International Journal on Advanced Science Engineering Information Technology

Vol.9 (2019) No. 3
ISSN: 2088-5334

Effect of Temperature on Synbiotic Sorghum Drinks Characteristics

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***Abstract*— Synbiotic drinks contain components of probiotics and prebiotics which can provide a functional effect for the body. Probiotics are resistant to stomach acid and bile salts so that they remain alive in the digestive tract. Prebiotics are a component of food that cannot be digested and can provide a growth effect for beneficial bacteria. Functional food in the form of synbiotic drinks can be made using sorghum which acts as prebiotic and Bifidbacterium bifidum acts as probiotic bacteria and characteristics of synbiotic drinks can be influenced by storage temperature. The purpose of this study was to determine the effect of storage temperature on the characteristics of sorghum-based symbiotic drink products (Sorghum bicolor L. Moench). The research method used is the experimental method with descriptive analysis, followed by regression analysis and correlation to determine the effect of storage temperature on the characteristics of symbiotic drinks. The treatment used in this study was the use of storage temperature of 15ºC ± 5ºC, 25ºC ± 5ºC, and 35ºC ± 5ºC. The results showed that total Lactic Acid Bacteria decreased at all temperatures storage with a very strong closeness. The total probiotic bacteria decreased at all temperatures storage with a very strong closeness. The pH value decreased at all temperatures storage with a very strong closeness. Viscosity increases at all temperatures storage with a very strong closeness and based on the calculation of shelf life prediction of synbiotik sorghum drinks, the rate of decline products due to the activity of the microorganisms will follow one order kinetics and the result is supposedly faster if storage temperatures offered up to room temperature.**

***Keywords*— characteristic of the product; synbiotic drinks; temperature storage.**

I. Introduction

The current issue of people lifestyle tends to be more aware of health, causing the widespread meaning of fulfilling food needs. Food is not limited to the fulfillment of nutrition or satisfaction of the mouthfeel but rather to maintain the health and fitness of the body, which is then called functional food. One functional food that is popular in the community and much developed by food experts is a drink with the addition of prebiotic substrates and probiotic bacteria known as synbiotic drinks products. Functional foods have beneficial effects on the human body for maintaining health and counteracting diseases. One of the functional foods product that can recover the human intestine is a synbiotic drink. Synbiotic drinks can maintain digestive system health and reduce the risk of colon cancer Synbiotic drinks contain prebiotics and probiotics, which are good for gut health [1].

Prebiotics are indigestible ingredients that have a good influence on the host by triggering selective growth activity against probiotic bacteria. Probiotics are non-pathogenic microorganisms that live as digestive microflora, which can have a positive influence on human health. Examples of probiotic bacteria are *Bifidobacterium* and *Lactobacillus*. The advantage of a combination of prebiotics and probiotics is to increase the survival of probiotic bacteria because specific substrates are available for fermentation [2].

To develop functional drinks innovation products, currently, many synbiotic drinks have been developed which are made by adding prebiotic sources derived from local raw materials. One of the potential local foods as a prebiotic source is sorghum. Sorghum (Sorghum bicolor L. Moench) is a cereal plant that can grow in a variety of environmental conditions so that it is potential to be developed, especially on dry climate marginal land in Indonesia. The advantages of sorghum lie in its extensive adaptability, tolerance to drought, high productivity, and is more resistant to pests and diseases compared to other food crops. In addition to easy cultivation, sorghum has extensive benefits, including for food, food, and industrial materials. Local cultivar Bandung sorghum can be used as a prebiotic. Sorghum seeds contain antioxidant, Fe, fiber, oligosaccharide, and β-glucan contents, which are included in carbohydrate non-starch polysaccharides (NSP) sorghum contains 8.8-11.1% dietary fiber [3]. Dietary fiber that not digested will undergo fermentation and promotes the growth of beneficial gut bacteria [4].

