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Effect of *trans*–Cinnamaldehyde and High Pressure Treatment on Physico–chemical and Microbial Properties of Milk during Storage Periods

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Abstract

This study was carried out to investigate the effect of *trans*-cinnamaldehyde and high pressure treatment on milk. Cinnamon oil milk was manufactured by high speed homogenization (3,000 rpm) and high pressure homogenization (500 and 2,000 bar) processing UHT milk and *trans*-cinnamaldehyde of various concentrations (0 to 0.1% (w/v)). Cinnamon oil milk was inoculated with *Escherichia coli* (6.4 Log CFU/mL) and kept at 7°C for 10 d to observe the antibacterial effect. The cinnamon oil milk containing 0.05% (w/v) *trans*-cinnamaldehyde initially began to show an antibacterial effect and *Escherichia coli* completely died in cinnamon oil milk added 0.1% (w/v) *trans*-cinnamaldehyde on the 6th day of storage. The result of the TBA value showed that the addition of 0.1% (w/v) *trans*-cinnamaldehyde was also effective to protect lipid oxidation. In the physical properties of cinnamon oil milk, particle sizes were enlarged in all samples during storage periods and the total color difference of cinnamon oil milk was slightly increased as level of high pressure. The surface tension of cinnamon oil milk treated 2,000 bar was remarkably higher than other samples. It seems that *trans*-cinnamaldehyde showed antibacterial activity and antioxidation effect at 0.05 and 0.1% (w/v) of concentration. Remarkably, high pressure treatment did not influence its microbial property but slightly affected the physical properties of cinnamon oil milk.

Key words: *trans*-cinnamaldehyde, high pressure homogenization, antibacterial activity, *Escherichia coli*, milk

Introduction

Food safety and hygiene are important issues in the food industry and food borne diseases have been major problems in both developed and developing countries. Therefore, drugs such as antibiotics have been used frequently, and over prescription of antibiotics has causes a continuous increase in the tolerances of the strains to which they are applied, causing an even greater potential threat to human health.

The over-prescription of antibiotics leads to a reduction of their effectiveness. Hence, it is necessary to find new antimicrobial agents for food hygiene. Essential oils from plants are attractive substances to consumers, because they are natural food stuffs. It has been reported that their antibacterial properties can help extend the shelf-life of food. Moreover, essential oils are important raw materials in the food, perfume, and the pharmaceutical industries. Molecular encapsulation of essential oils improves their

chemical properties and thermal stability. However, essential oils are very sensitive to the affect of light, temperature, oxygen, and humidity (Amrita *et al.*, 2009; Burt 2004; Cevallos *et al.*, 2010; Hossain *et al.*, 2011; Rahman *et al.*, 2011; Yossa *et al.*, 2010).

Trans-cinnamaldehyde (CIN, 3-phenyl-2-propenal) is organic compound that gives cinnamon flavor and odor, and accounts for the greater part (around 90%) of cinnamon oil. It is pale yellow in color and forms a low viscous liquid. It has been widely known that the most common application of CIN is as a flavoring agent of foods such as gum, ice cream, candy, and beverages. CIN is increasing in importance as the most effective antibacterial and antioxidant essential oil, and has been used in beverages such as fruit juice, milk and so on. Some researchers have evaluated the antibacterial or antioxidant properties of spice extract, i.e. eugenol, gingerol, thyme, capsaicin, CIN and so on, and among them, CIN was ranked quite highly (Amrita *et al.,* 2009; Hossain *et al.,* 2008; Hossain *et al*., 2011). Many researchers have studied the antimicrobial effect of CIN. A research team at the University of Illinois found that CIN inhibits oral bacteria growth by >50%; therefore, it could reduce production of volatile compounds that cause bad smells. It is particularly effec-

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tive against bacteria living at the back of tongue, and reduces anaerobic bacteria populations by about 43%. The combination of cinnamon and clove essential oil shows a synergistic antimicrobial effect, although the active concentration of the essential oil must be determined (Cevallos *et al.,* 2010; Chun 2012; Goñi *et al.,* 2009). CIN could be used to extend the stability of foods during storage.

