

Development of Job's Tears Yogurt

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Abstract

This research aimed to formulate the Job's tears yogurt using Job's tears beverage. Simultaneously, the effects of added Job's tears beverage on microbiological behavior of yogurt cultures, chemical and sensory properties of the product were investigated as well as consumer acceptance. The Job's tears beverage was used to replace the milk to the extent of 0, 25, 50, 75 and 100% in the yogurt formula. The presence of Job's tears affected the acid production of yogurt cultures during fermentation. Although yogurt containing 25% of Job's tears beverage obtained the highest scores for sensory properties, the texture of the product was poor. Therefore, additions of 5, 10 and 15% milk powder were studied to improve the yogurt texture. Yogurt made by 10% milk powder achieved the highest preference scores. Most of the consumers (89%) accepted the product with preference scores of color, texture, flavor and overall liking of 7.05, 7.03, 6.55 and 7.27, respectively. Addition of Job's tears remarkably increased fiber and protein contents and influenced the color, flavor and texture of the product.

Keywords: *Coix lacryma-jobi L., fermented dairy product, microbiological behavior, consumer acceptance, sensory property, milk powder.*

1. Introduction

Job's tears (*Coix lacryma-jobi* L.), commonly known as coix, are originally from India and now are native to the East and Southeast Asian region in China, Japan, Myanmar, the Philippines, and Thailand. Their seeds have been used to produce food products as well as ornamental products such as rosaries and necklaces. De-hulled mature seeds can be cooked and consumed together with cooked rice. The polished and milled flour can be sometimes mixed with water and consumed as cooling drink like barley or flour water. The pounded grain of Job's tears can also be used for brewing of beer in the Garo, Karbi and Naga tribes (Burkill 1935). In China, they are used as traditional medicine and supplementary medicinal foods. The beneficial health effects of Job's tears are: (i) reducing liver fat accumulation, (ii) protecting from tumor stimulating compounds, (iii) protecting against viral infection, (iv) reducing allergic reaction, (v) reducing coronary artery disease and atherosclerosis, and (vi) reducing osteoporosis

(Chang *et al.* 2003; Hung and Chang 2003; Shih *et al.* 2004; Yu *et al.* 2011). In Thailand, after the seed coats are removed, the seeds are cooked, dried, deep fried or baked and then consumed as a snack with different flavors. Job's tears flour can be obtained after polishing and milling and used as food ingredient. Addition of Job's tears flour to substitute the wheat flour in bakery products is also of interest. Dough made from Job's tears will not be raised during fermentation due to lack of gluten. A good mixture in bakery products is 70% wheat flour and 30% Job's tears flour in bread and cookies (Chawakorn 2006; Cheappensuk 2006; Reungkajorn *et al.* 2007). The seeds are also boiled with water to produce Job's tears beverage, which is available in the market as an alternative healthy cereal drink.

Fermented products such as yogurt made from Job's tears have not been reported elsewhere, therefore, this research aimed to develop Job's tears yogurt. Some characteristics of the product such as chemical and sensory properties as well as consumer acceptance were also investigated.

2. Materials and Methods

2.1 A Study of the Effect of Added Job's Tears in Yogurt Production

2.1.1 Preparation of Job's tears beverage: Job's tears seeds were washed, soaked in water for four hours, and boiled for 40 min. They were then blended with water in a ratio 1:2 of seed to water. The blended beverage was kept in the refrigerator for further processing.

2.1.2 Job's Tears Yogurt Production: Job's tears yogurt was produced by using ratios of milk to Job's tears beverage of 100:0, 75:25, 50:50, 25:75 and 0:100. The mixtures were then heated at 85°C for 15 min and cooled to 43°C. After that, 3% of yogurt cultures were added into the yogurt mixtures. Then, the yogurt mixtures were mixed well and incubated at 43°C for 4 hours to allow fermentation. The samples were collected every 30 min for determination of pH, acidity and collected hourly for lactic acid bacteria determination. The fermentation of yogurt was terminated when the pH was lower than 4.5. The yogurt was finally cooled and kept at 4°C for further analysis.