Probiotic bacteria should have antimicrobial properties and enhance metabolic activity. *Bifidobacterium bifidum* is one of the probiotic bacteria. These bacteria have properties that inhibit pathogenic microorganisms, anti-mutagenic, anti-carcinogens, preventing diarrhea, increasing immunity, and reducing cholesterol levels. Quality of food products requires to be kept. Quality changes in synbiotic drinks can be affected by storage temperature. B. bifidum is a heterofermentative non-pathogenic microbe, meaning that besides producing lactic acid, acetic acid which is beneficial for health as well as the beneficial effect of B. bifidum is its ability to produce stable bifidin antibiotics at 100 ° C for 30 minutes. In addition, these bacteria also have antibacterial and antagonistic activity against pathogenic microbes, including the genera Salmonella, Escherichia, Proteus, Shigella, and Candida. Microorganisms are very sensitive to the surrounding environment which results in the ease of damage. Microorganisms can be encapsulated to increase their viability. Microencapsulation aims to stabilize the core material, control the release of core material both its speed and release conditions, protect sensitive food components, reduce nutrient loss, add certain food components to other foodstuffs and convert liquid food to solid forms that are easier to handle. The changes are identified by the decrease of lactic acid bacteria and probiotic bacteria, changes in pH and viscosity [5]. The purpose of this study was to determine the effect of storage temperature on the characteristics of sorghum-based synbiotic beverage products (*Sorghum bicolor* L. Moench).

II. Materials and Methods

The materials used in this study are local cultivar Bandung sorghum seeds, pure culture *B. bifidum*, distilled water, melted sugar, and powdered skim milk. The media used is deMan Ragosa Sharpe Agar (MRSA). The chemicals used are 70% alcohol (Merck), 98% glacial acetic acid (Merck), pH 7 and 4 buffer solution, and physiological NaCl (Merck) 0.85%.

The research method used is the experimental method with descriptive analysis and using 3 treatments. The treatment applied is the use of storage temperature of 15ºC ± 5ºC, 25ºC ± 5ºC, and 35ºC ± 5ºC. Furthermore, regression and correlation analyses are carried out to determine the effect of analysis parameters on storage temperature.

Shell life prediction of product is done by collecting observation data every day. The products are stored at a temperature of 15°C ± 5°C observed once per day for 1 week, while the products are stored at a temperature of 25°C ± 5°C and 35°C ± 5°C observed every 2 hours to 10 hours of the first, the next data retrieval done on 24 hour. This shell life prediction using Arrhenius model.

A. Making of Synbiotic Drinks

1) Making of Sorghum Flour: The step is done by sieving course sorghum flour using a 100 mesh sieve. Sifted sorghum flour stored using zip-lock plastic.

2) Preparation Phase of B. bifidum Suspension: This step includes the making of *B. bifidum* liquid culture and bacterial inoculation.

- Making *B. bifidum* culture using MRS agar media tilted in a test tube and incubate at 37°C for 48 hours. The culture shed with sterilized distilled water. The turbidity of the bacterial suspension was checked according to McFarland 3 with a wavelength of 600 nm and absorbance of ± 0.616 equivalents as of the number of colonies $3x10^8$ CFU/ml using a spectrophotometer.
- Media used to create a suspension is 10% skim milk powder dissolved with distilled water and pasteurized at 62.8°C for 15 minutes using a water bath. Inoculation with the addition of 10% liquid culture B. bifidum after the temperature becomes ± 35°C to forming a suspension. The suspension was incubated at 37°C for 48 hours to calculate the number of bacteria.

3) Step of Making Synbiotic Drinks:

- Mix sorghum flour and sterile distilled water in a ratio of 1:10 and add 10% sugar, stir until homogeneous.
- Pasteurization at 60oC for 30 minutes using a water bath
- After temperature decrease until ± 35oC, add 12% (v/v) of probiotic bacteria B. bifidum
- Sterile 100 mL PET bottle with hot steam or UV light, used the bottle as products package.

