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THE FORTIFICATION WITH RED DRAGON FRUIT *(Hylocereus polyrhizuz)* PEEL EXTRACT EVAPORATED WITH MAE METHOD TOWARDS THE PHYSIC-CHEMISTRY QUALITY OF SYMBIOTIC YOGHURT

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ABSTRACT

This study aims at determining the optimum fortification with red dragon fruit peel extract evaporated with MAE method towards the physicchemistry characteristics of symbiotic yogurt. The research object is symbiotic yogurt made of pasteurized fresh milk, red dragon fruit peel extract evaporated with Microwave Assisted Extraction (MAE) method and evaporated with rotary vacuum evaporator, and yoghurt starter containing lactic acid bacteria: Streptococcus thermophilus and Lactobacillus bulgaricus (1:1). The study employs an experiment with Complete Random Design in four treatments including without fortification with red dragon fruit peel extract (P0) and with fortification with red dragon fruit peel extract at 10% (P1), 20% (P2), and 30% (P3) (v/v) with three replications. The variables to be analyzed include physical quality (pH, viscosity, syneresis), color (L*, a*, b*) and chemical quality (acid total, lipid and protein level). The data were analyzed using Analysis of Variance (ANOVA). If there was a difference, the analysis would be continued using Duncan multiple Range Test. The result indicates that the fortification with red dragon fruit peel extract shows significant differences on the pH level (P<0,05), viscosity (P<0,01), syneresis, color (L*, a*, b*) and chemical quality of symbiotic yoghurt. Fortification with red dragon fruit peel extract reduces the pH level and syneresis while it increases viscosity, total acid and protein level because of the fiber content i. e. pectin which functions as a stabilizer. Moreover, it also decreases the lipid profile and increases the turbidity (L*, b* and a*) values. It is concluded that the optimum fortification of 20% with red dragon fruit peel extract evaporated using MAE method results in symbiotic yoghurt observed from its physic-chemistry quality.

Key words: Fortification; red dragon fruit skin extract; symbiotic yoghurt; physic-chemistry quality.

INTRODUCTION

Yoghurt is a dairy product that undergoes Lactic Acid Bacteria (LAB) fermentation through the starter reaction of a mixture of Streptococcus salivarius subsp. thermophilus and Lactobacillus delbrueckii subsp. bulgaricus, and classified as functional food (Aswal et al., 2012). Natural functional components in yoghurt include bioactive peptide, conjugated linoleic acid, sphingolipid, fiber, butyrate and probiotic bacteria (Grajek et al., 2005). Milk as the raw material for making yogurt, which is also a protein source rich in essential amino acids, can be used as a source of bioactive peptides.

Therefore, yoghurt as the functional food is expected to prevent diseases, improve the human life quality and potentially increase health by supporting the body's immune system. During the storage, yoghurt often experiences defects i.e. the varied viscosity level and syneresis phase which result in the reduced quality of yoghurt thereby making the yoghurt less likely to be acceptable to the consumers (Mazloomi et al., 2011 and Damian, 2013). Fortification with prebiotics into the yoghurt formulation has become a consumers' lifestyle, resulting in a symbiotic, a combination of probiotics and prebiotics (Allgeyer et al., 2010), to stabilize the milk colloid system and prevent defects to occur. Prebiotics are carbohydrates which are beneficial for intestinal bacteria as a source of carbon and energy, stimulating the growth and survival rate of the limited bacteria in the intestine (Venter, 2007; Boeni and Pourahad, 2012). Moreover, red dragon fruit peel until now has not been used optimally. It is only seen as waste even though it has an antioxidant content of 16,181 ppm (Herdiani and Putri, 2018), phenol total of 651.76 mg GAE/100g, flavonoid total of 220.28 mg CE (Nur'aini and Sari Sari, 2016) and 46.7% food fiber, vitamins and a natural coloring agent (Sari, Adi and Andrias, 2015). As red dragon fruit peel extract still has a highwater content which will affect on the quality of the end symbiotic yoghurt product, thus, it is necessary to evaporate the extract to reduce its water content and volume to a certain extent without causing the loss of food nutrition (Chin and Hernandez, 1997).

