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Microflora of Pressurized Edam Cheese

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Abstract: Pressurization is a modern method of food processing and preservation. This paper discusses the effect of high pressure (200 and 400 MPa) on the microflora of ripening cheese. Cheese with a different ripening degree was subjected to a microbiological analysis which involved determination of the total bacteria count as well as the numbers of lactic streptococci, coli group bacteria, *Clostridium, Listeria* and *Salmonella*. After pressurization at 400 MPa, the number of lactic streptococci and total bacteria count decreased by 2-4 orders of magnitude. The high pressure did not result in the inactivation of technologically-undesirable bacteria.

Key words: High pressure technology, Edam cheese, microflora, ripening

Introduction

The high pressure technology is a modern method of food processing. It can be applied either as an alternative, non-thermal method of preservation of raw materials and food products, or as a means of modifying their organoleptic and technological properties. The term "HP technology" describes the application of pressures ranging from 100 to 1000 MPa at room temperature (Kolakowski *et al*., 1994; Trujillo *et al*., 2002), in industrial practice from 400 to 700 MPa (San Martin *et al*., 2002). This method can be modified, in order to inactive bacteria, through e.g. the application of high pressures at elevated temperatures or the addition of substances that lower a bacteria's resistance, e.g. lysozyme or nisin (Masschalck *et al*., 2000).

Food products subjected to high pressure treatment (e.g. jams, juices, sausages, ham, rice, cakes and desserts) emerged in the 1990s initially in Japan and then, successively, in the Member States of European Union and the United States (Kolakowski *et al*., 1994; Trujillo *et al*., 2002).

In the dairy industry, the high pressure technology may be applied for the preservation of milk and fermented drinks as well as for cheesemaking (Jankowska *et al*., 2005; Jankowska *et al*., 2003; Kolakowski *et al*., 1998; Trujillo *et al*., 2002). Pressurization of milk to be used for cheese making not only lowers the count of milk microflora (Buffa *et al*., 2001), but also affects the coagulation process. The selection of process conditions enables shortening the time of rennet coagulation and increasing the cheese yield (Huppertz *et al*., 2004; Lopez-Fandino *et al*., 1996). Cheeses produced from pressurized milk have been demonstrated to possess better organoleptic traits compared to those made of pasteurized milk (Buffa *et al*., 2001; Reps *et al*., 1998).

Pressurization accelerates the process of ripening, presumably due to the release of hydrolytic enzymes from bacterial cells inactivated upon pressure treatment (Kolakowski *et al*., 1998; O'Reilly *et al*., 2003; Saldo *et al*., 2000). It has also a considerable impact on the sensory and physicochemical properties of cheese (Messens *et al*., 1999; Messens *et al*., 2000; Saldo *et al*., 2000).

Materials and Methods

The object of the study was Edam cheese. Cheese samples, 6.5/5/6.5 cm in size, were cut out of cheese blocks after brining and paraffined. Prior to pressurization, the samples were packed under vacuum in additional barrier casings. Cheeses were pressurized for 30 min at 200 and 400 MPa, at room temperature, immediately after brining and after four, six and eight weeks of ripening. The non-pressurized cheese (control) and the pressurized cheese were subjected to microbiological analyses immediately after the pressurization and during ripening.

The cheeses were determined for:

- the presence of *Salmonella* and *Listeria* bacteria, with the use of TECRA UNIQUE *Salmonella* and TECRA UNIQUE *Listeria* tests;
- the total count of bacteria on plate count skim milk agar (Merck);
- the number of lactic fermentation streptococci on M17 agar according to Terzaghi (Merck);
- the number of coli group bacteria on the Chromocult coliform agar (Merck);
- the most probable number (MPN) of anaerobic proteolytic bacilli of the genus *Clostridium* on meat liver agar (Merck);
- the most probable number of anaerobic saccharolytic bacilli of the genus *Clostridium* on Bryant Burkey broth with resazurin and lactate (Merck) and with Durham tubes.

Microbiological analyses were carried out in triplicate.

Table 1: Total bacteria count in pressurized Edam cheese

Time of cheese ripening [weeks]	Control cheese	Period of cheese ripening before pressurization [weeks]							
		after brining		4		6		8	
		Pressure applied [MPa]							
		200	400	200	400	200	400	200	400
		[cfu/g]							
after brining	$2.3x10^9$	$2.6x10^9$	$1.3x10^6$	-	-	-	-	-	-
4	$4.2x10^8$	$4.0x10^7$	$1.4x10^7$	$1.4x10^{10}$	$4.2x10^6$	-	-	-	-
6	$9.2x10^9$	$8.8x10^7$	$1.2x10^6$	$5.1x10^8$	$7.5x10^5$	$2.5x10^9$	$9.0x10^5$	-	-
8	$1.8x10^9$	$5.0x10^8$	$1.6x10^6$	$4.0x10^8$	$1.8x10^6$	$4.6x10^8$	$1.4x10^7$	$9.8x10^8$	$1.5x10^5$

- not determined

Table 2: The number of lactic fermentation streptococci in pressurized Edam cheese