2.1.3 Chemical Analysis: The acidity of yogurt was determined by using titration method and pH value was determined by using calibrated pH meter in accordance with AOAC methods (AOAC 2000).

2.1.4 Microbiological Analysis: One gram or milliliter of sample was transferred into 9 mL of sterile 0.1% peptone solution. The decimal serial dilutions were performed. Lactic acid bacteria were enumerated using spread plate technique and MRS agar (Oxoid, USA). The petridishes were then incubated anaerobically at 37°C for 48 hours. The number of lactic acid bacteria was measured and expressed in cfu/g or mL of samples.

2.1.5 Sensory Analysis: All samples were evaluated one day after production by using 9-point hedonic scale and 20 panelists.

2.2 A Study of Texture Development of Job's Tears Yogurt

2.2.1 Yogurt Production: Job's tears yogurt was produced by using a ratio 75:25 of milk to Job's tears beverage. The mixture was

then heated at 85°C for 15 min and cooled to 43°C. After that, 3% of yogurt cultures were added into the yogurt mixtures. Then, the yogurt mixture was mixed well and incubated at 43°C for 4 hours to allow fermentation. The fermentation of yogurt was terminated when the pH was lower than 4.5. Finally, the yogurt was cooled and kept at 4°C for further analysis.

2.2.2 Just About Right Test: Formulation yogurt containing Job's tears beverage was carried out by using Just About Right (JAR) test with 30 panelists.

2.2.3 Improvement of Texture: Due to the result from JAR, the texture of Job's tears yogurt had to be improved. Therefore, milk powder was added at 5, 10 and 15% into the mixture of milk and Job's tears (25:75). The mixtures were then heated at 85°C for 15 min and cooled to 43°C. After that, 3% of yogurt cultures were added into the yogurt mixtures. Then the yogurt mixtures were mixed well and incubated at 43°C for 4 hours to allow fermentation. The samples were collected every 30 min for determination of pH, acidity and collected hourly for lactic acid bacteria determination. The fermentation of yogurt was terminated when the pH was lower than 4.5. Finally, the yogurt was cooled and kept at 4°C for further analysis.

2.2.4 Sensory Analysis: The samples were analyzed by using 9-point hedonic scale and 20 panelists.

2.2.5 Chemical Analysis: Fat, fiber and protein contents of the products were determined using the methods of AOAC (2000).

2.3 Consumer Acceptance Test

The consumer acceptance was conducted in four public places, three places in Bangkok and one place in suburbs of Bangkok. Two hundred consumers, who like yogurt, were voluntarily selected without compensation. They were asked to answer a questionnaire about their preferences and scored the products.

2.4 Statistical Analysis

A randomized complete block design (RCBD) with three replications was used in this experiment. The mean differences were determined using Duncant's Multiple Range Test.

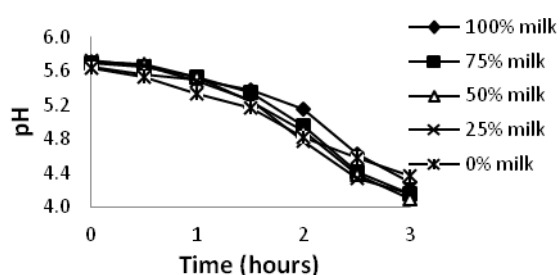
3. Results and Discussion

3.1 Effect of Job's Tears in Yogurt Production

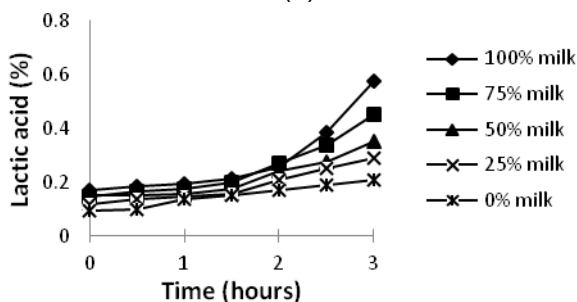
There were five different ratios of milk to Job's tears beverage used in yogurt production: 100:0, 75:25, 50:50, 25:0 and 0:100. The samples were collected every 30 min for determination of lactic acid and pH (Fig. 1).