The Microwave Assisted Extraction (MAE) method is employed with the advantages of better temperature control compared to conventional heating processes and a shorter time, which is done at a temperature of 76 °C (medium-high) for 10 minutes using a rotary vacuum evaporator. Because of this reason, fortification with red dragon fruit peel extract which functions as food fiber toward the symbiotic yoghurt will benefit the intestinal bacteria as a source of carbon and energy (Venter, 2007). Consumer acceptance of dairy products generally prioritizes physical and chemical qualities.

Therefore, the purpose of this study is to assess the optimum fortification with red dragon fruit peel extract in improving the physical quality (pH, viscosity, syneresis), color (L*, a*, b*) and chemical quality (acid total, lipid and protein levels) of symbiotic yogurt.

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MATERIALS AND METHODS

Materials

The materials used in this research include skim, *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, dragon fruit peel extract, aquades, buffer pH 4 and 7, NaOH, PP, petroleum ether, potassium oxalate monohydrate.

Research equipment

Beakerglass, measuring cup, pH meter, Viscometer, analytical balance, stirrer, pH meter, water bath, refrigerator, centrifugator, color reader, Confocal Laser Scanning Microscopy, incubator, laminar air flow, burette, soxhlet, and hot plate.

Research method

The research employed an experimental method with a complete random design, using 4 treatments and 3 replications. The treatments included without fortification with red dragon fruit peel extract (P0) and with fortification with red dragon fruit peel extract at 10% (P1), 20% (P2), and 30% (P3) (v/v). The research variables were the physical quality (pH, viscosity, syneresis), color (L*, a*, b*) and the chemical quality (acid total, lipid and protein levels) and microstructure.

Symbiotic yoghurt production

50 g of red dragon bark is added to 50 ml of distilled water. Extraction was performed in a microwave oven at 90 °C for 5 minutes. In addition, 50 mL of the extraction solution was placed in a 1 L vacuum, then placed in a microwave oven and evaporated at 70 °C for 10 min. Probiotic yogurt was made from skimmed milk (10%) by adding water, red dragon fruit peel extract (Hylocereus polyrhizus) added as treatment 10% (P1), 20% (P2), and 30% (P3). All samples were pasteurized at 72°C for 15 minutes, then the temperature was reduced to 42°C and the mixture was well mixed after inoculation with yogurt culture (3%). Incubation the sample at room temperature (25-28 °C) for 24 hours.

The Analysis of Physical and Chemical Qualities and Colors of Symbiotic Yoghurt

The analysis was carried out using different methods such as pH values employing the AOAC method (2005), viscosity following Thi and Ipsen (2009), syneresis referring to Conscious (2004), color (L *, a *, b *) according to the color reader test method (Yuwono and Susanto,1998) and acid total, lipid and protein profiles employing AOAC (2005) and microstructure.

Statistical Analysis

The data were analyzed using Analysis of Variance (ANOVA) software to test the optimum fortification with the red dragon fruit peel extract evaporated with MAE method towards the symbiotic yoghurt. If there were differences, the analysis would be continued using Duncan's Multiple Range Test.

RESULTS AND DISSCUSION

The result of physic quality of symbiotic yoghurt at different treatments

The means of pH, viscosity and syneresis of symbiotic yoghurt at different treatments are presented in table 1.

