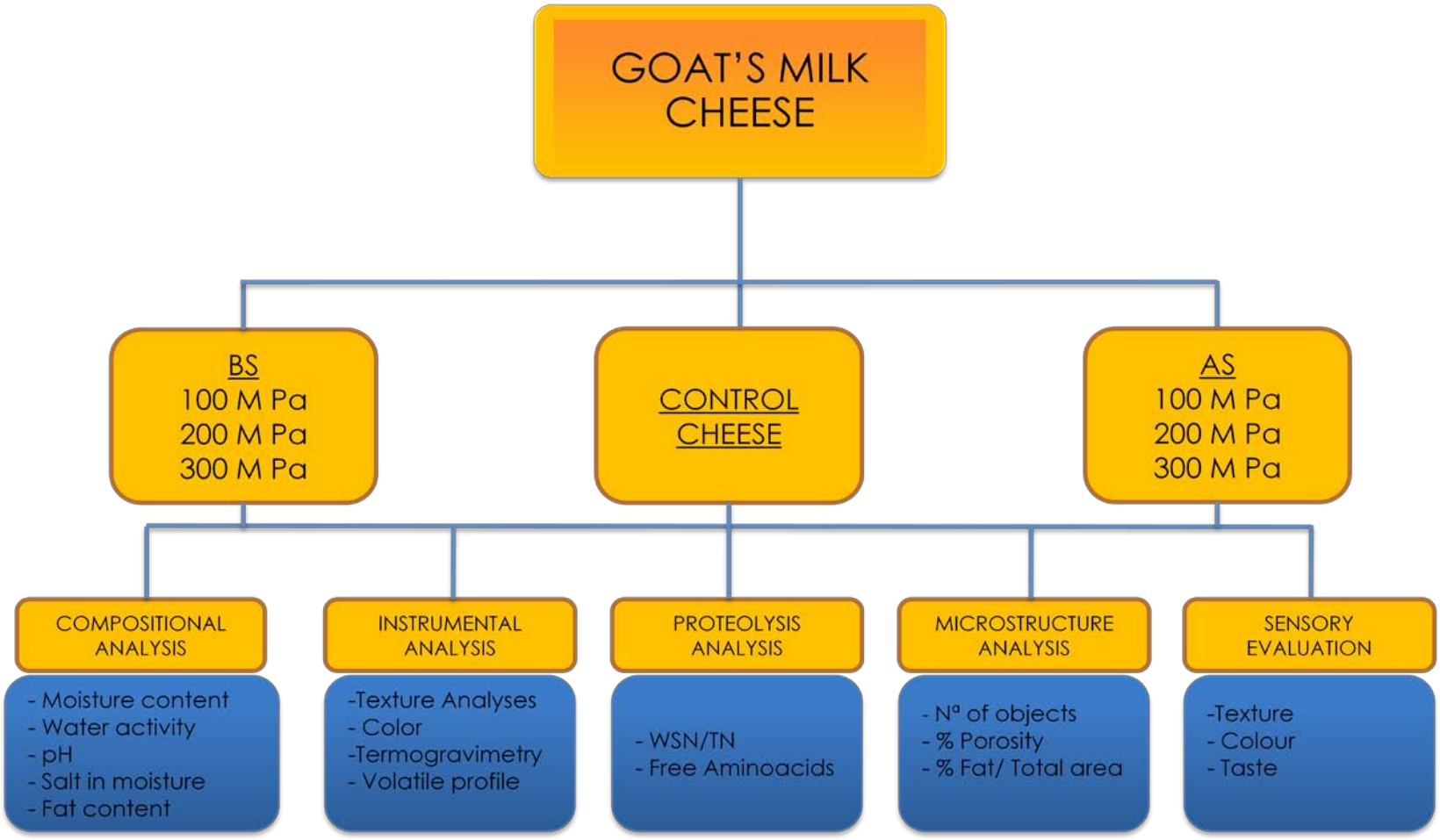

Figure 1.2 Diagram of the procedure followed to evaluate the modifications caused by HP treatments on different parameters of cheese.

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Chapter II

Literature review

Literature review

2.1 Goat's milk and cheese characteristics

2.1.1 Goat's milk and cheese production in the world, Europe and Spain.

Many animals are kept to produce milk for human consumption, among them the most important are cows, buffaloes, sheep, goats and camels. The strong commercial importance of cow's and buffalo's milks have caused them to be studied more extensively than others, such as goat's, sheep's and camel milks (Sturaro et al., 2013). Many researchers have focused on cheeses made using cow milk, and the production and processing of goat milk products have been ignored during years (Hayaloglu, Cakmakci, Brechany, Deegan, & McSweeney, 2007; Hayaloglu, Tolu, Yasar, & Sahingil, 2013). Thus, a lack of technology and research is also a characteristic of dairy products made with goat milk and somehow this fact limits its expansion in the market. In addition, the sensory aspects of goat products, such as a more intense flavor and aroma, also contribute to the low acceptance of these products by some consumers (J -P Dubeuf, de A. Ruiz Morales, & Castel Genis, 2010; J.-P. Dubeuf, Morand-Fehr, & Rubino, 2004; G F W Haenlein, 2004)

However, the growing consumption of dairy products made from goat's milk in recent years has required more knowledge of the raw materials. Cheeses made with goat milk are consumed world-wide and have been associated in recent years with increased goat milk production and demand in numerous countries (Queiroga et al., 2013; Sant'Ana & Bezerril, 2013). In recent years, demand from consumers and the recovery process of goat products sector due to state support policies and European development funds for rural areas development have encouraged the production of small ruminants and goat milk production is particularly encouraged by these aid programs (Hayaloglu et al., 2007, 2013; Poveda, Sánchez-Palomo, Pérez-Coello, & Cabezas, 2008).

Small scale goat production is of significant benefit to families all over the world living in a wide variety of climates and conditions. Worldwide goats are among the most popular and beneficial livestock for several segments of society with very limited resources. In fact, international studies indicate that goat farming is important on economic, cultural and traditional basis, helping the poor and especially women to raise goats. This activity can have a very significant impact on their income, social status and even on the local environment. Women play a major role in small ruminant production being the foremost tasks in small ruminant production milking, cutting and carrying grasses, grazing and mixing fodder (Yilmaz, Demircan, Gul, & Kart, 2014). It is important to note that unregulated and extensive use of pasture areas, bushes and moors for hair goat farming may damage floral ecosystems and thus, appropriately regulated goat grazing is needed and additionally have positive effects on soil erosion and bio-diversity.

The global domestic goat population has been estimated as 862 million. The countries having the highest percentage of goats are China (17.33 %), India (14.59 %), Pakistan (6.58 %), Bangladesh (6.54 %) and Nigeria (6.24 %), respectively (FAO, 2011). Southern European countries account for most of the production of ewe and goat's milk cheeses. Traditional cheese making procedures are strictly followed in some cases and there are also examples of cheese varieties in which they co-exist with modern industrial technology. Spain is the third largest producer of goat's milk in the European Union (EU), with 37.800 tons in 2013 (FAOSTAT, 2013). This rich heritage, dating in some cases from the middle ages, should be maintained for cultural and socio-economic reasons. Farming of goats and transformation of their milk into cheese contribute to the sustainable development of many regions, mostly in the Mediterranean countries.

Cheese production in Spain has increased by more than 14% since year 2000, currently accounting for 225.574 tons (FAOSTAT, 2013). This upward trend is set to continue in the coming years, unlike other European Union countries where both production and consumption of cheese have stabilized. One of the factors behind this increase has been the emergence of numerous artisanal cheese factories, where many producers process milk from their own farms. In some cases, this has enabled the recovery of traditional varieties, but in others, it has contributed to the loss of the original identity of cheeses.

The cheese making tradition in Spain is reflected in the existence of more than 100 different varieties of cheese, many of which are certified as being of distinctive quality through either the existing 26 Protected Designation of Origin (PDO) or 2 Protected Geographic Indication (PGI). The manufacture of many ewe's and goat's milk cheeses is regulated by these quality labels at a national level, established mainly in the Mediterranean countries to define and protect traditional products against imitations (Medina & Nuñez, 2004). This is of particular relevance in Spain, where a large tradition of goat's milk cheeses and consumption exist. These quality designations came into being as strategies to protect original and characteristic regional products from imitation and consequent fraudulent market competition (Diezhandino, Fernández, González, McSweeney, & Fresno, 2015). Some PDO cheeses are manufactured with goat's milk such as PDO Queso de Ibores, PDO Palmero, PDO Majorero, PDO Queso de Murcia and Queso de Murcia al vino, or with their mixtures, such as PDO Cabrales and PDO Gamoneu. There are also numerous types of goat's cheese that not account for a PDO but still very popular and with a high consumption in Spain like Sierra de Grazalema cheese, Queso de Benabarre, Gredos, Tiétar o La Vera, Cendrat del Montsec, Valdeteja, Armada y Cameros or Garrotxa cheese, which is about to obtain a PGI (Medina & Nuñez, 2004; Nicolau, Buffa, O'Callaghan, D. Guamis, & Castillo, 2015).

2.1.2 Goat's milk composition, nutritional benefits and coagulation properties.

Goat's milk cheeses have special tastes and flavors, very distinct from those of cheeses made from cow's milk. Genetic, physiological and environmental factors are responsible for variations in milk composition even within a single species. Further elements of variability are linked to flock management, climate, altitude or the botanical composition of pasture. Compositional differences of goat's milk respect to the cow's milk, mainly in proteins and fat,

account for differences in the sensory characteristics of the cheeses, as well. Thus, the influence of lactation stage, feeding regime, breeding conditions and milking system on the composition of goat's milk has been dealt with in numerous studies (George F W Haenlein, Park, Raynal-Ljutovac, & Pirisi, 2007; Juarez & Ramos, 1984; Morand-Fehr, Fedele, Decandia, & Le Frileux, 2007).

There is increasing research interest in goat milk due to inherent species-specific biochemical properties that contribute to its nutritional and sensory quality (Strzalkowska, Markiewicz-Keszycka, Bagnicka, Polawska, & Krzyzewski, 2013; Yang, Ding, Ma, & Jia, 2015). Additionally, these features are largely related to the chemical composition of goat milk, since proteins and fat are of fundamental importance, due to their contribution to the yield, flavor and sensory features of dairy products.

Milk proteins consist of caseins and whey proteins alpha-lactalbumin and beta-lactoglobulin (α-LA and β-LG). The average percentage of the four casein fractions (α_{s1}, α_{s2}, β and K) varies, and this influences the cheese making ability of the milk. Goat's milk protein fraction, consists of proteins with high biological value and lower allergenic potential. These characteristics are related to the quantity and structural differences of whey proteins (α-LA and β-LG) and to small-diameter of their fat globules, which allow for higher digestibility compared with cow milk. Many of the most distinctive properties of goat milk derive from its lipid fraction, being this additionally affected by several external factors such as season or feeding practices which can change the fat content and the fatty acid profile. Structural differences in triglycerides of goat's milk fat can affect the flavor of the produced cheese. For instance, goat milk contains minor branched chains, which have very low flavor threshold values (Scintu & Piredda, 2007). In addition, goat milk harbors a higher mineral content (calcium, iron, zinc and magnesium) a higher vitamin content (A and B complexes), which characterize it as a highly nutritious food for consumers (Morand-Fehr et al., 2007; Park, 2007; Sant'Ana & Bezerril, 2013). Goat milk has been reported to have higher digestibility and lower allergenic properties than cow milk, and also have certain therapeutic values, which makes it attractive to consumers (G F W Haenlein, 2004; Park, Juarez, Ramos, & Haenlein, 2007).

It is common knowledge that the composition of goat's milk casein is the main factor responsible for its technological limitations. Caprine casein contains a lower proportion of αs-casein, especially αs1-casein, higher degree of casein micelle dispersion and a higher proportion of β-casein than bovine casein. Thus, the renneting kinetics of goat's milk, conditioned by protein composition, is different from cow milk, and can explain the shorter coagulation time and the poor gelation properties. Previous studies have shown that this problem is closely related to the protein fraction of goat's milk. Differences in casein micelle composition, size, hydration, the mineral concentration, the mineral distribution in soluble phase and colloidal phase between two species can highly affect coagulation properties of goat's milk (Raynal-Ljutovac, Gaborit, & Lauret, 2005; Remeuf, Verdaletguzman, & Lenoir, 1995; Zhao et al., 2014). Therefore cheesemaking from goat's milk with a low αs1-casein content results in a less firm curd and lower protein retention and cheese yield that when milk of a high αs1-casein content is used (Medina & Nuñez, 2004).

The coagulation ability of goat milk is so poor that the gel has less hardness being more fragile and accounting for a higher whey separation than cow milk. Poor coagulation properties can lead to more particle loss in whey, lower cheese yield and less textural integrity. There are few types of goat milk cheeses than cow's milk cheeses, and most cheeses made from goat milk fall into the group of fresh or white unripened cheeses and soft cheeses. This is mostly related to the poor mechanical properties of goat milk curd, which is generally too soft to resist the applied mechanical forces during curd treatment in semi-hard and hard cheese manufacture (Medina & Nuñez, 2004).

The weaker mechanical properties of goat's milk curd constrain the manufacturing procedures used in goat's milk cheesemaking and limit the diversity of cheese types. Most cheeses made from goat's milk fall into the following groups according to Medina and Nuñez (2004):

- Fresh or white unripened cheeses, with a low DM content, usually less than 25%
- Soft cheeses, traditionally made from predominantly lactic curd, of small size, cylindrical or pyramidal in shape, and generally with mould growth or ash on the surface
- Semi-hard or hard cheeses, made from predominantly rennet-coagulated curd, or larger size than the soft cheeses, flat cylindrical-shaped and dry rind.

Although little research has been carried out on improving technological aptitudes of goat milk for cheese making or sensory characteristics of the end product, some studies appear in the scientific literature encompassing different topics (Table 2.1).

Multiple efforts have been done on evaluating the effect of feeding goats with several plants such as thyme, rosemary and artichoke with the goal of inhibit the lipids oxidation and as an strategy to reduce feeding costs and to take advantage of the waste from other industries (Boutoial, Ferrandini, Rovira, García, & López, 2013; Boutoial, Garcia, et al., 2013; Jaramillo Bustos, Valderrama, & Trujillo Mesa, 2013; Jaramillo et al., 2010). Another important feature of study has been the Somatic Cellules Count (SCC) on goat milk and its possible effect on technological aspects of milk such as production yield, total microbial count, milk physic-chemical composition, cheese making and the renneting properties of goat milk (Pazzola et al., 2012; Raynal-Ljutovac et al., 2005; Raynal-Ljutovac, Pirisi, de Crémoux, & Gonzalo, 2007). Several methods have been used to promote a stronger gel in goat curd and to avoid an excess of syneresis. Addition of whey protein concentrate (WPC) could enhance curd firmness and reduce syneresis. Other physical methods including heat treatment, high pressure treatment and ultrasound treatment have been applied to cow's milk to improve curd properties, but rarely to goat milk (Zhao et al., 2014).

As it is shown in Table 1 other studies were raised with regard to improve the specificity of the coagulant action. Even the most common rennet used is chymosin, recent characterizations of chymosins from different mammalian species like camel, goat, lamb, and buffalo were carried out. In respect to plant rennets, the only one effectively used in cheese making is obtained from the flower of *Cynara cardunculus*, demonstrating the high suitability of plant rennet for the production of sheep and goat cheeses. One of the most significant differences between cardoon and animal coagulants is the higher levels of the ripening index produced by the former (Esteves, Lucey, & Pires, 2002; Lucey, Johnson, & Horne, 2003; Roseiro, Barbosa, Ames,

& Wilbey, 2003) which could positively contribute to influence final goat cheese characteristics like flavor and/or texture (Almeida, Gomes, Faro, & Simoes, 2015). Microbial coagulants were as well studied and compared to plant coagulants being the former pointed as causing faster clotting (Garcia et al., 2012).

Table2.1. Strategies performed on milk coagulation and curd quality

Cheese variety	Encompassed topic	Goal-Impact	Reference
Goat, ewe and cow milk cheese	75-90 ºC Heating of milk 10 to 30 min	Increase of micelle size of 25 %, coagulation was slowed and curd whey draining was highly reduced in cow but less in goat.	Raynal-Ljutovac et al., 1998
Skimm milk powder	Comparision of animal-vegetal coagulation properties	Storage modulus *(G'), l*oss tangent *(tan* δ*)*and yield stress were higher to chymosin-induced gels than those of plant coagulants. Plant coagulants were slightly more proteolytic than chymosin gels and the former reached lower gel firmness.	Esteves et al., 2002
Imitation cheese	Blade speed	Increasing the blade speed led to hardness increase and decrease of the meltability	Noronha et al. 2008
Munster type cheese	Temperature (8, 12 and 16 ºC) and Relative Humidity (85, 93 adn 99 %)	Cheese weight loss was more influenced by the humidity than by temperature of ripening chamber.	Riahi et al. 2007
Goat's and ewe's milk	Somatic Celulles Count	High SCC was related to loss of yield, cheese making aptitude and to milk composition changes. Renneting properties were no affected by SCC. However SCC level was highly correlated with pH and protein content	Raynal-Ljutovac et al., 2007 and 2005; Pazzola et al. 2012
Ewe's semihard cheese	Artichoke Feeding	Decrease of fat and total free fatty acids content but high overall sensory scores compared with control cheese	Jaramillo et al., 2010 and 2013
	Supplemetation of pasture-fed ruminants	Improvement of the free fatty acids profile and enhancement of the level of minerals and vitamines	
Fresh cheese	Comparision of microbial-vegetal coagulation properties	Highest clotting time in microbial coagulant. Colour CIELab, protein and fat, cohesiveness and springiness, no differences were observed. However, dry matter content, hardness, gumminess and chewiness showed higher values being obtained in cheeses made with vegetable coagulants	Garcia et al., 2012
Goat milk gels	Ultrasounds	Reduced particle size in treated samples compared with control gels and increase of gel firmness, coagulum strength, final storage modulus, cohesiveness, water holding capacity and cross-linking of gels. Improvement of goat milk coagulation properties.	Zhao et al., 2014
Goat, cow and ewe's cheeses	Development of a new cardosin B-derived rennet	The secreted enzyme displays similar proteolytic properties, such as casein digestion profiles as well as optimum pH (pH 4.5) compared with those of native cardosin B	Almeida et al., 2015

Skeie (2014) has summarize technological aspects that could affect goat's milk properties concerning the cheese making such as genetic issues affecting polymorphisms in goat milk caseins (K and β), the presence of α_{s1}-CN in goat milk, health status of the herd, effect of lactation stage, the content of Free Fatty Acids (FFA) and the treatment of milk for cheese making. Strategies for optimizing these issues to improve goat milk curd are also mentioned. Thus, there are relationships among the characteristics of the composition of milk, the technology used, and the final products obtained that there are worth studying.

2.1.3 Goat cheese ripening characteristics

It is generally accepted that some modifications during semi hard goat's cheese making and ripening are needed to enhance the end product. In cheese manufacture, the process of whey being expelled out of curd is called syneresis. The rate and extent of syneresis strongly affects mechanical handling during the subsequent cheese-making steps, loss of fat and protein in whey, cheese moisture, ongoing acidification, and proteolysis, and therefore strongly influences cheese composition and quality (Everard et al., 2008; Lu & McMahon, 2015; Mellema, Walstra, van Opheusden, & van Vliet, 2002). Ercili-Cura et al. (2013) stated that the proneness of a gel network to spontaneous syneresis is related to the extent of network rearrangements during gel formation leading to contraction of the protein network. High rate of rearrangements lead to inhomogeneous network due to increasing size of the building blocks occurring large pores. Larger pores reveal increased propensity of a gel to show syneresis. The author observed that after microstructural evaluation, a reduction in syneresis correlated with an increase in the homogeneity of the gel network (decrease in size of clusters forming the network) and a decrease in pore size.

The ripening is the process through which specific characteristics of each cheese arise and confer to it its unique personality. The composition of the cheese matrix and its properties depend on physicochemical conditions developed during ripening. The biochemical reactions involved into ripening process are glycolysis, proteolysis and lipolysis affecting most constituents of cheese matrix, which are found in different physical state, namely as a solid matrix (paracasein), as a liquid phase (related with residual whey) or others such as fat, either solid or liquid according to cheese technology and temperature (Pierre, Michel, Le Graet, & Berrier, 1999). These biochemical reactions that take place during ripening predominantly proceed in the aqueous liquid fraction of the cheese, and water is often involved in their reactions (e.g. hydrolysis). Thus, characterization of a cheese by means of evaluation of its water types and the spatial organization of its constituents, would be helpful to understand and know better its microstructure, final characteristics and hence, the commercial behavior of cheese.

Many factors influence cheese ripening and many factors are described as influencing water content during ripening in cheese. Abellán et al. (2012) pointed out differences in most of the parameters studied during ripening concerning the size of goat cheese Murcia al Vino cheese. A chain of events took place in different sizes goat's cheeses during ripening. Cheeses of bigger size resulted in higher water content, which led to improved level of proteolysis and consequently of large peptides. Nevertheless, the concentration of aminoacids was not

affected by the size of cheese. Smaller cheeses showed higher amount of total solids, thus causing a shortening of marketing time of small cheeses due to their faster drying.

Water and salt have been described as playing a major role on the adequate ripening of cheese (Simal, Sanchez, Bon, Femenia, & Rossello, 2001). If cheese starts the ripening process with a non-adequate amount of salt and water, some strange fermentation could occur and the evolution of ripening could be negative in terms of sensory characteristics, such as, undesirable mouth-feel, texture or taste. Thus, is of main importance an appropriate control of salt and water content (Pandey, Ramaswamy, & St-Gelais, 2000). Other biochemical processes such as proteolytic breakdown of the casein matrix, may be an essential factor in the development of the cheese texture and flavor. These parameters and cheese proteolysis extent have been studied extensively. In contrast, much less is known about the state of water and its soluble components in cheese (M. R. Guo & Kindsted, 1995).

The water evaporation from the cheese surface during ripening generates the transfer of water from the core towards the surface by molecular diffusion. The best solutions found in scientific literature to predict water migration min cheese were obtained when diffusivities were made functions of porosity and salt concentration. Thus, a high correlation between the cheese mechanical consistency (state of the casein network) and the water retention capacity has been found by most of authors. In this sense, structural modifications in casein network of goat's milk cheeses could exhibit changes in ripening of cheese, leading to better water binding and restructuration of its water internal profiles.

2.2 WATER HOLDING CAPACITY OF CHEESE

2.2.1 Types of water existing and the spatial location of water in cheese

The total amount of moisture in a product is defined as the moisture content and it is expressed as a percentage of the total. Another factor to describe the availability of water in cheese could be the water activity (Aw) and it is described as the ratio between the vapor pressure of the food itself, when in a completely undisturbed balance with the surrounding air media, and the vapor pressure of distilled water under identical conditions. Nevertheless, water exists in various states in a material such as cheese and they can be described in terms of the spatial relationship between water and the solid constituents of the food (in cheese, these solids are predominantly protein although water interactions also exist between other cheese constituents). Water is believed to exist referred to as free (with mobility) and bound water (without mobility). The amount of water held by the food product, under a specific set of conditions, is traditionally referred to as the water-holding or water-binding capacity of the material.

The generalized moisture sorption isotherm for a hypothetical food system may be divided in three main regions. The first one represents strongly bound water with an enthalpy of vaporization considerably higher than that of pure water. This kind of water represents the first layer of water molecules (monolayer). Usually, water molecules in this region are unfreezable and are not available for chemical reactions. The second region represents water

molecules, which are less firmly bound, initially as multilayer's above the monolayer. In this area, water is held in the solid matrix by capillary condensation and impeded by the macrostructure of the protein matrix. This water is available as a solvent for low-molecular-weight solutes and for some biochemical reactions and is called entrapped water. The third area is the excess water present in macro-capillaries or as a part of the fluid phase in high moisture materials. This water exhibits nearly all the properties of bulk or free water and thus is capable of acting as a solvent being more loosely associated with the proteins even though it retains a large solvent capacity and it is freezable at -40 ºC. Microbial growth becomes a major deteriorative reaction in this region (Al-Muhtaseb AH, McMinn WAM, 2002). Thus, the distribution and movement of water in cheese may be characterized as being either bound to proteins, entrapped by those proteins, or expressible by centrifugation (McMahon, Fife, & Oberg, 1999).

2.2.2 Analytical methods used for measuring water-holding capacity of cheese

Several analytical techniques have been used to determine changes of phase during water loss, or mobility changes of water on foods, such as Differential Scanning Calorimetry (DSC), Nuclear Magnetic Resonance (NMR), capacitance measurement or Thermogravimetry (TGA), among others (Kneifel, Abert, Sendai, & Seiler, 1992; Kneifel, Paquin, Abert, & Richard, 1991)

Physico-chemical properties of dairy-food products can be studied by analytical tools such as Diferential Scanning Calorimetry (DSC). DSC has been used to measure macroscopic data such as the amount of unfreezable water in milk or in caseins (Ruegg, Luscher, & Blanc, 1974) or to characterize milk fat thermal properties, being a useful technique for determining the temperature of final melting and initial crystallization of fat or even to examine the thermal stability of ovine B-LG and the kinetic parameters of their heat denaturation (Calavia & Burgos, 1998; Lopez, Briard-Bion, Camier, & Gassi, 2006; Lopez, Lavigne, Lesieur, Bourgaux, & Ollivon, 2001).

Being the NMR a non-invasive method for cheese quality control, is useful to study the gross microstructure, including fissures and holes (Everett & Auty, 2008) providing the possibility of measure water mobility, diffusion of water molecules or consequences of freezing the bulk cheese. Le Dean (2001) characterized the relative influence of milk components on water using NMR and DSC. The spectroscopic technique of pulsed nuclear magnetic resonance is a promising technique for investigating the behaviour of water in foods It provides rapid, sensitive, noninvasive determination of non only the quantity of water present, but also the structure and dynamic characteristic of water in foods (Kuo, Gunasekaran, Johnson, & Chen, 2001; Padua, Richardson, & Steinberg, 1991).

Thermal gravimetric analysis (TGA) has been used to study kinetics of water loss in cheese due to its ability to measure devolatilization (or mass loss) history of a small cheese sample under conditions of minimal mass transfer limitation, especially under fast purging that sweeps off the volatiles from the sample surface (Shi, Liu, Guo, He, & Liu, 2014). Thermoanalytical analyses allow quantifying different types of water present in the cheese system and provide detailed information about the interactions between them (Buffa, Guamis, Saldo, & Trujillo,

2003; Jordi Saldo, Sendra, & Guamis, 2002). Water in cheese is bounded to the matrix at different levels, so when the matrix is heated, the water is lost in successive stages and at different temperatures depending on the activation energy required to break the bounds that link it to the matrix (Curtis et al., 1999). Each change of phase occurs at a specific temperature and corresponds to a water typology. Free water seems to be released at temperatures of 115 ºC, entrapped water at 120 ºC and finally bound water is released at temperatures around 130 ºC (Zamora, Ferragut, Juan, Guamis, & Trujillo, 2011). Results can be expressed by the weight loss by sample on each of these stages.

Thermogravimetric techniques represent a simple and rapid method for determining water content and for following its behavior during the various stages of ripening of cheese. Thermogravimetrical techniques provide detailed information on the cooperative activity of all the various water-matrix interactions and allow all the different water types present in a system to be accounted for. Different types of water include those with different energies of bonding to the matrix. Thermogravimetric measures can be used to make a detailed analysis of the water contained in the matrix, making both qualitative and quantitative distinctions among the various different types of water. It has been demonstrated that water can be bonded to the matrix with different energies and so, when the matrix is heated, the water is lost in successive stages, depending on the amount of activation energy required to break the bonds formed between the water and the matrix (Curtis et al., 1999).

Dielectric properties evaluation provides information about the response of materials to electromagnetic fields, thus, it is a convenient method for evaluating the food quality, especially for detecting the food moisture content. As concerning the dairy products, the studies about the dielectric properties and quality characteristics are relatively scarce. The dielectric measurement techniques have been used principally in the analysis of cheese composition and maturity (Velazquez-Varela, Fito, & Castro-Giraldez, 2014).

Recently, the rind percentage, ripening and moisture content of grated Parmigiano Reggiano cheese was predicted by means of waveguide spectroscopy measuring the capacitance of these samples. In addition differences between true Parmigiano Reggiano cheese and competitors were determined (Cevoli et al., 2015; Cevoli, Ragni, Gori, Berardinelli, & Caboni, 2012).

2.3 SALT CONTENT OF CHEESE

2.3.1 The role of salt on cheese and its effect on texture, microstructure, proteolysis and flavor

Cheese salting is one of the most important steps in cheese manufacture and the concentration of salt in cheese is considered one of the main physico-chemical quality attributes on cheese, which in turn will play a key role in the taste, flavor and texture of cheese.

Most natural cheese varieties contain added salt (Na^+ Cl^-). Salt is added to the curd in three different moments during manufacture. It may be: added to subdivided cheese curds

(cheddaring), applied by immersion of the formed cheese in brine (concentrated aqueous solution of Na^+ Cl^-), as in most of hard and semi-hard cheeses, or rubbed onto the surface after molding is complete, as in some Blue cheeses and ripened acid curd cheeses. The presence of salt in the cheese and the manner of its incorporation have a significant impact on the course of fermentation of lactose to lactic acid and on the microbiology, biochemistry, and final characteristics of the cheese (T.P. Guinee & Sutherland, 2011).

The use of salt in food technology can be explained through three general categories: processing, sensory (taste), and preservation (Arboatti et al., 2014; Man, 2007). In processing salt has an important role during the manufacture process largely affecting water content of cheeses which in turn modifies the enzyme activity of cheese, its proteolysis and hence, its texture and microstructure. In regards to sensory characteristics, salt confers its own specific flavor and modifies the flavor of other ingredients contributing to the overall aroma of cheese. Additionally, salt has a direct effect on water activity diminishing availability of water for microorganisms to growth. Consequently, it regulates the activity of starter culture microorganisms, retarding the growth of most bacteria and acting as a preservation agent on cheese (Arboatti et al., 2014; T.P. Guinee & Sutherland, 2011; Man, 2007; B M Paulson, McMahon, & Oberg, 1998). Therefore, water activity is influenced as well by the effect of Na Cl. (Winy Messens, Dewettinck, & Huyghebaert, 1999).

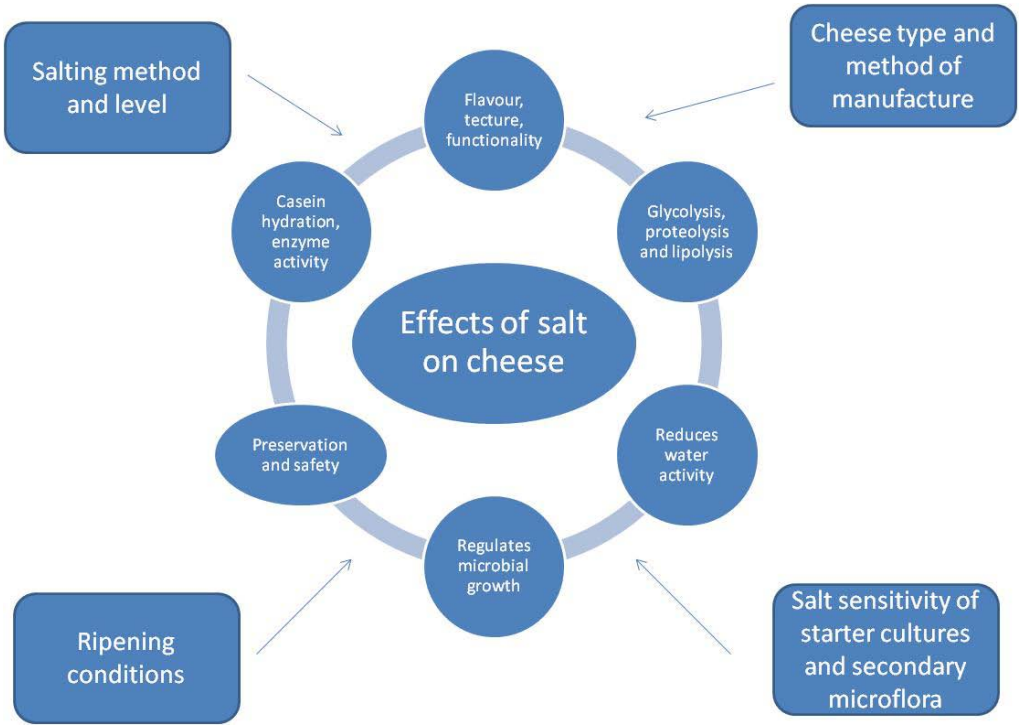

Figure 2.1. Roles of salt on cheese and external and internal factors affecting the content of salt on cheese. (adapted from Guinee and Sutherland 2011).

Among the main effects of salt in cheese, the concentration and distribution of salt have a major influence on various aspects of cheese quality including physical changes and texture(Figure 2.1). Numerous investigators have studied the effects of salt concentration, or

salt-in-moisture in rheological or textural parameters such as firmness, fracture stress and fracture strain. These studies have shown that increases in S/M within the range 0.4 - 12% resulted in increased firmness and fracture stress and sensory hardness for several types of cheese including Cheddar (Schroeder, Bodyfelt, Wyatt, & MC Daniel, 1988; Thakur, Kirk, & Hedrick, 1975), Gaziantep (Kaya, Kaya, & Oner, 1999) and Muenster (Pastorino, Hansen, & McMahon, 2003). On the other hand, the increase in strain may be attributed in part to the concomitant changes in composition such as reduction in moisture level and the effects of salt on proteolysis. Changes in the degree of protein hydration can be explained by the salting-inn salting-out effect, which cause subsequently alterations of the ratio viscous to elastic character on cheese. An increase in para-casein hydration, due to a salting in effect at S/M levels below 5 % occurs while at higher S/M values, due to the salting out effect, concomitant loss in casein hydration takes place. A higher degree in casein hydration would favor a more viscous character to the cheese showing higher strain in the fracture point. Conversely, a decrease of casein hydration at higher S/M values would impart shorter, firmer and a high brittleness cheese (T P Guinee, 2002; T.P. Guinee & Fox, 2004; T.P. Guinee & Sutherland, 2011).

