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Impact of coconut sugar syrup on quality of acidophilus milk during cold storage

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Abstract The influence of coconut sugar syrup on physico-chemical, microbiological and sensory properties of acidophilus milk during storage at 4°C for 8 days was investigated. Four formulations of acidophilus milk were tested with adding different coconut sugar syrup levels. Result showed that addition of coconut sugar syrup had significant effect on TSS of acidophilus milk formulations. However, there were no significant differences among formulations for pH, acidity and reducing sugar and DPPH radical scavenging activities during storage. Counts of *Lactobacillus acidophilus* TISTR 2365 were averaged 7.0 log cfu/ml throughout storage for all acidophilus milk formulations. *Escherichia coli* counts were less than 3 MPN/g and the counts of yeast and mold were less than 100 cfu/ml in all acidophilus milk formulations throughout storage period. The incorporation of coconut sugar syrup improved significantly sensory attributes. The surviaval of probiotic bacteria and sensory characteritics of acidophilus milks containg coconut sugar syrup were satisfactory for probiotic foods throughout cold storage.

Keywords: Acidophilus milk, Coconut sugar syrup, *Lactobacillus acidophilus*, Storage

Introduction

The consumer's demand for foods that supply significant nutrition and healthy benefits and/or prevent some diseases has been increasing attention. The dairy foods with functional properties have interested due to dairy foods with a good carries for probiotic and contain bioactive compounds (Yildiz, 2010; Machada *et al*., 2017). Probiotics are living microorganisms, when administered in adequate amounts, that have potential for improving the health of hosts (Oliveria *et al*., 2011). The probiotic *Lactobacillus acidophilus* helps to balance of the gut microbiota and prevents the growth of some enteropathogens such as *Escherichia coli* and *Salmonella* spp., boost immune system, decrease the risk of tumor development, reduce serum cholesterol level in the blood stream, prevent and relieve effects in several types of diarrhea, rotavirus

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diarrhea and antibiotic associated diarrhea (Yildiz, 2010; Kun, 2013; Ray and Montet, 2015). To obtain successful outcome of the probiotic therapy, US FDA recommended that the minimum probiotic counts should be at least 10^6 cfu/g or cfu/ml in probiotic foods at the time of consumption (Tripathi and Giri, 2014).

L. acidophilus strains are used in the processing of various dairy and non-dairy products such as acidophilus yogurt and acidophilus milk. Acidophilus milk is milk fermented by the probiotic *L. acidophilus.* It is claimed to be therapeutic and health-promoting properties (Amiri *et al*., 2010). During fermentation, *L. acidophilus* metabolizes lactose, and after fermentation it contains up to 0.6-0.7% lactic acid with no alcohol. This product is sour and not acceptable to the most consumers (Panesar and Marwaha, 2014). Sweeteners such as sucrose and/or flavoring agents are commonly added for development the sensory attribute. However, natural sweetener containing nutrients and bioactive ingredients could be used to improve food quality.

Coconut sugar is a natural sweetener that contains minerals including calcium, phosphorus, magnesium, chloride, sulfur and potassium, various short chain fatty acids such as acetate, propionate and butyrate including vitamins such as vitamin C and vitamin B complex, inulin and phytonutrients such as polyphenols and anthocyanidin. The vitamin C and phenolic compounds show antioxidant activity that protects biomolecules from the damage inducing by free radicals (Low *et al*., 2015). It contains volatile compounds including acetic acid, dodecanoic acid, 1,4 dimethyl-6-1 butyl-acetate and 2- butanol (Purnomo, 2007). Furthermore, coconut sugar is a low glycemic sweetener with a healthful glycemic index (GI) of 35 (Srikaeo and Thongta, 2015). Thus, it is good for diabetic and weight control. Low *et al*. (2015) reported that the supplementation of coconut sugar (15, 18 and 21%) in probiotic ice cream showed the improving of viability of *L. acidophilus* La-5, antioxidant activity and sensory attribute. Therev are no studied on coconut sugar syrup in acidophilus milk. The incorporation of coconut sugar syrup in a food product may improve their nutritional and sensory properties and the survivability of starter culture due to presence of prebiotic, inulin. Therefore, the objective of was to evaluate the addition of coconut sugar syrup at different levels on physico-chemical, microbiological and sensory properties of acidophilus milk during refrigerated storage for 8 days.

