UNIVERSITÀ DEGLI STUDI DI PADOVA

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DIPARTIMENTO DI INGEGNERIA INDUSTRIALE DII

CORSO DI LAUREA MAGISTRALE IN CHEMICAL AND PROCESS ENGINEERING

TESI DI LAUREA MAGISTRALE

NON-CONTACT ULTRASOUND FOR THE CHARACTERIZATION OF PHYSICOCHEMICAL PROPERTIES AND DEFECTS IN CHEESE

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Anno Accademico 2024/2025

A Marta, il mio punto fermo.
A Padova, infinitamente grata.

Abstract

One of the main objectives of the food industry is to guarantee high product quality to meet consumer demands. To this end, there are various industrial techniques for analysing food properties. This thesis focuses on the analysis of the physical-chemical properties and defects of cheese using non-contact ultrasound coupled with air. This is an innovative and modern technique that has grown significantly in recent years. It is reliable, low-cost, non-invasive and represents a leading solution in the field of food research. The main objective of the study is to monitor the evolution of cheese properties during the ripening process. Along with the ultrasound analysis, additional parameters such as cheese thickness, texture, and moisture content are also evaluated. The ultrasonic investigations are carried out using a relatively innovative methodology, which employs both pulse-echo (PE) and transmission-reception (TR) modes. During the study, various parameters are examined, with particular attention to the ultrasonic velocity, hardness and moisture content of the cheese. The results obtained show that ultrasonic velocity and hardness increase as the days of maturation progress, while moisture decreases. The results obtained are in line with expectations, as the progression of maturation leads to water loss, which results in greater compactness of the cheese matrix, thereby promoting both an increase in the propagation speed of ultrasonic waves and an increase in the mechanical strength of the product. The non-contact ultrasonic technique proves particularly effective as it allows rapid, reproducible and accurate measurements to be obtained without altering the product. Furthermore, its non-invasive and low-cost nature makes it suitable not only for research but also for large-scale industrial application. Possible future improvements include optimising the equipment to further reduce measurement noise and increase sensitivity, as well as integrating automated and intelligent data acquisition systems that enable real-time monitoring throughout the ripening process.

Riassunto

Uno degli obiettivi principali delle industrie alimentari è garantire un'alta qualità dei prodotti per soddisfare le richieste dei consumatori. A tal fine, esistono diverse tecniche industriali per analizzare le proprietà degli alimenti. Questa tesi si concentra sull'analisi delle proprietà fisico-chimiche e dei difetti del formaggio utilizzando gli ultrasuoni senza contatto accoppiati con aria. Si tratta di una tecnica innovativa e moderna, in forte crescita negli ultimi anni. È affidabile, a basso costo, non invasiva e rappresenta una soluzione di spicco nel settore della ricerca alimentare. L'obiettivo principale dello studio è monitorare l'evoluzione delle proprietà del formaggio durante il processo di maturazione. Oltre all'analisi ad ultrasuoni, vengono valutati anche altri parametri, come lo spessore del formaggio, la consistenza e il contenuto di umidità. Le indagini ultrasoniche si svolgono utilizzando una metodologia relativamente innovativa, che impiega sia la modalità pulse-echo (PE) sia la modalità trasmissione-ricezione (TR). Durante lo studio vengono esaminati diversi parametri con particolare attenzione alla velocità ultrasonica, alla durezza e al contenuto di umidità del formaggio. I risultati ottenuti mostrano che la velocità ultrasonica e la durezza aumentano con il progredire dei giorni di maturazione, mentre l'umidità diminuisce. Gli esiti ottenuti risultano in linea con le aspettative, poiché il progredire della maturazione comporta una perdita d'acqua che determina una maggiore compattezza della matrice casearia, favorendo di conseguenza sia l'incremento della velocità di propagazione delle onde ultrasoniche sia l'aumento della resistenza meccanica del prodotto. La tecnica a ultrasuoni senza contatto si dimostra particolarmente efficace poiché permette di ottenere misurazioni rapide, riproducibili e accurate senza alterare il prodotto. Inoltre, la natura non invasiva e low-cost la rende adatta non solo alla ricerca, ma anche a un'applicazione industriale su larga scala. Per il futuro, i miglioramenti possibili riguardano l'ottimizzazione delle apparecchiature, al fine di ridurre ulteriormente il rumore di misura e aumentare la sensibilità, nonché l'integrazione di sistemi di acquisizione dati automatizzati e intelligenti, che consentano un monitoraggio in tempo reale durante tutto il processo di maturazione.

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Introduction

Nowadays, the food industry is in continuous development, with increasingly advanced technologies and high standards of food quality and safety. One of the main sensory quality parameters is texture, a characteristic perceived through a more or less complex chewing process. Texture plays a fundamental role in the design and evaluation of the commercial quality of food products, as it represents one of the key aspects determining consumer acceptance and preference. Textural properties are strongly influenced by storage conditions, temperature, humidity, and the characteristics of food packaging. In both industrial and research fields, traditional methods are already used to evaluate texture, as in the case of a study conducted on Mahon cheese (J. Benedito *et al.*, 2000). Among these methods are uniaxial compression, puncture tests, and sensory analysis. Through these techniques, it is possible to analyse the evolution of texture parameters, such as hardness, during the ripening process of food, and to identify correlations between instrumental and sensory measurements.

Over the years, new and ambitious analytical techniques for food quality control have been developed, with the aim of replacing traditional methods that are often slow, expensive, and destructive (either totally or partially) to the analysed samples. Among these techniques are vibration rheometers, small displacement probes, visible/near/mid-infrared spectroscopy, Z-nose, electronic noses, and ultrasonic techniques (J. Benedito *et al.*, 2005). Small displacement probes are used to assess the ripening degree of various fruits such as apples, pears, and peaches (Arana *et al.*, 2001). The acoustic impulse-response technique and ultrasounds are applied to detect internal cracks in Manchego cheese (T. Conde *et al.*, 2008).

Ultrasound techniques represent one of the most innovative and promising approaches in the field of food analysis, as they are non-invasive, cost-effective, and easily applicable. They are classified into contact and non-contact (air-coupled) ultrasound. Numerous studies investigate the application of ultrasonic techniques for evaluating the ripening of fruits and vegetables (Mizrach, 2004), the composition of meat and fish (J. Benedito *et al.*, 2001), and the texture of different types of cheese (José V. García-Pérez *et al.*, 2002). Moreover, non-contact ultrasound has been successfully employed to detect foreign bodies in cheese, identify deliberate additives in chocolate, and determine the filling level and content of metallic food cans (P. Pallav *et al.*, 2008).

The main objective of this master's thesis project is to evaluate the evolution of the structural and textural properties of Oveja cheese, a traditional Spanish variety, during its ripening process. The study employs the non-invasive technique of non-contact (air-coupled) ultrasound to monitor structural changes throughout maturation. In order to obtain a comprehensive assessment of the cheese's texture, additional analyses are carried out using a texturometer and a moisture determination procedure. Together, these methods provide an integrated evaluation of the cheese's textural properties and their evolution during ripening. This project is inspired by a previous study conducted on Thierno cheese (A. Giacomozzi *et al.*, 2024). The data obtained from that research are analysed through various statistical techniques and mathematical correlations (equations).

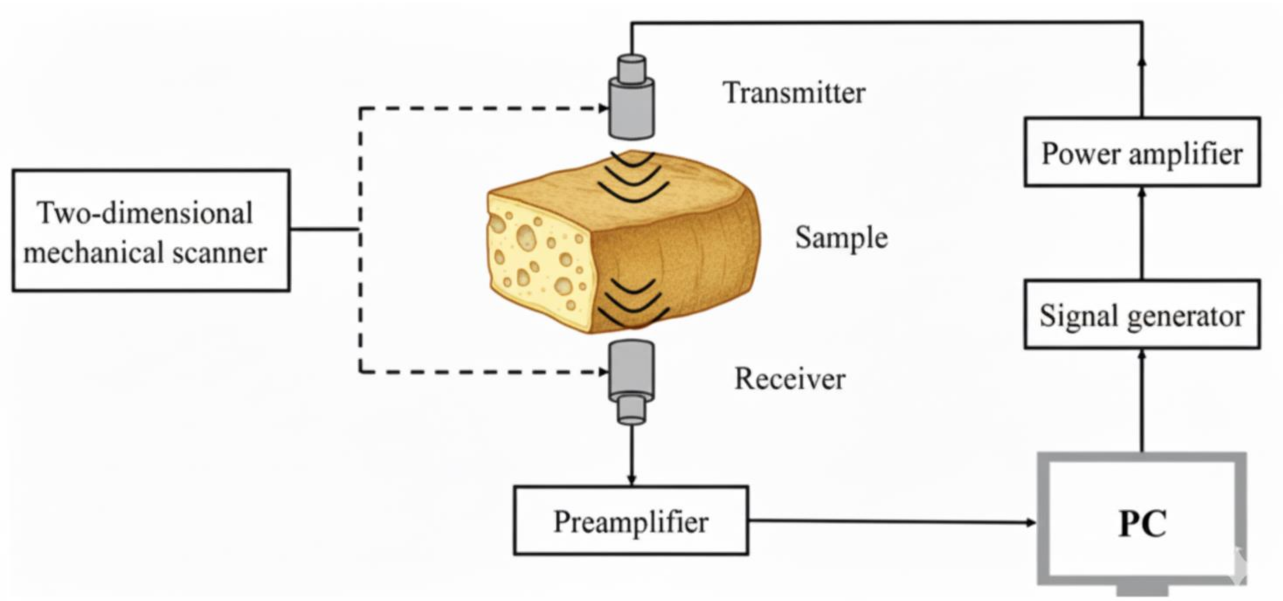

Figure I.1 *Schematic illustration of the setup of a non-contact ultrasound system applied to the analysis of a cheese wheel*

The first chapter presents a literature review, describing the different methodologies used in the food sector to assess consistency characteristics, with reference to cheese. The second chapter describes the methodology, the experimental steps, the instruments employed, and the materials with their preparation. Finally, the third chapter presents and discusses the results related to ultrasonic, texture, and moisture parameters analysed during the ripening process.

Chapter 1

State of the art

1.1 Seasoned cheese as case study

Global production of mature cheese has increased rapidly in recent years. According to Cognitive Market Research, the global specialty cheese market size was £141,251.2 million in 2024. It will expand at a compound annual growth rate (CAGR) of 5.20% from 2024 to 2031. Europe accounted for a market share of over 30% of global revenue with a market size of £42,375.36 million. This is because mature cheeses, compared to mass-produced cheeses, are distinguished by their unique flavours, textures, and appearances.

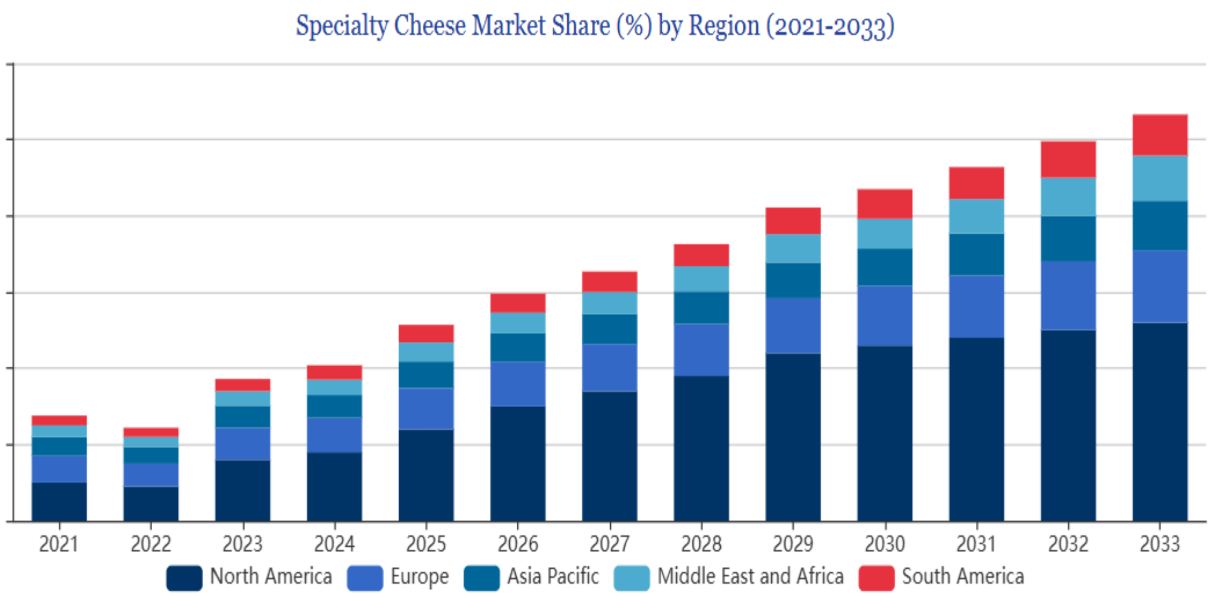

Figure 1.1 *Market share trend for specialty cheeses (%) by region in the period 2021-2033. The graph, for illustrative purposes only, shows the growth trend of the global market for aged and specialty cheeses (Based on data from Cognitive Market Research, 2024)*