Time of cheese ripening [weeks]	Control cheese	Period of cheese ripening before pressurization [weeks]							
		after brining		4		6		8	
		Pressure applied [MPa]							
		200	400	200	400	200	400	200	400
		[cfu/g]							
after brining	$3.5x10^9$	$1.7x10^9$	$1.4x10^5$	-	-	-	-	-	-
4	$7.3x10^8$	$7.3x10^8$	$2.8x10^6$	$2.2x10^9$	$3.5x10^6$	-	-	-	-
6	$9.0 x10^8$	$7.6x10^8$	$4.9x10^6$	$8.4x10^8$	$1.8x10^6$	$1.1x10^9$	$4.1x10^6$	-	-
8	$8.5x10^8$	$7.7x10^8$	$1.0x10^7$	$8.6x10^8$	$6.2x10^6$	$7.7x10^8$	$3.2x10^6$	$8.9x10^8$	$1.7x10^6$

- not determined

Results and Discussion

Total bacteria count: In the control Edam cheese, the total bacterial count ranged from 10^8 to 10^{10} cfu/g (Table 1). The number and morphology of colonies obtained during determinations indicate that the main microflora of the cheese examined were lactic streptococci.

During analyses, it was noted that the pressurization of Edam cheese at 200 MPa immediately after brining did not affect the total bacteria count. Yet, during its ripening, the number of microorganism was observed to decrease distinctly, i.e. after four weeks of ripening the decreased by 90% and after the subsequent two weeks by 99%, as compared to the control cheese. The pressure treatment of cheese after four weeks of ripening caused an increase in the number of bacteria, whereas pressurization after six and eight weeks of ripening decreased the number of microorganisms in cheese by 73 and 45%, respectively. It should be noted that in eight-week cheeses pressurized at 200 MPa, the count of microflora was lower than that reported in control cheeses.

These results are confirmed by the findings of Kolakowski *et al.* (1998) who demonstrated the possibility of reducing the total bacteria count in Gouda cheese by 24% after threefold, pulsatory pressurization at 200 MPa for 5 min.

The pressurization at 400 MPa resulted in a tangible decrease in the total bacteria count ranging from 99.00 to 99.99% depending on the degree of ripening of the pressurized cheese. Nevertheless, the pressurization after brining and that after six weeks of ripening produced an increase in the total bacteria count during subsequent ripening. The greatest reduction of microflora was observed in cheese pressurized after eight weeks.

It was confirmed that the degree of ripening of cheese subjected to a pressure treatment affected its yield. Kolakowski *et al.* (1996) demonstrated that a threefold 5-min pressurization of two- and six-week Gouda cheese at 200 MPa reduced the number of microflora by 43 and 40.5%, respectively, whereas that carried out at 400 MPa – by 95.2 and 92.7%, respectively.

Lactic streptococci: The number of lactic streptococci in the control cheese reached 3.5×10^9 cfu/g after brining, whereas during an eight-week ripening period it fluctuated between 7.3 10^8 to 9.0 10^8 cfu/g. It should be emphasized that lactic streptococci are relatively resistant to the pressure of 200 MPa, since 20-min pressurization at 200 MPa caused a reduction in their number that depended on strain susceptibility, yet not greater than one order of magnitude (O'Reilly *et al.*, 2002).

The pressure treatment of cheese at 200 MPa immediately after brining reduced the number of streptococci by 52% (Table 2). In the other cheeses, the

Table 3: The presence of coli group bacteria in pressurized Edam cheese *

Time of cheese ripening [weeks]	Control cheese	Period of cheese ripening before pressurization [weeks]							
		after brining		4		6		8	
		Pressure applied [MPa]							
		200	400	200	400	200	400	200	400
		[% of samples]							
after brining	67	100	0	-	-	-	-	-	-
4	67	67	33	0	33	-	-	-	-
6	0	67	0	0	0	0	33	-	-
8	67	33	33	33	0	100	100	67	100

* in 0.01 g of cheese. - not determined

Table 4: The number of anaerobic saccharolytic bacilli of the genus *Clostridium* in pressurized Edam cheese

Time of cheese ripening [weeks]	Control cheese	Period of cheese ripening before pressurization [weeks]							
		after brining		4		6		8	
		Pressure applied [MPa]							
		200	400	200	400	200	400	200	400
		[MPN]							
after brining	14	32	2.1	-	-	-	-	-	-
4	25	1.9	n	5.6	5.4	-	-	-	-
6	3.6	2.1	7.5	2.1	2.1	5.6	2.1	-	-
8	18	51	45	15	$1.1x10^2$	$1.2x10^2$	$2.5x10^2$	$6.3x10^2$	$5.7x10^2$

n: not present in 0.1g. - not determined

Table 5: The number of anaerobic proteolytic bacilli of the genus *Clostridium* in pressurized Edam cheese

Time of cheese ripening [weeks]	Control cheese	Period of cheese ripening before pressurization [weeks]							
		after brining		4		6		8	
		Pressure applied [MPa]							
		200	400	200	400	200	400	200	400
		[MPN]							
after brining	8.2	4.3	n	-	-	-	-	-	-
4	2.0	$1.2x10^{-2}$	n	n	$8.7x10^{-2}$	-	-	-	-
6	3.8	5.0	1.1	2.4	1.2	5.0	0.75	-	-
8	0.26	0.1	2.2	2.7	1.7	n	$4.0x10^{-2}$	$4.0x10^{-2}$	$2.4x10^{-2}$

n: not present in 0.1g. - not determined.

pressure of 200 MPa was found to exert no distinct effect on the counts of lactic streptococci.