Food producers continuously search for ways to improve color, taste, texture and nutritional value. Over the years, homogenization technology has evolved because consumers have demanded products with a longer shelf-life and better stability. From the early 1980s new technology was introduced for fine emulsion, and is based on high pressure capacity (Cook and Lagace, 1985; Paquin, 1999). In recent years, high pressure homogenization (HPH) considered advanced production techniques of the food industries because it causes unique changes in the physico-chemical and functional properties of food materials, such as increasing encapsulation efficiency, bioavailability and physical stability, without causing excessive turbidity. Moreover, dairy products result in much finer lipid droplets through the application of HPH (Ciron *et al.,* 2010; McClements and Qian, 2011; Van Hekken *et al.,* 2007). Milk or milk-based products are typically heat treated to remove microorganisms and to prolong self-life. However, thermal processing could deteriorate some of the nutritional properties, and off-flavor and easily occurred protein denaturation (Bouaouina *et al.,* 2006; Vassila *et al.,* 2002). The stability of milk could be improved, and activation of bacteria inhibited without thermal processing, which causes changes in the nutritional and sensory properties (Smiddy *et al.,* 2007; Wormbs *et al.,* 2004).

In this study, cinnamon oil milk (CM) was manufactured by using high speed homogenizer and high pressure homogenizer with various concentrations of CIN and UHT milk. CM samples were inoculated with *Escherichia coli* ATCC 10536 which has been typical index of food quality in food industry in order to observe the antibacterial activity of CIN. CM samples were analyzed through microorganism observation, TBA value, particle size, pH, total color difference, and surface tension, for a storage period of 10 d at 7°C.

Materials and Methods

Materials

Trans-cinnamaldehyde (CIN: 98%) was obtained from Alfa Aesar (A Johnson Mathey Company, USA). Ultra High Temperature milk was purchased from the market milk (Seoul Dairy Co-op., Korea). Tryptic soya broth (TSB), tryptic soya agar (TSA), and MacConkey agar (MAC) were provided from Difco (Becton Dickinson and Company Sparks, MD, USA). Trichloroacetic acid was bought from DAEJUNG (DAE JUNG CHEMICAL & METALS, Korea) and thiobarbituric acid was provided from VWR International (VWR Internaitonal Ltd., England).

Cinnamon oil-milk (CM) manufacture

CM (0% CM, 0.01% CM, 0.05% CM, and 0.1% CM) was prepared in various formulations. 0, 0.01, 0.05, and 0.1% (w/v) CIN was added into the UHT milk. UHT milk added CIN was performed by homogenization using a high speed homogenizer (Ultra Turrax® T25, IKA Labotechnik, Germany) at 11,000 rpm for 3 min. The CM emulsions were high pressure homogenized (HPH) under 0, 500 and 2,000 bar for 1 cycle by using the microfluidizer (Picomax® High Pressure Processor, MN400, Micronox, Korea). In every step of this process, the temperature of chamber was kept below 50°C through the use of an ice bath. The experiment was carried out to investigate the storage stability at 0, 1, 3, 6, and 10 storage d. During storage periods, CM was kept at 7°C.

Bacterial culture preparation

Gram-negative bacteria *E. coli* ATCC 10536 supplied by America Type Culture Collection (Manassas, USA) was used in this study to evaluate the activity of CIN *in vitro*. The *E. coli* was cultured on tryptic soya agar (TSA) at 37°C for 24 h for active microorganisms, and stored at -80°C in 50% sterile glycerol. Active cultures for experiments were prepared by transferring one loop full of cells from stock cultures to flask of tryptic soya broth (TSB), which were incubated at 37°C for 16-18 h.

Bacteria growth analysis

For the inoculation of CM with bacteria, *E. coli* were inoculated into 5 mL of TSB and grown for 18 h at 37°C in incubator to inoculate CM with approximately 1×10^6 CFU/mL of it. Amount of *E. coli* of the CM were analyzed between 10^3-10^5 CFU/mL and the average $\log_{10}$ counts obtained on MAC. The stock 1 mL of *E. coli* culture was diluted in the 0.85% NaCl solution 9 mL. 0.1 mL of bacteria solution was then spread on the surface of MAC plates. The plates were incubated at 37°C for 24 to 48 h. Detection of *E. coli* was based on enumeration of typical colonies according to the determination of colo-

nies, and counting bacteria colony as the colony forming unit (CFU/mL).

