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Proteolysis in cheddar-type cheese made from PEF (pulsed electric field) treated milk

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Abstract

Raw milk cheeses have been found to possess unique flavor and texture characteristics not obtainable in cheeses from pasteurized milk. However, cheeses made from pasteurized milk are widespread, primarily for public health reasons. Pulsed electric field (PEF) as a non-thermal pasteurization method has shown ability to keep the flavor and natural characteristics of food samples intact, thus providing advantage over conventional heat processing. In this study PEF treatment was performed in a continuous treatment chamber, consisting of two parallel stainless steel electrodes separated by a 5 mm thick insulator. A 30 kV/cm pulse generator was used to deliver bi-polar wave electric field to milk sample. Pulse width was 2 µs, pulse frequency was 2 Hz and up to 120 pulses were applied. Cheddar-type cheese curds were made from raw milk, pasteurized milk and PEF-treated milk and their proteolysis processes were compared using curd slurry under 30°C. The profiles of water-soluble peptides were measured using an RP-HPLC system. Results indicated that PEF treated milk has similar proteolysis profiles with raw milk in terms of peptides composition. Thus it showed the potential of making high quality and safe cheeses by PEF treatment without sacrificing the natural characteristics of the cheeses.

Proteolysis in cheddar-type cheese made from PEF (pulsed electric field) treated milk

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1. Introduction

Pasteurization of milk for cheese making became widespread after about 1940, primarily for public health reasons (Fox et al. 2000). However raw milk cheeses are known to possess unique flavor and texture characteristics not obtainable in cheeses from pasteurized milk. This may be due to the denaturation of indigenous enzymes, partial denaturation of whey proteins and their interaction with casein, and the destruction of some desirable non-starter lactic acid bacteria present in raw milk (McSweeney et al. 1993).

PEF involves the application of high voltage pulses at relatively low temperature to a food placed between two electrodes for very short time (normally less than 1 second). A great number of researches (Donn 1987; Qin et al. 1995 and Martin et al. 1997) have demonstrated the possibility of pasteurizing milk without sacrificing its quality. Dunn (1996) reported that milk treated with PEF suffered less flavor degradation. The author proposed the possibility of manufacturing dairy products such as cheese, butter and ice cream using PEF treated milk. No detailed information was given in the report. Sepulveda-Ahumada et al. (2000) evaluated the quality of cheese produced from PEF treated milk in terms of sensory and texture evaluation, and compared with the cheese made from heat pasteurized milk. They claimed that using milk pasteurized by PEF to obtain cheese appeared to be a feasible option to improve the product quality.

Knowledge of PEF effects on major cheese making steps, such as ripening is extremely crucial. However, scarce works have been reported so far on this aspect. Cheese ripening involves a complex series of biochemical events, which lead to the

characteristic taste, aroma and texture of each cheese variety. Major biochemical changes occurring in cheese ripening include proteolysis, glycolysis and lipolysis. Proteolysis is considered the most important issue in terms of flavor and texture development. It contributes to textural changes of the cheese matrix due to breakdown of the protein network. It also contributes to flavor and bitterness of cheese mainly through the formation of peptides and free amino acids (Sousa et al. 2001). Many cheese ripening studies focused on the proteolysis process (Farkye et al. 1995; O'Shea et al. 1996; Bütikofer et al. 1998; Albenzio et al. 2001; Benfeldt et al. 2001; Verdini et al. 2003).

Cheesemaking experiments, even on a pilot scale, are expensive and time-consuming. Kristoffersen et al. (1966) developed a rapid method for producing cheese flavor. In this method, cheese curd slurries (semisolid paste) were prepared and incubated at 30°C for five days, which could yield flavors similar to that of cheeses ripened for 3 months. Therefore, cheese curd slurries can be used as a quick tool to evaluate the contribution of different components into the cheese (Farkye et al. 1995). Briggs (2003) successfully used cheese slurries and studied the cheese ripening process accelerated by PEF treated lactic acid bacteria.

The objectives of this study were to evaluate the proteolysis process of cheddar-type cheese curd slurries made from PEF treated milk and compared the results with that from raw milk and heat pasteurized milk.

2. Materials and methods

2.1 Raw milk and heat pasteurized milk

Raw milk (3.14 ± 0.02 % (w/w) protein and 3.81 ± 0.06% (w/w) fat) was picked up at the dairy farm of Macdonald campus, McGill University (Ste. Anne de Bellevue, QC). Raw milk standard plate counts were < 5000 cfu/ml. Milk somatic counts were < 200,000 cells/ml.

