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Improved sensory acceptance and cytotoxicity to breast cancer cell line of instant germinated black rice yogurt supplemented with gellan gum

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Abstract-Various concentrations of gellan gum (e.g. 0.00, 2.50, 5.00, 7.50 and 10.00) were supplemented in instant germinated native black rice yogurt. After rehydration with the ratio of instant yogurt: water (1:10), then the properties of product were evaluated. Our results revealed that the concentration of gellan gum affected to the color (L*, a*,b*), pH and consistency of the yogurt. However, concentration of gellan gum did not affect to moisture content, total soluble solid and total viable count. From sensory evaluation with a 9-point hedonic scale, the gellan gum slightly increased color, odor, flavor and sharply increased texture and overall acceptability of yogurt. The addition of 10% (w/w) gellan gum resulted in the highest texture and overall acceptability scores ($p \leq 0.05$). Therefore, this treatment was selected to assess the properties compared with a control. Gellan gum did not affect the chemical composition including moisture content, protein, lipid, fiber, ash, total carbohydrate, γ-aminobutyric acid, DPPH antioxidant property, total soluble solid, total viable count and yeast and mold count. Moreover, results from Fourier Transform Infrared Spectroscopy (FTIR) analysis indicated that both control and instant germinated native black rice yogurt had similar transmission of spectra. Interestingly, this instant germinated black rice yogurt showed powerful toxicity to breast cancer cell line (MDA-MB-231) which was higher than in the control (without gellan gum). This finding suggested that these instant germinated native black rice yogurts could be served as a healthy alternative functional meal for general consumers, especially adults and elderly.

Keywords: Non-diary, germinated black rice, probiotic, gellan gum, yogurt

1. Introduction

Nowadays, vegetarian consumers are becoming increasingly aware towards healthy diets and longevity resulting in a growing market demand for new functional foods with a beneficial effect on health (Santos *et al.*, 2018; Ploll *et al.*, 2020). Black rice has been especially regarded as health food because of its high bioactive compound and anthocyanin content (Khalil & Elkot, 2020). The main compound is cyanidin-3-glucoside which has a black color. Previous studies showed that this substance had an inhibitory effect on cancer cell proliferation, reduction of total cholesterol, neurological and cardiovascular illness (Averilla *et al.*, 2019; Zawistowski *et al.*, 2009).

Farmers grow black rice in many areas of Thailand, including Chanthaburi province. Native black rice (Maepayatong dum rice) is the traditional rice variety in the district of Kao Kitchakut, Chanthaburi province. Moreover, Sangkitikomon *et al.* (2008) reported that anthocyanin from this black rice has higher antioxidant activity than red rice and rice berry. In addition, germinated black rice offers more considerable benefit especially as it has increased levels of γ-aminobutyric acid (GABA), dietary fiber, inositols, ferulic acid, phytic acid, tocotrienols, magnesium, potassium, zinc, γ-oryzanol, and makes the rice more absorbable by the body, tender and tastier. GABA is a neurotransmitter in the brain and in the spinal cord of mammals. This substance can lower hypertension, promote sleepiness, and has benefit for human health (Kushwaha, 2016).

For this reason, black rice a the famous ingredient used in many foods. For example, Ariyani *et al.* (2019) found that the addition of rice bran to yogurt improved the level of total phenol and antioxidant activity. Abbas (2016) and Haskito *et al.* (2020) showed that the addition of black rice bran flour to goat milk yogurt could increase the antioxidant activity and total lactic acid bacteria. Nontasan *et al.* (2012) made yogurt supplemented with black rice bran. They found that yogurt added with colorant powder 0.60%w/w had a purplish-pink color and could be kept under refrigeration storage (4°C) for up to 21 days. Furthermore, the incorporation of rice into yogurt could significantly improve the crude fiber contents of the yogurt. Furthermore, four rice varieties namely, At-309, At-405, MA-2 and Thai Jasmine (KDML 105) incorporated yogurt had higher water holding capacity compared to that of plain yogurt (without rice) during storage (Kumari *et al.*, 2015). Ningtnas and Haskito (2020) evaluated the comparison of acceptability analysis of goat milk yogurt fortification with various rice bran flour. The results showed that goat milk yogurt fortified with black rice bran flour was the most favored by the panelists. From our previously investigation, yogurt production from germinated native black rice was monitored. Germination (48 h) had highest GABA content and this condition was selected prepare black rice yogurt production. Highest overall acceptability was found in germinated black rice yogurt fermented with 20% Revon starter (Mongkontanawat *et al.*, 2018).

Gellan gum is an extracellular polysaccharide secreted by certain microorganisms including *Sphingomonas elodea* and *Pseudomonas elodea* by the fermentation process. This hydrocolloid is a nontoxic polysaccharide approved by the U.S. Food and Drug Administration

(USFDA) as a food additive with great potential to be employed in pharmaceutical industries and as biomaterial to promote oral colonization for probiotics that present oval candidiasis (Zhu *et al.*, 2015; Ribeiro *et al.*, 2020). It can gels at low concentration in hot solutions cooled in the presence of gel-promoting cation (Sworm & Stouby, 2021).The mechanism of gelation involves the formation of double helical junction zones from random coil chain followed by aggregation of double helical segments to form a three dimentional network with cations and hydrogen bonding with water (Grasdalen & Smidsrod, 1987). Gellan gum is used commercially in a wide range of food applications including water-based gels, bakery, dairy foods, beverages, confections and fruit products. In yogurt processing, gellan gum is typically added to the raw milk prior to homogenization and pasteurization. The concentration use is between 0.06 – 0.10% add a light texture and significantly reduces whey-off (Imeson, 2010).

