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In Vivo Activity Test of Yogurt Effervescant Combined of Jackfruit Seeds (*Artocarpus Heterophyllus*) and Red Dragon Fruit (*Hylocereus Polyrhizus*) in Kanaghurt Products As Laxative Agents

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ABSTRACT

Introduction: Today lifestyle has changes because of the influence of foreign culture such as eating habit which has different from real food into fast food. Fast food is considered unhealthy due to its high content of calories, protein, salt, and preservatives, but low dietary fiber, which may contribute to constipation. The urgency of this research lies in the utilisation of food ingredients with high nutritional potential, such as jackfruit seeds and red dragon fruit, which contain beneficial bioactive components, combined with yoghurt to improve digestive bacteria and promote healthy and prosperous living in line with the Sustainable Development Goals (SDGs).
Objective: This study aimed to formulate an optimized effervescent yogurt powder incorporating jackfruit seeds and dragon fruit (Kanaghurt) and to assess the in vivo laxative efficacy of the resulting product.
Methods: The method of this study was experimental, consisted of several stages, including the preparation of jackfruit seed powder, the development of three effervescent powder formulations, followed by physicochemical evaluation and in vivo laxative testing using Wistar rats.
Results: Organoleptic evaluation results showed that all formulations had a pink to pale pink colour, a distinctive yoghurt smell, and a slightly sour sweet taste. The average moisture content in F1, F2, and F3 was 1.20%, 0.94%, and 0.67%, respectively. The flow rate showed that F2 (1.68 seconds) had the best flow properties compared to F1 (7.99 seconds) and F3 (3.55 seconds). The dissolution time of all formulations was very fast, ranging from 45.46 to 50.96 seconds. However, the pH values of the preparations (F1: 8.04; F2: 8.14; F3: 8.29) were above the specified range (pH 6-7).
Conclusion: The conclusion of this study is that the three formulations of effervescent kanaghurt preparations meet most of the physical property test requirements except for pH. The strongest laxative effect with the lowest Norit Distance Ratio % value was formulation 2, which was 27.58%. The laxative effect produced was significantly different compared to the positive control.

Keywords : Effervescant Yogurt, Jackfruit Seeds, Laxative Test, Red Dragon Fruit

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INTRODUCTION

In the current era of globalization, the influx of Western culture into Indonesia has been overwhelming, with various aspects of life and Eastern values being greatly influenced by Western culture, including changes in eating patterns. There is a noticeable trend in urban communities towards consuming imported (modern) foods rather than traditional foods (Setyawati & Rimawati, 2016) . Free trade and increasingly fierce competition have led to the expansion of trade in goods and/or services, even across national borders, and the public's need for information has also increased. The basic need that humans require to sustain their lives is food (Fauzin, 2021). Consuming fast food has become one of the dietary choices of modern society today.

Fast food generally contains high levels of calories (mainly fats and simple sugar), salt, seasoning, preservatives and additive while being low in dietary fiber (Setyawati & Rimawati, 2016). Low in dietary fiber can causing constipation in result of increasing fecal mass and difficult in defecating, fiber has the ability to bind water in large intestine which increases the volume of faecaes and stimulates the rectal nerves (Claudina et al., 2018). According to data from the Indonesian Health Survey (2023), the national prevalence of insufficient fruit and vegetable consumption among individuals aged over 10 years is 96%. Fulfillment efforts to meet dietary and fiber intake can be achieved by utilizing unprocessed agricultural raw materials, thereby increasing value to the agricultural processing chain (Abbas & Suhaeti, 2016). One of the agricultural products that has not been widely processed is jackfruit seeds.

