UNIVERSITÀ DEGLI STUDI DI FOGGIA

DOTTORATO DI RICERCA IN
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COORDINATORE: PROF. FRANCESCO CONTÒ

TESI DI DOTTORATO

TITLE

Relationship between Food Waste Probability and Shelf Life

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SUMMARY

ABSTRACT

Obiettivo dello studio

L'allungamento della shelf life dei prodotti alimentari è un tema particolarmente sentito dal sistema agroindustriale italiano ed in particolare dall'industria lattiero-casearia a fronte della crescente richiesta da parte dei consumatori di prodotti freschi ed ultrafreschi.

Il presente studio si pone come obiettivo primario valutare l'esistenza o meno di una correlazione fra estensione della durabilità dei prodotti alimentari, ed in particolare dei latticini freschi prendendo a modello la mozzarella Fior di Latte, e riduzione dello spreco alimentare. Si eseguirà a tal scopo studio statistico di dati di mercato per cercare di correlare, nel comparto lattiero-caseario, la shelf life dei prodotti con lo spreco alimentare, adottando come parametro principale di valutazione la percentuale di prodotto reso da mercato sul prodotto venduto.

Si pone inoltre come ulteriori obiettivi lo studio sperimentale di strategie per l'allungamento della durabilità della mozzarella Fior di Latte (FdL).

Materiali e Metodi

Lo studio statistico è stato condotto utilizzando dati cortesemente messi a disposizione da una grande industria alimentare italiana, relativi a 640 diverse referenze di prodotti lattiero-caseari, che sono stati suddivisi in 17 classi in funzione della shelf life loro assegnata. Come variabili sono state utilizzate il valore di shelf life e la percentuale di reso da mercato (reso/venduto). I dati sono stati sottoposti dapprima ad analisi ANOVA per valutare eventuali differenze fra le 17 classi e il reso% e quindi a modellizzazione per analizzare la correlazione esistente fra le due variabili. Tutte le analisi statistiche sono state condotte con il pacchetto statistico IBM SPSS 20.

Diverse strategie di packaging e le loro relative combinazioni sono state utilizzate per valutare il loro impatto sulla durabilità della mozzarella Fior di Latte mantenuta alla temperatura costante di + 8°C (la temperature media dei frigoriferi domestici in Italia). In particolare si sono utilizzati coating attivi e confezionamento in atmosfera modificata (MAP) per valutarne l'effetto combinato. Ad intervalli regolari di tempo si sono prelevate unità campionarie che sono state valutate per la composizione del gas presente nello spazio di testa della confezione, per il pH del prodotto e del liquido di governo, per I contenuti microbici (conta microbica totale, conta dei batteri lattici e delle pseudomonadacee), nonchè per le caratteristiche sensoriali. Definiti i limiti di accettabilità microbica e sensoriale (MAL and SAL) rispettivamente a 10exp6 ufc/g per le pseudomonadacee e a punteggio 4,0 per la qualità globale dell'analisi sensoriale, è stato possibile calcolare la shelf life della mozzarella Fior di Latte sulla base del minimo valore ottenuto di MAL e SAL.

Risultati principali dello studio

All'analisi ANOVA il parametro reso% non mostra, per i prodotti freschi con shelf life inferiore o uguale a 30 giorni, differenze significative delle medie. Al contrario, per i prodotti aventi shelf life superiore a 30 giorni, le differenze fra i valori medi del reso% appaiono significative. L'ulteriore indagine condotta con un approccio di modellizzazione ha portato ad un modello esponenziale che spiega la correlazione fra shelf-life e reso% da mercato.

Dal lato della sperimentazione di tecniche protettive per l'estensione della shelf life si è evidenziato che la semplice ricopertura della mozzarella Fior di Latte con un strato di alginato polimerizzato in loco protegge solo relativamente il prodotto dallo sviluppo microbico. Il confezionamento in atmosfera modificata (MAP), specialmente ad alte proporzioni di CO_2, risulta invece efficace nell'inibizione dei microorganismi alteranti ma impatta negativamente la superficie del formaggio, ciò che non avviene se il formaggio è protetto dalla copertura di alginato. Caricando la copertura di alginato con composti ad azione antimicrobica (active coating) aumenta l'efficacia del sistema: i risultati sperimentali confermano che accoppiando MAP ed active coating (es. potassio sorbato o nanoparticelle di argento) si ottiene un sistema efficace nell'allungamento della shelf life della mozzarella Fior di Latte. Parimenti l'inclusione di cellule di *L. reuteri* e di glicerolo a formare un coating bioattivo migliora significativamente la shelf life del prodotto in liquido di governo se accoppiato a MAP.

Conclusioni

L'analisi statistica mostra una correlazione di tipo esponenziale fra percentuale di reso da mercato, uno degli indicatori di spreco alimentare, e shelf life assegnata ai prodotti quando questa è superiore a 30 giorni. La differenza fra le medie del reso% in funzione della shelf life non appare invece significativa per i prodotti freschi ed ultrafreschi (DLC < 30 gg). Il dato statistico sembra non concordare con quanto osservato sul campo dagli operatori alimentari, che rilevano benefici, a parità delle altre variabili, in termini di reso da mercato a seguito di allungamento della shelf life.

Il confezionamento in MAP ad elevate proporzioni di CO_2 è in grado di controllare lo sviluppo di *Pseudomonads* spp., il principale agente di alterazione di Fior di Latte e mozzarelle fresche in liquido di governo, ma altera le caratteristiche organolettiche del prodotto, in particolare degradandone la superficie. La presenza di una copertura di alginato protegge la superficie del formaggio dai danni causati dall'acidità del liquido di governo. L'utilizzo combinato di un coating attivo e del confezionamento in atmosfera modificata risulta in grado di allungare la shelf life del prodotto, confermando che è necessario un approccio ad ostacoli multipli per ottenere i risultati attesi.

INTRODUCTION

Food Loss & Food Waste

Food discard occurs at all stages of food life cycle, starting from harvesting, through processing, production and distribution, until domestic handling and final consumption (Lipinski et al., 2013; Schneider, 2008).

From an environmental point of view the European Union defines ‘‘*waste*’’ as ‘‘*any substance or object which the holder discards or intends or is required to discard*’’. This definition could be transferred to the food supply chain, but in the general practice the agro-food community makes use of two different terms, ‘‘**food loss**’’ and ‘‘**food waste**’’, according to the chain stages in which discard is generated (Beretta et al., 2013, Lipinski et al., 2013, UK Parliament, 2014). Incredibly, since there has been a long debate on where to put the division between loss and waste, there is still no agreement on their exact definitions.

According to Schneider (2008) the term ‘‘Food loss’’ should be used to indicate any food product discarded from the supply chain at primary production, processing and distribution steps. According to the Food and Agriculture Organization of the United Nations (FAO, 2015), food waste can be defined as ‘‘*the mass of food wasted in the part of food chains leading to edible products going to human consumption*’’. A further definition of food waste is provided by the Waste and Resources Action Programme (WRAP, 2014) as ‘‘*any food or drink produced for human consumption that has, or has had, the reasonable potential to be eaten, together with any associated unavoidable parts, which are removed from the food supply chain*’’.

Similarly, the FUSIONS project, a Pan-European initiative, working on standard food waste definition and measurement, defines food waste as ‘‘*any food, and inedible parts of food, removed from the food supply chain to be recovered or disposed*’’ (Östergen & Gustavsson, 2014).

These waste definitions actually refer to discards occurring along the whole food chain, including those generated at primary production level, which should be indicated as ‘‘food losses’’.

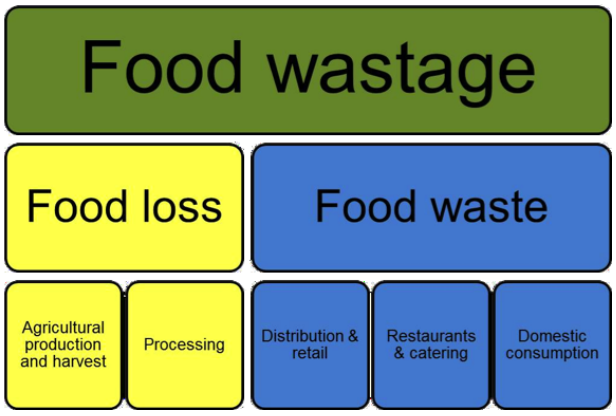
However, according to Gustavsson et al. (2011), ‘‘food waste’’ being the result of an intended decision, particularly in relation to consumers, the term ‘‘food waste’’ should refer to the end of the food chain, considering only purchase and final consumption.

The distinction, adopted in the present study, between ‘‘food loss’’ and ‘‘food waste’’ avoids the overlapping of the terms: the former should be used for the stages from primary production to raw materials transformation into food (i.e. end food product ready to distributed/consumed, in the

warehouse of the producer/manufacturer), the latter for all the stages from distribution to utilization by the final consumer.

According to FoodDrinkEurope (2015) the new new term of "Food Wastage" should be introduced.

Food Wastage is the decrease in edible food mass that was originally intended for human consumption. Inedible crop residues, animal parts and by-products should not be included.



Food Wastage includes Food Loss and Food Waste whose definition is exactly as described before, the discriminating point being the food product ready to be distributed/consumed (food can be wasted provided it is available to be consumed).

Food loss and food waste can be classified based on the supply chain steps where they are generated but they can be furtherly classified considering specific criteria, detailing a wide range of subcategories (Last Minute Market, 2012).

The most commonly used criterion is based on food category and is able to describe the whole food wastage all along the food chain. The discarded parts can be in turn classified according to the life cycle stage where the product becomes a loss/waste. Original food is the starting point, never employed for consumption. The "partly used food" represents what is left after using a part of product, considering processing or domestic handling (i.e. industry and kitchen by-products), while "leftovers" represent what remains on the plate or in the pot after a meal. Avoidability is mainly a domestic criterion: it allows to discriminate discarded food according to the possibility or not to prevent its generation (Beretta et al., 2013; Williams, 2012). The "possibly avoidable" is waste referring to food and drink that some people eat and others do not (e.g. cheese rind or bread crusts), or that can be eaten provided it is prepared following specific recipes (e.g. potato skins). The "unavoidable" waste is the discarded food part that is not edible under normal circumstances (e.g. pineapple skin) (Parfitt et al., 2010). Avoidability often relies on subjective choices, determined by social aspects. "Possibly reusable food" is food still suitable for consumption without further processing (e.g. products not responding to aesthetic specification). "Recyclable discards" can be used by industry for energy or value-added compounds recovery or transformation/synthesis (e.g. anaerobic digestion or extraction/creation of bioactive molecules), or composted at home. From an environmental point of view food discard is characterized by different resource content (i.e. land, water, energy and labour). For instance, the loss of environmental resources associated with waste of raw fresh vegetables, fresh-cut vegetables and

ready-to-eat vegetable meals is progressively higher, dramatically affecting the impact of discard on environment.

Food Loss

Food losses may arise at each supply chain level due to specific reasons. Local development impacts on Food losses according to available production and processing technologies, as well as on logistic control. In low-income countries food losses are generally higher due to the limited control of environmental parameters during primary production, storage, processing, distribution and retail (technical limitations, inadequate storage facilities and infrastructures, uncoordinated market systems) (Girotto et al., 2015).

Food losses depend thus on three global drivers (Parfitt et al., 2010):

- urbanization and contraction of the agricultural sector with extension of the food supply chains.
- diet shift towards vulnerable and shorter shelf life items.
- increased global trade of food coming from farther countries.

According to Nellman et al. (2009), between 25 and 50 % of produced food is lost along the supply chain. Crop losses at the primary production may vary from 5 to 50%. Similarly, loss varies significantly in post-harvest, from 20 to 75 % of harvested items, depending on product and situation (Gunders, 2012). Williams et al. (2012) reported that processing and packaging steps bring on the greater amount of food losses (70 kg/pro capita/year).

Food Waste

The risk of food waste increases with the number of passages along the food supply chain. To this regard, Kantor Scott et al. (1997) report that in the USA a typical food product is generally handled more than 30 times before it is displayed at the supermarket. This is not the case in Italy, especially for fresh dairy products. Williams et al. (2012) report that retailers are accountable only for a minor part of Food Waste (8 kg/pro capita/year).

Social development undeniably affects food wasted by consumers. Food wasting is facilitated by the almost constant food surplus availability in high-income countries, the major drop in prices and the growing alienation from food value (Ambler-Edwards et al., 2009; Smil, 2004). Individual reasons leading to food waste depend not only on product characteristics, but also on consumers' attitude (e.g. preference for fresh products and different taste, attention to healthy diets) and the excessive amount of incoming goods (e.g. offers, presents, unplanned purchase) often represent the root causes for food waste (Kranert, 2012). On the bases of these considerations, most of the

food wasted at home would be avoidable for sure (Beretta et al., 2013). In addition, media and public policy potentially pull domestic practices in conflicting directions, leading to opposite trends: on the one hand campaigns to reduce food waste, on the other hand agencies concerned with food safety. As a result, the domestic organization of daily life often ends with wasted food (Watson & Meah, 2013).

Private households discard the greater amount of food, wasting 76 kg/pro capita/year. This amount corresponds to 42 % of the food discarded along the whole supply chain (Waste Watcher, 2013; Williams et al., 2012). In Europe and USA, food wasted by consumers has been estimated to vary between the 15 and 30 % of all purchased food. According to the EPA (Environmental Protection Agency), the percentage of the purchased product that is wasted varies depending on food category (i.e. 50 % of salad; 25 % of fruit and vegetables such as potatoes, bananas and apple; 20 % of bread/bakery products; 10 % of meat/fish and dairy products). However, different studies provide different food waste estimates for each food category.

Fruits and vegetables are generally estimated to represent about 25–30 % of total food waste, followed by dairy and grain products. Waste percentages are significantly affected by geographical location. Fresh fruits and vegetables account for the largest portion of Turkish food waste, while in the Netherlands a high proportion of dairy products is wasted (Parfitt, 2010). Similarly, absolute estimates of total food waste often differ when obtained by applying different methodologies.

Even if consumers believe that industry and retailers generate most food discard, they are actually the main waste producers among all food chain actors. Food waste is significantly affected by household characteristics (Parfitt, 2010; Williams et al., 2012). Larger households waste more than smaller ones in absolute terms whereas per capita food waste is higher for small households and especially for single-person ones. Households with children tend to waste more than those without and youths waste more than older people, with retired ones wasting the least. It was also demonstrated that Hispanic households in the USA show lower food waste rates. Households with lower income and frequently purchasing food produce smaller amount of waste. Finally, consumer perception and awareness towards waste issues affect their food-wasting tendency. According to Waste Watcher, women usually charged with purchase and coming from larger households show the highest concern about food waste. However, 53 % of consumers declares that the global amount of wasted food is negligible, while 94 % of consumers recognizes that they are daily responsible for a remarkable food waste amount. These conflicting data indicate a significant consumers' confusion towards the waste issue.

Decreasing Food Wastage

A more sustainable management of food wastage starts from the adoption of a sustainable production and consumption approach, thus tackling food loss and waste throughout the global food supply chain (Papargyropoulou et al., 2014).

In particular, the so-called "waste hierarchy" orders possible management options according to their sustainability, intended as environmental impact as well as social and economic benefits. It also introduces the prevention concept, intended as reduction of discard generation. As a matter of fact disposal often represents the cheapest and easiest management way, but it is the less desirable disposal option, since biodegradable organic material does not return to its original state in nature (Fehr et al., 2001). On the contrary, discard reduction/prevention represents the most sustainable option although it is not always applicable, depending on the nature of discard (Papargyropoulou et al., 2014).

It should be noted that the waste hierarchy was actually developed to raise general awareness and encourage people to think beyond traditional management options (European Commission, 2014; Ohlsson, 2004; Tucker & Douglas, 2007), but although it represents a tool to identify the best management options, no quantitative data about its efficacy are currently available.

Food technology may exert a key role to face food discard, promoting technical solutions able to improve the overall quality of food products, in terms of safety, security as well as sustainability. To this purpose, it is essential to clearly identify the possibilities of reducing food products discard.

The "waste hierarchy" (Fig. 1), appoints the **reduction** of losses in the food industry as the most desirable option, avoiding the production of food surplus (Papargyropoulou, 2014; Zorpas & Lasaridi, 2013). The latter is physiologically implemented by food companies to accomplish the business goals and guarantee the required flexibility to meet market demand fluctuation.

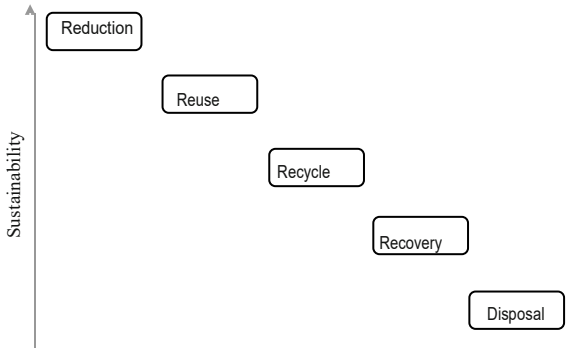


Fig. 1 Management options according to the waste hierarchy

Inadequate production planning and stock management are at the basis of food waste at the food industry level. Major savings could be generated by improving adherence to market demand through statistical prediction. In addition, processing losses could be minimized by modulating raw material selection and harmonizing stock supply with production cycles. However, primary production strictly depends on raw material variability and season. For this

reason, harmonization is not always feasible and is fraught with the risk of relocating waste generation from processing to primary production. When harmonization is not practicable, discard decrease may be obtained by complete processing of all raw material, even via production line diversification (e.g. chilled, minimal processing, canning, drying). It is evident that any corrective actions should be tested for effectiveness and eventual drawbacks. In the milk supply chain dairies are contractually obliged to collect all the milk produced by the farmers, regardless of the market demand. To face fluctuations they either purchase or sell raw milk from/to other dairies or/and diversify their production switching from fresh to long life products (e.g. butter, milk powder, UHT milk, extra hard cheese) when the offer is higher than the demand.

Since food losses (but also food wastes in the warehouses) may be due to processing errors or inadequate control of unit operations, **optimizing the existing technology** (e.g. adoption of in-line/on-line sensors) as well as developing new technologies can play a key role in wastage reduction. For instance, dough swarfs generated in the bakery industry could be minimized by properly designed rolling mills. Highly efficient ovens able to bake homogeneously the products, as well as handling systems lowering product damage, would reduce the percentage of items not complying with quality standards. No doubt that more research is needed to extend a preventive approach to several food industry fields, pursuing not only capital saving, but also environmental protection.

A second option to manage discards is to **reuse** outputs coming from a given unit operation to perform another one, desirably within the same industry. This means modifying the production process and/or implementing production diversification, to allow potentially discarded material to re-enter in the production cycle as raw material or semi-finished product. Discard characteristics may thus require only negligible changes, to make them suitable for the desired operation. By the way, reuse of discards represents a common practice in many industries. For instance, in the bakery industry, dough swarfs generated during lamination are kneaded again, while improperly cooked bakery products are grounded and reused in other formulations. Analogously, the meat/fish industry recovers processing swarfs and blood, converting them into structured products (e.g. frankfurters, surimi). Dairy industry generally recovers processing swarfs up to a defined percentage in the same production line and employs cheese whey to obtain other products such as ricotta cheese or whey protein concentrates. Wines and beer not fulfilling the requirements are generally directed to secondary production lines (e.g. distillates, vinegar). Thresh from beer production can also be employed to obtain bakery products. Similarly, fresh fruits and vegetables unsuitable for fresh consumption, due to inadequate characteristics (e.g. over-ripening, size, shape), are gainfully directed to canning and juice or jam production. Processing not only

avoids discards, but may also add value to them (Rolle, 2006), although this advantage may become negligible when transport to a different processing plant is required, increasing costs.

Even if processing is efficiently performed, by applying adequate prevention or reuse strategies and optimizing technological solutions, a huge amount of food is inevitably lost, e.g. due the presence of unusable and inedible parts and the impossibility for composite products discards (e.g. stuffed pastries, pizza) to be reused, since the separation of single components is hardly achievable and would be too expensive. It is thus necessary turning from prevention to management strategies choosing the best option along with the waste hierarchy, to guarantee the highest sustainability.

Donation, which is often a valuable option for consumers, can also be performed by producers. Substandard raw materials, products resulting from overproduction or items not sold due to low prices but still accomplishing legal requirements of food safety can be handed over to organizations supplying people in need (Schneider, 2013; Segrè & Falasconi 2011). The food surplus unfit for human consumption, including all the products withdrawn from the market for quality problems or simply because unsolded, can be addressed to livestock. This is one of the most traditional management practices performed for cereals and dairy discards, provided it is permitted by local relevant regulation, such as those hindering animal-based feed for livestock (EC Reg. No 999/2001; EC Reg. No 1234/2003; Otles et al., 2015).

Composting of food losses can also be performed by industries to produce fertilizers. On-site composting has a lower environmental impact, if compared with the centralized one, which requires transport to an external composting facility (Lundie & Peters, 2005).

Biofuel and bioenergy can be produced from losses by applying anaerobic digestion, pyrolysis and gasification, hydrothermal carbonization or incineration (Girotto et al., 2015). The residues from biofuels production can further be used as soil fertilizers (Notarnicola et al., 2012). Energy recovery would reduce the use of non-renewable resources, apparently decreasing global warming impacts. However, emissions and noise adversely affecting the environment, as well as about the high operative cost should be taken into account (Otles et al., 2015).