4) Observing of synbiotic drinks: Synbiotic drinks which stored at 15ºC ± 5ºC were observed once a day during the 7 days of storage, Synbiotic drinks which stored at 25ºC ± 5ºC and 35ºC ± 5ºC were observed once every 2 hours for the first 10 hours and the 24 hours of storage. The analyses in synbiotic drinks are total lactic acid bacteria using the Total Plate Count (TPC) method according to SNI 01-2897: 2008 [6]:

- Total probiotic bacteria using the Total Plate Count (TPC) method according to SNI 01-2897: 2008 [6]
- pH measurements using a pH meter [7]
- Measurement of viscosity using a viscometer [8]

III. Results and Discussion

A. Lactic Acid Bacteria (LAB)

Based on the test results, synbiotic drinks which stored at 15 ± 5ºC, 25 ± 5ºC, and 35 ± 5 º C decreased the total LAB. The storage temperature of 15ºC on day 7 (168 hour) shows $7.2x10^9$ CFU / mL lactic acid bacteria, while at 25°C on 24th hour present value of $7.1x10^9$ CFU / mL, and at 35ºC on 24th hours present $3.9x10^9$ CFU / mL. Table 1 shows that total LAB decreased significantly on the 6th day (144 hour), from 10^{10} to $9.5x10^9$ CFU / mL.

TABLE I
Total Lactic Acid Bacteria at 15ºC ± 5ºC

Time (hour)	Total Bacteria (CFU/mL)	Log CFU/mL
0	$4.4x10^{10}$	10.64
24	$3.8x10^{10}$	10.58
48	$3.1x10^{10}$	10.49
72	$2.5x10^{10}$	10.40
96	$2.0x10^{10}$	10.30
120	$1.5x10^{10}$	10.18
144	$9.5x10^9$	9.98
168	$7.2x10^9$	9.86

Based on Table 2, drinks stored at 25°C and 35°C LAB significantly decreased on the 10th-hour become $9.7x10^9$ CFU / mL and $5.1x 10^9$ CFU / mL respectively. LAB decreased due to reduced nutrition growth. LAB decrease due to reduced nutrition growth. This happened because the bacteria continue to metabolize, causing nutrients to decrease. According to Chramostová *et al.* [9], the temperature is also one of the factors that influence bacterial growth in a product. Based on the data in Table 1 and Table 2, the total decrease in BAL can be plotted on the graph so that the equation shown in Table 3.

TABLE II
TOTAL LACTIC ACID BACTERIA AT 25ºC ± 5ºC AND 35ºC ± 5ºC

Time (hour)	Total Bacteria (CFU/mL)		Log CFU/mL	
	25ºC	**35ºC**	**25ºC**	**35ºC**
0	$3.5x10^{10}$	$4.0x10^{10}$	10.54	10.60
2	$3.3x10^{10}$	$3.5x10^{10}$	10.52	10.54
4	$3.0x10^{10}$	$2.3x10^{10}$	10.48	10.36
6	$2.4x10^{10}$	$1.7x10^{10}$	10.38	10.23
8	$1.1x10^{10}$	$1.1x10^{10}$	10.04	10.04
10	$9.7x10^{9}$	$5.1x10^{9}$	9.99	9.71
24	$7.1x10^{9}$	$3.9x10^{9}$	9.85	9.59

TABLE III
REGRESSION EQUATIONS OF TOTAL LAB TOWARDS STORAGE TIME

Storage Temperature	Regression Equation	R^2 value	r value
15ºC±5ºC	y = -0.0047x + 10.701	0.9749	0.987
25ºC±5ºC	y = -0.0317x + 10.502	0.7644	0.874
35ºC±5ºC	y = -0.0443x + 10.496	0.8000	0.894

Based on Table 3, synbiotic drinks stored at 15ºC ± 5ºC, 25ºC ± 5ºC, 35ºC ± 5ºC has a value of r (correlation coefficient) close to 1, which is 0.89-0.99 (89%-99%). Correlation coefficient value indicates that total BAL with storage time has a very strong relationship closeness. The remaining 1% -11% difference influenced by other variables such as RH and storage temperature. According to Schlabitz *et al.* [10], bacterial growth factors consist of intrinsic factors (physical and chemical), extrinsic factors (storage environment conditions), food processing factors, and implicit factors (characteristics, interactions of microorganisms). The slope value stated that the decrease in total LAB during storage decreased by 0.00%-0.04%.

