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Manufacture and properties of purple waxy corn yogurt and its application in probiotic salad dressing production

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Abstract

This research was aimed to produce probiotic salad dressing with good acceptability. For this purpose, purple waxy corn milk samples prepared from corn kernels and water at four different ratios as 1:1, 1:2, 1:3 and 1:4 were used to produce purple waxy corn milk yogurt and it was found that corn kernels and water at 1:1 ratio gave the highest total soluble solid and viscosity which were higher than cow milk. Then, all corn milk ratios were used for yogurt production and the result showed that purple waxy corn milk yogurt made from purple waxy corn and water at 1:1 ratio (formula 1) provided the highest viscosity, also, this yogurt formula had the closest pH and titratable acidity values with a control yogurt from cow milk. Lactic acid bacteria were also investigated and it was shown that three strains, including Lactobacillus acidophilus, Streptococcus thermophilus and Bifidobacterium were growth during yogurt process, reaching the numbers of 8.45–8.65 log CFU/g. Finally, corn milk yogurt containing probiotic bacteria was used and mixed to salad dressing and it revealed that salad dressing made from corn milk yogurt formula 1 contributed to the highest viscosity and overall acceptability score. Viability of probiotic bacteria in salad dressing that mixed with purple waxy corn milk yogurt formula 1 was detected at the numbers of 6.70 ± 0.20 log CFU/g which were higher than the minimum therapeutic dose (6.00 log CFU/g). Consequently, probiotic salad dressing produced could be considered as one of the potential probiotic foods which could deliver benefits to consumers.

Keywords: Purple waxy corn, yogurt, probiotic, salad dressing

1. Introduction

The demand of consumers for healthier foods has greatly increased over the last decades, and was the impetus for the development of functional food products via the incorporation of ingredients believed to have one or more beneficial effects on human health. Maintaining gut health is one of the main targets of functional foods. Hence, a major development of functional food products is associated with the application of probiotics.Waxy or glutinous corn (Zea mays L. var. ceratina) is increasingly consumed in China, Korea, Vietnam, Taiwan, Laos, Myanmar and Thailand, and is harvested while immature and consumed on the cob as fresh food similar to sweet corn (Harakotr et al., 2014).

It is consumed as a boiled fresh corn on the cob due to its palatable glutinous texture. Purple waxy corn seed is an important source of anthocyanins with distinctive features regarding coloring of foods as well as the bio−functional components. Waxy corn has similar properties to sticky rice because its endosperm starch consists of mainly amylopectin. Small-scale farmers in Thailand and Southeast Asia have grown waxy corn as a cash crop next to rice for fresh consumption for more than a century (Ketthaisong et al., 2015).

Today, most consumers begin to be more interested in their health by focusing on healthy food. Fermented products are one of the healthiest products; especially yogurts, which are dairy products, produced by microbial fermentation processes. The predominant organisms in these dairy products are lactic acid bacteria (LAB), for example, Lactobacillus bulgaricus and Streptococcus thermophilus which are recognized as safe microflora (Generally Recognized as Safe; GRAS) (Tanganurat et al., 2014). The function of the starter cultures is to ferment lactose (milk sugar) to produce lactic acid. The increase in lactic acid decreases pH and causes the milk to clot, or form the soft gel that is characteristic of yogurt (Chaikulsareewath et al., 2015). Fermented dairy products have so far been the predominant commercial carriers of probiotics, however, health concerns like lactose intolerance, allergies to milk proteins, cholesterol and saturated fatty acid content have prompted research on the development of non−dairy carriers of probiotics (Srisuvor, 2016).

Probiotics are defined as living microorganisms, which when ingested in sufficient amounts, beneficially influence the health of the host by improving the composition of intestinal microflora (Yerlikaya, 2014). Probiotics market is steadily expanding which can be explained by growing health concern of consumers coupled with to the efficacy of probiotics (Novik and Savich, 2019). To convey health benefits, probiotic bacteria should be viable and available in sufficient numbers of at least 1×106 CFU ml-1or g-1 of product at the time of consumption (Isa and Razavi, 2017).

Salad dressings are semi−liquid, acidic, oil−in−water emulsions exhibiting a characteristic of creamy texture. The main emulsifiers of this type of emulsions are lipoproteins of egg yolk although in some commercial salad dressing products other emulsifiers such as milk proteins or gum arabic may also be employed. Polysaccharides (xanthan, pectins, etc.), are usually incorporated in commercial salad dressing products, especially those of low oil content, to stabilize the oil droplets against creaming and to compensate for the loss of thickening properties originating from their reduced oil content (Mantzouridou et al., 2013). Cooked starch may also be incorporated in these products since starch gelatinization results in the release of polysaccharides which may then act as emulsion stabilizers and thickeners (Mahmood et al., 2017). For this reason, the aim of this study was to investigate suitable corn kernels and water ratio for preparing purple waxy corn milk yogurt and use it in the manufacturing of salad dressing with functionality enhancement such as a probiotic cell. In addition, physical properties (color and viscosity), chemical properties (pH, titratable acidity, and total soluble solid), sensory acceptability and cell viability of lactic acid bacteria in the probiotic salad dressing were determined.