There have been many studies of black rice and yogurt as mentioned above. However, there is no information reported on production using instant germinated native black rice yogurt. Therefore, the aim of this study was to investigate the effect of gellan gum on some physic-chemical, microbiological properties and perfoform a sensory evaluation of germinated native black rice yogurt. The highest overall acceptability treatment was opted to investigate the chemical composition, GABA content, DPPH antioxidant assay, microbiological; and FTIR spectra and toxicity to breast cancer cell (MDA-MB-231) were compared between control sample and germinated black rice yogurt. Finally, the best treatment was chosen to evaluate effect of 16 weeks room storage on some properties of the instant black rice yogurt.

2. Materials and methods

2.1 Materials

Native black rice (Maepayatong dum rice) was purchased from a local farmer in the district of Kao Kitchakut, Chanthaburi province and then transported to the laboratory. Germinated native black rice was prepared according to modified methods described by Mongkontanawat *et al.* (2018). Briefly, the samples were soaked in water at the ratio of rice and water (1:10) at 40 ºC for 6 hours in a tray, after which the water was drained and samples incubated for 48 hours. The germination was stopped by drying using a hot air oven (Binder, Germany), at 55 ºC for 4.5 hours. The obtained germinated native black rice was stored at room temperature (37 ºC) and used for the next evaluation. For the starter culture, Revon soygurt (original flavor) which was a mixed culture composed of *Streptoccus thermophilus*, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus* and *Bifidobacterium animalis* subsp. *Lactis* was purchased from local convenice store in Thailand and used as inocula.

2.2 Production of powdered germinated black rice yogurt

Germinated black rice yogurt was prepared as followed. The 48h germinated native black rice was cooked in a rice cooker with a ratio of 1:2 (rice and water). The cooked rice was blended with water at a ratio of 1:2 (rice and water) using a blender with and then filtered with straining cloth. 3% (w/v) sugar and 5% (w/v) lactose was then added to the germinated native black rice milk. The rice media were prepared in a small polypropylene container (50 ml) which were pasteurized at 80°C for 5 minutes. Then, 20%(w/w) of Revon starter was mixed in the rice media and incubated at 42°C for 8 hours (Mongkontanawat *et al.,* 2018). Finally, the germinated fermented black rice yogurt was dried by using a hot air oven (Binder, Germany) at 80°C for 15 hours. The obtained dried yogurt was assayed for some properties including moisture content, %yield, bulk density and total viable count.

2.3 Effect of gellan gum on physic-chemical and microbiological of instant germinated black rice yogurt determination

The gellan gum was added in order to improve the viscosity of instant yogurt after rehydration. Five percent (%w/w) of gellan gum (Sisco Research Laboratories PLT. LTD., India) such as 0.00, 2.50, 5.00, 7.50 and 10.00 were mixed with powdered germinated black rice yogurt as prepared previously. Then, the rehydration of instant germinated black rice yogurt was started. Warm water (60 °C) was added to the instant germinated black rice yogurt with the ratio of 1:10 (powdered : water) and mixed well and then investigated for physico-chemical microbiological determination and sensory investigation. Experimental design used was completely block design (CRD) for properties determination and completely randomized block design (RCBD) for sensory evaluation.

2.4 Physical properties determination of rehydration of germinated black rice yogurt

The five treatments of instant germinated black rice yogurt were measured for color by using a color meter (Nippon Denshoku, ZE-2000, Japan). The equipment was calibrated with a standard plate. The color measurement was expressed in L*, indicating the lightness on a 0 to 100 scale from black to white and a* (+,-) indicated the redness or greenness whereas b* (+,-) indicated yellowness and blueness. The moisture content was evaluated by using a moisture analyzer (Sartorious MA 45, Germany). Finally, the rehydration of germinated rice was produced and monitored with Bostwick flow distances by using Bostwick consistometer (The Precision Equipment Company, United Kingdom).

2.5 Chemical properties determination

For chemical properties determination, the pH was measured with a pH meter (Subtex, Taiwan). And the total soluble solid was analysed by using a hand refractometer (Atago, Japan).

2.6 Microbiological properties determination

The standard plate count method with Plate Count Agar (PCA) medium after 48 h inoculation at 37ºC was used for total bacteria counts of samples and then reported as log CFU/ml (Yoon *et al.*, 2004).

2.7 Sensory evaluation

The five treatments of rehydrated instant germinated black rice yogurt were investigated for their sensory attributes (color, aroma, taste, texture, and overall liking) by using a 9-point hedonic scale (Watts *et al.*, 1989) with 50 untrained panelists from the staff and students of the Department of Food Innovation and Business (Faculty of Agro-industrial Technology, Rajamangala University of Technology Tawan-ok, Chanthaburi Campus, Chanthaburi, Thailand).

