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High Pressure Processing and its Application in Cheese Manufacturing: A Review

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ABSTRACT: Nowadays, high standard level of living has led to an increase in demand for foods which are minimally processed via high sensory and nutritional quality and also an extended shelf life. High pressure processing (HPP) is one of the techniques that was considered for food preservation at the first application in food industry. Pressures in the range of 300 to 650MPa have been found to affect the micro-organisms, although, there is an exception regarding spores inactivation. Many studies have proved that although the application of high pressure between 400-600MPa in raw milk does not result in a product with equivalent quality as sterilized milk, but, it gives the same quality as pasteurized milk. In addition, it has been proved that high pressure creates important roles in dairy processing. For example, it improves acid coagulation in order to accelerate cheese ripening. This review summarizes some significant studies concerned with the application of HPP for cheese ripening acceleration.

Keywords: *Cheese Manufacturing, Cheese Ripening, Innovative Techniques, High Pressure Processing*.

Introduction

The importance of food and its relation to health has led to an increase demand for food with high quality. This is the main reason for the development of some new technologies such as High Pressure Processing (HPP), Microwaves, Dielectric heating, Hurdle technology, Irradiation and particularly Nanotechnology as well as some other techniques to produce food with improved nutritional and sensory qualities. In this paper, HPP is the main subject to be developed. The application of this technology goes back to the 19th century where for the first time it was studied on some types of foods and the main object was to inactivate micro-organisms under high pressures at room temperature. Nevertheless, the duration of application of HPP was some few minutes however further experience, indicated that there are some limitations. For example, some enzymes which are effective in the quality of foods, displayed a baroresistancy that was more than few hundred megapascals while others may be resistant to pressures up to 1000MPa (Cano *et al*., 1997).

The effect of high pressure treatment also tightly depends on some factors such as pH, temperature and also substrate composition. Moreover, in terms of microbial destruction, high pressure affects cell membrane of microbes and makes complications between cell and components that surround it in order to lead to their death (Hendrickx *et al*., 1998). Sensitivity of microbial cells in the log phase is much more than when they are in the stationary or death phases.

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Furthermore, vegetative cells are also resistant to some extent of pressure. They are killed or inactivated in pressure range between 300 to 600MPa (Hoover *et al*., 1989). Seyderhelm and Knorr (1992) reported that a combination of heating and pressurization is required to inactivate the germinated cells instead of using much higher pressures. For example, spores will be killed at 400MPa and 60 °C.

One of the unique features of HPP is its uniform application to all parts of a food in comparison to other methods. According to isostatic principle, pressure is transmitted uniformly and instantaneously independent of size and geometry of food (Ramaswamy *et al*., 1999). Pressurization and de-pressurization take place rapidly and as a result, processing time is decreased as compared to the thermal processing (Fellows, 2000). Furthermore, there is a potential to have a combination of high pressure with other methods of processing that results in development of new processes and products (Fellows, 2000). HPP method causes the destruction of micro-organisms without having any destructive effects on valuable compounds such as vitamins, flavoring components as well as texture, thus, provides high quality products (Fellows, 2000). Beside all the benefits of HPP for food processing, some researches have focused on its applications in some foods such as cheese production where the pressure can accelerate cheese ripening and also improves the organoleptic properties of cheese (Reps *et al*., 1998).

Equipment and mechanism of high pressure processing

The history of HPP as an expensive operation goes back to late 20th century and reported that it is a costly operation as compared to the conventional processing for fruit juices (Manvell, 1996). The application of this technique in batch process of food industry is available. It requires vessels that can operate at pressures of 200 to 500 MPa with operating cycles of as little as two to three minutes. For example, it is reported that a throughput of 600 $1h^{-1}$ at 400 MPa is used in the commercial application of high pressure processing to produce pineapple juice in Japan (Palou *et al*., 1999).

The four main components of a high pressure system are:

A pressure vessel and its closure
A pressure generation system
A temperature control device
A materials handling system (Mertens, 1995).

The use of high tensile steel in pressure vessels let them withstand pressures up to 600MPa. In addition, pre stressed multi layers or wire wound vessels are used in higher pressures (Mertens, 1995). Vessel is closed by a thread of steel, a closure is positioned over the vessel by a sealed frame. A medium is required to control the temperature which is being pumped through the jacket of the pressure vessel for a constant temperature. Furthermore, in some conditions where the temperature is needed to be changed regularly, temperature control device response is installed. Therefore, a heat exchanger may be fitted in the system. The operation of a high pressure system is divided into two types of compression. While all air has been removed, water or oil as pressure transmitting mediums is pumped into the pressure vessel via an intensifier in order to make the desired pressure. This operation requires static pressure seals and is termed “indirect compression”. But, a “direct compression” requires dynamic pressure seals between the piston which is used to compress the vessel, and internal vessel surface. However, these are not used in commercial applications (Fellows, 2000).

