

Original Research Article

Effect of pectin and gellan gum on particles and added solids' stability in drinking fermented goat milk

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ABSTRACT

Initially, phase stability was studied of plain yogurt drink containing high-methoxy pectin (0%, 0.3%, 0.5%, 0.7% w/w) or gellan gum (0%, 0.03%, 0.05%, 0.07% w/w). Then, the uniform dispersion of suspended 15% (w/w) nata de coco was investigated based on the optimal components of high-methoxy pectin (0.7%) and an additional 0%, 0.2% or 0.4% or gellan gum (0.07%) and an additional 0%, 0.02% or 0.04% in order to enhance uniformity of the yogurt drink. The results showed that all yogurt drink samples containing HM-pectin had Bingham plastic behavior. However, only samples containing less than 0.09% gellan gum had Bingham plastic behavior while those containing more than this had Herschel-Bulkley behavior. For the study of solid particle dispersion, the samples containing 0.9% or 1.1% high-methoxy pectin and any addition of gellan gum produced good phase stability and solid particle dispersion. The results should assist the development of new beverages derived from goat milk.

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INTRODUCTION

Among Thai people, goat milk is less preferred to cow's milk due to its strong flavor (Pralomkarn et al., 2012). However, its consumption has been increasing during the last few years as seen from an increase of dairy goat production from 2012 to 2016 in many areas of Thailand (Department of Livestock Development, 2013). The driving forces for this trend are nutritional and health. Goat milk is considered as a nutraceutical health drink. It contains lower lactose content than cow milk and contains easily digestible fats and proteins. Moreover, it also contains lower content of alpha s-1 casein (allergic protein) than cow's milk. Therefore, it is suitable for lactose intolerant patients and it is an excellent alternative to cow's milk (Lad et al., 2017).

A variety of goat-milk products have been developed to meet consumer demands. Drinking yogurt is a probiotic beverage. Among non-bovine milks, goat milk is considered as the major probiotic carrier. Its properties such as appropriate pH, good buffering capacity and high nutrient contents could facilitate the long-term survival of probiotics. Thus, the satisfactory viability of probiotics in its products throughout storage time could achieved (Ranadheera et al., 2018).

Drinking yogurt using goat milk or drinking fermented goat milk can be found in farmer markets and some supermarkets in Thailand. Most products are home-made. Phase separation due to aggregation of caseins at low pH is commonly observed in yogurt drinks. This sedimentation problem caused by flocculation of casein particles can be solved by adding some hydrocolloids such as high-methoxy pectin (HM-pectin) (Jensen et al., 2010) and gellan gum into the product (Kaini et al., 2010a; 2010b).

Both pectin and gellan gum are thickening agents, gelling agents and colloidal stabilizers. Pectin is a complex mixture of polysaccharides derived from the cell walls of plants. It consists mainly of D-galacturonic acid (GalA) units joined in chains by means of a- $(1 \rightarrow 4)$ glycosidic linkage. The uronic acids contain carboxyl groups, some of which are naturally present as methyl esters while others are commercially treated with ammonia to produce carboxamide groups. HM-pectins are classified as having 60 to 75% degree of esterification. It is soluble in hot water and forms a gel at low pH (Raj et al., 2012). The mechanism of colloidal stability by pectin can be explained that when pectin molecules adsorb onto surfaces of casein particles, the negative charges of pectin and the positive charges of casein form steric repulsion. This repulsion suppresses the aggregation of casein particles and consequently maintain colloidal stability. However, the effectiveness of the stability depends on pectin concentration in the system. Too low a pectin concentration can cause instability due to one pectin molecule being attached to more than one casein particle; too high pectin concentration can cause loss of instability by depletion flocculation (Kaini et al., 2010a). Gellan gum is an extracellular anionic polysaccharide derived from an aerobic fermentation by the bacterium Sphingomonas elodea (ATCC 31461). It contains a linear tetrasaccharide repeating sequence of: \rightarrow 3)- β -D-Glcp-(1 \rightarrow 4)- β -D-GlcpA-(1 \rightarrow 4)- β -D-Glcp- $(1 \rightarrow 4)$ - α -L-Rhap- $(1 \rightarrow (Kaini et al., 2010a)$. Kaini et al. (2010a) found that a phase separation between an upper clear serum and a lower densely opaque layer in doogh (a yogurt-based Iranian drink) will be less, if a higher concentration of gellan gum or a combination of gellan gum and HM-pectin are added. Moreover, gellan gum can create a yield stress that can stabilize colloidal particles and extrinsic added solid particles in the fermented dairy drink (Kaini et al., 2010b).

