Development of Job's Tears Yogurt

By

Ms. Nontaon Keeratibunharn

ID: 511-2873

A special project submitted to the Faculty of Biotechnology,
Assumption University in part of fulfillment of the requirement
for the degree of Bachelor of Science

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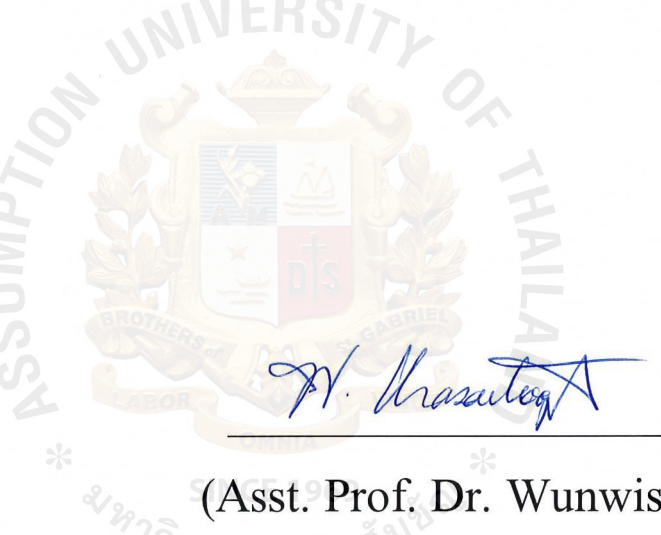
Title	: Development of Job's Tears (*Coix-lachryma-jobi* L.) Yogurt
By	: Ms. Nontaon Keeratibunharn
Advisor	: Asst. Prof. Dr. Wunwisa Krasaekoopt
Level of study	: Bachelor of Science
Faculty	: Biotechnology
Academic Year	: 2011



_____ Advisor

(Asst. Prof. Dr. Wunwisa Krasaekoopt)

Abstract

Job's tears yogurt is a fermented product that obtained from inoculation of yogurt starter to the mixture of milk and Job's tears beverage. The aim of this study was to investigate the effect of Job's tears beverage on microbiological, physicochemical and sensory attributes of Job's tears yogurt. pH, titratable acidity, viability of lactic acid bacteria, fat/ fiber/ protein content and sensory characteristics of produced samples were analyzed. The Job's tears beverage was used to replace the milk as 0, 25, 50, 75 and 100% in the formula of yogurt. During fermentation period, acidity and viability of lactic acid bacteria increased, while pH decreased due to lactic acid produced. Yogurt containing 25% of Job's tears beverage obtained the highest scores for sensory analysis and was selected for further development. Based on Just About Right test score, there was only one attribute as texture needed to be improved by increasing the addition of milk powder. Three levels of milk powder as 5, 10 and 15% were studied. The yogurt made by 10% milk powder achieved the highest preference scores. This product was also remarkably accepted by the consumers (89%), with the preference score of 7.3. Although the addition of Job's tears beverage affected the color, texture and flavor of the product, it tended to increase fiber (5.82%) and protein contents (6.16%) compared to control.

Acknowledgement

I would like to express the deepest appreciation and my sincere gratitude towards my advisor, Asst. Prof. Dr. Wunwisa Krasaekoopt from the School of Biotechnology, Assumption University for dedicating her precious time and giving advice throughout this project. It was a great opportunity to be experienced and gain knowledge in this research area. This project would not be successful without the great supports, this would be a great occasion to thank to A. Nootrudee Siriboon for guiding me the chemical analysis, and also Dr. Aussama Sontrunnarudrungsri for guiding me the sensory and statistical analysis. I would also like to thank to all my faculty members, teachers and technicians for lending a hand to assist me in this project

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Introduction

Job's tears, *Coix-lachryma-jobi* L. or also known as Chinese pearl barley, is one kind of cereal in China and spread to Indochina including Thailand (Katharine and Dorothy, 1999). Based on the researchers from credible institute, they have found out that Job's tear contains very high level of nutritive value, vitamin B, fiber and double amount of protein compared with medium grain brown rice (Diana Wind, 2009). Since it is considered as a nutritious health food, this cereal is very popular in Asian countries due to the significant consumption and the number of Asian foods made from Job's tears especially in Japan and China which are used as traditional medicine. From these reasons, it brings about an increase consumption and production of Job's tears in Thailand. This leads to the overflow of Job's tears supply in Thailand market, resulting in lower price of Job's tears (Vacharotayan et al., 1982). Although Job's tears have been used to produce many kinds of products, yogurt from Job's tear has not been reported elsewhere. Therefore, this project aimed to formulate yogurt form Job's tears.

Objectives

1. To study effect of Job's tears in yogurt production.
2. To study texture improvement and some properties of Job's tears yogurt.
3. To study consumer acceptance of Job's tear yogurt.

Literature Review

1. Job's tears

A Job's tears is a cereal in the family Gramineae, tribe Maydeae, genus Coix, scientific name *C. learyma-jobi* L. common name Job's tears. Job's tears came from Arabian language "Damu Duad" or "David's tears" (Thanaprachum, 1984).

The Job's tears was originally found in China 1,500 years ago. It was firstly brought into Thailand in 1983 to Saraburi, Lopburi, Nakornrachasima, and it was brought to the north in 1970 at Chaiyapume, Loei, Chieng rai, Phayao, and Lampang (Vacharotayan, 1982).

1.1) Characteristics of C. learyma-jobi L.

1. *C. learyma-jobi* L.var. typical has oval shape seed, bluish white kernel, hard and smooth outer kernel
2. *C. learyma-jobi* L.var. stenocarpa Stapf. Has long capsular seed and bluish white kernel.
3. *C. learyma-jobi* L. var. monilifer Stepf. Has flat and round shape seed, milky white, pink, brown, and black color. For this type of Job's tears, it is used for body adornment e.g. necklace.
4. *C. learyma-jobi* L. var. ma-yuen Stapf. Has groove along the length of the seed, very thin kernel, milky white and brown color. This type of Job's tears is being planted for human consumption (Vacharotayan et al., 1982)

1.2) Benefits of Job's tears

Job's tears can be used as a general health tonic, primarily benefitting the functions of the spleen, lungs, and skin. It improves the flow of water throughout the body, so it can be used by that experiencing water stagnation such as abdominal bloating and edema (excessive water weight). Coix is also commonly used in many formulas for arthritis and rheumatism since these conditions are associated with excess moisture. It is also said to be very nourishing to the skin, helping to clear blemishes and soften coarse skin, thus it is considered as a beauty aid in Asia. By grinding the seeds and mixing with honey, a paste can be made to smooth on problem skin, reducing blemishes and redness (Julia, 2007).

In terms of nutrients, Job's tears contain numerous amino acids, lysine, arginine, B1, adenosine, and thiamin.

Regarding preparation, Coix can be used as an extremely healthy and nutritious grain substitute in cooking, and there are currently a number of products on the market use Coix as the food base for diet cereals (Anonymous, 2011).

1.3) Cultivation and production

The commercial Job's tears type *C. learyma-jobi* L. var. ma-yuen can be cultivated by seed dropping only once a year during the period between May and June. After 4 months of growth, it gives the flowers and 2 more months for giving tears. The total period of cultivation from the first day of growing to the harvesting day is approximately 200 days (or 7 months). The general gross production per rai is 300-400 kg. The product after bran and husk polishing is 60/40, in which we will get about 60% of polished Job's tears and 40% of its bran and husk (Bonnie, 2012).