In-container and bulk processing are two methods of high pressure processing. It is known that the very high pressures result in a decrease in volume of foods. For example, there is a reduction of 15% of water volume at 600MPa. Therefore, packaging plays a significant role in in-container processing. On the other hand, bulk processing by requiring only pumps, pipes and valves is relatively simpler. Although, the cost of the equipment of high pressure processing is high, it is highly energy efficient. In addition, liquids by being under pressures by direct pumping with high pressure pumps lead to reduce the cost of the processing. Moreover, fast decompression through a small orifice causes high velocity and turbulent flow and increases the shearing force on microorganisms and the rate of the destruction (Earnshaw, 1992).

Figure 1 and Figure 2 show schematics of an indirect compression and a production unit for high pressure treatment respectively.

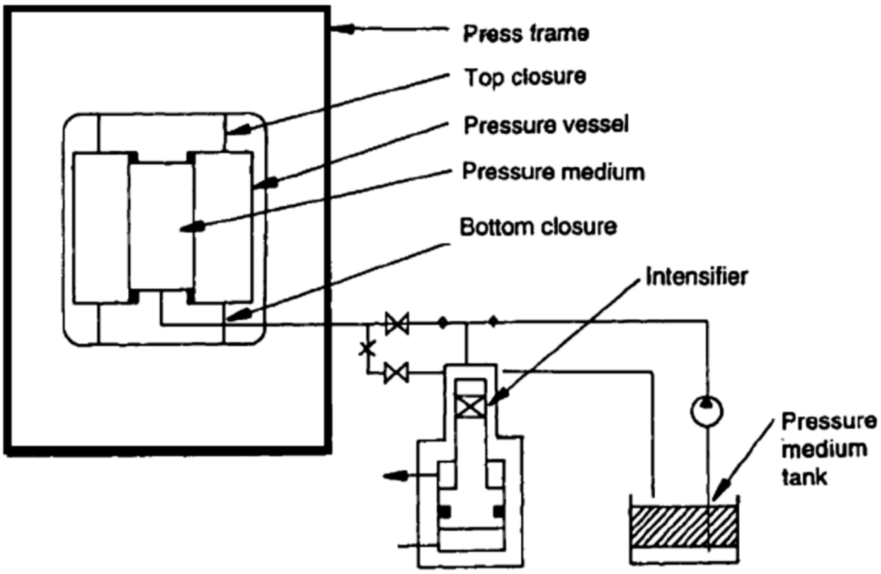

Fig. 1. A schematic of an indirect compression equipment for high pressure processing (After Mertens (1995).) (Fellows, 2000)

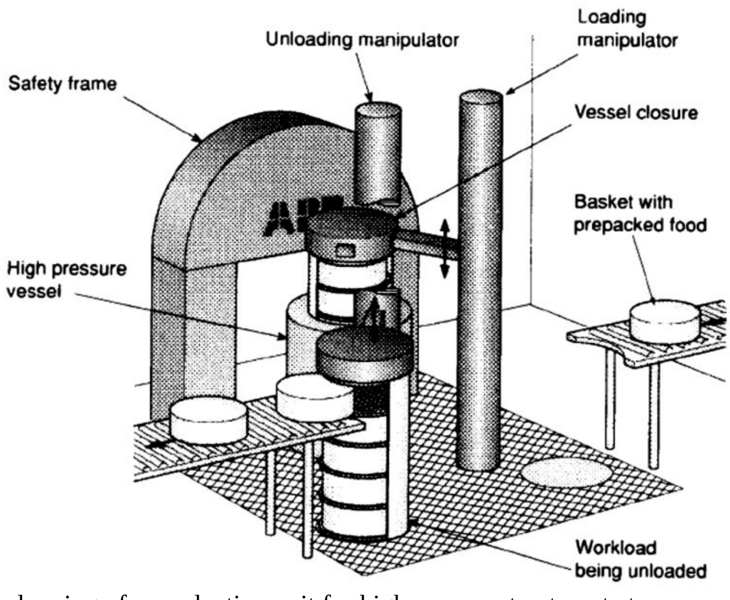

Fig. 2. A schematic drawing of a production unit for high pressure treatment at pressures between 400-800MPa (From Olsson (1995)) (Fellows, 2000)

