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PhD Thesis

The dairy industry between sustainability and 5.0: innovative strategies and life cycle assessments in the processing and packaging of stretched-curd cheeses

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All’amore

che mi ha sempre circondata.

Summary

Abstract

This doctoral thesis focuses on advancing food process engineering, particularly dairy plant design, integrating laboratory-scale experimentation while providing life cycle assessment (LCA) and industrial validation. The research aims to enhance cow's milk processing through systemic innovations within the dairy industry.

Curd freezing is proposed as a technological solution to improve raw material management (addressing milk surplus) and ensure greater production flexibility. This study explored the effect of freezing curd, produced from raw milk (RM) and pasteurized milk (PM), subjected to blast chilling and packaged in air (A), 100% nitrogen (N), and vacuum (V), on the quality of mozzarella obtained after 30, 60, and 90 days of storage at -18°C.

Furthermore, an LCA approach was conducted to assess the environmental impact of mozzarella production, accounting for different acidification methods, packaging systems, and shelf-life durations. This analysis led to the definition of mitigation and improvement strategies aimed at aligning curd management with environmental protection principles.

A technology transfer phase was also implemented, validating these protocols on a pilot scale within an industrial dairy to ensure scalability and industrial profitability processes.

The results confirm that combining curd freezing with optimized packaging systems is an effective strategy for reducing waste and enhancing supply chain sustainability. This approach provides small and medium-sized dairy enterprises with a scientifically validated protocol to improve both economic and environmental performance.

1. Introduction

1.1 Context and motivation of the study

This PhD project focuses on advancing food process engineering, specifically regarding dairy plant design and the optimization of processing workflows, ranging from pilot to industrial scale. The research activities prioritize process effectiveness, energy efficiency, and detailed system analysis. By focusing on energy saving and process analysis, the project seeks to enhance overall production sustainability.

Specifically, this research focuses on the cow's milk transformation process and improves the quality, environmental sustainability and added value of dairy products, with a particular focus on traditional southern products. Once the critical points in the various production processes have been identified, with attention to managing water and electricity consumption through parameter evaluation, optimized production protocols will be provided. Dairy farms, considering the increase in production and management costs, must be directed towards environmental and economic sustainability aimed at reducing waste and saving water and electricity resources. A significant amount of milk and by-products remains unused or unsold due to logistical issues, technical, and operational barriers. Curd freezing represents a technological solution that can improve raw material management and ensure greater production flexibility. This study explored the effect of freezing curds, produced from raw milk (RM) and pasteurized milk (PM), chilled to -18°C and conditioned in air (A), 100% nitrogen (N), and vacuum-packed (V), on the quality of Mozzarella cheese. The curd stretching phase occurred on day 0 and after 30, 60 and 90 days of storage at -18°C, following slow thawing at +4°C for 12 hours. The resulting Mozzarella cheeses were analyzed for chemical, physical, and mechanical parameters, both after production and after 6 days of refrigerated storage at 8°C. Based on this laboratory experiment, an LCA analysis was also conducted to evaluate the impact of Mozzarella production, considering different acidification methods, packaging systems, and different storage time intervals. These results confirm the potential of combining curd freezing with suitable packaging systems, proving to be an effective strategy for reducing waste and improving supply chain sustainability, offering new perspectives for more efficient management of resources and production processes. The analyses conducted using these technologies and protocols should highlight a reduction in waste materials, greater sustainability of production due to the lower use of resources, and therefore an increase in profits related to the reduction costs of production factors. Monitoring process production and adopting protocols for process management are essential for achieving an Industry 5.0 and should also be extended to small and medium companies in the dairy field, to make them increasingly sustainable.

1.2 Objectives of the research

This research focuses on enhancing the transformation processes of cow milk by introducing systemic innovations to the dairy industry. The primary goal is to optimize production efficiency, reducing environmental impact, specifically through the optimization of water and energy consumption, and ensuring high quality standards from raw material intake to final distribution.

To achieve these goals, the work is organized around the following specific objectives:

- Process Optimization: To evaluate current industrial processing standards and identify critical points within the production chain. This phase aims to minimize product losses and waste while defining benchmarks for resource management in dairy plants.
- Integration of Precision Technologies: To identify and adapt advanced technologies for the food industry, specifically calibrating their application to the dairy sector to enhance process reliability and consistency.
- Innovative Monitoring and Management Protocols: To investigate and implement rapid, non-destructive quality analysis tools alongside precision monitoring systems for water and energy consumption at critical processing stages. This includes developing strategies for waste reduction and resource recovery/reuse
- Impact and Technology Transfer: To evaluate the technical and environmental sustainability of the proposed innovations. By fostering a data-driven approach to process control in collaboration with industry partners, the research aims to bolster sectoral competitiveness through the direct application of findings.

The ultimate goal of this study is therefore to characterize and scientifically understand the proposed production approaches, which are part of the food waste recovery process. This approach will optimize the management of excess raw materials while promoting greater efficiency in the production of fresh stretched cheeses.

1.3 Structure of the thesis

This doctoral thesis is organized into seven main chapters, following a logical progression from the theoretical framework and literature review to experimental validation and life cycle impact analysis.

Chapter 1 provides the general introduction, outlining the context, the primary motivations of the study, and the specific research objectives.

Chapter 2 presents a comprehensive review of the state of the art, focusing on the dairy industry’s transition towards the Industry 5.0 paradigm. It examines the current challenges in sustainability, monitoring systems, and strategies for the optimization and recovery of energy and water resources.

Chapter 3 addresses the critical issue of milk overproduction and waste throughout the supply chain. It explores preservation strategies, with a specific focus on the use of frozen curd as a sustainable alternative to mitigate waste and improve production flexibility.

Chapter 4 details the first phase of the experimental work conducted at a pilot scale (Mac-Lab Unibas). This section describes the experimental design and the analytical evaluations performed on both curds and the resulting Mozzarella (Fiordilatte type, high moisture), discussing the technical feasibility of the proposed processes.

Chapter 5 applies the Life Cycle Assessment (LCA) methodology to evaluate the environmental impact of the production processes analyzed in the previous chapter. This chapter covers the literature background, the detailed methodology, and a discussion on study limitations and mitigation strategies to improve the environmental profile of the dairy chain analyzed.

Chapter 6 focuses on technology transfer and industrial validation. It presents a case study where the pilot-scale findings regarding frozen curd were implemented and tested within an industrial dairy plant, bridging the gap between academic research and real-world application.

Finally, Chapter 7 synthesizes the general results, discussing the scientific and industrial implications of the work. It also outlines the study's limitations and provides perspectives for future research and further technology transfer opportunities.

The thesis concludes with Chapter 8, which lists the references cited, Appendix A, containing supplementary data and supporting graphs, and Appendix B which provides a strategic analysis and preliminary technical design of automated curd production lines.

2. State of the art

This chapter analyzes the state of the art of the dairy industry, specifically focusing on the intersection between traditional processing and the emerging technological paradigms of Industry 5.0. The sector faces growing pressures due to the high perishability of raw materials and the environmental impact of transformation processes. Ecological transition strategies are examined, focusing on energy flow monitoring and advanced water resource management, essential tools for transforming operating costs into new opportunities within a circular economy.

2.1 The dairy industry: processes, products, and sustainability challenges

The dairy sector represents one of the most complex branches of the food industry, characterized by a wide array of transformation processes. From raw milk reception to the production of stretched-curd cheeses ("pasta filata"), the industry relies on a sequence of thermal, chemical, and mechanical unit operations (pasteurization, coagulation, syneresis, and stretching), each generating distinct environmental impacts (Singh et al. 2024). Each stage is critical not only for the organoleptic quality of the final product but also for its resource intensity. The diversity of products, ranging from fresh Mozzarella to aged cheeses, requires highly specific processing parameters, making the standardization of efficiency a significant challenge.

The dairy sector significantly impacts the environment through methane emissions from livestock and energy-intensive industrial processing; simultaneously, is increasingly vulnerable to climate-related risks, such as livestock heat stress and the depletion of essential water and soil resources (Marchi et al. 2025). These environmental pressures are compounded by the global challenge of food waste. Ranked as the most wasted food group after fruits and vegetables, dairy products suffer from short shelf-lives, consumer habits, and inadequate cold-chain management (Campbell and Feldpausch 2022). Furthermore, the industry must manage frequent fluctuations in milk supply and seasonal demand, which result in significant raw milk surpluses that are difficult to valorize without rapid processing or effective long-term storage solutions.

Regarding by-product management, whey from dairy production represents a huge global waste (160.7 million m^3 per year). Although partially reused as animal feed, a significant portion is often discharged directly into waterways, causing serious damage to ecosystems through eutrophication (Almeida et al. 2023). This waste generation translates directly into economic pressure, as dairy production is resource-intensive: up to 60% of total treatment costs in processing units are allocated to waste management (Udourioh et al. 2025). Furthermore, industry's energy consumption remains high due to thermal processing requirements for product safety and shelf life (Buchanan et al. 2025). In this context, waste valorization emerges as a critical strategy, offering the opportunity to convert dairy by-products into value-added resources such as biogas, bio-fertilizers, and biopolymers (Udourioh et al. 2025). By shifting from simple disposal to high-value recovery, the industry can address environmental regulations while transforming a significant cost burden into new revenue streams.

2.2 Industry 5.0: monitoring systems and automation in dairy processes

The transition from Industry 4.0 to Industry 5.0 represents a paradigm shift that emphasizes human-centric, sustainable manufacturing. While Industry 4.0 focused on automation and efficiency, Industry 5.0 prioritizes stakeholder value, human-technology symbiosis, and environmental responsibility (Ghobakhloo et al. 2024). In the dairy sector, this involves integrating continuous monitoring and precision technologies to ensure safety, quality, and profitability (Heema et al. 2022). On the international scene, in agri-food and dairy sectors, the use of precision technologies is starting to include use of sensors, machine/computer vision systems, elements of robotics, as long as there is a certain level of automation in the company (Kozub et al. 2020). Machining automation technology has acquired great potential to improve safety, quality and profitability by optimizing process parameters and control. These innovations require important investments that would be justified by large productions and large turnovers, however, on the market nowadays, there are sensors that allow to check numerous parameters and to keep production under control by improving the production process. An example of analytical control of milk and dairy products quality is given by the well-known near-infrared NIR technology along the production line capable of carrying out a rapid and non-destructive quality control. NIR, from initial applications on powder milk for moisture determinations (Nagarajan et al. 2006), to the subsequent determination of protein and lactose on milk, cheeses, and other products (Holroyd 2013; Yakubu et al. 2022), has good predictive capabilities also for fatty acids (saturated, monounsaturated, polyunsaturated) in milk and cheese (Llano Suárez et al. 2018; Coppa et al. 2014). In addition, NIR techniques can be implemented throughout the production process to evaluate parameters such as moisture, fat, and protein at different stages of cheese and/or other dairy production. The goal of every dairy farm should be to achieve maximum production, quality, maximizing profit. Today, with technological developments involving components, hardware, and software, NIR spectroscopy can be applied in the agri-food sector at different levels, providing the user with numerous parameters capable of evaluating the product and production process. Various problems could arise during cheese-making: milk not suitable for cheese-making, difficulties in controlling the temperature during the coagulation phase, defects in the maturation phase. It is therefore important to have tools capable of quickly and precisely indicating the quality of the raw material entering the dairy and minimizing critical issues during the production technology.

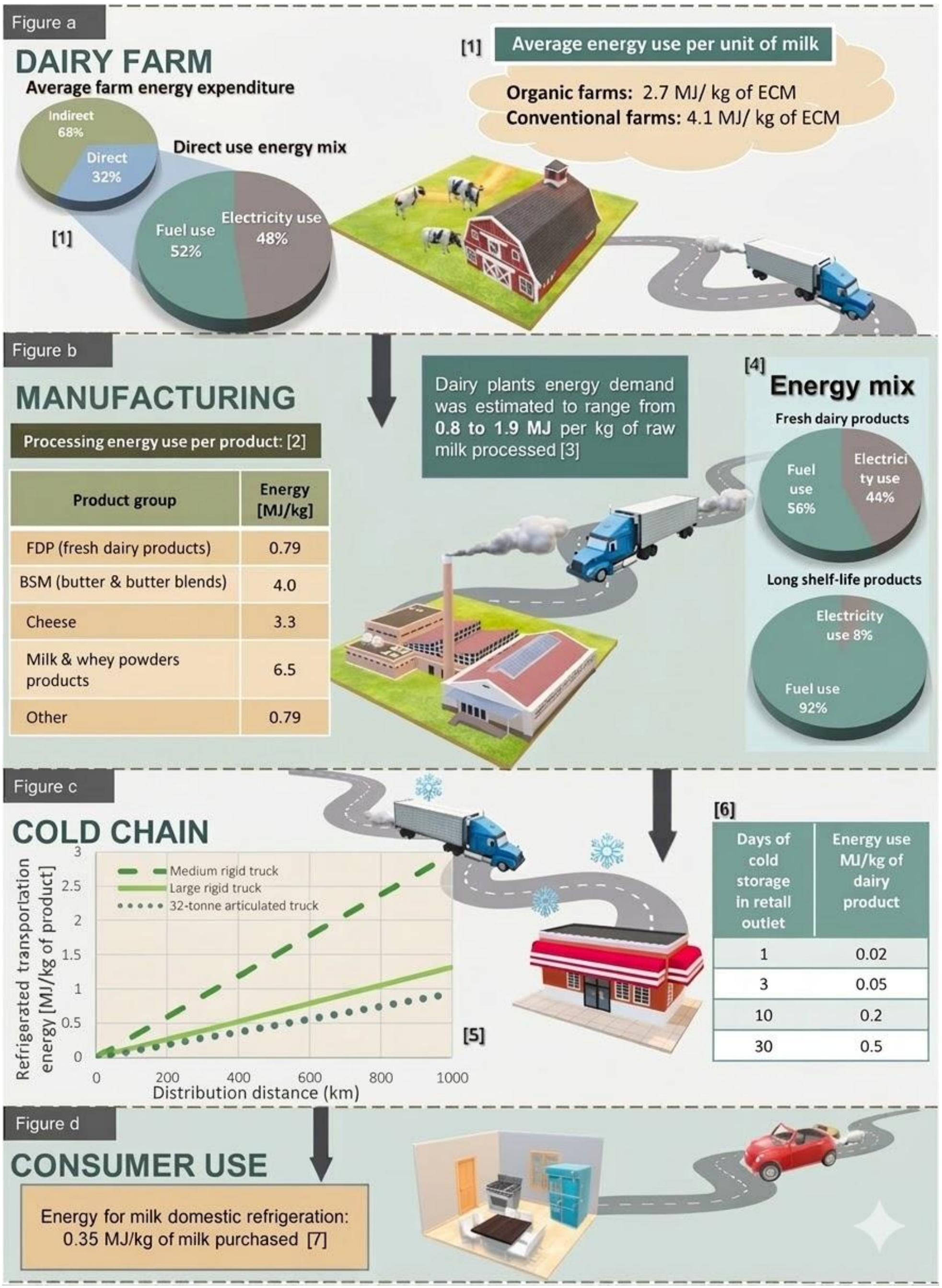
In dairy manufacturing, the Industry 5.0 transition manifests through the intelligent upgrading of hardware and data connectivity. This includes achieving precise temperature control for raw milk, automated pasteurization processes, and intelligent warehouse management systems (Wen et al. 2025). Manufacturing execution systems (MES) enhanced with AI (artificial intelligence), IoT (Internet of Things), and big data analytics provide real-time visibility and predictive capabilities addressing market fluctuations and regulatory changes (Boudjenoun et al. 2025). A Scandinavian organic dairy company (Mantravadi et al. 2023) implemented an IoT-driven Intelligent Manufacturing Execution System (IMES) across 23 factories to achieve real-time supply chain transparency and seamless IT/OT interoperability. Using digital twins and advanced data protocols, the company significantly enhanced operational efficiency, reduced changeover times, and established full product traceability to meet growing consumer demands for quality and sustainability. Digital twin technology enables virtual process simulation and optimization, supporting evidence-based decision-making while ensuring the model remains reconfigurable and flexible enough to respond to the physical manufacturing environment's conformational changes (Mo et al. 2024). Furthermore, Blockchain technology, integrated with smart contracts and decentralized autonomous systems, is increasingly used to improve supply chain transparency and product traceability (Leng et al. 2023).

In the current view and in dairy industry of Southern Italy, is increasingly essential for even small and medium-sized enterprises to adopt low-cost precision technologies. Finally, integrating these tools enables constant, automated monitoring of the entire production process, driving a reduction in waste and resource consumption while significantly increasing profitability and environmental sustainability.

2.3 Ecological transition: energy efficiency and water management

Ecological transition and sustainable development must necessarily concern the agri-food system, including dairy industry. Dairy industry in the South is characterized by a remarkable variety of products. Considering the increase in milk production costs, the supply of raw materials and energy costs it is necessary to direct production towards environmental and economic sustainability aimed at reducing waste and saving water and electricity resources (Rad and Lewis 2014). Therefore, modern dairy management must look beyond milk quality and yield. There are several objectives to take into consideration: precise control of process parameters, extension of product shelf-life, reduction of waste, process innovation, reduction of costs per kilogram of product and sales increase.

Energy consumption in the dairy industry is traditionally quantified as primary energy (kJ/kg of product, representing the combined total of electrical and thermal energy (ENEA, 1996). An overview of these energy dynamics is provided in Figure 1, which illustrates an energy map for the entire dairy supply chain.



Organic farms: 2.7 MJ/ kg of ECM
Conventional farms: 4.1 MJ/ kg of ECM

Dairy plants energy demand was estimated to range from **0.8 to 1.9 MJ** per kg of raw milk processed [3]

Product group	Energy [MJ/kg]
FDP (fresh dairy products)	0.79
BSM (butter & butter blends)	4.0
Cheese	3.3
Milk & whey powders products	6.5
Other	0.79

Days of cold storage in retail outlet	Energy use MJ/kg of dairy product
1	0.02
3	0.05
10	0.2
30	0.5

Energy for milk domestic refrigeration: 0.35 MJ/kg of milk purchased [7]

Figure 1: Infographic for the energy use in the dairy supply chain separated in 4 stages: the dairy farm (Figure a), manufacturing (Figure b), cold chain (Figure c) and consumption use (Figure d). Source: (Malliaroudaki et al. 2022); [1]: (Shine et al. 2020); [2]: (Flysjö et al. 2014); [3]: (Xu et al. 2009); [4]: (Ladha-Sabur et al. 2019); [5]: (Thoma et al. 2013); [6]: (Tassou et al. 2009); [7]:(Burek and Nutter 2020).

However, energy values are highly variable, influenced by product type, volume, processing equipment, and regional factors; therefore, energy benchmarks must be carefully calibrated to the specific context of the plant (Ramírez et al. 2006; Xu et al. 2009). In dairy industry, depending on the production type, industrial process may require heating and cooling, in fact the improvement of energy efficiency can verify on different levels, for example both through the reuse of heat recovered from outgoing milk from the pasteurization sections and reused to preheat incoming milk (Modi and Prajapat 2014; Giordano and Benedetti 2021) and on the cold chain (Marchi et al. 2022). The pasteurization process is the main process that consumes a significant amount of energy. Furthermore, changes in the performance of dairy processes could have a negative impact on the marketing of the product. Methods to reduce losses in the pasteurization process include CHP (Combined Heat and Power), the CIP process (Clean-In-Place) as well as solar energy for heating and cooling (Modi and Prajapat 2014). However, there are limitations to the pasteurization process that include high energy consumption, fuel, heat loss, and water waste. Dairies should aim to develop energy optimization protocols that allow adequate planning and continuous monitoring, with a view to a circular economy aimed at reducing waste, savings, and environmental sustainability. To reduce energy consumption and related greenhouse gas emissions, there are various examples of applications in cheese making. For example, different energy saving, and recovery technologies have been applied in the case of Cheddar cheese, including heat integration, anaerobic digestion of whey to produce biogas and fermentation of whey to produce bioethanol; saving of 0.34% of primary energy were achieved, as well as a reduction in negative greenhouse gas emissions and an increase in the added value of cheese (Gosalvitr et al. 2021). To identify opportunities for improvement of environmental and economic performance in the dairy industry, process modelling could be combined with sustainability assessment with LCA - Life Cycle Assessment (Gosalvitr et al. 2021).

Energy efficiency in a medium-sized dairy can also take place through continuous monitoring of energy flows at different levels with different measuring instruments (flow meter, thermal energy meter, temperature probe) (Cavazzini et al., 2019). A monitoring system can be easily integrated with other system already present in the company, to analyse the company's energy efficiency level and precisely identify the priority processes/plants in terms of energy consumption and any consumption anomalies. In previous works, data provided by the monitoring system supported assessments relating to efficiency measures, suggesting, for example, the adoption of a trigeneration plant that would allow for the simultaneous satisfaction of part of the electricity requirement, the thermal hot water utilities for process and space heating, and part of the product's refrigeration needs through electric chillers (Cavazzini et al., 2019). This system can be extended to any medium-small industry and makes it possible to evaluate it is convenient, based on production processes and costs, to adopt measures and interventions that can improve the company's energy efficiency.

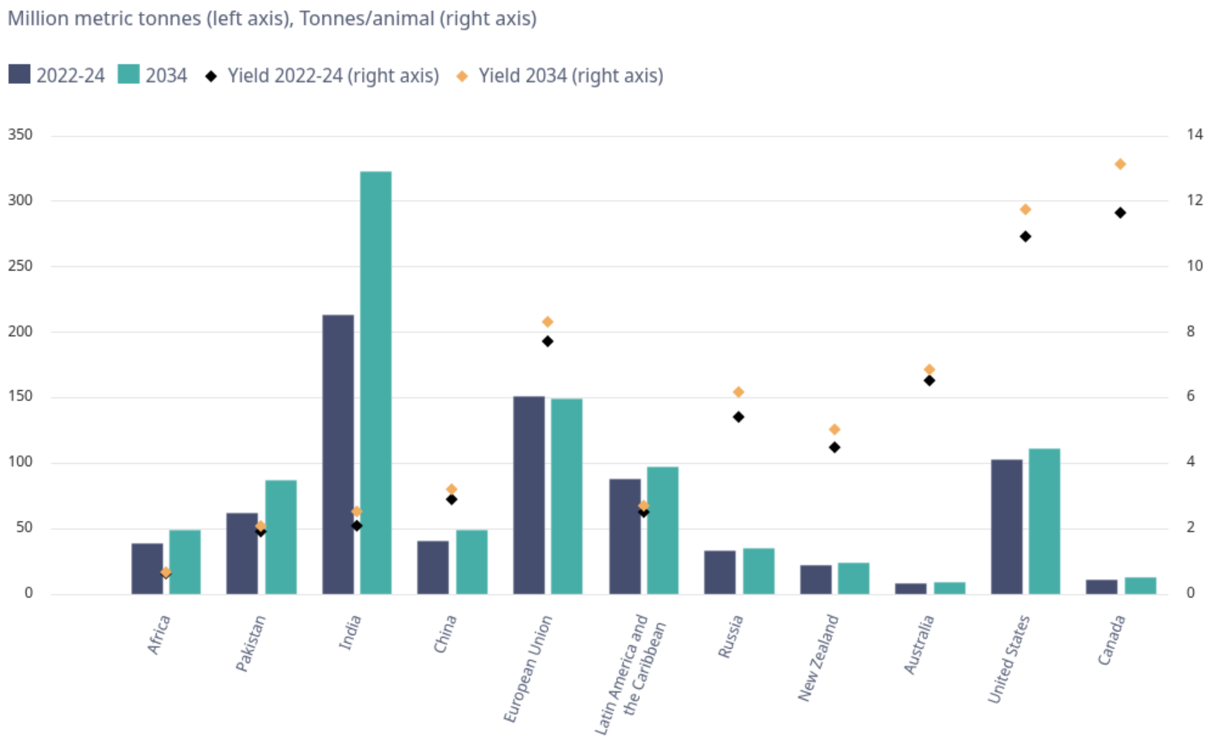
To date, water management has become a critical economic factor within the dairy sector, driven by stringent environmental legislation and the escalating costs of wastewater treatment. Reducing water consumption serves a dual purpose: it preserves a vital resource while simultaneously minimizing the volume of effluent requiring treatment (ENEA, 1996). This would lead to better use of resources and lower carbon emissions. Ultrafiltration and reverse osmosis processes can be useful in the dairy industry (El-Gazzar and Marth 1991). Membrane filtration, including ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO), has aroused considerable interest due to its low energy consumption and superior separation capabilities (Zhang and Feng 2022). These innovations offer promising integration into traditional processing schemes, enabling both water recovery and by-product valorization (Reig et al. 2021). By focusing on these strategies, the industry can reduce various forms of waste, such as surplus milk and whey. This approach not only facilitates the recycling of wastewater but also serves as a model to demonstrate the technical and economic benefits of such technologies to the wider dairy sector.

3. Milk overproduction and curd preservation strategy

Chapter 3 addresses the critical issue of milk overproduction and the resulting systemic waste within the global dairy supply chain. It examines the drivers of surplus, ranging from market saturation to intensified farming, and the environmental and ethical implications of disposing of such a resource-intensive raw material. Central to this chapter is the evaluation of preservation strategies designed to mitigate waste and decouple seasonal production from market demand. This section explores how frozen curd serves as a sustainable intermediate for stretched cheese industry.

3.1 Milk global paradox: overproduction and waste

The milk paradox refers to the simultaneous existence of global milk overproduction and widespread dairy product waste. This paradox reflects systemic inefficiencies in global food systems where production decisions are driven by profit maximization rather than demand alignment or nutritional needs. Waste in the dairy sector is not only about the milk that spoils. It is also about the wasted resources: the water, land, and energy consumed to produce milk that never reaches consumers. Despite climate challenges, global milk production is steadily growing. According to (OECD-FAO 2024) data, milk global production reached approximately 950 million tons in 2024, with India remaining the largest producer (Figure 2).



Note: The yield is calculated per cow/buffalo.

Figure 2: Milk production and yield in selected countries and regions; Million metric tonnes (left axis), Tonnes/animal (right axis); Source:(OECD-FAO, 2025).

There is a structural mismatch between where milk is overproduced and where demand is growing. Developed countries, e.g. United States and European nations, produce far more milk than they consume domestically, with substantial surpluses entering international trade; while developing countries rely on imports to meet consumption needs (OECD-FAO 2025; USDA, 2025). This trend suggests that overproduction in developed regions is driven not by local demand, but by the global market. Such saturation creates chronic price volatility, as abundant supply depresses prices when demand is weak. Farmers face pressure to increase production volume to maintain profitability in the face of low prices, creating a negative feedback loop where overproduction drives prices down, incentivizing further production increases. Furthermore, in developed regions, a significant structural shift has occurred in markets: dairy consumption has declined slightly due to the rising popularity of plant-based alternatives (Ammann et al. 2023). This trend reflects changing consumer preferences, driven by environmental concerns, perceived health benefits, and ethical considerations related to animal welfare.

Milk demand is increasing due to population growth, rising incomes and urbanization (Mottet et al. 2018), changing dietary preferences in emerging economies. Global milk production (81% cow milk, 15% buffalo milk, and 4% for goat, sheep and camel milk) is expected to grow at 1.8 % per annum over the next decade, primarily driven by higher yields per animal (OECD-FAO, 2025). Modern agriculture is increasingly defined by smart farming practices, where the integration of Automated Milking Systems (AMS), precision feeding, and genetic selection for high-producing dairy breeds has led to optimized production condition and enhanced animal health management (Dayoub et al. 2024; Si 2024; Didanna and Anja 2025). Production efficiency in PLF (Precision Livestook Farmin) has significantly improved through the optimization of herd management. By extending average lactation length, decreasing dry period durations, and shortening calving intervals, PLF have achieved substantially higher annual milk yields per animal. However, this intensification demands high capital investments that are only sustainable through increased yields and economies of scale. This has led to a structural shift toward larger, highly automated dairy farms that operate with a reduced workforce. While these changes maximize overall economic returns, they also introduce significant social and environmental concerns, such as the decline of rural employment and the environmental pressures of high-density livestock system (Clay et al. 2020).

Understanding the milk paradox overproduction-waste requires examining both the drivers of global milk production growth and the mechanisms through which loss occurs at different supply chain stages. Agricultural stage emerges as the most critical 'hotspot' for waste generation (Berlin 2002; Hospido et al. 2003). At the farm level, overproduction during periods of market saturation creates direct losses, as producers face decisions between dumping excess milk or incurring storage and transportation costs (Kashyap et al. 2023). Beyond logistical and market-driven surpluses, significant waste is generated by strict qualitative and safety standards. Milk containing traces of antibiotics or milk with high Somatic Cell Count cannot enter the food supply chain and must be discarded. The nature of milk waste varies significantly according to the regional level of infrastructure and supply chain (Kashyap et al. 2023). For instance, estimates indicate that in the United States, approximately 25% of dairy products are lost at the production level or wasted at the retail or consumer level annually (Martin et al. 2021). In Tunisia, collection inefficiency results in 18% of produced milk being lost before it even reaches processing facilities (Benabdallah et al. 2022).

Approximately 19% of global dairy waste volumes is linked to retail sector, food service, and private households (Campbell and Feldpausch 2022). Retail waste results from a combination of factors: over-purchasing, storage failures, misunderstanding of date labels, and spoilage due to temperature fluctuations in retail environments (Klein et al. 2024). Most consumers incorrectly interpret best-before dates as safety indicators rather than quality indicators, discarding products perceived as unsafe despite being nutritionally wholesome. Consequently, a significant waste reduction is achievable through improved information transparency and optimizing storage conditions and practices at home. Within this context, the "milk paradox" is not a logistical failure but an economic trap. The inability to flexibly manage these surpluses at the source leads to systemic waste, as the market cannot always absorb the excess output generated by this cycle of overproduction. The complex interactions between technological, economic, and policy-driven factors that fuel this cycle are summarized in Table 1.

Table 1: Synthetic overview of the primary drivers, mechanisms, and policy implications of the global milk overproduction-waste paradox.

Driver	**Mechanism**	**Impact on waste**	**Policy implication**
Technological intensification	Yield improvements (genetic, AMS); capacity increase	Structural oversupply	Manage adoption rates; support diversification
Precision feeding	Diet to maximize output per animal	Environmental degradation	Promote circular bioeconomics; incentivize forage-based system
Market Consolidation	Economies of scale incentivize farm expansion	Industry concentration and chronic overproduction	Strengthen antitrust measures; support small-scale farm resilience
Policy Support	Price guarantees disconnect production from real demand	Persistent overproduction	Align policy support with market needs
Cold Chain Development	Extension of shelf life increases marketable supply	Increased waste risk if infrastructure outpaces demand	Balance logistical investments with regional consumption capacity

The challenge is reconfiguring global dairy systems toward sustainability and sufficiency rather than maximum production and to improve supply chain management to reduce waste.

3.2 Curd Preservation Techniques: frozen curd as alternative strategy

Freezing is an effective preservation method that extends the shelf life of foods. It reduces the temperature below freezing point, causing crystallization and slowing biochemical reactions and microbial growth (Nájera et al. 2021). Curd, an intermediate product in cheese production obtained through the coagulation process, can be frozen to reduce costs and decrease the volume associated with milk transport. Freezing curd provides a sustainable and efficient approach for managing surplus production, reducing milk losses, and extending shelf life (Alinovi et al. 2021). The rationale for freezing curd instead of raw milk is based on the significant reduction in volume—since curd occupies approximately one-tenth of the volume of raw milk, thereby substantially cutting transport and storage emissions, and the enhanced stability achieved through the removal of whey. To maintain the qualitative characteristics of the final product, it is necessary to optimize freezing methods, thermal stability during storage, and effective thawing techniques (Nájera et al. 2021). Traditional freezing presents limitations within the cheese matrix due to the ice crystallization process, causing unwanted changes in the cheese's properties. To minimize structural damage caused by ice crystal formation, Individual Quick Freezing (IQF) techniques, such as blast chilling, are employed across various food supply chains (Digvijay et al. 2025). Additionally, cryogenic cooling (-20 °C) of the curd increased the cheese yield without major alteration in the semi-hard cheese quality, including sensory qualities (Richoux et al. 2009). Beyond traditional methods, innovative technologies are being explored. Isochoric freezing allows for preservation at sub-zero temperatures without the complete solidification of the sample and ice formation through pressure control, thereby minimizing mechanical damage (Nida et al. 2021; Fikiin et al. 2024). The effectiveness of this technique was demonstrated by (Maida et al. 2023) in raw milk stored at pressures (~77–96 MPa) and subzero temperatures (-5 °C to -10 °C), showing a reduction in microbial growth and quality degradation after 5 weeks. Similarly, Magnetic Field (MF) assisted freezing represents a significant advancement due to its capacity to generate smaller and more evenly distributed ice crystals. By influencing the physicochemical behavior of water molecules and enhancing the supercooling degree, MF technology fosters an accelerated nucleation phase that prevents the growth of large ice crystals (Yang et al. 2025). Furthermore, the scientific literature highlights several synergistic freezing approaches such as ultrasonication, cell alive freezing, glass transition temperature regulation, high-pressure freezing, and various pretreatments like pulsed electric fields, osmotic dehydration, and antifreeze proteins (Hu et al. 2022). However, freezing and subsequent thawing process may affect the chemical and physical properties of cheese, potentially affecting its final quality, in particular its texture and rheology (Alinovi and Mucchetti 2020; Conte et al. 2017; Kuo and Gunasekaran 2009; Bertola et al. 1996; Reid and Yan 2004). Several studies have evaluated the applicability of frozen curds for the production of various types of cheese. Different freezing and thawing processes for the production of a creamy cheese have shown that changes in texture are closely related to the rate of thawing of the curd (Hori 1982). At the laboratory scale, miniature cheesemaking trials were carried out to produce a young hard cheese (Reggianito Argentino cheese type) using curd and whey that had been separated and frozen. Freezing did not alter the pH, protein, moisture contents, sodium chloride levels, or thermophilic lactic bacteria counts of the cheeses made from the frozen components when compared with those produced from fresh materials (Vélez et al. 2015). To ensure a consistent supply of ovine cheese, various freezing and storage conditions were tested on pressed curd. Although vacuum packaging altered the external appearance, no changes in composition or moisture were reported, while a decrease in water activity and an increase in proteolysis were observed (Sendra et al. 1999). However, Teleme cheese produced from frozen curd and stored up to 6 months, showed poor quality due to marked proteolysis, which led to accelerated ripening, lower water retention and crumbly texture (Alichanidis et al. 1981). Despite these advantages, it must be noted that often the DOP production specifications of some cheeses (for example Mozzarella di Bufala Campana DOP) prohibit the use of frozen curd or milk. Additionally, adding curd freezing and storage stages could slightly increase energy consumption and emissions, which must be considered in the overall sustainability balance. Despite these challenges, several studies have investigated the use of frozen curd (both raw and pasteurized) from ewe’s milk (Alonso et al. 2013) and goat’s milk (Picon et al. 2013; Campos et al. 2011), mixed with fresh cow's milk curd for the production of Hispánico cheese type. The effects of frozen curd have also been examined in buffalo Mozzarella cheese, where different proportions of frozen and fresh curd were combined (Rinaldi et al. 2021). Additionally, curd obtained from homogenized milk and frozen for up to three months was shown to maintain its functional properties (Schmidt et al. 2024). These findings support freezing as a viable preservation strategy that does not compromise curd quality, reinforcing its potential application in cheese manufacture. To date, however, no studies have specifically analyzed the chemical-physical and rheological characteristics of stretched-curd cheese produced exclusively from frozen curd (i.e., 100% frozen curd), which is essential for industrial applications where flexible stock management is required. Moreover, the efficiency of curd preservation process depends strongly on both the curd's initial characteristics and the storage conditions. In the dairy sector, a considerable amount of product remains unused due to logistical, legal, or operational barriers that limit its valorization. Thus, new technological approaches are necessary for the recovery of excess raw materials and the optimization of semi-finished product management for fresh cheese production. In this regard, industrial interest in flexible curd handling is reflected by technological solutions such as the process described in patent WO2017109745A1 (Zambrini and Bernardi 2017), which employs individually quick-frozen (IQF) curd portions for Mozzarella manufacture. Despite growing industrial applications, scientific evidence regarding the effects of curd freezing on cheese functional properties and quality is limited. Our study addresses this gap by investigating the combined effects of blast chilling, freezing, and packaging technology of the curd on the quality of “Fiordilatte-type” Mozzarella cheese. The main objective was to evaluate the technological and qualitative implications of curd starting quality (raw vs. pasteurized milk), comparing the efficacy of different MAP conditions, in preserving the chemical, physical, and rheological properties of both the intermediate product and the final product.

Experimental part

The experimental section of this research is developed through an integrated approach that transitions from laboratory-scale optimization to environmental impact analysis and industrial validation. Chapter 4 details a pilot-scale study conducted to evaluate the technological properties of both curd and Mozzarella. The experimental design focuses on comparing raw and pasteurized milk as starting materials; these are processed into curd and subsequently preserved using blast chilling and various modified atmosphere packaging (MAP) conditions.

Building upon these findings, Chapter 5 introduces a comprehensive Life Cycle Assessment (LCA) to quantify the ecological footprint and resource efficiency of the proposed strategy. This analysis leads to the definition of mitigation strategies and improvements aimed at aligning curd management with the principles of environmental stewardship. Finally, Chapter 6 focuses on technology transfer, presenting the validation of the pilot-scale protocols within an industrial dairy plant to ensure the scalability and industrial viability of the processes.

4. Pilot scale at Mac-Lab

4.1 Experimental design and research phases

This research explores the potential of freezing curds to facilitate the flexible production of stretched-curd cheeses, enabling better management of surplus milk and semi-finished products. The comprehensive experimental workflow, including milk treatment, curd production, packaging system, and analyses on curd and Mozzarella cheese, is summarized in Figure 3.

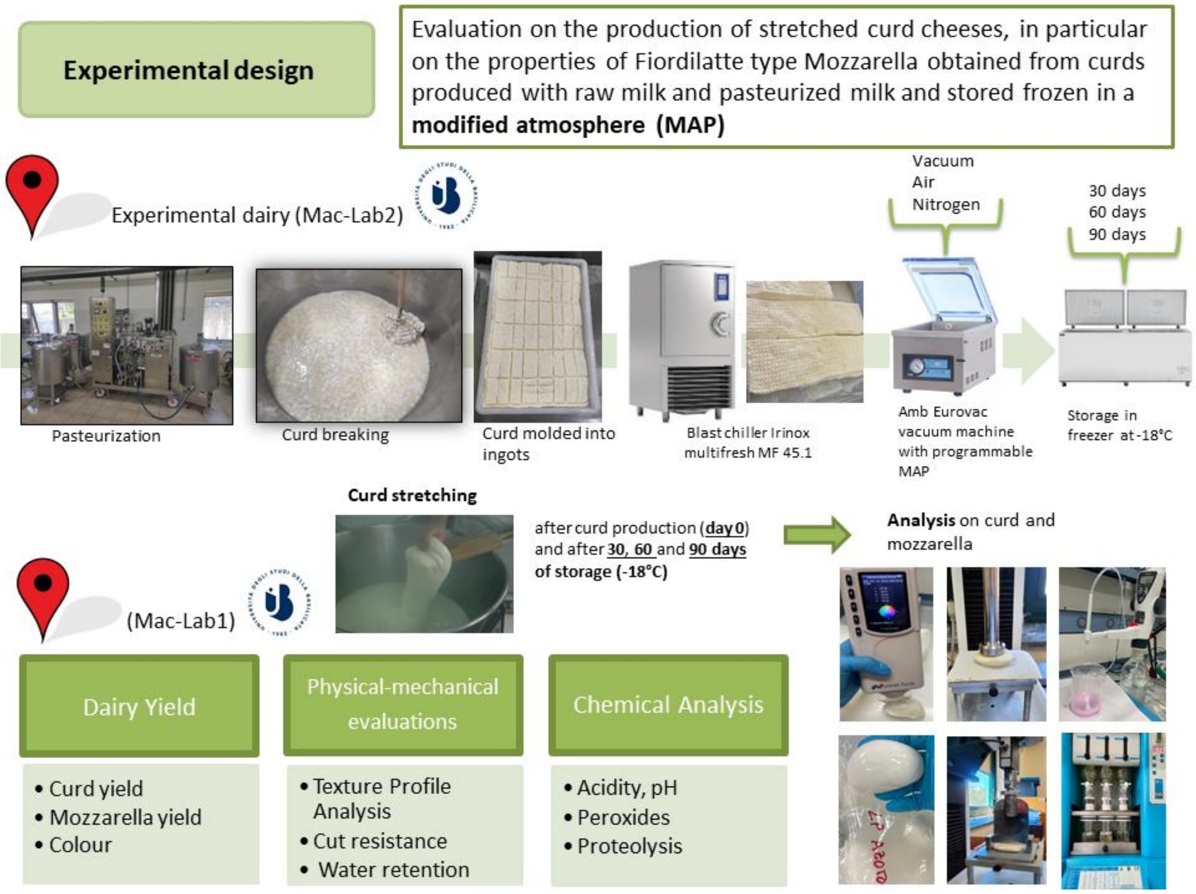


Figure 3: Schematic representation of the experimental design. The diagram illustrates the curd production phases from raw and pasteurized milk, blast-chilling, various packaging conditions (vacuum, air, and nitrogen), freezer storage (30, 60, and 90 days), curd stretching phase, and the chemical, physical, and rheological analyses performed on both the curd and the final Mozzarella.

Cheesemaking was carried out at the University of Basilicata’s experimental dairy (Mac-Lab2), where curds were produced in separate batches from raw milk and pasteurized milk. The curds were portioned, subjected to blast chilling, and subsequently packaged under different Modified Atmosphere Packaging (MAP) conditions (Vacuum, Air, 100% N2) and stored at -18 °C for 30, 60, and 90 days.

Subsequent analyses were conducted at the Mac-Lab (Laboratory of the DAFE, Department of Agricultural, Food and Forestry Sciences of the University of Basilicata). Specifically, frozen curds were analyzed for their chemical-physical and rheological properties, and the same analyses were performed on the Mozzarella cheese obtained after thawing and stretching. This study confirms the effectiveness of curd freezing, combined with appropriate packaging, in the production of stretched cheese, analyzing the technological and qualitative implications of this sustainable technique. The main objective was to evaluate the technological and qualitative implications of curd starting quality (raw vs. pasteurized milk), comparing the efficacy of different MAP conditions, in preserving the chemical, physical, and rheological properties of both the intermediate product (curd) and the final product (Mozzarella cheese).

4.1.1 The raw materials, curd production process, stretching phase

The milk used in this trial was sourced from an intensively managed herd of Holstein cows, with an average daily milk production of 35 kg. The animals were fed a total mixed ration (TMR) formulated as 60:40 F:C (forage: concentrate). The detailed composition of ration is reported in Table 2.

Table 2: Composition of the Total Mixed Ration (TMR).

Ingredient	**Quantity (kg, As-Fed)**	**DM (kg, Dry Matter)**
Forages		
Triticale silage	31.0	8.99
Alfalfa hay	3.00	2.73
Mixed hay	2.50	2.30
Total Forage (F)	**36.50**	**14.02**
Concentrates		
Water (added)	9.00	0.00
Finely ground corn	7.30	6.35
Concentrate Mixture (4772_/Relase M)"	5.20	4.62
Soybean meal	1.60	1.41
Total Concentrates (C)	**23.10**	**12.38**
Total Ration (F:C)	**59.60**	**26.40**

Proximal analysis of the raw milk was conducted using a Milkoscan FT1_Foss instrument (following the standard methods ISO 6662:2013 and IDF 141:2013). The average chemical composition of the milk was: fat 3.90 ± 0.14 g/100g and protein 3.38 ± 0.12 g/100g.

At the University of Basilicata’s experimental dairy, various types of curds were obtained from raw milk and pasteurized milk, using different methods of coagulation (Figure 3). Each trial was performed using a milk volume of 100 liters.

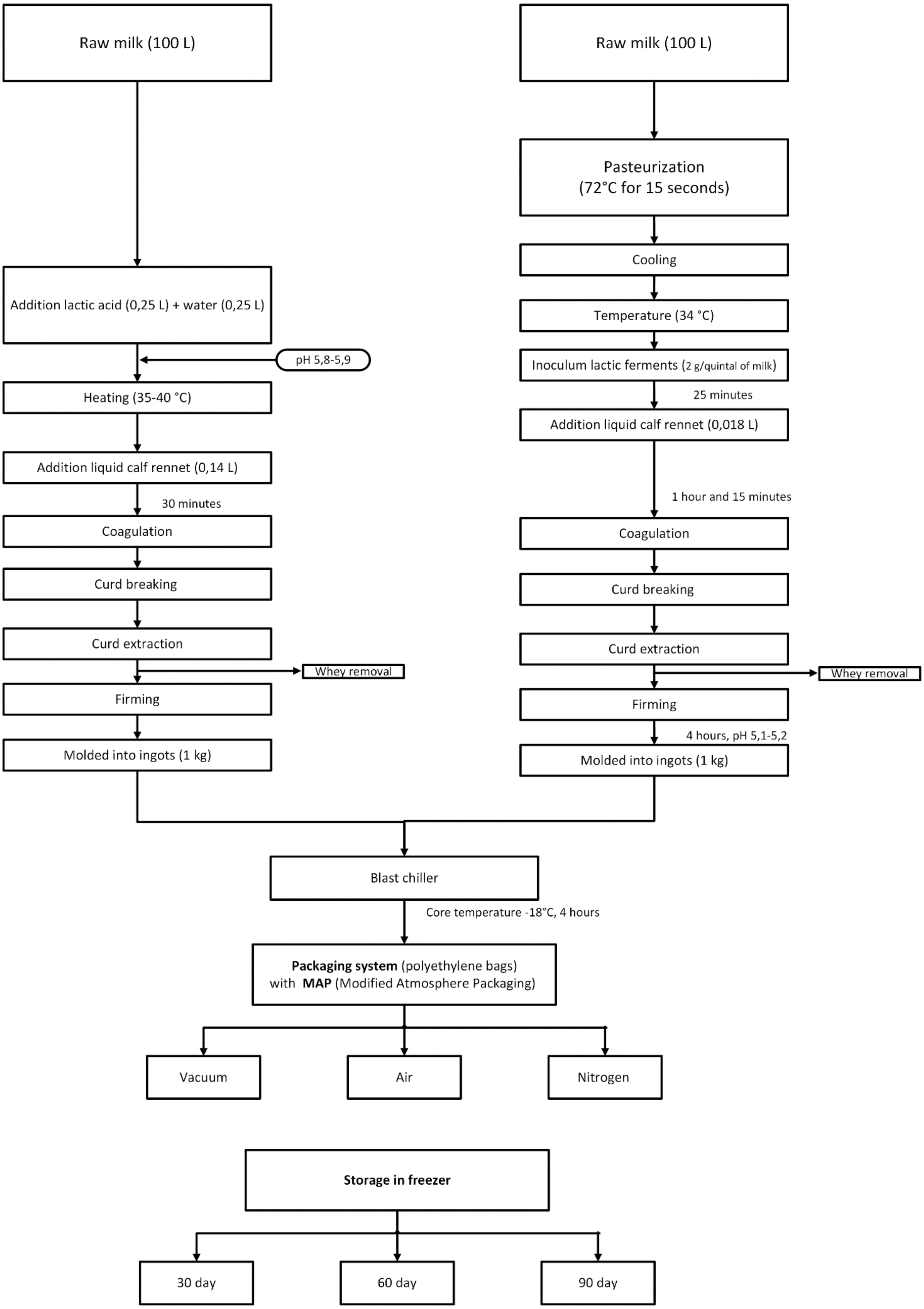


Figure 4: Curd production process with raw milk (left) and pasteurized milk (right), packaging system and storage.

For raw milk curd production, the raw filtered milk was first acidified by gradually adding a solution composed of 250 mL of lactic acid (LAFTL80, Galactic S.A) diluted in an equal volume of water, until the pH stabilized between 5.8 and 5.9. The milk was heated to 34-35 °C, and 14 mL rennet (liquid calf rennet, strength 1:10.000, Chimiosin 85%, SANTAMARIA Srl) was added. After 30 minutes, the curd was broken and mixed with a large whisk-like tool (“spino”) until the grains reached a size of 0.5-1 cm. The curd granules were lightly shaken to release trapped whey and subsequently extracted and shaped into rectangular blocks of uniform size.

For pasteurized milk curd production, the filtered raw milk was treated at 72 °C for 15 seconds using a lab-scale continuous-flow pasteurizer equipped with an indirect tubular heat exchanger (flow rate 60 dm^3/h) (Di Renzo et al., 2011). Post pasteurization, the milk was cooled to 34 °C, and lactic ferments (2 g per quintal of milk, LYOBAC MO3D, Alce group) were inoculated and subsequently, after 25 minutes, 18 mL rennet (liquid calf rennet, strength 1:10.000, Chimiosin 85%, SANTAMARIA Srl) was added. Coagulation required about 1 hour and 15 minutes, followed by cutting and mixing with the “spino” tool until the curd was reduced to 0.5-1 cm granules. The curd was gently shaken to release whey, pressed into rectangular pieces, and left to rest for 3-4 hours until reaching a pH of 5.1–5.2.