The pH of the finished products was in the range of 4.09-4.36 due to the conversion of lactose to lactic acid by enzyme β -galactosidase (also known as lactase) produced by yogurt cultures during fermentation. The initial drop of pH was caused by the activity of *Streptococcus thermophilus* until the pH of the mixture was approximately 5.0. The further decrease of pH to 4.0 was caused by *Lactobacillus bulgaricus* (Walstra *et al.* 2006).

Although the presence of Job's tears beverage in yogurt mixtures did not significantly ($p < 0.05$) effect the pH of the products, it influenced to the amount of lactic acid produced during fermentation. Yogurts containing Job's tears beverage at 25, 50 and 75% (w/w) showed a lower pH compared with yogurt containing either milk or Job's tears beverage alone.



(a)



(b)

Fig. 1. Acidity (a) and pH (b) value of yogurts produced by using 5 ratios of milk to Job's tears during fermentation.

This might be caused by glucose content in Job's tears drink. Job's tears contain starch, which might be converted to glucose during the production of Job's tears drink. In excessive glucose, homolactic LAB like *Lactobacillus* sp. and *Streptococcus* sp. catabolize one mole of glucose using the Embden-Meyerhof-Parnas pathway to yield two moles of pyruvate, resulting in more lactic acid (Salminen *et al.* 2004). While in 100% Job's tears, there was no lactose to enhance the growth of lactic acid bacteria, therefore, less lactic acid production, leading to higher pH. In addition, the rate of lactic acid production from glucose is slower than from lactose. Moreover, milk contains phosphate, which is a compound that has buffering properties; therefore, the pH of yogurt was not affected by the presence of Job's tears and lactic acid. Therefore, in 100% milk, the pH was higher than the mixtures containing Job's tears.

Microbial behavior of yogurt cultures was also investigated during the yogurt production (Fig. 2).

It was recognized that the presence of Job's tears in yogurt mixtures significantly ($p > 0.05$) reduced the growth of lactic acid bacteria due to less amount of lactose content, which was parallel to higher pH and low lactic acid in yogurt containing Job's tears. At the end of fermentation, the highest count of lactic acid bacteria (8.04 log cfu/mL) was observed in the sample made from 100% milk.

In contrast, the yogurt made from 100% Job's tears exhibited the lowest LAB count (6.83 log cfu/mL). The amount of added Job's tears also significantly ($p > 0.05$) affected the lactic acid bacteria count.

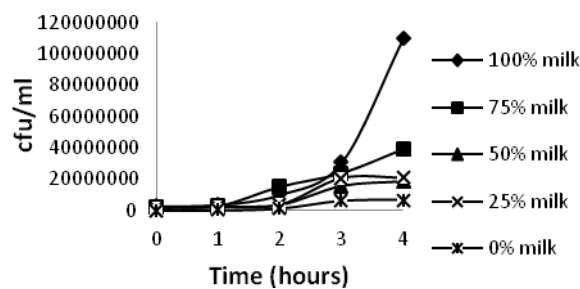


Fig. 2. Microbial behavior of lactic acid bacteria during Job's tears yogurt fermentation by using different amount of added milk.

This is due to the lactic acid bacteria need of specific nutrients to support their growth and milk contains these nutrients and provides a good medium for bacterial growth, especially carbohydrate as lactose and protein. Although the protein content in Job’s tears is as high as in milk, carbohydrates act as the primary source of energy which is easier to digest and therefore absorb more efficiently than proteins. Moreover, the carbohydrate in milk is simple carbohydrate (lactose) which is also easier to break down and absorb than in Jobs’ tears which is complex carbohydrate (starch).

Sensory properties of Job’s tears yogurt were also performed and the results are presented in Table 1.

Table 1. Sensory properties of Job’s tears yogurt produced by using different amount of added milk.

Attri- butes	Amount of added milk (%)				
	100	75	50	25	0
Overall	7.52 ^{a*}	6.67 ^b	6.20 ^{bc}	5.98 ^{cd}	5.53 ^d
Color	7.75 ^a	6.77 ^b	6.28 ^c	5.43 ^d	4.97 ^e
Texture	7.03 ^a	5.70 ^c	5.77 ^{bc}	6.27 ^b	7.05 ^a
Flavor	7.50 ^a	6.18 ^b	6.55 ^b	5.30 ^c	4.37 ^d

* The same letters mean there was no significant difference at $p < 0.05$.

