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Physico-chemical and microbiological properties of non-dairy yoghurt made from rice derivatives

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Abstract The results showed that the addition of glucose and gelatinized rice flour improved growth of the lactic acid bacteria, improved the organoleptic properties of the yogurt and accelerated the acidification rate, with the greatest effect observed with gelatinized rice flour. The most effective combination, of those tested, was found that rice milk containing 5%, glucose together with 50% gelatinized rice flour for both NYC-A and NYC-B cultures. Levels of carbohydrate, protein, lipid, pH, acidity and lactic acid bacteria count were similar to levels in commercial soy, almond and coconut yoghurts sold in Thailand. No contamination from other microflora was detected throughout the storage period for both NYC-A and NYC-B yogurts and they were successfully stored for 28 days at 4 ℃ without deterioration.

Keywords: Rice milk, Non-dairy product, Cereal yogurt, Fermented milks

Introduction

Yoghurt is amongst the oldest fermented milk products. Yogurts are consumed for its texture and flavour as well as being a valuable source of protein, calcium, vitamin B6 and vitamin B12 (Chandan *et al*., 2017). The manufacture of yoghurt involves the acid gelation of milk using lactic acid starter cultures that can be classified into standard and probiotic yoghurt cultures. Standard cultures consist of *Streptococcus thermophilus* or a mixture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. Different strains of S*treptococcus*, *Lactobacillus,* either singly or a mixed culture, are used for lactic acid fermentation. The raw material and different fermentation conditions such as temperature and culture concentration have been shown to influence on the growth of starter cultures, physicochemical characteristics and shelf-life of the yogurts (Ibrahim *et al*., 2019). Probiotic culture are standard cultures supplemented with probiotic bacterial strains

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including *Bifidobacterium* and *Lactobacillus acidophilus* (Corrieu and Béal, 2016; Nagpal *et al.,* 2012) showed that probiotic starter cultures promoted health benefits such as preventing gastrointestinal problems, reducing cholesterol and stimulating immune systems. Types of yoghurt may vary depending on processing conditions and sources of milk used including different animals and plants (Adewale *et al*., 2013).

Recently, consumer demand for non-dairy alternative products such as plant-based yoghurts, including soy, coconut, oat and almond (Alisa, 2020), has increased extensively. This is mainly as a result of the constantly increasing awareness of the need for sustainable food production and increasing of vegetarianism as well as medical reasons such as lactose intolerance and animal protein allergies (Chalupa-Krebzdak *et al*., 2018; Leahy *et al.*, 2010). Other plant material could also be considered to broaden the variety of yoghurt alternatives, for example rice (*Oryza sativa* L.), which is a staple food for half of the human population and is mainly cultivated in Asia (Kim and Dale, 2004). Thailand has long been a global leader in rice and rice derivatives production and export. In 2019, Thailand was ranked at 2th of the world in term of rice exports with 21% of the market share (Sowcharoensuk, 2019). Rice is an important source of nutrients required by the human body and is naturally gluten-free and hypoallergenic (Ito and Lacerda, 2019). Therefore, the development of rice-based yoghurt could be an avenue to increases the added value of Thai rice production, expand rice into a new market segment and provide a yogurt option for people who have allergies to animal milk, soy and almond based products. Plant based yoghurt production is made by using plant milk, a lactic acid starter culture and sometime the addition sweeteners, in a similar way to yoghurt made from animal milk (Bernat *et al*., 2015; Froiio *et al*., 2020). Diverse types of plant materials including seeds of lupin, barley, oat, peanut and wheat have been studied for developing yoghurt-like products (Coda *et al*., 2012; Luana *et al*., 2014; Salmerón *et al*., 2015; Bansal *et al*., 2016), but few studies have been reported on rice-based yoghurt preparations. Saccharified jasmine rice, rice flour mixed with red grape must and germinated brown rice flour mixed with sucrose and glucose have been tested for their suitability in making yoghurt-liked products. However, during the manufacturing processes, some of these products can take a long time, up to 2-5 days, from preparation to a finished product (Cáceres *et al*., 2019; Coda *et al*., 2012; Wongkhalaung and Boonyaratanakornkit, 2000). Moreover, rice based yoghurt formulations have been reported to generally have inferior nutritional levels than cow's milk or plant based yoghurts, which in addition to carbohydrate levels of 12-25% w/w, also contain small amount of protein (0.80% w/w) and fat (0.48% w/w) (Cáceres *et al*., 2019; Wongkhalaung and

Boonyaratanakornkit, 2000). In order to increase its nutritional value of rice based yogurt, it was decided to investigate the combination of rice derivatives (rice flour, rice protein concentrate, rice bran oil mixed with glucose) to develop novel rice-based yoghurt formulation and to characterize their physico-chemical and microbiological properties. Different commercial non-dairy starter cultures including standard and probiotic cultures and their shelf-life were assessed.