B. Probiotic Bacteria

Based on Table 4, stored drinks showed a significant decrease in total probiotic bacteria since day 1 (hour 24) from $3.2x10^{10}$ CFU / mL to $6,8x10^{9}$ CFU / mL.

TABLE IV
TOTAL PROBIOTIC BACTERIA AT STORAGE TEMPERATURE 15ºC ± 5ºC

Time (hour)	Total Bacteria (CFU/mL)	Log CFU/mL
0	$3.2x10^{10}$	10.51
24	$6.8x10^{9}$	9.83
48	$5.3x10^{9}$	9.72
72	$3.6x10^{9}$	9.56
96	$2.6x10^{9}$	9.41
120	$2.0x10^{9}$	9.30
144	$9.1x10^{8}$	8.96
168	$7.2x10^{8}$	8.86

Probiotic bacteria decreased significantly again when it reaches the 6th day of storage (144 hours) became $9,1x10^{8}$CFU / mL. Probiotic bacteria during storage at 15ºC (Table 4) decreases slowly compared to storage at 25ºC and 35ºC in Table 5.

TABLE V
TOTAL PROBIOTIC BACTERIA AT STORAGE TEMPERATURE 25ºC ± 5ºC AND 35ºC ± 5ºC

Time (hour)	Total Bacteria (CFU/mL)		Log CFU/mL	
	25ºC	**35ºC**	**25ºC**	**35ºC**
0	$3.1x10^{10}$	$3.0x10^{10}$	10.49	10.48
2	$2.8x10^{10}$	$2.7x10^{10}$	10.45	10.43
4	$2.6x10^{10}$	$1.8x10^{9}$	10.41	9.26
6	$4.6x10^{9}$	$1.3x10^{9}$	9.66	9.11
8	$4.0x10^{9}$	$9.5x10^{8}$	9.60	8.98
10	$2.5x10^{9}$	$6.2x10^{8}$	9.40	8.79
24	$1.3x10^{9}$	$3.4x10^{8}$	9.11	8.53

Based on Table 5, probiotic bacteria at a storage temperature of 25ºC decreased significantly at the 6th hour to $4.7x10^{9}$ CFU/mL. Probiotic bacteria at 35ºC decreased faster significantly, which happened at the 4^{th} hour ($2.0x10^{9}$ CFU/mL) and decreased again at the 10^{th} hour ($8.5x10^{8}$ CFU/mL) Based on Table 4 and Table 5; probiotic bacteria decrease faster at higher storage temperatures. At the storage temperature of 15ºC on the 7th day (168 hours) the number of probiotic bacteria was $7.2x10^{8}$ CFU/mL, while at the storage temperature of 25ºC on 24th hour the number was $1.3x10^{9}$ CFU/mL, and at the storage temperature of 35ºC on 24th-hours the number was $3.4x10^{8}$ CFU/mL.

