Original Research Article

The Effect of Stevia *(Stevia rebaudiana)* Sweetener Addition on Physical Quality of Synbiotic Yoghurt Containing Extract of Evaporated Red Dragon Fruit Peels *(Hylocereus polyrhizus)*

ABSTRACT

This research was conducted at the Laboratory of Animal Products Technology, Faculty of Animal Science, Universitas Brawijaya from August to October 2021. Synbiotic yoghurt is a combination of probiotics and prebiotics, so adding stevia sweetener can improve the physical quality. The purpose of this study was to determine the effect of adding stevia sweetener to synbiotic yoghurt of evaporated red dragon fruit peel extract in terms of physical quality. The research method used is a laboratory experiment using a Completely Randomized Design (CRD) pattern with four treatments and five replications. The percentage treatment of stevia sweetener carried out was the control treatment 0% (T0), 0.5% (T1), 2% (T2), 3.5% (T3). The analysis results showed that stevia sweetener concentration added a very significant difference (T<0.01) to pH, syneresis, and color L*a*b. However, it did not have a significant effect (P>0.05) on the water holding capacity. Based on the results of the research conducted, it can be concluded that the treatment with physical quality as the best treatment was T1 with the addition of 0.5% stevia sweetener with an average pH 4.15; syneresis 27.11%; water holding capacity 71.33%, and color L value 38.38; a value 13.46; b value 4.60. The physical quality of synbiotic yoghurt with the addition of 0.5% Stevia sweetener complies with SNI 2981:2009, and the use of 0.5% Stevia percentage is more efficient than the other percentage additions

Keywords: *Synbiotic Yoghurt, Red Dragon Fruit, Stevia, Physical Quality.*

1. INTRODUCTION

Functional food products have been widely circulated in the market, most of which are dominated by dairy products and their processed products [1]. One of the most popular today in the industry is synbiotic yoghurt. Synbiotic yoghurt is one of the functional foods which is the result of milk fermentation using lactic acid bacteria. The use of lactic acid bacteria in yoghurt such as *Lactobacillus bulgaricus* and *Streptococcus thermophillus* provides benefits for the body, namely it can suppress the presence of harmful microorganisms in the large intestine so that the digestive tract becomes healthier. Lactic acid bacteria can therefore be said to be probiotic bacteria, namely bacteria that are beneficial to the human body [2]. The presence of probiotics in the human colon is supported by the presence of prebiotics as an energy source for probiotic microorganisms. Prebiotics are carbohydrates that are resistant to human digestive enzymes so that they are available in the large intestine as an

energy source for probiotic bacteria [3]. One of the fruits that can be used as prebiotics is the peel of the red dragon fruit (*Hylocereus polyrhizus*) [4].

Red dragon fruit peel contains anthocyanins which are a group of red to blue pigments that are widely distributed in plants. Anthocyanins belong to pigments called flavonoids. Compounds of the flavonoid group are polar compounds and can be extracted with polar solvents as well. Some solvents that are polar include ethanol, water and ethyl acetate. This extraction process using Microwave-Assisted Extraction (MAE) is an extraction that utilizes microwave radiation to accelerate selective extraction by heating the solvent quickly and efficiently [5]. MAE increases the efficiency and effectiveness of extracting the active ingredients of various types of herbal plants and fruits [6]. This red dragon fruit peel extract can give yoghurt an attractive appearance.

The physical quality and variety of synbiotic yoghurt products can be improved by adding sweeteners. Sweeteners function are to improve taste and aroma, improve physical properties, as preservatives, improve as well as a source of calories for the body [7]such as the fiber content in stevia sweetener *(Stevia rebaudiana)* can increase the total solids content of synbiotic yoghurt. as well as binding water so that the more addition of stevia sweetener the more the amount of water bound, causing the water content to decrease and finally the synbiotic yoghurt will be thicker [8]. The addition of stevia sweetener *(Stevia rebaudiana)* is increasing, the total solids content in synbiotic yoghurt will increase and syneresis will decrease. Therefore, it is necessary to research the addition of stevia sweetener with the correct percentage in synbiotic yoghurt with evaporated red dragon fruit peel extract to improve physical quality.

