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Effects of Food Polysaccharides and Seaweed Calcium on the Physicochemical Properties of Prickly Pear Extract Fermented by *Lactobacillus rhamnosus* LS

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Abstract

Prickly pear extract (PPE) was fermented by *Lactobacillus rhamnosus* LS at 30°C for 2 days. To improve the physicochemical properties of fermented PPE, it was fortified with food polysaccharides (0.2%) or seaweed calcium before lactic acid fermentation. The viable cell counts, flow behavior, titratable acidity and color stability of fermented PPE were evaluated during 4 weeks of cold storage. Addition of xanthan gum or glucomannan increased the apparent viscosity and acid production, viable cell counts and red color of PPE were also well maintained during the cold storage. However, fermenting PPE with gellan gum resulted in a decrease in relative absorbance, indicating lower color stability. In particular, PPE fortified with carrageenan or alginic acid showed reduced acid production and lower viable cell counts. Addition of seaweed calcium at a 0.1% level had positive effects on color stability, and helped maintain viable cell counts of 4.1×10^9 CFU/mL. This study demonstrated that xanthan gum could be used as a good thickening agent and stabilizer for retaining viable cell counts and red color during the cold storage in PPE fermented by lactic acid bacteria.

Key words: prickly pear extract, lactic acid fermentation, polysaccharide, seaweed calcium

INTRODUCTION

Prickly pear (*Opuntia ficus-indica* var. *saboten* MAKINO) belongs to the Cactaceae family that is abundantly distributed in arid and semi-arid regions (1). Prickly pear fruit has been used for the production of processed foodstuffs such as jams, syrups or candies and is a rich source of calcium, vitamins and red pigment (2,3). In particular, the red pigments in prickly pear fruit are betalains that are similar to the betacyanins of red beets (4,5). The stability of red pigment was well maintained in acidic pH, even after long periods of cold storage (6). The water extract which included the red pigments of prickly pear fruit has been shown to be an excellent antioxidant for protecting against linoleic acid oxidation (7). The mucilage content from prickly pear fruit varies according to cultivar and harvesting season. Mucilage, a complex carbohydrate with a great capacity to absorb water, has been considered a potential source of industrial hydrocolloid (8). Amin et al. (9) reported that the mucilage is a neutral polysaccharide composed of arabinose, rhamnose, galactose, and xylose. Prickly pear cactus has been recognized as a therapeutic plant (10). The pectin-like mucilage from prickly pear lowers serum LDL cholesterol while leaving HDL cholesterol unchanged (11), and may reduce the need for insulin by diabetics (12). The mucilage and red pigment from prickly pear fruit have been isolated and their physicochemical properties characterized (6).

Prickly pear fruit with nutritional and functional value has been utilized for various applications in the field of food processing. An alcoholic beverage was successfully prepared by fermenting a mixture of prickly pear juice and grape juice (13). *Candida utilis* was cultured with prickly pear juice as a sole carbon source to produce a single cell protein source (5). The production of red pigment was carried out by *Monascus purpureus* grown on prickly pear juice (14). Recently, lactic acid fermentation of prickly pear extract was optimized by manipulating the solid content of prickly pear extract, temperature and nutrient components (1). To improve the quality of lactic fermented beverage, it is necessary to increase the viscosity and the viability of lactic acid bacteria in fermented PPE. In addition, the red color of PPE should be maintained during cold storage. The objectives of this study were to enhance the physicochem-

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ical properties, including viscosity and stability, of red pigment in PPE fermented by lactic acid bacteria, by fortification with food polysaccharides and seaweed calcium.

MATERIALS AND METHODS

Materials

Prickly pear fruit harvested in February of 2003 was obtained from a Jeju island market and stored at -20°C. Prickly pear fruit (200 g) was thawed, sliced, and then mixed with 800 mL distilled water. The mixture was shaken at 150 rpm for 2 hr at room temperature (20°C) using a Shaker (KMC-1205S, Vision Scientific Co.), and then its filtrate was obtained by passing through cotton cloth and then kept at 4°C. The food polysaccharides, carrageenan and glucomannan, were purchased from MSC Co. (Korea). Xanthan gum and gellan gum were obtained from Guigdao FTZ Co., LTD (China). Alginic acid, pectin and dextran were purchased from Sigma Chemical Co. (USA). Food polysaccharides were directly dissolved into the PPE heated before lactic acid fermentation at a 0.2% level. Seaweed calcium and calcium carbonate were purchased from Setalg (France) and Sankyo Seifun Co., LTD (Japan), respectively.

