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# The effects of different packaging and pressures on a dairy product treated with the High Pressure Processing

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## Abstract

High Pressure Processing is a recent non-thermal stabilization process, able to prolong foods shelf life without changing their nutritional and sensorial qualities. This work applies HPP, testing different pressures during the treatment (400MPa, 500MPa, 600MPa), on nutrient ricottas packaged in three solutions (round tray, basket and wrapping in vacuum bag). The aim of the research is to understand the influence of these parameters on the treated product (microbial abatement, syneresis, color, softness, nutritional changes), in order to optimize its shelf life without reducing the quality. Results show that the stabilization increases with the pressure: in particular 600MPa destroys pathogenic and spoilage microorganisms up to 30 days of shelf life, homogenizing the structure of the product that results softer and lighter, without nutritive losses. Not all the tested packaging solutions are suitable for the treatment: only the basket in vacuum bag can resist to the pressure and are user-friendly for the consumer who will buy treated ricottas at the supermarkets. Overall, this work contributes to the scientific literature regarding the benefits of HPP, and can be a starting point for food companies who want to test a new innovative and promising food stabilization method.

**Keywords:** High Pressure Processing, stabilization, food packaging, shelf life

## 1. Introduction

Today a food industry priority in the Parmigiano Reggiano area is the creation of safe, nutritious and tasty dairy products, with a long shelf life. For instance, to enhance the excess of the milk for the production of that cheese, a ricotta characterized by a high content of Omega 3, 6 and Conjugated Linoleic Acid (CLA) was created within a regional project "Dairy cow farming in Parmigiano Reggiano cheese area: innovation and tradition for a sustainable farming and for high-quality products" (European Commission, 2016). However, to sell it on the market, it was necessary to find its suitable stabilization and packaging method. Having discarded the use of heat treatment, effective in the microorganisms destruction but responsible of nutritional and sensorial changes, the Modified Atmosphere Packaging (MAP) was tried, but the reached shelf life was very short (Ugolotti & Vignali, 2018). Alternatively, High Pressure Processing (HPP) was tested: it is a non-thermal technology, widespread in America, Japan and Europe, applied on food products such as juices, ready meals, fish, meat, and dairy products. This technology is already known for its ability to extend the shelf life of products, breaking down the microbial flora, without changing foods' nutritional and qualitative characteristics (Pega, et al., 2018; Rossi Riberio, De Castro Leite Junior, & Cristianini, 2018; Calzada, Del Olmo, Picon, & Nunez, 2015; Devi, Buckow, Hemar, & Kasapis, 2013).

Therefore, HPP (600 MPa, 5 min) was tested on ricottas packaged in provisional trays, then observed for a month in refrigerated storage with some untreated ricottas (Stefanini, Vignali, & Coloretti, 2019). Observation and microbiological analysis showed that HPP is able to slow down chemical reactions, preserving the product's color and inhibiting the microbial growth up to 3 log CFU/g, prolonging the lag phase and reducing the maximum growth rate. Thanks to this, HPP ricottas reached 30 days of shelf life, while the untreated ones were not suitable for consumers after 10 days of storage. However, it remained to study the influence of pressure on microbial growth, analyzing different pressure parameters.

This article aims to end the research of the regional project, placing the high nutritional value ricottas on the market. Therefore, it is necessary to identify not only the best packaging for this new product (resistant to HPP and user-friendly), but also to understand the influence of the treatment's parameter on the product (microbial abatement, syneresis, color, softness changes), in order to optimize the shelf life without reducing the quality of the product. In the next chapter the state of the art about products and packaging tested with HPP is presented; then, methods and materials used in the research are explained. Finally, after the presentation of the results, the main conclusions of the work are drawn.

## 2. State of the art

HPP can be usually applied in a pressure range of 200-600 MPa at room temperature, with short time, from 3 up to 30 minutes. These parameters determine the efficiency of the treatment. The advantage of the pressure is its ability to ensure food safety thanks to the microorganisms membranes destruction, avoiding thermal degradation. In fact, it disrupts non covalent bonds in macromolecules, but has no effects on nutritional and flavour molecules (Datta & Deeth, 1999).