Table 1: Agriculture area and Job's tears product in Thailand (2526/2527)

Province	Agriculture area (Rais)	Total product (Tons)	Average total (kg/Rais)
Loei	123,082	44,432.6	361
Chaiyapume	13,450	4,277.1	318
Udonthani	3,406	6,64.2	195
Nongkhai	11,085	2,416.5	218
Sakonnakorn	Small area	-	181
Khonkaen	317	92.6	292
Petchaboon	7,452	1,356.3	182
Chiengmai	Small area	-	100
Chiengrai	3,146	629.2	200
Phayao	180	36	200
Total	**162,118**	**53,904.5**	**335.26**

Source: Uraikul (1984)

For Job's tears production in Thailand in 1984, total cultivation area was 162,118 rai with the gross production of 53,904.5 tons. The average production per rai was 335.26 kg/Rai. Loei was the largest area of production with 123,082 rais and 44,432.6 tons of the products (Table 1). The second largest area was Chaiyapume, 13,450 rais and 4,277.1 tons of products. There also were some cultivation areas scattering in some other provinces of the northern part of Thailand like Udonthani, Sakonnakorn, Khonkaen, Petchaboon, Chiengmai, Chiengrai and Phayao (Uraikul, 1984).

1.4) Chemical component of Job's tears

Sato and Miyata (1975) studied the chemical components of milled and polished Job's tear. It was found that both of them had not very much difference in chemical components. The milled Job's tears have total quantity of nitrogen 2.44%, fat 11.74%, ashes 2.53%, and starch 63.75%. When it was polished, to bring out the bran, the fat content decreased due to the loss of fat in the seed bran. The polished Job's tears had total nitrogen of 2.48%, fat 10.43%, ashes 2.73% and starch 67.75%.

Yang et al. (1978) also studied the chemical component of Job's tears after polishing. They reported that 7.90% of fat, 1.71% of ashes, and 53.60% of starch. These amounts were lower than the resulted reported by Sato and Miyata (1978). They also found that there were 14.60% of protein and 0.50% of fiber.

In addition, Sithibush (1983) analyzed the chemical components of Job's tears that grown in Phayao province. It was recognized that milling and polishing had an effect on the quantity of chemical component of Job's tears. Milling provides less quantity of ashes and fiber but higher quantity of protein, fat, and carbohydrate. Meanwhile, polishing of the bran made more carbohydrate but decrease in the quantity of protein, fat, ashes, and fiber. Therefore, the whole grain would have the quantity of protein 10.23%, fat 50.30%, ashes 7.95%, fiber 14.10%, and carbohydrate 56.46%. The milled and unpolished Job's tears had protein 14.46%, fat 7.90%, ashes 2.30%, fiber 0.95%, and carbohydrate 67.88%. For the polished Job's tears, they have protein 13.80%, fat 4.6%, ashes 2%, fiber 0.4%, and carbohydrate 71.39%.

Sato and Miyata (1975) studied the formation of blue complex of Job's tears and I_2. Amylase is a strengths chain mole all in helical structure. The helix composes of 6 glucose units per turn that can include one molecule of I_2 in the structure producing the blue complex. Upon

using spectrophotometer at 600-660 nm, it was found that Job's tears starch produced 0.04 and 0.02 transmission while the rice starch was 0.42 and 0.36 and the wax rice starch of 0. This result indicated that the starch from Job's tears may contain amylase similar to that of the wax rice starch than rice starch.

Sato and Miyata (1975) studied the amino acid content in Job's tears that are shown in Table 2. It was noticed that the amino acid in polished Job's tears consisted of essential amino acids such as leucine as much as 11.94%, isoleucine 2.28%, threonine 2.82%, phenylalanine 4.75%, lysine 2.21%, tryptophan 0.60% and non-essential amino acids as glutamic acid as much as 23.24%, proline 16.17%, alanine 9.95%, aspartic acid 7.04%, serine 4.44%, tyrosine 2.30%, and glycine 2.73%.

Table 2: Composition of amino acid in dehulled and polished Job's tears

Type of amino acid	% Amino acid
Essential amino acid	
Leucine	11.94
Valine	4.64
Methionine	1.75
Isoleucine	3.28
Threonine	2.82
Phenylalanine	4.75
Lysine	2.21
Tryptophan	0.60
Non-essential amino acid	
Cysteine	Less than 0.05
Histidine	2.25
Glutamic acid	23.24
Proline	16.17

Source: Sato and Miyata (1975)

Sato and Miyata (1975) determined the types of content of fatty acids in Job's tears. Job's tears lipid contained saturated fatty acids as palmitic acid 12.28%, stearic acid 1.52%, and unsaturated fatty acids as palmitileic acid 0.38%, oleic acid 60.06%, and 2 essential fatty acids which were linoleic acid 24.38% and linolenic acid 0.25%.

Table 3: Composition of fatty acids in polished Job's tears

Type of fatty acid	% Fatty acid
Saturated fatty acid	
Palmitic acid	12.38
Stearic acid	1.52
Unsaturated fatty acid	
Palmitoleic acid	0.38
Oleic acid	60.06
Linoleic acid	24.38
Linolenic acid	0.35

Source: Sato and Miyata (1975)

Sato and Miyata (1975) determined vitamin content in Job's tears and found out that polishing and affected vitamin content in the finished product 100 g of unpolished Job's tears could contain B1 76.8 μg , B2 218 μg , and the panthothenic acid 790 μg . When the bran was polished out, there would be fewer vitamins, B1 60.3 μg , B2 113 μg , and the panthothenic acid 640 μg .

Sithibush (1983) studied and analyzed the mineral content of whole kernel Job's tears, milled and polished as shown in Table 4. The milled Job's tears had more minerals than polished ones. There were 2.209% nitrogen, o.425% phosphorus, 0.352% potassium, 0.070% calcium, 0.238% magnesium, 0.10% sulfur and 55 ppm zinc. Except iron and chlorine content had increased to 87 ppm and 0.195%, respectively.

Table 4: Mineral content of Job's tears

Job's tears	Content of mineral (%)							Content of mineral (ppm)			
	N	P	K	Ca	Mg	S	Cl	Fe	Mn	Zn	Cu
Whole grain	1.673	0.355	0.410	0.115	0.198	0.170	0.121	65	-	50	-
Milled	2.318	0.485	0.317	0.110	0.240	0.160	0.142	80	-	60	-
Polished	2.209	0.425	0.352	0.070	0.238	0.100	0.195	87	-	55	-

Source: Sithibush (1983)

1.5) The use of Job's tears

Food

The variety of Job's tears used as food is *C. learyma-jobi* L. var. ma-yuen (Vacharotayan et. al., 1982). Schaafhausen (1952) ground the polished Job's tears into the flour and baked bread with wheat flour in the different portions. The result showed that at the portion of Job's tears flour 30% and wheat flour 70% provided the best formula bread, which was accepted by the consumers.

In Japan, Job's tears are used for beverage. The whole grain Job's tears were roasted like the coffee beans prepared as tea for refreshing to a soup like the European barley soup. Chinese used the polished Job's tears to cook as soup, bean curd, and desserts (Vacharotayan et al., 1982).

Wongkraisritong (1983) performed using Job's tears flour from dry milling to make cookies. It was found that the Job's tears cookies had sandy texture.

Banornaneksakul (1984) prepared Job's tears flour by wet milling and obtained the fine flour that passed 80 mesh sieves. Cookies from this flour had good acceptable flavor.