The analysis results of the evaporated red dragon fruit peel extract show a pH value of 5.46. The extract was fortified into milk of the raw material for the symbiotic yogurt, containing organic acids which can trigger the growth of Lactic Acid Bacteria (LAB) during the fermentation process. Harivaindaran et al. (2008) mentioned that the adding a red dragon fruit peel extract can stimulate the growth of LAB because it contains organic acids. During the fermentation process LAB will produce lactic acids, citric acids and acetic acids which decrease the pH level. The red dragon fruit peel also contains glucose, maltose and fructose as natural sugars that can trigger the growth of LAB. Wakhidah et al. (2017) stated that the increase in acidity in the yoghurt is caused by the activity of LAB during the fermentation process which breaks down the natural sugars from the dragon fruit peel into lactic acids, thus, the pH level decreases simultaneously with the addition of the given concentration of the red dragon fruit (Hylocereus polyrhizus) peel extract. LAB also changes the lactose in milk into lactic acids. Sawitri et al. (2008) stated that during the fermentation process, the lactose content in milk can be converted into lactic acids by LAB thereby decreasing the pH level and increasing acidity in the yoghurt.

Table 1. Means of pH, viscosity and syneresis of symbiotic yoghurt at different treatments

Treatment	pH	Viscosity (cps)	Syneresis (%)
P0	$4{,}01^{b} \pm 0.042$	$209{,}00^{v} \pm 7.43$	$45.62^{v} \pm 0.106$
P1	$3{,}91^{ab} \pm 0.051$	$291{,}83^{x} \pm 1.28$	$39.06^{x} \pm 0.203$
P2	$3{,}84^{a} \pm 0.082$	$346{,}33^{y} \pm 5.59$	$32.99^{y} \pm 0.217$
P3	$3{,}81^{a} \pm 0.090$	$418{,}08^{dz} \pm 3.01$	$30.16^{z} \pm 0.095$

^{a,b} : Different superscripts at the same column indicate a significant effect among the treatments ($P \leq 0{,}05$).
^{v,x,y,z} : Different superscripts at the same column indicate a very significant effect among the treatments ($P \leq 0{,}01$).

The results of the analysis of the evaporated red dragon fruit peel extract indicate a viscosity level of 29.23 cps. The extract was fortified into milk of the raw material for the symbiotic yogurt causing an increase in the viscosity of all treatments. In addition, the red dragon fruit peel contains 10.8% pectin, which is a fiber component in the middle lamella layer and primary cell wall in plants. Pectin contained in the red dragon fruit peel can form gel in acidic environment, thus making the yogurt thicker. Budiyanto and Yulianingsih (2008) claimed that pectin is essential in gel formation and as a stabilizer. Sirotek et al. (2004) mention that pectin has water-soluble properties, but when mixed with sugar and acids, it will form gel because pectin is a reversible colloid.

At pH of <5.3. the electrical changed part of pectin is absorbed on the casein micelle surface through electrostatic interactions while the other parts of the pectin interact with the serum phase (Maroziene and de Kruif, 2000; Tuinier et al., 2002). The adsorption of pectin to the casein micelle surface through the electrostatic interactions creates a complex which has negative electron, thereby forming an electrostatic repulsion and steric stabilization which can ultimately prevent flocculation and stabilize the fermented milk (Tromp et al., 2004).

The lowest mean of syneresis was obtained in the P_3treatment. The decreased syneresis is caused by the fortification with the evaporated red dragon fruit (Hylocereus polyrhizus) peel extract as the prebiotic. Aghajani and Pourahmad (2012) stated that the addition of fiber that functions as a prebiotic produces less syneresis compared to the addition of lactulose and oligofructose and the use of prebiotics to reduce the percentage of syneresis.

The decrease in the syneresis level is caused by the interactions among fiber, LAB and casein and this increases the bonds among protein particles. Nouri et al. (2011) reported that the reduction of the syneresis level possibly causes the occurrence of new tissues in the milk proteins due to some casein interaction, thus, increasing the number of bonds among the protein particles. Adequate levels of pectin are needed to influence the negative electron of the casein micelle surface and to stabilize the fermented milk.

However, if the pectin levels are insufficient, this results in the bridging flocculation. Moreover, if they are too over quantity, this results in too thick viscosity or phase separation (Glahn and Rolin, 1994).