TBA value

Lipid oxidation was also determined during storage. 1 mL of sample was mixed with 9 mL of 0.25 N HCl solution containing 15% trichloroacetic acid (TCA) and 0.375% thiobarbituric acid (TBA). Each sample was heated to 95°C water bath for 15 min and was then cooled to room temperature. After cooling, they were centrifuged at 3,000 rpm for 10 min, and the absorbance of the clear supernatant liquid was measured by a UV-Visible spectrophotometer (OPTIZEN, Korea) at 535 nm.

Measurement of particle size

Particle size of CM was determined by using a dynamic light scattering (DLS) instrument and the data was analyzed (Master sizer®, Malvern Instruments, UK). It does this by measuring the intensity of light scattered as a laser beam passes through a dispersed particulate sample. This data is then analyzed to calculate the size of the particles that created the scattering pattern by using dispersion technology software supplied by manufacturer. Particle size was determined three times at room temperature, and the mean values were used.

pH measurement

pH of CM was determined during storage period by using a pH meter (Mettler Toledo 340®, UK). The average three determinations taken at room temperature were used.

Total color difference measurement

Color was determined with a color reader (CR-10, Konica Minolta Sensing, Inc., Japan) which was calibrated using a white standard plate. The CIE L^*, a^*, and b^* values were designated as indicators of lightness, redness, and yellowness, respectively. The objective of this transformation is the creation of a color space to aid in the numerical classification of color differences. CIE $L^*a^*b^*$ Scale is recommended by Commission International de l'Eclairage (CIE). The samples were determined post-preparation, and three measurements were taken from the surface of each sample.

Interfacial tension measurement

The interfacial tensions of CM samples were determined using a Tensiometer® (Sigma 703D KSV, USA). This method was used to measure the maximum weight of the liquid, which was lifted with a Du Nouy ring (ring radius 9.545 mm, wire diameter 0.37 mm) pulled out of the liquid's surface or interface. The ring was put above the surface, and the balance was zeroed. Then, the ring touched the surface and was pushed through it into the liquid, becoming wet, resulting in the formation of positive and negative forces being applied to the ring. The maximum force that was needed to pull the ring from the liquid was proportional to the surface tension of the liquid. All tests were carried out in triplicate.

Statistical analysis

The data were analyzed by ANOVA using the statistical program SAS (2008). Differences among the means were compared using Duncan's Multiple Range test, and the correlations between independent variables and measured values were calculated as Pearson's correlation coefficients.

Results and Discussion

Effect of CIN or HPH on growth of *E. coli* during storage periods

CM (0% CM, 0.01% CM, 0.05% CM, and 0.1% CM) was manufactured by the additon of various concentrations [0 to 0.1% (w/v)] of CIN, and different levels (0, 500, and 2,000 bar) of HPH, and was kept at 7°C for 10 d. CM was inoculated with *E. coli* and its growth was observed to investigate the antibacterial activity of 0.05% CM and 0.1% CM. CIN were effective in inhibiting the growth of *E. coli* during the storage period. *E. coli* were dramatically reduced in 0.1% CM at 6 d. They were slightly decreased from 6.3 Log CFU/mL to 3.5 Log CFU/mL in 0.05% CM during the storage period (10 d). However, there was no antibacterial activity in either the 0% CM or 0.01% CM. Furthermore, HPH processing had no effect on antibacterial activity (Fig. 1). In recent studies, 200, 250 and 300 MPa was found to be effective in the inhibition of bacterial activation in bovine and soy milk (Cruz *et al.*, 2007; Pereda *et al.*, 2006; Thiebaud *et al.*, 2003). A combination of HPH processing and the addition of essential oils was supposed to be more effective than single treatments of the milk (Evrendilek and Balasubramaniam, 2011). However, in this study, there was no clear effect on milk treated with HPH at 500 or 2,000 bar.