The salt content of cheese may also affect cheese structure. Since salt also exerts more direct effects promoting changes in the degree of casein hydration and aggregation, it is capable to alter the ratio of viscous to elastic character in the cheese (T.P. Guinee & Fox, 2004). Guo et al. (1997) concluded that Na^+ Cl^- in the serum phase of Mozarella cheese promotes microstructural swelling, a concomitant increase in water-holding capacity and the solubilisation of intact caseins from the para-casein matrix. Increased salt content of cheese would promote solubilization of caseins causing the protein matrix to become more hydrated and to swell (L. Guo, Van Hekken, Tomasula, Tunick, & Huo, 2012; M. R. Guo & Kindsted, 1995; Brian M. Paulson, Mcmahon, & Oberg, 1998). Furthermore, Paulson et al. (1998) showed by scanning electron microscopy that unsalted cheeses had larger open channels with free serum (whey pockets) than the salted counterparts. As consequence of protein hydration promoted by salt addition, voluminosity of the cheese matrix also increases and distance between proteins in cheeses decreases which in turn, would enhance protein-protein interactions.

The other contribution of salt in cheese characteristics is the enhancing of sensory properties by means of salty taste. An appropriate salt level in cheese would improve the cheese flavor development as a result of combination of several compounds present in the correct ratio and concentrations and impair off-flavors as bitterness probably caused by undesirable fermentations (Hassan, Gawad, & Enab, 2013). Considered as a flavor enhancer, salt not only acts on salty perception but also on total flavor perception, which makes especially important to reach the optimum balance of salt content. It is widely known that at a given quantity of salt content, some enzymatic reactions in cheese are impeded due to the concomitant reduction of moisture content. As the main agents of lactose, lipid and protein catabolism are enzymes, and these are the aroma formation pathways, salt content in cheese could vastly influence on flavor development. Several strategies have been followed in the last years to reduce the salt content in cheese as excessive intake of sodium has been associated with harmful effects on human health and cheese has been pointed out as one of food products which mostly contribute to the daily salt intake in human diet (Arboatti et al., 2014; Floury et al., 2009; Lu & McMahon, 2015; Rulikowska et al., 2013).

2.3.2 Salt uptake and moisture loss during brining of cheese

When a cheese is placed in brine, salt and moisture gradients develop from the surface to the centre of the cheese (Luna & Chavez, 1992). It is generally considered that a net movement of the ions Na^+ Cl^- from the brine into the cheese, as a consequence of the concentration difference between the cheese moisture and the brine, takes place. At the same time, water from the curd interior moves to the surface because of osmosis (T.P. Guinee & Fox, 2004; Simal et al., 2001). Due to the principles of molecular transport a certain time is required in order to achieve an uniform salt distribution, which in turn will be function of cheese size, among other parameters (Gomes, Vieira, & Malcata, 1998).

The absorption of salt from the brine solution by cheese during brining, and the simultaneously water loss, result in reduction of cheese mass because the quantity of the salt absorbed is less than the water lost (Winy Messens, Dewettinck, et al., 1999). As salt from brine, and moisture from cheese, migrate in opposite directions during diffusion, salt uptake by cheese during the brine-salting process is accompanied by a simultaneous moisture loss. Consequently, there is an inverse relation-ship between the levels of salt and moisture in cheese. This is most readily observed in brine-salted cheeses immediately after salting, where a decreasing salt gradient from surface to the center is accompanied by a decreasing moisture gradient in the opposite direction. Furthermore, the quantity of moisture lost by cheese is related to the quantity of salt absorbed. The average over the whole region of salt and water diffusion in cheese has been found to be aprox. 2, indicating that the weight of water lost during brine salting is typically twice the weight of salt absorbed. This trend is consistent with the fact that the size of the diffusing hydrated Na^+ / Cl^- ion pair is approximately twice that of H3O / OH (T.P. Guinee & Sutherland, 2011).

Inverse correlation between moisture and salt content in cheese has been widely reported in literature. Generally, salting of cheese curd promotes syneresis and results in a decreased moisture level (Kindstedt, Larose, Gilmore, & Davis, 1996; Lu & McMahon, 2015; Pastorino et al., 2003). Based on previous studies, it is discernible the strong influence that salt exerts on moisture content of cheese. If the aim is an uniform ripening and optimum water content to be achieved, an even distribution of salt needs to be ensured on cheese during ripening.

2.4 HIGH-PRESSURE TREATMENTS APPLIED TO CHEESE

2.4.1 Description of the technology

High-pressure (HP) treatment is a non thermal technology, which generated effects do not rely on temperature, such as microbial inactivation or enzyme denaturation. HP is still being developed and applied as a minimal handling process for the production of a wide variety of safe and nutritious foods. The non-thermal pasteurization effect of high pressure on foods has been known since the 19th Century, when one of the first scientific reports on HP applications for food was written by Hite (1899) on shelf-life extension of milk. However, it was not until the 1990´s that the first HP products were developed. The first commercial HP products

appeared on the market in 1991 in Japan, where HPP is now being used commercially for products such as jams, sauces, fruit juices, rice cakes and desserts.

The potential application of HP in the food industry has gained popularity in recent years, due to developments in the construction of HP equipment which makes the technology more affordable. Since year 2000, high pressure processing has been successfully implemented in all type of food industries worldwide.

HP involves exposing a product to extremely high pressures in the range of 100 to 1000 MPa for a given time, although pressures used in commercial systems commonly range between 400 and 600 MPa and temperatures lower than 45ºC. Regarding to the time of treatments, for most commercial applications, products are pressurized for less than 10 min. HP effect is independent on the size or shape of the product (Hoover, Metrick, Papineau, Farkas, & Knorr, 1989; D Knorr, 1993), since pressure is applied isostatically (equally in all directions), and instantaneously, so solids foods retain their original shape.

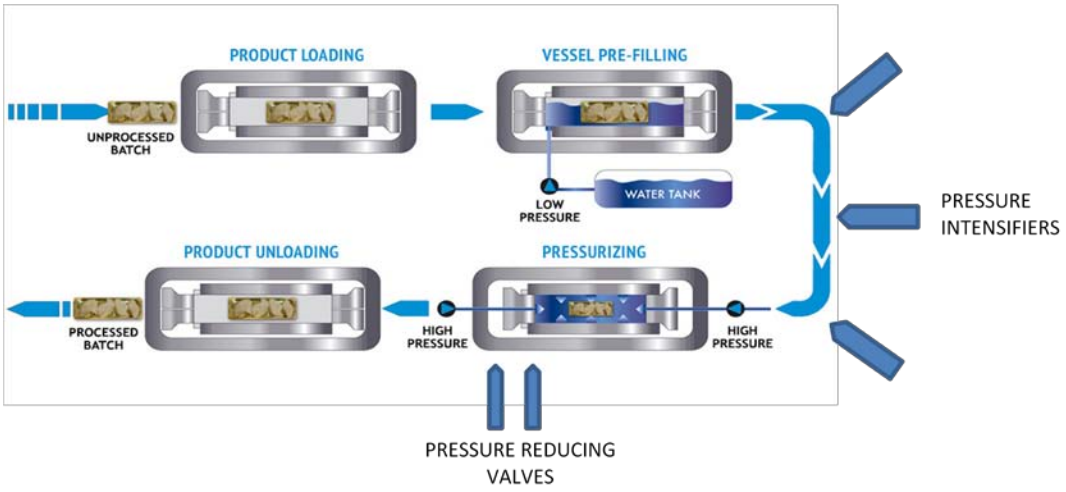

Figure 2.2. Outline of high-pressure processing on a commercial system (adapted from NC-Hyperbaric).

The industrial vessel capacity of HP equipments ranges from 35 to 525 L(Dietrich Knorr, Heinz, & Buckow, 2006). The HP treatments of foods at an industrial level are currently conducted in batch or semi-continuous systems of vertical or horizontal design. Packaged food is loaded into the pressure vessel of the equipment, the vessel is sealed, and water is pumped into the vessel to displace the air. The pumps start to inject the transmission fluid and bring the vessel to pressure process conditions. When the vessel is full, the pressure valve is closed, and water is pumped into the vessel until the process pressure is reached. Once the pressure into the vessel is maintained for the set time and the process time is completed, the pressure valve is opened and the water used for compression is allowed to expand and return to atmospheric pressure (Figure 2.2). Pressure is transmitted rapidly and uniformly throughout the fluid to the food non affecting the shape of food product being the pressure applied isostically. HP acts instantaneously and uniformly throughout a mass of food independent of size, shape, and food composition. Thus, package size, shape, and composition are not factors in process

determination. The work of compression during high-pressure treatment will increase the temperature of foods through adiabatic heating approximately 3 °C per 100 MPa, depending on the composition of the food. Following steps should be decompressing the vessel and removing the product. Chemical changes in the food generally will be a function of the process temperature and time selected in conjunction with the pressure treatment (FDA, 2014).

One of the main goals followed by HP application is food preservation. HP is capable to inactivate pathogens and reduce spoilage microorganisms' counts by means of pressure. Additionally, HP retains the most food nutrients and improves the food preservation with a slightly or non-existent effect on sensory quality of the HP-treated products (Delgado, González-Crespo, Cava, & Ramírez, 2012; Nguyen et al., 2010). Thus, some potential advantages of high pressure treated food are that products demonstrate better retention of nutrients, flavor, and color. One of the widest valuated advantages of high pressure is that many of the components responsible for the sensory and nutritional quality of foods, including flavor compounds and vitamins, are not damaged by high pressure treatment at the levels used in food processing (Patterson, 2005a).

HP technology can offer a high retention of sensory and nutritional attributes of food products, because the treatment can be performed near room temperature, while ensuring safety and stability during refrigerated storage. Therefore, final products with higher quality than those produced by conventional heat treatments can be obtained (Devi, Buckow, Hemar, & Kasapis, 2013a).

Although HP of food was initially developed to retain nutritional and sensorial aspects while ensuring safety and stability issues of perishable food products, this technology has attracted attention for modifying macromolecules arrangements such as protein denaturation, starch gelatinization, or other interactions between food ingredients. Tailoring the functional properties of food systems requires careful consideration of the processing variables in order to obtain the desired characteristics (Devi, Buckow, Hemar, & Kasapis, 2013b). Applying HP to food products result in modifications of interactions between individual components, rates of enzymatic reactions and inactivation of micro-organisms. Application of HP to milk has been shown to modify its gel forming characteristics as well as reducing its microbial load. HP offers as well the potential to induce similar effects to those generated by heat on milk protein.

2.4.2 Main applications and strategies of HP on cheese

HP studies on cheese have followed different goals mainly focused on the reduction of microbial counts, evaluation of cheese ripening concerning compositional, textural, microstructural and flavor characteristics of cheese and the study of the effect of HP on brining and on overall sensory characteristics of cheese.

HP is a useful tool to inactivate microorganisms as it induces changes in the cell membrane and wall of the microorganisms (Hoover et al., 1989). Due to the special attention focused on the safety of the food supply, many studies have dealt with that issue (Delgado et al., 2012; Martinez-Rodriguez et al., 2012a; C. O'Reilly & Kelly, 2001; Antonio J. Trujillo, Capellas, Saldo,

Gervilla, & Guamis, 2002). Some authors have been working on a wide range of microorganisms and others on specific spoilage bacteria, moulds and yeasts. Synergistic effect between pressure and microbial compounds like bacteriocines of LAB have been also evaluated (Arques, Garde, Gaya, Medina, & Nunez, 2006; Capellas, Mor-Mur, Gervilla, Yuste, & Guamis, 2000; Considine, Sleator, Kelly, Fitzgerald, & Hill, 2011; Daryaei, Coventry, Versteeg, & Sherkat, 2006; Lopez-Pedemonte, Roig-Sagues, De Lamo, Hernandez-Herrero, & Guamis, 2007). Many relevant factors, which have been also reported in the scientific literature account for HP inactivation of microorganisms. Temperature, strains and phase of growth, pH, water activity, cell membranes and wall and genetic mechanism and injured population are some of these factors (Cheftel, 1995; Hoover et al., 1989; Lopez-Pedemonte et al., 2007; C. O'Reilly & Kelly, 2001; Patterson, 2005b; Rendueles et al., 2011). It was found that HP is a technology that can be suitable for reduction of microorganisms affecting cheese. HP processing of cheese can ensure safety and extend product shelf-life when cheeses are not ripened. In the case of ripened cheeses, a good balance should be attained between ensuring microbial safety and maintaining traditional cheese quality characteristics (Martinez-Rodriguez et al., 2012b).

Other researchers focused their studies applying high-pressure treatments directly on cheese and evaluated the changing general characteristics throughout ripening. White-brined cheeses (Koca, Balasubramaniam, & Harper, 2011; Okpala, Piggott, & Schaschke, 2010; Van Hekken, Tunick, Farkye, & Tomasula, 2013), Hispánico and other ewe's milk cheese (Rocio Alonso et al., 2011; Arqués, Garde, Fernández-García, Gaya, & Nuñez, 2007; Avila, Garde, Gaya, Medina, & Nunez, 2006; Garde, Arques, Gaya, Medina, & Nunez, 2007; B. Juan, Trujillo, Guamis, Buffa, & Ferragut, 2007; A Picon, Alonso, Wely, & Nuñez, 2013), Gouda cheese (Kolakowski, Reps, & Babuchowski, 1998; Winy Messens, Van de Walle, Arevalo, Dewettinck, & Huyghebaert, 2000), Mozarella cheese (Johnston & Darcy, 2000; Sheehan et al., 2005), Cheddar cheese (Rynne et al., 2008; Serrano, Velazquez, Lopetcharat, Ramírez, & Torres, 2004), Camembert cheese (Kolakowski et al., 1998), blue vained cheese (Voigt, Chevalier, Qian, & Kelly, 2010) and Garrotxa or other types of goat cheese (Capellas, Mor-Mur, Sendra, & Guamis, 2001; Delgado et al., 2012; J Saldo, McSweeney, Sendra, Kelly, & Guamis, 2002; J. Saldo, Sendra, & Guamis, 2000; Jordi Saldo et al., 2003; A J Trujillo, Guamis, & Carretero, 2000), have been evaluated by many researchers. Physico-chemical and sensory characteristics of a cheese makes it unique and will influence consumer acceptance of this product, thus ensuring that the processing technologies applied, such as HP treatments do not affect cheese identity attributes in a negative fashion is of utmost importance. Studies related with these issues pointed out that deep changes in cheese texture and microstructure, pH, color, proteolysis index, and general ripening indicators were caused by HP processing. The magnitude of these changes was depending predominantly on the type and composition of cheese, but also on temperature, pressure and in a lesser extent on time of HP processing.

Some strategies have been carried out to improve brining of cheese, since it is a relatively long process, always depending on the size of the brined cheese. High-pressure treatments could play an important role on brining of cheeses, possibly shortening this step in the manufacture of cheese. Some researchers studied the transport of sodium chloride in HP Gouda cheeses (Winy Messens, Dewettinck, et al., 1999), the advantages of the high-pressure brining in Manchego-type cheese (Pavia, Jose Trujillo, Guamis, & Ferragut, 2000) and in Gouda cheese

(W Messens, Dewettinck, Camp, & Huyghebaert, 1998). Results revealed a better distribution of salt in HP cheeses, especially in medium and interior sectors of the cheese wedge, but no significant differences were observed concerning the salt uptake or salt diffusion when pressures between 50-500 MPa were applied.

Since ripening involves extended times of cheese in the ripening room before the optimum maturation point is reached and cheese can be placed on the market, this part of the cheese making process entail great associated costs and large capital investment is needed to keep the cheese factory afloat. Therefore, one of the main pursued objectives of researchers concerning the use of HP processing has been the acceleration of ripening. Several modifications on cheese matrix have been carried out following this objective. Increasing the water retention, releasing bacterial enzymes, and increasing enzyme activity under pressure (when not stopping it due to an over-pressure) are some of the effects found to be caused by high pressure and consequently shortening ripening time. Numerous research groups have assessed different conditions when working in this field such as holding HP for short times (5 to 20 min), apply low to moderate pressures for long periods of time (50 to 200 MPa for up to 82h) and combination of both, high pressure for short times and moderate pressures for long times. In general, acceleration or deceleration of proteolysis depended on high-pressure processing conditions like intensity of pressure and duration time of the treatment applied (Arqués et al., 2007; J. Calzada, Del Olmo, Picon, Gaya, & Nuñez, 2013; Garde et al., 2007; B. Juan, Ferragut, Guamis, Buffa, & Trujillo, 2004; Martinez-Rodriguez et al., 2012b; Winy Messens, Estepar-Garcia, Dewettinck, & Huyghebaert, 1999; J. Saldo et al., 2000; Antonio J. Trujillo et al., 2002; Yokohama, H., Sawamura, N. and Motobayashi, 1992). The changes induced by HP treatments in regard with the proteolysis of cheese revealed alteration in enzyme structure, conformational changes in the protein matrix making it more susceptible to the action of proteases and/or bacterial lysis enhancing the release of microbial enzymes that promote biochemical reactions.

Being ripening process that long, leading to consequently changes occurred in the cheese matrix over time, a number of studies have been carried out evaluating the effect of HP treatments applied at several stages of ripening (Arqués et al., 2007; Delgado et al., 2012; B Juan, Ferragut, Buffa, Guamis, & Trujillo, 2007; B. Juan, Trujillo, et al., 2007; O'Reilly, O'Connor, Murphy, Kelly, & Beresford, 2000). Even though HP treatments caused deep changes in physic-chemical, rheological and sensory properties of cheeses, they were minimized or even disappear during ripening.

However, alternative applications of HP technology are explored. Of particular importance in the manufacturing of dairy products is the modification of protein structure and functional properties resulting from high-pressure treatment. Many proteins are denatured by high-pressure because unfolding results in a reduction in molar volume (Needs, Stenning, Gill, Ferragut, & Rich, 2000). Protein denaturation under pressure is a complex phenomenon induced by the disruption of both hydrophobic bonds, sulfhydryl interactions and salt bridges. High pressure act by altering the balance of intramolecular and solvent-protein interaction. The impact of pressure-induced denaturation of protein depends on factors such as temperature of treatment, pH, ionic strength solvent, pressure and time level. Proteins modifications are mainly involved in high pressure functional properties modifications, thus

gelling, emulsifying or foaming properties change under the effect of high pressure. Although some works pointed out not desirable textures at the end of ripening, high pressure treatment has been widely so far recognized as a potential tool for the modification of cheese functional properties and could be used to create new cheeses or new textures (Johnston & Darcy, 2000; Winy Messens et al., 2000; Sheehan et al., 2005).

2.4.3. Effects of HP treatments on physic-chemical and ripening of cheese

Table 2.2 Effect of high-pressure treatments on pH and color (adapted from Martínez-Rodríguez et al., 2012)

Parameter evaluated	Cheese variety	Moment of application[a]	Treatment conditions P (MPa)/ t (min)/ T (ºC)[b]	Impact	Reference
pH	Camembert	D5, D10	0,1-500/4h/5	Higher pH in experimental cheeses compared with the control	Kolakowski et al., 1998
	Gouda	After brining, D5,D10	50,500/20-100/14		Kolakowski et al., 1998
		D14, D42, D1	50,225,400/59,34,14s/14		Messens et al., 1998, 1999
	Manchego	During brining	50,200/45/14		Pavia et al., 2000
	Mozarella	D1	200/60/20		Johnston and Darcy, 2000
	Garrotxa	D1	50,400,50+400/72h,5,5+72h/14		Saldo et al., 2000, 2002
	Fresh cheese	D1	400/20/20		Sandra et al., 2004
	Edam	D28,D42,D56	200,400//20		Iwanczak and Wisniewska 2005
	La Serena	D2, D50	300,400/10/10		Arqués et al.,2006; Garde et al.,2007
	Ewe's cheese	D1, D15	200-500/10/12		Juan et al. 2007,2008
	Cheddar	D1	400/10/8		Rynne et al., 2008
	Fresh Cheese	D2	9-291/1-29/20		Okpala et al., 2010
	Hispánico cheese	pressurized curds at day 1 to be added to final cheese	200-500/10/8		Picón et al., 2012, Alonso et al.,2011, 2012
	Ibores	D1, D30, D50	400,600/7/20		Delgado et al.,2012

[a] D: days of ripening; [b] P: pressure, t: time, T: temperature

Parameter evaluated	Cheese variety	Moment of application[a]	Treatment conditions P (MPa) / t (min)/ T (ºC)[b]	Impact[c]	Reference
Color	Mató	D1	500/5,15,30/10,25	L* and a* decreased whereas b* increased compared to control cheese	Capellas et al., 2001
	Garrotxa	D1	400/5/14	Lower lightness and higher chroma values than control cheese	Saldo et al., 2002
	Queso fresco	D1	400/20/20	More yellowish after 1d of treatment than control cheese but no after D8	Sandra, S. et al., 2004
	Cheddar	D1	400/10/25	a* decreased while b* increased compared to control cheese	Rynne et al., 2008
	Turkish white-brined	D1	50-400/5, 15/22	Increasing pressure intensity and holding time did not affect L, but a* decreased and b* increased compared to control cheese	Koca et al., 2011,
	Queso fresco	D1	300, 500/5/6	b* values were higher at 400MPa compared to the control	(Evert-Arriagada, Hernández-Herrero, Guamis, & Trujillo, 2014)

[a] D: days of ripening; [b] P: pressure, t: time, T: temperature; [c] a*, b* and L*: CIELab parameters

There are numerous factors that can play a dominant role in cheese consumer acceptance. Physic-chemical characteristics and all those derived from ripening in hard and semi-hard cheeses are particularly relevant in acquiring distinctiveness and unique characteristics by cheeses.

Generally, high pressure treatments did not change to large extent parameters like total solid, fat, ash, protein and nutrients contents in cheese. However, Saldo et al. (2000; 2002) and Delgado et al. (2012) studied semi hard goat's milk cheese and applying pressures between 400 and 600 MPa, reported higher values of moisture content in pressurized cheeses. Additionally, this increase led to a better water retention permitting water to be strongly bound. Similar results were reported by other authors in ewe's milk cheese HP-treated at 400 and 500 MPa (B Juan, Ferragut, et al., 2007) and in white brined cheese high-pressure treated at 400 MPa at the end of ripening (Koca et al., 2011)

Table 2.2 shows the effect of high-pressure on pH and color. Numerous studies concluded that high-pressure treatments caused a pH-shift increasing it. The magnitude of the gain depends upon the intensity of the pressure applied, the moment of high-pressure treatment application, time and temperature of treatments. Nevertheless, pH differences between pressurized and control cheeses become less significant during the ripening process.

HP treatments also alter color of cheese. Micelle disintegration induced by HP treatment also affects cheese matrix color (Antonio J Trujillo, Capellas, Saldo, Gervilla, & Guamis, 2002). As it is shown in Table 2.2, color followed different trends upon the cheese variety and the treatment conditions applied. Lower lightness was attributed to pressurized cheeses whereas higher values of b* were found in most of goat and other pressurized cheeses.

Many studies have been carried out to evaluate the effect of high-pressure on ripening and proteolysis of cheeses. A great variety of strategies have been undertaken concerning ripening and high-pressure. Pioneers researching acceleration of ripening by high-pressure treatments were Yokohama et al. (1992) who significantly reduced ripening times of Cheddar applying from 5 to 200 MPa for 72 h at 25 ºC. However, results obtained by these researchers have been questioned repeatedly by scientific community as Japanese researchers used 10 times more starter culture than usual, which could lead to increase the FAA values, equaling those of 6-months ripened Cheddar. Commercial Irish Cheddar has also undergone other high-pressure conditions for accelerating cheese ripening founding substantially different results than those obtained by Yokohama et al (1992). O'Reilly et al., (2000) applied 50 MPa for 72h at several stages of ripening (days 2,7,14,21 and 28) and found significant decrease in the levels of α_{s1}-casein and accumulation of α_{s1}-I-casein as well as increased pH 4.6 SN/TN two-fold compared to controls, when treatments were applied on days 2,7 and 14. Increased levels of FAA were also found by the authors when cheeses were treated on day 2 of ripening. The same author three years later (C. E. O'Reilly et al., 2003), found the same trend in α_{s1}-casein and α_{s1}-I-casein when pressures of 100 MPa during 72h were applied, and maximum levels of pH 4.6 SN/TN when pressures below 150 MPa were applied. Other research groups left out moderate pressures to apply higher pressures, such as 400 MPa for 10 min to the same cheese (Rynne et al., 2008). In this case the high-pressure treatments applied were useful to arrest or slow Cheddar ripening. Overall, data from these studies on Cheddar cheese clearly demonstrated

that low to moderate pressures treatment conditions are effective at accelerating proteolysis whereas higher pressure treatments may lead to slowdown of proteolysis. However, Saldo et al. (2002) applied different pressure conditions in Garrotxa cheese finding different effects than those described in Cheddar. When treatments of 50 MPa for 72 h at 14 ºC at day 1 little differences in levels of protelolysis appeared compared with the control. Additionally these differences trailed off during ripening and at day 28 were barely apparent. Conversely, treatments at 400 MPa for 5 min enhanced the production of FAA, reaching twice the value of control cheese at day 28. Those cheeses also revealed a high peptidase activity and consequently and acceleration of secondary proteolysis. A reduction of Garrotxa ripening time from 28 to 14 days was observed when a combination of these two high-pressure treatments was applied (shock high-pressure treatment at 400 MPa for 5 min at 14 ºC followed by a low-pressure treatment of 50 MPa for 72 h). The treatment at 400 MPa seemed to cause a release of microbial enzymes into the cheese matrix and the 50 MPa treatment enhanced enzyme activity. Delgado et al. (2012) studied the effect of HP on Ibores raw goat milk cheese, applying high-pressure treatments at different stages of ripening (days 1, 30 and 50). The author found that pH was higher in 400 MPa high-pressure treated cheeses (5.21) than in control cheese (5.14) while cheeses high-pressure treated at 600 MPa showed an intermediate value (5.18).

Similar results were obtained in other types of cheese such as ewe's milk cheese (Avila et al., 2006; Garde et al., 2007; B. Juan et al., 2004; A Picon et al., 2013) and mozarella cheese (Johnston & Darcy, 2000; C. E. O'Reilly et al., 2002; Sheehan et al., 2005).

In short, high-pressure treatments may slowdown or accelerate proteolysis of cheese upon the variety of cheese and intensity of pressure applied. It is of most importance to strike a compromise between the effect of high-pressure treatments whether on proteolysis whether on sensory attributes in order to not affect negatively any of the distinctive characteristic of the cheese studied, especially in goat cheeses which own such specific flavor appreciated by consumers.

2.4.4. Effects of HP treatments on water and salt content in cheese

Few literature has been found regarding to the effect of high-pressure on water binding of cheese and its salt distribution during ripening. Buffa et al., (2003) compared water distribution on cheeses made of raw, pasteurized (72 ºC, 15 s) and pressurized (500 MPa for 15 min) goat milk. Regarding the water loss, pasteurized cheese milk sample was the one which showed a larger rate during ripening being this cheese related at the same time to the major loss of W1 (free water) type of water. Internal water profiles were found to be similar between pressurized and raw milk cheeses, measured as W1 and W2 (bound water) levels.

Goat milk cheeses were HP processed (400 MPa for 5 min at 14 ºC) by Saldo et al., (2000; 2002) undergoing changes in the internal distribution of water in cheeses. Higher levels of moisture content and a decrease of water loss during ripening accompanied pressurized cheeses of these studies. Additionally, higher content of free water in these cheeses increased the output of the cheese-making process. The same authors in other study (2001) reported the same moisture contents for pressurized and control cheeses but a different water retention.

Pressurized cheeses (50 MPa for 72h at 25ºC) showed significant higher amount of bound water and lower values of free water compared with the control cheese.

In relation to salt content in cheese, Messens et al. (1998; 1999) studied the effect of high-pressure (100, 200, 300, 400 and 500 MPa) on brining and the transport of sodium chloride and water in Gouda cheese. The total amount of salt taken up by cheese was not influenced by pressure brining. However, brining under high-pressure conditions promoted disruption of the paracasein network, since more proteins, especially β-casein and peptides were found in pressurized cheese serum compared with serum of control cheese. Water loss of high-pressure cheeses was also affected by pressure brining. HP processing influenced cheeses diminishing water loss treated from 200-300 MPa upwards. The denser structure of HP cheeses may have prevented the water moving out as well. Based on these results, it is suggested that conversion of free water into protein-bound water took place in pressurized cheeses. Previous studies (Guamis et al., 1997) had focused its research in the control of Manchego type cheese brining by vacuum impregnation finding that impregnated cheeses had a higher water content as a consequence of less drying during the brine immersion period.

Since HP could modify the cheese matrix structure and thus may make it easier for Na^+ Cl^- molecules to pass through the cheese matrix, a faster achievement of salt diffusion and thus, distribution of salt throughout cheese wedge it is expected to be attained. A faster salting method in cheese, would allow an evenly distribution of salt in cheese, which in turn will reduce the risk of non desirable fermentations and may enhance the prevention of off-flavors occurrence. Necessarily, a proper salt uptake by cheese will have an impact on moisture content and influence the water binding of goat cheese.

2.4.5. Effects of HP treatments on texture and microstructure of cheese

The end product characteristics such as flavor, physicochemical, functional properties (texture properties) and quality of cheeses are significantly affected by the microstructure. The prediction or control of the properties of cheese require an understanding of the location of the various components and their interactions, which are made possible through the study of the microstructure, during manufacture, ripening and subsequent storage (El-Bakry & Sheehan, 2014). Accordingly, study of the cheese microstructure is of great importance to the cheese manufacturer and consumer.

The different mechanical processes involved in the manufacture of cheese are expected to produce differences in the protein matrix aggregates and free cavities that define product microstructure. For this reason is important to ascertain whether different production parameters lead to differences in cheese microstructure formation (Rovira, López, Ferrandini, & Laencina, 2011).

According to Koca, et al. (2011), generally one of the vastest effects of HP on cheese is related to the network structure. It depends on several factors such as composition, proteolysis, fat droplet size and distribution, casein-casein, casein-water and casein-fat interactions, the state of water (bulk or bound to casein matrix), pH and the state of calcium (ionic or bound to casein

matrix). Microstructure development of cheese begins with the rearrangement of casein micelles to form the micelle aggregates of the protein matrix. Then, HP disturb again the created network and leads to a restructuration of protein aggregates and formation of a new structure, probably closer, more homogeneous and entrapping more water, appearing as a swelled matrix (Boutrou et al., 2002; Rovira et al., 2011).

Table 2.3 describes several works on the effect of HP on rheological, textural and microstructure of cheeses. Serrano et al., (2004), Wick et al., (2004) and Rynne et al. (2008) applied several high-pressure treatments on Cheddar cheese founding different results upon to the intensity of the pressure applied. While 'low' pressure treatments up to 300 MPa and very high pressures such as 800 MPa did not showed significant differences, moderate high-pressure treatment increased the shredability of Cheddar. Mozzarella has been another cheese widely studied. Sheehan et al. (2005), O'Reilly et al. (2002) and Johnston and Darcy (2000) applying pressures from 200 to 400 MPa reached lower melting times and a less porous cheese matrix. However, Sheehan et al. (2005) did not find significant differences between pressurized and control cheeses related to rheological properties. Numerous authors have worked on ewe's milk cheese, whether if it is supported with a PDO (Garde et al., 2007) or not (R Alonso, Picon, Gaya, Fernández-García, & Nuñez, 2012; Avila et al., 2006; B. Juan, Trujillo, et al., 2007; A Picon et al., 2013).

Juan et al., (2007) found that cheeses high-pressure treated at day 15 were similar to the control cheese. Moderate pressures (200 to 300 MPa) enhanced firmness and cheeses treated at 500 MPa showed the highest deformability and the lowest fracturability. This fluidization of cheese matrix was also reported by Saldo et al., (2001) but when moderate pressures were applied (50 MPa for 72 h at 25 ºC) to Garrotxa goat's milk cheese. The author stated that the HP treatment made cheeses more fluid and less elastic than the control. In contrast, the control cheese became shorter and harder. When the same author (2000) applied higher pressures (400 MPa for 5 min at 14 ºC) a decrease in crumbliness and an increase in elasticity appeared in HP cheeses.

Delgado et al. (2012) studied the effect of HP on Ibores raw goat milk cheese, applying high-pressure treatments at different stages of ripening. All HP treated cheeses showed a reduction of hardness proportional to pressure intensity. Highest effects on instrumental texture were found by the author when goat cheeses were treated at the beginning of ripening (day 1).

Cheese structure has been studied at the molecular, macroscopic, and microscopic levels by several methods. Many of the studies in this area have focused on the observation of protein clusters and how they link together to make up the cheese protein matrix (Wium, Pedersen, & Qvist, 2003). In recent years, image analyses techniques have grown in number and improved in quality. Most uses of image software in this area have focused on identifying macro components of cheese and measuring their diameters, shapes and distribution. Studies at the microscopic level have described cheese microstructure and pore diameters during ripening in an indicative way based on the measurement of the horizontal diameter of pores although without taking into account its irregularities. However, such techniques do not allow a quantitative determination of microstructure parameters that would enable the characteristics of the cheese structure and its regularities to be described (Rovira et al., 2011).

Cheese microstructure can be examined using light microscopy (LM), confocal laser scanning microscopy (CLSM), scanning and transmission electron microscopy (SEM and TEM, respectively). The main advantage of electron microscopy techniques is that they allow for a much higher resolution imaging of the components of the cheese, in comparision to LM and CLSM techniques. However, CLSM is one of the most useful microscopy techniques for studying the microstructure of a wide variety of foods (Auty, 2013; Romeih, Moe, & Skeie, 2012). The microscopy technique is considered as a powerful tool since the laser scanning penetrates the cheese surface to visualize thin optical sections to obtain 3-dimensional analysis of the cheese microstructure without disturbing the internal structure by reconstructing the sequential sections of micrographs (El-Bakry & Sheehan, 2014).

It is known that protein content and the conformation of protein network is related to the bound water amount and all together will determine the final texture and palatability of cheese. The factors that impact upon cheese texture include fat globules occluded within the protein matrix, fat globules coated with casein micelle fragments that interact with the surrounding casein matrix, free pools of fat, casein matrix density, proteolysis, water content and the density of chains of fused casein micelles (Langton, Astrom, & Hermansson, 1996; Rovira et al., 2011). Moreover it is difficult to quantify the direct effect of any of these compositional components and factors separately as the concentrations of each tend to vary simultaneously and strong interactions between them take place during ripening (T P Guinee, 2002). High pressure treatments influences the structural properties perceived by consumers, such as texture, fragility, and elasticity enabling new cheese textures to be developed.