Currently, the utilization of jackfruit seed (*Arthocarphus heterophilus*) is limited to traditional boiling practices, planting the seeds, or discarded as waste, resulting in the suboptimal exploitation of their potential. The utilization of jackfruit seeds remains relatively low, at approximately 10% in the food sector is due to the lack of public interest processing jackfruit seeds (Akaso et al., 2021). Jackfruit seeds has potential nutritional compound such as protein, fat, carbohydrates, minerals (calcium, phosphorus, and iron) and also vitamins (vitamin A, vitamin C, and vitamin B1). Jackfruit seeds contain flavonoids, isoflavones, phenols, saponins, and lignan which contribute to its pharmaceutical properties such as antioxidant, antiinflammatory, and helps regulate blood sugar levels and promotes intestinal health. Jackfruit seeds contain high levels of oligosaccharides, which can be utilize as selective probiotics in the fermentation of microflora in the intestinal system and can suppress the growth of pathogenic microorganisms (Ye et al., 2025) .

Another potential plant as a natural remedy for supporting gut health is Red-fleshed dragon Fruit (*Hylocereus polyrhizus*). Red dragon fruit known for its rich fiber content such as oligosaccharides and also contain bioactive phytochemical compounds such as quersetin, rutin, carotenoids and hydroxycinnamic acids, which contribute to its antioxidant, anti-inflammatory, and anti-microbial properties. Pigments such as betalains and anthocyanins in red-fleshed dragon fruit can provide health benefits such as anti-diabetic, anti-cancer, dyslipidemia and obesity (Si-Yuan Chen, 2024). Red-fleshed dragon fruit has higher levels of sugar, protein, ascorbic acid, calcium, magnesium, and phosporus compared to white-fleshed dragon fruit (V.K. Lande*, 2024). Red-fleshed dragon fruit can also intensify metabolism, combat heart disease and treat dysentry (Puspawati et al., 2023).

The rapid development of technology and functional food products nowadays the value of jackfruit seeds and red-fleshed dragon fruit can be increased in the form of yoghurt. Yoghurt is popular among public because scientifically proven contain good nutrients and positive impact on health because it can balance the gut microbiota by suppressed harmful bacteria if it’s consumed regularly (Chakma et al., 2025). Yoghurt is fermented by probiotic such as Lactobacillus, a lactic acid bacteria, which has a function to acidifying the milk and produce lactic acid that will inhibit the growth of microorganism pathogens and also can be act as a preservative in food product (Barq et al., 2024). The ideal temperature for yoghurt storage in refrigerators is at 5°C, however in distribution process it’s commonly stored at 15-20°C which temperature fluctuations may affect the viability of lactic acid bacteria which can shorten the shelf life of yoghurt product (Oktavia et al., 2015). To

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overcome this situation, an innovation was developed by converting yoghurt into a powdered from which is more portable, acceptable to consumers and capable to extending product shelf life. To enhance colour, taste, and aroma, instant powder beverages are produced in effervescent form.

Effervescent granules are coarse granules that contains active pharmaceutical ingredients and excipients along with acids and bases in a dry mixture. When dissolved in water, the acids and bases react to generate carbon dioxide gas that imparts a refreshing sensation, it gives a well mixed and distribute more evenly than tablet so it's more simple to use (Adi-dako et al., 2021). The utilization of nutritional food materials such as jackfruit seeds and red dragon fruit in an effervescent powder form may contribute positively to lifestyle improvement, thereby promoting healthy and sustainable living, which aligns with sustainable development goals (SDGs). This research aimed to evaluate the physicochemical characteristics of yoghurt effervescent granules of jackfruit seeds (*Arthocarphus heterophilus*) and red-fleshed dragon fruit (*Hylocereus polyrhizus*) products namely as kannaghurt, as well as its in vivo laxative activity using Wistar rats.

MATERIALS AND METHODS

Equipment and Materials

The equipment used in this study included an analytical balance, glassware (Pyrex IWAKI), surgical instruments, mortar and pestle, ruler, stove, styrofoam, surgical scissors, cages, rat food and water, 3 mL and 1 mL syringes (Terumo), and an oral probe.