Materials and methods

UHT plain milk (Dutch Mill Ltd) was purchased locally in Chonburi, Thailand. Coconut sugar was obtained from Baan Farm Wan Ltd, Nonthaburi, Thailand. Coconut sugar syrup was prepared by mixing 150 ml sterile water and 200 g coconut sugar in order to adjust total soluble solid of 50°Brix, heated at 60°C for 5 min using water bath (Model 11DT-1, Heto, Denmark) and maintained at 4°C.

Milk media were prepared as 100 ml UHT milk poured in sterile beaker with the adding of 2% (w/v) yeast extract. Milk media was pasteurized at 85°C for 10 min using water bath (Chramostova *et al*., 2014).

Culture of the freeze dried probiotic, *L. acidophilus* TISTR 2365, was obtained from Microbiological Resources Centre, Thailand Institute of Scientific and Technological Research (TISTR), Thailand. Stock culture was prepared by adding 40% glycerol to the activated culture in deMan Rogosa Sharpe (MRS) broth (Difco Laboratories, India), stored at -18°C prior to thawing at 4°C before use (Sah *et al*., 2016).

Stock *L. acidophilus* culture (0.1 ml) was activated anaerobically in glass tubes using Oxoid gas jar and gas paks with MRS broth (9.9 ml) at 35°C for 24 h. Subsequently, milk media were inoculated with 5% (v/v) *L. acidophilus* culture and incubated at 35°C to a pH of 5.0 (or 12 h) (Chramostova *et al*., 2014). Concentration of *L. acidophilus* was defined as a final count approximately 8 log cfu/ml.

UHT milk supplemented with 1% (w/v) sucrose was pasteurized (85°C, 10 min). The milk was cooled at 45°C and inoculated with 5% (v/v) starter culture. Fermentation was carried out at 35°C until pH 5 was reached and to achieve approximately 8 log cfu/ml of *L. acidophilus*. Subsequently, the product was cooled to 4°C and the clot was broken by manual stirring with a glass rot. Coconut sugar syrup was added to the different formulations at 5 ml (termed CS5), 10 ml (termed CS10) or 15 ml (termed CS15) per 100 ml acidophilus milk under aseptic condition. Acidophilus milk without coconut sugar syrup (termed CS0) was used as control sample. The mixture was softly homogenized for 1 second (Yildiz, 2010; Machada *et al*., 2017). Finally, the product was divided in 50 ml sterile plastic containers, sealed and stored under refrigeration at 4°C for 8 days. The physico-chemical, microbiological and sensory properties of the samples were analyzed every 2 days throughout storage.

Physico-chemical analysis: pH value of the acidophilus milk was measured directly with a calibrated pH meter (Lab 850, Schott, Germany). Titratable acidity (as % lactic acid) was determined by titrating 9 g of sample with 0.1 N NaOH solation using phenolphthalein as an indicator. Total reducing sugar was determined using 3,5-dinitrosalicylic acid (DNS) according to Miller (1959). Total soluble solid content (°Brix) was measured with a handheld refractometer at 25°C (Master, Atago, Japan).

DPPH antioxidant analysis: 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was assessed as described by Gjorgievski *et al*. (2014) and expressed as:

$$\%\text{DPPH radical scavenging activity} = (\frac{A_0 - A_1}{A_0}) \times 100$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

Microbiological analysis: Samples (25 g) were homogenized with 225 ml of 0.1% sterile peptone and diluted in a 10-fold serial. *L. acidophilus* counts were carried out by pour plating in MRS agar medium at pH 5.4, adjusted with acetic acid and aerobic incubation at 37°C for 72 h (Oliveria *et al*., 2011). The yeast and mold counts were examined using compact dry yeast and mold, after incubated at 30°C for 4-5 days (Yousef and Carlstrom, 2003). Plates containing 25-250 colonies were counted and reported as colony-forming units per ml (cfu/ml) of sample. *E. coli* counts were analyzed using Most Probable Number (MPN) method (Yousef and Carlstrom, 2003).