2. Materials and Methods

2.1 Plant and ingredient materials and sample preparation

For this study, purple waxy corn variety ''Khao Kum Wan", purchased from Plant Breeding Research Center for Sustainable Agriculture, Khon Kaen University, Thailand was used. The whole corn was husked, silk removed, and finally washed with tap water. The corn kernels were separated from its cob and stored at −20°C until analysis. Sugar (Mitr phol, Thailand), skim milk powder (Windmill, Thailand), cooking oil (Angoon, Thailand), egg, salt (Prung thip, Thailand), vinegar (Aor Sor Ror, Thailand), and mustard (Best foods, Thailand) were purchased from the local market.

2.2 Different ratios of corn kernels and water to prepare purple waxy corn milk yogurt

Corn milk was prepared from corn kernels and water at the ratios of 1:1, 1:2, 1:3 and 1:4 (w/w). After that, the mixtures were blended for 5 min using a multi−function food processor mixer (Bear, LLJ−A12G1, China) and filtered through two layers of nylon to achieve purple waxy corn milk (PWCM). The corn milk was then analyzed for its physical (L*, a*, b* and viscosity) and chemical characteristics (total soluble solid, pH, and titratable acidity).

For yogurt preparation, corn milk at four different ratios was added separately with sugar (4%, w/v), and skim milk (9%, w/v), heated at 63°C for 30 min (low temperature long time pasteurization), cooled down to 43−45ºC and then 0.02% (w/v) commercial freeze-dried yogurt starter (YoFlex®:ABT−3, CHR HANSEN, Denmark) including *L. acidophilus, S. thermophilus* and *Bifidobacterium* was added. After that, they were incubated at 43ºC for 6−7 h until a pH of 4.0−4.6 was obtained. The purple waxy corn milk yogurts were prepared in triplicate for each treatment. The corn milk yogurt was then analyzed for its physical characteristics (L*, a*, b* and viscosity) at the end of the fermentation process and chemical characteristics (pH, and titratable acidity) during fermentation.

2.3 Salad dressing preparation

Salad dressing formulations consisted of different ingredients (g/100 g) are shown in Table 1 (Sarabood, 2009). All the ingredients used were purchased from a local supermarket. For each preparation, one batch (100 g) of salad dressing was produced. The sample preparation was as follows: the egg yolk and vinegar were first mixed together; then all the other ingredients, except the oil, were added and homogenized for 5 min in a mixer (Bear, DDQ−A40A1, China). Finally, the oil was added and additional homogenization for 2 min was done until homogeneous. The salad dressing samples were kept in a refrigerator at 4 ± 2°C until their analysis. All samples were analyzed for physical properties, chemical properties, microbiological properties, and sensory evaluation within one day of production.

Table 1 Ingredients for probiotic salad dressing production

Formula	Yogurt	Cooking oil	Sugar	Egg yolk	Salt	Vinegar	Mustard
Control	50 (CM)	30	5	8	1	5	1
PSD−1	50 (PWCM; 1:1)	30	5	8	1	5	1
PSD−2	50 (PWCM; 1:2)	30	5	8	1	5	1
PSD−3	50 (PWCM; 1:3)	30	5	8	1	5	1
PSD−4	50 (PWCM; 1:4)	30	5	8	1	5	1

Note: PSD = Probiotic Salad Dressing, CM = Cow Milk, PWCM = Purple Waxy Corn Milk; corn kernel: water

2.4 Color evaluation

The colors of all samples (purple waxy corn milk, purple waxy corn yogurt, and probiotic salad dressing) were evaluated using a colorimeter (ColorFlex Ez 45/0 L, USA) calibrated with a white calibration plate. The data was expressed in terms of L*, a* and b* parameters, where L* represents lightness (from 0 – black to 100 – white); a* and −a* for redness and greenness respectively; and b* and −b* for yellowness and blueness respectively. All samples were measured in triplicate.

2.5 Viscosity test

Sample apparent viscosity was measured using a Brookfield viscometer (Brookfield viscometer DV2T, USA). The analysis was carried out at 25°C by using spindle (No.63), and at rotational speed of 50 rpm for all samples. Results recorded in centipoises (cP) after 50 s of shearing. Purple waxy corn milk, purple waxy corn yogurt and probiotic salad dressing were tested under the same condition.

2.6 Measurement of pH, titratable acidity and total soluble solid value

The pH value was measured by pH meter (Sartorius, Docu−pH, Germany). The total soluble solid was measured by Digital Hand−held "Pocket" Refractometer PAL−1 (ATAGO, Japan) and recorded as °Brix. Titratable acidity was measured according to AOAC (2000); the samples were thoroughly mixed and 10 g of each sample was weighed accurately using an electronic balance. The samples were mixed with 100 ml of distilled water, added 2−3 drops of 1% phenolphthalein indicator solution, and then titrated with 0.1 N NaOH solutions until the substance reached a pH value 8.2, corresponding to the end point of the phenolphthalein. The amount of acidity was calculated based on the percentage of lactic acid and calculated according to the following formula:

$$\text{Titratable acidity (\% lactic acid)} = \frac{V \times N \times 90.08}{W \times 10}$$

Where V= volume of NAOH consumed, ml; N = normality of the NaOH; and W = weight of the test sample, g.

The pH and titratable acidity of yogurt production were evaluated during the fermentation process.