Aged cheeses are an excellent source of protein and rich in vitamins and minerals. They are characterised by high sodium content, but are also a source of calcium, phosphorus, potassium and zinc. The flavour, texture and appearance of cheese are influenced by many factors, such as the composition of the milk and seasonal and dietary variations in milk consistency (D.Fusco *et al.,*2020*).* The quality and safety are influenced by several factors, such as cheese-making techniques and maturing and storage conditions. All stages of the cheese production process must be carefully monitored to ensure that the correct levels of humidity and temperature are maintained (S.E. Focardi *et al*., 2022). In addition, the degree of cheese maturation, storage temperature and humidity influence protein decomposition and the formation of characteristic flavours during ageing (M. Tudor Kalit *et al*., 2020). As regards diseases directly linked to cheese production, there are not many, as the processes of cheese-making, cooking and maturing contribute to significantly reducing the presence of microorganisms. For example, in Emmental cheese, no microorganisms, except for *S. aureus*, are detected within 24 hours of production. This is because the cheese is produced using a high cooking temperature of around 53 °C. In Cheddar production, microorganisms such as *l'E. faecalis, E. coli* and *Salmonella* also decrease during the cheese maturing process at a temperature of 12 °C. (M. Tudor Kalit *et al*., 2020). Starter cultures play a vital role in cheese production, as they have a profound impact on the development of flavour and texture, as well as ensuring microbial safety. Starter cultures play a fundamental role in cheese production, as they significantly influence the development of flavour and texture, while ensuring microbiological safety. Starter cultures consist of carefully selected bacterial strains, generally belonging to lactic acid bacteria (LAB), such as *Lactococcus*, *Streptococcus* and *Lactobacillus* (M.C. Coelho *et al*., 2022). An equally important role is played by preservation methods, which aim to prevent deterioration caused by various microorganisms. These include high-pressure processing, the use of antifungal additives and the regulation of microbial proliferation using high levels of CO_2. All these techniques are adopted to maintain high product quality and meet consumer demands. In particular, it is essential to evaluate the physicochemical properties of cheese to meet these requirements. At an industrial level and in scientific research, various methodologies have been developed for this purpose, which are described in the following paragraphs. Among these techniques, the one on which this thesis focuses is non-contact (or air coupled) ultrasound analysis.

1.2 Non-destructive methods to assess food consistency

Below are some of the main non-destructive techniques used to characterise the textural properties of foods.

1.2.1 Acoustic impulse-response

The acoustic impulse response technique is a rapid, accurate and non-destructive measurement of consistency, in which the food is stimulated by a probe to obtain the complete spectrum of the recorded sound frequency. This technique is used to detect surface cracks in eggshells or voids in watermelons (Diezma-Iglesias *et al*., 2004); it is also applied to fruits such as peaches, apples and pears to quantify changes in texture during ripening (García-Ramos *et al*., 2006) and used to assess the texture of tomato fruit (Sarah Schotte *et al*., 1999).

In the latter case, acoustic impulse response allows a stiffness factor to be obtained, based on the first resonance frequency and the mass of the intact fruit, which allows the texture to be objectively assessed, eliminating the problems of subjective variability between operators. In addition, the technique is used to study changes in tomato consistency during storage and to analyse the influence of variety, producer, season, production method, degree of ripeness and storage conditions (Sarah Schotte *et al*., 1999). Regarding cheese, a study is being conducted to detect internal cracks in Manchego cheeses, both normal and defective, using the acoustic impulse response technique (J. Benedito *et al*., 2008).

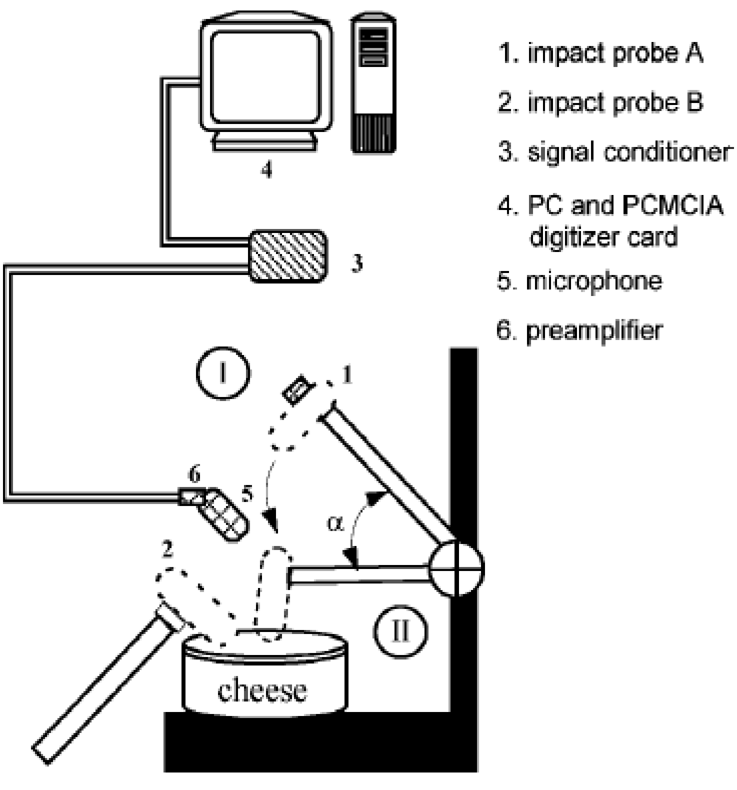

Figure 1.2 *Schematic diagram of the acoustic impulse-response system for probes A and B. (J. Benedito et al.,2008)*

Figure 1.2 shows the system used for the above-mentioned study on Manchego cheese. Two separate experiments were conducted: the first used an impact probe (probe A), while the second used a manual probe (probe B). The impact probe applies a controlled shock to the surface of the sample, allowing the acoustic and mechanical response generated by the shock to be recorded; it therefore allows for a more objective and reproducible measurement. In contrast, the manual probe is operated by the operator, who exerts direct pressure on the sample: this method is easier to use but can introduce greater variability in the results due to human intervention.

Overall, the impact probe provides more reproducible and quantitative measurements, while the manual probe is more sensitive to structural irregularities in the product. The results therefore confirm that acoustic analysis is an effective tool for the non-destructive detection of internal defects in Manchego cheese, highlighting differences in spectral responses, especially in the low (0–50 Hz) and medium frequencies (150–250 Hz), which are closely related to the textural quality of the product. In conclusion, the acoustic impulse response technique is effective overall, as it is non-invasive and can be applied automatically or in-line. However, the system requires accurate calibration and complex signal interpretation, which necessitates adequate spectral analysis and specific expertise.

1.2.2 Impact force-deformation

The Impact Force–Deformation technique is a non-destructive method used to characterise the mechanical and textural properties of foods by analysing the response of the sample to a controlled impact. This methodology is applied, for example, to evaluate certain mechanical properties and the susceptibility to bruising of nectarines (Prunus persica) (Refik Polat *et al.*, 2011). In this study, a pendulum (Figure 1.3) with a 50 cm long arm is used, and the tests are performed in two impact directions — from the bottom and side of the fruit — and at three different drop heights (30, 40 and 50 cm) to obtain different levels of impact energy.

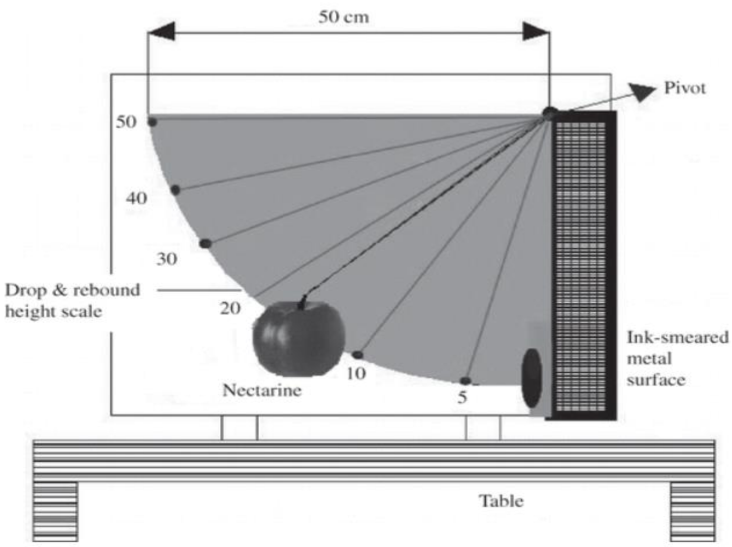

Figure 1.3 *Pendulum impactor for impact testing on nectarines. (Refik Polat et al., 2011)*

The Impact Force–Deformation method is also used to assess the firmness of peaches, obtaining parameters such as the impact force and the energy absorbed by the fruit during collision (Wang *et al*., 2009). Similarly, the technique is used in the study of tubers (Klaus Gottschalk *et al*., 2015), where it is used to analyse the effect of mechanical impact parameters on physical characteristics and impact damage through experimental analysis and finite element models.

Similarly, in the case of Manchego cheese (José V. García-Pérez *et al*., 2007), the application of Impact Force–Deformation allows for non-destructive evaluation of the product's mechanical response to impact, providing information on consistency, elasticity, the presence of internal defects and parameters closely linked to the textural quality of the cheese.

During the test (Figure 1.4), a probe is dropped or pushed with a specific force against the surface of the sample. At the moment of contact, an accelerometer records the acceleration signal generated by the impact, which is then digitised at a high sampling frequency (20 kHz) using a data acquisition card. The signal is filtered and conditioned to eliminate background noise and ensure an accurate reading. Based on the acceleration data, the force, velocity and deformation curves over time are calculated, along with a series of mechanical parameters characteristic of the material.

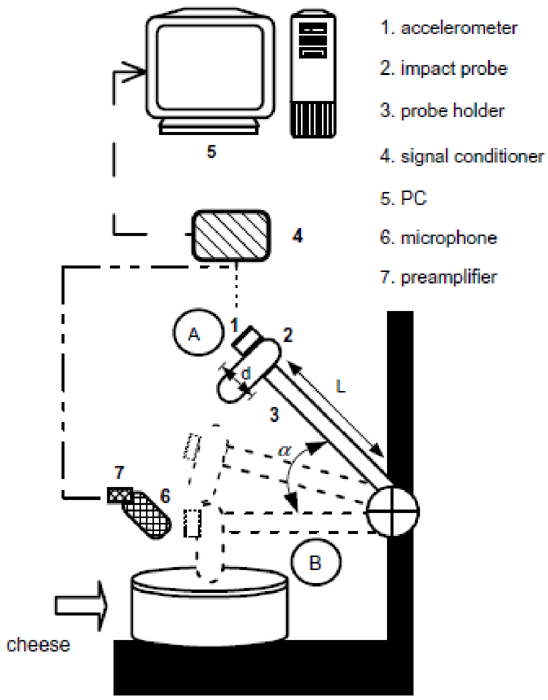

Figure 1.4 *Schematic diagram of the impact system for cheese texture (José V. García-Pérez al., 2007)*

In conclusion, the Impact Force–Deformation technique is an effective tool for the non-destructive evaluation of the consistency and structural uniformity of food, contributing to quality control and product classification.

1.2.3 Contact ultrasounds

Monitoring food production is essential to ensure a high level of product safety and quality, which directly impacts consumer health. This is why it's important to choose the right method for assessing food properties. Ultrasounds are acoustic wave with a frequency higher than human hearing (about 20 kHz). They can be distinguished into high or low frequency, having different applications in analysis, quality control, and food processing. Contact ultrasound method has various industrial applications, and it is a low-cost, non-destructive technique that can be applied in-line. Contact ultrasound involves direct contact between transducers and the measurement surface. It's essential that there are no air gaps between the transducers and the sample surface; for this reason, a coupling medium such as water or oil is used.

This technique is employed in several studies, such as the one conducted on apple tissue to analyze the influence of ultrasound on electrical conductivity, color, total polyphenols, and antioxidant activity (Artur Wiktor et al., 2015 It is also used to intensify the air drying of blackberry, where a comparison between airborne and contact ultrasound is carried out (Yang Tao et al., 2020).