Lactic acid fermentation

Lactobacillus rhanmosus LS previously isolated from soymilk curd residue (1), was used as a starter for lactic acid fermentation of PPE. *L. rhamnosus* LS was grown on MRS agar by incubating at 30°C for 24 hr, and then transferred to the sterilized PPE and fortified with 0.1% edible yeast extract (v/v), 0.1% glucose (v/v) and 0.1% edible calcium carbonate (v/v). The fermented PPE was then used as a starter culture for LAB fermentation of PPE.

For the lactic acid fermentation of PPE, the PPE was fortified with each polysaccharide and then pasteurized at 80°C for 10 min. The calcium carbonate was added at a 0.05% concentration prior to LAB fermentation. Also, calcium carbonate and seaweed calcium were added at 0.1~0.5% levels before LAB fermentation. The lactic acid fermentation of PPE was carried out by inoculating 3% seed starter that was cultured in PPE broth, and incubating at 30°C for 48 h. The fermented PPE was kept at 4°C for evaluating the viable cell counts, titratable acidity and color change during 4 weeks of cold storage.

Physicochemical properties

The pH and acidity of fermented PPE were evaluated by previously described methods (1). The red color of fermented PPE was evaluated by measuring the absorbance of diluted fermented PPE at 528 nm, and by using a Hunter color difference meter (CR-10, Minolta, Japan) and changes evaluated during the cold storage. Viable cell counts of lactic acid bacteria were determined by plating diluted cultures on MRS agar and incubating at 30°C for 48 h.

Viscosity of fermented PPE

Flow rheological experiments of natural and fermented PPE were performed using a RheoStress® 1 controlled stress rheometer (HAAKE, Thermo Electron Corporation, Germany) with a double gap concentric cylinder geometry adapted with a measuring cup DG43. The sample (13 mL) was loaded into the measuring cup and the shear stress (Pa) determined as the shear rate (1/s) in the range of 0.1 and 100. For determining the flow behaviors of the fluid, consistency index and flow behavior index were used as parameters of flow behavior and were determined based on the power law equation.

RESULTS AND DISCUSSION

PPE extracted from prickly pear fruit has characteristic water soluble mucilage and red pigment. The PPE had 14.8% (w/w) total solids and 0.5% (w/v) mucilage. Consistent with a previous report on the physicochemical properties of PPE (13), total acidity and pH were 0.35% and 4.0, respectively. The Hunter color redness value and total phenolic content were 14.8 and 5.7 mg%, respectively. The apparent viscosity of natural PPE was determined to be 10.3 cP by measuring at 60 rpm, but the viscosity of PPE was reduced after pasteurization at 80°C for 10 min. However, to upgrade the fermented PPE as valuable commercial beverage, it is necessary to enhance the viscosity of LAB fermented PPE. In addition, we anticipated that the addition of food polysaccharide would have positive effects on the physicochemical properties and lactic acid fermentation. Recently, various calcium ingredients have been used for the production of functional beverages. Although the PPE contained a relatively high content of calcium (15), the PPE fortified with calcium carbonate or sea weed calcium could be used for functional ingredients.

Effect of food polysaccharides on the acidity and relative absorbance

Plant polysaccharides such as pectin, carrageenan, glucomannan, alginic acid and microbial polysaccharides such as xanthan gum, dextran, gellan gum were added to the PPE at a 0.2% level. Previously, when a higher concentration of xanthan gum was used it did not completely dissolve because of its higher viscosity. Therefore, the concentration of polysaccharides added was fixed at 0.2%. As shown in Table 1, the acidities of

Table 1. Changes in titratable acidity and relative absorbance of PPE fermented after the addition of food polysaccharides

