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Study of Synbiotic Yoghurt Fortification with Red Dragon Fruit Peel Extract (*Hylocereus polyrhizus*) and Stevia Against Emulsion Properties and Color

(Kajian yoghurt sinbiotik fortifikasi ekstrak kulit buah naga merah (*Hylocereus polyrhizus*) dan stevia terhadap sifat emulsi dan warna)

Tama Mayna Kusuma Ningrum[1*], **Manik Eirry Sawitri** [1], dan **Abdul Manab**[1]
[1]Faculty of Animal Science, Brawijaya University, Malang, Indonesia

ABSTRACT. The research aimed to study the emulsion properties of the fortified synbiotic yoghurt fortification red dragon fruit peel extract (*Hylocereus polyrhizus)* and stevia as sweeteners. The research material is probiotic yoghurt, synbiotic fortified with red dragon fruit skin extract (*Hylocereus polyrhizus*) 20% made from 10% skim milk and yoghurt starter containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (1:1), and the addition of 0.5% stevia to synbiotic. The research method was an experimental completely randomized design with treatment T_1 = probiotic yoghurt, T_2 = synbiotic yoghurt fortified red dragon fruit (*Hylocereus polyrhizus*) peel extract 20% and $T_3 = T_2$ + 0.5% stevia, with 3 replications (v/v). . The variables observed were emulsion activity, emulsion stability, turbidity, whiteness index (WI) and yellowness index (YI). The results showed that fortification of evaporated red dragon fruit peel extract (*Hylocereus polyrhizus*) and stevia sweetener gave a significant difference ($P<0.05$) to the average emulsion activity, a very significant difference to the average of whiteness index and yellowish index ($P<0.01$) and did not give a significant difference ($P>0.05$) on the average of emulsion stability and turbidity of synbiotic yoghurt. It was concluded that 20% fortification of red dragon fruit skin (*Hylocereus polyrhizus*) and 0.5% stevia sweetener could improve the emulsion properties of synbiotic yoghurt.

Keywords: Colour, emulsion, red dragon fruit peel, synbiotic yoghurt, stevia

ABSTRAK. Tujuan penelitian adalah mempelajari profil emulsi dan warna yoghurt sinbiotik fortifikasi ekstrak kulit buah naga merah *(Hylocereus polyrhizus)* dan bahan pemanis stevia. Materi penelitian adalah yoghurt probiotik, sinbiotik yang difortifikasi dengan ekstrak kulit buah naga merah *(Hylocereus polyrhizus*) 20 % yang dibuat dari susu skim 10% dan starter yoghurt yang mengandung *Lactobacillus bulgaricus* dan *Streptococcus thermophilus* (1:1), serta penambahan stevia 0,5% pada yoghurt sinbiotik. Metode penelitian adalah percobaan dengan Rancangan Acak Lengkap dengan perlakuan T_1= yoghurt probiotik, T_2= yoghurt sinbiotik fortifikasi ekstrak kulit buah naga merah *(Hylocereus polyrhizus)* 20% dan $T_3 = T_2$ + 0,5% stevia, dengan 3 ulangan (v/v). Variabel yang diamati diantaranya aktivitas emulsi, stabilitas emulsi, turbiditas, serta warna ditinjau dari indeks keputihan (WI) dan indeks kekuningan (YI). Hasil penelitian menunjukkan bahwa fortifikasi ekstrak kulit buah naga merah *(Hylocereus polyrhizus)* evaporasi dan bahan pemanis stevia memberikan perbedaan yang nyata ($P<0,05$) terhadap rata-rata aktivitas emulsi, perbedaan yang sangat nyata terhadap rata-rata indeks keputihan dan indeks kekuningan ($P<0,01$) dan tidak memberikan perbedaan nyata ($P>0,05$) terhadap rata-rata stabilitas emulsi dan turbiditas yoghurt sinbiotik. Disimpulkan bahwa fortifikasi kulit buah naga merah ***(****Hylocereus polyrhizus)* 20% dan bahan pemanis stevia 0,5% dapat memperbaiki sifat emulsi yoghurt sinbiotik.