2.8 Comparision of chemical composition and some properties of final product and control (without gellan gum)

The best treatment from sensory evaluation was chosen in this section, the composition was assessed for moisture, protein, lipid, fiber, ash and carbohydrate content, respectively (AOAC, 1990). γ-Aminobutyric acid content was analysed by the Institute of Food Research and Product Development (IFRPD), Kasetsart University in Bangkok, Thailand. Briefly, a dried sample of 2.5 g was added to 18 ml of distilled water and 2 ml of 3% sulfosalicylic acid stirred for 30 minutes and centrifuged. The supernatant (0.1 ml) was mixed with 0.1 ml of $NaHCO_3$ and 0.40 ml of debsyl-C and these solutions were mixed together. The solution was heated in a water bath at 70°C for 10 minutes. The obtained solution was then mixed with 0.25 ml of ethanol and 0.25 ml of 0.025 M KH_2PO_2. The γ-aminobutyric acid (GABA) was detected using High Performance Liquid Chromatography (HPLC)(HPLC-UV detector:agilent, 1,200 serice; column:supercosil LC-DABS, 15 cm x 4.6 cm,3 um; flow rate 1 ml/min; mobile phase: gradient 80% CH_3COONa pH 6.80, acetonitrile inject volume 5 ul; column temperature 40 °C UV detector at 465 nm and standard GABA ≥ 99%) (Tadashi *et al.*, 1998)

For antioxidant activity, radical scavenging effect was analyzed by DPPH radical scavenging assay by modified the method of Zhu *et al.* (2006). Briefly, 2.00 ml of sample was mixed with 2.00 ml of 0.16 mM diphenyl picryhydrazyl (DPPH) in a test tube. Then, 2.00 ml of sample was also mixed with 2.00 ml of 95% ethanol in anothor test tube. Next, 2.00 ml of 95% ethanol was added with 2.00 ml of 0.16 mM Diphenyl picryhydrazyl (DPPH) in the last test tube. The mixture was shaken vigorously and incubated in dark conditions for 30 minutes. Finally, the absorbance was measured at 517 nm with a spectrophotometer. % Scavenging effect was calculated using the equation below.

DPPH radical scavenging activity (%) = [1-(Asample-Ablank)/Acontrol] x100

Asample = the absorbance of test compound

Ablank = the absorbance of test and blank reaction

Acontrol = the absorbance of control reaction

Microbiological properties were determined by assay for total plate count as previously mentioned and yeast and mold count were investigated by Potato Dextrose Agar medium after 48 hours inoculation at 37ºC and then used for viable cell counts of samples and reported as log CFU/ml (Yoon *et al.*, 2004).

The chemical structure of samples was analyzed by using Fourier Transform Infrared Spectroscopy (FTIR). The selected instant germinated black rice yogurt and control were sent to the Scientific and Technological Research Equipment Center and desiccated before FTIR analysis. Then, all samples were ground with potassium bromide and pressed at high pressure into a KBr pellet. An FTIR spectrophotometer (PerkinElmer,model NIRA,Massachusetts, USA) was used. The FTIR spectra of the samples were recorded in the range of 400 cm^{-1} to 4000 cm^{-1} at room temperature. The measuring resolution was 4 cm^{-1} and 32 interactions were assessed (Lui *et al.*, 2020)

Cytotoxicity assay by using MTT assay was measured by the Microbiology Department, Faculty of Science, Chulalongkorn University in Bangkok, Thailand. This method was performed following the method reported by Senthilraja and Kethiresom (2015) with the slight modifications. In brief, seeding cell MDA-MB-231 cell was seeded at 1.5 X 10^4 in a 96 well plate overnight (total volume 100 µl/well). For the cell treatment, mushroom beverage was prepared by using six different concentrations of bioactive compounds which were diluted in completed media and added to the well that contained the cells (100µl/well). Supernatant was removed, mixed completely with media that contained with sample solution or DMSO (vehicle control) and incubated for 24 h. For the measurement cell cytotoxicity, MTT solution (conc. 5 mg/ml) at 10 µl/ well was added and incubated at 37 °C for 4 h in CO_2 incubator. The purple formazan was dissolved by using isopropanol with HCl (100 µl/well) and mixed. Finally, the absorbance was monitored at wavenumber 540 nm by microplate reader.

2.9 Effect of room storage on some properties of the instant black rice yogurt

The best treatment from sensory evaluation is reported in this section, the powdered samples (50 g) were stored at room temperate (37 ºC) for 16 weeks in aluminium foid bags. Samples were taken at two weekly intervals. Then, the powdered samples were analyzed for some physical and microbiological properties. Physical property that were monitored in the samples were color parameters and moisture content as described previously. For the microbiological properties, viable cell counts (log CFU/ ml) were evaluated by the standard plate count method and yeast and mold count as mention previously.

2.10 Statistical analysis

All experiments were performed in triplicate using different lots of instant germinated powdered black rice yoghurt. Analysis of variance (ANOVA) ($p \leq 0.05$) was performed with the data. Significant difference among means within each experiment were separated by Duncan's multiple range test (DMRT) at a significance level of $\alpha = 0.05$ by using computer software (Helge, 2009).

