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HIGH PRESSURE TREATMENT OF SWISS CHEESE SLURRIES (I): INACTIVATION OF SELECTED MICROORGANISMS AFTER TREATMENT AND DURING ACCELERATED RIPENING

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Abstract: Cheese slurries, made from fresh Swiss cheese curd, were treated at 345 or 550 MPa for 10 or 30 minutes in an isostatic press at 25 ℃. The slurries were ripened at 30 ℃ for 0, 3 and 5 days. The growths of coliforms, yeasts and molds, starter bacteria (*Lactococci* and *Streptococci*), non-starter lactic acid bacteria (*Lactobacilli*), and presumptive coagulase-positive *Staphylococcus* were determined. An electronic nose was used to evaluate aroma development of the cheese slurries. Commercial Swiss cheeses with different ages were used as aroma references. The degree of inactivation of organisms was found to be a function of pressure intensity, exposure time, type of organism, and cheese slurry pH. In general, slurries treated at a higher pressure and with a longer exposure time showed a greater reduction in numbers and had less out-growth of organisms during ripening. *Coliforms*, yeasts and molds were completely inactivated at the pressures and time used. Starter bacteria appeared to be more resistant to being inactivated by high pressure treatment and had a greater out-growth rate than *Lactobacilli* and *Staphylococci*. Based on canonical analysis, nineteen samples for each batch were assigned to three groups. In general, higher intensity of pressure or longer exposure time caused less aroma development in the cheese slurries. When the cheese curd was incubated overnight prior to making the cheese slurries, stronger slurries with stronger aroma were observed. This study provided an explanation of the relative importance of relationships among high pressure treatment, starter bacteria, and aroma development during accelerated ripening.

Key words: high pressure, microorganisms, cheese slurry, aroma, electronic nose
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INTRODUCTION

Due to significant economic advantages, the acceleration of cheese ripening has been the goal of many researchers. Kristoffersen et al. (1967) reported on accelerated flavor production in a fresh curd cheese slurry. Intense, sharp and balanced cheese flavors developed within five days. They suggested that such slurries would have advantages over normally ripened mature cheese, including positive flavor control, lower cost and greater flexibility of supply. Shanley and Sutherland (1975) proposed that the most likely commercial application of the slurry technique would be the production of differently flavored slurries, which could be blended to produce any desired cheese flavor. Abdel Baky et al. (1982) found that the ripening period could be reduced from 4 months to 2 months by adding cheese slurry to cheese milk or to the cheese curd. Sutherland (1975) also demonstrated the feasibility of replacing the matured cheese component of processed cheese with blended curd slurries. The product is used in processed cheese formulations, snacks, crackers and imitation dairy products. However, the elevated temperature used for ripening the cheese slurry would be expected to accelerate the growth of undesirable flora. A wide range of microorganisms, including non-starter lactic acid bacteria (NSLAB), *Staphylococcus*, coliforms, yeasts and molds, had been reported to grow in cheese slurries (Dulley, 1976; Soda, 1993). Attempts to control the growth of undesirable microorganisms in cheese slurries by the addition of potassium sorbate or by heat treatment of the slurry resulted in a lack of flavor development (Grandhi et al., 1973).

Recently developed high-pressure technology is a non-thermal food processing methodology whereby foods are subjected to high hydrostatic pressure at or around room temperature. The treatment of foods by high pressure has been shown to reduce significantly the original microbial population, leading to a longer shelf-life, while the products retain natural taste and flavor (Vardag et al., 1995; Mertens, 1994). Pandya et al., (1995), and Tauscher (1995) reported on the use of high pressure to treat sauces, meat, yoghurt and fruit juices. Some researchers focused on the effect of high pressure on the microbial population or on a possible modification of enzyme activity (Hsin et al., 1995; Nakamura et al., 1994; Homma et al., 1994). Pressure inactivation of yeasts and molds was reported in citrus juices (Ogawa et al., 1990). Juices pressurized at 400 MPa for 10 min and at 40 ℃ did not spoil during 2 – 3 months of storage. A population of 106 cfu/ml of *Listeria monocytogenes* was inactivated by exposure to 340 MPa at 23 ℃ in UHT milk and the same population of *Vibrio parahaemolyticus* was killed by exposure to 170 MPa within 10 min at 23 ℃ in clam juice (Styles et al., 1991).

The primary objective of this work was to investigate the effect of high-pressure treatment on the inactivation rate of selected microorganisms in standard Swiss cheese slurries.