Kata kunci: Emulsi, kulit buah naga merah, stevia, warna, yoghurt sinbiotik

INTRODUCTION

Red dragon fruit (*Hylocereus polyrhizus*) has attracted interest due to its appealing appearance and red-violet colour as well as great nutritional and bioactive properties. Peels are the major by-products of fruit processing, and they contain high amounts of phenolic compounds such as flavonoids and antioxidants. The peel of this fruit contains useful chemical compounds such as vitamin C, vitamin E, vitamin A, alkaloid, terpenoids, flavonoids, thiamine, niacin, pyridoxine, cobalamin, phenolic, carotene, and phytoalbumin which are thought to have antioxidant benefits (Shinta and Hartono, 2018). Betalain pigments are divided into two groups, namely betacyanin which produces purplish red and betaxanthin which produce yellow-orange colors (Hendra *et al*., 2020). Betacyanin in red dragon fruit peel (*Hylocereus polyrhizus*) contains antioxidant activity with active ingredient flavonoid which functions as antifungal and antibacterial. Microwave-assisted extraction (MAE) method is employed for the extraction and the evaporation of the red dragon fruit peel (*Hylocereus polyrhizus*), which has potential as dietary fibre.

Yoghurt is made from fermented milk using lactic acid bacteria. The most widely used in yoghurt is the combination of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Lactic acid bacteria, such as *Lactobacillus bulgaricus*

**Email Korespondensi*: tama.kusuma@student.ub.ac.id
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and *Streptococcus thermophilus* are probiotic strains that were used in dairy industries and could be used as a biological agent for the reduction of aflatoxin M1 in milk (Zakaria *et al*., 2019). Synbiotic yoghurt was composed of probiotics in the form of lactic acid bacteria and prebiotics which are generally in the form of dietary fibre, which in this research was from red dragon fruit peel extract (*Hylocereus polyrhizus*). Red dragon fruit peel (*Hylocereus polyrhizus*) contains pectin that can function as a prebiotics, gelling agent, thickener, stabilizer, and emulsifier. Pectins are complex heteropolysaccharides made up of a linear main chain of repeated units of galacturonic acid that can promote different biological activities (Santos *et al*., 2021). Pectin represents a structurally highly complex and chemically heterogeneous family of molecules, being used as a prebiotic potential that has shown favorable effects on human health (Chung *et al*., 2017).

The fortification of the red dragon fruit peel extract (*Hylocereus polyrhizus*) is 20% (v/v) was used as dietary fibre in yoghurt (Tambunan and Sawitri, 2020). Synbiotic yoghurt with red dragon fruit peel extract (*Hylocereus polyrhizus*) has an unfavorable aftertaste for consumers. Consumers prioritize yoghurt products that are “natural” and sweet without the additional calories of sugar. The calorific value of sugar can affect the properties of functional yoghurt, so it is advised to use low-calorie sweeteners such as stevia. *Steviol Glycosides* in stevia contains no calorie because the sugar is connected to steviol scaffolding by a glycosidic bond (Jooken *et al*., 2012). Stevia as a sweetener will stay stable when combined with foods that are processed with high temperature, acidic, and fermentation products (Furlán and Campderrós, 2017). Stevia leaf extract can be used as the source of functional compounds in yoghurt (Yoon *et al*., 2016). Stevia, a sweetener that replaces sugar, is extracted from the plant Stevia rebaudania and it is safe for consumption following the dosage of 3 mg/kg body weight per day (Purwaningsih *et al*., 2021).

The product of yoghurt as the probiotic source the red dragon fruit peel extract (*Hylocereus polyrhizus*) as a prebiotic source and stevia as sweetener become one of the functional food which is beneficial to society. The objective of the current study was to use red dragon fruit peel extract (*Hylocereus polyrhizus*) and stevia in the manufacture of yoghurt. Food processing into functional food is very important in the Covid-19 pandemic time because consuming probiotics and prebiotic sources can increase human body immunity(Aulia *et al*., 2021)