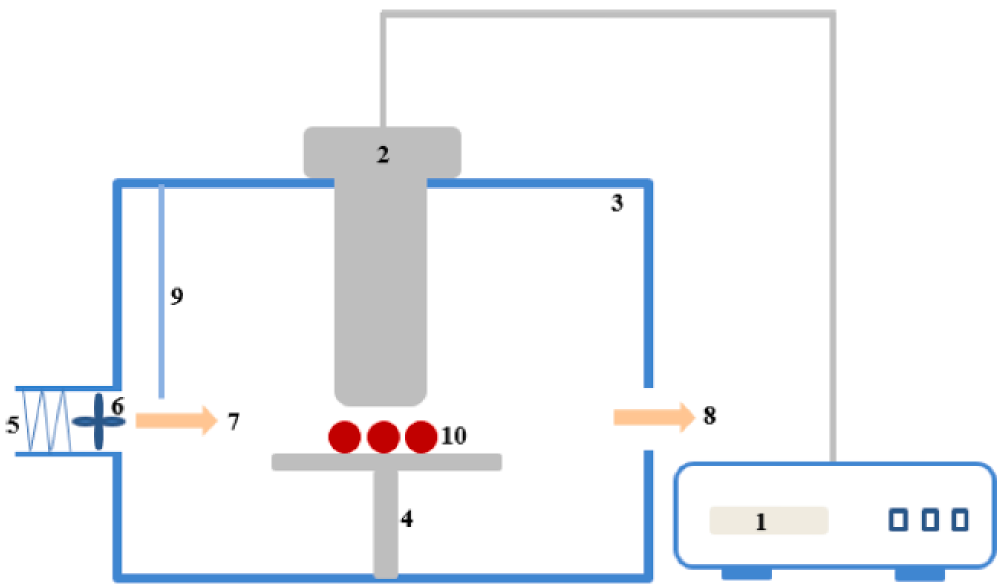

Figure 1.5 *Experimental setup for ultrasound-assisted air drying of blackberry. 1: ultrasound generator; 2: ultrasound probe; 3: air dryer; 4: sample holder; 5: electrical heater; 6: electrical fan; 7: air inlet; 8: air outlet; 9: thermometer; 10: blackberry (Yang Tao et al., 2020)*

This study shows that both airborne ultrasound and contact ultrasound accelerate the drying process, reducing energy consumption and increasing the preservation of anthocyanins and organic acids in blackberries compared to traditional air drying. However, contact sonication is found to be more effective than air sonication because:

- Blackberries receive a greater amount of ultrasonic energy (0.299 W) compared to air sonication (0.245 W).
- There is a greater reduction in temperature and humidity gradients within the berry, leading to more homogeneous distribution.
- Total energy consumption is 27.0% lower than that of air-assisted ultrasound drying.
- Blackberries dried by contact sonication retain greater amounts of anthocyanins and organic acids than those treated with air sonication, indicating superior product quality.

In conclusion, contact ultrasound offers greater efficiency in blackberry drying, both in terms of energy and quality.

1.2.4 Non-contact (or air coupled) ultrasounds

The main topic of this thesis concerns the use of non-contact (or air-coupled) ultrasound for the evaluation of physical-chemical properties and for the detection of possible defects in cheese .In recent years, this technique has attracted growing interest because, unlike conventional ultrasonic methods, it does not require the use of an acoustic coupling medium between the piezoelectric transducers and the sample under examination. In traditional methods, in fact, to ensure good acoustic energy transfer, a coupling fluid or gel, such as oil or grease, is generally used, or the material to be tested is immersed in a water tank or a spray system is used. However, the presence of such media can introduce errors in measurements, particularly in the calculation of the transit time of ultrasonic waves. Furthermore, the transmission and partial reflection of energy within the coupling layer can alter the waveform and frequency spectrum, compromising the accuracy of attenuation measurements.
Non-contact ultrasonic techniques, on the other hand, allow acoustic waves to be generated and detected in the air without physically interacting with the sample. This approach minimises distortions in the detected signal and allows analyses to be carried out on materials in difficult conditions, such as samples that are very hot or very cold, or located in hostile or difficult-to-access environments. Furthermore, these systems make it possible to take measurements at a distance from the surface of the material, while maintaining high sensitivity and reliability of results. (Robert E. Green Jr, 2004)

Non-contact ultrasound uses air as the coupling medium between the transducers and the sample to be analysed. However, the main problem with this technique is the discrepancy between the acoustic impedance of air and that of food samples. This problem can be solved using various methods, such as the pulse compression technique and the air instability compensation method (Mahdi Faramarzi *et al*., 2015).

The impulse compression technique, combined with the use of non-contact ultrasound, was used to monitor the physical and chemical changes in milk-based products contained in glass containers (S. Meyer *et al*., 2006). Two broadband capacitive transducers and a glass container were used to carry out the experiment. In addition to serving as a test cell, the glass container also allowed direct visual observation of the samples. The application of the impulse compression method made it possible to evaluate the agglomeration processes in dairy products, providing useful data on the structural evolution of the material over time.

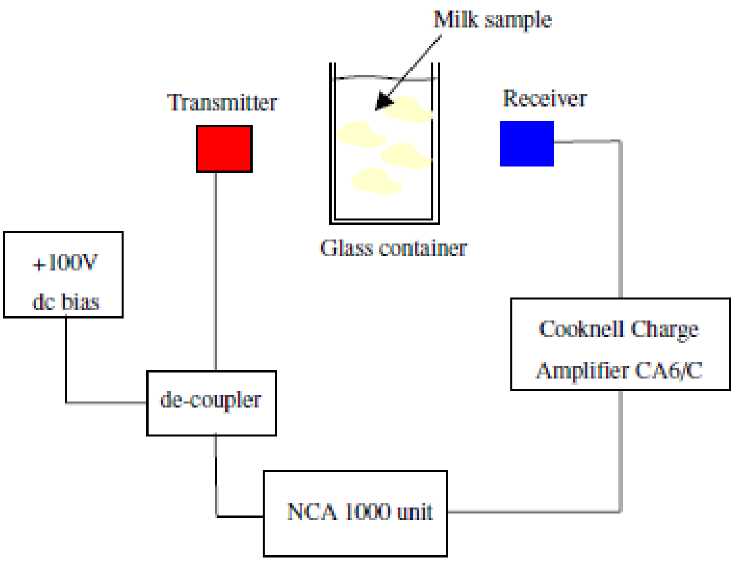

Figure 1.6 *Diagram of the non-contact ultrasonic measurement system for milk samples. The NCA 1000 unit generates and receives the modulated signal, within which pulse compression takes place (S. Meyer et al., 2006)*

The results showed that combining the two techniques ensures a high signal-to-noise ratio during ultrasonic transmission, allowing for more stable and reliable measurements. The sensitivity of the method was confirmed by preliminary experiments, in which different types of dairy products — such as whole milk, semi-skimmed milk and chocolate drinks — showed different acoustic properties due to their different compositions. In particular, it was observed that pH variations influence the amplitude of the transmitted signal, suggesting a direct relationship between the chemical state of the product and the ultrasonic response.

Overall, the results of this study demonstrate that non-contact ultrasound is a non-destructive, economical and reliable technique for analysing the physical and chemical characteristics of food. This gives the method considerable potential for application in the food industry, both for quality control and for monitoring production processes. However, it is also important to highlight some limitations. Air-coupled measurements are still sensitive to environmental noise and can be affected by variations in temperature, humidity and air pressure, which alter the propagation of the ultrasonic wave.

1.3 Destructive methods to assess food consistency

The sensory and structural properties of food can also be assessed using destructive techniques. These methods involve direct contact with the product, causing it to deform and, in many cases, resulting in the partial or total destruction of the sample.

1.3.1 Surface probes tests

The term *surface probe tests* refers to the application of a mechanical probe that comes into contact with the surface of the food, applies a force and measures the mechanical response. These probes are used for penetration, compression and adhesion tests to measure parameters such as hardness, cohesion and breakability of the product.

1.3.1.1 Puncture and penetration tests

Puncture and penetration tests are types of localized mechanical tests that involve a point contact between the probe and the surface of the food product. During the test, a probe-conical, cylindrical, spherical, or needle-shaped-is pushed into the surface of the sample until a specific depth is reached or until the material fractures. The applied force is recorded as a function of displacement, allowing the calculation of characteristic parameters such as:

- Maximum force (Fmax) → an indicator of hardness or resistance to penetration.
- Area under the force–displacement curve → the work required for penetration, which reflects the compactness and cohesiveness of the product.

These tests are widely used in the food industry for the objective evaluation of texture. For instance, Yulu Mou *et al.* (2025) perform puncture tests on 156 germplasm samples of crispy pears to analyze the variability in texture characteristics, investigate the correlation between sensory and instrumental evaluations, and explore the effects of ripening, fruit size, and storage duration on the mechanical properties of the pulp.

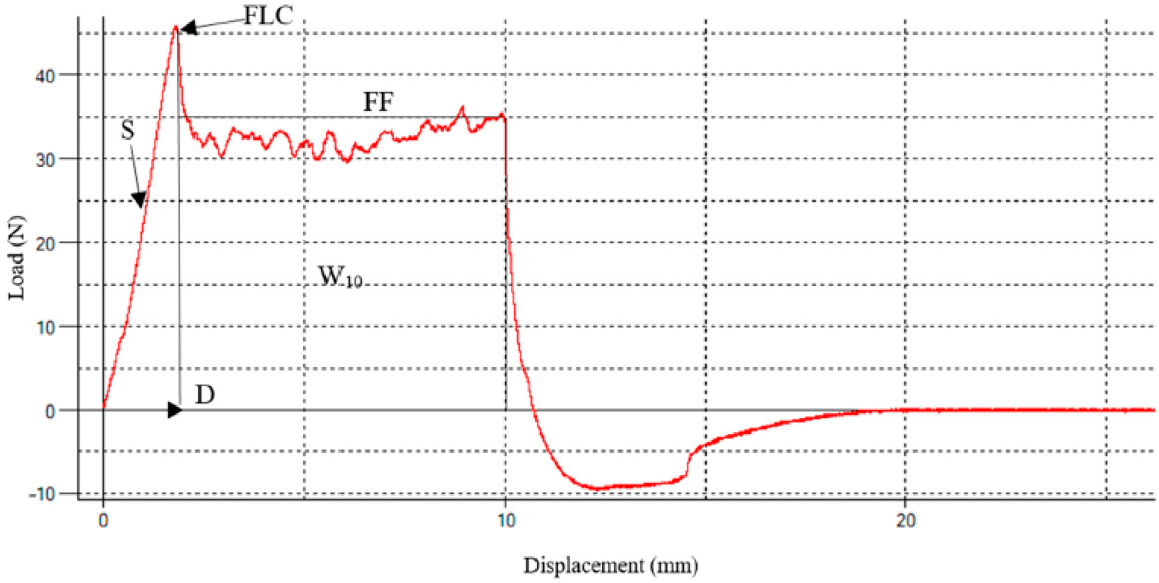

Figure 1.7 *Load–displacement curve obtained by puncture test of crisp pear with texture analyzer ((Yulu Mou et al., 2025)*

Each previously peeled fruit is pierced both anteriorly and posteriorly, ensuring that the puncture point is central. Puncture tests are performed every 4 days, using 20 randomly selected fruits per time point, and the results are averaged. The parameters obtained are shown in Figure 1.7: FLC (flesh boundary compressive force), FF (flesh firmness), S (slope of the force-deformation curve), D (deformation associated with the flesh boundary compressive force), and W10 (work required to achieve a 10 mm flesh deformation).

The results of this study show that the coefficient of variation of most puncture parameters is high, indicating the great diversity in the texture of pear varieties. These findings demonstrate that this method is reliable, low-cost, and easy to apply. However, its localized measurement, sensitivity to probe shape and size, temperature and humidity conditions, and destructive nature offer limited information on the internal structure and bulk mechanical behavior, which may lead to difficulties when comparing products with different compositions or geometries.

1.3.1.2 Uniaxial compression tests

Uniaxial compression is a type of volumetric test, commonly applied to semi-hard and pressed foods. The sample (cylindrical, cubic, or parallelepiped) is compressed along one axis between two flat plates, measuring the force-deformation relationship. This method allows the product's overall deformability and internal strength to be estimated.

In the food sector, the uniaxial compression test finds wide application, particularly in the dairy industry. Numerous studies are carried out on different types of cheese, such as the investigation on mold-ripened cheeses for the evaluation of rheological properties (George Ipate *et al.*, 2024), the analysis of the textural properties of Manchego cheese (José V. García-Pérez *et al.*, 2006), and the study on the universal compression behavior of semi-hard cheeses (Jolien de Boer *et al.*, 2025). However, the use of this technique is not limited to dairy products. Other foods are also analysed through uniaxial compression testing. For example, three ready-to-eat snacks—deep-fried batter droplets made from chickpea flour, extruded corn balls, and puffed rice—are subjected to uniaxial compression to evaluate their mechanical properties (R. Ravi *et al.*, 2005).