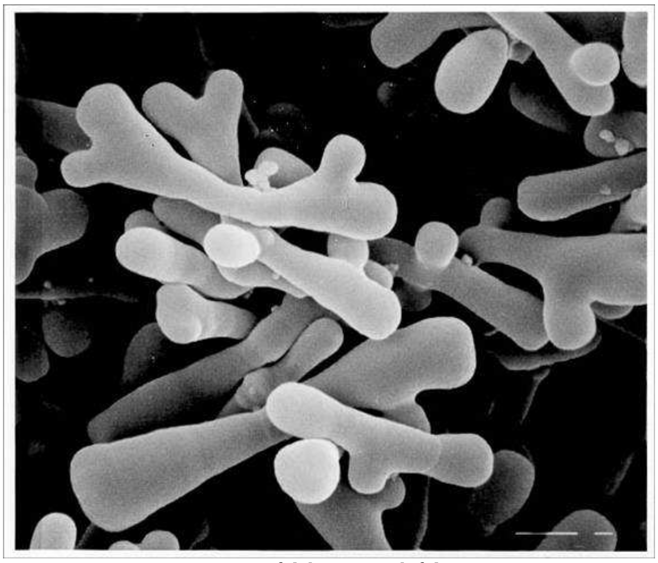

Fig. 1 *Bifidobacterium bifidum*

The decrease speed difference of probiotic bacteria due to the storage temperature difference. Higher storage temperatures will boost the metabolic rate and growth faster. Therefore, nutrients decrease and the number of bacteria decreases faster Table 6 plotted regression equation of data in Table 4 and Table 5. The graph shows the relationship between temperature and storage time. Synbiotic drinks stored at 15ºC ± 5ºC and 25ºC ± 5ºC, 35ºC ± 5ºC has an r-value close 1, equal to 0.78-0.96 (78%-96%). The value of r (correlation coefficient) shows that the stored of total probiotic bacteria has a very strong relationship. The difference between 4-22% influenced by intrinsic factors, extrinsic factors, food processing factors, and implicit factors. The slope value states that the decrease in total probiotic bacteria during storage will decrease by 0.00% - 0.08%.

TABLE VI
REGRESSION EQUATIONS OF TOTAL PROBIOTIC BACTERIA TOWARDS STORAGE

Storage Temperature	Regression Equation	R^2 value	r value
15**ºC**±5ºC	y = -0.0086x + 10.24	0.9273	0.963
25**ºC**±5ºC	y = -0.0612x + 10.348	0.7387	0.859
35**ºC**±5ºC	y = -0.076x + 9.9546	0.6048	0.778

According to Kailasapathy *et al.*[11], *B. bifidum* will grow faster at its optimal temperature (37°C-41°C). B. bifidum was growing faster in the synbiotic beverage that stored at 35ºC in accordance with the results. In contrast to the synbiotic drinks stored at 15°C, the *B. bifidum* growth slower due to slow bacterial metabolic activity [5], besides *B. bifidum* also hard to grow below the limit of optimal temperature (22ºC). The storage of synbiotic drinks at 25ºC growth faster than storage at 15°C.

C. pH Value

Based on Table 7 and Table 8, the higher storage temperature means the lower the pH value will decrease faster, influenced by the optimal bacterial growth temperature. The optimal formation of lactic acid was the cause of it. If B. bifidum added to drinks, it will simpler the nutrients and produce lactic acid [9].

TABLE VII
PH VALUE OF SYNBIOTIC DRINKS AT 15ºC ± 5ºC

Time (hour)	pH
0	6.8
24	6.7
48	6.4
72	5.5
96	4.6
120	4.5
144	4.4
168	4.1

TABLE VIII
PH VALUE OF SYNBIOTIC DRINKS AT 25ºC ± 5ºC AND 35ºC ± 5ºC

Time (hour)	pH	
	25ºC	35ºC
0	6.7	6.9
2	6.5	6.8
4	6.5	6.6
6	6.4	6.4
8	5.8	5.3
10	5.5	4.7
24	4.2	3.0

According to Queiroz, *et al.* [4] and Bernat[12], the mechanism for the formation of lactic acid starting from the breakdown of the substrate into simple sugars produces energy for the activity of bacteria and probiotics, then producing lactic acid. The more lactic acid be formed the lower pH value. In these conditions, the more H^+ ions produced, the lower pH value and give a sour taste to the product.

Based on Table 8, storage period at 35°C and 25°C in 24 hours can produce pH 3.0 and pH 4.2. Meanwhile, based on Table 9, storage period at 15°C produce pH 4.1 in 7 days (168 hours). This statement compatible with a research by Schlabitz et al. [10], during storage in refrigerator fermented synbiotic milk drinks, pH will change slowly. According to Burnside [13], probiotic drinks made from cereals will undergo significant acid formation after 8 hours. Liu et al. [14] stated that one of the probiotic bacteria, Lactobacillus plantarum in synbiotic drinks, can produce pH up to 3.9 after 16 hours.