2. MATERIALS AND METHODS

2.1 Materials

The materials used in the research were skimmed milk powder, a starter culture of standard yoghurt containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, red dragon fruit peel (*Hylocereus polyrhizus*), stevia sweetener (Tropicanaslim), aluminum foil, aquadest, NaOH 0.1N, 40% formalin, indicator PP, H_2SO_4. microwave (Sharp), beaker glass, erlenmeyer, buret, centrifugator, centrifuge tube, analytical scale, hotplate and magnetic stirrer (SBS-06), thermometer, higrometer, pH meter (Ohaus), refrigerator (Modena), colorimeter.

2.2 Methods

The research method used is a Laboratory Experiment by using a Completely Randomized Design (CRD) pattern with four treatments and five replications. The treatments that were tried were the addition of Stevia percentage 0% (T0), 0.5% (T1), 2% (T2); 3.5% (T3). Data were analyzed using Analysis of Variety (ANOVA), and if there were significant differences, it would be further testing with *Duncan's Multiple Range Test (DMRT)*. Parameters observed were pH by pH meter [9], syneresis by centrifugation method [10], water holding capacity by centrifugation [11] and color L*a*b by colorimeter.

2.2.1 Evaporated Red Dragon Fruit Peel Extraction

The dragon fruit peel was cleaned and cut into 1cm squares, weighed 50gr of dragon fruit peel pieces, added 50ml of distilled water, and put into an erlenmeyer. Then put in the microwave at high temperature (90^oC) for 5 minutes. Furthermore, the extract was filtered and measured again as much as 50ml and put into a beaker glass. After that, it was put into a modified microwave with a rotary evaporator at medium-high temperature (75^oC) [12].

2.2.2 Manufacture of Synbiotic Yoghurt

In making synbiotic yoghurt, weighing 50gr skimmed milk powder, added 100ml red dragon fruit peel extract, and put into an Erlenmeyer. Then add 350ml aquadest and homogenize. It was pasteurized at 75^{o}C for 15 minutes. After that, the temperature was lowered to 40^{o}C and inoculated with a 3% starter, then incubated at room temperature (26-27^{o}C) for 21 hours. Finally, Stevia is added after the fermentation process according to the treatment. Finally, Stevia is added after the fermentation process according to the treatment.

3. RESULTS AND DISCUSSION

3.1 pH

The results of the analysis of variance showed that the addition of different concentrations of stevia sweetener gave a very significant difference (T<0.01) to the average pH of synbiotic yoghurt containing extract of evaporated red dragon fruit peel. Table 1 shows that the average pH of synbiotic yoghurt containing extract of evaporated red dragon fruit peel increased with the addition of stevia sweetener concentration. The lowest to highest average pH was T0 (4.11±0.008), T1 (4.15±0.007), T2 (4.16±0.008), T3 (4.16±0.008). The lowest average pH was produced by T0 and the highest average pH was produced by T3. T0 shows a very significant difference with T1, T2 and T3. While T1 showed no significant difference with T2 with T3.

The increase in the average pH of synbiotic yoghurt with red dragon fruit peel extract with the addition of stevia sweetener was caused by the quality of the raw materials used. The raw material used in the manufacture of synbiotic yoghurt with red dragon fruit peel extract is skimmed milk powder with a pH of 6.8 added to the evaporated dragon fruit peel extract with a pH of 3.4 and a pH of stevia sweetener 6.20 thereby increasing the average pH of synbiotic yoghurt red dragon fruit peel extract, with the addition stevia sweetener. Increasing the pH of synbiotic yoghurt with red dragon fruit extract with stevia sweetener indicates that the addition of stevia sweetener causes less organic acid to be formed. The sugar content in stevia sweetener cannot be used optimally in the growth of lactic acid bacteria to form organic acids. According to previous research, the stevioside content in stevia leaves cannot be used to support bacterial growth, such as in yoghurt starter bacteria [13]. The amount of acid content formed during the growth process of bacteria cultured on media containing stevioside was lower than the acid yield obtained from bacteria grown on media containing sucrose, glucose, or fructose. Stevia sweetener also acts as a sweetener, generally reducing acidity and increasing the pH of fermented beverages [14].