Sensory evaluation of acidophilus milk formulations was conducted by 30 un-trained student panelists from Department of Food Science, Burapha University, Thailand. Panelists received each of the four acidophilus milk formulations in randomized sequence on small white glass at 4-5°C using a blind method. Acceptability of appearance, consistency, color, taste, aroma, flavor and overall acceptance were evaluated using a 9-point hedonic test (1=extremely dislike to 9=extremely like) (Ranadheera *et al*., 2012; Machada *et al*., 2017).

Statistical analysis: the physico-chemical and microbiological properties were subjected to two-way analysis of variance (ANOVA) depending on the variables: treatments (0, 5, 10 and 15 ml coconut sugar syrup per 100 ml acidophilus milk) and storage time. One way ANOVA was carried out to determine the statistical significance of sensory properties of the different acidophilus milk formulations and where appropriate, Randomized Complete Block Desisn (RCBD) was performed for comparison of all treatments. Differences of the mean values were analyzed by Duncan's multiple range tests and was considered significant at $p<0.05$.

Results

The results of pH, titratable acidity, reducing sugar and TSS presented in Table 1. The addition of coconut sugar syrup did not significantly affect pH or level of lactic acid in each acidophilus milk formulation ($p\geq0.05$). pH of the acidophilus milks decreased, while lactic acid concentration increased, but not significantly throughout storage period at 4°C for 8 days ($p \geq 0.05$). Additionally, as the amount of coconut sugar syrup increased, reducing sugar content in acidophilus milks increased insignificantly ($p \geq 0.05$), while the significant increases of TSS observed ($p < 0.05$). The reducing sugar content in each acidophilus milk formulation was similar throughout storage as well as TSS ($p \geq 0.05$).

Table 1. Physico-chemical properties of acidophilus milks containing coconut sugar syrup at different levels or not added during refrigerated storage for 8 days.[a]

Parameters	Days	Acidophilus milk formulations			
		CS0	CS5	CS10	CS15
pH	0	4.82±0.08 ^{ns,NS}	4.81±0.08 ^{NS}	4.82±0.08 ^{NS}	4.79±0.11 ^{NS}
	2	4.62±0.23 ^{ns}	4.70±0.09	4.69±0.15	4.75±0.07
	4	4.61±0.08 ^{ns}	4.62±0.12	4.58±0.12	4.63±0.13
	6	4.52±0.04 ^{ns}	4.60±0.02	4.49±0.09	4.58±0.07
	8	4.48±0.02 ^{ns}	4.50±0.16	4.48±0.12	4.54±0.12
Acidity (% lactic acid)	0	0.53±0.10^{ns,Ns}	0.53±0.04^{NS}	0.49±0.07^{NS}	0.48±0.05^{NS}
	2	0.61±0.10^{ns}	0.57±0.06	0.55±0.06	0.52±0.07
	4	0.60±0.07^{ns}	0.56±0.09	0.55±0.06	0.53±0.08
	6	0.65±0.03^{ns}	0.58±0.05	0.57±0.08	0.54±0.05
	8	0.69±0.05^{ns}	0.65±0.13	0.62±0.09	0.58±0.13
Reducing sugar (g/ml)	0	0.061±0.032^{ns,NS}	0.069±0.020^{NS}	0.077±0.012^{NS}	0.084±0.017^{NS}
	2	0.080±0.010^{ns}	0.080±0.014	0.082±0.005	0.085±0.005
	4	0.071±0.007^{ns}	0.078±0.010	0.078±0.009	0.084±0.014
	6	0.073±0.002^{ns}	0.080±0.005	0.078±0.006	0.083±0.008
	8	0.070±0.007^{ns}	0.088±0.022	0.089±0.014	0.080±0.002
TSS (°Brix)	0	7.37±0.14^{d,NS}	10.47±0.47^{c,NS}	12.93±0.09^{b,NS}	16.33±0.57^{a,NS}
	2	7.10±0.52^{c}	10.47±0.66^{b}	12.63±0.80^{b}	16.43±0.61^{a}
	4	7.37±0.42^{d}	10.67±0.09^{c}	12.93±0.09^{b}	16.37±0.52^{a}
	6	7.10±0.05^{c}	10.33±0.47^{b}	12.53±0.85^{b}	16.47±0.66^{a}
	8	7.10±0.14^{d}	10.27±0.61^{c}	12.50±0.38^{b}	16.30±0.90^{a}