Medicines

Job's tears are highly nutritional food for patients during their recuperation period. It also has many medicinal effects such as urinal expellant, spleen nourishing, lung nourishing, cold killer. Moreover, it can be the medicine to cure the rheumatism if fermented in alcohol (Tanwiwat, 1978). The pharmaceutical research reported that the coixonolide or 1-methyl-2 (cis-

9-hexadecenyloxy) propyl-trans-11-octadecenoate which is extracted from Job's tears has an effect in restraining the tumor growth (Chaicharntippayut, 1981).

Animal feeds

Both Job's tears pulp and bran after polishing can be used as feed for swine and other livestock. It can be used directly or mixed with other ingredients for chicken for example (Yamsonrat, 1960).

Ornaments

In some Western and Asian countries including the northern of Thailand, Chiengmai for example, have been using Job's tears seeds as the body ornament since ancient time by threading the beautiful necklaces and bracelets, even making the fancy clothing, because of the glossy and shiny of Job's tears seeds (Vacharotayan et. al., 1982).

2. Yogurt

Yogurt is one of the most popular fermented dairy products all over the world. The production of yogurt has shown tremendous increase during the past few years mainly due to the introduction of wide variety of flavors to the yogurt, and the convenience of it as a ready-made break-fast food and the image of yogurt as a low fat healthy food (Fatih Yildiz, 2009).

Yogurt is a fermented milk product which was apparently brought to Turkey by the mongols millenia ago. It is produced by adding a "starter" of active yogurt containing a mixed culture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. These produce lactic acid during fermentation of lactose. The lactic acid lowers the pH, makes it tart, causes the milk protein to thicken and acts as a preservative since pathogenic bacteria cannot grow in acid conditions. The partial digestion of the milk when these bacteria ferment milk makes yogurt easily digestible. (David, 2010)

2.1) Types of yogurt

Yogurt is typically classified into the following groups (John, 2012):

1. **Set yogurt**

   This type of yogurt is incubated and cooled in the final package and is characterized by a firm jelly like texture.

2. **Stirred yogurt**

   This type of yogurt is incubated in a tank and the final coagulum is broken by stirring prior to cooling and packing. The texture of stirred yogurt will be less firm than set yogurt somewhat like a very thick cream. There is some slight reformation of the coagulum after the yogurt has been packed, however this is slight and cannot be relied upon.

3. **Drinking yogurt.**

   This type of yogurt is very similar to stirred yogurt, having the coagulum broken prior to cooling. In drinking yogurt the agitation used to break the coagulum is severe. Little if any reformation of the coagulum will reoccur after packing.

4. **Frozen yogurt.**

   Frozen yogurt is inoculated and incubated in the same manner as stirred yogurt. However, cooling is achieved by pumping through a whipper/ chiller/ freezer in a fashion similar to ice cream. The texture of the finished product is mainly influenced by the whipper/ freezer and the size and distribution of the ice crystals produced.

5. **Concentrated yogurt**

   This type of yogurt is inoculated and fermented in the same manner as stirred yogurt. Following the breaking of the coagulum, the yogurt is concentrated by boiling off some of the water, which is often done under vacuum to reduce the temperature required. Heating of low pH yogurt can often lead to protein being totally denatured and producing

rough and gritty textures. This is often called strained yogurt due to the releasing of liquid fat from the coagulum upon heating, which was similar to manner used for making soft cheese.

6. Flavored yogurt

Yogurts with various flavors and aromas have become very popular. Common additives are fruit or berries, usually as a puree or as whole fruit in syrup. These additives often have as much as 50% sugar in them. However with the trend towards healthy eating gaining momentum; many manufacturers offer a low sugar and low fat version of their products. Low or no sugar yogurts are often sweetened with saccharin or more commonly aspartame (Neil, 2001).

2.2) Benefits of yogurt

The primary benefits of natural yogurt are simply a result of the potassium, calcium, B12 and other B vitamins and protein that it contains (Elaine, 2012).
The claimed benefits of yogurt include:

- Reduced gastrointestinal infections
- Improved immune system (resistance to illness and infection)
- Greater resistance to cancer
- Reduced incidence of osteoporosis (weakened bone strength)

Additional benefits include

- Treatment of, and reduction of the symptoms of, thrush
- Lactose intolerant people can often consume yogurt because the enzymes in the yogurt help to reduce the lactose levels in the intestines - thus gaining the benefits contained in all milk based products (above all calcium)
- Improved absorption of calcium in the intestine because the lactic acid in the yogurt provides the perfect environment for this absorption to occur (Neil, 2001).

3. Milk

A white fluid secreted by the mammary glands of female mammals for the nourishment of their young, consisting of minute globules of fat suspended in a solution of casein, albumin, milk sugar, and inorganic salts.

Worldwide, cow's milk is most commonly used to make yogurt, but milk from water buffalo, goats, sheep, camels and yaks is also used in various parts of the world. In theory, the milk of any mammal could be used to make yogurt (Anonymous, 2001).

Table 5: Composition of milk

Milk Composition	%
Protein	3.5
Fat	4
Mineral matter	0.75
Water	87.25
Lactose	4.75

Source: Fannie (2010)

The total protein component of milk is composed of numerous specific proteins. The primary group of milk proteins is the caseins. There are 3 or 4 caseins in the milk of most species; the different caseins are distinct molecules but are similar in structure. All other proteins found in milk are grouped together under the name of whey proteins. The major whey proteins in cow milk are beta-lactoglobulin and alpha-lactalbumin.

Caseins have an appropriate amino acid composition that is important for growth and development of the nursing young. This high quality protein in cow milk is one of the key reasons why milk is such an important human food (Walter, 2010).

Traditionally, yogurt has been made by heating the milk to partially remove water. When the temperature of the milk reaches 85°C, one particular micellar protein (kappa-casein) at the surface of the casein micelles reacts with one particular whey protein (beta-lactoglobulin). This interaction produces minute 'bumps' on the casein micelle surfaces. The beta-lactoglobulin-kappa-casein complex later prevents other casein micelles from getting attached at these sites.

Later, when yogurt bacteria metabolize lactose and produce lactic acid and the milk starts to coagulate (Bryan, 1997).

4. Manufacturing process

4.1) Standardization process

Standardization or milk composition modifying process is to modify the milk composition before it is used to make yogurt. This standardization process typically involves reducing the fat content and increasing the total solids. The fat content is reduced by using a standardizing clarifier and a separator (a device that relies upon centrifugation to separate fat from milk). From the clarifier, the milk is placed in a storage tank and tested for fat and solids content. For yogurt manufacture, the solids content of the milk is increased to 16% with 1-5% fat and 11-14% solids-not-fat (SNF). This is accomplished either by evaporation of some water, or adding concentrated milk or milk powder. Increasing the solids content improves the nutritional value of the yogurt, makes it easier to produce a firmer yogurt and improves the stability of the yogurt by reducing the tendency for it to separate on storage (John, 2012).

4.2) Heat treatment

The heat treatment of the milk prior to fermentation is generally considered essential in commercial manufacturing. After the solids composition is adjusted, stabilizers are added and the milk is pasteurized. This step has many benefits. First, it will destroy all the microorganisms in the milk that may interfere with the controlled fermentation process. The presence of unknown numbers of unknown organisms in the raw milk would make the fermentation too unreliable and unpredictable for commercial operations. As many ingredients used these days are dry powders, the heat treatment must be capable of coping with the large numbers of fungal and bacterial spores associated with dry powders. In order to ensure that the flavor, aroma and texture of the product is optimized the growing conditions for the starter culture must be as near perfect as possible. Second, the heat treatment will have a physiochemical effect on the proteins and other additives within the mix. It will denature the whey proteins in the milk which will give the final yogurt product better body and texture. Moreover, the heating may be necessary for some of the ingredients to achieve the required state to form gels and protein lattice that lead to the products final viscosity and texture (John, 2012). Third, it will not greatly alter the flavor of the milk.