Raw milk was filled in sterile plastic bottles and stored at 4^{o}C for less than 4 hrs prior to PEF or heat pasteurization. Heat pasteurization was carried out by batch heating raw milk at 63 ^{o}C for 30 minutes in water bath.

2.2 PEF treated milk

A 30 kV pulse generator with a matched output impedance of 100 Ω and a continuous treatment chamber system were used in the experiment. The output voltage profile was bi-polar instant reversal square waveform. The treatment chamber consists of two parallel stainless steel electrodes separated by a 5 mm thick polyoxymethylen Derlin® insulator, with 85 mm^2 of surface area. The voltage and current across the treatment chamber were captured simultaneously using a 2 channel digital oscilloscope (TDS3000, Tektronix, Wilsonville, OR).

The samples were treated following the procedure described by Yu and Ngadi (2005).

The parameters and selected levels were: electric field intensity of 30 kV/cm; outlet temperature of 50 ± 1°C; pulse number of 80 and 120 pulses. The pulse width was 2 μs and the frequency of pulses was set at 2 Hz.

2.3 Preparation of cheese curd slurries

The Cheddar-type cheese curd was made following the revised procedure of Farkye et al. (1995). The starter culture (2% v/v) and liquid rennet (0.02% wt/v) were added in sequence to 500 ml warm milk samples (30°C). The rennet-treated milk was then left to coagulate under still conditions. The coagulum was cut and cooked to 39°C for over 30 min and held at this temperature for 15 min. The whey was drained at pH 5.3 and the curd was kept for slurry preparation.

The slurry was prepared by blending a 50 g of cheese curd and sterile distilled water in 1:1 ratio in a sterile blender jar (Hamilton, Beach, model C54252, Mexico). The diluted slurry (100 g) was then transferred aseptically into sterile tubes, capped loosely and incubated at 30°C for 5 days.

2.4 Peptides analysis by RP-HPLC

Water Soluble Fraction (WSF) was extracted from slurries as described by Altemueller and Rosenberg (1996). A sample (30 g) of cheese slurry was homogenized with 150 ml of distilled water at maximum speed on a vortex at 25°C. The homogenate was centrifuged for 20 min at 10000 X g. The fat layer was discarded and the supernatant

was filtered through Whatman No. 1 filter paper. The pH in the supernatant was adjusted to 4.6 using 1M HCI. The WSF extracted at PH 4.6 was then filtered through 0.22 µm pore-size micro-filter for peptide index analysis by RP-HPLC.

The HPLC analysis was carried out by RP-HPLC using a Varian system (Varian Associates, CA, USA) equipped with a photodiode array detector (model 330), an auto-sampler (model 410) and a quaternary solvent delivery module (model 240). The system is connected to a personal computer and data was processed using Varian Star Workstation 5.5 software (Varian Associates, CA, USA). Separations were carried out using a 150 X 4.6 mm, C-18, 90^{o} pore size column (Varian Associates, CA, USA). A guard column (Supelguard LC-18-DB, 20 x 4.6 mm I.D) was used in all cases.

A binary multi-step elution gradient was used (Altemueller et al. 1996). The composition of solvent A was 0.1% trifluoroacetic acid (TFA) in water and that of solvent B was 0.1% TFA in acetonitrile: water (75:25, v/v). A stepwise gradient elution in the order: 0% B (100% A) for 5 min; 30% B over 40 min; 65% B over 15 min; 80% B over 10 min at a flow rate of 0.75 ml/min was used. The column was then rinsed and allowed to equilibrate for 25 minutes between injections. Aliquot volume of 100 µl was injected into a 100 µl HPLC loop. The eluate was monitored at 220 nm. All chemicals and solvents were HPLC grade. Acetonitrile and trifluoroacetic acid were purchased from Fisher Scientific (Nepean, ON). Milli-Q (Millipore, Bedford, MA) water was used throughout the experiment.

HPLC test for each WSF extract was performed in triplicate. The total integration area of peptides detected at 220nm during the HPLC run was determined. The UV absorption peaks observed for the HPLC runs were arbitrarily divided into two groups to allow a quantitative hydrophobic-hydrophilic index analysis (Lau et al. 1991). The first group consisted of the peaks with retention times from 5 to 50 minutes, which was assumed as the hydrophilic peptide portion. The second group of peptides with retention time from 50 to 70 minutes was the more hydrophobic peptide portion. The ratio of hydrophobic to hydrophilic peptides was obtained by dividing the total peak area of the hydrophobic peptide portion by that of the hydrophilic peptide portion.