Considerable amounts of high value-added compounds can also be recovered through fermentation, biochemical processing or chemical extraction of most production losses (biorefinery). Developing new products having a considerable market value from food discards is costly and requires an operative context where production and discard management strategies are efficiently interconnected.

Shelf Life of Food Products

In the literature several definitions of shelf life of a food product can be found. The most shared are the following:

- Shelf Life is the length of time that a food product can be stored keeping acceptable characteristics of flavour, color, smell, aroma, texture, nutritional value and safety of consumption without becoming unfit for use, consumption, or sale;
- the Shelf Life of a food product is the length of time between the moment when the product is manufactured and packed and the one when it becomes unacceptable, under well defined and controlled keeping conditions;
- Shelf Life means the period of time during which a food product keeps its characteristics in terms of specific attributes, e.g. such as vitamin fortification level (Anonymous, 1974; Labuza, 1982; Marsh, 1986; Lee et al., 2008).

From a regulatory point of view in EU a food product shelf-life must be expressed either in terms of "date of minimum durability" or "use by date" (Reg EU No 1169/2011) and it must be present of the food label, being one of the mandatory particulars.

The "date of minimum durability" of a food means the date until which the food retains its specific properties when properly stored. In the case of foods which, from a microbiological point of view, are highly perishable and are therefore likely after a short period to constitute an immediate danger to human health, the date of minimum durability shall be replaced by the "use by" date. After the "use by" date a food shall be deemed to be unsafe in accordance with Article 14 (2) to (5) of Reg (EC) No 178/2002.

Based on the above-mentioned definitions it is clear that a food shelf-life setting may not simply be an objective task, because its own concept goes beyond the simple meaning of spoilage and/or microbiological food safety (Porretta, 2008). Shelf-life setting lays on the interaction product-consumer and basically on his acceptance level, the latter depending on his expectations on a food product in contextual conditions. As a consequence shelf-life setting should not be considered an exclusive technical task.

In literature, several studies concerning the determination of the best shelf-life of different food products have largely demonstrated the subjective value of the shelf-life concept. Thus it is clear that for a food product the shelf-life must be associated to a defined market. This means that the same product could have different shelf-life according to the country of destination.

The concept of shelf-life is very often linked to pre-packed products offered to consumer through a logistic and distribution chain. In other words, shelf-life of raw materials or unpacked foods are very rarely mentioned, whereas it is very common to deal with the shelf-life of a pre-packed food

making reference to the specific packaging system adopted and the distribution conditions used. On these bases, the definition of the optimal shelf-life of a food product leans on the knowledge of the length of time that will allow to satisfy the consumers needs and expectations.

A pre-packed food product shelf-life is mainly influenced by three factors: the type of food, the packaging system and the keeping conditions (Lee et al., 2008). These factors are often intercorrelated: e.g. some foods require special packaging materials and some types of pack are only fit for specific foods. Furthermore, food stability is influenced by storage conditions (often determined by logistic needs), hence improvements of storage conditions positively impact on product shelf-life. Last but not least the package characteristics must be adapted to the distribution conditions and viceversa.

As defined by Labuza in his pioneeristic studies (Waletzko and Labuza, 1976; Labuza, 1982) and in the following ones (Labuza, 1984; Labuza and Schmidl, 1985 and 1988; Labuza and Taoukis, 1990; Labuza et al., 1992; Labuza and Fu, 1993; Fu and Labuza, 1993 and 1997; Nelson and Labuza, 1994; Labuza and Szybist, 2001), the speed of quality decay is the result of an integrated effect involving the food recipe, the manufacturing process, the packaging system and the storage conditions.

New food technologies for shelf-life extension

In recent years food science and technology interest has been driven to innovate in the field of food shelf-life extension as a consequence of the need for the food business operators to adapt to new distribution systems and to the change in the feeding habits of the consumers.

An extended shelf-life means for businesses a differentiating attribute capable to meet modern trends of dynamic life styles where consumers are less and less engaged with food courses and meal preparation (Darian and Cohen, 1995; Verlegh and Candel, 1999; Candel, 2001; De Boer et al., 2004; Scholderer and Grunert, 2005; Buckley et al., 2007, Marie et al., 2007; Olsen et al., 2009). Shelf-life extension can be considered an innovation positively leading to improvements of products quality and to more efficient production and logistic management along the supply chain. Food durations being equal, shelf-life extension is correlated to better quality and food safety (mainly for perishable foods). From a logistic point of view extending products shelf-life may allow a more efficient scheduling of manufacturing, a reduced frequency of sourcing and the possibility of reaching consumers geographycally far from production sites.

From the side of technological innovation several solutions have been studied to extend food shelf-life, in terms of food formulation, new processes and packaging systems. A huge scientific literature is therefore available concerning food shelf-life.

Although the positive impact of food shelf-life extension is generally recognized and confirmed by the scientific literature, nonetheless not much attention has been given to the most important element of interest of the food business operators, i.e. the real impact of SLE on food waste. The importance of a larger knowledge on this issue has been recalled in several studies, e.g. Wikstrom and Williams who focalized their interest on a deeper knowledge of food wastage, their environmental impact and their causes (Wikstrom and Williams, 2010).

Food waste and shelf life

Extending a food product shelf-life may play a significant role in hindering food waste. Food wastage is a topic politically touching, since food wastage has impact on society, economy and environment. In other words it concerns sustainability.

A recent resolution of the European Union (http://www.europarl.europa.eu, 2011) highlights that every year at home, in supermarkets, restaurants and all along the food supply chain more than 50% of available food products is wasted, when 79 million citizens live under the poverty threshold and 16 millions depend on the aid charity institutions. The European parliament has pushed for a resolution on urgent measures to halve food wastage within 2025 and to facilitate the access to food by indigent European citizens. The same study asserts that in case no action is taken food wastage will increase by 40% within 2020. This is a real, not only ethic but also economic and social problem, with huge implications for the environment as well. Impressively the EU Commission has reported 89 million tons per year food wastage, forecasting 126 million tons/year in (+ 40%).

Since wastage is created in any phase of the food supply chain (primary producers, manufacturers, normal traders, retailers, restaurant owners and consumers) it seems necessary at least to measure the amount of wastage created in each food chain branch and level in order to identify a coordinated strategy, combining national and trans national strategies, capable to improve the efficiency of each food production and consumption chain.

No doubt that quantifying the extent of food wastage is a complex task. Different methodological approaches have been used, based on evaluations, data collected through interviews, indirect statistical measures and even assessment of the garbage produced by families, coffee shops and restaurants. In any case it appears as a priority to be able to improve our capability to evaluate food wastage with the aim of defining better protocols and procedures and identify actions focused to educate consumers not to waste food (Scott et al. 1997; Schneider, 2007).

Shelf Life Extension Strategies

Food technology can indirectly affect food wasted during retailing and at household level. The application of novel technologies to extend the ingredient/product shelf life has been claimed to potentially reduce food loss and waste generated upon distribution and purchase. Among these technologies are innovative active/intelligent packaging and non-thermal decontamination techniques such as those based on electromagnetic (e.g. UV light, pulsed light), mechanic (e.g. ultrasounds, high pressure processing, high-pressure homogenization) or chemical stresses (e.g. ozone, non-thermal plasma). However, discard reduction by implementation of these technologies may result in a sale decrease, potentially limiting company investments in these technologies. Companies obviously tend to focus on avoiding food discard before sale but care less for product destiny after sale. In addition, the relation between shelf life extension and discard reduction does not appear to be straightforward according to Amani and Gadde (2015), and it would be therefore necessary to monitor the effectiveness of the application of shelf life extending interventions on the actual food discards. A product with a longer shelf life will probably be stored by consumers for a longer time, running a higher risk of being forgotten in the pantry and exceed the expiration date. The latter has probably an important responsibility for domestic waste generation, especially for shelf stable products. Most of them are generally attributed a best before date that is sometimes selected based on the necessity to increase product turnover on the shelves and not following a real safety or quality risk. Identifying the optimal turnover frequency would allow using products still suitable for consumption even if no more appealing the standard consumer, products that could be allocated on appropriate markets for substandard products (Girotto et al., 2015). Being generally the choice of expiration date a specific task of the producer, the waste responsibility is often not directly attributable to consumers, but might rely on the producer itself. In this context, legislation on expiration dates, that has inadvertently increased food waste, should be re-examined within a more inclusive competing-risk framework (Godfray et al., 2015). The evolution of expiration date from a simple consumer protection to the wider concept of the protection of a sustainable food-consumer relation could significantly reduce food waste generation. Expiration dates should thus be defined considering not only product safety and quality, but also environmental and social impact. These aspects should be merged with food technology through a pioneering interdisciplinary approach, in order to develop a methodology for defining shelf life values able to concomitantly satisfy consumers and minimize food waste, may be defining two expiry dates, the former for quality and the latter for safety and security.

Fresh dairy products preservation

Fresh dairy products preservation is generally achieved, where possible, by heat treatment (e.g. Thermo-quarg, ricotta, mascarpone) and hot filling coupled to keeping at low temperature (4-6°C) during all the shelf life. Deep freezing is increasingly used for fragile dairy products to reach very far destinations (e.g. Oceania and Far East). Deep frozen products may be sold either as such or as chilled products after defrosting, provided the target country regulation admits this practice.

Among fresh dairy products fresh pasta filata cheeses are considered extremely perishable; due to their pH, high moisture and fat content, their spoilage is mainly ascribed to microbial growth. Currently, their packaging consists of rigid or flexible films of multilayer materials or trays made up of polyethylene/paper laminated films and tetrapack-packages (Robertson, 1993). These systems do not represent strategic solutions to assure a long shelf life.

The packaging of Burrata (an ultrafresh Italian cheese made from fresh mozzarella and cream), in particular, represents a very important cost item since it is really time-consuming, requires very skilled workers being very complex and not yet mechanized. Traditionally, this cheese was wrapped in leaves of a local vegetable (*Asphodelus* spp.), but due to hygienic concerns, such type of package is no longer allowed.

Actually, the product is sold after packaging in a double bag of plastic material, the first of which contains the cheese, the second envelops the previous and mimics the *Asphodelus* leaves.

Various efforts are made to extend fresh cheese shelf life; in particular the attention has been focused on fresh Fior di Latte and Mozzarella cheese, which are generally packed under a light brine and whose expiry date may vary from 3 to 27 days at 4-6°C. Some solutions concern the use of packaging systems with antimicrobial properties (Lopez-Rubio et al., 2006; Coma, 2008). Conte et al. (2007) successfully investigated a release system based on lemon extract to prolong shelf life of Mozzarella cheese. Gammariello et al. (2008) demonstrated that selected essential oils, properly dissolved in the brine of cheese, can exert an inhibitory effect on microorganisms responsible for spoilage of Fior di latte cheese, thus promoting a longer microbial stability. The most effective natural compounds were also combined according to a Central Composite Design and successfully enhanced Fior di Latte microbial quality (Gammariello et al., 2010). The addition of an enzyme as lysozyme in the packaging brine also prolonged product storability (Sinigaglia et al., 2008).

Data in the literature indicate that substitution of brine with a natural hydrogel or a bio-based coating could represent another interesting strategy for fresh cheese preservation (Laurienzo et al.,

2006, 2008; Del Nobile et al., 2010). Relevant results were achieved when an active coating was applied to the product prior to packaging under modified atmosphere conditions (MAP) (Conte et al., 2009). The potential of MAP in extending dairy products shelf life was demonstrated by various authors (Floros et al., 2000; Pantaleao et al., 2007; Papaioannou et al., 2007). The gases normally used for MAP include CO_2, O_2 and N_2. Eliot et al. (1998) reported that shredded mozzarella cheese packaged under MAP (75% CO_2) was well protected from undesirable microorganisms attack and gas formation. Alves et al. (1996) also found that microbial growth in sliced mozzarella cheese, packaged under MAP and stored at 7°C, was delayed by using high concentrations of CO_2 that represents the most important gas from a microbiological point of view (Daniels et al., 1985).

More recently, Incoronato et al. (2010) and Gammariello et al. (2011) investigated the application of silver nanoparticles to Fior di Latte cheese, showing a marked increase in shelf life, due to the ability of silver cations to control microbial proliferation, without affecting the functional dairy microbiota and the sensory characteristics of the product.

A very few works in the literature were devoted to packaging of Stracciatella and Burrata, compared to packaging of Mozzarella and Fior di Latte. The only two examples are the works of Gammariello et al. (2009) and Conte et al. (2011). In the first study, the authors showed that packaging of Stracciatella under a protective atmosphere based on different CO_2:N_2 gas mixtures, delayed microbial growth and prolonged sensory acceptability. In the second, lysozyme/Na_2-EDTA, combined with sealing of product under MAP, positively affects shelf life of Burrata cheese.

Due to the relevance of fresh pasta filata cheese both for domestic consumption and for export, it is important to investigate further new technical possibilities to extend the shelf life of these products.

The study will focus on Fior di Latte cheese, a typical Mediterranean fresh pasta filata product, made from cow's milk and usually packaged under a light brine. Its high moisture (from 55% to 64%) and high fat content (>45% fdm; Salvadori del Prato, 2001) makes it very susceptible to microbial spoilage, particularly under temperature abuse (T > 6°C). Although milk is generally pasteurized and a further heat treatment is applied during curd stretching, post processing contamination may occur, causing FdL cheese spoilage and eventually safety risks to consumers (Spano et al., 2003). Undesirable microorganisms such as pseudomonads, coliforms, yeasts, and molds may cause defects in flavor, texture, and appearance and result in economic losses (Gammariello et al., 2008; Conte et al., 2009; Del Nobile et al., 2009). Fior di Latte shelf life also depends on the quality of raw material and on the process conditions (Brody, 2001).

Current technologies for the preservation and shelf life extension of food products include heat processing, chemical preservatives, modified atmosphere packaging or refrigeration. Unfortunately, these strategies do not fully control spoilage bacteria. The great availability of nutrients in foods

may enable bacteria to repair damaged cells (Gill et al., 2002). Both the intrinsic (fat, protein, water content, antioxidants, pH, salt and other additives) and the extrinsic properties (temperature, packaging in vacuum⁄gas⁄air, characteristics of micro-organisms) of the food can influence bacterial sensitivity to natural and chemical preservatives (Shelef, 1983; Tassau et al., 2000). At present, FdL cheese shelf life is approximately 5-7 days, and many efforts are in progress to prolong this shelf life by means of process innovation and raw materials quality improvement. Good opportunities came from the use of antimicrobial compounds during milk transformation (Del Nobile et al., 2009).

The high consumer attention to food safety aspects justifies increased research interest in using active agents derived from natural sources, as plant essential oils or plant extracts, considered suitable for food application, able to reduce the microbial count and to control the cell growth during the different steps of the product life (Conte et al., 2007; Gammariello et al., 2008b; Gammariello et al., 2010). Efforts to prolong the shelf life of FdL cheese are also made by the optimization of storage and packaging conditions (Conte et al., 2009; Del Nobile et al., 2009; Del Nobile et al., 2010). The potential of modified atmosphere packaging (MAP) and active packaging to extend the shelf life of different dairy products has been proposed by various authors (Floros et al., 2000; Pantaleao et al., 2007; Papaioannou et al., 2007).

On the other end the great demand for fresh-like products still promotes the search for new technologies to preserve food. One of the most recent potential approaches to prolong the shelf life of fresh products is the use of bio-preservation systems. Bio-preservation consists in the use of natural or controlled microbiota or antimicrobials as a way of preserving food and extending its shelf life (Ananouet al., 2007). Beneficial bacteria or their fermentation products are used in bio-preservation to control spoilage and render pathogens inactive in food (Yousef et al., 2003). Lactic acid bacteria (LAB) have antagonistic properties which make them particularly useful as bio-preservatives. When LAB compete for nutrients, their metabolites often include active antimicrobials such as lactic and acetic acid, hydrogen peroxide, and peptide bacteriocins. Bio-preservative bacteria must be harmless to humans. LAB bacteriocins are used as an integral part of hurdle technology. Using them in combination with other preservative techniques can effectively control spoilage bacteria and other pathogens, and can inhibit the activities of a wide spectrum of organisms, including inherently resistant Gram-negative bacteria. Angiolillo et al. (2013) showed that the addition of *Lactobacillus rhamnosus* in an edible sodium alginate coating applied on the surface of FdL cheese exerted an antimicrobial activity against *Pseudomonas* spp. and *Enterobacteriaceae.*

Lactobacillus reuteri is a hetero fermentative lactobacillus recognized as normal inhabitant of the human and animal gut (Reuter, 2001). It is also frequently found in fermented and probiotic foods

(Vollenweider and Lacroix, 2004). *L. reuteri* as a food supplement is accepted and widely used to improve gastrointestinal health and has been granted qualified presumption of safety (QPS) by the European Food Safety Authority (EFSA). Probiotic effects of *L. reuteri* have been proposed due to the ability of some strains to produce reuterin (b-hydroxypropionaldehyde; b-HPA) during anaerobic metabolism of glycerol (Rodríguez et al., 2003). Reuterin is an antimicrobial compound soluble in water, resistant to heat and stable over a wide range of pH values, that inactivates Gram-negative and Gram-positive bacteria (Vollenweider et al., 2003). Direct addition of reuterin to control food-borne pathogens such *Salmonella sp., Escherichia coli* O157:H7*, Listeria monocytogenes* and *Staphylococcus aureus* has been investigated in milk and dairy products (Arqués et al., 2008a; Arqués et al., 2008b) but the gap of these studies consisted in the fact that reuterin was used as a food additive. This limit has been overcome developing a new biopreservation system consisting in a sodium alginate coating containing *L. reuteri* in combination with glycerol (registered in the European Union as food additive E 422), applied on the surface of FdL cheese in order to extend its shelf life by means of in situ production of reuterin.

OBJECTIVE OF THE THESIS

This thesis, considering what has been highlighted up to this point, being of interest the identification the determinants of the food waste as far as possible, will focus in particular on the relation between food shelf-life and waste. To this aim a double approach will be developed.

Initially, thanks to the availability of real market data kindly offered by a domestic dairy industry a statistical analysis will allow to identify possibly a mathematical function correlating products shelf-life with an important food waste component, i.e. the product returned from the market expressed as percentage on sold quantity.

A second objective of this thesis will be, based on the above mentioned market data, to evaluate if actions put in place to extend the shelf-life of specific products have actually reduced food waste.

The third and last objective of this thesis will be to assess experimentally the possible application of new specific packaging strategies to extend the shelf-life of Fior di Latte cheese, a product characterized by a strong microbiological fragility and consequently a very short shelf-life.

The basic strategy will be of associating modified atmosphere packaging (MAP) to a bio-based coating, eventually charged with antimicrobial components, in order to achieve a significant shelf-life extension of Fior di Latte (FdL) cheese. A few experimental case studies will be illustrated.

The first case study is designed to evaluate the combined effect of MAP and plain/active coating; the second one aims to optimize such combined effect (MAP + anti microbial compound charged coating), whereas the third one intends to assess the efficacy of a combination MAP + silver-nanoparticle charged coating.

The fourth and final case study deals with a new biopreservation system developed including into the bio-based coating living cells of a probiotic lactic acid bacterium (*Lactobacillus reuteri*) and glycerol.

Materials and Methods

Samples

Fior di Latte samples (approximately 150 g each) were kindly provided by a local cheese factory "Capurso Azienda Casearia S.p.A." (Gioia del Colle, Bari, Italy) and brought to our laboratory under refrigerated conditions (4°C).

Microbiological Analyses

Twenty grams of mozzarella cheese were diluted in 180 mL of a sterile saline solution (0.9%) and homogenised in a blender (Stomacher, International PBI, Milan, Italy). After, decimal dilutions of cheese homogenates were made in saline solution and plated on selective media for determination of mesophilic lactic acid bacilli, lactococci, total bacterial count, *Enterobacteriaceae* and *Pseudomonas* spp.

Microbiological analyses for Total Viable Count (TVC), *Pseudomonas* spp., *Enterobacteriaceae*, lactic acid bacteria, lactococci were performed according to the International Standard ISO 8261:2001. Media and conditions used for the enumerations were as follows: Plate Count Agar (PCA, Oxoid, Milan, Italy) incubated at 30°C for 24–48 h for TVC; Pseudomonas Agar Base (PAB, Oxoid), added with CFC selective supplement, incubated at 25°C for 48 h for *Pseudomonas* spp. count; for *Enterobacteriaceae*, Violet Red Bile Glucose Agar (VRBGA, Oxoid) incubated at 37°C for 18–24 h; de Man Rogosa Sharpe agar (MRS, Oxoid) incubated anaerobically in the jars HP 11 (Oxoid) at 37°C for 2–4 days for lactic acid bacteria (LAB); M17 agar (Oxoid) incubated anaerobically in the jars HP 11 (Oxoid) at 37°C for 48 h for lactococci. The microbiological analyses were carried out twice on two different batches.

At each sampling time the pH was also measured by a pH meter (Crison, Barcelona, Spain). The measurements were done in duplicate on two different samples.

To determine the effectiveness of the packaging strategy the microbial acceptability limit (MAL), defined as the storage time at which microbial counts of selected spoilage group reached the threshold value permitted, was calculated as reported by Del Nobile et al. (2009a).