Lipid oxidation

TBA values were slightly increased from 0.145 to 0.191 in whole milk initially, upon treatment with CIN
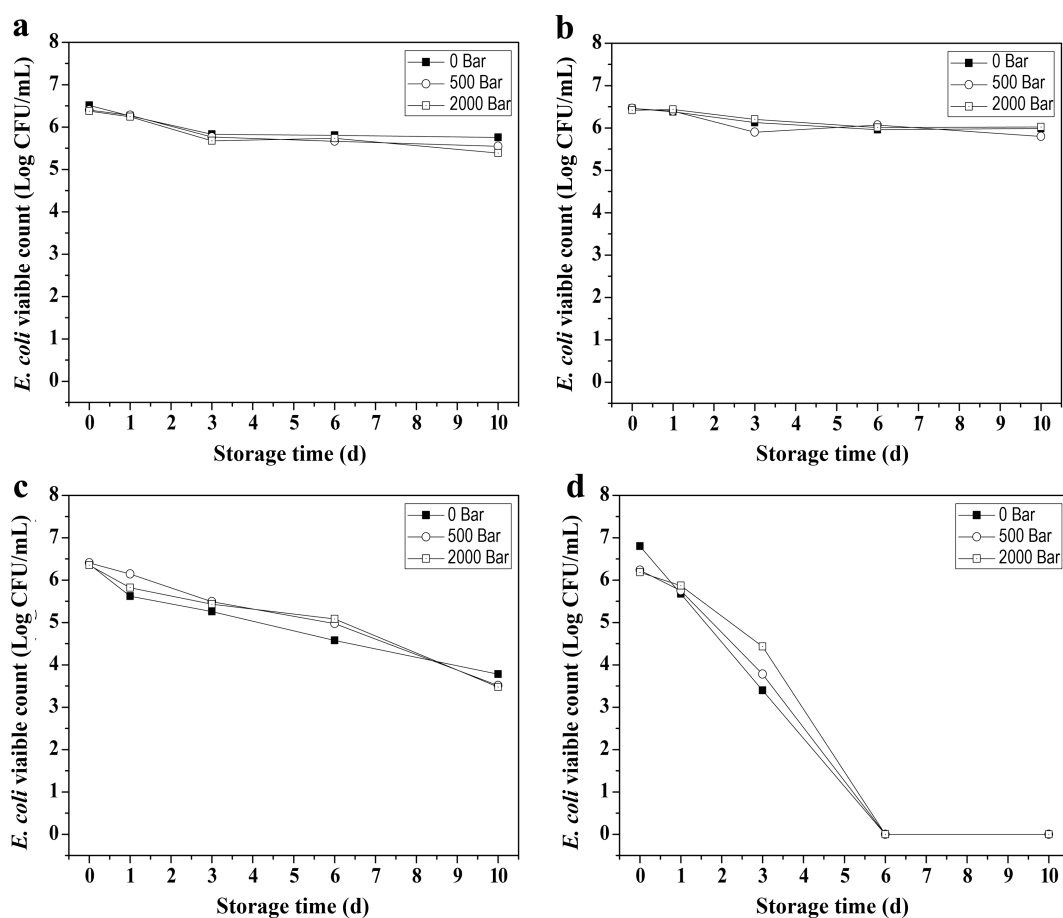
Fig. 1. Effect of CIN or high pressure homogenization to *E. coli* growth in CM during storage periods. CIN concentration: a, 0%; b, 0.01%; c, 0.05%; d, 0.1% (w/v)

(Fig. 2). All milk samples showed similar trends of lipid oxidation. Among the all CM, 0.05% CM and 0.1% CM treated 500 bar HPH had a slightly lower TBA value of 0.159 and at the end of the storage period, 0.1% CM treated 2,000 bar HPH had the lowest TBA value of 0.161. The addition of CIN was more effective in increasing the TBA value than HPH treatment. HPH milk had higher TBA values than raw milk, because HPH processing generated heat, resulting in temperatures of up to 50°C, which is near the denaturation temperature of milk proteins. Moreover, HPH increased the surface area of milk, with the result that HPH treated milk was more easily oxidized than raw milk. However, over 0.1% (w/v) CIN was effective in inhibiting lipid oxidation of milk. As Fig. 2 shows, specially, the variation was big end of storage periods. It was expected that TBA values were influenced by flocculation of milk droplet during storage periods. Flocculation of milk droplet generated, but it might did not affect particle size appreciably. However, oxidation could be occurred by exposure of CIN which was broken by excessive flocculation of cinnamon oil milk's droplets.