Sugar at a certain level has an inhibitory effect on yoghurt bacteria, so the level of added sugar should not exceed the level of 10-11% [15]. The higher the level of stevia administration, it will cause the decrease in the activity of the starter bacteria so that the formation of lactic acid from lactose also decreases. Bacteria, yeast, and molds are placed in a concentrated sweetener solution, then the water in the cells will come out through the membrane and flow into the sweetener solution, this event is known as osmosis, and in this condition, the microorganism cells undergo plasmolysis so that their reproduction is inhibited [8]. If the glucose concentration is higher, the fermentation speed will decrease and inhibit the activity of *Lactobacillus bulgaricus* and *Streptococcus thermophillus* microbes so that the fermentation process will take longer. This happens because of the glucose concentration is too large, plasmolysis will occur in the cell wall of the microorganism causing the cell wall to break [16].

Table I. The average pH, syneresis, and water holding capacity of synbiotic yoghurt on the percentage of stevia sweetener

Treatment	Analysis		
	pH	Syneresis (%)	Water Holding Capacity (%)
T0	4.11[a]±0.008	29.01[b]±0.12	69.77±1.77
T1	4.15[b]±0.007	27.11[a]±0.53	71.33±3.01
T2	4.16[b]±0.008	26.80[a]±0.67	71.87±3.22
T3	4.16[b]±0.008	26.03[a]±0.79	72.31±3.48

3.2 Syneresis

The results of the analysis of variance showed that the addition of different concentrations of stevia sweetener gave a very significant difference ($P<0.01$) to the syneresis mean of synbiotic yoghurt with red dragon fruit peel extract. Table 1 can be seen that the syneresis means of synbiotic yoghurt with red dragon fruit peel extract decreased with the addition of stevia sweetener concentration. The average syneresis from the highest to the lowest was T0 (29.55±0.12), T1 (27.11±0.53), T2 (26.80±0.67), T3 (26.03±0.79). The highest syneresis average was produced by T0 and the lowest syneresis average was produced by T3. T0 shows a very significant difference with T1, T2, and T3. While T1 shows the same as T2 with T3.

Syneresis occurs due to the shrinkage of the three-dimensional structure of the protein network which causes a decrease in the bond strength of whey protein so that it is separated from yoghurt [17]. The occurrence of syneresis is probably caused by changes in the solubility of casein and shrinkage of casein particles. At lower pH conditions, the solubility of protein and casein will decrease which causes more protein-protein interactions and lower protein-water interactions. The low protein-water interaction will result in precipitation so that the protein network tends to release water. Increased protein-protein interactions and decreased protein-water interactions cause shrinkage or protein gel contractions that promote curd formation along with whey separation. If the building block of the protein network shrinks, then all the tissue will shrink proportionally so that the protein's ability to bind or trap water can be lost [18]. The occurrence of syneresis during the storage of yoghurt products is an important appearance defect for yoghurt manufacturers to pay attention to [19].

Yoghurt is easily damaged, one of which is syneresis, namely the separation between solids and liquids. Syneresis occurs as a result of the decreased ability of protein networks to bind water, the higher the syneresis, the lower the quality [20]. Syneresis affects consumer acceptance. The separation will be higher when stored at 25^{o}C and 45^{o}C for 25 hours. The activity of lactic acid bacteria is faster if the storage temperature used is higher [21]. One way to prevent syneresis is to add materials that can bind water and increase the viscosity of yoghurt [22].