The addition of Job’s tears in the yogurt mixture significantly ($p > 0.05$) affects the sensory properties of the finished product in all attributes: higher in Job’s tears proportion, lower in the preference scores of attributes except for texture score.

It was recognized that the highest scores of color, flavor and overall liking were observed in the yogurt made from 100% milk, followed by those of 75% milk, while the lowest value was found in yogurt made from 100% Job’s tears. In terms of texture, the highest score was observed not only in the yogurt from 100% milk, but also in yogurt made from 100% Job’s tears due to high protein content of both sources. The increase of the proportion of Job’s tears in the formula reduced the score of texture until 50% of Job’s tears was used. When the proportion of Job’s tears was higher than milk, the texture score increased. It might be caused by low protein contents when 25% and 50% of milk was

replaced with Job’s tears, resulting in low viscosity. Also, Job’s tears contained high amount of starch that increases the viscosity of the product when 75% and 100% Job’s tears were used.

Moreover, yogurt made from 25% Job’s tear had higher overall score than the others except for control (100% milk), therefore, this proportion was chosen for further experiments.

3.2 Texture Improvement of Jobs Tears Yogurt

Yogurt containing 25% of Job’s tears was produced and Just About Right Test was also performed with 30 panelists. There were 5 attributes used for analysis such as yogurt flavor, Job’s tears flavor, sweetness, sourness and texture. The result is shown in Table 2. It was noticed that every attribute received just the right percentage of more than 50%, except for texture, indicating that this attribute required further improvement.

Therefore, milk powder of 5, 10 and 15% was added to the yogurt mixture in order to improve the texture. The acidity and pH during fermentation were also investigated (Fig. 3) as well as the microbiological behavior of yogurt cultures (Fig. 4).

It was noticed that the amount of milk powder affected the change of pH and acidity during fermentation due to different levels of lactose. Higher milk powder and higher lactose content resulted in lower pH and higher acidity, except for 15% milk powder. The pH and acidity of yogurt made from 10% and 15% milk powder were similar to each other. Higher amount of milk powder in 15% yogurt did not increase lactic acid production due to the same amount of yogurt starter used in both 10% and 15% cases. The sample with 15% milk powder might have lactose remaining after fermentation, resulting in high post-acidification during the refrigerated storage. For all yogurt samples, there was a decrease in pH value from 0 h to 3 h. The initial pH ranges from 5.54 to 5.60 and the final pH ranges from 4.11 to 4.43 for all samples.

Concerning the microbiological behavior of yogurt cultures, it was noticed that the amount of added milk powder significantly influenced the lactic acid bacteria count ($p >$

0.05). The highest count of lactic acid bacteria (7.46 log cfu/mL) was observed in yogurt containing 5% milk powder; in contrast, the yogurt sample containing 10% and 15% milk powder were not significantly different ($p < 0.05$) and exhibited the lowest viability, 6.93 and 6.92 log cfu/mL, respectively.

Table 2. JAR score of yogurt containing 25% Job's tears.

Attributes	Percentage of Response				
	Moderately too less	Somewhat too less	Just right	Somewhat too much	Moderately too much
Yogurt flavor	3	13	84	0	0
Job's tears flavor	0	23	70	7	0
Sweetness	13	17	70	0	0
Sourness	0	20	67	10	3
Texture	17	60	23	0	0