Although the pressure of 400 MPa has a considerable impact on the survivability of lactic streptococci strains, the degree of bacteria inactivation may be strain-dependent (Wick *et al.*, 2004).

After the pressure treatment at 400 MPa, the number of lactic streptococci decreased in the cheese by 99.5 to 99.99%, depending on its degree of ripening.

In the case of cheese pressurized after brining, the population number of streptococci was observed to increase during ripening. After eight weeks of ripening, it was noted that the extent of streptococci reduction became more distinct along with extended period of cheese ripening before pressurization, as compared to the control cheese.

Pathogenic and technologically-undesirable bacteria: The presence of pathogenic bacteria in milk and cheeses (De Buyser *et al.*, 2001) is likely to pose a risk to consumer health. Literature data indicate the possibility of a substantial reduction in the number of *L. monocytogenes* in cheese as a result of pressurization. Szczawiñski *et al.* (1997) demonstrated that the pressure treatment of cheese at 500 MPa for 15 min decreased the number of bacteria by six orders of magnitude. There are no data on the effect of pressure on *Salmonella* bacteria in cheese, however,

pressurization of milk at 350 MPa/10 min has been reported to cause their inactivation (Bozoglu *et al.*, 2004). In the study reported, bacteria of the genera *Salmonella* and *Listeria* were not detected in cheese after brining. Therefore, they were not determined in further stages of the experiment.

The growth of undesirable microflora may lead to numerous defects in cheese, the most common of which is the so-called "early blowing of cheeses". This defect is due to the growth of coli group bacteria (Wuytack *et al.*, 2002). In contrast, the so-called "late blowing of cheese" is probably caused by the growth of saccharolytic bacilli of the genus *Clostridium* (Su and Ingham, 2000).

The Gram-negative bacteria, including the coli group bacteria, are more susceptible to the effect of high pressure. Threefold 5-min pressurization of Gouda cheese at 200 MPa reduced the number of coli group bacteria by 90%, and that carried out at 400 MPa caused their complete inactivation (Kolakowski *et al.*, 1996). In the case of *Escherichia coli,* the susceptibility to high pressure may be a strain-specific trait, and individual strains are likely to demonstrate a considerable diversification in this respect (Linton *et al.*, 2001).

In the cheeses examined, it was impossible to determine the number of coli group bacteria. Their presence could only be detected in 0.01 g of cheese.

During analyses, in the control cheese the presence of coli group bacteria in 0.01 g of cheese was detected in 50% of the samples, on average, whereas in the cheeses pressurized at 200 and 400 MPa it was detected in 47 and 33% of the samples, respectively.

The spore-forming bacteria are likely to occur in pressurized products due to the considerable resistance of resting spores to high pressures. Hence, the application of combined methods is suggested, e.g. pressures over 600 MPa together with high temperatures (Jankowska, 2001). Simultaneous application of pressurization and nisin addition is also possible - the high pressure produces a change in the permeability of cellular membranes, which increases the efficiency of the activity of that bacteriocin (Buffa *et al.*, 2001).

The cheese examined contained a negligible (no more than 10 cfu/g) amount of proteolytic bacilli of the genus *Clostridium* (Table 5). Subjecting cheese to a pressure treatment at 200 MPa and 400 MPa has no impact on the number of these bacilli (fluctuations in the number of bacilli are within the standard deviation of the mean); only cheeses pressurized at 400 MPa and determined in the fourth and eighth week of ripening contained a smaller population of proteolytic bacilli compared to the control cheeses.

The control cheese, with a different degree of ripening, contained slightly more saccharolytic bacilli of the genus *Clostridium* (max. 25 cfu/g), (Table 4). Due to the low number of gas-producing bacteria it is difficult to characterize the effect of pressure on this group of bacteria. The high pressure positively affects the growth of anaerobic saccharolytic bacilli during the ripening of pressurized cheese. This results from the sporulation of the resting spores after pressurization.

The results obtained point to the need for further investigations into the effect of high pressures on cheese microflora.

Conclusions: Pressurization at 200 MPa for 30 min had no significant effect on either the total bacteria count or the number of lactic streptococci. After a pressure treatment at 400 MPa the number of those bacteria decreased by ca. 2-4 orders of magnitude. In the cheeses pressurized at 400 MPa, irrespective of the degree of their ripening, the total bacteria count and lactic streptococci after eight weeks of ripening was lower by 2-3 orders of magnitude than that in the control cheeses.

Pressures of 200 and 400 MPa did not result in the complete inactivation of technologically-undesirable microflora in cheeses, i.e. coli group bacteria and spore-forming bacteria.

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