Materials and methods

Materials

Rice flour was obtained from Thai Flour Industry Co. Ltd., rice protein concentrates from Fenchem Co. Ltd., rice bran oil from Thai Edible Oil Co. Ltd., and food grade glucose powder from Bkkchemi Co. Ltd. Two commercial non-dairy yoghurt cultures (NYC A-B) were purchased from Berli Jucker Public Co. Ltd. and Tinnakorn Chemical and Supply Co. Ltd. (Thailand) Public. NYC-A was the standard non-dairy yoghurt culture consisting of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* and NYC-B was the probiotic non-dairy yoghurt culture consisting of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Bifidobacterium animalis* subsp. *lactis*, *Lactobacillus acidophilus.* All other chemicals and reagents were of analytical grade.

Rice based yoghurt fermentation

Plengsaengsri *et al*. (2019) previously described the basic components of rice-based yoghurt used in these experiments as gelatinized rice flour, rice protein concentrate, rice bran oil, glucose, calcium lactate and stabilizer. Gelatinized rice flour and glucose were used to examine their influences on acidifying activity. The mixtures were homogenized at 8,000 rpm and sterilized at 121 ℃ for 20 minutes. After cooling to 42 ℃, the starter culture was added according to the manufacturer’s instructions and fermented at 43 ℃ without shaking. After fermentation, the products were stored at 4 ℃ for 28 days.

Microbiological analysis

Twenty-five grams of rice-based yoghurt samples were weighed in a sterile stomacher bag and 225 mL of 0.1% peptone water (Merk) was added. Each sample was then homogenized in the stomacher (IUL Masticator, Spain) at room temperature (about 30^{o}C) for 1 min. Numbers of *S. thermophilus* was determined using pour plate M17 agar (Hi-media Laboratories, India), after incubation at 37 ℃ for 48 hours (Sert *et al*., 2017). *L. bulgaricus* and *L. acidophilus* were determined using pour plate MRS (de man, Rogosa and Sharp) and *B. lactis* was determined using pour plate Bifidobacterium agars (Hi-media Laboratories, India), after incubation under anaerobic conditions at 37℃ for 72 hours. Results were expressed as Log CFU/g (Turgut and Cakmakci, 2017). *E. coli* 3M™ Petrifilm ™ was used for 24 h at 37 °C ±1°C for *Escherichia coli*. Enumeration of *Staphylococcus aureus* was performed on 3M™ Petrifilm™ Staph Express Count Plate after incubation for 24 hours at 35±1℃ (AOAC, 2000). The growth of yeasts and molds was also accessed using spread plate potato dextrose agar (Hi-media Laboratories, India), after incubated at 23-25℃ for 5-7 days (Mohammadi-Gouraji *et al*., 2019).

Physico-chemical analysis

Proximate analysis for moisture, total solids, protein (N*6.25), fat, ash, fibre and carbohydrate content were performed according to standard AOAC methods (2000). The pH of samples was determined using a glass electrode pH meter (METTLER TOLEDO Inlab® Expert Pro-ISM). For titratability acidity determination, 10 g of samples was mixed with 30 mL of distilled water and titrated with a standard solution of 0.1 M NaOH using phenolphthalein as the indicator. Total acidity was calculated as % lactic acid (AOAC, 2000).

Statistical analysis

Experiments and measurements were carried out in triplicate. Data were reported as mean ± standard deviation and analysed were using the SPSS package (IBM SPSS statistics version 24 for window 10). One-way analysis of variance (ANOVA) was performed and a Duncan's Multiple Range Test was applied on the individual variables to compare the mean and to assess their significance differences (at $p < 0.05$).