The result of L*, a*, b* colors of symbiotic yoghurt at different treatments

The means of L*, a*, b* colors of symbiotic yoghurt at different treatments are illustrated in Table 2.

The lowest mean of the L* color was obtained in the P_3 treatment. The color change in the symbiotic yoghurt is caused by the fortification with red dragon fruit (Hylocereus polyrhizus) peel extract which has a red dye resulting in a decrease in the color of L* or a decrease in the cleanness of yoghurt. Harjanti (2016) stated that there are anthocyanin substances in the peel of the red dragon fruit. Anthocyanin acts to give red color to the symbiotic yoghurt and has the potential to become a natural coloring for food that is safer for health when fortified in its processing. Jeon (2007) also states that color is an indication of a treatment in food, where color affects the level of consumer acceptance.

Table 2 Means of L*, a*, b* colors of symbiotic yoghurt at different treatments

Treatment	Color			*Chroma* (C)	*Hue*/H (rad)	*Hue*/H (°)
	L	a (+)	b (+)			
P_0	92,81^{d}±0,09	0,17^{a}±0,01	7,65^{d}±0,03	7,65	1,54	88,73
P_1	90,40^{c}±0,08	2,90^{b}±0,04	6,15^{c}±0,03	6,80	1,13	64,75
P_2	87.21^{b}±0,04	12,19^{c}±0,03	5,50^{b}±0,02	13,37	0,42	24,28
P_3	85,94^{a}±0,58	33,59^{d}±0,07	4,10^{a}±0,07	33,84	0,12	6,96

Notes: different letters in the same column shows that there is a significant difference at (P<0,01)