Both raw-milk and pasteurized-milk curds into 1-kg blocks, were subjected to blast chilling (Multifresh MF 45.1, Irinox SpA). The internal temperature of each block dropped to –18 °C in four hours, ensuring fast freezing while limiting the development of large ice crystals and preserving the curd’s qualitative traits. After chilling, the blocks were packaged in 80-micron polyethylene bags using an Amb Eurovac vacuum machine with programmable MAP (Modified Atmosphere Packaging). Three packaging atmospheres were applied: vacuum (V), Air (A: 78.2% N_2, 20.8% O_2, 0.03% CO_2), and nitrogen (100% N_2). The packaged curds were subsequently stored at –18 °C.

The stretching phase was performed on fresh curd (day 0) and after storage intervals of 30, 60, and 90 days. Twenty-four hours prior to processing, 1 kg of each curd batch was moved to a 4°C cold room for gradual thawing. The curd, sliced into strips and dry-salted (25 g/kg), was manually stretched in hot water (80–90°C) to form Mozzarella cheese (spherical shape, 50–70 g each). A group of Mozzarella samples was analyzed immediately after stretching, while the other portions were stored in the preserving liquid at 8 °C and evaluated 6 days later.

4.1.2 Analytical evaluations on curds and Mozzarella obtained

Analytical determinations on curds and Mozzarella cheese were performed in triplicate. The parameters assessed included: yield, freezing rate, pH, titratable acidity, color, texture profile analysis (TPA), cutting test, melting behavior test, proteolysis, and peroxide value. Freezing kinetics were tracked using K-type thermocouples and TC-08, Pico® (Pico Technology ltd, UK), logging data every second with PicoLog 6® software. An IP57 model pHmeter (XS instruments, Italy) was employed for pH readings, while titratable acidity was evaluated by titration with 0.1 N NaOH and phenolphthalein as a colorimetric indicator, and results were expressed as lactic acid percentage. Color measurements according to CIELAB system (L* (brightness), a* (red trend) and b* (yellow trend) on three different points of each curd and Mozzarella sample was assessed using a portable SA130 colorimeter (SAMA, Italy).

Rheological characterization was performed using a TA texture analyzer (Instron 3343 single column universal, Instron Corp, Canton, Massachusetts, USA). A double compression test, as described by (Alinovi and Mucchetti 2020), was performed using with a cylindrical stainless steel probe (35 mm diameter) and a crosshead speed of 1.5 mm/s was applied to compress the samples (slice-shaped, thickness 13 mm, diameter 34 mm). During this test, water-release behavior was also quantified using a paper towel. Additionally, a cutting test as described by (Reid and Yan 2004), was performed on 6 mm thick slices with a constant speed of 0.5 mm/s. Meltability was assessed through a modified version of the Schreiber test (Drake et al. 1999; Reid and Yan 2004). Curd and Mozzarella samples (7 mm thick) were placed on aluminum foil, measuring the change in diameter before and after heating at 100 °C for 10 minutes. Proteolytic activity was quantified using the o-phthaldialdehyde (OPA) method (Church et al. 1983). A sample (5 g) was homogenized with 2 mL of water and 25 mL of 0.75 N trichloroacetic acid (TCA) and filtered through cotton wool. The filtrate (100 µL) was mixed with 2 mL of OPA reagent in quartz cuvettes, after 2-minute incubation, absorbance at 340 nm was measured (Ultrospec 2100 Pro, Biochrom Ltd, Cambridge, UK).

Total fat was extracted from desiccated samples (105°C, 24 h) using Soxhlet system (SER 148/3, VELP Scientifica, Italy), as described by (Tolve et al. 2018). Peroxide value (PV) was measured according to the official method (AOCS, 1989, Cd 8–53 of the American Oil Chemists' Society). Extracted oil was mixed with chloroform: acetic acid (2:3, v/v) and a saturated solution of potassium iodide. Titration was performed with 0.01 N sodium thiosulfate, and the PV was expressed in meq O_2/kg.

Statistical analysis of the experimental data was conducted using MATLAB (Version R2023a, The MathWorks, Inc., Natick, MA, USA). The technological properties of curd and Mozzarella were evaluated using linear mixed-effects (LME) models to assess the influence of fixed effects, including milk type, storage time, packaging treatment, and their interactions. Analysis of variance (ANOVA) was performed, followed by Tukey’s Honest Significant Difference (HSD) test to identify significant differences ($p < 0.05$). To explore the relationships between chemical markers and rheological attributes, Principal Component Analysis (PCA) was also performed.

4.2 Results

The results are presented in two subsections, categorized by the product type: the first part focuses on the characterization of the curd, while the second part details the findings related to the Mozzarella cheese.

4.2.1 Curd

Curd Freezing Rate was calculated using the formula

$$FR = \frac{T2 - T1}{t2 - t1}$$

where T1 is the initial freezing temperature, T2 is the final freezing point, and $(t2 - t1)$ is the time elapsed between the start and end of freezing. Figure 5 shows the freezing graph for curds obtained from RM and PM, showing that FR1 is -0.27 ± 0.005 °C/min, while FR2 is 0.32 ± 0.05 °C/min. FR1 was lower than FR2; therefore, curds obtained from raw milk froze more slowly than those obtained from pasteurized milk.

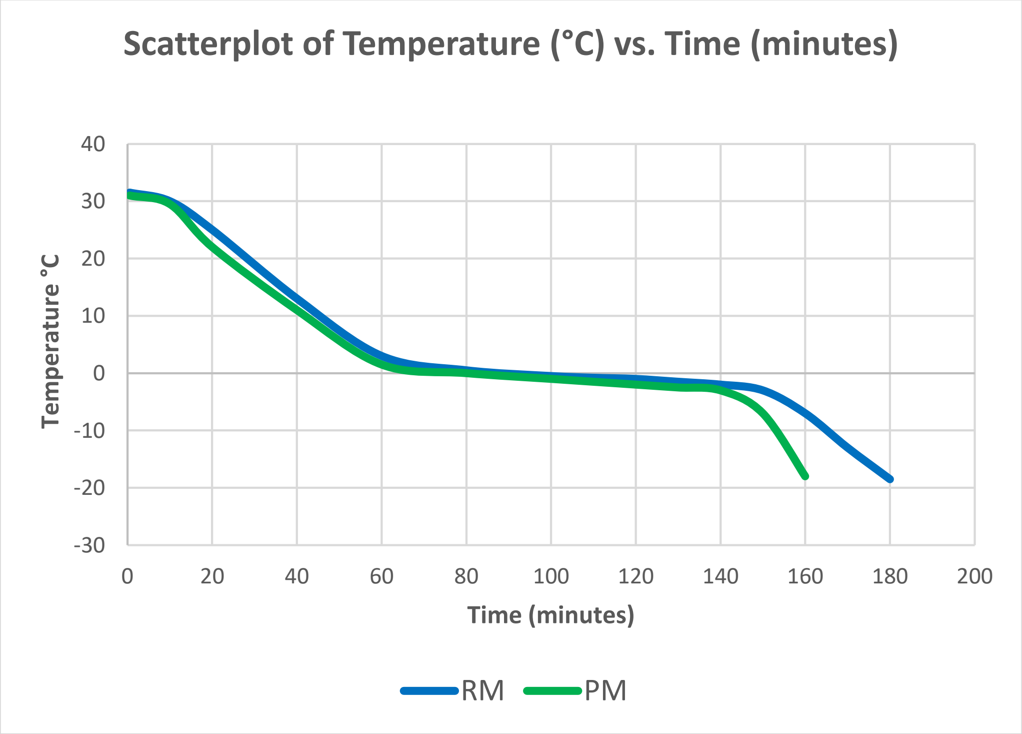
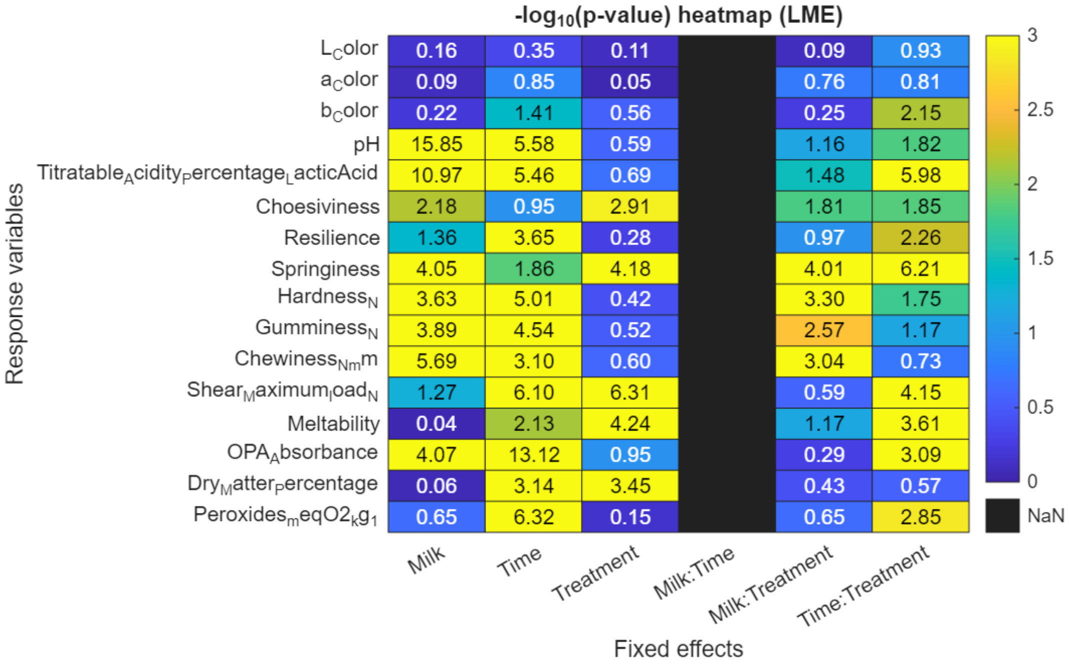


Figure 5: Freezing of curds obtained from raw milk (RM) and pasteurized milk (PM).

Analysis of variance applied to linear mixed-effects (LME) models showed that both chemical and rheological parameters were significantly influenced by the main factors (Milk, Time, Treatment) and their interactions (Figure 6). Specifically, storage time was the most influential factor significantly impacting pH, acidity, OPA, and most rheological parameters. Milk type significantly affected acidification dynamics (pH and acidity), the majority of the textural attributes, peroxide levels, and proteolysis (OPA). Additionally, significant interactions between

Time and Treatment were observed for acidity, springiness, shear strenght and OPA, suggesting that the choice of packaging significantly alters the rate of proteolysis and structural degradation over the 90-day period.



	Milk	Time	Treatment	Milk:Time	Milk:Treatment	Time:Treatment
$L_C$olor	0.16	0.35	0.11		0.09	0.93
$a_C$olor	0.09	0.85	0.05		0.76	0.81
$b_C$olor	0.22	1.41	0.56		0.25	2.15
pH	15.85	5.58	0.59		1.16	1.82
Titratable$_A$cidity$_P$ercentage$_L$acticAcid	10.97	5.46	0.69		1.48	5.98
Choesiviness	2.18	0.95	2.91		1.81	1.85
Resilience	1.36	3.65	0.28		0.97	2.26
Springiness	4.05	1.86	4.18		4.01	6.21
Hardness$_N$	3.63	5.01	0.42		3.30	1.75
Gumminess$_N$	3.89	4.54	0.52		2.57	1.17
Chewiness$_{Nm}$m	5.69	3.10	0.60		3.04	0.73
Shear$_M$aximum$_l$oad$_N$	1.27	6.10	6.31		0.59	4.15
Meltability	0.04	2.13	4.24		1.17	3.61
OPA$_A$bsorbance	4.07	13.12	0.95		0.29	3.09
Dry$_M$atter$_P$ercentage	0.06	3.14	3.45		0.43	0.57
Peroxides$_m$eqO2$_k$g$_1$	0.65	6.32	0.15		0.65	2.85

Figure 6: Heatmap illustrating the statistical significance of fixed effects (Milk, Time, Treatment) and their interactions on the chemical and rheological parameters of the curd. The values displayed in each cell represent the negative logarithm of the p-value (-log$_{10}$(p)). A value of 1.30 corresponds to a significance level of $p = 0.05$; higher values (moving towards yellow) indicate greater statistical significance. Black cells represent missing or non-calculable interaction terms (NaN).

Principal component analysis (PCA) was applied to explore multivariate relationships among chemical and rheological variables (Figure 7). The first two principal components explained 53.8% of the total variance, with PC1 accounting for 36.3% and PC2 for 17.5%.

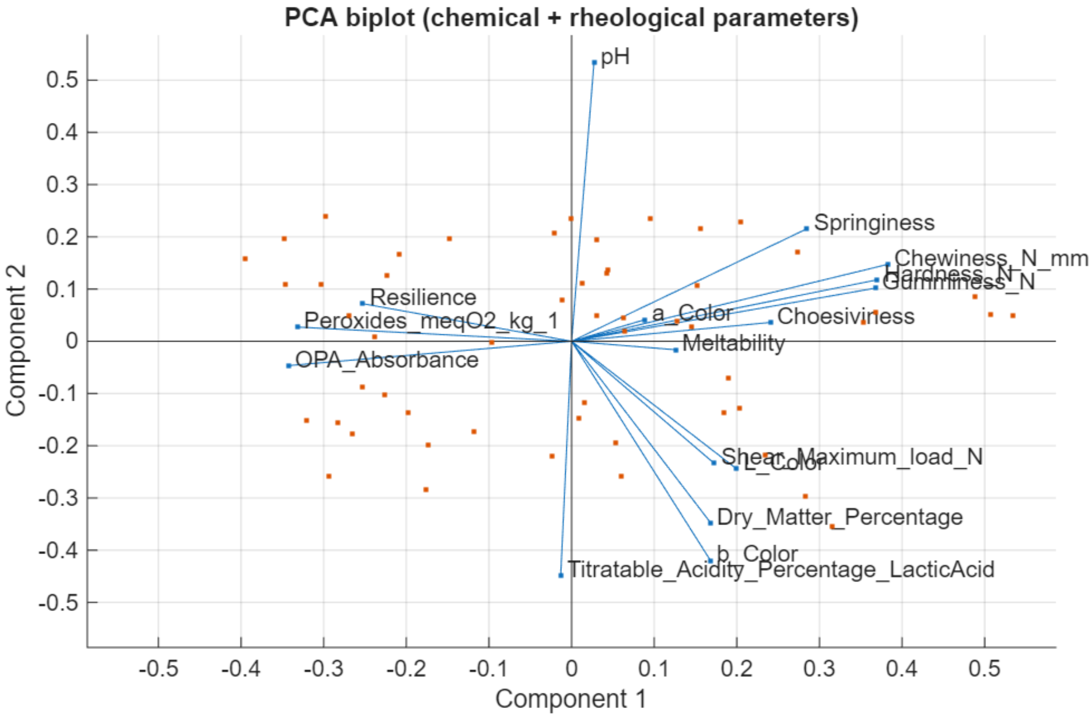


Figure 7: PCA biplot of chemical and rheological variables in curd samples. Blue vectors indicate the direction and magnitude of each parameter's contribution to the principal components, showing the relationship between specific variables and the sample clusters.

PC1 clearly discriminated samples according to storage duration, showing a progressive shift from 30 to 90 days (Figure 8). Storage time emerged as the dominant variable profoundly altering the product's structural integrity. At 30 days, the curd exhibits improved structural characteristics, while with increasing storage time, proteolysis and lipid oxidation deteriorate. This suggests that proteolysis breaks down the protein network, reducing mechanical compactness and resulting in greater softness or loss of structure.

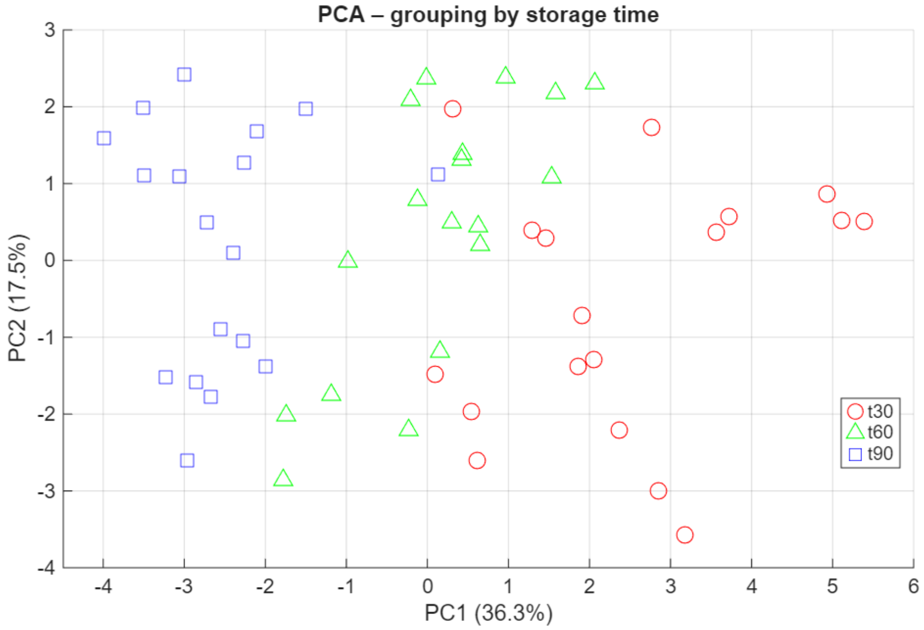


Figure 8: PCA score plot in curd samples grouped by storage time.

PCA grouped by packaging treatment (Figure 9) shows overlapping clusters, indicating that atmospheric treatment is not the primary driver of overall variance, which is instead dominated by storage time. However, LME analysis confirms that packaging significantly influences specific technological properties, such as meltability and springness structure.

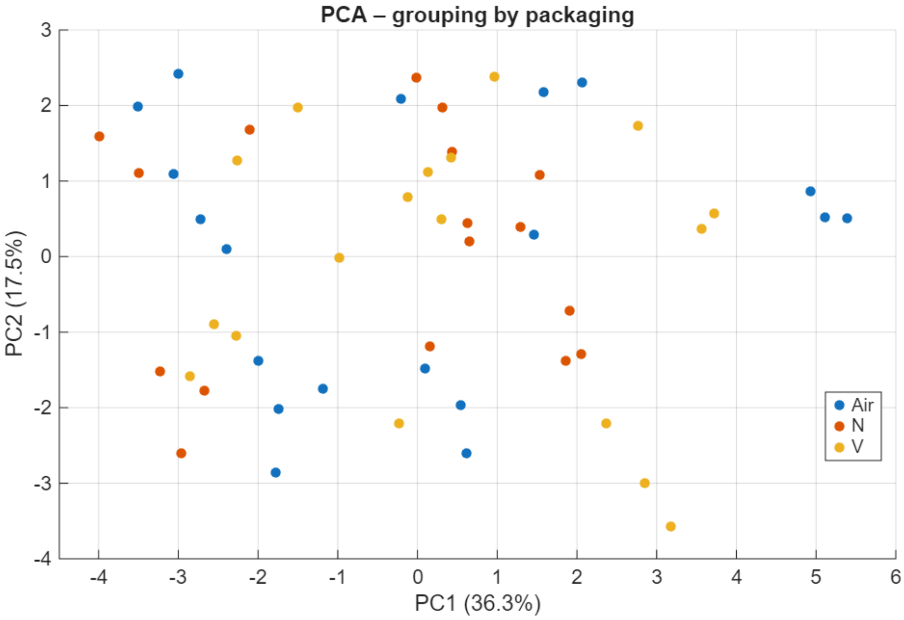


Figure 9: PCA score plot in curd samples grouped by packaging treatment.

As dry matter increases, hardness increases (Figure 10). However, the dispersion of data at t60 and t90 suggests that, after the first 30 days, the loss of hardness is probably not due to moisture alone, but to the enzymatic breakdown (proteolysis) of caseins, as confirmed by the increase in OPA values over time.

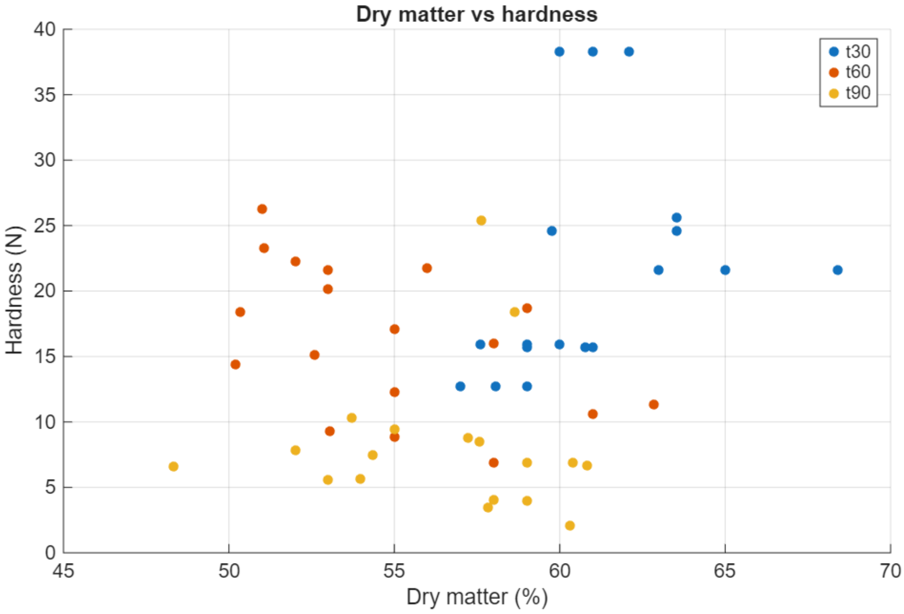


Figure 10: Relationship between Dry Matter and Hardness in curd samples. Scatter plot correlating the percentage of dry matter with mechanical hardness (N), grouped by storage interval (t30, t60, t90).

The lipid degradation appeared to influence technological properties, particularly meltability. Figure 11 demonstrates the impact of lipid degradation processes on the technological melting properties of the product under different storage atmospheres.

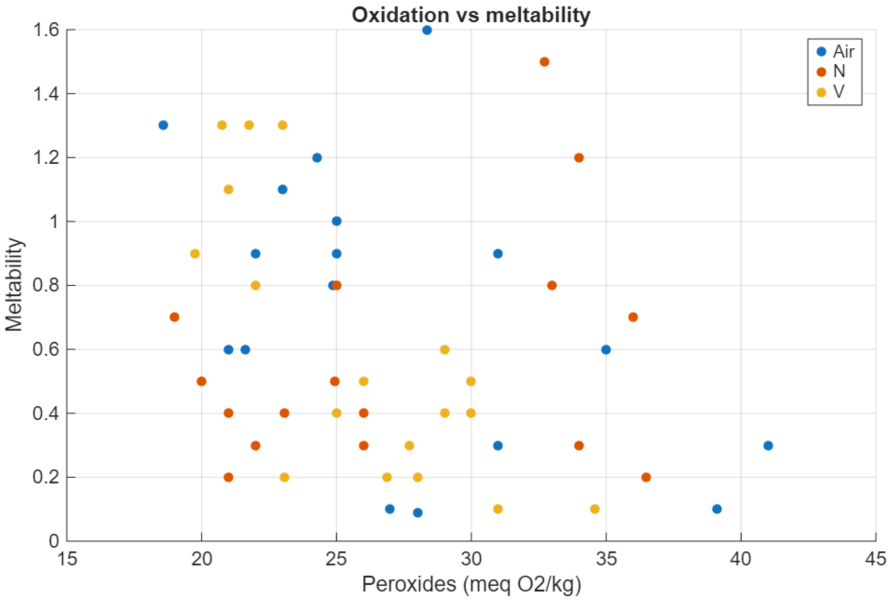


Figure 11: Scatter plot relating peroxide values to the curd's meltability, grouped by packaging treatment.

Univariate analysis of the most significant parameters confirmed the influence of storage time on curd quality. Hardness remained relatively stable between 30 and 60 days but decreased significantly after 90 days (Figure 12).

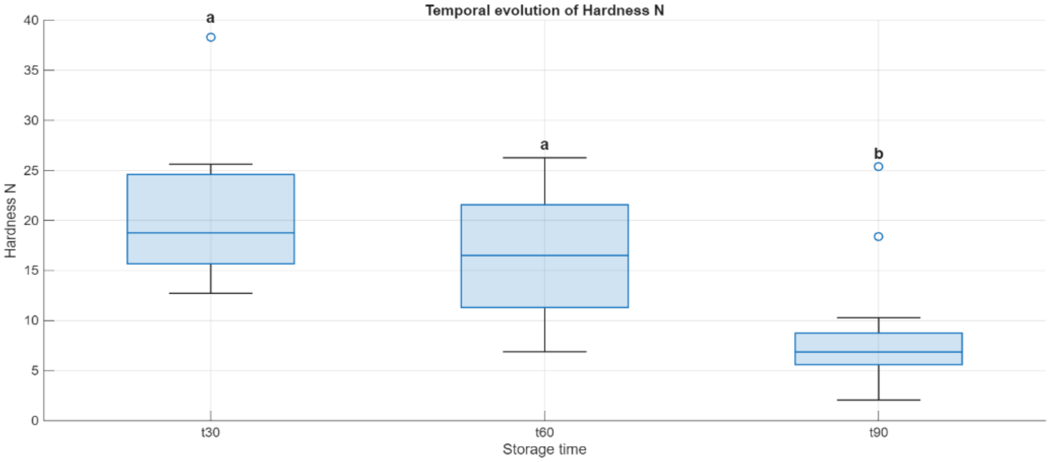


Figure 12: Temporal evolution of Hardness in curd samples. Different letters (a-b) indicate significant differences ($p < 0.05$).

This structural reduction was mirrored by the dry matter percentage, which dropped significantly after the first 30 days (Figure 13).

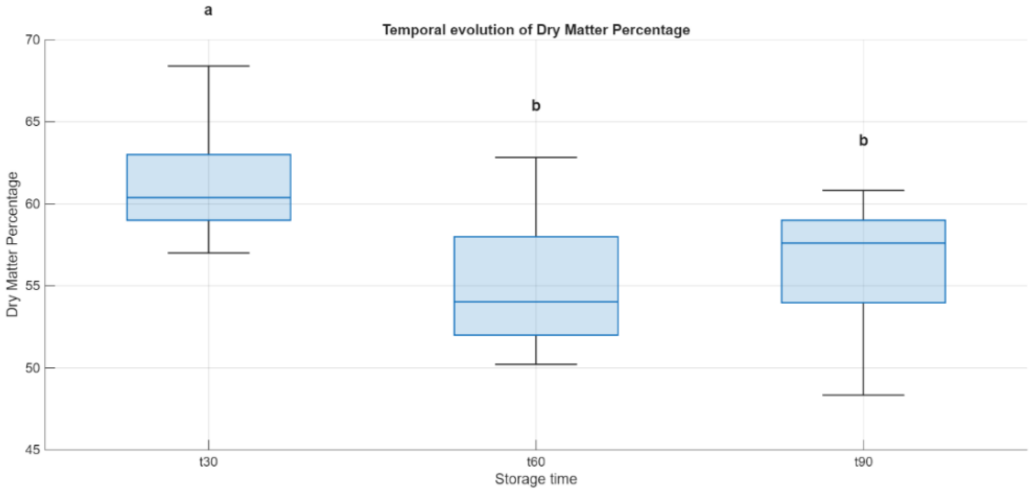


Figure 13: Temporal evolution of Dry Matter Percentage in curd samples. Different letters (a-b) indicate significant differences ($p < 0.05$).

Peroxide levels showed a consistent and significant increase throughout the entire observation period, reaching their maximum at t90 (Figure 14).

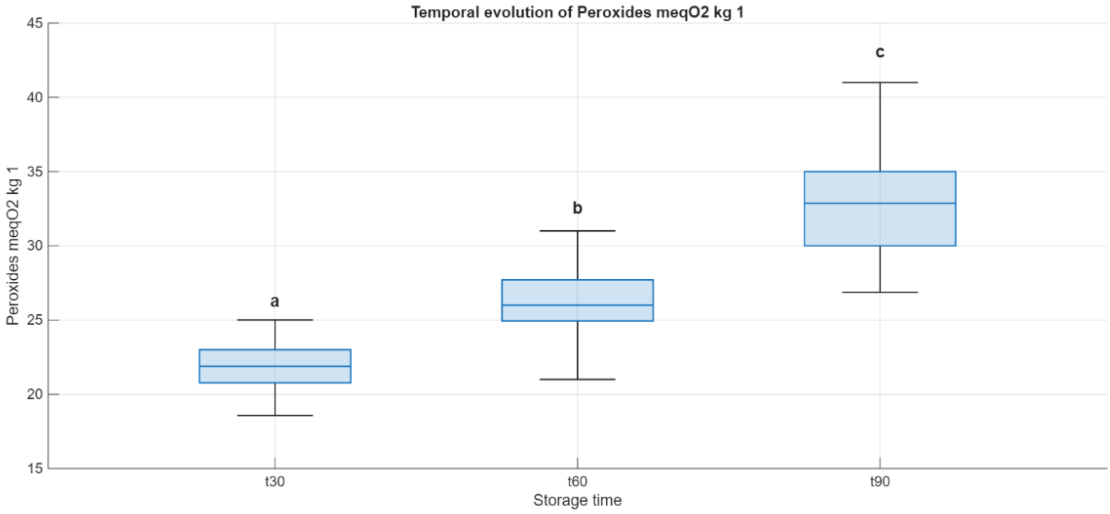


Figure 14: Temporal evolution of Peroxides in curd samples. Different letters (a-c) indicate significant differences ($p < 0.05$).

A similar trend was observed for proteolysis (OPA), which increased significantly at each sampling point, indicating continuous proteolysis during storage (Figure 15).

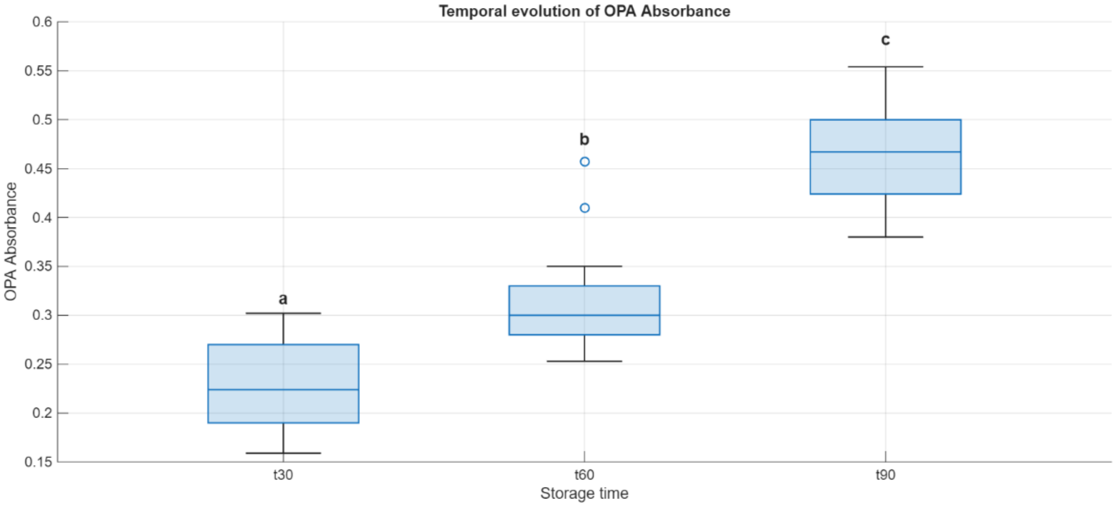


Figure 15: Temporal evolution of Proteolysis (OPA) in curd samples. Different letters (a-c) indicate significant differences (p < 0.05).

4.2.2 Mozzarella

Figure 16 shows a clear distinction in Mozzarella yield between the two milk type, obtained throughout the entire 90-day storage period. Pasteurized milk (PM) consistently achieved higher yields than raw milk (RM)type, starting at 85% compared to 70% on day 0. While RM showed a linear decline in yield, reaching a low of 62.35% on day 90, the PM group peaked at 86.32% on day 30, before declining and subsequently recovering to 81.21% at the end of the study.

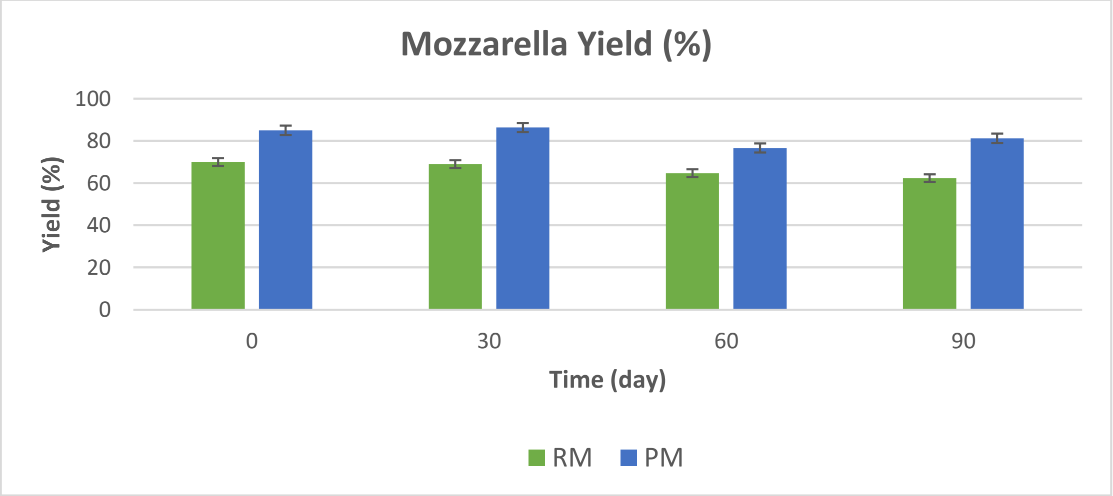


Figure 16: Evaluation of yield in Mozzarella as a function of milk and time storage [RM: Raw Milk; PM: Pasteurized milk].

Pasteurization treatment positively affects cheese yield, as evidenced in studies on Cheddar (Lau et al. 1990; San Martín-González et al. 2007), Egyptian soft Domiati cheese (Salwa et al. 2002), spreadable goat cheese (Frau et al. 2014) and Mexican pasta filata cheese (Morales-Celaya et al. 2012), while ultra-heat treatments (denaturing up to 35% of whey proteins) have been shown to increase Mozzarella yield by 3.4% (Schafer and Olson 1975). This enhancement, which can range from 0.1 to 0.4 kg (Lau et al., 1990), is probably linked to higher moisture levels within the cheese matrix (Tadjine et al. 2019) and the heat-induced denaturation of whey proteins. Specifically, intense thermal treatments promote the formation of complexes between denatured whey proteins and micellar κ-casein (Frau et al. 2014). These interactions modify the surface characteristics of casein micelles, leading to extended coagulation times and the development of a curd with superior water-holding capacity due to the hydrophilic nature of the incorporated whey fractions (Dalgleish 1990; Singh and Waungana 2001). Nevertheless, further studies are needed to study microbiological quality and assess whether heat treatment affects other components of milk or changes in protein structures.

In Figure 17, yields are also discriminated by treatment. Storage time influences yields, which decrease after just 30 days of storage.

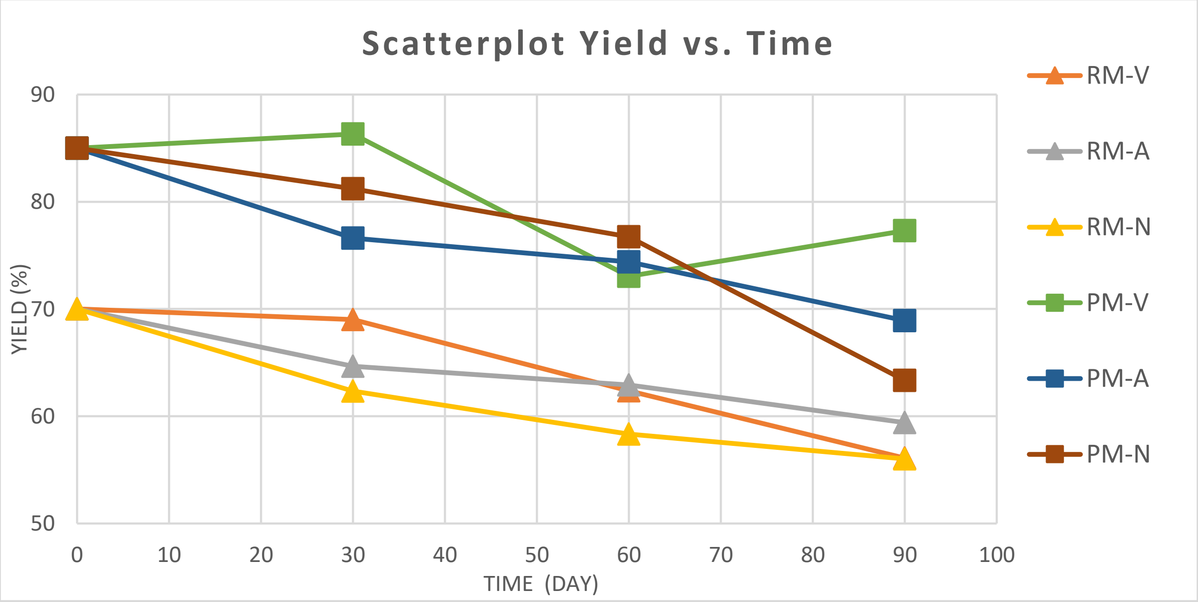
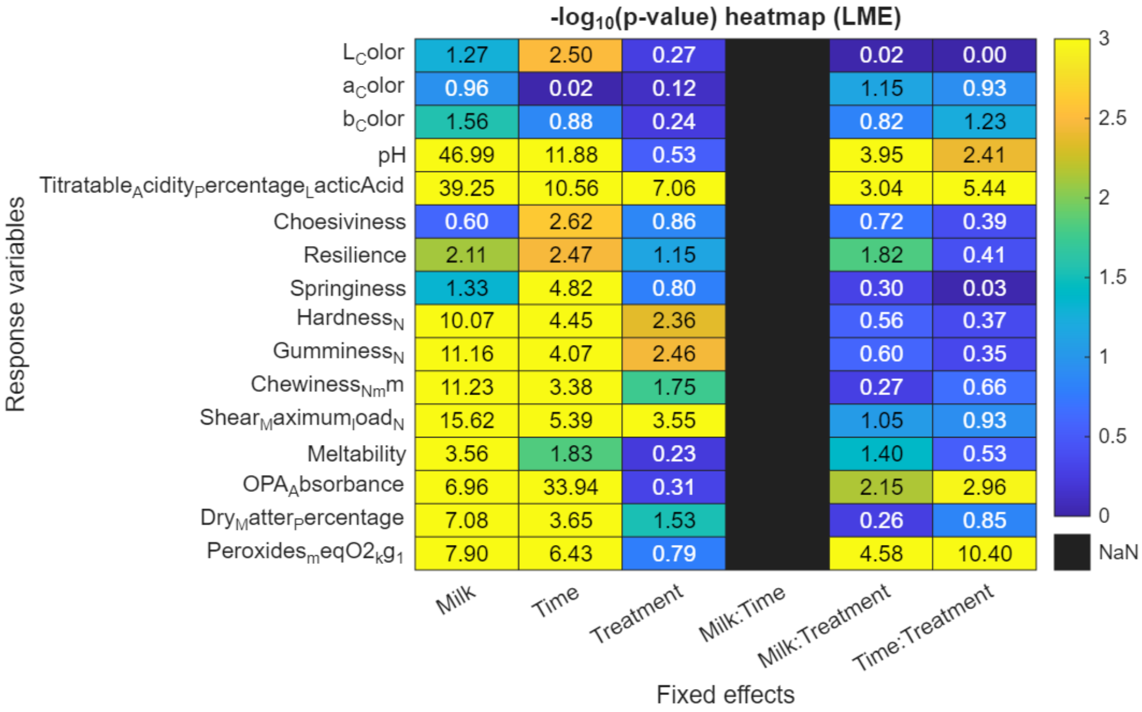


Figure 17: Scatterplot of the relationship between yield and time, as a function of milk type and treatment. [RM: Raw Milk; PM: Pasteurized milk; MAP packaging - V: Vacuum; A: Air; N: Nitrogen.]

Data indicates that pasteurized milk results in higher Mozzarella yields compared to raw milk, though heat treatment is not the only contributing factor. The different production technologies (Figure 3) also report the method of curd acidification: direct acidification by chemical means versus indirect acidification by microbiological processes. The influence of milk heat treatment, as indicated by Morales-Celaya et al. (2012) probably has a greater impact than the type of acidification. However, in processing with indirect acidification, , the longer period during which the curd undergoes demineralization and the greater amount of rennet used (acting on k-casein) may contribute to higher moisture retention, which can positively affect yield.

Linear mixed-effects (LME) modeling applied to Mozzarella samples revealed that almost all chemical and rheological parameters are profoundly influenced by the main factors Milk and Time, and not by their iterations, even considering the Treatment factor (Figure 18).



-$\log_{10}$(p-value) heatmap (LME)

Response variables	Milk	Time	Treatment	Milk:Time	Milk:Treatment	Time:Treatment
L$_C$olor	1.27	2.50	0.27		0.02	0.00
a$_C$olor	0.96	0.02	0.12		1.15	0.93
b$_C$olor	1.56	0.88	0.24		0.82	1.23
pH	46.99	11.88	0.53		3.95	2.41
Titratable$_A$cidity$_P$ercentage$_L$acticAcid	39.25	10.56	7.06		3.04	5.44
Choesiviness	0.60	2.62	0.86		0.72	0.39
Resilience	2.11	2.47	1.15		1.82	0.41
Springiness	1.33	4.82	0.80		0.30	0.03
Hardness$_N$	10.07	4.45	2.36		0.56	0.37
Gumminess$_N$	11.16	4.07	2.46		0.60	0.35
Chewiness$_{Nm}$m	11.23	3.38	1.75		0.27	0.66
Shear$_M$aximum$_l$oad$_N$	15.62	5.39	3.55		1.05	0.93
Meltability	3.56	1.83	0.23		1.40	0.53
OPA$_A$bsorbance	6.96	33.94	0.31		2.15	2.96
Dry$_M$atter$_P$ercentage	7.08	3.65	1.53		0.26	0.85
Peroxides$_m$eqO2$_k$g$_1$	7.90	6.43	0.79		4.58	10.40

Figure 18: Heatmap illustrating the statistical significance of fixed effects (Milk, Time, Treatment) and their interactions on the chemical and rheological parameters of the Mozzarella. The values displayed in each cell represent the negative logarithm of the p-value (-log10(p)). A value of 1.30 corresponds to a significance level of p = 0.05; higher values (moving towards yellow) indicate greater statistical significance. Black cells represent missing or non-calculable interaction terms (NaN).

Milk type exerted a critical effect, greater than that observed for curd, especially on acidification dynamics (pH and titratable acidity; Figure 19) and on mechanical structure, particularly on shear maximum load and gumminess, chewiness, and hardness.

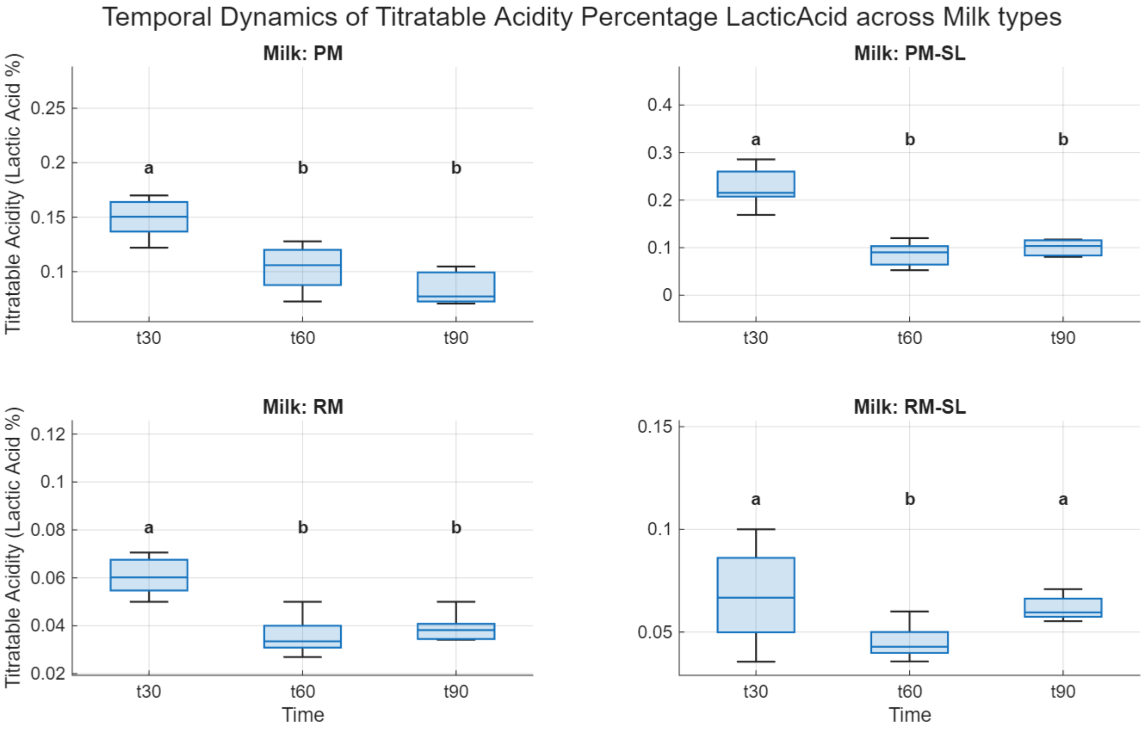


Figure 19: Temporal evolution of Tritable Acidity (Lactic Acid %) in Mozzarella samples as a function of Milk type. Different letters (a-c) indicate significant differences ($p < 0.05$).

A reduction in pH values was observed in all RM and PM Mozzarella samples, as well as in PM-SL Mozzarella, analyzed after 6 days of shelf life. Samples derived from pasteurized milk (PM and PM-SL) showed significantly higher acidity levels (up to 0.3-0.4%) than raw milk samples (RM and RM-SL), which generally remained below 0.1%. Between 60 and 90 days, acidity tended to stabilize, except in the case of RM-SL.

Principal component analysis (PCA) was applied to explore multivariate relationships among chemical and rheological variables (Figure 20). The first two principal components explained 46% of the total variance, with PC1 accounting for 30.2% and PC2 for 15.8%.

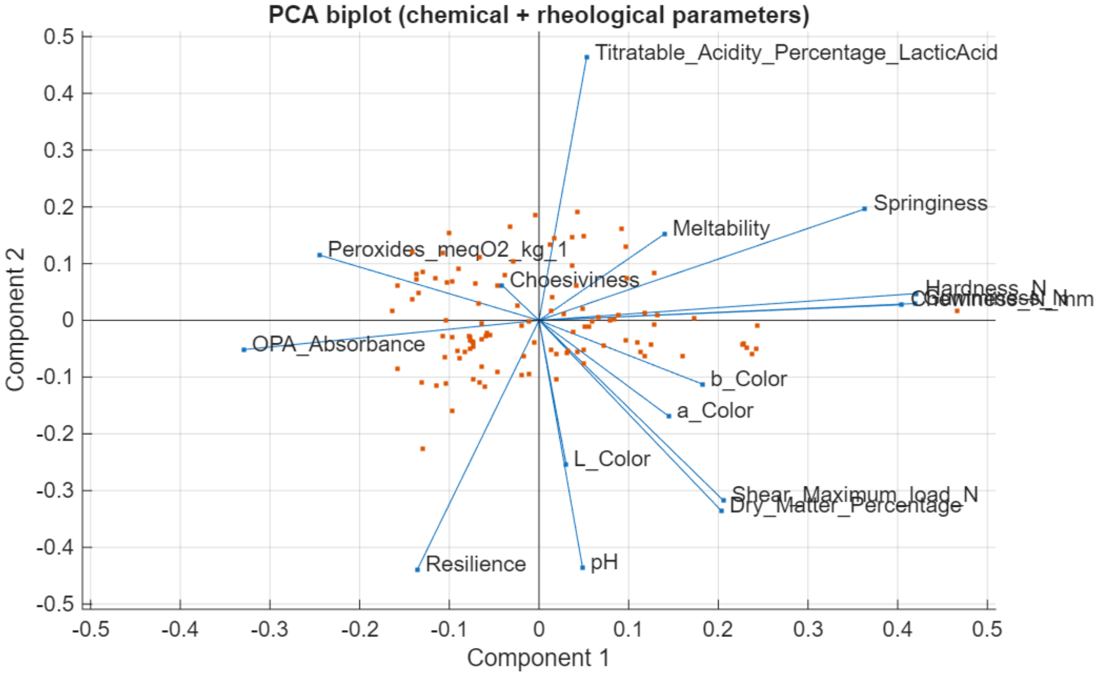


Figure 20: PCA biplot of chemical and rheological variables in Mozzarella samples. Blue vectors indicate the direction and magnitude of each parameter's contribution to the principal components, showing the relationship between specific variables and the sample clusters.

