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Characteristics of Hyloceregurt: Fermented Milk from Stems and Peel of Dragon Fruits

(*Karakteristik Hyloceregurt: Susu Fermentasi Dari Batang Tumbuhan dan Kulit Buah Naga*)

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ABSTRACT

Background: Fruitghurt, the result of fruit or fruit by-product fermentation, is a functional food. Functional foods are foods that are rich in benefits. A formulation of hyloceregurt has been derived from the stem and peel of the dragon fruit (Hylocereus polyrhizus), with potential as an immunomodulatory agent. **Objectives:** This study is aimed at determining the quality of hyloceregurt. **Material and Methods:** Hyloceregurt formula variations are made from fermented stem powder, fruit peel powder, or dragon fruit peel juice. The test include physical characteristics, chemical analysis, microbiology analysis, and hedonic test. **Results:** Hyloceregurt formula from the stems of the plant and the peel of the dragon fruit has almost the same physical characteristics that include a distinctive smell, an acidic taste with a pH ranging from 3-5, and a thick liquid consistency. The color of the hyloceregurt follows the waste of the dragon fruit used (pink and green). The chemical characteristics of hyloceregurt have met the SNI standard, including fat content with a range of 3.1-3.6% and protein content with a range of 4.95–6.74% b/b. The microbiological characteristics of hyloceregurt indicate that the value of the total plate figure is in the range of 1.1-3.3 x 10^9 CFU/mL. Panelists on the hedonic test preferred three formulas: 1% hyloceregurt from the peel powder or fresh peel fruit juice, and dragon fruit plant stems based on smell, taste, color, and consistency. **Conclusions:** All formulas in 1% fruit or by-fruit were the most preferred formulas, and based on the best physical characteristic tests and chemical content analysis, met the requirements of SNI 2981 of 2009. However, microbiologically, they still did not meet the requirements of SNI 2981 of 2009, so further tests still need to be carried out.

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INTRODUCTION

Food's function has evolved from just satisfying taste and nutrition as primary demands to becoming active elements to promote human health through additional functions. Functional foods are foods that have such functions. Although the term "functional food" has never been widely accepted, it has become a widely held concept that the food consumed gives health advantages (Sørensen et al., 2022). The market for functional foods increased by 5% between 2020 and 2021, compared to only 0.3% between 2019 and 2020. This is most likely owing to the COVID-19 pandemic's increased focus on Health.

During the COVID-19 pandemic, people competed to keep their immune systems healthy. The immune system (immunity) is a system in the body that is tasked with responding or responding to "attacks" by microorganisms from outside the body such as bacteria, viruses, fungi, and various germs that cause disease. When the immune system does not work optimally, the body will be susceptible to disease. The body needs immunomodulators, namely agents that can modulate the immune response, to optimize the body's defenses. These immunomodulators consist of immunostimulants and immunosuppressants. In infectious conditions, immunostimulants play a role in increasing the immune response to fight the infector (Owen, 2013).

The majority of immunomodulators have a wide range of effects on the immune system, which can occasionally lead to harmful consequences for patients. The increase in cases related to the immune system encourages researchers to find new immunomodulator alternatives that are safe and effective. The sources of immunostimulants are very diverse, and one that is being widely developed is probiotics. Yogurt is a probiotic drink created from milk that has been fermented with the inclusion of beneficial lactic acid bacterial (LAB) cultures, one of which is *Lactobacillus acidophilus*. Fruitghurt, which is a variant of yogurt, is produced through the fermentation of fruit juice or fruit byproducts, including peels and seeds (Roza et al., 2018).

Exopolysaccharides are polysaccharides that are weakly attached to the outside surface of cells or discharged into the surrounding environment. Extracellularly, these compounds can be generated through the action of enzymes released by bacteria, or they might be internally created and subsequently secreted beyond the cell (Jurášková et al., 2022). The physicochemical features of exopolysaccharides (EPS) give them great potential, as they can also protect microbial cells from abiotic or biotic stresses, competition, pH, and temperature. Therefore, EPS produced by LAB can be used as an alternative source of new polysaccharides and can become future polysaccharides. Polysaccharides are a type of natural polymers. Extensive research by scientists all around the world has revealed the potential of several synthetic and natural polymers to have immunostimulatory properties. These polymers stimulate and

engage the immune system by acting as pattern recognition receptor (PRR) agonists, especially in gut-associated lymphoid tissue (Eswar et al., 2023; Mundiri, Megantara, and Anggaeni, 2020).