Nata de coco is a transparent jelly-like food. It is a product from culturing the bacterial cellulose called *Acetobacter xylinum* through

fermentation with coconut water. It is a good source of natural fibers and has been consumed as a dessert and it is also served with fruit cocktails (Halib et al., 2012).

The idea behind this research was to develop a new product from goat milk. The plan was to produce 'goat-milk drinking yogurt' containing a dispersion of chewing particles so that consumers will not only gain health benefits from the beverage but also enjoy drinking the beverage. Therefore, this research aimed to study the effects of HM-pectin and gellan gum on rheological properties and physical stability of goat milk drinking yogurt containing solid particle dispersion. The solid particles were suspended *nata de coco*. It is to be expected, the result would be useful for the development of goat's milk beverage.

MATERIALS AND METHODS

Materials

Raw goat's milk (2.46% fat, 3.84% lactose, 7.81% total solids and 8.77% water) was delivered from a local farm in Kamphaeng Saen, Nakorn-pathom, Thailand. The starter culture mixture consisting L. bulgaricus and S. thermophilus was purchased from Brenntag Ingredients (Thailand) Public Company Limited, Bangkok, Thailand. HM-pectin was from Cargill Siam Limited, Bangkok, Thailand. Gellan gum was from Winner Group Enterprise Public Company Limited, Bangkok, Thailand. Nata de coco particles ((7x7x7 mm) were purchased from Sri-Muang Market, Ratchaburi, Thailand. Glass bottles with plastic caps for packing drinking yogurt samples were purchased from JJ Glass Thailand, Bangkok, Thailand.

Preparation of yogurt curd

Raw goat milk was pasteurized at 85°C for 5 min and cooled rapidly to 43°C. The starter culture was added to the pasteurized milk at a concentration of 0.14% (w/v). The mixture was incubated at 43°C for 6 hr to obtain yogurt curd. Then, the curd was cooled to 4 °C and stored at 4 °C for 24 hr prior to drinking yogurt preparation.

Study 1: Effects of HM-pectin and gellan gum on plain drinking yogurt

The yogurt curd was ground using a kitchen grinder. Each sample of drinking yogurt was prepared by mixing the plain yogurt solution with a mixture of 13% (w/w) sucrose solution and a hydrocolloid either HM-pectin or gellan gum. Pectin is commonly used for creating stability in milk is around 0.1-1%. HM-pectin of 0.25% (w/w) is required to ensure stability in acid milk drinks (Kaini et al., 2010a). According to Kaini et al. (2010a), gellan gum concentrations to form weak gel are dependent on salt concentration in solutions; the concentration can be 0.005-1% (w/w). They used gellan gum of 0-0.05% for their yogurt-based Iranian drink. Therefore, HM-pectin (0%, 0.3%, 0.5%, 0.7% w/w) or gellan gum (0%, 0.03%, 0.05%, 0.07% w/w) were used in this study. After the mixing, the drinking yogurt samples were poured into glass bottles and closed with plastic caps. They were stored at 4°C. Rheological properties, pH values and phase separation of the samples were investigated during 15 days of storage. The Phase separation of the samples was investigated by observed by storing a set of samples in test tubes at 4°C. The height of the clear serum on the top of each test tube indicating phase separation was measured. The best concentrations of HM-pectin and gellan gum from this step (showing no phase separation) were selected to continue to the next study.