The most impact of Time (Figure 21) was visible on OPA values, indicating high proteolysis over the 90 days, in all RM and PM Mozzarella samples, as well as in RM-SL and PM-SL Mozzarella, analyzed after 6 days of shelf life (Figure 22).

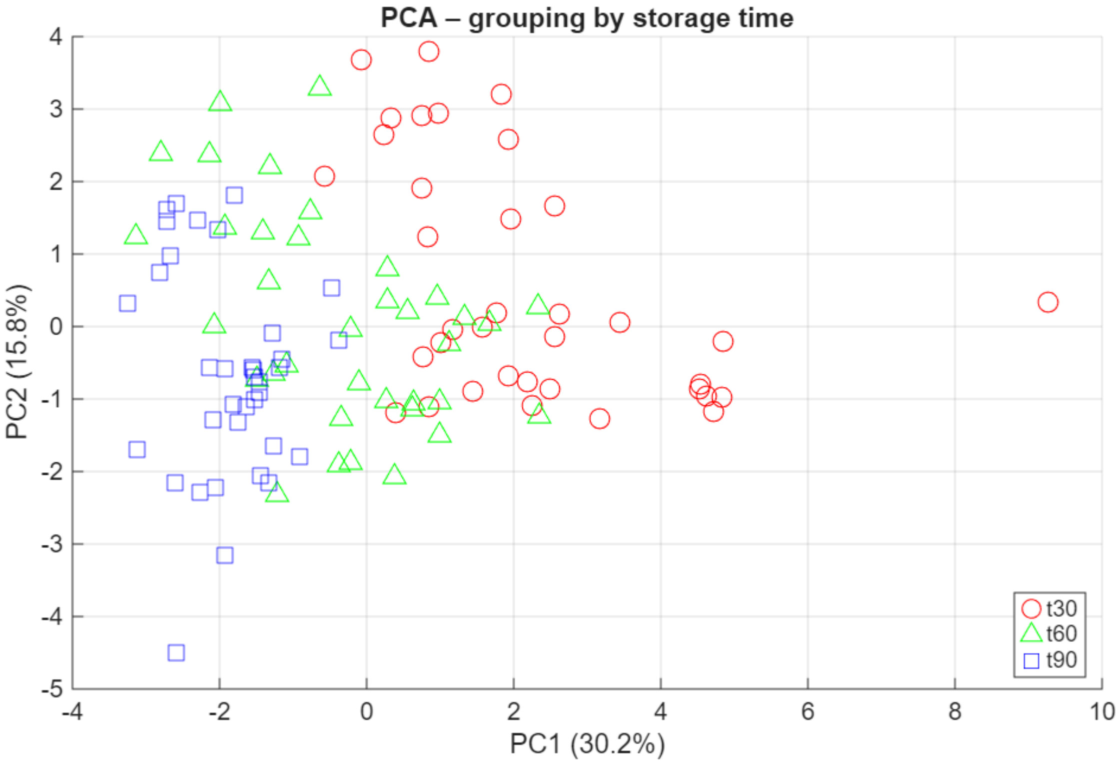


Figure 21: PCA score plot in Mozzarella samples grouped by storage time.

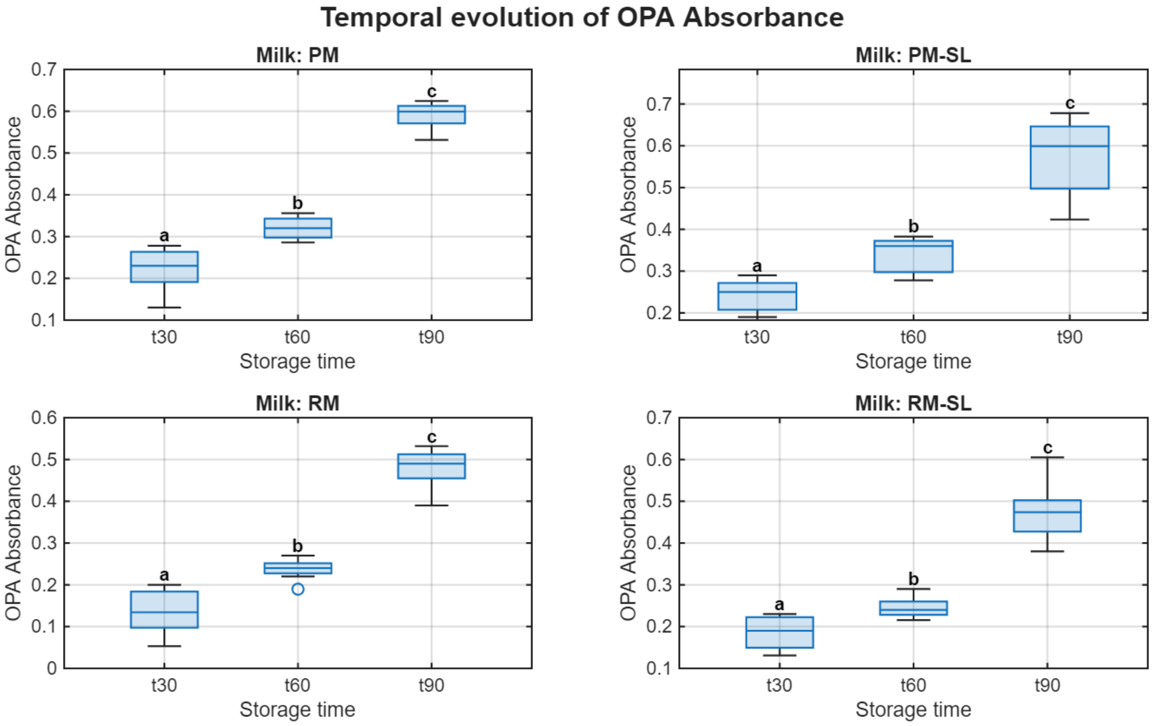


Figure 22: Temporal evolution of Proteolysis (OPA) in Mozzarella samples as a function of Milk type. Different letters (a-c) indicate significant differences ($p < 0.05$).

Unlike curd, the effect packaging was particularly evident on titratable acidity (Figure 18). However, the most significant aspect is the Time*Treatment interaction, which is extremely significant for Peroxides. This finding, supported by the dispersion in Figure 23, demonstrates that packaging choice could influence the rate of lipid oxidation during storage (Figure 24).

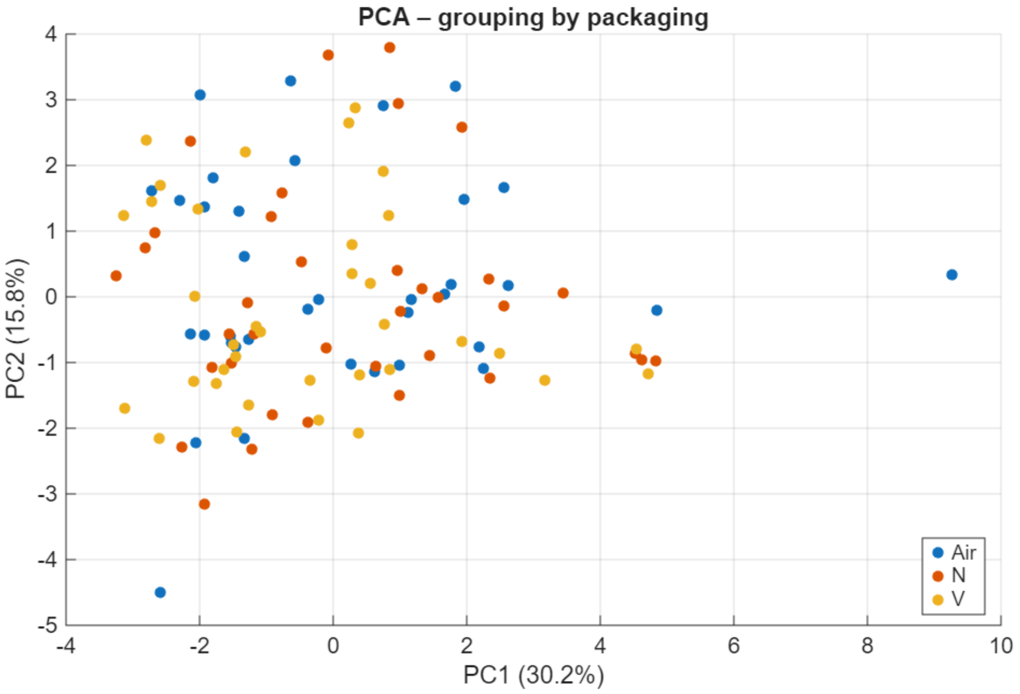


Figure 23: PCA score plot in Mozzarella samples grouped by packaging treatment.

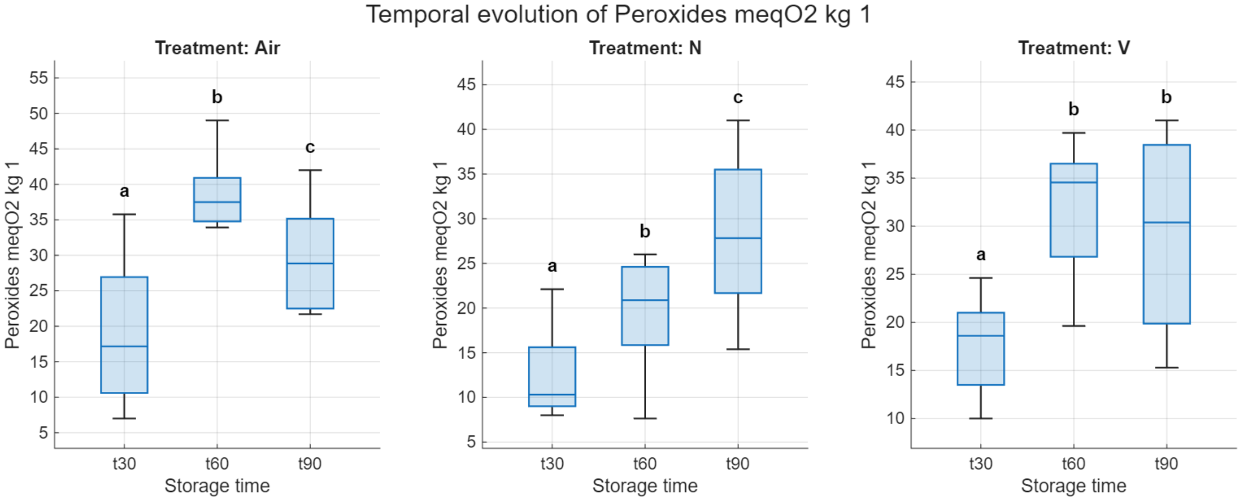


Figure 24: Temporal evolution of Peroxides value in Mozzarella samples as a function of treatment. Different letters (a-c) indicate significant differences (p < 0.05).

4.3 Conclusion

Curd results confirms that the physicochemical and rheological evolution of the curd is driven by a complex synergy between raw material properties and storage conditions. Storage duration (Time) emerged as the primary factor influencing structural decay, as evidenced by the progressive shift in PCA space from 30 to 90 days. This shift is characterized by a significant increase in proteolytic markers (OPA) and a concomitant loss of mechanical resistance (Hardness and Chewiness).

The application of Principal Component Analysis (PCA) successfully discriminated samples based on their maturation stage, highlighting that while Dry Matter contributes to initial firmness, the subsequent softening is largely mediated by enzymatic breakdown of the protein matrix. In summary, to preserve the optimal textural and technological profile of the curd, storage should not exceed 60 days, as the advanced stages of lipid oxidation and proteolysis beyond this threshold lead to an irreversible loss of product identity.

Similarly, the study on Mozzarella samples highlights that storage time remains the dominant driver of structural integrity, accounting for a significant portion of the total variance. However, in Mozzarella, the raw material (Milk type) exerts a more critical influence than in the curd, particularly regarding acidification dynamics and mechanical parameters. Specifically, pasteurized milk samples (PM and PM-SL) displayed a more intense acidification profile compared to raw milk variants. The choice of packaging (treatment) may be influenced by degradation processes. The significant interaction between time and treatment for peroxide levels indicates that protective packaging (vacuum or nitrogen) is essential to control the rate of lipid oxidation, which otherwise accelerates in samples packaged in air

Finally, the progressive decline in hardness and the stabilization of acidity after 60 days suggest that, much like the curd, Mozzarella reaches a technological tipping point at this stage. Beyond two months of storage, the cumulative effects of intense proteolysis and oxidative stress compromise the functional and rheological identity of the product, regardless of the milk base or packaging used.

5. LCA (Life Cycle Assessment) approach

The PhD project explored the effectiveness of curd freezing in stretched-curd cheese production, enabling better management of excess milk and semi-finished products. Initial experimental results show that this technology, especially when combined with appropriate packaging systems (vacuum, air, N_2), has significant effects on product yield and quality, and opens potential benefits in terms of sustainability and resource optimization. In particular, the technological properties of Mozzarella obtained from curds produced with raw cow milk and pasteurized cow milk, frozen and stored at -18°C in controlled atmospheres such as air, nitrogen and vacuum packaged in MAP (Modified Atmosphere Packaging), were studied and analyzed.

A Life Cycle Assessment (LCA) was conducted using pilot-scale experimental data to compare the various alternatives analyzed. The environmental impact was evaluated, considering the milk and the different coagulation methods, as well as the curd packaging system in the production of stretched curd cheeses, specifically Fiordilatte Mozzarella cheese.

This comparison will enable the objective quantification of the benefits and challenges in terms of resource savings and management efficiency, thereby providing a scientific basis for strategic and policy decisions aimed at promoting more sustainable practices in the dairy sector.

These results are expected to help enhance the environmental performance not only to small artisanal businesses, which often have more flexible and limited waste management, but also to medium-sized businesses or groups of small businesses that could benefit from sharing infrastructure and resources, resulting in cost reductions and greater operational efficiency.

5.1 Literature Background and Methodological Approaches

LCA represent a standardized international methodology for quantifying the environmental impacts of products and services throughout their entire life cycle (Nemecek et al. 2024). The environmental sustainability of the dairy sector has been extensively investigated by means of life cycle assessment (LCA), as this approach allows for the identification of the production phases with the greatest environmental impact and facilitates comparisons between different production systems. However, the current literature highlights a wide variability in environmental impacts, mainly due to the diversity of cheese varieties, manufacturing processes - including pasteurization, refrigeration and ripening - and methodological choices adopted (Silvério et al. 2025).

An essential step of conducting LCA investigations is the accurate definition of the Functional Unit (FU), which must be strictly aligned with scope and objectives of the research (Pérez et al. 2024). In the dairy context, the most prevalent units refers to a “kilogram of milk” (Nunes et al.

2020; Cabral et al. 2020; Silva et al. 2023; Alessandro Dalla Riva et al. 2015) - often corrected for fat and protein content (FPCM) (Rossi et al. 2023; Berlese et al. 2019; Bava et al. 2018; Pirlo et al. 2014) – and “kilogram of cheese” (Alessandro Dalla Riva et al. 2018; Vergé et al. 2013; Mondello et al. 2018; Santos et al. 2022; Tarighaleslami Amir Hossein et al. 2019), while more specific scenarios may also employ nutrient-based functional units (McLaren et al. 2021; Gislon et al. 2023), including nutritional indices (Kovanen et al. 2025) or micronutrient-based units (Saarinen et al. 2017).

Regarding system boundaries, the selected approach is equally critical. LCA studies may adopt a "cradle-to-farm-gate" perspective, the perspective when assessing milk production, encompassing all stages from cow feed production to milk packaging at the processing facility, or a more extensive perspective that also includes distribution of the final product. Alternatively, in the dairy sector, a “gate-to-gate” perspective may be adopted, whereby the analysis begins at the entry gate (i.e., the reception of raw materials or intermediate goods) and ends at the exit gate (i.e., completion of the production process) of a facility. Recent reviews focused on the environmental impacts derived from the dairy sector (Moja et al. 2025; Silvério et al. 2025) highlight a significant shift away from limited "gate-to-gate" assessments toward more comprehensive "cradle-to-grave" or "cradle-to-retail" analyses. This expansion of system boundaries reflects a growing recognition that a holistic environmental assessment requires consideration of all upstream processes and impacts across the entire product life cycle.

Table 3 provides a concise overview of the state of the art in LCA studies applied to cheese and milk production with a particular focus on works conducted in Italy. It summarizes each study by specifying the cheese variety, geographical context, and key methodological parameters, such as system boundaries and functional units.

Table 3. Overview of LCA studies on cheese and milk (period 2009-2024), with a particular focus on works conducted in Italy.

Dairy product	**Country**	**Functional Unit**	**System boundaries**	**Methodology**	**Software**	**Main results**	**Reference**
Mozzarella cheese	Denmark	1 ton of Mozzarella with fixed fat and protein content.	Cradle-to-grave	Eco-indicator 95 v.2.1 equivalency factors	SimaPro 7.1.8	Phospholipase is an enzyme which is able to increase the yield of cheese. The use of phospholipase in the production of Mozzarella and other pasta filata products reduces the potential contribution to global warming total annual CO_2 reduction potential is in the order of 7×10^8 kg CO_2 equivalents.	(Nielsen and Høier 2009)
Dairy products (raw milk, Cheddar, Mozzarella, butter, powdered milk)	Canada	1 kg of product	Integrated cradle-to-gate model (field to processing plant)	ULICEES model, based on the IPCC methodology (IPCC 2000, 2006)	SimaPro 7.3.2	The on-farm emissions are mainly related to farm practices. Off-farm emissions are mainly linked to the use of energy, primarily electricity. Carbon footprint: 0.9383-1.12 kg CO_2eq/L raw milk (depending on climatic conditions and herd management); 5.3 kg CO_2eq/kg cheese (Cheddar, Mozzarella, specialty cheese, and processed cheese); 7.3 kg CO_2eq/kg butter; 10.1 kg of CO_2eq/kg powdered milk.	(Vergé et al. 2013)
Cheddar and Mozzarella cheeses and Whey	USA	1 ton of cheddar cheese 1 ton of Mozzarella cheese 1 ton of dry whey	Cradle-to-grave; Farm gate-to-gate	ReCiPe Midpoint, ReCiPe Endpoint, IPCC GWP 100a, Cumulative Energy Demand, USEtox	SimaPro 7.3	Raw milk is the major contributor to most impact categories. Climate change and cumulative energy demand impacts are closely linked to fossil fuel consumption. Carbon Footprint: Cheddar: 8.60 kg CO_2e/kg cheese consumed; Mozzarella: 7.28 kg CO_2e/kg Mozzarella consumed Energy consumption: Mozzarella 45.4 MJ/kg Cheddar 48.5 MJ/kg	(Kim et al. 2013)
Buffalo milk	Italy	1 kg of FPCM	Cradle-to-farm gate	LatteGHG calculation tool developed to estimate the carbon footprint of dairy cattle milk in typical Italian dairy systems	Electronic worksheet	Greater emissions were due to on-farm activities (enteric fermentation and the animal feed). Carbon footprint: 3.75 kg CO_2eq/1 kg of FPCM	(Pirlo et al. 2014)

Raw cow milk	Italy	1 kg of raw milk	Cradle-to-dairy farm gate	ReCiPe Midpoint (H) v1.11, Cumulative Fossil Energy Demand	SimaPro 8	Purchased feed production was the largest source of emissions, followed by agricultural crop production, livestock, and manure management. Climate Change: 1.80-2.19 kg CO_2eq/kg raw milk; Cumulative Fossil Energy Demand: 8.84-10.78 MJ/kg raw milk.	(Dalla Riva et al. 2015)
Mozzarella cheese	Italy	1 kg of high-moisture (HM) Mozzarella	Cradle-to-grave	ReCiPe Midpoint (H) Cumulative Energy Demand	SimaPro 8.1	Animal feed production and raw milk production were hot spots for all impact categories except ozone depletion. Mozzarella produced from purchased curd had larger effects than Mozzarella produced directly from raw milk, due to the transportation and additional operations required to transform the curd into Mozzarella. Climate change Mozzarella: 6.6 kg CO_2e/kg Mozzarella (HM) consumed; 45.1 MJ cumulative energy demand/kg	(Dalla Riva et al. 2017)
Mozzarella cheese and whey	Italy	123 g of Mozzarella cheese made from 1 L of cow milk.	Cradle-to processing gate boundary (dairy gate)	CMLe IA baseline (v. 3.01/EU 25) method	Not specified	Raw milk production is the greatest impact phase along the supply chain. Three cow diets were compared: A diet, with traditional feeding hay and no liquid whey; the B diet, with silages but no liquid whey; the C diet, including both silages and liquid whey. Recycling the whey can have environmental benefits.	(Palmieri et al. 2017)
Grana Padano cheese	Italy	1 kg of FPCM; 1 kg of PDO Grana Padano cheese 12-month ripened without packaging	Cradle-to-cheese factory gate	ILCD 2011 Midpoint V1.03.	Not specified	Milk production was responsible of more than 93% of environmental impacts of cheese. Excluding milk production from the system boundary, milk transport and use of electricity were the main responsible of the environmental impact of cheese-making process. The values obtained from climate change category were 1.46 kg CO_2 eq/ kg FPCM and 10.3 kg of CO_2 eq/kg cheese.	(Bava et al. 2018)
Two artisanal cheeses (“Franxón” and PDO “Casín”)	Spain	1 kg of cheese	Cradle-to-retail store	Greenhouse Gas Protocol V1.01 ReCiPe Midpoint (H) V1.12	SimaPro 8.0	Raw milk was the most relevant source of environmental impacts in all the categories. Energy consumption and boiler emissions in cheese factories significantly impact ecotoxicity and ionising radiation categories. However, repurposing whey as animal feed effectively offsets environmental burdens. Carbon footprint:	(Canellada et al. 2018)

						10.2 kg CO_2 eq/ kg of cheese In the system analysed, the carbon footprint is reduced in 1.7 kg CO_2 eq/ kg cheese on behalf of the use of whey to feed pigs.	
Pecorino Toscano PDO	Italy	1 kg of cheese,	Cradle-to-gate	ReCiPe Midpoint (H)	SimaPro 8.0.2	The environmental hot-spots are mainly connected to direct emissions from the sheep enteric fermentation and to the production of feed used in sheep breeding, as well as, to waste water treatment and electricity consumption during cheese production. Climate Change 22.13 kg of CO_2eq/kg cheese	(Mondello et al. 2018)
Dairy industry	New Zealand	1 kg cheese	Cradle-to-grave.	ReCiPe 2014 Endpoint (H) Cumulative Exergy Demand	OpenLCA 1.7.4	Integrating one-third biomass into the energy mix significantly reduces the environmental footprint of dairy production. Cumulative Exergy Demand (CExD) of Cheese production process: Natural gas based scenario 3.85×10^{-1} MJ-Eq; Coal 4.73×10^{-4} MJ-Eq; Biomass based scenario 1.05×10^{-5} MJ-Eq.	(Tarighaleslami et al. 2019)
Beira Baixa PDO, Portugal	Portugal	1 kg raw sheep milk; 1 kg of cheese	Cradle-to-gate	ReCiPe Midpoint 2008	GaBi	Greatest impacts occur within the milk production process (fodder cultivation to produce animal feed, enteric fermentation and manure management). Climate Change 14,96 kg CO_2eq/kg cheese	(Nunes et al. 2020)
Goat cheese	Brazil	1 L of goat milk; 1 kg of goat cheese	Cradle-to-gate	International Reference Life Cycle Data System - ILCD 2011 Midpoint + V1.09/EC-JRC Global	SimaPro 8.3.	Milk production contributes most significantly to the environmental impacts (specifically soybean production, which is the main ingredient of goat feed). It is recommended to replace soy with hay and grass in goat feed and to use by-products such as whey to reduce environmental impact. 6.19 kg CO_2eq/kg cheese	(Cabral et al. 2020)

Milk and cheese employing two different systems (semi-confinement and pasture-based cows)	Spain	1 kg of FPCM 1 kg of cheese	Cradle-to-farm gate	Greenhouse Gas Protocol V1.01	SimaPro 8	Feed production and enteric emissions are the primary drivers of milk's carbon footprint. Pasture-based systems reduce this footprint by 18% for milk and 11% for cheese compared to semi-confined systems. Additionally, increasing meat co-production helps distribute and mitigate the overall environmental burden. Carbon Footprint: 1.22 and 0.99 kgCO_2eq / kg FPCM in the semi-confinement and the pasture-based dairy farms. 16.6 and 14.7 kgCO_2eq/ kg of cheese for milk produced in semi-confinement and pasture-based systems, respectively.	(Laca et al. 2020)
Organic Parmigiano Reggiano cheese,	Italy	1 kg of organic cheese	Gate-to-gate; Cradle-to-gate; Cradle-to-grave.	IPCC 2013, IPCC 2013 incl. CO2 uptake, ILCD 2011 Midpoint + ReCiPe 2016 AWARE	SimaPro 9.1.1	Agricultural and livestock farming have a significant impact on the environment. The application of different methods gives different impact results for Organic Parmigiano Reggiano cheese. Global Warming: 7.24 kg CO_2eq/kg cheese (IPCC 2013) Climate change: 4.15 kg CO_2eq/kg cheese (IPCC 2013 incl. CO2 uptake) Climate change 1.24 kg CO2eq/kg cheese (ILCD 2011) Global warming 8.12 kg CO2eq/kg cheese (ReCiPe 2016)	(Borghesi et al. 2022)
Minas frescal cheese and cured minas cheese	Brazil	1 L of milk; 1 kg of cheese	Cradle-to-gate	ILCD MidPoint	SimaPro 8.3	Greater emissions were due to on-farm activities (enteric fermentation and the animal feed). The cured Minas cheese presented higher impacts when compared to Minas Frescal cheese due to the larger volume of milk used in the manufacturing process. Four feed mix scenarios were evaluated to identify the dairy farm diet with the lowest environmental footprint.	(Silva et al. 2023)
Local Dairy Supply Chain: fresh raw milk, yogurt, fresh cheese, Mozzarella cheese, and aged cheese (2-3 months ripened).	Italy	1 kg of fresh raw milk, 1 kg of yogurt, 1 kg of fresh cheese, 1 kg of Mozzarella cheese 1 kg of aged cheese (2-3 months ripened).	Cradle-to-grave	ReCiPe Midpoint (H) IPCC GWP 100a	SimaPro 9.1.1	The greater emissions for each dairy product were due to on-farm activities, whereas the contribution of cheese making, selling, and consumption stages was significantly lower. Carbon Footprint were 4.39, 5.10, 9.82, 8.40, and 15.34 kg CO_2 eq. for fresh raw milk, yogurt, Mozzarella cheese, fresh cheese, and aged cheese, respectively.	(Rossi et al. 2024)

The table shows wide variability in results, mainly attributable to differences in farming practices, system boundaries, different cheese types and methodologies applied.

Based on the analyzed studies, the raw milk production phase consistently emerges as the primary environmental hotspot (Dalla Riva et al. 2017;Dalla Riva et al. 2018; Kim et al. 2013; Palmieri et al. 2017; Rossi et al. 2024, 2023; Vergé et al. 2013; Nunes et al. 2020; Canellada et al. 2018; Cabral et al. 2020; Santos et al. 2022; Silva et al. 2023; Borghesi et al. 2022; Pirlo et al. 2014; Palmieri et al. 2017), often accounting for over 90% of the total impact (Bava et al. 2018). Within the farm boundaries, the main drivers are enteric fermentation and animal feed production, particularly when relying on soybean (Cabral et al. 2020) or intensive crops. Regarding the industrial processing stage, the environmental burden is primarily linked to electricity (Vergé et al. 2013; Bava et al. 2018) and fossil fuel consumption for heating, refrigeration, and wastewater treatment (Dalla Riva et al. 2018; Mondello et al. 2018). Furthermore, products type and feeding management significantly influence the final environmental results. Mozzarella cheese generally has a lower carbon footprint (ranging from 6 to 15 kg CO_2e/kg (Dalla Riva et al. 2017; Santos et al. 2022)) than aged or hard cheeses (Rossi et al. 2024), such as Pecorino Toscano (Mondello et al. 2018), Grana Padano (Gislon et al. 2023) due to the energy-intensive nature of long-term maturation. Buffalo Mozzarella has a significantly higher impact (reaching up to 33.9 kg CO_2eq/kg) than cow Mozzarella, due to different biological efficiency and nutritional requirements of buffaloes compared to dairy cows (Berlese et al. 2019; Rossi et al. 2023). The transition from semi-confined to pasture-based systems can reduce the carbon footprint of milk by approximately 18% and that of the resulting cheese by 11% (Laca et al. 2020). Finally, the literature highlights effective mitigation strategies, such as the valorization of whey as animal feed to offset burdens (Canellada et al. 2018; Cabral et al. 2020; Santos et al. 2022; Palmieri et al. 2017) the use of specific enzymes to increase yield (Nielsen and Høier 2009) and the transition to biomass or renewable energy in processing plants (Tarighaleslami et al. 2019).

5.2 Methodology

In line with the most established approaches identified in the literature (Table 1), LCA was adopted as the core method to evaluate the environmental impacts associated with the different alternative processes used to produce “Fiordilatte” Mozzarella, as examined during the experimental phase of the PhD project.

The methodology adopted for this study complies with the ISO 14040 and 14044 series (ISO 2006a; ISO 2006b), which structure the LCA framework into four distinct phases:

I. Goal and scope definition;
II. Life cycle inventory (LCI) analysis;
III. Life cycle impact assessment (LCIA);
IV. Interpretation.

These documents provide a robust global framework for LCA but are designed to be versatile, enabling customization based on the specific context of the investigation.

5.2.1 Goal and Scope Definition

This study evaluates the environmental performance of employing different alternative processes (including the use of frozen curd and different coagulation and packaging methods) for producing Fiordilatte Mozzarella using a LCA methodology. All curd and Mozzarella cheeses were produced, packaged, and stored in strict accordance with the protocols and procedures already detailed in Chapter 4, section “Experimental design and research phases”, summarized in Figure 3. The objective is to identify critical points, determine the phases with the highest energy consumption, and propose improvement actions. Specifically, the LCA approach was applied to evaluate the environmental impacts associated with cheese production at pilot scale at the experimental dairy of the University of Basilicata, in southern Italy. The study established 1 kg of Mozzarella as the functional unit (FU). This approach enables a clear comparison of material and energy use, as well as associated environmental impacts, thereby facilitating the evaluation of alternative processes and potential technological improvements. A cradle-to-gate perspective was considered, including the following phases: milk production, milk transport and reception at the pilot plant, milk treatment (pasteurization), curd coagulation and formation, curd processing (cutting, salting, stretching), energy and water consumption during processing and Ricotta cheese formation. In addition, wastewater, solid waste and emissions generated were also considered in the LCA. The following operations were excluded: packaging for distribution and sale to the final consumer, since the Mozzarella produced was used exclusively for laboratory analyses. Based on the different milk coagulation methods and the different curd production and storage processes (Figure 4, Chapter 4, section 4.1.1.) a total of 20 scenarios were identified for analysis (Table 4).

Table 4: Overview of the analyzed Mozzarella production scenarios and their key parameters.

Scenario	Milk	Packaging	Storage time (day)
A: RM-t0	Raw Milk	No packaging	0
B: PM-t0	Pasteurized Milk	No packaging	0
C1: RM-V-t30	Raw Milk	MAP – Vacuum	30
C2: RM-A-t30	Raw Milk	MAP – Air	30
C3: RM-N-t30	Raw Milk	MAP – Nitrogen	30
D1: PM-V-t30	Pasteurized Milk	MAP – Vacuum	30
D2: PM-A-t30	Pasteurized Milk	MAP – Air	30
D3: PM-N-t30	Pasteurized Milk	MAP – Nitrogen	30
E1: RM-V-t60	Raw Milk	MAP – Vacuum	60
E2: RM-A-t60	Raw Milk	MAP – Air	60
E3: RM-N-t60	Raw Milk	MAP – Nitrogen	60
F1: PM-V-t60	Pasteurized Milk	MAP – Vacuum	60
F2: PM-A-t60	Pasteurized Milk	MAP – Air	60
F3: PM-N-t60	Pasteurized Milk	MAP – Nitrogen	60
G1: RM-V-t90	Raw Milk	MAP – Vacuum	90
G2: RM-A-t90	Raw Milk	MAP – Air	90
G3: RM-N-t90	Raw Milk	MAP – Nitrogen	90
H1: PM-V-t90	Pasteurized Milk	MAP – Vacuum	90
H2: PM-A-t90	Pasteurized Milk	MAP – Air	90
H3:PM-N-t90	Pasteurized Milk	MAP – Nitrogen	90

MAP: Modified Atmosphere Packaging.

5.2.2 Life cycle inventory (LCI) analysis

Inventory data, from real production processes conducted at pilot scale, were collected directly from the experimental dairy plant. Secondary data from literature were used to estimate inputs and outputs where direct measurement was not feasible, specifically regarding atmospheric emissions from natural gas boiler operation. Generic input processes and flow were obtained from Agribalyse v3.2 (Colomb et al. 2015), Ecoinvent v3 (Wernet et al. 2016), and Agri-Footprint 7.0 databases (Blonk et al. 2025). These databases are essential tools for assessing and improving environmental sustainability. Agribalyse and Agri-footprint in particular, specialize in agriculture and food supply chains, enabling the modeling and assessment of environmental impacts from primary production to the final consumer. Ecoinvent covers a wide range of sectors beyond agriculture, including energy supply, transportation, building materials, chemicals, and waste management.

Following the definition of system boundaries, inputs and outputs were identified (Table 5) and linked to the functional unit.

Table 5: Data sources and database used for the Life Cycle Inventory (LCI).

Parameter	**Unit of measure**	**Data source**	**Database/ Reference**
Raw Milk	kg	Dairy cow, Calf and Milk raw, at farm gate, {IT}, Mass.	Agri-Footprint 7.0
Milk Transport	tkm	Transport, Agri-Footprint.freight, lorry 3.5-7.5 metric ton, EURO5 RER.	Ecoinvent v3
Tap water	L	Tap water production, conventional treatment Europe without Switzerland.	Ecoinvent v3
Electrical energy	kWh	Electricity, low voltage {IT}| market for electricity, low voltage | Cut-off, S.	Ecoinvent v3
Heating system (Natural gas)	kg	Natural gas, from medium pressure network (0.1-1 bar), at service station {GLO}| market for | Conseq, S.	Ecoinvent v3
Minor ingredients: Lactic Acid	kg	Lactic acid {RER}| lactic acid production | Cut-off, S.	Ecoinvent v3
Minor ingredients: Salt	kg	Salt {GLO}| market for salt | Cut-off, S.	Ecoinvent v3
Cleaning Products: Alkaline detergent	kg	Sodium hydroxide, without water, in 50% solution state {RER}| market for sodium hydroxide, without water, in 50% solution state | Cut-off, S.	Ecoinvent v3
Cleaning Products: Hydrochloric acid	kg	Hydrochloric acid, without water, in 30% solution state {RER}| market for | APOS, S.	Ecoinvent v3
MAP-Gas (Nitrogen)	kg	Nitrogen, via cryogenic air separation, production mix, at plant, gaseous EU-27 S.	ELCD, JRC
MAP-Gas (Air)	m^3	Compressed air, 800 kPa gauge {RER}| compressed air production, 800 kPa gauge, >30kW, optimized generation | APOS, S.	Ecoinvent v3
Packaging materials: Polyethylene bags	kg	Polyethylene low density granulate (PE-LD), production mix, at plant RER.	ELCD, JRC
Packaging materials: Paper	kg	Rolls of paper towels, at plant {RER} S. Agribalyse v3.2	Agribalyse v3.2
Wastewater	L	Wastewater, average {Europe without Switzerland}| treatment of wastewater, average, wastewater treatment | Cut-off, S. Ecoinvent v3.	Ecoinvent v3
Organic Waste	kg	Municipal solid waste {IT}| treatment of municipal solid waste, sanitary landfill | Cut-off, S. Ecoinvent v3.	Ecoinvent v3
Plastic Waste	kg	Waste polyethylene, for recycling, sorted {Europe without Switzerland}| treatment of waste polyethylene, for recycling, unsorted, sorting_obsolete | Cut-off, S. Ecoinvent v3.	Ecoinvent v3
Gas natural emissions	kg	Carbon dioxide, Methane, Dinitrogen monoxide, Carbon monoxide	(IPCC, 2006; EMEP/EEA, 2023)

The LCA included all the inputs and outputs involved in Mozzarella cheese production organized in the following subsystems: raw materials, water, electricity, energy use, cleaning agents, packaging materials, transportation, and boiler gaseous emissions. Raw milk (at farm gate[1]) was

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[1] The dataset represents average Italian raw milk production (cradle-to-farm gate) within a mixed crop-livestock system. It includes fodder production, grazing management, and animal husbandry for dairy cows and replacements, with manure treated via a residual approach. The inventory accounts for all crop and livestock inputs (e.g., fertilizers, energy, feed, water, transport) and direct land use. Calculated emissions include enteric fermentation (CH_4), manure management, and nutrient losses to air, soil, and water (e.g., N_2O, NH_3, NO_3^-, heavy metals)."

delivered by the supplier to the experimental dairy, travelling approximately 40 km (transport freight lorry 3.5-7.5 metric ton, Euro 5). The impact of minor ingredients and cleaning products (e.g. lactic acid, salt, alkaline detergent, and hydrochloric acid) were included. However, other minor ingredients, such as starter cultures and rennet were excluded due to their negligible contribution in terms of contribution and the lack of specific inventory data. For packaging, two materials were considered: low-density polyethylene (LDPE) plastic bags and paper rolls. The dairy plant used electricity for lighting and equipment operation, and thermal energy generated by a natural gas boiler with an efficiency of 85%, providing hot water and steam for processing operations. Thermal energy demand was therefore covered by natural gas combustion, which represents the main source of direct CO_2 emissions at the dairy plant. Gaseous emissions were calculated following the Intergovernmental Panel on Climate Change (IPCC) guidelines for greenhouse gas (GHG) emissions. Emission factors for natural gas combustion, used to estimate the release of CO_2, CO, CH_4, and N_2O, are listed below:

- 56.100 kg CO_2/TJ (IPCC, 2006)[2];
- 5 kg CH_4/TJ (IPCC, 2006)[2];
- 0.1 kg N_2O/TJ (IPCC, 2006)[2];
- 24 kg CO/TJ (EMEP/EEA, 2023)[3]

In some scenarios, gas consumption for MAP (Modified Atmosphere Packaging) was included in the inventory, specifically for air and nitrogen packaging (both supplied as compressed gas in cylinders). Tap water consumed during the process was treated as effluent and discharged to a public wastewater treatment system. Plastic packaging materials were sorted for recycling, while organic waste was managed as municipal solid waste and sent to landfill. The whey obtained from the production of Mozzarella cheese was processed into Ricotta cheese, which was considered a co-product. The resulting spent whey fraction (also called “Scotta” or Secondary Whey) was recovered to feed pigs on a nearby farm; therefore, so it processing as waste was not included in the analysis.

The inventory data, as reported in Tables 6 and 7, were structured in accordance with the system boundaries previously defined and showed in Figure 25. This block flow diagram facilitates the visualization of the system boundaries for each individual scenario and enables the systematic identification of all relevant input and output flows.

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[2] Table 2.5 Default emission factors for stationary combustion in the residential and agriculture/forestry/fishing/fishing farm, kg of greenhouse gas per GJ on a Net Calorific Basis, 2006 IPCC Guidelines for National Greenhouse Gas Inventories

[3] Table 3-26 Tier 2 emission factors for non-residential sources, Agriculture/forestry/fishing, EMEP/EEA air pollutant emission inventory guidebook 2023

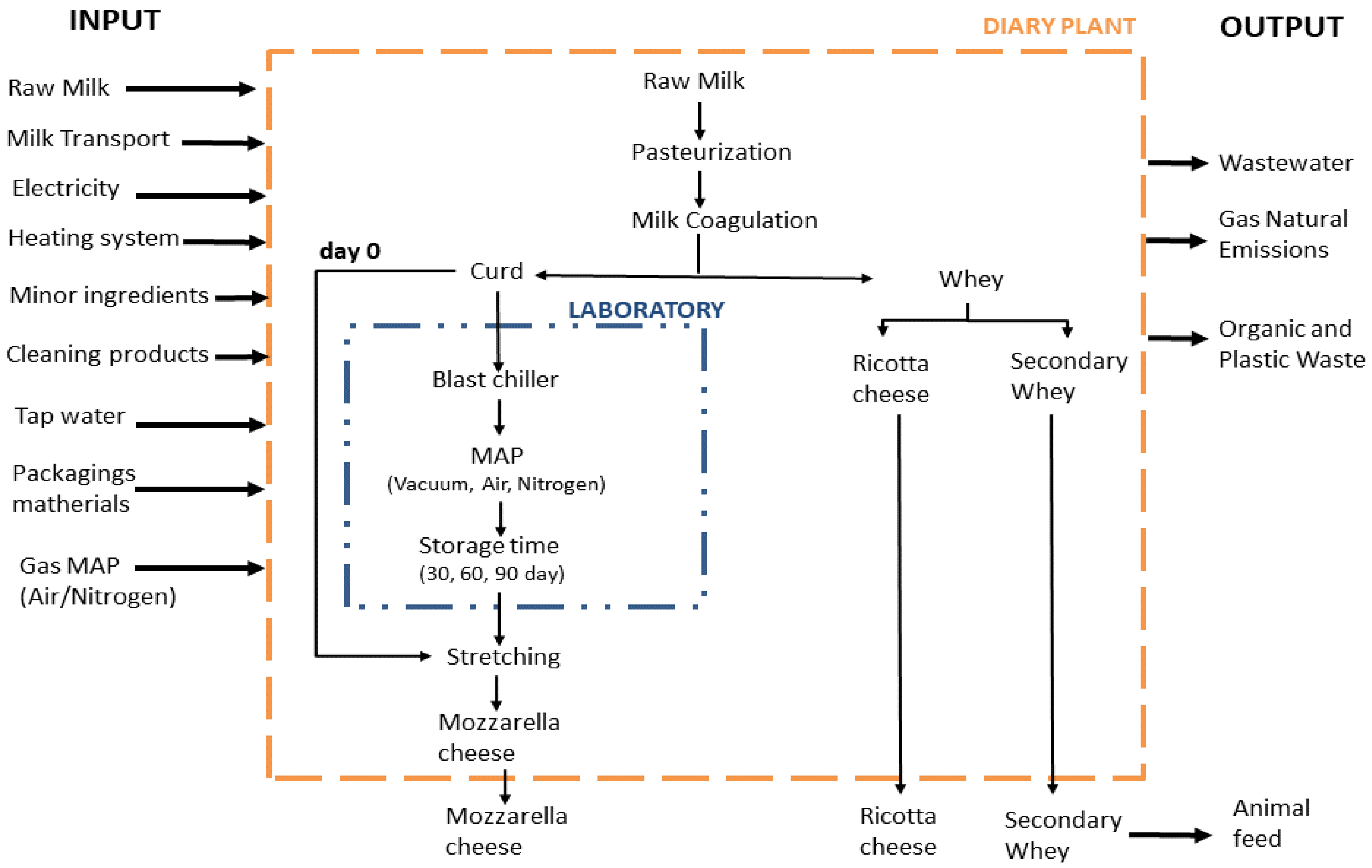

Figure 25: System boundaries.

Table 6: Inventory data of the experimental trials carried out employing raw milk expressed per functional unit (FU: 1 kg of Mozzarella cheese).

RM- M; Raw Milk Mozzarella		**SCENARIO**									
	Unit of measure	**A**	**C1**	**C2**	**C3**	**E.**	**E2**	**E3**	**G1**	**G2**	**G3**
Milk	kg	10.21863	10.21863	10.21863	10.21863	10.21863	10.21863	10.21863	10.21863	10.21863	10.21863
Milk Transport (40 km)	tkm	0.4087	0.4087	0.4087	0.4087	0.4087	0.4087	0.4087	0.4087	0.4087	0.4087
Tap water	kg	23.810	29.762	29.762	29.762	29.762	29.762	29.762	29.762	29.762	29.762
Electricity	kWh	0.2302	1.3519	1.3536	1.3519	1.3661	1.2748	1.3653	1.3774	1.3762	1.3704
Heating system (kg natural gas)	kg	0.1070	0.1451	0.1451	0.1451	0.1451	0.1451	0.1451	0.1451	0.1451	0.1451
Minor ingredients	kg	0.065	0.065	0.065	0.065	0.065	0.065	0.065	0.065	0.065	0.065
1. Lactic Acid	kg	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025
2. Salt (25 g per kg curd)	kg	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Cleaning products	kg	0.015	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
1. Alkaline detergent (NaOH 0,4%)	kg	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
2. Hydrochloric acid (HCl 0,5%)	kg	0.005	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Gas MAP (Nitrogen)	kg				0.009			0.009			0.009
Gas MAP (Air)	m3			0.007608			0.007608			0.007608	
Packaging materials		0.072	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09
1. Polyethylene bags	kg	0.022	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
2. Paper	kg	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Output											
Wastewater	kg	23.810	29.762	29.762	29.762	29.762	29.762	29.762	29.762	29.762	29.762
Organic and Plastic Waste	kg	0.072	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09
1. Plastic Waste	kg	0.022	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
2. Organic Waste	kg	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Natural Gas emissions (total)	kg	0.32257639	0.43756375	0.43756375	0.43756375	0.43756375	04.3756375	0.43756375	0.43756375	0.43756375	0.43756375

1. CO_2	kg	0.32240967	0.43752467	0.43752467	0.43752467	0.43752467	0.43752467	0.43752467	0.43752467	0.43752467	0.43752467
2. CH_4	kg	0.00002874	0.00003900	0.00003900	0.00003900	0.00003900	0.00003900	0.00003900	0.00003900	0.00003900	0.00003900
3. N_2O	kg	0.00000006	0.00000008	0.00000008	0.00000008	0.00000008	0.00000008	0.00000008	0.00000008	0.00000008	0.00000008
4. CO	kg	0.00013793	0.00018718	0.00018718	0.00018718	0.00018718	0.00018718	0.00018718	0.00018718	0.00018718	0.00018718
Electrical and thermal energy consumption	MJ	6.576	12.666	12.672	12.666	12.717	12.388	12.714	12.757	12.753	12.733
Secondary Whey	kg	8.3845	8.3845	8.3845	8.3845	8.3845	8.3845	8.3845	8.3845	8.3845	8.3845
Ricotta cheese (Co-product)	kg	0.211	0.211	0.211	0.211	0.211	0.211	0.211	0.211	0.211	0.211

Table 7:Inventory data of the experimental trials carried out employing pasteurized milk expressed per functional unit (FU: 1 kg of Mozzarella cheese).

PM- M; Pasteurized Milk Mozzarella	**Unit of measure**	**SCENARIO**									
		B	**D1**	**D2**	**D3**	**F1**	**F2**	**F3**	**H1**	**H2**	**H3**
Milk	L	8.80341	8.80341	8.80341	8.80341	8.80341	8.80341	8.80341	8.80341	8.80341	8.80341
Milk Transport (40 km)	tkm	0.3521364	0.3521364	0.3521364	0.3521364	0.3521364	0.3521364	0.3521364	0.3521364	0.3521364	0.3521364
Tap water	kg	20.513	27.350	27.350	27.350	27.350	27.350	27.350	27.350	27.350	27.350
Electricity	kWh	1.6581	4.0601	4.0613	4.0584	4.0653	4.0700	4.0683	4.0782	4.0806	4.0703
Heating system (kg natural gas)	kg	0.1586	0.2025	0.2025	0.2025	0.2025	0.2025	0.2025	0.2025	0.2025	0.2025
Minor Ingredients	kg										
1. Salt (25 g per kg curd)	kg	0.032	0.032	0.032	0.032	0.032	0.032	0.032	0.032	0.032	0.032
Cleaning Products	kg	0.013	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026
1. Alkaline detergent (NaOH, 0,4%)	kg	0.009	0.017	0.017	0.017	0.017	0.017	0.017	0.017	0.017	0.017
2. Hydrochloric acid (HCl 0,5%)	kg	0.004	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009
Gas MAP (Nitrogen)	kg				0.009			0.009			0.009

Gas MAP (Air)	m^3			0.00932			0.00932			0.00932	
Packaging materials	kg	0.062	0.077	0.077	0.077	0.077	0.077	0.077	0.077	0.077	0.077
1. Polyethylene bags	kg	0.019	0.034	0.034	0.034	0.034	0.034	0.034	0.034	0.034	0.034
2. Paper	kg	0.043	0.043	0.043	0.043	0.043	0.043	0.043	0.043	0.043	0.043
Output											
Wastewater	kg	20.513	27.350	27.350	27.350	27.350	27.350	27.350	27.350	27.350	27.350
Organic and Plastic Waste	kg	0.062	0.077	0.077	0.077	0.077	0.077	0.077	0.077	0.077	0.077
1. Plastic Waste	kg	0.019	0.034	0.034	0.034	0.034	0.034	0.034	0.034	0.034	0.034
2. Organic Waste	kg	0.043	0.043	0.043	0.043	0.043	0.043	0.043	0.043	0.043	0.043
Natural Gas emissions (total)	kg	0.52771512	0.66001816	0.66001816	0.66001816	0.66001816	0.66001816	0.66001816	0.66001816	0.66001816	0.66001816
1. CO_2	kg	0.52744238	0.65967705	0.65967705	0.65967705	0.65967705	0.65967705	0.65967705	0.65967705	0.65967705	0.65967705
2. CH_4	kg	0.00004701	0.00005879	0.00005879	0.00005879	0.00005879	0.00005879	0.00005879	0.00005879	0.00005879	0.00005879
3. N_2O	kg	0.00000008	0.00000010	0.00000010	0.00000010	0.00000010	0.00000010	0.00000010	0.00000010	0.00000010	0.00000010
4. CO	kg	0.00022564	0.00028221	0.00028221	0.00028221	0.00028221	0.00028221	0.00028221	0.00028221	0.00028221	0.00028221
Electrical and thermal energy consumption	MJ	15.371	26.357	26.379	26.369	26.394	26.411	26.405	26.440	26.449	26.412
Secondary Whey	kg	7.2232	7.2232	7.2232	7.2232	7.2232	7.2232	7.2232	7.2232	7.2232	7.2232
Ricotta Cheese (Co-product)	kg	0.182	0.182	0.182	0.182	0.182	0.182	0.182	0.182	0.182	0.182

5.2.3 Life cycle impact assessment (LCIA)

Impact assessment was performed with the LCA software SimaPro 10.2.0.3 (PRé Sustainability, 2025), using the ReCiPe 2016 Midpoint (H) V1.11 method.