Finally, it helps release the compounds in milk that will stimulate the growth of the starter culture. Pasteurization can be a continuous-or batch-process. Both of these processes involve heating the milk to a relatively high temperature and holding it there for a set amount of time. One specific method for batch process pasteurization is to heat a large, stainless steel vat of milk to 185° F (85°C) and hold it there for at least 30 minutes.

4.3) Homogenization process

While the milk is being heat treated, it is also homogenized. Homogenization is a process in which the fat globules in milk are broken up into smaller, more consistently dispersed particles. This produces a much smoother and creamier end product. In commercial yogurt making, homogenization has the benefits of giving a uniform product, which will not separate. Homogenization is accomplished using a homogenizer or viscolizer. In this machine, the milk is forced through small openings at a high pressure and fat globules are broken up due to shearing forces (John, 2012).

4.4) Fermentation process

When pasteurization and homogenization are completed, the milk is cooled to between 109.4-114.8 F (43-46°C) and the fermentation culture is added in a concentration of about 2%. It is held at this temperature for about three to four hours while the incubation process takes place. During this time, the bacteria metabolize certain compounds in the milk producing the characteristic yogurt flavor. An important byproduct of this process is lactic acid (John, 2012).

Depending on the type of yogurt, the incubation process is done either in a large tank of several hundred gallons or in the final individual containers. Stirred yogurt is fermented in bulk and then poured into the final selling containers. Set yogurt, also known as French style, is allowed to ferment right in the container it is sold in. In both instances, the lactic acid level is used to determine when the yogurt is ready. The acid level is found by taking a sample of the product and titrating it with sodium hydroxide. A value of at least 0.9% acidity and a pH of about 4.4 are the current minimum standards for yogurt manufacture in the United States. When the yogurt reaches the desired acid level, it is cooled, modified as necessary and dispensed into containers (Helferich and Westhoff, 1980).

Materials and Methods

1. To study effect of added Job's tear in yogurt production

1.1 Preparation of Job's tears beverage

Job's-tears seeds were washed, soaked in water for 4 hours, and boiled for 40 minutes. It was then blended with water in a ratio of seed to water as 1:2. The blended beverage was kept in the refrigerator for further processing.

1.2 Job's tears yogurt production

Job's tears yogurt was produced by using ratio of milk to Job's tears beverage as 100:0, 75:25, 50:50, 25:75 and 0:100. The mixtures were then heated at 85°C for 15 minutes for pasteurization 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 minutes 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.

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).

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. The plates were then incubated anaerobically at 37°C for 48 hours. Subsequently, the number of lactic acid bacteria was

measured and expressed in CFU. All measurements were the averages of triplicate samples.

1.5 Sensory analysis

All samples were evaluated 1 day after production by 20 panelists to select the sample of Job's tears yogurt with the most general acceptability. The sensory attributes were color, texture, flavor and overall liking. The acceptability values were scored by using 9-point hedonic scale.

2. To study texture development of Job's tear yogurt

2.1 Yogurt production

Job's tears yogurt was produced by using ratio of milk to Job's tears beverage as 75:25. The mixture was then heated at 85°C for 15 minutes for pasteurization 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 Just About Right Test (JAR)

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

2.3 Improvement of texture

Due to the result from JAR, the texture of Job's tear yogurt was needed to be improved; therefore, milk powder was added at 5, 10 and 15%. The mixtures were then heated at 85°C for 15 minutes for pasteurization 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 minutes 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.

2.4 Sensory analysis

The samples were analyzed by using 9-point hedonic scale and 20 panelists.

2.5 Chemical analysis prototype product

After product prototype was developed, chemical analysis was performed. Fat, fiber and protein contents were determined (AOAC, 1999) and compared with the control (yogurt without Job's tear).

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 and scored the products based on their preferences.

4. Statistical analysis

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

Result and Discussion

1. Effect of Job's tears in yogurt production

There were 5 different ratios of yogurt mixtures produced which were 100:0, 75:25, 50:50, 25:0 and 0:100 of milk to Job's tear beverage. Each of them was collected every 30 minutes during fermentation to observe the change in pH value and acidity. The determination of pH value and acidity was performed by using calibrated pH meter and titration method, respectively (AOAC, 2000). All determinations were conducted in triplicate. The average data were then statistically analyzed (Figure 1).

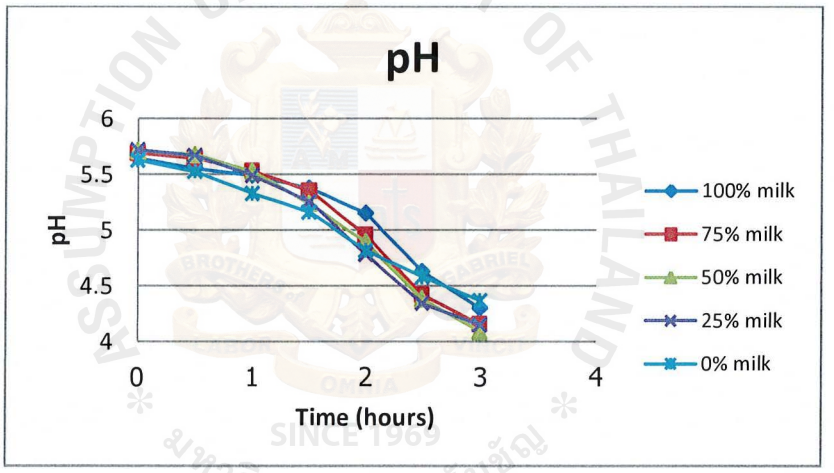

Figure 1: pH value of yogurt produced by using 5 ratios of milk to Job's tears during fermentation

During fermentation, part of the lactose in milk which act as the main carbon source that provides energy for growth of the bacteria was converted to lactic acid, catabolized by the enzyme β-galactosidase (also known as lactase), decreasing the pH values to a range of 4.09 to 4.36. The streptococci are responsible for the initial pH drop of the yogurt mix to approximately 5.0. The lactobacilli are responsible for a further decrease to pH 4.0 (Pieter Walstra et. Al., 2005).

Yogurt fermentation involves the conversion of lactose to lactic acid by bacteria, resulted in pH reduction. Figure1 showed the pH changed during fermentation with different concentrations of milk and Job's tears beverage in yogurt. For all yogurt samples, there was a decrease in pH value from 0 h to 3 h. The initial pH ranged from 5.62 to 5.72 and the final pH ranged from 4.09 to 4.36 for all samples.

According to statistical result from Table7, it was recognized that there were no significant differences of pH value obtained from different ratios of yogurt mixture ($p < 0.05$) until the last hour of fermentation period, yogurt containing Job's tears beverage at 25%, 50% and 75% (w/w) showed a lower pH compared with yogurt containing either 100% milk or 100% Job's tears beverage alone. This might be caused by glucose content in Job's tear drink. Job's tear contains starch, which might be converted to glucose during the production of Job's tear drink. In excessive glucose, homolactic LAB like *Latobacillus* sp. and *Streptococcus* sp. catabolize one mole of glucose in the Embden-Meyerhof-Parnas pathway to yield two moles of pyruvate. Intracellular redox balance is maintained through the oxidation of NADH, concomitant with pyruvate reduction to lactic acid (Salminen et al., 2004). On the other hand, higher pH was observed when Job's tear alone was used. This might be caused by low lactose content, leading to low lactic acid production. In addition, the rate of lactic acid production from glucose is slower than from lactose.