2.7 Determination of Total Lactic Acid Bacteria (LAB)

Viable counts of lactic acid bacteria were enumerated using a pour plate technique and Lactobacillus deMan Rogosa and Sharpe (MRS) Agar (Himedia, India). For the microbiological assessments, 25 g of sample were added to 225 mL of sterile 0.1% peptone water (Himedia, India) and blended in a stomacher (Seward, 400 Circulator Lab Blender, UK) for 1 min. The samples were serially diluted and 1 mL of an appropriate dilution was used to inoculate a plate in duplicate. The plates were incubated at 37°C for 48−72 h. The samples were examined for lactic acid bacteria in agreement with the procedure described by Srisuk (2016). All of the results were expressed in Colony Forming Unit (log CFU/g) (AOAC, 2000) and calculated according to the following formula:

$$\text{Lactic acid bacteria (log CFU/g)} = \log_{10} [(n \times d) / V]$$

Where n = number of colonies on the plate within the countable range of 30 to 300 colonies per plate; d = dilution factor; and V = sample volume of cultured plate.

2.8 Sensory Evaluation

The sensory evaluation was carried out in a sensory laboratory. Sensory analysis based on a 9−point hedonic scale of 1 to 9 (where scale 9 = Like Extremely, 8 = Like Very Much, 7 = Like Moderately, 6 = Like Slightly, 5 = Neither Like nor Dislike, 4 = Dislike Slightly, 3 = Dislike Moderately, 2 = Dislike Very Much, 1 = Dislike Extremely) was carried out. Sensory evaluation was performed by an untrained sensory panel consisting of 30 untrained assessors. Sensory evaluation was conducted on the samples after one day cold storage at 4 ± 2°C. The samples were tempered at 10 ± 1°C before tasting. The assessors were given representative probiotic salad dressing of 10 g placed in white plastic glass fitted with lids; all samples labeled with three−digit random numbers were randomly served to panelists. Five salad dressing samples were served at the same time, furthermore drinking water was provided to rinse the palate between samples (Naknaen *et al*., 2018). The assessed sensory characteristics were color, appearance, viscosity, texture, odor, flavor, taste and overall acceptability. The sensory evaluation was carried out in a testing area with properly controlled environmental conditions. Lighting of the room was also the same throughout the experiment with daylight white tone.

2.9 Statistical analysis

All data were reported as means ± standard deviation. Statistical analysis was performed using SPSS statistical software program version 19. The physical properties, chemical properties and probiotic counts of samples were analyzed by analysis of variance (ANOVA) using Completely Randomized Design. The results from sensory evaluation were analyzed by analysis of variance using Randomized Complete Block Design. The experimental data were subjected to an ANOVA and the Duncan's multiple range test (DMRT) ($P \leq 0.05$) was used to identify significant differences between group means.

3. Results and Discussion

3.1 Physiochemical properties of purple waxy corn milk

In order to get purple waxy corn milk that was suitable for yogurt production process; it was needed to study the amount of water to be mixed with corn kernels to produce corn milk. The results showed that the water content affected the properties of corn milk, which was statistically and significantly different (P≤0.05). The results shown were as follows:

Color values of cow milk and purple waxy corn milk (PWCM) prepared from the corn kernels with water at different ratios of 1:1, 1:2, 1:3 and 1:4 had L*, lightness value of 41.53−87.70, a*, redness to greenness value, of (−3.91) −0.30 and b*, yellowness to blueness value of 1.28-8.37, which were significantly different based on the statistical analysis (P≤0.05) (Table 2). It was found that the cow milk had the lightest color while PWCM 1:1 had the lowest lightness value of 41.53. This result was due to the white color of cow milk. Purple waxy corn, a plant in a family of Poaceae, is an important source of anthocyanin. Purple waxy corn seed is an important source of anthocyanin with distinctive features regarding coloring of foods as well as the bio−functional components (Polthum and Ahromrit, 2014). Moreover, anthocyanin is the pigment that shows the color of red, purple and blue (Simla *et al*., 2016). Therefore, purple waxy corn milk had a* value that was positive, which indicated red color, while as for b* parameter, cow milk had more yellow color than PCWM samples. Consequently, the purple waxy corn milk that had been mixed with large quantities of water produced lighter color than that formulated with a lower amount of water.

Table 2 Color values of cow and purple waxy corn milks prepared from corn kernels with water at different ratios

Treatments	L*	a*	b*
cow milk	87.70 ± 0.00^{a}	-3.91 ± 0.00^{d}	8.37 ± 0.01^{a}
PWCM; 1:1	41.53 ± 0.00^{d}	0.30 ± 0.02^{a}	3.40 ± 0.01^{c}
PWCM; 1:2	45.02 ± 0.02^{c}	0.11 ± 0.01^{b}	3.87 ± 0.04^{b}
PWCM; 1:3	45.71 ± 0.00^{b}	0.08 ± 0.02^{b}	3.41 ± 0.02^{c}
PWCM; 1:4	45.72 ± 0.02^{b}	0.02 ± 0.01^{c}	1.28 ± 0.03^{d}

Note: Values are mean ± SD (n = 3). Different superscripts in the same column indicate significant differences at P≤0.05, PWCM = Purple Waxy Corn Milk; corn kernel: water

For total soluble solid, pH and titratable acidity of corn milk, it was found that the ratio of water used to prepare corn milk had a significant impact on the chemical properties of corn milk. The total soluble solid of PWCM; 1:1 sample had the highest value of 13.30°Brix and was higher than cow milk (Table 3). The total soluble solid values of PWCM tended to be reduced when higher amount of water was mixed to the corn kernel. The total soluble solid is an indicator of the amount of dissolved solid in water, which refers to various kinds of sugars, including organic acids (Munoz−Robredo *et al.*, 2011). These substances could help to promote microbial growth during fermentation of yogurt process (Akin *et al*., 2007; Mantzourani *et al*., 2018).