The ReCiPe method, developed in 2008 by RIVM, CML, Pré Consultants, Radboud University Nijmegen, and CE Delft, combines the advantages of the CML 2001 and Eco-indicator 99 methods. The main objective of ReCiPe is to transform the extensive list of life cycle inventory results into a limited number of indicator scores. ReCiPe 2016 is an updated and extended version of ReCiPe 2008 and, like its predecessor, allows for the determination of indicators at two levels: Midpoint and Endpoint (Huijbregts et al. 2017). ReCiPe Midpoint (problem-oriented) calculates environmental impacts in 18 categories, while ReCiPe Endpoint (damage-oriented) estimates 3 indicators (Figure 26, Table 8), both available for three different perspectives:

- Individualist (I): based on short-term interest, on types of impact that are indisputable, on technological optimism regarding human adaptation.
- Hierarchical (H): based on the most common political principles regarding timing and general issues.
- Egalitarian (E): long term, based on the principle of caution, taking into account longer timescales.

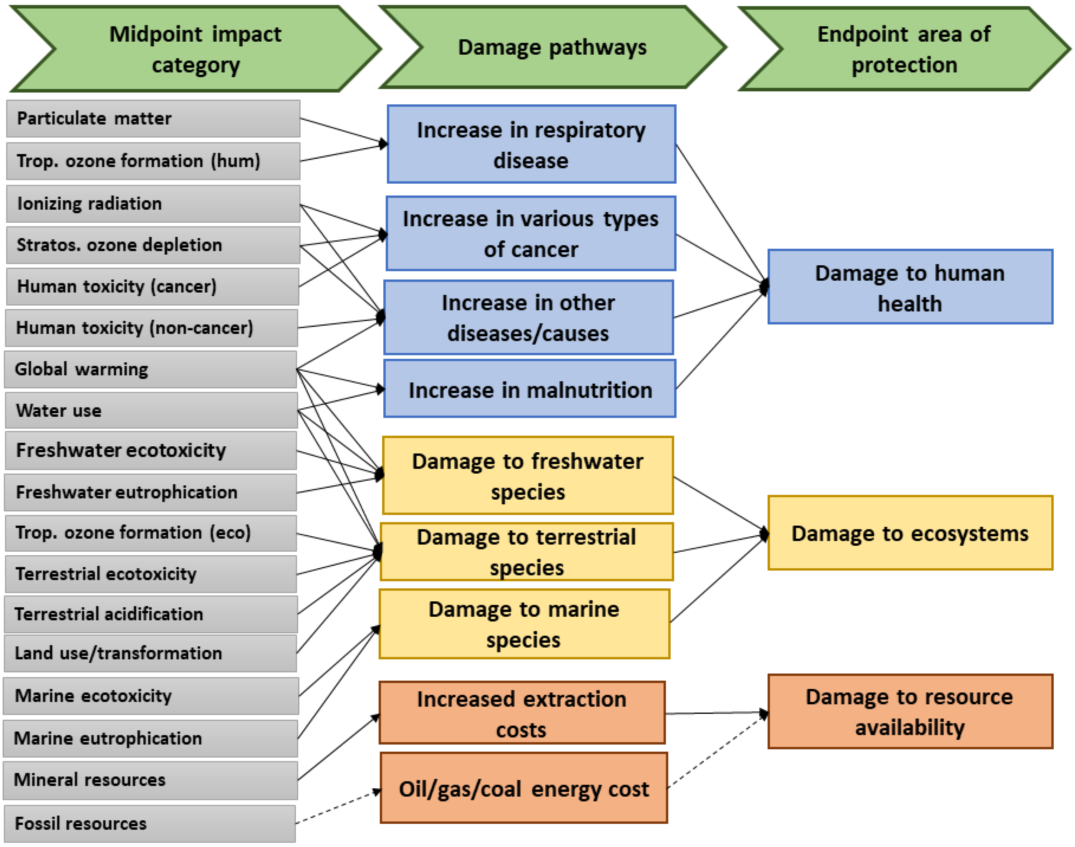

Figure 26: Overview of the impact categories that are covered in theReCiPe2016 methodology and their relation to the areas of protection (adapted from Huijbregts et al. 2017).

Table 8:Midpoint impact categories and related indicators (Huijbregts et al. 2017).

Midpoint impact category	**Indicator**	**Midpoint characterisation factors**	**Unit of measure**	**Reference**
Climate change	Infrared radiative forcing increase	Global warming potential (GWP)	kg CO_2-eq to air	(IPCC 2014; Joos et al. 2013)
Ozone depletion	Stratospheric ozone decrease	Ozone depletion potential (ODP)	kg CFC-11-eq to air	WMO, 2011
Ionizing radiation	Absorbed dose increase	Ionising radiation potential (IRP)	kBq Co-60-eq to air	(Frischknecht et al. 2000)
Fine particulate matter formation	PM2.5 population intake increase	Particulate matter formation potential (PMFP)	kg PM2.5-eq to air	(Van Zelm et al. 2016)
Photochemical oxidant formation: terrestrial ecosystems	Tropospheric ozone increase	Photochemical oxidant formation potential: ecosystems (EOFP)	kg NOx-eq to air	(Van Zelm et al. 2016)
Photochemical oxidant formation: human health	Tropospheric ozone population intake increase	Photochemical oxidant formation potential: humans (HOFP)	kg NOx-eq to air	(Van Zelm et al. 2016)
Terrestrial acidification	Proton increase in natural soils	Terrestrial acidification potential (TAP)	kg SO_2-eq to air	(Roy et al. 2014)
Freshwater eutrophication	Phosphorus increase in freshwater	Freshwater eutrophication potential (FEP)	kg P-eq to freshwater	(Helmes et al. 2012)
Marine eutrophication	Nitrogen increase in marinewater	Marine eutrophication potential (MEP)	kg N-eq to seawater	(Woods et al. 2016)
Human toxicity: cancer	Risk increase of cancer disease incidence	Human toxicity potential (HTPc)	kg 1,4-DCB-eq to urban air	(van Zelm et al. 2009)
Human toxicity: non-cancer	Risk increase of non-cancer disease incidence	Human toxicity potential (HTPnc)	kg 1,4-DCB-eq to urban air	(van Zelm et al. 2009)
Terrestrial ecotoxicity	Hazard-weighted increase in natural soils	Terrestrial ecotoxicity potential (TETP)	kg 1,4-DCB-eq to industrial soil	(van Zelm et al. 2009)
Freshwater ecotoxicity	Hazard-weighted increase in freshwaters	Freshwater ecotoxicity potential (FETP)	kg 1,4-DCB-eq to freshwater	(van Zelm et al. 2009)
Marine ecotoxicity	Hazard-weighted increase in marine water	Marine ecotoxicity potential (METP)	kg 1,4-DCB-eq to marine water	(van Zelm et al. 2009)

Land use	Occupation and time-integrated land transformation	Agricultural land occupation potential (LOP)	m^2 × yr annual cropland-eq	(de Baan et al. 2013)
Water use	Increase of water consumed	Water consumption potential (WCP)	m^3 water-eq consumed	(Hoekstra and Mekonnen 2012)
Mineral resource scarcity	Increase of ore extracted	Surplus ore potential (SOP)	kg Cu-eq	(Vieira et al. 2017)
Fossil resource scarcity	Upper heating value	Fossil fuel potential (FFP)	kg oil-eq	(Huijbregts et al. 2017)

The characterization factors are representative of the global scale, for this reason, ReCiPe 2016 has been moved from the European to Global category. ReCiPe 2016 is currently the most widely used LCA method, both in research and industry (Segovia-Guerrero et al. 2025), in food production processes (Eslami et al. 2024; Priyadarshini et al. 2022), in agricultural production (Giuliana et al. 2024; Fridrihsone et al. 2020), just as in the dairy industry (see Methodology in Table 3).

Additionally, Global Warming Potential (GWP100) of greenhouse gases (D. Kim et al. 2013; Rossi et al. 2024, 2023a; Berlese et al. 2019) and Cumulative Energy Demand (CED) (Alessandro Dalla Riva et al. 2015; A. Dalla Riva et al. 2017; D. Kim et al. 2013; Tarighaleslami Amir Hossein et al. 2019) were selected to specifically evaluate the energy and carbon-related impacts.

IPCC 2021 GWP100 method (V1.03) provides a standardized approach to quantifying the GWP of greenhouse gases over a 100-year time horizon (Enarevba and Haapala 2023). It expresses the impacts in kg CO_2-eq. across fossil, biogenic, and land transformation categories, providing also an overall damage assessment. While shorter timeframes tend to overemphasize immediate effects, the 100-year perspective ensures alignment with long-term climate stabilization goals and international policy frameworks (UNFCCC, Paris Agreement, 2015). This timeframe encompasses multiple generations, allowing for a comprehensive assessment of both short- and long-term climatic responses to atmospheric greenhouse gas concentrations.

CED method quantifies the total primary energy consumed throughout the entire life cycle of a product or service. It is the sum of different forms of energy (expressed in MJ), typically classified as renewable (biomass, wind, solar, geothermal, water) and non-renewable (fossil, nuclear, biomass). This categorization allows for a more detailed analysis of energy dependence and helps identify areas where a product's life cycle relies heavily on non-renewable sources, thus indicating potential areas for improvement (Dalla Riva et al. 2017).

The characterization results discussed in the following Section Interpretation did not consider allocation between Mozzarella and Ricotta, since Ricotta production is similar across all scenarios. However, to evaluate the environmental impacts associated with this by-product, the characterization results were subsequently subject to mass allocation and are presented in Section Allocation with particular focus on the carbon footprint.

5.3 Interpretation

LCIA transforms inventory data into environmental indicators using characterization factors. To provide a comprehensive environmental assessment, the different production scenarios were classified and evaluated using a dual approach that combines individual scenario baselines with comparative analyses. This methodology allows a targeted investigation of critical process stages, focusing specifically on the trade-offs between processing methods, packaging technologies, and storage durations.

The detailed evaluations are structured as follows:

- Influence of Pasteurization.
- Influence of MAP System.
- Influence of Pasteurization, MAP System and storage time.

Tables 9 to 14 present the results expressed per FU (1 kg of Mozzarella), using the ReCiPe 2016 Midpoint (H) V1.11 method (Table 9 and 10), GWP100 (Table 11 and 12), and CED (Table 13 and 14) methods, distinguishing in each case between production from RM (Raw Milk) and PM (Pasteurized Milk).

Table 9: Environmental impact assessment results (ReCiPe 2016 Midpoint (H) V1.11 method) for 1 kg of Mozzarella produced from raw milk under different scenarios.

Impact category	**Unit**	**Case A**	**Case C1**	**Case C2**	**Case C3**	**Case E1**	**Case E2**	**Case E3**	**Case G1**	**Case G2**	**Case G3**
GW	kg CO2 eq	18.70487	19.30272	19.30392	19.30353	19.30782	19.27561	19.30834	19.31188	19.31204	19.31018
SOD	kg CFC11 eq	0.00012	0.00012	0.00012	0.00012	0.00012	0.00012	0.00012	0.00012	0.00012	0.00012
IR	kBq Co-60 eq	0.20404	0.26192	0.26228	0.26203	0.26259	0.25857	0.26266	0.26312	0.26334	0.26290
OFHH	kg NOx eq	0.03826	0.03905	0.03905	0.03905	0.03906	0.03901	0.03906	0.03907	0.03907	0.03906
FPMF	kg PM2.5 eq	0.06851	0.06902	0.06903	0.06903	0.06903	0.06900	0.06903	0.06903	0.06903	0.06903
OFTE	kg NOx eq	0.06274	0.06358	0.06358	0.06358	0.06359	0.06353	0.06359	0.06359	0.06359	0.06359
TA	kg SO2 eq	0.46469	0.46603	0.46604	0.46604	0.46605	0.46596	0.46605	0.46606	0.46606	0.46606
FE	kg P eq	0.00250	0.00265	0.00265	0.00265	0.00265	0.00264	0.00265	0.00265	0.00265	0.00265
ME	kg N eq	0.02263	0.02267	0.02267	0.02267	0.02267	0.02267	0.02267	0.02267	0.02267	0.02267
TEC	kg 1,4-DCB	27.06595	27.97640	27.97788	27.97649	27.98813	27.91274	27.98757	27.99748	27.99656	27.99178
FEC	kg 1,4-DCB	0.98267	1.02876	1.02883	1.02876	1.02934	1.02561	1.02931	1.02981	1.02976	1.02952
MEC	kg 1,4-DCB	0.55228	0.60968	0.60976	0.60968	0.61040	0.60575	0.61036	0.61098	0.61092	0.61062
HTC	kg 1,4-DCB	0.15634	0.20121	0.20128	0.20121	0.20180	0.19804	0.20177	0.20226	0.20221	0.20198
HNTC	kg 1,4-DCB	13.81323	14.36134	14.36214	14.36134	14.36800	14.32516	14.36763	14.37330	14.37275	14.37002
LU	m2a crop eq	16.27083	16.28525	16.28529	16.28525	16.28542	16.28435	16.28541	16.28556	16.28556	16.28547
MRS	kg Cu eq	0.01393	0.01554	0.01554	0.01554	0.01556	0.01543	0.01556	0.01557	0.01557	0.01556
FSR	kg oil eq	1.29438	1.50658	1.50693	1.50677	1.50827	1.49755	1.50837	1.50962	1.50962	1.50897
WC	m3	0.31912	0.32572	0.32574	0.32572	0.32579	0.32534	0.32579	0.32585	0.32585	0.32582

GW: Global Warming potential; SOD: Stratospheric ozone depletion; IR: Ionizing Radiation; OFHH Ozone formation, Human health; FPMF Fine particulate matter formation; OFTE Ozone formation, Terrestrial ecosystems; TA: Terrestrial acidification; FE: Freshwater eutrophication; ME: Marine eutrophication; TEC: Terrestrial ecotoxicity; FEC: Freshwater ecotoxicity; MEC: Marine ecotoxicity; HCT: Human carcinogenic toxicity; HNCT: Human non-carcinogenic toxicity; LU: Land use; MRS: Mineral resource scarcity; FRS: Fossil resource scarcity; WC: Water consumption.

Table 10: Environmental impact assessment results (ReCiPe 2016 Midpoint (H) V1.11 method) for 1 kg of Mozzarella produced from pasteurized milk under different scenarios.

Impact category	**Unit**	**Case B**	**Case D1**	**Case D2**	**Case D3**	**Case F1**	**Case F2**	**Case F3**	**Case H1**	**Case H2**	**Case H3**
GW	kg CO2 eq	16.84159	17.91449	17.91552	17.91469	17.91636	17.91865	17.91825	17.92100	17.92246	17.91897
SOD	kg CFC11 eq	0.00010	0.00010	0.00010	0.00010	0.00010	0.00010	0.00010	0.00010	0.00010	0.00010
IR	kBq Co-60 eq	0.23992	0.35784	0.35818	0.35788	0.35808	0.35859	0.35834	0.35869	0.35908	0.35843
OFHH	kg NOx eq	0.03371	0.03526	0.03526	0.03526	0.03526	0.03527	0.03527	0.03527	0.03527	0.03527
FPMF	kg PM2.5 eq	0.05956	0.06055	0.06055	0.06055	0.06055	0.06056	0.06056	0.06056	0.06056	0.06056
OFTE	kg NOx eq	0.05483	0.05647	0.05647	0.05647	0.05647	0.05648	0.05648	0.05648	0.05648	0.05648
TA	kg SO2 eq	0.40167	0.40425	0.40426	0.40425	0.40426	0.40426	0.40426	0.40427	0.40427	0.40427
FE	kg P eq	0.00230	0.00259	0.00259	0.00259	0.00259	0.00259	0.00259	0.00259	0.00259	0.00259
ME	kg N eq	0.01949	0.01955	0.01955	0.01955	0.01955	0.01955	0.01955	0.01955	0.01955	0.01955
TEC	kg 1,4-DCB	24.37539	26.34927	26.35034	26.34796	26.35357	26.35754	26.35614	26.36424	26.36630	26.35780
FEC	kg 1,4-DCB	0.90218	1.00063	1.00068	1.00056	1.00085	1.00104	1.00097	1.00137	1.00147	1.00105
MEC	kg 1,4-DCB	0.54487	0.66750	0.66756	0.66741	0.66776	0.66801	0.66792	0.66842	0.66855	0.66802
HTC	kg 1,4-DCB	0.18227	0.28021	0.28026	0.28014	0.28042	0.28062	0.28055	0.28095	0.28105	0.28063
HNTC	kg 1,4-DCB	12.49992	13.65193	13.65250	13.65113	13.65437	13.65658	13.65578	13.66042	13.66155	13.65671
LU	m2a crop eq	14.03453	14.06436	14.06440	14.06434	14.06442	14.06450	14.06446	14.06458	14.06463	14.06448
MRS	kg Cu eq	0.01382	0.01720	0.01720	0.01720	0.01721	0.01722	0.01721	0.01723	0.01723	0.01722
FSR	kg oil eq	1.33363	1.70061	1.70090	1.70060	1.70123	1.70194	1.70178	1.70277	1.70320	1.70202
WC	m3	0.28148	0.29445	0.29447	0.29445	0.29448	0.29451	0.29450	0.29454	0.29456	0.29451

GW: Global Warming potential; SOD: Stratospheric ozone depletion; IR: Ionizing Radiation; OFHH Ozone formation, Human health; FPMF Fine particulate matter formation; OFTE Ozone formation, Terrestrial ecosystems; TA: Terrestrial acidification; FE: Freshwater eutrophication; ME: Marine eutrophication; TEC: Terrestrial ecotoxicity; FEC: Freshwater ecotoxicity; MEC: Marine ecotoxicity; HCT: Human carcinogenic toxicity; HNCT: Human non-carcinogenic toxicity; LU: Land use; MRS: Mineral resource scarcity; FRS: Fossil resource scarcity; WC: Water consumption.

Table 11: Environmental impact assessment results (IPCC 2021 GWP100 method (V1.03)) for 1 kg of Mozzarella produced from raw milk, in different scenarios.

Impact Category	**Unit**	**Case A**	**Case C1**	**Case C2**	**Case C3**	**Case E1**	**Case E2**	**Case E3**	**Case G1**	**Case G2**	**Case G3**
GWP100 - fossil	kg CO2-eq	7.212	7.796	7.797	7.796	7.801	7.769	7.801	7.805	7.805	7.803
GWP100 - biogenic	kg CO2-eq	7.593	7.594	7.594	7.594	7.594	7.594	7.594	7.594	7.594	7.594
GWP100 - land transformation	kg CO2-eq	1.831	1.831	1.831	1.831	1.831	1.831	1.831	1.831	1.831	1.831
GWP100 -damage category	**kg CO2-eq**	**16.637**	**17.221**	**17.223**	**17.222**	**17.226**	**17.195**	**17.227**	**17.230**	**17.231**	**17.229**

Table 12:Environmental impact assessment results (IPCC 2021 GWP100 method (V1.03) for 1 kg of Mozzarella produced from pasteurized milk, in different scenarios.

Impact Category	**Unit**	**Case B**	**Case D1**	**Case D2**	**Case D3**	**Case F1**	**Case F2**	**Case F3**	**Case H1**	**Case H2**	**Case H3**
GWP100 - fossil	kg CO2-eq	6.928	7.976	7.977	7.976	7.977	7.980	7.979	7.982	7.983	7.980
GWP100 - biogenic	kg CO2-eq	6.543	6.545	6.545	6.545	6.545	6.545	6.545	6.545	6.545	6.545
GWP100 - land transformation	kg CO2-eq	1.578	1.578	1.578	1.578	1.578	1.578	1.578	1.578	1.578	1.578
GWP100 -damage category	**kg CO2-eq**	**15.049**	**16.099**	**16.100**	**16.099**	**16.101**	**16.103**	**16.102**	**16.105**	**16.107**	**16.103**

Table 13: Environmental impact assessment results (CED method) for 1 kg of Mozzarella produced from raw milk, in different scenarios.

Impact category	**Unit**	**Case A**	**Case C1**	**Case C2**	**Case C3**	**Case E1**	**Case E2**	**Case E3**	**Case G1**	**Case G2**	**Case G3**
Non renewable, fossil	MJ	59.127	68.816	68.832	68.825	68.893	68.405	68.898	68.955	68.955	68.925
Non-renewable, nuclear	MJ	3.785	4.970	4.977	4.977	4.983	4.907	4.989	4.993	4.997	4.994
Non-renewable, biomass	MJ	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002
Renewable, biomass	MJ	1.562	1.744	1.745	1.744	1.746	1.733	1.746	1.748	1.748	1.747
Renewable, wind, solar, geothe	MJ	1.996	3.551	3.554	3.552	3.571	3.446	3.570	3.586	3.585	3.577
Renewable, water	MJ	2.404	3.524	3.526	3.525	3.537	3.450	3.537	3.548	3.548	3.542
Total	**MJ**	**68.875**	**82.608**	**82.637**	**82.624**	**82.733**	**81.943**	**82.742**	**82.832**	**82.836**	**82.787**

Table 14:Environmental impact assessment results (CED method) for 1 kg of Mozzarella produced from pasteurized milk, in different scenarios.

Impact category	**Unit**	**Case B**	**Case D1**	**Case D2**	**Case D3**	**Case F1**	**Case F2**	**Case F3**	**Case H1**	**Case H2**	**Case H3**
Non renewable, fossil	MJ	60.897	77.640	77.653	77.639	77.668	77.700	77.693	77.738	77.758	77.704
Non-renewable, nuclear	MJ	4.461	6.772	6.778	6.777	6.776	6.786	6.786	6.788	6.795	6.788
Non-renewable, biomass	MJ	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002
Renewable, biomass	MJ	1.550	1.921	1.922	1.921	1.922	1.923	1.923	1.924	1.925	1.923
Renewable, wind, solar, geothe	MJ	3.702	7.015	7.017	7.013	7.022	7.029	7.027	7.040	7.044	7.029
Renewable, water	MJ	3.457	5.809	5.811	5.808	5.814	5.819	5.817	5.826	5.829	5.819
Total	**MJ**	**74.069**	**99.158**	**99.183**	**99.160**	**99.204**	**99.260**	**99.247**	**99.318**	**99.353**	**99.265**

5.3.1 Influence of Pasteurization

The environmental impacts of producing 1 kg of Mozzarella from raw milk (Case A) and pasteurized milk (Case B) were assessed to establish baseline values. Subsequently, a comparative analysis (Case A *vs.* Case B) was conducted to quantify the specific environmental burden introduced by the pasteurization process. Comparisons with existing literature are primarily possible for Cases A and B. This is because their production workflows are consistent with standard Mozzarella production processes using raw or pasteurized milk, which are typically analyzed in literature studies. In contrast, the other subsequent scenarios are difficult to compare, as there is no LCA literature on Mozzarella that includes blast chilling of curd, MAP packaging systems, and long-term frozen storage (up to 90 days).

In Mozzarella produced from raw milk (Figure 27), the subsystem with the greatest environmental impact was clearly milk production (including both agricultural and livestock farming phases), contributing more than 60% across all 18 impact categories analyzed.

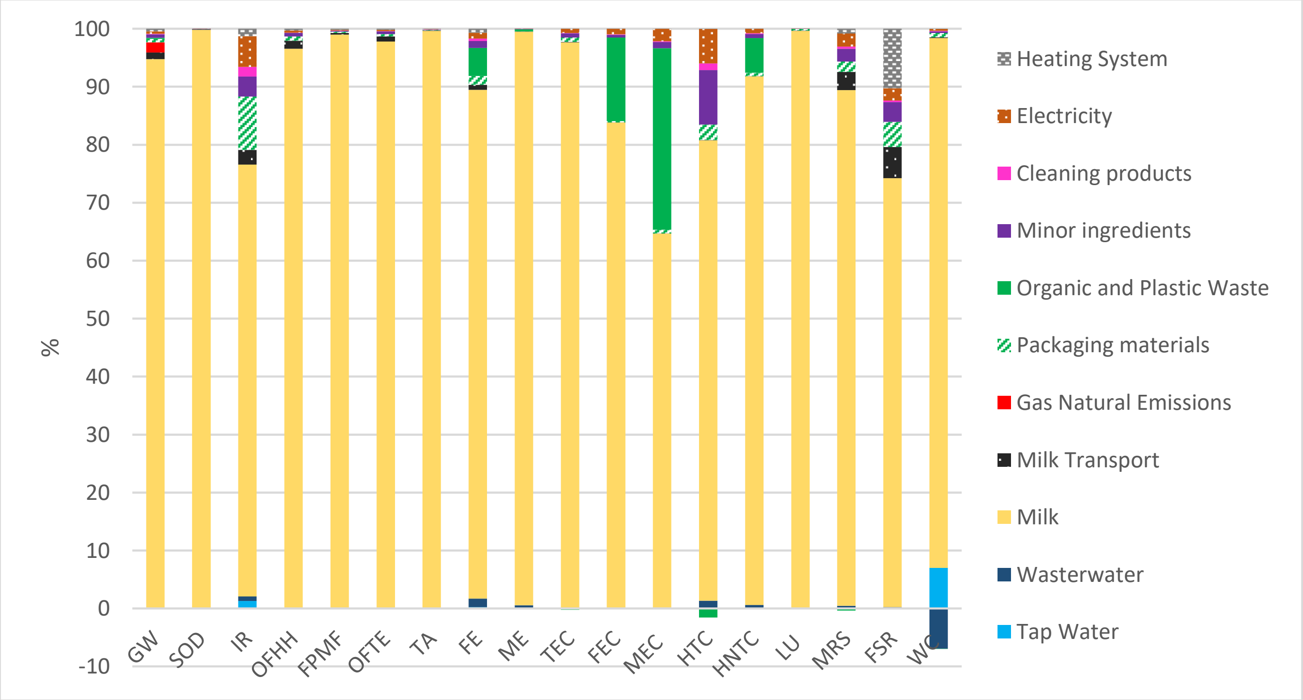

Figure 27: Characterization results obtained for Case A (RM-t0) (Mozzarella obtained from raw milk at time 0), using ReCiPe 2016 Midpoint (H) V1.11 method (FU: 1 kg Mozzarella). [GW: Global Warming potential; SOD: Stratospheric ozone depletion; IR: Ionizing Radiation; OFHH Ozone formation, Human health; FPMF Fine particulate matter formation; OFTE Ozone formation, Terrestrial ecosystems; TA: Terrestrial acidification; FE: Freshwater eutrophication; ME: Marine eutrophication; TEC: Terrestrial ecotoxicity; FEC: Freshwater ecotoxicity; MEC: Marine ecotoxicity; HCT: Human carcinogenic toxicity; HNCT: Human non-carcinogenic toxicity; LU: Land use; MRS: Mineral resource scarcity; FRS: Fossil resource scarcity; WC: Water consumption].

Plastic waste recycling had a beneficial impact on the human carcinogenic toxicity category, while the wastewater subsystem positively influenced the water consumption category due to the treatment and discharge of effluent into the environment.

Individual analysis of case B (Figure 28) identifies milk production as the dominant hotspot, with values consistently above 54%, for all impact categories within the ReCiPe method. Electricity consumption, which increased due to the pasteurization step, was particularly harmful in several impact categories: human carcinogenic toxicity (37%), ionizing radiation (32%), mineral resource scarcity (16%) and marine ecotoxicity (15%). While electricity usage does not directly produce ionizing radiation (UNSCEAR, 2019), it influences the Ionizing Radiation and Human Toxicity categories through processes associated to the generation and life cycle of energy systems (Ma et al. 2025). The severity of these impacts depends on the specific power generation technology employed (Perčić et al. 2025; Aisyah et al. 2025). As in case A, plastic recycling reduced the burden on human carcinogenic toxicity, and the wastewater subsystem provided environmental benefits regarding water consumption category.

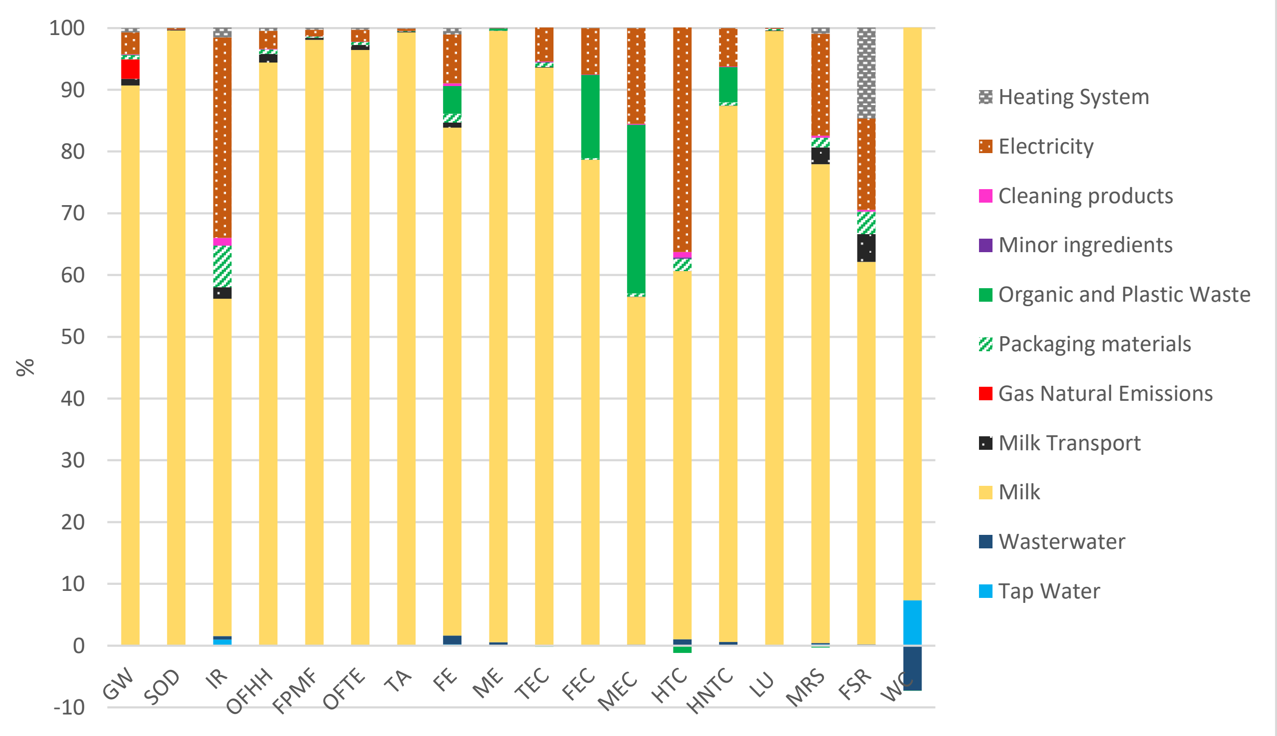

Figure 28: Characterization results obtained for Case B (PM-t0) (Mozzarella obtained from pasteurized milk at time 0), using ReCiPe 2016 Midpoint (H) V1.11 method (FU: 1 kg Mozzarella). [GW: Global Warming potential; SOD: Stratospheric ozone depletion; IR: Ionizing Radiation; OFHH Ozone formation, Human health; FPMF Fine particulate matter formation; OFTE Ozone formation, Terrestrial ecosystems; TA: Terrestrial acidification; FE: Freshwater eutrophication; ME: Marine eutrophication; TEC: Terrestrial ecotoxicity; FEC: Freshwater ecotoxicity; MEC: Marine ecotoxicity; HCT: Human carcinogenic toxicity; HNCT: Human non-carcinogenic toxicity; LU: Land use; MRS: Mineral resource scarcity; FRS: Fossil resource scarcity; WC: Water consumption].

When comparing Case A vs. Case B (Figure 29), Mozzarella obtained with raw milk (Case A) exhibit higher impacts in 15 of the categories analyzed, including Global Warming (18.70 vs. 16.84 kg CO2 eq), Land Use (16.27 vs. 14.03 m²a crop eq) and Water Consumption (0.32 vs. 0.28 m³).

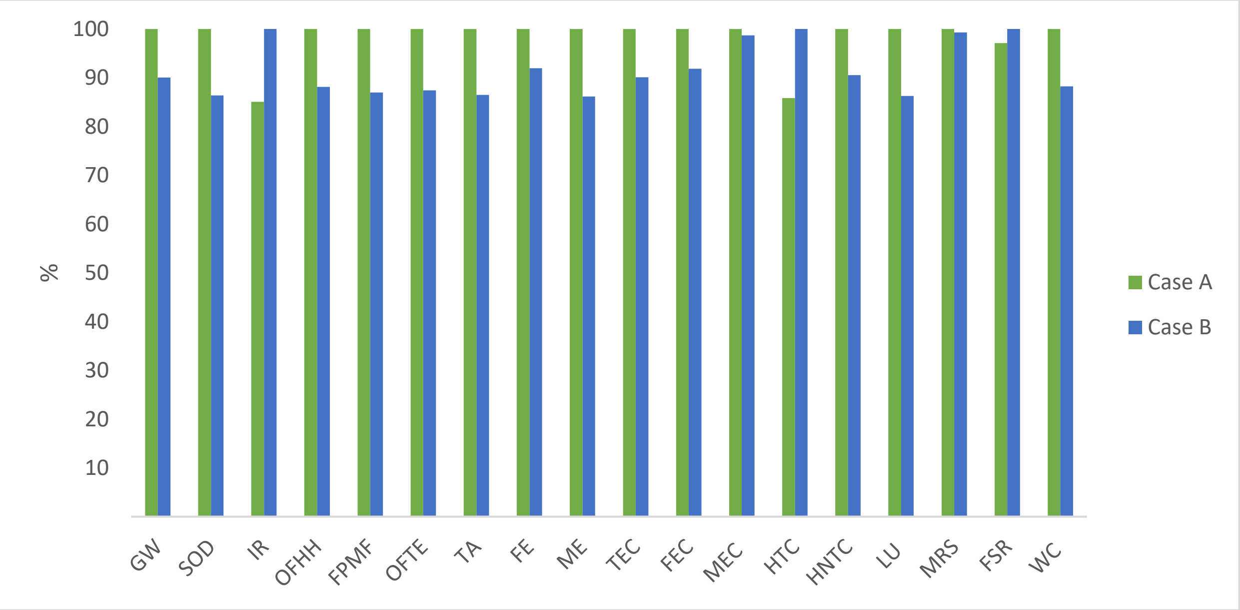

Figure 29: Comparative environmental impact assessment between Case A and Case B, using ReCiPe 2016 Midpoint (H) V1.11 method; (FU: 1 kg Mozzarella). [GW: Global Warming potential; SOD: Stratospheric ozone depletion; IR: Ionizing Radiation; OFHH Ozone formation, Human health; FPMF Fine particulate matter formation; OFTE Ozone formation, Terrestrial ecosystems; TA: Terrestrial acidification; FE: Freshwater eutrophication; ME: Marine eutrophication; TEC: Terrestrial ecotoxicity; FEC: Freshwater ecotoxicity; MEC: Marine ecotoxicity; HCT: Human carcinogenic toxicity; HNCT: Human non-carcinogenic toxicity; LU: Land use; MRS: Mineral resource scarcity; FRS: Fossil resource scarcity; WC: Water consumption].

The observed reduction in environmental impacts for Case B is directly linked to its higher transformation yield. The data indicate that producing 1 kg of Mozzarella requires 8.80 kg of pasteurized milk, whereas the raw milk process (Case A) requires 10.21 kg of raw milk. Since milk production represents the dominant hotspot across all impact categories and scenarios analyzed, the higher milk input in Case A significantly amplifies upstream environmental load. However, the thermal treatment in Case B introduces specific burdens associated with industrial energy consumption. Consequently, Case B shows higher impact values in those categories typically linked to electricity and fossil fuel use, i.e., fossil resource scarcity (1.33 *vs.* 1.29 kg oil eq), ionizing radiation (0.24 *vs.* 0.20 Co-60 eq), and human carcinogenic toxicity (0.18 *vs.* 0.15 kg 1,4-DCB). In conclusion, while pasteurization (Case B) increases energy-related impacts and human toxicity, it effectively reduces the greenhouse gas (GHG) emissions and land use. As it has been previously commented this improvement is fundamentally driven by the optimized milk-to-cheese conversion rate, which reflects a higher production efficiency for Case B in terms of yield.

Most GHG emissions in cheese production are attributable to the farm phase, particularly activities related to cow feed production (Mondello et al. 2018; Carvalho et al. 2022). Similarly, in our study, the primary environmental burden in GW category is driven by milk production. Excluding the farm phase clearly reduces the overall environmental impacts; however, the processing stage still requires a high energy input due to energy-intensive operations such as refrigeration, pasteurization (Nunes et al. 2020), and ripening (Silva et al. 2023). Therefore, to provide a deeper understanding of the environmental trade-offs, the comparison between Raw Milk (Case A) and Pasteurized Milk (Case B) was extended using two complementary methods: IPCC 2021 GWP100 to assess climate change impact and Cumulative Energy Demand (CED) to evaluate energy efficiency.

The analysis with IPCC 2021 GWP100 method (Figure 30) confirms the trend observed previously in the results obtained by ReCiPe method, i.e., Case A generates a higher carbon footprint (damage category) (+10%) compared to Case B. This differential is consistent across all specific sub-categories (fossil, biogenic, and land transformation) where Case A systematically exhibits higher impact values than Case B.

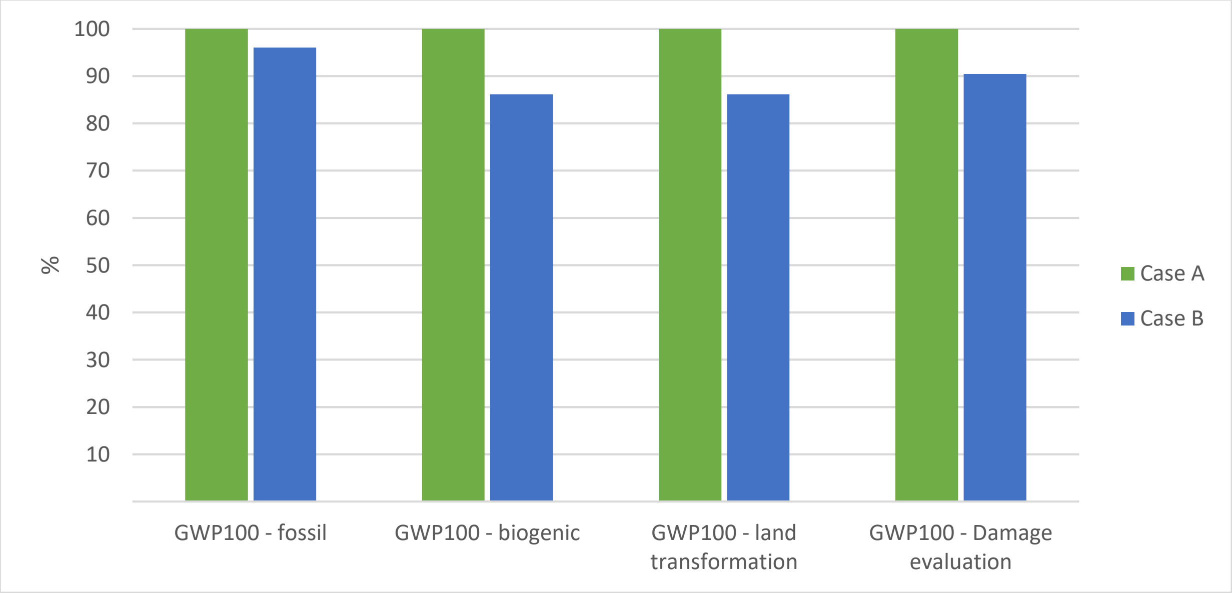

Figure 30: Comparative Global Warming Potential between Case A and Case B, using the IPCC 2021 GWP100; (FU: 1 kg Mozzarella).

The comparison with CED method (Figure 31 and 32) highlights the "energy cost" of pasteurization process, specifically Case B requires 74.1 MJ/FU (+7.5 %) compared to Case A (68.9 MJ/FU).

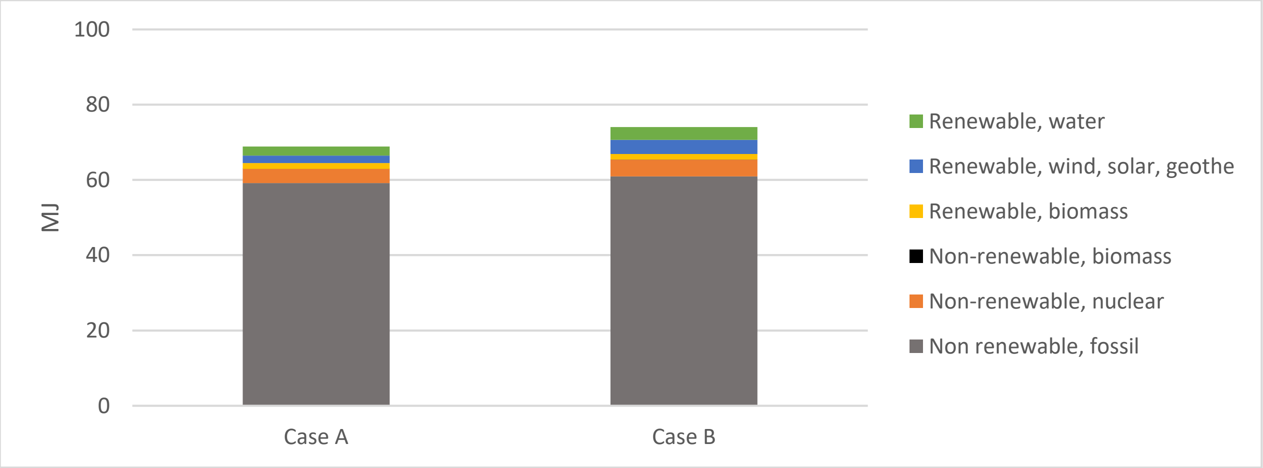

Figure 31: Comparative Energy Demand between Case A and Case B, using CED Method; (FU: 1 kg Mozzarella).

In terms of energy sources (Figure 32), Case A maintains a larger share of renewables (hydro, wind, solar), instead Case B shifts the demand toward non-renewable sources.

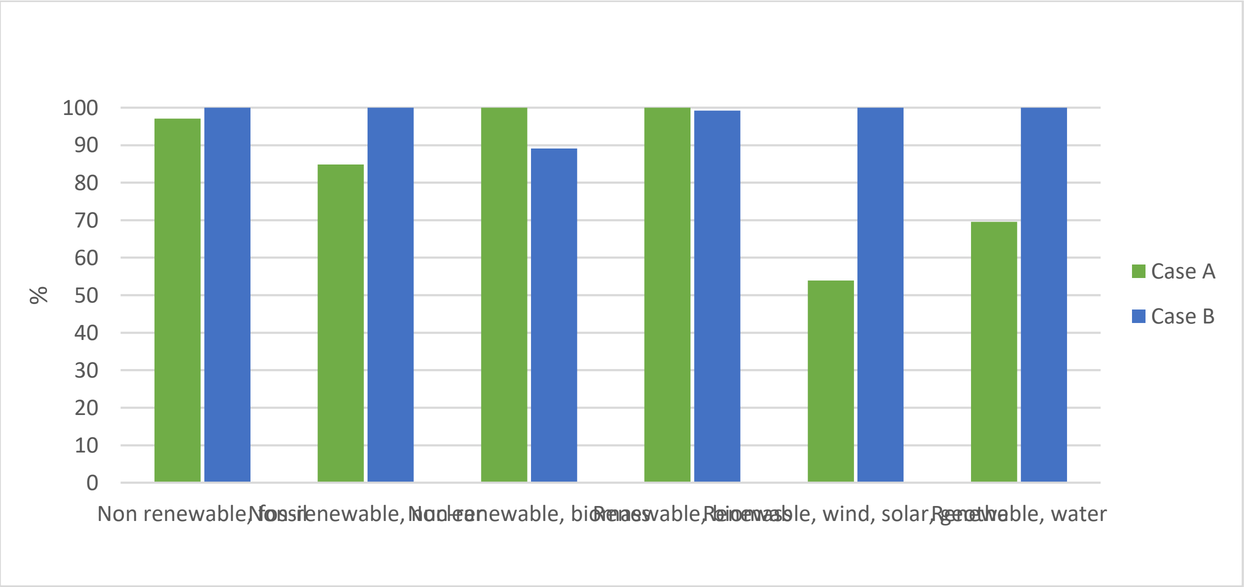

Figure 32: Comparative Energy Demand by specific energy sources for Case A and Case B, using CED Method; (FU: 1 kg Mozzarella).

The industrial pasteurization process results in a higher consumption of fossil and nuclear energy to support the electrical and thermal demands of the heating and cooling cycles. While pasteurization (Case B) reduces the Carbon Footprint (GWP100) through better efficiency, it simultaneously drives up the Cumulative Energy Demand (CED) because of the energy-intensive industrial process (Table 15).

Table 15: Summary of environmental trade-offs: Global Warming Potential vs. Cumulative Energy Demand for Case A and Case B (FU: 1 kg Mozzarella).

Impact Category	**Method**	**Unit**	**Case A (Raw Milk)**	**Case B (Pasteurized Milk)**	**Variation (%)**	**Trend**
Carbon Footprint	IPCC GWP100	kg CO2 eq	16.64	15.05	- 9.6 %	Improvement (Lower Impact)
Energy Demand	CED	MJ	68.9	74.1	+ 7.5 %	Increase (Higher Impact)

As summarized in Table 16, the results obtained here from Carbon Footprint (GWP100) and Cumulative Energy Demand (CED) methods were compared with a selection of relevant international studies on Mozzarella cheese production, which reflect the study's distinct positioning relative to the state of the art.

Table 16: Comparison of the present study results with international literature on Mozzarella cheese (FU: 1 kg Mozzarella).

Topic	**System boundaries**	**Pasteurization**	**Methodology**	**Results**	**Ref.**
Present study (Mozzarella by Raw Milk)	Cradle-to-gate	*NO*	*ReCiPe Midpoint (H);* *IPCC GWP100a;* *CED*	16.6 kg CO_2eq/kg 68.9 MJ/kg	-
Present study (Mozzarella by Pasteurized Milk)	Cradle-to-Gate	*YES*	*ReCiPe Midpoint (H);* *IPCC GWP100a;* *CED*	15.0 kg CO_2eq/kg 74.1 MJ/kg	-
Mozzarella, Italy	Cradle-to-grave	YES	ReCiPe midpoint (H); CED	6.6 kg CO_2eq/kg 45.1 MJ/kg	(Dalla Riva et al. 2017)
Mozzarella, USA	Cradle-to-grave	NO	ReCiPe Midpoint; ReCiPe Endpoint; IPCC GWP 100a; CED; SEtox	7.28 kg CO_2eq/kg (5.13-9.89 CO_2eq/kg, variability due to regional and system management) 45.4 MJ/kg	(Kim et al. 2013)
Mozzarella, Italy	Cradle-to-grave	NO	ReCiPe Midpoint (H) IPCC GWP 100a	9.82 kg CO_2eq/kg	(Rossi et al. 2024)
Mozzarella, Brazil	Cradle-to-gate	YES	Database Ecoinvent 3; CML-IA baseline V3.06/World 2000 methodology	16.31 kg CO_2eq/kg	(Santos et al. 2022)
Buffalo Mozzarella, Italy	Cradle-to-grave	NO	ReCiPe Midpoint (H); IPCC GWP 100a	12.0 kg CO_2eq/kg	(Rossi et al. 2023)
Buffalo Mozzarella, Italy	Cradle-to-gate	NO	CML-IA baseline V3.05 method; IPCC GWP 100a	33.9 kg CO_2eq/kg	(Berlese et al. 2019)

The Global Warming Potential results obtained in this study (16.6 kg CO_2e/kg for RM Mozzarella and 15.0 kg CO_2e/kg for PM Mozzarella) are within the range described in the literature for this type of cheese (5.13 – 36.7 kg CO_2e/kg) and are in the same order of magnitude with the findings (16.3 kg CO_2e/kg) reported by (Santos et al. 2022), in Brazil. However, considering methodological variations in system boundaries (cradle-to-gate vs. cradle-to-grave), our values are significantly higher than those observed in North Italy (6.6 and 9.82 kg CO_2e, (A. Dalla Riva et al. 2017; Rossi et al. 2024), or North America (7.28 kg CO_2e; (Kim et al. 2013). This trend reflects the specific production context of Basilicata (Southern Italy), where lower milk yields contrast with the optimized industrial systems of Northern Italy and America. Thus, the higher impact can be attributed to the specific inefficiencies inherent to our pilot-scale production compared to full industrial processing. As regard energy performance, Cumulative Energy Demand (CED) calculated in this study (74.1 MJ/kg) exceeds the literature average of approximately 45 MJ/kg (Dalla Riva et al. 2017; Kim et al. 2013). This reflects the lower efficiency of our small-scale equipment (e.g., small boiler, low-flow pasteurizer) compared to the large-scale industrial machinery typically assessed in existing literature.