In order to quantitatively determine the microbial acceptability limit, a modified version of the Gompertz equation was fitted to the experimental data, as reported in previous works (Conte et al., 2009; Del Nobile et al., 2009).

$$\log(N(t)) = \log(N_{max}) - A \cdot \exp\left\{-\exp\left\{\left[(\mu_{max} \cdot 2.71) \cdot \frac{\lambda - MAL}{A}\right] + 1\right\}\right\} + A \cdot \exp\left\{-\exp\left\{\left[(\mu_{max} \cdot 2.71) \cdot \frac{\lambda - t}{A}\right] + 1\right\}\right\} \quad (1)$$

where N(t) is the viable cell concentration at time t, A is related to the difference between the decimal logarithm of maximum bacterial growth attained at the stationary phase and decimal logarithm of the initial value of cell concentration, μmax is the maximal specific growth rate, λ is the lag time, Nmax is the microbial threshold value, MAL is the microbiological acceptability limit [i.e., the time when N(t) is equal to Nmax], and t is the storage time. The value of Nmax for *Pseudomonas* spp. was set to 10^6 cfu/g of cheese and 10^5 CFU/g for Enterobacteriaceae ($MAL^{Enterobacteriaceae}$) according to other works dealing with same dairy product (Conte et al., 2009).

Sensory Analysis

Sensory analysis was conducted according to a method reported in the literature (Chiavari et al., 2006). A quantitative descriptive analysis was used for the comparison of samples (UNI 10957:2003 Sensory analysis – Method to define the sensory profile of foods and beverages).

In accordance with the standard UNI 10957:2003, seven/eight testers of the Food Packaging laboratory were selected on the basis of international standards ISO 8586-1:1993 and ISO 8586-2:1994. Nine sessions of one hour each were required to define the sensory profile, with the frequency of three meetings a week. The sessions were used to familiarize the testers with the characteristics of Fior di Latte samples in terms of odor, color, texture and overall quality. After training, FdL samples were presented to each panelist without brine (Angiolillo et al., 2013). The panelist was asked to evaluate odor, color, texture and overall quality every day for the entire period of observation, by using a scale from 0 to 7, where 4 was the minimum threshold for cheese acceptability. To judge the overall quality of cheese the following product characteristics were also taken into account: white porcelain, smooth surface, tight shut-off, elastic release of buttermilk after cutting, lack of holes and typical milk smell. The analysis of the texture was performed by touching the surface of the products with fingers and evaluating the degree of surface fraying with movements from top to bottom of the surface. Each taster evaluated a set of four samples each labeled with a random three-digit code. Samples have been stored in the sensory analysis laboratory at room temperature before tasting and the order of presentation was different for each accepted

subject, to avoid mutual interference. Before evaluating, each coated FdL cheese was deprived of the coating and immersed in water at room temperature for a few minutes, in order to tie these samples to wet uncoated cheese. The tasters used individual tasting booths in the hall of sensory analysis of the Food Packaging laboratory. The quantitative analysis was performed with a number equal to three replicates sessions on different days at the same hour of each day. In order to determine the sensory acceptability limit (SAL), that represents the storage time to reach the sensory attribute threshold, a modified version of the Gompertz equation was fitted to the experimental data (Conte et al., 2009; Del Nobile et al., 2009).

$$SA(t) = SA_{min} - A^{SA} \cdot \exp\left\{-\exp\left\{\left[\left(\mu_{max}^{SA} \cdot 2.71\right)\frac{\lambda^{SA} - SAL}{A^{Q}}\right] + 1\right\}\right\} + A^{SA} \cdot \exp\left\{-\exp\left\{\left[\left(\mu_{max}^{SA} \cdot 2.71\right)\frac{\lambda^{SA} - t}{A^{SA}}\right] + 1\right\}\right\} \quad (2)$$

where SA(t) is the sensory attribute at time t, SA_{min} is the sensory attribute threshold value, A is related to the difference between the sensory attribute attained at the stationary phase and the initial value of sensory attribute, μ_{max} is the maximal rate at which SA(t) decreases, λ is the lag time, SAL is the sensory acceptability limit (i.e., the time at which SA(t) is equal to SA_{min}), and t is the storage time. As reported above, the value of SA_{min} was set equal to 4.

Shelf Life Calculation

The shelf life of a packed product is, by definition, the time at which one of the product quality sub-indices reaches its threshold value. In this work, the shelf life of each tested samples was calculated as the lowest value among $MAL^{Pseudomonas}$ and SAL^{OQ}, as also reported in other works (Gammariello et al., 2011).

Headspace Gas Composition

Before opening the FdL boxes, headspace O_2 and CO_2 composition was determined using a Checkmate 9900 gas analyzer (PBI Dansensor, Ringsyed, Denmark). To avoid modifications in the headspace gas composition due to gas sampling, each package was used only for a single measurement. Two boxes were used for each test.

Statistical Analysis

Experimental data of all tested samples were compared by one-way Anova analysis. A Duncan's multiple range test, with the option of homogeneous groups ($P < 0.05$), was used to determine

significance among treatments. To this aim, Statistica 7.1 for Windows (StatSoft Inc., Tulsa, OK, USA) was used.

Case study 1: optimization of a protecting packaging system applied to FdL cheese

FIRST TRIAL: FDL UNDER MAP

Samples were aseptically removed from their original packages and packaged in polypropylene trays (two pieces each) with pot water as covering liquid in air (CNTR) and modified atmosphere. The top film was a PET/12+CAST/50 with an Oxygen Transmission Rate (OTR) of 190.32 cc/(m^2·day) and Carbon Dioxide Transmission Rate (CDTR) of 707.94 cc/(m^2·day). The samples were sealed by means of a thermosealer ORVED Mod. VGP (ORVED S.p.A. Musile di Piave, Italy). The gas combinations used were the following: MAP1 20% CO_2, 80% N_2, MAP2 50% CO_2, 50% N_2, MAP3 80% CO_2, 20 %N_2. All samples were stored at 8±1°C.

SECOND TRIAL: FDL WITH COATING AND MAP

FdL samples (approximately 150 g each) were dipped into a sodium alginate solution prepared by dissolving sodium alginic acid powder (2% w/v) in sterile distilled water tempered to 50°C (Lucera et al., 2014). A crosslinking solution of calcium chloride (5% w/v) was used to promote the alginate gel forming process by dipping the product for 1 minute. After the treatment the coated FdL samples (COAT) were air-dried for 2 minutes. Then, the coated samples packaged in polypropylene trays (two pieces each) with the covering liquid in MAP with the same gas combinations used in the first trial. Uncoated samples were used as control (CNTR) and packaged in air. All samples were stored at 8±1°C.

THIRD TRIAL: FDL WITH ACTIVE COATING AND MAP

FdL samples (appr.150 g each) were dipped into a sodium alginate solution prepared by dissolving sodium alginic acid powder (2% w/v) and potassium sorbate (3% w/v) in sterile distilled water tempered to 50°C (Lucera et al., 2014). A crosslinking solution of calcium chloride (5% w/v) was used to promote the alginate gel forming process by dipping the product for 1 minute. After the treatment the coated FdL samples (COAT_PS) were air-dried for 2 minutes. Then, the coated samples were packaged in polypropylene trays (two pieces) with the covering liquid in air (COAT-PS_air) and MAP3 (COAT-PS_MAP3). The MAP3 was chosen as the best among the atmospheres tested in the previous trial. The FdL samples coated with sodium alginate solution without potassium sorbate were also packaged in air and MAP3 (COAT_air, COAT_MAP3). Moreover,

uncoated samples were used as control and packaged in air (CNTR). All samples were stored at 8±1°C.

Case study 2: sodium alginate coating containing *antimicrobials* applied to FdL cheese

First Trial: FdL with Active (PS/SB/CL/CA) Coating and MAP

The coating solution was prepared by dissolving sodium alginic acid (2% wt/vol) in distilled sterile water. Different active substances, as potassium sorbate (0.1% wt/vol) (PS), sodium benzoate (0.5% wt/vol) (SB), calcium lactate (0.5% wt/vol) (CL) and calcium ascorbate (0.5% wt/vol) (CA) were added to the alginate solution. After complete dissolution of the active compounds, the coatings were obtained by immersing cheese samples first in the sodium alginic acid solution and then in a calcium chloride solution (5% wt/vol) for 1 min. For each treatment, two coated samples were prepared and then packaged in polypropylene trays containing brine.

The investigated samples were labelled as follow: PS-Coat, SB-Coat, CL-Coat, CA-Coat (sample coated with a solution of sodium alginic acid containing potassium sorbate, sodium benzoate, calcium lactate and calcium ascorbate, respectively);

Second Trial: FdL with Active Coating (PS) and MAP

In the second trial, three different concentrations of potassium sorbate (1%, 2%, 3% wt/vol) in the coating were tested on mozzarella cheese. These samples were prepared as described above and packaged in the same conditions. In both the experimental trials uncoated mozzarella cheese was used as the control (Cnt). In the second trial coated cheese without any active compound were also prepared. All the investigated cheese samples were stored at 8±1°C.

The investigated samples were labelled as follow: PS-1%, PS-2%, PS-3% (sample coated with a solution of sodium alginic acid containing potassium sorbate at 1%, 2%, 3% wt/vol, respectively).

The sodium alginic acid and the calcium chloride, as well as all the active compounds were purchased from Farmalabor (Canosa di Puglia, Italy).

Case study 3: active sodium alginate coating containing Silver Nanoparticles

Coating and packaging of FdL samples

FdL samples were dipped into a sodium alginate solution prepared by dissolving sodium alginic acid powder (2% w/v) in sterile distilled water tempered to 50°C. A crosslinking solution of calcium chloride (5% w/v) was used to promote the alginate gel forming process by dipping the product for 1 minute. Then, the coated FdL samples were packaged in plastic vessels (two pieces) with the covering liquid in air (COAT-air) and MAP (COAT-MAP). The top film was a

PET/12+CAST/50 with an Oxygen Transmission Rate (OTR) of 190.32 cc/(m^2·day) and Carbon Dioxide Transmission Rate (CDTR) of 707.94 cc/(m^2·day). The samples were sealed by means of a thermosealer ORVED Mod. VGP (ORVED S.p.A. Musile di Piave, Italy). The gas combination used is the following: 50% CO_2, 50% N_2. In addition, the active coating was prepared by dissolving sodium alginic acid (2% w/v) and 0.25 mg/ml of silver nanoparticles (Ag-NP) in sterile distilled water. Silver nanoparticles were prepared as reported in the previous paper of Incoronato et al. (2010). Transparent coatings were obtained by dipping the FdL samples first in the active sodium alginate solution and then in a calcium chloride solution (5% w/v) for 1 minute. After coating, the samples were packaged in plastic vessels (two pieces) with the covering liquid in air (COAT-Ag_air) and MAP (COAT-Ag_MAP). In addition to the samples packaged in the covering liquid the coated samples were also packaged in air and MAP without the covering liquid. Moreover, uncoated samples were used as control and packaged in air (CNTR). All samples were stored at 8±1°C.

Case study 4: active sodium alginate coating containing *Lactobacillus reuteri*

Once transported to the lab, the samples were dipped into three different sodium alginate solutions. The first one was prepared by dissolving sodium alginate acid (Farmalabor, Canosa di Puglia, Italy) (2% wt/vol) in distilled water; the second one was prepared by dissolving sodium alginate acid (2% wt/vol) in a solution made of 2% (wt/vol) of pure concentrated freeze dried *L. reuteri* and distilled water and the third solution was made of 2% (wt/vol) of pure concentrated freeze dried *L. reuteri*, 0.6% of glycerol (Sigma) and distilled water. The coated samples were immersed into a 5% (wt/vol) calcium chloride ($CaCl_2$) (Sigma–Aldrich Milan, Italy) for 1 min, to allowed creating a stable coating on the cheese surface. All samples were dried at room temperature for 2 min and packaged in commercially available polypropylene bags with brine (0.2% wt/vol of NaCl solution). The control samples consisted in FdL cheese without coating, packaged in trays with brine. All the samples were stored at 9°C. The experimental analyses were conducted in two different trials, using two different production batches of samples.

First Trial: FdL with Active (L. reuteri) Coating prepared on the same day and MAP

In the first trial, Fior di Latte samples were dipped into the sodium alginate solutions prepared on the same day of their production.

Second Trial: FdL with Active (L. reuteri) Coating prepared on 48 h before and MAP

In the second trial, samples were dipped in sodium alginate solutions prepared 48 hours before cheese production. Sodium alginate solutions used in the second trial were prepared in bottles with

hermetic seals in order to avoid evaporation. Immediately after their preparation, sodium alginate solutions were incubated at 25 °C for 48 hours.

Samples will be named as follows: CNT (control sample consisting in Fior di Latte cheese without coating), COAT (Fior di Latte cheese with a 2% sodium alginate coating without *L. reuteri*), ACT-COAT (Fior di Latte cheese with a 2% sodium alginate coating containing *L. reuteri*) and ACT-COAT-GLY (Fior di Latte cheese with a 2% sodium alginate coating containing *L. reuteri* and glycerol). The sampling was performed immediately after the production, and subsequently every day at the same time for a week.

Statistical Analysis of data from the domestic market

Data utilized in the current study were provided by a large Italian food company. Data of a total 640 dairy products pertaining to different categories (tipology and size) were taken into account. Specifically, 640 typologies of products were considered: baby food (cream, milk, snack and yogurt), butter (with and without lactose, in different sizes), various typologies of milk (flavoured, lactose-free, biological, with extended shelf life, high quality, UHT), cream (fresh, UHT, lactose-free), goat UHT milk, yogurt and ricotta cheese, various typologies of yogurt and dessert, spreadable cheese in different sizes, mozzarella cheese (different sizes, traditional, for pizza, light, lactose-free), stretched curd, mascarpone cheese (different sizes), ricotta cheese (light, biological, lactose-free and mix), various typologies of soft and semi-hard cheese made of cow's, sheep and goat's milk, extra-hard cheese as Grana Padano and Parmigiano Reggiano in different sizes and at different ripening time.

The variables considered for each product were shelf life and a standardized variable as follows

returned_goods_ratio = returned goods (tonnes) / delivered goods (tonnes).

The shelf life is discretized into 17 ranges, in order to consider together even different types of products, which share a common range of shelf life.

Firstly, the one-way ANOVA was used for testing the eventually differences in *returned_goods_ratio* with respect to 17 range of shelf life. Consequently, a model based approach was proposed to analyse the relationship between *shelf life and returned_goods_ratio*. All statistical analyses were conducted with the IBM SPSS 20, Statistical Package for the Social Sciences Program.

Results and Discussion

Case study 1: optimization of a protecting packaging system applied to FdL cheese

The work is divided into three parts that contributed to the final result. The first trial attempts to investigate the effect of modified atmosphere packaging (MAP) on the overall quality of FdL cheese. Then, in the second trial the combined effect of the selected MAP and edible coating on the shelf life of the cheese was investigated. Finally, in the third trial the coating was loaded with potassium sorbate and its combined effect with MAP on the shelf life of FdL was studied.

It is worth noting that since cheese samples were taken from three different productions of the same month, a certain level of variability in the quality characteristics of FdL was expected. Therefore, to overcome this problem, the shelf life values of tested samples were compared with the related control samples prepared in the same experimental trial.

Headspace Gas Composition

Figure 1 highlights the evolution of headspace gas composition of FdL packaged in MAP. As can be inferred from the figure, the FdL samples packaged under MAP show a quick decrease of the CO_2 concentration (Fig. 1a). This behavior was probably due to the gradual solubilization of the CO_2 in the covering liquid. Specifically, the FdL packaged with the highest CO_2 concentration (MAP3) presented a CO_2 level that decreased down to 20% after the first day of storage. Likewise, the CO_2 concentration reached a value of about 13% for the MAP2 and 7% for MAP1. Moreover, the oxygen concentration increased in all samples due to the film permeability until it reached a steady-state after about 3 days of storage (Fig. 1b). In particular, a value of about 11% for MAP3 and 5 and 4% for MAP2 and MAP1, respectively, was observed.

It is worth noting that the headspace gas composition for the second and third trial did not show differences compared to the first trial (data not shown).

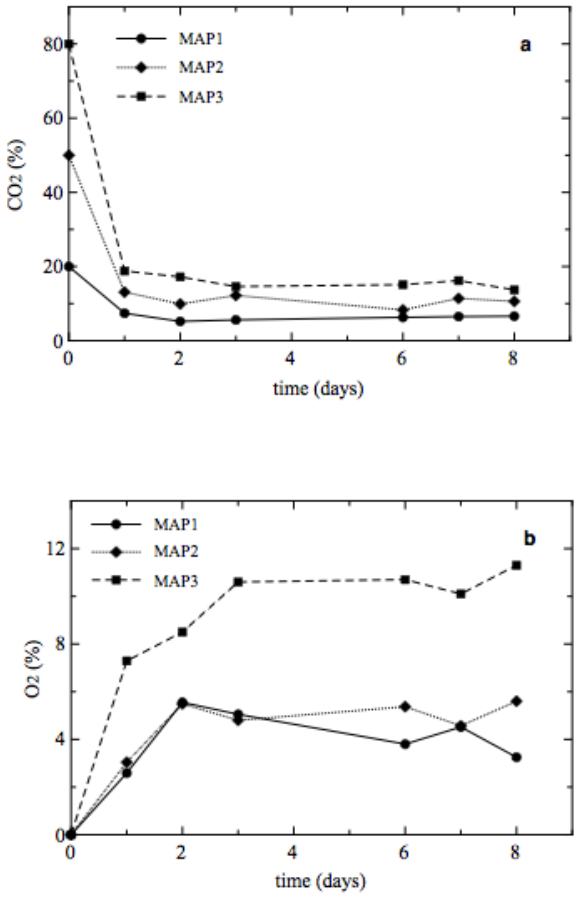


Figure 1 Evolution of the CO_2 (a) and O_2 concentration (b) in the headspace composition of Fior di Latte samples.

MICROBIOLOGICAL QUALITY

Fresh dairy products are ready-to-eat foods easily contaminated by undesirable microorganisms. Some of them are spoilage microorganisms which may produce unwanted visual appearance and diminish the commercial value of cheese, other ones are pathogens that affect product safety.

Figures 2 show the evolution of *Pseudomonas* spp. cell load plotted as a function of storage time for the FdL cheese samples. The horizontal solid line marks the microbiological threshold value. Concerning the first trial, the packaging of FdL under MAP with high carbon dioxide concentration (MAP2 and MAP3) was able to keep the *Pseudomonas* spp. cell load below the microbiological limit for the entire storage period (Fig 2a). This was probably due to the specific effect of CO_2 as confirmed in previous studies (Eliot et al., 1998). The lowest CO_2 concentration (MAP1) resulted only in a slight delay in reaching the threshold value respect to the control samples. The evolution of *Pseudomonas* spp. for the second trial highlighted that the CNTR samples reached first the threshold value followed by the coated samples packaged in MAP1 and MAP2 (Fig. 2b). The presence of high CO_2 concentration (MAP3) in the headspace gas composition determined a slower growth of the *Pseudomonas* spp. that reached the microbiological limit at about 7 days of storage. Finally, the effect of active coating and MAP on the growth of *Pseudomonas* spp. is shown in Figure

2c. As can be seen, the CNTR samples quickly reached the microbiological limit respect to the other samples.

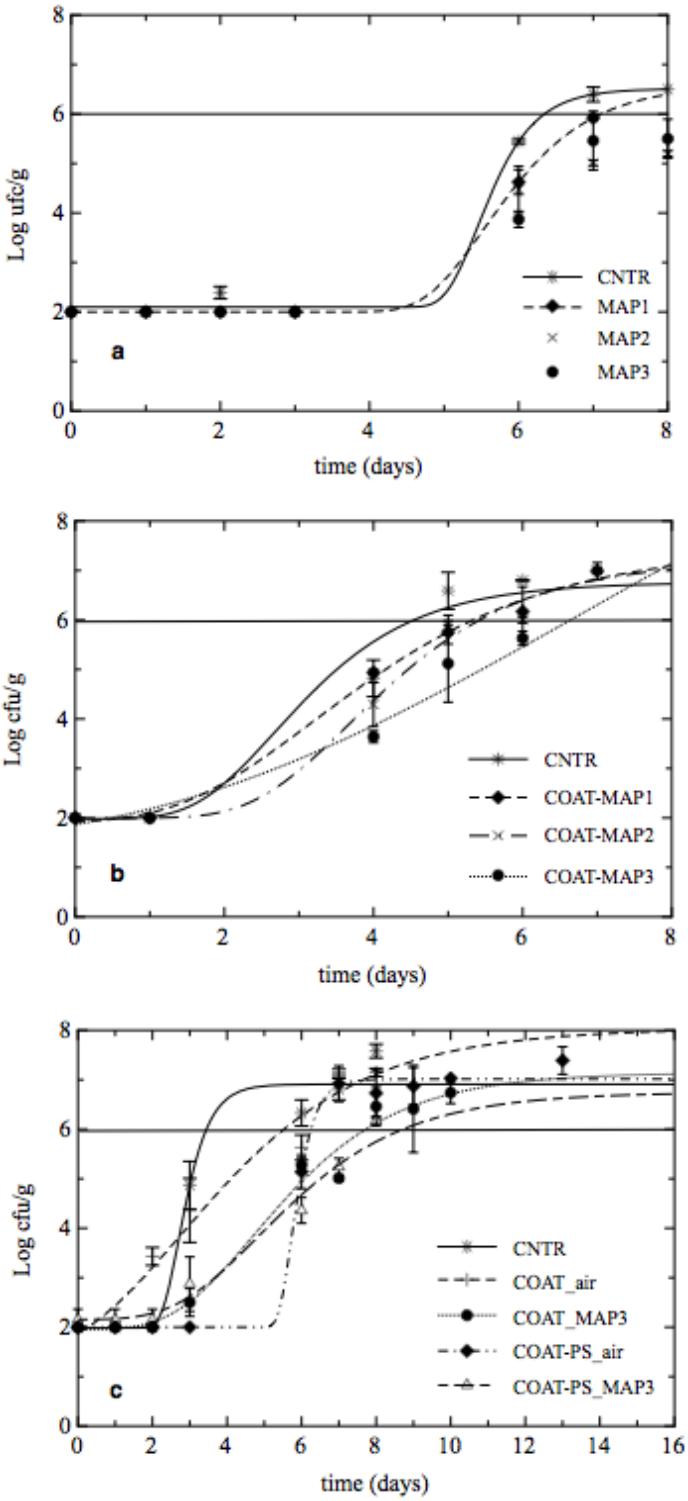


Figure 2 Evolution of *Pseudomonas* spp. cell load of Fior di Latte of the first (a), second (b) and third (c) experimental phase.