The decrease in syneresis of red dragon fruit peel extract with the addition of stevia sweetener is thought to be due to the higher amount of solids due to the addition

of stevia sweetener. The factors that influence the syneresis of synbiotic yoghurt include acidity and pH, total solids, and water holding capacity [20]. The addition of stevia sweetener is increasing, the total solids content in synbiotic yoghurt will increase and syneresis will decrease. The decreased activity of lactic acid bacteria due to the addition of stevia sweetener will inhibit the breakdown of lactose into lactic acid, thereby inhibiting the decrease in the pH value of yoghurt. The pH condition that is not too acidic in yoghurt makes the water that binds to the protein remains bound, thereby reducing the occurrence of syneresis [22].

3.3 Water Holding Capacity

The results of the analysis of variance showed that the addition of different concentrations of stevia sweetener did not make a difference ($P>0.05$) to the average water holding capacity of synbiotic yoghurt with red dragon fruit peel extract. Table 1 shows that the average water holding capacity of synbiotic yoghurt with red dragon fruit peel extract increased with the addition of stevia sweetener concentration. The average water holding capacity from the highest to the lowest is T3 (72.31±3.48), T2 (71.87±3.22), T1 (71.33±3.01), T0 (69.77±1.77). The highest average water holding capacity was produced by T3 and the lowest average water holding capacity was produced by T0.

The increase in the average water holding capacity of synbiotic yoghurt with red dragon fruit peel extract with the addition of stevia sweetener is suspected as stevia sweetener in synbiotic yoghurt can increase viscosity, because sugar can bind water which can cause the solution to become more viscous. Viscosity or viscosity is the ability to hold fluid to flow [24]. Stevia sweetener can bind water, along with decreasing water content due to being bound by sugar, the fluid flow rate in synbiotic yoghurt will also decrease, this can cause viscosity to increase. The addition of sugar will increase the total dissolved solids in synbiotic yoghurt, it can make the viscosity and water binding capacity of synbiotic yoghurt higher [25].

The water-holding capacity of yoghurt has a positively charged slope, where the higher the concentration of addition of stevia sweetener, the water-holding capacity also increases. The value of water holding capacity is relatively high in yoghurt which has an average total lactic acid of 1.24% and an average pH of 4.17, considering that in general fermentation products will experience the release of water molecules due to protein denaturation activity by low pH so that the water holding capacity is low. decreased [26]. Casein in an environment with an isoelectric pH range will form a water molecule binding system that is weaker than when casein is in an environment with a normal pH.

The casein molecule undergoes a change in ionic charge from negative to positive because it interacts with stevia sweetener when the pH of the synbiotic yoghurt reaches the isoelectric point of casein so that casein and lactic acid cannot bind to each other because they have the same ionic charge [27]. Stevia sweetener can increase the binding power of synbiotic yoghurt by preventing hydrogen bonds between casein molecules and lactic acid, as well as triggering changes in the charge of casein ions and maintaining bonds between protein molecules so that the tendency of negatively charged

casein molecules to bind with positively charged lactic acid molecules through hydrogen bonding can be prevented. The addition of stevia sweetener causes an increase in the hydrophilic nature of the protein so that its ability to bind water increases [28].

Table 2. The average color L*a*b of synbiotic yoghurt on the percentage of stevia sweetener

Treatment	Color analysis			Chroma	Hue
	L	a	b		
T0	41.12^{d}±1.31	14.18^{b}±0.79	5.53^{c}±0.27	15.22	21.31
T1	38.38^{bc}±0.36	13.46^{a}±0.50	4.60^{b}±0.11	14.22	18.87
T2	37.30^{ab}±0.34	12.93^{a}±0.45	4.28^{ab}±0.15	13.62	18.32
T3	37.64^{a}±0.17	12.72^{a}±0.54	4.04^{a}±0.08	13.35	17.62