The materials used in this study were red dragon fruit, jackfruit seeds, citric acid (food grade), sodium bicarbonate (food grade), CMC-Na (food grade), yoghurt (food grade), sugar, tartaric acid (food grade), Dulcolax, distilled water, norit suspension, and 0.5% Na.CMC (PT. Brataco).

Research Methods

Preparation of jackfruit seed powder

1 kg of jackfruit seeds were washed thoroughly. The seeds were boiled for 30 minutes to remove the sap and soften the texture. The skin of the jackfruit seeds was peeled after boiling, then the seeds were put into a blender and distilled water was added. The blender contents were filtered to obtain jackfruit seed juice. The jackfruit seed juice was powdered using the crystallization method. The resulting powder is sieved using a 100-mesh sieve.

Production of Kanaghurt Effervescant

Formulation of Kanaghurt Effervescant

The Kanaghurt Effervescent formula used in this study is presented in Table 1.

Table 1. Kanaghurt Effervescent Formulation

Ingredient	Composition (%)		
	F1	F2	F3
Jackfruit Seed Powder	3,125	3,125	3,125
Red Dragon Fruit Powder	10	10	10
Yoghurt Powder	3,125	3,125	3,125
Citric acid	2	0.3	0.3
Sodium bicarbonate	25	25	50
CMC-Na	0,25	0,25	5
Tartaric acid	0,1	0,1	0,1
Sugar	Ad 100	Ad 100	Ad 100

The acidic components, namely citric acid and tartaric acid, and the basic components, namely sodium bicarbonate, were individually weigh and prepared separately. Jackfruit seed powder, red-fleshed dragon fruit powder, and sodium-CMC were weighed and added to the sodium bicarbonate

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container, and mixed until homogeneous. The pre-weighed sugar was sieved using a 100-mesh sieve and stored in a separate container. The acidic components, the basic component, and sugar were then mixed until homogeneous and dried in an oven at 40°C for 30 minutes. Subsequently, yogurt powder was added, mixed thoroughly to achieve uniformity, and stored in an airtight container.

Physicochemical Properties Test of Kanaghurt Preparation

Organoleptic Test

The organoleptic test was conducted by observing the colour, odour, taste, and shape of the kannaghurt granules.

Moisture Content Test

Weigh 2 g of powder and place it in a metal dish, then place it in a moisture balance at 105°C for 15 minutes, then weigh it to obtain the percentage value. The moisture content test is considered satisfactory if it is below 5%.

Flow Rate and Angle of Repose Test

A total of 100 g of powder was placed in a funnel with a cover at the bottom. The funnel cover was slowly opened until all the powder had fallen out of the funnel and formed a pile on the paper. The flow time of the powder is determined from the moment the powder begins to flow until it stops flowing, using a stopwatch. The angle of repose is obtained by measuring the height and diameter of the pile of granules formed, repeated three times. If the flow time of 100 g is ≤ 10 seconds, the granules have good flow velocity, and if the angle of repose of the granules is ≤ 40°, the powder exhibits good flowability.

pH Test

4 g of granules are dissolved in 150 ml of distilled water, then the pH is measured using a pH meter, with three replicates. The pH value of good effervescent granules ranges between 6-7.

Dissolution Time Test

A total of 7 g of granules are placed in 200 mL of distilled water.The acceptable dissolution time for effervescent preparations is less than 5 minutes, measured from the moment the powder is immersed in water until it is completely dissolved and effervescence has ceased. This evaluation is three replicates. Good granules have a dissolution time of < 5 minutes.