Results

Preliminary study

The pH and % lactic acid acidity levels during the 24 h fermentation process of rice milk inoculated with *S. thermophilus* and *L. bulgaricus* (NYC-A) or *S. thermophilus*, *L. bulgaricus*, *B. lactis*, *L. acidophilus* (NYC-B) is shown in Figure 1. No significant changes ($p < 0.05$) in pH (5.86 ± 0.04 to 5.83 ± 0.03) and lactic acid acidity (0.11 ± 0.01 to 0.12 ± 0.01%) was observed in rice milk inoculated with NYC-A during 24 h fermentation, whereas the milk inoculated with NYC-B had significant reduced ($p < 0.05$) pH from 5.84 ± 0.04 to 4.36 ± 0.05 and lactic acid acidity increased significantly ($p < 0.05$) from 0.11 ± 0.02 to 0.37 ± 0.03% after 24 h fermentation.

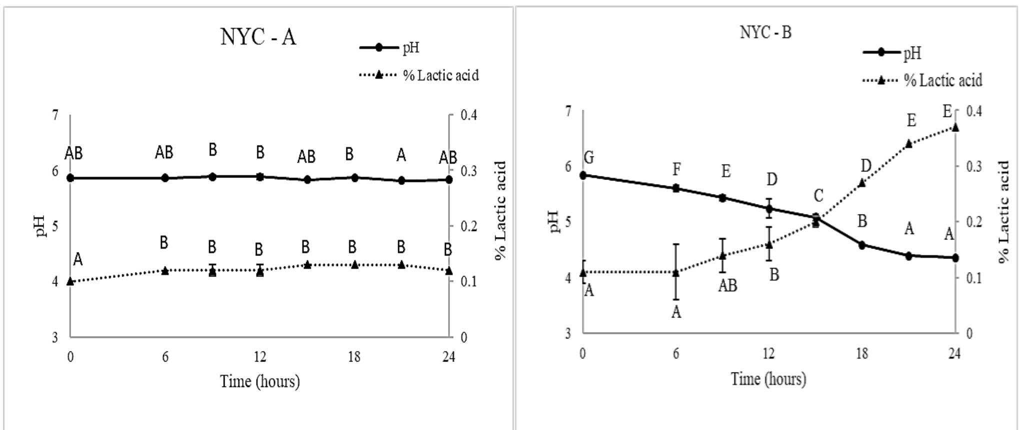

Figure 1. pH (●) and % lactic acid acidity (▲) in basic rice milk inoculated with NYC-A or NYC-B during 24 h fermentation. Data points are means of three independent fermentations (n=3). Bar on data points represent standard errors. The means with different letters in each experiment differ significantly ($p < 0.05$)

Neither *S. thermophilus* or *L. bulgaricus* (NYC-A) grew in the rice substrate during 24 h fermentation (Figure 2). The initial populations of *S. thermophilus* and *L. bulgaricus* showed log 6.52 ± 0.32 cfu/g and log 6.64 ± 0.18 cfu/g, respectively. However, after 24 h fermentation, the number of both *S. thermophilus* and *L. bulgaricus* decreased significantly ($p < 0.05$) to log 5.8 ± 0.21 cfu/g and log 5.67 ± 0.12 cfu/g, respectively. In contrast to NYC-B, *S. thermophilus*, *Lactobacillus* sp. (*L. bulgaricus* and *L. acidophilus*) and *B. lactis* strains used in this study grew in the rice milk substrate and their populations stabilized after 18, 21 and 24 h of incubation, respectively. After 24 h incubation, the number of *S. thermophilus*, *Lactobacillus* sp. (*L. bulgaricus* and *L. acidophilus*) and *B. lactis* were log 7.54 ± 0.49 cfu/g, log 7.54 ± 0.21 cfu/g and log 7.94 ± 0.48 cfu/g, respectively.