MATERIALS AND METHODS

1. Cheese slurry preparation

Fresh curd was manufactured by a conventional Baby Swiss cheese procedure (Sohn, 1996). The curd was removed from the fat immediately after they drained and froze. Commercial starter cultures were used for acid production and flavor development. These organisms included *Lactococcus*, *Streptococcus*, and *Lactobacillus*. Two batches of Baby Swiss cheese curd were used in this study. Batch 1 was made at pH 6.3 in our pilot plant without additional incubation. Batch 2 was obtained fresh from the Brewster Dairy Inc., Brewster, OH, and was incubated at 22 ℃ overnight to decrease the pH to 5.3 before making slurries. Cheese slurries were prepared by mixing standard curd with a 10% by weight sterile solution of sodium chloride to give a final salt in moisture (salt concentration in the water phase of the cheese, S/M) of around 5%. The curd and brine were mixed in a stomached mixer for 3 min, then poured into plastic bags (ca. 50g each), and vacuum-packaged.

2. High pressure treatment

Cheese slurries vacuum packaged in plastic bags were stored and shipped overnight in wet ice to Oregon State University. All samples were warmed to ambient temperature (25 ℃). They were pressure-treated at 345 MPa or 550 MPa for 10 minutes or 30 minutes, respectively in an Engineered Pressure Systems (Erie, PA) 100 mm diameter by 250 mm long isostatic pressure unit, within 24 – 48 hours of vacuum packaging and refrigerating.

3. Microbiological analysis

The cheese slurries were ripened at 30 ℃ and were analyzed at 0, 3 and 5 days for the presence of several types of microorganisms. Samples were assayed for colony forming units (cfu) by standard pour-plate procedures with serial dilutions in 0.1% peptone water, except for *Staphylococcus*, which was obtained by surface plating. The cfu were counted on two petri plates having 30 – 300 colonies with a Quebec colony counter. M17 agar enriched with lactose was used to count *Lactococcus* and *Streptococcus*. Selective *Lactobacillus* agar in anaerobic incubation was used to count the starter *Lactobacillus* and adventitious non-starter *Lactobacillus*. No attempt was made to differentiate the injured from uninjured cells in this study.

4. Chemical analysis

The moisture and salt contents of the slurries were analyzed according to Standard Methods for the Examination of Dairy Products (Richardson, 1985). The pH of curds and slurries was determined with a Fisher Accument pH Meter (Model 25).

5. Statistical analysis

Data were analyzed using analysis of variance by the general linear models procedure (GLM) of Statistical Analysis System (SAS, 1985). Least Square Means was employed to separate the means when analysis of variance indicated a significant ($P < 0.05$) effect. For statistical analysis purposes, the microbial cell numbers

less than 10 or log 1 cfu were treated as 10 or log 1 cfu.

RESULTS AND DISCUSSION

There were less than 10 coliforms in control and treated samples in the pH 6.3 slurry under all conditions and times of incubation. For the pH 5.3 slurry , the control had 720 cfu of coliforms at 0 day and the cfu decreased to 60 after 5 days; all treated slurries had less than 10 cfu at 0 day and no out-growth was noted after 3 or 5 days.

Similar results were observed for yeasts and molds. Except for the control at pH 6.3, which had low levels of molds and yeasts (13, 15 and 30 molds at 0, 3 and 5 days, respectively), all other samples had less than 10 cfu at 0, 3 or 5 days.

Table 1 shows the inactivation of starter bacteria, *Lactobacilli*, and *Staphylococci* after high-pressure treatments and before incubation (0 day). All the high-pressure treatments significantly inactivated the three organisms ($P < 0.05$). Among the four treatments, the effects of inactivation of organisms were dependent on intensity of pressure, time of exposure, type of organisms, and pH of the slurries. Increasing the pressure resulted in higher cell mortality. The effect of pressure was very significant for all three groups of organisms ($P < 0.001$). In general, increasing time of exposure lowered counts. The differences between 10 min and 30 min treatments were significant for *Lactobacilli* ($P < 0.05$) and *Staphylococci* ($P < 0.001$), but not for Starter culture numbers ($P > 0.05$). Among those three groups of organisms, *Lactobacilli* were the most sensitive to high-pressure treatments, next were *Staphylococci* in pH 6.3 with respect to the reduction of cfu/g. However, in pH 5.3's samples, *Staphylococci* were completely inactivated by any high-pressure treatment. Also, at higher pressure, the cells of starter cultures and Lactobacilli were reduced more in Batch 2 than in Batch 1. Ronner (1994) found that higher pressure was needed to inactivate gram-positive bacteria than gram-negative bacteria. Yeasts and molds are more sensitive to high pressure than Gram-negative bacteria. They indicated that a treatment of 200 MPa (2 kBar) for 10 min sufficed to inactivate 1 to 3 log cycles of yeasts and molds. Their results agree with our observations.