Polysaccharides	Titratable acidity (%)						Relative absorbance (%)					
	Fermentation			Storage (days)			Fermentation			Storage (days)		
	0	2	7	14	21	28	0	2	7	14	21	28
Blank	0.33[1)]	1.06	1.21	1.31	1.38	1.40	100	94.8	92.3	76.9	68.3	55.0
Pectin	0.34	1.18	1.21	1.34	1.39	1.40	100	94.3	99.0	75.4	66.0	54.5
Carrageenan	0.31	0.76	0.76	0.78	0.77	0.77	100	96.9	89.6	86.5	81.2	69.6
Glucomannan	0.30	1.09	1.20	1.25	1.39	1.40	100	93.9	91.1	91.0	71.8	60.4
Xanthan gum	0.29	1.18	1.21	1.23	1.30	1.40	100	105.5	90.9	89.9	86.1	78.2
Alginic acid	0.28	0.70	0.70	0.73	0.75	0.88	100	88.2	83.8	78.7	76.9	76.9
Gellan gum	0.25	0.94	1.10	1.11	1.12	1.30	100	93.7	84.6	71.5	58.0	47.4
Dextran	0.27	0.94	1.11	1.12	1.20	1.37	100	92.6	90.3	83.4	75.5	67.8

[1)]Data were presented as mean±SD (n=3).

fermented PPE in the presence of polysaccharides, except for carrageenan and alginic acid, were similar to PPE fermented without polysaccharide for 2 days. After fermentation for 2 days, the titratable acidity of fermented PPE was over 0.94%. But the addition of carrageenan and alginic acid as acidic polysaccharides resulted in the inhibition of acid production, indicating about 0.8% acidity. In particular, the acidity of PPE fermented by *L. rhamnosus* LS with/without polysaccharides gradually increased during the 4 weeks of cold storage, showing 18~38% increases in acidity. It turns out that *L. rhamnous* LS can produce acid even at 4°C. However, PPE fermented with carrageenan did not indicate any change in acidity during the cold storage.

When the thermal stability of betalain pigment of prickly pear fruits grown in Southeastern Spain was evaluated it was found that this pigment was very sensitive to temperature, with a loss of almost 75% of the initial value by heating at 70°C and by 90% at 90°C (16). In our studies the stability of PPE red pigment could be successfully maintained, indicating 84% retention (1). The relative red color of fermented PPE, as measured by absorbance, decreased during the 4 weeks of cold storage, but when xanthan gum and alginic acid were added it showed greater stability in red color with more than 70% retention. The PPE with carrageenan, glucomannan and dextran had moderate color stability, exhibiting more than 60% retention value. But, the PPE with pectin and gellan gum had similar color retention values to that of fermented PPE without polysaccharide. We conclude that the addition of certain polysaccharide may enhance the stability of red color during the cold storage of fermented PPE. It has been reported that the stability of red pigment of betalains can be affected by pH, temperature, light, oxygen, water activity and enzyme (17), and that anthocyanin-DNA copigmentation complexes result in the mutual protection against oxidative damage (18). Generally, copigments are colourless substances which can form a coloured cluster with colourless forms of anthocyanins. Copigments include a large variety of structurally unrelated compounds, such as flavonoid and non-flavonoid phenols, amino acids and organic acid (19). In addition, the thermal stability of anthocyanins is enhanced by copigmentation such as pullulan-anthocyanin mixture (20).

The mucilage, pectin-like polysaccharide, isolated from PPE by alcohol precipitation forms a complex with red the pigment; the red pigment could be removed by repeating solubilization in water and precipitation in alcohol (unpublished results). This implies that the PPE polysaccharide somehow reacts with the red pigment, contributing to the increased stabilization of the red pigment.

To elucidate the hypothesis that polysaccharides can improve color stability, various polysaccharides were added to PPE before lactic acid fermentation. Among them xanthan gum and alginic acid were the best stabilizers for preserving the red pigment during cold storage. During the cold storage of fermented PPE, the surface color was determined by a Hunter colorimeter. As shown in Table 2, the lightness (L) value of fermented PPE was about 19 and did not change during the cold storage. In the PPE fortified with/without polysaccharide, the redness (a) value of PPE was decreased by lactic acid fermentation. During cold storage for 4 weeks, the redness value of PPE with xanthan gum, alginic acid or dextran decreased the least, but the red color in PPE with gellan gum and dextran increased slightly at the end of the storage period. However, the effect of polysaccharides on color stability was not statistically significant. The color appearance was well preserved in all the fermented PPE, with and without polysaccharides, retaining the original red pigment after fermentation and during cold storage for 4 weeks.