3. Results

The effect of gellan gum on the physicochemical and microbiological properties of rehydration of instant germinated black rice yogurt has been studied. Results revealed that the properties of dried black rice yogurt including moisture content,%yield, bulk density and total plate count showed

4.90±0.16% w/w, 0.71±0.04% w/w, 10.67±0.58 g/mg, <1.00±0.00 log CFU/ml; respectively. Then, the effect of gellan gum on the physico-chemical and microbiological properties of rehydration of instant black rice yogurt was investigated. Results showed that the addition of gellan gum trended to be increase lightness (L*), while, a*(redness) b*(yellowness) were slightly decreased. In contrast, the concentration of gellan gum did not effect the moisture content, total soluble solid and viable cell count (Table 1,2). Nevertheless, the concentration of gellan gum dramatically significant decreased the Bostwick flow distances and increased thickness and consistency of rehydrated germinated black rice yogurt. In the other hand, the addition of gellan gum tended to slightly significantly increase the pH values.

Table 1. The effect of various concentration of gellan gum on some physical properties of instant germinated black rice yogurt

Gellan gum(%w/w)	Color parameters			Moisture content (%)^{ns}	Bostwick flow distances (cm)
	L*	a*	b*		
0.00	58.78±0.01^{e}	6.64±0.09^{a}	13.70±0.12^{ab}	2.89±0.16	16.00±0.35^{a}
2.50	59.00±0.08^{d}	6.58±0.11^{b}	13.72±0.03^{a}	2.87±0.19	8.89±0.49^{b}
5.00	59.23±0.03^{c}	6.60±0.08^{b}	13.73±0.03^{a}	2.88±0.29	8.33±0.35^{c}
7.50	59.30±0.02^{b}	6.58±0.05^{b}	13.64±0.05^{bc}	2.86±0.30	7.33±0.35^{d}
10.00	59.47±0.03^{a}	6.57±0.05^{b}	13.62±0.07^{c}	2.89±0.19	5.17±0.43^{e}

Each data represents mean of three replications with standard error. Values with a different letter are significantly different ($p \leq 0.05$) according to Duncan's multiple range test.

Table 2. The effect of various concentration of gellan gum on some chemical and microbiological properties of instant germinated black rice yogurt

Gellan gum(%w/w)	Total soluble solid (°Brix)^{ns}	pH	Total count bacteria (log CFU/ml)^{ns}
0.00	7.00±0.00	4.16±0.09^{b}	<1±0.00
2.50	7.00±0.00	4.21±0.11^{ab}	<1±0.00
5.00	7.00±0.00	4.22±0.08^{ab}	<1±0.00
7.50	7.00±0.00	4.27±0.05^{a}	<1±0.00
10.00	7.00±0.00	4.28±0.05^{a}	<1±0.00

Each data represents mean of three replications with standard error. Values with a different letter are significantly different ($p \leq 0.05$) according to Duncan's multiple range test.

The effect of gellan gum on the sensory evaluation of rehydration of instant germinated black rice yogurt was determined. It was found that the sensory attributes of rehydrated germinated black rice yogurt supplemented of the various concentration of gellan gum were as shown in Table 3.

Table 3. Sensory attributes of instant germinated black rice yogurt with various supplemented of gellan gum as various concentration

Gellan gum(%w/w)	Likeness scores				
	Color[ns]	Aroma[ns]	Taste[ns]	Texture	Overall liking
0.00	6.77±0.68	6.77±0.73	6.60±0.67	6.57±0.63 [b]	6.77±0.63[b]
2.50	6.77±0.57	6.87±0.97	6.63±0.67	6.60±0.62 [b]	6.77±0.43[b]
5.00	6.73±0.64	6.77±0.77	6.63±0.72	6.87±0.78 [ab]	6.73±0.52[b]
7.50	6.87±0.82	6.80±0.66	6.63±0.81	6.90±0.76 [ab]	6.87±0.78[ab]
10.00	6.90±0.80	6.90±0.76	6.80±0.76	7.17±0.70 [a]	7.13±0.63[a]

Values with a different letter are significantly different ($p \leq 0.05$) according to Duncan's multiple range test. Acceptability was evaluated using a structured hedonic scale of 9 points, from 1 (dislike very much) to 9 (like very much).

Color, aroma, and taste slightly increased but did not differ significantly ($p \leq 0.05$) among the samples. Moreover, the concentration of gellan gum also slightly significantly increased in texture and overall liking and a significant difference ($p \leq 0.05$) was found. Indeed, 10% w/w of gellan gum showed the highest texture and overall liking with a score of 7.17±0.70 and 7.13±0.63 (moderately liked). However, there were non-significant differences with the addition of gellan gum 5.00 and 7.50% w/w. Furthermore, the addition of 10% w/w gellan gum also showed the highest likeness score of color, aroma, taste. Thus, it was chosen for continuing to determine phyco-chemical, microbiological properties compared with the control group (without gellan gum) and then the influence of shelf life room storage (37°C) on the aboved mention properties at 0, 2, 4, 6,8,10,12,14 and 16 weeks of storage were evaluated in the next assessment.

Some phyco-chemical and microbiological properties were compared with the control. Results showed that there was no significant difference between the control and the experimental germinated black rice yogurt in the chemical composition and other parameters. The moisture, protein, lipid, fiber, ash and carbohydrate content levels were 2.61±0.14, 19.60±0.40, 0.00±0.00, 2.47±0.16, 0.56±0.07 and 74.75±0.11% for control and 2.62±0.18, 19.42±0.09, 0.00±0.00, 2.43±0.13, 0.57±0.06 and 74.95±0.12% for experimental germinated black rice yogurt, respectively. The values of γ-aminobutyric acid, DPPH radical scavenging activity, total count of bacteria and yeasts and mold were 0.69±0.06 mg/100g, 2.47±0.16%,<1±0.00 log CFU/g and <1±0.00 log CFU/g for control and 0.64±0.07 mg/100g, 2.43±0.13%, <1±0.00 log CFU/g and <1±0.00 log CFU/g for experimental germinated black rice yogurt, respectively. Germinated black rice yogurt supplemented with 10% gellan gum slightly increased in moisture content, ash and carbohygrate content. On the other hand, protein, fiber, γ-aminobutyric acid, DPPH radical scavenging activity were not significantly different in germinated black rice yogurt supplemented with 10% gellan gum.