Table 1 Inactivation of microorganisms by high pressure treatment (log cfu/g)[1,2]

Treatment	Organism					
	Starter		Lactobacilli		Staphylococci	
	Batch 1 (pH 6.3)	Batch 2 (pH 5.3)	Batch 1 (pH 6.3)	Batch 2 (pH 5.3)	Batch 1 (pH 6.3)	Batch 2 (pH 5.3)
Control	7.7	8.8	4.0	5.1	2.3	3.3
345 MPa 10 min	7.0	7.7	3.1	3.9	1.3	1.0
345 MPa 30 min	6.9	7.5	2.8	3.8	1.0	1.0
550 MPa 10 min	6.0	2.0	1.0	1.8	1.5	1.0
550 MPa 30 min	6.0	2.0	1.0	1.0	1.0	1.0

[1] Means with the same letter within a column are not significantly different ($P > 0.05$)

[2] Values shown as 1.0 indicate less than 10 cfu/g and indicate complete inactivation when measured directly after treatment

During 3 and 5 days incubation (ripening), all three groups of organisms grew to some extent (Table 2,3 and 4). The extent of outgrowth was related to intensity of pressure, exposure time, type of organism, and pH of the slurries. Lower outgrowth was found in slurries treated at higher pressure and longer exposure time. Growth of *Staphylococci* did not occur in the slurries treated with 550 MPa for 10 or 30 min. It appeared that higher pressures were needed to completely inhibit the growth of presumptive coagulase-positive *Staphylococci*. High-pressure treatment reduced outgrowth more in Batch 2 than Batch 1. The only exception was for the treatment with 550 MPa for 10 min in which the starter cultures in Batch 2 had higher outgrowth. The similar growth of organisms after high-pressure treatments (recovery) was also observed by Metrick

et al. (1989) in strained chicken. They found that recovery of *Salmonella* occurred in chicken, but did not occur in buffer because of its lack of available nutrients. Our data indicated that cheese slurry might be a good medium for the recovery of microorganisms.

Table 2 Growth of starter bacteria during incubation at 30 ℃(log cfu/g)[1]

pH of Slurry	Treatment	Incubation time (day)		
		0	3	5
Batch 1	Control	7.7	8.7	8.9
(pH 6.3)	345 MPa 10 min	7.0	9.1	9.1
	345 MPa 30 min	6.9	8.2	9.1
	550 MPa 10 min	6.0	8.5	9.0
	550 MPa 30 min	6.0	7.5	8.9
Batch 2	Control	8.8	8.6	7.8
(pH 5.3)	345 MPa 10 min	7.7	8.7	7.9
	345 MPa 30 min	7.5	7.9	7.4
	550 MPa 10 min	2.0	3.8	5.5
	550 MPa 30 min	2.0	2.0	2.2

[1]Means with the same letter within a column are not significantly different ($P > 0.05$)

Table 3 Growth of *lactobacilli* during incubation at 30 ℃(log cfu/g)[1,2]

pH of Slurry	Treatment	Incubation time (day)		
		0	3	5
Batch 1	Control	4.0	8.6	8.8
(pH 6.3)	345 MPa 10 min	3.1	8.7	9.1
	345 MPa 30 min	2.8	7.4	8.9
	550 MPa 10 min	1.0	7.1	7.9
	550 MPa 30 min	1.0	1.0	7.7
Batch 2	Control	5.1	8.7	8.7
(pH 5.3)	345 MPa 10 min	3.9	8.3	8.7
	345 MPa 30 min	3.8	7.3	8.2
550 MPa 10 min	1.8	3.0	4.0	
550 MPa 30 min	1.0	1.0	1.0	

[1]Means with the same letter within a column are not significantly different ($P > 0.05$)

[2]Values shown as 1.0 indicate less than 10 cfu/g and indicate complete inactivation when measured directly after treatment

Table 4 Growth of *Staphylococci* during incubation at 30 ℃(log cfu/g)[1,2]

pH of Slurry	Treatment	Incubation time (day)		
		0	3	5
Batch 1	Control	2.3	6.4	6.6
(pH 6.3)	345 MPa 10 min	1.3	5.3	4.6
	345 MPa 30 min	1.0	5.5	5.2
	550 MPa 10 min	1.5	2.5	1.0
	550 MPa 30 min	1.0	1.0	1.0
Batch 2	Control	3.3	3.5	3.6
(pH 5.3)	345 MPa 10 min	1.0	2.3	2.6
	345 MPa 30 min	1.0	1.3	1.0
	550 MPa 10 min	1.0	1.0	1.0
	550 MPa 30 min	1.0	1.0	1.0

[1]Means with the same letter within a column is not significantly different ($P > 0.05$)

[2]Values shown as 1.0 indicate less than 10 cfu/g and indicate complete inactivation when measured directly after treatment

The difference in pressure sensitivity of the organisms in Batch 1 and Batch 2 may be explained by the difference in pH. pH is one of the factors affecting the inactivation rate in high-pressure treatment (Ronner, 1994). Pandya (1995) also stated that lowering pH enhanced the effectiveness of pressure inactivation and injury of yeast. The reaction occurring under high pressure involves mainly hydrogen bonds, electrostatic links and hydrophobic interactions. At lower pH or higher hydrogen ion concentration, the reaction may be promoted. Also, the lower pH environment may not favor the recovery or repair of stressed cells.