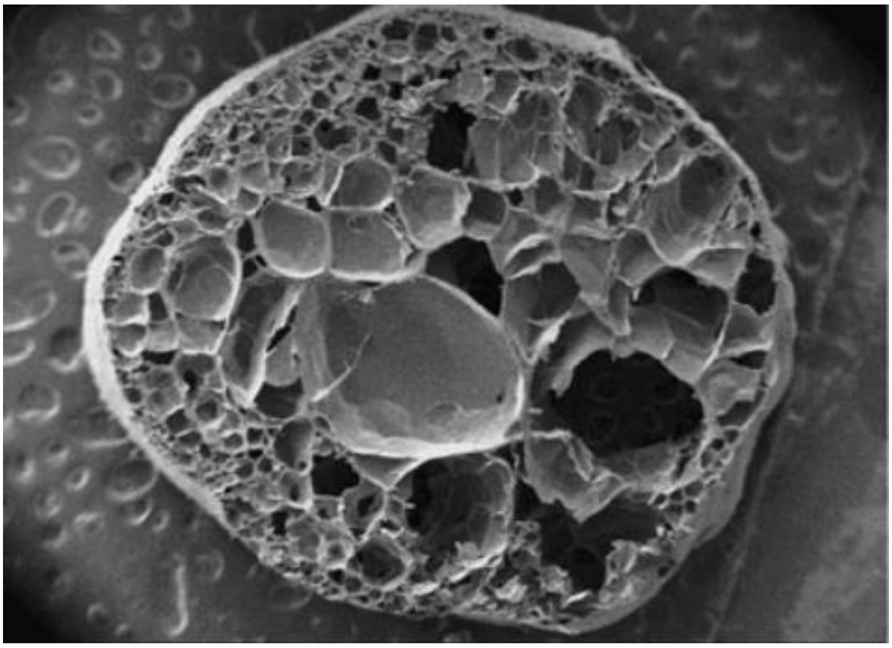

Figure 1.8 *Microstructure of the cross section of puffed rice (R. Ravi et al., 2005)*

In this study, the three types of snacks are compressed uniaxially at different crosshead speeds ranging from 0.01 to 10 mm/s. The compression curves show that the product initially undergoes a linear elastic deformation, followed by a nonlinear plastic deformation zone that may exhibit major and minor fractures. The type and number of fracture points in the plastic region depend on several factors, such as the nature and structure of the material, moisture content, and the extent and speed of compression. The figure 1.8 shows the microstructure of the cross section of puffed rice.

In conclusion, this technique, like the one described in the previous subparagraph§1.3.1.1, is relatively simple and low-cost, allowing a quantitative and reproducible evaluation of the mechanical properties of the product, although it is destructive and sensitive to the test conditions (temperature, humidity, etc.).

Chapter 2
Materials and methods

2.1 Raw materials

The type of cheese employed is called "Queso Suave de Oveja". Oveja is a cheese produced in different parts of Spain. The one involved in this study is processed in the company "*Quesería Artesana Los Corrales"* (Castellón, España). It is a cheese made from pasteurised sheep's milk and matured for forty days. It is presented in a cylindrical format of around 1,200 kg. The coagulation is enzymatic with lamb rennet and contains probiotic bacteria of the Bifidus type. The rind is greyish due to oiling. The dough is firm, light yellow in colour, with mechanical eyes irregularly distributed throughout the dough. The texture is firm and elastic. It has a pleasant lactic smell and a flavour of sheep's milk with a sweet undertone, very typical of Mediterranean cheeses. The aftertaste is long and reminiscent of nuts.

Over the course of six consecutive weeks, a total of 18 cheese wheels are analysed, three per week. The samples are sent by the producing company to the analysis laboratory at the: Escuela Técnica Superior de Ingeniería Agronómica y del Medio Natural (ETSIAMN), Universitat Politècnica de València (UPV), Edificio 3F, Valencia, Spain.

Transport takes place in cardboard packaging and at controlled room temperature (20-25 °C).

Figure 2.1 *Original packaging containing the Oveja cheese forms used in this study*

2.2 Controlled ripening

The controlled transport conditions indicated in section §2.1 ensure that the cheeses sent in the first weeks retain the state of ripeness corresponding to the production process, i.e. they present the conditions described above (section §2.1). The cheeses sent in the last weeks of analysis show an advanced state of maturation, with mould formation on the surface and a harder texture to the touch. During the analyses, the various wheels are stored in a refrigeration chamber at 4ºC. Therefore, the post-ripening times studied are 5, 12, 19, 26, 33, 40 days, corresponding to t1, t2, t3, t4, t5, t6 respectively.

2.3 Cheese sample preparation

In the following sections §2.4, §2.5, §2.6 and §2.7 respectively, the procedures for thickness, ultrasonic, texture and moisture analysis of cheese are described. Thickness and ultrasonic tests are conducted using whole cheeses. Afterwards, each wheel is wrapped using a transparent wrapper and labelled indicating the wheel number and ripening time. Example: OR1-t1, OR2-t1 and OR3-t1 respectively represent the three cheeses analysed during the first week (where "O" indicates Oveja, "R" indicates repetition and "t1" time 1).

For the texture and moisture studies, carried out the day after the thickness and ultrasound studies, each cheese wheel is cut lengthwise into three 1 cm thick slices. A device called a "double-arm knife press" or "double-arm laboratory cutter" (Figure 2.1) is used to obtain the pieces.

Immediately after being cut, the three slices of each cheese are stored in plastic containers: one piece on the top and two on the bottom, separated by a metal grid. Each container is labelled as reported above. Between each analysis, the containers are stored in a refrigeration chamber at 4°C, as mentioned in section §2.2.

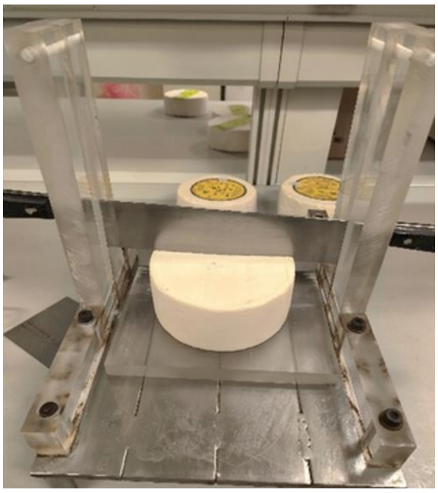

Figure 2.2 *Whole wheel of Oveja cheese placed on a metal platform and cut with a double-arm knife press to obtain samples for analysis*

2.4 Thickness measurement

The first step in this project is to measure the thickness of each cheese wheel. Prior to this, the weighing and measurement of the diameter of each sample took place. Thickness is a key parameter, as it serves to determine the ultrasonic velocity (section §2.5.3) through the cheese measured at different storage times.

2.4.1 Equipment and experimental procedure

The equipment used for thickness testing is shown in Figure 2.3. It consists of:

- a metal scaffold (A);
- a laser sensor (Baumer, OXH7-11161809) (B);
- a feeder connected to the sensor (C).

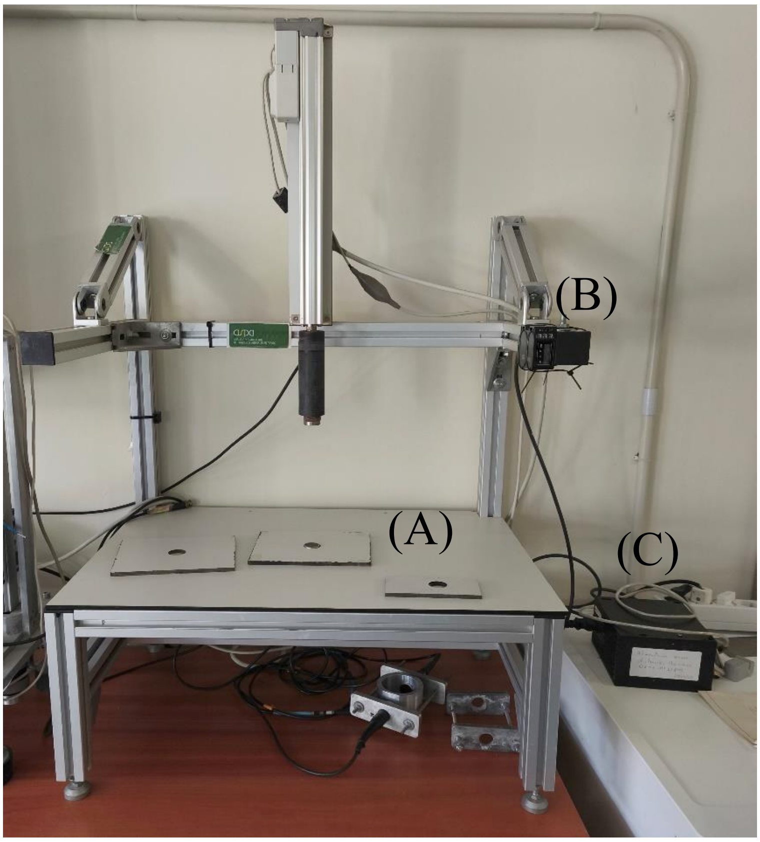

Figure 2.3 *Equipment used to measure the cheese thickness. It consists of a metal scaffold (A), a laser sensor (B), and a feeder connected to the sensor (C)*

Each shape is positioned on the metal scaffold and the thickness is measured at 21 different points using the laser sensor.

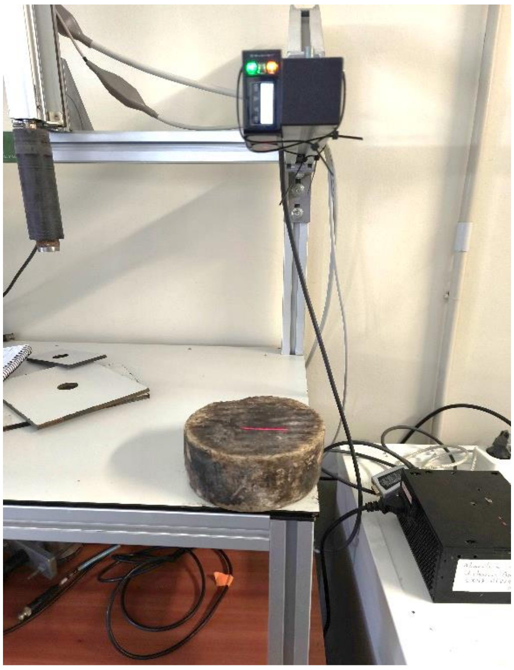

Figure 2.4 *Practical measurement example: the laser sensor detects the thickness of the Oveja cheese placed on the metal scaffold*

The Baumer OXH77-11161809 sensor is a 2D laser profile sensor from the PosCon OXH7 series, designed to accurately measure profiles and distances in industrial applications. The main features are:

- Functions: **Light-section sensor**
- Light Color: **Red**
- Wavelength: **650 nm**
- Shape: **Rectangular body**

The thickness of the object is measured reliably thanks to the OX sensor, which is unaffected by distance variations or minor misalignments. As shown in Figure 2.5, the object can move within the measurement range without affecting the result. In addition, the sensor is able to compensate for inclinations of up to ±30°, maintaining the same accuracy as if it were mounted in a perpendicular position (Figure 2.6).

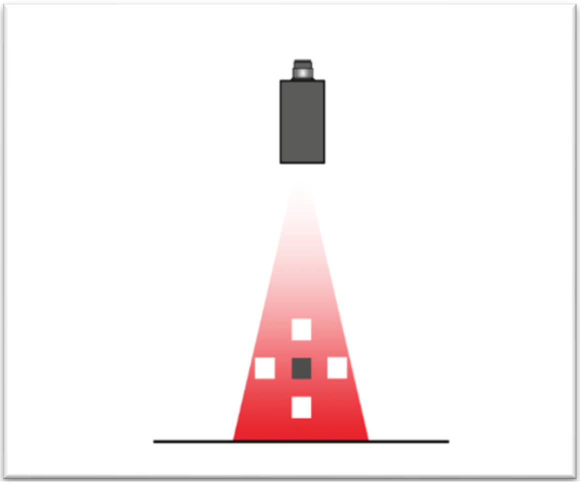

Figure 2.5 *The sensor compensates for variations in the distance of the object, ensuring reliable measurements even in the presence of movement*

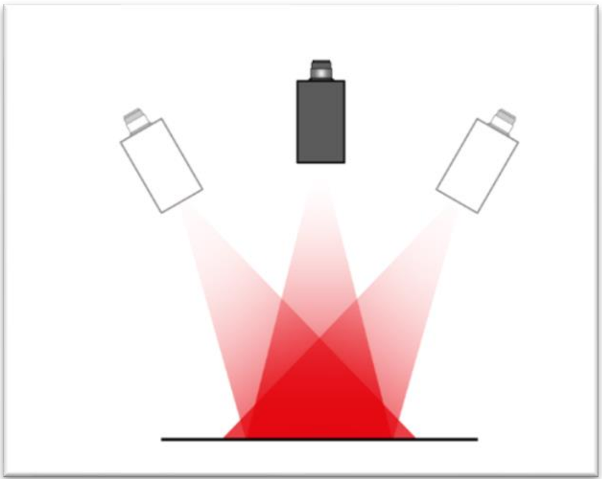

Figure 2.6 *The sensor compensates for mounting angles of up to ±30°, automatically correcting dynamic position variations*

2.5 Non-contact ultrasonic measurement

Ultrasonic measurements are carried out using a relatively novel technique. It consists of using both pulse-echo (PE) and transmission-reception (TR) modes.