The pH of corn milk at different formulas was statistically and significantly different with values in the neutral pH range, which were between 6.79 and 6.90. This value was much closed to cow milk and the corn milk acidity was lower than the cow milk acidity, which indicated that purple waxy corn milk could be a good substrate for microbial growth during fermentation. Trikoomdun and Leenanon (2016) had found that sweet corn milk prepared from corn kernels and water at ratios of 1:1, 1:2, and 1:3 (w/w) had a pH range of 6.6−6.7. The result indicated that pH of corn milk was near the optimal pH for the thermophilic lactic acid bacterium *S. thermophilus* which was between 6.5 and 7.5 (Hutkins and Nannen, 1993; Rault *et al*., 2009; Zhang *et al*., 2011). Furthermore, the lactic acid produced by *S. thermophilus* reduced the pH of the milk to an optimal level for *L. bulgaricus* (Mchiouer *et al*., 2017).

In the part of the titratable acidity value, it was found that increasing the amount of water used to prepare corn milk caused the milk acidity to be significantly declined ($P \leq 0.05$). Increasing the amount of water was able to dilute organic acids in corn milk that led to lower acid content in the samples.

Table 3 Total soluble solid, pH and titratable acidity of cow and purple waxy corn milks prepared from the corn kernels with water at different ratios

Treatments	Total Soluble Solid (°Brix)	pH	Titratable acidity (% lactic acid)
cow milk	12.22 ± 0.09^{b}	6.90 ± 0.01^{a}	0.09 ± 0.02^{a}
PWCM; 1:1	13.30 ± 0.08^{a}	6.79 ± 0.02^{d}	0.07 ± 0.02^{ab}
PWCM; 1:2	8.35 ± 0.05^{c}	6.82 ± 0.01^{c}	0.06 ± 0.03^{ab}
PWCM; 1:3	6.40 ± 0.08^{d}	6.86 ± 0.01^{b}	0.06 ± 0.01^{ab}
PWCM; 1:4	3.32 ± 0.09^{e}	6.90 ± 0.01^{a}	0.03 ± 0.01^{b}

Note: Values are mean ± SD (n = 3). Different superscripts in the same column indicate significantly differences at $P \leq 0.05$, PWCM = Purple Waxy Corn Milk; corn kernel: water

The viscosity of the corn milk was reduced according to the volume of water added to produce purple waxy corn milk. It was found that PWCM 1:1 had the highest viscosity value equaled to 1,938.00 cP and PWCM 1:4 had the lowest viscosity of 36.00 cP ($P \leq 0.05$) (Table 4). It was assumed that viscosity of yogurt could be improved by PWCM that had high viscosity. The physical attributes of yogurts, including perceived viscosity, are crucial aspects for yogurt quality, prevention of whey separation (wheying−off) and overall sensory consumer acceptance of yogurts. Increased yogurt viscosity was observed when the total solids content of sample was increased (Lee and Lucey, 2010). The chemical compositions of starch in corn kernels are amylose and amylopectin, and some intermediate materials that retain characteristics of both fractions (Ketthaisong *et al*., 2015). When purple waxy corn milk was heated, the treatment caused a gelatinization process that increased the viscosity in corn milk (Mahmood *et al*., 2017). It was suggested that yogurt production from purple waxy corn milk might contribute to higher viscosity of fermented corn milk. If compared with yogurt from cow milk, corn yogurt with formulas 3 and 4 had lower viscosities than the cow yogurt (control), while corn yogurt with formulas 1 and 2 had higher viscosities (Table 4). Consequently, yogurt with high viscosity resulted in a stronger network, and less syneresis (Karam *et al.*, 2013). A suitable yogurt viscosity depended on yogurt types and applications of yogurt (Ramaswamy *et al.*, 2015, Saleh *et al.*, 2020). In this experiment, yogurt was added as an ingredient of salad dressing. In order to make a good property salad dressing, one attribute of yogurt that was important was viscosity.

The viscosity value of corn milk yoghurt was decreased at higher ratios of water to corn kernel. The results showed that purple waxy corn yogurt formula 1 had the highest viscosity value equaled to 1,260.00 cP while formulas 3 and 4 had the lowest viscosities of 75.00 cP (Table 4). During cow milk yogurt process, milk heated at temperature higher than 70°C causes denaturation of whey protein; as a result the disulfide bond is formed between κ−casein and denatured whey proteins which makes a firmer gel (Mahomud *et al.*, 2017). In general, corn contains high amount of starch. The average content of starch in corn was estimated around 10−11%, yet the content of starch in sweet corn was lower than other varieties of corn. The starch content in corn is expected to play a role as natural stabilizer in product (Yasni and Maulidya, 2014). Furthermore, starch was preferred in yogurt industry, because it was a good thickener and had an ability to reduce yogurt flaws by improving texture and make the product more appealing to consumers (Saleh *et al.*, 2020). For these reasons, purple waxy corn milk formulas 1 and 2 had higher viscosity than the control sample. Starch in purple waxy corn milk produced a positive effect on the viscosity of yogurt, but increasing the ratio of water to corn kernels resulted in reducing the amount of starch in corn milk.