Laxative Test

In vivo laxative test preceded by research ethic approval from Universitas Ngudi Waluyo Ethics commission to obtain Ethical Clearance. The procedure for laxative test using twenty wistar rats which were adapted for 7 days prior to the study. Before testing, the rats were weighed and randomly divided into 5 groups, each consisting of 4 rats. The rats were induced with loperamide for 2 days. The rats were fasted for 18 hours and given water. The groups were divided as follows:

a. Group I (negative control) was given 0.5% Na.CMC
b. Group II (positive control) was administered dulcolac 0.9 mg/kgBW
c. Group III: Formula 1 at a dose of 3.24 mg/kgBW
d. Group IV: Formula 2 at a dose of 3.24 mg/kgBW
e. Group V: Formula 3 at a dose of 3.24 mg/kgBW

After treatment administration, the animals in each group were left undisturbed for 45 minutes, after which all rats received 1 mL of a 0.5% norit suspension as a marker. Twenty-five minutes following norit administration, the rats were sacrificed and dissected; the intestines were removed, carefully excised from the pylorus to the rectum, stretched, and measured for the total length and the distance traversed by the marker. Evaluation was performed by comparing the percentage ratio of marker distance to intestinal length between the test and control groups. The ratio of marker distance to intestinal length was calculated using the formula:

$$\frac{\text{Length of intestine traversed by norit}}{\text{Total length of the intestine}} \times 100\%$$

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Data Analysis

Data analysis was performed using SPSS Version 27 with homogeneity testing using the Levene test and normality testing using *the Shapiro-Wilk test*. If the data obtained was normal and homogeneous, parametric testing (ANOVA) was performed with LSD post-hoc testing.

Ethical Clearance

This research has been approved by the ethics commission and obtained ethical clearance with number 196/KEP/EC/UNW/2025.

RESULTS

Physichochemical Characteristics

To enhance the pharmacological effects of jackfruit seeds and red dragon fruit, a granulated effervescent yogurt preparation (Kanaghurt) was formulated, followed by physicochemical characterization and in vivo activity evaluation. Organoleptic testing was conducted to assess the visual characteristics of the formulations as presented in Table 2.

Table 2. Organoleptic test results for Kanaghurt Effervescent

Formula	Colour	Odour	Taste
1	Light pink	Yoghurt typical	Sweet and slightly sour
2	Pale pink	Yoghurt typical	Sweet and slightly sour
3	Pale pink	Yoghurt typical	Sweet and slightly sour

Another physicochemical characterization of the effervescent granules was performed to assess the stability and shelf-life of the formulation such as moisture content, flow rate, angle of repose, pH, and dissolution time. The result are presented in Table 3. Moisture within the granules is necessary to facilitate interparticle bonding, resulting in a more compact mass (Widya & Rahmawati, 2025). However, excessive moisture content may promote particle aggregation and agglomeration, leading to poor flow properties (Syaputri & Saila, 2023).

Table 3. Results of Physical Property Tests of Effervescent Preparations

Test	Formulation			Requirements
	1	2	3	
Water content (%)	1.20	0.94	0.67	<5%
Flow rate (seconds)	7.99	1.68	3.55	<10 seconds
Angle of repose (degrees)	6.31	5.97	6.81	$\leq 40^{O}$
pH	8.04	8.14	8.29	6–7
Dissolution time (seconds)	50.96	45.46	45.68	< 5 minutes

Laxative Activity Test

The laxative activity assay in this study employed norit as a marker, which is a highly adsorptive compound capable of non-specifically absorbing bacterial toxins and gases, as well as drugs, nutrients, and enzymes within the gastrointestinal tract. Dulcolax was used as the positive control; it is a stimulant laxative that enhances colonic muscle contractions to accelerate bowel movement. () The test results are presented in Table 4.