Particle size

The Particle size of CM was measured by $D_{4,3}$ of size parameter (Table 1) which gave average size of the disperse phase of milk diluted emulsions. In $D_{4,3}$ parameter measurement, the particle size of milk was determined to range from 0.40 μm to 8.92 μm during storage periods. Particle size of CM was decreased with higher pressure levels or increasing CIN concentration. The particle size of CM was increased significantly as storage periods progressed for all CM ($p<0.05$). In Cruz *et al.* (2007) study, the particle size of raw soy milk was 0.55 μm, and that of ultra high pressure (200 MPa) treated soy milk was 0.14 μm according to in $D_{4,3}$ size determinations. It was supposed that treatment with high pressure was effective in decreasing the particle size of bovine or soy milk. In this study, HPH was initially effective in decreasing the size of milk droplets, but milk droplets became larger over time.
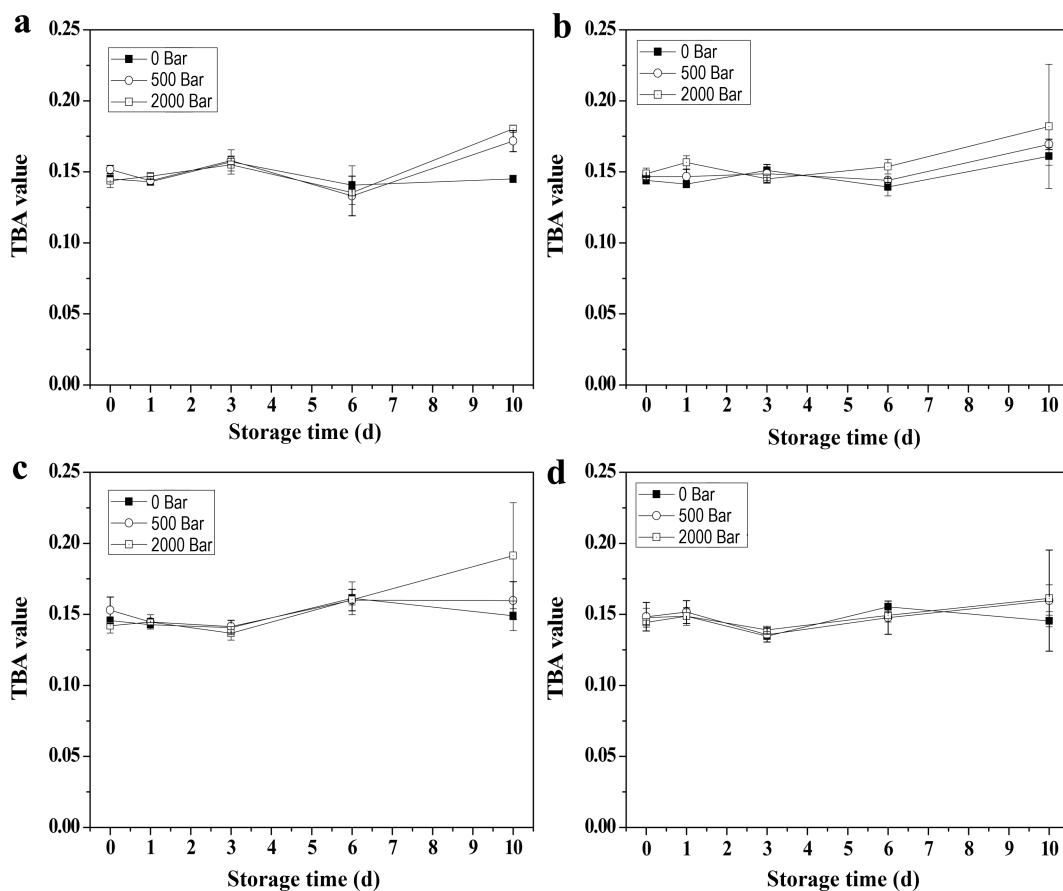
Fig. 2. Effect of CIN or high pressure homogenization to lipid oxidation in CM during storage periods. CIN concentration: a, 0%; b, 0.01%; c, 0.05%; d, 0.1% (w/v)