The chemical composition and cytotoxicity properties of instant germinated black rice yogurt with 10% gellan gum supplemented were compared with those of the control (without gellan gum). Determination of chemical composition employed FTIR for structural analysis of chemical structure. The FTIR spectra shows the molecular vibrations of covalent bonds at the infrared region range (4000-400 cm^{-1}).

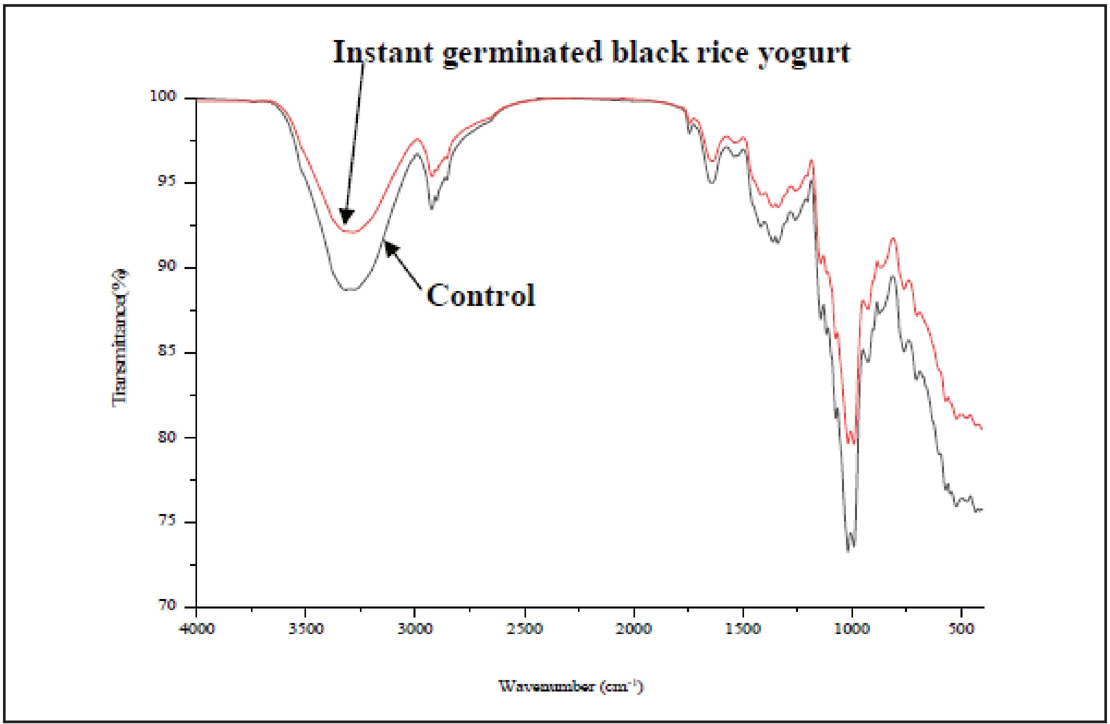

Figure 1. FTIR spectra of instant germinated black rice yogurt compared with control

Figure 1 shows the FTIR transmission spectra of experimental instant germinated black rice yogurt and the control (without gellan gum). The infrared transmission characteristics of samples indicated that both the spectra of two samples affected the chemical structure with a similar spectral pattern. Highest transmission intensity was found in polysaccharide regions (900-1200 cm^{-1}) in both samples. The transmission bands at 3300 and 1610 cm^{-1} could be due to bound water (Rojas *et al.*, 2011). On the other hand, the intensity of FTIR transmission of instant germinated black rice yogurt was lower than that control. This demonstrated that the addition (10%w/w) of gellan gum could interfere with the transmission spectra in the carbohydrate and polysaccharide region, allowing the transmission spectra was lower than control.

Regarding cytotoxicity, Figure 2 shows that high concentrations for both samples (control and instant germinated black rice yogurt) tended to be toxic to breast cancer cells MDA-MB-231. The percentage of viability of the cancer cell line decreased when the concentration of both samples was higher. Interestingly, instant germinated black rice was dramatically toxic to cancerous cell line (MDA-MB-231) at higher level than found with the control (without gellan gum).