The coated samples packaged in air with (COAT-PS_air) and without active compound (COAT_air) reached the threshold value at the same time. However, the potassium sorbate loaded in the coating prolonged the lag phase of the *Pseudomonas* spp. of about 5 days.

Potassium sorbate is widely used in many types of foods ranging from cheese and yogurt to dried fruit and meat. When added to water, potassium sorbate breaks down into ionic sorbate and potassium (K). It is the sorbic acid that is active as an anti-microbial (Ricke, 2003). The antimicrobial effect of the potassium sorbate on spoilage bacteria such as *Pseudomonas flourescens* in soft cheese is well documented (Stanojevic et al., 2009; Azza & Ahmed, 2010). Authors stated a percent reduction of *P. fluorescens* count at the end of storage period by about 94-99% for cheese containing potassium sorbate at the increasing concentrations (0.02-0.2%). The antimicrobial effect is based on the increase in proton concentration thereby lowering the external pH. Organic acid such as sorbic acid may affect the integrity of microbial cell membrane or cell macromolecules or interfere with nutrient transport and energy metabolism, causing bactericidal effect (Ricke, 2003). The packaging under MAP combined with active coating (COAT-PS_MAP3) slowed down the growth of the *Pseudomonas* spp. that reached the threshold limit a day after the samples without potassium sorbate (COAT_MAP3). Results confirm literature data on the effect of active coating in combination with MAP on the spoilage microorganisms of dairy product (Del Nobile et al., 2009a; Del Nobile et al., 2009b; Conte et al., 2009). Some studies have recorded the efficacy of natural compounds, alone or in combination with other preservation methods, when directly applied to milk (Cava et al., 2007) or to cheese by spraying, immersing, or dusting the products. Antimicrobials may also be spread onto the packaging materials that come in contact with the cheese or incorporated into the plastic films used for packaging (Conte at al., 2007).

Regarding the other microbial groups investigated in the first trial, the modified atmosphere packaging at the highest CO_2 levels (MAP3) affected the growth of total viable count. In particular, at the end of storage the final cell load of MAP3 sample were about 2 log cycle lower (4 log cfu/g) compared to the control and the other MAP sample (6 log cfu/g) (data not shown). The *Enterobacteriaceae* cell load remained below the detection limit for the entire storage period (data not shown). The most important gas from a microbiological point of view is CO_2, used alone or in a mixture with N_2 and/or O_2, which inhibit the growth of many microorganisms including spoilage bacteria (Daniels et al., 1985). The absence of O_2 from the package headspace and the bacteriostatic properties of CO_2 may explain this observation. Gammariello et al. (2009 a, b) explained that the gas mixture of 95% CO_2/5% N_2 for Apulian fresh cheeses and 75% CO_2/25% N_2 for Stracciatella, respectively, were the most effective for the inhibition of spoilage microorganisms.

This supported the results obtained in Gonzales-Fandos et al. (2000), which showed that CO_2 had an inhibitory effect on coliforms on Cameros cheese.

The effect of high CO_2 levels (MAP3) on the total viable count was confirmed also in the second trial in which the FdL with coating was packaged under MAP. In fact, for MAP3 sample a slower increase of the total viable count was obtained if compared with the FdL packaged under MAP with low CO_2 concentration (data not shown). The *Enterobacteriaceae* cell load was greatly influenced by the MAP conditions. Particularly, the microbial growth decreased with the increase of the CO_2 concentration (Fig. 3a).

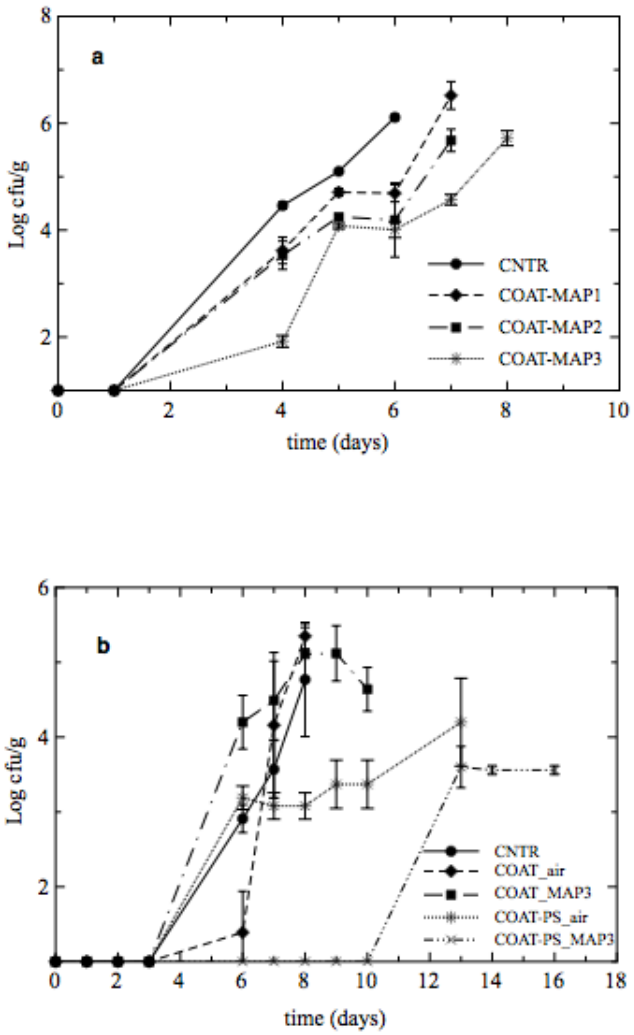


Figure 3

Evolution of *Enterobacteriaceae* cell load of Fior di Latte (FdL) of the second (a) and third (b) experimental phase.

Finally, in the third trial the active coating in combination with MAP was able to slow down the growth of the total viable count more than the MAP alone (data not shown). This effect was more evident on the *Enterobacteriaceae* cell load (Fig. 3b). It is worth noting that for the COAT-PS_MAP3 sample an extension of the lag phase of about 10 days was observed. After this period an increase of the enterobacteria cell load was detected but the final concentration did not exceed 4 log cfu/g. For the sample with active coating packaged in air (COAT-

PS_air) no extension of the lag phase was observed. In this case, the effect of the active coating was highlighted by a lower growth of the *Enterobacteriaceae* that remained below 4 log cfu/g until 10 days and then increased at 13 days. Literature data reported the antimicrobial effects of potassium sorbate on the same microorganisms (Stanojevic et al., 2009). The FdL with the coating alone in air and MAP3 did not lead to a slow down in the growth of *Enterobacteriaceae*. In most fresh or processed foods, microbial contamination occurs at a higher intensity on the food surface, thus requiring an effective microbial growth control (Padgett et al., 1998). Edible antimicrobial films and coatings have shown to be an efficient alternative in controlling food contamination (Debeaufort et al., 1998). Several natural substances are suitable to develop an active packaging. Conte et al. (2007) successfully tested the effectiveness of a lemon extract release system on Mozzarella cheese. Moreover, the substitution of brine with a natural hydrogel or a bio-based coating could represent another interesting strategy for fresh cheese preservation (Del Nobile et al., 2010; Laurienzo et al., 2006, 2008). Relevant results were achieved when an active coating was applied to the product prior to packaging under MAP (Conte et al., 2009). The typical dairy microorganisms play an important role in food fermentations, allowing to create the desired flavour and exerting preservative effects on the finished product. In this case study, the applied strategies in all the experimental phases did not affect the growth of typical dairy microorganisms. In particular, the microbial cell load of lactococci and lactic acid bacteria did not differ significantly between control and treated samples (data not shown). The $MAL^{Pseudomonas}$ values for the three experimental phases are listed in Table 1.

Table 1 Microbial ($MAL^{Pseudomonas}$), sensory acceptability limit ($SAL^{O.Q.}$) and shelf life value of FdL samples.

First trial	**$MAL^{Pseudomonas}$**	**$SAL^{O.Q.}$**	**Shelf-life**
CNTR	6.35±0.33[a]	7.88±0.50[b]	6.35±0.33[c]
MAP1	7.10±0.00[b]	7.18±0.81[b]	7.10±0.00[c]
MAP2	>8	4.00±0.96[a]	4.00±0.96[b]
MAP3	>8	2.79±0.07[a]	2.79±0.07[a]
Second trial			
CNTR	4.54±0.07[a]	5.06±0.92[a]	4.54±0.07[a]
COAT-MAP1	5.33±0.21[b]	5.27±0.23[a]	5.27±0.23[b]
COAT-MAP2	5.42±0.37[b]	5.60±0.67[a]	5.42±0.37[b]
COAT-MAP3	6.64±0.43[c]	6.88±0.08[b]	6.64±0.43[c]
Third trial			
CNTR	3.45±0.62[a]	6.81±0.25[a]	3.45±0.62[a]
COAT_air	5.52±0.52[b]	6.73±0.14[a]	5.52±0.52[b]
COAT_MAP3	7.72±0.38[c]	8.87±0.12[b]	7.72±0.38[c]
COAT-PS_air	6.25±0.15[b]	11.23±0.30[c]	6.25±0.15[b]
COAT-PS_MAP3	8.72±0.33[d]	12.28±0.50[d]	8.72±0.33[d]

[a-d]Data in the same column with different superscript letters are significantly different (P<0.05).
MAP1: 20% CO_2, 80 %N_2; MAP2: 50% CO_2, 50 %N_2; MAP3: 80% CO_2, 20 %N_2.

In the first trial, the $MAL^{Pseudomonas}$ value for the CNTR and MAP1 samples was about 6 and 7 days, respectively. Whilst, the microbial acceptability limit for the MAP2 and MAP3 samples was higher than the monitored period. In the second trial, the Fior di Latte packaged with the highest CO_2 level (COAT-MAP3) showed the highest $MAL^{Pseudomonas}$ value (~ 6.6 days). No difference for the $MAL^{Pseudomonas}$ values between the packaging with 20% and 50% of CO_2 (MAP1 and MAP2) was observed. For these samples a value of about 5 days was recorded. Moreover, for the CNTR sample the lowest $MAL^{Pseudomonas}$ value of about 4.5 days was obtained. In the third trial the packaging under MAP reached the highest value of the microbial acceptability limit. In particular, a value of about 8 and 9 days for coating (COAT_MAP3) and active coating (COAT-PS_MAP3), respectively, was observed. Under air conditions, the presence of active compound in the coating did not affect the microbial acceptability that showed a value of about 6 days. The $MAL^{Pseudomonas}$ value for the CNTR samples was about 3.5 days.

SENSORY QUALITY

The evolution of *overall quality* of Fior di Latte samples is shown in Figure 4.

The solid horizontal line in the figure represents the *overall quality* threshold value. In the first trial, the packaging of FdL under MAP caused a faster decrease of the *overall quality* probably due to the gradual solubilization of the CO_2 in the covering liquid (Fig. 4a). Particularly, the development of carbonic acid in the covering liquid negatively affected the texture of FdL that tended to strip on the surface. The *overall quality* decreased more rapidly with the increasing of the CO_2 concentration in the package. In particular, for the CNTR and MAP1 samples a SAL^{OQ} value of about 8 days was obtained, whereas at higher CO_2 levels in the package (MAP2 and MAP3) a SAL^{OQ} value of 3-4 days was recorded (Table 1). The MAP technique has a number of advantages such as quality retention of fresh products, image improvement of the product, extension of shelf life and minimum use of additives and preservatives (Garabal et al., 2010). However, the use of carbon dioxide presents some disadvantages related to the sensorial characteristics such as flavour-related problems.

The presence of CO_2 is expected to result in a pH drop, which is thought to be associated with the formation of carbonic acid, acidic amino acids and free fatty acid production during proteolysis and lipolysis, respectively (Dermiki et al., 2008).

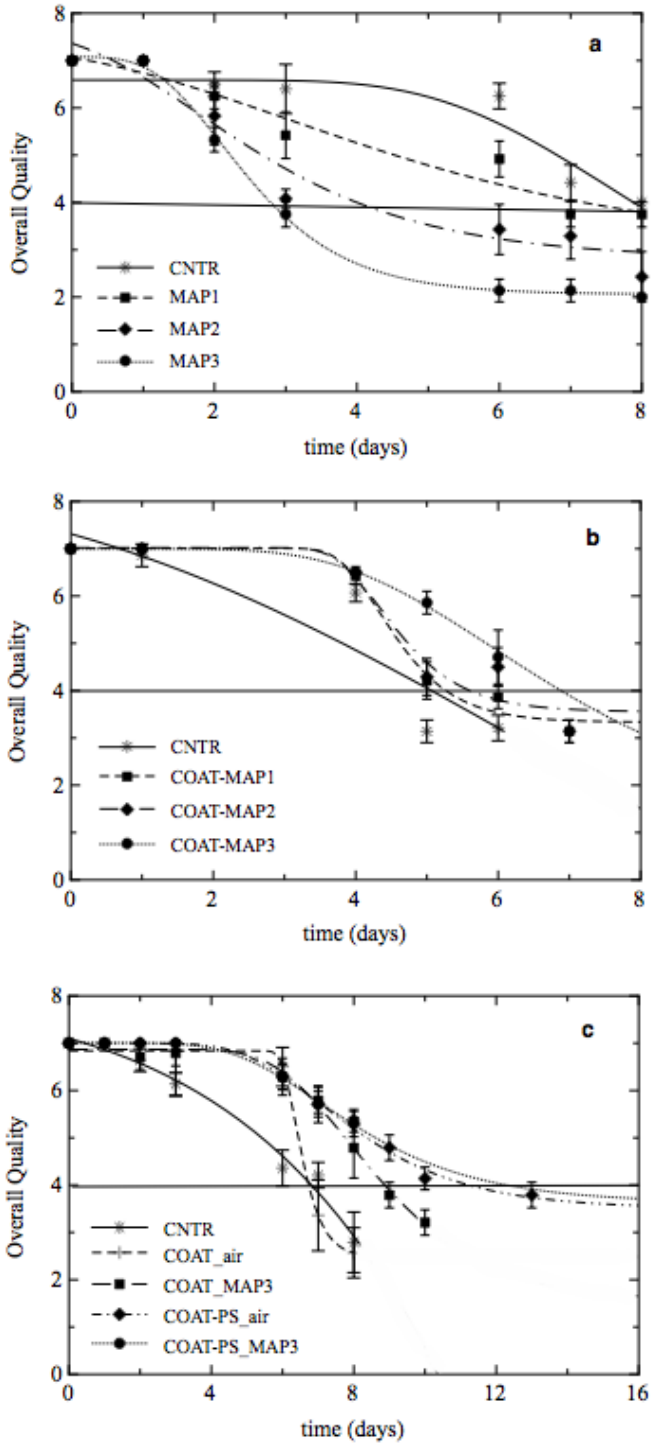


Figure 4 Evolution of *overall quality* of Fior di Latte of the first (a), second (b) and third (c) experimental phase

The presence of the coating on the FdL cheese surface reduced the damage due to the solubilization of the CO_2 in the covering liquid (Fig. 4b). In fact, in this case the texture did not represent the factor limiting the *overall quality* that was compromised by the odor attribute. In particular, the *overall quality* for the CNTR samples rapidly decreased, whereas the MAP1 and MAP2 samples had a high score until 4 days of refrigerated storage and then the score suddenly decreased. The FdL cheese packaged with high CO_2 concentration (COAT-MAP3) reached later the sensorial acceptability limit. A SAL^{OQ} value for this samples of about 7 days compared to 5 days for CNTR and samples under low CO_2 concentration (COAT-MAP1 and COAT-MAP2) was achieved (Table 1).

Finally, the figure 4c shows the evolution of the *overall quality* of the third trial of FdL cheese. As can be seen the CNTR and COAT_air samples reached first the sensorial threshold value followed by the COAT-MAP3 samples. Then, the active coating both in air and MAP (COAT-PS_air and COAT-PS_MAP3) improved the *overall quality* of FdL samples that remained above the threshold value up to 10 days. The factor limiting the *overall quality* for the third trial was the odor attribute. The SAL^{OQ} values recorded were 7 days for CNTR and COAT_air samples, 9 days for coated FdL under MAP (COAT-MAP3) and 11 and 12 days for the active coating in air and MAP, respectively (Table 1).

SHELF LIFE

The shelf life of the fdL cheese for the three experimental phases was calculated as the lowest value among $MAL^{Pseudomonas}$ and SAL^{OQ} (Table 1). It is worth noting that the CNTR samples for the three trials showed a different shelf life value due to the variability of the Fior di Latte cheese making. FdL cheese, recognized in Italy as a Guaranteed Traditional Speciality, is manufactured throughout the traditional processing mainly chemical acidification of the curd or using commercial or natural whey starter cultures (Faccia et al., 2013). As can be seen, in the first trial the factor that limited the shelf life of FdL cheese was the microbial quality for CNTR and MAP1 samples and the sensorial quality for MAP2 and MAP3 samples. The antimicrobial effect of MAP with high CO_2 concentrations on the shelf life of dairy products was widely demonstrated (Gammariello et al., 2009a; Conte et al. 2009; Del Nobile et al., 2009a; Hotchkiss et al., 2006). Researchers have reported that different dairy products were well protected from undesirable microorganisms attack and gas formation using high concentrations of carbon dioxide that represents the most important gas from a microbiological point of view (Eliot et al., 1998; Alves et al., 1996). However, the use of MAP for packaging of FdL cheese in traditional brine is limited due to its detrimental effect on the product surface. For this reason, no work concerning the application of the MAP on the FdL cheese in brine has been made to the best of our knowledge. In particular, table 1 shows that no significant differences between FdL packaged in air (CNTR) and those under MAP with low CO_2 concentration (MAP1) was observed in terms of shelf life, with values of about 6-7 days. The samples packaged under high CO_2 levels (MAP3) showed the lowest shelf life value (3 days) together with the MAP2 sample (4 days).

In the second trial, in order to protect the surface of FdL from the damage of the CO_2 solubilisation, the cheese was covered with coating before packaging in MAP. This approach improved significantly the sensorial acceptability. In particular, the shelf life of FdL samples was compromised by the microbial quality except for the COAT-MAP1 sample. Therefore, the combination of the coating and MAP3 was able to improve the FdL shelf life that reached a value of about 6.6 days compared with the CNTR sample (4.5 days). The samples packaged with 20% and 50% of CO_2 (COAT-MAP1 and COAT-MAP2) did not show significant difference between them, and a value of about 5 days was reached. Edible coatings have properties that make them suitable for increasing food shelf-life. Coatings are mainly employed to extend the shelf life of fresh food improving the structural integrity of the product, reducing modifications in color, flavor and taste (Volpe et al., 2010). Moreover, the semi-permeable barrier provided by edible coatings is aimed to extend shelf life by reducing moisture and solute migration, gas exchange and oxidative reaction rates.

Finally, in the last experimental phase the microbial quality was the factor that limited the shelf life of the FdL cheese. It is worth noting that the combined use of active coating and MAP (COAT-PS_MAP3) was able to prolong the shelf life by 157%. In fact, the CNTR samples showed a value of about 3.5 days compared to a shelf life of about 9 days for the COAT-PS_MAP3 sample. Valid synergistic effects between lysozyme/Na_2EDTA carried to the product surface by the coating and modified atmosphere packaging on FdL cheese were demonstrated (Conte et al. 2009). Moreover, the silver-nanoparticles loaded into the edible coating in combination with MAP were able to control the spoilage bacteria proliferation on Fior di Latte cheese (Gammariello et al., 2011).

The coating alone with the packaging under MAP (COAT_MAP3) allowed to obtain a shelf life value of about 8 days. Whilst the active coating (COAT-PS_air) did not provide improvement compared to the coating alone (COAT_air) when the FdL was packaged in air. In fact, the same shelf life value for COAT-PS_air and COAT_air samples was obtained (5.5-6 days). Coatings can increase the shelf life of foods by acting as carriers of food additives and antimicrobial agents. Several antimicrobial coatings have been developed to minimize growth of spoilage microorganisms that may contaminate the surface of fresh product (Volpe et al., 2010; Conte et al., 2009; Del Nobile et al., 2009a; Del Nobile et al., 2009b; Aloui et al., 2014). However, the combined strategies used in this study highlighted the possibility of extending the shelf life of FdL in the covering liquid by means a pre-packaging with the purpose of protecting the product surface and carrying the antimicrobial compounds.