Finally, comparisons with buffalo Mozzarella (33.9-36.7 kg CO_2e (Berlese et al. 2019)) confirm that our results, while higher than standard industrial bovine Mozzarella, remain significantly lower than buffalo derivatives, due to different metabolic processes, different feed utilization efficiencies and lower efficiency in converting it into milk (Berlese et al. 2019; Rossi et al. 2023).

5.3.2 Influence of MAP System and storage time

To evaluate the environmental influence of the packaging system, a comparative analysis of different short-term (30-day) storage scenarios was conducted:

- Raw Milk: Comparison of C1 (Vacuum) vs. C2 (Air) vs. C3 (Nitrogen).
- Pasteurized Milk: Comparison of D1 (Vacuum) vs. D2 (Air) vs. D3 (Nitrogen).

The 30-day storage interval was selected to isolate the packaging variable, as cumulative electricity consumption for refrigeration is lower than in the 60- or 90-day scenarios. As a result, observed variations could be attributed exclusively to different packaging systems, specifically the energy required for the production and injection of technical gases (nitrogen and compressed air).

Analyzing the individual scenarios C1, C2, and C3, as shown in Figures 33, 34, and 35, respectively, the milk production subsystem stands out as the main environmental hotspot, contributing more than 50% to the total impact across all the categories analyzed. Furthermore, plastic waste recycling and wastewater treatment provided beneficial effects in the human carcinogenic toxicity and water consumption categories, respectively, across all scenarios.

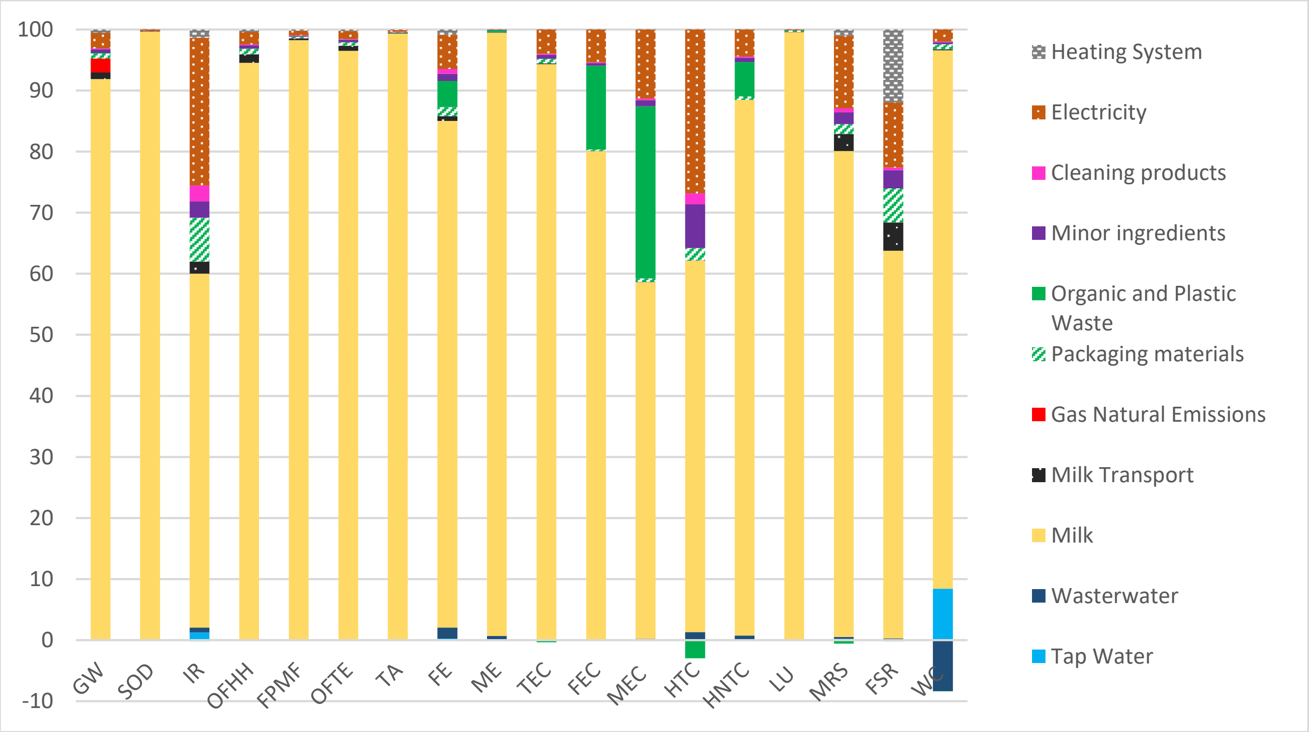

Figure 33: Characterization results obtained for Case C1 (RM-V-t30; Mozzarella by Raw Milk, MAP-Vacuum, storage time 30 day), using ReCiPe 2016 Midpoint (H) V1.11 method (FU: 1 kg Mozzarella). [GW: Global Warming potential; SOD: Stratospheric ozone depletion; IR: Ionizing Radiation; OFHH Ozone formation, Human health; FPMF Fine particulate matter formation; OFTE Ozone formation, Terrestrial ecosystems; TA: Terrestrial acidification; FE: Freshwater eutrophication; ME: Marine eutrophication; TEC: Terrestrial ecotoxicity; FEC: Freshwater ecotoxicity; MEC: Marine ecotoxicity; HCT: Human carcinogenic toxicity; HNCT: Human non-carcinogenic toxicity; LU: Land use; MRS: Mineral resource scarcity; FRS: Fossil resource scarcity; WC: Water consumption].

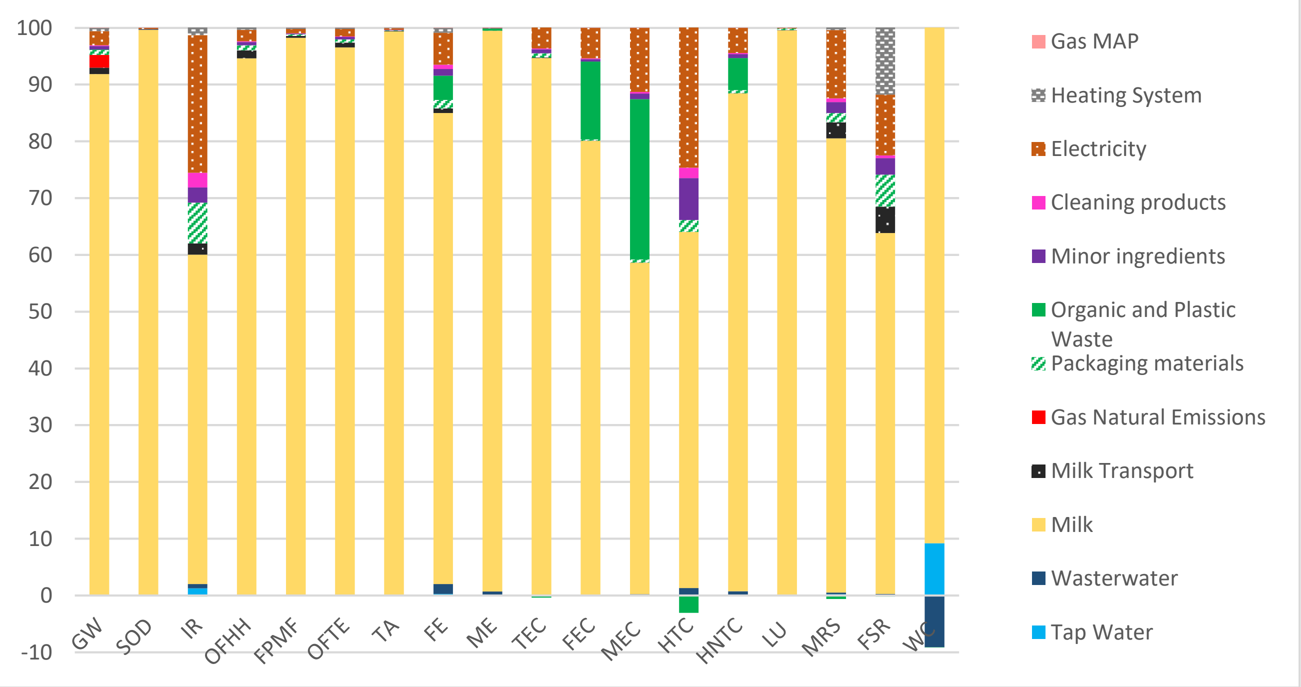

Figure 34: Characterization results obtained for Case C2 (RM-A-t30; Mozzarella by Raw Milk, MAP-Air, storage time 30 day), using ReCiPe 2016 Midpoint (H) V1.11 method (FU: 1 kg Mozzarella). [GW: Global Warming potential; SOD: Stratospheric ozone depletion; IR: Ionizing Radiation; OFHH Ozone formation, Human health; FPMF Fine particulate matter formation; OFTE Ozone formation, Terrestrial ecosystems; TA: Terrestrial acidification; FE: Freshwater eutrophication; ME: Marine eutrophication; TEC: Terrestrial ecotoxicity; FEC: Freshwater ecotoxicity; MEC: Marine ecotoxicity; HCT: Human carcinogenic toxicity; HNCT: Human non-carcinogenic toxicity; LU: Land use; MRS: Mineral resource scarcity; FRS: Fossil resource scarcity; WC: Water consumption].

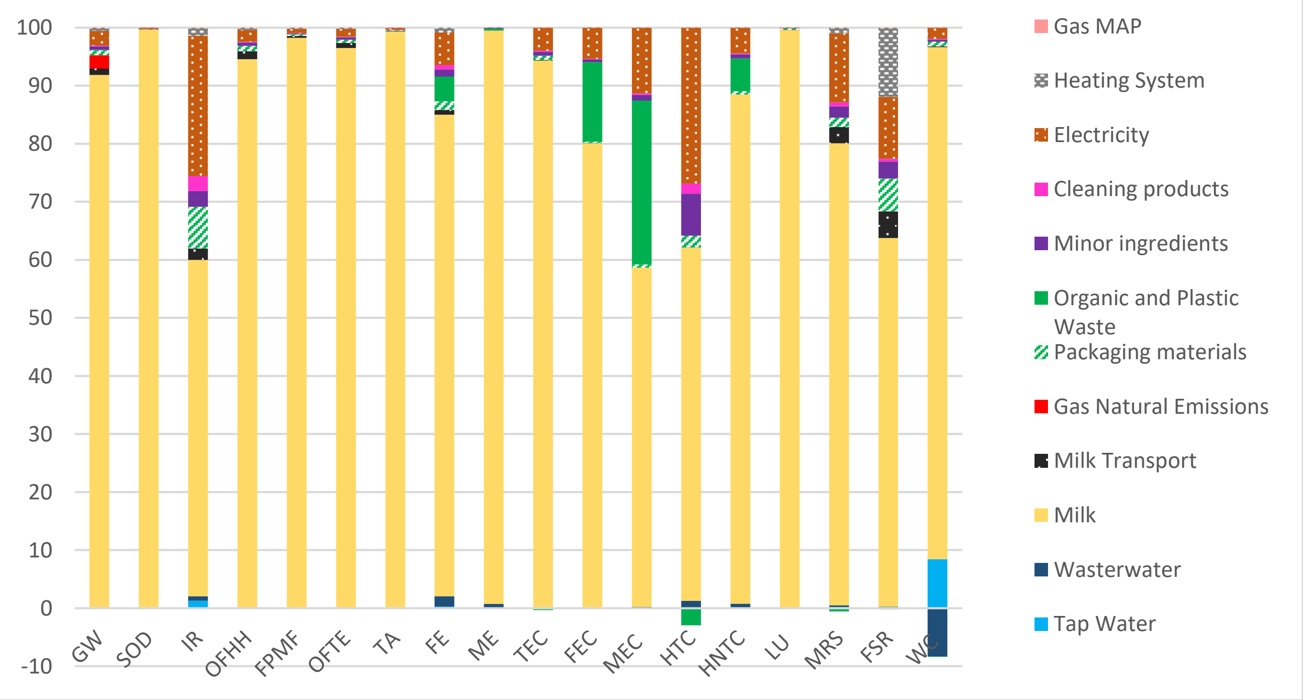

Figure 35: Characterization results obtained for Case C3 (RM-N-t30; Mozzarella by Raw Milk, MAP-Nitrogen, storage time 30 day), using ReCiPe 2016 Midpoint (H) V1.11 method (FU: 1 kg Mozzarella). [GW: Global Warming potential; SOD: Stratospheric ozone depletion; IR: Ionizing Radiation; OFHH Ozone formation, Human health; FPMF Fine particulate matter formation; OFTE Ozone formation, Terrestrial ecosystems; TA: Terrestrial acidification; FE: Freshwater eutrophication; ME: Marine eutrophication; TEC: Terrestrial ecotoxicity; FEC: Freshwater ecotoxicity; MEC: Marine ecotoxicity; HCT: Human carcinogenic toxicity; HNCT: Human non-carcinogenic toxicity; LU: Land use; MRS: Mineral resource scarcity; FRS: Fossil resource scarcity; WC: Water consumption].

Similarly to the previous RM scenario analysis, and as shown in Figures 36, 37, and 38, cases D1, D2, and D3, identified milk production as the dominant hotspot for most of the impact categories. Nevertheless, when employing pasteurized milk, electricity consumption, was particularly impactful in several categories, i.e., human carcinogenic toxicity (58%), ionizing radiation (53%), mineral resource scarcity (32%), marine ecotoxicity (30%) and fossil resources scarcity (28%). As in the raw milk scenarios, plastic waste recycling and wastewater treatment generated environmental benefits in the human carcinogenic toxicity and water consumption categories, respectively.

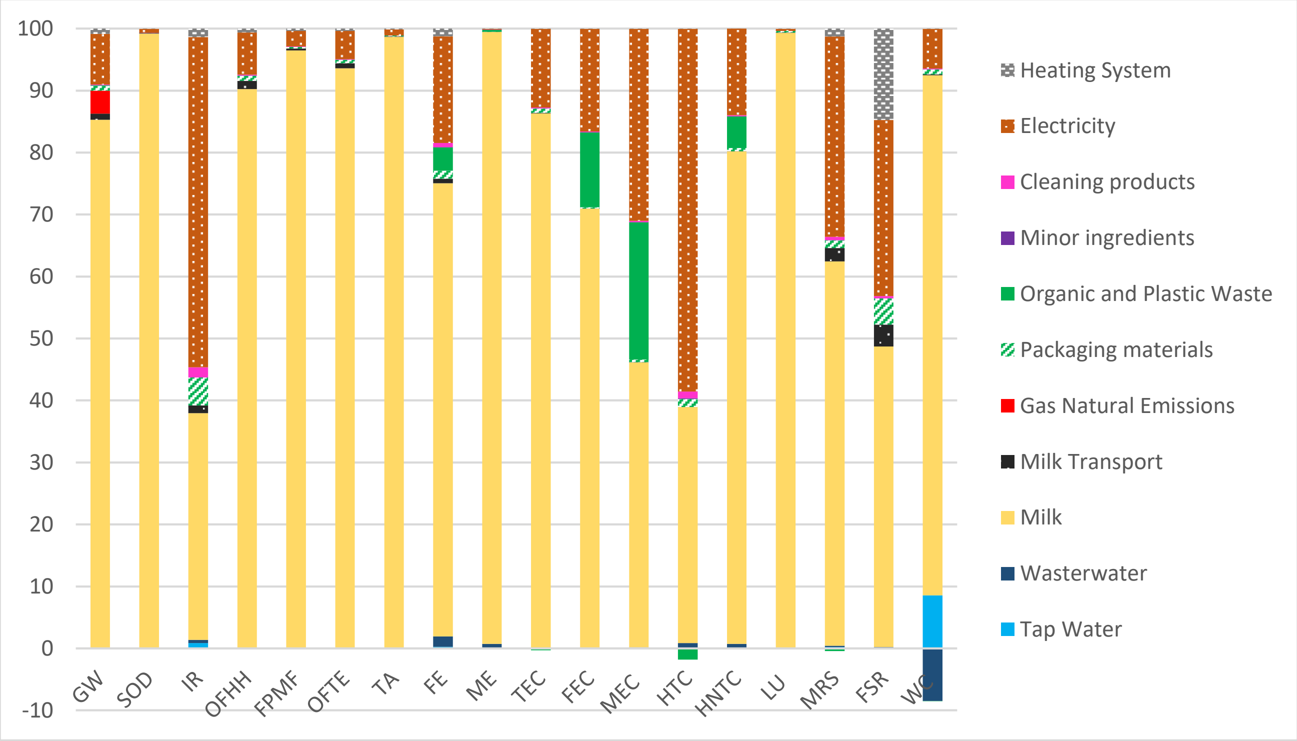

Figure 36: Characterization results obtained for Case D1 (PM-V-t30; Mozzarella by Pasteurized Milk, MAP-Vacuum, storage time 30 day), using ReCiPe 2016 Midpoint (H) V1.11 method (FU: 1 kg Mozzarella). [GW: Global Warming potential; SOD: Stratospheric ozone depletion; IR: Ionizing Radiation; OFHH Ozone formation, Human health; FPMF Fine particulate matter formation; OFTE Ozone formation, Terrestrial ecosystems; TA: Terrestrial acidification; FE: Freshwater eutrophication; ME: Marine eutrophication; TEC: Terrestrial ecotoxicity; FEC: Freshwater ecotoxicity; MEC: Marine ecotoxicity; HCT: Human carcinogenic toxicity; HNCT: Human non-carcinogenic toxicity; LU: Land use; MRS: Mineral resource scarcity; FRS: Fossil resource scarcity; WC: Water consumption].

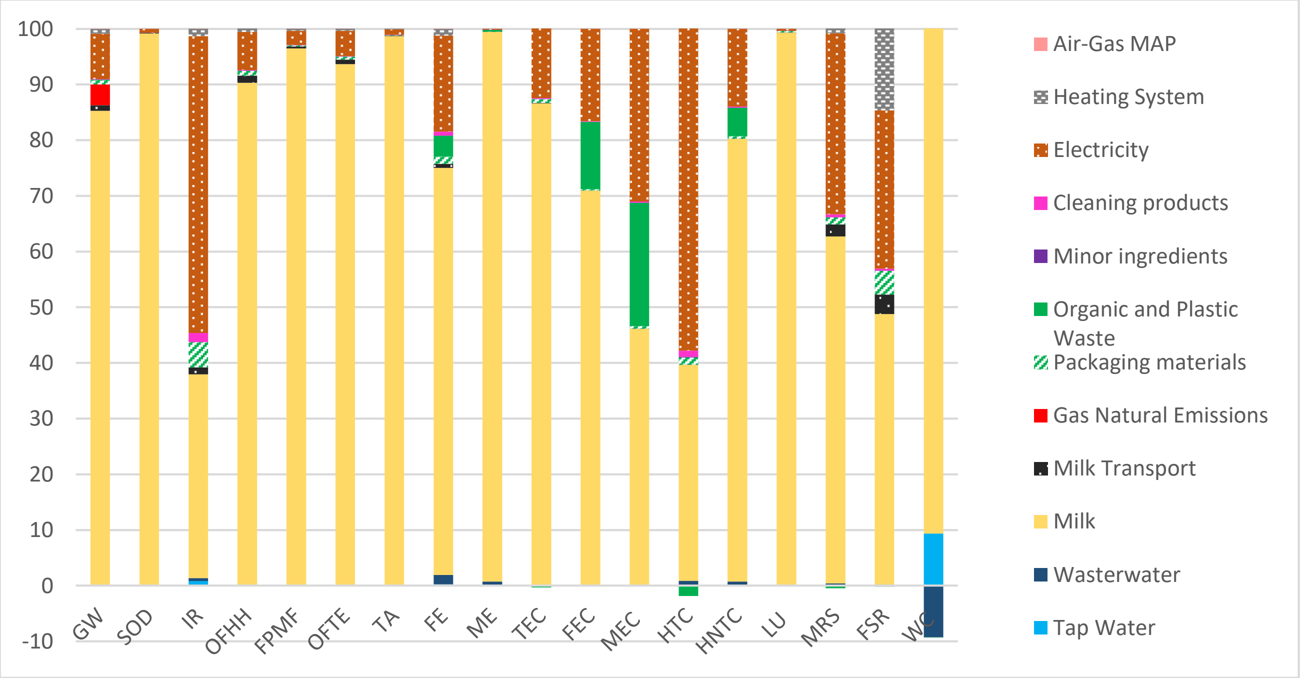

Figure 37: Characterization results obtained for Case D2 (PM-A-t30;, Mozzarella by Pasteurized Milk, MAP-Air, storage time 30 day), using ReCiPe 2016 Midpoint (H) V1.11 method (FU: 1 kg Mozzarella). [GW: Global Warming potential; SOD: Stratospheric ozone depletion; IR: Ionizing Radiation; OFHH Ozone formation, Human health; FPMF Fine particulate matter formation; OFTE Ozone formation, Terrestrial ecosystems; TA: Terrestrial acidification; FE: Freshwater eutrophication; ME: Marine eutrophication; TEC: Terrestrial ecotoxicity; FEC: Freshwater ecotoxicity; MEC: Marine ecotoxicity; HCT: Human carcinogenic toxicity; HNCT: Human non-carcinogenic toxicity; LU: Land use; MRS: Mineral resource scarcity; FRS: Fossil resource scarcity; WC: Water consumption].

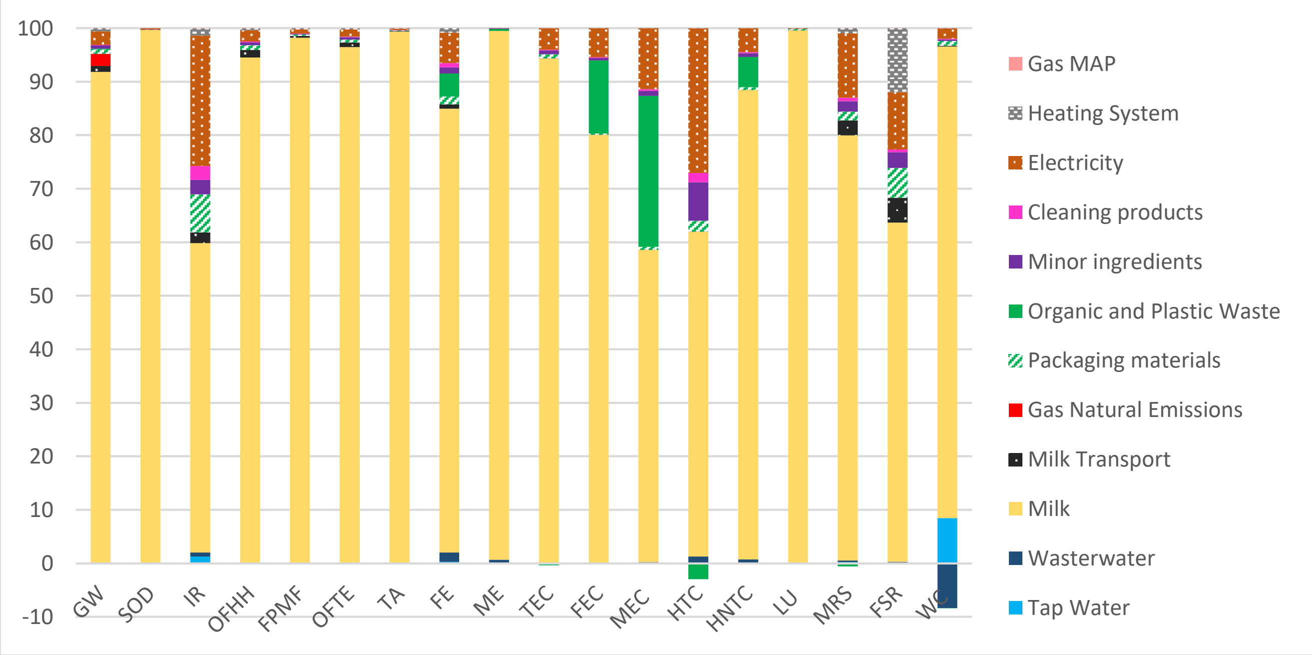

Figure 38: Characterization results obtained for Case D3 (PM-N-t30; Mozzarella by Pasteurized Milk, MAP-Nitrogen, storage time 30 day), using ReCiPe 2016 Midpoint (H) V1.11 method (FU: 1 kg Mozzarella). [GW: Global Warming potential; SOD: Stratospheric ozone depletion; IR: Ionizing Radiation; OFHH Ozone formation, Human health; FPMF Fine particulate matter formation; OFTE Ozone formation, Terrestrial ecosystems; TA: Terrestrial acidification; FE: Freshwater eutrophication; ME: Marine eutrophication; TEC: Terrestrial ecotoxicity; FEC: Freshwater ecotoxicity; MEC: Marine ecotoxicity; HCT: Human carcinogenic toxicity; HNCT: Human non-carcinogenic toxicity; LU: Land use; MRS: Mineral resource scarcity; FRS: Fossil resource scarcity; WC: Water consumption].

Consistent with the findings for scenario groups C and D, the individual analysis of the remaining scenarios (E1, E2, E3; F1, F2, F3; G1, G2, G3; H1, H2, H3) confirmed the trends previously discussed, for raw and pasteurized milk, respectively. Detailed impact profiles for these scenarios are available in the Appendix A (Figures 61–72; Section 9 - Appendix A).

The comparative analysis, including the 30-day storage time and the different MAP configurations (Vacuum, Air, and Nitrogen) revealed no substantial differences across any impact categories, either for Mozzarella obtained from Raw Milk (Scenarios C1-C2-C3) or for Pasteurized Milk (Scenarios D1-D2-D3). Moreover, irrespective of the assessment method applied (ReCiPe, GWP, or CED), the results reported in Tables 7 to 12 show no meaningful differences, with variations below 0.2% in most categories. Similar trends are observed in the comparative analysis of Mozzarella obtained from Raw Milk after 60 days of storage (scenarios E1-E2-E3) and after 90 days (scenarios G1-G2-G3), as well as from Pasteurized Milk after 60 days of storage (scenarios F1-F2-F3) and after 90 days (scenarios H1-H2-H3). Therefore, from an environmental perspective, no MAP packaging option can be identified as superior, since the performance differences among vacuum, air, and nitrogen packaging – under the same storage time and acidification method - are negligible. Although MAP configurations influence the emission profile, the dominant environmental burden is associated with raw milk production; in pasteurized milk scenarios, electricity consumption also represents a significant contribution.

In recent years, LCA studies have explored the environmental footprint of food packaging (Kim et al. 2023; Hemachandra et al. 2024). Within the dairy sector, scientific literature has focused primarily on liquid milk, analyzing various packaging solutions: HDPE bottles, glass bottles (virgin or recycled), and multi-layer cartons (Brock and Williams 2020). Bottle with 50% opaque R-PET(recycled polyethylene terephthalate) bottles offer environmental advantages across all impact categories compared to 100% virgin PET packaging, confirming the benefits of adopting recycled materials in UHT milk packaging (Garzoni et al. 2026). For the packaging of yogurt, the environmental impact was examined by considering the packaging, the weight of the packages and the material compositions of polypropylene (PP) and polystyrene (PS), and the use of additional paper wrappers (Köck et al. 2025). For dairy products, particularly the hard cheese Parmigiano Reggiano, the potential of R-PET as a sustainable alternative for its outer packaging was specifically evaluated (Borghesi et al. 2022).

To date, no specific LCA studies comparing different modified atmospheric packaging conditions for cheese or curd have been identified in the literature. More recent research has focused on the development of biodegradable and sustainable packaging materials for dairy products (Coltelli et al. 2025); however, this work primarily evaluate the barrier and mechanical properties of the materials (specifically PLA/PBSA-based films for soft cheeses under high-humidity conditions), not the impact of different storage atmospheres.

5.3.3 Influence of Pasteurization, MAP System and storage time

A global comparative analysis to quantify the relative impact of milk heat treatment, packaging system, and storage time was conducted. Figure 39 shows the environmental impact profile of the RM (raw milk) cases compared to the PM (pasteurized milk) cases under vacuum conditions (Vacuum), monitored over a time horizon of 30, 60 and 90 days.

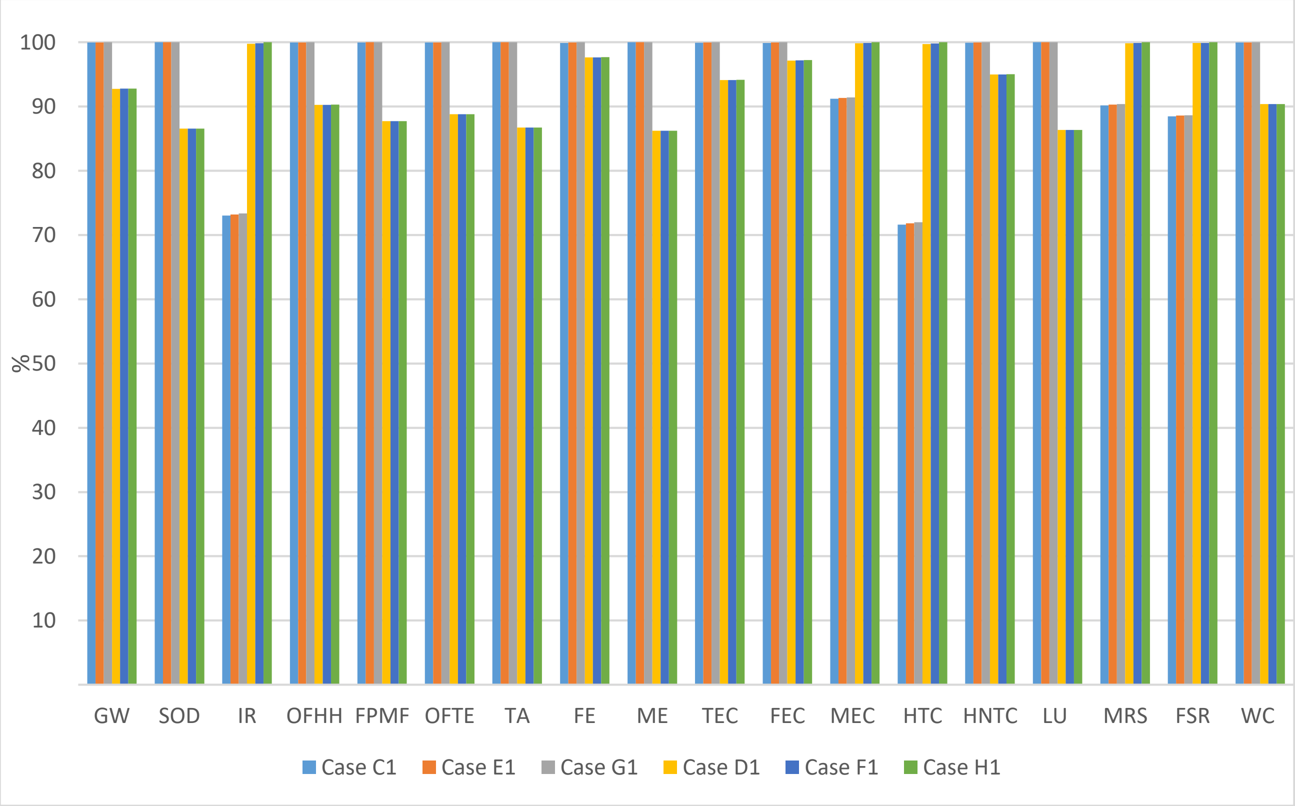

Figure 39: Comparative environmental impact assessment MAP-Vacuum (RM Case C1-E1-G1 vs PM Case D1-F1-H1) across different storage time intervals (30-60-90 day), using ReCiPe 2016 Midpoint (H) V1.11 method; (FU: 1 kg Mozzarella). [GW: Global Warming potential; SOD: Stratospheric ozone depletion; IR: Ionizing Radiation; OFHH Ozone formation, Human health; FPMF Fine particulate matter formation; OFTE Ozone formation, Terrestrial ecosystems; TA: Terrestrial acidification; FE: Freshwater eutrophication; ME: Marine eutrophication; TEC: Terrestrial ecotoxicity; FEC: Freshwater ecotoxicity; MEC: Marine ecotoxicity; HCT: Human carcinogenic toxicity; HNCT: Human non-carcinogenic toxicity; LU: Land use; MRS: Mineral resource scarcity; FRS: Fossil resource scarcity; WC: Water consumption].

Across the impact categories analyzed, the distribution of the environmental burden is clearly driven by the milk processing method (RM vs. PM) rather than by the storage time (30, 60, and 90 days). While the RM scenarios (C, E, and G) showed greater impacts in 13 of the 18 categories analyzed - including categories such as Global Warming, Stratospheric Ozone Depletion and Water Consumption - the PM scenarios (Scenarios D-F-H) exhibited a marked increase in Ionizing Radiation, Human Carcinogenic Toxicity, Marine ecotoxicity, Mineral and Fossil Resource Scarcity. This is probably due to the higher electricity consumption associated with the pasteurization process. As previously noted, the environmental advantage of the pasteurization scenarios results from higher processing yields (8.80 kg of RM *vs.* 10.21 kg of PM per kg of Mozzarella).

Figure 40 presents the Carbon Footprint analysis using the IPCC 2021 method, confirming that raw milk scenarios (RM) generate a higher total Carbon Footprint and a 7% higher Damage Evaluation than pasteurized milk scenarios (PM).

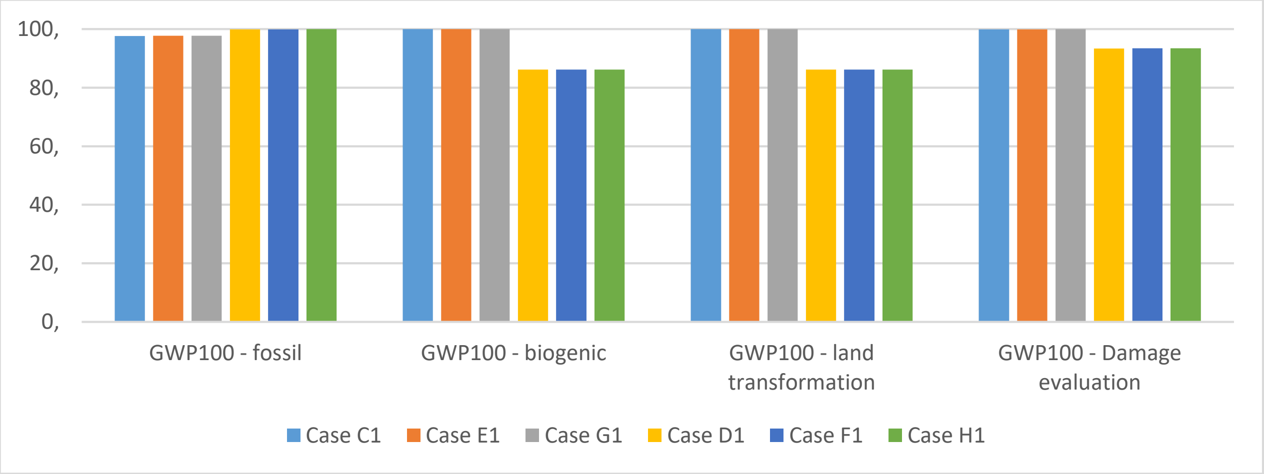

Figure 40: Comparative Global Warming Potential between MAP-Vacuum (RM Case C1-E1-G1 vs PM Case D1-F1-H1) across different storage time intervals (30-60-90 day), using the IPCC 2021 GWP100; (FU: 1 kg Mozzarella).

Specifically, the RM process shows a 13% higher impact in GWP biogenic and land transformation categories due to the lower yields obtained when raw milk is employed, while the PM process exhibits a higher GWP fossil impact (+3%) due to a higher electricity demand originated by the pasteurization step. In addition, the PM scenarios (D1, F1, and H1) show higher energy demand (Figure 41), primarily from non-renewable fossil and nuclear sources, employed to obtain electricity.

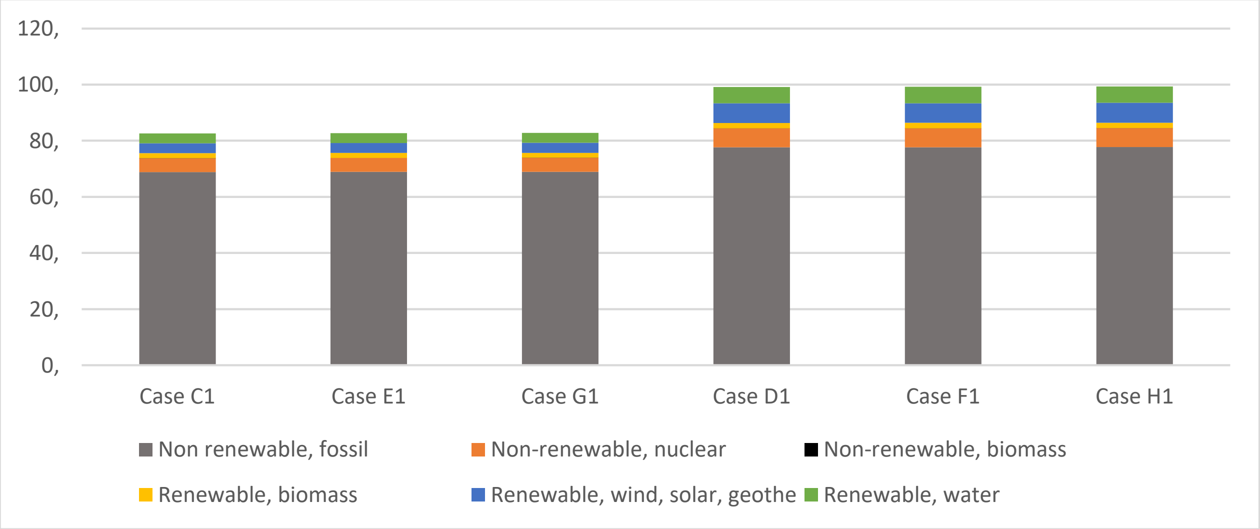

Figure 41: Comparative Energy Demand MAP-Vacuum (RM Case C1-E1-G1 vs PM Case D1-F1-H1) across different storage time intervals (30-60-90 day), using CED Method; (FU: 1 kg Mozzarella).

The environmental trends identified for MAP-Vacuum configurations (Figures 39–41) are consistently replicated across the MAP-Air (Figures 73-75; Section 9 - Appendix A) and MAP-Nitrogen (Figures 76-78; Section 9 - Appendix A) scenarios. Irrespective of the gas composition in MAP, the primary environmental driver remains the milk acidification method (RM vs. PM), while the influence of both the packaging gas and the storage duration (30, 60, and 90 days) remains statistically insignificant. These results confirm that the environmental profile is mainly related to the coagulation method and the resulting processing yield, rather than to the specific packaging system employed.

5.4 Allocation

If the system is treated as a multi-output process generating Mozzarella and Ricotta, environmental burdens can be allocated between the two products based on their respective masses relative to the total production output (Tab. 17).

Table 17:Mass allocation factors applied to Mozzarella and Ricotta for Scenarios by Raw Milk and Scenarios by Pasteurized Milk (FU: 1 kg Mozzarella).

Parameter	Scenarios by Raw Milk	Scenarios by Pasteurized Milk
Mozzarella	1 kg	1 kg
Ricotta	0.211 kg	0.182 kg
Allocation Factor Mozzarella	82.58 %	84.60 %
Allocation Factor Ricotta	17.42 %	15.40 %

The application of mass allocation, using different allocation factors depending on the scenario analyzed, significantly redefines the environmental profile of the final product. This procedure shifts a portion of the total environmental impact (17.42 % for the raw milk and 15.4% for pasteurized milk scenarios) to the Ricotta co-product, thus reducing the specific impact on Mozzarella. Similar values were observed by (Berlese et al. 2019) where the Mozzarella allocation factor was equal to 76%, in a scenario where in addition to Mozzarella, the production of ricotta and aged cheese was considered. For instance, regarding Global Warming potential category (Method ReCiPe), comparing Cases A and B (Figure 42) reveals that the impact for Case A decreases from 18.70 to 15.45 kg CO_2 eq, while for Case B it decreases from 16.84 to 14.25 kg CO_2 eq.

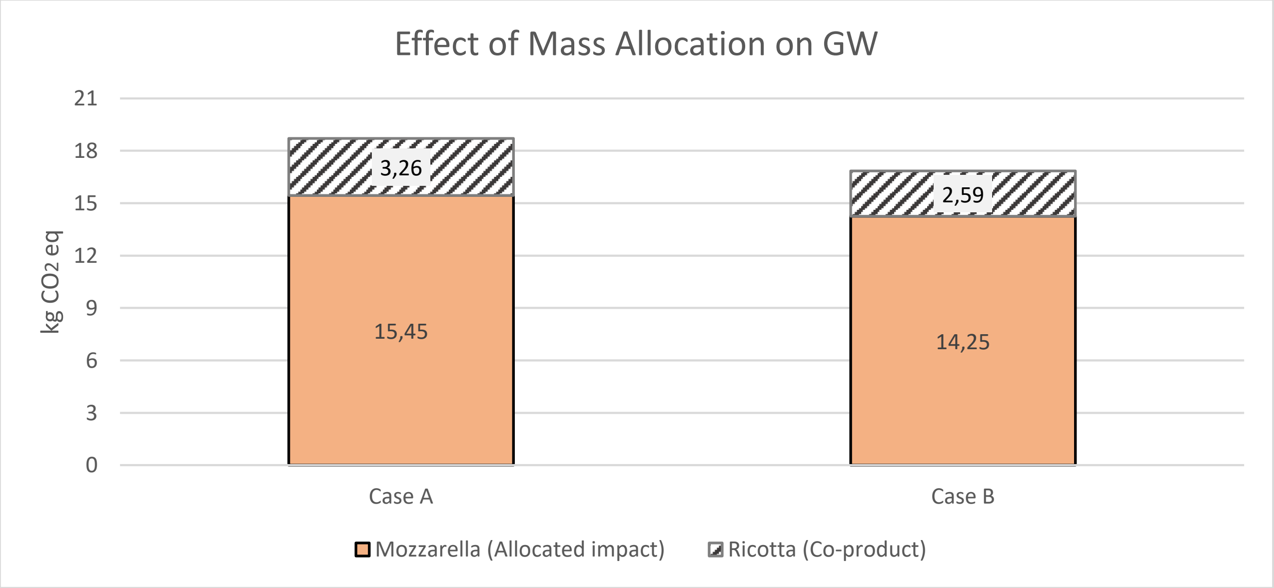

Figure 42: Distribution of Global Warming potential (ReCiPe 2016 Midpoint (H) V1.11 method) between Mozzarella and Ricotta co-product using mass allocation (Case A vs. Case B). The stacked bars illustrate the share of impact assigned to the functional unit (Mozzarella; orange bar) and the share reallocated to the co-product (Ricotta; black and white hatched bar).

Our Global Warming potential result for Ricotta (3.26 and 2.59 kgCO2eq for RM and PM, respectively) are intermediate between the values observed in the literature, which range from 0.26 kgCO2eq/kg (Ferronato et al. 2025) and 8.33 kg CO2eq/kg (Đekić et al. 2025).

As the mass allocation factor is applied uniformly across the inventory, a consistent downward trend is evident in all categories. To prevent graphical redundancy, the allocated results are summarized in tables for the ReCiPe (Tables 18 and 19), GWP100 (Tables 20 and 21), and CED (Tables 22 and 23) Methods. All tables distinguish between Raw Milk (RM) and Pasteurized Milk (PM) scenarios.

Table 18: Allocated environmental impact assessment results (ReCiPe 2016 Midpoint (H) V1.11 method) for 1 kg of Mozzarella produced from raw milk under different scenarios.

Impact category	**Unit**	**Case A**	**Case C1**	**Case C2**	**Case C3**	**Case E1**	**Case E2**	**Case E3**	**Case G1**	**Case G2**	**Case G3**
GW	kg CO2 eq	15.44648	15.94018	15.94118	15.94085	15.94440	15.91780	15.94483	15.94775	15.94789	15.94634
SOD	kg CFC11 eq	0.00010	0.00010	0.00010	0.00010	0.00010	0.00010	0.00010	0.00010	0.00010	0.00010
IR	kBq Co-60 eq	0.16850	0.21629	0.21659	0.21639	0.21684	0.21353	0.21691	0.21728	0.21747	0.21711
OFHH	kg NOx eq	0.03159	0.03905	0.03905	0.03905	0.03906	0.03901	0.03906	0.03907	0.03907	0.03906
FPMF	kg PM2.5 eq	0.06851	0.06851	0.06851	0.06851	0.06851	0.06851	0.06851	0.06851	0.06851	0.06851
OFTE	kg NOx eq	0.05181	0.05250	0.05250	0.05250	0.05251	0.05246	0.05251	0.05252	0.05252	0.05251
TA	kg SO2 eq	0.38374	0.38485	0.38485	0.38485	0.38486	0.38479	0.38487	0.38487	0.38487	0.38487
FE	kg P eq	0.00207	0.00219	0.00219	0.00219	0.00219	0.00218	0.00219	0.00219	0.00219	0.00219
ME	kg N eq	0.01869	0.01872	0.01872	0.01872	0.01872	0.01872	0.01872	0.01872	0.01872	0.01872
TEC	kg 1,4-DCB	22.35106	23.10291	23.10413	23.10298	23.11260	23.05034	23.11213	23.12031	23.11956	23.11561
FEC	kg 1,4-DCB	0.81149	0.84955	0.84961	0.84955	0.85003	0.84695	0.85001	0.85041	0.85037	0.85018
MEC	kg 1,4-DCB	0.45607	0.50347	0.50354	0.50347	0.50407	0.50023	0.50403	0.50454	0.50449	0.50425
HTC	kg 1,4-DCB	0.12910	0.16616	0.16622	0.16616	0.16665	0.16354	0.16662	0.16703	0.16699	0.16679
HNTC	kg 1,4-DCB	11.40697	11.85959	11.86026	11.85959	11.86510	11.82971	11.86479	11.86947	11.86901	11.86676
LU	m2a crop eq	13.43645	13.44836	13.44840	13.44836	13.44850	13.44762	13.44849	13.44861	13.44862	13.44854
MRS	kg Cu eq	0.01150	0.01283	0.01283	0.01283	0.01285	0.01274	0.01285	0.01286	0.01286	0.01285
FSR	kg oil eq	1.06890	1.24413	1.24442	1.24429	1.24553	1.23668	1.24561	1.24664	1.24665	1.24611
WC	m3	0.26353	0.26898	0.26899	0.26898	0.26904	0.26866	0.26904	0.26909	0.26909	0.26906

GW: Global Warming potential; SOD: Stratospheric ozone depletion; IR: Ionizing Radiation; OFHH Ozone formation, Human health; FPMF Fine particulate matter formation; OFTE Ozone formation, Terrestrial ecosystems; TA: Terrestrial acidification; FE: Freshwater eutrophication; ME: Marine eutrophication; TEC: Terrestrial ecotoxicity; FEC: Freshwater ecotoxicity; MEC: Marine ecotoxicity; HCT: Human carcinogenic toxicity; HNCT: Human non-carcinogenic toxicity; LU: Land use; MRS: Mineral resource scarcity; FRS: Fossil resource scarcity; WC: Water consumption.

Table 19:Allocated environmental impact assessment results (ReCiPe 2016 Midpoint (H) V1.11 method) for 1 kg of Mozzarella produced from pasteurized milk under different scenarios.