MATERIALS AND METHODS

The materials for this research are yoghurt made of skim milk, starter cultures standard yoghurt which contains *Lactobacillus bulgaricus* and *Streptococcus thermophilus* 1:1 (v/v). 20% of red dragon fruit peel extract (*Hylocereus polyrhizus*) was added into synbiotic yoghurt (Tambunan, and Sawitri, 2020). Red dragon fruit (*Hylocereus polyrhizus*) was obtained in Malang City, East Java, and the peel was taken. Extraction of red dragon fruit peel (*Hylocereus polyrhizus*) using Microwave-assisted extraction (MAE). The red dragon fruit peel (*Hylocereus polyrhizus*) then be cut into pieces, 50g of red dragon fruit peel added to 50 ml of distilled water. Extraction was carried out in a microwave at 90°C for 5 minutes. Furthermore, 50 ml of the extraction solution was put into a 1 L glass beaker then put into the microwave, evaporated at 70°C for 10 minutes. Probiotic yoghurt (T_1) was made from skim milk (10%) with the addition of aquades, as for synbiotic yoghurt (T_2 and T_3) was added the red dragon fruit peel extract (*Hylocereus polyrhizus*) 20%. All samples were pasteurized at 72°C for 15 minutes, then the temperature was lowered to 42°C, and the mixture was well stirred after inoculating starter yoghurt (3%). Incubation at room temperature (25-28°C) for 24 hours. In treatment T_3, the stevia 0,5% was added. Stevia sweetener used is commercial stevia powder that can be found in supermarkets. The yoghurt formulation is shown in Table 1.

Table 1. Formulation sample (Modification, Sawitri *et al*., 2020)

Materials (%, w/v, v/v)	Treatment		
	T1	T2	T3
Skim milk	10	10	10
Aquades	87	67	66,5
Evaporated red dragon fruit peel extract	0	20	20
Starter	3	3	3
Stevia			0,5
Total		100	

Note :
T_1: Fortification of Evaporated red dragon fruit peel extract 0% (v/v)
T_2: Fortification of Evaporated red dragon fruit peel extract 20% (v/v)
T_3: Fortification of evaporated red dragon fruit peel extract 20% + 0,5% stevia (v/v)

Sample analysis was conducted in Biomol Laboratory, Faculty of Sciences, Universitas Brawijaya, Malang. Variables observed include emulsion activity, emulsion stability (Arioui *et al*., 2018), turbidity (Liu *et al*., 2017), whiteness index, and yellowness index (Mehdizadeh *et al*., 2012). Sample preparation for emulsion activity and stability test is 5 ml soybean oil, then it is mixed with 15 ml sample. After that homogenized, take 0,1 ml, and then dissolved in a 0,1 (v/v) SDS solution. Sample read used spectrophotometer on 500 nm absorbance. Sample for turbidity test is dissolved in aquades in 1:20 ratio, then sample read using spectrophotometer on 650 nm absorbance. Colour measurement is carried out using a colour reader (OEM CHNSPEC CS-10 Colorimeter). The Hunter L, a, and b values correspond to lightness (L) and red-green (a) and yellow-blue (b). Test for whiteness and yellowness index follows the formula :

$$YI = 142{,}86 \; x \; L^{-1}$$
$$WI = 100 - [(100 - L)^2 + a^2 + b^2]^{0,5}$$

Statistical Analysis

The model used for the analysis was a completely randomized design with 3 treatments and 3 replications. Differences among groups were assessed using a one-way ANOVA followed by Duncan's Multiple Range Test. A value of P< 0,05 or P<0,01 was considered statistically significant.

RESULTS AND DISCUSSION

Table 2. The average of emulsion stability, emulsion activity and synbiotic yoghurt turbidity in various treatments

Treatment	Emulsion activity (m^2/g)	Emulsion stability (min)	Turbidity (OD)
T_1	7,68±0,70^a	21,96±2,12	0,43±0,33
T_2	8,84±0,49^b	25,45±4,75	0,63±0,40
T_3	9,40±0,49^b	25,93±3,70	0,53±0,29

Note: a,b,c: different superscript in the same column shows the significant difference (p<0.05)

Emulsion Activity

The average of yoghurt emulsion activity was increased following the addition of dragon fruit peel extract (*Hylocereus polyrhizus*) and stevia (p<0,05). The addition of red dragon fruit extract increases the emulsion activity. It shows the occurrence of fat globule droplet size reduction, hence it is more uniform dan reduce coagulation. Pectin is capable of stabilizing emulsions, largely owing to its ability to elevate the apparent viscosity of the continuous phase in emulsions, and reduce the interfacial tension between oil and the aqueous phase (Wang *et al*., 2021). The emulsion activity depends on the amount of pectin and protein which is bound to its structure (Bayar *et al*., 2018). In general, the polysaccharide is negatively charged between pH 7 and 3, meanwhile, casein micelle does not have a charge approaching pH 5 and it will positively charge in low pH (Tuinier *et al*., 2002).