2.5.1 Instrumentation and image acquisition

The equipment used to collect the ultrasound signal is illustrated in the Figure 2.7 and consists of a computer (A), a perforated platform (B), a pulser-receiver instrument (C) (DifraScope®/AirScope®, DASEL Sistemas, Madrid, Spain), a preamplifier (D) and two unfocused piezoelectric transducers (E,F) (emitter and receptor) (ITEFI-CSIC, Madrid, Spain).

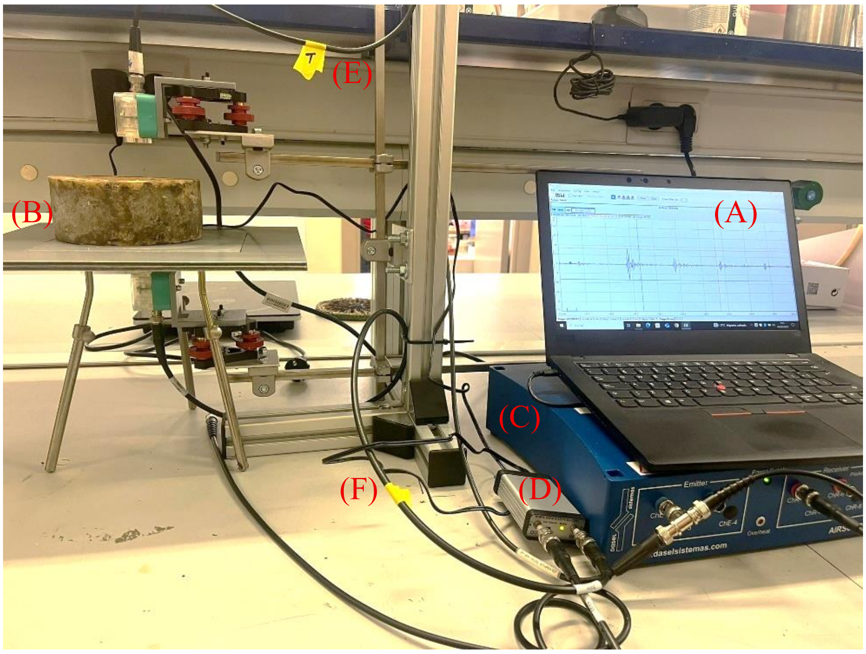

Figure 2.7 *Equipment used to collect the non-contact ultrasound signal. It consists of a computer (A), a perforated platform (B), a pulser-receiver device (C), a preamplifier (D), and two unfocused piezoelectric transducers (E, F), used as emitter and receiver*

According to the TR methodology, the pulse-receiver instrument sends an electrical signal to the emitter transducer, which converts it into an ultrasonic wave. This wave passes through the air and the surface of the sample, propagating through its interior until it reaches the receiver transducer, which converts it back into an electrical signal. This signal is then sent to a preamplifier, then amplified and digitised by the pulse receiver. In the PE technique, a single transducer is used as both emitter and receiver. The emitted ultrasonic wave bounces off discontinuities or internal surfaces of the sample and returns to the same transducer. This allows measurements to be made on only one side of the sample. In this study, measurements in Pulse-Echo mode are made on both sides of the sample: first using the upper transducer as emitter/receiver; then by repeating the measurement with the lower transducer, reversing its function.

2.5.2 Experimental procedure

The steps for collecting ultrasonic signals are as follows:

1) The signal through air is measured using the UT-View programme, to obtain reference signals. A receiver gain of 0 dB is used to capture this signal.
2) The cheese sample is placed on a perforated platform with a circular hole (60 mm in diameter), designed to mechanical support while minimizing interference with the ultrasonic beam.
3) The sample is analysed with both ultrasonic techniques TR and PE, as described in section §2.5.1. The two strategies are applied at the same point on the cheese, and this procedure is repeated at 10 different spots on the sample. The acquired ultrasonic signals are collected with the UT-View software and saved directly on the computer.

During the measurements, a reception gain of 45 dB is set for the TR technique and 32 dB for the PE technique. The unfocused piezoelectric transducers, with a central frequency of 0.25 MHz and active diameter of 25 mm, are perfectly aligned with each other and positioned 122.5 mm apart. The pulser-receiver instrument emits a semi-cycle square wave with an amplitude of 400 V, matched to the central frequency of the transducers. The signal of the receiver transducer is amplified and digitalized at 10 Mpoints/s.

2.5.3 Signal processing

Through signal analysis, carried out in the time domain, two key parameters in the evaluation of the cheese ripening process can be calculated: thickness and speed of non-contact ultrasounds.

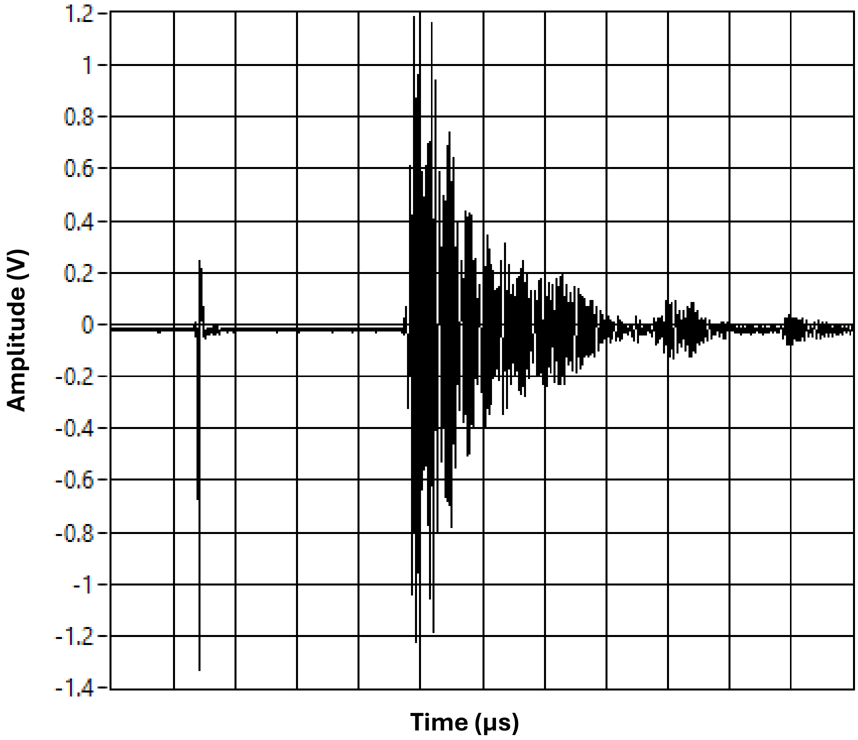

Figure 2.8 *Example of acquired signal represented in the time domain*

The thickness of the cheese sample is used to calculate the ultrasonic velocity. The traditional (direct) method of determining this thickness is described in paragraph §2.4. However, this approach can be a limitation in many industrial environments. In contrast, PE time-of-flight (ΔTOF_{PE}) measurements offer the advantage of not requiring direct measurement of the sample's thickness (Garcia Perez et al., 2019). Consequently, the thickness obtained by the method described in paragraph §2.4 is not used for the calculation of the ultrasonic velocity but only used to compare the values obtained with those calculated using the PE methodology.

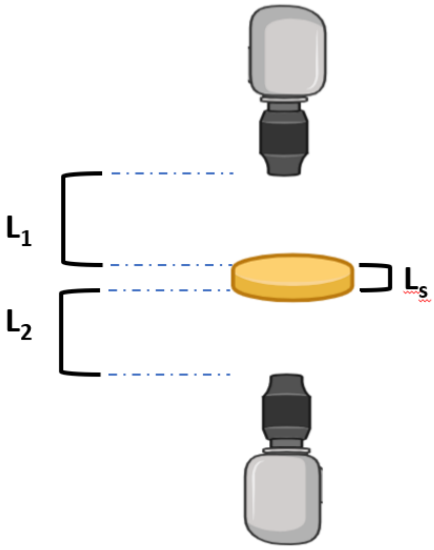

Figure 2.9 *Illustrative scheme of the ultrasonic measurement configuration using the PE technique:* L_1 *represents the distance between the transducer and the sample,* L_s *is the thickness of the sample, while* L_2 *is the distance between the sample and the receiver*

The distances between each transducer and the sample surfaces are determined as follows:

$$L_i = \frac{\Delta TOF_{PEi}\, x\, v_{air}}{2} \qquad (i = 1{,}2) \tag{2.1}$$

Where:

- ΔTOF_{PEi} is the time-of-flight difference between the first and second echo in PE mode, measured respectively for the upper and lower surfaces.
- v_{air} is the ultrasonic velocity in air.
- L_1corresponds to the upper surface, and $L_2$to the lower surface.

The total distance between the transducers, L, is known a priori. From the determined $L_1$and L_2, the sample thickness L_s can be obtained as: $L_s = L - L_1 - L_2$.

Finally, the ultrasonic velocity (v) through the cheese samples can be calculated with the TR mode, using the following equation (Álvarez-Arenas et al., 2009):

$$v = \frac{L_s}{\frac{L_s}{v_{air}} + \Delta TOF_{TR}} \tag{2.2}$$

ΔTOF_{TR} represents the difference between the time it takes for the ultrasonic signal to pass through the cheese sample and the time it takes for the same signal to pass through the air only (i.e. without the sample).

To calculate the flight time ΔTOF_{TR} , the cross-correlation method is used with LabVIEW software. This procedure is based on comparing the reference signal (air) with the signal acquired from the sample. Both signals are loaded into the software, checking that they have the same sampling frequency [Fs (Hz)] and that they are recorded under the same time conditions. The necessary parameters are then set:

- Signal start point index or time instant at which the useful pulse in the signal to be analysed begins.
- Longitude (length): window, expressed in number of samples, containing the main pulse, avoiding late reflections or external noise.
- Ref cut-off point: reference signal window, selected using the same criterion.

In this way, the comparison is limited to the portions of the signal containing the useful burst. Once the parameters have been defined, the software calculates the cross-correlation function and identifies the correlation peak, which corresponds to the best alignment between the two signals. The output is represented by the ΔTOF_{TR} value (in µs): a positive ΔTOF_{TR} indicates that the analysed signal is delayed with respect to the reference, while a negative ΔTOF_{TR} indicates an advance.

The ΔTOF_{PE} is always obtained using the cross-correlation technique, as the difference between the signals of the two echoes: the one reflected on the lower face of the sample (after passing through the sample twice) and the one reflected on the upper face.

2.6 Textural measurement

2.6.1 Compression-relaxation test

After ultrasonic analysis, the texture of the slices reserved for this purpose is analysed, as described in paragraph §2.3.

A texturometer (TA.XT2i, Stable Micro Systems, Surrey, UK) connected to a computer is used. Each slice is placed on the rectangular platform of the texturometer and 10 holes are drilled through a cylindrical probe in the centre, following a compression-relaxation mechanism. The curves obtained from these tests are analysed using Exponent-Lite software.

The parameters used are shown below:

- Cylindrical flat probe ø 6 mm.
- Compression speed 0.20 mm/sec.
- Strain 40% (deformation of 0.4 cm).