Abbreviation: CS0, acidophilus milk without coconut sugar syrup; CS5, acidophilus milk containing added coconut sugar syrup at 5 ml/100 ml acidophilus milk; CS10, acidophilus milk containing added coconut sugar syrup at 10 ml/100 ml acidophilus milk; CS15, acidophilus milk containing added coconut sugar syrup at 15 ml/100 ml acidophilus milk; TSS, total soluble solid; [a], Means±Standard deviation; values with different small letters in a row for each property are significantly different at $p < 0.05$; [ns], not significant in a row for each property; [NS], not significant in a column for each property.

Acidophilus milks containing different coconut sugar syrup levels showed similar antioxidant activities relative to that without coconut sugar syrup ($p \geq 0.05$) (Figure 1). Major decreases in DPPH radical scavenging activities in acidophilus milk samples occurred during storage. DPPH radical scavenging activities of acidophilus milk formulations including CS0, CS5, CS10 and CS15 were 46.5, 47.0, 48.8, and 47.5%, respectively at the initial of storage, while these values were 24.9, 21.7, 27.0 and 25.9%, respectively after cold storage for 8 days. Decreases in antioxidant activities in acidophilus milks were ranged of 22-25%.

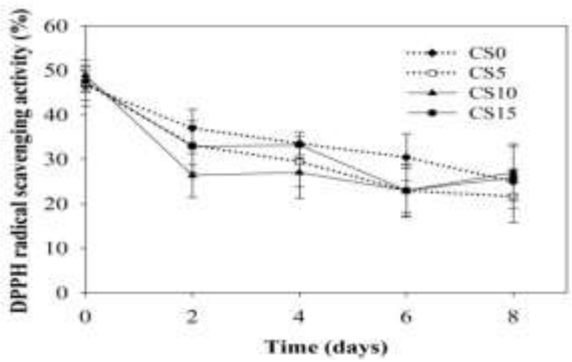

Figure 1. Changes in DPPH radical scavenging activities of acidophilus milks containing coconut sugar syrup at different levels or not added during refrigerated storage for 8 days

Counts of viable *L. acidophilus* TISTR 2365 in acidophilus milk without or with any of the 3 levels of coconut sugar syrup did not differ significantly ($p \geq 0.05$) at the same storage time (Table 2). Additionally, a slight reduction in the viability of *L. acidophilus* was observed in all acidophilus milk samples at the end of the storage period ($p \geq 0.05$), except CS15 showed a significant decline in viability of *L. acidophilus* ($p < 0.05$). The results of hygienic sanitary microbiological determination indicated that *E. coli* counts were <3 MPN/g, and the counts of yeast and mold were <100 cfu/ml in all acidophilus milk formulations throughout refrigerated storage for 8 days.

Table 2. Viable counts of *L. acidophilus* in acidophilus milks containing coconut sugar syrup at different levels or not added during refrigerated storage for 8 days.[a]

Days	Viable counts of *L .acidophilus* TISTR 2365 (cfu/ml)			
	CS0	CS5	CS10	CS15
0	$7.8 \pm 0.1^{ns,NS}$	7.7 ± 0.1^{NS}	7.7 ± 0.1^{NS}	7.7 ± 0.0^{A}
2	7.7 ± 0.1^{ns}	7.6 ± 0.0	7.5 ± 0.0	7.6 ± 0.1^{AB}
4	7.6 ± 0.0^{ns}	7.5 ± 0.1	7.6 ± 0.0	7.4 ± 0.0^{AB}
6	7.5 ± 0.1^{ns}	7.4 ± 0.1	7.5 ± 0.0	7.5 ± 0.1^{AB}
8	7.5 ± 0.2^{ns}	7.4 ± 0.1	7.4 ± 0.1	7.3 ± 0.2^{B}

Abbreviation: CS0, acidophilus milk without coconut sugar syrup; CS5, acidophilus milk containing added coconut sugar syrup at 5 ml/100 ml acidophilus milk; CS10, acidophilus milk containing added coconut sugar syrup at 10 ml/100 ml acidophilus milk; CS15, acidophilus milk containing added coconut sugar syrup at 15 ml/100 ml acidophilus milk; [a], Means±Standard deviation; values with different capital letters in a column are significantly different at $p<0.05$; [ns], not significant in a row; [NS], not significant in a column.