Table 4 Viscosity of purple waxy corn milk and yogurt prepared from corn kernels with water at different ratios

Purple waxy corn milk	Viscosity (cP)	Purple waxy corn yogurt	Viscosity (cP)
cow milk	48.00±2.10^{d}	control	367.50±169.70^{b}
PWCM; 1:1	1,938.00±8.48^{a}	formula 1	1,260.00±42.42^{a}
PWCM; 1:2	511.20±0.00^{b}	formula 2	502.50±10.60^{b}
PWCM; 1:3	355.20±3.39^{c}	formula 3	75.00 ±21.20^{c}
PWCM; 1:4	36.00±1.02^{e}	formula 4	75.00±10.60^{c}

Note: Values are mean ± SD (n = 3). Different superscripts in the same column indicate significantly differences at P≤0.05, PWCM = Purple Waxy Corn Milk; corn kernel: water

3.2 Physiochemical properties of purple waxy corn yogur

The purple waxy corn milk of PWCM 1:1, 1:2, 1:3 and 1:4 were used to produce purple waxy corn yogurt formulas of 1, 2, 3 and 4, respectively. The yogurt sample with cow milk was used as a control sample.

The results showed that purple waxy corn yogurt made from different purple waxy corn milk affected L*, a* and b* values of product (Table 5). The highest L* value was found in cow milk yogurt (control) followed in order by purple waxy corn milk yogurt formulas 4, 3, 2 and 1. The a* value of yogurt formula 1 had the highest value, while the b* value of yogurt control had the highest value. Moreover, the a* value of all corn milk yogurt was positive values

(+a* indicated redness) while the control formula had a negative value (−a* indicated greenness). Purple waxy corn milk had a purple color, while fermentation of the milk into yogurt formed more acidic condition, resulting in the changing of anthocyanin compounds from purple to magenta (Yang *et al*., 2009). The pH of corn milk yogurt was in the range of 4.04−4.07 (Fig 1) related to anthocyanin turns red-pink in acids (pH 1−6) (Ibrahim *et al*., 2011). Wahyuningsih *et al.* (2017) reported that anthocyanins are red at low pH (acidic conditions), at higher pH value, anthocyanins will provide color fading of colorless, yellow purple and blue. Therefore, yogurt made from purple waxy corn milk containing a large amount of corn kernels had a reduced brightness value while the redness value increases.

Table 5 Color values of purple waxy corn yogurt prepared from different formulas of purple waxy corn milk

Treatments	L*	a*	b*
control	92.69 ± 0.02[a]	-1.74 ± 0.02[e]	13.59 ± 0.02[a]
formula 1	50.40 ± 0.02[e]	14.48 ± 0.02[a]	4.29 ± 0.02[c]
formula 2	52.83 ± 0.02[d]	13.18 ± 0.01[b]	4.24 ± 0.02[d]
formula 3	58.18 ± 0.04[c]	12.18 ± 0.03[c]	2.55 ± 0.04[e]
formula 4	61.07 ± 0.04[b]	10.25 ± 0.01[d]	4.61 ± 0.01[b]

Note: Values are mean ± SD (n = 3). Different superscripts in the same column indicate significantly differences at P≤0.05.

The pH and titratable acidity of yogurt production were evaluated during the fermentation process. The results showed that as the fermentation period increased, resulting in lower pH values and higher titratable acidity values (Fig 1). The pH value of yogurt was between 4.04 and 4.29. Trikoomdun and Leenanon (2016) studied the production of corn milk yogurt supplemented with probiotics and it was found that during incubation at 42°C, pH tended to decrease whereas % acidity tended to increase particularly from 2 to 6 h. The titratable acidity (calculated as lactic acid) was between 0.38 and 0.79%. It was remarkable that the use of purple waxy corn milk to ferment yogurt could be carried out. It showed that in purple waxy corn milk, there were enough nutrients necessary for microorganism grow, promoting fermentation processes. Moreover, skim milk is good sources of protein for microbial growth, because it contains relatively high concentration of protein. The addition of sucrose, a disaccharide, also has the potential to be used as energy source for the growth of LAB (Pato *et al*., 2019). Especially, yogurt formula 1 had an acidity content equaled to 0.67%, which was closed to the cow milk yogurt. Before the fermentation process, cow and purple waxy corn milks were inoculated with starter culture (YoFlex®:ABT−3). The microorganisms in the culture grow well in substrate namely, cow milk. However, this study displayed that there was a potential for the cultures to ferment purple waxy corn milk. The result indicated that the lactic acid bacteria exploited the purple corn milk that contained carbohydrates or fermentable sugar during fermentation to produce lactic acid. Lactic acid was produced causing the milk pH level to be decreased and the acidity to be increased (Sandra *et al*., 2019). The titratable acidity of normal fermented milk products was within the range of 0.7−1.2% (Choi *et al*., 2016) and the minimum of titratable acidity, expressed as percent lactic acid (%, w/w) of yogurt was 0.6%

(Surono and Hosono, 2011); therefore, the fermentation was considered to have proceeded in a normal way. This experimental result suggested the possibility of applying purple waxy corn milk as a raw material in fermentation to provide healthy probiotic products.

All yogurt formulas were used to produce 5 probiotic salad dressing formulas in order to evaluate the effect of purple waxy corn yogurt on the salad dressing quality and survival of lactic acid bacteria in salad dressing samples after production process.