3. Results and discussions

3.1 Concentration of peptides in WSF

McGugan et al. (1979) fractionated a mild and an aged Cheddar cheese into a WSF, a fat fraction, and a residue fraction. They identified the nonvolatile WSF as the major contributor of cheese flavor intensity. They indicated that the volatiles in the fat fraction might have some influence on flavor quality, but not on flavor intensity. Aston et al. (1986) confirmed that the nonvolatile WSF of cheese is an essential fraction for flavor intensity in mild and aged Cheddar cheese. HPLC has successfully used to analyze the nonvolatile WSF of cheese (Lau et al. 1991, McSweeney et al. 1993).

The present study used HPLC analysis to compare the peptide profile in the WSF extracted from Cheddar type cheese curd slurries made from raw milk (RM), pasteurized milk (PM) and PEF treated milks (120 pulses (PEF120) and 80 pulses (PEF80)).

Figure 1 shows the typical HPLC profiles of Cheddar cheese curd slurry. At 220 nm, the total area under the peaks on the HPLC profile represents the light absorbed by aromatic amino acids and peptide bonds present in the WSF of cheese (Lau et al. 1991). As cheese ages, more caseins and high molecular weight peptides are being broken down into smaller peptides that may be water soluble. Therefore, it was expected that as the cheese aged, the total water-soluble peptide content increased.

Figure 2 shows the total peak areas of HPLC profiles of Cheddar type cheese curd slurries made from RM, PM, PEF120 and PEF80.

As expected, with increased incubation time, the total peak areas increased. This implies that the total water-soluble peptide content increased. When comparing the results on the day 0 and the day 3, it can be found that RM samples had the largest peak areas, followed by the PEF80, PEF120 and PM samples. McSweeney et al. (1993) compared HPLC profiles of peptides in WSF of Cheddar cheeses made from raw and pasteurized milk and reported the differences between the two profiles. They deduced that the difference could be due to the non-starter Microflora present in the raw milk. Although PEF treatment could kill the non-starter Microflora, the indigenous enzymes could survive since they require more severe PEF treatment to obtain a significant reduction (Ho et al. 1997). These enzymes could function in cheese aging and may have

resulted in production of more water-soluble peptide. Peak areas of PEF80 were found more than that of PEF120, which was probably due to the less severity of PEF treatment.

Lau et al. (1991) used the same HPLC approach as McSweeney et al. (1993), and reported that the amounts of water-soluble peptides were the same for RM and PM Cheddar cheeses.

On the day 5, the trend was slightly different. RM still gave the largest peak area, while the peak areas of PM, PEF80 and PEF120 were not much different. It was interesting that for RM and PM, the peak area increased fast with the increase of the incubation time, while PEF treated milk increased relatively slow. This needs more investigation probably in terms of protein structure change during PEF treatment.

3.2 Analysis of hydrophilic and hydrophobic peptides

Figure 3 and 4 show the amounts of hydrophilic and hydrophobic peptides in WSF of Cheddar cheese curd slurries made from RM, PM, PEF80 and PEF120.

It was found that both hydrophilic and hydrophobic peptides increased with the increase of the incubation time in all the test samples. This was consistent with the findings by Lau et al. (1991), who reported that hydrophilic and hydrophobic peptides increased during the Cheddar cheese aging for both raw and pasteurized milk cheeses.

Champion and Stanley (1982) stated that extracts of bitter Cheddar cheese contained a high proportion of hydrophobic peptides. Lau et al. (1991) found a high proportion of hydrophobic peptides present in the WSF of Cheddar cheese made from pasteurized milk than in cheese made from raw milk. He believed that a proper balance among the various water-soluble components is important for typical Cheddar cheese flavor. He proposed that the difference in the ratio of hydrophobic to hydrophilic peptides present in the WSF in Cheddar cheeses made from pasteurized and raw milk may cause flavor differences.

Figure 5 shows the ratio of hydrophobic to hydrophilic peptides in RM, PM, PEF80 and PEF120 Cheddar cheese curd slurries.

It was found that PM gave the significant high ratio of hydrophilic to hydrophobic peptides during incubation. Compared with PM, PEF treated milk gave similar ratios as

RM, especially PEF80, which could imply that PEF treated milk could give similar flavor to Cheddar cheese as RM.