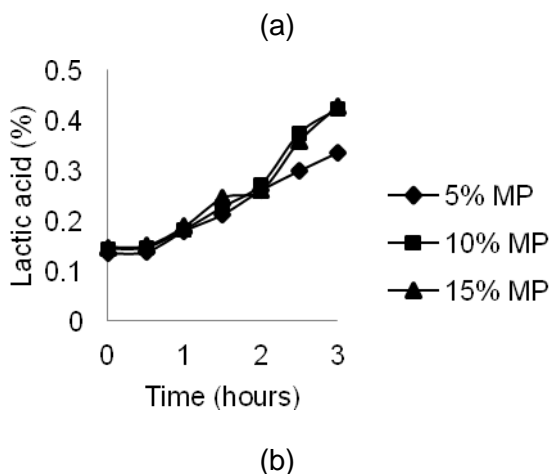
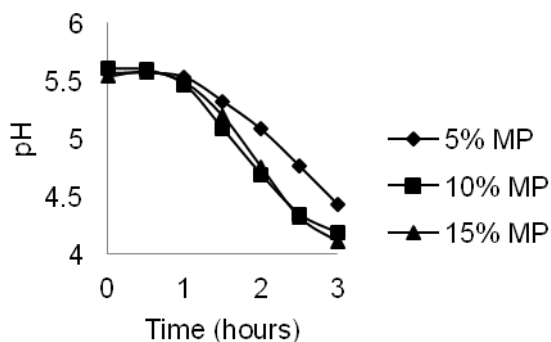


Fig. 3. The pH and acidity of Job's tears yogurt containing different levels of milk powder.

Although 10% and 15% milk powder had higher lactose content to enhance the growth of yogurt cultures, the lowest count of lactic acid bacteria was observed. A possible explanation in accordance with previous studies is that the addition of milk powder $> 5%$ (w/w) did not accelerate the bacterial growth, but instead prolonged the fermentation period, probably due to changes in osmotic pressure (Kim *et al.* 2009; Watson 2013) and too high acidic condition, since more milk powder resulted in more lactose converted to lactic acid and thus additional pH reduction, which is not suitable for bacterial growth. Moreover, the termination of fermentation at $pH < 4.7$ inhibited the production of lactic acid, which has detrimental effect on the viability of the bacteria.

Sensory properties of Job's tears yogurt containing different levels of milk powder were also analyzed. The preference scores are shown in Table 3.

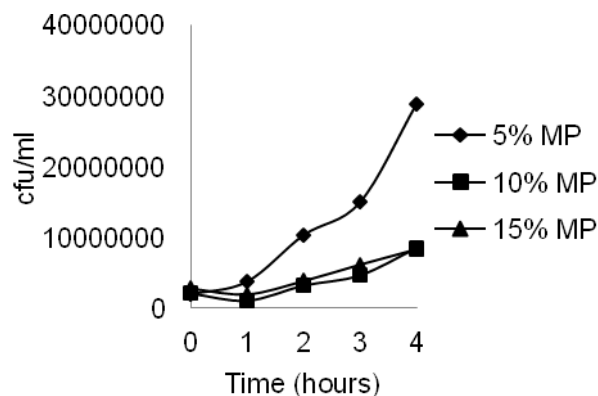


Fig. 4. Microbiological behavior of yogurt cultures during fermentation of in Job's tears yogurt containing different levels of milk powder.

Table 3. Sensory properties of Job's tears yogurts containing different levels of milk powder.

Attributes	% Milk powder		
	5	10	15
Overall	6.40 ^{c*}	7.52 ^a	7.13 ^b
Color	7.60 ^a	7.45 ^{ab}	7.25 ^b
Texture	5.87 ^c	7.48 ^b	7.88 ^a
Flavor	6.33 ^c	7.73 ^a	7.02 ^b

* The same letters mean there was no significant difference at $p < 0.05$.

Addition of milk powder in yogurt mixture had significantly ($p > 0.05$) affected the sensory qualities of the products in all attributes. The increase of milk powder reduced the preference score of product color and conversely increased the texture score. It might be caused by higher amount of milk protein, especially casein, which is responsible for color and texture of product. High level of milk protein provides better texture (Sodini *et al.* 2004; Peng *et al.* 2009).

On the other hand, too much or too low milk powder influence differently the preference scores of flavor and overall acceptance. High milk powder as high as 15% gave the powdery flavor to the product, while a lack of milk flavor was detected in 5% milk powder. Job's tears yogurt containing 10% milk powder had the highest scores for flavor and overall liking, 7.73 and 7.52, respectively. Therefore, 10% milk powder was chosen for the consumer acceptance test.