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Table 4. Percentage ratio of Norit Distance to Intestinal Length

Group	Ratio of Norit Distance to Intestinal Length (%)				Average (%) ± SD
	Replication				
	1	2	3	4	
Negative Control	67,87	69,07	59,01	36,84	58,19±14,93
Positive Control	41,36	25,47	14,69	63,31	36,21±21,13
F1	27,23	49,43	61,21	46,82	46,17±14,09
F2	24,75	22,62	26,32	36,63	27,58±6,22
F3	26,36	41,86	28,13	30,43	31,70±6,98

p-value analysed with post hoc test (ANOVA) using LSD: [a]Data significantly different from F3, p-value < 0.05, [b]Data not significantly different from F3, p-value > 0.05, [c]Data not significantly different from F2, p-value > 0.05

DISCUSSION

Organoleptic of formulas 1, 2, and 3 of effervescent powder preparations containing red-fleshed dragon fruit and jackfruit seeds as active ingredients. Of the three formulas, the organoleptic test results for each formula showed a characteristic yoghurt odour, which was due to the addition of yoghurt to the effervescent preparation. In this preparation, yoghurt has the function of stimulating peristaltic movement in the human digestive tract, thereby increasing the processes of digestion, absorption, faecal excretion, and the elimination of bacteria (Hendarto et al., 2019). The taste of the three formulas is sweet and slightly sour, due to the addition of ingredients used as acid sources, namely citric acid and tartaric acid, as well as yoghurt, which also produces a sour sensation. The sweet taste, of course, comes from the addition of sugar. Regarding the colour produced, formula 1 produced a brighter pink colour, while formulas 2 and 3 produced a paler colour. This is due to the addition of more acidic and alkaline ingredients compared to formula 1.

The moisture content of effervescent granules can influence the quality and stability of the formulation. Moisture content evaluation obtained for formulas 1, 2 and 3 were 1.19%, 0.94% and 0.67%, respectively and met the requirement for effervescent granules preparations is <5% ((BPOM RI, 2023). The moisture level is affected by the manufacturing process, in which production room humidity was maintained at 25%. Citric acid and tartaric acid, both of which are hygroscopic, may be affected by elevated humidity. Excessive humidity can also promote particle aggregation and agglomeration, resulting in poor flow properties of the granules (Rani et al., 2020). The highest moisture content was found in formula 1, which may be due to the higher use of citric acid compared to formulas 2 and 3, as citric acid is hygroscopic and therefore has the potential to absorb water vapour in the air. This aligns with the research conducted by Syaputri & Saila (2023), which found that the moisture content in the effervescent granules differed due to the varying amounts of citric acid added to each formula.

The measurement of flow rate and angle of repose was conducted to determine the flow characteristics of the powder. Flow behaviour is a critical parameter, as it influences content uniformity and affects the ability of granules to be filled into packaging. Good flow properties are characterized by the ability of particles to resist clumping and to flow freely under the influence of gravity (Pradhan et al., 2023) . The average flow rates produced for the three formulas were 7.99, 1.68, and 3.55 seconds, respectively. These results indicate that each formula has a flow rate that meets the requirement of ≤ 10 seconds with p value .

The angle of repose is measured by the slope of the powder cone formed against the horizontal plane after passing through the funnel. The smaller the angle formed, the better the powder will flow (Megawati et al., 2025) . The results of the angle of repose evaluation show that the results for each formula are 6.31^{O} ; 5.97^{O} ; 6.81^{O}, respectively. From these results, all formulas meet the requirements because their values are ≤ 40^{O} . If the result obtained is >40^{O}, it means that the powder has high

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moisture content and can cause large cohesive forces. Non-cohesive granules will spread out to form a lower pile, resulting in a smaller angle (Octavia et al., 2021) .

The pH measurement aims to determine the acidity level of the effervescent yoghurt granule solution combining red dragon fruit and jackfruit seeds. An effervescent solution that is too acidic can cause stomach irritation, while one that is too alkaline can cause a bitter and unpleasant taste (Rani et al., 2020) . The pH evaluation results showed that the three formulas had pH values of 8.04, 8.14, and 8.29. The pH requirement for effervescent preparations is 6-7, so none of the three formulas meet the requirements. This may be due to the fact that the basic components used in the formulation are too much, while the acidic components are too little, so that after the reaction is complete, there will be unreaction basic components remaining, causing the final solution to be basic with a pH above 7.