Table 1. Particle size of CM during storage periods in $D_{4,3}$ size parameter

Pressure (bar)	CIN (%, w/v)	Storage time (d)				
		0	1	3	6	10
0	0	$0.507\pm0.001^{1)bB}$	0.517 ± 0.001^{aA}	0.500 ± 0.000^{bB}	0.523 ± 0.001^{aA}	0.453 ± 0.001^{cF}
	0.01	0.510 ± 0.000^{bAB}	0.500 ± 0.000^{bC}	0.510 ± 0.000^{bA}	0.510 ± 0.000^{bB}	5.620 ± 0.195^{aC}
	0.05	0.500 ± 0.000^{bC}	0.513 ± 0.006^{aAB}	0.500 ± 0.000^{bB}	0.510 ± 0.000^{aB}	0.510 ± 0.000^{aE}
	0.1	0.500 ± 0.000^{bC}	0.490 ± 0.000^{cD}	0.510 ± 0.000^{aA}	0.510 ± 0.000^{aB}	0.510 ± 0.000^{aE}
500	0	0.513 ± 0.006^{bA}	0.500 ± 0.000^{bC}	0.493 ± 0.006^{bC}	0.527 ± 0.006^{bA}	5.647 ± 0.311^{aC}
	0.01	0.510 ± 0.000^{bAB}	0.510 ± 0.006^{bAB}	0.49 ± 0.0006^{bC}	0.490 ± 0.006^{bC}	0.880 ± 0.025^{aD}
	0.05	0.490 ± 0.000^{bD}	0.510 ± 0.000^{bB}	0.490 ± 0.000^{bC}	0.493 ± 0.006^{bC}	8.923 ± 0.415^{aA}
	0.1	0.493 ± 0.006^{aD}	0.500 ± 0.000^{aC}	0.500 ± 0.000^{aB}	0.497 ± 0.000^{aC}	7.720 ± 0.139^{aB}
2,000	0	0.400 ± 0.000^{eG}	0.410 ± 0.000^{bG}	0.410 ± 0.000^{bF}	0.420 ± 0.000^{bE}	5.650 ± 0.231^{aC}
	0.01	0.400 ± 0.000^{eG}	0.400 ± 0.000^{cH}	0.420 ± 0.000^{bE}	0.420 ± 0.000^{bE}	0.430 ± 0.000^{aE}
	0.05	0.450 ± 0.000^{eE}	0.450 ± 0.000^{bE}	0.450 ± 0.000^{bD}	0.453 ± 0.006^{bD}	0.523 ± 0.006^{aE}
	0.1	0.420 ± 0.000^{eF}	0.440 ± 0.000^{bF}	0.420 ± 0.000^{cE}	0.457 ± 0.006^{aD}	0.440 ± 0.000^{bE}

[1)]Average±SD (n=3).
[a-e]Means that different superscript within the same row are significantly different ($p<0.05$).
[A-F]Means with different superscript within same column are significantly different ($p<0.05$).

pH measurement

The pH of CM was measured from 6.40 to 6.61 during storage periods (Table 2). The pH of samples did not changed over the storage period, and there was no signif-

icant difference according to the level of pressure applied (Table 2, $p>0.05$). pH was increased slightly with increasing CIN concentration, but there was no significant difference ($p>0.05$). This level is similar to that of fresh milk

Table 2. Change of pH value during storage periods

Pressure (bar)	CIN (%, w/v)	Storage time (d)				
		0	1	3	6	10
0	0	$6.47\pm0.02^{1)cF}$	6.50 ± 0.01^{bF}	6.60 ± 0.02^{aB}	6.61 ± 0.01^{aAB}	6.61 ± 0.02^{aAB}
	0.01	6.63 ± 0.01^{aA}	6.51 ± 0.01^{cF}	6.61 ± 0.01^{abA}	6.61 ± 0.01^{bAB}	6.48 ± 0.03^{dD}
	0.05	6.63 ± 0.01^{aAB}	6.63 ± 0.01^{aC}	6.61 ± 0.01^{bAB}	6.59 ± 0.01^{cBC}	6.59 ± 0.01^{cB}
	0.1	6.63 ± 0.01^{aA}	6.63 ± 0.01^{aC}	6.60 ± 0.01^{bAB}	6.60 ± 0.01^{bABC}	6.62 ± 0.01^{aA}
500	0	6.49 ± 0.02^{cE}	6.65 ± 0.01^{aB}	6.60 ± 0.01^{bB}	6.48 ± 0.02^{cE}	6.45 ± 0.01^{dE}
	0.01	6.63 ± 0.01^{bA}	6.65 ± 0.01^{aAB}	6.61 ± 0.01^{cAB}	6.60 ± 0.01^{cABC}	6.48 ± 0.01^{dD}
	0.05	6.61 ± 0.01^{aCD}	6.60 ± 0.01^{aE}	6.60 ± 0.01^{aB}	6.57 ± 0.01^{bD}	6.40 ± 0.01^{cF}
	0.1	6.61 ± 0.01^{bCD}	6.63 ± 0.00^{aC}	6.61 ± 0.01^{bA}	6.62 ± 0.01^{abA}	6.59 ± 0.01^{cB}
2000	0	6.50 ± 0.01^{cE}	6.66 ± 0.01^{aA}	6.58 ± 0.01^{bC}	6.59 ± 0.01^{bC}	6.46 ± 0.01^{dE}
	0.01	6.60 ± 0.01^{dD}	6.63 ± 0.01^{aC}	6.60 ± 0.01^{cAB}	6.62 ± 0.01^{bA}	6.60 ± 1.09^{dcAB}
	0.05	6.61 ± 0.01^{abCD}	6.62 ± 0.01^{aD}	6.60 ± 1.09^{bAB}	6.60 ± 0.01^{bABC}	6.51 ± 0.01^{cC}
	0.1	6.61 ± 0.01^{abBC}	6.62 ± 0.01^{aCD}	6.61 ± 0.01^{aA}	6.60 ± 0.01^{bcABC}	6.60 ± 0.01^{cAB}