HPP was experimented on dairy products as milk, cheese, ricotta, yogurt, and ice-cream. Applied to fresh cheese it improves cheese structure, texture and yield, reducing the bacterial count (Okpala, Piggott, & Schaschke, 2010). For instance, it is able to change the metabolism of lactic acid bacteria and inhibit their acidifying activity (Pega, et al., 2018). HPP on cheeses from unpasteurized milk affects some Volatile Organic Compound (VOC) such as ketones, hydrocarbons and aldehydes, but also the colour parameters, increasing L* and decreasing a*. The concentration of total Free Fatty Acids (FFA) does not change as well as the odour quality and intensity (Calzada, Del Olmo, Picon, & Nunez, 2015).

On ricotta, HPP creates more homogenous particles (0.04-30⊠m) in comparison to the heat processed (0.04-100⊠m) (Rossi Riberio, De Castro Leite Junior, & Cristianini, 2018). However, in some dairy products, part of the whey is expelled from the cheese curd (syneresis), because of the pressure (Devi, Buckow, Hemar, & Kasapis, 2013).

The choice of the packaging material is essential to ensure the integrity of the packaging during and after the treatment and consequently ensure the quality, safety and consistency of the treated product. Firstly, packaging must be flexible: plastic structures that exhibit elastic behaviour are most suitable for the treatment of food pre-packaged with HPP (Juliano, Koutchma, Sui, Barbosa-Cànovas, & Sadler, 2010). In fact, the pressure compresses the food product up to 12%, but it also reduces the volume of the package, which must be able to resist and recover the initial volume after the pressure has been released (Largeteau, Angulo, Coulet, & Demazea, 2009). This is important to avoid deformation, creases, splits, cracks or other defects in the packaging. Moreover, welding must be efficient to avoid subsequent recontamination of the product.

Besides, the headspace in the packaging should be kept to a minimum: this maximizes the use of the pressure vessel capacity and reduces the time required to reach the pressure. A maximum headspace of 30% was recommended. (Largeteau, Angulo, Coulet, & Demazea, 2009).

Although the HPP treatment is non-thermal, it is important to consider the adiabatic heating of 2-3°C for each compression of 100 MPa, when choosing the packaging, so that the differences in temperature do not affect the barrier and mechanical properties of the package. Finally, the material must not allow the migration of water used for compression or food components through the packaging, or the transfer of packaging components into the food during processing or storage (Caner, Hernandez, & Harte, 2004).

However, not all plastic materials have the same behaviour with HPP: some laminated materials show delamination after the treatment, such as PP/PET based films, due to the discrepancy of the mechanical properties of the laminated films (Mensitieri & Fraldi, 2016). Instead, polypropylene (PP) based materials have good temperature and impact resistance, low water permeability but high oxygen and good weld ability: they are excellent for HPP treatment (Mensitieri & Fraldi, 2016; Sand, 2017).

## 3. Materials and Methods

First of all, a market survey was carried out in the Parmigiano Reggiano area to understand the consumers' interest in the consumption of an high quality product treated innovatively. Although ricotta is still a niche product, consumers are well disposed to buy it for its nutritional peculiarities (Mantione & Vignali, 2017). Subsequently, a second survey was carried out in supermarkets to discover the current types of ricotta's packaging. The research was carried out in nine Parma's stores: Conad, Coop, Esselunga, Eurospin, Famila, In's, Lidl, Panorama and Sigma. Two main packaging have been identified: plastic round trays (used by 43 ricotta's brands in the supermarket), and plastic conic baskets (used by 7 brands). As for the trays, 56% of them were made of PP, 21% in Polystyrene (PS) and for the remaining 23% the material was not specified. The portions were made up in 44% by two trays of 100g each joined by a cardboard cluster, in the other 44% ricottas were sold in a single portion of 250g, while the remaining percentage of trays was in various weights. Finally, information about ricottas' prices were collected to have a reference in establishing the future price of ricotta with high nutritional value, once it will be placed on the market. The average price of ricotta's trays was 5.32 €/kg, while the conic baskets on the market, usually in PP, had an average price of € 6.30/kg.