Inactivation of micro-organisms

HPP has a significant role in inactivation of microorganisms without any destructive effects on organoleptic characteristics of cheese. For example, cheese treatments include application of pressures of 450MPa at 10min or 500MPa at 5min. It is reported that such pressure utilization caused reduction of more than 5.6 logarithmic units of Listeria monocytogenes in cheese made from raw goat milk (Gallot-Lavalee, 1998). Naturally, it is also known that the destruction of microorganisms increases with the pressure increase (Reps *et al*., 1998). The other application of HPP to inactivate the harmful microorganisms in fresh cheese has been studied by some authors and have shown that high pressure treatment at 400MPa at 2-25°C for 5min led to 7 logarithmic units of reduction in Escherichia coli population (Capellas *et al*., 1996). In addition, some counts of E.coli that had not been completely inactivated under pressure, have not been observed after preservation for 1 week at 4°C in vacuum packaging. Furthermore, after 2 months of storage at 4°C, any of the microorganisms that had survived under pressures were not detected. Milk pasteurization at the temperature ranging from 72 to 74 degrees Celsius and 15S (HTST) or at equivalent conditions (LTLT) also leads to inactivate pathogenic microorganisms in order to provide a microbiological safety for being used in cheese production. However, it is reported that pasteurization of milk has some negative effects due to sensory properties or texture defects as well as delaying cheese ripening (Grappin *et al*., 1997). It is known that microbiological quality of cheese produced from milk under pressure of 500MPa for 15S at 20°C is similar to the pasteurized milk cheeses (Buffa *et al*., 2001). On the other hand, there are some differences between the composition of cheeses made by the application of high pressure or produced from pasteurized milk.

The effects of high pressure processing on different stages in cheese manufacturing

- *Rennet coagulation time*

An early study in 1994 showed that RCT was remained unchanged after treatment at pressures below 150MPa. It was also obtained that coagulation time decreases at pressure range between 200 to 670MPa (Desobry-Bonan *et al*., 1999). On the other hand, it is known from most studies that there is a reduction in RCT following whole of pressure treatment < 200MPa and also pressures between 200 to 400 as compared to the untreated milk (Shibouchi et al., 1992 ; Lopez-Fandino *et al*., 1996 ; Lopez-Fandino *et al*., 1997 ; Lopez-Fandino *et al*., 1998 ; Johnston *et al*., 1998 ; Needs *et al*., 2000). Although, their studies resulted in lower RTC at 200MPa in comparison with higher pressures, a study in 1999 showed that rennet coagulation time in treated milk at pressures above 200MPa was similar to that was treated at 200MPa (Trujillo *et al*., 1999). Furthermore, a study in 1997 indicated that pressures around 200MPa that resulted in lower rennet coagulation time, led to a reduction in the rate of the released caseinomacropeptides (Lopez-Fandino et al., 1997). Whey proteins are reported to be denatured at pressure treatment above 100MPa and among serum proteins, β-lactoglobulin is the most susceptible to denaturation. For instance, pressure treatment at 200 or 300MPa leads to 20 or 80% denaturation of β-lactoglobulin. As a result, a decrease in the level of β-lactoglobulin has occurred while the α-lactoglobulin level remained relatively unchanged (Lopez-Fandino *et al*., 1996; Felipe *et al*., 1997; Brooker *et al*., 1998). A reduction of around 30% in the whey proteins contents has been reported as compared to the untreated samples (Brooker *et al*., 1998). The diameter of casein micelles that is reduced by high pressure treatment might affect rennet coagulation time in order to accelerate the aggregation (Needs *et al*., 2000). It is also known that a delay on the rate of casein micelles aggregation in the second stage of rennet coagulation at pressures above 300MPa is due to the association of increased denatured β-lactoglobulin with casein micelle involved in the aggregation (Ohmiya *et al*., 1987; Lopez-Fandino *et al*., 1997; Trujillo *et al*., 1999).

As the reduction in the contents of whey proteins paralles the increasing pressures during rennet coagulation that results in reduced whey proteins in curd drainage, cheese yield and moisture contents are increased (Lopez-Fandino *et al*., 1996; Pandey *et al*., 1998).