Study 2: Effects of HM-pectin and gellan gum on solid particle dispersion in drinking yogurt

Plain drinking yogurt was prepared in the same way as mentioned in the previous study. The formulae here were 0% hydrocolloid; an additional 0%, 0.2% or 0.4% to the best concentration of HM-pectin from the previous study; an additional 0%, 0.02% or 0.04% to the best concentration of gellan from the previous study. After the drinking yogurts were cooled to room temperature, 15% (w/w) of *nata de coco* was mixed into each sample. Then, the samples were poured into glass bottles, sealed with their capped and stored at 4°C. The samples were stored and investigated similarly to the study in the previous step. The uniform dispersion of suspended *nata de coco* was also investigated.

Rheological characteristics of drinking yogurt

Rheological characteristics explain flow behavior of fluid foods. The rheological properties of drinking yogurts from both studies were measured using a Haake rheometer (RheoStress 600) with a concentric cylinder geometry under a shear rate range of 1-1,000 s⁻¹. For the study 2, the liquid phase of the samples (mixtures of drinking yogurt and *nata de coco*) was used for this measure. Stress values using for shearing a sample and shear rate values of the samples were recorded. These values were fitted by the Excel function with a suitable mathematical model. The model mostly used to explain flow behavior of food containing additives are Bingham plastic model (Steffe, 1996):

$$\sigma = \sigma_o + \eta_B \gamma \tag{1}$$

and Herschel-Bulkley model:

$$\sigma = \sigma_o + K \gamma^n \tag{2}$$

for which σ is shear stress (Pa), $\dot{\gamma}$ is shear rate (s⁻¹), σ_o is yield stress (Pa), η_B is Bingham viscosity (Pa s), *K* is consistency index (Pa sⁿ) and ⁿ is flow behavior index.

Statistical analysis

The data were based on 5 replicates. The rheological model parameters were subjected to ANOVA and Duncan's multiple range tests using SPSS 17.0 for Windows. The significance level of P < 0.05 was considered significant different.

RESULTS AND DISCUSSION

Study 1: Effects of HM-pectin and gellan gum on plain drinking yogurt

The same trend of pH values was observed in all drinking yogurt samples. The fresh samples had pH values around 3.7-3.9 and the values decreased to 3.4-3.6 after 5-8 days of storage and stayed fairly constant. A lower pH value in yogurt could infer to a greater probiotic activity (Fergusson, 2017). Thus, it could be hypothesized that the bacteria culture was still surviving by the end of the storage time. As explained elsewhere, lactic acid bacteria convert sucrose and lactose into lactic acid, which consequently raise the acidity of yogurt. The pH values of these samples were similar to those of plain goat milk yogurts containing pectin and carrageenan in the research of Posecion et al. (2005).

For rheological properties, the apparent viscosity values ($\eta = \sigma/\dot{\gamma}$) of the fresh samples are shown in Figure 1. As expected, an increase of HM-pectin or gellan gum additions elicited an increase in viscosity of the samples. This confirms that both hydrocolloids are thickening

agents. However, a smaller quantity of gellan gum resulted less viscous drinks. The same trends were observed in the samples during 15 days of storage. The best fit of rheological data for all samples was Bingham plastic model. The model parameters are shown in Tables 1 and 2.



Figure 1. Apparent viscosity of plain drinking yogurts containing (a) HM-pectin (b) gellan gum at 0 days of storage.

Table 1. Rheological	model parameters	of plain drii	ıking yogurt
containing 0 – 0.7% l	HM-pectin.		

HM-pectin	Days of storage	$\eta_{\scriptscriptstyle B}$ (mPas)	$\sigma_{_{\! o}}$ (mPa)	R ²
0%	0	5.8±0.2ª	33.0±7.1°	0.976±0.003
	8	6.1±0.2ª	16.4±9.8ª	0.972±0.003
	15	6.3±0.1ª	24.2 ± 2.5^{ab}	0.972±0.004
0.3%	0	7.4±0.1 ^b	74.0±16 ^d	0.969±0.003
	8	7.9±0.1 ^b	76.0±7.9 ^d	0.971±0.003
	15	7.9±0.2 ^b	16.8±5.9ª	0.971±0.003
0.5%	0	8.6±0.1°	74.1±5.0 ^d	0.979±0.003
	8	8.8±0.1°	36.8±2.1°	0.970±0.004
	15	8.5±0.1°	12.4±8.4ª	0.967±0.002
0.7%	0	10.6±0.2 ^d	44.4±2.5°	0.990±0.003
	8	10.2 ± 0.1^{d}	27.0 ± 6.8^{ab}	0.980±0.001
	15	10.1±0.1 ^d	40.8±3.4 ^c	0.978±0.001

Means \pm SD in the same column identified by different letters are significantly different (P < 0.05).