Table 2.3 Effect of HP treatments on rheological, textural and microstructure characteristics of cheese (adapted from Martínez-Rodríguez et al., 2012)

Cheese variety	Moment of application[a]	Treatment conditions P (MPa) / t (min) / T (ºC)[b]	Impact	Reference
Mozarella	D1	200/60/20	Pressurized Mozarella showed less porosity in cheese matrix	Johnston and Darcy, 2000
Mató (goat's milk)	D1	500/5,15,30/10,25	HP treatments revealed no significant differences between HP and control cheeses	Capellas 2001
Garrotxa	D1	50/72h/25	Pressurized cheese was more fluid and less elastic than control	Saldo et al., 2001
Gouda	D3	50,225,400/1h/14	Less rigid and solid-like, more viscoelastic, and had less resistance to flow at longer times than the control	Messens et al., 2000
Low-moisture Mozarella	D1, D5	400/20/25	Reduced time required to attain satisfactory cooking performance (by day 15). Increased fluidity, flowability and stretchability, and reduced melting time on heating at 280 ºC	O'Reilly et al., 2002
Stirred and milled-curd Cheddar	D1	345,483/3,7/NS[c]	Accelerated shredability (microstructure and sensory properties of 27-day-old commercial cheese obtained in 1 day)	Serrano et al., 2004
Cheddar	1 and 4 months	200-800/5/25	Pressures up to 300 MPa applied to 1-month-old cheese had no significant effect. At 800 Mpa, cheese had similar fracture stress and Young's modulus as control cheese. Pressure applied to 4-month-old cheese increased fracture stress.	Wick et al., 2004
	D1	400/10/25	Increased fracture strain and fracture stress values, lower fluidity, flowability and stretchability increased up to 21 d, but to a lesser extent than in control cheese	Rynne et al., 2008
Reduced-fat Mozzarella	D1	400/5/21	No significant effect on rheological properties	Sheehan et al., 2005
Hispánico	D15	400/5/10	Softening of the texture in HP cheeses	Avila et al., 2006
La Serena	D2 or D50	300,400/10/10	Highest fracturability, hardness and elasticity in cheese treated on day 2	Garde et al., 2007
Ewe's milk cheese	D1 or D15	200-500/10/12	Moderate pressures applied on day 1 enhanced fracture stress and cheese treated at higher pressures showed highest deformability, lowest fracturability, and rigidity.	Juan et al., 2007
White brined cheese	D1	50- 400/5,15/22	Unpressurized cheeses showed sponge-like matrix, fat globules of different size and large mechanical holes. HP cheeses showed a more compact and continuous matrix. A decrease in fracture stress and springiness occurred in pressurized cheeses.	Koca et al., 2011

Hispánico cheese	pressurized curds at day 1 to be added to final cheese	200-500/10/8	Control cheese resulted in a firmer texture (higher fracture stress). A denser and more compact structure and no differences were found in overall porosity in HP cheeses	Picón et al., 2012, Alonso et al., 2011, 2012
Torta del Casar	D1, D30, D50	400,600/7/20	All HP cheeses showed a reduction of fracture stress proportional to pressure intensity and a rise on springiness. All differences were more evident in treatments carried out at day 1.	Delgado et al., 2012
Brie	D14, D21	400, 600/5/9	Firmer texture (higher fracture stress) was found in cheeses HP at day 14	Calzada et al., 2014

[a] D= day; [b] P= pressure, t= time, T= temperature; [c] NS= no specified

2.4.6 Effects of HP on flavor and sensory characteristics of cheeses

Cheese is a biochemically active dynamic product that undergoes many changes during ripening. Cheese flavor development is one of the consequences of these chemical changes occurring during this period. Flavor compounds are produced through the principal biochemical degradation pathways: glycolysis, proteolysis and lipolysis. Depending on the variety, technology, microflora and ripening conditions, flavor compounds are produced to give unique sensory characteristics to each cheese variety. Resulting from basic biochemical transformations, a background flavor seems common to all cheese varieties. However, the characteristic aroma of most cheeses results from the subtle combination of a large number of odorous volatile compounds present in the correct concentration ratios. In this context, off-flavor may possibly result from the breaking of the fragile equilibrium that constitutes the right flavor balance (Le Quéré, 2004).

Studies on goat cheese flavor are fewer than those on cow cheese flavor and most of them relate only to goat cheese aroma (Ha & Lindsay, 1991; Le Quere, Pierre, Riaublanc, & Demaizieres, 1998a). Some authors have shown that the goat flavor intensity is linked to the genetic polymorphism of the caprine α_{S1}-casein, leading also to differences in the cheese composition and texture, making goat's milk cheeses very special sensory products (Chilliard, Ferlay, Rouel, & Lamberett, 2003; Pierre et al., 1999).

Most of the studies carried out to evaluate the effect of HP on cheeses have been focused on reduction of microbial counts, although not always collateral damage on sensory properties of cheese has been considered. It is known that application of severe HP treatments could hinder the formation of certain volatile compounds and lower enzymatic activity (Martinez-Rodriguez et al., 2012b). However, Norton and Sun (2008) reported negligible effect on flavor characteristics due to HP processing, which makes it a suitable technology for fresher and minimally processed foods. Calzada et al., (2014) tried to evaluate HP treatments as a tool to control the lipolysis, volatile compounds and off-odours in Torta de Casar cheese. The conditions applied were 400 and 600 MPa for 5 min after 21 or 35 days of ripening. Control cheese of this study undergone excessive formation of some impact flavor compounds which affected negatively its sensory characteristics. In contrast, HP cheeses maintained their characteristics throughout a prolonged refrigerated storage period.

Other ewe's milk cheeses were also studied concerning to the effect of HP on cheeses sensory attributes (R Alonso et al., 2012; Rocio Alonso et al., 2011; Arqués et al., 2007; Avila et al., 2006; B Juan, Barron, Ferragut, & Trujillo, 2007; B. Juan, Trujillo, et al., 2007; Antonia Picon et al., 2010). Results following different trends were found by Juan et al. (2007) suggesting that HP treatments may act on bacterial enzymes by different manners depending on the intensity of pressure applied and the enzyme involved, thus enhancing or hindering the formation of volatile compounds. In other study from the same authors , cheeses high-pressure treated at first stages of ripening obtained lower punctuations in quality (odor and aroma) by panelists compared to the control and the HP-treated cheeses at day 15 (Bibiana Juan, Barron, Ferragut, Guamis, & Trujillo, 2007). Pressurized ewe's milk curds (200, 300, 400 and 500 MPa) were added during the manufacturing

of Hispánico cheese and volatile and sensory profile of these cheese were evaluated. No significant differences between cheeses of the same age were found due to high-pressure processing. Differences in umami and bitter taste were also negligible and moreover, sensory characteristics did not appear to be influenced by the differences recorded for the concentrations of peptides or FAA (R Alonso et al., 2012; Rocio Alonso et al., 2011; Antonia Picon et al., 2010). Neither Arqués et al., (2007) found significant differences in Hispánico and La Serena high-pressured treated cheeses. Furthermore, little changes occurred in cheeses by means of HP processing disappeared throughout ripening.

Other studies have been carried out on Cheddar cheese. Serrano et al., (2004) observed that pressure-treated (345 and 483 MPa) cheeses at day 1 showed similar sensory attributes to those observed in their unpressurized counterparts at day 27. Similar results were obtained by Rynne et al., (2008) which high-pressure Cheddar (400 MPa) showed greater intensity of some sensory attributes compared to the control cheese at day 90 of ripening. However, pressurized cheeses became less intensely flavored at day 180 of ripening.

The specific aroma of goat cheese has been well identified by different authors (Engel et al., 2002; Ha & Lindsay, 1991; Le Quere, Pierre, Riaublanc, & Demaizieres, 1998b; Salles et al., 2002). 4-Methyloctanoic and 4-ethyloctanoic acids have been found to be the main volatile compounds responsible for the goat flavor and they are perceived at very low concentration (Salles et al., 2002).

Several authors have studied the effect of high-pressure treatments on volatile profile and sensory characteristics of goat's milk cheeses. Delgado et al., (2011; 2012) applied 400 and 600 MPa at different stages of ripening (days 1, 30 or 50) of Ibores cheese. High-pressure induced major changes when applied at first stages of ripening related to sensory attributes such as appearance, odour and texture). In regards to volatile compounds, HP treatment enhanced the formation of ketones, hydrocarbons and δ-decalactone. Saldo et al., (2003) stated that new cheese varieties regarding to the volatile profile could possible to be developed by means of high-pressure technology, even the pressures applied (400 MPa) seemed to decrease lipolysis level on HP goat's milk cheese.

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Chapter III

Material and methods

Material and methods

The first experiments of this work were focused towards the set up of the cheese making protocol Goat milk's cheeses were produced with pasteurized milk and added in a determinate amount of calcium chloride, rennet and starter culture. The coagulation, pressing, brining and ripening conditions were also studied before choosing the optimum option to develop the required cheese (Appendix I).

3.1 Milk supply pasteurization and starter culture preparation

Raw whole goat milk was obtained by mechanical milking from a local goat herd situated in Cànovas, Catalunya, Spain (Granja Josep Illa). The transport and storage of milk was carried out in refrigeration conditions until the thermal treatment. Milk was pasteurized (72 ºC 15 s) in a 500L / h plate heat exchanger (Talleres Garvía, Barcelona, Spain) the same day of the cheese making passing through directly from the thermal treatment equipment to the cheese vat. Cheese was manufactured in a stainless steel 250 L mechanized vat (Talleres Garvía, Barcelona, Spain) with two arms provided both of cutting and stirring accessories. Bulk set starter culture was prepared the day before to the cheese making, adding to 2 L of milk mesophilic starter mix from a frizzed dried culture stored in freezing conditions (Choozit, MA 11 LYO 125 DCU, Danisco, DuPont Nutrition and Health, Wilmington, Delaware, USA) composed of *Lactococcus lactis ssp cremoris*, *Lc. lactis ssp lactis*. The milk was fermented during 16h at 30 º C obtaining a pH of 4'50 approximately starter culture ready to add to the vat.

3.2 Cheese manufacture and high pressure treatment

Milk was placed in the vat (Figure 3.1a) and warmed under mechanical stirring until 20 º C. At this point the starter culture (2 % v/v) was added. The pH of the milk was measured with a portable pH-meter (PH 25, electrode 50 54 with automatic temperature compensation, Crison, Alella, Spain). Rennet (0.02 % Laboratorios Arroyo, Santander, España) and calcium chloride commercial solution (0.02 % Laboratorios Arroyo S.A., Santander, España) were added at the same time at a temperature of 35ºC. After 1 min of gently stirring and 35 min of coagulation (Figure 5 a)), the curd was first manually and then automatic cut to about korn size. The used molds were the baby Portuguese type (Industrias Plasticas Arroyo S.A., Santander, España) and they were filled resulting on 370 ± 20 g of curd. Filled molds were placed in a ten pistons vertical press (Talleres Garvía, Barcelona, Spain) at 0.5 kPa for 1 hour and at 2 kPa for 2 hours (Figure 3.1 b and c). Cheeses were salted by immersion in brine (1.143 Kg NaCl / L) at 13 ºC for 60 min and then they were first soft vacuum packaged and then treated by high pressure (100, 200 and 300 MPa for 5 min at 14 ºC). The high pressure treatments were performed in batch isostatic equipment (GEC Alsthom ACB,

Nantes, France). One group of cheeses was treated by high pressure before the brine (100BS, 200BS and 300BS) and the rest were salted after the high pressure treatment (100AS, 200AS and 300AS). Control cheeses (C) did not receive any high pressure treatment. After the pressure treatment all cheeses were placed in a climatic chamber (ATP line KBF, Binder GmbH, Tuttlingen, Germany) for its ripening developed at 13ºC and 90% R.H. for 30 days. The whole experiment was performed 3 times.

a)

b)

c)

Figure 3.1 Cheese making process. a) goat's milk curd ready to be cut; b) and c) vertical pressing of cheeses

3.3 Sampling of cheeses during ripening

Samples of each batch of cheese were separated in untreated (control; C) and HP treated cheeses at 100, 200 or 300MPa for 5 min at 14 º C, applied before (100BS, 200BS and 300BS) or after salting (100AS, 200AS and 300AS).

The analyses of cheeses were performed one day after HP treatments and also at 7, 15 and 30 days of ripening, excepting for fat and volatile compounds that were analyzed at day 1 and 30 of ripening. All samples were properly homogenized prior to their analysis.

3.4 Physicochemical analysis

Triplicate samples were assayed for moisture content by the gravimetric method according to the International Dairy Federation (IDF) standard (IDF4A, 1982). The pH was measured per duplicate potentiometrically with a pH meter (Crison Micro-pH 2001, Crison, Alella, Spain) on cheese distilled water (1:1) slurry. Water activity (a_w) was measured per duplicate in a water activity analyzer (Aqualab®, Model Series 3 TE, Decagon Devices, Inc., Pullman, WA). Triplicate samples were assayed for fat using the Van Gulik method (IDF 222: 2008, n.d.). Sodium Chloride was determined by three measures with a Chloride Analyzer (926 Chloride Analyzer, Sherwood Scientific Ltd., Cambridge, England) in two parts of cheese at each sampling day, an inner (in.) and outer (out.) area of cheese (Figure 3.2). Salt uptake rate was calculated as the mean of measurements performed of the difference between inner and outer part of cheese at each sampling point.

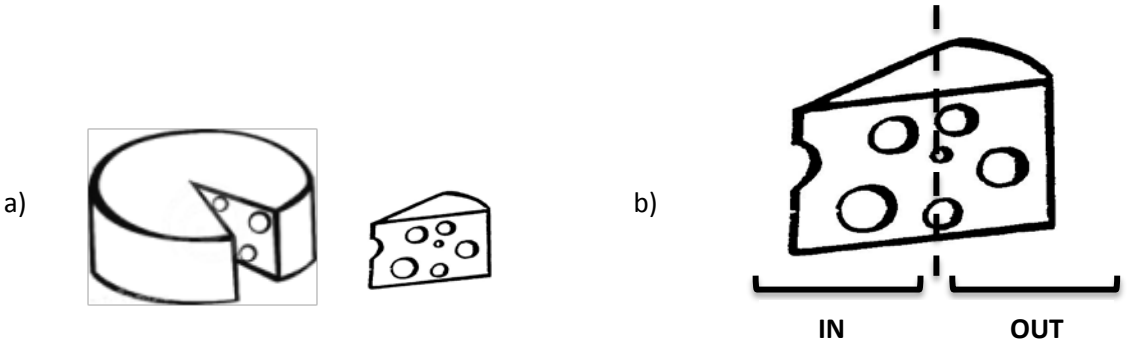

Figure 3.2 Image representing the sampling of cheese for each analysis performance (a), and sampling for sodium chloride analysis (b).

3.5 Nitrogen fractions and total free amino-acids assessment

Total nitrogen (TN) was measured in duplicate by the Dumas combustion method IDF Standard 185 (IDF 185, 2002). Water soluble extracts of cheese were prepared according to the method described by (Kuchroo & Fox, 1982). From the water soluble extracts at pH 4.6 water soluble nitrogen (WSN) fraction was obtained and the nitrogen was determined by Dumas combustion method (IDF185: 2002, n.d.). The nitrogen content of WSN fraction was expressed as a percentage of total nitrogen (WSN/TN, %) which is described as the ripening index. The determination was performed in duplicate.

For FAA determination, water-soluble extract was prepared according to Cd-ninhydrinn method described by Folkertsma and Fox (1992) as follows: 30 g of grated cheese were added to 60 mL distilled water and homogeneized for 5 min with an Ultraturrax (Diax 900, Heidolph, Germany) at

5000 rpm. After 1 hour at 40 ºC in slow, but steady movement, the homogenate was centrifuged at 5697 g for 30 min at 10 ºC. The supernatant was filtered through glass wool and the caseinic fraction was collected in eppendorf tubes. A sample (80µL for samples of days 1 and 7 and 40 µL for samples of days 15 and 30) of water soluble extract was diluted to 1 mL with distilled water and 2 mL Cd-ninhydrin reagent were added (0.8 g ninhydrin dissolved in a mixture of 80 mL of 99.5 % ethanol and 10 mL acetic acid, followed by the addition of 1 g Cd Cl_2 dissolved in 1 mL of distilled water). The mixture was heated at 84 º C for 5 min, cooled and the absorbance at 507 nm was determined in a spectrophotometer (UV 2310, Dinko Instruments, Barcelona, Spain). Analysis of 0.006 to 0.078 mg L-leucine solutions allowed constructing the standard curve. The analyses were made in duplicate and the results were expressed as mg leucine released per g of cheese.

3.6 Texture analysis

Texture analysis was performed in a texturometer TA-XT2 (Stable Micro Systems LTD, Surrey, UK). After removing the external part of cheeses, a total of 5 cubes sized at 1.5 x 1.5 x 1.5 cm were diced from each cheese sample and held at 20ºC for 2h before the assay. The uniaxial compression test was carried out using a 245 N load cell and a compression cylinder of 36 mm of diameter. Cheese cube samples were compressed to 80 % of their original height at a constant temperature of 20 º C with a crosshead speed og 80mm min^{-1} as described by Juan et al. (2007). True stress and true strain were calculated according to Calzada and Peleg (1978) by the following equations:

True stress: $$\sigma_{(t)} = \frac{F_{(t)}}{A_{(t)}}$$ **Eq. 1:**

Where $\sigma_{(t)}$ (Nm^{-2}) is the true stress at time (t); $F_{(t)}$ (N) is the force at time (t); and $A_{(t)}$ (m^2) is the area at time (t).

The true strain (e) was calculated according to the Eq. (2) of Calzada and Peleg (1978):

True strain: $$\varepsilon = \ln \frac{H_0}{H_0 - \Delta H}$$ **Eq. 2:**

where **ε** (-) is the true strain; H_0 (m) is the original height and ΔH the height differential.
Fracture stress (σ) and fracture strain (**ε**) parameters were calculated from the true stress-strain curves (Calzada & Peleg, 1978).

3.7 Color determination

To measure the color a portable Hunter Lab spectrocolorimeter (MiniScan™ XETM, Hunter Associates Laboratory INC., Reston, Viriginia, USA) was used. Cheeses were measured under Fcw illuminant (cool white fluorescent), with a 10º observer. CIE *L**-, *a**- and *b**- values from the

CIELab scale were read at six different points of inner surface of cheeses paste cut in two halves. L* value describes lightness-darkness (ranges between 100-0), a* value indicates greenness (negative values) to redness (positive values) and b* values reflects blueness (negative values) to yellowness (positive values). Total color differences (ΔE) were calculated by the following equation (Eq. 1) to compare Control and HP cheeses and differences in color during the ripening.

Total color differences: $\Delta E = \sqrt{(\Delta L^2 + \Delta a^2 + \Delta b^2)}$ **Eq. 3**

Measurements were taken on different points of the surface of cheeses cut into two halves. Six consecutive measures were taken of every cheese (MartínN Buffa, Trujillo, Pavia, & Guamis, 2001).

3.8 Thermogravimetrical analysis

The thermogravimetric analyses (TGA) were performed on a TGA/SDTA851^e thermobalance (Mettler-Toledo GmdH Analytical, Schwerzenbach, Switzerland). Aproximately 15 mg of cheese was place in the thermobalance alumina sample pan and heated from 25 to 250 ºC as the method established, at a scanning rate of 5 ºC min^{-1} (Figure 3.3 c). A flow of nitrogen as a protector gas of 60-80 mL min^{-1} was used. Analyses of all samples were performed in triplicate. Different types of water were found by use of the Mettler-Toledo STARe software to convert the output signal, and their content was expressed as loss of weight between each temperature stage (M Buffa, Guamis, Saldo, & Trujillo, 2003).

a) b) c)

Figure 3.3 a) Images of the TGA instrumentation, b) alumina pans prepared to be filled with the cheese sample and alumina pan already filled, closed and holey, c) alumina pan placed in the thermobalance of the TGA instrument.

3.9 Microstructure analysis

Evaluation of cheese microstructure was performed by confocal laser scanning microscopy (CLSM). Cheese samples were cut with a scalpel 1 mm thick and placed immediately in a 0.2% (w/v) Nile Blue A solution containing Nile Red traces (Sigma, Steinheim, Germany) for 5 min in order to stain

them. The objective of this double dye was to stain the fat and the protein network separately. After that, slices of cheese were washed twice in separate clear distilled water containers remaining 5 min in each one. Slices of cheese were placed on a microscope welled slide and covered with non-fluorescent observation medium Fluoprep (Fluoprep, Biomerieux, Lyon, France) and a cover slip on them. Images were captured by a Leica TCS SP2 AOBS (Heidelberg, Germany) using a 63 x magnification objective lens with a numerical aperture of 1.4. Fluorescence from samples was excited with the 488 nm line of an argon laser. Images were acquired in 2 channels simultaneously (488 and 633 nm aproximately) as 1024 x 1024 pixel slides (6 slides per sample approximately) in x-y dimension along the z plane at constant gain and offset. Each slide had a thickness of 1,5 µ. Images were captured per duplicate on each sample, and 5 different points on each duplicate were analysed.

Quantification of images was carried out by use of Metamorph sowtfare (Metamorph Microscopy Automation & Image Analysis Software, Molecular Devices LLC, California, United States). Final micrographs were transformed into 8-bit binary images and then normalized in the same conditions to guarantee the correct and standard contrast for all the images. Before quantification, all the micrographs were calibrated according to the magnification of the image.

Porosity and the total fatty area were calculated as the percentage of black areas (free space in the protein matrix) and red areas with respect to the total image area, respectively. The number of objects of each image area was calculated as well with Metamorph sowtfare.

3.10 Sensory analyses

Selection of panelists

Sensory evaluation of control and HP-treated cheeses was carried out by 12 trained panelists from the Centre Especial de Recerca Planta Tecnologia dels Aliments (CERPTA). All panelists had a large expertise in cheese and dairy products sensory testing (Figure 3.4 b)

Sensory tests

A profile of 6 sensory attributes of cheese grouped in appearance, mouth texture and taste were analyzed. All sessions were conducted at ambient temperature in a prepared for sensory evaluations room equipped with fluorescent lighting. The panel sessions were held mid-morning, about 3 h after breakfast. Cheeses were cut in rectangular shape (Figure 3.4 a) and 0.3 cm thick. Mineral water and bread biscuits were supplied in each session for mouth washing and neutralize the taste. A quantitative descriptive test was applied and panelist marked responses on a 7-point intensity scale situating each different sample on the scale up or down punctuated respect the control that was placed in the middle. The samples were identified using random three-digit codes (Figure 3.4 a). Sensory tests were carried out at day 30 of ripening, coinciding with the expected optimum ripening point.

a) b)

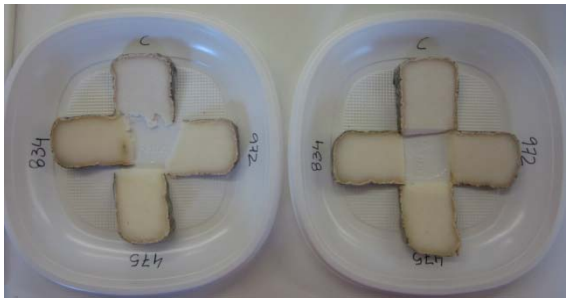

Figure 3.4 a) rectangular pieces of cheese samples properly codified for the sensory evaluation. b) panelists evaluating goat's milk cheeses

The attributes evaluated by panelists were: appearance (color –from whitish to yellow-), texture (firmness, moisture feeling and granularity) and taste (acidity and bitterness) (Table 3.1) (Appendix III).

Table 3.1 Sensory attributes evaluated on HP and Control cheeses

Appearance	color
Texture	firmness, moisture mouthfeel, granularity
Taste	acidity, bitterness

Respect to the color, the descriptive scale used by panelists indicated lower values on it as whiteness of sample whereas higher values in the scale were related to browning or darkening of cheeses.

3.11 Analysis of volatile compounds in cheese by solid-phase microextraction

Several preliminary tests were carried out in order to determinate the appropriate type of fiber, its coating and thickness, the time and temperature of extraction and the running time of the method (Appendix II).

Preparation of cheese samples

A wedge of cheese from days 1 and 30 of ripening was cut, wrapped in alumina paper and kept in a freezer at -80 ºC until the day before analyses. Cheese sections were thawed at 4ºC overnight

before volatile analyses. The external part of cheese surface was removed in order to minimize the presence of volatile compounds that could have migrated from the environment. Cheese samples were grated resulting all of a uniform size. Two and a half grams of cheese were placed in a 10 mL (1:4 w/v) vial, which was immediately afterwards sealed with PTFE / silicone septa (Supelco, Bellefonte, PA, USA) (Juan, Barron, Ferragut, & Trujillo, 2007). Cheese samples were previously tempered at 50 ºC for 10 min.

SPME (Solid Phase Micro Extraction) and GC-MS

Before each analysis, the fiber should be cleaned so as to remove contaminants that could give a high background in the chromatogram. It was preconditioned for 30 min at 250ºC. Volatile compounds extraction was carried out by pressing down the 50 / 30 µm Divinylbenzene / Carboxen / Polidymethylsiloxane (DVB/CAR/PDMS) SPME fiber (Supelco, Bellefonte, PA, USA) into the syringe needle and then lowered into the vial (which was sealed with a septum type cap) by pressing the plunger. It was exposed during 30 min at 50 ºC. Desorption of the extracted volatiles was carried out on a chromatography system (HP 6890 Series II, Agilent, Santa Clara, CA, USA) mass spectrometry (MS) system (HP 5972 Agilent, Santa Clara, CA, USA). The adsorbed volatiles were desorbed in the gas chromatograph (GC) injector port in splitless mode. During desorption, the fiber remained in the injector for 10 min at an initial temperature of 40 ºC with helium as the carrier gas at a flow rate of 1 mL / min. Finally, the split valve was opened after 5 min. The detector was used in electron impact ionization mode with a mass range of 30-250 *m/z*. The column used was 0.25 µm in a 60 m x 0.25 mm (HP-Innowax-GC, 19091N-136E, Agilent Technologies). The temperature was programmed in three stages. The initial temperature was 40 º C and then was increased to 110 ºC at a rate of 5 ºC / min followed by 10 ºC / min to 240ºC to give a runtime of 41 min.

Identification of volatile compounds

Identification of volatile compounds was based on comparison of the spectra with those of the NIST08 library (NIST/EPA/NIH, National Institute of Standards and Technology/Environmental Protection Agency/National Institutes of Health). Signals were processed using Agilent MSD Productivity ChemStation Enhanced Data Analysis software (Agilent Technology, Santa Clara, CA). Finally, confirmation of the identity of some volatile compounds was achieved by comparison of the retention times and mass spectra of individual components with those of authentic reference compounds injected under the same conditions. These are specified as ST in Tables of chapter VI.

The limit of detection (LOD) corresponds to the minor amount of a compound which signal can be distinguished from the noise of the chromatogram. LOD value was obtained from the mean of 10 blank areas plus three times the standard deviation of the reached values.

For comparison of samples to show differences between the varying treatments, data values were expressed as area of peak / 10^5.

3.12 Statistical analyses

The complete experiment was repeated on 3 independent occasions. Data was processed to evaluate differences between the HP-treated (BS and AS) and the control cheeses by multifactor analysis of variance (ANOVA). General linear models procedure of Statgraphics (Statgraphics, Inc., Chicago, IL, USA) was used taking into account both treatment and production factors, as well as their interaction. LSD test was applied to compare sample data and evaluations were based on a significance level of $P < 0.05$. Principal Component Analysis was performed to reduce the data in two dimensions and identify patterns of variation in the results obtained in the present study. Statistica software (7.0 version, Statsoft Inc., Tulsa, OK) was used for this purpose.

3.13 References

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Chapter IV

Changes in physicochemical, textural, microstructural and sensory properties of pressurized goat's milk cheeses before and after brining

Changes in physicochemical, textural, microstructural and sensory properties of pressurized goat's milk cheeses before and after brining.

4.1 Introduction

Among parameters studied in cheeses, probably the texture, and thus the microstructure, become crucial factors in consumer acceptance of cheese (Kataoka, Lord, & Pawliszyn, 2000). These quality parameters gain importance in goat cheeses due to their different ripening compared to cow's or ewe's cheeses. The moisture loss on goat's milk cheeses during ripening is tightly related to their microstructure and might determine the final texture of cheese. It is well known that texture is one of the most important quality parameters in cheese, especially in ripened cheeses. The assessment of texture normally includes the consistency of cheese mass related to the interaction between its macro components. Many factors influences the final texture of cheese, some of them related to the manufacture process in fresh cheeses, and some of them, related to the ripening process in the case of matured cheese. Moisture content, pH, salt content, proteolysis, lipolysis and fat content, among others, contribute to the biochemical phenomenon that take place during the ripening of cheese.

High hydrostatic pressure has been widely described as a very useful technology because of its impact on pathogens and spoilage microorganisms enhancing food preservation. However, few studies in the last times have been focused on texture and microstructure of pressurized food products. Several authors evaluated the texture and microstructure on pressurized goat cheeses and reported reduced crumbliness and increased elasticity and mouth feel. A fall of hardness proportional to pressure intensity and a rise of gumminess and chewiness were also reported (Delgado, González-Crespo, Cava, & Ramírez, 2012; Saldo, Sendra, & Guamis, 2001). Other authors investigated further the microstructure of pressurized cheeses and pointed out that a more continuous and smoother protein matrix was obtained (Capellas, Mor-Mur, Sendra, & Guamis, 2001; Picon, Alonso, Wely, & Nuñez, 2013; Pierre, Michel, Le Graet, & Berrier, 1999). The authors mentioned above indicated HP treatments as a potential technology to use in the dairy industry to create cheese types and develop novel textures in dairy products.

Regarding to sensory characteristics of HP cheeses, several authors reported relevant results. Delgado et al. (2012) applied 400 and 600 MPa cheeses for 7 min at 10 º C on raw goat milk cheeses and found significant ($P < 0.05$) differences in the appearance, odor and texture between control and HP-cheeses while flavor and taste remained unchanged. Traits like hardness and friability were significantly lower in pressurized cheeses while springiness was higher in HP-treated cheeses. Similar results were observed by Juan et al. (2007) who studied sensory properties of high-pressure semi-hard ewe's cheese and found that high-pressure treatments applied at day 1 caused major changes compared to control cheeses than high-pressure treatments carried out at day 15. Furthermore, cheeses HP-treated at day 15 presented higher scores in the hedonic test than cheeses treated at day 1. Other authors (Saldo, Sendra, & Guamis, 2000) found some

negative sensory notes on HP cheeses. Pressurized goat's cheeses exhibited bitter notes in their sensory test, especially 400 MPa HP-treated cheeses. This fact could be associated to the presence of short hydrophobic peptides. However, the texture of those cheeses was improved compared to control, as previously has been mentioned in the effects of HP on texture.

In order to further investigate these parameters on pressurized goat's milk cheese, this study examines the effect of moderate high-pressure (100, 200 or 300 MPa for 5 minutes at 14ºC) treatments applied on goat's milk cheeses by analyses of cheese physico-chemical properties (pH, moisture content, fat, protein), color, proteolysis, texture, microstructure and sensory attributes throughout ripening. Furthermore, since the content of salt at the moment of HP treatment application may have a significant effect on key microstructure parameters, the relationship between the salt content at the moment of the HP treatment and the microstructure was also investigated.

4.2 Results and discussion

4.2.1 Cheese composition

Main physic-chemical and composition parameters of control and HP goat's milk cheeses analyzed throughout ripening are shown in Table 4.1. Cheese pH was significantly affected by HP treatment. There was a notable decrease in pH at very first stages of ripening (day 1 and day 7) in all cheeses, which subsequently increase steadily until reaching maximum values between days 15 and 30 (Table 4.1). This behavior is in line with results observed in other type of HP-cheeses such as ewe's milk cheese (B. Juan, Ferragut, Guamis, Buffa, & Trujillo, 2004), goat's milk cheese (Delgado et al., 2012) or Mozarella cheese (Johnston & Darcy, 2000).

At the beginning of ripening, significant ($P < 0.05$) differences could be observed between samples regarding to the HP treatments. While 100 and 200 MPa HP-treated samples showed lower or similar values to the control cheese, 300MPa HP-treated cheeses showed higher pH values. The mean pH values for the 300 HP-treated cheeses were at least 0.23 pH units higher than the values for the control cheese at each sampling day attaining a maximum at day 15 being this difference of 0.44 pH units. Based in these results, two groups can be well differentiated in Table 4.1 regarding to the level of pH: control, 100 and 200MPa < 300 MPa.

Other researchers found similar pH changes to those found in this study caused by HP treatments. Regarding to goat's milk cheese, Delgado et al. (2012) also observed a slight increase of pH values at pressures of 400 MPa applied on semihard Ibores goat's milk cheese, although the effect of HP was less intense than in the present study. Saldo et al. (2000) apply several high-pressure treatments and it was not until 400 M Pa pressure-treatment was performed that the author detected a pH increasing shift in goat's milk cheese. Other type of cheeses were also affected by means of high-pressure regarding to pH levels, such as Gouda (W Messens, Dewettinck, Camp, & Huyghebaert, 1998; Winy Messens, Estepar-Garcia, Dewettinck, & Huyghebaert, 1999),

Camembert (Kolakowski, Reps, & Babuchowski, 1998) or ewe's milk cheese (B. Juan et al., 2004, 2007). The increase of pH could be due to the release of colloidal calcium phosphate into the soluble phase during the HP treatment (Johnston & Darcy, 2000), the inactivation of the starter culture as a result of HP treatment (Saldo, McSweeney, Sendra, Kelly, & Guamis, 2002), the higher protein hydrolysis in pressurized cheeses with consequent formation of hydrolysis products that could rise the pH (Guerzoni et al., 1999; W Messens et al., 1998) and the general increase in dissociation degree of ionizable groups, like carbonyl groups in protein systems, which may change concentration of free hydrogen ions, and hence, pH values (Sheehan et al., 2005). Needs et al. (2000) reported denaturation of casein micelles and release of micellar calcium due to high-pressure treatments. The fact that 100 and 200 MPa (and no 300 MPa cheeses) HP-cheeses showed similar pH values to the control cheese, suggests that probably these pressures are not capable to induce structural changes enough to cause such modification on protein matrix, by contrary, 300 MPa samples were much more affected by pressure, raising their pH values and maintaining them throughout ripening.