Table 3. Mean scores of sensory properties of acidophilus milks containing coconut sugar syrup at different levels or not added during refrigerated storage for 8 days.[a]

Days	Sample	Sensory properties (scores)						
		Appearance	Consistency	Color	Taste	Aroma	Flavor	Overall acceptance
0	CS0	7.6±1.0^{NS}	7.6±0.7^{A}	7.2±1.0^{NS}	6.1±1.1^{B}	6.8±1.0^{NS}	6.3±0.8^{B}	6.5±0.8^{C}
	CS5	8.2±0.7	7.0±0.8^{AB}	7.1±0.9	6.9±0.9^{A}	7.0±0.8	6.9±1.0^{A}	7.6±0.6^{AB}
	CS10	7.7±0.7	6.5±1.1^{B}	7.0±1.0	7.0±0.8^{A}	7.1±1.0	6.9±1.0^{A}	7.9±0.7^{A}
	CS15	7.8±0.7	7.0±1.2^{B}	7.2±1.0	7.0±1.1^{A}	6.9±1.0	7.0±1.1^{A}	7.4±1.0^{B}
2	CS0	7.6±1.1^{A}	6.6±0.7^{NS}	7.5±1.0^{A}	4.8±1.4^{B}	6.2±1.6^{B}	5.4±1.9^{C}	5.7±1.4^{C}
	CS5	6.9±1.3^{B}	6.9±1.3	7.0±1.0^{B}	6.5±1.4^{A}	6.9±1.0^{A}	6.6±1.1^{B}	6.6±1.2^{B}
	CS10	6.8±1.3^{B}	6.9±1.1	6.9±1.0^{B}	7.3±1.1^{A}	7.2±1.2^{A}	7.4±0.9^{A}	7.5±0.8^{A}
	CS15	7.2±1.3^{AB}	6.7±1.0	7.2±0.9^{AB}	7.2±1.2^{A}	7.4±0.9^{A}	7.4±0.9^{A}	7.4±0.9^{A}
4	CS0	7.3±1.3^{A}	6.5±1.2^{NS}	7.0±1.2^{A}	4.3±1.3^{C}	5.9±1.3^{B}	4.8±1.4^{C}	4.9±1.1^{C}
	CS5	6.5±1.5^{B}	6.3±1.1	6.5±1.3^{B}	5.7±1.4^{B}	6.1±1.2^{AB}	5.7±1.4^{B}	5.9±1.2^{B}
	CS10	6.7±1.5^{AB}	6.4±1.3	6.5±1.3^{B}	6.6±1.4^{A}	6.3±1.4^{AB}	6.6±1.4^{A}	6.8±1.3^{A}
	CS15	7.2±1.1^{AB}	6.6±1.3	6.6±1.1^{AB}	7.2±1.3^{A}	6.7±1.3^{A}	6.9±1.1^{A}	7.1±1.2^{A}
6	CS0	5.7±0.9^{AB}	6.0±0.9^{A}	5.6±0.9^{A}	3.9±1.0^{C}	4.6±1.1^{B}	4.9±1.1^{B}	4.8±1.2^{C}
	CS5	5.2±1.0^{B}	5.5±0.9^{B}	5.2±1.0^{B}	4.9±0.8^{B}	5.1±1.0^{A}	5.4±0.8^{A}	5.2±0.8^{BC}
	CS10	5.6±0.8^{B}	5.7±1.0^{AB}	5.3±0.8^{AB}	5.5±0.9^{A}	5.4±0.9^{A}	5.2±0.9^{AB}	5.6±1.0^{AB}
	CS15	6.2±1.0^{A}	5.5±0.8^{B}	5.5±0.9^{AB}	5.8±1.3^{A}	5.3±1.1^{A}	5.3±1.0^{AB}	5.8±1.3^{A}
8	CS0	6.5±1.5^{AB}	5.4±1.9^{B}	7.2±1.0^{A}	3.4±1.6^{C}	5.6±1.5^{B}	4.1±2.1^{B}	4.8±1.1^{C}
	CS5	6.5±1.7^{B}	5.5±1.7^{B}	6.6±0.8^{B}	4.2±1.5^{B}	6.4±2.0^{A}	4.9±1.8^{B}	5.4±1.4^{B}
	CS10	6.8±1.7^{AB}	5.7±1.7^{AB}	6.7±1.0^{B}	6.2±1.4^{A}	6.4±1.8^{A}	5.9±1.5^{A}	6.6±1.5^{A}
	CS15	6.9±1.5^{A}	6.0±2.0^{A}	6.7±1.0^{B}	6.7±1.6^{A}	6.3±1.6^{A}	6.3±1.8^{A}	6.9±1.5^{A}