[1]Average±SD (n=3).
[a-c]Means that different superscript within the same row are significantly different (*p*<0.05).
[A-E]Means with different superscript within same column are significantly different (*p*<0.05).

Table 3. Total color difference of CM as CIN concentration or high pressure level during storage periods

Treatments		Storage time (d)				
CIN (%, w/v)	Pressure (bar)	0	1	3	6	10
0	0	51.31±0.26	51.48±0.49	51.46±0.09	51.45±0.11	51.71±0.11
	500	51.32±0.31	51.71±0.14	51.52±0.28	51.61±0.05	51.09±0.06
	2000	50.99±0.22	51.77±0.11	51.51±0.09	51.03±0.16	50.70±0.05
0.01	0	51.87±0.18	51.71±0.18	51.41±0.22	51.74±0.06	50.81±0.01
	500	51.15±0.29	51.73±0.14	51.74±0.05	51.75±0.05	51.28±0.05
	2000	50.95±0.63	51.32±0.10	51.42±0.24	51.59±0.11	50.92±0.05
0.05	0	51.97±0.42	50.33±0.14	51.01±0.05	51.74±0.13	51.66±0.04
	500	52.02±0.35	50.87±0.64	51.39±0.09	51.47±0.05	51.48±0.06
	2000	51.77±0.20	50.75±0.63	51.26±0.19	51.64±0.04	51.14±0.05
0.1	0	51.45±0.18	50.85±0.10	51.37±0.18	51.25±0.11	50.81±0.06
	500	52.26±0.07	50.48±0.11	51.42±0.06	51.88±0.11	51.10±0.10
	2000	52.26±0.11	50.82±0.17	51.53±0.11	51.55±0.05	51.02±0.01

(pH 6.6-6.8) (Kim, 2007). In Kim *et al.* (2007) study, it was not influenced significantly by high pressure (200 MPa) with low temperature treatment. They suggested that high pressure processing was not responsible for the pH change. Hayes *et al.* (2005) also prepared milk treated high pressure at 1,500 bar, 2,000 and 2,500 and then milk were kept at 4°C for 14 d. The pH of milk treated at 1,500 bar was decreased from 6.65 to 6.0; however, there was no decrease in the pH of milk treated at 2,000 or 2,500 bar. It was suggested that high pressure treatment did not affect pH of milk according several researches including this study.

Total color difference

Total color ranged from 50.33 to 52.26 (Table 3). Total color difference of raw milk was slightly increased pres-

sure increased. However, the color of CM samples varied irregularly during the storage period. And the reason that 0.1% CM had a rather higher value than others was the original color of CIN.