Referring to the moment of high-pressure treatment application, no significant differences (before or after salting) could be observed not at day 1 neither at the end of ripening in pH values. These results indicate that the pressure has a major effect than the moment of its application.

In relation to moisture content, it can be observed how moisture content decreased gradually by age in all samples (Table 4.1). As it was expected, this parameter was significantly ($P < 0.05$) affected by HP-treatment. Experimental cheeses obtained different values of moisture content than the control, especially 300MPa HP-treated cheeses which had higher ($P < 0.05$) moisture content in all sampling days. Differences in cheeses were dramatically greater at the end of ripening, especially in 300 MPa, which obtained values of BS: 37.881 g / 100 g cheese and AS: 33.234 g / 100 g cheese compared to the control cheese (25.70 g / 100 g cheese). These results are in line with those found by other authors in goat's milk cheese (Delgado et al., 2012; Saldo, McSweeney, et al., 2002) and other types of cheese like Camembert (Kolakowski et al., 1998), or ewe's milk cheese (B. Juan et al., 2007). They pointed out that different HP-treatments affected cheese, rising its pH and moisture content, just after the treatment and throughout the ripening. Further discussion about moisture content is developed in chapter V.

Generally, cheeses HP-treated before brining (BS) finished their ripening with higher moisture content than their counterparts after brining (AS), regardless to the intensity of pressure applied. Although the moisture content was similar or lower to their counterparts AS at days 1, 7 or 15, experimental cheeses HP-treated before brining (BS), at last stage of ripening, were capable to retain more water.

Results of fat and protein content are showed in Table 4.1 being both values expressed on dry basis. At the beginning of ripening, fat in dry matter of control cheese was 61.64 %, whereas mean fat content of HP-treated cheeses at day 1 ranged from 59.25 to 65.28 %. At day 30, control cheese contained approximately 66.53 % whereas fat content of pressurized cheeses ranged from 63.28 to 68.96 %. Oscillations on fat/DM content could be attributed to water removal due to HP-

treatments at day 1. It has been reported that alterations on cheese matrix could provoke differences in fat/DM content without a clear relation-ship with moisture content of each sample (Huppertz, Fox, & Kelly, 2004; Needs et al., 2000). Total protein content did not seem to be affected by ripening and neither by high pressure treatments. Protein values were similar whether at day 1 and day 30 and between samples undergone pressurization and the control cheese. Values of protein content at day 30 ranged from 33.30 to 41.87 % in pressurized cheeses and the control cheese showed 36.10 % of protein content.

4.2.2 Color analysis

Instrumental measurements of color in pressurized and control cheeses during ripening are shown in Table 6. CIE L* (lightness) and a* (redness) color values decreased in all samples by age. Nevertheless, b* value, related to yellowness, increased in all cheeses as ripening progressed. After the HP treatment, while 200 and 300 MPa samples remained with lower L* values, 100 MPa samples showed similar values than the control. Cheeses HP-treated at 100 AS MPa were the lightest cheeses throughout ripening (Table 4.2). However, no significant differences between the rest of pressurized and control cheeses could be observed at day 30. This fact could be related to the pH of samples. It is known that lower is the pH of cheese, whiter is the sample. Post-acidification manufacturing defects during ripening usually lead to whiter cheeses in core, which in fact, suppose lighter cheeses (Sheehan et al., 2005). Lower values of pH and moisture content of these cheeses, gave rise to changes in light scattering resulting the lightest cheeses.

HP-treatments seemed to distribute cheese samples in regards to redness/greenness (a*) values. Samples HP treated at 200 MPa obtained lower levels of a* value, 100 MPa cheeses were similar to the control and cheeses HP-treated at 300 MPa, showed up an increase of a* just after the HP treatment. Redness/greenness (a*) values in control cheese experimented an increase throughout ripening, whereas experimental cheeses viewed increased the values of this parameter until the period of time between days 7 and 15 and then decreased at day 30. While control cheeses ended the ripening showing the highest values (-0.656 *± 0.169*), 300 MPa cheeses viewed decreased significantly a* parameter reaching values of -1.250 *± 0.206* and -1.360 *± 0.123* in BS and AS moments, respectively. A similar trend during ripening was followed by pH in 300 MPa cheeses revealing maximum levels at day 15 of ripening. Therefore, a possible relation-ship between a* values and pH could exist on experimental cheeses. Higher pH values could be related to increased casein hydration altering light-scattering properties of HP cheeses (Rynne et al., 2008).

In general, there were significant differences in b* color value, leading to a yellowing effect because of HP-treatments (Table 4.2). The b* values of 300MPa treated-cheeses showed higher levels compared with control cheeses at the beginning of ripening and almost at each sampling day during ripening. Similar increasing values of b* were also reported by other authors (Capellas et al., 2001; Bibiana Juan, Ferragut, Guamis, & Trujillo, 2008; Okpala, Piggott, & Schaschke, 2010; Saldo, Sendra, & Guamis, 2002; Sheehan et al., 2005) in experimental HP-cheeses. As time passes, the curd absorb interstitial serum drops and occupies the now empty pockets before filled by

serum, decreasing incident light scattering and appearing darker (Johnston & Darcy, 2000; Paulson, McMahon, & Oberg, 1998; Rynne et al., 2008). Changes in b* value could be attributed to changes in the refractive index of cheeses leading to a color intensification.

Differences in color parameters such as increased yellowness or greenness could indicate that high-pressure treatments induced structural changes on the protein network of cheeses, promoting a denser matrix and hence, causing different refraction indices altering light-absorbing properties of pressurized cheeses. In this sense, several authors have attributed as well changes in color of goat cheeses to a vast effect of HP-treatments on the microstructure of cheese (Capellas et al., 2001; Saldo, Sendra, et al., 2002) (see section 2.4). According to the authors, a more hydrated and continuous protein matrix has been found in pressurized cheeses, fact that could explain the increase in yellowness of the cheese surface and thus different refraction index found between those samples and the control cheese.

Regarding to the moment of application of high-pressure treatments, BS cheeses generally showed lower values of a* (except for 300 MPa cheeses) and higher values of b* showing increased values of greenness and yellowness compared to the control cheese and the rest of pressurized cheeses at the end of ripening. These differences were significant ($p > 0.05$) in most of cases.

Total color differences were calculated between each sample and the control cheese at day 1 and at day 30 (Table 4.2). It has been reported that ***ΔE**** values from 3 upwards are obvious for the human eye and conversely, values downwards 1 are not that obvious by naked eye. Table 4.2 shows that both 300 MPa (BS and AS) samples at day 1 were the only ones cheeses which accounted values above 1 regarding total color differences (1.13 and 1.60, respectively). Therefore it can be concluded that 300 MPa cheeses were the most different in regards to color differences compared with the control cheese. At day 30, was the difference between 100BS and the control, which showed the higher value (5.03). However, 300MPa (BS and AS) showed again high values of ***ΔE*,*** close to 4, which is in line with significant differences found at day 30 between these cheeses and the control in color parameters studied.

4.2.3 Water soluble nitrogen (WSN) and free amino-acids (FAA) assessment

Measurements of water-soluble nitrogen (WSN/TN) and free amino acids (FAA) performed on pressurized and control cheeses throughout maturation are shown in Table 4.3.

Primary proteolysis results mainly from the action of plasmin, chymosin, and to a lesser extent by pepsin, which are responsible for the initial hydrolysis of caseins in milk. This parameter is measured as the water soluble nitrogen fraction and includes proteins (excluding all caseins), peptides, amino acids and smaller N compounds, such as amines, urea and ammonium. The ratio of water soluble nitrogen and total nitrogen (WSN/TN) has frequently been used as a 'ripening index' for cheese as it reflects the proteolysis extent (Kuchroo & Fox, 1982).

The level of pH 4.6 SN expressed as percentage of total N, showed increasing values of WSN/TN throughout ripening, either in control and HP-treated cheeses. Generally, pressure applied influenced WSN/TN of cheeses at the end of ripening showing significant differences in all samples (except for 200 AS). During first and mid stages of ripening (days 1, 7 and 15) oscillating values of WSN/TN were found respect to the intensity of the pressure applied. The mean level of WSN/TN in the experimental cheeses over the 30-day ripening period was higher than that found in the control cheese (BS: 20.10 % and AS: 19.74 % compared to 16.49% in control cheese). While at day 1 the samples that showed major values were 100 BS and AS (8.41 *± 1.20* and *8.51 ± 1.54,* respectively*),* at days 7, 15 and at the end of ripening were 300 MPa samples the ones that showed highest values. It is noticeable, that 300 MPa AS HP-treated cheese changed its tendency during ripening from containing the lowest value (7.84 ± *1.24*) among pressurized cheeses at day 1, to show the highest level of WSN/TN at day 30 (22.96 ± *1.06*) compared with all samples. Additionally, 300 MPa BS cheese also showed a great change between day 1 and 30, increasing its WSN/TN value more than a 135 %. At the end of ripening, experimental cheeses (except for 200 MPa AS) reached higher values of WSN/TN than the control cheese, especially 300 MPa cheeses. As it is shown in Table 7, at day 30, 100 and 300 MPa AS obtained higher values than their counterparts' pressurized cheeses before brining (BS). However, no clear tendency was observed in the ratio WSN/TN respect to the moment of brining. Pressurized cheeses did not seem to be influenced by the moment of brining respect to the HP application.

Results in this study match with those obtained in other works. Delgado et al. (2012) who studied the effect of HP on goat's milk cheese found that both maturation and HP treatments affected the ratio WSN/TN increasing these values compared to the control cheese. Juan et al. (2004, 2007) also observed an increase of this ratio in all cheeses, especially in HP-treated samples applying a wide spectrum of pressure conditions on ewe's milk cheese, including those used in the present study. This author observed that moderate pressure treatments (300 and 400 MPa) enhanced primary proteolysis (WSN/TN ratio). Levels of pH 4.6 SN/TN in pressurized Cheddar blocks (50 MPa, 72h at 25ºC) at several days after the manufacture, obtained as well higher levels than control cheeses in the study carried out by O'Reilly et al. (2000). Kolakowski et al. (1998) and Rynne et al. (2008) also found slightly increased values of WSN in HP-treated Camembert cheese (50MPa, 4h) and in HP-treated cheddared cheese-blocks (400 MPa, 10min), respectively.

At the beginning of ripening process, proteins are degraded to macropeptides by rennet or its substitutes. In the next step, microbial peptidases degrade macropeptides down to low-molecular peptides and amino acids. In the present study several factors could influence the enhancing of primary proteolysis in pressurized goat's milk cheeses. Since high pressure can influence proteolysis process throughout destabilization of casein micelles, which can enhance its sensibility to proteolytic enzymes, it is not surprising that higher WSN/TN values were obtained in pressurized samples compared with the control cheese. It is known that high-pressure could have weakened hydrophobic interactions, which might have led to an increased exposure of susceptible bonds that are cleavable by proteolytic enzymes. Besides, the changes in protein conformation

(see section 2.4), enzyme activation by pressure due to higher amount of moisture content in pressurized cheeses, could be some of the factors accelerating proteolysis.

Secondary proteolysis results mainly from the action of starter peptidases, which degrade peptides and produce free amino acids (FAA). A significant increase of FAA levels was found in all samples, including the control, during the ripening of cheeses (Table 4.3). This increase was greater ($p < 0.05$) in pressurized than in control cheeses, attaining mean values at day 30 of 3.23 and 2.75 mg leucine/g, respectively.

As it can be observed in Table 4.3, immediately after the pressure treatment was applied, the FFA values of 300 MPa HP-treated cheese samples were higher and proved significant ($p < 0.05$) compared to the rest of pressurized and non-treated cheeses samples. As cheese aged, 300 MPa BS and AS cheese samples followed the same FAA profile, showing higher levels than the control cheese at the end of ripening (day 30). Two groups of samples can be identified based on results of FAA throughout ripening. While 100, 200 MPa and control cheeses seemed to increase their FAA values, and hence start the proteolysis at day 15, 300 MPa cheeses reached at day 7 values even higher than those of the rest of samples at day 15. Based in these results, it can be drawn that proteolysis in the first group did not start until day 15 of ripening while cheeses 300 MPa HP-treated started this process 7 days before. The higher moisture content reached in cheeses of this study could account for high FAA in experimental cheeses (Saldo, McSweeney, et al., 2002).

Additionally, it is noticeable that 300 MPa seemed to attain different values at the end of ripening regarding to the moment of HP application whether before or after brining (Table 4.3). Cheeses HP-treated at 300 MPa BS showed lower values in each sampling day than its counterpart HP-treated after brining (300 MPa AS) which reached 1.42–fold FAA level. This fact could lead to suggest a synergistic effect between the content of salt at the moment of pressurization and the intensity of the pressure applied.

According to the scientific literature, it seems that HP processing at a given pressure induces rising values of FAA on cheeses. In this sense, Juan et al. (2007) pointed out that higher amount of FAA were found in cheeses treated at moderate pressures (300MPa) compared with the control cheese. However, other authors working at higher pressures have reported kind of deceleration of secondary proteolysis cheeses by means of high-pressure.

Most of the enzymes capable to degrade peptides into amino acids require autolysis of starter bacteria to be released into the cheese matrix, since they are intracellular enzymes. The effect of HP treatments on microorganisms, causing structural and functional alterations, and disrupting or increasing permeability of the membrane would enhance the release of intracellular enzymes and enhance proteolysis of studied cheeses. Several authors (B Juan et al., 2007; Rynne et al., 2008; Saldo, McSweeney, et al., 2002) have pointed out the acceleration of proteolysis of cheese due to the HP treatments which is consistent with the results found in this study.

4.2.4 Texture analysis

Table 4.4 shows the results of the uniaxial compression test applied to control and HP cheeses to study both fracture stress ($\sigma_{(t)}$) and fracture strain (ε) values and determinate texture changes in goat's milk cheese induced by HP treatments. All samples were influenced by age ($P < 0.05$) increasing its fracture stress ($\sigma_{(t)}$) values as maturation progressed. At day 15, all samples (except 300MPa) increased almost 3 times their values of fracture stress ($\sigma_{(t)}$) compared to day 1. These values continued rising until the end of ripening.

Immature cheese is more sensitive to changes induced by pressure treatments, therefore it is not surprising that at day 1, a clear effect of pressure can be observed in goat's milk cheeses. At the beginning of ripening, HP-processing seemed to classify all samples in two groups. Pressure seemed to not affect fracture stress ($\sigma_{(t)}$) values in 100 MPa samples at the beginning of ripening (days 1, 7 and 15), as their values were very similar to control cheeses. On the other hand, higher-pressure treatments (200 and 300 MPa) affected fracture stress values modifying them since day 1 onwards. Samples HP-treated at 200 MPa showed increasing values from the beginning of ripening, being the highest ones at day 30. Samples HP-treated at 300 MPa invested their trend showing higher values of fracture stress than the control at day 1 and 7 and change it into the lowest ones at days 15 and 30. Samples of 300MPa both BS and AS got the lowest values of fracture stress (84.453 *± 25.688* and 86.974 *± 7.965*, respectively) at day 30 while 100 and 200 MPa AS reached the higher ones, even more than the control (Table 4.4). At the end of ripening, all pressurized cheeses showed significant differences compared with the control cheese, that is, 100 and 200 MPa HP-treated samples attained higher values and in contrast, 300 MPa samples showed stress values below the control values. Significant differences referring to high-pressure treatments at different moments of brining appeared in 100 and 200 MPa cheeses. In both cases HP-treated samples after brining (AS) resulted in lower fracture stress (σ_{t}) values at day 30 of ripening.

Several authors (B. Juan et al., 2007; Saldo et al., 2000) have found general increases of stress values when high-pressures were applied, attributing this fact to the removal of water just after the high-pressure treatment. In this study, the contrary effect occurs since pressurized cheeses retained major amounts of water and showed a better rearrangement of internal water profile (see chapter V). In agreement with results found in the present study in 300 MPa HP-treated cheeses, other researchers have found a decrease in cheese hardness as pressure was increased. Delgado et al. (2012) applied pressures of 400 and 600 MPa on Ibores raw goat milk's cheese, and observed how cheeses pressurized at day 1, after 60 days of ripening, became less firm than the control. Koca et al., (2011) also observed a significant decrease in hardness, when applied pressures from 200 MPa (15 min) to 400 MPa (5 and 15 min) on cheese, and Serrano et al. (2005) found similar lower hardness results applying pressures of 345 and 483 MPa (3 and 7 min) to Cheddar cheese. Thus, water loss could not explain the results and pressure seemed not to have the same effect applied at different scales. Intensity pressure at which the HP-processing is applied may determine increase or decrease of fracture stress of cheeses compared with the control. In the present study, at the end of ripening, pressures of 100 and 200 MPa caused higher

stress values than the control cheese, while 300 MPa HP-treatments led to lower values of stress on goat's milk cheeses. The change of tendency in 300 MPa samples could be related to the different moisture content in these cheeses between day 1 and day 30. At the beginning of ripening, 300MPa BS samples were the cheeses with lower moisture content (Table 5), and also the most affected by high-pressure in stress value (Table 4.4). Probably the compacting effect by pressure and the consequent water expulsion, could contribute to the higher fracture stress compared to the control cheese at day 1. Nevertheless at the end of ripening those samples seemed to show the highest water content and at the same time seemed to be the less fractural (lower values of $\sigma_{(t)}$). In this sense, cheeses with higher moisture content (300 MPa cheeses) could undergo significant modifications on textural properties when they were pressurized. Pressures above 200 MPa induced rupture of non-covalent interactions within protein molecules, which result in denaturation and re-aggregation of proteins in a new conformation resulting of a lower stress. Positive correlations were found in Cheddar cheese between the content of intact casein and fracture stress (Guinee, Auty, & Fenelon, 2000) so it is expected to find lower fracture stress values on pressurized samples.

Table 4.4 shows the strain (ε) values of HP-treated and control samples during ripening. Strain (ε) values decreased significantly in all samples over the 30-day ripening period. This fact is related to the water loss during ripening leading to less deformable cheeses. Evidence of dramatic differences between samples caused by the HP treatment can be observed at day 1. Comparison of pressurized cheeses to the control showed how samples HP-treated at 100 and 200 MPa showed similar strain (ε) values, while 300 MPa treatments caused increase of strain (ε) values by 26 %. Most of scientific studies observed that possible differences on textural parameters caused by pressure treatments at day 1, disappear throughout ripening (M. N. Buffa, Trujillo, Pavia, & Guamis, 2001; Delgado et al., 2012). However, this does not occur in the present work, where HP effect is maintained until the end of ripening affecting the final texture characteristics of cheese. Same tendency can be observed at day 30 of ripening, when strain (ε) values of 300 MPa cheeses differ significantly from the rest of samples including the control cheese, being dramatically higher. Cheeses high-pressure treated at 300 MPa only decreased their values by 27.1 % (300 BS) and 41.42 % (300 AS) along ripening while 100 and 200 MPa cheeses and control cheeses at day 30 only attained less than 50% strain values compared to day 1. Significant moisture, pH and FAA values of 300 MPa cheeses could be related to high strain values of these cheeses whether at day 1 or at the end of ripening.

No significant differences could be attributed to the moment of HP treatment application in 100 and 200 MPa pressurized samples. However, 300 MPa cheeses, which showed a significant effect on strain caused by pressure, were likely to display differences upon the moment of HP application. Cheeses pressurized before brining (BS) showed higher strain values during all ripening (except for day 15) compared to those pressurized after salting (AS). Again, pH and moisture could be the fact that boosted increased values of strain in BS cheeses.

An increase in strain (ε) following HP treatment of cheese has previously been reported for goat and Garrotxa-type cheese (M. N. Buffa et al., 2001; Saldo et al., 2000) and ewe's milk cheese (B.

Juan et al., 2007). However, this phenomenon attained dramatically higher values in the present study compared with those reported on aforementioned works, both at day 1 and throughout ripening.

Phenomenons occurring during ripening have a profound effect on cheese texture. Several factors could determine variations in strain (ε) texture parameter. Cheese softening over time can be defined in a simple manner by two processes that take place during ripening; the solubilisation of calcium bonds and proteolysis. Additionally, free oil coming from fat globules rupture due to high pressure treatment, confers specific functional properties to cheese like enhanced meltability, spreadability and better mouthfeel. Proteolysis and solubilisation of calcium leads to higher strain values in cheese, which in turn results in higher level of deformability. This is consistent with pH, moisture content and free amino acids (FAA) values reached by 300 MPa cheeses, which obtained the highest scores in all cases. Higher levels of pH could be caused by slow solubilisation of colloidal calcium phosphate or by formation of alkaline compounds derived by the breakdown of protein (Koca et al., 2011). Creamer and Olson (1982) already pointed out that an increase in the pH from 4.9 to 5.4 resulted in a linear increase in strain (ε). Moisture is another important factor that, being redistributed after HP treatment could modify as well texture of cheese, promoting the softening of the paste (Sheehan et al., 2005). Buffa et al. (2001) already pointed out that strain decreases as ripening progresses probably due to a loss of elastic structural elements and to the decrease of the amount of available water for solvation of protein. Higher values of moisture content have negative correlations with fracture stress and are positively correlated to strain (ε) (Guinee, 2002). One of the major effects derived from proteolysis is the formation of FAA, the weakening of bond strength in protein matrix and the reduction of density diminishing cross-links and interactions between the casein structures (Everett & Auty, 2008). The lessening of molecular interactions and the reduction in bond calcium lead as well to higher values of strain and the softening of 300 MPa cheeses texture.

Great values of strain indicate more deformable cheeses, a softening in the paste and better texture characteristics. As well as 100, 200 and control cheeses showed a high rate of hardening, conversely, 300 MPa cheeses showed low stress values related to firmness of cheese and high strain values, which are related to this softening of the paste throughout ripening. This fact could be specially appreciated in goat's milk cheeses which are usually involved in very fast drying processes during ripening resulting in dry and hard cheeses, sometimes rejected by consumers.

According to our results, pressure effect seems to be much more important than the moment of brining, which is completely diluted at the end of ripening. However, cheeses showing lowest values of strain (ε) or shortest textures, (e.g. 100 and 200 MPa samples) are also those showing lowest moisture, FAA content and pH values. Nevertheless, in spite of differences found referring to the pressure treatment intensity, no changing values seemed to appear between cheeses HP-treated before and after brining related to textural parameters studied (($\sigma_{(t)}$) and (ε)).

All samples, (excep for 300 MPa) appeared as firmer, harder and shorter than those pressurized at 300 MPa, which showed a softer, more deformable and longer texture. Pressures of 100 and 200

are likely unable to provoke enough changes in protein matrix to augment its elasticity and modify enough textural parameters compared to the control cheese. The ratio of hardening was also higher in control, 100 and 200 MPa samples while cheeses high-pressure treated at 300 MPa followed a progressive ripening attaining optimum values of stress and strain.

4.2.5 Microstructure analysis

The confocal laser scanning microscopy (CLSM) was applied to study the effect of HP-treatments on microstructure of goat's cheeses. Micrographs of 100, 200, 300 MPa treated-cheeses before (BS) and after salting (AS) and ripened for a 30-day period are shown in Figure 4.1. Additionally to the visual observation of micrographs, quantification of several parameters such as total number of objects, percentage of total lipidic area or percentage of porosity was carried out (Table 4.5).

As it was expected, ripening had a profound effect on goat's cheese microstructure. Generally, at day 1 micrographs showed a continuous phase of protein matrix (green area) with more or less fat globules of different shape (red objects) (Figure 4.1). At days 7 and 15 of ripening (data no shown), protein matrix looked more heterogeneous varying between samples and being more open giving rise to the apparition of first cavities and larger fat globules, probably due to a fat coalescence phenomenon. Then, at day 30, protein matrix appeared well defined, revealing a denser structure by compaction of protein aggregates. An open structure was revealed to appear at the end of ripening due to the loss of moisture. Other authors found similar results respect to the uniformity of protein fraction of cheese during ageing (M. Buffa, Trujillo, Pavia, & Guamis, 2001; Koca et al., 2011; Picon et al., 2013).

Observation of micrographs (Figure 4.1), indicated that high-pressure had a significant effect on the microstructure of goat's milk cheese. It is likely that after the HP application (day 1), 100 MPa showed up a microstructure quite similar to that of control cheese, with a disperse protein matrix and numerous entrapped fat globule within. However, micrographs of 200 and 300 MPa were similar between them, showing a denser protein matrix with larger fat drops. Some cavities can be observed in these samples images, probably due to serum removal just after the HP-treatment. Micrographs changed by 30-day period in all samples, showing an increase of cavities and large pores compared with the beginning of ripening. The trend to formation of amorphous voids (black areas) with ageing of cheese has been described in earlier studies (El-Zeini, 2006; Romeih, Moe, & Skeie, 2012). An increase of the cavities size occurs with ageing of cheese probably because of a weakening of paracasein matrix caused by proteolysis or CO_2 production by microorganisms.

At day 30 control accounts for a sponge-like and soft structure and the 100 and 200 MPa cheese samples showed a coarse and porous matrix. The sample which appeared as the most different was 300 MPa cheeses giving rise to a more homogeneous matrix, more porous (300 MPa BS) and more compact (300 MPa AS).

Number of objects. Results of the number of objects counts did not show any clear trend respect to the high-pressure treatment applied at day 1. Quantification results (Table 4.5) does not show significant differences at day 1 compared to the control cheese except for 200 BS and 100 AS samples, which showed higher and lower values, respectively. However, at day 30, the values found in 300 MPa AS cheese became significant (20189 *± 1277.*87), and showed a great increase compared to the control cheese and the rest of pressurized samples.

Comparing results of cheeses treated before and after salting, a tendency of more numerous objects could be observed in BS cheeses at day 1. However, these differences trailed off with ripening and were no longer observed at day 30. According to Everett and Auty (2008) unsalted cheese has larger pockets of serum phase. Pressure effect is closely linked to moisture content in cheese matrix, indeed the higher is the moisture content, the higher is the pressure effect. Therefore, a dryer protein network of AS cheeses during the HP processing could explain the lesser effect of HP on them. This confirms the macroscopic visual evidence that can be observed in Figure 4.2. Applying pressure treatments before the brining gave rise to a brilliant, smooth, and compacted surface of cheeses, while samples pressurized after brining appeared with a wrinkled, plenty of mechanical holes and cavities and porous surface. At day 1, BS cheeses are likely they have been dramatically affected by pressure softening their surface while AS cheeses appeared very similar and as rough-surfaced as the control cheese. Serrano et al., (2004) already observed in Cheddar cheese that an increase in surface smoothness due to HP-treatments, could enhance shredding ability of cheese, which in turn could be related to the higher fracture strain values obtained in the present studies.

No significant differences could be observed in regards to the number of objects and the moment of high-pressure treatments application. Pressure intensity caused a major effect on cheeses than their condition of salted or unsalted cheeses at the moment of HP processing.

Total lipidic area. Red areas of micrographs were identified and quantified giving rise to the parameter total lipidic area of control and pressurized cheeses expressed in percentage of each image (Table 4.5). At day 1, a general reduction of the total area of lipids seemed to appear in pressurized samples, although only 200 and 300 MPa BS showed significant differences. These results are in line with CLSM micrographs (Figure 4.1) which showed a decrease of red areas, which correspond to fat globules, in 200 and 300 MPa cheeses. As figure 4.1 shows, high pressure caused an apparent disappearance of fat, especially in 200 and 300 MPa treated samples while control and 100 MPa cheeses showed large areas of fat globules distributed by all area of image. Probably 100 MPa treatment application was not enough to disrupt fat globules whereas 200 and 300 MPa caused the rupture of fat globule membrane and crumbled them giving rise to posterior smaller size fat globules formation becoming those ones hardly visible by this analysis technique (Everett & Auty, 2008).

Since fat globules physically interfere with the whole structure of the cheese casein matrix, smaller globules are more likely to fit into small voids and are better retained within the curd structure. Larger globules are easier to deform and to rupture, producing free oil drops not bind to the

matrix (Everett & Auty, 2008). On the contrary, small globules are much harder to deform and rupture and less likely to disrupt casein matrix, leading to firmer cheeses. In this sense, the fact that higher percentages of total lipidic area were found in 300 MPa cheeses at day 30 (Table 4.5), could be related to strain values and a plasticization effect caused by fat. These results are in line as well with the higher fat content obtained by these samples. Ong et al., (2011) have suggested that chemical bonds may exist between the fat globule membrane components and the protein matrix. This might allow for more rearrangement to take place in the cheese matrix, favoring the formation of a more homogeneous and compact structure in cheeses owing higher lipidic content.

A possible effect of the moment of HP treatment application could arise at day 1 in 200 and 300 MPa cheeses, revealing BS samples with significant lower percentage of total lipidic area. However, these differences were trailed off during ripening, when the effect of the moment was diluted and the pressure intensity appeared as having larger influence on total lipidic area percentage.

Porosity. Porosity (black areas) of samples was measured respect to the total area of image. Percentage of porosity increased in all samples during ripening showing different percentage of increment upon the treatment applied (Figure 4.1). There is a positive relation-ship between the loss of moisture and the increase of porosity during ripening in goat's cheeses. Loss of water, increase of proteolysis and concomitant weakness of protein matrix and CO_2 production by microorganisms could be the origin of gradual pockets formation (Aminifar & Emam-Djomeh, 2014; Romeih et al., 2012). Furthermore, results derived from visual observation of micrographs and quantification of porosity by image analyses at day 30 are fully aligned which confirms the reliability of the used technique.

Numerical results did not show a clear tendency at day 1 regarding to the porosity of samples, not related to the effect of HP on cheeses and neither to the moment of HP application (Table 4.5). High porosity of cheeses could lead to more heterogeneous matrix, which could account for the high standard deviations obtained (Rovira, Garcia, Laencina, & Belen Lopez, 2013). However, studying the percentage increment of porosity between days 1 and 30, a noteworthy phenomenon was observable. Samples HP-treated at 300 MPa, reached lowest values of porosity at day 30 compared to the rest of samples including the control cheese. A synergistic effect between pressurization and passage of time could be observed, especially in 300 MPa cheeses. Although 300 MPa cheeses showed little more porosity than other cheeses at day 1, probably due to the empty holes previously occupied by serum pockets, at the end of maturation (day 30) these samples changed their trend and got the lowest values of porosity. It means that final microstructure of 300 MPa cheeses became denser, much more compact and with a regular well-formed protein network. Picón et al., (2013) studied microstructural characteristics of Hispánico cheese made using HP-treated (200-500 MPa for 10 min) curds. According to our results, the authors found that the distribution of pores in 200 MPa HP-treated curds resembled that of non-pressurized curd, but higher pressures (300 and 400 MPa) gave rise to a more homogeneous distribution and a continuous protein network. In the present study, further visual inspection of micrographs (Figure 4.1) indicated that 100 and 200 MPa had an irregular distribution of pores

similar to this showed by the control, and a coarse and open structure was obtained in these samples. Nevertheless, 300 MPa HP-treated cheeses, especially AS cheese, revealed a homogeneous systematic protein aggregate network. The protein matrix appeared as a smooth continuous phase of aggregated micelles, characterized by a compact fusion and a dense structure. Control, 100 and 200 MPa cheeses, which showed high values of porosity at the end of ripening also account for a great moisture loss.

According to Rovira et al., (2013), porosity is one of the parameter most related with visible differences in curd microstructure. Therefore, results obtained in this study in regards to microstructure, indicates that it is likely high-pressure treatments caused a compaction of the protein matrix reducing its porosity, and leading to a denser network, especially in 300 MPa high-pressure treated cheeses. Quantification analyses confirm the visual evidence previously showed in Figures 4.1 and 4.2. The effect of the pressure intensity seemed to be larger than the condition of salted or unsalted cheeses at the moment of HP processing.

4.2.6 Sensory analysis

The mean scores awarded by panelists to the sensory attributes evaluated in pressurized and control goat's milk cheeses are shown in Table 4.6. Organoleptic properties were evaluated in 3 groups; appearance: described by the color, texture: described by firmness, mouthfeel (moisture feeling on mouth) and granularity and the taste: described by sourness and bitterness. These parameters were only evaluated at day 30, being that time the optimum ripening point expected for this kind of cheese.

Generally, pressurized cheeses showed increasing values of yellowness compared with the control, although only significant ($P < 0.05$) differences were obtained by 300 MPa cheeses in panelists observations. It is noticeable, that correlation between sensory assessment and instrumental determination of color parameters was high. Instrumental assessment of color showed up a general increase of b* value (yellowness) (Table 4.6) in pressurized cheeses and pointed 100 MPa AS as the lightest sample, which in turn was as well punctuated by panelists with lower scores of color intensity.

Additionally, samples of 100 BS and 300 BS and AS were the ones which showed highest scores of color by panelists and also were the samples which showed higher negative values of a* parameter, which could be related to this darkening effect of cheeses. Delgado et al (2012) have found an increase of yellowness in pressurized Ibores goat's milk cheese as well. The author pointed out that issue as a negative aspect for consumer acceptance taking into account the appreciated white color in goat's milk cheeses. However, in our study any panelists indicated it as a negative characteristic probably because cheeses had the appearance more likely to a more ripened cheese compared to control cheese.

Cheeses high-pressure treated at 300 MPa received a significantly higher overall grade than did other samples, mostly with respect to textural parameters. Textural attributes, namely firmness mouthfeel and granulosity, showed dramatic ($P < 0.05$) differences scored by panelists in 300 MPa samples compared to the control cheese. Firmness and granulosity showed very low values in these cheeses while the mouthfeel notes increased respect to the rest of cheeses including the control (300 BS: 5.75 ± 1.65; 300 AS: 5.33 ± 1.55). In other studies, control cheeses also obtained higher values of hardness or firmness, and lower elasticity scores (Alonso et al., 2011).

In the present study this fact could be attributed to two phenomenon that take place during ripening; the major loss of water and at the same time, lower values of proteolysis index found in control and 100 and 200 MPa cheeses. High values of hardness and firmness could be related to the strengthening effect of moisture loss and the concomitant concentration of cheese solids and compaction of texture. Higher strain values could contribute to high results of mouthfeel, thus high elasticity sensation, creating a longer texture. Proteolysis has been reported as the main cause of loss of integrity of the casein network, with the subsequent weakening effect on the cheese texture (Alonso et al., 2011). Higher values of proteolysis in 300 MPa cheeses could enhance as well mouthfeel attribute and softening of cheese.