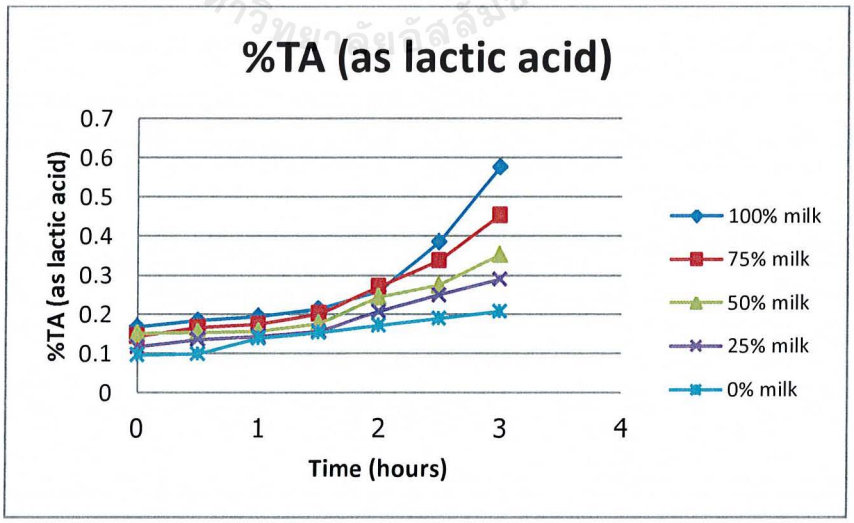

Figure 2: Acidity (as lactic acid) of yogurt produced by using 5 ratios of milk to Job's tears during fermentation

Fermentation resulted in decreasing the pH value, whereas the titratable acidity increased. The highest final titratable acidity was observed in the samples containing 100% milk (control) and the lowest final titratable acidity was related to the samples made from 100% Job's tears beverage this was due to the lack of lactose content which was the major carbohydrates present in milk, thus less lactose was converted to lactic acid.

Microbial behavior of yogurt cultures was also investigated during the yogurt production (Figure 3).

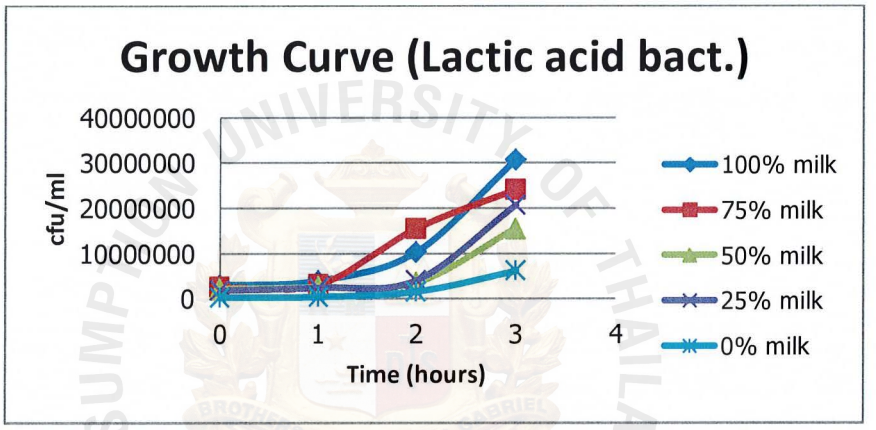

Figure 3: Microbial counts of 5 different ratios of milk and Job's tears in yogurt measured every 60 minutes of fermentation period (average)

It was recognized that the presence of Job's tear 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 tear. 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 (w/w); in contrast, the sample made from 100% Job's tears (w/w) exhibited the lowest viability (6.83 log cfu/mL). The amount of Job's tears beverage added significantly ($p > 0.05$) affected the lactic acid bacteria count. This is due to the lactic acid bacteria need 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 but carbohydrates acts as the primary source of energy which is easier to digest and therefore absorbed 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 tear yogurt were also performed and the results were presented in Table 6.

Table 6: Sensory properties of Job's tears yogurt

Attributes	100% milk	75% milk	50% milk	25% milk	0% milk
Overall	7.5^{a}	6.7^{b}	6.2^{bc}	6.0^{cd}	5.5^{d}
Color	7.8^{a}	6.8^{b}	6.3^{c}	5.4^{d}	4.9^{e}
Texture	7.0^{a}	5.7^{c}	5.8^{bc}	6.3^{b}	7.0^{a}
Flavor	7.5^{a}	6.2^{b}	6.6^{b}	5.3^{c}	4.4^{d}

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

Table 6 demonstrates the sensory analysis results for the 5 samples. The score of color, flavor, texture and overall liking of the samples were observed. The findings showed that the highest score of color, flavor and overall liking was observed in the yogurt samples made from 100% milk. The lowest value was related to the sample made from 100% Job's tears beverage. In term of texture, the highest score observed in the yogurt samples made from 100% milk and also observed in samples made from 100% Job's tears beverage, while the lowest values were remarked in the sample containing 25% Job's tears beverage and also in the sample containing 50% Job's tears beverage.

Based on the result, the amount of milk and Job's tears beverage added significantly ($p > 0.05$) affected all of yogurt attributes. It showed that the sample made from 100% milk (control) achieved the highest score for every attribute. The preference score were decreased when more Job's tears beverage was added. However, in term of texture, not only yogurt made from 100% milk got the highest score but also yogurt made from 100% Job's tears beverage. This is due to the viscous texture of Job's tears beverage

itself, not resulting from the protein coagulation, since the result based on microbial activities. It also showed the lowest amount of acid produced and the number of viable cell counts. Hence, protein coagulation could not be performed very well in this mixture of yogurt.

2. Texture improvement of Jobs tear yogurt

Yogurt containing 25% of Job's tear was produced and Just About Right Test was also performed with 30 panelists. There were 5 attributes for analysis as yogurt flavor, Job's tear flavor, sweetness, sourness and texture. The result was shown in Table 7

Table 7: JAR score of yogurt containing 25% Job's tears (n=30)

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

Although, there were high percentages of just right in most attributes (more than 50%), it was noticed that there was low score as 23% in texture attribute. Therefore, to improve the texture of the product, milk powder was added as 5%, 10% and 15%. pH, acidity as well as microbial behavior were also investigated during the yogurt fermentation.

Figure 4 showed the pH changes during fermentation with different concentrations of milk powder contained in yogurt. 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. It was recognized that at the end of fermentation period, there were no significant ($p < 0.05$) differences of pH value obtained

from yogurt containing 10% and 15% of milk powder which were significantly ($p > 0.05$) lower than pH of yogurt containing 5% milk powder.