Pectin is a polygalacturonic acid that has a negative charge (Ajani and Okunlola, 2021). Pectin reacts with positively charged macromolecules. Pectin is a hydrophilic colloid that has a negative charge (from the free ionized carboxyl group) and has no isoelectric point. Casein is an amphoteric compound that can react with both acids and bases (Hasenhuettl, 2019). Casein molecules have both positive and negative charges. When proteins and polysaccharides carry opposite charges, they can form agglomerations of either soluble or insoluble complexes (Wusigale *et al*., 2020). Pectin will bind to casein to form an emulsion activity. Protein-pectin complexes occur at a pH below the isoelectric point of casein (pH 4,5-4,8), where casein has a positive charge and will interact with negatively charged pectin to form a stable electrostatic complex. Pectin adsorbs via electrostatic interactions at pH 5 between the carboxylic groups of pectin and the cationic amino acid residues of casein in fermented milk systems (Tuinier *et al*., 2002). Pectin covered the casein micelles in layers and so the attraction among the particles is lowered. At high pH, the pectin conformation is more extended as almost all carboxyl groups are ionized, pectin is adsorbing to droplets in a loop-tail way and stabilizes the droplet in an electrostatic way (Verkempinck *et al*., 2018).

High total dissolved solids can increase the stability of the emulsion because it minimizes the presence of deposits. The addition of stevia as much as 0,5 % gains a total of solid yoghurt (Widodo *et al*., 2015). The high total of dissolved solid can increase emulsion stability, sweetener changes the flow power because it can increase the viscosity, so it can trigger the enhancement of emulsion activity and emulsion stability (Aewsiri *et al*., 2013).

Emulsion Stability

Emulsion stability is the ability of a protein to maintain its emulsion structure for a certain period. The emulsion stability index (ESI) shows that the emulsion ability prevents changes in its structure during the specified period (Rodsamran and Sothornvit, 2018). The average of yoghurt emulsion stability was increased following the addition of dragon fruit peel extract (*Hylocereus polyrhizus*) and stevia (p>0,05). Dragon fruit peel extract (*Hylocereus polyrhizus*) contains pectin known as a polysaccharide that is the main component of plant cell walls. The addition of pectin can influence pH, and ionic power that influences the stability of the fermented milk (Umam *et al*., 2018). Milk protein hydrolysis becomes a smaller peptide which shows the active site of the protein which play a role in increasing the emulsifying properties (Castro *et al*., 2015). Pectin adsorbs via electrostatic interactions between carboxylate groups of pectin and cationic amino acid residues of casein in diluted yoghurt below the casein isoelectric pH. Steric stabilization could be responsible for the stabilizing mechanism of yoghurt, where pectin electrostatically adsorbs to the casein aggregate surface. Pectin could interact with protein during acidic conditions. Emulsion stability is primarily influenced by protein solubility and surface hydrophobicity (Indu *et al*., 2019).

Pectin is able to inhibit the movement of the scattered oil droplets, so it hinders or minimized their tendency to migrate and merge. On emulsification, the whole complex is absorbed strongly on the oil/water interface through protein anchor. Meanwhile, the loaded polysaccharide units spread to the watery solution. The carbohydrate chains in pectin generate films coating oil droplets and protecting the droplets from coalescence (Pi *et al*., 2019).

The increased bacterial activity due to the presence of prebiotics can affect the stability of the yoghurt emulsion. The EPS produced by lactic acid bacteria during fermentation and the formation of the casein curd of milk are capable of water retention and inhibit syneresis and sedimentation in yoghurt. In addition, by reacting with proteins, they may contribute to the reinforcement of the casein network, which improves the rheological properties (Pluta *et al*., 2019). The improvement of yoghurt emulsion stability is probably due to electrostatic interactions between the anionic EPS and the positively charged molecules of casein, which strengthen the casein network.

Turbidity

Turbidity is an important index for mirroring protein aggregation and coagulation in yoghurt. These methods directly detect particles in solution by the attenuation of the incident light due to the light scattered (Pignataro *et al*., 2020). Synbiotic yoghurt turbidity does not show an improvement (p>0,05). The highest turbidity found in treatment T2 is 20% fortification of red dragon fruit peel synbiotic yoghurt. The addition of dragon fruit peel extract (*Hylocereus polyrhizus*) which is a heteropolysaccharide is thought to increase the activity of lactic acid bacteria in producing organic acids that increased the turbidity of synbiotic yoghurt. Organic acids are formed from the breaking of pectin polymer and become simpler monomers by lactic acid bacteria (Suharyono *et al*., 2012). The addition of stevia into synbiotic yoghurt causes the decrease of yoghurt turbidity, this might be caused by lactic acid bacteria's inability to degrade glycoside in stevia. Lactic acid bacteria do not have enzymes to break glycoside contained within stevia leaf extract (Jooken *et al*., 2012).