All tests are performed at room temperature (25°C). Between measurements, the slices are stored as explained in paragraph §2.3

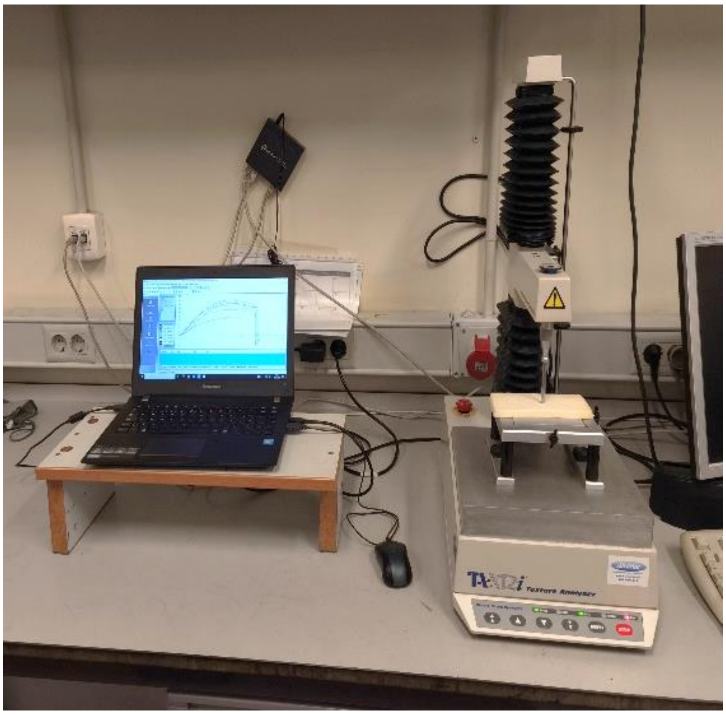

Figure 2.10 *Equipment used for measuring consistency: a texturometer connected to a computer*

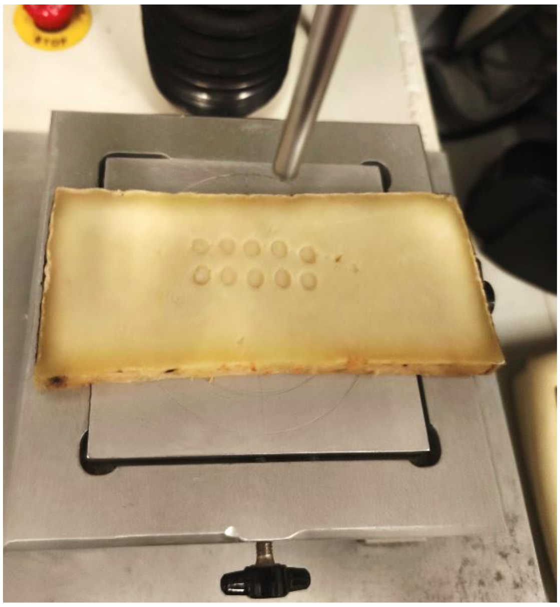

Figure 2.11 *Cheese after the perforation test with the cylindrical probe: the 10 holes made following the compression-relaxation mechanism can be observed*

2.6.2 Textural parameters

Through the compression-relaxation mechanism, performed under the conditions mentioned in the paragraph, the force is recorded as a function of time, generating curves like the one in the figure 2.12. The compression phase lies between the coordinate origin (0,0) and point 2, while the relaxation phase lies between points 2 and 3. From the analysis of this curve, the following two parameters are calculated:

- Maximum compressive force (Fmax, [N]): force recorded at point 2.
- Gradient of the force (∇F,[N/mm]): it corresponds to the derivative of the force-time curve at the point of tangency (point 1).

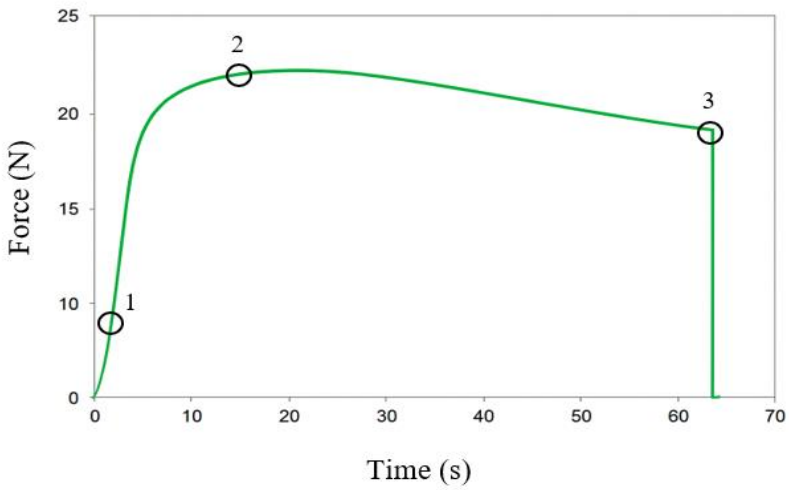

Figure 2.12 *Characteristic curve of the compression-relaxation test.Force (F) on the y-axis [N] versus time (t) on the x-axis [s]. Point 1 represents the point of tangency used to calculate the force gradient (∇F), Point 2 indicates the maximum compressive force (Fmax) and Point 3 marks the end of the test*

2.7 Moisture analysis

Finally, a moisture analysis is performed. The instruments used are a cutting board, a knife, metal ramekins, a balance and laboratory spoons. From each slice, the central part, i.e. the portion previously pierced during the texture analysis, is taken out and cut into small pieces. For each slice, two metal ramekins are filled, each containing approximately 3 grams of cheese. As 9 slices per week are analysed, this results in a total of 18 metal containers.

Figure 2.13 *Metal ramekins filled with small cheese pieces taken from the central, perforated area of each slice, prepared for moisture analysis*

All jars are placed in a natural convection incubator (BINDER™ 9010-0323) at a temperature of 105 °C. After 24 hours, an initial measurement of the weight of the jars is taken. The measurement is repeated 48 hours after the start of treatment, i.e. 24 hours after the first measurement.

Figure 2.14 *Metal ramekins containing cheese samples after 48 h in the incubator (second weighing)*

2.8 Statistical analysis

The influence of ripening time on texture, ultrasonic and moisture parameters is analysed using two complementary statistical approaches. First, a one-way analysis of variance (ANOVA) is performed using Statgraphics Centurion XVII software (Statgraphics Technologies Inc., VA, USA), followed by Fisher's LSD (Least Significant Difference) test with a 95% confidence level, to verify the presence of significant differences between the means of the different ripening times and to identify homogeneous groups.

Secondly, simple linear regressions are applied using the least squares method. For each model, the regression line equation is calculated, as well as the coefficient of determination (R^2), which expresses the amount of variability explained by the ripening time, and the p-value associated with the angular coefficient, which allows the statistical significance of the relationship to be assessed. When $p < 0.05$, the relationship between ripening time and the parameter considered is statistically significant.

The combined use of ANOVA with Fisher's LSD test and linear regression allows both to highlight the differences between individual ripening times and to describe the overall trend of the parameters over time.

Chapter 3
Results and discussion

In this chapter, the results of the moisture, texture and ultrasound tests obtained by statistical analysis are reported.

3.1 Evolution of moisture parameters during maturation

Through the moisture analysis it is possible to obtain a graph showing the evolution of the cheese moisture percentage as a function of ripening time (Figure 3.1). Each point on the graph represents the average moisture content calculated from the cheese samples analysed on that day. The days shown on the x-axis represent the maturation time. The first value is recorded on day 5, as the experimental work begins five days after the cheeses arrive at the laboratory.

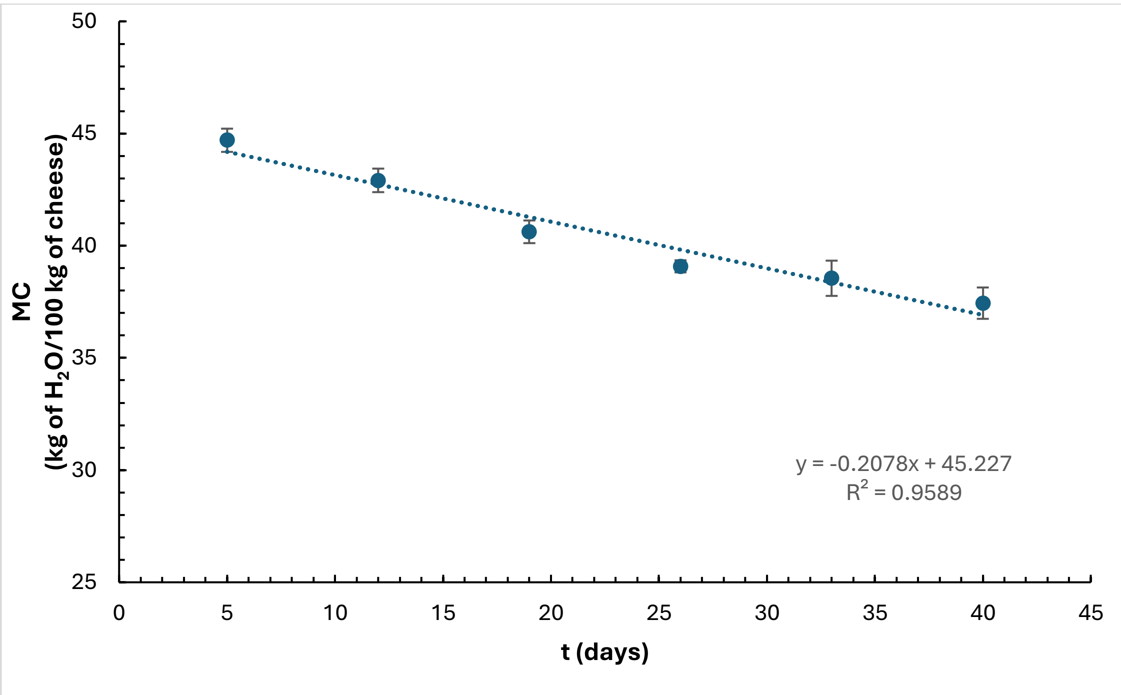

Figure 3.1 *Evolution of moisture content as a function of ripening time*

The analysis of the data reveals a clear downward trend in moisture ($p<0,05$) as ripening time increases: this behaviour is in line with the natural ripening process of cheese, during which the water contained in the product gradually evaporates, making it more compact and drier.

The trend is modelled by means of a linear regression line having, as indexed in the figure, the following equation:

$$y = -0.2078x + 45.227$$

where:

- y represents the percentage of moisture
- x the ripening days
- the negative angular coefficient (-0.2078) indicates that, on average, moisture is reduced by about 0.21% for each ripening day
- the intercept (45.227) represents the estimated value of moisture at the beginning of the process (day 0)

The coefficient of determination $R^2 = 0.9589$ shows a strong linear correlation between ripening days and moisture reduction. In particular, the model justifies around 95.9% of the variability observed in the data, confirming the effectiveness of linear regression as a descriptive tool for the phenomenon analysed. The graph also shows vertical error bars, calculated as the standard deviation of the repeated measurements. These bars highlight the experimental variability around the mean value and allow the accuracy of the measurements to be assessed. The standard deviations are low, confirming the good repeatability of the measurements.

Table 3.1 displays the results of the ANOVA analysis, highlighting the mean values and standard deviations of moisture content. Fisher's LSD analysis reveals five homogeneous groups, referred to as apices (A, B, C, D, E). No statistically significant differences are observed between days 26 and 33 (D).

Table 3.1 *Table reporting moisture content mean ± standard deviation. Letters indicate homogeneous groups established from LSD intervals (95%)*

Time (days)	MC (kg of H_2O/100 kg of cheese) + SD
5	44.70 ± 0.52^{A}
12	42.91 ± 0.52^{B}
19	40.62 ± 0.51^{C}
26	39.08 ± 0.27^{D}
33	38.55 ± 0.79^{D}
40	37.44 ± 0.70^{E}

3.2 Evolution of textural parameters during maturation

The textural parameters examined are maximum compressive strength (N) and gradient (N/mm) as a function of curing time.

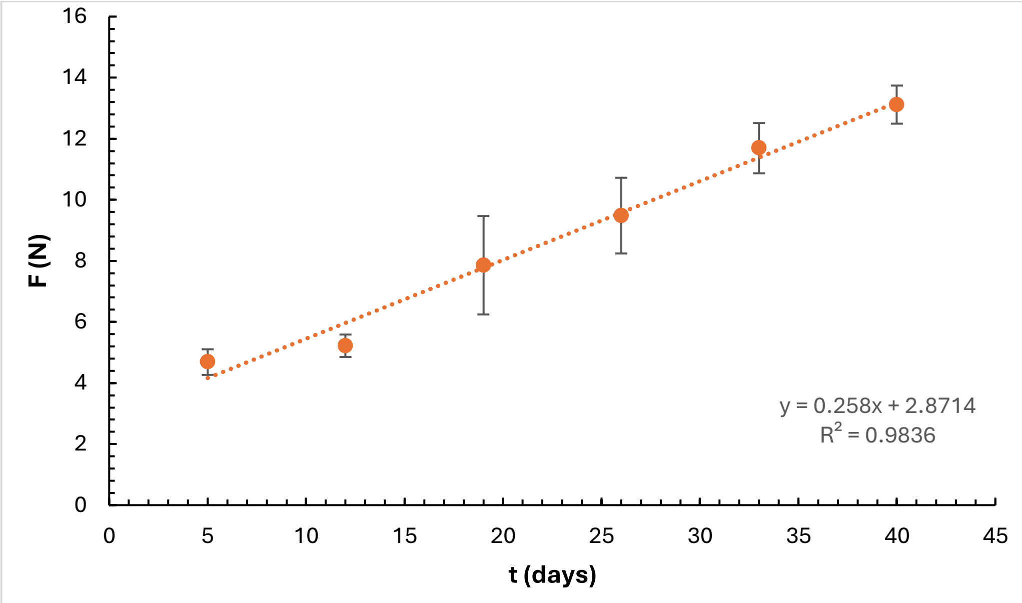

Figure 3.2 *Evolution of maximum compression force as a function of ripening time*

Figure 3.2 illustrates the evolution of maximum compression force as a function of the maturation time of aged cheese. The force values (F) vary from approximately 3 to 12 N and increase progressively ($p<0,05$) as the days pass. This trend is well described by the regression line ($y = 0.258x + 2.8174$), whose positive slope indicates an increase in force as the maturation time increases. The high coefficient of determination ($R^2 = 0.9836$) confirms the excellent linear correlation between the two variables. From a physical point of view, the increase in the force required to cut the sample is attributable to the structural changes that occur during ageing: progressive loss of water, greater compactness of the casein matrix, reorganisation of proteins and crystallisation of mineral salts. These phenomena cause the cheese to harden and therefore become more resistant to deformation.