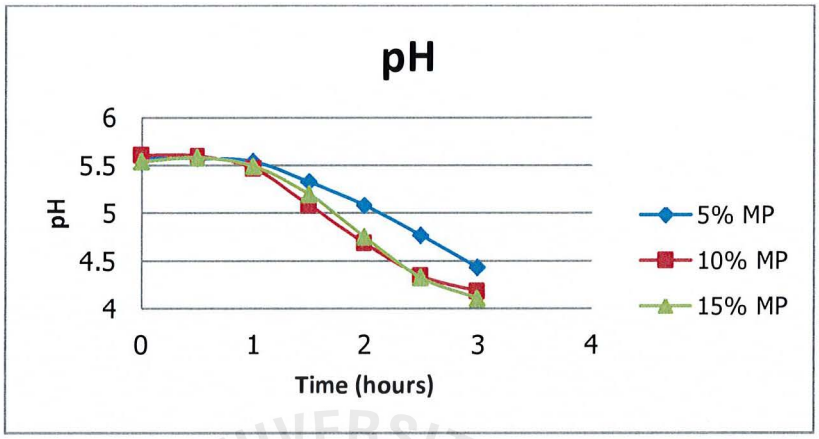

Figure 4: pH value of 3 different ratios of milk powder in yogurt containing 25% Job's tear

As yogurt fermentation involves in the conversion of lactose to lactic acid which further results in pH reduction. More lactic acid should be produced when more lactose are present. Since lactose can be found in milk or milk powder. The addition of milk powder will directly affect to the amount of lactose containing in yogurt. Hence, the more milk powder added, the more lactose converted to lactic acid and finally the lower pH value should be observed.

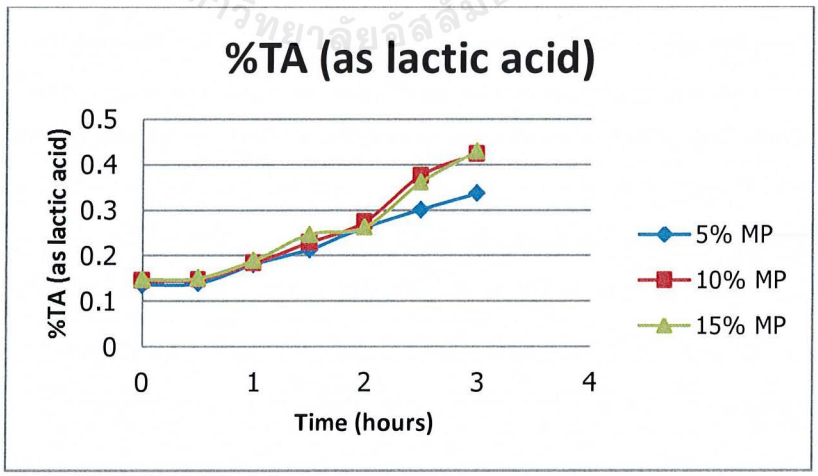

Figure 5: Acidity (as lactic acid) of 3 different ratios of milk powder in yogurt containing 25% Job's tear

Fermentation resulted in decreasing the pH value, whereas the titratable acidity increased (Figure 5). There were no significant differences of pH value obtained from yogurt containing 10% and 15% of milk powder ($p < 0.05$) which were significantly higher than pH of yogurt containing 5% milk powder ($p > 0.05$). This was due to the lack of lactose content which was the major carbohydrates present in milk powder, thus less lactose was converted to lactic acid.

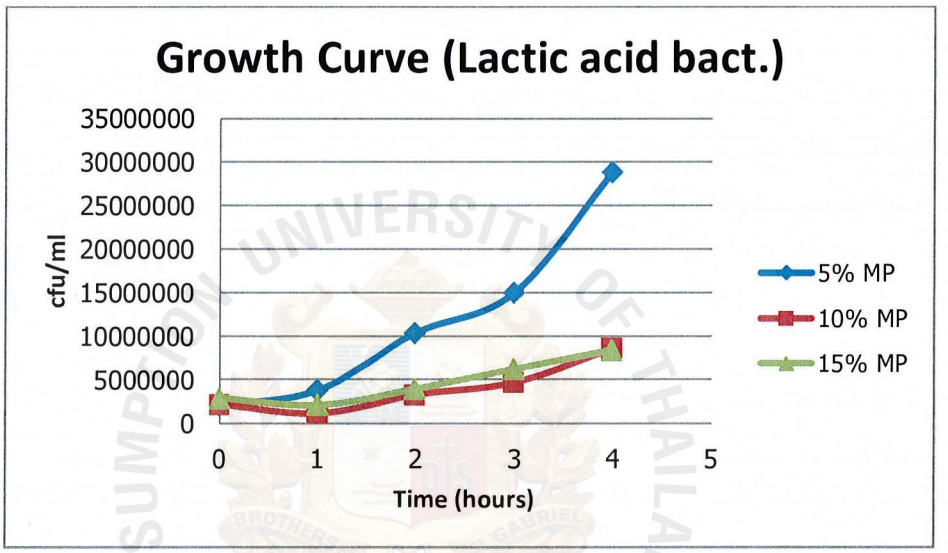

Figure 6: Microbial counts of 3 different ratios of milk powder in yogurt measured every 60 minutes of fermentation period (average)

As indicated in Figure 6, it shows the counts of lactic acid bacteria of the samples. It was noticed that the amount of milk powder added 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 the sample made from yogurt containing 5% milk powder; in contrast, the yogurt sample containing 10% and 15% were not significantly different ($p < 0.05$) and exhibited the lowest viability which are (6.93 log cfu/mL) and (6.92 log cfu/mL), respectively.

Although 10% and 15% milk powder will give more nutrients as lactose to support bacterial growth, it showed the lowest count of lactic acid bacteria. This could be explained by the previous study 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 (Sung-han Kim et. al., 2008; John, 2012) and too high acidic condition, since more milk powder will result in more lactose converted to lactic acid produced and thus more pH reduction, that is not suitable for bacterial growth. Moreover, from previous research, it was found that the termination of full fermentation at <pH 4.7 will inhibit the production of lactic acid, which has detrimental effect on the viability of the bacteria. The statistical results obtained by Yate's algorithm (Box et. al., 1978)

Based on the chemical and microbial analysis, yogurt sample containing 5% milk powder showed the lowest amount of lactic acid produced but the highest the counts of lactic acid bacteria. This can be explained that the amount of lactic acid produced was strongly affected by the amount of lactose contained in yogurt, not mainly affected by the number of lactic acid bacteria.

Sensory properties of Job's tear yogurt containing different levels of milk powder were also analyzed. The preference scores were shown in Table 8.

Table 8: Sensory properties of Job's tears yogurt sample (n=30)

Attributes	% Milk Powder		
	5	10	15
Overall	6.4^{c}	7.5^{a}	7.1^{b}
Color	7.6^{a}	7.5^{a}	7.3^{a}
Texture	5.9^{c}	7.5^{b}	7.9^{a}
Flavor	6.3^{c}	7.7^{a}	7.0^{b}

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

The score of color, flavor, texture and overall liking of the samples were observed. It was noticed that the addition of milk powder at 5%, 10% and 15% were not significantly different with the color score ($p < 0.05$) but significantly different with the texture, flavor and overall liking score ($p > 0.05$). The more milk powder added, the more yogurt viscosity increased and perceived thickness. This was caused by an increase in

total solids content of milk which helps thicken yogurt texture (Skriver et al., 1999; Sodini et al., 2004; Peng et al., 2009). Although samples made from 15% milk powder achieved the highest texture score, milk powder can also give the powdery off-flavors to yogurt, thus affect to the flavor score. As a result, the yogurt samples containing 10% milk powder achieved the highest score of flavor and overall liking was selected as product prototype.

In addition, crude fiber, fat and protein contents of Job's tear yogurt were determined and compared with yogurt without Job's tear (Table 9).