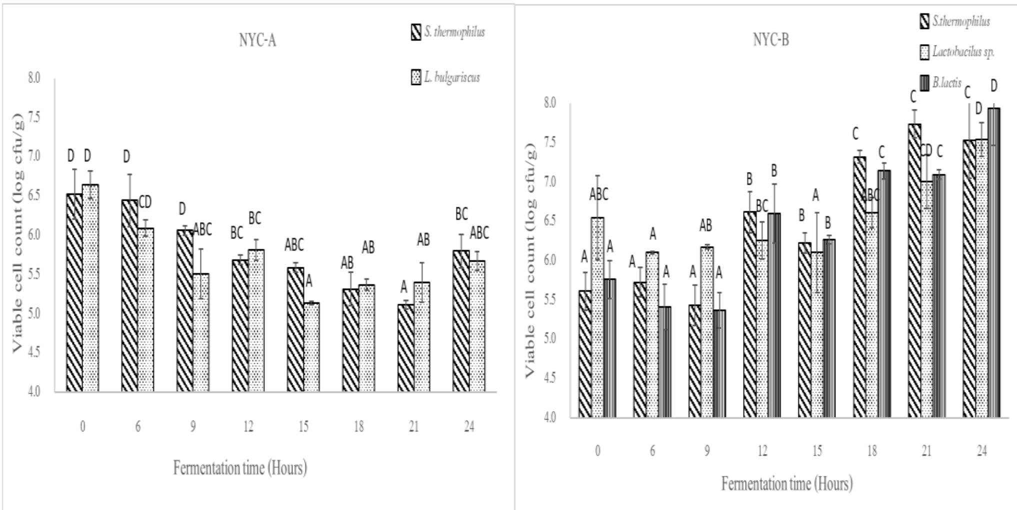

Figure 2. Changes in viable microbial counts of *S. thermophilus, L. bulgaricus, Lactobacillus* sp. (*L. bulgaricus* + *L. acidophilus*) and *B. lactis* in basic rice milk inoculated with NYC-A or NYC-B during 24 h fermentation. Data are the means of three independent fermentations (n=3). Bars on data points represent standard errors. The means with different letters in each strain differed significantly ($p < 0.05$)

Rice based yoghurt preparations

The effect of rice milk supplementation using the combination of glucose and gelatinized rice flour on the acidifying activity of the commercial non-dairy yoghurt cultures (NYC-A and NYC-B) is shown in Tables 1 and 2. The rice milk culture NYC-A, supplementation with different final concentration of glucose (5-15% w/w) did not significantly ($p < 0.05$) altered the pH value, whereas supplementation with different final concentration of gelatinized rice flour (30-70% w/w) significantly ($p < 0.05$) increased the acidification rate after 6 h fermentation. As NYC-A grew, they generated lactic acid, reducing the pH during the fermentation. The greatest effect of supplementations was seen in combination with the final concentration of glucose 5-10% (w/w) and gelatinized rice flour 50-70% (w/w). These formulations reached a pH $\leq$ 4.5 and had % lactic acid acidity higher than 0.2% (w/w) after 15 h of fermentation (data not shown). Rice milk culture, NYC-B (Table 2), containing different concentrations of glucose 5-15% (w/w) did not significantly ($p < 0.05$) alter the ability of the microbial culture to change the pH, whereas samples containing different concentration of gelatinized rice flour (% w/w) significantly ($p < 0.05$) changed the pH values. As NYC-B grew, they produce lactic acid which decreased in pH levels. The pH level in the supplemented samples was progressively reduced during the 24 h of fermentation. Only the rice milk containing glucose 5-10 % (w/w) together with gelatinized rice flour 50-70% (w/w) reached a pH ≤ 4.5 and had % lactic acid acidity 0.37% (w/w) (data not shown) after 12 h of incubation.

Table 1. Changes in pH as a function of incubation time during the acidification of rice milk containing different concentrations of glucose combined with different concentrations of gelatinized rice flour using culture NYC-A