Regarding to the taste parameters, namely acid taste and bitterness, again 300 MPa cheeses revealed significant differences compared with the rest of samples including control cheese. These samples obtained the lowest scores in acid taste by panelists in both BS (1.83 *± 0.93*) and AS (2.75 *± 1.91*) samples. Higher pH and proteolysis values are two factors that contribute utterly to decrease of acid taste. Bitterness viewed decreased their values in pressurized cheeses (except for 100 BS) compared with the control cheese (Table 4.6). However, these differences were only significant in 300MPa cheeses, showing lower values than the rest of cheeses including the control. Saldo et al. (2000), reported bitter notes in HP-treated cheeses, especially in 400 MPa treated-cheeses that were evidenced by panelists. An excessive concentration of low-molecular-weight, mainly hydrophobic peptides, which are accumulate during ripening, has been reported as a cause of bitter notes on cheeses (Le Quéré, 2011). According to our results, it seems like enhanced ripening, especially in 300 MPa cheeses did not conferred bitter notes to these cheeses, on the contrary those ones showed the lowest values of this sensory attribute. Pressure intensity could be a key element to control in order to avoid the accumulation of peptides conferring bitter notes.

Panelists were asked for indicate some general comments they found noteworthy in cheeses. In general terms they described control, 100 MPa and 200 MPa BS and AS as firm, acid, crumble, chalky and poor flavored samples while 300 MPa obtained better overall impression respect to the texture and flavor. Panelists pointed out the enhanced characteristics of these cheeses giving less importance to the fact that they did not release specific but strong goat's cheese odor anymore. Low firmness, high mouthfeel and the lack of acidic aftertaste were the sensory attributes that contributed more to the rating of 300 MPa cheeses.

4.3 Conclusions

The application of HP technology in goat's milk cheeses modified some of the physico-chemical properties of goat's milk cheeses. Especially 300 MPa HP-treatments, which rose up the pH value and the moisture content of cheese throughout ripening. Additionally, this HP treatment caused the highest color differences leading to a yellowing effect of goat's milk cheeses, which in turn appeared as much more ripened cheese than others. Proteolysis of goat's milk cheeses was increased by HP-treatments, especially when 300 MPa treatments were applied, which caused a higher amount of WSN/TN and FAA leading to much more proteolyzed cheeses compared with the control and the rest of pressurized cheeses at the same point of ripening.

In relation to textural parameters, pressurized cheeses at 300 MPa were less firm and account for higher strain values compared with the control and the rest of pressurized cheeses, resulting in less hard and more elastic goat's milk cheeses. Significant differences referring to high-pressure treatments at different moments of brining appeared in 100 and 200 MPa cheeses. In both cases HP-treated samples after brining (AS) resulted in lower fracture stress (σ_t) values at day 30 of ripening.

Microstructure of goat's milk cheeses could be studied by quantification of micrographs and well correlated to visual observations. At the end of ripening, higher lipidic area and lower porosity were two characteristics attributed to 300 MPa cheeses compared to the rest of cheeses including the control, which could account for an enhanced texture, lower fracture stress, higher strain and better mouthfeel as punctuated by panelists in sensory analyses.

Sensory analyses results confirmed instrumental measurements of color, moisture content and texture of cheese.

Although the intensity pressure effect was higher than the moment of application, higher moisture content, strain and porosity could be observed in BS samples. This fact could be probably due to a higher effect of HP on these cheeses because of an absence of salt and the possible dumping effect of it during HP processing.

In conclusion it seems that novel textures and flavors could be developed by HP processing applying 300 MPa HP-treatment more specifically. This technology may provide new textures to traditional cheeses or even the possibility to create novel types of cheese.

Table 4.1. Mean values ± *standard deviation* (n = 6) of physic-chemical composition (pH, moisture, fat and protein content) of control and pressurized (100, 200 or 300 MPa for 5 min at 14 º C) goat's milk cheeses before (BS) or after (AS) salting, analyzed during ripening (days 1, 7, 15, 30)

	Day	C	100 BS	200BS	300BS	100AS	200AS	300AS
pH	1	4.885 ± *0.151*^{b}	4.838 ± *0.106*^{a}	4.832 ± *0.122*^{a}	5.122 ± *0.193*^{c}	4.830 ± *0.109*^{a}	4.882 ± *0.138*^{b}	5.127 ± *0.109*^{c}
	7	4.690 ± *0.164*^{d}	4.585 ± *0.067*^{a}	4.623 ± *0.049*^{b}	5.023 ± *0.170*^{e}	4.657 ± *0.109*^{c}	4.632 ± *0.124*^{b}	5.030 ± *0.242*^{e}
	15	4.822 ± *0.107*^{b}	4.778 ± *0.059*^{a}	4.885 ± *0.153*^{c}	5.298 ± *0.250*^{e}	4.768 ± *0.105*^{a}	4.782 ± *0.100*^{a}	5.260 ± *0.260*^{d}
	30	4.848 ± *0.017*^{a}	4.895 ± *0.057*^{b}	4.870 ± *0.032*^{a,b}	5.118 ± *0.217*^{d}	4.888 ± *0.076*^{b}	4.897 ± *0.089*^{b}	5.123 ± *0.218*^{d}
Moisture	1	51.089 ± *1.894*^{c}	51.274 ± *1.784*^{c}	51.474 ± *1.976*^{d}	50.118 ± *0.419*^{a}	50.048 ± *0.743*^{a}	50.430 ± *2.709*^{b}	51.901 ± *1.908*^{e}
	7	43.807 ± *1.826*^{b,c}	42.476 ± *2.672*^{a}	43.697 ± *2.602*^{b,c}	46.611 ± *0.886*^{d}	43.147 ± *2.688*^{a,b}	44.219 ± *2.214*^{c}	46.555 ± *4.726*^{e}
	15	39.037 ± *3.271*^{d}	35.869 ± *2.130*^{a}	38.996 ± *3.475*^{d}	42.317 ± *1.047*^{e}	37.351 ± *1.645*^{b}	38.278 ± *0.231*^{c}	42.549 ± *1.079*^{e}
	30	25.704 ± *2.405*^{b}	29.620 ± *8.037*^{d}	26.203 ± *4.139*^{c}	37.881 ± *6.852*^{f}	24.795 ± *2.786*^{a}	25.714 ± *3.614*^{b}	33.234 ± *2.822*^{e}
Fat / DM	1	61.640 ± *1.35*	59.250 ± *2.96*	64.620 ± 3.*90*	59.465 ± *7.34*	59.385 ± *1.07*^{a}	65.289 ± 5.*35*	62.154 ± 6.*49*
	30	66.530 ± *7.41*^{b}	65.080 ± 1.*40*^{b}	68.967 ± *3.84*^{b}	68.493 ± 2.*05*^{b}	66.732 ± 2.*02*^{b}	63.280 ± *5.37*^{a}	67.190 ± *1.72*^{b}
Protein / DM	1	35.149 ± *2.659*^{a,b,c}	36.460 ± *3.071*^{c,d}	37.595 ± *2.522*^{d,e}	36.287 ± *1.477*^{b,c,d}	34.014 ± *3.488*^{a}	34.882 ± *4.947*^{a,b}	38.559 ± *1.857*^{e}
	7	35.553 ± *2.121*^{b}	33.978 ± *3.916*^{a}	36.024 ± *3.739*^{b}	36.808 ± *2.896*^{b}	35.641 ± *3.205*^{b}	36.084 ± *3.057*^{b}	39.180 ± *7.701*^{c}
	15	36.716 ± *4.212*^{b}	36.537 ± *2.422*^{b}	36.893 ± *2.875*^{b,c}	37.521 ± *3.202*^{b,c}	35.263 ± *3.991*^{a}	37.903 ± *1.413*^{c}	36.875 ± *3.784*^{b,c}
	30	36.100 ± *1.627*^{b,c}	40.258 ± *2.055*^{d}	33.300 ± *4.829*^{a}	41.872 ± *4.300*^{d}	35.080 ± *2.498*^{a,b}	33.880 ± *2.197*^{a}	36.352 ± *1.988*^{c}

^{a,b,c,d,e} : Superscript letter in the same row indicates significant ($p < 0.05$) differences.

Moisture (g / 100 g cheese). Fat / DM: fat in dry matter (g / 100 g DM). Protein / DM: protein in dry matter (g / 100 g DM)

BS: before salting. AS: after salting. C: control.

Table 4.2. Mean values ± *standard deviation* of color in control and pressurized (100, 200 or 300 MPa for 5 min at 14 º C) goat milk cheeses before (BS) or after brining (AS) analyzed during ripening (days 1, 7, 15 and 30). Delta of color (*ΔE*), calculated as differences in color measures between indicated samples, is also indicated at the end of the table.

	DAY	C	100 BS	200BS	300 BS	100 AS	200 AS	300 AS
CIE L*	1	94.222 ± *0.122*^{c}	94.320 ± *0.246*^{c}	93.820 ± *0.252*^{b}	93.700 ± 0.277^{b}	94.240 ± *0.074*^{c}	93.604 ± *0.280*^{a,b}	93.286 ± *0.411*^{a}
	7	93.504 ± *0.235*^{d}	92.928 ± *0.503*^{c,d}	92.268 ± *0.260*^{a,b}	92.176 ± 0.148^{a,b}	92.042 ± *0.994*^{a,b}	92.416 ± *0.249*^{b,c}	91.784 ± *0.232*^{a}
	15	91.422 ± *0.527*^{e}	90.804 ± *0.443*^{d}	90.354 ± *0.340*^{b,c}	90.646 ± 0.105^{c,d}	91.550 ± *0.221*^{e}	89.940 ± *0.214*^{a,b}	89.690 ± *0.178*^{a}
	30	82.180 ± *0.702*^{a,b,c}	81.248 ± *0.965*^{a}	83.052 ± *0.879*^{c}	81.886 ± 1.067^{a,b}	84.268 ± *0.430*^{d}	82.842 ± *1.028*^{b,c}	81.524 ± *0.455*^{a}
CIE a*	1	-1.252 ± *0.022*^{b}	-1.264 ± *0.048*^{b}	-1.340 ± *0.020*^{a}	-1.126 ± *0.018*^{c}	-1.268 ± *0.015*^{b}	-1.324 ± *0.011*^{a}	-1.136 ± *0.023*^{c}
	7	-0.812 ± *0.043*^{c}	-0.862 ± *0.054*^{b,c}	-0.898 ± *0.028*^{a,b}	-0.890 ± *0.019*^{a,b}	-0.936 ± *0.071*^{a}	-0.842 ± *0.036*^{b,c}	-0.930 ± *0.067*^{a}
	15	-0.630 ± *0.039*^{c}	-0.832 ± *0.113*^{b}	-0.848 ± *0.050*^{b}	-0.888 ± *0.019*^{a,b}	-0.652 ± *0.024*^{c}	-0.900 ± *0.025*^{a,b}	-0.940 ± *0.111*^{a}
	30	-0.656 ± *0.169*^{d}	-1.172 ± *0.134*^{b}	-1.112 ± *0.121*^{b}	-1.250 ± *0.206*^{a,b}	-0.862 ± *0.068*^{c}	-0.806 ± *0.142*^{c,d}	-1.360 ± *0.123*^{a}
CIE b*	1	10.008 ± *0.112*^{b,c}	9.400 ± *0.597*^{a}	10.434 ± *0.284*^{c}	10.994 ± *0.092*^{d}	9.890 ± *0.223*^{b}	10.426 ± *0.093*^{c}	11.320 ± *0.520*^{d}
	7	10.294 ± *0.063*^{a}	10.710 ± *0.213*^{b}	11.126 ± *0.110*^{c}	12.704 ± *0.105*^{e}	11.600 ± *0.384*^{d}	11.610 ± *0.146*^{d}	12.962 ± *0.242*^{e}
	15	11.812 ± *0.271*^{a}	12.736 ± *0.245*^{c}	12.964 ± *0.167*^{c}	14.726 ± *0.141*^{f}	12.134 ± *0.170*^{b}	14.084 ± *0.169*^{d}	14.458 ± *0.193*^{e}
	30	15.378 ± *0.463*^{a}	17.646 ± *0.216*^{d}	17.512 ± *0.408*^{d}	16.682 ± *0.804*^{c}	16.152 ± *0.439*^{b,c}	15.892 ± *0.370*^{a,b}	15.976 ± *0.404*^{a,b}
*ΔE**			100 BS vs C	200 BS vs C	300 BS vs C	100 AS vs C	200 AS vs C	300 AS vs C
	1		0.66	0.58	1.13	0.16	0.70	1.60
	30		5.03	3.64	3.88	1.81	2.73	4.02

^{a,b,c,d,e}: superscript letters in the same row indicate significant statistical differences (LSD test, $p < 0.05$)

BS: before salting. AS: after salting. C: control. n = 6. *ΔE*: color delta

Table 4.3. Mean values ± *standard deviation* of the evolution of water soluble nitrogen (WSN/TN, expressed as percentage of total nitrogen) and free amino acids (FAA, expressed as mg Leu g^{-1} cheese) during ripening (days 1, 7, 15 and 30) of pressurized (100, 200 or 300 MPa for 5 min at 14 ºC) before (BS) or after (AS) brining and control goat milk cheese

	DAY	C	100 BS	200BS	300BS	100AS	200AS	300AS
WSN/TN	1	7.75 ± 1.94 ^{a}	8.41 ± 1.20 ^{b,c}	7.99 ± 0.42 ^{a,b,c}	8.36 ± 1.03 ^{b,c}	8.51 ± 1.54 ^{c}	8.30 ± 1.01 ^{a,b,c}	7.84 ± 1.24 ^{a,b}
	7	12.60 ± 1.60 ^{b,c}	10.88 ± 2.44 ^{a}	13.92 ± 2.59 ^{d}	14.06 ± 1.90 ^{d}	12.73 ± 1.68 ^{c}	12.20± 2.65 ^{b}	18.27 ± 7.26 ^{e}
	15	13.01± 0.48 ^{b}	12.71 ± 2.16 ^{b}	13.67 ± 0.34 ^{c}	16.24 ± 2.61 ^{d}	13.71 ± 3.94 ^{c}	12.10 ± 1.44 ^{a}	16.40 ± 2.13 ^{d}
	30	16.49± 2.42 ^{a}	18.27 ± 1.65 ^{b}	18.46± 1.78 ^{b}	19.72 ± 1.12 ^{c}	19.37 ± 2.66 ^{c}	16.15 ± 1.66 ^{a}	22.96 ± 1.06 ^{d}
FAA	1	0.44 ±0.08 ^{a}	0.45 ± 0.02 ^{a}	0.47 ± 0.08 ^{a}	0.55 ± 0.21 ^{b}	0.44 ± 0.06 ^{a}	0.46 ± 0.07 ^{a}	0.60± 0.09 ^{b}
	7	0.62 ± 0.24 ^{b,c}	0.44 ± 0.21 ^{a}	0.74± 0.09 ^{c}	0.92 ± 0.35 ^{d}	0.68 ± 0.14 ^{c}	0.48 ± 0.06 ^{a,b}	1.52 ± 0.12 ^{e}
	15	0.73 ± 0.14 ^{a}	0.78 ± 0.21 ^{a,b}	0.85 ± 0.28 ^{a,b}	1.67 ± 0.70 ^{c}	1.10 ± 0.37 ^{b}	0.88 ± 0.05 ^{a,b}	2.30 ±0.89 ^{d}
	30	2.75 ± 0.82 ^{a}	3.04 ±0.10 ^{b}	3.06 ± 0.07 ^{b,c}	3.45 ± 0.18 ^{d}	3.20 ± 0.94 ^{c}	2.74 ± 0.48 ^{a}	3.91 ± 0.36 ^{e}

^{a,b,c,d,e}: different superscript letters in the same row indicate significant statistical differences (LSD test, $p < 0.05$).

BS: before salting. AS: after salting. C: control. n = 6

Table 4.4 Mean values ± *standard deviation* of stress ($\sigma_{(t)}$) expressed in KPa and strain (ε) (dimensionless) parameters on pressurized (100, 200 or 300 MPa for 5 min at 14 ºC) before (BS) or after brining (AS) goat's milk cheeses during ripening (days 1, 7 , 15 and 30)

	DAY	C	100 BS	200 BS	300 BS	100 AS	200 AS	300 AS
STRESS	1	21.822 ± *5.787*^{d}	28.275 *± 4.816*^{cd}	52.507 *± 13.232*^{a}	44.045 *± 11.231*^{b}	24.655 *± 11.60* ^{d}	33.194 *±7.789*^{c}	35.018 ± 10.242^{c}
	7	51.999 ± *5.674*^{d}	67.654 *± 9.386*^{bc}	108.556 *± 26.045*^{a}	62.458 *± 12.157*^{c}	49.523 *± 7.388*^{d}	73.528 *± 8.885*^{b}	72.957 ± 12.161^{b}
	15	94.200 ± *51.671*^{c}	100.562 *± 47.794*^{c}	115.936 *± 44.977*^{b}	56.966 *± 22.373*^{f}	100.940 *± 36.754*^{c}	136.729 *± 45.686*^{a}	77.167 ± 22.226^{e}
	30	149.594 ± *11.84*^{d}	240.178 *± 46.33* ^{b}	268.852 *± 34.63* ^{a}	84.453 *± 25.688*^{e}	198.164 *± 31.57* ^{c}	217.147 *± 18.846*^{c}	86.974 ± 7.965^{e}
STRAIN	1	0.653 ± 0.*074*^{c}	0.694 *± 0.117*^{c}	0.695 *± 0.087*^{c}	0.830 *± 0.086*^{a}	0.638 *± 0.092*^{c}	0.655 *±0.031*^{c}	0.816 ± 0.068^{b}
	7	0.391 ± 0.*057*^{c}	0.368 *± 0.058*^{cd}	0.366 *± 0.041*^{cd}	0.755 *± 0.082*^{a}	0.397 *± 0.089*^{c}	0.337 *± 0.029*^{d}	0.674 ± 0.073^{b}
	15	0.360 ± 0.*069*^{bc}	0.367 *± 0.110*^{b}	0.310 *± 0.029*^{c}	0.758 *± 0.076*^{a}	0.313 *± 0.054*^{bc}	0.335 *±0.044*^{bc}	0.714 ± 0.066^{a}
	30	0.280 ± 0.*042*^{c}	0.217 *± 0.018*^{d}	0.255 *± 0.018*^{cd}	0.653 *± 0.075*^{a}	0.258 *±0.056*^{cd}	0.271 *± 0.028*^{c}	0.577 ± 0.045^{b}

^{a,b,c,d,e,f}: different superscripts letters in the same row indicate significant statistical differences (LSD test, $p < 0.05$)

BS: before salting. AS: after salting. C: control. n = 9

Table 4.5. Mean values ± *standard deviation* of microstructure parameters (nº objects, total area of lipids and porosity in percentage) measured on control and pressurized (100, 200 or 300 MPa for 5 min at 14 º C) goat's milk cheeses during ripening (day 1, 7, 15 and 30)

	DAY	C	100BS	200BS	300BS	100AS	200AS	300AS
Nº OBJECTS	1	9125 ± *128.65*^{b,c}	7599 ± *323.11*^{b}	10469 ± *513.43*^{d}	10922 ± *247.27*^{c,d}	4662 ± *248.21*^{a}	7309 ± *281.79*^{b}	8635 ± *195.57*^{b}
	7	5079 ± *241.94*^{a}	5399 ± *186.56*^{b}	9911 ± *341.64*^{c}	5141 ± *156.56*^{a}	8730 ± *272.39*^{b,c}	8150 ± *247.14*^{b,c}	3630 ± *354.34*^{a}
	15	6480 ± *485.19*^{a}	6722 ± *342.22*^{a}	5226 ± *261.10*^{a}	6681 ± *235.16*^{a}	8758 ± *298.09*^{a,b}	10023 ± *657.12*^{c}	9650 ± *234.74*^{b}
	30	10411 ± *410.88*^{a}	9950 ± *1593.69*^{a}	9746 ± *1094.65*^{a}	10070 ± *518.78*^{a}	9698 ± *711.84*^{a}	8603 ± *482.53*^{a}	20189 ± *1277.87*^{b}
% TOTAL AREA OF LIPIDS	1	23.40 ± *4.51*^{c,d}	28.20 ± *6.48*^{d}	11.01 ± *5.88*^{a}	14.80 ± *6.85*^{a,b}	18.10 ± *7.51*^{b,c}	19.29 ± *4.15*^{b,c}	17.94 ± *3.55*^{b,c}
	7	9.81 ± *3.71*^{a}	14.68 ± *4.43*^{a,b}	16.55 ± *4.84*^{b}	14.99 ± *1.24*^{a,b}	16.95 ± *4.52*^{b}	14.85 ± *6.05*^{a,b}	12.77 ± *11.59*^{a,b}
	15	13.40 ± *2.32*^{a,b}	16.46 ± *7.39*^{b}	13.79 ± *1.84*^{a,b}	10.02 ± *7.42*^{a}	9.88 ± *3.68*^{a}	11.34 ± *4.62*^{a,b}	10.71 ± *1.30*^{a}
	30	9.29 ± *3.00*^{a,b}	6.87 ± *1.84*^{a}	8.40 ± *1.56*^{a,b}	11.03 ± *2.45*^{b}	7.16 ± *2.23*^{a}	6.14 ± *1.88*^{a}	19.12 ± *7.21*^{c}
% POROSITY	1	2.82 ± *2.98*^{a}	2.20 ± *1.73*^{a}	9.33 ± *4.21*^{b}	5.52 ± *4.87*^{a,b}	5.17 ± *7.21*^{a,b}	6.22 ± *5.55*^{a,b}	4.85 ± *6.08*^{a,b}
	7	15.21 ± *10.48*^{c}	16.74 ± *12.59*^{c}	5.69 ± *4.32*^{a,b}	9.22 ± *4.43*^{a,b,c}	1.69 ± *2.15*^{a}	9.86 ± *2.65*^{a,b,c}	14.50 ± *14.16*^{b,c}
	15	12.83 ± *6.12*^{c,d}	5.04 ± *4.12*^{a,b}	17.61 ± *6.73*^{d}	1.21 ± *0.49*^{a}	7.50 ± *6.10*^{b,c}	7.42 ± *5.05*^{b,c}	3.40 ± *2.53*^{a,b}
	30	18.34 ± *4.67*^{c,d}	19.75 ± *9.51*^{c,d}	22.36 ± *5.74*^{d}	12.64 ± *5.37*^{b}	13.40 ± *5.66*^{b,c}	15.25 ± *4.42*^{b,c}	3.56 ± *1.20*^{a}

^{a,b,c,d,e}: different superscripts letters in the same row indicate significant statistical differences (LSD test, $p< 0.05$)

BS: before salting. AS: after salting. C: control. n = 7 micrographs of each cheese at each sampling sampling point.

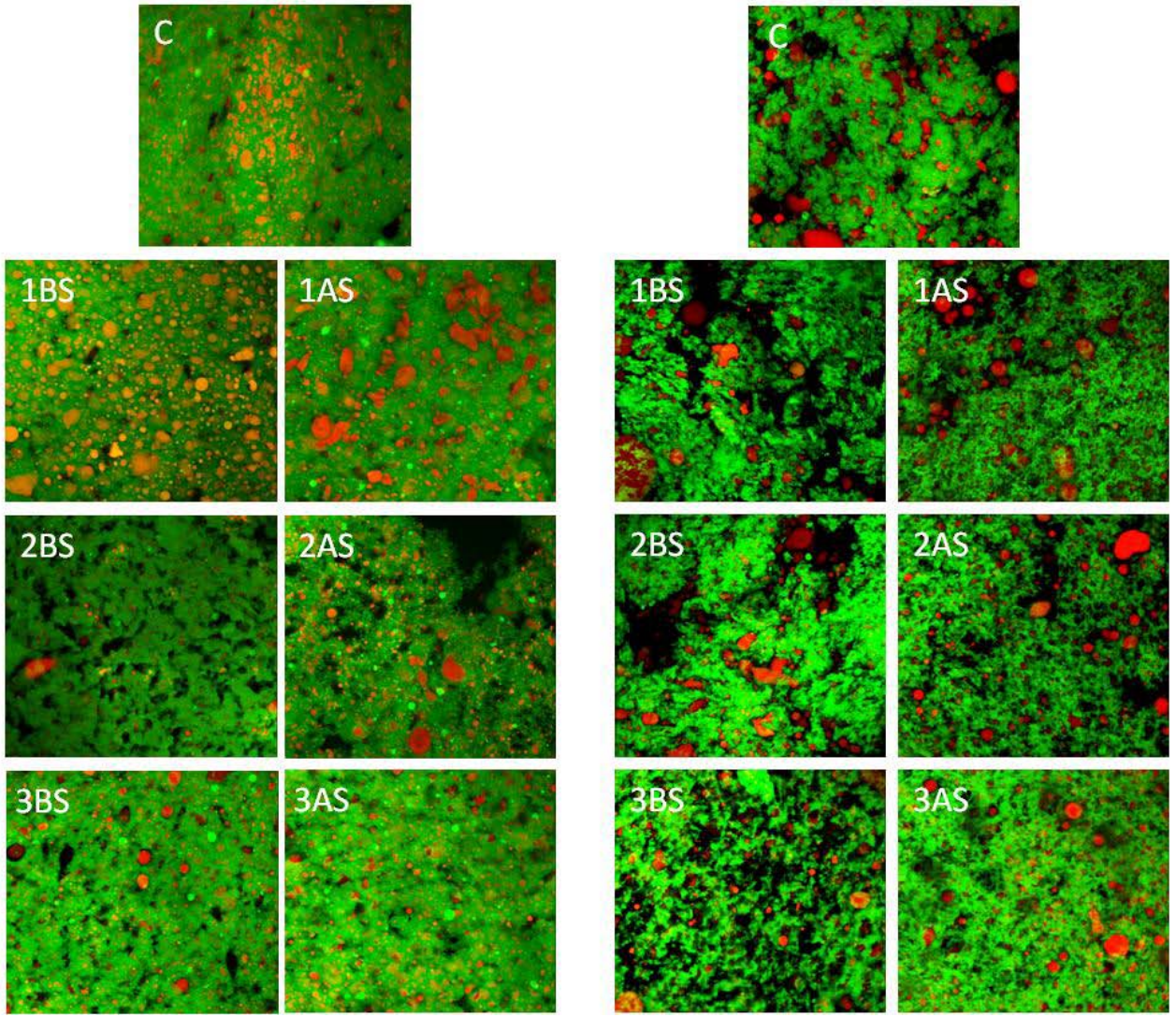

Figure 4.1. Confocal laser scanning micrographs of day 1 (left two columns) and day 30 (right two columns) of goat's milk cheese ripening. Micrographs represent HP treatments (5 min at 14 ºC) separated by pressure and moment of HP-treatment application (C: control cheese; 1BS: 100 MPa before salting; 2BS: 200 MPa before salting; 3BS: 300 MPa before salting; 1AS: 100 MPa after salting; 2AS: 200 MPa after salting; 3AS: 300 MPa after salting).

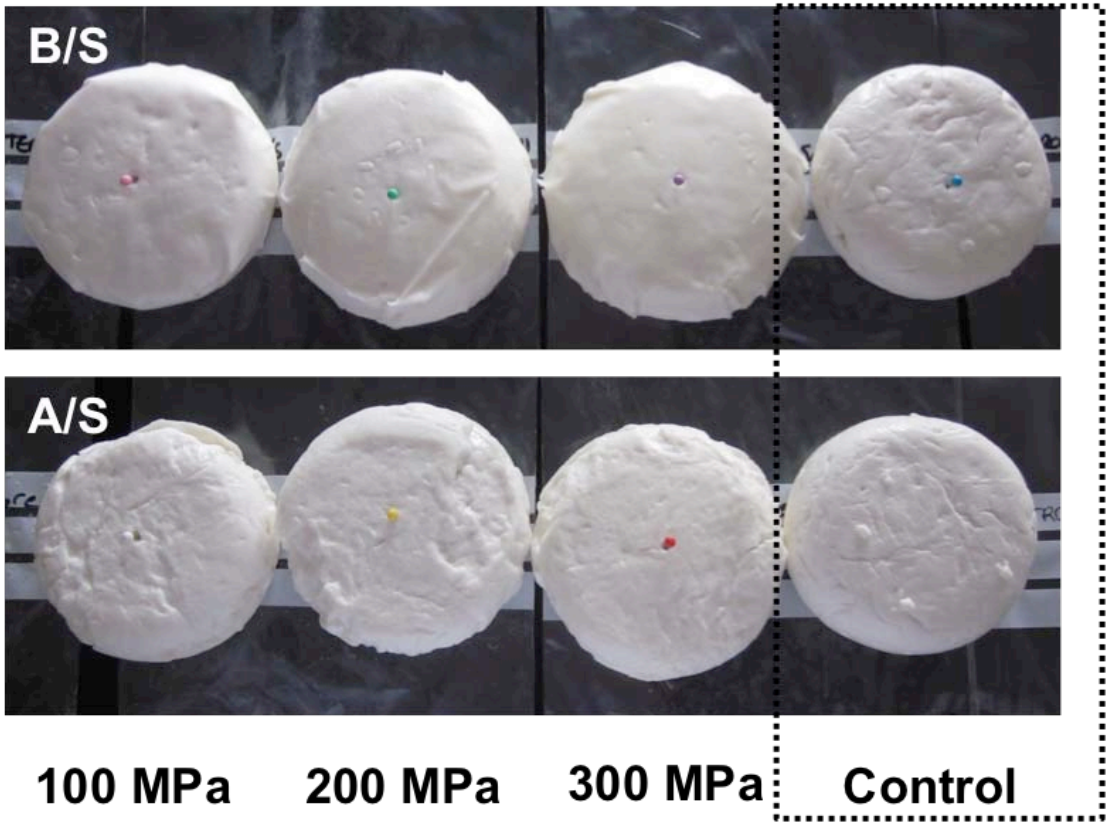

Figure 4.2. Picture of 1 day-old cheeses after the HP-treatment pressurized before (BS) and after (AS) the brining and control cheeses.

Table 4.6. Mean values ± *standard deviation* of the sensory attributes evaluated at day 30 in pressurized (100, 200 or 300 MPa for 5 min at 14 º C) before (BS) or after (AS) brining and control goat's milk cheeses.

	CONTROL	100BS	200BS	300BS	100AS	200AS	300AS
COLOR	$4.00^{a,b}$	$4.91 \pm 0.90^{b,c}$	$4.83 \pm 1.03^{b,c}$	5.00 ± 1.35^{c}	3.80 ± 0.91^{a}	$4.00 \pm 1.65^{a,b}$	5.16 ± 1.52^{c}
FIRMNESS	4.00^{b}	5.61 ± 1.07^{c}	5.28 ± 1.48^{c}	1.33 ± 0.49^{a}	5.42 ± 1.17^{c}	4.38 ± 1.56^{b}	1.41 ± 0.66^{a}
MOUTHFEEL	4.00^{c}	$3.95 \pm 1.37^{a,b}$	$3.14 \pm 1.71^{a,b}$	5.75 ± 1.65^{d}	3.05 ± 1.25^{a}	$3.90 \pm 1.37^{b,c}$	5.33 ± 1.55^{d}
GRANULOSITY	$4.00^{b,c}$	4.81 ± 1.28^{c}	$4.76 \pm 1.50^{b,c}$	1.58 ± 0.79^{a}	$4.38 \pm 1.50^{b,c}$	3.90 ± 1.33^{b}	2.50 ± 1.24^{a}
ACIDITY	4.00^{c}	4.14 ± 1.35^{c}	3.80 ± 1.05^{c}	1.83 ± 0.93^{a}	4.21 ± 1.13^{c}	3.85 ± 1.08^{c}	2.75 ± 1.91^{b}
BITTERNESS	4.00^{b}	4.00 ± 1.00^{b}	3.76 ± 1.13^{b}	2.83 ± 1.40^{a}	3.81 ± 0.87^{b}	3.90 ± 1.13^{b}	3.25 ± 1.76^{a}

^{a,b,c,d,e}: different superscript letters in the same row indicate significant statistical differences (LSD test, $p < 0.05$)

BS: before salting. AS: after salting

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Chapter V

Effect of HP treatments applied before and after brining on water holding capacity and salt content of goat's milk cheese

Effect of HP treatments applied before and after brining on water holding capacity and salt content of goat's milk cheese

5.1 Introduction

The sensory and physical properties of cheeses will mostly determine their consumer acceptance. Although goat's milk cheese has become very popular in the last years, there are still some determinant issues of their commercial characteristics in regards to consumer preferences that need further investigation. Frequently these cheeses reach the final ripening point without the desired characteristics in terms of moisture content and texture leading to impoverished sensory quality.

Ripening is a main step in cheese manufacture since the final texture, taste, odor and color are developed throughout this period. This process is influenced by multiple factors like technological issues used in cheese manufacture, cheese-maker experience, ripening room conditions, and composition of cheese before ripening, such as bacteriology and surface flora, endogenous enzymes and water content, among others. It is important to note that gel characteristics of rennet curd such as water holding capacity will influence the ripening characteristics of cheese since improper moisture content would result in poor body and textural properties, and will influence yield and textural attributes of cheese. Additionally it has been noted that cheese with poor texture has poor flavor (Everett & Auty, 2008). Diffusivity, that controls the movement of water from the interior to surface of cheese during ripening affects bacterial and mold growth and determinate the internal moisture profiles. Thus, special attention should be paid to moisture content control to obtain desired characteristics in cheese (Pandey, Ramaswamy, & St-Gelais, 2000).

Numerous studies have been conducted to determine the importance of water loss with special regard to ripening process (Buffa, Guamis, Saldo, & Trujillo, 2003; Castell-Palou, Rosselló, Femenia, Bon, & Simal, 2011; Curtis et al., 1999; Hickey, Guinee, Hou, & Wilkinson, 2013; Moro García, Bartolomé, & Alvarez, 1993). The migration and evaporation process through the cheese rind during the ripening process produces weight-loss. During this drying process, water is vaporized from the wet surface to the stream of air and at the same time water is removed from the surface, it diffuses from the interior of the cheese paste towards the surface (Castell-Palou et al., 2011).