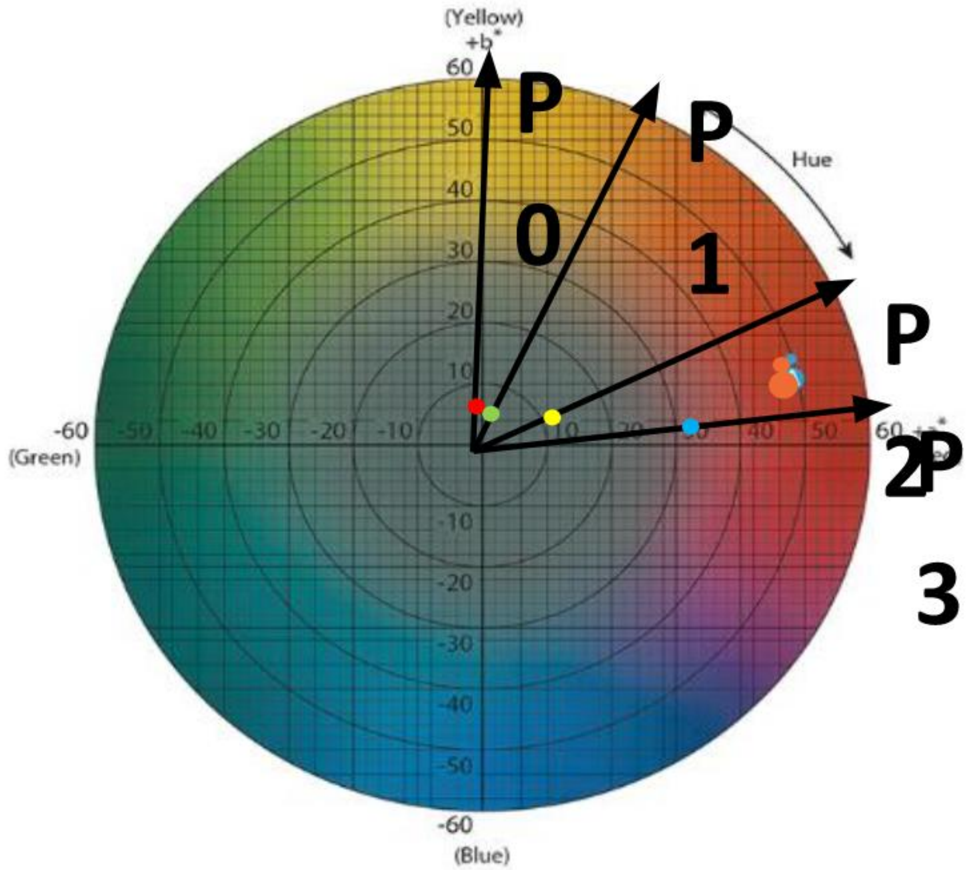

Figure 1. Color Spectrum (L*,a*,b*) of Symbiotic Yoghurt Sinbiotik in Different Treatments

The highest mean of the a* color was obtained in the P_3 treatment. The increase in the a* color in the symbiotic yoghurt is caused by the fortification with the evaporated extract of the red dragon fruit (Hylocereus polyrhizus) peel. The peel of the red dragon fruit (Hylocereus polyrhizus) has anthocyanin and betasianin red dyes which function to bring a red color and a source of antioxidants. Rebecca, Boyce and Chandran (2010) stated that the peel of the red dragon fruit (Hylocereus polyrhizus) contains the natural dye of betacyanin which belongs to the betalain group which gives the red color and is a source of antioxidants including the phenol compounds which are able to bind to free radicals. Therefore, the color pigments in the red dragon fruit (Hylocereus polyrhizus) peel functions as a substitute of synthetic dyes and an antioxidant.

The lowest mean of the b* color was obtained in the P_3 treatment. The decrease in the b* color in the symbiotic yoghurt is caused by the fortification with the red dragon fruit (Hylocereus polyrhizus) peel extract which contains a red dye, thus reducing the intensity of the b* color caused by the interaction of fresh milk as the raw material for the yoghurt. Zanhi and Jideani (2012) stated that the white color intensity of the milk casein is caused by the fermentation process which decreases the lactose content thereby increasing the yellowish intensity as in the b* value. However, fortification with red dragon fruit (Hylocereus polyrhizus) peel extract decreases the yellowish color intensity. Puteri et al. (2014) stated that adding additives to yoghurt such as red dragon fruit peels cause a decrease in the intensity of the b* color. Thus, the red dye following the color of the dragon fruit peel dominates the colors.

The result of chemical quality of symbiotic yoghurt at different treatments

The means of acid total, lipid profile and protein level in the symbiotic yoghurt in different treatments are provided in the Table 3.

The result of the acid total analysis of the evaporated red dragon fruit peel extract indicates a value of 1.08% and the lactose content of the milk as the raw material of the symbiotic yogurt shows that as much as 3.86% of it can stimulate LAB and metabolize into lactic acids causing an increase in the acid total levels in all treatments. The highest was found in the P_3treatment. Khuriyati et al. (2018) stated that the concentration of organic acids in the red dragon fruit peel ranges from 0.34 to 3.33%. The acidity level of the dragon fruit peel was affected by the accumulation of organic acids i. e. malic acids. Malic acid is an organic acid which becomes the major factor in the metabolism of Crassulacean Acid Metabolism (CAM) plants such as red dragon fruit. Dianaasaril et al. (2018) stated that lactose is converted to glucose and galactose-6-phosphate and then to lactic acids by LAB. This LAB activity influences the acidity of the yoghurt because of the metabolite products in the form of lactic acids (Gad, Kholif and Sayed 2010).

Table 3. Means of Acid Total, Lipid Profile and Protein Level in The Symbiotic Yoghurt Ii Different Treatments

Treatments	Acid Total (%)	Lipid Profile (%)	Protein Level (%)
P0	$1.31^{a} \pm 0.034$	$3.57^{a} \pm 0.040$	$4.23^{a} \pm 0.042$
P1	$1.39^{ab} \pm 0.021$	$3.30^{b} \pm 0.100$	$4.59^{b} \pm 0.083$
P2	$1.43^{bc} \pm 0.032$	$3.06^{c} \pm 0.032$	$4.85^{c} \pm 0.106$
P3	$1.49^{c} \pm 0.024$	$2.85^{d} \pm 0.061$	$4.98^{d} \pm 0.210$

^{a,b,c,d} different letters in the same column shows that there is a significant difference ($P \leq 0,01$).