Impact category	**Unit**	**Case B**	**Case D1**	**Case D2**	**Case D3**	**Case F1**	**Case F2**	**Case F3**	**Case H1**	**Case H2**	**Case H3**
GW	kg CO2 eq	14.24798	15.15566	15.15653	15.15583	15.15724	15.15917	15.15884	15.16116	15.16240	15.15945
SOD	kg CFC11 eq	0.00008	0.00009	0.00009	0.00009	0.00009	0.00009	0.00009	0.00009	0.00009	0.00009
IR	kBq Co-60 eq	0.20297	0.30273	0.30302	0.30276	0.30294	0.30336	0.30316	0.30345	0.30378	0.30324
OFHH	kg NOx eq	0.02852	0.03905	0.03905	0.03905	0.03906	0.03901	0.03906	0.03907	0.03907	0.03906
FPMF	kg PM2.5 eq	0.06851	0.06851	0.06851	0.06851	0.06851	0.06851	0.06851	0.06851	0.06851	0.06851
OFTE	kg NOx eq	0.04638	0.04777	0.04777	0.04777	0.04778	0.04778	0.04778	0.04778	0.04778	0.04778
TA	kg SO2 eq	0.33982	0.34200	0.34200	0.34200	0.34200	0.34201	0.34201	0.34201	0.34202	0.34201
FE	kg P eq	0.00195	0.00219	0.00219	0.00219	0.00219	0.00219	0.00219	0.00219	0.00219	0.00219
ME	kg N eq	0.01649	0.01654	0.01654	0.01654	0.01654	0.01654	0.01654	0.01654	0.01654	0.01654
TEC	kg 1,4-DCB	20.62158	22.29148	22.29239	22.29037	22.29512	22.29848	22.29730	22.30414	22.30589	22.29870
FEC	kg 1,4-DCB	0.76325	0.84654	0.84658	0.84648	0.84672	0.84688	0.84682	0.84716	0.84725	0.84689
MEC	kg 1,4-DCB	0.46096	0.56470	0.56476	0.56463	0.56493	0.56513	0.56506	0.56548	0.56559	0.56514
HTC	kg 1,4-DCB	0.15420	0.23706	0.23710	0.23700	0.23724	0.23740	0.23734	0.23769	0.23777	0.23741
HNTC	kg 1,4-DCB	10.57493	11.54953	11.55001	11.54886	11.55159	11.55347	11.55279	11.55672	11.55767	11.55358
LU	m2a crop eq	11.87321	11.89845	11.89848	11.89843	11.89850	11.89857	11.89853	11.89863	11.89868	11.89855
MRS	kg Cu eq	0.01169	0.01455	0.01455	0.01455	0.01456	0.01457	0.01456	0.01457	0.01458	0.01456
FSR	kg oil eq	1.12825	1.43872	1.43896	1.43871	1.43924	1.43984	1.43971	1.44054	1.44091	1.43991
WC	m3	0.23813	0.24911	0.24912	0.24910	0.24913	0.24916	0.24914	0.24918	0.24920	0.24915

GW: Global Warming potential; SOD: Stratospheric ozone depletion; IR: Ionizing Radiation; OFHH Ozone formation, Human health; FPMF Fine particulate matter formation; OFTE Ozone formation, Terrestrial ecosystems; TA: Terrestrial acidification; FE: Freshwater eutrophication; ME: Marine eutrophication; TEC: Terrestrial ecotoxicity; FEC: Freshwater ecotoxicity; MEC: Marine ecotoxicity; HCT: Human carcinogenic toxicity; HNCT: Human non-carcinogenic toxicity; LU: Land use; MRS: Mineral resource scarcity; FRS: Fossil resource scarcity; WC: Water consumption.

Table 20: Allocated environmental impact assessment results (IPCC 2021 GWP100 method (V1.03)) for 1 kg of Mozzarella produced from raw milk, in different scenarios.

Impact Category	**Unit**	**Case A**	**Case C1**	**Case C2**	**Case C3**	**Case E1**	**Case E2**	**Case E3**	**Case G1**	**Case G2**	**Case G3**
GWP100 - fossil	kg CO2-eq	5.956	6.438	6.439	6.438	6.442	6.416	6.442	6.445	6.445	6.444
GWP100 - biogenic	kg CO2-eq	6.270	6.272	6.272	6.272	6.272	6.271	6.272	6.272	6.272	6.272
GWP100 - land transformation	kg CO2-eq	1.512	1.512	1.512	1.512	1.512	1.512	1.512	1.512	1.512	1.512
GWP100 -damage category	**kg CO2-eq**	**13.738**	**14.221**	**14.222**	**14.222**	**14.226**	**14.200**	**14.226**	**14.229**	**14.229**	**14.228**

Table 21: Allocated environmental impact assessment results (IPCC 2021 GWP100 method (V1.03)) for 1 kg of Mozzarella produced from pasteurized milk, in different scenarios.

Impact Category	**Unit**	**Case B**	**Case D1**	**Case D2**	**Case D3**	**Case F1**	**Case F2**	**Case F3**	**Case H1**	**Case H2**	**Case H3**
GWP100 - fossil	kg CO2-eq	5.861	6.747	6.748	6.748	6.749	6.751	6.750	6.753	6.754	6.751
GWP100 - biogenic	kg CO2-eq	5.535	5.537	5.537	5.537	5.537	5.537	5.537	5.537	5.537	5.537
GWP100 - land transformation	kg CO2-eq	1.335	1.335	1.335	1.335	1.335	1.335	1.335	1.335	1.335	1.335
GWP100 -damage category	**kg CO2-eq**	**12.731**	**13.620**	**13.620**	**13.620**	**13.621**	**13.623**	**13.623**	**13.625**	**13.626**	**13.623**

Table 22: Allocated environmental impact assessment results (CED method) for 1 kg of Mozzarella produced from raw milk, in different scenarios.

Impact category	**Unit**	**Case A**	**Case C1**	**Case C2**	**Case C3**	**Case E1**	**Case E2**	**Case E3**	**Case G1**	**Case G2**	**Case G3**
Non renewable, fossil	MJ	48.827	56.828	56.842	56.836	56.892	56.488	56.896	56.943	56.943	56.919
Non-renewable, nuclear	MJ	3.125	4.105	4.110	4.110	4.115	4.052	4.120	4.123	4.127	4.124
Non-renewable, biomass	MJ	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002
Renewable, biomass	MJ	1.290	1.440	1.441	1.440	1.442	1.431	1.442	1.443	1.444	1.442
Renewable, wind, solar, geothe	MJ	1.648	2.933	2.935	2.933	2.949	2.846	2.948	2.961	2.961	2.954
Renewable, water	MJ	1.985	2.910	2.912	2.911	2.921	2.849	2.921	2.930	2.930	2.925
Total	**MJ**	**56.877**	**68.217**	**68.241**	**68.231**	**68.321**	**67.668**	**68.329**	**68.403**	**68.406**	**68.366**

Table 23: allocated environmental impact assessment results (CED method) for 1 kg of Mozzarella produced from pasteurized milk, in different scenarios.

Impact category	**Unit**	**Case B**	**Case D1**	**Case D2**	**Case D3**	**Case F1**	**Case F2**	**Case F3**	**Case H1**	**Case H2**	**Case H3**
Non renewable, fossil	MJ	51.519	65.683	65.694	65.683	65.707	65.734	65.728	65.766	65.783	65.737
Non-renewable, nuclear	MJ	3.774	5.729	5.734	5.733	5.733	5.741	5.741	5.743	5.749	5.742
Non-renewable, biomass	MJ	0.001	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002
Renewable, biomass	MJ	1.311	1.625	1.626	1.625	1.626	1.627	1.627	1.628	1.628	1.627
Renewable, wind, solar, geothe	MJ	3.132	5.935	5.937	5.933	5.941	5.947	5.945	5.956	5.959	5.947
Renewable, water	MJ	2.924	4.914	4.916	4.913	4.918	4.923	4.921	4.929	4.932	4.923
Total	**MJ**	**62.662**	**83.888**	**83.909**	**83.889**	**83.927**	**83.974**	**83.963**	**84.023**	**84.053**	**83.978**

5.5 Study Limitation, Improvements and Mitigation strategy

The quality of the Life Cycle Inventory depends on the availability of site-specific primary data collected directly from the production facilities, complemented by secondary data. Variability can be attributed to several structural factors, including differences in livestock feed management, regional energy mixes, transportation distances for milk and finished products, and variations in allocation methodologies and functional units (Vergé et al. 2013; Moja et al. 2025). According to ISO 14044 guidelines, co-products, when present, require impact allocation, which can substantially influence the environmental load assigned to the dairy product under study (Silvério et al. 2025).

The present work is based on pilot scale data, with limited processing volumes (100 L of Raw Milk yielding 10.08 kg of Mozzarella, and 100 L of Pasteurized Milk yielding 11.7 kg). A 'cradle-to-gate' approach was adopted, excluding the distribution and consumption phases. Consequently, impacts associated with transportation to retailers, consumer refrigeration, and packaging end-of-life are not quantified, as the Mozzarella was intended solely for laboratory analysis.

To further reduce the overall environmental impact, a more sustainable approach could involve valorizing whey and/or secondary whey by producing high-value derivatives (e.g., whey powder or whey-based beverages) and managing whey permeate as a by-product. However, this strategy requires additional processing steps and dedicated equipment, such as ultrafiltration, reverse osmosis, spray drying, and bottling systems.

Ultimately, the goal of an LCA is to translate analytical results into concrete sustainability actions and improvements. In the case of Mozzarella supply chain, mitigation strategies can be implemented at three distinct levels (Dalla Riva et al. 2017; Tarighaleslami n et al. 2019; Verduna et al. 2020):

- Farm-Level: improved milk yield and meat co-production, optimized manure management;
- Dairy-Level: enhancement of energy efficiency through the upgrade of cooling technologies (e.g., heat pumps, thermal recovery systems), investment in renewable energy;
- Supply Chain: optimization of transport logistics and adoption of sustainable packaging solutions to reduce the burden of the final distribution phase.

5.6 Conclusion

This LCA study provides an environmental assessment on Mozzarella production by evaluating strategic interventions such as curd freezing, alternative packaging systems, and varying storage durations. These strategies aim to promote sustainable production, particularly in the context managing milk surpluses.

The results identify the raw milk production phase as the primary environmental hotspot, contributing most significantly to the carbon footprint across all analyzed scenarios. Additionally, the milk-to-cheese conversion rate significantly influences sustainability, specifically, the optimized processing yield of pasteurized milk (requiring 8.80 kg of milk per kg of Mozzarella compared to 10.21 kg for raw milk) results in a 10% lower carbon footprint and a 13% reduction in biogenic and land-use impacts.

While the pasteurization process entails a specific “energy cost,” reflected by a 7.5% increase in Cumulative Energy Demand (CED) and higher impacts in electricity-sensitive categories such as Ionizing Radiation, this is offset by a reduction in the environmental footprint of the raw milk supply. Specifically, the higher milk-to-cheese conversion rate of the pasteurized process reduces the total amount of milk required per unit of final product, thereby lowering the impact associated with the upstream agricultural phase (dairy farming).

Given the impact of electricity consumption, strategic investments in renewable energy—such as photovoltaic systems—and the adoption of high-efficiency thermal recovery systems are strongly recommended to mitigate the energy penalty associated with heat treatments. Implementing these measures could substantially reduce the carbon footprint of Mozzarella production from pasteurized milk.

With respect to the packaging stage, impact categories remained largely unchanged regardless of the modified atmosphere packaging (MAP) configuration (Vacuum, Air, or Nitrogen). This indicates that the packaging phase contributes negligibly to the overall environmental impact compared to the upstream milk acidification process.

Even when extending storage times from 30 to 90 days, no differences were observed in the environmental impacts of frozen curd, likely due to the small volumes used in our pilot study. Furthermore, this study fills a significant gap in the current LCA literature, as no studies have investigated the environmental implications of different packaging atmospheres in curd and Mozzarella production. Consequently, producers can optimize—and, if necessary, select—the MAP packaging system based on sensory quality and food safety requirements without causing significant environmental trade-offs.

However, it is important to recognize that these findings are derived from a pilot-scale study. While the results provide a clear environmental direction, further scaling up of production on an industrial scale should be considered. The transition from the pilot phase to large-scale production could introduce different energy efficiencies, logistical complexities and potential economies of scale that could reshape the magnitude of the identified impacts.

In conclusion, these findings offer concrete insights for industry stakeholders and researchers committed to supply chain sustainability. As resource efficiency becomes a global priority, this study provides a solid scientific basis for guiding decision-making in dairy production, balancing technological innovation and environmental responsibility.

6. Technology Transfer and Validation of Pilot-Scale Protocols in an Industrial Dairy Plant

This chapter focuses on the technology transfer phase, presenting the validation of the protocols developed at the pilot scale within an industrial setting. The study was conducted at "Società Cooperativa Agricola Coprover" (Baragiano, PZ, Italy) to assess the scalability and industrial viability of the optimized curd production processes. The primary objective of this validation was to characterize fresh and frozen curd samples and study their shelf life in relation to production parameters. Specifically, the study aimed to (Figure 43):

- Evaluate chemical and physical characteristics: Analyze the rheological parameters and oxidation/proteolysis indicators of fresh curd (stored up to 14 days) and frozen curd (stored up to 3 months);
- Propose an optimized protocol: Define guidelines for curd production, storage, and distribution to enhance the final product's quality and ensure the economic sustainability of the process.

Technology Transfer, validation of Pilot-Scale Protocols in an Industrial Dairy Plant

OBJECTIVES

Characterization of fresh and frozen curd samples
Propose an optimized protocol

1. Evaluate chemical and physical characteristics.
2. Define guidelines for curd production, storage, and distribution to enhance the final product's quality and ensure the economic sustainability of the process.

Figure 43: Overview of the processing steps and equipment used to optimize the curd supply chain at the COPROVER dairy plant.

6.1 Experimental design and sampling

On May 5, 2025, the dairy plant "Coprover" processed approximately 6,000 liters of cow's milk. The milk was subjected to thermization (68°C for 15 s), followed by inoculation with LYOBAC-D MO3D Rotation 085 (Alce Group) and the addition of Chimax Supreme 200 Rennet (Hensen - 200 IMCU; 20 ml/100 kg).

Approximately 10 kg of curd, prepared according to the dairy's standard operating procedures and ready for stretching (pH 5.24), were collected and transported, under suitable conditions, to the MacLab, DAFE (Department of Agricultural, Food and Forestry Sciences), University of Basilicata.

Upon arrival at the laboratory, the curd was divided into different experimental groups to evaluate various storage conditions and packaging systems. The sampling plan and storage durations are detailed in Table 24.

Table 24: Experimental design and sampling schedule for fresh, refrigerated, and frozen curd validation.

	Description	**Packaging System**	**Storage Temperature**	**Sampling Times (day)**
Fresh	Fresh (control)	None	N/A (immediate)	t0
R-	Refrigerated	None	5±1°C.	t3; t7; t14
R-V	Refrigerated Vacuum	V - Vacuum	5±1°C.	t3; t7; t14
F-V	Frozen Vacuum	V – Vacuum	-18°C	t30; t60; t90

Analyses on fresh curd were performed at t0. Refrigerated samples (R and R-V) were analyzed after 3, 7, and 14 days of shelf life. The frozen samples (F-V) were stored for 30, 60, and 90 days. Prior to analysis, the frozen curd was subjected to a slow thawing process at +4°C for 12 hours.

6.1.1 Analytical Methods

All analyses were conducted in triplicate on the samples described in the experimental design.

Various parameters were evaluated:

- Chemical composition;
- pH evolution and titratable acidity;
- Color;
- Curd consistency, compressive and shear strength (with Texture Analyzer), extensibility;
- Proteolytic activity;
- Fat/peroxide rancidity;
- Melting test;
- Microbiological screening.

The chemical composition of the curd samples (moisture, protein, fat, lactose, ash, carbohydrates, salt) and the fatty acid profile, specifically saturated fatty acids (SFA), unsaturated fatty acids

(UFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA), were determined using FoodScan (FOSS Analytical, Italy). Chemical composition values are expressed as a percentage (g/100g of cheese), while fatty acids and peroxide values are expressed as a percentage of fat. The dry matter content was determined gravimetrically (105°C, 24 h). The pH value was determined using a portable IP57 pH meter (XS Instruments, Italy) equipped with a combined electrode and integrated temperature probe (Model 2 POREF Temp BNC DHS). Color measurements according to CIELAB system: L* (brightness), a* (red trend) and b* (yellow trend) on three different points of each curd and Mozzarella sample was assessed using a portable SA130 colorimeter (SAMA, Italy).

Rheological characterization was performed using a TA texture analyzer (Instron 3343 single column universal, Instron Corp, Canton, Massachusetts, USA). A double compression test, as described by (Alinovi and Mucchetti 2020), was performed using with a cylindrical stainless steel probe (35 mm diameter) and a crosshead speed of 1.5 mm/s was applied to compress a cylindrical samples of 25 mm diameter. Additionally, a cutting test as described by (Reid and Yan 2004), was performed on a rectangular sample (25x50x25 mm) with a constant speed of 0.5 mm/s.

Using a tensile dynamometer equipped with clamps, the extensibility of the curd was assessed by determining the maximum load (N) required to break the sample. A standard rectangular curd sample (1,5 × 4 cm) was defined and immersed in hot water at 90°C for 3 minutes. The sample was positioned and clamped to the measuring instrument and subjected to extensibility analysis.

Meltability was assessed through a modified version of the Schreiber test (Drake et al. 1999; Reid and Yan 2004). Cylindrical samples (8 mm thick; 35 mm in diameter) were placed on aluminum foil, measuring the change in diameter before and after heating at 100 °C for 10 minutes. Proteolytic activity was quantified using the o-phthaldialdehyde (OPA) method (Church et al. 1983). A sample (5 g) was homogenized with 2 mL of water and 25 mL of 0.75 N trichloroacetic acid (TCA) and filtered through cotton wool. The filtrate (100 µL) was mixed with 2 mL of OPA reagent in quartz cuvettes, after 2-minute incubation, absorbance at 340 nm was measured (Ultrospec 2100 Pro, Biochrom Ltd, Cambridge, UK).

For microbiological analyses, curd samples were homogenized with 90 mL of sterile sodium citrate (2% w/v) using a Stomacher 400 Lab Blender (International PBI, Milan, Italy) for 2 minutes. Serial decimal dilutions were subsequently prepared in the same solution. To evaluate the starter culture (LYOBAC MO3D- Streptococcus thermophilus, Alce group), thermophilic streptococci were evaluated on M17 agar supplemented with 10 g/L of lactose (LM17) and subsequently incubated at 42°C for 48 hours, under anaerobic conditions (AnaeroGen and AnaeroJar, Oxoid, Basingstoke, Hampshire, UK). Total mesophilic bacteria (Plate Count Agar, PCA; 30°C, 48 hours, aerobic) and coliforms (Violet Red Bile Agar, VRBA; 30°C, 24 hours, anaerobic; ISO 4832 (ISO, 2006c)) were also evaluated. After incubation, colonies were counted using a digital colony counter (EasyCount 2, bioMérieux Italia).

Data processing was performed using JASP Team (2025) [JASP (Version 0.95.1)]. The objective of the statistical processing was to evaluate the quality of the curd considering different qualitative, chemical-physical, rheological and microbiological parameters, measured at different times and following the application of different treatments. The statistical analysis considered two factors:

- Treatment factor (4 levels: Fresh; R, Refrigerated; R-V, Refrigerated-vacuum; F-V, Frozen vacuum);
- Time factor (7 levels: 0, 3, 7, 14, 30, 60, and 90 days after treatment).

A descriptive analysis was performed, with graphs showing the trends for each variable (parameters analyzed). An inferential analysis was then performed to verify whether treatment, time, and their interaction had significant effects on each parameter ($p < 0.05$).

6.2 Results

The graphs presented in this section show the curd analytical parameters measured. Two types of graphical representations were generated:

- Bar plots, which depict the trends of each parameter as a function of time and treatment;
- Box plots, which illustrate the distribution of each parameter in relation to the specific treatment.

Statistically significant differences are indicated in the graphs using uppercase letters (e.g., A, B). Values marked with different letters indicate significant statistical differences ($p < 0.05$) between samples subjected to the various treatments and storage times.

6.2.1 Color Analysis

The variation in brightness (L*) is reported in Figure 43 and 44. Sample of curd F-V t90, (curd which after production was chilled, then vacuum-packed and finally stored frozen for 90 days) showed statistically significant values compared to fresh curd (Fresh t0) (Figure 44).

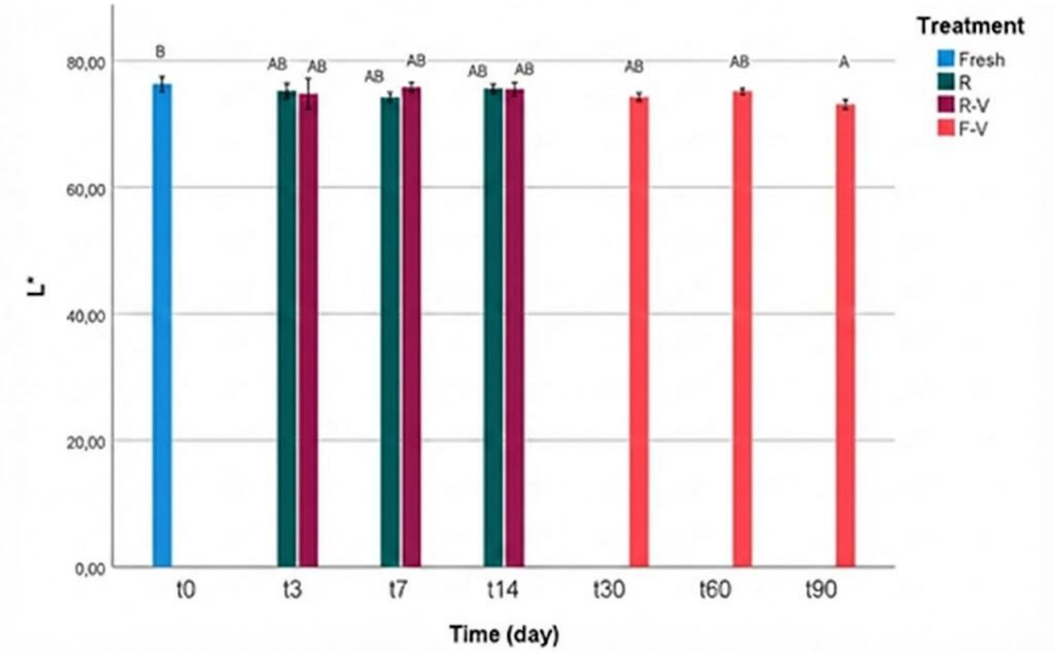

Figure 44: Variation of brightness (L) as a function of Time and Treatment.*

By evaluating the brightness parameter L* in relation to the treatment (Figure 45), significant differences emerged between the Fresh curd compared to the frozen F-V curd. In general, the fresh samples showed higher L* values than the frozen ones.

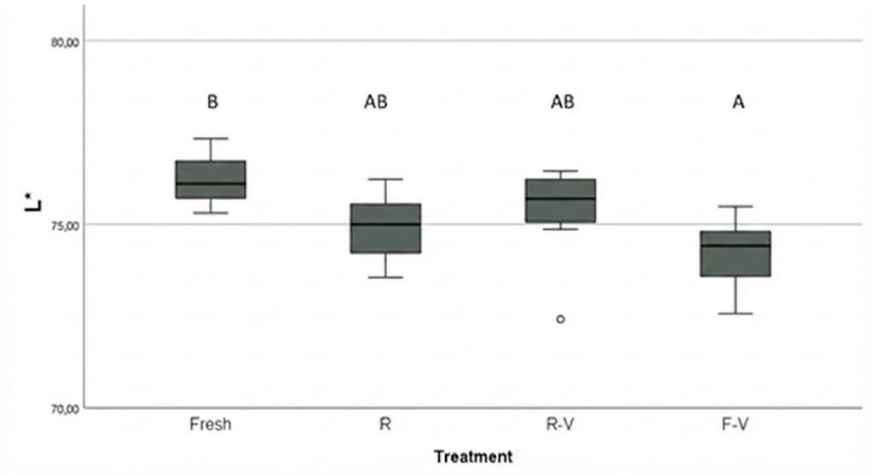

Figure 45: Variation of brightness (L) as a function of Treatment.*

No significant differences were observed in the other colorimetric parameters of the curd (a*, red/green; b*, blue/yellow). This indicates that the color, an important characteristic for the visual acceptability of the product, does not undergo marked alterations either due to the effects of treatments or time, with the exception of a slight darkening after prolonged freezing.

6.2.2 Chemical Parameters

The initial pH value of fresh curd-t0 was 5.23 (Table 25).

Table 25: pH evolution as a function of treatment (values expressed as mean ± standard error).

Treatment	pH value
Fresh	5.23 ± 0.009^{A}
R	5.16 ± 0.005^{B}
R-V	5.18 ± 0.012^{B}
F-V	5.19 ± 0.007^{B}

Values in the same column with different letters (A-B) differ significantly ($P < 0.05$).

Figure 46 show that pH varied with both the type of treatment and the storage time.

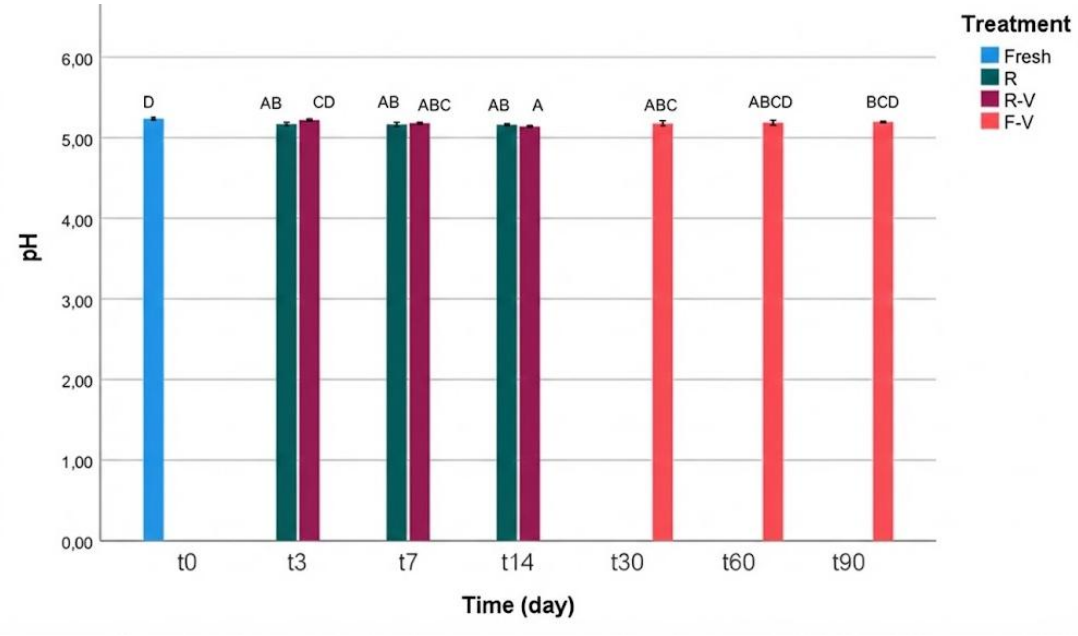

Figure 46: pH variation as a function of time and treatment.

A slight decrease in pH over time was observed in the refrigerated samples (R and R-V). This phenomenon can be attributed to the metabolic activity of residual acidifying microorganisms. In contrast, the frozen-vacuum treatment (F-V) maintained stable pH values throughout the entire storage period. Therefore, when evaluating the overall effect of the Treatment (Figure 47), the pH of the fresh curd (Fresh-t0) differed statistically from all other treatments.

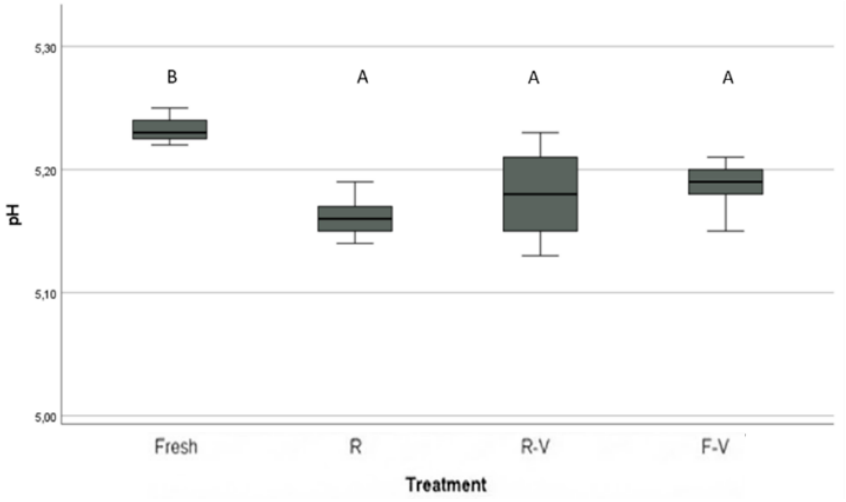

Figure 47: pH variation as a function of treatment.

This trend differs from that reported by (Rinaldi et al. 2021). In their study evaluating the impact of mixing frozen and fresh curd on buffalo Mozzarella quality, they observed significantly different pH values between fresh (4.97) and frozen curd (5.13). Furthermore, a study on buffalo milk curds (Pandolsook and Tungjaroenchai 2023) highlighted a correlation between curd pH and its structural characteristics; specifically, Mozzarella obtained from curd stretched at pH 5.1 exhibited greater hardness, chewiness, and extensibility compared to that stretched at pH 4.9.

Titratable acidity is an important indicator for assessing curd shelf life and appears to play a crucial role in flavor development and microbial stability (Akhtar et al. 2025). Figure 48 shows the evolution of titratable acidity, expressed as a percentage of lactic acid, over time and as a function of different storage treatments.

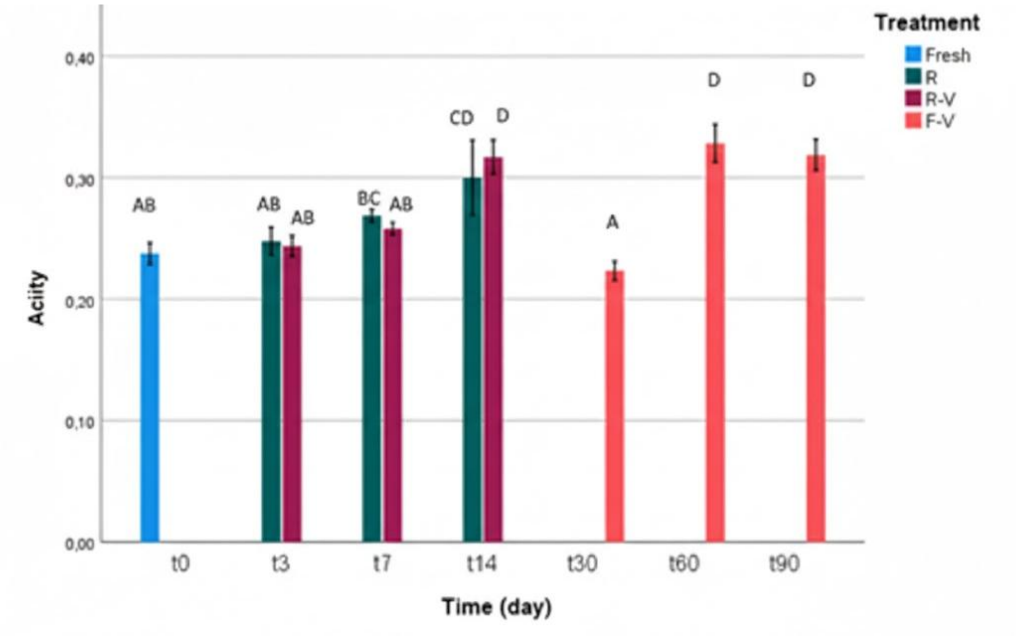

Figure 48: Variation of titratable acidity (% lactic acid) as a function of time and treatment.

Samples stored under refrigeration, both with and without vacuum packaging, showed a progressive increase in acidity over the 14 days of storage. Freezing, however, effectively halted the acidification process only in the first 30 days. However, at 60 and 90 days, a significant increase in acidity was observed, likely related to residual enzymatic activity at low temperatures or reactivation after thawing. The increase in lactic acid may be due to the growth of microorganisms that break down lactose (Marrella et al. 2023). Acidity and pH are often correlated: the decrease in pH could be due to the breakdown of lactose residues by lactic acid bacteria (Ruiz et al. 2025). The late increase in acidity in frozen samples is interesting, as it suggests that, while freezing is effective for short- and medium-term storage, prolonged storage may still lead to a deterioration in quality.

Dry matter content analysis revealed significant differences both as a function of time and treatment (Figure 49), with opposing trends depending on the treatment (Figure 50).

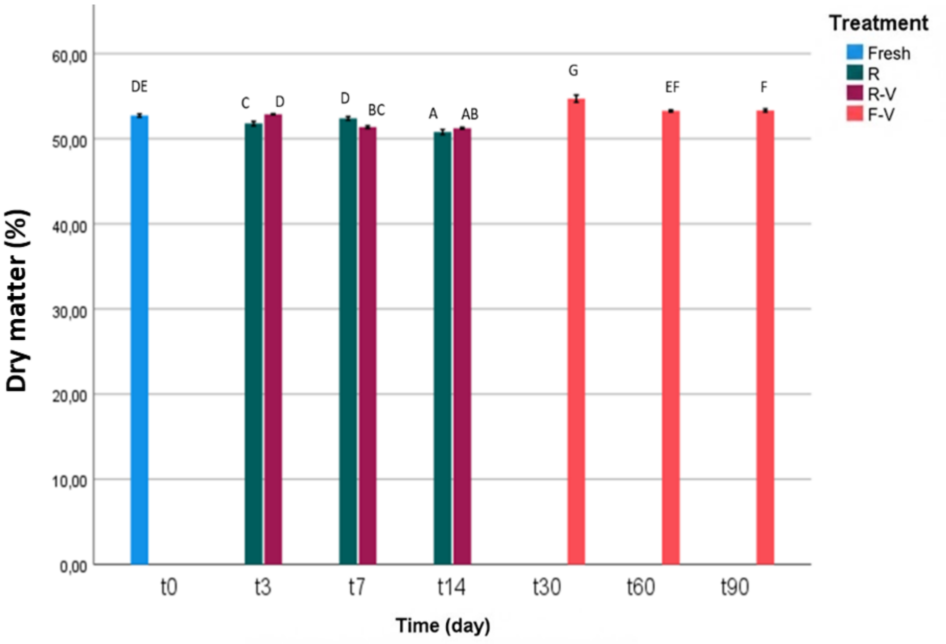

Figure 49: Variation in dry matter (%) as a function of time and treatment.

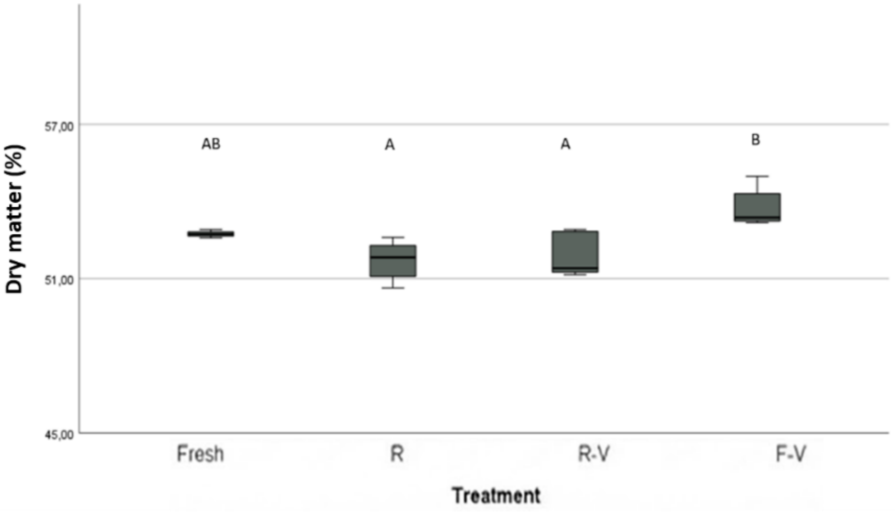

Figure 50: Variation of dry matter (%) as a function of treatment.

In refrigerated samples (R), dry matter decreases after 14 days, while in samples refrigerated under vacuum (R-V), the decrease is evident after just 7 days. In samples subjected to freezing, a slight increase in dry matter is observed at 30 days, followed by a decrease at 60 and 90 days. Considering the effect of the treatment (Figure 50 and Table 26), the frozen samples have higher dry matter values than the refrigerated ones, but not significantly different from the fresh curd.

Table 26: Evolution of Dry matter (%), Moisture (%), Protein (%), Fat (%), Ash (%) as a function of treatment (values expressed as Mean ± Standard Error).

Treatment	Dry matter (%)	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
Fresh	52.74 ± 0.245^{AB}	47.26 ± 0.092^{AB}	21.94 ± 0.213^{B}	28.05 ± 0.031^{A}	0.633 ± 0.117^{A}
R	51.67 ± 0.093^{A}	48.33 ± 0.243^{A}	21.26 ± 0.187^{AB}	27.89 ± 0.054^{A}	0.533 ± 0.138^{A}
R-V	51.83 ± 0.265^{A}	48.17 ± 0.265^{A}	20.79 ± 0.118^{A}	27.97 ± 0.106^{A}	0.711 ± 0.059^{A}
F-V	53.78 ± 0.245^{B}	46.22 ± 0.245^{B}	21.34 ± 0.107^{AB}	29.02 ± 0.087^{B}	1.156 ± 0.028^{B}

Values in the same column with different superscript letters (A-B) differ significantly ($P < 0.05$).

This trend differs from the findings reported by (Rinaldi et al. 2021), who observed a decrease in dry matter between fresh and frozen curds (56.6% vs. 53.4%). In our experiment, freezing instead led to an increase in dry matter, resulting in a reduction in curd hardness and changes in relative fat content. According to (Alichanidis et al. 1981), the lower moisture content in cheese made with frozen curds could be due to changes in the structure of casein micelles. These changes reduce the curd's ability to retain whey, partially explaining the observed decrease in moisture.

The analyses performed showed that the preservation treatment significantly influenced the protein content of the curd (Table 26) . In particular, the fresh curd (21.94%) showed higher values than all preservative treatments, significantly different from the R-V curd (20.79%) and still higher than the R (21.26%) and F-V (21.34%) samples.

Reduction observed in the protein content of the stored samples can be attributed to proteolysis, which causes the degradation of complex proteins into peptides and aminoacids. Regarding protein content, our data confirm the observations of Rinaldi et al. (2021), who reported a reduction in protein in frozen curd compared to fresh curd.

Fat content was significantly affected by the storage treatments (Table 26) and storage time.

The oxidation of fats and the potential for rancidity were measured by the peroxide value in all samples. The values measured were always below the detection limit (<0.01), regardless of storage treatment or time. This indicates that during the period analyzed, no detectable quantities of primary lipid oxidation products accumulated. However, it should be noted that the peroxide value. However, the peroxide value describes only the initial phase of oxidation, while in more advanced stages, values may decrease due to the degradation of peroxides into secondary compounds (e.g., aldehydes, ketones). Therefore, the absence of changes in peroxide values does not necessarily imply the absence of oxidative processes, but rather indicates that primary lipid degradation was not detected with this method. Looking ahead, the use of complementary markers (e.g., TBARS for secondary oxidation products) could provide a more comprehensive assessment of rancidity status.

Ash content, a key parameter for the nutritional and functional quality of the product, provides an indication of the total amount of minerals and other inorganic components present in the curd. Frozen samples show higher ash levels compared to all others (Table 26). This result is related to the moisture content of the samples, which directly influences the relative ash concentration. In fact, the vacuum-frozen samples (F-V) showed lower moisture values (46.22%) than the fresh (47.26%) and refrigerated (R: 48.33%; R-V 48.17%) samples. The reduction of water in the frozen samples could have determined a higher apparent concentration of minerals, explaining the increase in ash content observed over time. However, our data differ from what reported by (Rinaldi et al. 2021), who instead observed a decrease in fat and ash content in the frozen curd compared to the fresh one.

Analysis of fatty acid content showed no statistically significant differences as a function of time or treatment. The stability of these values suggests that during the different treatments (R, R-V, F-V), oxidative degradation processes during storage were minimal, without compromising the nutritional quality of the curd. Statistical analyses revealed no significant differences in salt, carbohydrate, and calcium content as a function of treatment and storage time.

6.2.3 Rheological and texture parameters

Cohesiveness represents the ability of the product to resist deformation and maintain its internal structure. Refrigerated curd showed a general decreasing trend over time, more evident from t7 day of storage (Figure 51). This decline in cohesiveness in refrigerated samples may be linked to progressive acidification. Regarding the F-V curd, a decrease was observed after 60 and 90 days of storage compared to the fresh curd.

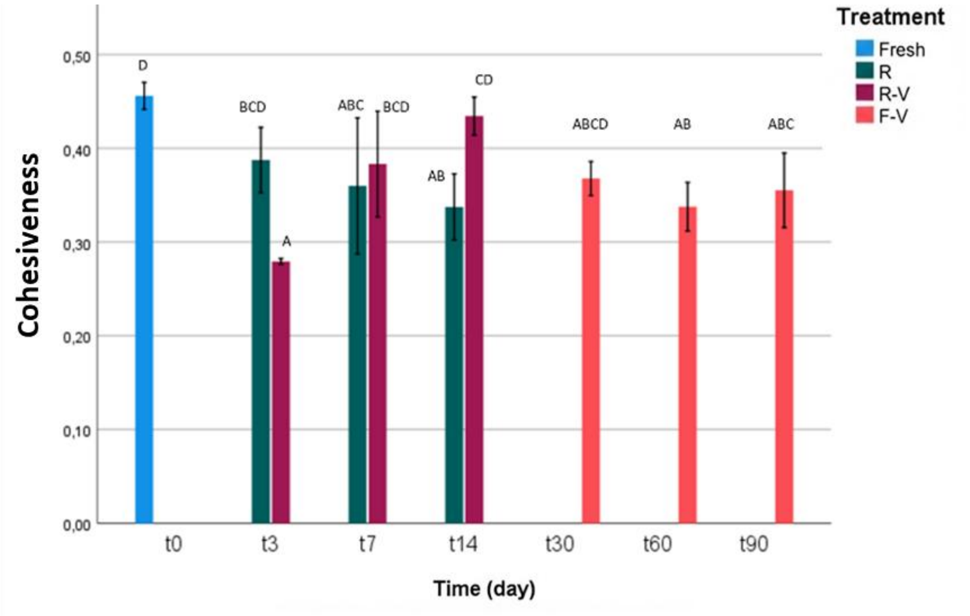

Figure 51: Variation of Cohesiveness as a function of time and treatment.

Evaluating the effect of the Treatment (Figure 52), the cohesiveness values of fresh curd were statistically different from all other treatments; therefore, both refrigeration and freezing negatively influence the structural stability of the curd and the quality of the final product.

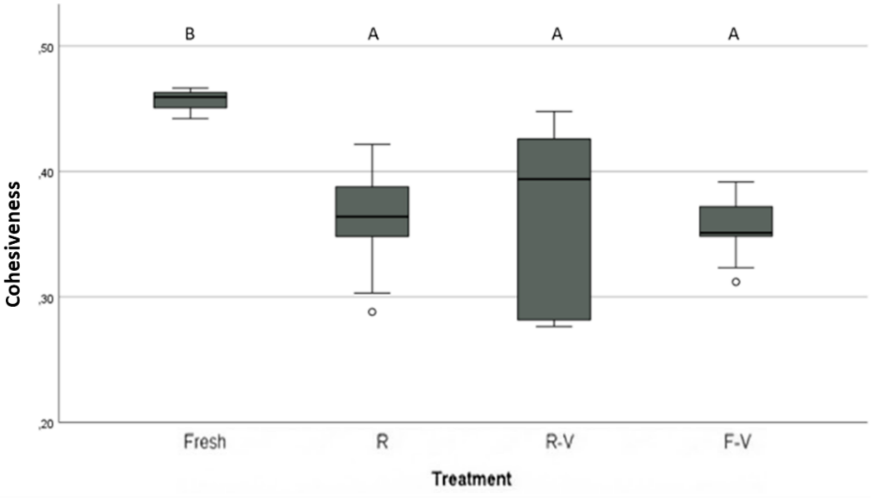

Figure 52: Variation of Cohesiveness as a function of treatment.

Hardness reflects the force required to deform the curd and is expressed as the maximum peak force at the first compression. A significant increase in hardness was observed in R-V curd samples analyzed after 3 days of storage compared to all other samples (Figure 53).

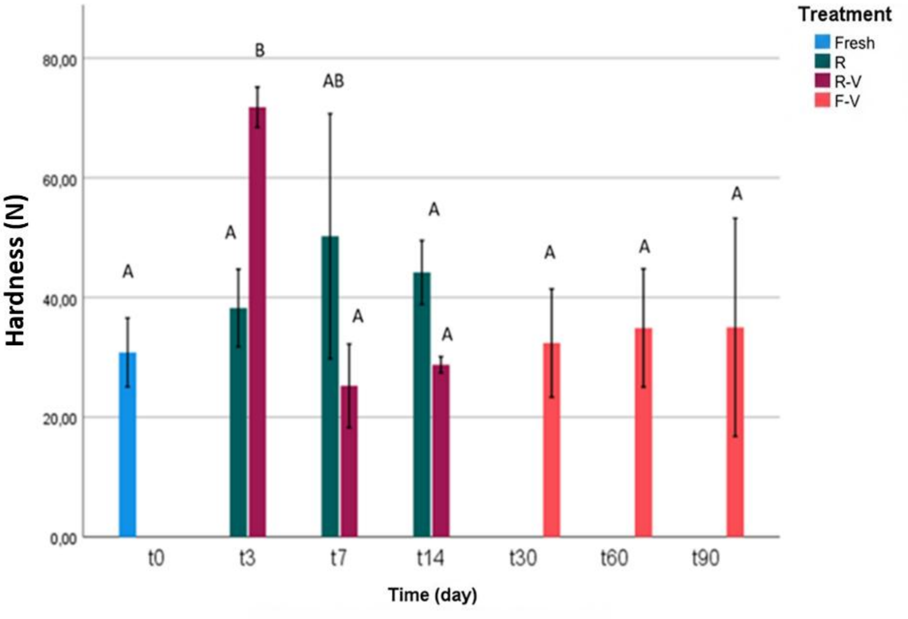

Figure 53: Evaluation of Hardness (N) as a function of time and treatment.

This hardness value could suggest that vacuum packing, combined with refrigeration, may rapidly induces a change in the curd structure, making it stiffer and more compact. However, no significant differences emerged when evaluating the hardness parameter across the different treatments.

Texture tests on the curd showed that elasticity and chewiness were not significantly affected by either the preservation treatment (fresh, refrigeration, vacuum refrigeration, vacuum freezing) or the storage time. These parameters, which describe the product's ability to return to its original shape after deformation and the energy required to disintegrate it to a state ready for swallowing, respectively, remained unchanged in all samples analyzed.

Chewiness measures the energy required to chew a solid food to a state ready for swallowing. After 3 days, R-V samples showed a significant increase in chewiness compared to R-V samples analyzed on days 7 and 14 of storage, and compared to all F-V samples at all different storage times (Figure 54).

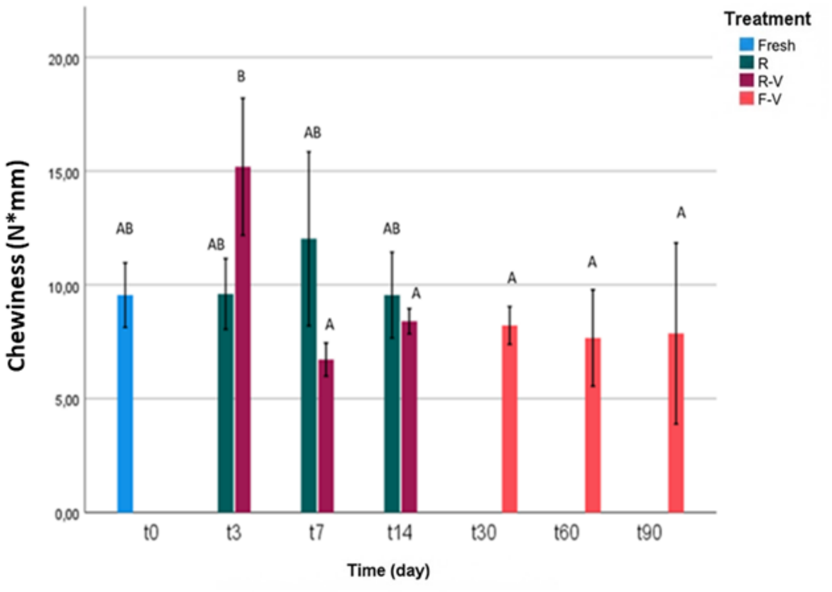

*Figure 54: Evaluation of Chewiness (N*mm) as a function of time and treatment.*

Frozen samples showed greater long-term chewiness stability compared to refrigerated samples. However, no significant differences emerged when evaluating the chewiness parameter as a function of the different treatments.

Extensibility measures the curd's ability to be stretched without breaking. It is a key parameter for evaluating the workability of curds intended for the production of pasta filata cheeses. The fresh curd showed significantly higher extensibility values than all other curds subjected to the various treatments (Figure 55) and analyzed at different times (Figure 56).

