Total lactic acid bacteria, antioxidant activity, and acceptance of synbiotic yoghurt with red ginger extract 
(Zingiberofficinale var. rubrum)

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Abstract Antioxidant related to oxidative stress can caused the metabolic disorders. A functional food that high in antioxidant can be use as the alternative prevention. The addition of red ginger extract in yoghurt could form a functional food, that high in antioxidant, synbiotic and fiber. The influence of red ginger extract on yoghurt synbiotic against lactic acid bacteria, antioxidant activity and acceptance were analyzed. This was an experimental research with one factor complete randomized design, specifically the addition of red ginger extract 0%; 0,1%; 0,3% and 0,5% into synbiotic yoghurt. Total plate count method used to analyze the lactic acid bacteria, 1,1-diphenyl-2-picrylhydrazyl (DPPH) method for antioxidant activity, and acceptance analyzed with hedonic test. The higher the dose of extract added to synbiotic yoghurt, the antioxidant activity got significantly increased (ρ=0,0001), while the lactic acid bacteria got insignificantly decreased (ρ=0,085). The addition of 0,5% red ginger extract obtained the antioxidant activity of 71% and 4,86 x 10 13 CFU/ml on lactic acid bacteria, which the requirement for probiotic on National Standard of Indonesia is >10⁷ CFU/ml. The addition of extract had a significant effect on acceptance (ρ=0,0001) in flavor, color, and texture, but not aroma (ρ=0,266). The optimal product in this research was the yoghurt synbiotic with addition of 0,1% red ginger extract. To summarize, the addition of red ginger extract in synbiotic yoghurt had significant effect on antioxidant activity, flavor, color, and texture, but no significant effect on lactic acid bacteria and aroma.

Keywords: yoghurt, synbiotic, red ginger, lactic acid bacteria, antioxidant activity.

1. Introduction
The National Cholesterol Education Program’s Adult Treatment Panel III (NCEP-ATP III), identified metabolic syndrome as a condition in which there are 3 or more of the risk factors such as central obesity, elevated triglycerides, decreased HDL cholesterol, elevated blood pressure, and fasting glucose.[1] Research about the prevalences of metabolic syndrome, in the elderly obtained rates of 18,2% in elderly women and 6,6% in elderly men.[2] Meanwhile, in studies with adolescent subjects, obtained the prevalence by 34% of 50 obese adolescents, aged 10-19 years old.[3] Studies on adolescents with overweight and obesity, obtained rates of 23%.[4]

Metabolic syndrome, can be treated with a regulation of diet, physical activity, and weight.[1] The administration of functional food, in form of synbiotic yoghurt is one of the example of diet regulation. Synbiotic is a combination of probiotic and prebiotic.[5] In correlation with metabolic syndrome, probiotic in form of lactic acid bacteria, can help lower the serum cholesterol levels because of the Bile
Salt Hydrolase (BSH) enzyme, which deconjugates bile salts that synthesized from cholesterol. In addition, the consumption of probiotic could help lower the blood pressure.[7]

Inulin, which is a prebiotic, known to elevate the growth and viability of lactic acid bacteria.[8] The consumption of inulin has effect on glycemic status and lipid profile, by lowering HbA1c level, fasting blood sugar, total cholesterol, triglycerides, LDL cholesterol, LDL/HDL cholesterol ratio, triglyceride/HDL cholesterol ratio and increasing HDL cholesterol.[9]

Oxidative stress has an important role in the incidence of metabolic syndrome, because the increasing of reactive oxygen species (ROS).[10] Diet high in antioxidants can help to prevent ROS. Red ginger (Zingiber officinale var. Rubrum) is high in antioxidants, with phenol content (95.34 mg/100 g) and flavonoid (53.67 mg/100 g), that significantly higher (p < 0.05) than white ginger (61.89 mg/100 g and 34.55 mg/100 g).[11] In addition, there was study about the administration of red ginger, which stated that by given 3.2 ml/kg BB of red ginger for 21 days, can lower the cholesterol levels in women with dyslipidemia as much as 8.64%.[12]

Beside the addition of red ginger extract, the diversification of functional food in form of synbiotic yoghurt, can also be done by adding carrageenan. Carrageenan is a soluble fiber, that extracted from red seaweed or Rhodophyceae, which can be used for thickening, gelling, and emulsifying.[13] Carrageenan also can reduce the level of total cholesterol and LDL, and lower the biomarker for chronic inflammation, which are leukocytes, fibrinogen, and c-reactive protein (CRP).[14] There was a study stated that administration of carrageenan in diet can improve the blood plasma lipid profile in mouse, by lowering the total cholesterol, LDL, and triglycerides, while also elevating the HDL cholesterol.[15]

The innovation of functional food in form of synbiotic yoghurt with the addition of red ginger extract (Zingiber officinale var. rubrum) and carrageenan, which is the combination of synbiotic, high in antioxidant, and fiber, is expected to be an alternative product that could treat metabolic syndrome. The total lactic acid bacteria, antioxidant activity, and acceptance in synbiotic yoghurt with the addition of red ginger extract will be analyzed.