Case study 2: sodium alginate coating containing *antimicrobials* applied to FdL cheese

During the first experimental step (S_1), different active coatings were tested to find the most suitable to preserve the quality characteristics of cheese during storage. Subsequently, the best active coating (i.e. potassium sorbate loaded alginate) was tested at three different concentrations to select the optimal amount to improve FdL cheese shelf-life (S_2).

FIRST TRIAL: FDL WITH ACTIVE (PS/SB/CL/CA) COATING AND MAP

Figure 5 illustrates the evolution during storage of *Pseudomonas* spp. viable cell concentration for tested samples.

The initial microbial count for all the samples of mozzarella was approximately 3.13 log cfu/g and increased gradually during the entire observation period. The final cell load did not differ significantly between active coated and control cheese samples.

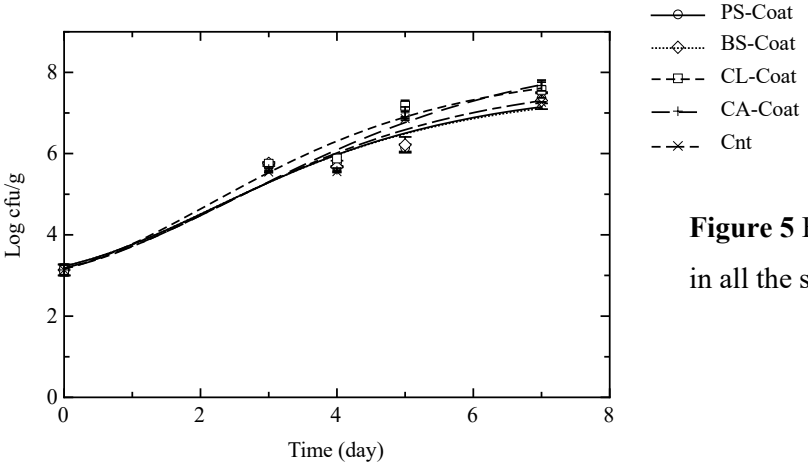


Figure 5 Evolution of *Pseudomonas* spp. in all the samples investigated in the S_1

However, a certain influence of selected substances was observed. On the fifth day of storage a difference accounting for about one logarithmic cycle was observed between PS-Coat (6.14 log cfu/g), SB-Coat (6.21 log cfu/g) and CL-Coat (7.1 log cfu/g) compared to CA-Coat (7.0 log cfu/g) and Cnt (7.0 log cfu/g) samples.

For *Enterobacteriaceae*, the cell load increased for Cnt, CL-Coat and CA-Coat more rapidly than PS-Coat and SB-Coat samples (Figure 6).

Furthermore, while *Pseudomonas* spp. growth was showed to be negatively affected by PS and SB at the two last storage times, the *Enterobacteriaceae* growth was shown to be affected already after three days of storage.

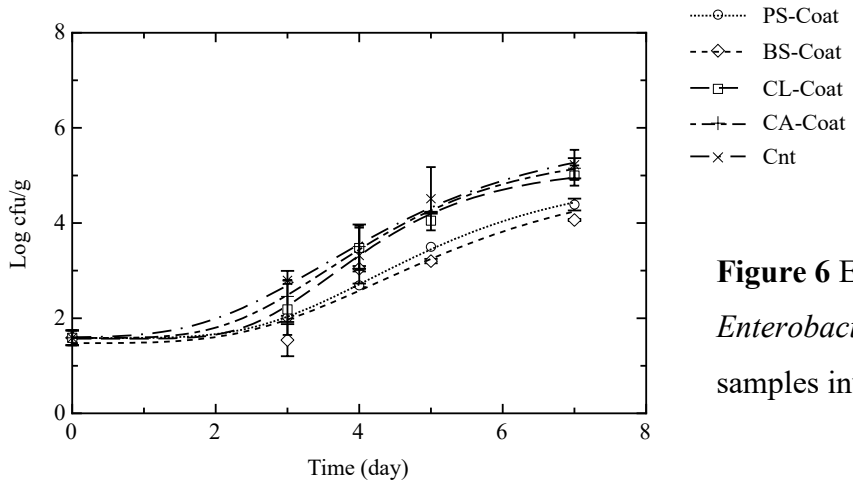


Figure 6 Evolution of *Enterobacteriaceae* in all the samples investigated in the S_1

In particular, at the end of the storage period PS-Coat and SB-Coat showed a concentration of *Enterobacteriaceae* of about 4.4 log cfu/g and 4.06 log cfu/g, respectively. In the literature, examples of antimicrobial effects of potassium sorbate and sodium benzoate on the same microorganisms were also reported (Stanojevic, Comic, Stefanovic, & Solujc-Sukdolak, 2009).

The functional microorganisms of cheese grew during storage without marked differences between the control and the coated samples. In particular, for mesophilic lactic acid bacilli the cell concentration reached a maximum value of about 5 log cfu/g. For lactococci, the initial cell load was about 3.3 log cfu/g and increased to about 7 log cfu/g for all the samples (data not shown). Therefore, the presence of active compounds did not influence the growth of typical dairy micro-organisms, that generally play an important role in food fermentations, allowing creating the desired flavour changes and exercising preservative effects on the product. The cell load of total bacteria showed a trend similar to that found for lactococci bacteria (data not shown).

The pH of FdL cheese, monitored during the entire observation period, ranged between 6.50 and 6.30 in all the investigated samples (data not shown).

Sensory evaluation

The overall quality of cheese samples is shown in Figure 7.

The horizontal line represents the acceptability threshold. As can be seen, the products treated with coating containing potassium sorbate and sodium benzoate showed values of sensory quality above the threshold (score = 4) for the entire observation period. On the

contrary, all the other samples (Cnt, CA-Coat and CL-Coat) became unacceptable between the fifth and seventh day.

In particular, for Cnt and CA-Coat all the sensory parameters (e.g. odour, colour, firmness) were compromised after 5 days of storage, whereas, for the CL-Coat sample the odour was the factor that mainly affe cted the acceptability.

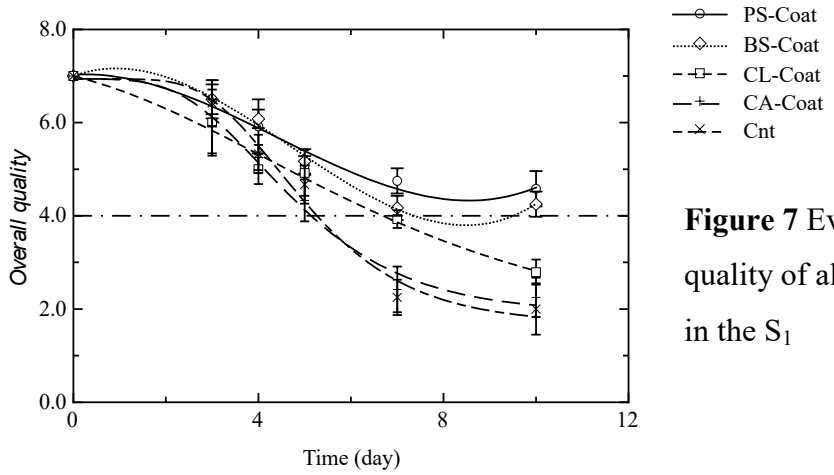


Figure 7 Evolution of the overall quality of all the samples investigated in the S_1

SECOND TRIAL: FDL WITH ACTIVE COATING AND MAP - Optimization of potassium sorbate concentration in the coating

The evolution of *Pseudomonas* spp. plotted as a function of storage time, for treated and untreated FdL cheese, is represented in Figure 8.

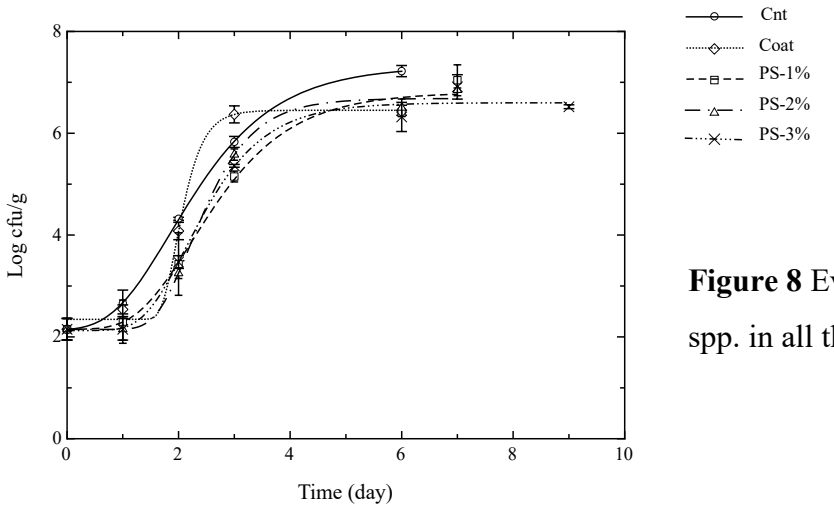


Figure 8 Evolution of *Pseudomonas* spp. in all the samples investigated

Pseudomonas spp. play an important role in dairy products spoilage, due to the great production of lipolytic and proteolytic enzymes that affect the quality and, consequently,

provoke consumer unacceptability (Wiedmann, Weilmeier, Dineen, Ralyea, & Boor, 2000; Dogan & Boor, 2003).

Previous researchers (Cantoni, Marchisio, & Galli, 2000; Cantoni, Stella, Ripamonti, & Marchese, 2001; Bishop & White 1986) reported that a microbial load equal to 10^6 cfu/g may represent the contamination level at which the alterations of the product start to appear.

As can be seen from data of Figure 8, the microbial cell load of all the investigated samples rapidly increased during the first three storage days; a slight slowdown was observed for PS-1%, PS-2%, PS-3% with respect to the control samples (Cnt and Coat), according to what reported by Deeb & Ahmed (2010).

With regards to *Enterobacteriaceae*, the antimicrobial effect of PS grew as its concentration increased (Figure 9).

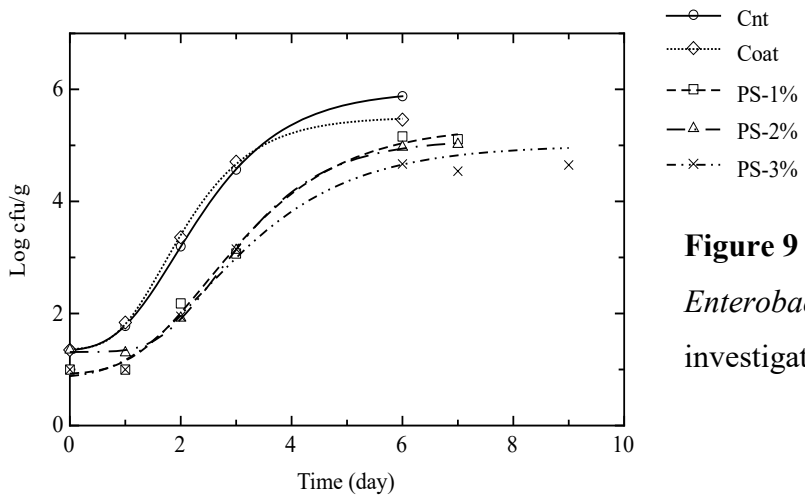


Figure 9 Evolution of *Enterobacteriaceae* in all the samples investigated

In fact, the PS-3% sample showed always the lowest cell load among the investigated samples and especially with respect to Cnt sample. The control samples recorded the highest microbial load (5.8 and 5.4 log cfu/g, respectively), whereas the samples coated with PS attained a final microbial concentration ranging from 5.1 to 4.6 log cfu/g, depending on the amount of active agent incorporated in the coating. The trend of mesophilic lactic acid bacilli and lactococci was similar in all the samples (data not shown), thus confirming the lack of antimicrobial effects of PS on these microbial groups.

There was a slight increase of microbial load but it then remained relatively stable.

These results are in agreement with findings of other researchers (Doğruer, Gürbüz, & Nizamlioğlu, 1996; Ozdemir & Demirci, 2006) who reported that inhibitory effect of PS on lactic bacteria was very limited. The total bacterial counts showed an increasing trend in all the samples. The initial microbial count was about 4.5 log cfu/g, and then it rose in the control samples slightly faster than in the other cheese coated with PS (data not shown).

Sensory evaluation

Figure 10 shows the FdL cheese overall quality during the storage time. As can be inferred from the Figure, the overall quality for the Cnt and Coat gradually decreased and then levelled off, thus reaching values below the acceptability limit after the third storage day. The overall quality of PS-1% slumped during the whole storage period, while PS-2% and PS-3% show a similar trend differing only at the last storage day when the PS-2% declined more rapidly in comparison to PS-3%.

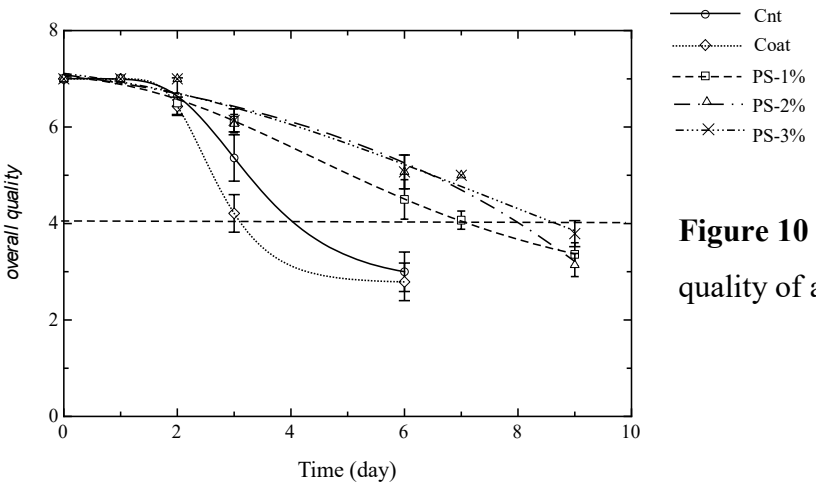


Figure 10 Evolution of the overall quality of all the samples investigated

Data confirmed that coating without active compound (Coat) cannot improve significantly the sensory properties of cheese. On the contrary, for sample with PS there was a relevant sensory improvement. Results recorded also demonstrate that the effectiveness noticeably increased as the concentration of PS increased. As a fact, the PS-2% and PS-3% samples doubled the time at which the overall quality reached its threshold value (about 8 days) when compared to both control samples.

Case study 3: sodium alginate coating containing Silver Nanoparticles

Microbiological Analysis

The evolution of *Pseudomonas* spp. cell load in FdL cheese samples packaged with covering liquid during the storage time is shown in Figure 11a. The initial cell load of *Pseudomonas* spp. was 2 log cfu/g and quickly increased for CNTR and COAT-air samples until it reached the threshold value at about 4 days of storage. With the packaging of the FdL cheese under MAP (COAT-MAP) a longer lag phase was obtained. As can be inferred from the figure, the *Pseudomonas* spp. cell load increased after the third day and it took one day more to reach the microbiological acceptability limit if compared to the air packaged sample (about 5 days).

A slower growth of the *Pseudomonas* spp. for FdL samples packaged with active coating in air and MAP was observed.

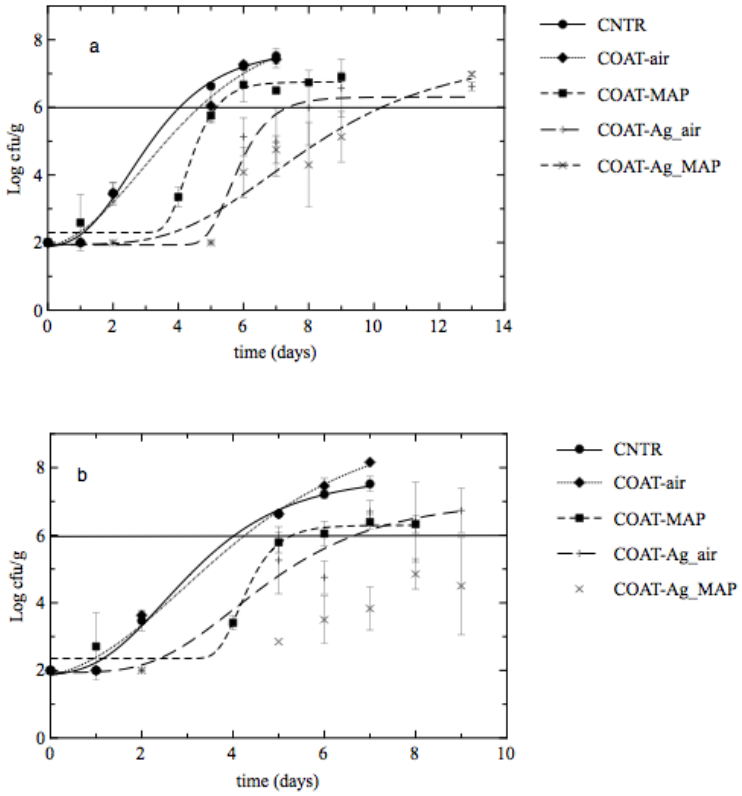


Figure 11 Evolution of *Pseudomonas* spp. cell load in Fior di Latte cheese samples packaged with (a) and without the covering liquid (b).

In particular, the Ag-NP loaded in the edible coating prolonged the lag phase and a slower exponential growth, mostly for samples packaged under MAP, was observed. The microbiological threshold value at about 7 and 10 days for COAT-Ag_air and COAT-Ag_MAP, respectively, was reached. A similar evolution of the *Pseudomonas* spp. cell load for FdL samples packaged without covering liquid was observed (Fig. 11b).

It is worth noting that the samples with a Ag-NP coating and packaged in MAP (COAT-Ag_MAP) without covering liquid did not exceed the microbiological threshold value during the entire storage time. The microbiological analysis for this sample was stopped at 9 days as it has became unacceptable from a sensory point of view at about 6 days of storage. Studies on the deterioration of fresh dairy cheeses assert that the predominant microflora affecting FdL cheese quality is represented by the *Pseudomonas* spp. (Gammariello, Di Giulio, Conte, & Del Nobile, 2008; Gammariello, Conte, Di Giulio, Attanasio, & Del Nobile, 2009; Sinigaglia, Bevilacqua, Corbo, Pati, & Del Nobile, 2008; Conte el al., 2009; Faccia, Mastromatteo, Conte, & Del Nobile, 2012). Among compounds with antimicrobial properties, silver nanoparticles have been received considerable attention because of their strong toxicity against a wide range of microorganisms (Sondi & Salopek-Sondi, 2004; Gammariello et al., 2011). Bacterial growth in the presence of a given concentration of

silver has been found to be dependent on the initial number of cells (Sondi & Salopek-Sondi, 2004; Pal, Tak, & Song, 2007). Very few applications of Ag-NP applied to several foods are reported in literature (An et al., 2008; Fernandez et al., 2009). Also, Costa, Conte, Buonocore, & Del Nobile (2011) and Costa, Conte, Buonocore, Lavorgna, & Del Nobile (2012) investigated the possibility to improve the shelf life of fresh-cut carrots and fresh fruit salad. Regarding to the application of Ag-NP applied to dairy foods, Gammariello et al. (2011) evaluated the possibility to replacing the brine with an alginate edible coating (8% w/v) containing very high concentrations of silver-nanoparticles (5, 10, 15 mg/ml) for prolonging the shelf life of FdL cheese. The silver-nanoparticles loaded into the edible coating in combination with MAP (30% CO_2, 5% O_2, 65% N_2) were able to control the spoilage bacteria proliferation. However, the highest silver concentrations worsened the sensory quality of Fior di Latte and thus its shelf life.

MAL values for all samples were also obtained (Table 2).

Table 2 Microbial ($MAL^{Pseudomonas}$), sensory acceptability limit ($SAL^{O.Q.}$) and shelf life of FdL samples.

	Samples	$MAL^{Pseudomonas}$	SAL^{OQ}	Shelf-life
	CNTR	4.04±0.32a	6.48±0.005a,b	4.04±0.32b
No covering liquid	COAT-air	4.26±0.36a,b	5.74±0.17a	4.26±0.36b
	COAT-MAP	5.30±0.36c	5.89±0.55a	5.30±0.36a,c
	COAT-Ag_air	6.67±0.60d	5.80±0.36a	5.80±0.36a
	COAT-Ag_MAP	>9e	5.67±0.32a	5.67±0.32a
With covering liquid	COAT-air	4.63±0.44a,b,c	>7b	4.63±0.44b,c
	COAT-MAP	5.16±0.18b,c	7.80±0.53c	5.16±0.18a,c
	COAT-Ag_air	7.36±0.67d	8.61±0.41d	7.36±0.67d
	COAT-Ag_MAP	10.2±0.96f	11.01±0.87e	10.2±0.96e

[a-f]Data in the same column with different superscript letters are significantly different ($P<0.05$).