4. Conclusion

During the incubation of Cheddar cheese curd slurry, PEF treated milk gave more total peak areas in HPLC profiles of the water-soluble fraction than pasteurized milk at the beginning stage, and similar at the latter stage. Raw milk had even higher total peak areas. The analysis of hydrophobic and hydrophilic portions in HPLC profiles showed that PEF treated milk had the similar hydrophobic and hydrophilic proportion as raw milk, which was significantly different from that of pasteurized milk. Results implied that PEF treated milk could give similar flavor to Cheddar cheese as raw milk. More studies are needed to investigate the mechanism behind this.

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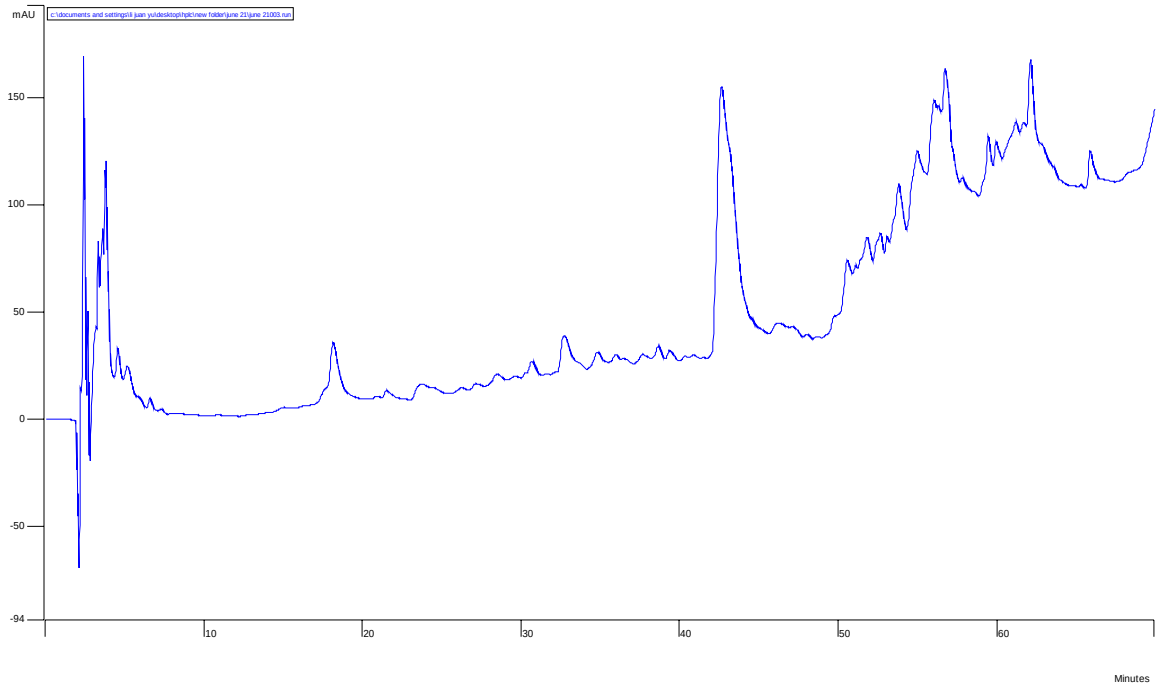

Figure 1 Typical HPLC profiles of Cheddar cheese curd slurry

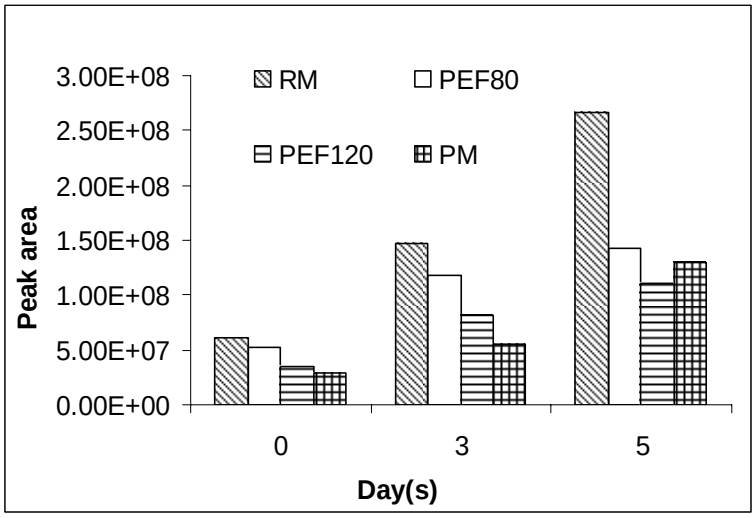

Figure 2 Total peak areas of HPLC profiles of Cheddar type cheese curd slurries made from RM, PM, PEF120 and PEF80.