It is widely accepted that water exist in three different ways in cheese: free water in the serum channels with a weak bounding to the matrix, entrapped water, considered the water closer in proximity to the casein matrix, and the bound water, strongly linked to the matrix and adsorbed to the caseins, thus unavailable as a solvent for biological functions (Curtis et al., 1999; Kneifel, Seiler, Dewit, & Kindstedt, 1993; McMahon, Fife, & Oberg, 1999). On the other hand, entrapped water is that embedded by the macrostructure of protein matrix, weakly associated with the proteins and that can migrate depending upon its mobility within the protein matrix. Entrapped water cannot

be expressed by centrifugation. In contrast, free or expressible water is not impeded by the protein matrix and may be expressed by centrifugation. Although it keeps its solvent capacity, it has lower a_w compared with that of pure water, because of the presence of Na^+ Cl^-, lactose and other low molecular weight solutes (Hickey et al., 2013). Bound water is considered as non solvent water, so it is no available for microbiological growing; it is unfreezable water and associated to the solids as the thin layer that coats the protein or simply as the water is more closely located near them. This definition is done in terms of the spatial relationship between water and the solid constituents of the cheese, which are mainly proteins.

Besides to the moisture content and the internal water profiles, by examining the factors implied in the ripening of cheese, and thus, in the final texture, taste and odor characteristics, salt content should give some means to choose manufacturing variables and ripening conditions, which will give rise to the desired type of cheese. Salt in cheese has an effect on microorganisms and influences the gel formation time, ripening, cheese surface (rind), cheese mass (consistency, structure and texture), cheese flavor and shelf life of cheese. During brining, salt is absorbed from the brine solution by cheese and simultaneously, water is lost. As diffusion is the reached equilibrium between 2 phases with different concentrations, after immersion of cheese into brine (> 10% Na Cl), there is an exchange between cheese mass and the lesser concentrated whey in the interior of cheese.

Regarding to the improvement of water-holding capacity and brining process on final characteristics of cheese, several authors have been worked applying high-pressure treatments both in milk and cheese chasing different aims. Most of studies carried out regarding the analysis of water holding capacity and high-pressure treatments showed a decrease of total water content as cheese aged, indicating different moisture profiles depending on whether they were pressurized cheeses or control cheeses. Buffa et al., (2003) compared water types of goat cheeses made of raw, pasteurized and pressurized milk at 500 MPa for 15 min. The author found that in 1 day-old cheeses, free water was the predominant water-type and additionally, it was the water type that decreased in a greater manner throughout ripening, especially in pasteurized milk's goat cheeses. Water loss and moisture internal pattern of raw and pressurized goat's milk cheeses were very similar probably due to their similar structure and their physic-chemical characteristics. According to Saldo et al. (2002), some changes in the internal distribution of water were attributable to high-pressure treatments on goat's milk cheese applying pressures of 400 MPa for 5 min. The authors reported that higher moisture content and less weight loss during ripening was found in pressurized cheeses compared with the control, enhancing enzymatic and microbial activity in goat's milk cheese.

Some other works have been carried out to study the mechanism of penetration of brine into the cheese, not only by capillary action but also due to the pressure gradient imposed to the system by high pressure treatments or vacuum application (Chiralt et al., 2001; Gonzalez-Martinez, Chafer, Fito, & Chiralt, 2002; Guamis, Trujillo, Sendra, Buffa, & Saldo, 2000; W Messens, Dewettinck, Camp, & Huyghebaert, 1998; Winy Messens, Dewettinck, & Huyghebaert, 1999; Pavia,

Jose Trujillo, Guamis, & Ferragut, 2000). However, the authors reported conflicting or no conclusive results.

Based in previous studies, it seems like salt uptake neither salt distribution were highly dependent of high-pressure treatments. In -depth studies should be performed in order to examine the effect of high-pressure on salt content of goat's milk cheeses. As the cheese mass structure shows large differences before and after salting, and it is widely affected by high pressure treatments, in this work it could be an interesting approach to study the application of high-pressure treatments before and after brining of cheeses. Hence, the objective of the current study was to evaluate the effect of several high pressure treatments (100, 200 or 300 MPa for 5 min at 14 ºC) on the water holding capacity of goat's milk cheese, before (BS) or after (AS) salting of cheeses. An additional goal chased in this study will be to evaluate the differences in the penetration and homogenization of salt along cheese wedge during cheese ripening of control and pressurized cheeses.

5.2 Results and discussion

5.2.1 Moisture and Aw

In order to evaluate the moisture profile of pressurized and control cheeses, moisture content, activity of water and thermogravimetrical analyses were performed.

As it was expected moisture content decreased gradually by age in all samples and was significantly ($P < 0.05$) affected by HP-treatment. Experimental cheeses obtained higher values of moisture content, especially 300MPa HP-treated cheeses, which had higher ($P < 0.05$) moisture content compared with the control, being greater these differences at the end of ripening (see chapter IV).

Water activity (Aw) is an index of the free water that is available to contribute to water vapor pressure (Hickey et al., 2013) and its measurement enables examining the water available in food for microbial and chemical processes. Additionally, it is related to free water concept (Saldo et al., 2002).

As it was expected, Aw decreased ($P < 0.05$) in both pressurized and control cheeses throughout ripening (Table 5.1). Generally, at first stage of ripening all samples reached similar results ranging between 0.979 *± 0.002* and 0.983 *± 0.002* in pressurized cheeses and 0.979 *± 0.003* in control cheese. However, as time passes differences between samples became evident. It is not until the day 7 when the pressure effect appeared. Pressurized cheeses at 100 MPa BS obtained significant values below the control cheese and these differences were maintained until the end of ripening. However, no significant differences could be observed in the rest of samples at days 7 and 15. Indeed, it is not until day 30 when well defined Aw profiles could be observed. Cheeses high-pressure treated at 300 MPa reached the highest values compared with the rest of samples including the control cheese.

In regards to the moment of pressurization, it did not seem to cause significant changes in Aw profiles of pressurized cheeses. Although at day 30 slight increase of Aw values could be observed in after salting pressurized cheeses compared to those which pressure treatment was performed before salting (Table 5.1).

5.2.2 Thermogravimetrical analyses

The evaluation of the pressurized and control cheeses water profile by thermogravimetical analysis resulted in a general model curve (Figure 11) divided into three segments which in turn correspond to each water typology. As it is shown in Figure 11, the temperatures of total phase change ranged from 115 to 125 º C and different temperatures matched with each interval, namely 25 - 115 º C for free water (W1), 115 – 125 º C for entrapped water (W2) and 125 – 260 º C for bound water (W3). These partially overlapping stages, are in agreement with other authors (Buffa et al., 2003; Moro García et al., 1993; Zamora, Ferragut, Juan, Guamis, & Trujillo, 2011) who stated successive water loss stages determined by weight loss in cheese, separated in several phases corresponding to free, entrapped or bound water. Thus, the loss of cheese mass related to the loss of water, was calculated for W1, W2 and W3 between each of these temperature intervals. Activation energy required to break the bounds formed between the water and the matrix of cheese is reached at similar temperatures found by other authors (Curtis et al., 1999; Saldo et al., 2002; Zamora et al., 2011).

Table 11 shows the effect of different pressures applied before and after salting on weight loss of cheeses, expressed as percentage, when heated by TGA and separated by three weight loss steps. In general terms, the greater amount of released water corresponds to W1 typology followed by W3 and W2, being the latter the scarcer water-type released in all samples at all sampling points (overall mean W1: 27.08 *± 9.10;* W3*:* 8.05 *± 2.03* and W2: 5.91 *± 1.97*, respectively*).* Different trends were followed by each water typology throughout ripening. As ripening progressed, very much less W1 was released, while values of W2 and W3 seemed to remain more stable up to day 30 of maturation. In this sense, it can be confirmed the statement of Curtis et al. (1999) who indicates that free water is affected by a much larger number of parameters than those affecting bound water. That could be the reason because of free water resulted in a larger mass loss variation between days 1 and 30 than W2 or W3 were.

The mechanism of water loss during ripening period is well known. As ripening progresses water is vaporized from the wet surface of cheese to the stream of air. At the same time that water is removed from the surface, it diffuses from the interior of the cheese paste towards the surface (Castell-Palou et al., 2011; McMinn & Magee, 1999). Diffusivity causes redistribution of water in cheese during ripening and creates internal moisture profiles. However, there are some factors limiting the overall water transfer. Probably the progressive decrease of moisture content in cheese, affects the diffusivity gradient expelling every time less serum throughout storage time. In this sense and in line with results from Mc Mahon et al. (1999), the gradient depletion could be the cause for the less W1 expressed in the present study at day 30 compared with first stages of

ripening. Although total W1 showed dramatic high released values compared with W2 and W3, at day 30, free water was still the most abundant water-type that could be found in all cheeses. In contrast, values of W2 and W3, namely entrapped and bound water respectively, were not affected by ripening time and kept stable over time, remaining virtually the same throughout ripening.

The results in the present work agree with those found by Buffa et al., (2003) who reported declining values of W1 during the first 30 days of ripening, although these differences were no significant anymore at the end of ripening. Curtis et al., (1999) observed how total water content was reduced by age. This loss was certainly due to migration-evaporation processes, which are much stronger on the peripheral part of the whole cheese. However, the authors reported that bound water followed a similar trend than total water, decreasing in the course of ripening. Conversely, in the present work, W3 was less variable throughout time than free water was (Table 11).

Respect to the high-pressure effect, the thermogravimetrical analysis revealed some differences on the water typology of cheeses. High pressure treatments increased free water content (W1) revealing significant differences in 300 MPa samples compared with the control cheese and the rest of pressurized cheeses at all sampling points (except for 300 MPA AS at day 1) (Table 11). It is known that cheese mass under pressure conditions suffers a slight compaction reducing its volume. Juan et al. (2007) already observed water removal just after HP treatments (200-500 MPa) on ewe's milk cheese. However, results of the present work could indicate that the higher is the high-pressure treatment applied, the higher amount of water released corresponds to W1. These differences between studies could be attributed to the different intensity of HP treatment applied on cheese. Possibly, pressures of 100 and 200 MPa, performed in this study, caused slight water removal just after the high-pressure treatment, since no large differences have been observed in these samples compared with the control cheese. On the contrary, 300 MPa high-pressure treatments retained much more water in cheeses, certainly belonging to free water pool, which could suggest that effect of high-pressure on protein matrix caused a profound restructuration of internal water profile retaining larger amounts of free water on cheese.

It is known that high-pressure treatments cause reorganization in cheese matrix affecting water distribution enabling better absorption of water in the protein, and water absorption in cheeses (Juan et al., 2007; Okpala, Piggott, & Schaschke, 2010; Trujillo et al., 2000). The new structure comprised of protein and fat in a different manner after 300 MPa high-pressure treatments were applied, possibly led to better retention of water (free water) and to an enhanced ripening through a slow water loss. Cheeses 300 MPa high-pressure treated were also the samples which reached higher moisture values at the end of ripening (see chapter IV), suggesting that although a high amount of free water is loss during the ripening in 300 MPa cheeses, a desirable drying process took place in these cheeses.

In general, values in percentage of weight loss of W2 in pressurized cheeses are similar or below to those found in control cheese during all ripening. This fact could be attributed to the large amount

of free water found in these samples, since the internal water profile of each sample is determined by the balance between the three water types. Inverse trends could be observed between W1 and W2 types of water; 300 MPa samples which showed higher values of W1, at the same time showed lower values of W2. In this sense, it is important to note that the diffusion gradient could contribute to the movement of W2 and its transformation in any of the other water types, either in W3 by absorption of protein network or in W1 by migration out of the protein matrix as suggested by Curtis et al., (1999). Higher mobility of W2 compared with W3 could cause its removal mostly migrating to W1 location.

In the case of bound water (W3) a clear effect of HP treatments could be observed at day 1. Control and 300 MPa cheeses obtained the highest values of bound water while 100 and 200 MPa samples seemed to bind less water. However, at the end of ripening 300 MPa samples showed significant differences compared with the control. Cheeses pressurized at 300 MPa retained a major amount of water strongly bonded to the protein matrix. Similarly, Saldo et al. (2002) did indicate that bound water was not very much affected by ripening and obtained higher values of this water type in HP-cheeses (400 MPa for 5 min).

Regarding to the moment of HP-treatment, any special trend could be observed related to water distribution profiles in goat cheeses. However, cheeses high-pressure treated before salting (BS) showed slightly higher values of bound water (W3) than AS samples at the end of ripening (BS: 8.78 and AS: 6.98 %). Even these differences between samples treated before and after salting in some cases were no significant; Table 11 shows how at day 30 the moment of application of high-pressure treatments actually had an effect, causing a major water loss of W3 typology on cheeses HP-treated before salting.

Pressure seemed to play a more important role in rearrangement of protein matrix and consequently internal water profiles than the moment of its application. In the present work the higher the pressure applied, the higher effect on water distribution and hence, on cheese microstructure (see chapter IV). Treatments of 300 MPa seemed to cause a great release of free water, which could be expelled after pressure application at the beginning of ripening. However, at the same time this pressure treatment might cause the formation of a new network which enables water to be more strongly linked to the cheese matrix. Additionally, significant higher values of bound water compared to the control cheese in 300 MPa samples at the end of ripening, could be observed. The fact that cheeses treated at a higher pressure showed minimum values of entrapped water (W2) may be related to an internal movement of water molecules after the pressure application. Probably there was a redistribution of water from less-to more-mobile fraction, decreasing entrapped water and increasing free water.

Results obtained in the present work are related to the types of water measured by thermogravimetrical analyses and total moisture content, which showed that 300 MPa high-pressure treated cheeses expelled much more water at the beginning of ripening, but retained a major humidity in the optimum ripening point. This fact is in line with major amounts of bound

water found in 300 MPa cheeses at the end of ripening (Table 5.2). Sensory results also revealed higher moistness sensation in mouth of 300 MPa pressurized cheeses (see chapter IV).

5.2.3 Salt content

Cheeses pressurized at 100, 200 or 300 MPa before and after brining were analyzed to evaluate the influence of high-pressure treatments on penetration and homogenization of salt between the inner and outer part of cheese during ripening (Table 13). As it was expected, all samples gained salt in moisture during ripening ranging from 1.7-2.6 at day 1 to 4-6.6 at the end of ripening. After the high-pressure treatments all samples showed different salt uptakes between the outer and the inner part of cheese. These differences were declining throughout ripening until day 30, when values in inner and outer parts of cheese were almost equilibrated.

Salt ions were transported through the cheese mass giving rise to different salt in moisture patterns upon the area of the cheese analyzed. At day 1, all cheeses showed lower levels in the inner area than in the outer area of cheese. Then, as ripening progressed, cheeses reached their equilibrium point, in regards to penetration of salt, at some point of ripening between days 15 and 30. At day 30 all cheeses reached equilibrium between inner and outer parts of cheese except for 200 MPa and control cheeses. From Table 13, it can be derived that at the end of the ripening period the salt became almost evenly distributed through the cheese coinciding with the study of Messens et al. (1999). When a cheese is placed into the brine, a portion of salt is dissolved in moisture located on the surface of cheese, taking place an inward diffusion of sodium and chloride ions. Due to the difference of salt and moisture concentration, a movement of water from the interior of the curd to the surface occurs because of osmosis, enabling a homogeneous distribution of salt and moisture throughout the cheese wedge during ripening.

The influence of both pressure treatment (100, 200 or 300 MPa) and salting moment (BS or AS) in the salt uptake of cheeses was examined as well (Table 13). At first stages of ripening, control cheese showed relative lower values in the inner part of the cheese compared with most of pressurized cheeses. However, at days 15 and 30, this sample changed the tendency and attained similar values to some of the pressurized cheeses. Respect to the outer part of cheese wedge, control cheese showed intermediate values during ripening. It was noticeable the effect of 300 MPa on salt distribution of cheeses at day 1 and during the whole ripening period. Those cheeses reached highest values of salt in moisture in the inner part of cheese at day 1 (Table 13). Therefore, it can be derived that 300 MPa high-pressures, have an effect just after the HP-treatment promoting the diffusion of salt from the outside to the inner side. These results are consistent to those found by Guamis et al., (1997) who salted Manchego type-cheese by brine vacuum impregnation and observed that experimental cheeses reached higher values in the inner parts of the cheese just after the treatment. In our study high-pressure caused a similar effect than vacuum brine impregnation. At day 1 after high-pressure treatment, 300MPa pressurized cheeses showed a stronger penetration of salt and additionally this effect was sustained over time. Lower salt values in the outer parts and higher salt content in the inner parts of cheese compared

with their pressurized counterparts and with the control cheese could be observed in 300 MPa cheeses whether at day 1 or 30.

By examining salt in moisture results at day 30, the following cheeses; 200MPa BS, 100MPa AS and control, exhibited highest salt-in-moisture values in both parts of cheese. Such high values can be related with higher water loss during ripening, revealing all these samples dry matter values above 73% at day 30.

Table 14 shows the level of distribution of salt on pressurized and control cheeses, as measured by the difference of salt concentration between inner and outer part of cheeses at each sampling point. Therefore, values close to 0 mean an even penetration of salt throughout the cheese wedge and negative values indicate that distribution of salt has been completed exhibiting even more salt in the inner part than in the outer part of the cheese. As it is shown in Table 14, salt equilibrium between inner and outer parts of cheese is also reached before in 300 MPa cheeses compared with the control and the rest of pressurized cheeses. Pressurized cheeses at 300 MPa obtained negative values of rate of salt uptake already at day 7, while negative values in the control cheese did not appear until day 30 of ripening (Table 14). These results indicate that 300 MPa treated-cheeses reached faster the uptake equilibrium obtaining an even distribution of salt and attaining higher results in inner than in outer part of cheese at the optimum ripening point. Samples HP-treated at 100 and 200 MPa followed similar patterns than the control cheese in regards to rate of salt uptake, in some cases without reaching the salt uptake equilibrium between inner and outer part of wedge cheese, even not at day 30.

Saldo et al., (2002) described similar equilibrium times for pressurized cheeses 300g-weighted. These authors pointed out that salt diffusion in HP-treated cheeses appeared faster than in control cheese. In our study only 300MPa HP-treated cheeses, seemed to diffuse faster the salt content through cheese matrix compared to the rest of samples.

There are several structural elements that could account for the slower salt penetration in control cheese. Since this cheese showed a very porous and heterogeneous protein matrix and more protein aggregates, it is reasonable to state that sodium and chloride ions found it difficult to cross through the cheese structure in this cheese more than in others (see chapter IV). The same salt profile was observed in the cheeses evaluated by Pavia et al. (2000) compared with cheeses in the present study; at the beginning of ripening, the more external the zone, the higher the salt content. However, the authors applied 50 and 200 MPa for 45 min on Manchego type-cheese and this high pressure processing during brining did no accelerate neither the rate of salt uptake nor the rate of salt diffusion in Manchego-type cheese. Messens et al., (1999) had already observed that high-pressure brining did not accelerate salting in Gouda cheese. Based on the results of this study, it seems like high pressure treatment before and after the brining had a more effective impact on cheeses, especially at 300 MPa HP-conditions, than high-pressure brining performed by the aforementioned authors,.

Observing differences caused by the moment of HP application (Table 13), it seemed like cheeses HP-treated at 100 or 200 MPa after salting showed lower mean levels of salt in moisture than their

counterparts HP-treated before salting during ripening while cheeses HP-treated at 300 MPa showed higher mean levels in AS cheeses. Probably a slight damping effect of salt in front of HP processing could account for lower levels of salt in moisture in AS cheeses. The addition of Na^+ Cl^- to the cheese increase the interactions between proteins and the surrounding water, thus creating a stronger and better linked network that could exert a protective effect on cheeses in front of high-pressure impact on cheese matrix. It appears that diffusion of Na^+ Cl^- into cheese binds the casein together into a more homogeneous matrix as the chloride anion is a kosmotropic ion which promotes hydrophobic interactions (Madadlou, Khosrowshahi asl, Mousavi, & Farmani, 2007). Cheeses treated before salting did not take advantage of this protective effect and viewed modified their values of salt in moisture by means of high pressure treatments. However, pressures of 300 MPa probably were able to overcome the dumping effect of salt promoting the better penetration of salt in AS cheeses.

5.3 Conclusions

TGA analyses resulted in a model curve which showed 3 overlapping stages corresponding to W1 (free water), W2 (entrapped water) and W3 (bound water) at given temperatures.

Internal moisture profiles of goat's milk cheeses were affected in a great manner by pressure intensity applied and in a lesser extent, by the moment of HP application. 300 MPa cheeses showed greater amounts of W1 (free water) released at all sampling points during ripening.

Although little oscillations of W3 were found at first stages of ripening, 300 MPa samples finished their ripening with major amounts of W3, together with the control cheese, showing both samples a better binding of water at the optimum ripening point compared with 100 and 200 MPa cheeses.

Analyzing moisture profiles of goat's milk cheeses, W1 was the larger water type found in all cheeses during ripening, followed by W3, and finally by W2, which was the scarcest water-type existing in goat's milk cheeses of this study. W1 and Aw values were well correlated showing higher values of both parameters in 300 MPa cheeses. Part of W2 water type could have been migrated to other fractions of water type due to HP processing, such as free water, increasing W1 values in pressurized cheeses.

No large effect can be observed in high-pressure treated-samples respect to the moment of brining and the salt content. However, it is likely that pressure application influenced salt uptake and penetration of salt throughout the wedge of cheese.

Most of samples reached the equilibrium in regards to the salt content between inner and outer parts of cheese at the determinate optimum ripening point for cheeses in this study (day 30). Pressurized samples, especially 300 MPa cheeses revealed a greater penetration of salt at day 1, showing higher values than control and the rest of pressurized cheeses in the inner part of cheese. While control or other pressurized cheeses did not reach salt uptake equilibrium before day 30,

300 MPa cheeses revealed a faster diffusion of salt during ripening exhibiting similar values between both cheese parts studied (inner and outer) at day 7 of ripening.

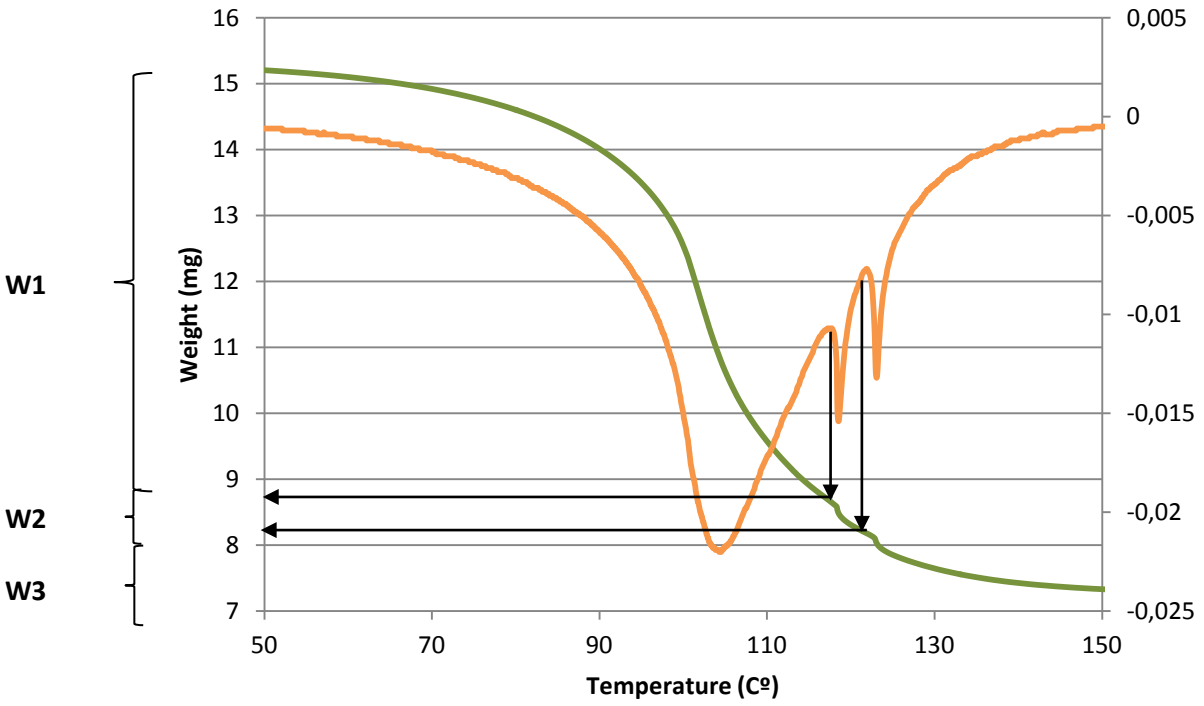

Figure 5.1 Model curve ( ) and first derivative ( ) indicating weight loss in function of temperature in control and pressurized (100, 200 or 300 MPa for 5 min at 14 º C) goat's milk cheeses before (BS) or after (AS) brining obtained by thermogravimetrical analyses.

Table 5.1 Mean values and *± standard deviation* of activity water (A_w) values of control and pressurized (100, 200 and 300 MPa for 5 min at 14 º C) cheeses before (BS) and after (AS) salting

	DAY	C	100BS	200BS	300BS	100AS	200AS	300AS
Aw	1	0.979 ± *0.003*^{a,b}	0.979 ± 0.002^{a}	0.981 ± 0.003^{b,c,d}	0.981 ± 0.004^{c,d}	0.982 ± 0.003^{c,d}	0.983 ± 0.002^{d}	0.982 ± 0.002^{c,d}
	7	0.976 ± 0.001^{b,c}	0.972 ± 0.*004*^{a}	0.977 ± 0.003^{b,c,d}	0.978 ± 0.002^{c,d}	0.974 ± 0.004^{a,b}	0.974 ± 0.003^{a,b}	0.978 ± 0.002^{c,d}
	15	0.972 ± 0.001^{b}	0.967 ± 0.004^{a}	0.973 ± 0.001^{b,c}	0.977 ± 0.002^{b,c}	0.968 ± 0.*006*^{a}	0.972 ± 0.001^{b}	0.972 ± 0.002^{b,c}
	30	0.950 ± 0.*022*^{b,c,d}	0.936 ± 0.*016*^{a}	0.948 ±0.012^{a,b}	0.960 ± 0.002^{d,e}	0.945 ± 0.*013*^{a,b}	0.961 ± 0.020^{c,d,e}	0.961 ± 0.004^{e}

^{a,b,c,d,e,f}: different superscript letters in the same row indicate significant statistical differences (LSD test, $P < 0.05$). n= 6. C: control

Table 5.2 Mean values and *± standard deviation* of percentage of mass loss and of control and pressurized (100, 200 or 300 MPa for 5 min at 14 º C) cheeses before (BS) or after (AS) brining at days 1, 7, 15 and 30 upon the three different types of water (W1, W2 and W3) characterized by TGA

			W1	W2	W3
DAY 1	C		37.03 *± 2.86* ^{a,b,c}	5.17 *± 2.27* ^{a,b}	5.88 *± 1.84* ^{a}
	BS	100	38.10 *± 2.77* ^{b,c}	9.12 *± 2,85* ^{c}	9.75 *± 2.04* ^{b}
		200	37.30 *± 2.72* ^{a,b}	5.73 *± 1.84* ^{b}	6.94 *± 2.61* ^{a,b}
		300	44.45 *± 1.10* ^{d}	3.15 *± 1.05* ^{a}	6.22 *± 2.25* ^{a}
	AS	100	34.39 *± 1.77* ^{a}	5.83 *± 1.46* ^{b}	7.79 *± 2.74* ^{a,b}
		200	38.18 *± 2.64* ^{b,c}	6.06 *± 0.38* ^{b}	6.45 *± 2.51* ^{a,b}
		300	41.28 *± 6.23* ^{c,d}	4.77 *± 1.97* ^{a,b}	6.16 *± 3.50* ^{a}
DAY 7	C		28.30 *± 4.31* ^{b}	8.40 *± 1.81* ^{b}	11.06 *± 1.87* ^{c}
	BS	100	24.74 *± 3.56* ^{a}	11.79 *± 2.20* ^{c}	7.38 *± 2.59* ^{b}
		200	26.47 *± 3.54* ^{a,b}	4.47 *± 2.20* ^{a}	11.12 *± 5.81* ^{c}
		300	36.16 *± 2.36* ^{c}	4.70 *± 1.52* ^{a}	5.29 *± 0.43* ^{a}
	AS	100	26.36 *± 3.30* ^{a,b}	7.09 *± 1.18* ^{a,b}	9.45 *± 3.31* ^{c}
		200	27.86 *± 4.03* ^{b}	8.10 *± 1.84* ^{b}	8.13 *± 2.11* ^{b}
		300	34.90 *± 1.81* ^{c}	5.77 *± 0.80* ^{a}	8.20 *± 0.59* ^{b}
DAY 15	C		22.73 *± 3.68* ^{a}	10.20 *± 1.51* ^{b}	5.97 *± 3.44* ^{a}
	BS	100	22.07 *± 4.31* ^{a}	5.55 *± 1.98* ^{a}	10.65 *± 5.80* ^{c}
		200	20.09 *± 5.58* ^{a}	4.72 *± 2.44* ^{a}	13.63 *± 7.45* ^{d}
		300	32.73 *± 3.79* ^{c}	4.31 *± 1.06* ^{a}	6.32 *± 0.19* ^{a}
	AS	100	21.73 *± 6.08* ^{a}	4.84 *± 1.40* ^{a}	7.10 *± 2.96* ^{a,b}
		200	21.37 *± 5.13* ^{a}	5.34 *± 1.36* ^{a}	10.53 *± 4.68* ^{c}
		300	28.43 *± 3.11* ^{b}	3.88 *± 0.86* ^{a}	8.25 *± 4.20* ^{b,c}
DAY 30	C		15.95 *± 1.88* ^{b}	6.57 *± 2.07* ^{b}	5.98 *± 0.80* ^{a}
	BS	100	12.96 *± 3.38* ^{a}	4.95 *± 0.94* ^{a,b}	7.76 *± 1.71* ^{c}
		200	14.83 *± 2.80* ^{a,b}	4.97 *± 1.59* ^{a,b}	9.36 *± 1.88* ^{d}
		300	20.52 *± 4.70* ^{c}	5.02 *± 2.34* ^{a,b}	9.22 *± 0.99* ^{d}
	AS	100	14.22 *± 3.42* ^{a,b}	4.76 *± 1.51* ^{a,b}	5.96 *± 1.13* ^{a}
		200	13.69 *± 2.93* ^{a,b}	4.52 *± 0.86* ^{a}	7.18 *± 0.72* ^{a,b}
		300	21.49 *± 2.77* ^{c}	5.62 *± 2.35* ^{b}	7.81 *± 1.46* ^{b,c}

^{a,b,c,d}: different superscript letters in the same column indicate significant statistical differences (LSD test, $P < 0.05$).

Table 5.3 Mean values and *± standard deviation* of salt in moisture (%) values for control and HP cheeses (100, 200 or 300 MPa for 5 min at 14 º C) before (BS) or after (AS) salting during ripening (days 1, 7, 15 and 30) analyzed in the outer and inner area of cheese

	DAY	1				7				15				30			
		OUT		INN		OUT		INN		OUT		INN		OUT		INN	
C		2,282	*±0,790*^{d}	1,321	*±0,428*^{b}	2,400	*±0,259*^{b}	2,145	*±0,386*^{a}	3,643	*±0,619*^{d}	3,169	*±0,382*^{d}	6,306	*±0,960*^{d}	5,654	*±0,782*^{d}
BS	100	2,643	*±0,135*^{f}	1,484	*±0,130*^{d}	3,237	*±0,904*^{g}	2,877	*±0,744*^{f}	4,098	*±0724*^{e}	3,299	*±0,660*^{e}	5,297	*±0,886*^{e}	5,842	*±1,238*^{e}
	200	1,757	*±0,968*^{a}	1,464	*±0,26e*^{d}	2,776	*±0,480*^{d}	2,512	*±0,288*^{e}	4,008	*±0,235*^{e}	3,170	*±0,476*^{d}	6,630	*±1,814*^{f}	6,165	*±1,784*^{f}
	300	2,298	*±0,715*^{d}	1,729	*±0,843*^{f}	2,208	*±0,745*^{a}	2,346	*±0,259*^{c}	2,735	*±0,238*^{a}	2,703	*±1,160*^{b}	3,629	*±1,060*^{a}	4,604	*±0,406*^{a}
AS	100	2,202	*±1,159*^{b}	1,358	*±0,501*^{c}	2,956	*±0,716*^{f}	2,230	*±0,407*^{b}	3,260	*±0,508*^{b,c}	2,828	*±0,666*^{c}	5,959	*±0,306*^{f}	6,136	*±1,272*^{f}
	200	2,596	*±0,706*^{e}	1,266	*±0,407*^{a}	2,854	*±0,666*^{e}	2,395	*±0,579*^{d}	3,310	*±1,030*^{c}	2,526	*±0,408*^{a}	5,116	*±0,435*^{b}	4,990	*±0,575*^{b}
	300	2,244	*±0,409*^{c}	1,573	*±0,477*^{e}	2,479	*±0,344*^{c}	2,334	*±0,395*^{c}	3,197	*±0,410*^{b}	3,461	*±0,475*^{f}	4,059	*±0,446*^{c}	5,197	*±1,008*^{c}

^{a,b,c,d,e,f}: different superscript letters in the same column indicate significant statistical differences (LSD test, $P < 0.05$).

OUT: outer part of cheese. INN: inner part of cheese. C: control. n = 6

Table 5.4 Mean values and *± standard deviation* of rate of salt uptake (%) on pressurized cheeses (100, 200 or 300 MPa for 5 min at 14º C) before (BS) or after (AS) brining and control cheeses during ripening (days 1, 7, 15 and 30).