Gellan gum	Days of storag	$\eta_{\scriptscriptstyle B}$ (mPas)	$\sigma_{_{\! o}}$ (mPa)	R ²
0 %	0	5.8±0.2ª	33.0±7.1°	0.983±0.007
	8	6.1±0.2ª	16.4±9.8ª	0.967±0.002
	15	6.3±0.1ª	24.2±2.5 ^{ab}	0.965±0.002
0.03%	0	7.0±0.1 ^b	199.5±19.3 ^g	0.982±0.006
	8	8.5±0.3 ^d	126.4±18.9 ^d	0.982±0.003
	15	8.0±0.1°	144.4±18.9º	0.982±0.001
0.05%	0	7.9±0.1°	137.6±17.8 ^{de}	0.983±0.007
	8	8.9 ± 0.4^{e}	231.6 ± 12.9^{h}	0.984±0.001
	15	8.5±0.2 ^d	175.5±6.9 ^f	0.984±0.001
0.07%	0	7.9±0.1°	137.6±18.8 ^{de}	0.983±0.005
	8	9.0 ± 0.4^{e}	403.0±13.9 ^j	0.985±0.004
	15	8.8 ± 0.2^{de}	263.1±19.9 ⁱ	0.988±0.003

Table 2. Rheological model parameters of plain drinking yogurt containing 0 – 0.07% gellan gum.

Means \pm SD in the same column identified by different letters are significantly different (P < 0.05).

Overall, the samples containing HM-pectin or gellan gum elicited higher Bingham viscosity than the samples that did not contain any hydrocolloid. The Bingham viscosity also increased with an increase in hydrocolloid concentration. For the samples containing HM-pectin, their Bingham viscosity values were not significantly different during the 15 days of storage. In contrast to the samples containing gellan gum, their Bingham viscosity values tended to increase with storage time, especially on the first 8 days of storage. Then, the decreasing tendency of the values was observed at the end of shelf life (15 days of storage). The samples containing hydrocolloids tended to have higher yield stress values than the samples that did not contain any hydrocolloid. However, they were not consistent with the hydrocolloid concentrations or the storage times. It is obvious that these hydrocolloids effectively function as thickening agents. The inconsistency of yield stress values might due to inconsistency of milk protein's particle sizes. Pectin molecules adsorbing onto surfaces of casein particles created an increase in milk protein's particle sizes (Phan Thi and Ipsen, 2009). And, gellan gum could affect inhomogeneity of milk protein's particles (Kaini et al., 2010a). Gellan gum gave a range of Bingham viscosity values near to those obtained from HM-pectin. However, yield stress values in the samples containing gellan gum were a lot higher than those containing HM-pectin. Low viscosity but high yield stress is a typical result of gellan gum in order to create high consistency of a fluid gel network (Kaini et al., 2010b).

Phase separation during 15 days of storage was observed by measuring the clear serum on the top of the test tube of each sample (Figure 2). Comparing with others, the sample containing no hydrocolloid showed the highest rate of separation. Adding 0.3-0.5% HM-pectin or 0.03-0.07% gellan gum to the drinking yogurt would not stop phase separation of drinking yogurt but would result less separation. It can be seen that adding at least 0.7% HM-pectin or 0.07% gellan gum to the drinking yogurt would create its phase stability. The mechanisms of phase stability function of HM-pectin and gellan gum are different. Enough HM-pectin adsorbing and coating onto casein surfaces create steric repulsion in the system so that phase stability occur (Tromp et al 2004). Gellan gum adsorbs onto casein surfaces creating different charges attraction similar to HM-pectin. But, it also forms crosslinking network with the serum. This supports well dispersion of casein in the serum (Kaini et al., 2010a;b).