2. Methods
This was a food production research, that took place in Laboratory Terpadu, Diponegoro University, from August until September 2016. Materials needed to make synbiotic yoghurt with the addition of red ginger extract are fresh cow’s milk from Faculty of Animal Husbandry and Agriculture, Diponegoro University, starter Lactobacillus bulgaricus and Streptococcus thermophilus from PAU Gadjah Mada University, Yogyakarta, red ginger from Pasar Bulu, Semarang, inulin from PT DPO Indonesia, carrageenan from UKM Suket Segoro, and stevia from market in Semarang.

This was an experimental research with one complete randomized design, which is the addition of red ginger extract with 3 treatment levels; 0.1%; 0.3% and 0.5% and one control group without the addition of red ginger extract. Repitition did 3 times for calculating total lactic acid bacteria and antioxidant activity, and no repetition for acceptance.

A preliminary research was conducted to determine the percentage of stevia and carrageenan added to synbiotic yoghurt, whereas the addition of inuline was based on the research of functional beverage product jelly drink yoghurt srikaya. Extraction of red ginger conducted on preliminary research, with maceration method for 36 hours using 95% ethanol as much as 125 mL. The 95% ethanol need to be replaced with another 125 ml of 95% ethanol every 12 hours during the maceration process.

The process of making synbiotic yoghurt with the addition of red ginger extract, begins with the pasteurization process of cow’s milk up to ± 70°C and keep at that temperature for 15 seconds, then cooled down to 40°C. After that, added 4% inulin, 0.2% stevia, and 10% starter Lactobacillus bulgaricus and Streptococcus thermophilus to the cow’s milk, then incubated for approximately 24 hours using an incubator at 37°C. Last, added 6% carrageenan and red ginger extract to the yoghurt.

In this study, the test used for determine the total lactic acid bacteria was Total Plate Count (TPC) method, DPPH method for antioxidant activity, and hedonic test for acceptance, on 30 panelist semi-
trained that were students from Nutrition Sciences, Diponegoro University. The normality test used was Shapiro-Wilk, because the sample size was <30. The total lactic acid bacteria used One way Anova test. Antioxidant activity used One Way Anova, followed by Duncan test. Acceptance used Friedman, followed by Wilcoxon test.

3. Results
3.1 Total Lactic Acid Bacteria (LAB)

The addition of red ginger extract in synbiotic yoghurt, had no significant effect on total lactic acid bacteria ($\rho = 0.085$). However, as the dose of red ginger extract increased, there were some decreased on total lactic acid bacteria in synbiotic yoghurt. All tables, figures and illustration should be written in the main text.

| Dose | Total LAB ($10^3$ CFU/ml)$^a$ | $\rho = 0.085$ |
|------|-------------------------------|------------------|
| 0%   | 90.37±65.02                   |                  |
| 0.1% | 81.65±23.68                   |                  |
| 0.3% | 27.19±41.35                   |                  |
| 0.5% | 4.86±6.02                     |                  |

$^a$ = One Way Anova Test

3.2 Antioxidant Activity

The higher the dose of red ginger extract added to synbiotic yoghurt, the antioxidant activity also got higher. The addition of red ginger extract had significant effect on antioxidant activity with $\rho = 0.0001$. The highest antioxidant activity was the synbiotic yoghurt with the 0.5% addition of red ginger extract.