Various studies on EPS production that have been carried out show that the type of LAB and fermentation conditions used will influence EPS production results, therefore fermentation conditions must be optimized to obtain the best EPS production results at an effective and efficient cost. (Nguyen et al., 2020). An alternative method for maximizing EPS efficiency involves utilizing dragon fruit byproducts, such as peels or stems, to produce a type of yogurt known as hyloceregurt. Because it forms an antioxidant network, the combination of probiotics and chemicals in dragon fruit peels or stems in a formulation is expected to have a better effect on the body's defenses. Currently, research has been carried out to determine the quality of the Hyloceregurt formula based on physical, chemical, and microbiological properties, as well as test the taste preferences of the Hyloceregurt formula.

MATERIAL AND METHODS

Materials

Plant stem and peels powder of dragon fruit obtained from Wonokerto Village, Bancak District, Semarang Regency, pasteurized fresh milk, yogurt starter, saline phosphate buffer solution (PBS), ammonia, ether, magnesium powder, hydrochloric acid, toluene, chloroform, Dragendorff's reagent, Mayer's reagent, hydrochloric acid, amyl alcohol, iron(III) chloride, gelatin, Steasny's reagent, sodium hydroxide, sulfuric acid, anhydrous acetic acid, deMan Rogosa and Sharpe Agar (MRSA) medium, sodium chloride, 96% alcohol, glucose, phenol 5%, concentrated sulfuric acid, 70% alcohol, crystal violet, iodine, safranin, malachitegreen, and 3% H_2O_2.

Methods

The research was carried out through four stages of testing, among others: 1) physical characteristic testing that includes color, aroma, flavor, and consistency. 2) microbiological testing on the standard number of microbes in the formula of hyloceregurt; 3) fat and protein analysis of the formula; 4) hedonic test with 25 respondents. The rating is based on the scoring previously set by Rahayu (1998), with modifications. The tested Hyloceregurt formulation is detailed in Table 1, and the control for testing consisted of a formulation in which dragon fruit byproduct was not added.

Table 1. Hyloceregurt Formulation

Materials	Hyloceregurt Formula								
	DP			FP			DS		
	I	II	III	I	II	III	I	II	III
Fresh milk (L)	1	1	1	1	1	1	1	1	1
Dragon fruit byproduct (%)	1	2	3	1	2	3	1	2	3
Yogurt Starter (%)	10	10	10	10	10	10	10	10	10

Hyloceregurt Preparation

The plant stems and peel of dragon fruit are sorted wet, then washed with running water and drained. Samples of dragon fruit stem were dried in a drying cabinet at a temperature of around 60°C until they were dry and could be broken. Meanwhile, some fruit peels are dried, and some are used fresh. Dried simplicia was sorted and powdered with a blender, then sieved. Ensure that all tools and materials that will be used have been washed thoroughly beforehand. Pasteurized fresh milk is heated at 90°C for around 2–5 minutes. The milk is then added to 5% plain yogurt (as a starter, which is a culture of *S. thermophilus, L. bulgaricus, L. acidophilus, and Bifidobacterium*), and each additional ingredient (plant stem powder, dragon fruit peel powder, or dragon fruit peel fresh juice). Finally, the solution was incubated for 48 hours at 37°C. This fermentation technique adheres to the methodology employed by Nurhasanah et al. (2019).

Physical properties assay

Sensory tests include color, aroma, taste, and viscosity. This test is carried out organoleptically, namely by testing the quality of a material or product using the five human senses. Organoleptic is usually carried out by describing the color, smell, and consistency of the preparation (Barel, Paye, and Maibach, 2005).

Microbiology assay

Testing includes determining the total number of lactic acid bacteria using the Total Plate Count method. The hyloceregurt sample was diluted using 9 ml of distilled water (this solution is a 10^1 dilution), then continued dilution up to 10^3 (Novia, Dharmayanti, and Khoiriya, 2022).

Chemical properties assay

Chemical tests include measuring pH, protein, fat, and lactic acid content. pH measurements are carried out using a pH meter. Crude protein content in yogurt samples was measured using the Kjedahl method according to SNI 2981 (2009) standards. Fat content analysis was carried out using the soxhletation method. Total acid analysis was calculated as lactic acid according to SNI 2981 (2009) standards using the titration method (Nurhasanah et al., 2019).

Hedonic test

Taste preference testing for the hyloceregurt formula was carried out involving 25 participants (without age or disease history restrictions).