Hour	Glucose content (% w/w)	Gelatinized rice flour content (% w/w)			
		10	**30**	**50**	**70**
0	5	5.89^{aA} ±0.05	5.88^{aA} ±0.02	5.82^{aA} ±0.05	5.87^{aA} ±0.05
	10	5.86^{aA} ±0.17	5.85^{aA} ±0.02	5.80^{aA} ±0.03	5.86^{aA} ±0.02
	15	5.83^{aA} ±0.04	5.82^{aA} ±0.16	5.88^{aA} ±0.14	5.80^{aA} ±0.09
6	5	5.92^{cA} ±0.03	5.36^{bA} ±0.02	5.27^{aA} ±0.09	5.39^{bA} ±0.08
	10	5.94^{cA} ±0.04	5.43^{bB} ±0.02	5.31^{aA} ±0.05	5.45^{bA} ±0.01
	15	5.88^{bA} ±0.12	5.60^{aC} ±0.03	5.57^{aB} ±0.02	5.44^{aA} ±0.12
9	5	5.86^{bA} ±0.05	5.24^{aA} ±0.06	5.23^{aA} ±0.07	5.30^{aA} ±0.06
	10	5.86^{cA} ±0.08	5.15^{aA} ±0.00	5.24^{bA} ±0.16	5.28^{bA} ±0.04
	15	5.80^{bA} ±0.13	5.27^{aA} ±0.09	5.34^{aA} ±0.01	5.35^{aA} ±0.04
12	5	5.85^{bA} ±0.04	5.03^{aA} ±0.06	5.11^{aA} ±0.08	5.08^{aA} ±0.05
	10	5.90^{bA} ±0.08	5.09^{aAB} ±0.00	5.18^{aA} ±0.06	5.16^{aA} ±0.06
	15	5.84^{bA} ±0.13	5.25^{aB} ±0.09	5.29^{aA} ±0.22	5.32^{aB} ±0.07
15	5	5.84^{cA} ±0.07	4.92^{bA} ±0.01	4.54^{aA} ±0.03	4.55^{aA} ±0.04
	10	5.89^{cA} ±0.12	4.96^{bA} ±0.03	4.56^{aA} ±0.04	4.54^{aA} ±0.03
	15	5.74^{cA} ±0.09	5.10^{bA} ±0.03	4.80^{aB} ±0.02	4.82^{aB} ±0.05
18	5	5.76^{cA} ±0.02	4.94^{bA} ±0.01	4.56^{aA} ±0.07	4.48^{aA} ±0.10
	10	5.87^{cA} ±0.06	5.00^{bA} ±0.02	4.52^{aA} ±0.05	4.49^{aA} ±0.07
	15	5.85^{cA} ±0.1	5.12^{bB} ±0.06	4.79^{aB} ±0.02	4.67^{aB} ±0.06
21	5	5.63^{cA} ±0.05	4.65^{bA} ±0.06	4.46^{aA} ±0.02	4.50^{abAB} ±0.13
	10	5.83^{cB} ±0.04	4.87^{bB} ±0.03	4.44^{aA} ±0.05	4.43^{aA} ±0.03
	15	5.87^{cB} ±0.15	4.95^{bB} ±0.01	4.68^{bB} ±0.02	4.63^{aB} ±0.08
24	5	5.58^{cA} ±0.04	4.64^{bA} ±0.05	4.49^{aA} ±0.03	4.48^{aA} ±0.10
	10	5.78^{cB} ±0.02	4.85^{bB} ±0.03	4.48^{aA} ±0.02	4.50^{aA} ±0.05
	15	5.84^{bB} ±0.14	4.88^{bB} ±0.11	4.70^{aB} ±0.04	4.67^{aA} ±0.11

Data are the mean of three independent fermentations (n=3). Means with different letters (eg. a, b) in each line and in each column (e.g. A, B) in the same fermentation hour differ significantly ($p<0.05$).

Table 2. Change in pH as a function of incubation time during the acidification of rice milk containing the different concentrations of glucose together with different concentrations of gelatinized rice flour using culture NYC-B