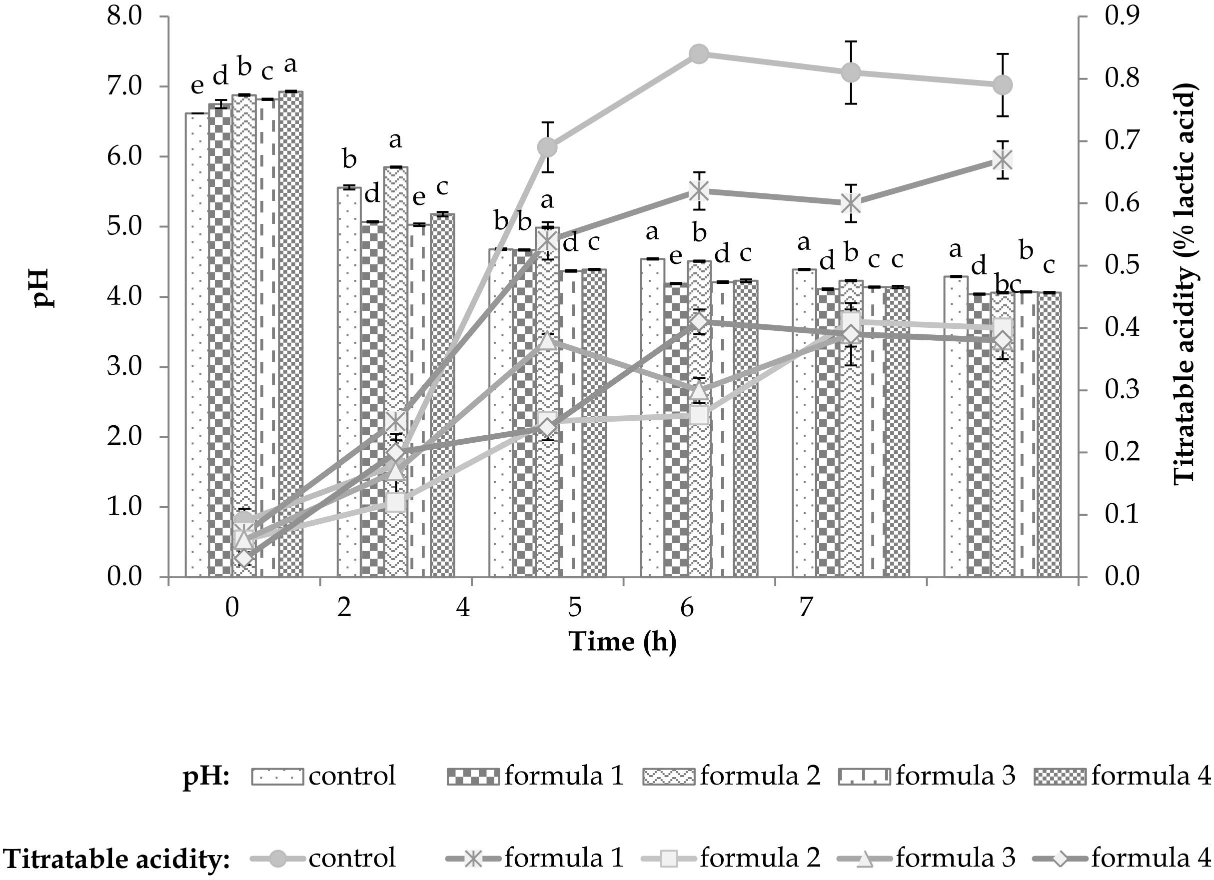

Fig 1 The pH (vertical axis on the left) and titratable acidity (vertical axis on the right) of purple waxy corn yogurt during fermentation time at 43°C for 7 h.
Note: Values are mean ± SD (n = 3). Different superscripts in the same fermentation time indicate significantly differences at P≤0.05.

3.3 Physiochemical properties, sensory evaluation and microbiological properties of probiotic salad dressing

The salad dressing samples produced from purple waxy corn yogurt formulas 1, 2, 3 and 4 were named as probiotic salad dressing (PSD) formulas PSD−1, PSD−2, PSD−3 and PSD−4, respectively. The salad dressing sample with cow milk yogurt was used as a control sample. Then, physiochemical properties, sensory evaluation and microbiological properties of all probiotic salad dressing samples were evaluated.

The values of color parameters (L*, a* and b*) of the salad dressing made with different formulas of purple waxy corn milk yogurt are shown in Table 6.

The highest L* and b* values were found in the control followed in order by probiotic salad dressing formulas PSD−4, PSD−3, PSD−2 and PSD−1. The a* value of probiotic salad dressing formula PSD−1 had the highest value, while the control had the lowest value. These differences in color values could be related to the color of purple waxy corn milk that used to produce yogurt.

Table 6 Color values of probiotic salad dressing prepared from different formulas of purple waxy corn milk yogurt

Treatments	L*	a*	b*
Control	84.73 ± 0.02^{a}	0.91 ± 0.01^{e}	37.35 ± 0.01^{a}
PSD−1	73.36 ± 0.02^{e}	6.55 ± 0.04^{a}	21.61 ± 0.03^{e}
PSD−2	73.95 ± 0.09^{d}	5.65 ± 0.03^{b}	25.29 ± 0.02^{d}
PSD−3	74.46 ± 0.06^{c}	5.16 ± 0.02^{c}	28.29 ± 0.05^{c}
PSD−4	74.75 ± 0.04^{b}	3.94 ± 0.01^{d}	33.56 ± 0.02^{b}

Note: Values are mean ± SD (n = 3). Different superscripts in the same column indicate significantly differences at $P \leq 0.05$, PSD = Probiotic Salad Dressing