RESULTS AND DISCUSSION

This is preliminary research to find out whether yogurt with a formula combined with fresh peel (FP), dried peel (DP), and dried stem (DS) of dragon fruit has the potential to be developed into a yogurt formula rich in exopolysaccharides. These additional ingredients were chosen because they contain nutritional elements that are good for our bodies. According to Rochmawati (2019), the contents of vitamins A, C, and E in each of these components are respectively 102.13 µg, 540.27 µg, and 105.67 µg per 100 g of dry weight.

Sulistyarini et al. (2020) carried out a phytochemical screening of dragon fruit stem powder and reported that it contained flavonoids, saponins, and steroids. Meanwhile, according to Naga and Nizori (2020), the peel of dragon fruit contains vitamin C, vitamin E, vitamin A, alkaloids, terpenoids, flavonoids, thiamine, niacin, pyridoxine, cobalamin, phenolics, carotene, and phytoalbumin. Dragon fruit peel is rich in polyphenols, which are bioactive sources of antioxidants and immunomodulators. Several research support polyphenols' immune regulation effect: certain polyphenols influence immune cell populations, modify cytokine production, and pro-inflammatory gene expression (Yahfoufi, 2018). The antioxidant activity in dragon fruit peels is greater than that in pulp.

The hyloceregurt formula is made using a starter containing the lactic acid bacteria *S. thermophilus, L. bulgaricus, Bifidobacterium, and L. acidophilus*. In the fermentation process, *S. thermophilus* and *L. bulgaricus* bacteria carry out mutually beneficial metabolism (mutualistic symbiosis). *Lactobacillus bulgaricus* will produce amino acids and short peptides, which can trigger the growth of *S. thermophilus*. Meanwhile, *S. thermophilus* produces formic acid, which helps the growth of *L. bulgaricus*. These two bacteria have different roles in the yogurt fermentation process: *L. bulgaricus* plays a role in forming the aroma, while *S. thermophilus* forms the taste and acidity level. *Lactobacillus acidophilus* is a natural microflora in the human digestive tract that can produce lactic acid as the main product of sugar fermentation. These bacteria produce bacteriocins, which can stimulate the body's formation of antibodies (C. Li et al., 2023).

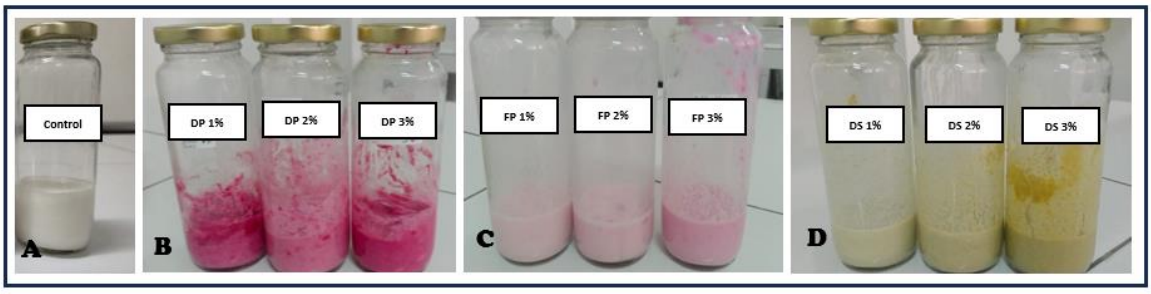

Figure 1. Hyloceregurt formula orientation. A) Control; (B) 1%, 2%, 3% DP formula; (C) 1%, 2%, 3% FP Formula; (D) 1%, 2%, 3% DS Formula.

The orientation results showed that the consistency of DS, DP, and FP yogurt formulas with concentrations of 1%, 2%, and 3% after incubation produced a consistency that was too thick or dense (Figure 1), exceeding the density of the control. This is influenced by additional ingredients in the form of dried stems, fresh peels, and dried peels of dragon fruit, which contain a lot of fiber. Dragon fruit contains 50.4% fibre with a ratio of soluble to insoluble dietary fibre of 1:3.8 (Arivalagan et al., 2021). Fibre binds water very easily, so the water content in milk is bound by the fibre from the additional ingredients. The formula showed improved thickness (Figure 2) after the concentration of additional ingredients was reduced to 0.5, 1, and 2%.