3.3 Acceptance

Acceptance was done with hedonic test, in terms of flavor, color, aroma, and texture of synbiotic yoghurt with the addition of red ginger extract.

| Dose | Flavor | Color | Aroma | Texture |
|------|--------|-------|-------|---------|
|      | Mean   | Exp   | Mean  | Ket     | Mean  | Exp   | Mean  | Exp   |
| 0%   | 2.80±0.66$^a$ Like | 3.10±0.61$^a$ Like | 3.07±0.74 Like | 2.80±0.71$^c$ Like |
| 0.1% | 2.77±0.77$^b$ Like | 3.03±0.49$^b$ Like | 2.80±0.71 Like | 3.07±0.69$^a$ Like |
| 0.3% | 2.30±0.75$^c$ Dislike | 2.37±0.62$^d$ Dislike | 2.77±0.77 Like | 2.93±0.58$^b$ Like |
| 0.5% | 1.80±0.66$^d$ Like | 2.80±0.66$^c$ Like | 2.80±0.76 Like | 2.30±0.79$^d$ Like |

$^a, b, c, d$ = One Way Anova Test

Explanation: Numbers followed by different superscript letter (a, b, c, d) showed the real different.
3.3.1 Flavor. Synbiotic yoghurt with the addition of red giger extract had significant effect on acceptance in term of flavor ($\rho = 0.0001$). As the dose get higher, there were some decreases on panelists’ acceptance in flavor, whereas the synbiotic yoghurt with doses of red ginger extract as much as 0.3% and 0.5% were not preferred.

3.3.2 Color. The addition of red giger extract on synbiotic yoghurt had significant effect on acceptance in color ($\rho = 0.0001$). Panelists liked all the color of synbiotic yoghurt with the addition of red giger extract, except the 0.3% one.

3.3.3 Aroma. In term of aroma, there were no significant effect on synbiotic yoghurt with the addition of red giger extract ($\rho = 0.266$). Panelists liked the aroma of synbiotic yoghurt with the addition of red giger extract in every dose.

3.3.4 Texture. The most liked yoghurt in term of texture were the synbiotic yogurts with the addition of red giger extract of 0.1% and 0.5%. The addition of red giger extract had significant effect on texture of synbiotic yoghurt ($\rho = 0.0001$). Panelists liked all the texture of synbiotic yoghurt except the one with 0.5% red giger extract addition.

4. Discussion

4.1 Total Lactic Acid Bacteria

The addition of red giger extract in synbiotic yoghurt, had no effect on total lactic acid bacteria. However, there was a decrease on total lactic acid bacteria from synbiotic yoghurt, as the dose of red extract get higher. Despite the degression, the amount of lactic acid bacteria in synbiotic yoghurt with the addition of red giger extract, still fulfilled the amount of probiotic needed accoring to Standar Nasional Indonesia (SNI), in order to have benefits on health ($>10^7$ CFU/mL) The degression of total lactic acid bacteria, might happened because of the antibacterial activity, possessed by ginger. The antibacterial activity, derived from the chemical components in ginger, which are sesquiterpenoid with zingiberene as its main component. There are also $\beta$-sesquiphellandrene, bisabolene, and farnesene, which are still part of sesquiterpenoid.[17]

The decrease in total lactic acid bacteria, as the dose of red giger extract get higher, is compatible with another study about the addition of ginger juices in various levels into yoghurt, which proved that as the dose of ginger juice added to yoghurt got higher, the growth of S. thermophilus and L. bulgaricus would be more inhibited.[18] In other fermented product with addition of ginger extract, there was also a decrease on total lactic acid bacteria.[19]

The strength level of antibacterial activity in ginger, also influenced by the treatment process on ginger. In this study, the extraction process of ginger used maceration method with 95% ethanol as the solvent. Extraction with ethanol as the solvent, will provide a stronger antibacterial activity, compared to the extraction with water as the solvent.[17] Before the maceration process, red gingers used in this study were sun-dried. There were no significant difference between using fresh ginger or dried ginger, in related to total lactic acid bacteria. The significant difference or decrease in total lactic acid bacteria, more related to the dose of ginger extract added to product.[20]

4.2 Antioxidant Activity

The addition of red giger extract had a significant effect on antioxidant activity in synbiotic yoghurt ($\rho = 0.0001$). The higher the dose of red giger extract, the higher the antioxidant activity would be. The increasing of antioxidant activity could happen because ginger has some main components, which are 6-gingerol, 6-shogaol, and 10-gingerol, that have strong antioxidant activities.[21]

In the preliminary study, it is known that the red giger extract had a very high antioxidant activity, which was 92.5%. It was compatible with another study about the high antioxidant activity in ginger, which was 90.1%, whereas the ginger is dried and also extracted with ethanol as the solvent.[22],[23] Eventhough the value of antioxidant activity in red giger extract was pretty high, when it added to the synbiotic yoghurt, the antioxidant activity value got decreased compared to the raw ginger extract.
4.3 Texture. α, with sesquiterpenoids synbiotic yoghurt (yoghurt. Red ginger has its own signature aroma that comes from volatile oil, which contained a lot of all kinds of doses. There were no significant differences of aroma in every dose added to synbiotic yoghurt. In this study, combination of bioactive components in synbiotic yoghurt with the addition of red ginger extract, caused antagonistic effect, indicated by the decrease in antioxidant activity.[29]