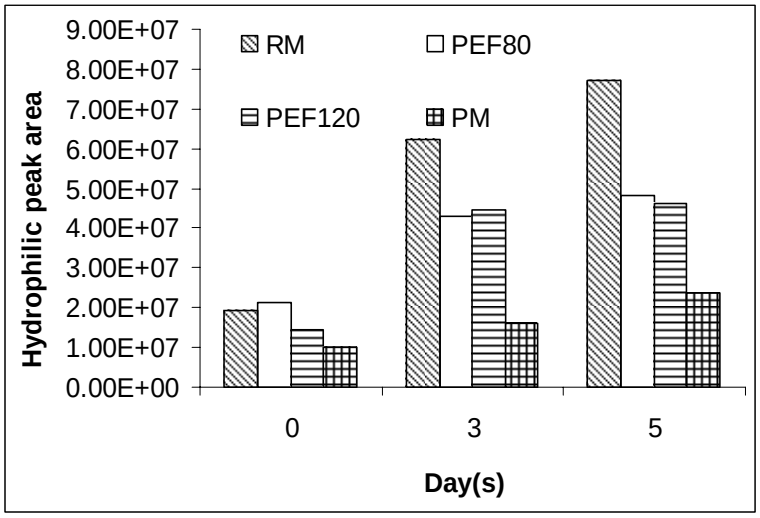

Figure 3 The amounts of hydrophilic peptides in WSF of Cheddar cheese curd slurries made from RM, PM, PEF80 and PEF120.

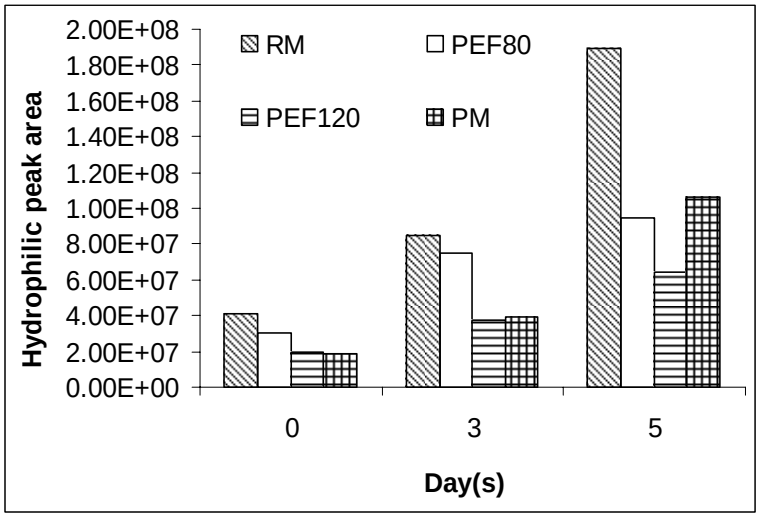

Figure 4 The amounts of hydrophobic peptides in WSF of Cheddar cheese curd slurries made from RM, PM, PEF80 and PEF120.

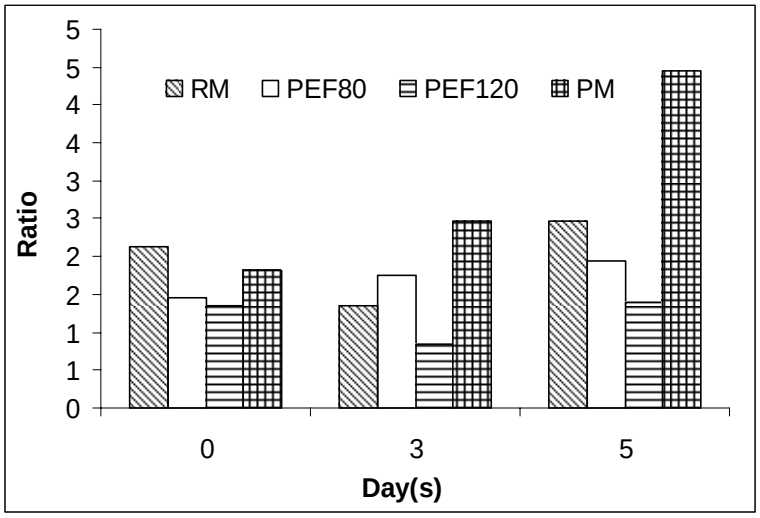

Figure 5 The ratio of hydrophobic to hydrophilic peptides in RM, PM, PEF80 and PEF120 Cheddar cheese curd slurries