The data obtained from Table 7 and Table 8 are plotted on the graph of the pH value towards storage time then the equation as shown in Table 9.

TABLE IX
REGRESSION EQUATIONS OF PH VALUE TOWARDS STORAGE TIME

Storage Temperature	Regression Equation	R^2 value	r value
15**ºC**±5ºC	y = -0.0086x + 10.24	0.9273	0.963
25**ºC**±5ºC	y = -0.0612x + 10.348	0.7387	0.859
35**ºC**±5ºC	y = -0.076x + 9.9546	0.6048	0.778

Based on Table 9, pH value of synbiotic drinks at a temperature of 15ºC ± 5ºC, 25ºC ± 5ºC, 35ºC ± 5ºC has a value of r (correlation coefficient) close to 1, which is 0.96-0.98 (96% -98%). The correlation coefficient indicates that the pH value with storage time has a very strong closeness. The slope value states that the decrease in pH value during storage will decrease by 0.02%-0.18%.

D. Viscosity

The observation of viscosity values in synbiotic drinks at several storage temperatures are given in Table 10 and Table 11.

TABLE X
THE VISCOSITY OF SYNBIOTIC DRINKS TEMPERATURE AT 15ºC ± 5ºC

Time (hour)	Viscosity (mPas)
0	27
24	30
48	35
72	40
96	59
120	75
144	78
168	90

TABLE XI
SYNBIOTIC DRINKS VISCOSITY AT 25 ± 5ºC AND 35 ± 5ºC

Time (hour)	pH	
	25ºC	35ºC
0	24	25
2	25	30
4	29	32
6	33	36
8	38	43
10	42	62
24	45	116

Based on Table 10 and Table 11, the viscosity of synbiotic drinks has increased during storage at every three storage temperatures. High viscosity signifies increasing volatility. The increase in viscosity is affected by the increase in lactic acid which is known from the decreasing pH value due to storage temperature. The viscosity of synbiotic drinks also influenced by several factors including the type of culture, use of temperature, and the use of stabilizers [11]. The data in Table 10 and Table 11 are plotted on the graph of viscosity towards storage time. The equation is given in Table 12.

TABLE XII
REGRESSION EQUATIONS OF VISCOSITY TOWARDS STORAGE TIME

Storage Temperature	**Regression Equation**	**R^2 value**	**r value**
15**ºC**±5ºC	y = 0.4067x + 20.083	0.9531	0.9762
25**ºC**±5ºC	y = 0.9157x + 26.651	0.7808	0.8836
35**ºC**±5ºC	y = 3.9488x + 18.681	0.9717	0.9857

Based on Table 12, the viscosity of the synbiotic drink at a temperature of 15ºC ± 5ºC, 25ºC ± 5ºC, 35ºC ± 5ºC has a value of r (correlation coefficient) close to 1, which is 0.88-0.99 (88%-99%). The correlation coefficient indicates that the viscosity towards the storage time has a very strong closeness. The slope value states that the increase in viscosity during storage increase by 0.41% -3.95%.

Increased viscosity values can be affected by total dissolved solids. The use of sorghum flour and skim milk will increase the total dissolved solids. According to Kechagia et al. [15], an increase in viscosity also caused by protein coagulation. The protein content of sorghum flour in the manufacture of synbiotic drinks is 10-12%. According to Teixeira et al [16] skim milk containing casein─which is the main protein of milk─in acidic conditions becomes unstable and coagulating to gel form. Coagulation of proteins will increase the viscosity.