Hour	Glucose content (% w/w)	Gelatinized rice flour content (% w/w)			
		10	**30**	**50**	**70**
0	5	5.82^{aA} ±0.05	5.86^{aA} ±0.02	5.85^{aA} ±0.05	5.84^{aA} ±0.05
	10	5.86^{aA} ±0.02	5.85^{aA} ±0.02	5.82^{aA} ±0.03	5.82^{aA} ±0.02
	15	5.85^{aA} ±0.06	5.87^{aA} ±0.10	5.86^{aA} ±0.14	5.80^{aA} ±0.10
6	5	5.45^{bA} ±0.06	5.33^{abA} ±0.08	5.24^{abA} ±0.03	5.08^{aA} ±0.22
	10	5.42^{cA} ±0.04	5.32^{bA} ±0.05	5.47^{cB} ±0.00	5.19^{aA} ±0.04
	15	5.26^{abB} ±0.04	5.36^{cb} ±0.01	5.33^{bB} ±0.03	5.15^{aA} ±0.17
9	5	5.28^{bA} ±0.00	5.03^{abA} ±0.06	4.92^{aB} ±0.02	4.95^{aA} ±0.30
	10	5.35^{aB} ±0.05	5.04^{abA} ±0.07	4.78^{aA} ±0.07	5.09^{abA} ±0.31
	15	5.42^{bB} ±0.03	5.14^{aA} ±0.08	4.95^{aB} ±0.09	4.99^{aA} ±0.23
12	5	4.62b^{A} ±0.08	4.60b^{A} ±0.07	4.36^{aA} ±0.06	4.36^{aA} ±0.06
	10	4.95^{cB} ±0.10	4.57^{bA} ±0.08	4.39^{aA} ±0.05	4.28^{aA} ±0.07
	15	5.07^{cB} ±0.05	4.75^{bB} ±0.04	4.51^{aB} ±0.03	4.44^{aA} ±0.12
15	5	4.72^{cA} ±0.11	4.38^{abA} ±0.06	4.28^{aA} ±0.04	4.44^{bA} ±0.06
	10	5.10^{cC} ±0.12	4.34^{aA} ±0.06	4.18^{aA} ±0.04	4.35^{abA} ±0.17
	15	4.95^{bB} ±0.10	4.42^{aA} ±0.07	4.28^{aB} ±0.05	4.43^{aA} ±0.14
18	5	4.12^{aA} ±0.03	4.09^{aA} ±0.04	4.07^{aA} ±0.03	4.07^{aA} ±0.10
	10	4.37^{dB} ±0.02	4.17^{cB} ±0.04	4.10^{bA} ±0.03	4.02^{aA} ±0.04
	15	4.65^{dC} ±0.08	4.46^{cC} ±0.03	4.29^{bB} ±0.07	4.14^{aA} ±0.06
21	5	4.36^{bAB} ±0.10	4.10^{aA} ±0.03	4.06^{aA} ±0.03	4.14^{aA} ±0.12
	10	4.32^{bA} ±0.05	4.09^{aA} ±0.04	4.04^{aA} ±0.03	4.16^{aA} ±0.12
	15	4.51^{cB} ±0.07	4.18^{bB} ±0.04	4.06^{aA} ±0.03	4.18^{bA} ±0.07
24	5	4.04^{bA} ±0.03	4.03^{abA} ±0.02	4.02^{abA} ±0.01	3.97^{aA} ±0.06
	10	4.22^{dB} ±0.02	4.10^{cB} ±0.03	4.03^{bA} ±0.03	3.96^{aA} ±0.03
	15	4.41^{cC} ±0.09	4.31^{cC} ±0.04	4.18^{bB} ±0.06	3.96^{aA} ±0.02

Data are the means of three independent fermentations (n=3). Means with different letters (e.g. a, b) in each line and in each column (e.g. A, B) in the same fermentation hour differ significantly ($p < 0.05$).

Physico-chemical and microbiological properties of rice-based yoghurts

Physico-chemical and microbiological properties of rice-based yoghurt NYC-A and NYC-B cultures obtained after 15 h and 12 h of fermentations are shown in Table 3. Carbohydrates were the main component in both of rice-based yoghurts (8.03-8.49% w/w) followed by protein (3.29-3.49% w/w) lipids (2.80-2.90% w/w), fiber (0.90-2.22) and ash (0.39-0.40). The product of culture NYC-A contained 0.22±0.03% lactic acid, pH 4.5±0.02 and viable cell counts of *S. thermophilus* and *L. bulgaricus* of log 7.72 cfu/g and log 7.64 cfu/g, respectively. NYC-B contained 0.36% lactic acid, pH 4.38 and viable cell counts of *S. thermophilus*, *Lactobacillus* sp. (*L. bulgaricus* + *L. acidophilus*) and *B. lactis* of log 7.52 ± 0.13 cfu/g, log 7.43 ± 0.18 cfu/g and log 7.4 ± 0.21 cfu/g respectively. Based on the results, the physico-chemical and microbiological properties NYC-A, NYC-B yogurts were in the ranges demonstrated in selected commercial soy, almond and coconut yoghurts sold in Thailand (Table 3).

