Original Research Article

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

ABSTRACT

The research was held from August 2021 until October 2021 in Laboratory Livestock Product Technology Universitas Brawijaya, Malang, Indonesia. Synbiotic yoghurt is a combination of probiotics and prebiotics, so adding stevia sweetener can improve the chemical 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 chemical 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). Data analysis used Analysis of Variance (ANOVA); if the results obtained were significant, the analysis will continue with Duncan's Multiple Range Test (DMRT). The analysis results showed that stevia sweetener concentration added a very significant difference ($P<0.01$) to total lactic acid, protein content, fat content, and antioxidant activity. Furthermore, based on SNI 2981:2009 regarding the quality of yoghurt, the treatment that has the best chemical quality is T1 with the addition of stevia sweetener 0.5%, with an average total value of lactic acid 1.33%; protein content 3.95%; 2.41% fat content; and 10.14% antioxidant activity.

Keywords: *Synbiotic Yoghurt, Red Dragon Fruit Peels Extract, Stevia, Chemical Quality.*

1. INTRODUCTION

Fermented milk products are functional foods that are beneficial for health. The fermented milk using lactic acid can increase the nutritional value, especially vitamin B complex, folic acid, pantothenic acid, and biotin [1]. The advantage of the fermentation process is that it allows specific substrates that can increase the viability of bacteria so the body can get better benefits [2]. *Lactobacillus* is a probiotic microorganism that can maintain and improve human health, so the use of lactic acid bacteria to develop functional food is beneficial for the bacterial host [3]. Prebiotics are materials that the body cannot digest, but probiotic bacteria can utilize them as an energy source [4]. The combination of probiotics and prebiotics acts as a symbiotic.

Red dragon fruit skin *(Hylocereus polyrhizus)* is very beneficial for health

because it has a rich content of fiber, phosphorus, vitamin C, and calcium. The edible portion of crude fiber in red dragon fruit is 10.1 g per 100 g; besides, it contains high antioxidant vitamins such as vitamins A, C, and E, namely 102.13 µg, 540.27 mg, and 105.67 µg per 100 g dry weight [5]. Red dragon fruit peel contains polyphenols and good sources of antioxidants, including total phenol 39.7 mg/100g, total flavonoids (catechins) 8.33 mg/100g, betacyanin 13.8 mg [6], pectin yield 25,79% [7]. The benefits of the red dragon fruit skin can be achieved using the Microwave Assisted Extraction method.

Synbiotic yoghurt products' chemical quality and variety can be improved by adding sweeteners. Sweeteners have many functions, such as improving taste and aroma, improving physical properties, as preservatives, and improving chemical properties [8]. The addition of Stevia Sweetener *(Stevia rebaudiana)* it also referred as Stevia affects a decrease in total lactic acid in synbiotic yoghurt, an increase in protein content, and a decrease in fat content along with the addition of stevia sweetener concentration [9]. Therefore, it is necessary to research the addition of stevia sweetener with the correct percentage in synbiotic yoghurt evaporated red dragon fruit peel extract to improve chemical quality.

2. MATERIALS AND METHODS

2.1 Materials

The materials used in the research were skimmed milk powder, starter culture of standard yoghurt containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (1:1), red dragon fruit peel (*Hylocereus polyrhizus*), stevia sweetener (Tropicanaslim), aluminum foil, aquadest, NaOH 0.1N, formaldehyde 40%, indicator PP 1%, H_2SO_4. microwave (Sharp), beaker glass (Pyrex), erlenmeyer (Pyrex), buret, centrifugator, centrifuge tube, analytical scale (I-3000), hotplate and magnetic stirrer (SBS-06), thermometer, higrometer (HTC-1), colorimeter (CS-10), pH meter (Ohaus), refrigerator (Modena).

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 total lactic acid by titration method [10], protein content by titration method [11], fat content by lactescent and antioxidant activity by DPPH [12].

2.2.1 Evaporated Red Dragon Fruit Peel Extraction

The dragon fruit peel was cleaned and cut into 1cm squares, weighed 50g 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).

2.2.2 Manufacture of Synbiotic Yoghurt

In making synbiotic yoghurt, weighing 50gr skim milk, added 100ml red dragon fruit peel extract, and put into an Erlenmeyer. Then add 350ml aquadest and homogenize. It was pasteurized at 75^oC for 15 minutes. After that, the temperature was lowered to 40^oC and inoculated with a 3% starter, then incubated at room temperature (26-27^oC) 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

The average total Lactic Acid, Protein Content, Fat Content, and Antioxidant Activity of synbiotic yoghurt on the addition of different percentages of Stevia sweetener 0% (P0); 0.5% (P1), 2% (P2); 3.5% (P3) can be seen in Table 1

Table I. Average total lactic acid test, protein content, fat content and antioxidant activity of synbiotic yoghurt on the percentage of stevia sweetener