Figure 2. Phase separation of drinking yogurts after 15 days of storage. The sample contained (a) 0% hydrocolloid (b) 0.3% HM-pectin (c) 0.5% HM-pectin (d) 0.7% HM-pectin (e) 0.03% gellan gum (f) 0.05% gellan gum (g) 0.07% gellan gum.

Study 2: Effects of HM-pectin and gellan gum on solid particle dispersion in drinking yogurt

From the previous study, adding 0.7% HM-pectin or 0.07% of gellan gum gave the best phase stability in goat milk drinking yogurt. Thus, this condition was the basis for developing a goat milk drinking yogurt containing *nata de coco* in suspension. The objective here was to let the *nata de coco* fully disperse in the drinking yogurt. Therefore, two more amounts of HM-pectin were used in this section of the study; 0.9% and 1.1%. Also, two more amounts of gellan gum were used; 0.09% and 0.11%.

The apparent viscosity values of the liquid phase of drinking yogurt samples are shown in Figure 3.



Figure 3. Apparent viscosity of plain drinking yogurts containing nata de coco with (a) HM-pectin (b) gellan gum at 0 days of storage.

As expected, higher viscosity values than those from the previous study were observed. Higher concentrations of hydrocolloid elicited fairly different flow characteristics. The samples containing HM-pectin followed the Bingham plastic model. The samples containing less than 0.09% gellan gum followed the Bingham plastic model; those containing 0.09% gellan gum and higher followed the Herschel-Bulkley model. The rheological model parameter parameters are shown in Tables 3 and 4

Table 3. Rheological model parameters of plain drinking yogurtcontaining 0 – 1.1% HM-pectin.

HM-pectin	Days of storag	$\eta_{\scriptscriptstyle B}$ (mPas)	$\sigma_{_{\! o}}$ (mPa)	R ²
0%	0	12.2±1.9°	550.9±144.1°	0.976±0.020
	8	10.0 ± 1.0^{a}	288.7±126.9 ^b	0.989±0.003
	15	9.7 ± 1.0^{a}	231.5±34.5ª	0.988±0.001
0.7%	0	12.5±0.9°	564.5±230.4°	0.987±0.004
	8	11.4 ± 0.4^{b}	252.7±57.2ª	0.992±0.000
	15	11.7 ± 0.7^{b}	289.9 ± 87.9^{b}	0.990±0.000
0.9%	0	17.9±1.1°	929.0±129.3 ^d	0.969±0.006
	8	14.3±3.5 ^d	721.7±245.3 ^{cd}	0.975±0.006
	15	16.8 ± 2.1^{de}	673.9±135.2°	0.975±0.006
1.1%	0	23.7±1.5 ^g	1126.7±217.4 ^e	0.962±0.008
	8	22.0±0.5 ^f	910.2±109.5 ^d	0.972±0.004
	15	21.3±1.5 ^f	959.7±165.7 ^{de}	0.997±0.063

Means \pm SD in the same column identified by different letters are significantly different (P < 0.05).

Similar to the previous study, the Bingham viscosity and yield stress increased with an increase in hydrocolloid concentration. The values were also higher than those observed from the previous study. High syneresis of *nata de coco* (Okiyama et al., 1992) might play an important role of the drinking yogurt's texture. Again, gellan gum gave much higher values of yield stress than HM-pectin and the values tended to increase during storage. This implied that gellan gum is very effective for giving yield stress to a fluid food. It has high ability to either bond with caseins or stay free in the fluid and build a consistent structure to prevent phase separation of the fluid food (Kaini et al., 2010b).