The error bars associated with the strength values, calculated as the standard deviation of the replicates, are wider than those for moisture content. This indicates greater experimental variability in the consistency measurements, probably due to the structural heterogeneity of mature cheese and the nature of the mechanical test itself, which is more affected by local differences in the matrix than the measurement of water content.

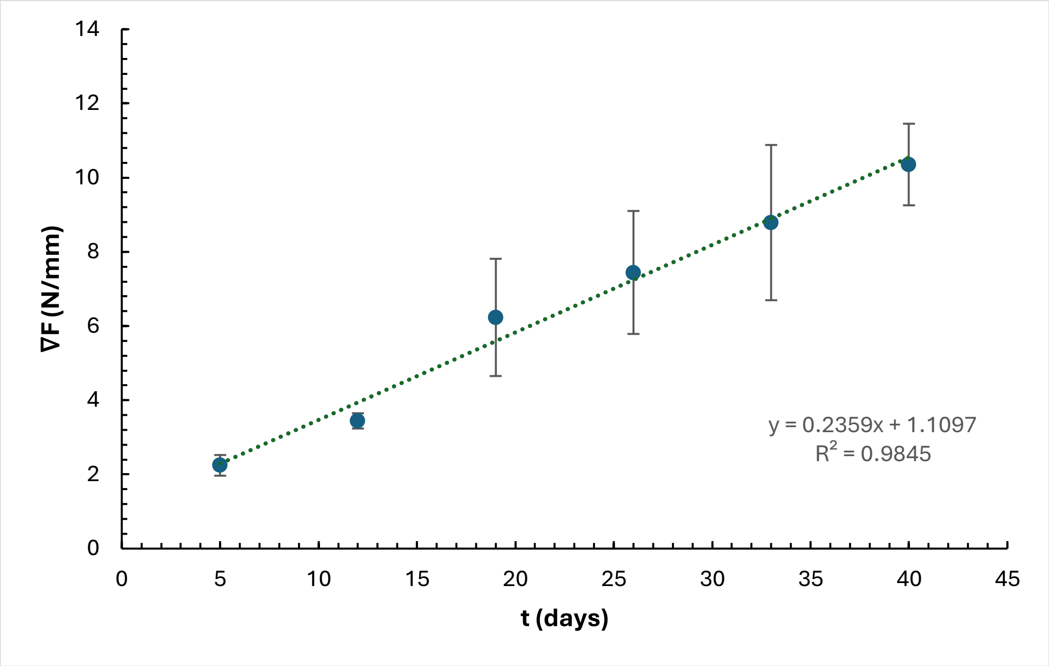

Figure 3.3 *Evolution of the force gradient as a function of ripening time*

The force gradient (N/mm) represents the variation in force per unit length. A high value means that a small change in position results in a large change in force. In the case of an elastic material, the force gradient is a measure of its stiffness. As displayed in the graph (Figure 3.3), there is a significant increase in the gradient as a function of curing time ($p<<0,05$), with values ranging from 2 to 10 (N/mm). This indicates that during the ripening process, the food becomes more resistant to breakage, suggesting hardening and greater structural rigidity. As in the previous graphs, this one also includes error bars indicating the standard deviations.

The results of the ANOVA analysis are shown in Table 3.2, which presents the values of maximum compressive strength and gradient during the curing period (expressed in days). For both parameters, the mean values are presented together with their respective standard deviations. The letters in superscript identify the homogeneous groups determined by Fisher's LSD analysis. Five homogeneous groups (A, B, C, D, E) are identified for maximum compressive strength; no statistically significant differences are observed between days 5 and 12. As regards the gradient, four homogeneous groups are identified (A, B, C, D). Again, no significant difference is observed between days 5 and 12. Of particular interest is the result observed on day 26, which belongs to group BC: it shows no statistical differences with either day 19 (B) or day 33 (C).

Table 3.2 *Table reporting the results for force and force gradient mean ± standard deviation. Letters indicate homogeneous groups established from LSD intervals (95%)*

Time (days)	F (N) + SD	∇F (N/mm) + SD
5	4.69 ± 0.42[A]	2.24 ± 0.28[A]
12	5.22 ± 0.37[A]	3.44 ± 0.21[A]
19	7.86 ± 1.61[B]	6.23 ± 1.58[B]
26	9.48 ± 1.24[C]	7.44 ± 1.66[BC]
33	11.69 ± 0.82[D]	8.79 ± 2.09[C]
40	13.12 ± 0.62[E]	10.35 ± 1.10[D]

3.3 Evolution of ultrasonic parameters during maturation

Figure 3.4 shows the trend in ultrasonic velocity (m/s) as a function of ripening time. It can be seen that the velocity values increase as the ripening time increases, fluctuating between 1580 and 1900 m/s.

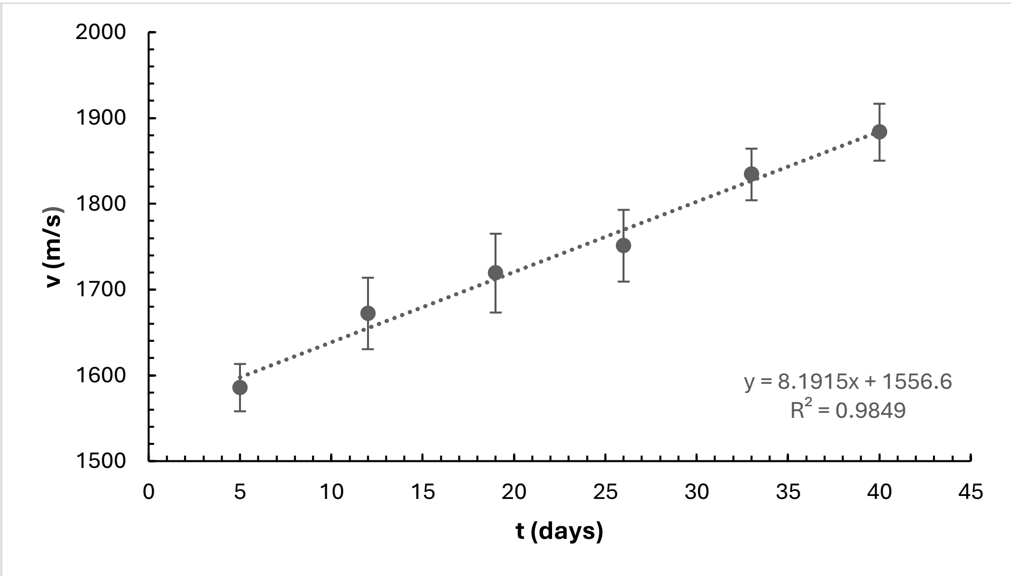

Figure 3.4 *Evolution of the ultrasonic velocity as a function of ripening time*

The speed of propagation of ultrasound in a material depends on:

$$v = \sqrt{\frac{E}{\rho}} \quad (3.1)$$

Where:

- E = elastic modulus.
- ρ = material density.

As the cheese matures, its water content decreases (as described in paragraph §3.1), making the protein matrix more compact. The casein network becomes denser, increasing the cohesion and rigidity of the tissue. These factors cause a significant increase in E, while ρ varies minimally. As a result, the increase in ultrasonic velocity with maturation time is statistically significant ($p \ll 0,05$).

For ultrasonic velocity, the ANOVA analysis provides the results presented in the table 3.3. In this case, six homogeneous groups (A, B, C, D, E, F) are identified, showing how each value, corresponding to a different time (day), is statistically significant.

Table 3.3 *Table reporting velocity mean ± standard deviation. Letters indicate homogeneous groups established from LSD intervals (95%)*

Time (days)	v (m/s) + SD
5	1585.65 ± 27.57^{A}
12	1672.13 ± 41.65^{B}
19	1719.13 ± 45.95^{C}
26	1751.08 ± 41.82^{D}
33	1834.17 ± 30.09^{E}
40	1883.43 ± 33.16^{F}

3.4 Correlations between ultrasonic, textural and moisture parameters

Table 3.1 summarises the regression equations, coefficients of determination (R^2) and p-values obtained for the parameters analysed during maturation, as described in paragraphs§3.1, §3.2, §3.3. All parameters show highly significant trends ($p \ll 0.05$), confirming that the changes observed over time are not due to random variations and are statistically significant. The coefficients of determination (R^2), all greater than 0.95, indicate that the linear regression models account for over 95% of the variability in the experimental data, thus validating the robustness of the trends represented in the graphs. Moisture content decreases with time (negative slope), whereas force, force gradient and ultrasonic velocity increase (positive slopes), in agreement with the physical phenomena associated with cheese ripening.

Table 3.4 *Linear regression equations, coefficients of determination (R^2) and p-value for the parameters analysed during ripening. X value refers to the maturation time*

Parameter	Regression equation	R^2	p-value
MC	y = -0.2078x + 45.227	0.9589	0.000641
F	y = 0.258x + 2.8714	0.9836	0.000101
∇F	y = 0.2359x + 1.1097	0.9845	9E-05
v	y = 8.1915x + 1556.6	0.9849	8.59E-05

The following figures show the linear relationships between ultrasonic velocity and the textural and moisture parameters analysed.

- Figure 3.5: shows moisture content (expressed kg of H_2O/ 100 kg of cheese) on the x-axis and velocity (m/s) on the y-axis. The correlation is inverse, as velocity decreases as moisture increases.
- Figure 3.6: represents the relationship between the force gradient and ultrasonic velocity, highlighting a direct linear correlation.
- Figure 3.7: illustrates the correlation between force and ultrasonic velocity, which is also direct linear.

Moreover, in all three graphs the points are coloured differently and labelled as t1, t2, t3, t4, t5 and t6, to indicate that each point corresponds to a specific ripening time.