Treatment	Analysis			
	Lactic Acid	Protein Content	Fat Content	Antioxidant Activity
T0	$1,44^{w}$±0,09	$3,66^{a}$±0,12	$3,06^{c}$±0,01	$6,77^{a}$±0,26
T1	$1,33^{x}$±0,09	$3,95^{b}$±0,10	$2,41^{bc}$±0,11	$10,14^{c}$±0,22
T2	$1,29^{x}$±0,10	$4,09^{b}$±0,09	$2,31^{b}$±0,08	$9,58^{b}$±0,27
T3	$1,24^{x}$±0,07	$4,13^{b}$±0,09	$2,15^{a}$±0,05	$10,21^{c}$±0,17

3.1 Lactic Acid

The acidity level in synbiotic yoghurt with red dragon fruit peel extract was calculated as a percentage of the total lactic acid produced. The analysis of variance showed that the addition of different concentrations of stevia sweeteners gave a very significant difference (P<0.05) to the average of total lactic acid synbiotic yoghurt red dragon fruit peel extract. Table 1 shows that the mean total acidity of synbiotic yoghurt red dragon fruit peel extract decreased with the addition of stevia sweetener concentration. The highest to lowest total acid averages are T0 (1.44±0.09), T1 (1.33±0.09), T2 (1.29±0.10), T3 (1.24±0.07). The highest average total acid was produced by T0, and T3 produced the lowest total acid average.

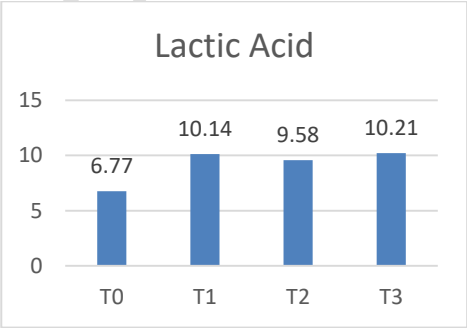

Fig. 1. Lactic Acid

The increase in the average pH of synbiotic yoghurt red dragon fruit peel extract with stevia will be followed by a decrease in the average total lactic acid because the average total lactic acid is inversely proportional to the average pH. The average pH is closely related to the average level of total lactic acid produced [13]. The decrease in average total lactic acid, along with the addition of Stevia, is thought to be due to its role as buffering capacity, which is a solvent that can withstand changes in pH either by absorbing or desorbing ions H^+ and OH^-. Stevia contributes to an OH^- cluster, which adds more sugar means more OH^- clusters are provided, increasing the average pH and decreasing the average total lactic acid [14]. Stevia sweetener contributes to the number of OH^- clusters, which means the more sweetener added, the more OH^- cluster is provided. So that the average pH of synbiotic yoghurt red dragon fruit peel extract with Stevia produced is higher, resulting in lower average total lactic acid.

3.2 Protein Content

The analysis of variance showed that the addition of different concentrations of stevia sweetener gave a very significant difference (P<0.01) to the mean protein content of synbiotic yoghurt red dragon fruit waste extract. Table 1 shows that the average protein content of synbiotic yoghurt red dragon fruit extract increased with the addition

of stevia sweetener concentration. The average protein content from lowest to highest are T0 (3.66±0.12), T1 (3.95±0.10), T2 (4.09±0.09), T3 (4.13±0.09). The lowest average protein content was produced by T0, and T3 produced the highest average protein content.

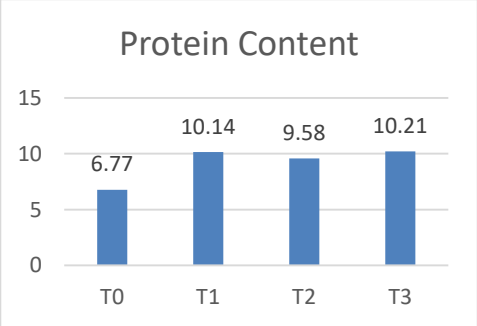

Fig. 2. Protein Content

The Formol Titration method was used to determine the dissolved protein content in this research. Determination of protein content using formol titration is to hydrolyze the protein in the sample [15][16]. During the hydrolysis process, insoluble proteins will be converted into soluble nitrogen compounds [17]. The increase in protein content is thought to be caused by the hydrolysis process of the protein itself, which is one way to increase the protein content in a food ingredient. The increased protein hydrolysis will result in an increasing number of free carboxyl groups, thus causing the dissolved nitrogen content (%N) in the formol titration results to increase [18]. The protein hydrolysis technique breaks down protein structure into peptide fragments and amino acids, causing an increase in dissolved nitrogen content. These peptide and amino acid fractions will be detected as total protein levels [19].

3.3 Fat Content

The analysis of variance showed that the addition of different concentrations of stevia sweetener gave a very significant difference (P<0.01) to the mean fat content of synbiotic yoghurt red dragon fruit waste extract. Table 1 shows that the average fat content of synbiotic yoghurt red dragon fruit waste extract decreased with the addition of stevia sweetener concentration. The average protein content from highest to lowest are T0 (3,06±0,01), T1 (2,41±0,11), T2 (2,31±0,08) T3 (2,15±0,05). The highest average fat content was produced by T0, and T3 produced the lowest average fat content.