Data highlighted that the active coating could enhance microbial stability by inhibiting the proliferation of *Pseudomonas* spp. for both in air and MAP. In particular, a MAL value of about 7 days for samples packaged in air with and without covering liquid was obtained. Whilst, the combination of MAP and active coating recorded the best results with a MAL value of about 10 days for samples with covering liquid and higher than 9 days for samples without covering liquid. The free-silver nanoparticles coated samples packaged under MAP showed a MAL value of only one day (about 5 days) longer compared to the same packaged in air and the control samples (4 days). Polymeric systems containing silver represent a viable approach to avoid the direct contact of nanoparticles with food. In fact, it must be noted that the developed coating containing nanoparticles needs to be removed before eating Fior di Latte. This is particularly important to satisfy the European Union safety regulation that

regulates the presence of silver ions in food matrices and limits the amount to 0.05mg of Ag/kg, which is not biocidal in food (Fernandez et al., 2009).

The silver nanoparticles loaded into the coating exhibited a strong antimicrobial activity against the enterobacteria (Fig. 12).

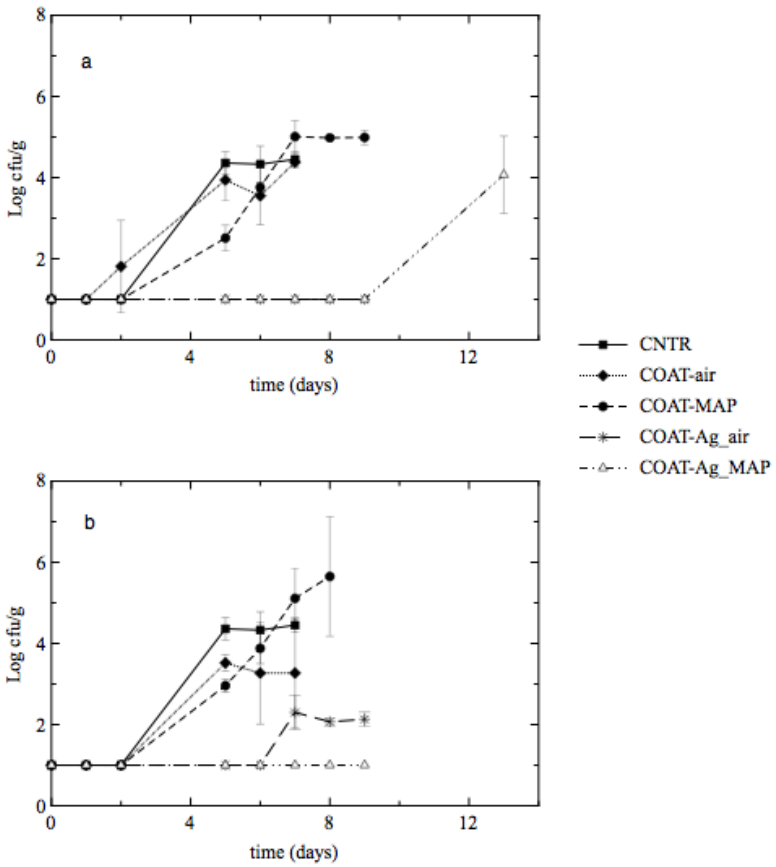


Figure 12: Evolution of *Enterobacteriaceae* cell load in FdL cheese samples packaged with (a) and without the covering liquid (b).

As can be seen, the *Enterobacteriaceae* cell load in the FdL samples with active coating did not exceed 2 log cfu/g until the ninth day of storage for both samples packaged with and without the covering liquid. For active samples under MAP and packaged with the covering liquid (COAT-Ag_MAP) the *Enterobacteriaceae* cell load increases from the nine day until it reaches a final cell load of about 4 log cfu/g (Fig. 12a). It is worth noting that the silver nanoparticles exerted their antimicrobial effect in both samples: in air and MAP.

A significant antibacterial activity against model bacteria, *E. coli*, was also reported for silver nanoparticles embedded in cellulose acetate, a traditional polymeric film used in the food packaging industry (Varsha, Bajpai, & Navin, 2010). Therefore, silver nanoparticles embedded into an agar hydrogel was tested with success on FdL cheese (Incoronato et al., 2011). In this study the active tubes were prepared by pouring on the bottom of the tubes an agar-water solution (0.8%) containing different concentrations of silver-nanoparticles. Then, the FdL samples were introduced in the active tubes and the traditional brine was poured

into each tub to cover the cheese samples. The active packaging system increased the shelf life of cheese, due to the ability of silver ions to control the microbial proliferation.

The antimicrobial effect of the active packaging system was also observed on the total viable count (Fig. 13).

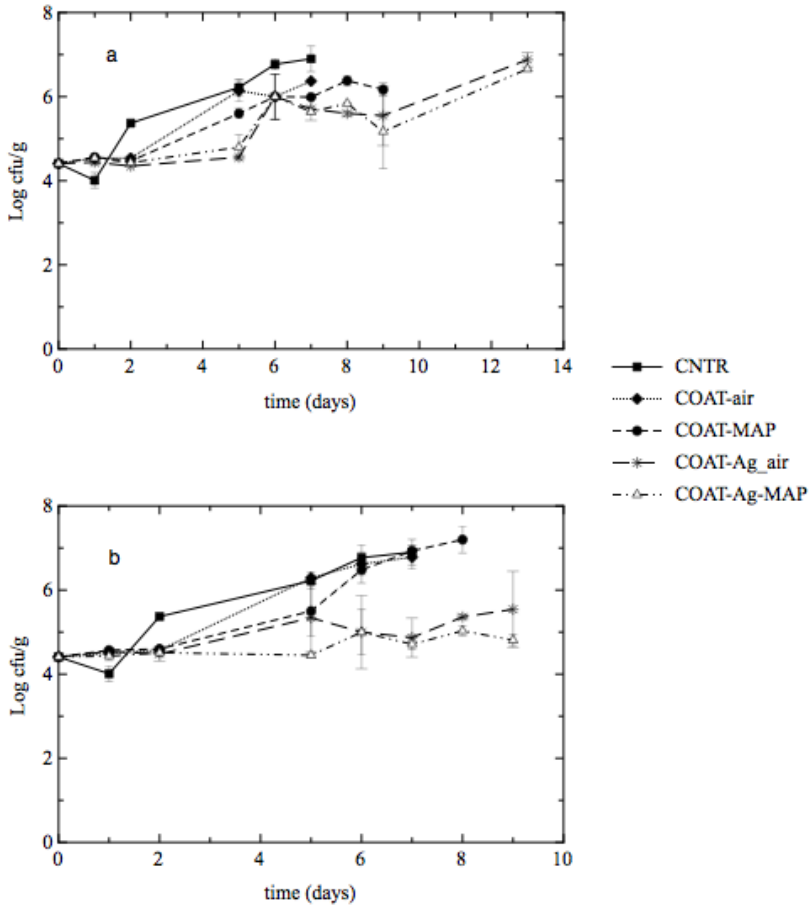


Figure 13 Evolution of TVC cell load in FdL cheese samples packaged with (a) and without the covering liquid (b).

In fact, for the Fior di Latte packaged with active coating, both in air and MAP, the TVC cell load did not exceed 6 log cfu/g at 9 days of storage. After this period the cell load increased for the samples packaged in the covering liquid until it reached a value of about 7 log cfu/g at 13 days of storage (Fig. 13a). The coating alone, for samples packaged both in air and MAP, was not able to control the microbial proliferation of the product.

Moreover, for all FdL samples an increase of the lactic acid bacteria and lactococci cell load was observed during the storage. However, the final cell load was lower for samples with active coating respect to the samples packaged with free-Ag-NP coating (data not shown).

Sensory Analysis

Figure 14 shows the overall quality plotted as a function of the storage time for Fior di Latte samples packaged with (a) and without the covering liquid (b).

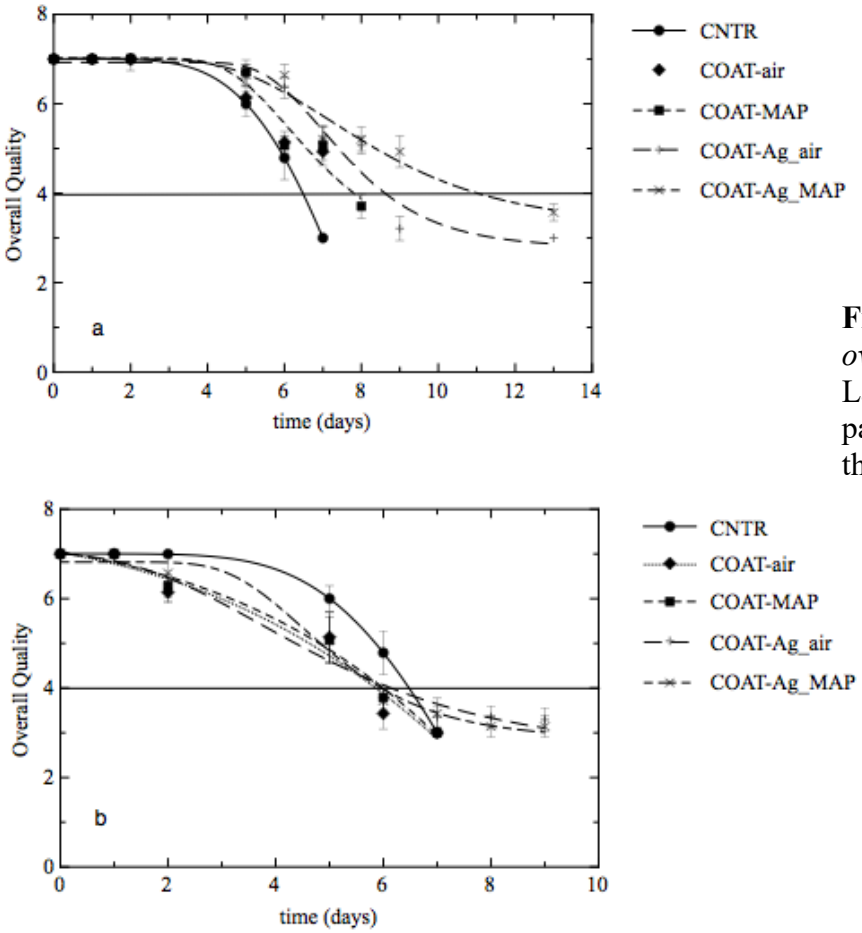


Figure 14 Evolution of *overall quality* of Fior di Latte cheese samples packaged with (a) and without the covering liquid (b).

As can be seen from figure 14a, the control samples showed a fast decrease in overall quality while the coated samples in air (COAT-air) did not exceed the threshold value for the entire observation time. In fact, the analysis for this samples were stopped due to the achieving of the microbiological threshold value. Moreover, the packaging under MAP improved the overall acceptability of the coated samples (COAT-MAP). Fior di Latte with active coating showed a better overall quality above all when the MAP packaging (COAT-Ag_MAP) was used. This demonstrated the efficacy of the system in preserving the product without compromising sensory attributes.

FdL samples packaged without the covering liquid showed a different evolution of the overall quality parameter (Fig. 14b). In particular, all the coated samples reached the threshold acceptability limit at the same time (6 days) compared to the control samples (about 6.5 days). The absence of the covering liquid dried the edible coating applied on the FdL surface worsening the overall quality of the product. The SAL^{OQ} values for the FdL samples were also calculated (Table 2). Data show that all the treatments applied to the FdL samples packaged in the covering liquid improved the sensorial quality of the product if compared to the control sample. In fact, CNTR sample recorded a SAL^{OQ} value of about 6.5

days while for all the treated samples a value ranging between 7 and 11 days was observed. It is worth noting that when the coating treatment was combined with packaging in MAP a higher sensorial acceptability limit was obtained if compared with the same samples packaged in air. The sensory attribute limiting the overall quality of the products packaged in the covering liquid was the odour for the coated samples and the odour and colour for the active coated samples as the silver nanoparticles tended to change the colour of the product over time (data not shown). The packaging of FdL without covering liquid damaged the efficacy of the applied treatments. For this samples all the sensorial attributes were limiting the overall quality as the absence of the covering liquid tended to dry the product by causing hardening and yellowing of the FdL surface (data not shown).

Headspace Gas Composition

The evolution of the O_2 and CO_2 in the headspace of packages sealed under air and MAP is shown in Figure 15.

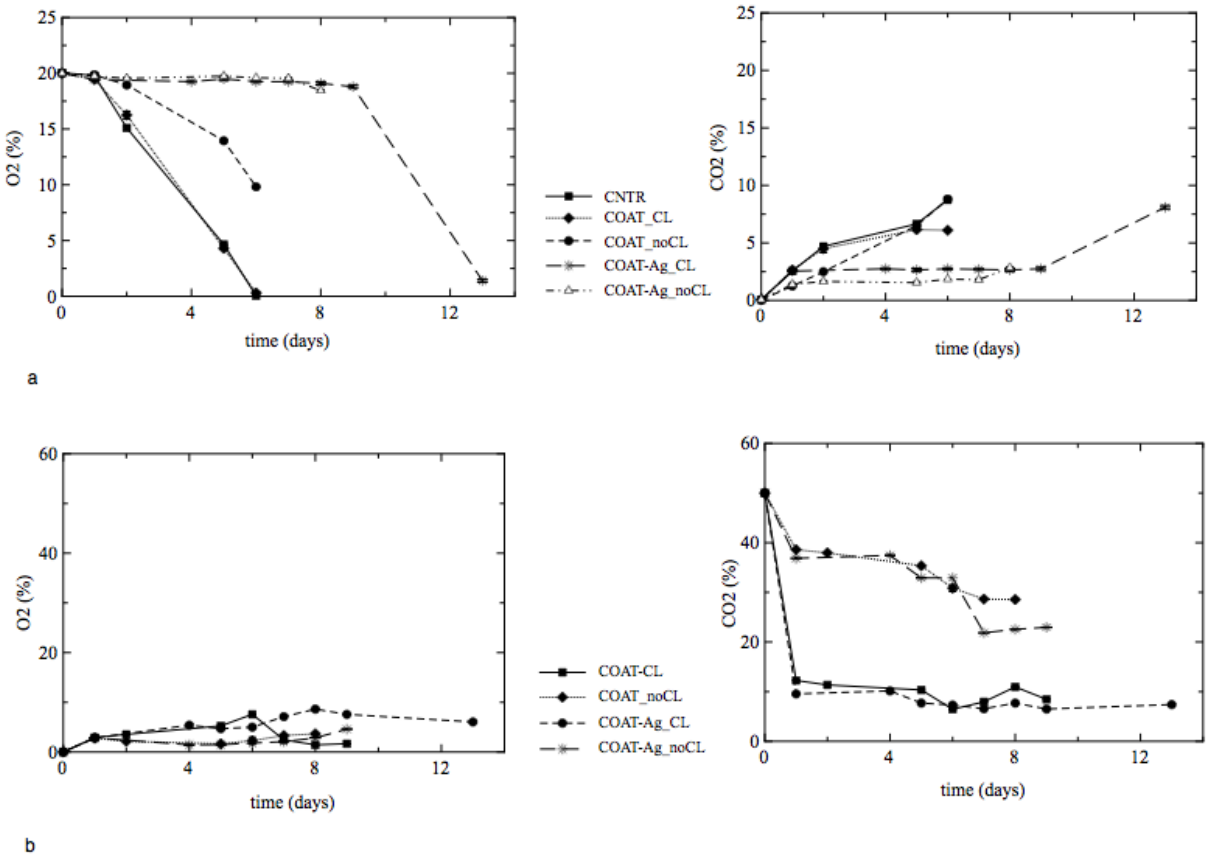


Figure 15 Evolution of the O_2 and CO_2 concentration in the headspace composition of the Fior di Latte cheese samples packaged in air (a) and MAP (b).

As can be seen, when the FdL samples were packaged in air the O_2 levels tended to decrease, especially for FdL packaged with free-Ag-NP coating and control samples (Fig 5a). The increase of CO_2 seems to be closely correlated to a decrease in the O_2 concentration in the headspace of these samples.

The abovementioned evidences suggest that the O_2 decrease could be due to microbiological respiration and chemical reactions involving O_2. It is worth noting that when FdL was packaged without the covering liquid (COAT_noCL) a slower O_2 decrease was observed. On the contrary, the gas composition for samples with active coating remained constant until to 8 days of storage. This was supported by the microbiological data that show a higher antimicrobial effect against the spoilage bacteria for samples with active coating. The O_2 levels decreased to a value of about 1.4% and CO_2 increased until to 8.0% at the end of storage period for samples packaged with covering liquid.

The packaging of the FdL under MAP highlighted that the presence of the covering liquid caused a CO_2 solubilisation up to 10% after one day of storage (Fig. 15b). Then, the CO_2 level remained constant until the end of storage period. The FdL samples packaged without the covering liquid showed a slow decrease of the CO_2 concentration over time. In particular, a CO_2 final level of about 29 and 23% for COAT_noCL and COAT-Ag_noCL, respectively, was observed. Moreover, the O_2 levels in the headspace of the package showed a slight increase over time probably due to oxygen permeation through the packaging film.

Shelf life evaluation

The shelf life values of FdL, calculated as the lowest between MAL and SAL, are listed in Table 3.

Table 3 Microbial ($MAL^{Pseudomonas}$), sensorial acceptability limit ($SAL^{O.Q.}$) and shelf life value of the FdL samples.

	Samples	$MAL^{Pseudomonas}$	SAL^{OQ}	Shelf-life
	CNTR	4.04±0.32a	6.48±0.005a,b	4.04±0.32b
No covering liquid	COAT-air	4.26±0.36a,b	5.74±0.17a	4.26±0.36b
	COAT-MAP	5.30±0.36c	5.89±0.55a	5.30±0.36a,c
	COAT-Ag_air	6.67±0.60d	5.80±0.36a	5.80±0.36a
	COAT-Ag_MAP	>9e	5.67±0.32a	5.67±0.32a
With covering liquid	COAT-air	4.63±0.44a,b,c	>7b	4.63±0.44b,c
	COAT-MAP	5.16±0.18b,c	7.80±0.53c	5.16±0.18a,c
	COAT-Ag_air	7.36±0.67d	8.61±0.41d	7.36±0.67d
	COAT-Ag_MAP	10.2±0.96f	11.01±0.87e	10.2±0.96e

[a-f]Data in the same column with different superscript letters are significantly different ($P<0.05$).

As it can be seen, a significant prolongation of shelf life was recorded with treatments applied to the samples packaged with covering liquid. Particularly, the active coating based on Ag-NP improved the shelf life of FdL, that reached a value of about 7 and 10 days for packaging in air and MAP, respectively. The free-Ag-NP coating applied to FdL packaged in air and MAP only prolonged the shelf life of one day (about 5 days) compared to the control samples (4 days). Moreover, for all the treatments the shelf life limiting factor was the microbiological quality, thus demonstrating that the adopted packaging solutions did not affect the sensorial characteristics of the FdL cheese. On the contrary, when the treatments were applied to FdL packaged without the covering liquid a shorter extension of the shelf life was observed. In particular, a shelf life value of about 6 days for FdL with active coating was observed (COAT-Ag_air and COAT-Ag_MAP). For this samples the sensorial quality was the factor that limited the shelf life of the product. The combination of the free-Ag-NP coating with the packaging in MAP only determined a shelf life extension of one day (5 days) compared to the control samples (4 days). Whilst the same treatment combined with packaging in air (COAT-air) was not sufficient to enhance product shelf life. For these samples the shelf life limiting factor was the microbial quality. Therefore, the developed nanocomposite system improved the microbial quality of FdL without affecting the sensorial properties of the product packaged in traditional brine, thus prolonging its shelf life.

Case study 4: sodium alginate coating containing *Lactobacillus reuteri*

Biopreservation was studied to preserve the microbiological and sensory quality of FdL cheese. In particular, the system consisted in the application of two kinds of active coatings on the product surface: the first containing *L. reuteri* and the second containing the microorganism plus glycerol. Furthermore, these active solutions were applied to cheese in two different ways: without any fermentation by the microorganism (first trial) and after 48 hours of fermentation (second trial). This technological strategy was chosen because it has been demonstrated that *L. reuteri* produces the reuterin as an intermediate metabolite during the anaerobic fermentation of glycerol (Rash et al., 2002). To prove the effectiveness of the treatments, microbial and sensory attributes were monitored.

Microbiological Quality

Figure 16 shows the evolution of *Pseudomonas* spp. count in FdL cheese during the two experimental trials: without fermentation of the sodium alginic solution (a) and after 48 hours of microbial fermentation (b). Bishop and White (1986) stated that a *Pseudomonas* spp.

microbial load equal to 10^6 cfu/g of cheese represents the contamination level at which the alterations of the product start to appear.