Table 3. Physico-chemical and microbiological properties of rice-based yoghurt NYC-A and NYC-B cultures in comparison with commercial non-dairy yoghurts sold in Thailand

Physico-chemical Microbiological properties	NYC-A Yoghurt[a]	NYC-B Yoghurt[a]	Soy Yoghurt[b]	Almond Yoghurt[b]	Coconut Yoghurt[b]
Protein (%)	3.29	3.49	6	4	4
Lipid (%)	2.80	2.90	0.5	8	2.5
Fiber (%)	2.22	0.90	7	1	-
Ash (%)	0.40	0.39	-	-	-
Carbohydrate (%)	8.03	8.49	12	9	13
pH	4.50	4.38	4.26	4.21	4.22
% Lactic acid	0.22	0.36	0.47	0.55	0.51
S. thermophilus	7.72	7.52	5.31	7.13	7.41
Lactobacillus sp.	7.64	7.43	4.91	8.33	7.25
B. lactis	-	7.58	5.40	7.58	5.40

[a] Proximate analysis of rice based yoghurt.
[b] Non-dairy yoghurts was explored from the market in Thailand.

Shelf-life study of rice-based yoghurts

The shelf-life study showed that during 7 days of storage at 4 ℃, the pH of the rice-based yoghurts producing from NYC-A significantly ($p < 0.05$) decreased from 4.50 ±0.02 to 4.35 ±0.01 and remained unchanged during 28 days storage, whereas the pH of the rice-based yoghurts producing from NYC-B gradually decreased and stabilized during 21 days storage.Acidification in rice-based yoghurts inoculated with NYC-A or NYC-B cultures showed the similar trends, with acidification gradually increasing throughout 14 days of storage and remained unchanged during 28 days subsequent storage (Figure 3).

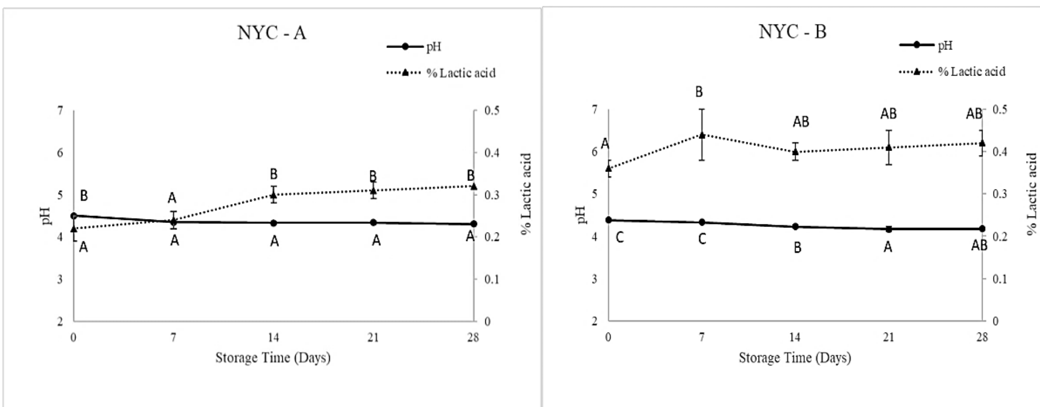

Figure 3. pH (●) and % lactic acid acidity (▲) in rice-based yoghurt culture NYC-A or NYC-B during 28 days of storage at 4 ℃. Data points are means of three independent fermentations (n=3). Bars on data points represent standard errors. The means with different letters in each experiment differed significantly ($p < 0.05$)

With rice-based yoghurt culture NYC-A, the number of *S. thermophilus* significantly ($p < 0.05$) increased from log 7.72 ± 0.13 cfu/g to log 7.95 ± 0.10 cfu/g after 7 days of storage then remained stable for up to 28 days (log 7.80 ± 0.04 cfu/g), whereas the number of *L. bulgaricus* (day 0 = log 7.64 ± 0.10 cfu/g) did not significantly ($p < 0.05$) change during 28 days of storage at 4 ℃ (log 7.42 ±0.05 cfu/g) (Figure 4). With rice-based yoghurt culture NYC-B, the number of *S. thermophilus* and *Lactobacillus* sp. (*L. bulgaricus* and *L. acidophilus*) significantly ($p < 0.05$) decreased from log 7.52 ±0.13 cfu/g to log 6.47 ±0.17 cfu/g and log 7.43 ± 0.18 cfu/g to log 6.22 ±0.18 cfu/g after 21 days of storage and stabilized for up to 28 days, respectively, whereas the number of *B. lactis* significantly ($p < 0.05$) decreased from log 7.40 ±0.21 cfu/g to log

6.57±0.16 cfu/g after 7 days of storage then stabilized during 28 days. Yeast mold, *E. Coli* and *S. aureus* were not detected in any sample during 28 days storages.