Effect on viable cell counts

To evaluate the LAB fermentation in the presence of

Table 2. Changes in the Hunter color values of the PPE fermented with polysaccharides during cold storage

Polysaccharides		Fermentation storage (days)					
		0	2	7	14	21	28
Blank	L	18.80±0.00[1)]	18.80±0.00	19.00±0.00	19.06±0.04	18.63±0.00	18.99±0.00
	a	16.10±0.00	13.36±0.04	10.63±0.04	10.73±0.04	14.30±0.00	11.60±0.00
	b	2.03±0.04	0.33±0.04	1.02±0.04	0.80±0.00	0.26±0.04	0.56±0.04
Pectin	L	19.23±0.04	18.70±0.00	19.20±0.00	19.00±0.00	18.30±0.00	18.90±0.00
	a	16.13±0.04	11.90±0.00	9.86±0.04	9.26±0.04	12.83±0.04	10.46±0.04
	b	1.96±0.04	0.26±0.04	0.76±0.04	0.66±0.04	-0.30±0.00	0.70±0.00
Carrageenan	L	19.20±0.00	18.70±0.00	18.90±0.00	19.23±0.04	18.23±0.04	18.90±0.00
	a	15.66±0.04	13.43±0.04	10.56±0.04	9.83±0.04	13.76±0.04	10.36±0.04
	b	2.16±0.04	0.83±0.04	1.40±0.00	1.50±0.00	0.30±0.00	0.30±0.00
Glucomannan	L	19.30±0.00	18.90±0.00	19.10±0.00	19.20±0.00	17.40±0.00	18.90±0.00
	a	15.50±0.00	12.23±0.04	10.23±0.04	9.86±0.04	17.83±0.04	10.83±0.04
	b	2.00±0.00	0.70±0.00	1.13±0.04	1.00±0.00	-1.73±0.04	0.70±0.00
Xanthan gum	L	19.26±0.04	19.06±0.04	19.40±0.00	19.40±0.00	18.50±0.00	19.46±0.04
	a	14.66±0.04	11.20±0.00	11.83±0.04	11.13±0.04	16.03±0.04	10.50±0.00
	b	2.53±0.04	-0.90±0.08	0.26±0.04	0.60±0.00	-0.90±0.00	0.16±0.04
Alginic acid	L	19.60±0.00	19.46±0.04	19.20±0.00	19.50±0.00	18.63±0.04	18.90±0.00
	a	14.90±0.00	11.46±0.04	10.30±0.14	10.66±0.04	12.06±0.04	12.03±0.04
	b	2.80±0.00	1.40±0.00	1.30±0.00	1.43±0.00	0.70±0.00	0.86±0.00
Gellan gum	L	19.53±0.04	19.70±0.00	19.83±0.04	19.80±0.00	19.20±0.00	19.70±0.00
	a	13.33±0.04	11.63±0.04	10.76±0.04	10.80±0.00	13.10±0.00	13.23±0.04
	b	2.26±0.04	0.73±0.04	0.33±0.04	0.60±0.00	0.50±0.00	0.40±0.00
Dextran	L	19.80±0.00	19.70±0.00	19.70±0.00	19.70±0.00	19.03±0.04	19.10±0.00
	a	15.40±0.00	11.63±0.04	11.40±0.00	11.40±0.00	13.26±0.04	12.73±0.04
	b	2.90±0.00	0.73±0.04	0.73±0.04	0.73±0.04	0.23±0.00	0.53±0.04

[1)]Data were presented as mean±SD (n=5).