- *Curd formation*

Application of high pressure processing by Molina *et al*. (2000) indicated that the treatment at 400MPa for 15min leads to a considerable increase in curd firmness relative to the untreated milk. Although, Ohmiya *et al*. (1987) in an early study had perceived an accelerated rate of curd formation at 400MPa, the rate of curd firming was also observed to be increased following the treatment at pressures below 200MPa. But, by the way of contrast, there was a decrease in the curd formation rate of milk during treatment at 200-400MPa that resulted in the reduction of RCT (Lopez-Fandino *et al*., 1996 ; Lopez-Fandina *et al*., 1997). The highest rate of curd firming was reported at HP treatment at 200MPa (Needs *et al*., 2000).

A comparison between the gels made from untreated milk and those were treated at 600MPa indicated that HP treatment results in a compact gel structure and also much more interrupted network over a long distance that are direct consequences of disruption of casein micelles and β-lactoglobulin following an increase in the number of protein particles after pressure processing while gels made from untreated milk have an uninterrupted network with longer interstitial spaces and a coarser structure (Needs et al., 2000).

- *Cheese ripening*

Application of high pressure processing concerning acceleration of cheese ripening was first studied in 1992 on cheddar cheese (Yokoyama et al). Treatment at 50MPa resulted in 26.5mg/g of free amino acid while untreated cheese (6 months old) contained 21.3mg/g of free amino acid. Both of those cheeses were found to have the same taste. A study in the year 2000 showed that FFA and soluble nitrogen at the pH of 4.6 increased immediately in early days of aging after HP treatment. However, these were found to be less affected by HP with cheese age (O'Reilly *et al*., 2000). Other studies on Gouda cheese in 1998 and 1999 showed that although by increasing the holding time (20-100 min or 3 days) and pressure ranging between 50 to 500MPa have no effect on the index of proteolysis during ripening, but lead to an increase in pH shift and difference between pH in HP treated cheese and those are not pressurized (Kolakowski *et al*., 1998; Messens *et al*., 1999). On the other hand, higher level of nitrogen in serum was observed in cheese produced from HP treatment (300MPa).

Most of the studies showed that HP treatment resulted in accelerated ripening by affecting the action of chymosin on αs_1-casein. It was also found that the increasing rate of proteolysis might be caused by a combination of pressure and temperature (Yokoyama *et al*., 1992; O'Reilly *et al*., 2000). Furthermore, it is reported that pressures up to 800MPa have no considerable effect on enzymatic activity of chymosin whereas it was observed to be still active at 800MPa (Scollard *et al*., 2000). In addition, pressures below 300MPa resulted in higher rate of hydrolysis of αs_1-casein that is affected by increased surface of casein micelle and higher availability for proteolysis as compared to the control group. However, higher pressures were found to be an ineffective way to liberate some of the peptides (Scollard *et al*., 2000). On the other hand, Voigt *et al*. (2012) in a study concerned with cheddar cheese reported that HP treatment and time of ripening significantly affected the enzymatic activity of plasmin. It is also known that plasmin is inactivated at temperature above 80°C that leads to less hydrolysis of αs_2-casein and β-casein (Benfeldt *et al*., 1997; Benfeldt *et al*., 2001) while chymosin resulted in primary separation of β-casein and αs_1-casein during ripening. Some authors investigated that accelerated hydrolysis of β-casein by plasmin during ripening resulted by the application of HP treatment (Messens *et al*., 1998) (Urea-PAGE analysis). Although, the diffusion rate of salt during brining was found to be independent of HP treatment, loss of moisture during ripening was considerably reduced after treatment above 300MPa (Messens *et al*., 1999).

High pressure processing is known to be ineffective in some ripening characteristics such as lipolysis in Gouda cheese while lipolysis in comembert cheese is reported to be slightly affected by HP treatment (500MPa and 4hr) (Kolakowski *et al*., 1998). On the other hand, no increase was observed in proteolysis of Saint Paulin cheese following HP treatment at 50MPa for 8hr at 20°C (Messens *et al*., 2000).

- *Cheese composition*

A comparison between HPP treated milk cheeses and raw or pasteurized milk cheeses showed that although the contents of some compositions such as moisture, salt and total free amino acids are higher by the use of high pressures than in pasteurized or raw milk cheeses, the level of lipolysis in cheeses resulted from milk treated by this method and raw milk is also higher than cheeses produced from pasteurized milk. This might be caused by thermal processing while lipase is reported to be partially resistant to high pressure treatment (Messens *et al*., 1999). Therefore, high pressure processing results in higher contents of free fatty acids in milk in comparison to the pasteurized milk (Trujillo *et al*., 1999). HPP also creates new texture by reducing the content of water that is variable between blocks of cheeses that leads to a viscoelastic texture (Torres-Mora *et al*., 1996; Messens *et al*., 1999).