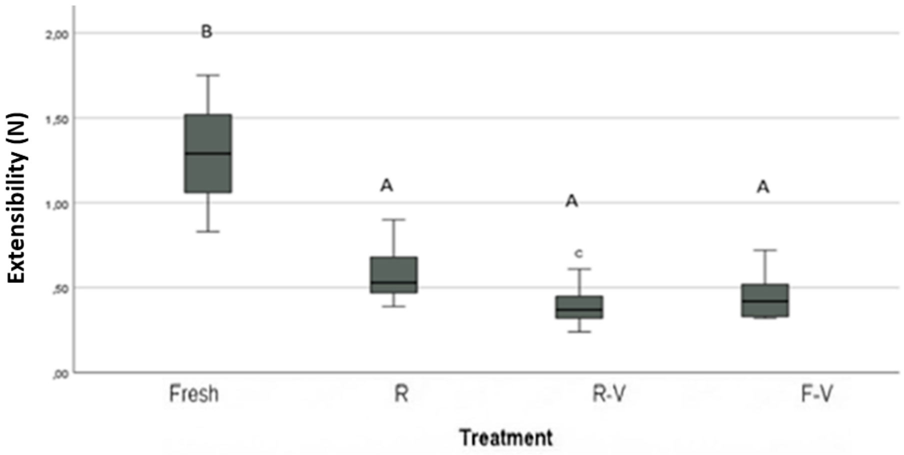

Figure 55:Evaluation of Extensibility (N) as a function of treatment.

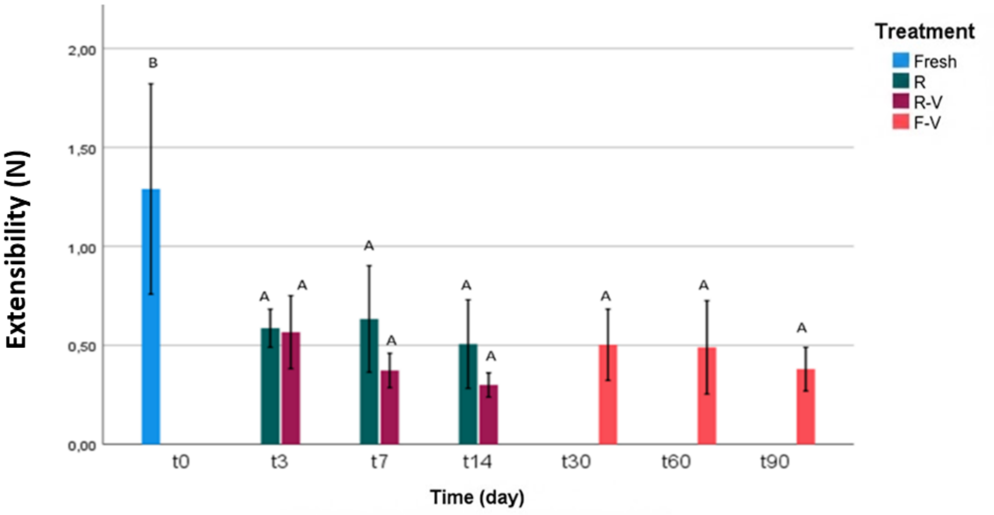

Figure 56:Evaluation of Extensibility (N) as a function of time and treatment.

The t0-Fresh curd was visually more elastic, reaching greater heights before reaching the breaking point, which was characterized by progressive fraying. In contrast, the R, R-V, and F-V samples were structurally harder, more compact, and less elastic; consequently, fracture occurred abruptly with evident cracks throughout the sample. This suggests that both refrigeration and freezing negatively affect the curd's structural stability, its ability to stretch, and therefore the quality of the final product.

The shear test measures the maximum force required to cut a curd sample, providing an indication of its internal cohesion and resistance to fracture. The fresh curd showed significantly higher shear resistance values compared to all other curds subjected to the different treatments (Figure 57) and analyzed at different times (Figure 58).

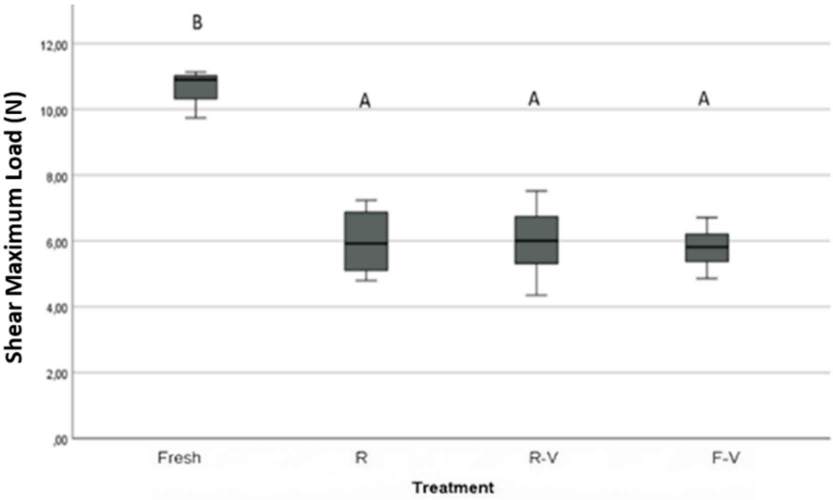

Figure 57: Variation of Shear Maximum Load (N) as a function of treatment.

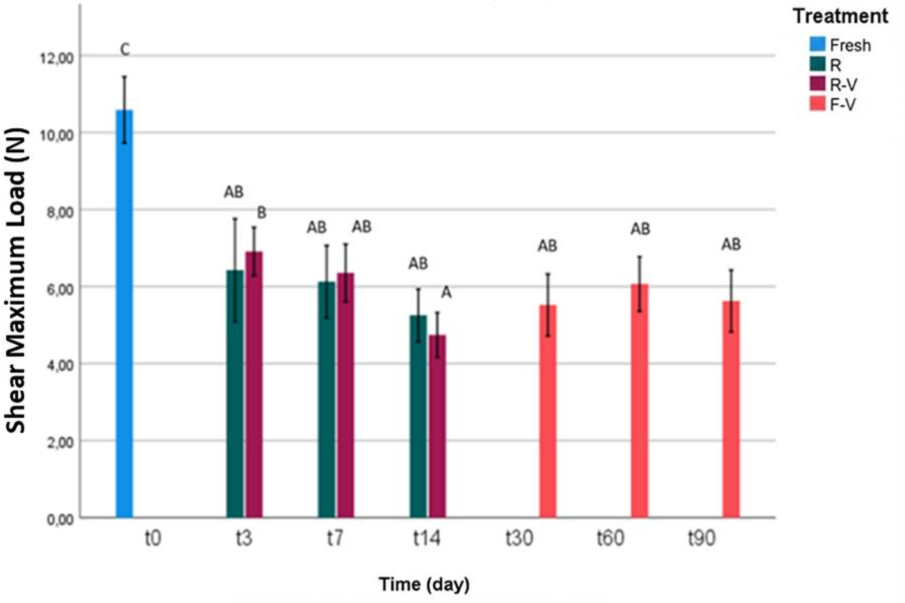

Figure 58: Variation of Shear Maximum Load (N) as a function of time and treatment.

The progressive loss of shear strength, evident over time especially in the R and R-SV samples, could be linked to the continued acidification that made the curd softer and more fragile. The frozen samples showed a different trend, with shear strength values that, although lower than those of the fresh curd, remained relatively stable over time.

Table 27 summarizes the main textural parameters analyzed, as a function of treatment.

Table 27: Evolution of the main rheological parameters as a function of treatment (values expressed as Mean ± Standard Error).

	Fresh	**R**	**R-V**	**F-V**
Water Retention (%)	1.733 ± 0.278^{BC}	0.674 ± 0.077^{A}	0.981 ± 0.157^{AB}	1.736 ± 0.230^{C}
Cohesiveness	0.457 ± 0.00 ^{A}	0.361 ± 0.014^{B}	0.366 ± 0.024^{B}	0.352 ± 0.009^{B}
Hardness (N)	30.83 ± 2.871^{A}	44.24 ± 3.632^{A}	41.95 ± 7.573^{A}	34.11 ± 3.292^{A}
Chewiness (N*mm)	9.550 ± 0.709 ^{A}	10.396 ± 0.771A	10.107 ± 1.372^{A}	7.919 ± 0.665^{A}
Springiness (mm/mm)	0.683 ± 0.006 ^{A}	0.658 ± 0.018^{A}	0.713 ± 0.022^{A}	0.670 ± 0.030^{A}
Gumminess (N)	14.02 ± 1.108 ^{A}	15.70 ± 0.811^{A}	14.02 ± 1.591^{A}	11.88 ± 0.901^{A}
Extensibility (N)	1.290 ± 0.266 ^{A}	0.576 ± 0.056^{B}	0.413 ± 0.050^{B}	0.458 ± 0.050^{B}
Shear test (N)	10.590 ± 0.211 ^{A}	5.938 ± 0.310^{B}	6.006 ± 0.363^{B}	5.740 ± 0.211^{B}

Values in the same row with different superscript letters (A-C) differ significantly ($P < 0.05$).

The Schreiber melting test, which evaluates a cheese's ability to melt and stretch, revealed that the diameter of the melted curd disk remained virtually unchanged in all samples analyzed. Melting tests conducted on the curd did not show any significant influences due to either the storage treatment (fresh, refrigeration, vacuum refrigeration, vacuum freezing) or the storage time. The stability of this parameter indicates that, regardless of the storage method and duration, the curd maintained a uniform melting ability.

6.2.4 Proteolytic Activity

The proteolytic activity of the curd, assessed using the OPA method, provides information on protein degradation during storage. The average initial OPA value for the fresh curd was approximately 0.10, indicating a low level of protein degradation at the start of the experiment (Figure 59).

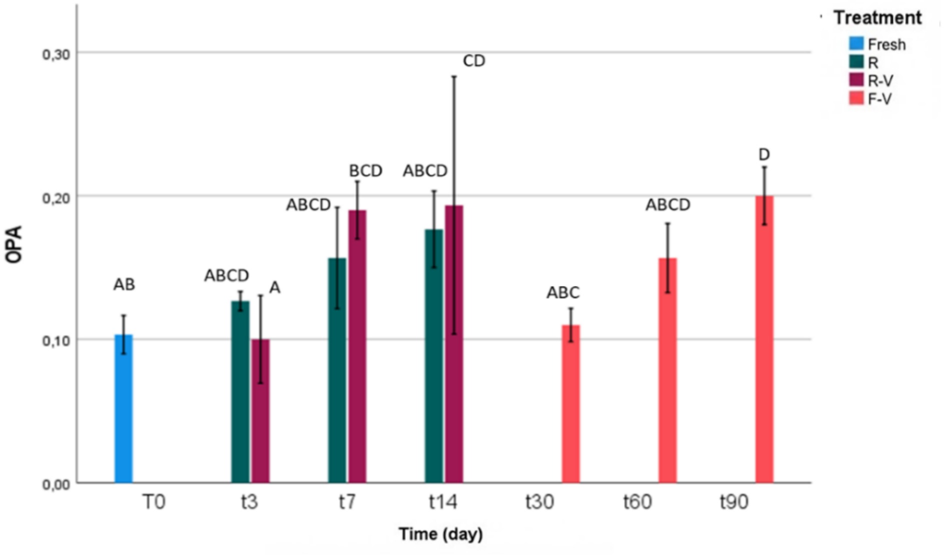

Figure 59: Evaluation of Proteolysis (OPA) as a function of time and treatment.

Samples stored under refrigeration, both with and without vacuum, with the exception of analyses performed after 3 days of storage, showed a tendency for proteolytic activity to increase with increasing storage time. The F-SV treatment maintained protein degradation levels similar to the fresh product for up to 60 days of storage, with significant differences only after 90 days. However, no significant differences emerged when evaluating the OPA parameter as a function of the different treatments.

6.2.5 Microbiological Parameters

Microbiological analyses were conducted to evaluate the effect of different packaging methods on the bacterial flora of the curd.

The results demonstrated effective hygienic management of the production process, as coliforms were not detected in any analyzed sample (< 99 CFU/mL). Regarding the starter culture (Streptococcus thermophilus), its survival was influenced differently by the treatments (Figure 60).

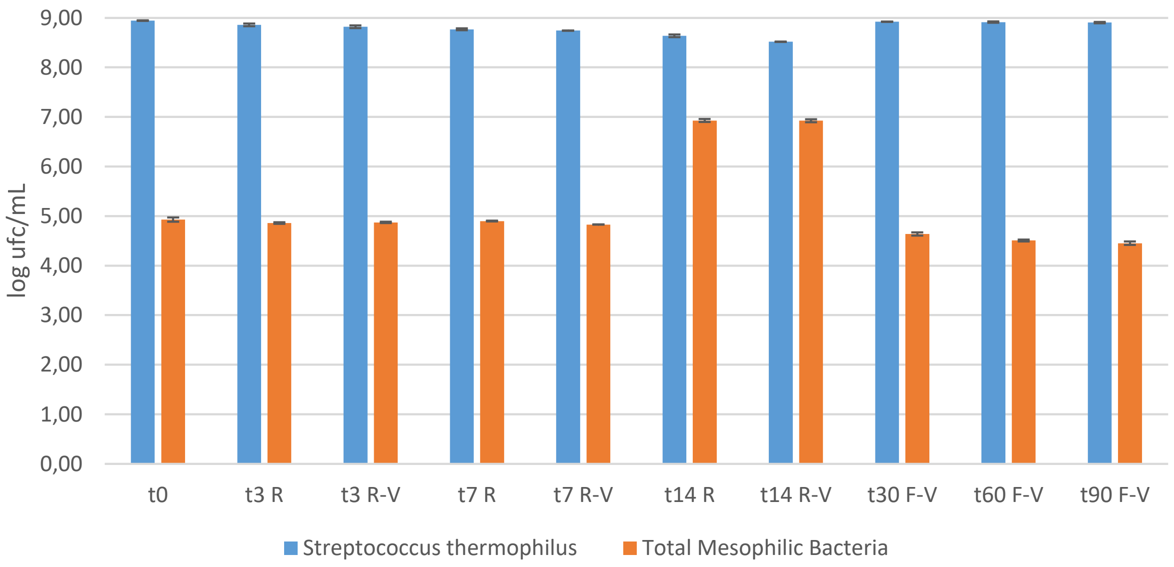

Figure 60: Evolution of the starter culture (Streptococcus thermophilus, blue bars) and Total Mesophilic Bacteria (orange bar), expressed as log10 cfu/g in the different curd samples.

Refrigeration, with or without vacuum packaging, slightly reduced the survival of the starter culture. A reduction of 0.31 log cycles was observed for the t14 R sample, while a reduction of 0.43 log cycles was observed for the t14 R-V sample. No reduction in starter culture survival was observed for the curd samples chilled, vacuum-packed, and stored at -18°C for 30, 60, and 90 days (t30 F-V, t60 F-V, t90 F-V). Although a slight reduction in Streptococcus thermophilus population (starter culture) was evident, the viability of the starter culture was not significantly affected by the preservation methods used.

The total mesophilic bacterial population showed opposite trends between the two preservation methods. In the refrigerated samples, the population increased significantly (2 log cycles) after 14 days (t14 R and t14 R-V), likely due to the extended storage time at refrigeration temperature.

In contrast, in the F-V treatment, a progressive reduction in the total mesophilic bacterial population was observed, specifically a reduction of 0.29 log cycles at 30 days, 0.42 log cycles at 60 days, and 0.48 log cycles at 90 days.

6.3 Conclusion

The experimental analysis allowed for the evaluation of the impact of preservation methods on curd quality, with the aim of identifying suitable preservation strategies under the specific conditions investigated. The results highlighted a significant influence of both storage time and treatment on the physicochemical, rheological, and microbiological characteristics of the curd.

Compared to Fresh untreated curd, the treatment designated as F-V (blast chilling, vacuum packaging, and storage at -18°C) showed the most favorable product characteristics over the long term among the methods tested. Specifically, despite a reduction in rheological parameters, vacuum freezing ensured good structural uniformity, limited microbial activity, and stabilized pH and lactose content. Furthermore, peroxide values remained consistently below the detection limit, suggesting substantial lipid stability, although integrating analyses with secondary oxidative compounds is advisable for a more complete assessment of rancidity.

The structural and texture properties of fresh curd remain superior to those of any stored sample, whether refrigerated or frozen. Refrigeration, even when combined with vacuum packaging, did not prove adequate for prolonged preservation: degradation of structural properties was observed as early as the seventh day, characterized by increased acidity, hardness, and chewiness, and reduced cohesiveness, extensibility, and shear resistance.

Based on these results, it can be concluded that frozen-vacuum (F-V) preservation represents an effective solution within the experimental framework of this study for extending the shelf-life of curd for potential long-distance distribution or prolonged storage.

The adoption of a protocol involving rapid thermal blast chilling and vacuum packaging offers notable benefits in terms of maintaining quality, preventing deterioration, and optimizing the supply chain. The use of refrigeration can be considered, but only for short-term curd management, i.e., for a maximum of 3 days, during which values remain acceptable. This approach not only improves the quality of the final product but also contributes to the economic sustainability of the production process by reducing waste and expanding market opportunities.

7. General conclusion and future perspective

This doctoral research provides a comprehensive framework for advancing dairy process engineering, integrating laboratory-scale experimentation, life cycle assessment (LCA), and industrial validation. The study successfully demonstrates that blast chilling, MAP packaging, and storage at -18°C represent a robust technological solution for managing surplus milk and improving supply chain flexibility. The integration of thermal blast chilling with vacuum packaging not only preserves product quality but also aligns with Industry 5.0 goals, reducing food waste and optimizing resource efficiency. This data-driven approach offers small and medium-sized dairy farms a scientifically validated protocol for improving economic and environmental sustainability. While these findings provide a robust analytical basis, further research incorporating sensory analysis will be essential to validate the commercial applicability of these protocols and ensure consumer acceptance. Such evaluations will represent a key step in future developments to confirm that the observed physicochemical stability translates into a profile compatible with high-quality final products.

7.1 Experimental Findings

The comparative analysis of curd and Mozzarella reveals a complex synergy between raw material properties and storage conditions:

- Structural degradation: Shelf life is the main factor in structural degradation. For both intermediate products (curd) and final products (Mozzarella), a "technological tipping point" occurs at 60 days. Beyond this threshold, intense proteolysis (OPA) and lipid oxidation occur, worsening rheological characteristics.
- Influence of raw material: While time dominates the variance, milk type (raw vs. pasteurized) significantly determines acidification dynamics and mechanical parameters, especially in Mozzarella. Pasteurized milk variants (PM and PM-SL) exhibit a more intense acidification profile than raw milk samples.
- Packaging effectiveness: Protective packaging, particularly vacuum (V) or 100% nitrogen (N), better controls lipid oxidation rates. Samples packaged with air show accelerated peroxide degradation, compromising the functional quality of the cheese.

7.2 Life Cycle Assessment (LCA)

The LCA study highlights that the raw milk production phase is the main environmental critical point. Key sustainability insights include:

- Process efficiency: Although pasteurization requires an "energy cost," the increased processing yield translates into a 10% lower carbon footprint thanks to the reduced upstream impact of dairy farming.

- Impact of packaging and storage: The choice of modified atmosphere packaging (vacuum, air, or nitrogen) and the extension of shelf life from 30 to 90 days contributed negligibly to the overall environmental impact. This allows producers to prioritize sensory quality and food safety when choosing a packaging system, without significant environmental compromises.

7.3 Industrial Validation and Protocol Optimization

The transition to industrial scale at dairy “Coprover" validated the pilot-scale findings:

- Long-term Preservation Strategy: Under the experimental conditions, the Frozen-Vacuum (FV) method (blast chilling, vacuum packaging, and storage at -18°C) demonstrated the highest stability for long-term storage. This protocol effectively maintained structural uniformity, limited microbial activity, and stabilized pH and lactose content compared to the other tested methods.
- Short-term handling: Refrigerated storage, even when vacuum-packed, proved suitable only for short-term management (maximum 3 days). Significant structural degradation, increased acidity, and loss of shear strength were observed as early as the seventh day of refrigeration, limiting its shelf-life.

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9. Appendix A: Supplementary Data and Supporting Graphs

This section contains graphical representations of the data analyzed during the research. Since these data exhibit trends and dynamics similar to those already discussed and presented in Chapters 5 (LCA), this grouping ensures better greater flow in the main text while providing a comprehensive overview for in-depth consultation.

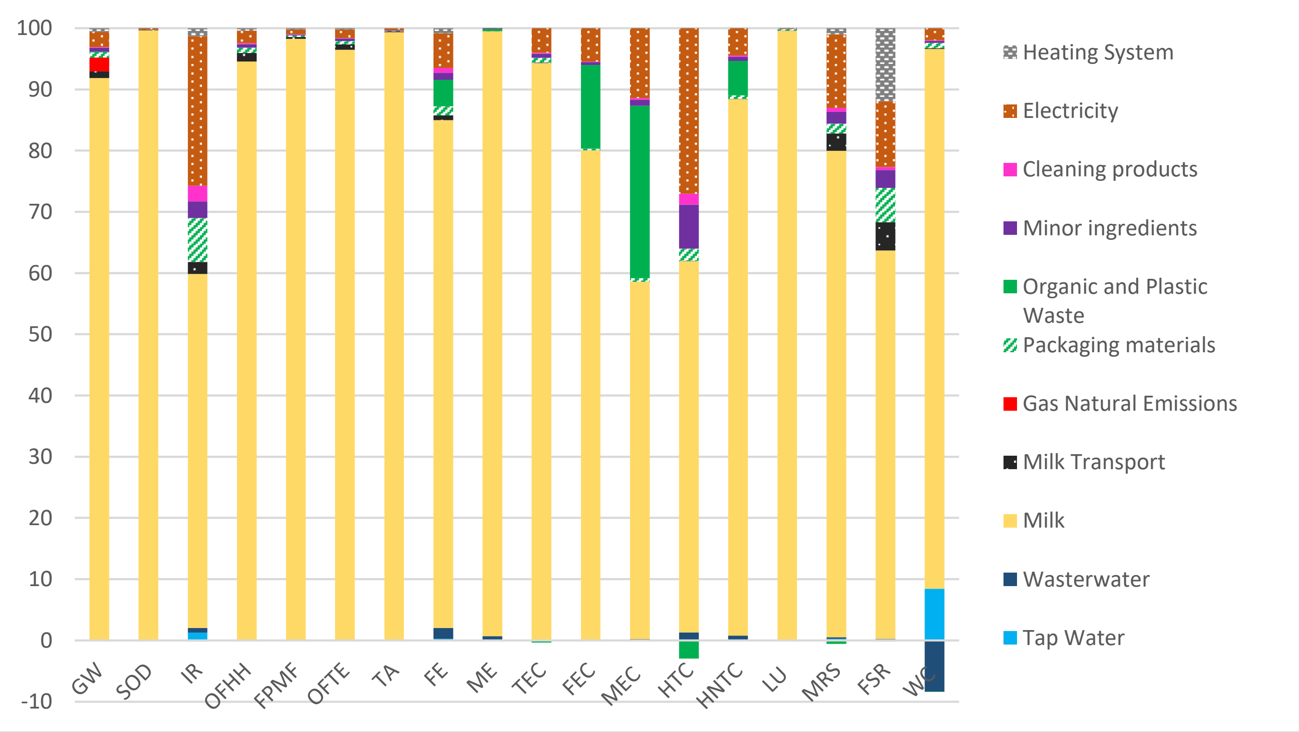

Figure 61: Characterization results obtained for Case E1 (RM-V-t60), Mozzarella by Raw Milk, MAP-Vacuum, storage time 60 day), using ReCiPe 2016 Midpoint (H) V1.11 method (FU: 1 kg Mozzarella). [GW: Global Warming potential; SOD: Stratospheric ozone depletion; IR: Ionizing Radiation; OFHH Ozone formation, Human health; FPMF Fine particulate matter formation; OFTE Ozone formation, Terrestrial ecosystems; TA: Terrestrial acidification; FE: Freshwater eutrophication; ME: Marine eutrophication; TEC: Terrestrial ecotoxicity; FEC: Freshwater ecotoxicity; MEC: Marine ecotoxicity; HCT: Human carcinogenic toxicity; HNCT: Human non-carcinogenic toxicity; LU: Land use; MRS: Mineral resource scarcity; FRS: Fossil resource scarcity; WC: Water consumption].

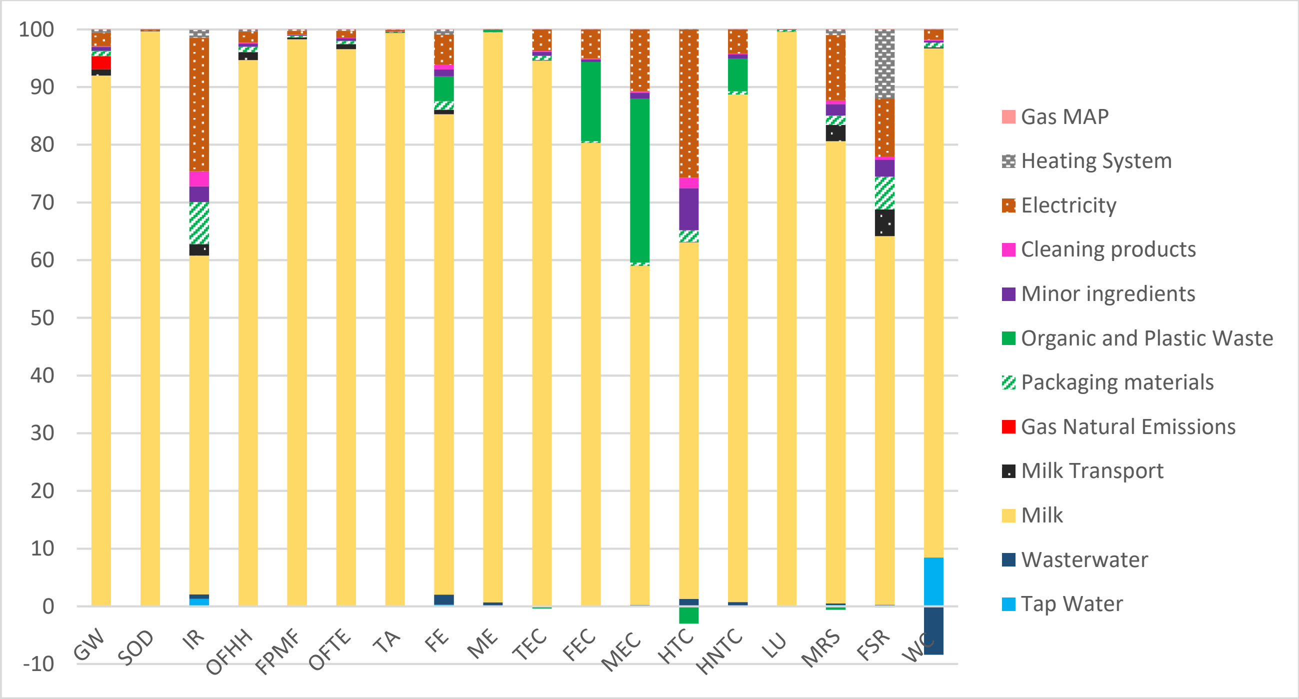

Figure 62: Characterization results obtained for Case E2 (RM-Air-t60), Mozzarella by Raw Milk, MAP-Air, storage time 60 day), using ReCiPe 2016 Midpoint (H) V1.11 method (FU: 1 kg Mozzarella). [GW: Global Warming potential; SOD: Stratospheric ozone depletion; IR: Ionizing Radiation; OFHH Ozone formation, Human health; FPMF Fine particulate matter formation; OFTE Ozone formation, Terrestrial ecosystems; TA: Terrestrial acidification; FE: Freshwater eutrophication; ME: Marine eutrophication; TEC: Terrestrial ecotoxicity; FEC: Freshwater ecotoxicity; MEC: Marine ecotoxicity; HCT: Human carcinogenic toxicity; HNCT: Human non-carcinogenic toxicity; LU: Land use; MRS: Mineral resource scarcity; FRS: Fossil resource scarcity; WC: Water consumption].

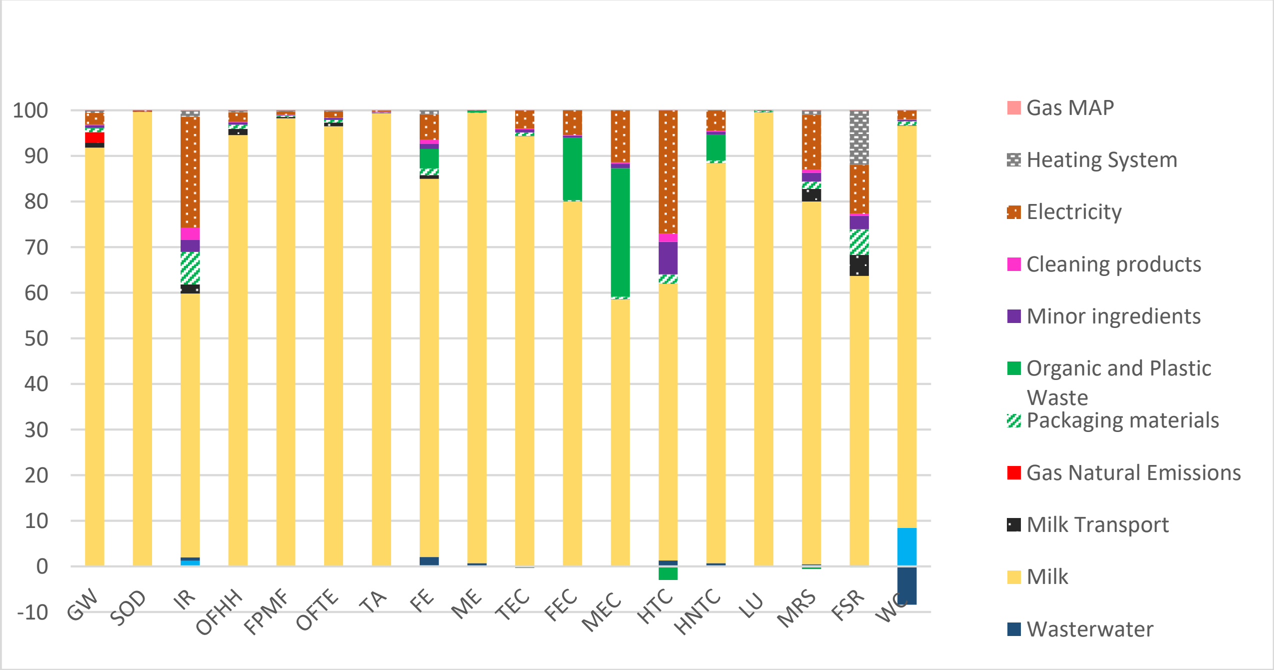

Figure 63: Characterization results obtained for Case E3 (RM-N-t60), Mozzarella by Raw Milk, MAP-Nitrogen, storage time 60 day), using ReCiPe 2016 Midpoint (H) V1.11 method (FU: 1 kg Mozzarella). [GW: Global Warming potential; SOD: Stratospheric ozone depletion; IR: Ionizing Radiation; OFHH Ozone formation, Human health; FPMF Fine particulate matter formation; OFTE Ozone formation, Terrestrial ecosystems; TA: Terrestrial acidification; FE: Freshwater eutrophication; ME: Marine eutrophication; TEC: Terrestrial ecotoxicity; FEC: Freshwater ecotoxicity; MEC: Marine ecotoxicity; HCT: Human carcinogenic toxicity; HNCT: Human non-carcinogenic toxicity; LU: Land use; MRS: Mineral resource scarcity; FRS: Fossil resource scarcity; WC: Water consumption].

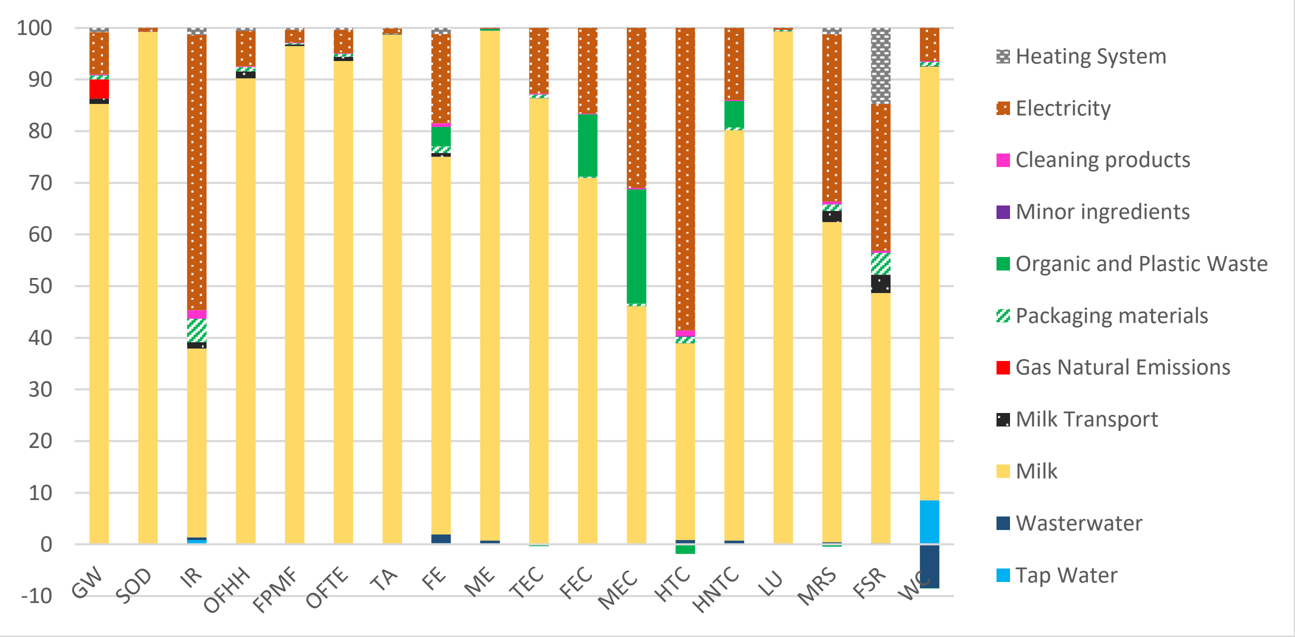

Figure 64: Characterization results obtained for Case F1 (PM-V-t60), Mozzarella by Pasteurized Milk, MAP-Vacuum, storage time 60 day), using ReCiPe 2016 Midpoint (H) V1.11 method (FU: 1 kg Mozzarella). [GW: Global Warming potential; SOD: Stratospheric ozone depletion; IR: Ionizing Radiation; OFHH Ozone formation, Human health; FPMF Fine particulate matter formation; OFTE Ozone formation, Terrestrial ecosystems; TA: Terrestrial acidification; FE: Freshwater eutrophication; ME: Marine eutrophication; TEC: Terrestrial ecotoxicity; FEC: Freshwater ecotoxicity; MEC: Marine ecotoxicity; HCT: Human carcinogenic toxicity; HNCT: Human non-carcinogenic toxicity; LU: Land use; MRS: Mineral resource scarcity; FRS: Fossil resource scarcity; WC: Water consumption].

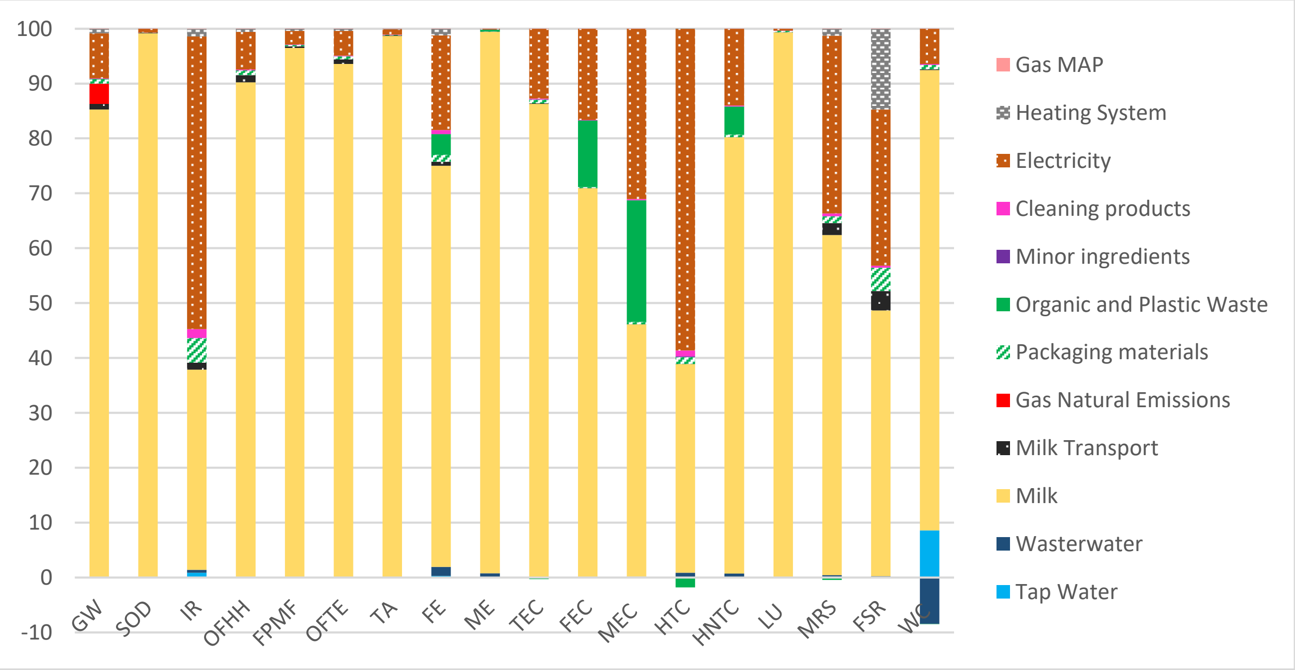

Figure 65: Characterization results obtained for Case F2 (PM-A-t60), Mozzarella by Pasteurized Milk, MAP-Air, storage time 60 day), using ReCiPe 2016 Midpoint (H) V1.11 method (FU: 1 kg Mozzarella). [GW: Global Warming potential; SOD: Stratospheric ozone depletion; IR: Ionizing Radiation; OFHH Ozone formation, Human health; FPMF Fine particulate matter formation; OFTE Ozone formation, Terrestrial ecosystems; TA: Terrestrial acidification; FE: Freshwater eutrophication; ME: Marine eutrophication; TEC: Terrestrial ecotoxicity; FEC: Freshwater ecotoxicity; MEC: Marine ecotoxicity; HCT: Human carcinogenic toxicity; HNCT: Human non-carcinogenic toxicity; LU: Land use; MRS: Mineral resource scarcity; FRS: Fossil resource scarcity; WC: Water consumption].

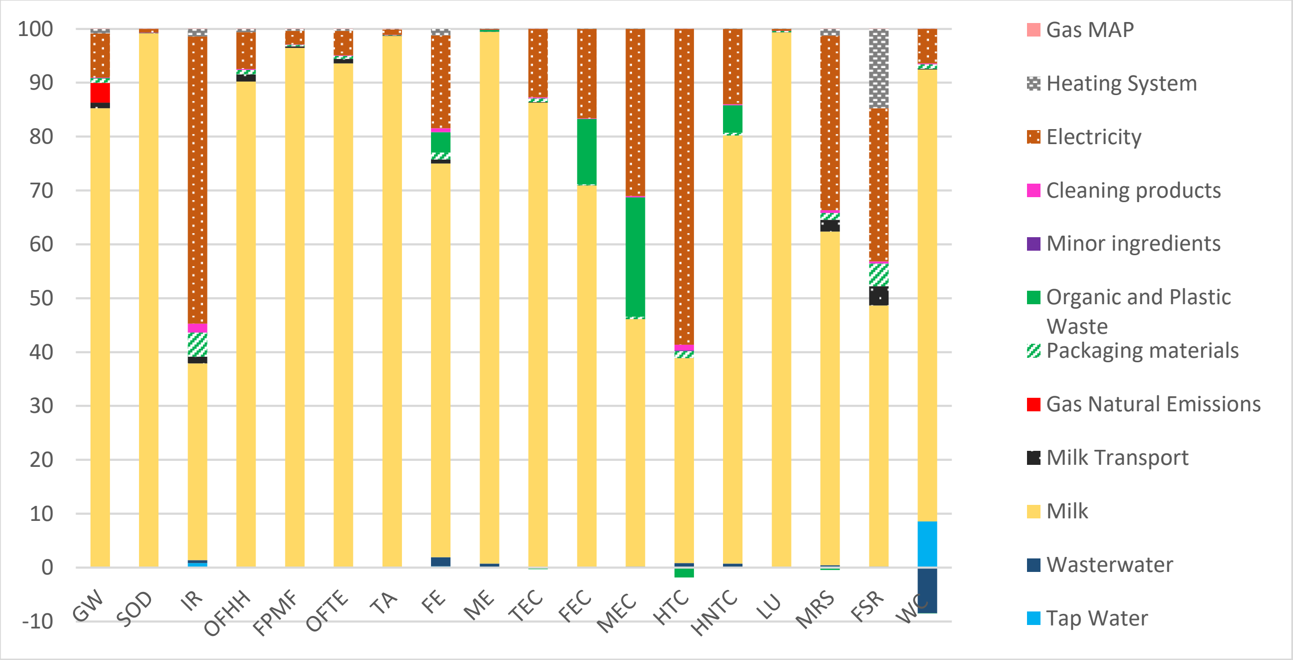

Figure 66: Characterization results obtained for Case F3 (PM-N-t60), Mozzarella by Pasteurized Milk, MAP-Nitrogen, storage time 60 day), using ReCiPe 2016 Midpoint (H) V1.11 method (FU: 1 kg Mozzarella). [GW: Global Warming potential; SOD: Stratospheric ozone depletion; IR: Ionizing Radiation; OFHH Ozone formation, Human health; FPMF Fine particulate matter formation; OFTE Ozone formation, Terrestrial ecosystems; TA: Terrestrial acidification; FE: Freshwater eutrophication; ME: Marine eutrophication; TEC: Terrestrial ecotoxicity; FEC: Freshwater ecotoxicity; MEC: Marine ecotoxicity; HCT: Human carcinogenic toxicity; HNCT: Human non-carcinogenic toxicity; LU: Land use; MRS: Mineral resource scarcity; FRS: Fossil resource scarcity; WC: Water consumption].

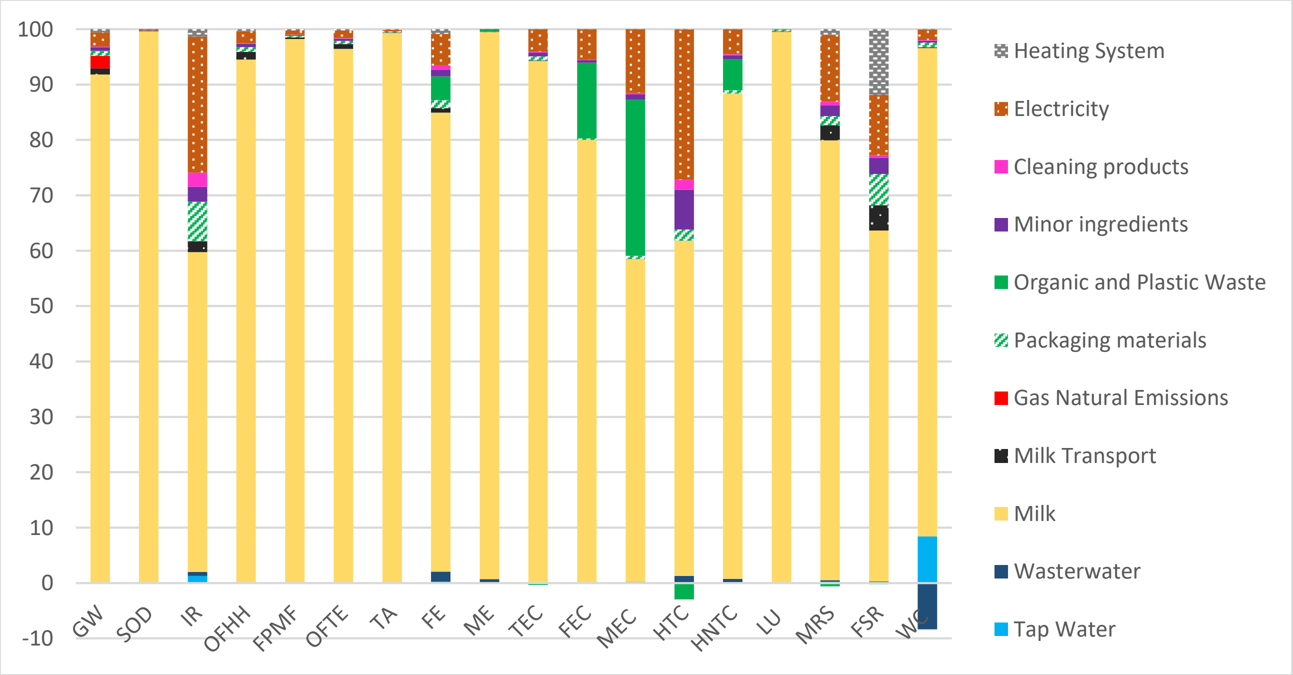

Figure 67: Characterization results obtained for Case G1 (RM-V-t90), Mozzarella by Raw Milk, MAP-Vacuum, storage time 90 day), using ReCiPe 2016 Midpoint (H) V1.11 method (FU: 1 kg Mozzarella). [GW: Global Warming potential; SOD: Stratospheric ozone depletion; IR: Ionizing Radiation; OFHH Ozone formation, Human health; FPMF Fine particulate matter formation; OFTE Ozone formation, Terrestrial ecosystems; TA: Terrestrial acidification; FE: Freshwater eutrophication; ME: Marine eutrophication; TEC: Terrestrial ecotoxicity; FEC: Freshwater ecotoxicity; MEC: Marine ecotoxicity; HCT: Human carcinogenic toxicity; HNCT: Human non-carcinogenic toxicity; LU: Land use; MRS: Mineral resource scarcity; FRS: Fossil resource scarcity; WC: Water consumption].

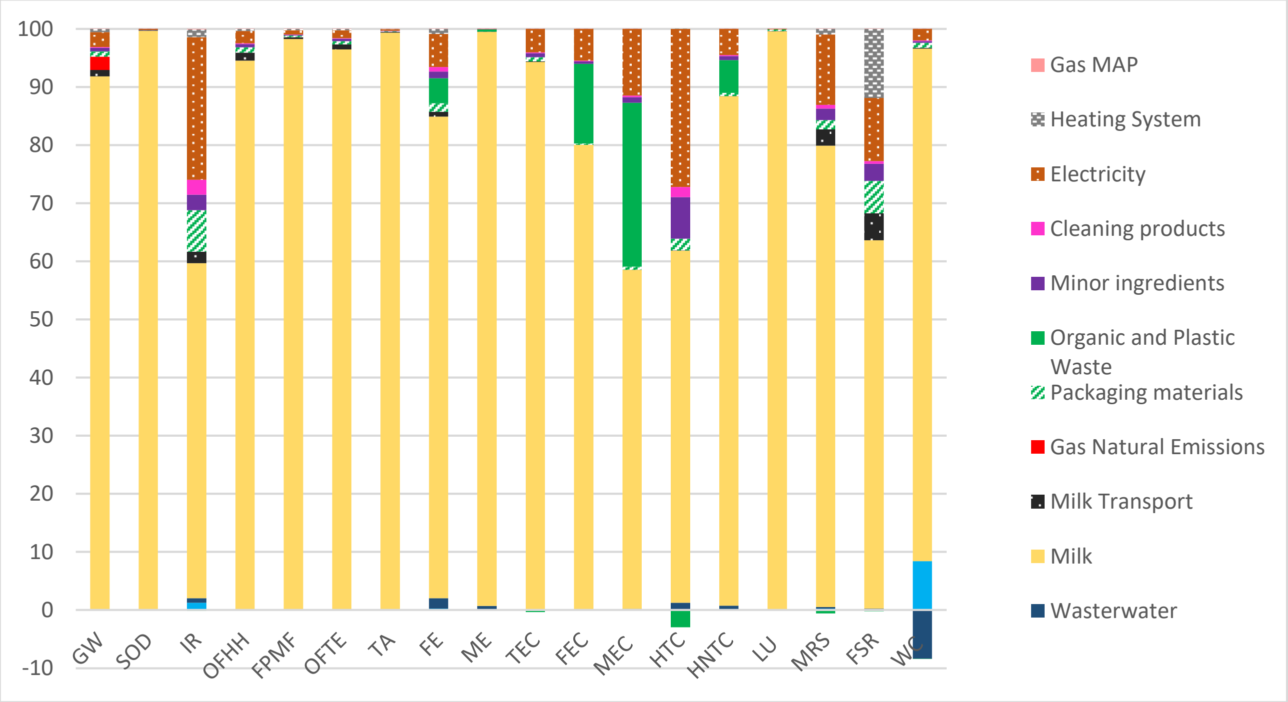

Figure 68: Characterization results obtained for Case G2 (RM-V-t90), Mozzarella by Raw Milk, MAP-Air, storage time 90 day), using ReCiPe 2016 Midpoint (H) V1.11 method (FU: 1 kg Mozzarella). [GW: Global Warming potential; SOD: Stratospheric ozone depletion; IR: Ionizing Radiation; OFHH Ozone formation, Human health; FPMF Fine particulate matter formation; OFTE Ozone formation, Terrestrial ecosystems; TA: Terrestrial acidification; FE: Freshwater eutrophication; ME: Marine eutrophication; TEC: Terrestrial ecotoxicity; FEC: Freshwater ecotoxicity; MEC: Marine ecotoxicity; HCT: Human carcinogenic toxicity; HNCT: Human non-carcinogenic toxicity; LU: Land use; MRS: Mineral resource scarcity; FRS: Fossil resource scarcity; WC: Water consumption].