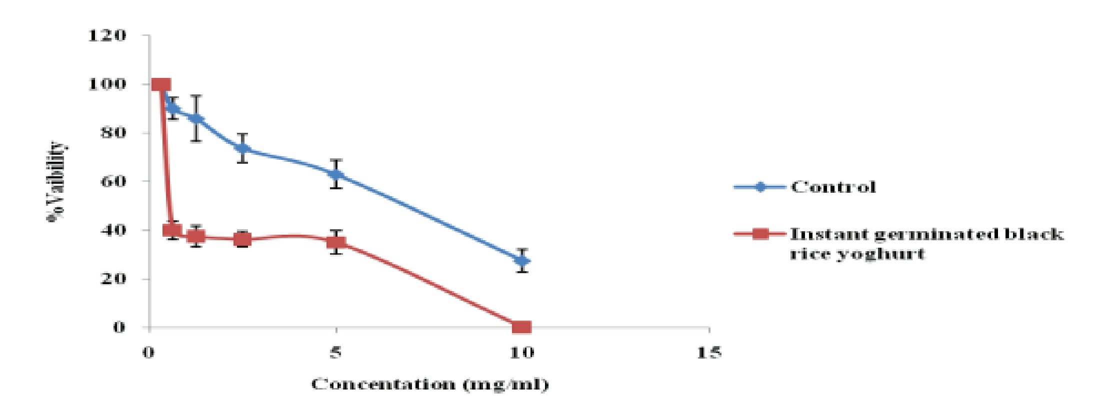

Figure 2. Effect of instant germinated black rice yogurt to percentage of viability of breast cancer cell MDA-MB-231

Finally, the influence of shelf-life by room storage (37°C) on some properties after 16 weeks of storage was determined. The shelf-life storage of the experimental germinated black rice yogurt was continued to evaluate when stored at room temperature for 16 weeks. Our result revealed a slightly significant ($\rho < 0.05$) increase in moisture content, total viable count, yeast and mold during room storage period. For total viable count and yeast and mold count, 10 weeks and 14 weeks storage was detected the numbers of microorganism. On the other hand, the long-time storage did not effect lightness (L*) as shown in Table 4.

Table 4. Effect of shelf-life room storage (37°C) on some chemical and microbiological properties of developed instant germinated black rice yogurt

Storaged time (weeks)	Color parameters			Moisture content (%)	Total count bacteria(log CFU/g)	Yeast and mold(log CFU/g)
	L* ns	a*	b*			
0	59.47±0.03	6.57±0.06 abc	13.62±0.07 ab	2.89±0.19 b	<1±0.00 e	<1±0.00 c
2	59.43±0.04	6.60±0.02 ab	13.63±0.07 ab	3.02±0.15 g	<1±0.00 e	<1±0.00 c
4	59.41±0.04	6.59±0.02 abc	13.65±0.04 a	3.18±0.14 f	<1±0.00 e	<1±0.00 c
6	59.38±0.05	6.62±0.03 ab	13.63±0.06 ab	3.32±0.12 e	<1±0.00 e	<1±0.00 c
8	59.36±0.05	6.63±0.04 b	13.59±0.05 b	3.38±0.10 de	<1±0.00 e	<1±0.00 c

Table 4. Effect of shelf-life room storage (37°C) on some chemical and microbiological properties of developed instant germinated black rice yogurt (cont.)

Storaged time (weeks)	Color parameters			Moisture content (%)	Total count bacteria(log CFU/g)	Yeast and mold(log CFU/g)
	L* ns	a*	b*			
10	59.33±0.02	6.59±0.05 abc	13.62±0.05 ab	3.48±0.10 d	0.66±0.13 d	<1±0.00 c
12	59.29±0.03	6.60±0.05 abc	13.61±0.05 ab	3.64±0.05 e	2.88±0.02 c	<1±0.00 c
14	59.26±0.03	6.56±0.03 e	13.58±0.03 b	3.79±0.05 b	4.09±0.01 b	0.54±0.19 b
16	59.22±0.02	6.60±0.05 abc	13.60±0.06 ab	3.95±0.04 a	5.31±0.01 a	1.18±0.09 a

Each data represents mean of three replications with standard error. Values with a different letter are significantly different ($p \leq 0.05$) according to Duncan's multiple range test.

4. Discussion and conclusion

We studied the effect of gellan gum on the physico-chemical and microbiological properties of rehydration of instant germinated black rice yogurt. Results indicated that the addition of gellan gum increased lightness (L*) and pH. This could be gellan gum being white so lightness increased depending on the concentration of the gum added. For pH, this could be because of the pH of gellan gum was approximately 5.00-7.50 so the pH increased when mixing high concentration of gellan gum. Nevertheless, the concentration of gellan gum sharply significantly decreased Bostwick flow distance of rehydration of germinated black rice yogurt. Because gellan gum is a gelling agent the gel was formed by interaction with water resulting in increased stickiness. Therefore, the Bostwick flow distance shortened whereas the consistency and viscosity tended to increase as they were dependent on the concentration of gellan gum that was added.

For sensory evaluation, germinated black rice yogurt supplemented with gellan gum exhibited slightly increasing color, aroma, and taste attributes; especially, the attributes of texture and overall liking were sharply significantly increased with the score moderately liked (Table 3). This could be due to gellan gum being a gelling-polymer, therefore, the texture was improved by the gelation process. This involved reactions of double helical junction zones from the random coil chains, followed by aggregation of double helical segments to form a three-dimensional network by complexation with cations and hydrogen bonding with water (Grasdalen & Smidrod, 1987). So, the sedimentation decreased and lead to high product acceptability. Our results are in agreement with Krasaekoopt (2011), who reported that the addition of hydrocolloids improved the sensory quality of fermented whey beverage from different types of milk. To our knowledge, this is the first study that has used gellan gum to improve stability of black rice yogurt by gelling process and could be used in non-diary product instead gelatin.