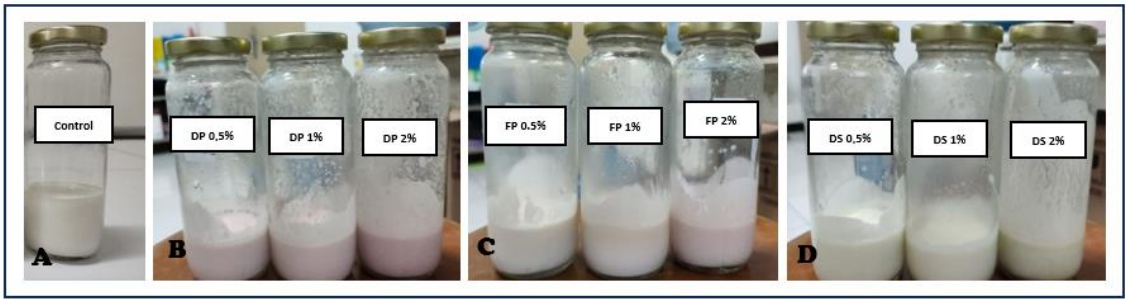

Figure 2. Hyloceregurt Formulation (A) Control; (B) 0.5%, 1%, 2% DP Formula; (C) 0.5%, 1%, 2% FP Formula; (D) 0.5%, 1%, 2% DS Formula.

Physically, the three hyloceregurt formulas produce almost the same thickness/consistency, and homogeneity (Table 2). The difference occurs in terms of colour; the DS formula is light green, while the DP and FP are pink. In terms of taste characteristics, all formulas produced a less sour taste than the control. This is associated with the inhibition of the fermentation process due to additional ingredients. Inhibition of the fermentation process results in a decrease in acidity levels. The exception is the 0.5% FP formula, which produces a sweet taste, possibly because the fermented milk taste is more dominant.

Table 2. Physical Characteristics of Hyloceregurt Formula

Formula	**Physical characteristics**			
	Smell	Taste	Consistency	Colour
FP 0,5%	Specific	slightly sweet	Thick and homogeneous liquid	Pink
FP 1%	Specific	slightly sour	Thick and homogeneous liquid	Pink
FP 2%	Specific	slightly sour	Thick liquid, not homogeneous	Pink
DP 0,5%	Specific	slightly sour	Thick and homogeneous liquid	Pink
DP 1%	Specific	slightly sour	Thick and homogeneous liquid	Pink
DP 2%	Specific	slightly sour	Thick and homogeneous liquid	Pink
DS 0,5%	Specific	slightly sour	Thick and homogeneous liquid	Light green
DS 1%	Specific	slightly sour	Thick and homogeneous liquid	Light green
DS 2%	Specific	slightly sour	Thick liquid, not homogeneous	Light green
Control	Specific	Sour	Thick and homogeneous liquid	White

The incubation times used in this study were 2 and 24 hours. On an industrial scale, making kefir using a 1-5% starter in pasteurised milk requires a fermentation time of 12–15 hours at a temperature of 20–25 °C. In this research, an incubation time of 2 hours indicates that the fermentation process has begun, which is indicated by the production of lactic acid. Meanwhile, an incubation time of 24 hours is given in the hope that the additional ingredients containing high fibre can be broken down better.

After incubation, the pH and lactic acid levels in the formula will indicate how successfully the fermented milk was made. The optimum conditions for the growth of *L. bulgaricus* are pH 5.5 with a temperature of 37°C, while the optimum conditions for the growth of *S. thermophilus* are pH 6.8 with a temperature of 37°C. Both bacteria are thermoduric bacteria; that is, they are able to grow optimally at a temperature of 20–37 °C with a minimum growth temperature of 5–10 °C. This is in accordance with the conditions produced in the hyloceregurt fermentation process of dragon fruit stem bark and dragon fruit peels in this study.

Table 3. pH and Lactic Acid Levels of Hyloceregurt Formula

Formula	pH		Lactic Acid Levels (%)	
	2 h	24 h	2 h	24 h
Control	5	4	4,02	6,96
FP 2 %	5	3	10,23	7,45
FP 1 %	6	3	8,75	16,4
FP 0,5%	6	4	8,75	24,56
DP 2%	5	3	13,3	11,07
DP 1 %	5	3	9,24	18,56
DP 0,5%	6	3	15,43	15,9
DS 2%	5	4	10,57	29,71
DS 1%	6	5	3,65	33,59
DS 0,5%	6	3	6,08	24,02

The pH after 2 hours of incubation ranged from 5 to 6 (Table 3). At the end of 24 hours of incubation, the pH of all formulations, including the control, appeared to have decreased relative to the second hour. Increasing the incubation time increases microbial activity and the number of microorganisms, resulting in a greater degradation of the substrate and a decrease in the pH of the medium (Ayuti et al., 2016). According to Food and Drug Supervisory Agency Regulation No. 34 of 2019 regarding Food Categories, fermented milk products made with *Lactobacillus kefiri*, *Lactococcus*, and *Acetobacter* starter cultures must contain at least 0.6% lactic acid. Overall, the formulas for hyloceregurt contain lactic acid and still satisfy these criteria.