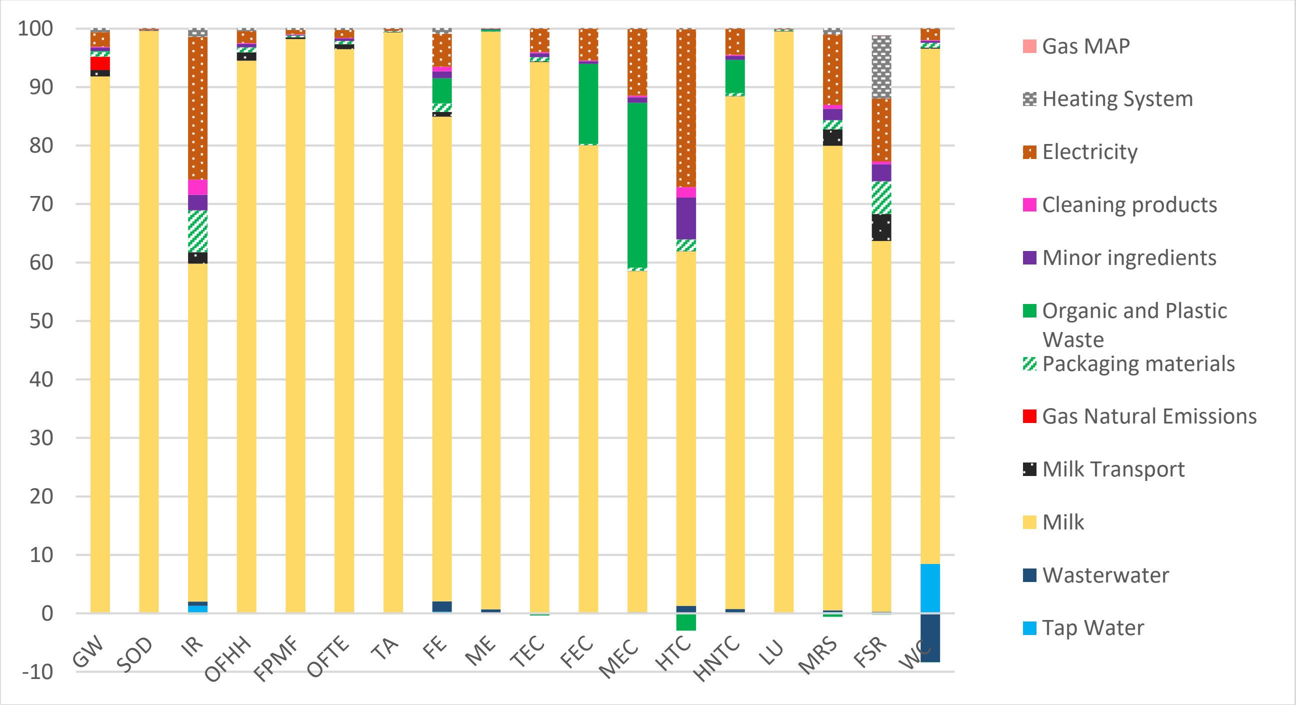

Figure 69: Characterization results obtained for Case G3 (RM-N-t90), Mozzarella by Raw Milk, MAP-Nitrogen, storage time 90 day), using ReCiPe 2016 Midpoint (H) V1.11 method (FU: 1 kg Mozzarella). [GW: Global Warming potential; SOD: Stratospheric ozone depletion; IR: Ionizing Radiation; OFHH Ozone formation, Human health; FPMF Fine particulate matter formation; OFTE Ozone formation, Terrestrial ecosystems; TA: Terrestrial acidification; FE: Freshwater eutrophication; ME: Marine eutrophication; TEC: Terrestrial ecotoxicity; FEC: Freshwater ecotoxicity; MEC: Marine ecotoxicity; HCT: Human carcinogenic toxicity; HNCT: Human non-carcinogenic toxicity; LU: Land use; MRS: Mineral resource scarcity; FRS: Fossil resource scarcity; WC: Water consumption].

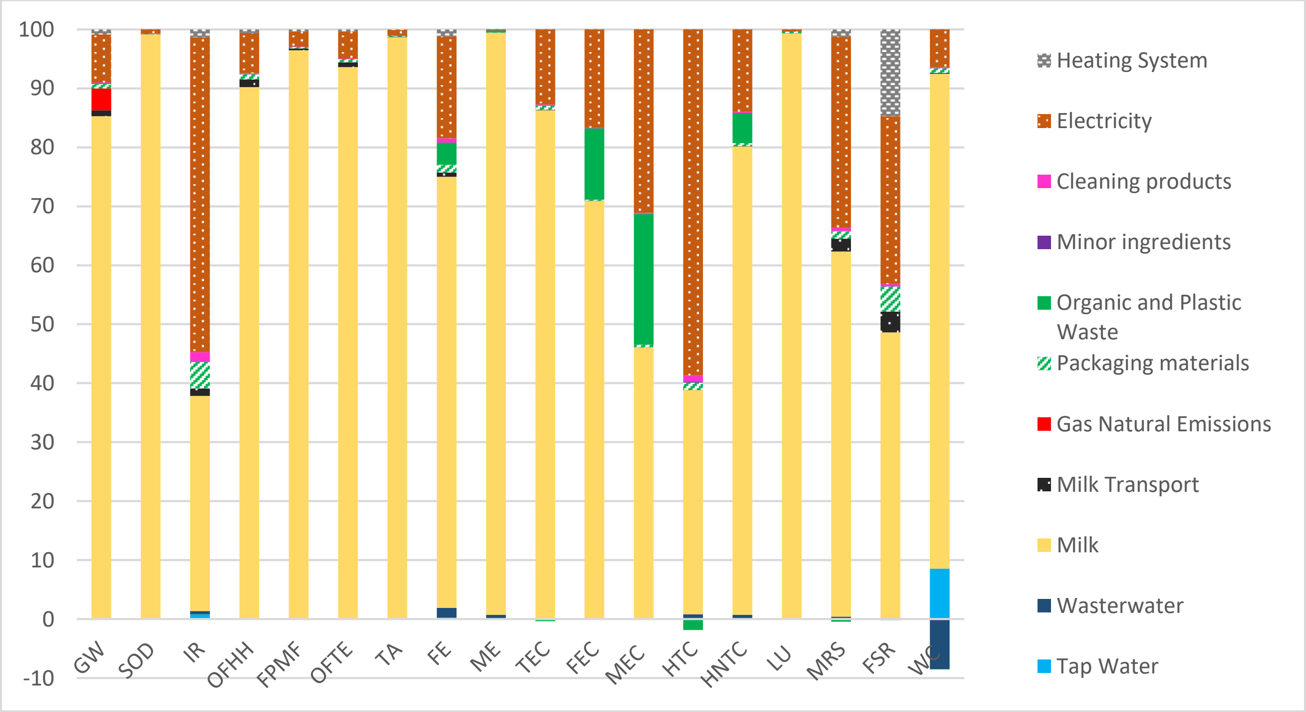

Figure 70: Characterization results obtained for Case H1 (PM-V-t90), Mozzarella by Pasteurized Milk, MAP-Vacuum, storage time 90 day), using ReCiPe 2016 Midpoint (H) V1.11 method (FU: 1 kg Mozzarella). [GW: Global Warming potential; SOD: Stratospheric ozone depletion; IR: Ionizing Radiation; OFHH Ozone formation, Human health; FPMF Fine particulate matter formation; OFTE Ozone formation, Terrestrial ecosystems; TA: Terrestrial acidification; FE: Freshwater eutrophication; ME: Marine eutrophication; TEC: Terrestrial ecotoxicity; FEC: Freshwater ecotoxicity; MEC: Marine ecotoxicity; HCT: Human carcinogenic toxicity; HNCT: Human non-carcinogenic toxicity; LU: Land use; MRS: Mineral resource scarcity; FRS: Fossil resource scarcity; WC: Water consumption].

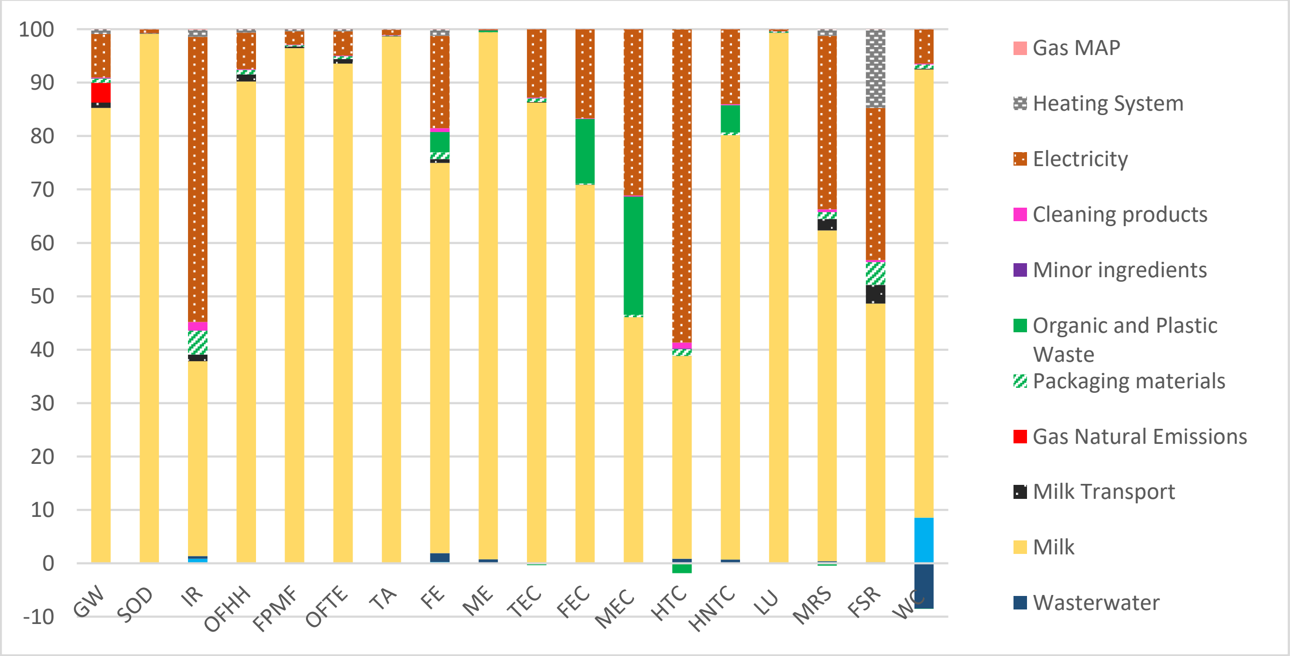

Figure 71: Characterization results obtained for Case H2 (PM-A-t90), Mozzarella by Pasteurized Milk, MAP-Air, storage time 90 day), using ReCiPe 2016 Midpoint (H) V1.11 method (FU: 1 kg Mozzarella). [GW: Global Warming potential; SOD: Stratospheric ozone depletion; IR: Ionizing Radiation; OFHH Ozone formation, Human health; FPMF Fine particulate matter formation; OFTE Ozone formation, Terrestrial ecosystems; TA: Terrestrial acidification; FE: Freshwater eutrophication; ME: Marine eutrophication; TEC: Terrestrial ecotoxicity; FEC: Freshwater ecotoxicity; MEC: Marine ecotoxicity; HCT: Human carcinogenic toxicity; HNCT: Human non-carcinogenic toxicity; LU: Land use; MRS: Mineral resource scarcity; FRS: Fossil resource scarcity; WC: Water consumption].

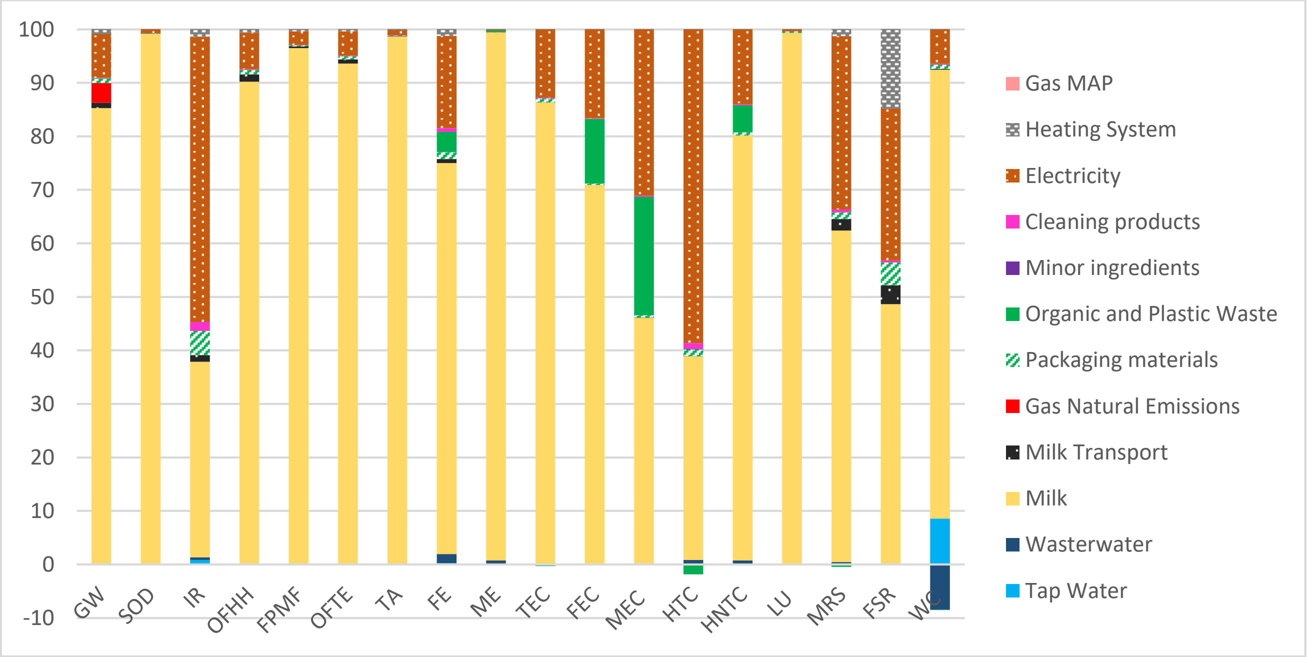

Figure 72: Characterization results obtained for Case H3 (PM-N-t90), Mozzarella by Pasteurized Milk, MAP-Nitrogen, storage time 90 day), using ReCiPe 2016 Midpoint (H) V1.11 method (FU: 1 kg Mozzarella). [GW: Global Warming potential; SOD: Stratospheric ozone depletion; IR: Ionizing Radiation; OFHH Ozone formation, Human health; FPMF Fine particulate matter formation; OFTE Ozone formation, Terrestrial ecosystems; TA: Terrestrial acidification; FE: Freshwater eutrophication; ME: Marine eutrophication; TEC: Terrestrial ecotoxicity; FEC: Freshwater ecotoxicity; MEC: Marine ecotoxicity; HCT: Human carcinogenic toxicity; HNCT: Human non-carcinogenic toxicity; LU: Land use; MRS: Mineral resource scarcity; FRS: Fossil resource scarcity; WC: Water consumption].

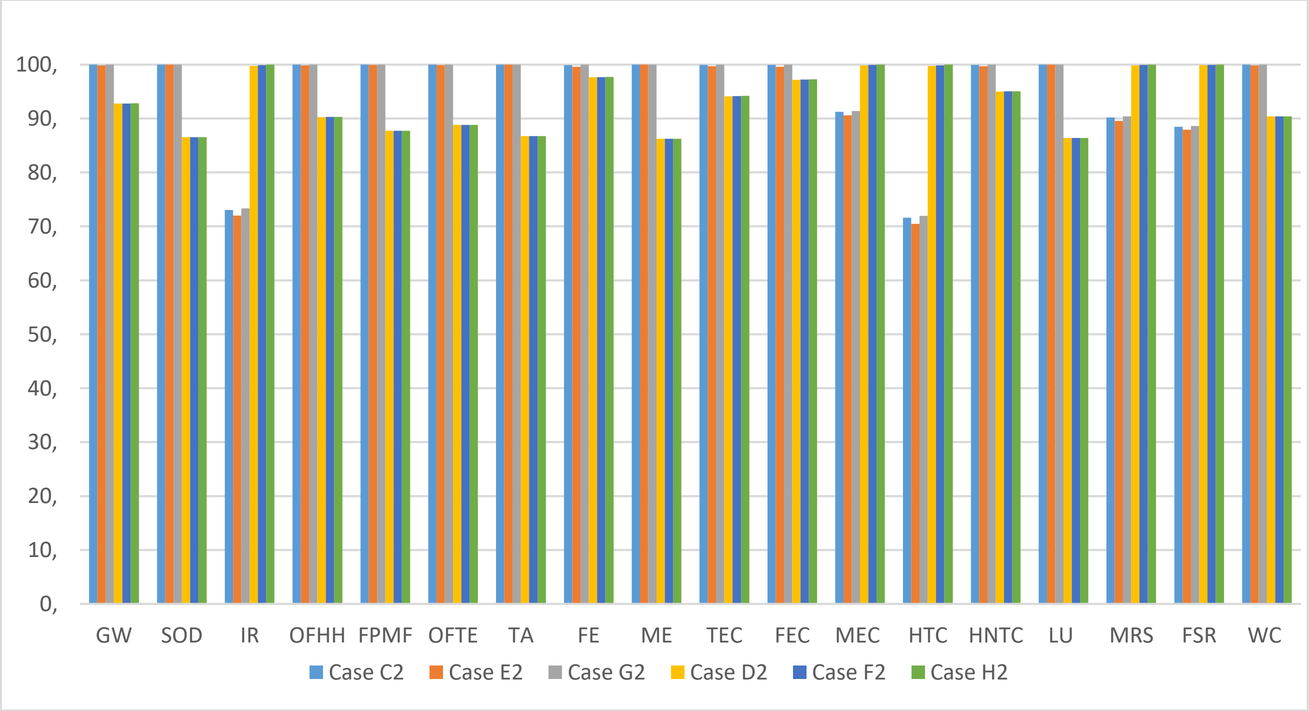

Figure 73: Comparative environmental impact assessment MAP-Air (RM Case C2-E2-G2 vs PM Case D2-F2-H2) across different storage time intervals (30-60-90 day), using using ReCiPe 2016 Midpoint (H) V1.11 method; (FU: 1 kg Mozzarella). [GW: Global Warming potential; SOD: Stratospheric ozone depletion; IR: Ionizing Radiation; OFHH Ozone formation, Human health; FPMF Fine particulate matter formation; OFTE Ozone formation, Terrestrial ecosystems; TA: Terrestrial acidification; FE: Freshwater eutrophication; ME: Marine eutrophication; TEC: Terrestrial ecotoxicity; FEC: Freshwater ecotoxicity; MEC: Marine ecotoxicity; HCT: Human carcinogenic toxicity; HNCT: Human non-carcinogenic toxicity; LU: Land use; MRS: Mineral resource scarcity; FRS: Fossil resource scarcity; WC: Water consumption.].

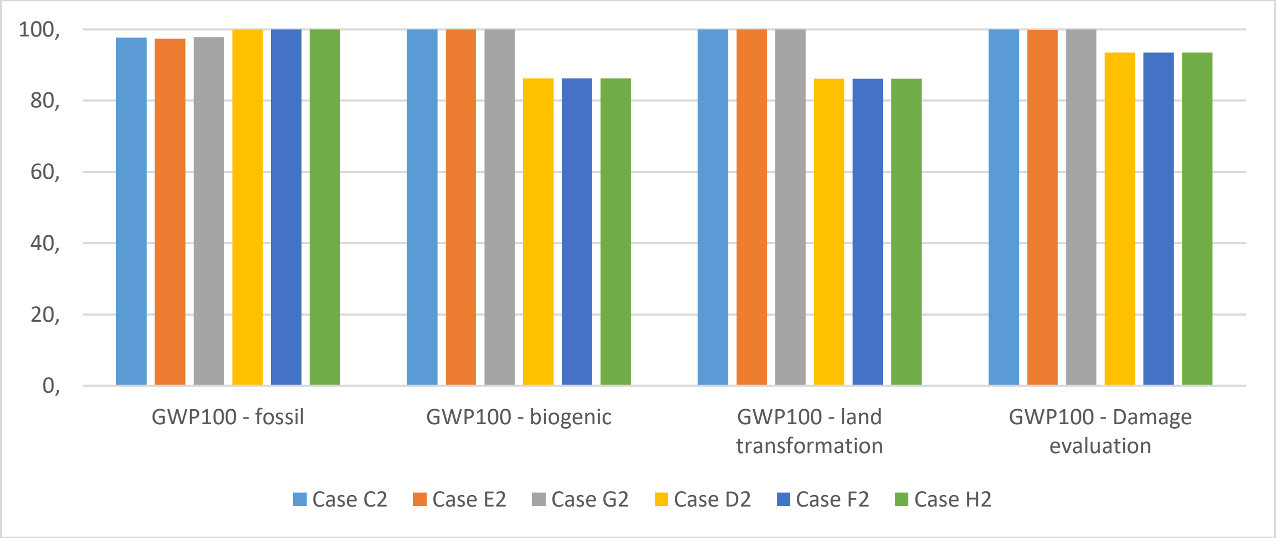

Figure 74: Comparative Global Warming Potential between MAP-Air (RM Case C2-E2-G2 vs PM Case D2-F2-H2) across different storage time intervals (30-60-90 day), using the IPCC 2021 GWP100; (FU: 1 kg Mozzarella).

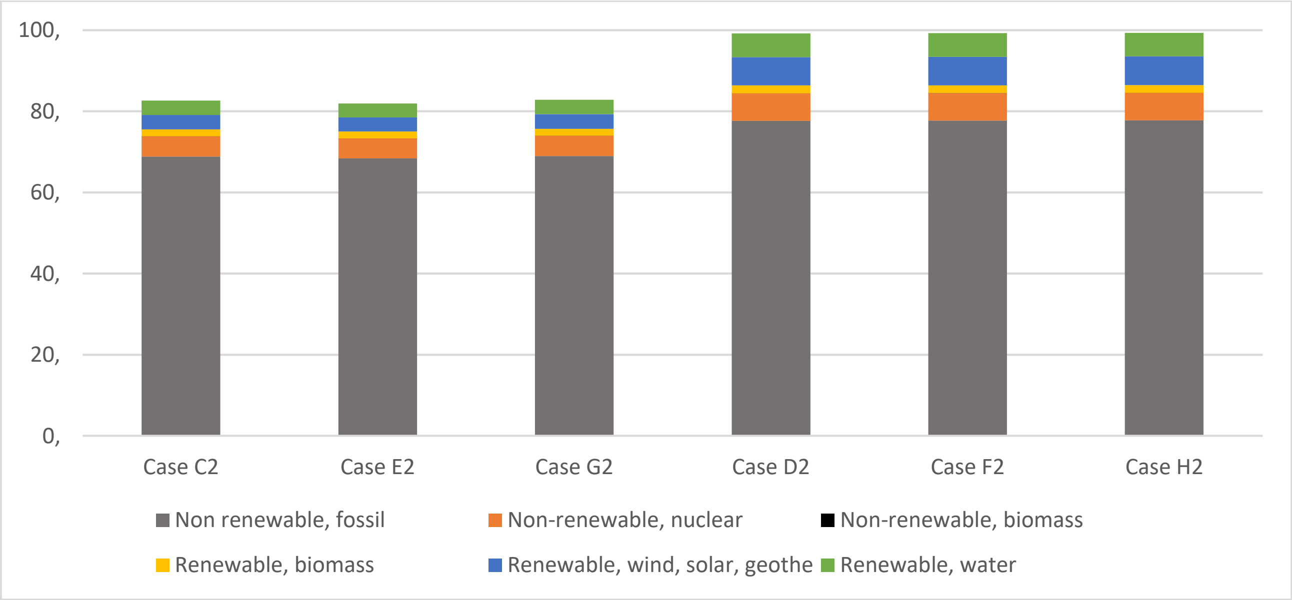

Figure 75: Comparative Energy Demand MAP-Air (RM Case C2-E2-G2 vs PM Case D2-F2-H2) across different storage time intervals (30-60-90 day), using CED Method; (FU: 1 kg Mozzarella).

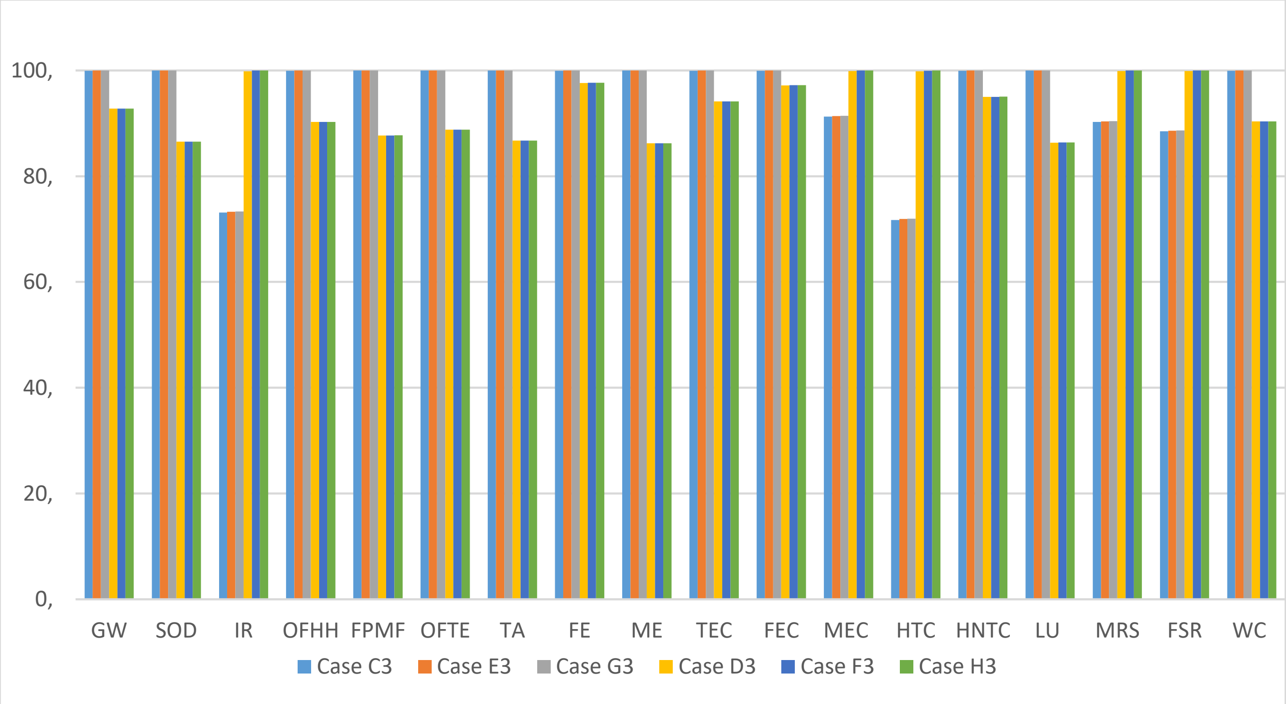

Figure 76: Comparative environmental impact assessment MAP-Nitrogen (RM Case C3-E3-G3 vs PM Case D3-F3-H3) across different storage time intervals (30-60-90 day), using using ReCiPe 2016 Midpoint (H) V1.11 method; (FU: 1 kg Mozzarella). [GW: Global Warming potential; SOD: Stratospheric ozone depletion; IR: Ionizing Radiation; OFHH Ozone formation, Human health; FPMF Fine particulate matter formation; OFTE Ozone formation, Terrestrial ecosystems; TA: Terrestrial acidification; FE: Freshwater eutrophication; ME: Marine eutrophication; TEC: Terrestrial ecotoxicity; FEC: Freshwater ecotoxicity; MEC: Marine ecotoxicity; HCT: Human carcinogenic toxicity; HNCT: Human non-carcinogenic toxicity; LU: Land use; MRS: Mineral resource scarcity; FRS: Fossil resource scarcity; WC: Water consumption.].

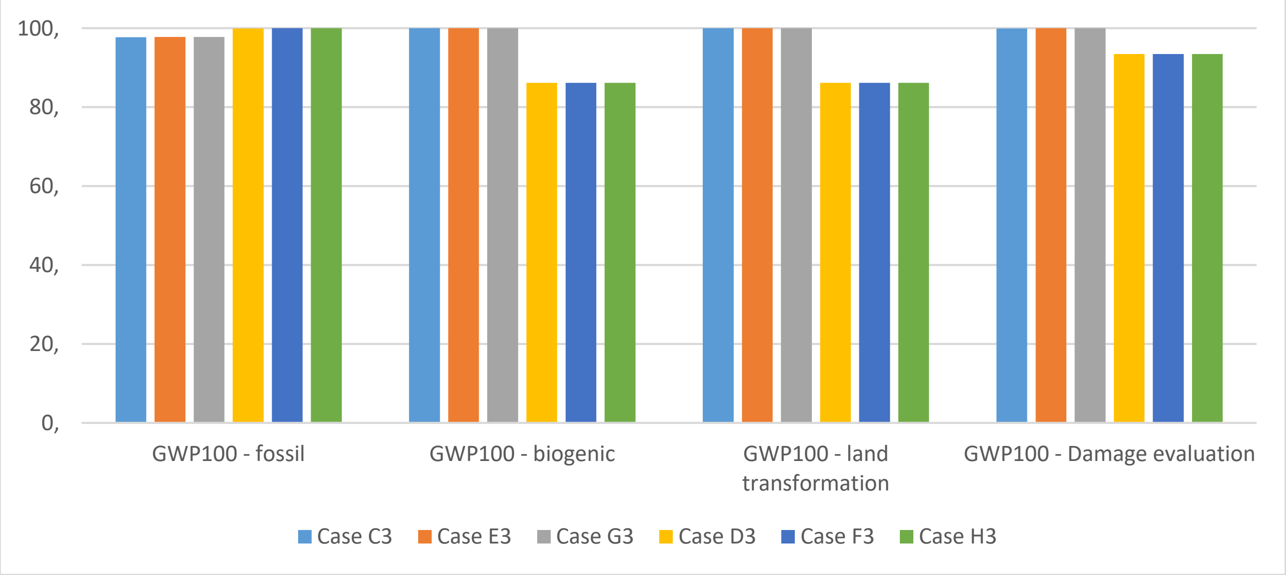

Figure 77: Comparative Global Warming Potential between MAP-Nitrogen (RM Case C3-E3-G3 vs PM Case D3-F3-H3) across different storage time intervals (30-60-90 day), using the IPCC 2021 GWP100; (FU: 1 kg Mozzarella).

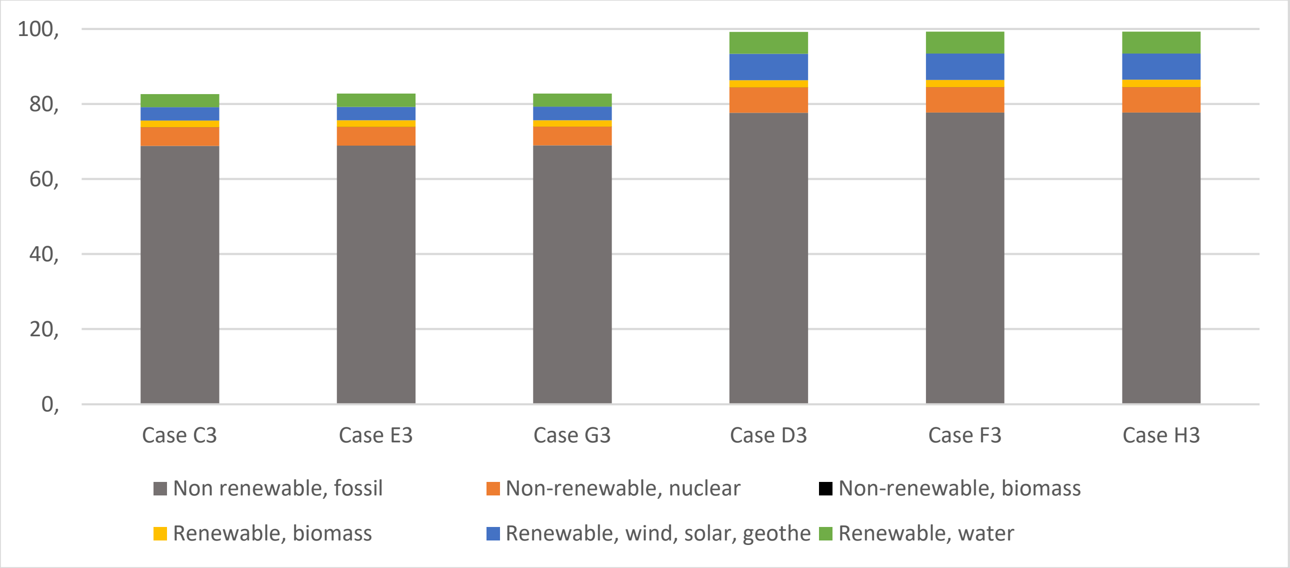

Figure 78: Comparative Energy Demand MAP-Nitrogen (RM Case C3-E3-G3 vs PM Case D3-F3-H3) across different storage time intervals (30-60-90 day), using CED Method; (FU: 1 kg Mozzarella).

10. Appendix B: Strategic analysis and preliminary technical design of an automated curd production line

10.1 Market analysis, strategic drivers for curd production and business models

In 2024, global milk production reached 982 million tonnes (+1.4%), supported by a moderate expansion in international dairy trade to 86 million tonnes (milk equivalent). Trade volume of cheese remained positive, world trade in butter rebounded (+3,1 %) while trade volumes of milk powders declined (FAO, 2025). Within the European landscape, Germany, France, and Italy remain the leading producers. Italy, specifically, produced approximately 1.21 million tonnes of cow's milk cheese in 2024 (CLAL, 2024), and ranked as the world’s second-largest exporter by value, reaching €5.4 billion (ISMEA, 2025).

Curd is a semi-finished product, an intermediate product obtained from the coagulation of milk, which can be used for various purposes: mozzarella, pizza cheese, processed cheeses, and protein ingredients. While specific trade data is often scarce, curd produced in Eastern Europe is frequently utilized by other nations for final cheese processing. For istance, Foodcom S.A. markets "Cagliata 48%" cheese, a pasteurized cow's milk curd designed for industrial applications and cheese blends. The curd is packaged in blocks, in hygienic and sealed packaging, labeled and protected for refrigerated transport (+6°C), at a price of 3450 € /MT (€ 3.45/kg) (Foodcom, 2026).

Dairy sector has seen significant technological advancements, with automated production lines from Germany, Denmark, Poland, and Bulgaria. These lines represent a major innovation in dairy sector, enabling greater mechanization and automation of production processes, characterized by high levels of technological integration, process control, and final product quality standardization (Alekseeva et al. 2021). Modern technologies also allow real-time monitoring of milk coagulation properties, a fundamental aspect for optimizing the yield and quality of the products obtained (Mota et al. 2022).

The dairy industry is moving toward specialization. By separating the production of raw ingredients (like curd, protein concentrates, or casein) from the final cheese-making process, companies can optimize their costs and improve overall efficiency. This versatility allows companies to adapt quickly to market demand and sell their products to different segments of the food industry (ready-to-eat foods, sports supplements, pharmaceutical industry).

Companies may opt to specialize in industrial curd production as intermediate suppliers based on several strategic drivers:

- *Supply chain specialization*: Curd production allows for focus on the initial stages of milk processing, including milk reception and treatment, coagulation, whey separation, and curd maturation. These stages require a high level of raw material consumption, so specialization allows for economies of scale and reduced unit processing costs;
- *Reduction of industrial investments*: The full production of finished cheeses, particularly stretched cheeses, requires heavy investment in stretching, molding, cooling, and packaging lines. Curd specialization reduces technical requirements and costs, allowing for simpler management of the production plant;
- *Economic advantages of intermediate products:* Trading intermediate dairy products simplifies the supply chain, relocating primary production to where the raw material (milk) is more advantageous and competitive. Logistically, transporting curd allows for a drastic reduction in weight and volume compared to transporting milk.

Curd production and marketing can be categorized into three main strategic configurations:

- Integrated Dairy Plants: Facilities that manage the entire value chain, producing both curd and finished cheeses, offering the flexibility to process the curd or sell it based on market demand;
- Specialized Intermediate Plants: Facilities dedicated exclusively to semi-finished products. They supply industrial curd to other food manufacturers who lack the primary processing capacity or are located in regions where raw milk is more expensive or less available;
- Dairy Cooperatives: Centers that centralize raw milk from multiple producers and process it into curds to achieve economies of scale.

10.2 Preliminary design of an automated line for the production of curd from cow's milk

Curd production represents a fundamental step in the dairy supply chain, particularly for the production of “pasta filata” cheeses such as mozzarella, provola, scamorza, and similar products for the food processing market. Modern mozzarella manufacturing frequently adopts a decentralized model, where curd production and final processing occur at geographically distinct sites.

The following describes the preliminary design of an automated line for the production of cow's milk curd. The process includes the following phases: milk reception and storage, heat treatment, enzymatic coagulation, curd cutting, whey drainage, curd maturation at pH 5.5, packaging, storage, logistic and transport. Two alternative system configurations are analyzed, comparing them from a technological, energy, logistical, and economic perspective.

- **Line A** – Complete maturation and refrigerated storage;
- **Line B** – Arresting maturation by rapid freezing.

10.2.1 Curd production process

Curd is the product of milk protein coagulation, primarily casein, achieved through the combined action of coagulating enzymes and lactic acid bacteria (Fusco et al. 2022). During the coagulation process, casein micelles undergo destabilization, leading to the formation of a three-dimensional network that traps water, fats, and soluble substances present in the milk. This transformation results in a protein gel (Lucey 2002) which, through subsequent cutting and drainage operations, progressively separates from the whey (Amalfitano et al. 2019).

Curd in stretched cheese is subjected to an acidification phase during which residual lactose is converted into lactic acid by lactic acid bacteria (Yazhini et al. 2025). This process results in a progressive decrease in pH to values between 5.2 and 5.5, an essential condition for the subsequent stretching phase.

The industrial curd production process can be divided into the following main phases:

1. Milk reception and storage (refrigerated silos);
2. Standardization and heat treatment;
3. Enzymatic coagulation;
4. Curd cutting and whey separation;
5. Drainage and maturation of the curd;
6. Packaging;
7. Logistics and Transport.

Modern production lines integrate these operations using automated systems equipped with temperature sensors, pH meters, automatic conveyors, PLC (Programmable Logic Controller) controls and SCADA (Supervisory Control and Data Acquisition) supervision.

1. Milk Reception and Storage

Raw milk from farms is transported in tankers and discharged at dedicated reception stations. During this phase, several quality controls are performed, including fat and protein content, Total Bacterial Count (TBC), presence of antibiotics, titratable acidity and delivery temperature. Subsequently, the milk is transferred to refrigerated stainless steel silos, generally maintained at temperatures between 4 °C and 6 °C. The storage capacity is typically sized to ensure 12–24 hours of production autonomy.

2. Standardization and heat treatment

Before coagulation, the milk undergoes heat treatment, typically through HTST (High Temperature Short Time) pasteurization. Typical parameters: temperature of 72–75°C for a holding time of 15–20 seconds. The process is conducted using plate heat exchangers (PHEs), followed by cooling to the specific coagulation temperature, typically between 32°C and 35°C

3. Enzymatic coagulation

Coagulation takes place in vats equipped with agitators and automated cutting systems. During this stage, rennet (coagulating enzymes) and starter cultures (lactic acid bacteria) are added. The coagulation time generally ranges from 20 to 40 minutes, resulting in a firm curd that is subsequently cut to facilitate whey separation.

4. Curd cutting and whey separation

Curd cutting is performed using agitators fitted with blades or wires. The objectives of this operation are to increase the contact surface area, promote whey expulsion (syneresis) and control the size of the curd particles (grains). The curd-whey mixture is then transferred to drainage systems.

5. Drainage and maturation of the curd

In modern industrial plants, whey drainage and curd maturation are performed using automated systems consisting of drainage belts, vibrating tables, maturation tunnels, and screw conveyors. Key process parameters monitored during this phase include temperature, residence time, drainage efficiency, and pH evolution. Maturation typically lasts between 3 and 5 hours.

6. Packaging

Once the target pH is reached, the curd is transferred to the packaging section. The product can be packed in: food-grade plastic tubs, stainless steel crates or bulk containers.

7. Logistics and Transport

The packaged curd is moved to cold storage rooms and then loaded on refrigerated trucks.

10.3 Comparative Analysis: Line A vs. Line B

As illustrated in the process layout (Figure 79), the plant is designed to handle the production flow linearly, from primary milk transformation to curd packaging.

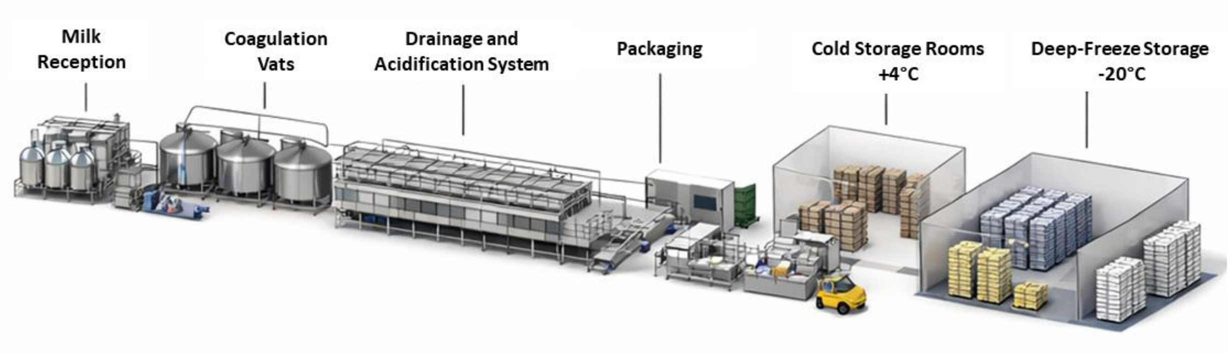

Figure 79: Schematic layout of the automated curd production line, illustrating the complete process flow from raw milk reception to final storage in chilled (+4 °C) or deep-freeze (-20 °C) environments.

The selection between the two analyzed configurations (Table 28) depends strictly on the strategic objectives of the facility. While Line A focuses on complete maturation and refrigerated storage, Line B introduces the arresting of maturation by rapid freezing.

Table 28:. Comparative analysis of Line A (standard maturation and refrigeration) versus Line B (acidification arrest and rapid freezing): evaluation of process parameters, energy impact, and logistical flexibility.

Line	**A (Refrigerated)**	**B (Frozen)**
Process Focus	Full maturation at the production site	Arrested maturation via rapid freezing
Storage Temperature	+4 °C	−20 °C
Key Operations	Packaging → Cold Storage → Transport	Rapid cooling → Tunnel freezing → Deep-freeze storage
Shelf Life	Limited (Short-term)	Extended (Long-term)
Energy Impact	Lower consumption (Standard cooling)	High consumption (Freezing & Maintenance)
Investment	Lower (Standard refrigeration plant)	Higher (Blast freezer & specialized tunnels)
Logistics	Just-in-time delivery required	High flexibility; production buffering possible
Product Quality	Better structural integrity of the curd	Potential structural changes due to ice crystals

Based on the process flow (Figure 79) and the configurations compared in Table 28, the following section provides a preliminary design for a high-capacity industrial plant dedicated to bovine curd production.

1. Production Capacity and Sizing

Working from a capacity of 100,000 liters/day with an estimated curd yield of 20%, the plant will achieve a daily output of 20,000 kg/day. Operational production is supported by a 20-hour duty cycle, enabling an hourly output of 1,000 kg/h. This volume necessitates a dynamic maturation system capable of managing approximately 4,000 kg of product in transit.

2. Facility Layout and Area Allocation

The plant (covering a total area of approximately 2,600–2,800 m²), following Lean Manufacturing principles that minimize the movement of raw materials and staff, can be divided into several areas (Figure 80):

- Reception and processing Area (400 m²), where milk is standardized and undergoes HTST (High Temperature Short Time) pasteurization.
- Coagulation and maturation Area (700 m²), with a belt drainage system (20–30 m in length) to ensure the 4-hour residence time required to reach the pH target.
- Storage and logistics: the required cold storage capacity is approximately 60-70 m³ (considering daily production and pallet transport, 33 units will be needed (average pallet weight of 600 kg; 1.5-2 m^3). To optimize storage management and ensure at least 3 days of autonomy, a capacity of 250-300 m^3 per cells should be considered." In particular a chilled storage room at +4 °C for Line A and a blast freezing system (tunnel at -35 °C and storage cells at -20 °C) for Line B.

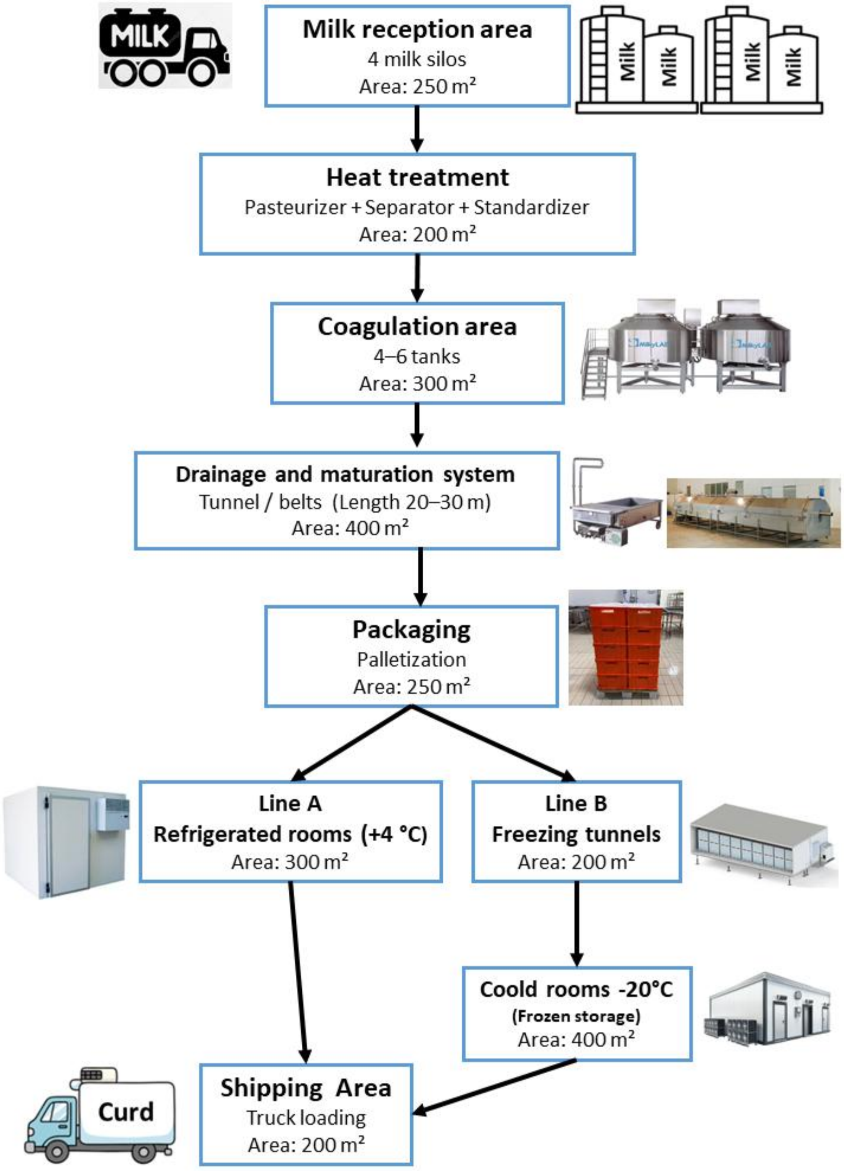

Figure 80: Process flow diagram and dimensional layout of a dairy plant for curd production (Capacity: 100,000 L/day). The diagram illustrates the functional areas, their respective surfaces (m^2), and the dual post-packaging storage lines: Line A for fresh products (+4°C) and Line B for frozen products (-20°C).

The total investment for a production line of this scale ranges between €2.7 million and €5.6 million, depending on the level of automation and the specific refrigeration technologies selected (companies specializing in dairy technology: GEA Group, Tetra Pak, DIMA Sintex Group and ALMAC Srl). Operational energy costs are estimated at approximately €0.01/kg for Solution A and €0.09/kg for Solution B. In European retail, cow's milk mozzarella is typically priced between 8-17 €/kg, depending on the brand and quality. In the industrial channel, curd is generally sold at lower values (e.g., Foodcom at €3.45/kg). If a dairy produces 20,000 kg/day of curd and sells it at an average price of €4/kg, it would generate a daily turnover of €80,000 and an annual turnover of approximately €29 million.

Designing an industrial curd production line requires an integrated assessment of technological, energy, and logistical aspects. Analyzing the overall investment for the entire line and specific storage solutions, Line A is more efficient in terms of both energy and equipment. Instead, Line B offers greater flexibility in logistics and inventory management. The final choice will essentially depend on three critical factors: the geographic distance between production sites, the variability of market demand, and the strategic need for production accumulation.

10.4 Reference

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