various polysaccharides, LAB were counted in fermented PPE before and after cold storage. As shown in Table 3, carrageenan and alginc acid showed the lower viable cell counts after fermentation for 2 day. This result coincides with the lower acid production. Therefore, the inhibition of cell growth resulted in lower acid production during lactic acid fermentation. When PPE was fermented with glucomannan and xanthan gum the viable cell counts were highest at 4.01×10^9 and 4.08×10^9 CFU/mL, respectively. The higher bacterial counts may be due to some unknown nutrient component existing in crude polysaccharide used as commercial food polysaccharide. These results suggest that carrageenan and alginic acid may interact strongly with inorganic compounds such as calcium, resulting in the low availability. Except for alginic acid and carrageenan, the PPE with other polysaccharides showed similar viable cell counts to those of fermented PPE without polysaccharide. The viable cell counts in fermented PPE gradually decreased during cold storage for 4 weeks. After cold storage for 4 weeks, the fermented PPE with glucomannan, xanthan gum, or gellan gum had the higher viable cell counts with 1.75×10^9, 1.33×10^9, 1.37×10^9 CFU/mL, respectively. It can be reasonably assumed that the addition of polysaccharide can enhance the viability of LAB during cold storage. In conclusion, the red pigment and viable cell counts

Table 3. Changes in viable cell counts of PPE fermented with polysaccharides during cold storage (Unit: 10^8 CFU/mL)

Polysaccharides	Storage periods (days)				
	After fermentation	7	14	21	28
Blank	21.0±5.29[1)]	14.5±4.82	9.3±1.15	15.0±0.15	11.8±1.60
Pectin	21.5±5.89	13.5±1.32	9.3±1.15	7.0±0.32	11.8±5.92
Carrageenan	3.5±5.00	1.0±0.02	1.5±0.03	1.4±0.25	0.2±0.03
Glucomannan	40.1±3.32	13.8±0.28	12.5±0.50	25.0±8.66	17.5±2.18
Xanthan gum	40.8±3.71	20.7±0.02	18.0±1.00	30.9±9.13	13.3±1.15
Alginic acid	5.7±0.57	2.8±0.53	3.0±0.20	2.4±0.03	5.6±2.88
Gellan gum	20.8±2.02	16.7±5.77	23.3±2.88	14.2±1.44	13.7±1.52
Dextran	20.0±0.00	18.0±8.88	9.5±0.00	10.5±0.70	10.0±0.00

[1)]Data were presented as mean±SD (n=3); seed starter: 20.3×10^8 CFU/mL.

from fermented PPE by *L. rhamnosus* LS can be well maintained. The addition of food polysaccharide affected on the stability of red pigment and the viability of LAB.

Effect on viscosity

Among the food polysaccharides tested, gellan gum and xanthan gum greatly increased the viscosity of fermented PPE. As shown in Fig. 1, the addition of xanthan

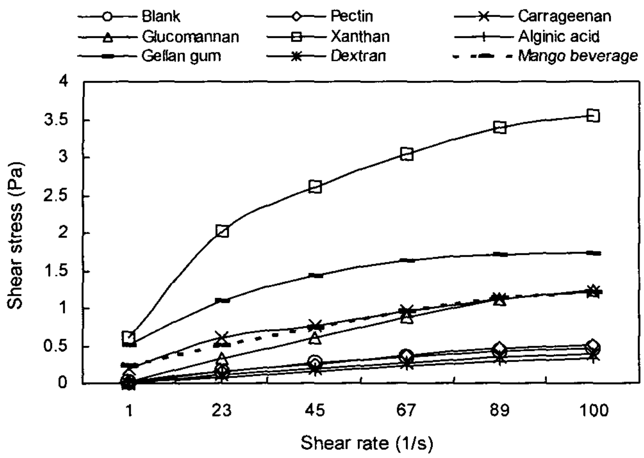

Fig. 1. Flow patterns of PPE fermented with food polysaccharides. Mango beverage is a commercial beverage of Haetae Co.