			RATE Salt in Moisture (%)
1	C		0,890 ± 0,508 ^{d,e}
	BS	100 BS	1,260 ± 0,208 ^{e}
		200 BS	-0,090 ± 0,098 ^{a}
		300 BS	0,326 ± 0,322 ^{b}
	AS	100 AS	0,441 ± 0,277 ^{b,c}
		200 AS	1,068 ± 0,072 ^{d,e}
		300 AS	0,741 ± 0,033 ^{c,d}
7	C		0,302 ± 0,153 ^{b}
	BS	100 BS	0,229 ± 0,090 ^{b}
		200 BS	0,143 ± 0,073 ^{b}
		300 BS	-0,440 ± 0,175 ^{a}
	AS	100 AS	0,310 ± 0,075 ^{b}
		200 AS	0,212 ± 0,112 ^{b}
		300 AS	0,033 ± 0,654 ^{b}
15	C		0,158 *± 0,297* ^{a,b}
	BS	100 BS	0,708 *± 0,414* ^{c,d}
		200 BS	0,906 *± 0,544* ^{d}
		300 BS	0,507 *± 0,787* ^{b,c,d}
	AS	100 AS	0,308 *± 1,158* ^{b,c}
		200 AS	0,023 *± 1,058* ^{a,b}
		300 AS	-0,273 *± 0,328* ^{a}
30	C		-0,010 *± 0,124* ^{b,c,d}
	BS	100 BS	-0,443 *± 0,447* ^{b,c}
		200 BS	0,464 *± 0,154* ^{d}
		300 BS	-1,299 *± 0,842* ^{a}
	AS	100 AS	0,385 *± 1,183* ^{c,d}
		200 AS	0,029 *± 0,104* ^{b,c,d}
		300 AS	-0,552 *± 0,137* ^{a,b}

^{a,b,c,d}: different superscript letters in the same column indicate significant statistical differences (LSD test, $P < 0.05$).

5.4 References

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Chapter VI

Evaluation of volatile compounds on goat's milk cheeses HP-treated before and after brining

Evaluation of volatile compounds on goat's milk cheeses HP-treated before and after brining

6.1 Introduction

Cheese aroma consists of a multitude of compounds which formation is due to complex biochemical reactions that take place during ripening. During the step of ripening, the cheese undergoes multiple changes since it is a biochemically dynamic product. These processes are determined by physic-chemical characteristics of cheese which in turn define texture, microstructure, sensory and flavor profiles of cheese. All these factors influence on evolution of cheese during ripening and contribute to the development of the unique aroma of each cheese variety. A multitude of factors such as the aromatic richness of milk cheese, which in turn depends on its animal species (bovine, ovine, caprine or buffalo) or the animals housing conditions (if they are in stall or grazed) are some of the features that determine volatile profile composition on cheese. The technology used in the cheese making, the coagulation pattern followed and the ripening conditions applied also have a strong influence on volatile profile (Yang, Ding, Ma, & Jia, 2015).

Metabolism of a large number of compounds and the interaction between them, generate the food flavor. The main chemical families to which aroma compounds consist are alcohols, aldheydes, ketones, carbonic acids, esters, sulphur and nitrogen compounds. Those compounds are perceived by the consumer during eating food as being released in every beat, so mostly flavor and texture will determine the acceptance of a cheese by the consumer (Attaie, 2009). Since concentration of one component can significantly affect aroma of cheese, it is so critical the balance between aroma compounds in cheese and needs not to be disturbed.

The agents involved in cheese flavor are enzymes from milk, rennet, starter cultures and secondary flora, which degrade proteins, fat, citrate and carbohydrates by different metabolic pathways. In short, creation of aroma compounds in ripened cheeses is shown as in Table 6.1.

Goat's milk cheeses are sensory special products. Their strong smell and characteristic taste makes each of them unique. The most characteristic compounds found in goat's milk cheese belong to main chemical families such as acids, alcohols, esters and ketones being the carbonic acids the group which account for the major part of the volatile profile in goat's cheeses (Chilliard, Ferlay, Rouel, & Lamberett, 2003; Engel et al., 2002; Le Quere, Pierre, Riaublanc, & Demaizieres, 1998).

Some fatty acids are widely recognized as belonging to goat's milk cheese volatile profile such as hexanoic, octanoic and decanoic acids, popularly termed caproic, caprilic and capric, respectively (Justa María Poveda, Sánchez-Palomo, Pérez-Coello, & Cabezas, 2008). Branched-chain fatty acids (BCFA) has been also highlighted by several studies as contributing to characteristic active odor compounds in goat's cheese volatile profile (Delgado, González-Crespo, Cava, & Ramírez, 2011a;

Yang et al., 2015). Other branched-chain of octanoic acid such as 4-methyl and 4-ethyl has been considered to largely contribute to specific characteristic of goat cheeses as well (Table 6.2). Among alcohols, 2-heptanol has been used to be pointed out like one of the most commonly found in goat cheeses. The presence of some branched-chain alcohols, such as 1butanol, 3-methyl also denotes a common characteristic of goat cheese. Esters are also common volatile compounds on goat cheese, specifically butanoic and hexanoic acid ethyl ester (Delgado et al., 2011a). These compounds derived from their corresponding carboxylic acids, softens the strong goat aroma changing it into sweet and conferring some fruity or floral notes to cheese. Ketones family is one of the most abundant in dairy products and in goat's milk cheese most of them are methylketones, such as 2-butanone, 2-heptanone, 2-octanone, 2-nonanone and 2-undecanone (Table 6.3). The presence of aldheydes, hydrocarbons, sulfur compounds and terpenes enriches and completes the volatile profile of goat cheeses.

A large portion of goat's milk cheese production is made by acidification obtaining acid curds which can be ripened or intended for fresh consumption. There are few semi hard goat cheeses in the market compared with acid curd goat cheeses, probably due to its difficulty to retain water during ripening and its reduced sensory characteristics when it is finally placed to the selling point. Trends towards consumption of improved in nutritional quality products and healthier eating habits provide increasing interest in emerging technologies. High pressure processing is a technology which involves advantages of non thermal procedures and at the same time is modifying some physic-chemical properties of cheese conferring to it new sensory characteristics including texture, taste and aroma. This technology appears as an alternative to develop desired sensory properties for semi hard goats' milk cheeses.

In the literature exists many reports where high pressure technology has been successfully applied to milk and cheese pursuing several purposes. Generally, no large differences in volatile profile of pressurized and control cheeses have been found (Delgado et al., 2011a; Evert-Arriagada, Hernández-Herrero, Gallardo-Chacón, Juan, & Trujillo, 2013; Saldo et al., 2003). However, the modification of goat's milk cheeses aroma by means of high-pressure treatments, depends mostly on high-pressure processing conditions (intensity of pressure, time and temperature), on the moment of HP-treatments application during ripening and on the type of cheese and its physic-chemical characteristics (Juan, Barron, Ferragut, & Trujillo, 2007). These parameters predetermine the extent of the effect of HP- treatments on final volatile profile of goat's milk cheese.

Little information exists involving application of HP treatment at different moments during cheese making and its consequences on volatile profile of cheeses. Therefore, the main purpose of this study was to evaluate if the moment (BS: before salting and AS: after salting) of different high-pressure treatments (100, 200 or 300 MPa) application during the salting process affected the volatile profile of cheeses. Additionally, the effect of aging on overall flavor of pressurized goat's cheeses was considered and analyzed on cheeses at day 1 and 30 of ripening, evaluated by SPME/GC-MS analysis (see Chapter III).

6.2 Results and Discussion

Table 6.4 shows volatile compounds (Area units (Au) x 10^5) grouped according to their nature, measured in the headspace of high-pressure treated cheeses (100, 200 or 300 MPa) before and after brining (BS and AS) at day 1 and at day 30 of ripening. In general, compounds identified in control and pressurized cheeses belonged to the most common chemical families found in cheeses (Delgado et al., 2011a; Delgado, González-Crespo, Cava, & Ramírez, 2011b; Evert-Arriagada et al., 2013; Juan et al., 2007; Le Quéré, 2011). Ketones and acids were by far the most abundant volatile compounds isolated from the headspace of cheese, representing more than 80 % of total found volatile compounds. Other volatile compounds classified as miscellaneous compounds were also identified in hard goat's cheese like ethyl esters, terpenes, aromatic hydrocarbons and phenol.

The level of ketones, acids and alcohols were affected by ripening time, since their values increased ($P < 0.05$) at day 30 in all samples. However, values of aldheydes were lower at day 30 than those at the beginning of ripening in all cheeses (Table 1). Conversely, other compounds like ethyl esters, aromatic hydrocarbons and terpenes did not showed a clear tendency due to the ripening time.

The transformed (area units / 10^5) mean concentrations of each volatile compound identified in cheeses are shown in Tables (6.5 – 6.8). A total of 36 volatile compounds were detected in the headspace of goat's milk cheeses studied in the present work, which consisted of control and HP-treated cheeses before and after brining. The volatiles included nine ketones (Table 6.5), six acids (Table 6.6), five alcohols (Table 6.7), five aldheydes (Table 6.8) and eleven miscellaneous compounds that could not be classified in any of the former groups (Table 6.9).

6.2.1 Effect of HP-treatment on volatile profile of cheeses at day 1 and 30 of maturation

As it is shown in Table 6.4, no significant differences could be observed in main chemical family's values due to HP-treatments, nor at day 1 neither at day 30. However, there was a slight effect of high-pressure treatment on some of the samples studied. Relative abundances at day 1 of high pressure treated-cheeses showed an increase compared with the control in acids (C: 164.27 x 10^5 Au and HP: 349.56 x 10^5 Au) and aldheydes (C: 38.39 x 10^5 Au and HP: 117.15 x 10^5 Au) measured as mean values regardless of the moment of HP application. In contrast, mean alcohols value at day 1 was lower than the control after the HP treatments. Although these differences, as commented above, were not significant at day 30, the pressure effect was more evident especially in 300 MPa HP-treated cheeses, which showed higher values of acids (1086.05 Au) and ketones (1899.99 Au) compared with the control (acids: 878.41 and ketones: 1600.20 Au) cheese.

Regarding to the moment of high-pressure application, cheeses HP-treated before salting (BS) seemed to reach greater values than cheeses high-pressure treated after salting (AS). As it is shown in Table 6.4, pressure intensity and the moment of HP treatment application influenced the total sum of observed compounds. Samples HP-treated at 300 MPa before brining (BS) attained

higher scores of total volatile compounds sum of area units than their counterparts high-pressure treated at lower pressures (100 or 200 MPa) and cheeses HP-processed after brining (AS). Nevertheless, the impact of pressure intensity was larger than this caused by the different moment of HP application.

6.2.2 Ketones. Ketones were the largest group of volatile compounds identified in goat's milk cheeses (Table 6.5). Nine ketones were detected, being most of them aliphatic linear ketones or methyl ketones owning between 7 and 18 carbon atoms, as described by Guillén et al., (2004). Among ketones, 2-nonanone and 2-heptanone, followed by far by 2-undecanone, 2-pentanone, 8-nonen-2-one and 2-butanone, 3-hydroxy, were the most abundant. Only one ketone (2-butanone, 3-hydroxy) isolated in the present study, suffered a relative decrease of its abundance between day 1 (441.60 x 10^5 Au) and day 30 (300.06 x 10^5 Au), while most of ketones experienced a great increase throughout ripening. Similar results regarding to the methyl ketones isolated in this study were reported by Juan et al. (2007) who studied the effect of high pressure treatments on ewe's milk cheese at different stages of ripening. In this study the main methylketones found throughout ripening were 2-nonanone and 2-heptanone, whereas 2-octanone, 2-decanone and 8-nonen-2-one were detected, although to a lesser extent.

It is widely known that the synthesis of ketones is related to the enzymatic activity of moulds, especially in surface-mould ripened cheeses (Curioni & Bosset, 2002). Since goat's milk cheeses studied were ripened allowing the growth of moulds in their surface, it is not surprising that large amount of odd-chain alkan-2-ones from C_3 to C_{15} were detected. Usually, ketones are the main fraction in constituents of cheese aroma and they are mostly produced from FFA by an alternative pathway of beta-oxidation (Delgado et al., 2011b; Le Quéré, 2004).

Regarding to HP treatments, some changes in ketones values can be observed (Table 6.5). Several ketones showed an increase at day 30 in 300MPa samples respect to the control cheese, nevertheless these differences were only significant ($p < 0.05$) in 8-nonen-2-one. Several authors have pointed out the application of high-pressure as a method of reducing microbial counts, thus changing dramatically the volatile profile of cheese (Capellas, Mor-Mur, Gervilla, Yuste, & Guamis, 2000; Delgado, González-Crespo, Cava, & Ramírez, 2012; Jose Delgado, Delgado, Gonzalez-Crespo, Cava, & Ramirez, 2013; Lopez-Pedemonte, Roig-Sagues, De Lamo, Hernandez-Herrero, & Guamis, 2007; Rynne et al., 2008; Trujillo, Capellas, Saldo, Gervilla, & Guamis, 2002). However, Juan et al. (2007) observed that the treatment applied at 300 MPa allowed a recovery of lactic acid bacteria, explaining why these samples reached similar ketones values compared to the control cheeses. In this study, there should be another factor explaining higher level of ketones in 300 MPa and lower levels in 100 and 200 MPa, playing an important role in ketones development during ripening, but microorganisms. Although formation of ketones is mainly a result of the lipolytic action by microbiota in cheeses, again higher levels of moisture, pH and proteolysis could enhance the formation of ketones in 300 MPa cheeses (see chapter IV). In agreement to our results, Delgado et al. (2011a) found higher levels of ketones in HP-treated (400 and 600 MPa) goat cheeses compared with the control cheese. Additionally, total ketones were significantly increased when

cheese was HP-treated at 600 MPa after 3 and 5 weeks of manufacture (Calzada, del Olmo, Picon, Gaya, & Nunez, 2014). Features related to degradation of methylketones into alcohols were described by Juan et al. (2007) reporting that pressures above 400 MPa could impede this transformation, thus indirectly increasing ketones values in HP cheeses.

In this study the moment (BS or AS) of HP-treatment application did not influenced the ketones volatile composition. The fact that levels of ketones were not largely ($p > 0.05$) affected by HP treatments, has been previously reported by some authors as a positive issue (Delgado et al., 2011b; Saldo et al., 2003). Ketones are common constituents of most dairy products and due to their typical odors and their low perception thresholds are indispensable in cheese aroma. Floral and fruity notes are generally given by ketones, therefore increasing values of ketones in 300 MPa HP-treated cheeses compared with the control can be considered as a valuable factor in these cheeses.

Low levels of 2-butanone, 3-hydroxy (acetoine) were identified in this study and also in other goat cheese varieties (Bintsis & Robinson, 2004; Carunchia Whetstine, Karagul-Yuceer, Avsar, & Drake, 2003; Engel et al., 2002; J.M. Poveda & Cabezas, 2006). The absence of detection of 2,3-butanedione (diacetyl) and the low levels found of 2-butanone, 3-hydroxy (acetoine) throughout ripening could be due to a possible transformation of 2,3-butanedione (not detected) into 2-butanone,3-hydroxy by starter bacteria. According to Juan et al. (2007), this is consistent with the recovery of BAL because of moderate high-pressure treatments of 100, 200 or 300 MPa were applied and growing of microorganisms was not compromised by high-pressure treatments.

It is important to highlight that several ketones were isolated only when some HP treatments were applied. For instance, 4-cycloheptenone or cyclohexanone, 3-methyl appeared only in 100 MPa or 3-octanone, cyclohexanone, 3-methyl and 2,5-octanedione, which appeared only when the HP-treatment applied was of 300 MPa. However, these results are not shown in tables because the amounts detected of these compounds were under the limit of detection (LOD).

Finally, some of the isolated ketones could be legitimized as markers of maturation since they mostly appeared at day 30 of ripening. Some of these compounds have been also described by other authors to be part of volatile profile of goat cheeses, such as 2-nonanone, 2-undecanone and 2-nonen-2-one (Attaie, 2009; Delgado et al., 2011a; Justa María Poveda et al., 2008; Saldo et al., 2003). Abundance of these compounds seemed to be unaffected at day 30 neither by pressure intensity, nor by moment of HP-treatment application. Indeed, only significant differences were obtained analyzing 8-nonen-2one, when 300 MPa pressure was applied, which values were higher than the rest of cheeses, including the control. This fact could suggest that 300 MPa cheeses attained higher ripening index by showing higher amounts of compounds classified as markers of maturation.

6.2.3 Acids. Acids are one of the larger chemical group isolated generally from cheeses and also in this study.

Some free fatty acids may be originated from lipolysis of milk fat or protein hydrolysis. However, it is also important to note that, some of the fatty acids, specifically those having between 4 and 20 carbon atoms, mostly come from the lipolysis of triglycerides by moulds (Curioni & Bosset, 2002). In the present study, as cheeses remain 30 days in the ripening room and moulds were allowed to grow in their surface, carboxylic acids, specifically those of 4-20 carbon atoms largely contributed to the volatile profile of goat's milk cheeses.

All acids detected in this study at day 1, were also detected at day 30 exhibiting and increase of their abundance, except for acetic acid, which did show a depletion in most of cheeses during the aging of cheese (Table 6.6). Significant productions of hexanoic, butanoic and octanoic acids were noticed, being these FFA the most abundant acids found in this study. Levels of these acids increased substantially during ripening whether in HP cheeses and control cheeses (Table 6.6). Hexanoic was the most abundant acid detected in this study, both at day 1 and day 30, with a total relative abundance up to $2.01 \cdot 10^9$ Au. Other compounds were detected in large abundance corresponding to octanoic at day 1 and to butanoic and octanoic acids at day 30. Butanoic acid suffered the major changes in relative mean abundance between day 1 and day 30 of all samples increasing its value during ripening in more than 500%. Although high-pressure treatments modified relative abundances of carboxylic acids and amounts of these acids increased or decreased up to the high-pressure treatment applied, none of these differences were significant at day 1.

In the case of 300 MPa, the combined effect of ripening and HP-treatments boosted higher values of most of carboxylic acids, especially hexanoic, octanoic, and n-decanoic acids, being these acids usually found at high concentration in goat milk cheeses (Hayaloglu, Tolu, Yasar, & Sahingil, 2013). However, while treatments at 300 MPa increased values of most acid compounds at day 30, this HP treatment, caused the decrease of acetic acid like at day 1. Acetic acid is not produced by lipolysis, but it is considered to be originated from the fermentation of lactate by microorganisms (Delgado et al., 2011a). It is not surprising that the higher the pressure applied the lowest the levels of acetic acid obtained. This fact suggests that probably 300MPa HP-treatment is affecting to the microorganisms involved in acetic acid formation, decreasing their activity and thus this acid relative abundance. Generally, at the end of ripening 300 MPa HP-treatments increased most of acids values (except for acetic acid), while cheeses treated at 100 or 200 MPa showed a decrease or similar values of abundances in volatile compounds compared with the control cheese.

The compounds hexanoic octanoic and butanoic acids are important in the aroma formation of cheese, not only by themselves but they are also precursors of methyl ketones, alcohols, lactones and esters (Le Quéré, 2004). These compounds have been previously described in goat cheeses as rancid cheese-like odour, goat odour and pungent notes (Delgado et al., 2011b; Juan et al., 2007) and widely recognized as the responsible for the characteristic aroma of goat cheeses giving sensory notes such as goat odor, rancid and pungent odour, and sour or aged cheese odour, respectively (J.M. Poveda & Cabezas, 2006). Several authors studied the effect of HP (400 and 600 MPa) on goat (Delgado et al., 2011b; Saldo et al., 2003) cheeses. Lower levels of carboxylic acids

and more specifically, typical goaty acids were reported by authors in pressurized cheeses. This fact was attributed to the lower lactococci counts and inactivation of bacterial enzymes by the HP-treatment. In the present study, while 100 and 200 MPa cheeses showed moderate or lower values of acids compared with the control, higher amounts in 300 MPa cheeses, especially in the case of goaty acids were reported during ripening. In this sense, results suggest another cause for this depletion of acids values in 100 and 200 MPa treated cheeses but the lower microbial counts. Probably, high amounts of moisture and FAA in 300 MPa cheeses (see chapter IV) are the key elements which enriched 300 MPa acids values, since proteolysis is one of the main pathways of formation of carboxylic acids during ripening. Higher values of acids in HP treated cheeses could be also due to the restructuration of cheese matrix caused by the high pressure (see chapter IV), and the consequent better interaction between enzymes and subtracts. Differences in results obtained applying several pressures can be attributed to the different inactivation level achieved depending on the pressure applied. Delgado et al. (2011b) also stated that the application of lower pressures than those performed in his studies (400-600MPa) might enhance certain biochemical lipolytic pathways, thus giving rise to changes in volatile profile of cheeses.

Taking into account sensory results, some remarkable characteristics of 300 MPa cheeses have to be noticed. On one hand, after HP treatments, a better texture is achieved (see chapter IV) compared with the control cheese that remains dry, hard and mouth feel-less, as it is usual in ripened semi-hard goat cheeses. The different texture of 300 MPa cheeses includes better water binding (see chapter V) within the cheese matrix, a softer and more continuous paste and a melting mouthfeel (see chapter IV). Moreover, 300 MPa treated-cheeses keep and even have increased their values of caproic, caprilic and capric carboxylic acids. The modification of carboxylic acids could lead to cheese volatile profile changes, as these acids are precursors of other volatiles compounds causing any changes at the end of maturation in the sensory profile as well. Thus, 300 MPa cheeses maintain their goaty sensory notes, their personality and their typical goat cheese aroma characteristics originating a new type of cheese but preserving its distinctiveness.

6.2.4 Alcohols. Alcohols have been described as one of the main chemical groups in goat's milk cheeses (Hayaloglu, 2013). These compounds can be originated by lactose metabolism by the pentose phosphate pathway, some chemical reactions and the activity of lactic acid bacteria dehydrogenase which leads to the reduction of aldheydes or methyl ketones that form their corresponding alcohols.

In this study a total of 5 alcohols were identified showing amounts up to the level of detection and being the third major chemical group isolated from headspace in goat's milk cheeses (Table 6.7). However, the amount of alcohols found was relatively low compared with other's flavor compound like acids or ketones. Alcohols isolated in this study were also reported by other authors as relevant compounds in cheese aroma. Molina et al., (1999) and Massouras et al. (2006) also studied goat milk volatile profile cheeses observing that the presence of, 1-butanol, 3-methyl is more usual in goat milk cheeses than in cow's cheeses.

Most of alcohols, namely 1-butanol, 3-methyl, 1-hexanol, 2-heptanol and 2-nonanol were only detected at day 30 of ripening of cheeses. Only ethanol was isolated both at day 1 and day 30. The amount of ethanol fluctuated during ripening in all cheeses however, this compound together with 2-nonanol were the most abundant alcohols in all samples obtaining more than 4.24 x 10^8 and 6.09 x 10^8 total Au, respectively, calculated as a measure regardless of the day or ripening, the pressure applied and the moment of pressure application. Ethanol has been already described as an important alcohol in other goat's milk cheeses (Attaie, 2009; Bontinis, Mallatou, Pappa, Massouras, & Alichanidis, 2012; Hayaloglu, Cakmakci, Brechany, Deegan, & McSweeney, 2007; Sable, Letellier, & Cottenceau, 1997). It is important to note also that high amounts of ethanol in some samples could be tightly related to the high amount of acids obtained by these cheeses and its posterior transformation.

The compound 2-nonanol is one of the principal secondary alcohols, which along with ketones, are considered to be the most important compounds in the aroma of mould-ripened cheeses (Le Quéré, 2004) , which is the case of goat's milk cheeses evaluated in the present study. Hayaloglu et al., (2013) observed different trends in ketones values during ripening of goat milk's cheese Gokceada, however depletion of these compounds were usually reported, probably due to the transformation of methyl ketones into secondary alcohols. Our results are in line with these previous experiences, as 2-nonanone is the most abundant methyl-ketone and 2-nonanol showed major values of alcohols, suggesting that there has been a reduction of the former into the latter.

Relative abundance values of major alcohols oscillated when different pressure treatments were applied. In general, the level of alcohols was slightly affected by high-pressure treatments. While no significant differences were detected related to HP-treatment in most of samples, 300 MPa BS HP-treated cheeses showed a different trend compared with control and the rest of pressurized (100 and 200 MPa) cheeses (Table 6.7). Those cheeses showed an increase of their values at day 30 in ethanol, 1-hexanol ($p < 0.05$), 1-butanol, 3-methyl and a great increase in 2-nonanol ($p < 0.05$), which is again expected, as 300 MPa BS cheese was the sample which obtained the major level of 2-nonanone at day 30 (Table 6.5).

While ethanol and 2-nonanol showed great amounts of abundance at day 30, 1-hexanol, 1-butanol, 3-methyl and 2-heptanol had minor contribution to the total amount of alcohols in HP-treated and control cheeses. According to Moio et al. (1993), the lower level of branched-chain primary alcohols such as 1-butanol, 3-methyl could reduce the pleasant aroma of cheese in all samples, but this is not the case of the present study, where 300 MPa BS HP-treated cheese showed 27.26 x 10^5 Au (Table 6.7), being the only sample where this compound could be isolated above the limit of detection.

The compound 2-heptanol was detected in all cheeses at day 30, except for 300 MPa and 200 AS cheeses, where was not detected. The compound 2-heptanol has been already detected in other goat's milk cheeses and additionally it has been identified as contributing to the volatile profile with mushroom notes (Delgado et al., 2011b; Justa María Poveda et al., 2008). The absence of this

compound in 300 MPa treated cheeses, could be related to a softening of the goaty and mouldy odour in cheeses, which could be positively valued and become a desirable effect.

Other alcohols were found only at day 30 in several samples, for instance 2-tridecanol. Additionally, 2- tetradecanol only appeared in control samples and 1-dodecanol and 1-pentanol, 4-methyl appeared in 300 BS and 300 AS, respectively. These compounds have been not included in the Table 6.6 as their values were under the established limit of detection.

In this study, some synergistic effect seemed to appear at the end of ripening between 200 and 300 MPa and BS moment. At day 30, these cheeses reached higher values than their counterparts pressurized after brining. Additionally, a clear relation-ship is established between ketones and alcohols, since increasing amounts of ketones due to high-pressure treatments have been also showed in alcohols. Therefore, 300 MPa HP-treatments and BS-moment influence amounts of ketones and thus, of alcohols.

6.2.5 Aldheydes. Table 6.8 shows mean values of aldheydes for pressurized (100, 200 or 300 MPa) before (BS) and after (AS) brining and control cheeses during ripening. According to Guillen et al. (2004), three of the 5 total aldheydes isolated in this study were aliphatic (hexanal, heptanal and 2-hexanal, (E)) and the other two belonged to the aromatic fraction (benzaldheyde, benzaldheyde, 2,5-bis[(trimethylsilyl)oxy]-) of aldheydes.

Aldheydes formed from amino acids by Strecker degradation are not normally found in cheese in great amounts. They are transitory compounds being immediately reduced to the corresponding alcohols (Adda, Gripon, & Vassal, 1982; Dunn & Lindsay, 1985). Thus, lower levels of aldheydes compared with other volatile compounds found in this study, suggest a rapidly transformation of aldheydes into alcohols, which in turn is a common fact in goat's milk cheeses (Hayaloglu et al., 2013).

Hexanal was the most abundant aldheyde in goat's milk cheeses at day 1 followed distantly by heptanal, which was only detected in BS and control cheeses. 2-hexanal (E), benzaldheyde, benzaldheyde, 2,5-bis[(trimethylsilyl)oxy]- were only found at day 1 in 200 BS cheese (Table 6.8). A high concentration of aldheydes may cause off-flavours, consequently a low level of aldheydes may be an indication of good maturation of cheese (Luigi Moio & Addeo, 1998). Thus, as it was expected, no aldheydes were detected up to the detection level at day 30.

Common aldheydes detected in cheese are hexanal, heptanal, nonanal, 2-methyl-propanal, 2-methyl-butanal, 3-methyl-butanal and benzaldheyde (Le Quéré, 2011). Hexanal and 2-hexanal (E) give the green note of immature fruit (Le Quéré, 2004) and herbaceous aromas (Curioni & Bosset, 2002). Most of them were isolated in the present work and especially in 200 BS cheeses, suggesting that probably these cheeses have not an appropriate volatile component balance. As commented before, high amounts of aldheydes could lead to an impeded desirable ripening. These results are consistent with the sensory punctuations of this sample, being evaluated as one

of the worse by panellists. Cheeses HP-treated at 200 MPa obtained high levels of firmness, acidity and granulosity and a very dry texture (see chapter IV).

No ethanal was found in any samples, not at day 1 and neither at day 30, even it is the most common aldheyde found in fermented dairy products and it is derived from lactose fermentation and breakdown of threonine (Lees & Jago, 1976; Marshall, 1987). Aldheydes are rapidly reduced to primary alcohols or even oxidized to the corresponding acids so the great amounts of ethyl alcohol in all samples could explain the absence of ethanal.

Based on these results, some relationship between pressure and the moment of its application regarding to the volatile profile of goat's cheeses could be drawn. Again, like in acids, ketones and alcohols, BS cheeses showed a major effect of pressure being modified their aldheydes abundance values, especially in 200 MPa cheeses. Salt inhibitory effect, high concentration of FAA, water holding capacity that allow transformation of volatile compounds, are some of the factors that could be involved in these changing dynamics giving rise to differences in volatile profile of pressurized cheeses compared with the control cheese (see chapter IV and V).

6.2.6 Miscellaneous compounds. Table 6.9 shows 11 compounds that could not be classified in any of the former groups, namely miscellaneous compounds. Several compounds like 2 ethyl esters, 3 aromatic hydrocarbons, 1 terpene, 3 pyrazynes and 1 butanamide were identified in goat's milk cheeses within this group.

Esters are important contributors to cheese aroma due to their low perception threshold (Molimard & Spinnler, 1996) and their high volatility at room temperatures. The presence of ethyl esters have been attributed to esterification between alcohols derived from lactose fermentation (ethanol) or amino acids catabolism and short to medium-chain fatty acids (Le Quéré, 2004). Most esters encountered in cheese are described as having sweet, fruity and floral notes. Esters appear during the early stage of ripening, and the microorganisms involved in their formation seem to be mainly yeasts (Le Quéré & Molimard, 2004; Le Quéré, 2011). Furthermore these compounds can contribute to the aroma of cheese by minimizing the sharpness and the bitterness imparted by fatty acids and amines, respectively (Gallois & Langlois, 1990). Hexanoic acid - ethyl ester seemed to decrease in pressurized at 200 MPa BS and control cheeses during ripening while it increased in other samples like 300 MPa HP-treated cheeses. The fact that hexanoic acid, ethyl esther was not detected at day 30 in control neither in 200 MPa HP-treated cheeses, could be related to low punctuations given by panelists in sensory analyses. These cheeses were described as acidic and sharped-taste cheeses, which can be explained by the absence of this compound that acts as a volatile softener compound of cheese flavor, leading to cheeses of strong taste and a broken volatile component balance.

Aromatic hydrocarbons such as toluene, styrene and benzene-1,3-dimethyl were only detected at day 1 in 200 BS treated cheeses. The presence of these compounds could be positive at a determinated concentration, but tend to release unpleasant sensory notes as their value reach

greater levels. Again, the presence of these compounds in 200 BS HP-treated samples could account for obtaining negative punctuations by panelists in sensory analyses.

Terpenes in cheese originate from plants that constitute the forage mixture of the pastures and they are transferred to the milk of the grazing animals and ultimately to the cheese (Mariaca et al., 1997). Thus, these compounds are important in these cheeses which are manufactured in regions where animals graze, like Alps or the Pyrenees areas, where cheeses are produced in an artisanal way. The only terpene isolated from our cheeses is α-pinene, which is the most frequently terpene identified in cheeses (Guillén et al., 2004; Jung, Ganesan, Lee, & Kwak, 2013). Only one sample (200 MPa BS) showed values of alfa-pinene at day 1, however at day 30 were 300 MPa AS and the control cheeses which account for 23.80 x 10^5 and 23.55 x 10^5 Au, respectively. Several authors have observed the presence of this compound in cheeses like Cheddar (Curioni & Bosset, 2002), Tulum cheese (Hayaloglu et al., 2007) and fresh cow's cheese (Evert-Arriagada et al., 2013), however, their importance in the formation of cheese flavour remains uncertain (Dunn & Lindsay, 1985).

Another group of volatile compounds isolated from cheeses in this study is pyrazynes and pyridines. Few information has been found about these compounds in goat's milk cheese, although Curioni and Bosset (2002) already described their contribution to cheese flavour.

It is remarkable that the only butanamide derived compound isolated in this study appeared in BS (100, 200 and 300 MPa) and control samples at day 1. This compound was affected by pressure and by the moment of its application although oscillating values were found at the end of ripening. Few references have been found referring to butanamide compounds in cheeses, nevertheless Alewijin et al. (2003) isolated butanamide in Gouda and Danish blue cheese and included it within the group of miscellaneous compounds as well, due to its difficult classification into the usual volatile compounds groups in cheese.

6.3 Conclusions

Thirty six volatile compounds were isolated in goat's milk cheese. The aroma profile was characterized primarily by acids and ketones compounds. Alcohols, aldehydes and other miscellaneous compounds were also identified although in lesser amounts.

In this study, HP-treatments caused several changes on the overall amount of volatile compounds found in goat's milk cheeses. While pressures of 300 MPa seemed to increase total amount of volatile compounds, cheeses treated at 100 and 200 MPa revealed lower levels compared with the control cheese. Results evidenced that high-pressure treatments up to 300 MPa, allowed the formation of aroma of cheese, indeed, equaling or even exceeding volatile compounds abundances of the control cheeses.

Some of the volatile chemical families, such as acids, ketones and alcohols substantially increased their presence when 300 MPa HP-treatments were applied on goat's milk cheeses. However, 100 and 200 MPa treated cheeses did show similar or even lower amounts of volatile compounds than control cheese in most of volatile compounds leading to an impoverished volatile profile. The absence of several compounds (2-heptanol and 2-hexenal-E) in 300 MPa treated-cheeses modified its volatile profile enhancing it and achieving a reduction of mouldy and herbaceous notes. On the other hand, the presence of compounds like hexanoic acid ethyl ester could minimize the sharpness and goaty notes changing the volatile profile of 300 MPa treated cheeses towards less pungent becoming softer cheeses.

The pressure and its moment of application revealed a synergistic effect in some fractions of the volatile profile, showing an enrichment of it especially in the case of BS cheeses.

Changes in volatile profile due to HP-treatments could be beneficial in the search for a new goat cheese-type, slightly modifying typical goaty flavor, thus softening its pungent flavor, and maintaining an appropriate volatile component balance.