3.4 Color L*a*b

The results of the analysis of variance showed that the addition of different concentrations of stevia sweetener gave a very significant difference (P<0.01) to the average color of L*a*b synbiotic yoghurt with red dragon fruit peel extract. Table 2 shows that the average color of L*a*b synbiotic yoghurt with red dragon fruit peel extract with stevia sweetener decreased with the addition of the percentage of stevia sweetener. The lowest to highest average L values are T3 (37.64±0.17), T2 (37.30±0.34), T1 (38.38±0.36) and T0 (41.12±1.31). The highest average L value was produced by T0 and the lowest average L value was produced by T3. The average value from the lowest to the highest is T3 (12.72±0.54), T2 (12.93±0.45), T1 (13.46±0.50) and T0 (14.18±0.79). The highest average a value is T0 and the lowest a value average is T3. The lowest to highest average b values were T3 (4.04±0.08), T2 (4.28±0.15), T1 (4.60±0.11) and T0 (5.53±0.27). The highest average b value is T0 and the lowest average b value is T3. The CIE L*a*b color spectrum can be seen in Figure 1.

Lightness indicates the darkness of the color. The greater the lightness value, the lighter the color, and the smaller the lightness value, the darker the color. The L notation states the brightness parameter which has a value of 0 for black to 100 which represents white [29]. The L value in this study ranged from 37.64 to 41.12 indicating low brightness, this is because the added dragon fruit peel extract indicates the product has a low brightness level (dark) and produces a purplish red color that is getting darker [10]. The addition of the percentage of stevia sweetener in each treatment reduced the lightness of the product, this indicates that the stevia sweetener has a cloudy white color which makes the product's lightness decrease.

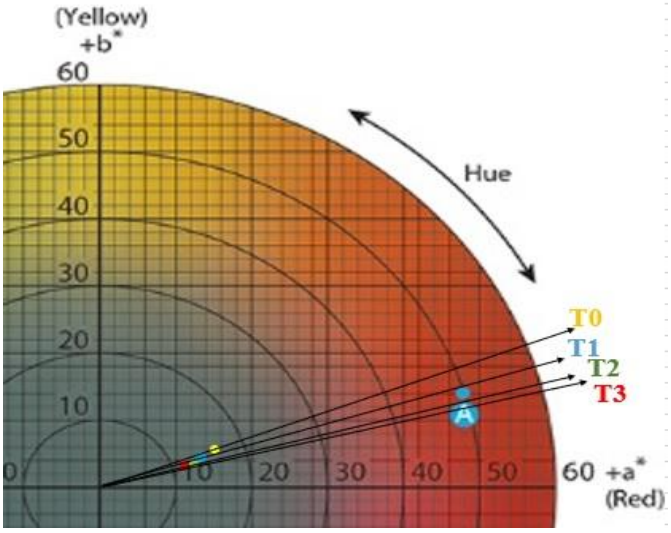

Figure 1. The CIE L*a*b color spectrum

The average value for a* color can be seen in Table 2. Color a* indicates a reddish or greenish color, a positive a* color indicates a red color, while a negative a* color indicates a green color. The decrease in a* color occurs

due to the processing process, so that the formation of the red color is hindered because the dragon fruit peel extract which is heated will change its color to pink because the betacyanin in the red dragon fruit peel extract reacts when exposed to heat to fade [30]. The reddish color also fades because of the addition of stevia sweetener, this is because the basic color of this stevia sweetener is cloudy white so that it decreases with each addition of the percentage of stevia sweetener in each treatment. The average value for color b* can be seen in Table 2. Color b* indicates a yellowish or bluish color, positive b* color indicates yellow, while negative b* color indicates blue. The yellowish color is caused by beta carotene contained in milk, beta carotene in milk will be more visible as the viscosity of yoghurt increases. However, the addition of this red dragon fruit extract covers the resulting yellowish color so that the measured color tends to be bluish followed by the addition of stevia sweetener which will cause the color to tend to bluish [31].

4. CONCLUSION

Based on the results of the research conducted, it can be concluded that the treatment with physical quality as the best treatment was T1 with the addition of 0.5% stevia sweetener with an average pH 4.15; syneresis 27.11%; water holding capacity 71.33%, and color L value 38.38; a value 13.46; b value 4.60. The physical quality of synbiotic yoghurt with the addition of 0.5% Stevia sweetener complies with SNI 2981:2009, and the use of 0.5% Stevia percentage is more efficient than the other percentage additions.

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