The supplementation of 10% w/w gellan gum did not affect the functional composition, γ-aminobutyric acid, DPPH radical scavenging activity(%), total count of bacteria and yeast (Figure 1). This could be because a minor amount of gellan gum was added, therefore, the chemical composition and other properties were not changed. Since, in the FTIR spectrum of pure gellan gum, the bands appearing at 1627.81 and 1409.87 cm^{-1} were due to asymmetric and symmetric stretching of carboxylate group. The band at 2927.74 cm^{-1} were due to the stretching vibrations of $–CH_3$ group, whereas, those appearing at 1153.35 and 1024.13 cm^{-1} were due to ethereal hydroxylic C-O stretchings. Bending vitration of C-H appeared at 891.05 cm^{-1}. The band at 3427 cm^{-1} was due to the presence of OH group of glucopyranose ring (Dixit *et al.*, 2011). However, those peaks did not show in this study. So, the structural deconvolution analysis could be of help to indentify overlapping peaks in further evaluation. The fortified yogurt with 10% gellan gum in this study contained more than is generally added during diary yogurt production (Imeson, 2010). However, based on the different production conditions and ingredients in the formula, the concentration could be different.

Interestingly, instant germinated black rice exhibited a dramatic toxic effect on the cancerous cell line (MDA-MB-231) higher than those control (Figure 2). This is the first research that tested black rice yogurt supplemented with gellan gum for its effects on cancer cell lines. The results of this research corresponded with the studies of Arrigo *et al.* (2014) and Liang *et al.* (2019), which revealed that gellan gum nanohydrogel and black rice anthocyanin could inhibit the growth of cancer cell lines by apoptosis. Overall, this cytotoxicity could be a synergistic interaction amongst the gellan gum, probiotic microorganisms and anthocyannin in black rice by inducing cancerous cell apoptosis. Since gellan gum has a negative charge causing electrostatic reaction with cancerous cell line, it might inhibit cell growth and induce tumor cell death.

In conclusion, the addition of gellan gum increased consistency, viscosity and sharply increased the sensory attributes in texture and overall liking and slightly increased color, aroma, and taste, whereas, it did not affect moisture content, total soluble solid, total viable count, γ-aminobutyric acid, DPPH radical scavenging activity (%). Interestingly, the cytotoxicity to breast cancer cell MDA-MB-231 of the instant germinated black rice was higher than in the control (without gellan gum). These results suggested that the germinated black rice yogurt could serve as an alternative healthy non-dairy product for vegetarian consumers in the future. However, the taste should be further improved in order to increase the acceptability of the product. Furthermore, the cytotoxicity should be examined by comparing with the normal cell line before further application.

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6. References

Abbas, H.M., Hussien, A.M.S., & Ibrahim, G.E. (2016). Changes in antioxidant activity and volatile compound of functional yoghurt fortified with rice bran during storage. *Journal of Chemical and Pharmaceutical Research,8*(7), 761-766. http://jocpr.com/vol8-iss7-2016/CPR-2016-8-761-766.pdf.

Averilla, J.N., Oh, J., Kim, H.J., Kim, J.S. & Kim, J.S. (2019). Potential health benefits of phenolic compounds in grape processing by-product. *Food Science and Biotechnology, 28*(6), 1607-1615.

AOAC.(1990). Official method of analysis vol 1 (15th Ed). Association of Official Analytical Chemist Inc.

Ariyani, O., Nisa, F.Z., Apriyanto, M., & Aprilia, V. (2019) .Total phenol and antioxidant activities of rice bran yoghurt. *Indonesian Journal of Nutrition and Dietetics,7*(2),37-40. http://dx.doi.org/10.21927/ijnd.2019.7(2).37-40.

Arrigo, G., Navarro, G., Meo, C.D., Matricardi, P., & Torchilin, V. (2014). Gellan gum nanohydrogel containing anti-inflamatory and anti-cancer drugs: a multi-drug delivery system for a vombination therapy in cancer treatment. *European Journal of Phamaceuticals and Biopharmaceutics, 87*(2), 208-216. https://doi.org/10.1016/j.ejpb.2013.11.001.

Dixit, R., Verma, A., Singh, U.P., Soni, S., Mishra, A.K., Bansai, A.K., & Pandit, J.K. (2011). Preparation and characterization of gellan-chitosan polyelectrolyte complex beads. *Latin American Journal of Pharmacy, 30*(6), 1186-1195.

Grasdalen, H., & Smidsrod, O. (1987). Gelation of gellan gum. *Carbohygrate Polymer, 7*, 371-393.

Haskito, A.E.P., Mahdi, C., & Noviatri, A. (2020). Comparision of antioxidant activity and total lactic acid bacteria (LAB) of goat milk yoghurt, goat milk yoghurt fortified by related red and black rice bran flour. *IOP Conference Series : Earth and Environmental Science, 465*, 1-7.

Helge, T.S.(2009). *Statistical analysis of designed experiments* (3rd Edition). Springer.

Imeson, A. (2010). *Food stabilizers, thickeners and gelling agents*. Blackwell Publishing.

Khalil, R.A.M. & Elkot, W.F.(2020). Functional properties and nutritional quality of processed cheese spreads enriched with black rice powder. *Egyptian Journal of Food Science, 48*(2), 281-289. http://10.21608/EJFS.2020.36261.10 68.