Based on the premises found in literature, considering the results of the market analysis as well as the availability of packaging in companies that collaborated on the project, three types of packaging for ricottas have been tested in the HPP treatment:

- conic basket (PP) in a vacuum bag (PP)
- wrapping and vacuum PP bag
- round tray (PP) without modified atmosphere

**Figure 1:** The three tested packaging for ricottas

Many portions of 250g of ricotta have been created to have enough packaged samples (treated with HPP and untreated) on which perform different tests over 4 times: T0 = packaging and treatment time, T1 = 10 days, T2 = 20 days, T3 = 30 days. The first two types of packaging were created at Pascoli Alti (Pascoli Alti, 2020), while the last was created at The Experimental Station for the Food Preserving Industry (SSICA, 2020) thanks to a FoodPack 400 heat sealer (ILPRA, 2020). The packs were filled as much as possible to limit the headspace. To perform the high pressure treatment on the packaging, an Avure Technologies machine was used in an Italian company near Parma (HPP Italia - Food Safety, 2020). The treatment cycle is the same performed in a previous research (Stefanini & Vignali, 2020). The wrapped ricotta and the conic basket were treated at three different pressures: 400, 500 and 600 MPa. The treatment time has been set to 3 minutes in order to understand how ricottas changed from a microbiological and aesthetic point of view (colour and syneresis) when the only pressure changed. As for the ricotta packaged in trays, the treatments were carried out at 400 MPa and 600 MPa for 3 minutes.

The colorimetric variations of the samples were calculated using the CIELab 1976 colour space, thanks to the Minolta CM2600d colorimeter with illuminating setting D65, observer angle of 10° and average MAV mask. For each sample, four colorimetric measurements were made and the average value was calculated with the respective standard deviation. Every 10 days this tool was used to calculate the L *, a * and b * parameters of the ricottas. The colour analyses were carried out on ricotta packaged in basket and wrap, and in less detail on those packed in trays since most of them, as will be explained later, broke during the treatment.

For each sample analysed, the amount of liquid released from the ricotta (syneresis) was taken into account in ml. Furthermore, the homogeneity and the softness of the product were considered macroscopically.

The VOC were evaluated at T0 on untreated and treated ricottas at 600 MPa. The extraction of the volatile fraction was carried out using the Solid Phase Micro-Extraction (SPME) technique, with the same method and parameters of the previous research (Stefanini & Vignali, 2020).

Moreover, microbiological analyses were carried out on the samples to evaluate the effectiveness of the treatment in terms of shelf life extension comparing the non processed ricottas with the HPP ones. For reasons of cost and better resistance to HPP, the analyses were carried out only on the baskets, considering the following microorganisms: lactic acid bacteria, faecal coliforms, negative nuclease cocci, yeasts and molds and finally the total bacterial count. Data were obtained for untreated ricotta and treated at 400, 500 and 600 MPa: every 10 days, 20g of each sample were diluted with 9 parts of citrate buffer (trisodium citrate 20 g/L), then homogenized for 120'' with Stomacher. Also the pH trend over time was analysed, using a Crison pH-meter equipped with penetration electrode. After the homogenization, the suspension was used for serial dilutions in physiological solution (NaCl 9g/L). Finally, dilutions were inoculated in different breeding grounds in order to search for the cited microbial groups.

## 4. Results and Discussion

The treatment results were different, based on the type of packaging and the pressure used.

All the round trays treated at 600 MPa broke, due to the delamination of the material: the treatment water entered into the product, making it unsuitable for consumption. Also some trays treated at 400 MPa showed breakages, deformations and defects. Probably, the trays were not enough flexible to withstand the treatment, or the material was not pure PP, but a PP/PET multilayer, which delaminated confirming the results mentioned in the literature. Consequently, due to the poor resistance to treatment, this packaging option was discarded although the ricotta had few syneresis and good aesthetic quality.

The ricottas packaged in vacuum wraps resisted to the treatment without break. However, this solution was not user-friendly: the wrapping got completely wet due to the serum released during the treatment: it was difficult to handle and remove from the surface of the product. Furthermore, these ricottas showed a lumpier and drier consistency than the others and a higher serum release than ricottas in baskets (Figure 2). Even this option was therefore discarded for the placing of ricottas on the market.