Surface tension

The range of surface tension was from 43.05 to 50.06 mN/m during the storage period (Fig. 3). CM treated HPH was higher than non-treated CM. Interestingly, surface tension of 0 % CM treated 2000 bar was remarkably increased from 3 d of the storage period up to around 50.06 mN/m. HPH processing slightly affected the interfacial tension of CM. Some researchers have reported modification of protein denaturation by high hydrostatic pressure. When pressure is released from the emulsion system the unfolded protein could partly return to its orig-
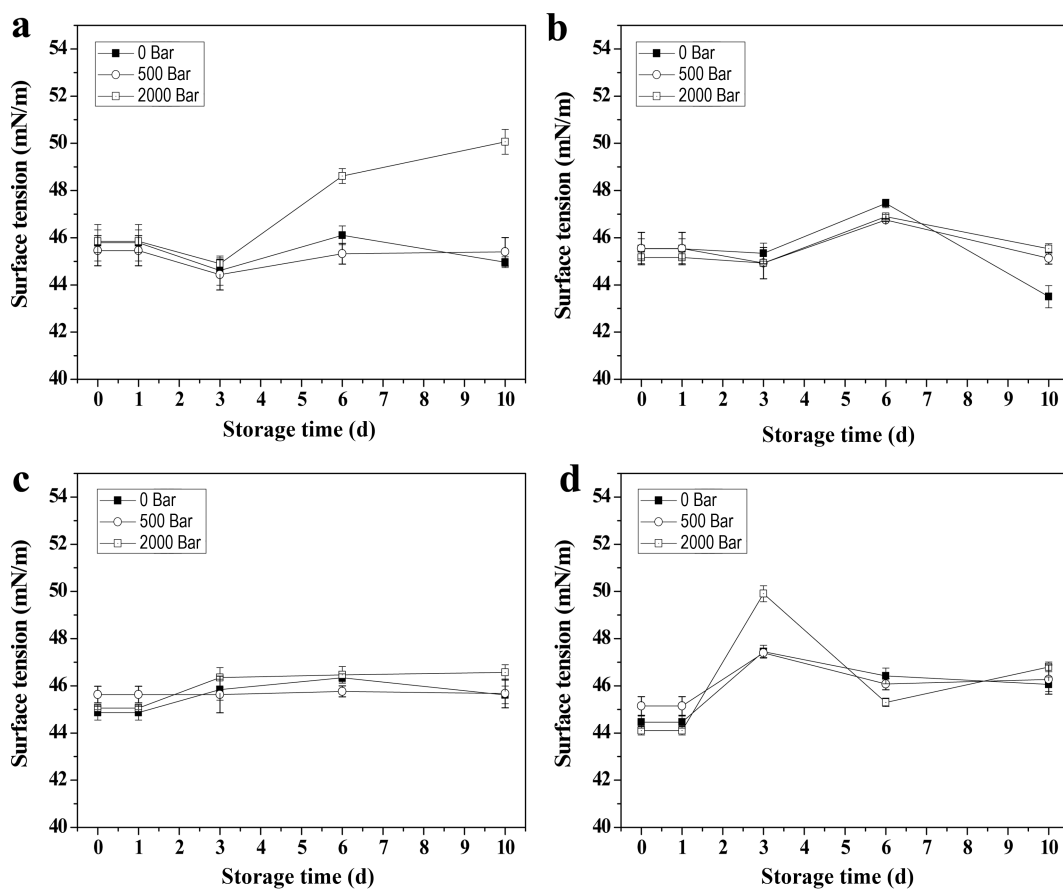
Fig. 3. Effect of CIN or high pressure homogenization to surface tension in CM during storage periods. CIN concentration: a, 0%; b, 0.01%; c, 0.05%; d, 0.1% (w/v)

inal protein structure, noted as reversible protein denaturation. Furthermore, the unfolded protein can be adsorbed onto the surface of the oil droplets and reform, to adhere more strongly by pressurization (Iordache and Jelen, 2003; Surassmo, 2011).

Conclusion

This study was carried out in order to investigate the effects of *trans*-cinnamaldehyde and HPH treatment on the physio-chemical and microbial properties of milk. The addition of over 0.05% (w/v) *trans*-cinnamaldehyde had antimicrobial activity in milk, and 0.1% (w/v) of *trans*-cinnamaldehyde had a high efficacy in decreasing the number of *E. coli* CFUs in the milk. Moreover, it was also effective in inhibiting lipid oxidation by TBA value. It was determined that the addition of *trans*-cinnamaldehyde could improve the chemical and microbial stability of milk during storage. Although HPH treatment was not effective on antimicrobial activity in this study as expected, HPH did decrease the particle size of CM, and

furthermore, the reduced size of particles of CM increased the rate of lipid oxidation, due to the increase in surface area. It was suggested that the addition of *trans*-cinnamaldehyde had more influence on the stability of milk than high energy physical processing.

Acknowledgement

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