The contents of non-ionic calcium and phosphorus have also been increased in pressure treatment. This is caused by micelles that are fragmented under pressure (Trujillo *et al*., 2000). Trujillo *et al*. (1999) in a study on cheese made from HPP treated goat milk, had presented a comparison between pressurization and pasteurization. They showed that high pressure processing causes a reduction in whey proteins solubility that leads to lower amount of proteins in whey. No differences were found in term of microbiological safety. On the whole, rennet coagulation and curd firming at cutting in cheeses made by HPP are improved as compared to pasteurization. However, rennet clotting time in pressures above 300MPa will be increased (Lopez-Fandino *et al*., 1997). Application and the effects of high pressures between 50 to 1000MPa on inactivation of microorganisms and cheese enzymes was studied by Kolakowski *et al*. (1998). They reported that proteolytic enzymes are inactivated by the application of pressures above 800MPa. It was also observed that high pressure processing leads to higher organoleptic acceptability of cheese.

According to the results of San Martin-Gonzalez *et al*. (2006), there is an increase in the content of protein in dry cheese (PDC) with pressure treatment. For instance, higher PDC was obtained in HP treated at 483MPa and 30°C than in cheeses made from pressurization at 483MPa and 10°C for raw or pasteurized milk. Protein recovery was

81.55% in dry matter at 483MPa and 30°C while PDC at the same pressure but lower temperature was 77.28%. Also, 77.90% of protein was recovered following increased pressure to 676MPa at the same temperature. They did not observe any considerable distinction in terms of protein contents between high pressure treatment at 483 and 676MPa at the same temperature. Therefore, higher content of protein is required for a reasonable combination of pressure and temperature. Fat contents differed from 91.22 in cheeses from raw milk to 95.62 for HP treated at 676MPa and 10°C. However, there were not any noticeable differences by increasing both pressure and temperature. For example, application of high pressure at 483MPa and the temperature of 30°C resulted in lower fat (90.97%) than in treatment at the same pressure but lower temperature (94.76%).

4.5. The effect of HPP on the ripening acceleration, moisture contents and the yield efficiency of some types of cheeses

In terms of cheese ripening acceleration, different pressure conditions such as 400-600MPa for short times (5-15 min) or lower pressures (50Mpa) for long time (72hr) have been studied for different cheese varieties. For example, high pressure treatment at 50MPa and 72hr on Garrotxa goat cheese resulted in improved solute diffusion, water holding capacity and salt distribution (Saldo *et al*., 2001). In addition, Cheddar cheeses produced from HP treated milk showed higher yield and moisture contents. San Martin-Gonzales *et al*. (2006) reported increased moisture contents of cheddar cheeses were made from high pressure treated milk at 483MPa at 30°C and 676MPa at 10°C while pressurization at 483MPa and 10°C did not lead to any noticeable changes in cheeses. The method of cheddar manufacturing was substantially different from conventional cheddar manufacturing. In particular, starter addition to the milk was at least 10 fold higher than conventional inoculation rates. In certain cheese varieties such as Mozzarella and Gouda, increased rate of proteolysis on exposure to pressure treatment of 400-600MPa for 5-15 min was observed (San Martin-Gonzales *et al*., 2004). Cheeses made from ewe's milk and treated with HPP also showed enhanced proteolysis (Juan *et al*., 2006). Denaturation of whey proteins are also known to be increased at higher temperature, which is shown synergistic effects of both temperature and pressure (Tauscher, 1995; Lopez-Fandino *et al*., 1998; Wick *et al*., 2004). Increased contents of moisture might be caused by fat globuls and casein micelles that are not closely aggregated and allowed the moisture to be trapped and also denatured proteins caused the exposure of hydroxyl groups (Johnston *et al*., 1992; Lopez-Fandino *et al*., 1996; Drake *et al*., 1997). The highest percentage of wet weight yield was related to HP treatment at 483MPa at 30°C and 676MPa at 10°C (11.54%, 11.40%, in order). In addition, a study by Drake *et al*. (1997) on cheese produced from high pressure treated milk at 586MPa and 5°C resulted in similar wet yield (11.30%). It is obvious that temperature plays a significant role beside high pressures.

Conclusion

The great advantages of high pressure processing in cheese manufacturing are reduction of RCT, accelerated rate of curd formation and also increased yield of cheese, depending on cheese variety. However, all these changes require appropriate combinations of pressure, time and temperature. Application of high pressure processing in cheese manufacturing also results in acceleration of proteolysis during ripening. Therefore, advanced researches are required to investigate optimized operation conditions for HP treatments in order to achieve positive results.

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