Rahmani et al. (2021) proved that the addition of Lactobacillus acidophilus and Bifidobacterium in yogurt preparations increased protease activity so that many proteins were broken down into amino

acids, both essential and non-essential. Apart from that, it also increases the activity of the lipase enzyme, which breaks down fat into fatty acids, both saturated and unsaturated. Lipolytic activity is controlled by the lipase enzyme produced by lactic acid bacteria as the pH decreases. Lipase will also free fatty acids from milk fat molecules. Consequently, the lipid content of yogurt will be reduced when it is supplemented with the probiotic starter germs *Lactobacillus acidophilus* and *Bifidobacterium*. The results of the study revealed that the lipid content of the treated yogurt was greater than 3% (Table 4). This is in accordance with the provisions of SNI 2981 of 2009 regarding yogurt (BSN, 2009), which stipulate a minimal fat content of 3%. The protein content of hyloceregurt was lowest at FP 1% and highest at DS 1%, as determined by scientific research. The protein content of hyloceregurt satisfies SNI 2981 of 2009 (minimum 2.7% protein) requirements for yogurt (BSN, 2009).

Table 4. Lipid Content and Protein Content of Hyloceregurt Formula

Formula	Lipid (%)	Protein (%)
FP 1%	3,109	4,951
DP 1%	3,643	5,904
DS 1%	3,425	6,742
control	3,321	5,343

This microbiological study of the features of hyloceregurt (Table 5) showed that the amount of microbes that produce lactic acid does not meet the requirements of SNI 2981 of 2009 (BSN, 2009). There should be 10^7 CFU/mL of microbes in yogurt. The lack of lactic acid-producing microorganisms is most likely due to the extra material's high cellulose concentration, which causes the water in the substrate to bond and create a gel. Microbial growth can be inhibited by the gel. Hence, to fulfill the established provisions, additional study is required to ascertain an optimal and advantageous composition for probiotics.

Table 5. Total Number of Lactic Acid Bacteria Hyloceregurt Formula

Formula	ALT (CFu/mL)
FP 1%	$3,3 \times 10^4$
DP 1%	$3,0 \times 10^4$
DS 1%	$1,0 \times 10^4$
Control	$2,9 \times 10^4$

According to the hedonic test (Table 6), the most popular shade of hyloceregurt is the one made using the DP formula (1 and 2%). In terms of smell, the panelists gave the nod to the 1% DP formulation since it was the closest to the scent of unprocessed starter or yogurt. The smell that is produced by different hyloceregurt formula still comes from the plants themselves. The DP 1% hyloceregurt flavour was voted the best by the focus group since it is less overpowering than the others. On the other hand, DP 1% is most often picked for consistency. The high uniformity of the dried peel of dragon fruit powder in the

media may change how the 1% DP formula is mixed. As a result of the hedonic test, the three formulas picked by the panelists based on organoleptics (colour, taste, smell, and consistency) were yogurt with 1% fresh fruit peel, 1% dried fruit peel, and 1% dry stem added to it.

Table 6. Hedonic Test Score of Formula Hyloceregurt

Formula	Scoring			
	Colour	Smell	Taste	Consistency
DS 1%	2,84	2,04	1,68	2,36
DS 0,5%	2,88	2,12	1,92	2,64
FP 1%	2,92	2,44	2,08	1,64
FP 0,5%	2,96	2,48	1,96	2,6
DP 2%	3,44	2,68	2	2,2
DP 1%	3,44	2,88	2,6	2,8
DP 0,5%	2,88	2,76	2,4	2,52
Control	2,96	2,8	2,36	2,36

CONCLUSION

The formula consisting of 1% dried fruit peels, known as Hyloceregurt, has been determined to be the most optimal based on evaluations of its physical and chemical features, as well as hedonic testing. According to the findings of microbiological analyses, it is evident that the formula fails to meet the stipulated criteria outlined in the 2009 SNI 2981 standard.

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CONFLICT OF INTEREST

The authors state that there are no potential conflicts of interest.

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