In addition, crude fiber, fat and protein contents of Job's tears yogurt containing 10% milk powder were determined and compared with the control (100% milk) (Table 4). Addition of Job's tears in yogurt significantly increased ($p > 0.05$) the fiber and protein contents of the product from 0.08% to 5.82% and 5.58% to 6.16% for crude fiber and crude protein, respectively, resulting in the high nutritional value of the product.

Table 4. Some chemical properties of Job's tears yogurt containing 10% milk powder.

Chemical properties	Samples	
	Control	Job's tears
Crude fat (%)	2.60 ^a	2.40 ^a
Crude fiber (%)	0.08 ^b	5.82 ^a
Crude protein (%)	5.58 ^b	6.16 ^a

* The same letters mean there was no significant difference at $p < 0.05$.

3. Consumer Acceptance of Job's Tears Yogurt

Job's tears yogurt was produced by using the following ingredients: 75% milk, 25% Job's tears beverage, 3% sugar, 10% milk powder, and 3% yogurt starter culture. After fermentation, the yogurt was kept in the refrigerator overnight. The consumer

acceptance was then performed using two hundred consumers. They answered an initial questionnaire before to be given the samples for the preference test. Consumer demographics and consumer behavior data were also collected.

The male (33%) and female (67%) participants in this test aged under 18 (6%), 18-25 (57%), 26-35 (20%), 36-45 (12%), 46-55 (4%), and above 55 (1%). The majority of the participants were students (71%) while the second largest group consisted of office staffs (20%). Most of them had income between 5,001-10,000 Baht/month (43%), while another 17% earned between 10,001-15,000 Baht/month and 17% received income from 15,001 to 20,000 Baht/month. A few (6%) of the participants received more than 25,000 Baht/month.

Most of the participants consumed yogurt once a month (43%). Some participants consumed yogurt 2-3 times a week (16%), others consumed it once a week (31%) and the remaining ones indicated a lack of consumption (10%).

For the price of a cup of yogurt (110 g), 96% of them preferred 10-15 Baht, while some (3%) were willing to pay 16-20 Baht and only 1% were willing to pay more than 20 Baht.

The locations at which most participants bought yogurt were the minimarts/convenient stores (47%), supermarkets/shopping malls (36%) and the university campus (17%).

Most of the participants (89%) moderately liked the product with scores of 7.1, 7.0, 6.6 and 7.3 for color, texture, yogurt flavor and overall liking, respectively (Figure 5). However, only 11% of them rejected it, which implied that there could be a potential market for this product (Fig. 6).

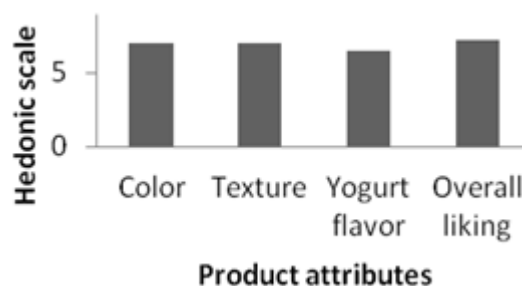


Fig. 5. Preference score of product attributes ($n = 200$).

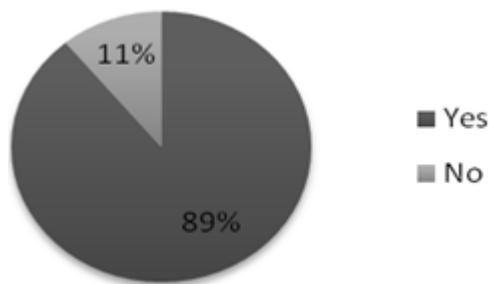


Fig. 6. Consumer acceptance of Job's tears yogurt ($n = 200$).

4. Conclusion

The suitable formula of Job's tears for yogurt was: 25% Job's tears beverage, 75% milk, 3% sugar, 10% milk powder, and 3% yogurt starter culture. Addition of Job's tears remarkably increased fiber and protein contents and influenced the color, flavor and texture of the product. Most of the consumers (89%) accepted the product with preference scores of color, texture, flavor and overall liking of 7.05, 7.03, 6.55 and 7.27, respectively.

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