The higher the dose of red ginger extract added to synbiotic yoghurt, the antioxidant activity got increased, but compared to the antioxidant activity in raw ginger extract, it got decreased. The result was compatible with one study about jelly drink product with the addition of ginger essence. There was an increase in antioxidant activity of jelly drink, as the concentration of ginger essence got higher, but compared to the antioxidant activity value in raw ginger essence, it got decrease.[30]

4.3 Acceptance
4.3.1 Flavor. As the dose of red ginger extract got increased, the acceptance level in term of flavor got decreased. Panelists described the flavor of synbiotic yoghurt with the addition of red ginger extract as spicy and strong. The flavor of synbiotic yoghurt became bitter with the addition of 0.3% and 0.5% red ginger extract, that might caused the decrease in acceptance of flavor.

Synbiotic yoghurt has a sour taste, that happened because fermentation process of lactose that becomes acid by starter Lactobacillus bulgaricus and Streptococcus thermophilus, that causes the sour taste in yoghurt.[31] The spicy and strong flavor in synbiotic yoghurt with the addition of red ginger extract, came from the components named gingerol and shogaol in ginger.[32] Gingerol is the main component in ginger that caused the spicy flavor, because of the 6-dehydrogingerdione, the liquid oil that contained in gingerol.[33]

The red gingers that used in this study were sun-dried. The drying process can bring out the spicy flavor, that comes from shogaol, which is the nonvolatile phenylpropanoid-derived component from gingerol. Beside those two components, there also a component named zingerol which produced by gingerol, that brings out the slightly spice taste.[33]

For that reasons, the higher the dose of red ginger extract added to synbiotic yoghurt, the flavor of synbiotic yoghurt would become more spicy and bitter, that might lead to decrease in acceptance level of flavor.

4.3.2 Color. The addition of red ginger extract had significant effect on the color of synbiotic yoghurt. The color of synbiotic yoghurt with the addition of red ginger extract was pale yellow, and the more of extract added to synbiotic yoghurt, the color would become more yellow-ish. The yellow color came from the pigment in ginger.[18]

There are 3 main components of pigment that bring out the yellow in ginger, which are 6-dehydrogingerdione, curcumin, and demethoxycurcumin, with 6-dehydrogingerdioneas the main pigment, that becomes the base of pale yellow color in ginger. 6-dehydrogingerdione oxidized from 6-gingerol, which is the main component in ginger. The more of 6-gingerol contained in an extract, the more of 6-dehydrogingerdione will be, so the color will become more popped.[34]

In term of color, the product that got dislike from panelists was the synbiotic yoghurt with the the addition of 0.3% red ginger extract. That might happened because the red ginger extract, in form of liquid gel, not mixed throughly into synbiotic yoghurt, so the color didn’t perfectly showed.

4.3.3 Aroma. The addition of red ginger extract had no correlation with the acceptance level of aroma in synbiotic yoghurt (p = 0.266). Panelists like the synbiotic yoghurt added with red ginger extract, in all kind of doses. There were no significant differences of aroma in every dose added to synbiotic yoghurt. Red ginger has its own signature aroma that comes from volatile oil, which contained a lot of sesquiterpenoids, with α-zingibereneas its main component.[35]

4.3.4 Texture. The acceptance level of texture, influenced by the addition of red ginger extract into synbiotic yoghurt (p = 0.0001). In term of texture, the only product that got dislike from panelists was

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the synbiotic yoghurt with 0.5% addition of red ginger extract. It might happened because the higher the dose of extract added to synbiotic yoghurt, the water content would increase that might affect the consistency of synbiotic yoghurt. This result was compatible with another study of organoleptic in yoghurt with the addition of ginger juice, whereas the panelists’ acceptance of texture got decreased along with the increased of the dose of ginger juice added into synbiotic yoghurt.[18]

5. Conclusion (please add more fact in conclusion part)
The addition of red ginger extract in synbiotic yoghurt, had no effect on lactic acid bacteria and aroma, but had effects on antioxidant activity, and acceptance in flavor, color, and texture. The optimal product from this research was the synbiotic yoghurt with the addition of 0.1% red ginger extract.

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