The analysis results of the lipid profile of the milk as the raw material of the symbiotic yoghurt is 4.92%. As the evaporated red dragon fruit peel extract does not contain lipid, the fortification with the evaporated red dragon fruit peel extract which has a prebiotic characteristic is metabolized as an energy source by LAB. The process produces short chain fatty acids which then affects the lipid profile in the symbiotic yoghurt. The lowest mean was found in the P_3treatment. Grajek et al. (2005) stated that prebiotics are fermented by probiotics to produce short-chain fatty acids in the form of acetate, butyrate and propionate which are used by microorganisms as an energy source. Franck and De Leenheer (2005) stated that the minimum standard lipid profile of the symbiotic drinks with whole milk as a raw material is at least 0.2% and this lipid profile depends on the raw material used, as for skim milk or fresh milk the lipid profile is less than 0.05%.

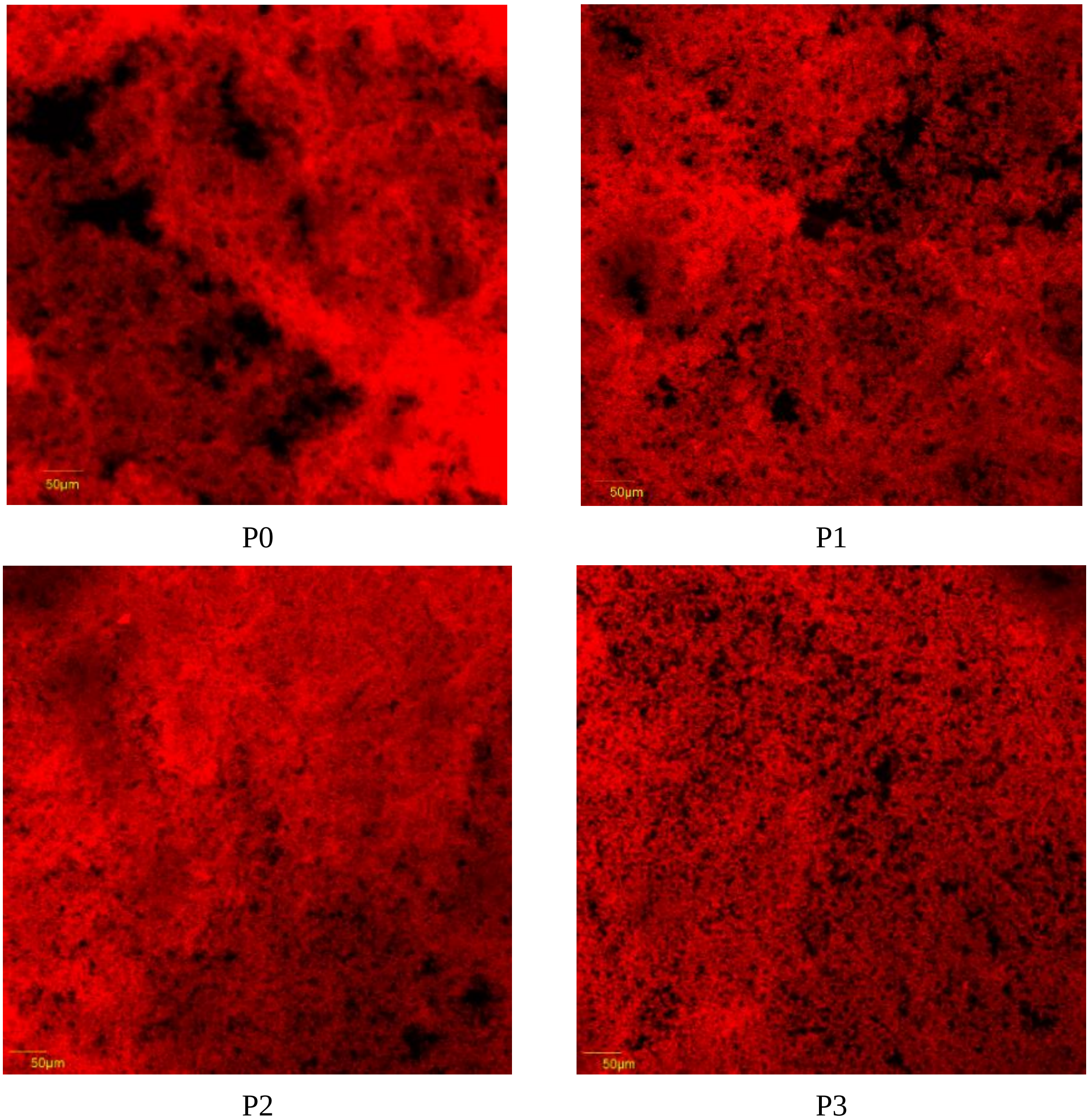

Figure 2. Microstructure of symbiotic yoghurt at different treatments

The analysis result of the protein level of the milk as the raw material of the symbiotic yoghurt is as much as 2.73%. This indicates that that value will affect the end product protein levels because LAB utilizes nitrogen and carbon contents to live and develop as most of their constituent is protein. Herawati and Wibawa (2003) stated that the increase in the protein levels of the symbiotic yoghurt can also be caused by LAB activities as LAB utilizes nitrogen and carbon sources to live and develop. Some constituent components of bacteria are protein thus it can affect the protein content of the yoghurt. Dianasaril et al. (2018) stated that during the fermentation process lactic acids are formed as the product of milk lactose metabolism by the LAB starter. The more LAB content produced during the fermentation proces, the protein content will increase because most of the LAB contains protein.