Table 9: Some chemical properties of product prototype compared to control

Chemical properties	Samples	
	Control (plain yogurt)	Job's tears yogurt
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 $p < 0.05$

It was noticed that addition of Job's tear drink significantly ($p > 0.05$) influenced the fiber and protein contents of yogurt and did not significantly ($p < 0.05$) influenced the fat content. The yogurt made by using Job's tear drink had higher fiber and protein contents up to approximately 5.74% and 0.58%, respectively (compared to control). This implied that addition of Job's tear increased the nutritional value of the product.

3. Consumer acceptance of Job's tears yogurt

The yogurt prototype was produced by using the following ingredients as 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 were given a set of questionnaire to answer before the samples were given to test for their preferences. Consumer demographic, consumer behavior data were also collected.

Male (33%) and female (67%) were participated in this test with the age in the range of 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 lady/boy (20%). Most people had income between 5001-10,000 Baht/month (43%), while another 17% was 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 consumers consumed yogurt once a month (43%). Some consumers consumed yogurt 2-3 times a week (16%). Few people consumed it once a week (31%) and even fewer consumers never consume it (10%).

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

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

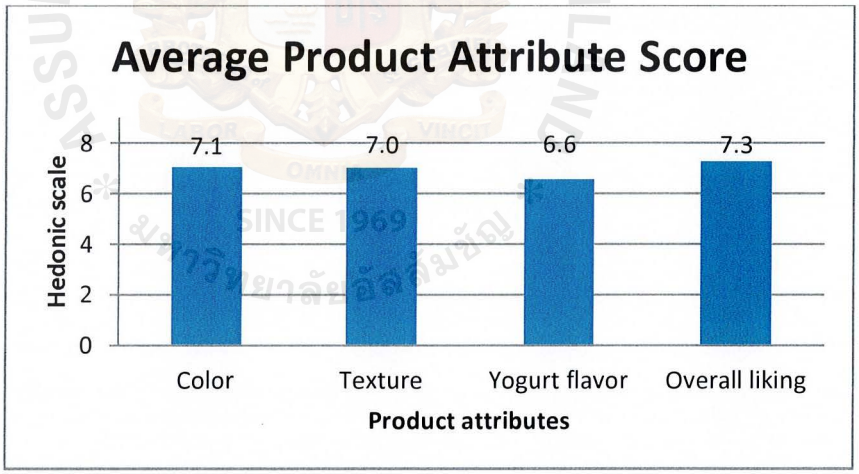

Figure 7: Average product attributes score of product prototype

After the yogurt containing Job's tear was served, it was noticed that most of the consumers (89%) moderately like the product with the scores of 7.1, 7.0, 6.6 and 7.3 for color, texture, yogurt flavor and overall liking, respectively (Figure 7). However, only 11%

of consumer rejected this product, which was implied that there was a potential market for this product (Figure 8).

Figure 8: Job's tears yogurt acceptability

Table15: Demographic information for consumer acceptance

Criteria		Percentage (%)
Gender	Male	66
	Female	134
Age	Under 18	12
	18-25	115
	26-35	41
	36-45	23
	46-55	7
	56 and above	2
Education	Less than high school	3
	High school	56
	Bachelor	97
	Higher than bachelor	44
Occupation	Student	141
	Lecturer	5
	Office lady/boy	39
	Employee	10
	Other	4
Income	5,000 or less	19
	5,001-10,000	86
	10,001-15,000	34
	15,001-20,000	34
	20,001-25,000	16
	above 25000	11

Conclusion

This research revealed that the presence of Job's tear in the yogurt formula remarkably influenced the color, flavor and texture of the product. The suitable amount of Job's tear for yogurt production was 25%, providing yogurt with 2.4% fat, 5.82% fiber and 6.16% protein contents. Most of the consumers (89%) accepted the product with the preference scores of color, texture, flavor and overall as 7.05, 7.03, 6.55 and 7.27, respectively. Additions of Job's tear drink increased fiber and protein contents of the product.

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Appendix I

pH

(Vary milk and Job's tears)

T = 0

ANOVA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.02164000	0.00541000	0.21	0.9259
Error	10	0.25533333	0.02553333		
Corrected Total	14	0.27697333			

T = 0.5

ANOVA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.05850667	0.01462667	0.68	0.6221
Error	10	0.21546667	0.02154667		
Corrected Total	14	0.27397333			

T = 1

ANOVA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.08297333	0.02074333	3.77	0.0403
Error	10	0.05500000	0.00550000		
Corrected Total	14	0.13797333			

T = 1.5

ANOVA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.09489333	0.02372333	1.37	0.3114
Error	10	0.17306667	0.01730667		
Corrected Total	14	0.26796000			

T = 2

ANOVA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.25497333	0.06374333	1.51	0.2718
Error	10	0.42240000	0.04224000		
Corrected Total	14	0.67737333			

T = 2.5

ANOVA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.19344000	0.04836000	3.65	0.0439
Error	10	0.13233333	0.01323333		
Corrected Total	14	0.32577333			

T = 3

ANOVA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.15622667	0.03905667	10.31	0.0014
Error	10	0.03786667	0.00378667		
Corrected Total	14	0.19409333			

Appendix II

Acidity

(Vary milk and Job's tears)

T = 0

ANOVA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.30000000	0.07500000	3.95	0.0356
Error	10	0.19000000	0.01900000		
Corrected Total	14	0.49000000			

T = 0.5

ANOVA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.38000000	0.09500000	4.56	0.0235
Error	10	0.20833333	0.02083333		
Corrected Total	14	0.58833333			

T = 1

ANOVA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.20066667	0.05016667	1.51	0.2708
Error	10	0.33166667	0.03316667		
Corrected Total	14	0.53233333			

T = 1.5

ANOVA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.26333333	0.06583333	3.11	0.0663
Error	10	0.21166667	0.02116667		
Corrected Total	14	0.47500000			

T = 2

ANOVA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.60766667	0.15191667	13.60	0.0005
Error	10	0.11166667	0.01116667		
Corrected Total	14	0.71933333			

T = 2.5

ANOVA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	2.13433333	0.53358333	43.86	<.0001
Error	10	0.12166667	0.01216667		
Corrected Total	14	2.25600000			

T = 3

ANOVA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	7.67166667	1.91791667	74.72	<.0001
Error	10	0.25666667	0.02566667		
Corrected Total	14	7.92833333			

Appendix III

Sensory test

(Vary milk and Job's tears)

Overall liking

ANOVA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	136.8466667	34.2116667	17.84	<.0001
Error	295	565.8333333	1.9180791		
Corrected Total	299	702.6800000			

Color

ANOVA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	289.8866667	72.4716667	49.85	<.0001
Error	295	428.8333333	1.4536723		
Corrected Total	299	718.7200000			

Texture

ANOVA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	103.5466667	25.8866667	11.33	<.0001
Error	295	673.8500000	2.2842373		
Corrected Total	299	777.3966667			

Flavor

ANOVA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	344.5133333	86.1283333	49.69	<.0001
Error	295	511.3666667	1.7334463		
Corrected Total	299	855.8800000			

Appendix IV

pH

(Vary milk powder)

T = 0

ANOVA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.00668889	0.00334444	2.26	0.1852
Error	6	0.00886667	0.00147778		
Corrected Total	8	0.01555556			

T = 0.5

ANOVA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.00086667	0.00043333	0.25	0.7888
Error	6	0.01053333	0.00175556		
Corrected Total	8	0.01140000			

T = 1

ANOVA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.00762222	0.00381111	2.30	0.1812
Error	6	0.00993333	0.00165556		
Corrected Total	8	0.01755556			