Abbreviation: CS0, acidophilus milk without coconut sugar syrup; CS5, acidophilus milk containing added coconut sugar syrup at 5 ml/100 ml acidophilus milk; CS10, acidophilus milk containing added coconut sugar syrup at 10 ml/100 ml acidophilus milk; CS15, acidophilus milk containing added coconut sugar syrup at 15 ml/100 ml acidophilus milk; [a], Means±Standard deviation; values with different capital letters in a column for each storage interval are significantly different at $p<0.05$; ^{NS}, not significant in a column for each storage interval.

Mean scores of sensory attributes including appearance, consistency, color, taste, aroma, flavor and overall acceptance in acidophilus milks containing coconut sugar syrup at different levels or not added during storage are shown in Table 3. The addition of coconut sugar syrup impacted positively the sensory attributes of acidophilus milk, except color scores. Acidophilus milks containing coconut sugar syrup (CS5, CS10 and CS15) were scored higher than control sample (CS0) in terms of taste, aroma, flavor and overall acceptance, possibly reflecting the presence of sugar compounds as well as other satisfying flavoring agents in coconut sugar syrup. At the beginning of storage (0 day), CS5 and CS10 indicated high scores for overall acceptance ($p<0.05$). However, when storage time increased, CS10 and CS15 showed high scores for overall acceptance ($p<0.05$). It is possible that the sour taste in product may be developed resulting from the increasing acidity during storage, thus adding sweetness at high concentration could be improved sensory attributes. Moreover, sensory attributes of samples decreased during storage. Overall acceptance scores of CS0, CS5, CS10 and CS15 throughout storage were varied within the range of 4.8-6.5, 5.2- 7.6, 5.6-7.9 and 5.8-7.4 scores, respectively.

Discussion

The pH and acidity are considered one of the crucial parameters influencing the viability of *L. acidophilus*. The pH below 4 is considered harmful to survival viability of probiotic microorganisms (Ranadheera *et al*., 2012; Ranadheera *et al*., 2016). In this study, pH of each acidophilus milk formulation remained higher than 4.5 during storage and important to the viability of probiotic *L. acidophilus*. The significant increased TSS observed in acidophilus milks containing added coconut sugar syrup were associated with the presence of sugar components such as glucose (2-3%), fructose (1-4%) and sucrose (78-89%) in coconut sugar (Philippine Coconut Authority, 2015). Additionally, TSS of each acidophilus milk formulation decreased little during storage suggesting *L. acidophilus* hydrolyzed minor quantities of lactose and produced minor quantities of lactic acid during cold storage.

The supplementation of coconut sugar syrup in acidophilus milks did not affect their DPPH radical scavenging activities. Beside, DPPH radical scavenging activites of acidophilus milks were unstable during storage in which may be due to the slight activity of probiotic bacteria in refrigerated temperature and presence of dissolved oxygen in samples (Nematollahi *et al*., 2016). This result agreed with the finding of Najgebayer-Lejko (2014), who found that the antioxidant capacities, ferric-reducing antidant power (FRAP) of probiotic milks such as bioyogurts and acidophilus milks decreased by 2-22%

after storage at 4°C for 21 days. Our finding on benefit of acidophilus milk is consistent with earlier studies. Demirci *et al.* (2017) found that yogurts supplemented with rice bran exhibited DPPH radical scavenging activityies within the range of 3.52-12.75%. Low *et al.* (2015) reported that DPPH radical scavenging activities of probiotic ice creams supplemented with cane sugar and coconut sugar at concentration of 15-21% were 32-45% and 11-24%, respectively.