Red dragon fruit peel (*Hylocereus polyrhizus*) contains pectin ± 10,8% (Yati *et al*., 2017). Pectin within red dragon fruit peel (*Hylocereus polyrhizus*) extract can interact with casein to form a stronger and uniform structure. During fermentation reduction of pH from 5 to 4.3, the particle size was then increased due to the fast adsorption of pectin before the aggregation of casein micelles took place. Pectin as prebiotics has been shown to additionally stabilize casein gel network as a filler (Kieserling *et al*., 2019). Increased turbidity due to variations in particle size caused by enzyme induced cross-linking among the casein micelles (Chen *et al*., 2018). Pectin binds to casein to prevent aggregates in yoghurt. The fewer aggregates formed increases the more adsorption, so the turbidity of yoghurt increases. Electrostatic interaction caused the formation of a cross bond between pectin with casein micelle which negatively charged and prevent the formation of casein aggregate (Bonnaillie *et al*., 2014). Proteins can form biofilms on their surfaces to make solid structures and prevent coalescence to form larger molecules. Forming protein trapped inside the pectin network in low pH in yoghurt, it's formed a solid structure in yoghurt (Khubber *et al*., 2021).

Table 3. The average of synbiotic yoghurt whiteness and yellowness index in various treatments

Treatment	Whiteness index	Yellowness index
T_1	44,02±0,19^x	28,34±0,63^x
T_2	61,57±0,02^y	9,03±0,04^y
T_3	81,49±0,46^z	9,58±2,01^y

Note: x,y,z : different superscript in the same column shows a significant difference (p<0.01)

Whiteness Index

The average of yoghurt Whiteness index (WI) was increased following the addition of dragon fruit peel extract (*Hylocereus polyrhizus*) and stevia (p>0,05). The addition of stevia showed the highest whiteness index. Stevia has a white colour where the brightness value (L*) is 100. The high brightness value also increases the whiteness index value in synbiotic yoghurt. The higher the L* value, the higher the degree of whiteness index (Sailah and Miladulhaq, 2021)

The whiteness index can indicate the nature of the aggregation of particle components in yoghurt. The changes in WI can be linked to the changes in structure, so it resulted in a different scattering of light (Truong *et al*., 2016). Synbiotic yoghurt has higher emulsion stability compared to control yoghurt which the size of the particles is more uniform and scattered. The enhancement of milk acidity cause casein complex destabilization and then the protein fraction is changed from micelle condition to disperse condition (Teichert *et al*., 2020). The small and uniform particle size increases the surface area resulting in higher light scattering. Particle aggregation cause the scattering of light to decrease and it also decreases the brightness (Milovanovic *et al*., 2021).

Yellowness Index

Yellowness Index (YI) shows the degree of yellowness to change the colour of the yoghurt into yellow and linked with b value* (its shows positive value for yellowness colour with CIELAB coordinate) (Barros *et al*., 2020). Yellowness index decrease with the addition of dragon fruit peel (*Hylocereus polyrhizus*) extract and stevia (p<0,01). Dragon fruit peel extract (*Hylocereus polyrhizus*) yoghurt synbiotic and stevia have a pink colour, so they have a higher redness value (a*) than yellowness value (b*) (the data are not shown). It is because red dragon fruit peel (*Hylocereus polyrhizus*) contains betacyanin that gives red-violet pigment colour.

Control yoghurt without the addition of dragon fruit peel (*Hylocereus polyrhizus*) extract and stevia have a higher yellowness index. Visually, the colour of plain yoghurt is yellowish-white (Noprianto *et al*., 2020). Milk carotenoid responsible for yellow colour (higher b* value) in yoghurt. Carotene in milk fat and lactoflavin (riboflavin) in milk whey causes the yellow colour in yoghurt (Chudy *et al*., 2020).

CONCLUSIONS

It can be concluded that 20% fortification of red dragon fruit peel (*Hylocereus polyrhizus*) and 0,5% stevia can improve the synbiotic yoghurt profile emulsion and colour properties. It is suggested to further research microbiology quality considering red dragon fruit peel (*Hylocereus polyrhizus*) functions as prebiotics that support the development and viability of probiotics in synbiotic yoghurt.

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