Table 6.1 Main biochemical pathways of aroma compounds formation

Agents involved	Biochemical reaction	Metabolite formed	Consequences
Milk enzymes Starter cultures Secondary flora	Fermentation of lactose, lactate and citrate	Acids, mainly L-,D- and DL-lactic acid, sometimes acetic or carbonic and propionic acid.	Formation of butyric acid leads to taste and aroma defects.
	Protein decomposition (Proteolysis)	Free amino acids, biogenic amines, final ammonia	
	Fat hydrolisis (Lipolysis)	Free fatty acids	Goaty flavours
	Fission and oxidation of free amino acids and free fatty acids	Formation of special aroma compounds	

Table 6.2 Taste groups of amino-acids and flavor characteristics of free fatty acids (Adapted from (Kammerlehner, 2009)

Amino acids	Taste
Leucine, Lysine and Tyrosine	Neutral, nearly tasteless
Proline	Sweet
Threonine and Phenylalanine	Sweet-bitter
Tryptophane and histidine	Bitter
Aspariginic acid	Acidic-bitter
Glutamic acid	Acidic

Fatty acid (common name and chemical formula)	Flavour
Acetic acid *(Ethanoic acid, CH_3COO)* Propionic acid *(Ethane carboxylic acid, CH_3CH_2COOH)* Formic acid *(Methanoic acid, HCOOH)*	Biting
Butyric acid *(Butanoic acid, $C_4H_8O_2$)* Valeric acid *(Pentanoic acid, $CH_2(CH_2)_3COOH$)*	Sweaty
Capronic acid *(Hexanoic acid, $C_6H_{12}O_2$)*	Rancid
Caprylic acid *(Octanoic acid, $C_8H_{16}O_2$)* Capric acid *(Decanoic acid $C_{10}H_{20}O_2$)* Lauric acid *(Dodecanoic acid $C_{12}H_{24}O_2$)* Myristic acid *(Tetradecanoic acid $C_{14}H_{28}O_2$)* Palmitic acid *(Hexadecanoic acid, $C_{16}H_{32}O_2$)* Stearic acid *(Octadecanoic acid $C_{18}H_{36}O_2$)*	Wax-like (molten)
Oleic acid [9(Z)-Octadecanoic acid, $C_{18}H_{34}O_2$] Linoleic acid [(Z,Z) -9,12 Octadienoic acid $C_{18}H_{32}O_2$]	Odourless

Table 6.3. Sensory attributes of aroma identified with methyl ketones isolated from goat cheeses

KETONES	CORRESPONDENT ODOUR
2-pentanone	fruity
2-heptanone	mushroom or herbaceous
2-octanone	mouldy
2-nonanone	sour
2-undecanone	cooked
2-butanone, 3-hydroxy	butter
8-nonen-2one	cooked

(Le Quéré & Molimard, 2004; Justa María Poveda et al., 2008)

Table 6.4. Total volatile compounds[a] (Area units x 10^5) and total sum (Σ)[b] grouped in the main chemical families detected at day 1 and 30 in control and high-pressure-treated (100, 200 or 300 MPa) cheeses before or after salting (BS or AS).

	C	100BS	200BS	300BS	100AS	200AS	300AS
DAY 1							
Acid	164.27	317.27	888.63	202.76	167.56	268.02	253.13
ketone	85.29	135.38	109.39	53.41	40.12	72.95	86.20
alcohol	80.33	46.38	226.97	40.50	<LOD	32.02	39.54
aldheyde	38.39	102.12	367.97	50.18	50.60	70.72	61.31
miscellaneous compounds	184.89	66.81	282.89	38.49	<LOD	36.73	47.90
TOTAL Σ	3388.58	4059.40	11174.60	2598.29	2062.50	3098.64	3176.13
DAY 30							
acid	878.41	782.49	988.60	1069.39	776.95	812.13	1102.71
ketone	1600.20	1260.63	1627.91	2063.62	1613.02	1569.41	1736.37
alcohol	169.70	105.74	155.95	274.43	166.28	85.10	164.98
aldheyde	<LOD	<LOD	<LOD	26.95	<LOD	<LOD	<LOD
miscellaneous compounds	140.11	105.84	126.98	236.32	216.28	89.16	158.61
TOTAL Σ	17082.19	13818	17712.51	22200.15	16916.97	15637.43	19281.73

[a] Values(Area Units x 10^5) are given as means. [b] Total sum of abundances for each cheese sample.
Values within a row with different superscripts differ significantly (LSD test, $P < 0.05$). <LOD=under the limit of detection

Table 6.5. Abundance[a] (area units x10^5) of ketones detected at day 1 and 30 in the volatile fraction of control and pressure-treated cheeses (100, 200 or 300 MPa) before (BS) or after (AS) brining.

	ID[b]	DIA	C	100 BS	200 BS	300 BS	100 AS	200 AS	300 AS
2-Pentanone	**MS, ST**	**1**	<LOD	24.48	<LOD	<LOD	<LOD	<LOD	<LOD
		30	105.26	130.58	121.10	44.71	81.02	103.59	87.06
2-Heptanone	**MS, ST**	**1**	<LOD	<LOD	74.83	<LOD	<LOD	<LOD	27.93
		30	422.23	376.79	491.48	307.10	515.75	394.47	439.52
2-Butanone, 3-hydroxy-	**MS, ST**	**1**	68.98	132.77	38.09	43.98	49.98	56.63	51.17
		30	<LOD	<LOD	<LOD	43.03	163.17	27.39	66.47
2-Nonanone	**MS**	**1**	<LOD	<LOD	41.18	<LOD	<LOD	<LOD	<LOD
		30	931.66	686.60	877.62	1433.61	648.54	931.80	975.61
2-Undecanone	**MS**	**1**	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
		30	110.68	71.28	92.05	137.79	118.71	95.20	90.08
2-Heptanone, 3-methyl-	**MS**	**1**	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
		30	<LOD	30.39	<LOD	<LOD	<LOD	<LOD	<LOD
2-Decanone	**MS**	**1**	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
		30	<LOD	27.29^{a,b}	<LOD	86.06^{b}	<LOD	<LOD	<LOD
2-Octanone	**MS**	**1**	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
		30	26.93	<LOD	<LOD	<LOD	26.22	<LOD	23.44
8-Nonen-2-one	**MS**	**1**	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
		30	27.45^{a}	27.56^{a}	107.70^{a,b}	301.02^{c}	174.59^{c}	86.00^{a,b}	213.22^{b,c}
Total ketones[c]		**1**	85.29	135.38	109.39	53.41	40.12	72.95	86.20
		30	1600.20	1260.63	1627.91	2063.62	1613.02	1569.41	1736.37

[a] Values(Area Units x 10^5) are given as means. Values within a row with different superscripts differ significantly (LSD test, $P < 0.05$). [b]ID: Identification method; MS=mass spectra. Tentatively identified on the basis of the NIST libraries, ST= positively identified by comparison with MS of authentic standards. [c]Total expressed as mean values. <LOD=under the limit of detection

Table 6.6. Abundance[a] (area units x10^5) of acids detected at day 1 and 30 in the volatile fraction of control and pressure-treated cheeses (100, 200 or 300 MPa) before (BS) or after (AS) brining.

	ID[b]	DIA	C	100 BS	200 BS	300 BS	100 AS	200 AS	300 AS
Acetic acid	**MS**	**1**	40.52	55.85	285.89	23.43	23.35	38.20	37.08
		30	26.26	36.15	61.95	<LOD	31.25	66.66	26.25
Butanoic acid	**MS**	**1**	36.33	38.72	196.84	33.80	36.67	33.54	34.43
		30	292.62	279.90	319.55	249.44	237.51	310.11	479.05
Hexanoic acid	**MS, ST**	**1**	83.81	87.79	278.43	59.70	43.60	69.82	86.00
		30	300.71[a,b]	278.39[a]	340.71[a,b]	353.70[a,b]	289.59[a]	291.53[a]	402.90[b]
Octanoic acid	**MS, ST**	**1**	67.70	79.44	155.92	61.83	46.21	75.52	64.16
		30	192.65[a,b,c]	154.85[a]	179.56[a,b]	256.15[c]	169.84[a,b]	160.71[a]	235.99[b,c]
n-Decanoic acid	**MS**	**1**	44.43	54.20	102.30	43.81	29.98	46.93	40.47
		30	110.94[a,b]	83.84[a,b]	107.76[a,b]	188.05[c]	96.35[a,b]	70.99[a]	125.99[b]
Total acids[c]		**1**	164.27	317.27	888.63	202.76	167.56	268.02	253.13
		30	878.41	782.49	988.60	1069.39	776.95	812.13	1102.71

[a] Values(Area Units x 10^5) are given as means. Values within a row with different superscripts differ significantly (LSD test, $P < 0.05$). [b]ID: Identification method; MS=mass spectra. Tentatively identified on the basis of the NIST libraries, ST= positively identified by comparison with MS of authentic standards.
[c] Total expressed as mean values . <LOD=under the limit of detection

Table 6.7. Abundance[a] (area units x10^5) of alcohols detected at day 1 and 30 in the volatile fraction of control and pressure-treated cheeses (100, 200 or 300 MPa) before (BS) or after (AS) brining

	ID[b]	DIA	C	100 BS	200 BS	300 BS	100 AS	200 AS	300 AS
Ethanol	**MS, ST**	**1**	79.56	93.37	445.80	88.77	<LDD	52.56	34.06
		30	<LDD	57.85	81.60	95.44	183.51	71.81	96.89
1-Hexanol	**MS, ST**	**1**	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
		30	<LOD	<LOD	<LOD	71.06[b]	<LOD	27.70[a]	<LOD
2-Heptanol	**MS, ST**	**1**	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
		30	32.35	26.27	28.99	<LOD	26.72	<LOD	<LOD
1-Butanol, 3-methyl	**MS**	**1**	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
		30	<LOD	<LOD	<LOD	27.26	<LOD	<LOD	<LOD
2-Nonanol	**MS**	**1**	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
		30	<LOD	166.73[a]	176.27[a]	358.90[b]	122.10[a,b]	96.54[a]	165.91[a]
Total alcohols[c]		**1**	80.33	80.33	226.97	40.50	<LOD	32.02	39.54
		30	169.70	169.70	155.95	274.43	166.28	85.10	164.98

[a] Values(Area Units x 10^5) are given as means. Values within a row with different superscripts differ significantly (LSD test, $P < 0.05$). [b]ID: Identification method; MS=mass spectra. Tentatively identified on the basis of the NIST libraries, ST= positively identified by comparison with MS of authentic standards. [c] Total expressed as mean values. <LOD=under the limit of detection

Table 6.8. Abundance[a] (area units x10^5) of aldheydes detected at day 1 and 30 in the volatile fraction of control and pressure-treated cheeses (100, 200 or 300 MPa) before (BS) or after (AS) brining.

	ID[b]	DIA	C	100 BS	200 BS	300 BS	100 AS	200 AS	300 AS
Hexanal	**MS, ST**	**1**	59.06	71.88	308.25	33.64	32.70	48.72	47.88
		30	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Heptanal	**MS**	**1**	25.55	37.30	24.76	30.93	<LOD	<LOD	<LOD
		30	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
2-Hexenal, (E)	**MS, ST**	**1**	<LOD	<LOD	33.35[b]	<LOD	<LOD	<LOD	<LOD
		30	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Benzaldheyde	**MS, ST**	**1**	<LOD	<LOD	52.03[b]	<LOD	<LOD	<LOD	<LOD
		30	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Benzaldheyde, 2,5-bis[(trimethylsilyl)oxy]-	**MS**	**1**	<LOD	<LOD	345.18	<LOD	<LOD	<LOD	<LOD
		30	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Total aldehydes[c]		**1**	38.39	102.12	367.97	50.18	50.60	70.72	61.31
		30	<LOD	<LOD	<LOD	26.95	<LOD	<LOD	<LOD

[a] Values(Area Units x 10^5) are given as means. Values within a row with different superscripts differ significantly (LSD test, $P < 0.05$). [b]ID: Identification method; MS=mass spectra. Tentatively identified on the basis of the NIST libraries, ST= positively identified by comparison with MS of authentic standards. [c] Total expressed as mean values. <LOD=under the limit of detection

Table 6.9 Abundance[a] (area units x10^5) of miscellaneous compounds detected at day 1 and 30 in the volatile fraction of control and pressure-treated cheeses (100, 200 or 300 MPa) before (BS) or after (AS) brining.

	ID[b]	DIA	C	100 BS	200 BS	300 BS	100 AS	200 AS	300 AS
Ethyl esters									
Hexanoic acid, ethyl ester	**MS**	**1**	42.68	<LOD	91.28	<LOD	<LOD	<LOD	<LOD
		30	<LOD	<LOD	<LOD	30.60	26.23	<LDD	31.51
2-Hydroxymandelic acid, ethyl ester	**MS**	**1**	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
		30	<LOD	37.14	<LOD	<LOD	<LOD	<LOD	<LOD
Aromatic hydrocarbons									
Toluene	**MS, ST**	**1**	<LOD	<LOD	109.97	<LOD	<LOD	<LOD	<LOD
		30	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Styrene	**MS**	**1**	<LOD	<LOD	33.99[b]	<LOD	<LOD	<LOD	<LOD
		30	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Benzene, 1-3-dimethyl-	**MS**	**1**	<LOD	<LOD	204.60[b]	<LOD	<LOD	<LOD	<LOD
		30	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Terpene									
α-Pinene	**MS**	**1**	<LOD	<LOD	23.95[b]	<LOD	<LOD	<LOD	<LOD
		30	23.55	<LOD	<LOD	<LOD	<LOD	<LOD	23.80
Others									
Trichloromethane	**MS**	**1**	<LOD	<LOD	113.01[b]	<LOD	<LOD	<LOD	<LOD
		30	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Butanamide, 2,2,3,3,4,4,4-heptafluoro-N-[2-[(trimethylsilyl)oxy]-2-[4-[(trimethylsilyl)oxy]phenyl]ethyl]-	**MS**	**1**	24.67[a,b]	32.53[a,b]	157.69[b]	26.18[a,b]	<LOD	<LOD	<LOD
		30	<LOD	<LOD	<LOD	41.59[b]	41.98[a,b]	<LOD	<LOD
Pyridine, 2,6-dimethyl-	**MS**	**1**	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
		30	58.47[b]	<LOD	<LOD	<LOD	29.43[a,b]	<LOD	26.19[a,b]
Pyrazine, 2,5-dimethyl	**MS**	**1**	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
		30	47.03[b]	<LOD	27.87[a,b]	<LOD	42.67[b]	<LOD	<LOD
2-Methyl-6-propylpyridine	**MS**	**1**	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
		30	47.80[a,b]	34.02[a]	77.41[a,b]	182.00[b]	101.45[a,b]	<LOD	<LOD
Total miscellaneous compounds[c]		**1**	184.89	66.81	282.89	38.49	<LOD	36.73	47.90
		30	140.11	105.84	126.98	236.32	216.28	89.16	<LOD

[a] Values(Area Units x 10^5) are given as means. Values within a row with different superscripts differ significantly (LSD test, $P < 0.05$). [b]ID: Identification method; MS=mass spectra. Tentatively identified on the basis of the NIST libraries, ST= positively identified by comparison with MS of authentic standards. [c]Total expressed as mean values <LOD=under the limit of detection

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Chapter VII

Final conclusions

Final conclusions

1. The application of High Hydrostatic Pressure (HP) treatments at 100, 200 or 300MPa before (BS) or after (AS) salting, modified some of the physico-chemical properties of goat's milk cheeses. Changes were especially noticeable at 300 MPa, which rose up the pH value and the moisture content of cheeses. Additionally, this pressure caused the highest color differences, leading to a yellowing effect, and increased the amount of WSN/TN and FAA. Thus, 300 MPa goat's milk cheeses appeared as much more ripened than their counterparts probably due to color characteristics and proteolysis values.

2. In relation to textural parameters, cheeses treated at 300 MPa were less firm and showed a more elastic behavior than control and the other HP cheeses. Textural characteristics of 300 MPa cheeses could be attributed to their intensive proteolysis and the high pH and moisture values. Significant differences referring to pressurized at different moments of brining were also observed. At the end of ripening, cheeses treated at 100 and 200 MPa after brining resulted in lower fracture stress (σ_t) than the observed in cheeses pressurized before brining.

3. Depending on the pressure applied to cheeses different microstructure were obtained. Whereas control cheeses had a sponge-like structure, 100 and 200 MPa showed a coarse matrix and 300 MPa gave the most homogeneous and regular structure. The 300 MPa cheeses were characterized by their low porosity and elevate lipidic area at the end of ripening.

4. Sensory analyses supported instrumental measurements of color, moisture content and texture of cheeses and were able to differentiate 300 MPa cheeses from the rest based on appearance, textural and taste attributes. High scores of mouthfeel, closely related to moisture sensation, were obtained by these cheeses, whereas granulosity, firmness, acid and bitter taste were significantly undervalued compared to the rest of samples. Cheeses high-pressure treated at 300 MPa received higher overall grade than other samples, mostly with respect to textural parameters, leading to better mouthfeel cheeses and reducing release of pungent or goaty notes. Panelists were not able to identify differences between BS and AS cheeses.

5. Thermogravimetrical analyses revealed some differences on water typology of cheeses. The W1 (free water) was the larger type of water found in all cheeses during ripening, followed by W3 (bound water) and by W2 (entrapped water), which was the scarcest water-type found in this work. Internal water profile of cheeses changed during ripening mainly depending on the HP applied. The 300 MPa cheeses showed greater amounts of W1 and to a

lesser extent of W3, indicating good water binding capacity compared with control or HP cheeses.

6. Most of samples reached the equilibrium of salt content between inner and outer parts of cheese at the end of ripening. Regarding the pressure effect, HP samples, especially 300 MPa cheeses, revealed a greater penetration of salt at day 1, showing higher values than control and the rest of pressurized cheeses in the inner part of cheese. The 300 MPa cheeses revealed a faster diffusion of salt during ripening obtaining similar values between both cheese parts studied (inner and outer) at day 7 of ripening. No large effect can be observed in HP samples respect to the moment of brining. However, it is likely that pressure application influenced salt uptake and penetration of salt throughout the wedge of cheese.

7. HP-treatments changed the overall amount of volatile compounds of goat's milk cheeses, increasing total amount of values when 300 MPa treatments were applied and decreasing it at 100 and 200 MPa revealing the latter an impoverished volatile profile. The pressure and its moment of application revealed a synergistic effect in some fractions of the volatile profile, showing an enrichment of it in the case of BS cheeses. The presence or absence of several compounds in 300 MPa HP-treated cheeses modified its volatile profile enhancing it leading to a reduction of mouldy notes and minimizing sharpness and goaty attributes in those cheeses becoming softer and less pungent in regards to their volatile profile.

8. Among the studied variables, pressure intensity was able to produce major changes on goat's milk cheeses, with especial regards to 300 MPa HP-treaments. In general, the moment of HP application did not show significant differences except for some of the attributes analyzed like moisture content, fracture stress, strain, porosity and volatile profile at some of the pressures applied. HP processing at 300 MPa may provide new textures to traditional cheeses or even the possibility to create novel types of cheese enhancing their commercial characteristics being more appealing to consumers and providing beneficial factors, economically speaking.

Chapter VIII

Appendix

Appendix I

8.1 Preliminary adjustments of cheese making conditions

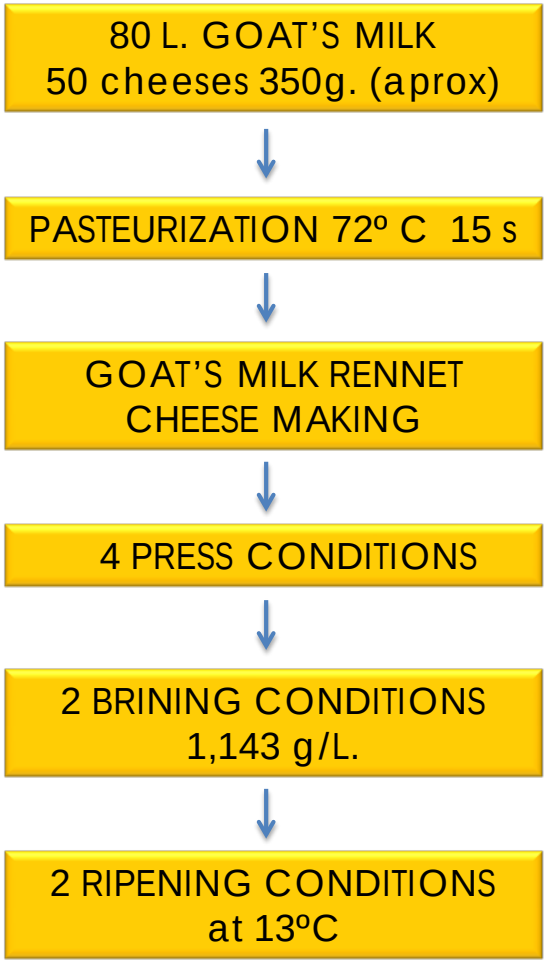
Preliminary tests were carried out to determine optimal conditions of cheese making. Since one of the main goals of this study was to enhance the moisture content of cheese, several conditions during cheese manufacture were modified in order to assure the maximum water binding in cheese. Pressing, brining and different relative humidity in the ripening room conditions were tested in order to determine moisture content, salt in moisture, pH and Aw of cheeses.

The figure 13 shows the experimental design followed to test four different pressing conditions involving several pressures and times and combinations between them.

Table 23 shows physico-chemical results of cheeses pressed at several conditions of pressure and time. Sample number 4 obtained higher moisture content at every point of sampling during ripening. Higher moisture content of this cheese, probably led to higher values of pH and appropriate values of salt in moisture at the end of ripening. Values of Aw also contribute to the choice of condition number 4 as the optimal to retain maximum water into cheese matrix.

Table 8.1 Physic-chemical results of goat milk cheeses manufactured under several pressing conditions.

Day	Sample	Moisture	Aw	pH	Cl (g/100 g de formatge)	S/M (salt in moisture)
1	1	53,059 *± 0,063*	0,981*± 0,002*	5,100 *± 0,035*	1,218 *± 0,000*	2,296*± 0,007*
	2	52,143 *± 0,294*	0,982*± 0,002*	5,050 *± 0,010*	0,894 *± 0,013*	1,715*± 0,044*
	3	52,601 *± 0,323*	0,981*± 0,000*	5,080 *± 0,025*	1,002 *± 0,006*	1,905*± 0,035*
	4	52,395 *± 0,163*	0,983*± 0,002*	5,100 *± 0,032*	1,013 *± 0,000*	1,933*± 0,009*
10	1	50,344 *± 0,088*	0,978*± 0,000*	4,900 *± 0,021*	1,068 *± 0,012*	2,121*± 0,023*
	2	50,130 *± 0,151*	0,982*± 0,001*	4,850 *± 0,023*	1,081 *± 0,006*	2,156*± 0,002*
	3	49,636 ± *0,063*	0,982*± 0,001*	4,840 *± 0,026*	0,917 *± 0,000*	1,848*± 0,036*
	4	50,033 ± *0,048*	0,984*± 0,000*	4,950 *± 0,042*	0,827 *± 0,005*	1,653*± 0,001*
15	1	43,907 *± 0,073*	0,970*± 0,001*	4,840 *± 0,021*	1,792 *± 0,007*	4,081*± 0,002*
	2	43,371 ± *0,195*	0,973*± 0,001*	4,770 *± 0,010*	1,045 *± 0,008*	2,409*± 0,001*
	3	45,203 *± 0,033*	0,976*± 0,000*	4,860 *± 0,017*	1,168 *± 0,000*	2,584*± 0,032*
	4	46,424 ± *0,020*	0,977*± 0,002*	4,800 *± 0,021*	1,193 *± 0,007*	2,570*± 0,014*
30	1	32,415 *± 0,025*	0,951*± 0,001*	5,11 *± 0,044*	1,710 *± 0,006*	5,275*± 0,026*
	2	33,671 ± *0,086*	0,954*± 0,001*	5,47 *± 0,006*	1,761 *± 0,012*	5,230*± 0,034*
	3	34,954 ± *0,083*	0,961*± 0,001*	5,36 *± 0,026*	1,600 *± 0,019*	4,577*± 0,009*
	4	36,756 *± 0,090*	0,959*± 0,001*	5,77 *± 0,049*	1,723 *± 0,006*	4,688*± 0,012*



PRESS CONDITION	PRESSING PHASE 1		PRESSING PHASE 2	
	Pressure	Time	Pressure	Time
1	0,5Kg	0,5h	1Kg	1h
2	0,5Kg	0,5h	1Kg	2h
3	0,5Kg	1h	1Kg	1h
4	0,5Kg	1h	1Kg	2h

BRINING CONDITION	BRINING TIME
1	48 min
2	60 min

RIPENING CONDITION	RELATIVE HUMIDITY
1	80 %
2	90 %

Figure 8.1. Cheese making process and several conditions of pressing applied on goat's milk cheese.

It is known that the salt content of a cheese affect in a direct manner water binding and moisture content of it. As the main goal of this study were to evaluate the influence of HP on water and salt distribution within the cheese matrix, another batch of cheeses was manufactured applying 2 different times of brining. Sensory results were carried out to evaluate which brining time was better suited to achieve the objectives of the study. Cheeses were brined at 48 and 60 min in a brining solution 1.139 g/L concentrated. Panelists found too salty taste in cheeses salted during 60 min even defining it as a negative attribute. No off-flavours and no lack of salt was observed in cheeses brined for 48 min, thus this latter condition was selected to proceed to the HP experiments.

Respect the relative humidity selected in the ripening room, 90 % R.H. was chosen because of the better growing of moulds on surface of cheese after visual examination, leading to a possible better retention of moisture within the mass of cheese.

Appendix II

8.2 Preliminary tests for choosing volatile profile analyses conditions

Preliminary tests for choosing fiber coating, temperature and time extraction for SPME analyses of cheeses volatile compounds were carried out. Different running times of the GC-MS method were also assayed.

Table 8.2 Conditions tested for the tune up of volatile compounds analyses of goat cheeses

Tested fiber coatings	Temperature Extraction (ºC)	Time Extraction (min)	Running time (min)
65μm PDMS / DBV (Supelco)	40	20	53
85μm CAR / PDMS (Supelco)	50	30	42
50 / 30μm DBV / CAR / PDMS (Supelco)	60		

In order to choice the most appropriate fiber, three different coatings were studied (Table 24), taking into account the extraction method, polarity of compounds and thickness of coating.

Extraction by headspace was chosen to extract volatile compounds of cheeses. Static headspace sampling was applied for extraction because of the simplicity and the appropriate sensitivity of the method.

Regarding to the fiber coatings and its matching with volatile compounds, some of them like divinyl benzene (DVB) are very efficient in extracting polar compounds because of its polar porous solid coating properties. The rule 'like dissolves like' applies in the selection for the appropriate SPME fiber for extraction. Other fiber coatings like PDMS or CAR/PDMS-coated fibers could be more efficient extracting organic compounds containing only carbon and hydrogen atoms (alkanes). A combination of a non-polar material like PDMS and a polar material like PDMS or a DVB/CAR/PDMS fiber coating could be an appropriate choice to extract substrates having a mixture of polar and non-polar materials (bipolars) like alcohols, aldheydes, ketones, ethers, and carboxilic acids. Several fiber coatings were tested and best results were obtained with a DVB/CAR/PDMS-coated fiber compared to a CAR/PDMS and a PDMS/DVB. More compounds were detected with this fiber and a better signal from the equipment showing greater amounts of abundance seemed to be performed. Thus, a DVB/CAR/PDMS-coated fiber was chosen to extract volatile compounds of cheeses.

The sensitivity of the extraction time required for the SPME method could be determined by the fiber coating thickness. Thicker coatings promote greater sensitivity of the method but longer times for equilibration. Hence, thinner coatings speed up extraction time but offers at the same

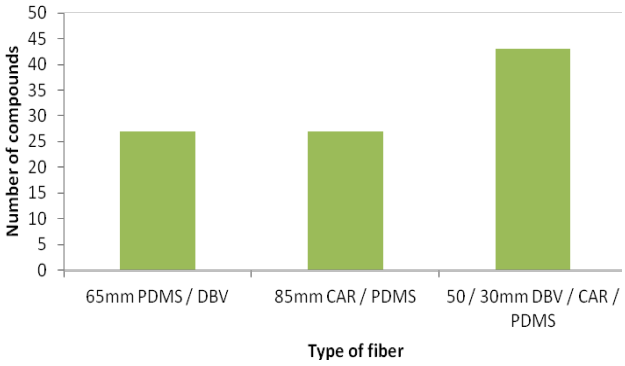
time the required sensitivity. A 50/30 μm fiber-thickness was chosen to proceed with the SPME analyses.

Three different extraction temperatures were assayed (Table 24), being 50ºC the optimal equilibration temperature for the fiber chosen. At this temperature, major number of compounds was detected and a greater abundance of those was counted.

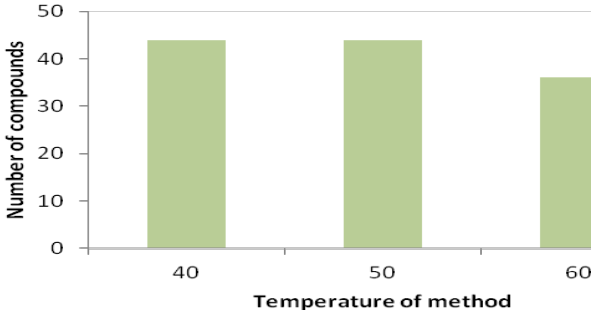
In order to achieve reproducibility, careful timing and constant convection conditions by sample stirring have to be maintained. Time of extraction is given by the optimal equilibrium reached between compounds migration from the sample matrix to the gaseous phase (headspace) and from the headspace to the fiber coating. Preliminary test were carried out during 20 and 30 minutes of extraction. Technical issues and adaptation of sampling extraction to running time in the equipment were determinant to choose 30 minutes as the optimal extraction time.

Two different methods (Table 24) with temperature ramps were loaded and tested in GC-MS equipment. After analyzing obtained chromatograms, shorter running time was chosen (42 min), optimizing the total time of analyses and taking into account that the retention time of most of volatile compounds detected was between 11 and 35 minutes.

a)



b)



c)

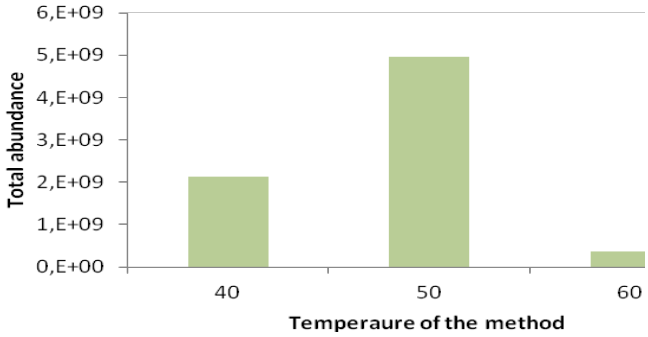


Figure 8.2 Results of number of compounds obtained with different tested types of fiber a); at different temperatures of extraction b); and total abundance of compounds found at different temperatures of extraction.

Therefore, the optimum conditions chosen for the SPME analyses, considering efficiency and technical issues on the volatile profile of goat's milk cheeses, were DVB/CAR/PDMS of 50/30 μm fiber thickness, selecting a 50 º C equilibration temperature, 30 min of extraction time and 42 min of running time method.

Appendix III

8.3 Profile sheet of tasting notes used to perform the sensory analyses

NOM DEL CATADOR:

DATA: MOSTRA Nº:

Heu de catar les mostres i apuntar les diferències entre la mostra control i la resta.

Es tracta de situar dintre de l'escala donada, les mostres respecte al control.

En el cas que trobeu algún comentari important a fer, el podeu anotar en la casella d'observacions.

+/-1: desviació LLEUGERAMENT perceptible de l'atribut a valorar respecte al control

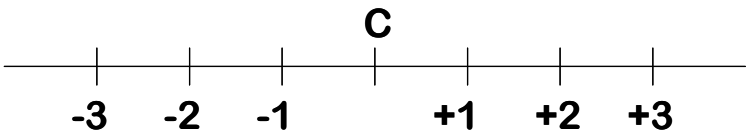
+/-2: desviació MODERADAMENT perceptible de l'atribut a valorar respecte al control

+/-3: desviació MOLT perceptible de l'atribut a valorar respecte al control

TEXTURA

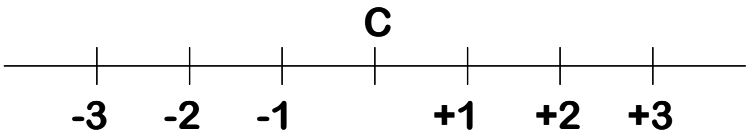
FERMESA

(Dits i Boca)



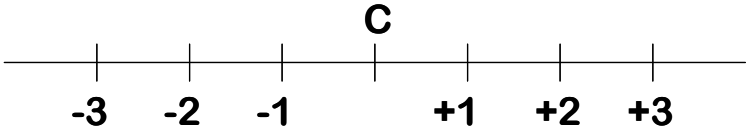
SENSACIÓ D'HUMITAT

(En boca)



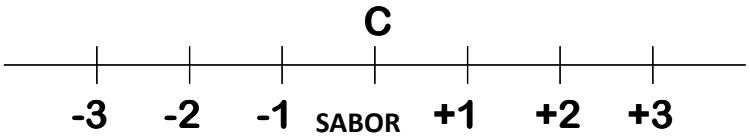
GRANULOSITAT

(En boca)

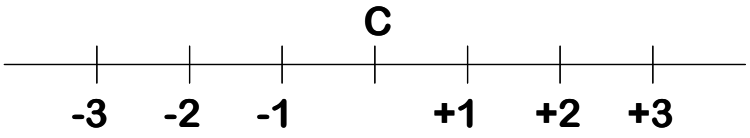


PASTOSITAT/

ADHERÈNCIA

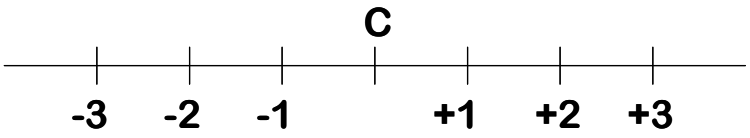


SABOR



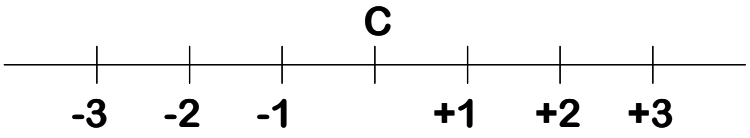
ACIDESA

AMARGOR



ASPECTE

COLOR



QUINA MOSTRA T'HA AGRADAT MÉS?

OBSERVACIONS