The result suggested that decreased in *L. acidophilus* counts of high sugar concentration (15 ml coconut sugar syrup per 100 ml acidophilus milk) may be due to osmotic stress. However, high viability of *L. acidophilus* was obtained in all acidophilus milks at the completion of storage in term of *L. acidophilus* were 7.3-7.5 log cfu/ml. The counts of *L. acidophilus* after the storage in acidophilus milks with or without coconut sugar syrup were higher than the recommended minimum therapeutic level (>10^6 cfu/ml) to qualify the probiotic foods (Tripathi and Giri, 2014). Result was in agreement with Junaid *et al*. (2013) who found slightly decreasing total viable counts of *L. acidophilus* in flavored probiotic acidophilus milk during storage at 4°C for 6 days. This finding suggested that *L. acidophilus* can survive under acidic condition and low temperature (4°C) in acidophilus milk during storage, and viability of *L. acidophilus* in acidophilus milk during storage was negatively influenced by incorporation of high coconut sugar syrup level and *L. acidophilus* counts were above 6.0 log cfu/ml during storage time in all acidophilus milk formulations. Moreover, the counts of *E. coli*, yeast and mold were done according to Notification of the Ministry of Public Health, No. 353, Re: fermented milk, Thailand (Ministry of Public Health, 2013). The results revealed that all acidophilus milk formulations were safe for consumption throughout cold storage for 8 days.

The incorporation of a natural sweetener through addition of coconut sugar syrup was a crucial factor in improving consumer acceptance of acidophilus milk. This result showed clearly that acidophilus milks containing coconut sugar syrup maintained high scores of consumer acceptance despite their high acidity, likely reflecting the presence of sugars and flavoring compounds in coconut sugar syrup. Similarly, Machada *et al.* (2017) found that the addition of stingless bee honey enhanced flavor and acceptance of goat yogurt during refrigerated storage for 14 days and suggesting a positive effect of the natural saccharides found in stingless bee honey on sensory evaluation. In addition, Junaid *et al.* (2013) reported the decreasing in sensory attributes of flavored probiotic acidophilus milk after 6 days of cold storage.

In conclusion, the incorporation of coconut sugar syrup in acidophilus milk formulations affected significantly TSS, but not for the other physico-chemical properties, namely pH, acidity and reducing sugar, and DPPH radical scavenging activities during refrigerated storage. The counts of *L. acidophilus* TISTR 2365 in all acidophilus milk formulations remained adequate (>6 cfu/ml) to improve health benefits to the consumer throughout the shelf life. Moreover, the addition of coconut sugar syrup appeared to improve sensory attributes of acidophilus milk. The coconut sugar syrup can be added to acidophilus milk successfully at level of 10 ml per 100 ml of acidophilus milk. Finally, the study showed a successful incorporation of coconut sugar syrup as ingredient in acidophilus milk with gratifying probiotic counts and sensory characteristics.

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References

Amiri, Z. R., Khandelwal, P. and Aruna, B. R. (2010). Development of acidophilus milk via selected probiotics and prebiotics using artificial neural network. Advances in Bioscience and Biotechnology, 1:224-231.

Chramostova, J., Mosnova, R., Lisova, I., Pesek, E., Drbohlav, J. and Nemeckova, I. (2014). Influence of cultivation conditions on the growth of *Lactobacillus acidophilus*, *Bifidobacterium* sp., and *Streptococcus thermophiles*, and on the production of organic acids in fermented milks. Czech Journal of Food Science, 32:422-429.

Demirci, T., Aktas, K., Sozeri, D., Ozturk, H. I. and Akin, N. (2017). Rice bran improve probiotic viability in yoghurt and provide added antioxidantive benefits. Journal of Functional Foods, 36:396-403.

Gjorgievski, N., Tomovska, J., Dimitrovska, G., Makarijoski, B. and Shariati, M. A. (2014). Determination of the antioxidant activity in yogurt. Journal of Hygienic Engineering and Design UDC637, 272:88-92.

Junaid, M., Javed, I., Abdullah, M., Gulzar, M., Younas, U., Nasir, J. and Ahmad, N. (2013). Development and quality assessment of flavored probiotic acidophilus milk. Journal of Animal and Plant Science, 23:1342-1346.