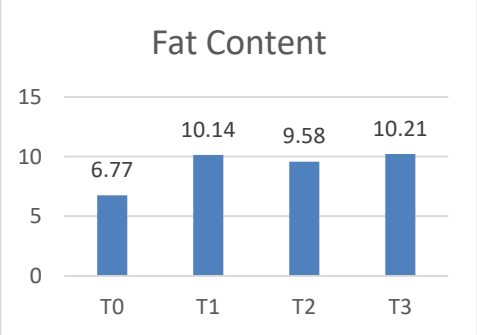

Fig. 3. Fat Content

The decrease in fat content was due to the hydrolysis of fats containing short C-atom fatty acids to form free fatty acids and glycerol. Meanwhile, unsaturated fatty acids will be oxidized to form aldehydes, ketones, alcohols, and organic acids with short C atoms. These compounds play a role in the flavor of the resulting yoghurt [20]. Hydrolysis of fat can occur due to lipase enzymes produced by yoghurt bacteria. The factor that affects fat hydrolysis during storage is the fat content of yoghurt because the higher the fat content of yoghurt, the more likely it is for fat to be hydrolyzed [21]. Lactic acid bacteria produce lipase enzymes so that fat is hydrolyzed and causes a decrease in fat content from raw materials into yoghurt. The decrease in fat content is also due to the fact that lactic acid bacteria use fat for energy sources and flavor formation [22].

Table 1 shows that the more stevia sweetener added, the lower the fat content. The fat content produced in this study ranged from 2.15-3.06%. Yoghurt fat content can be divided into full-fat yoghurt (fat content more than 3%), half-fat yoghurt (fat content 0.5-3.0%), and low-fat yoghurt (fat less than 0.5%).

The fat content difference is based on the type of milk and the mixture of ingredients used in its manufacture. According to SNI 2981:2009, the maximum fat content contained in yoghurt is 3.3%. The fat content in synbiotic yoghurt ranges from 2.15-3.06%. It happens because the raw material used is stevia sweetener which has low-fat content. Meanwhile, according to SNI 2981:2009, the yoghurt that uses cow's milk as raw material has higher fat content than the fat content found in synbiotic yoghurt red dragon fruit peel extract with the addition of stevia sweetener with skim milk as a base. It means the fat content in the manufacture of synbiotic yoghurt in this study can have met SNI 2981:2009.

3.4 Antioxidant Activity

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 antioxidant content of synbiotic yoghurt red dragon fruit waste extract. Table 1 shows that the average antioxidant content of synbiotic yoghurt red dragon fruit extract is increasing. The average of the highest to lowest antioxidant levels was T3 (10.21±0.17), T1 (10.14±0.22), T2 (9.58±0.27), and T0 (6.77±0.26). The highest mean antioxidant content was obtained from treatment T3, and the lowest average antioxidant was obtained from treatment T0.

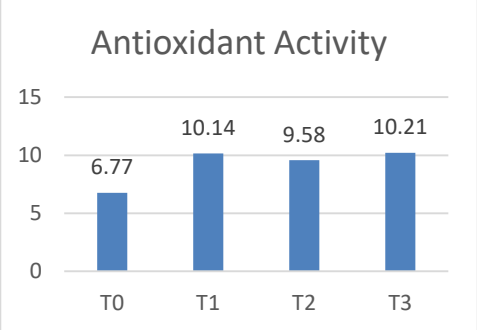

Fig. 4. Antioxidant Activity

The addition of red dragon fruit peel extract increased antioxidant levels in synbiotic yoghurt, because it contains polyphenols and good source of antioxidants; including total phenol 39.7 mg/100g, total flavonoids (catechins) 8.33 mg/100g, and betacyanin 13.8mg [7]. Increased antioxidant activity is also associated with an increase in total lactic acid bacteria, with high antioxidant activity. These lactic acid bacteria have the ability to break down proteins into bioactive peptides [23].

The addition of stevia sweetener increases antioxidant levels and increases the percentage of stevia sweetener addition. Stevia sweetener can increase the antioxidant activity of synbiotic yoghurt with a phenol content of 15.50mg/g in it [24]. Other ingredients that are a source of antioxidants include skim milk. β-lactoglobulin in skim milk acts as an antioxidant which has an antioxidant activity of 26.41% [25]. In addition, flavonoid and polyphenolic compounds that act as antioxidants in stevia sugar have antioxidant activity of 22.61%.

4. CONCLUSION

Based on the results of the research conducted, it can be concluded that the treatment with chemical quality as the best treatment was T1 with the addition of 0.5% stevia sweetener with an average total value of lactic acid 1.33%; protein content 3.95%; fat content is 2.41%, and antioxidant activity is 10.14%. The chemical quality of synbiotic yoghurt with the addition of 0.5% Stevia sweetener complies with SNI 2981:2009, and the use of Stevia is more efficient than the other percentage additions.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

Author RDN wrote the draft of manuscript and performed the statistical analysis. Author MES and AM designed the study, managed the analysis study and the literature searches. All authors read and approved the final manuscript.

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