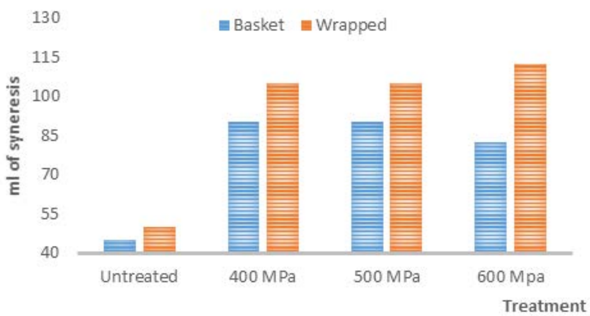


**Figure 2:** Syneresis in ricotta's baskets and trays depending on the pressure treatment

The best results have been obtained with baskets: they resisted to the treatment, they were user-friendly and ricottas appeared homogeneous and soft. Furthermore, in the colour tests ricottas in baskets showed a higher brightness than the wrapped ones (Figure 3).

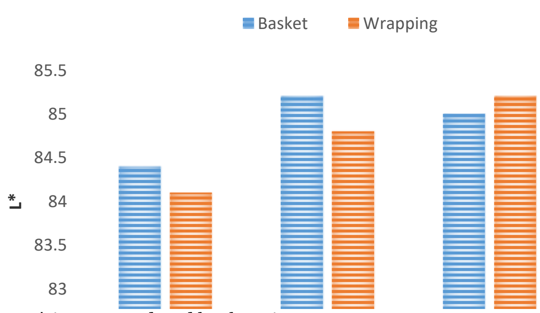


**Figure 3:** L* in wrapped and basket ricotta

The only disadvantage that cannot be overcome is the syneresis, which also occurred in this type of packaging and which increased exponentially over time in all treated ricottas, but slightly less in those treated at 400 MPa (Figure 4).

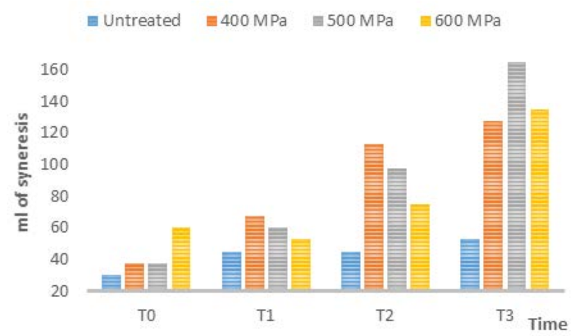


**Figure 4:** Syneresis during time in basket's ricotta

The microbiological analyses carried out at the initial time T0 on the untreated samples and HPP ricottas for 3 minutes at pressures of 400, 500, 600 MPa, showed that the effect of HPP was evident and proportional to the applied pressure. In fact, although a microbial reduction also occurred with the treatment at 400 MPa, the best results were obtained with a higher pressure, in particular 600 MPa (Table 1).

**Table 1:** Microbiological analyses results at T0 [CFU/g]

| **Sample** | Total bacterial count | Lactic acid bacteria | Fecal coliforms | Negative Nuclease Cocci | Yeasts and moulds |
|---|---|---|---|---|---|
| Untreated | 4.08 | 3.94 | <1 | <2 | 2.40 |
| 600 MPa | 2.70 | 1.60 | <1 | <2 | <2 |
| 500 MPa | 3.70 | 3.00 | <1 | <2 | <2 |
| 400 MPa | 3.83 | 2.85 | <1 | <2 | <2 |

Over time, measurements of the microbial load have been made in ricottas treated at different pressures, to identify the parameter that guarantees greater product food safety and a longer shelf life. The results showed that pH decreased over 30 days, especially in ricotta treated with 400 MPa (Figure 5).

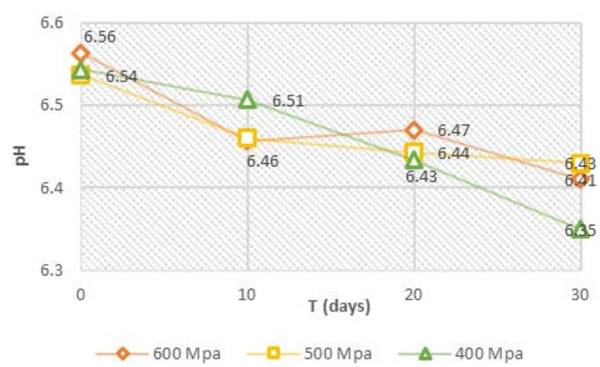


**Figure 5:** pH test results

One of the main causes of acidification was the growth of bacteria, in particular lactic acid bacteria which ferment acidifying the food product. The greatest development of these bacteria occurred once again in ricottas treated at 400 MPa (Figure 6).