The dispersion of nata de coco in the drinking yogurts was investigated for 15 days of storage and the appearances on day 15 are shown in Figure 4. It is clear that, without hydrocolloid addition, nata de coco particles were not fully dispersed in the drinking yogurt. The particles were floating on the top close to the clear serum phase. The mechanism of this instability may be explained in a similar way to creaming, namely that one layer is lifted up by gravitational separation (Taherian, 2006). The nata de coco particles had lower density than the drinking yogurt so they were lifted up. At 0.7% addition of HM-pectin, phase separation of the milk system occurred and could be seen as a thin clear ring at the top of the bottle. Adding HM-pectin higher than 0.7% seemed to be more stable for nata de coco dispersion. In the cases of gellan gum addition, adding at least 0.07% of gellan gum could provide good dispersion of *nata de coco* in the drinking yogurt. This confirms that high yield stress values from gellan gum could provide good stability for solid particles dispersion. Gellan gum is not only prevent phase separation of a fluid food but also trap particles in the weak gel network of that fluid food. That prevents either flocculation or sedimentation (Kaini et al., 2010b). However, sensory evaluation should be conducted for confirm better selection of the beverage formulae.

Table 4. Rheological mo	odel parameters of	plain drinking yogurt	containing 0 - 0.11%	6 gellan gum
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Gellan gum	Days of	Bingham plas	stic		 Hersch	Herschel-Bulkley	Herschel-Bulkley	Herschel-Bulkley
sto	storage	$\eta_{\scriptscriptstyle B}$ (mPas)	$\sigma_{_{\! o}}$ (mPa)	R ²	К (1	K (mPas ⁿ)	K (mPas ⁿ) n	K (mPas ^a) n σ_o (mPa)
0%	0	12.2±1.9°	550.9±144.1°	0.976±0.020		-		
	8	10.0 ± 1.0^{a}	288.7±126.9 ^b	0.989±0.003		-		
	15	9.7 ± 1.0^{a}	231.5±34.5ª	0.988±0.001		-		
0.07%	0	11.5±0.4 ^b	1684.5±186. ^d	0.918±0.010		-		
	8	11.4±1.3 ^b	1886.9±428.9 ^e	0.901±0.029		-		
	15	11.6 ± 1.7 b	1878.0±520.5 ^e	0.900±0.030		-		
0.09%	0	-	-	-	533	533.3±45.6	533.3±45.6 0.27±0.02	533.3±45.6 0.27±0.02 2477.3±247.7 ^a
	8	-	-	-	565	565.9±19.6	565.9±19.6 0.26±0.01	565.9±19.6 0.26±0.01 2705.5±284.1 ^{bc}
	15	-	-	-	548	548.7±30.1	548.7±30.1 0.25±0.01	548.7±30.1 0.25±0.01 2896.8±338.0°
0.11%	0	-	-	-	 598	598.2±47.3	598.2±47.3 0.26±0.01	598.2±47.3 0.26±0.01 2720.2±133.2 ^b
	8	-	-	-	581	581.9±48.9	581.9±48.9 0.27±0.01	581.9±48.9 0.27±0.01 2431.5±331.8ª
	15	-	-	-	549	549.2±41.7	549.2±41.7 0.24±0.03	549.2±41.7 0.24±0.03 3212.2±188.9 ⁴

Means \pm SD in the same column identified by different letters are significantly different (P < 0.05).



Figure 4. Dispersion of *nata de coco* in the drinking yogurts after 15 days of storage. The sample contained (a) 0% hydrocolloid (b) 0.3% HM-pectin (c) 0.5% HM-pectin (d) 0.7% HM-pectin (e) 0.03% gellan gum (f) 0.05% gellan gum (g) 0.07% gellan gum.

CONCLUSIONS

Physical stability of plain goat milk drinking yogurt can be considered as phase stability between serum and dense protein. Adding 0.7% HM-pectin or 0.07% gellan gum to the drinking yogurt provides good phase stability to the drink. For solid particle dispersion, the stability can be considered as phase stability between suspended solid particles and the liquid yogurt phase. A higher amount of hydrocolloid addition is required. For *nata de coco* particles which have lower density than the drinking yogurt, adding more than 0.7% HM-pectin or upwards of 0.07% gellan gum could provide good dispersion. However, sensory evaluation should be conducted for establishing a suitable goat milk beverage for the market.

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