Microstructure of the Red Dragon Fruit Extract Synbiotic Yoghurt in various addition concentration

Micelles casein in P0 and P1 form a protein network with thin strands, so the protein structure is more open because of the not compact interaction between micelles casein. As shown by the bigger pores in P2 and P3 treatment In P2 treatment, the microstructure is more solid whereas the micelles casein is more compact and homogeneous. it forms smaller pores and whey restrained firmly inside the gel matrix The more uniform the casein aggregation inside yoghurt, the better the gelation trait, to produce higher hardness and viscosity of the yoghurt (Li, et al., 2021). The tighter microstructure caused less reconstruction of the protein and reduce dehydration as well as shrinkage. Sawitri et al. (2020) state that overall, fermented milk synbiotic can hinder excessive syneresis and permeability as well as a blockage in the part that forms the pores. Red dragon fruit peel contains pectin. Increased addition of low methoxyl pectin (LMP) to manage yoghurt caused the increase of cross tie with casein or cavity filling and finally the disruption of casein tissue at the maximum level of pectin (Khubber et al. 2021). This shows the electrostatic interaction of casein with pectin in the contribution of yoghurt gel formation. Pectin inside passion fruit fiber might have interacted with protein and bacterial exopolysaccharide, forming a more compact structure with a greater real viscosity and the skill to trap the whey phase (Espírito-Santo et al., 2013).

CONCLUSION

It is concluded that the fortification with 20% red dragon fruit peel extract evaporated with MAE method produced the optimum physic-chemical quality and color of symbiotic yoghurt in terms of the means of the pH at 3,84, viscosity at 346.33 cps, syneresis at 32.99, acid total at 1.43%, lipid at 3.06% and protein content at 4.85%, and color (L* at 87.21, a* at 12,19, b* at 5,50).

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