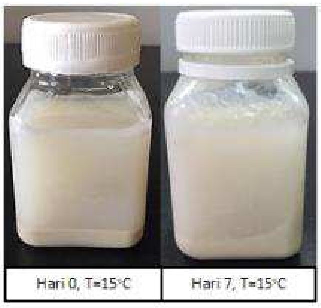

Fig. 2 Synbiotic Drinks at 15ºC: D-0 (left); D-7 (right)

Fig. 3 Synbiotic Drinks at 25ºC: D-0 (left); D-1 (right)

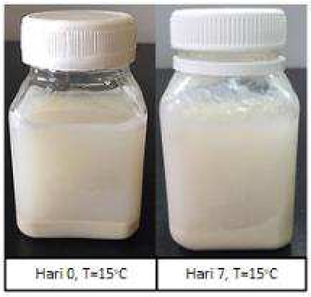

Fig. 4 Synbiotic Drinks at 15ºC: D-0 (left); D-7 (right)

E. Shell Life Prediction

The rate of decline in the quality of food products due to the activity of the microorganisms will follow one order kinetics.

TABLE XIII
DETERMINATION OF THE ORDER OF REACTION

Parameter	**Temperature**	**R^2 Ordo 0 value**	**R^2 Ordo 1 value**
Probiotic	15^0C±5^0C	0.7780	0.9273
	25^0C±5^0C	0.5042	0.7387
	35^0C±5^0C	0.6368	0.6084
Lactic Acid Bacteria	15^0C±5^0C	0.9896	0.9749
	25^0C±5^0C	0.6857	0.7644
	35^0C±5^0C	0.6510	0.8000
pH Value	15^0C±5^0C	0.9265	0.9353
	25^0C±5^0C	0.9659	0.9720
	35^0C±5^0C	0.9391	0.9651
Viscosity	15^0C±5^0C	0.9531	0.9676
	25^0C±5^0C	0.7808	0.7375
	35^0C±5^0C	0.9717	0.9633

TABLE XIV
ACTIVATION ENERGY

Parameter	**Equation**	**Slope (-Ea/R)**	**Ea (Kcal/mol)**
Probiotic	y =-9752.3x + 30.208	-9752.3	19.3798
Lactic Acid Bacteria	y =-9992x + 30.416	-9992	19.8561
pH value	y = -10658x + 31.504	-10658	21.1796
Viscosity	y = -10046x + 33,864	-10046	19.9634

Arrhenius equations that are used based on the critical point of probiotic bacteria

$$\mathbf{K = 1.3 \text{ x } 10^{13} \text{ x } e^{-\frac{\frac{19,3798\ kkal}{mol}}{\frac{1,9872x10^{-3}kkal}{mol}.K} x\frac{1}{T}}} \tag{1}$$

The value of the lowest to the highest consecutive Ea found in probiotic bacteria parameters of 19.3798 kcal/mol, lactic acid bacteria of 19.8561 kcal/mol, the viscosity of 19.9634 kcal/mol, and a pH of 21.1796 kcal/mol

Shelf life of probiotic bacteria sinbiotik drinks was 23 days at 4^{o}C, storage temperature for 14 days at a temperature of 8^{o}C storage, 8 days at a temperature of 12^{o}C storage, 5 days at a temperature of 16^{o}C storage, 3 days at a temperature of 20^{o}C and 2 days of storage at a temperature storage of 24^{o}C

IV. Conclusion

Based on the research of synbiotic drinks stored at a temperature of 15 ± 5ºC, 25 ± 5ºC, and 35 ± 5ºC, the conclusions are total Lactic Acid Bacteria (LAB), total probiotic bacteria and pH value decreased at all temperatures are decreasing at all storage temperatures with a very strong closeness, but viscosity increases at all temperatures storage with a very strong closeness. Shelf life drink sorghum sinbiotik supposedly faster if storage temperatures offered up to room temperature

Acknowledgment

We thank all those who have helped in carrying out the research. We thank our Faculty of Agricultural Industrial Technology (FTIP) UNPAD who provided insight and expertise that greatly assisted the research, although they may not agree with all of the interpretations/conclusions of this paper. We are also immensely grateful for their comments on an earlier version of the manuscript, although any errors are our own and should not tarnish the reputations of these esteemed persons. We would like to thank the Rector University of Padjadjaran that has funded this research through the Competence Research Lecture's University of Padjadjaran 2017-2018 fiscal year

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