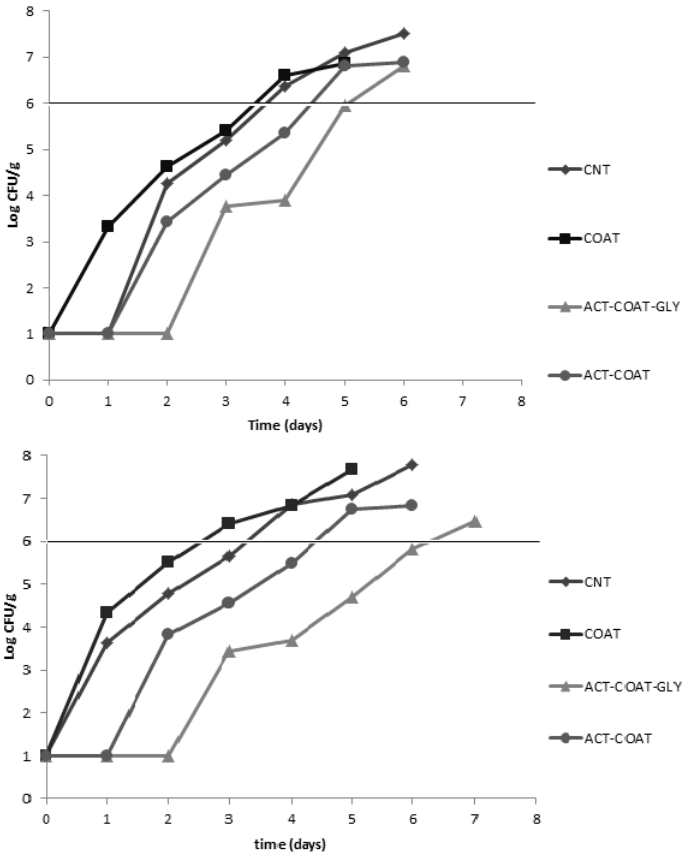


Figure 16. Evolution of *Pseudomonas* spp. count in Fior di Latte cheese during the two experimental trials: without fermentation of the active sodium alginate solution (a), with a fermentation period of 48 hours at 25°C of the active sodium alginate solution (b).

As it can be seen in the figure a, there is an immediate increase in microbial count for COAT sample while the microbial population for CNTR and ACT-COAT samples started to grow one day later and in the case of ACT-COAT-GLY, two days later. COAT and CNTR samples reached the microbiological acceptability limit ($MAL^{pseudomonas}$) at day 3.3 and 3.5 respectively and so, faster than the other two active samples ACT-COAT and ACT-COAT-GLY that have match the limit at day 4.3 and 5, respectively. In Figure b the precedent findings were confirmed and emphasized, in fact as in the first trial, COAT and CNTR samples were the first ones started to grow and to reach the microbial limit at day 2.5 and 3, respectively. Also in this case, the microbial count for the two active samples ACT-COAT and ACT-COAT-GLY, started to grow one and two days later respect to the COAT and CNTR samples. The gap between the two active samples in reaching the $MAL^{pseudomonas}$ was more evident during this second trial; in fact, ACT-COAT sample match the limit at day 4.5, while ACT-COAT-GLY sample overlapped the microbial limit at day 6.2 and then 1.7 day later respect to the ACT-COAT and more than 3 days later respect to the CNTR and COAT samples. Making a comparison between the two trials, we can say that the addition of the *L.*

reuteri with glycerol to the alginate solution improves the microbiological quality but, allowing the microorganism to ferment the solution for 48 hours, further improves the result. The delay in reaching the MAL in the two active samples (ACT-COAT and ACT-COAT-GLY) may be explained by the presence of the active coating on the product surface. The addition of *L. reuteri* exerted a sort of antimicrobial action, probably due to the acidification of the substrate and the production of some metabolites such as organic acids and bacteriocins (Angiolillo et al., 2013). The combination of the microbial strain with glycerol extended the microbial quality because it has been demonstrated that this probiotic microorganism produces the antimicrobial component reuterin as an intermediate metabolite during the anaerobic fermentation of glycerol (Rash et al., 2002). Therefore, allowing the microorganism to ferment the active solution for 48 hours further improved the microbial quality of FdL samples because the microorganism produced reuterin.

These findings were also confirmed by the enumeration of lactic acid bacteria (data not shown) in the film making solution after 48 hours fermentation. *L. reuteri* dry powder had a load of 9.21 log cfu/g. The lactic acid bacteria count in the active sodium alginate solution (with *L. reuteri* and glycerol) was 7.52 log cfu/g, after 48 hours fermentation no lactic acid bacteria were enumerated in the same alginate solution, proving that probably the fermentation process was effective in the production of reuterin but at the same time this antimicrobial component was letal for the survival of the lactic acid bacteria population.

A reduction of pH value was recorded for both trials in the brine liquid (data not shown) and this is a commonly observation in dairy products enriched with probiotics because of the continuous production of organic acids that lower the pH of the environment and consequently inhibit bacterial pathogens growth (Pithava et al., 2011).

Figure 17 shows the evolution of Enterobacteriaceae population in FdL cheese during the two experimental trials: the first (a) and the second (b). Also for this microbial group the addition of probiotic and its combination with glycerol in the coating, proved to be effective in slowing down bacterial count in the final product. In figure (a) there was an immediate increase of the microbial group for COAT sample while for CNTR sample, from day 2. These two samples reached the acceptability limit ($MAL^{Enterobacteriaceae}$) at days 3.5 and 5, respectively. While for the two active samples, the microbial population started to grow at day 2 for ACT-COAT and at day 3 for the ACT-COAT-GLY sample. The two active samples matched the microbial limit at day 6 and so later respect to COAT and CNTR samples.

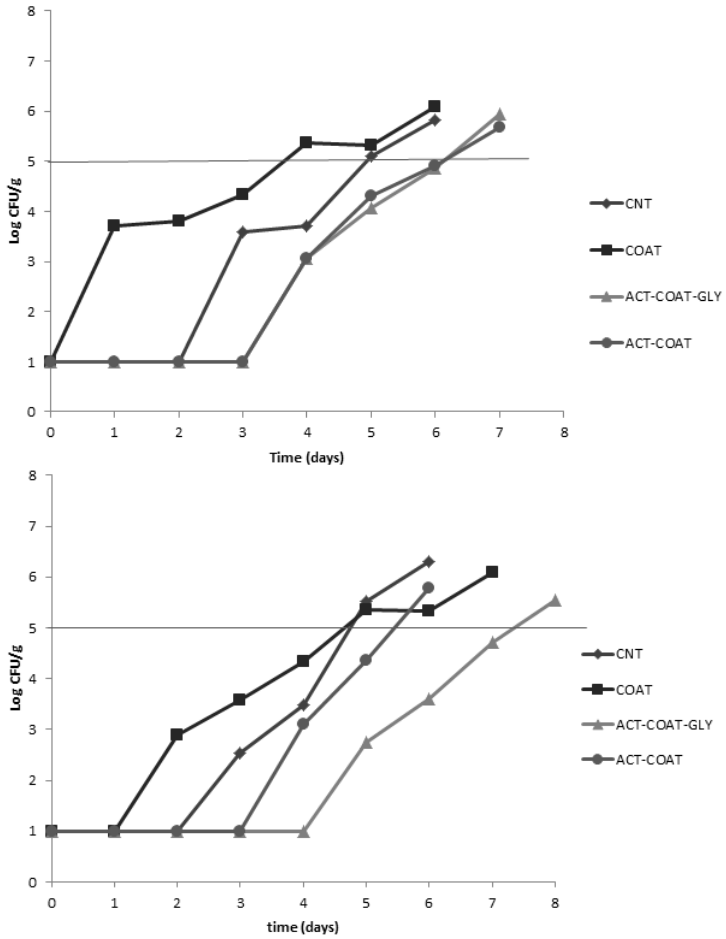


Figure 17. Evolution of Enterobacteriaceae count in Fior di Latte cheese during the two experimental trials: without fermentation of the active sodium alginate solution (a), with a fermentation period of 48 hours at 25°C of the active sodium alginate solution (b).

In the second trial (b), the 48 hours fermentation of active alginate solution proved to be effective in the slowing down the microbial deterioration, in fact in this case, COAT and CNTR samples reached the microbial limit together at day 4.5 while the ACT-COAT at day 5.3. On the contrary, the ACT-COAT-GLY sample started to deteriorate 1 day later respect to the active sample without glycerol (ACT-COAT) and 3 days later respect to samples without any active addition (COAT and CNTR), overlapping the microbial limit at day 7. Also in this case the combination of the probiotic microorganism with glycerol improved the microbial quality and the 48 hours fermentation of the alginate solution further prolonged the microbial quality by one day respect to the first trial. It can be suggested, on the basis of the microbial considerations, that the addition of probiotic and its combination with glycerol in the coating surface of the FdL cheese is an optimal and innovative way to preserve the product and at the same time, with a combination of an optimal fermentation time of the active alginate solution, to guarantee a significant microbial control.

Sensory Quality

Edible films and coatings are usually consumed with the coated product. Therefore, the incorporation of compounds such as probiotics should not affect consumer acceptance. The

addition of probiotics to obtain antimicrobial edible films and the effect of this addition to the food product has been barely studied (Rojas-Grau et al., 2009). The taste of these nutraceutical ingredients has been regarded as a particularly important factor since several authors found that probiotics cause the acidification of the substrate and the production of some metabolites such as organic acids and bacteriocins (Pithava et al., 2011). Furthermore, the increase of fermentation processes can also lead to a change in the product structure. FdL cheese is considered a traditional Italian product with characteristic sensorial properties linking it to the concept of "natural-traditional-product". Thus, it is necessary to preserve these attributes also when probiotic and/or prebiotic are added. It is important that treatments applied to functionalize also allow maintaining the appearance (i.e. color and integrity) and the flavor characteristics, being the first factors that the consumer perceives as product quality (Faccia et al., 2013).

Figure 18 shows the evolution during storage of the overall quality of Fior di Latte cheese during the two experimental trials: without fermentation (a) and with a fermentation of 48 hours (b). The curves were obtained by fitting Eq. (2) to the experimental data, whereas the horizontal dashed line is the sensory threshold.

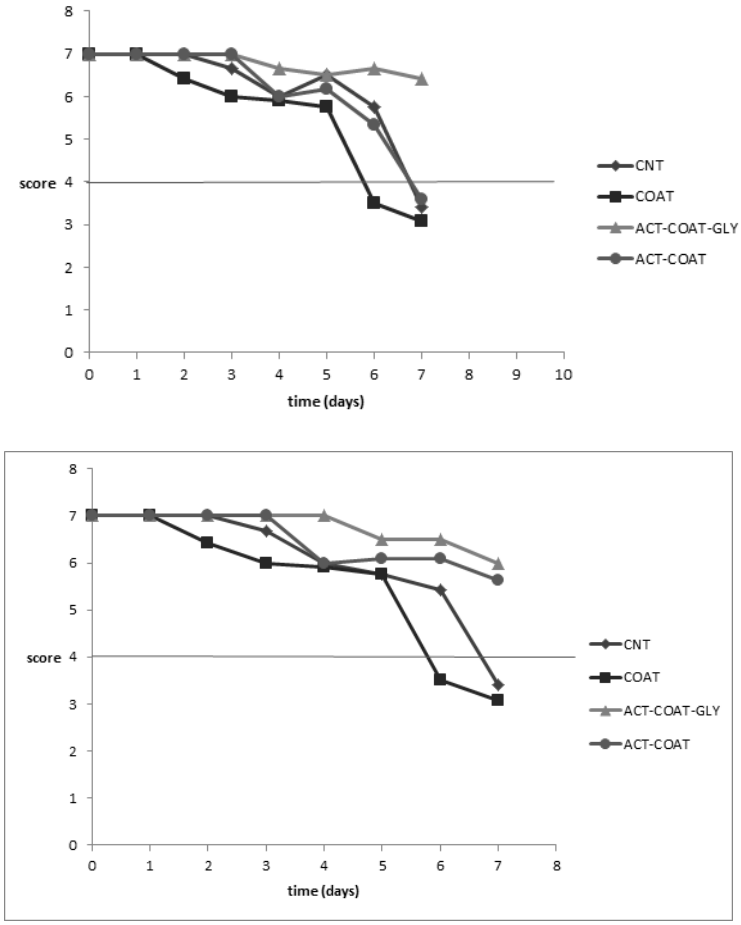


Figure 18. Evolution of the overall quality in Fior di Latte cheese during the two experimental trials: without fermentation of the active sodium alginate solution (a), with a fermentation period of 48 hours at 25°C of the active sodium alginate solution (b).

In the first trial (a), there was a steadily decrease of overall quality more pronounced in the COAT samples which matched the sensory acceptability limit ($SAL^{o.q.}$) at day 6, while CNT and ACT-COAT samples reached the limit both at day 7. Sample ACT-COAT-GLY did not match the threshold for the entire observation period and for this reason the $SAL^{o.q.}$ was set > 7 days (Table 4).

Table 4. Shelf life (days) of FdL samples as the lowest value between $MAL^{Pseudomonas}$, $MAL^{Enterobacteriaceae}$, and SAL

Samples		Microbial quality (day)		Sensory quality (day)	Shelf life
		$MAL^{Pseudomonas}$	$MAL^{Enterobacteriaceae}$	$SAL^{O.Q.}$	
T=9°C (TRIAL I)	CNT[1]	3.5	5	7	3.5
	COAT[2]	3.3	3.5	6	3.3
	ACT-COAT-GLY[3]	5	6	> 7	5
	ACT-COAT[4]	4.3	6	7	4.3
T=9°C (TRIAL II)	CNT	3	4.5	7	3
	COAT	2.5	4.5	6	2.5
	ACT-COAT-GLY	6.2	7	> 7	6.2
	ACT-COAT	4.5	5.3	> 7	4.5

[1]CNT = control sample consisting in Fior di Latte cheese without coating.
[2]COAT= Fior di Latte cheese with a 2% sodium alginate coating without *L. reuteri*.
[3]ACT-COAT-GLY[3]= Fior di Latte cheese with a 2% sodium alginate coating containing *L. reuteri* and glycerol.
[4]ACT-COAT= Fior di Latte cheese with a 2% sodium alginate coating containing *L. reuteri.*

The same trend was observed in the second trial (b) with the only difference that the ACT-COAT sample in this case recorded the same overall quality trend of the ACT-COAT-GLY, probably due to the fact that the fermentation time allowed the microorganism to increase its fermentative action, contributing to improve the microbial quality and, as a consequence, also the sensory quality of the final product. The trend of the overall quality coincided with that of the odor (Figure 18), thus proving that this attribute represented the factor limiting cheese storability. Texture and color (Figure 18) attributes did not affect the overall quality of the product; indeed, the panelists expressed their judgments with a score above the acceptability limit during the entire storage time. The panelists highlighted a typical milk odor of samples coated with probiotic (ACT-COAT and ACTCOAT-GLY), which conceded with the natural

characteristics of this Italian traditional product. This consideration probably determined the higher $SAL^{O.Q.}$ for the two active coated samples. A study conducted by Mirzaei et al. (2012) confirmed that the addition of free or encapsulated probiotics had no significant effect on sensory properties of a probiotic Iranian cheese, in fact the total evaluation in terms of color, texture and taste of samples were good and did not have any marked off-flavor during the storage and samples enriched with probiotics recorded an overall quality better than that with no probiotic cells. Therefore, sensory evaluation confirmed the considerations of the microbial quality: the use of *L. reuteri* into the sodium alginate solution was an active way to biopreserve the Fior di Latte cheese and its combination with glycerol and with a 48 hours-fermentation time was more effective in slowing down the microbial deterioration and consequently improving the sensory properties.

Shelf Life

The FdL shelf life is listed in Table 4 for each sample tested in this case study. It was calculated as the lowest value between $MAL^{Pseudomonas}$, $MAL^{Enterobacteriaceae}$ and $SAL^{O.Q.}$ (Conte et al., 2009). It can be emphasized from data that microbial quality limited the shelf life of the three samples with a major contribute of *Pseudomonas* spp. growth. In the first trial (without any fermentation), the shelf life of CNT and COAT was set at 3.5 day, while the two active sodium alginate coated samples (ACT-COAT and ACT-COAT-GLY) revealed a longer storability, about 4.3 and 5 days, respectively, probably for the improvement of the microbial and sensory characteristics of the active addiction. With the 48 hours of microbial fermentation there was a further increase of both microbial and sensory quality, in fact ACT-COAT-GLY shelf life was set at 6.2 days and then 1.2 days later respect to the first trial. The shelf life for CNTR, COAT and ACT-COAT samples was approximately equal to the first trial. To sum up, the application of an edible active sodium alginate coating enriched with *L. reuteri* and glycerol to Fior di Latte cheese improved its microbial and sensory characteristics, contributing to extend its shelf life.

Statistical Analysis of data from the domestic market

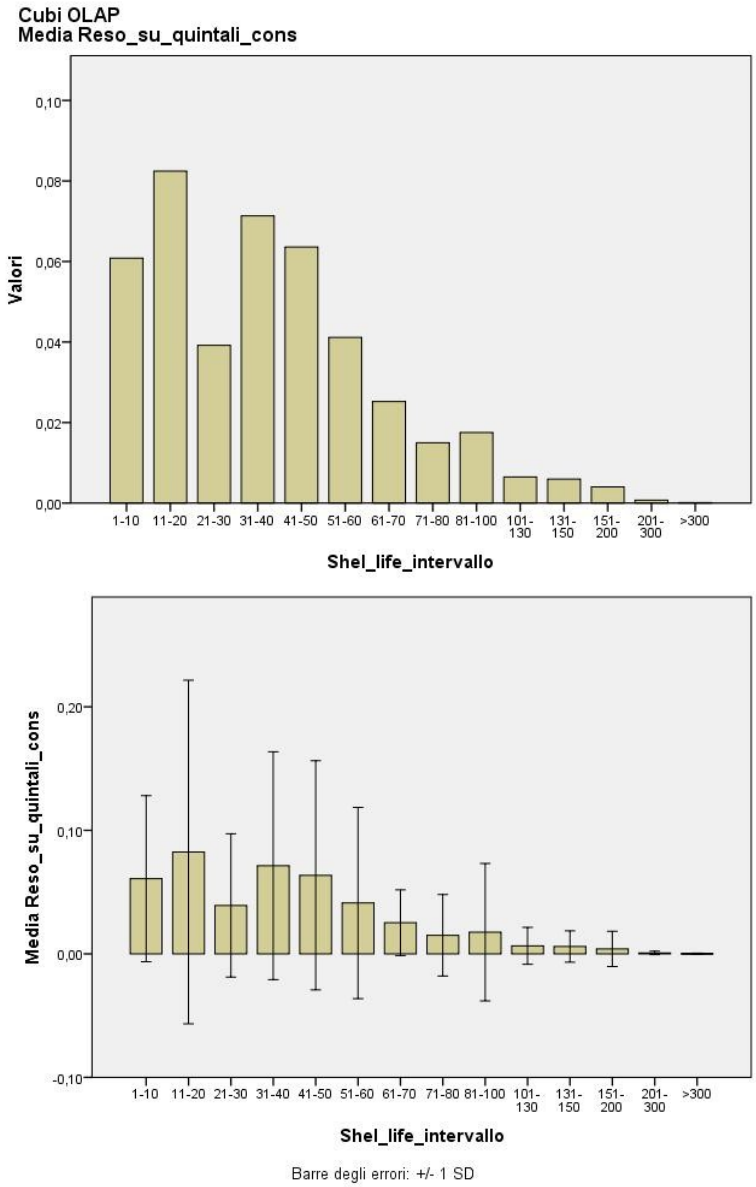


Figure 19. Distribution of average RGR as a function of Shelf-Life

Figures 19 illustrate the distribution of the RGR according to the products Shelf-life range.

The distribution is clearly far from being normal and the box-plot diagram highlight the wide variation of some classes (e.g. 11-20 days shelf-life). The box plot (a.k.a. box and whisker diagram) is a useful standardized way of displaying the distribution of data based on five numbers: minimum, first quartile, median, third quartile, and maximum. In the simplest box plot the central rectangle spans the first quartile to the third quartile (the *interquartile range* or *IQR*). A segment inside the rectangle shows the median and "whiskers" above and below the box show the locations of the minimum and maximum. Not uncommonly real datasets

display surprisingly high maximums or surprisingly low minimums called *outliers*. John Tukey has provided a precise definition for two types of outliers:

- **Outliers** are either 3×*IQR* or more above the third quartile or 3×*IQR* or more below the first quartile.
- **Suspected outliers** are are slightly more central versions of outliers: either 1.5×*IQR* or more above the third quartile or 1.5×*IQR* or more below the first quartile.

If either type of outlier is present the whisker on the appropriate side is taken to 1.5×*IQR* from the quartile (the "inner fence") rather than the max or min, and individual outlying data points are displayed as unfilled circles (for suspected outliers) or filled circles (for outliers). Outliers are not necessarily "bad" data-points; indeed they may well be the most important, most information rich, part of the dataset. Under no circumstances should they be automatically removed from the dataset. Outliers may deserve special consideration: they may be the key to the phenomenon under study or the result of human blunders.

Figure 20 reports a box-plot diagram of products with range of shelf life not exceeding 50 days. It highlights the presence of outliers in each range and of suspected outliers for shelf-life ranges over 10 days.