Viscosity and pH of probiotic salad dressing are shown in Table 7. The experimental results showed that PSD−1 had the highest viscosity (1,967 cP) when compared with the other samples ($P \leq 0.05$). The other salad dressing formulas had lower viscosity values in the range of 181−552 cP. In addition, increasing the proportion of water towards corn kernel in the yogurt formulation significantly decreased the apparent viscosity of the salad dressing ($P \leq 0.05$). Polysaccharides are amongst the most widely used stabilizers in the industry to stabilize oil−in−water emulsions and control their rheological properties (Mantzouridou *et al.*, 2013). By using polysaccharides (corn starch) in corn milk, which adsorb onto the surface of the droplets and prevent aggregation by steric and/or electrostatic forces, enhancement of stability against flocculation and coalescence can be achieved (Paraskevopoulou *et al.*, 2005). According to previous studies, an increase in viscosity due to high concentration of starch probably causes the oil droplets to become smaller and unflocculated (Abedinzadeh *et al.*, 2016). Moreover, high levels of oil and egg yolk resulted in salad dressings with more pronounced viscous characteristics (Ma *et al.*, 2016). *Naknaen et al.* (2018) studied enhancing the quality attributes of salad dressing by incorporating Gac aril paste (Gac aril; red aril surrounding the seeds of Gac fruit) as a biologically active ingredient and it was found that as the level of Gac aril addition increased, the viscosity of the salad dressing tended to be decreased. Additionally, waxy corn showed as an important source of amylopectin, which were able to potentially waxy (glutinous) taste in food samples (Simla *et al.*, 2010). The pH of the salad dressing containing purple waxy corn yogurt was lower than the salad dressing containing cow milk yogurt. Moreover, pH values of all probiotic salad dressings were determined according to the standard specifications of Thai Industrial Standard no 1402−2540: mayonnaise and salad cream (Thai Industrial Standard, 1997) that acidity of less than 4.1 was one of the qualifications needed.

Table 7 Viscosity and pH of probiotic salad dressing prepared from different formulas of purple waxy corn milk yogurt

Treatments	Viscosity (cP)	pH
Control	547.20 ± 23.75[b]	4.04 ± 0.01[a]
PSD−1	1,967.00 ± 25.45[a]	3.86 ± 0.01[c]
PSD−2	552.00 ± 6.78[b]	3.87 ± 0.01[c]
PSD−3	238.80 ± 8.48[c]	3.88 ± 0.01[c]
PSD−4	181.20 ± 11.87[d]	3.92 ± 0.01[b]

Note: Values are mean ± SD (n = 3). Different superscripts in the same column indicate significantly differences at P≤0.05, PSD = Probiotic Salad Dressing

The sensory qualities of the salad dressing samples were evaluated in the terms of color, appearance, viscosity, texture, odor, flavor, taste and overall acceptability, as shown in Fig 2. The color and appearance scores of probiotic salad dressing (PSD) 1−4 samples showed significantly (P≤0.05) lower values when compared to the control due to the color of PSD 1−4 was quite light brown which was different from the color of the control sample. It should be noted from this study that the PSD−1 containing purple waxy corn yogurt formula 1 showed the highest score of viscosity, texture, flavor and taste compared to other treatments. Regarding the overall acceptability, it was found that PSD−1 had the highest acceptance with the score of 7.60 ± 0.20. This value was not significantly different from the control, PSD−2 and PSD−4. Consequently, the PSD−1 was accepted by the panelists, indicating that the sensory attributes of salad dressings containing purple waxy corn yogurt were considered acceptable.

For viable lactic acid bacteria cells in purple waxy corn yogurt and probiotic salad dressing shown in Fig 3, it was found that all purple waxy corn yogurt treatments maintained between 8.45−8.65 log CFU/g. This meant that purple waxy corn helped to support the growth of bacteria during the fermentation process, also making high levels of the lactic acid bacteria cells. Moreover, sugar and skim milk powder were added to corn milk yogurt process to provide an additional carbon source for the lactic acid bacteria. Yogurt made with skim milk and sugar was the preferred yogurt due to its mild acidic taste and medium thick consistency (Agustinah *et al*., 2019). In part of salad dressing, there was an approximately 1− to 2−log loss in the total number of viable cells for lactic acid bacteria in both salad dressing-control and PSD−1 samples during salad dressing production process. However, PSD−1 sample still contained high amount of lactic acid bacteria equaled to 6.70 log CFU/g as compared with the control (6.35 log CFU/g). In general, the lactic acid bacteria were derived from yogurt samples, whereas other ingredients used in salad dressing process did not contain these bacteria. In addition, the total minimum of lactic acid bacteria in probiotic food was 6 log CFU/g of viable cells to be accepted as the therapeutic minimum (Terpou *et al*., 2019). Moreover, purple waxy corn also contains a mixture of amylopectin, which is considered as a resistance starch (RS). Resistance starch is a prebiotic that helps support the survival of the probiotic microorganisms in food products (Fuentes−Zaragoza *et al*., 2011).