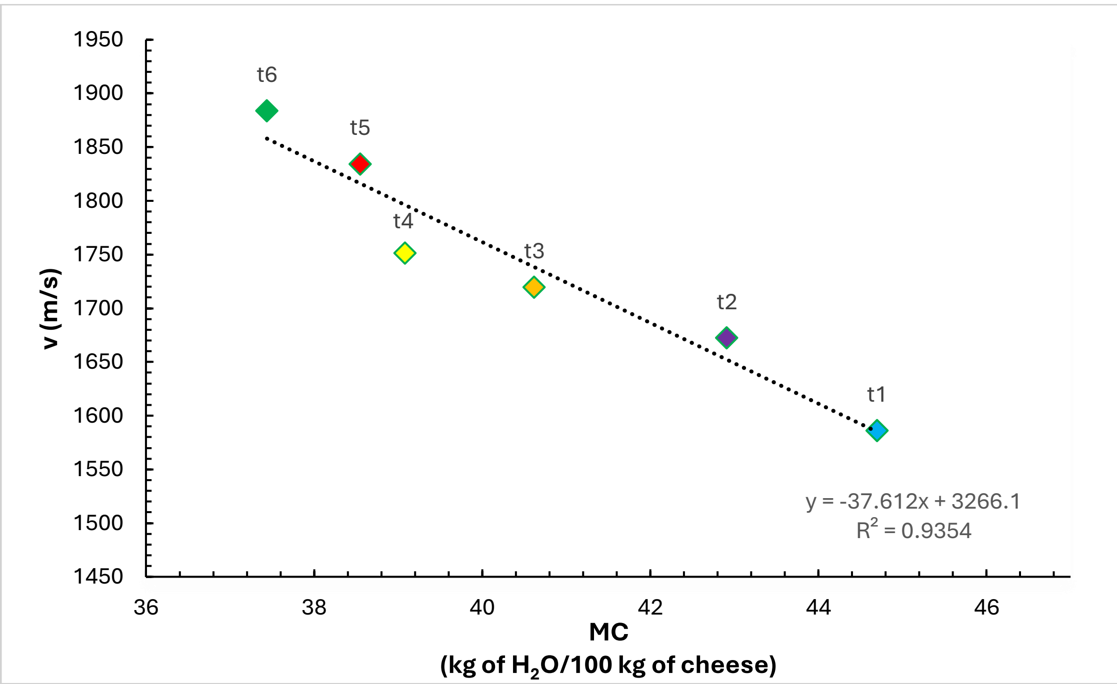

Figure 3.5 *Comparison between moisture content and ultrasonic velocity*

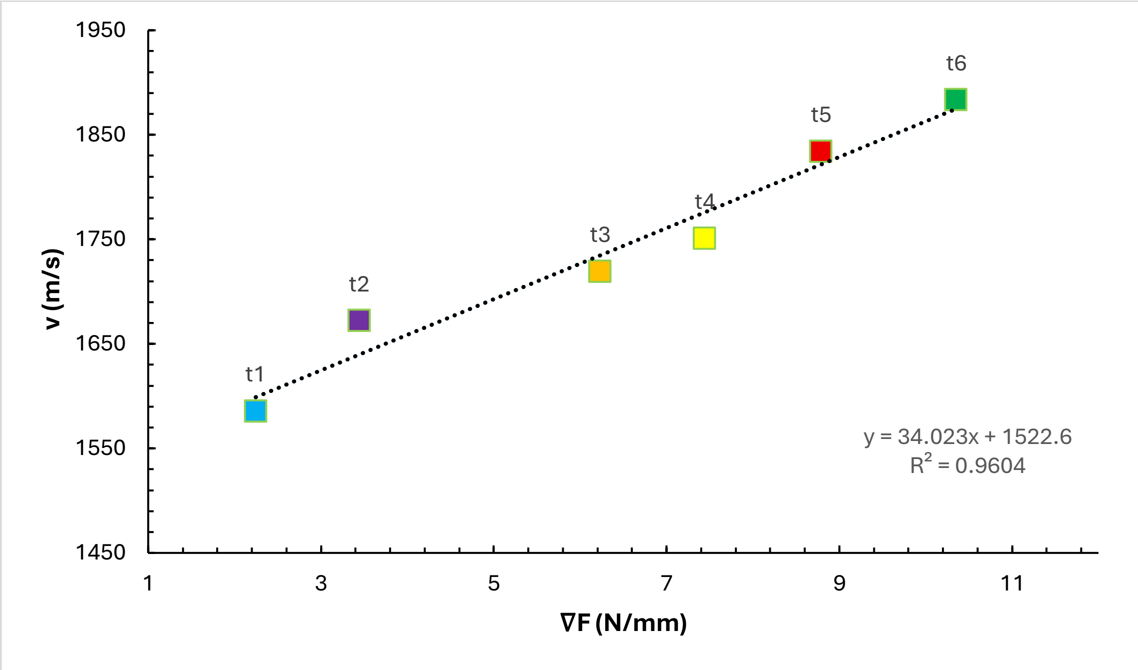

Figure 3.6 *Comparison between gradient of the force and ultrasonic velocity*

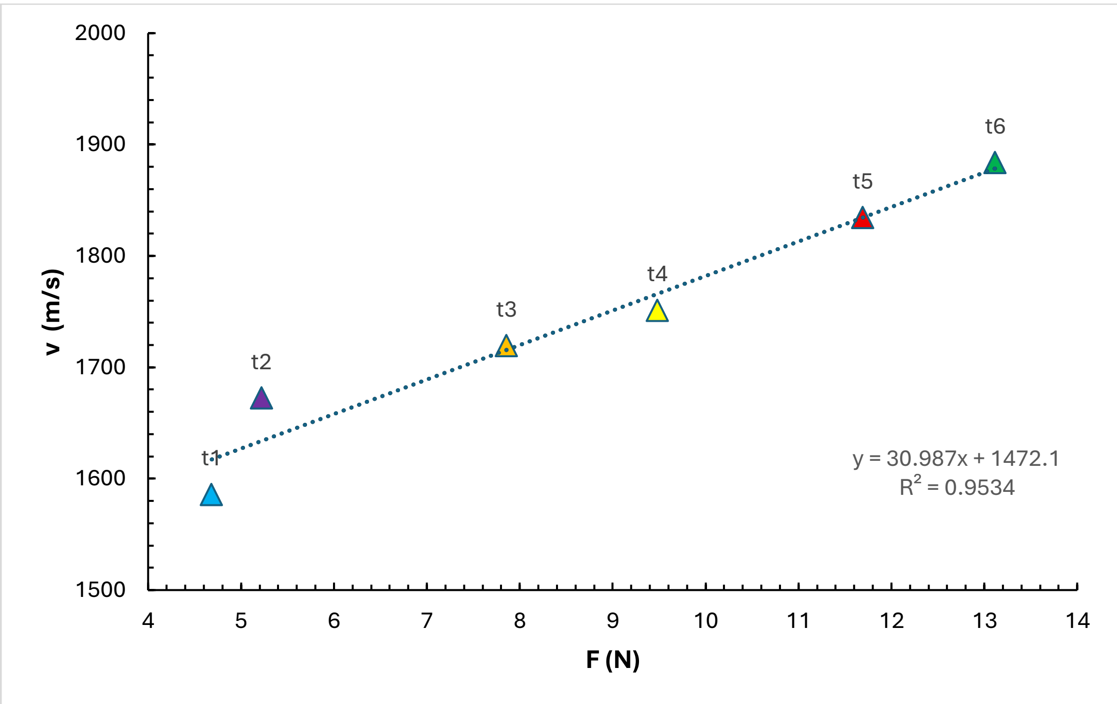

Figure 3.7 *Comparison between force and ultrasonic velocity*

Table 3.2 lists the results of the linear regressions relating to the three figures (3.5, 3.6 and 3.7). In all relationships, the linear model excellently describes the experimental data ($R^2 > 0.93$; $p << 0.05$), which means that the independent variables considered (humidity, slope, force) almost completely explain the variability observed in speed. The angular coefficients (m) of the regressions show how speed varies according to the different parameters analysed. Speed decreases as relative humidity increases (m = -37.612) and increases as the gradient (m = 34.023) and force (m = 30.987) increase. The intercept values (q) are high. However, these are derived from the linear model fit and should not be considered as real physical values outside the experimental range.

Table 3.5 *Linear regression equations, coefficients of determination (R^2) and p-values relating to the comparative graphs described above. (Figure 3.5, Figure 3.6, Figure 3.7)*

	Regression equation	R^2	p-value
v vs MC	y = -37.612x + 3266.1	0.9354	0.0016
v vs ∇F	y = 34.023x + 1522.6	0.9604	0.000596
v vs F	y = 30.987x + 1472.1	0.9534	0.000827

Conclusions and future outlooks

The main objective of this master's thesis is to study and evaluate the variations in the physical and chemical properties of cheese during the ripening process. In particular, the focus is on analysing the textural properties, moisture content, and ultrasonic velocity, as these parameters provide an in-depth understanding of the structural and qualitative evolution of the product during ageing.

The results highlight the reliability and effectiveness of the non-contact ultrasonic technique, which is non-invasive, non-destructive, and low cost. The accuracy of the results is attributable not only to the use of innovative signal analysis techniques but also to the appropriate storage and maintenance conditions of the samples. Statistical analysis of the collected data (ultrasonic velocity, texture, and moisture) shows an overall accuracy between 93% and 98%, confirming the validity of the method and the robustness of the models used.

However, a significant limitation also emerges: the transmission of the ultrasonic signal is affected by the nature and surface of the food. In the specific case of whole cheese samples, the amplitude of the acquired signal is not particularly high. This highlights the importance of optimising experimental conditions and signal acquisition parameters to ensure consistent and reliable measurements. For future developments and to extend the application of this methodology to other foods, a number of innovations can be introduced:

- Advanced signal processing techniques, such as transforms, adaptive filters, and wavelets, which are useful for improving sensitivity and better isolating the useful signal from noise.
- More sophisticated time-of-flight estimation algorithms, not limited to cross-correlation, but including approaches such as STFT (Short-Time Fourier Transform), zero-crossing-based methods, or adaptive models.
- Hardware improvements, such as transducers with more suitable frequencies, greater power, more sensitive detectors, and optimisation of the sensor–sample distance, to increase the signal-to-noise ratio, especially in the presence of irregular surfaces or thin samples.

In conclusion, from the analysed parameters, it is possible to observe that during cheese maturation, hardness tends to increase while moisture content decreases. This behaviour can be explained by the increase in density and the reduction in water content in the matured product, which is also reflected in a more intense flavour compared to that of fresh cheese. At the same time, the ultrasonic velocity increases with maturation time, indicating that the medium becomes more compact and transmits the acoustic wave more efficiently. These results confirm that non-contact ultrasound represents a promising tool for non-destructive food analysis. Nevertheless, further research on other materials and products is necessary to verify its effectiveness and promote its real-time industrial application.

APPENDIX A

Other non-contact ultrasound results

In this section, the procedure used to calculate the sample thickness, based on the Pulse-Echo technique already introduced in paragraph §2.5.3 of Chapter 2, is described in more detail. For comparison and verification purposes, the thickness of the sample is also measured manually using the procedure reported in paragraph §2.4, to compare the direct measurement with the one obtained by ultrasonography.

The procedure is based on the measurement of the time of flight of the ultrasonic pulse reflected at the interfaces inside the sample, according to the following steps:

I. **Measurement of the time of flight**

The time of flight corresponding to the reflections from the upper and lower surfaces of the sample ($\Delta TOF_{\text{upper}}$ and $\Delta TOF_{\text{lower}}$) is recorded.

II. **Calculation of the partial thicknesses**

The thicknesses of the upper and lower regions are calculated using the following relationship:

$$l = \frac{v_{air} \cdot \Delta TOF}{2} \times 10^{-6}$$

where:

- l is the section thickness (m)
- v_{air} is the ultrasonic velocity in air (m/s)
- ΔTOF is the measured time of flight (µs)
- the factor 10^{-6} converts microseconds to seconds
- the division by 2 accounts for the two-way travel of the ultrasonic wave (forward and back)

III. **Calculation of the total sample thickness**

The total sample thickness (l_{tot}) is obtained as the difference between the total known distance between the transducer and the receiver (L_{tot}) and the sum of the upper and lower thicknesses:

$$l_{\text{tot}} = L_{\text{tot}} - (l_{\text{upper}} + l_{\text{lower}})$$

This procedure allows isolating the effective thickness of the cheese sample, excluding the contribution of the air layers above and below the sample.

Table A.1 *Comparison between the average sample thickness measured manually (§2.4) and obtained using the Pulse-Echo technique (§2.5.3) for different maturation times (t1-t6)*

Maturation time	Manual thickness (m)	Pulse-Echo thickness (m)	Difference (m)	Relative error (%)
t1	0.0574	0.0533	-0.0041	7.1429
t2	0.0559	0.0517	-0.0042	7.5134
t3	0.0607	0.0577	-0.0030	4.9423
t4	0.0572	0.0526	-0.0046	8.0420
t5	0.0557	0.0503	-0.0054	9.6948
t6	0.0592	0.0530	-0.0062	10.4730

The table A.1 shows the comparison between the average thickness measured manually and that obtained using the Pulse-Echo technique for the different maturation times. It can be observed that the thickness values measured with the Pulse-Echo technique are slightly lower than those obtained manually. However, the relative error between the two measurements ranges between 5% and 10%, indicating a good agreement between the two methods. This demonstrates that the Pulse-Echo technique is reliable and provides measurements with a low margin of error.

Nomenclature

ANOVA	Analysis of variance
∇F	Gradient of force
CAGR	Compound annual growth rate
D	Deformation
E	Elastic modulus
F	Force
FF	Flesh firmness
FLC	Flesh boundary compressive force
Fmax	Maximum force
F_s	Sampling frequency
L	Total distance between the transducers
L_1	Distance between the transducer and the sample
L_2	Distance between the sample and the receiver
L_s	Sample thickness
LSD	Least Significant Differences

m	Angular coefficients
MC	Moisture content
p	P-value
PE	Pulso-echo
q	Intercept
R^2	Coefficient of determination
S	Slope of the force-deformation curve
STFT	Short-Time Fourier Transform
t	Time
TR	Transmission-reception
v	Ultrasonic velocity
v_{air}	Ultrasonic velocity in air
W_{10}	Work required to achieve a 10 mm flesh deformation
ΔTOF_{PE}	Time-of-flight in PE mode
ΔTOF_{TR}	Time-of-flight in TR mode
ρ	Material density

Symbols

µs	Microsecond
cm	Centimeters
dB	Decibel
Hz	Hertz
MHz	Megahertz
mm	Millimeters
Mpoints	Mega points
N	Newton
Ø	Diameter
s	Seconds
V	Volt
W	Watt

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Acknowledgements

I would first like to express my sincere gratitude to my supervisor, Professor Sara Spilimbergo, for her constant support, availability, and valuable advice throughout the entire course of this work. My heartfelt thanks also go to my Spanish tutors, Professor José Javier Benedito Fort and Professor José Vicente García-Pérez, for giving me the opportunity to carry out my thesis at the Universitat Politècnica de València, and for their guidance and support during this experience. A special thank you goes to Dr. Anabella Soledad Giacomozzi, with whom I shared the project and the experimental activities in the laboratory. Thank you for your kindness, patience, support, and for all the moments we shared, which made this experience even more meaningful. It has truly been an honor and a great pleasure to be part of such a passionate, skilled, and welcoming research team.

I would like to thank my family for the love and support they have always given me, for the opportunities I have had, and for helping me become the person I am today.

A special thanks goes to Vitto, Marti, and Lisa, who have been part of my life in Padua and made it truly special, and to all the people I had the chance to meet during these almost two years in Veneto.

I would also like to thank my housemates, Domenico and Alessandro, for putting up with me, supporting me, and sharing both the joys and challenges of life at Via Agordat 16.

Heartfelt thanks to all my friends who have been by my side throughout these years.

Finally, a special thank you goes to Aurora, my “best Erasmus friend,” without whom my experience in Valencia would not have been the same.

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