Table 4. Changes in flow behavior indexes of PPE fermented in the presence of polysaccharides

Polysaccharides	After fermentation (2 days)		
	a	b	r
Blank	0.0171	0.7229	0.9994
Pectin	0.0103	0.8484	0.9999
Carrageenan	0.1158	0.5045	0.9925
Glucomannan	0.0196	0.9019	0.9999
Xanthan gum	0.6031	0.3842	0.9999
Alginic acid	0.0047	0.9262	0.9997
Gellan gum	0.0303	0.6708	0.9962
Dextran	0.0068	0.8800	0.9999
Mango beverage[1]	0.1007	0.5361	0.9884

$\tau = ar^b$ (power law equation, a: consistency index, b: flow behavior index, r: shear rate, τ: shear stress).
[1]Mango beverage is a commercial beverage of Haetae Co.

gum in PPE resulted in the highest viscosity, showing the distinguished flow pattern compared to those of other polysaccharides. As shown in Table 4, flow behavior index and consistency index indicated the pseudoplastic flow. Xanthan gum has the highest consistency index value, carrageenan and Mango beverage had similar consistency index values. The apparent viscosity of PPE with xanthan gum or gellan gum was also higher than that of commercial Mango beverage. However, addition of other polysaccharides, except for dextran, alginic acid, or pectin, showed only a slight increase in apparent viscosity. Therefore, xanthan gum is the best thickener for enhancing the viscosity of acidic beverage such as fermented PPE by LAB. Considering the overall effects of polysaccharides on the LAB fermentation in PPE, xanthan gum is the best polysaccharide for use in PPE fermented by *L. rhamnosus* LS.

Effect of calcium

To determine the effects of calcium on the lactic acid fermentation of PPE, calcium carbonate and seaweed calcium were added to PPE at levels ranging from 0~0.5% before lactic acid fermentation. As shown in Table 5, the addition of calcium resulted in an increase in viable cell counts in PPE fermented for 2 days. With the addition of 0.5% calcium carbonate or 0.5% seaweed calcium, the viable cell counts of fermented PPE were 4.2×10^9 and 4.8×10^9 CFU/mL, respectively. The acidity of fermented PPE was increased by the addition of up to 0.3% calcium, but was slightly reduced at the 0.5% level, which may be due to excess calcium in PPE consuming some of the lactic acid to form calcium lactate.

Relative absorbance was increased to well over 100% by the addition of both calcium carbonate and seaweed calcium. This suggests that seaweed calcium may facilitate the growth of lactic acid bacteria because of the presence of various inorganic compounds. It was reported that PPE is composed of sugar, mucilage, pigment and abundant calcium (15). In spite of the presence of calcium in PPE, the additional calcium as a nutrient may

Table 5. Effect of calcium carbonate and seaweed calcium on the lactic acid fermentation of PPE

Calcium	Before fermentation			After fermentation (2 days)			
	%	pH	Titratable acidity (%)	pH	Titratable acidity (%)	Viable cell counts (Unit: 10^8 CFU/mL)	Relative absorbance (%)
$CaCO_3$	Blank	3.88	0.34	3.12	0.76	16.0±2.16	87.85
	0.1	4.42	0.21	3.10	0.80	41.0±9.34	94.39
	0.3	6.28	0.04	3.46	0.80	42.6±8.99	111.63
	0.5	6.71	0.03	3.85	0.60	42.0±8.31	109.68
Sea weed calcium	0.1	4.56	0.13	3.30	0.96	41.3±9.56	111.95
	0.3	6.65	0.05	3.49	0.80	41.6±8.95	120.31
	0.5	7.04	0.03	3.72	0.71	48.3±9.21	116.05

Seed starter: 22.5×10^8 CFU/mL.

Table 6. Change in Hunter color value of PPE fermented in the presence of calcium sources during storage