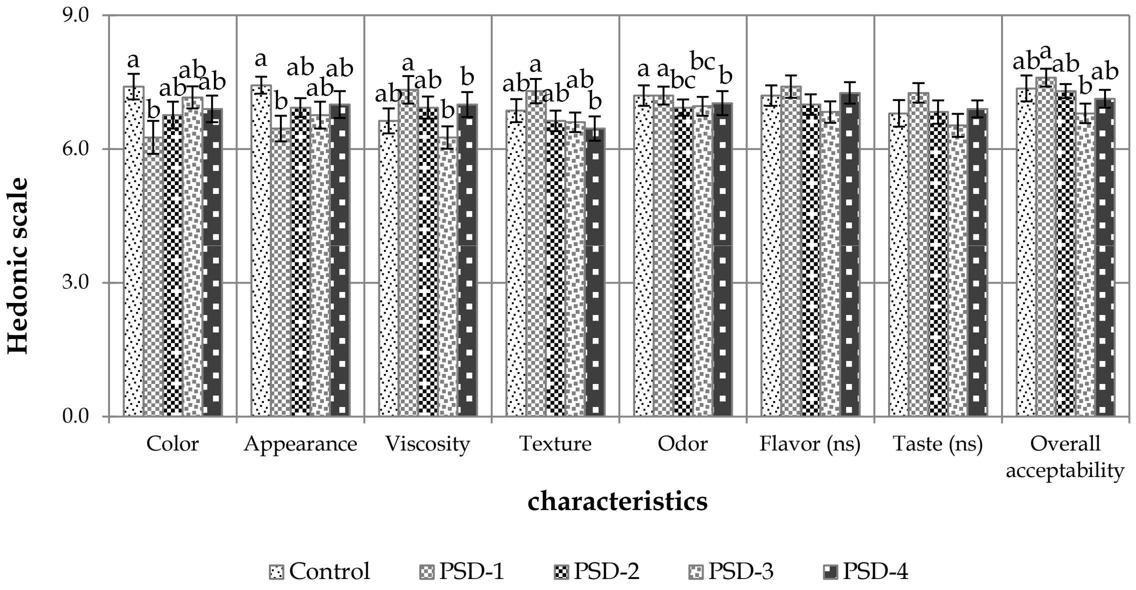

Fig 2 Sensory evaluation of probiotic salad dressing (PSD) samples prepared from different formulas of purple waxy corn yogurt. Note: Values are mean ± SD (n = 3). Different superscripts in each sensory characteristic indicate significantly differences at P≤0.05, PSD = Probiotic Salad Dressing. ns = non−significant differences (P>0.05).

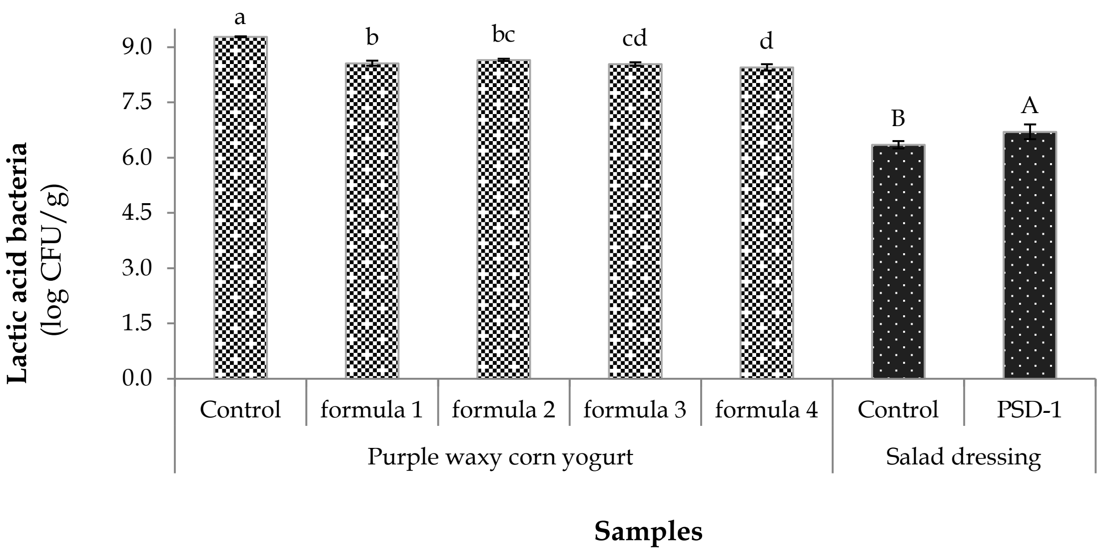

Fig 3 Cell viability of lactic acid bacteria in purple waxy corn yogurt samples and probiotic salad dressing (PSD) samples. Note: Values are mean ± SD (n = 3). Different superscripts (a−d) in the purple waxy corn yogurt sample and (A−B) in salad dressing sample indicate significantly differences at P≤0.05

4. Conclusion

This study demonstrated that purple waxy corn could be used as an alternative source of bio−functional components to fortify salad dressings. The values of their color parameters depended on the ratio of corn kernels with water as well as on the addition of yogurt. Purple waxy corn milk prepared from corn kernels and water at a ratio of 1:1 was used to produce purple waxy corn milk yogurt which contributed to the highest viscosity. Additionally, it was the suitable level for yogurt production which had the pH and titratable acidity closed to the control yogurt from cow milk. Yogurt formula 1 that made from purple waxy corn milk, containing corn kernels (1): water (1) could be a good ingredient for salad dressing production. Based on the results of physical properties, chemical properties and consumer acceptance, the highest accepted product was the salad dressing fortified with yogurt formula 1. Consequently, probiotic salad dressing produced could be considered as one of the potential probiotic foods which contribute benefits to consumers. Further research should be carried out regarding the survival of probiotic bacteria during storage.

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