T = 1.5

ANOVA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.08895556	0.04447778	31.03	0.0007
Error	6	0.00860000	0.00143333		
Corrected Total	8	0.09755556			

T = 2

ANOVA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.26586667	0.13293333	48.83	0.0002
Error	6	0.01633333	0.00272222		
Corrected Total	8	0.28220000			

T = 2.5

ANOVA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.37348889	0.18674444	82.39	<.0001
Error	6	0.01360000	0.00226667		
Corrected Total	8	0.38708889			

T = 3

ANOVA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.17242222	0.08621111	63.60	<.0001
Error	6	0.00813333	0.00135556		
Corrected Total	8	0.18055556			

Appendix V

Acidity

(Vary milk powder)

T = 0

ANOVA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.00023400	0.00011700	3.25	0.1106
Error	6	0.00021600	0.00003600		
Corrected Total	8	0.00045000			

T = 0.5

ANOVA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.00023400	0.00011700	4.33	0.0685
Error	6	0.00016200	0.00002700		
Corrected Total	8	0.00039600			

T = 1

ANOVA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.00012600	0.00006300	0.54	0.6094
Error	6	0.00070200	0.00011700		
Corrected Total	8	0.00082800			

T = 1.5

ANOVA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.00163800	0.00081900	7.58	0.0228
Error	6	0.00064800	0.00010800		
Corrected Total	8	0.00228600			

T = 2

ANOVA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.00028800	0.00014400	2.29	0.1828
Error	6	0.00037800	0.00006300		
Corrected Total	8	0.00066600			

T = 2.5

ANOVA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.00945000	0.00472500	65.62	<.0001
Error	6	0.00043200	0.00007200		
Corrected Total	8	0.00988200			

T = 3

ANOVA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.01625400	0.00812700	64.50	<.0001
Error	6	0.00075600	0.00012600		
Corrected Total	8	0.01701000			

Appendix VI

Sensory test

(Vary milk powder)

Overall liking

ANOVA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	38.6333333	19.3166667	24.37	<.0001
Error	177	140.3166667	0.7927495		
Corrected Total	179	178.9500000			

Color

ANOVA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	3.7000000	1.8500000	1.94	0.1463
Error	177	168.5000000	0.9519774		
Corrected Total	179	172.2000000			

Texture

ANOVA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	136.8111111	68.4055556	94.52	<.0001
Error	177	128.1000000	0.7237288		
Corrected Total	179	264.9111111			

Flavor

ANOVA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	58.8111111	29.4055556	35.64	<.0001
Error	177	146.0500000	0.8251412		
Corrected Total	179	204.8611111			

Appendix VII

Fat/Fiber/Protein determination

Fat content

ANOVA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.06201667	0.06201667	1.21	0.3328
Error	4	0.20471667	0.05117917		
Corrected Total	5	0.26673333			

Fiber content

ANOVA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	49.39270417	49.39270417	6676.57	<.0001
Error	4	0.02959167	0.00739792		
Corrected Total	5	49.42229583			

Protein content

ANOVA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.50560970	0.50560970	16.80	0.0149
Error	4	0.12036969	0.03009242		
Corrected Total	5	0.62597940			

Appendix VIII

Ballot form for sensory analysis,

Questionnaire for consumer acceptance

Batch_____

Panelist no._____

Date__/__/__

Preference test

Tested product: Job's tears yogurt

Instruction: Please evaluate the sample relating to you preference according to 9-point hedonic scale

1 = Dislike extremely
2 = Dislike very much
3 = Dislike moderately
4 = Dislike slightly
5 = Neither like nor dislike
6 = Like slightly
7 = Like moderately
8 = Like very much
9 = Like extremely

Product Attributes	Sample				
	922	394	862	594	408
Overall liking					
Color					
Texture					
Yogurt flavor					

Comments_____

_____

_____

Thank you for your cooperation

Batch_____

Panelist no._____

Date__/__/__

Preference test

Tested product: Job's tears yogurt

Instruction: Please evaluate the sample relating to you preference according to 9-point hedonic scale

1 = Dislike extremely
2 = Dislike very much
3 = Dislike moderately
4 = Dislike slightly
5 = Neither like nor dislike
6 = Like slightly
7 = Like moderately
8 = Like very much
9 = Like extremely

Product Attributes	Sample		
	571	639	423
Overall liking			
Color			
Texture			
Yogurt flavor			

Comments_____

_____

_____

Thank you for your cooperation

Panelist no._____

Date__/__/__

Just About Right test (JAR)

Tested product: Job's tears yogurt

Instruction: Please indicate your opinion about the following characteristics of the sample

Product Attributes	Response				
	Moderately too less	Somewhat too less	Just Right	Somewhat too much	Moderately too much
Yogurt flavor					
Job's tears flavor					
Sweetness					
Sourness					
Texture					

Comments_____

_____

_____

Thank you for your cooperation

Consumer no._____
Date____/_____/____

Questionnaire

(Consumer test)

Product: Job's tears yogurt

Instruction: Please read carefully and answer each of the following questions by writing in or choosing the best answer

Part1

1. Gender

   ☐ Male ☐ Female

2. Age group

   ☐ Under 18 ☐ 18-25 ☐ 26-35 ☐ 36-45

   ☐ 46-55 ☐ 56 and above

3. Education

   ☐ Less than high school ☐ High school ☐ Bachelor ☐ Higher than bachelor

4. Occupation

   ☐ Student ☐ Lecturer ☐ Office lady/boy

   ☐ Government officer ☐ Employee ☐ Entrepreneur

   ☐ Other_____(please specify)

5. What is your salary per month?

   ☐ ≤ 5,000 baht ☐ 5,001-10,000 baht ☐ 10,001-15,000 baht

   ☐ 15,001-20,000 baht ☐ 20,001-25,000 baht ☐ ≥ 25,001 baht

Part2

1. How often do you consume yogurt?

   ☐ Everyday ☐ 2-3 times a week ☐ Once a week

   ☐ Once a month ☐ Never

2. Would you like a healthier option to yogurt?

   ☐ Yes ☐ No

   Why?_____

3. What is the most important reason to buy yogurt?

☐ Healthy ☐ Tasty ☐ Reasonable price

☐ Comfortable environment of shop ☐ Good service provided

☐ Other_____(please specify)

4. How much would you be willing to pay for a small serving of yogurt? (110 g)

☐ 10-15 baht ☐ 16- 20 baht ☐ 21- 25 baht ☐ 26- 30 baht

☐ Other_____(please specify)

5. What flavors of yogurt would you prefer?

☐ Plain ☐ Strawberry ☐ Grape ☐ Mango

☐ Raspberry ☐ Blueberry ☐ Mixed fruit ☐ Chocolate

☐ Green tea ☐ Other_____(please specify)

6. Where would you like to buy yogurt?

☐ Shopping mall/super market ☐ Minimart ☐ University campus

☐ Cinema ☐ BTS/MRT stations ☐ Airport

☐ Other_____(please specify)

7. Do you accept this Job's tears yogurt?

☐ Yes ☐ No

Why?_____

Part3

Instruction: Please evaluate the sample relating to your preference according to 9-point hedonic scale

1 = Dislike extremely
2 = Dislike very much
3 = Dislike moderately
4 = Dislike slightly
5 = Neither like nor dislike
6 = Like slightly
7 = Like moderately
8 = Like very much
9 = Like extremely

Product Attributes	Score
Color	
Texture	
Yogurt flavor	
Overall liking	

Comments_____

_____

_____

Thank you for your cooperation