CONCLUSIONS

The evidence provided in this study suggests that high pressure has the potential to inactivate undesirable organisms (*Coliforms*, *Staphylococcus*, *Lactobacillus*, yeasts and molds) in cheese slurries. The inactivation rate of selected microbes and aroma development of cheese slurries treated by high pressure is mainly dependent on the intensity of pressure, exposure time, and condition of cheese curds. Use of hydrostatic pressure in the acceleration of cheese slurry seems feasible. Additional work is needed to find optimal conditions for maximal aroma development with minimal undesirable microbial growth in cheese slurries.

References

Abdel Baky, A. A., El Fak, A. M., Rabie, A. M. et al., 1982. Cheese slurry in the acceleration of Cephalotyre "Ras" cheese ripening. *J. Food Prot*. **45** (10): 894.

Dulley, N. R., 1976. The utilization of cheese slurries to accelerate the ripening of Cheddar cheese. *The Australian Journal of Diary Technology*. **31**:143.

Homma, N., Ikeuchi, Y., Suzuki, A., 1994. Effects of high-pressure treatment on the proteolytic enzymes in meat. *Meat Science*. **38**(2): 219.

Hsin, T. L., Gow, C. Y., 1995. Effect of high hydrostatic pressure on the inactivation of enzymes and sterilization of guava juice. *Journal of the Chinese Agricultural Chemical Society*. **33**(1): 18.

Kristoffersen, T., Mikolajcik, E. M., Grould, I. A., 1967. Cheddar cheese flavor. IV. Directed and accelerated ripening process. *Journal of Dairy Science*. **50** (3): 29.

Mertens, B., 1994. Hydrostatic pressure treatment of food: equipment and processing. *In*: Food Preservation by Combined Processes., L. Leistner and L. G. M. Gorris, ed. EUR 15776 En, p.135

Metrick, C., Hoover, D. Farkas, D., 1989. Effects of high hydrostatic pressure on heat-resistant and heat-sensitive strains of Salmonella. *J. of Food Science*. **54** (6): 1547.

Nakamura, K., Enomoto, A., Fukushima, H. et al., 1994. Disruption of microbial cells by the flash discharge of high-pressure carbon dioxide. *Bioscience, Biotechnology, and Biochemistry*. **58**(7): 1297.

Ogawa, H., Fukuhisa, K., Kubo, Y. et al., 1990. Pressure inactivation of yeast, molds, and pectinesterase in Satsuma mandarin juice: effects of juice concentration, pH, organic acid, and comparison with heat sanitation. *Agri. biol. Chem*. **54**: 1219.

Pandya, Y., Jewett, F. F., Hoover., Jr., D. G., 1995. Concurrent effect of high hydrostatic pressure, acidity and heat on the destruction and injury of yeasts. *Journal of Food Protection*. **58**(3): 301.

Richardson, G. H., ed., 1985. Standard Methods for the Examination of Dairy Products. 15th ed. Am. Publ. Health Associ., Inc., Washington, DC.

Ronner, Ulf., 1994. Food preservation by ultrahigh pressure. *In*: Food Preservation by Combined Processes. L. Leistner and L. G. M. Gorris, ed. EUR 15776 En, p.31

SAS, 1985. Statistical Analysis System. SAS Institute Inc., Cary, NC.

Shanley, R. M., Sutherland, B. J., 1975. CSIRS Dairy Res. Rep. No.13.

Soda, M.E., 1993. Accelerated maturation of cheese. *Int. Dairy Journal*. **3**:531.

Sohn, S., 1996. Texture and microstructure of low-fat Swiss cheese. Ph.D. Diss., Ohio State Univ. Columbus.

Style, M. F., Hoover, Jr. D. G., Farkas, D. F., 1991. Response of Listeria monocytogenes and Vibrio parahaemolyticus to high hydrostatic pressure. *J. Food Science*. **56**:1404.

Sutherland, B.J., 1975. Rapidly ripened cheese curd slurries in processed cheese manufacture. *The Australian Journal of Dairy Technology*. **30**(3): 138.

Tauscher, B., 1995. Pasteurization of food by hydrostatic high pressure: chemical aspects. Zeitschrift-fuer-Lebensmittel-Untersuchung-und-Forschung. **200**(1): 3.

Vardag, T. and Koerner, P., 1995. High pressure: a real alternative in food processing. *Food Marketing and Technology*. **9**(1): 42.