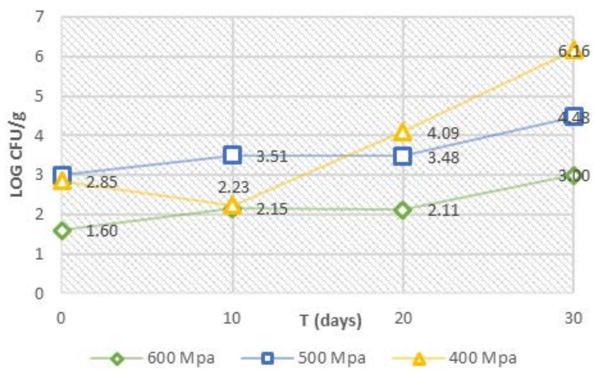


**Figure 6:** Acid Lactic Bacteria over time

Moreover, in the chart is clearly visible the difference between 600 MPa and the other pressure at T0 and T1 (Figure 7). Finally, at T3 the result of this treatment was very similar to that obtained with the pressure of 500 MPa.

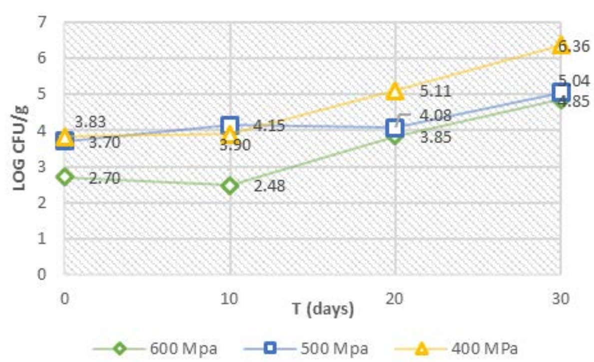


**Figure 7:** Total bacterial count during time

Therefore, among the three treatments investigated, the best was the one at 600 MPa for 3 minutes. To ensure the safety of the product, a test on the presence of toxigenic and pathogenic bacteria was carried out on the ricotta treated at 600 MPa after 30 days. The results show that the content of Listeria, Staphylococci, Clostridia and Escherichia Coli in ricotta was absent or within the limits imposed by regulations. Therefore, the product can be consumed even after 30 days from the packaging and treatment time. Moreover, to check that the HPP treatment did not change the nutritional content of omega 3 and CLA of the ricotta, tests were carried out by comparing the content of phospholipids and fatty acids on the untreated T0 samples and on the HPP samples treated at 600 MPa. The results showed no difference between the two types of ricottas.

Finally, in the tests on the volatile fraction of the ricotta treated at 600 MPa and not treated at T0 (Table 2), the only difference observed is related to the aldehyde content which is higher in the untreated ricotta compared to the one treated with high pressure at 600 MPa. Among the aldehydes, the one that showed the greatest difference is the hexanal, which in the untreated ricotta is present twice than in HPP ones.

**Table 2:** VOC at T0 in untreated and treated ricottas at 600 MPa [unit area/1000000]

| VOC | Untreated | 600 MPa |
|---|---|---|
| Ketones | 19.94 | 22.02 |
| Aldehydes | 20.54 | 11.74 |
| Alcohols | 1.30 | 1.15 |
| Esters | 0.32 | 0.42 |
| Acids | 8.64 | 8.50 |

## 5. Conclusions

According to the obtained results regarding shelf life, visual appearance, colour, homogeneity, microbiological content and volatile components, it can be stated that HPP technology is very promising on ricottas with high nutritional value. However, by varying the treatment's pressure, the effects on the product change, in particular the microbial stabilization. The treatment at 600 MPa for 3 minutes is able to extend more the ricotta's shelf life, preserving its nutritional characteristics homogenizing it and making it soft on the palate. Among the three types of packaging analysed, basket is the best from an aesthetic point of view, mechanical resistance to treatment and it is very user-friendly.
Therefore, the combined effect of appropriate packaging and treatment parameters manage to transform an extremely perishable product into a food with a shelf life greater than 30 days. The results of this research will not only contribute to the scientific literature regarding the benefits of HPP, but can be a starting point for food company to try the use of a new, innovative and highly promising food technology stabilization.

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