Ca	%	Color	Storage periods (days)					
			0	2	7	14	21	28
$CaCO_3$	0	L	19.37±0.04[1)]	18.67±0.04	19.07±0.04	19.57±0.04	19.40±0.00	18.93±0.04
		a	12.10±0.08	11.80±0.00	12.93±0.04	12.10±0.00	13.33±0.04	12.56±0.04
		b	0.43±0.04	0.30±0.00	-0.30±0.00	0.90±0.00	0.50±0.00	0.20±0.00
	0.1	L	18.97±0.04	19.20±0.00	18.57±0.04	19.60±0.00	19.37±0.00	19.80±0.04
		a	15.73±0.04	14.03±0.04	15.40±0.00	13.67±0.04	14.20±0.00	14.20±0.00
		b	1.27±0.04	0.20±0.00	-1.43±0.04	0.60±0.00	0.43±0.04	-0.50±0.00
	0.3	L	19.00±0.05	19.30±0.00	18.70±0.05	19.80±0.05	19.40±0.00	19.80±0.05
		a	14.60±0.05	13.80±0.00	15.10±0.05	12.30±0.05	12.90±0.00	12.90±0.08
		b	-0.10±0.00	-0.30±0.00	-0.40±0.00	-0.20±0.00	-0.10±0.00	-0.70±0.00
	0.5	L	20.00±0.00	19.40±0.05	19.00±0.00	19.70±0.05	19.40±0.00	19.40±0.05
		a	13.13±0.09	14.50±0.00	14.23±0.04	12.57±0.04	13.53±0.04	13.17±0.04
		b	-2.00±0.00	0.00±0.00	-0.43±0.04	-0.47±0.04	0.16±0.04	-0.27±0.04
Sea weed calcium	0.1	L	19.23±0.04	19.30±0.00	18.93±0.04	18.47±0.04	19.43±0.04	19.50±0.00
		a	14.67±0.04	12.40±0.00	14.27±0.04	13.00±0.00	15.33±0.04	13.53±0.04
		b	1.60±0.00	-0.07±0.04	-0.80±0.00	0.06±0.04	-0.47±0.04	-0.33±0.04
	0.3	L	19.40±0.05	19.30±0.00	19.30±0.00	19.60±0.05	19.30±0.00	19.40±0.05
		a	12.10±0.08	12.60±0.05	13.00±0.05	12.70±0.00	16.00±0.00	15.50±0.04
		b	0.43±0.00	-0.10±0.00	-1.00±0.00	-0.10±0.00	-1.30±0.00	-1.20±0.00
	0.5	L	19.20±0.05	19.60±0.00	19.40±0.05	19.40±0.00	19.30±0.00	19.60±0.05
		a	14.67±0.04	12.80±0.00	13.50±0.00	12.67±0.04	13.53±0.04	13.23±0.04
		b	1.60±0.00	-0.63±0.04	-0.73±0.04	-0.57±0.04	-0.83±0.04	-1.33±0.04

[1)]Data were presented as mean±SD (n=5).

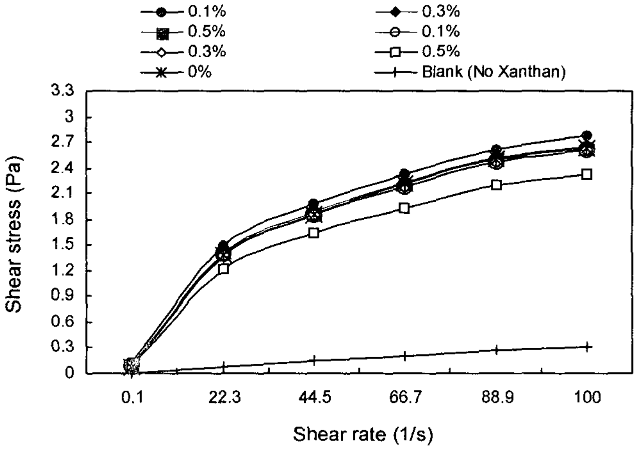

Fig. 2. Change in flow patterns of the PPE fermented in the presence of calcium (Open symbol: seaweed calcium; closed symbol: $CaCO_3$).

have enhanced the cell growth resulting in higher viable cell counts. There were no significant differences in viable cell counts between PPE fermented with calcium carbonate or seaweed calcium. To assess the eye-appeal of the color of fermented PPE before and after cold storage, Hunter color value was determined. As shown in Table 6, fermented PPE fortified with calcium showed a higher redness value (a value) compared with that of PPE fermented without calcium, and the redness remained stable during cold storage. Also, the lightness value (L) was not changed after fermentation and during cold storage.

In conclusion, the addition of calcium as calcium carbonate and seaweed calcium up to the 0.5% level enhanced the color stability after fermentation and during cold storage. In the fermented PPE with xanthan gum and 0.1% calcium carbonate, the apparent viscosity of fermented PPE was higher than that fermented with a higher concentration of calcium. In particular, 0.5% seaweed calcium resulted in a decrease in apparent viscosity. However, in the presence of calcium, PPE fortified with xanthan gum had typical pseudoplastic flow patterns compared with natural PPE without polysaccharide (Fig. 2).

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