The dissolution time test aims to determine the time required for the granules to dissolve completely, which is indicated by the cessation of carbon dioxide gas production in water. Effervescent granules contain acidic and basic components. When the basic and acidic components react with water, the two compounds are released into the solution. The dissolution time evaluation results show that all three formulas meet the dissolution time requirements for effervescent granules, which are 50.96 seconds, 45.46 seconds, and 45.68 seconds. F2 has a faster dissolution time than F1 and F3 because F2 produces more$_{CO_2}$ gas. The more CO_2 produced, the faster the dissolution time required. The rapid release of CO_2 gas from the solid mass creates bubbles that help break down the effervescent granule particles into smaller particles. The gas bubbles rising to the surface of the water create rapid agitation in the liquid. This agitation significantly increases the dissolution rate of the active ingredient and other excipients.

Results of laxative activity testing using the intestinal transit method. This laxative effect testing uses the intestinal transit method. This method works by observing the passage of norit through the entire intestine of a rat using norit as a marker. Norit is an adsorbent compound that cannot be digested. Norit is administered to test animals and functions as a marker that is not absorbed by the intestine. Laxative activity is assessed based on how far norit is able to be pushed by intestinal peristalsis. The smaller the percentage ratio of norit distance to intestinal length (meaning norit moves further relative to the total intestinal length), the greater the laxative effect of the preparation, because intestinal peristalsis increases. The results obtained in this study showed that the negative control (CMC-Na) gave a % ratio value of 58.19%, which can be used as a benchmark for normal intestinal peristalsis in test animals. In the positive control, a % ratio value of 36.21% was obtained, indicating that bisacodyl can increase intestinal peristalsis. In formula 2, the percentage ratio of the distance between the norit and the length of the intestine was 27.58%, which was better than the percentage ratios obtained in formulas 1 and 3. Kanaghurt effervescent can have a laxative effect due to the content of red dragon fruit, which contains fibre and prebiotics. Fibre serves to increase the volume and weight of faeces, which stimulates intestinal muscle contractions (peristaltic movement). Stronger and more regular peristaltic movement accelerates the movement of food residues through the intestines, thereby facilitating bowel movements and preventing constipation. When fibre mixes with water in the digestive tract, soluble fibre forms a gel-like substance. This gel softens the consistency of stool, making it easier and more comfortable to pass (Sharma et al., 2024) . Similarly, jackfruit seeds, which are also high in fibre, act as a prebiotic. Jackfruit seeds contain resistant starch, a type of fibre that is not easily digested in the small intestine. This resistant starch then moves to the large intestine and acts as a prebiotic, a food source for beneficial gut bacteria. The fermentation of resistant starch by these bacteria produces short-chain fatty acids, which nourish the cells of the large intestine and maintain gut health (Celestial et al., 2023) .

The laxative effect of this effervescent Kanaghurt formula is also optimised by the presence of yoghurt. Yoghurt contains probiotics such as *Lactobacillus* and *Bifidobacterium*. These beneficial bacteria work by replacing and increasing the number of good bacteria in the gut. With an increase in the population of good bacteria, the growth of harmful bacteria that can cause infections or digestive disorders (such as diarrhoea and intestinal inflammation) can be suppressed, allowing the

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digestive tract to function normally. Probiotics in yoghurt help facilitate bowel movements (peristalsis) and increase the frequency of bowel movements, which is effective in treating constipation (Savaiano, 2014) .

CONCLUSIONS

All three formulations of the effervescent kanaghurt preparation met most of the physical property test requirements except for pH. The strongest laxative effect with the lowest Norit Distance Ratio to Intestinal Length value was found in formulation 2, which was 27.58%. The laxative effect produced was significantly different compared to the Positive Control. Further research is needed to explore this topic in greater depth using a larger sample size and varied methodologies.

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