Krasaekoopt, W. & Cabraal, T.L.(2011). Effect of hydrocolloid on sensory properties of the fermented whey beverage from different types of milk. *AU Journal of Technology, 14*(4), 253-258.

Kumari, A.G.I.P., Ranadheera, C.S., Prasanna, P.H.P., Senevirathne, N.D. & Vidanarachchi, J.K.(2015). Development of a rice incorporated symbiotic yogurt with low retrogradation properties. *International Food Research Journal, 22*(5), 2032-2040. http://www.ifrj.upm.rdu.my.

Kushwaha, U.K.S. (2016). *Health benefit of black rice*. Springer.

Liang, T., Zhang, Z., & Jing, Pu. (2019). Black rice anthocyannins embedded in self-assembled chitosan/ chondroitin dulfate nanoparticles enhance apoptosis in HCT-116 cells. *Food Chemistry, 301*, 1-9. https://doi.org./10.1016/ j.foodchem.2019.125280.

Mongkontanawat, N., Wongekalak, L., Nonmuang, W., & Thumrongchote, D. (2018). Yoghurt production from germinated native black rice (Maepayatong Dum Rice). *International Journal of Agricultural Technololgy*, 14, 543-558.

Ningtas, C.K., & Haskito, A.E.P.(2020). Comparison of acceptability analysis of goat milk yogurt fortification with various rice bran flour. *Proceeding of the IOP Conference Series : Earth and Environmental Science, 465*,1.-7. https://doi:10.1088/1755-1315/465/1/012012.

Nontasan, S., Moongngarm, A., & Deeseenthum, S.(2012). Application of functional colorant prepared from black rice bran in yogurt. *APCBEE Procedia, 2*,62-67. https://doi:10.1016/j.apcbee.2012.06.012.

Ploll, U., Petritz, H., & Stern, T. (2020). A social innovation perspective on dietary transitions: diffusion of vegetarianism and veganism in Austria. *Environmental Innovation and Social Transition, 36*, 164-176.

Ribeiro, F.C., Junqueira, J.C., Santos, J.D., Barros, P.P., Rossoni, R.D., Shukla, S., Fuchs, B.B., Shukla, A., & Mylonakis, E. (2020). Delvelopment of probiotic formulations for oral candidiasis prevension: gellan gum as a carrier to deliver *Lactobacillus paracasei* 28.4. *Antibacterial Agents and Chemotherapy, 64*(6), 1-16.

Rojas, J., Uribe, Y., & Zuluaga, A. (2011). Powder and compaction characteristics of pregelatinized starches. *Pharmazie, 67*, 513-517. https://doi.10.1691/ph.2012.1118.

Sahoo, S.K., Sahoo, SU.K.,Behera, A., Patil, S.V., & Panda, S.K. (2013). Formulation, in vitro drug release study and anticancer activity of 5- fluorourацil loaded gellan gum microbeads. *Pharmaceutical Technology, 70*(1), 123-127.

Sangkitikomon, W., Tentumnou, T., & Rodchanasasod, A. (2008). Comparisions of total antioxidants of red rice, black rice and black sticky rice. *Journal of nutrition, 43*, 16-21.

Santos, G., Nunes, T.P., Silva, M.A.A.P., Rosenthal, A., & Pageni, A.A.C. (2018). Development and acceptance of freeze-dried yogurt "powder yogurt". *International Food Research Journal, 25*(3), 1159-1165. http://www.ifrj.upm.rdu.my.

Senthilraja, P., & Kathiresan, K. (2015). In vitro cytotoxicity MTT assay in Vero, HepG2 and MCF-7 cell lines study of Marine Yeast. *Journal of Applied Pharmaceutical Science, 5*, 080-084.

Sworm, G., & Stouby, L. (2021). Chapter 28-Gellan gum. In Phillips G. & Williams P. (Eds.). *Handbook of hydrocolloids* (3th ed., pp.855-885). Woodhead Publishing series in food science.

Tadashi, A., Yoshiatsu, K., Wen Bin, & Toshihiko, Y. K.(1998). High performance liquid chromatographic determination of β-alanine, β-aminoisobutyric acid and γ-aminobutyric acid in tissue extracts and urine of normal and caminooxy acetate-treated rats. *Journal of Chromatography B,712*, 43-49.

Watts, B.M., Yumaki, C.L., Jeffery, L.E., & Elais, L.G.(1989). *Basic sensory methods for food evaluation*. The International Development Research Centre.

Yoon, K.Y., Woodams, E.E., & Hang, Y.D.(2004). Probiotication of tomato juice by lactic acid bacteria. *Journal of Microbiology, 42*, 315-318.

Zawistowski, J., Kopec, A., & Kitts, D.D. (2009). Effects of a black rice extract (Oryza sativa L., indica) on cholesterol levels and plasma lipid parameters in Wistar Kyoto rats. *Journal of Functional Foods*, 1(1), 50-56.

Zhu, K., Zhou, H., & Qian, H.(2006). Antioxidant and free radical scavenging activities of wheat germ protein hydrolysates (WGPH) prepared with catalase. *Process Biochemistry, 41*, 1296-1302.

Zhu, L., Ao, J., & Li. P.(2015). A novel in situ gel base of deacetylase gellan gum for sustained ophthalmic drug delivery of ketotifen: in vitro and in vivo evaluation. *Drug Design, Development and Therapy, 9*, 3943-3949.