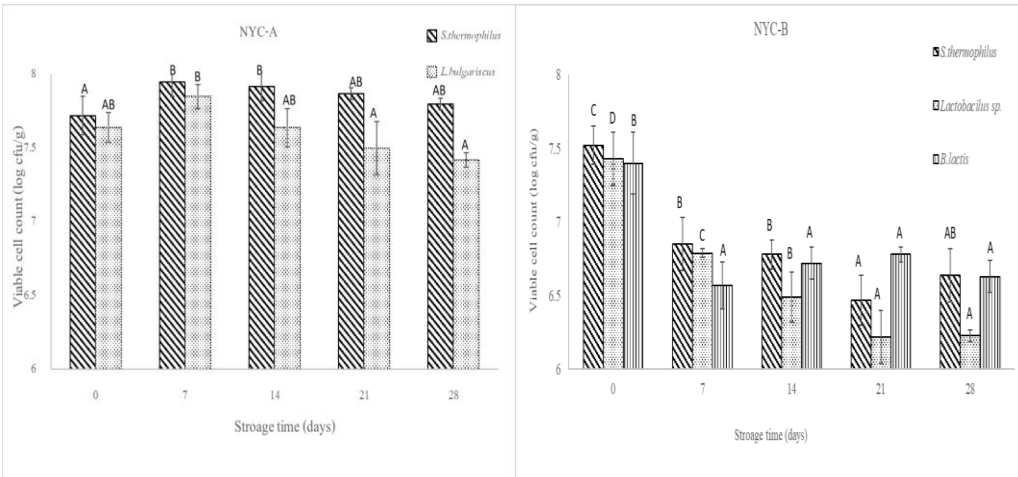

Figure 4. Changes in viable microbial counts of *S. thermophilus, L. bulgaricus, Lactobacillus* sp. (*L. bulgaricus* + *L. acidophilus*) and *B. lactis* in rice-based yoghurt culture NYC-A or NYC-B during 28 days of storage at 4 ℃. Data are the mean of three independent fermentations (n=3) Bars on data points represent standard errors. The means with different letters in each strain differ significantly ($p < 0.05$)

Discussion

This preliminary study was conducted to determine the suitability of basic rice milk substrate, prepared according to Plengsaengsri *et al*. (2019), for rice-based yoghurt production. Amrane (2001) showed that the growth rate of lactic acid bacteria is correlated to acidification and our results show that the basic rice formulation was not suitable for the growth or for enhancing the acidification rate of commercial non-dairy yoghurt cultures. The acidification rate of the fermented milk is mainly affected by the composition of starter cultures (Sodini *et al*., 2002) and several methods have been developed to improve the growth of lactic acid bacteria by acting on milk fortifications, such as adding sugar, skim milk or pulse extracts (Sodini *et al*., 2002; Kim *et al.*, 2009; Zare *et al*., 2011). In this study, the addition of glucose and gelatinized rice flour was shown to improve growth of the lactic acid bacteria and improved the organoleptic properties of the yogurt as previously reported by

Gallo *et al*. (2019). Also, the addition of glucose together with gelatinized rice flour significantly ($p < 0.05$) accelerated the acidification rate, with the greatest effect observed with gelatinized rice flour. The most effective combination, of those tested, was found to be rice milk containing 5% (w/w) glucose together with 50% (w/w) gelatinized rice flour for both NYC-A and NYC-B cultures. These values were in the ranges of previously reports for rice flour-based yoghurts. However, the difference in pH, % lactic acid acidity and fermentation time observed in this study compared to previous reports and could be due to fermentation conditions (starter culture strains, inoculation size, temperature) and substrate compositions (Gallo *et al*., 2019; Cáceres *et al*., 2019; Magala *et al*., 2015; Coda *et al*, 2012). Our rice-based yoghurts had similar levels to those of carbohydrate, protein, lipid, pH, acidity and lactic acid bacteria count to those found in selected commercial soy, almond and coconut yoghurts sold in Thailand. NYC-A grew slower than NYC-B, even when inoculated at the same concentration, which could be due the strain of NYC-A used did not adapt well to the environment. No contamination from other microflora was detected throughout the storage period. Our rice-based yoghurts both NYC-A and NYC-B cultures kept at least 28 days at 4 ℃ without deterioration.

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