Kun, L. Y. (2013). Microbial biotechnology principles and applications. Singapore, World Scientific Publishing.

Low, R. H. P., Baba, A. S. and Aboulfazli, F. (2015). Effects of different levels of refined cane sugar and unrefined coconut palm sugar on the survivability of *Lactobacillus acidophilus* in probiotic ice cream and its sensory and antioxidant properties. Food Science and Technology Research, 21:857-862.

Machada, T. A. D. G., Oliveira, M. E. G. D., Campos, M. I. F., Assis, P. O. A. D., Souza, E. J. D., Madruga, S., Pacheco, M. T. B., Pintada, M. M. E. and Queiroga, R. D. R. D. E.

(2017). Impact of honey on quality characteristics of goat yogurt containing probiotic *Lactobacillus acidophilus*. LWT-Food Science and Technology, 80:221-229.

Miller. G. L. (1959). Use of Dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry, 31:426-428.

Ministry of Public Health (2013). Notification of the Ministry of Public Health (No. 353) B.E. 2556 RE: Fermented milk. Thailand. Retrieved from http://extwprlegs1.fao.org/docs/pdf/tha159846.pdf.

Najgebayer-Lejko, D. (2014). Effect of green tea supplementation on the microbiological, antioxidant, and sensory properties of probiotics milks. Dairy Science and Technology, 94:327-339.

Nematollahi, A., Sohrabvandi, S., Mortazavian, A. M. and Jazaeri, S. (2016). Viability of probioctic bacteria and some chemical and sensory characteristics in cornelian cherry juice during cold storage. Electronic Journal of Biotechnology, 21:49-53.

Oliveria, R. P. D. S., Florence, A. C. R., Perego, P., Oliveira, M. N. D. and Converti, A. (2011). Use of lactulose as prebiotic and its influence on the growth, acidification profile and viable counts of different probiotics in fermented skim milk. International Journal of Food Microbiology, 145:22-27.

Panesar, P. S. and Marwaha, S. S. (2014). Biotechnology in Agriculture and Food Processing Opportunities and Challenges. New York, Taylor and Francis Group.

Philippine Coconut Authority (2015). Coconut sap sugar. Food Product Development Division (FPDD) Guide No.5-Series of 2015. Retrieved from http://www.pca.da.gov.ph/coconutrde/images/coconut%20sugar%20final.pdf.

Purnomo, H. (2007). Volatile components of coconut fresh sap, sap syrup and coconut sugar. ASEAN Food Journal, 14:45-49.

Ranadheera, C. S., Evans, C. A, Adams, M. C. and Baines, S. K. (2012). Probiotic viability and physic-chemical and sensory properties of plain and stirred fruit yogurts made from goat’s milk. Food Chemistry, 135:1411-1418.

Ranadheera, C. S., Evans C. A., Adams, M. and Baines, S. K. (2016). Co-culturing of probiotics influences the microbial and physio-chemical properties but not sensory quality of fermented dairy drink made from goats’ milk. Small Ruminant Research, 136:104-108.

Ray, R. C. and Montet, D. (2015). Microorganisms and Fermentation of Traditional Foods. New York, Taylor and Francis Group.

Sah, B. N. P., Vasiljevic, T., McKechnie, S. and Donkor, O. N. (2016). Physicochemical, textural and rheological properties of probiotic yogurt fortified with fibre-rich pineapple peel powder during refrigerated storage. LWT-Food Science and Technology, 65:978-986.

Srikaeo, K. and Thongta, R. (2015). Effects of sugarcane, palm sugar, coconut sugar and sorbitol on starch digestibility and physicochemical properties of wheat based foods. International Food Research Journal, 22:923-929.

Tripathi, M. K. and Giri, S. K. (2014). Probiotic functional foods: survival of probiotics during processing and storage. Journal of Functional Foods, 9:225-241.

Yildiz, F. (2010). Development and Manufacture of Yogurt and Other Functional Dairy Products. New York, Taylor and Francis Group.
Yousef, A. E. and Carlstrom, C. (2003). Food Microbiology: A Laboratory Manual. New Jersey, John Wiley and Sons.

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