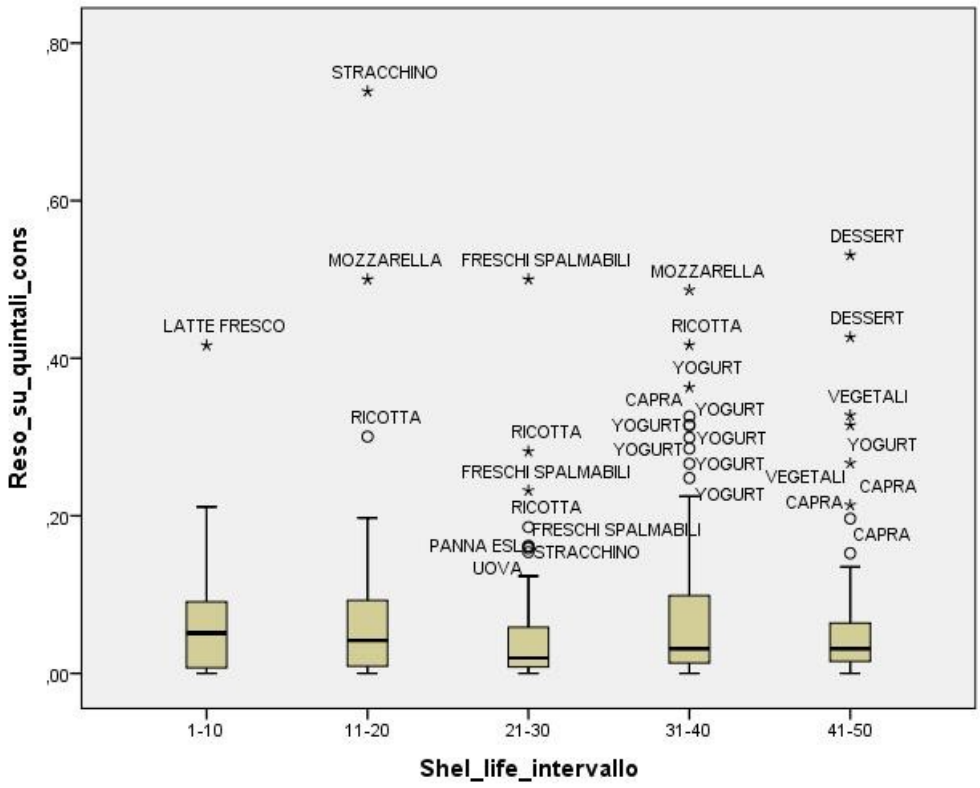


Figure 20. Box-plot Diagram RGR for SLR from 1 to 50 days.

Table 5 and 6 report the details of the descriptive statistics used for the above mentioned graphs and the results of the ANOVA analysis.

Table 5. Descriptive statistics of *returned_goods_ratio* for shelf life's range

Range Shelf life (days)	Mean	95% Confidence Interval for Mean		Std. Deviation	Minimum	Maximum	Skewness
		Lower Bound	Upper Bound				
1-10	0,061	0,045	0,077	0,067	0,000	0,416	2,576
11-15	0,080	0,028	0,132	0,090	0,000	0,301	1,323
16-20	0,084	0,022	0,146	0,160	0,000	0,739	3,415
21-25	0,037	0,028	0,045	0,037	0,000	0,161	1,376
26-30	0,041	0,026	0,057	0,072	0,000	0,500	4,016
31-40	0,071	0,056	0,087	0,092	0,000	0,486	2,084
41-50	0,064	0,043	0,084	0,093	0,000	0,531	3,032
51-60	0,041	0,018	0,065	0,077	0,000	0,438	3,650
61-70	0,025	0,015	0,035	0,027	0,000	0,105	1,212
71-80	0,015	0,000	0,030	0,033	0,000	0,152	3,686
81-100	0,018	0,007	0,028	0,056	0,000	0,409	4,646
101-130	0,006	0,004	0,009	0,015	0,000	0,082	3,335
131-150	0,006	0,001	0,011	0,013	0,000	0,056	2,776
151-200	0,004	0,001	0,007	0,014	0,000	0,104	5,828
201-300	0,001	0,000	0,001	0,001	0,000	0,005	2,029
301-700	0,000	0,000	0,000	0,001	0,000	0,004	5,655
>700	0,000	0,000	0,000	0,000	0,000	0,000	0,000

Table 6. Anova results

Source	SS	df	MS	F	Prob
Between groups	.714333567	16	.044645848	10.27	0.0000
Within groups	418.937.097	964	.004345821		
Total	490.370.453	980	.00500378		

The *returned_goods_ratio* (RGR) has not shown in the short-term (shelf life from 1 to 30 days) significant mean changes, as well as also shown in Table 5 of Multiple Comparisons.
Instead the differences between the average values of *returned_goods_ratio* are significant between the range of shelf life with inferior limit greater then 30.
Investigating further, by means of a modeling approach it has come to highlight an exponential model, shown in Fig. 21, illustrating the relation between the two variables for shelf life> 30.

Table 7.

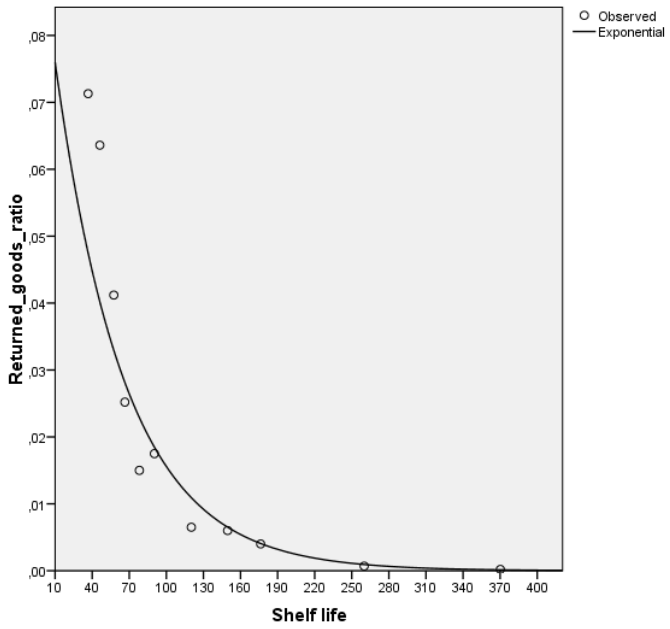
Model Summary and Parameter Estimates

Dependent Variable: Returned_goods_ratio

Equation	Model Summary					Parameter Estimates	
	R Square	F	df1	df2	Sig.	Constant	b1
Exponential	,967	260,753	1	9	,000	,091	-,018

The independent variable is Shelf life.

Fig.21. Relation between *returned_goods_ratio* and shelf life >30 days.



The result of the modelling approach correlating RGR% to shelf-life of dairy products having a shelf-life higher than 30 days fits very well with an exponential curve. A negatively more than proportional correlation was in any case expected due to the commercial restrictions on goods return generally applied by food manufacturers and accepted by retailers.

On the contrary no similar correlation was found between the two variables for fresh products, i.e. having a shelf-life shorter than 30 days. This may appear curious to dairy fresh food manufacturers since they know that improving hygienic practices and, even better, acting on technological innovation, is definitely possible to extend the shelf life of some products.

One of the best example of a successful technological innovation in the field of market milk is represented by the ESL Milk, i.e. a milk pasteurized at high temperature (125-129°C) for a very short time (1 s) in a steam infusion plant and aseptically packed in cartons or plastic

bottles. ESL Milk is similar to fresh pasteurized milk in terms of organoleptic and nutritional characteristics but its microbiological residual load is so poor that its shelf-life in the refrigeration chain (4-6°C) easily reaches 25 days (VS 7 days for fresh pasteurized milk).
The impact on food waste is reduced to one third, expressed as RGR (Returned Goods Ratio), consumer complaints being equal.
Improving at premium level milk quality and manufacturing hygiene makes possible to extend ESL Milk shelf-life from 26 to 30 days (see unpublished results for the years 2013-2015, where Pd return % stands for RGR and CPM for Complaints per Million Sold Units).

Table 8. Extended Shelf Life Milk: KPIs 2013-2015, with shelf-life extension (from 26 to 30 days)

ESL Milk	2013	2014	2015
Pd return %	1,7	1,4	1,6
CPM	3,5	3,10	1,10
Shelf life (d)	26	26/30	30

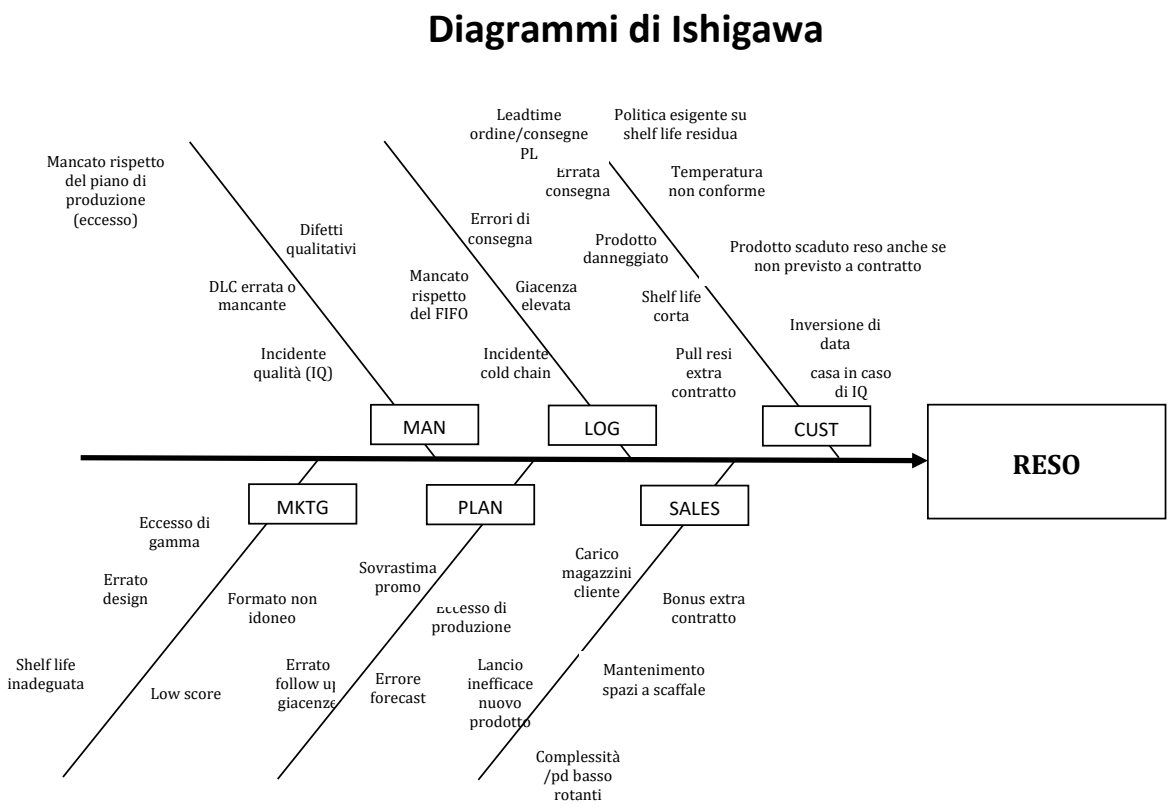
Similarly, working on pre heat-treatment of the milk and improving hygienic process conditions and manufacturing planning, it was possible to increase the shelf-life of water-buffalo mozzarella cheese by more than 10%, while decreasing RGR and consumer complaints ratio.

Table 9. Buffalo Mozzarella: KPIs 2013-2015, with shelf-life extension (from 18 to 20 days)

Buffalo Mozzarella	2013	2014	2015
Pd return %	9,4	6,7	3,4
CPM	4,5	5,3	4,0
Shelf life (d)	18	18	20

In this thesis a statistical analysis carried out on Italian market data has highlighted an inverse correlation between quantities of products returned from the market and shelf-life of the same products. A model approach has shown a significative exponential curve explaining the

relation between the two variables only for dairy products whose shelf-life exceeds 30 days. For fresh dairy products this does not apply, may be due to the fact that for fresh products the variable shelf-life is only one of the factors having impact on food waste (see Ishigawa diagram here below).



Conclusions

Food waste is a significant contributor to the overall EU negative environmental impacts, responsible for 17% of direct greenhouse gas emissions and 28% of material resource use. The amount of annual food waste in Europe is estimated to increase from over 100 million tons in 2014 to about 126 million tons by 2020 (European Commission, 2015) if no action is taken. The target set at the European level aims at 50% prevention of avoidable food waste by 2025 (European Parliament, 2012).

There is potential for spoilage of food products at any stage of the supply chain when the products reach their "best before" or "salable date". As a key to the food waste problem, solutions have been developed intending to allow products to last longer through a wide range of preservation techniques including packaging solutions, chilling, freezing, pasteurization, sterilization, drying, etc. (Gould, 1996). Other studies confirm the potential role of such technologies including packaging in providing a solution to other aspects of global food wastage through extending the shelf-life of fresh foods and thus preventing food spoilage (Williams et al. 2012, anonymous 2013a, b, Christiansen 2014, Bowling 2013). However, there is lack of evidence whether a longer shelf life necessarily reduces waste in terms of guaranteeing consumption before reaching the best before date. This uncertainty is due to the complexity of food supply chains (Van der Vorst et al., 2005, van Donk et al., 2008, van Donselaar et al., 2006, Verdouw et al., 2010, Christopher et al., 2009, Taylor and Fearne, 2009, Gedenk et al., 2010, Roth et al. 2008) as well as consumption behaviors (WRAP, 2013, EC, 2010, EPRS, 2014, Gjerres & Gaiani, 2013, Grunert, 2014).

From the food manufactures side it appears that the food sector has to manage a number of complexities generally dealt with in supply chain management, besides product perishability (van Donk, 2000, Van der Vorst et al., 2005). These complexities include the handling of large volumes and the more and more increasing product variety in terms of packaging sizes and recipes (Van der Vorst et al., 2005, van Donk et al., 2008). Moreover the supply chain features variation in terms of production and delivery lead times for various products, although lead times in general are short (van Donselaar et al., 2006, Verdouw et al., 2010). The supply uncertainty leads to low predictability and stability of supply (van Donk et al., 2008, Christopher et al., 2009) and the demand is often uncertain, largely caused by high and increasing frequencies of promotional activities (Taylor and Fearne, 2009, Gedenk et al., 2010).

All these features impact on inventory management. The cost of lost sales is often higher than inventory-carrying costs (Ketzenberg and Ferguson, 2008) and therefore firms prefer to profit from increasing product storage in order to reduce the amount of lost sales. Keeping products in storage for a longer time is problematic when it comes to perishable products. A major trend in food supply chains has therefore been to rely on shelf life extension solutions.

There are more or less unwritten rules by supermarket chains that they require products delivered to their shops to retain a substantial amount of shelf life (generally 2/3 of remaining shelf-life, while others demand at least 75-80%; a large Italian retailer requires for fresh products, i.e. those whose shelf-life is equal/less than 30 days, to be delivered to their selling point within "shelf-life – 5 days"). Consequently, the amount of remaining shelf life has to be taken into account in the preceding stages of the supply chain, ensuring that at the point of delivery, the respective date for this retailer has not been exceeded.

Data from the warehouse of Swedish dairy producer showed a considerable reduction in waste when shelf life for cream and yoghurt was extended three times (Amani and Gadde, 2015). This was confirmed also in Italy by the introduction of ESL (Extended Shelf Life) Milk, with characteristics similar to fresh pasteurized milk but a shelf-life three times as much, that shows a level of product returns from the market lower than 1/3 in percentage on sales. According to Amani and Gadde interviews with personnel working at production warehouses, as well as retail stores, revealed that they are more inclined to control the storage levels of products with shorter shelf life and prioritize their handling in comparison with long-life products. This means that products with longer shelf life stay in storage longer. Moreover, the longer shelf life of these products also leads to less frequent production. Accordingly, the production frequency is linked to the shelf life. These conditions may be part of the explanation why the increase in the shelf life didn't completely remove the waste at the storage: the flow of goods was slowed down from production to distribution. There is a clear correlation between shelf life and the time products are kept in storage: the longer the shelf life, the longer the time in storage.

Based on these data, it can be argued that ***the prolongation of shelf life does not necessarily reduce waste***. Yet it may contribute to supply chain efficiency through increasing economies of scale.

The consumer's primary point of contact with shelf life extension is through packaging. While packaging covers a range of other functions, it also has a significant role in reducing food waste. Some achievements in this regard are mentioned by WRAP (2013), including reclosing packs to prevent dehydration in the fridge; small sized and/or subdivided packaging, microfiltered fresh milk, vacuum-packed fresh meat, and intelligent packs for fresh fruit & vegetables, which helps stop them over-ripening. Although the public perception of packaging is dominated by end-of-life aspects, when the packaging becomes waste (Barlow and Morgan, 2013), packaging is considered a "lesser evil". Furthermore, it is stressed that food losses are seldom included in life cycle analyses of the food packaging system, nor are they included in the debate on sustainable packaging. This might then indicate a packaging less prone to food waste as a favorable alternative (Wikström and Williams, 2010).

It is suggested that packaging attributes, such as desired quantity, mechanical protection, easy opening, and food safety/freshness information should be included in simple scenario techniques when deciding on packaging (Wikström, et al., 2014). Packaging could have an essential role to play in preserving the value invested in products by ensuring that they can deliver their designed service with minimum wastage. However, considering the complexities in supply chain and consumption behavior, solutions to the food waste problem must be critically examined to determine whether they can fulfill their promises of reducing food waste, considering the whole chain in the entire life cycle of the product. Only when these aspects are confirmed, such solutions can be described as more sustainable.

In conclusion, ***the relation between shelf life extension and food waste reduction does not appear to be straightforward***. Complex consumption patterns (e.g. shopping in larger volume results in longer storage periods at households) in combination with a long supply chain and several storage points, imply that shelf life extension may not guarantee consumption before products have reached the "best before date". Another factor is the increasing demand for so-called "fresh products", which may lead to the perception that products with longer shelf life are considered less fresh.

As an attempt to find new ways for extending the shelf-life of extremely perishable dairy products the packaging system combining MAP and active coating has been studied within the perimeter of this thesis, using Fior di Latte (FdL) cheese as a model and monitoring microbial and sensorial parameters to determine the quality loss during storage at 8°C.

In Case study 1 (optimization of a protective packaging system) results highlighted that the packaging at high CO_2 levels was able to control the growth of *Pseudomonas* spp., even though it compromised the sensorial quality of the investigated cheese. The presence of the coating protected the FdL surface by the damage caused by the solubilization of the CO_2 in the covering liquid. Moreover, the investigated edible coating improved the esthetic appearance and physical stress. The use of active compounds, such as potassium sorbate, loaded with coating combined with MAP reduced physiological changes and microbial growth. In fact, the antimicrobial effect of the potassium sorbate against spoilage bacteria was able to increase the shelf life of the product. Moreover, the combined use of active coating and modified atmosphere packaging was able to prolong the product shelf life of about 157%.

In Case study 2 (active packaging system containing antimicrobials) the influence of different active coatings on quality decay of FdL cheese was assessed. In the first experimental step, a screening of active coatings was carried out highlighting that from the microbiological point of view the compounds exerted similar effects, whereas from a sensory point of view, the samples PS-Coat and SB-Coat maintained a better quality if compared to the other samples. In the second experimental trial the best performing compound (potassium sorbate) was used at three concentrations to assess the best amount to control microbial and sensory quality: the PS-3% sample exhibited a good quality because the microbial proliferation was slowdown and the sensory properties were visibly preserved.

In Case study 3 (active packaging system including MAP & Silver Nanoparticles active coating) silver nanoparticles incorporated into a bio-based coating in combination with MAP were used to prolong the shelf life of Fior di Latte packaged with or without the covering liquid. The results of this study have suggested that the combination of coating with silver nanoparticles controlled microbial growth better than the sole coating treatment. In particular, the active coating allowed a significant improvement of the shelf life of FdL that reached a value of about 10 days when the active coating was combined with MAP. It is worth noting that the treatments were more effective when FdL cheese was packaged with the covering liquid compared to the samples without it. This strategy could be adopted by the industrial sector because of its simple application and could allow the diffusion of dairy products beyond the local market.

Finally in the Case study 4 (bioactive packaging system including *L.reuteri* and glycerol) the combination of the probiotic microorganism with glycerol (ACT-COAT-GLY) improved the microbial quality by one day compared to the same active solution without glycerol

(ACT-COAT) and by about one and a half day compared to the FdL sample without any active coating (CNTR). The 48 hours fermentation of the active alginate solution (second trial) further prolonged the microbial quality respect to the first trial. In fact, in this case, COAT and CNTR samples reached the microbial limit together at day 4.5 while the ACT-COAT at day 5.3. On the contrary, the ACT-COAT-GLY sample started to deteriorate 1 day later respect to the active sample without glycerol (ACT-COAT) and 3 days later respect to samples without any active addition (COAT and CNTR), overlapping the microbial limit at day 7. It can be suggested, on the basis of the microbial considerations, that the addition of the probiotic *L. reuteri* and its combination with glycerol in the coating applied on FdL surface is an optimal and innovative way to biopreserve the product and at the same time, with a combination of an optimal fermentation time, to prolong its microbial quality. The sensory evaluation confirmed the considerations of the microbial quality: in fact, panelists highlighted a typical milk odor in the sample coated with probiotic (ACT-COAT and ACT-COAT-GLY), that promoted better scores for the two active coated samples. Therefore, the application of an active coating enriched with *L. reuteri* and glycerol to FdL cheese improved its microbial and sensory characteristics, contributing also to extend its shelf life.

In conclusion MAP at high CO_2 levels is able to control Pseudomonads growth but compromises the sensorial quality of FdL cheese. The coating presence protects the cheese surface from structural damages. The combined use of active coating with MAP is able to prolong the product shelf life: a multiple hurdle approach is necessary to achieve the expected results.

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