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FULL PAPER

Re-formulating *Monascus* fermented durian seeds yogurt with strawberry (*Fragaria x ananassa*) puree to enhance its microbiological, physicochemical and organoleptic properties

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Abstract

Healthy lifestyle and consumption of fermented products, is the trend that is popular among health-conscious individuals. *Monascus* fermented durian seed (MFDS) is a fermented product of durian seed using the *Monascus purpureus* culture. Even though MFDS possessed specific bioactive properties, such as competent antioxidant, anti-diabetes and anti-hypercholesterolemia. However, the addition of MFDS extract to yogurt has some limitations [e.g.: reduction of colour and taste preference]. Thus, there is a need for an innovative food technology method to enhance key organoleptic characteristics of a newly formulated yogurt product made of MFDS and strawberry puree. The aim of this investigation was to determine the effect of difference concentrations of strawberry puree on microbiology, physicochemical, and organoleptic properties of MFDS yogurt product. The results showed that all concentrations of strawberry puree [5%, 10%, 15%, and 20% (w/v) of the total mixture] have affected the microbial, physicochemical and sensory properties of re-formulated yogurt product. Strawberry purees caused an acidity increase of yogurt with pH value between 4.287 and 4.475. The recorded titratable acidity was 0.74% to 1.17%. The colour parameters such as lightness and yellowness also decreased, however, the values of redness, chroma, and hue increased. The shelf-life experiment (maximum of 7 days) of the re-formulated yogurt revealed a maximum syneresis of 22.52%. Based on the sensory evaluation preferences, re-formulated yogurt with 10% strawberry puree was the most favourable product, with a preferred value of the colour at 5.6 (rather like), the flavour at 5.8 (rather like), and mouthfeel (rather like).

1. Introduction

Yogurt is a fermented milk product that involves Lactic acid bacteria (LAB). Yogurt is coined as a healthy food product that is not just highly nutritive but also contains lactic acid bacteria as its bioactive component. Red yeast rice is a solid-state fermented product that is produced by the fungus *Monascus purpureus* (Arunachalam and Narmadhapriya, 2011). Some Asian countries like Indonesia, red yeast rice is used as a natural food colouring, preservatives as well as functional foods. Red yeast rice is generally produced using rice as a medium for growth through solid state fermentation, based on research conducted by Srianta *et al*. (2012), durian seeds have the potential as a suitable medium for red yeast rice fermentation. According to Nugerahani *et al*. (2017) the bioactive properties contained in *Monascus* fermented durian seeds (MFDS) are antioxidant, anti-diabetes and anti-hypercholesterolemia properties.

Many yogurt products were further developed by adding other food ingredients that have positive health impacts. One form of such re-formulation at this time was to combine the yogurt with other fermented products such as red yeast rice, to improve the appearance of colour and functional properties of yogurt. According to Romulo *et al*. (2017), yogurt with the addition of red yeast rice extract did not inhibit the growth of lactic acid bacteria involved in yogurt fermentation but the addition of red yeast rice affected the level of preference for yogurt, especially because of its dark red colour and bitter taste. Hence the addition of fruit (such as

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strawberries) would be an alternative to cover these deficiencies by enhancing the taste, colour, and texture. The addition of strawberry can also increase lactic acid bacteria activity, which will have an impact on changes in yogurt acidity (pH and total lactic acid). Such increase is due to the phytochemical content of the fruit mainly the phenolic compounds, flavonoids, vitamins, dietary fibre, and simple sugars (Ozcan *et al*., 2013; Vijaya Kumar *et al*., 2015).

Therefore, this study aimed to evaluate the effect of different concentrations of strawberry puree on microbiological, physicochemical, and organoleptic properties of MFDS yogurt product. The employed concentration of MFDS was 7.5% (v/v) and the varied concentrations of strawberry puree were 0%, 5%, 10%, 15%, and 20% (w/v) of the total volume of UHT milk used.

2. Materials and methods

2.1 Materials

Full cream ultra-high temperature milk, strawberry (*Fragaria x ananassa*), skim milk powder, refined sugar was obtained from local supermarkets in Surabaya, Indonesia. Commercial freeze-dried yogurt starter was used (Yogourmet, Lyo-San Inc., Lachute, Québec, Canada) containing *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, and *Lactobacillus acidophilus*. MFDS powder was prepared from solid state fermentation of durian seed (*Durio Zibethinus* cultivar Petruk) with *Monascus purpureus M9* starter.

2.2 Monascus purpureus M9 culture preparation

Pure culture of *Monascus purpureus* M9 was routinely cultured every 14 days on Potato Dextrose Agar (PDA) (Merck) slant, then incubated at 30^{o}C for 7 days and preserved at 4^{o}C to create stock cultures. The starter culture for making MFDS was prepared by inoculating 8 loops of the culture scrabbed from the PDA slant (culture stock) to the 150 mL Potato Dextrose Broth (PDB), then incubated at 30^{o}C for 10 days. Finally, the starter culture was used for the durian seed fermentation.

2.3 Monascus fermented durian seed powder production

MFDS powder was produced according to Puspitadewi *et al*. (2016) with some modifications. A durian seeds (*Durio zibethinus* cultivar Petruk) were obtained from a durian fruit processing unit in Surabaya. Durian seeds were boiled in 5% (w/v) of $Ca(OH)_2$ solution at 90^{o}C for 10 mins to remove the mucus. After that, the seed coat was peeled off and the seeds were cut into smaller sizes (1 cm^3). Approximately 50 g of small size durian seeds were transferred into a 250 mL Erlenmeyer, and sterilized at 121^{o}C for 15 mins. The sterile durian seeds were left to cool down at room temperature before being inoculated with 5% (v/w) *Monascus purpureus* M9 culture. Subsequently The Erlenmeyer's of durian seeds were incubated at 30^{o}C for 14 days with manual shaking daily. MFDS was dried in an oven at 45^{o}C for 24 hrs and finally grounded into a fine powder.

2.4 Monascus fermented durian seed extract production

MFDS extract was produced according to Puspitadewi *et al*. (2016) with some modifications. MFDS powder was dissolved in sterile water at a ratio 1:50. Subsequently the mixture was homogenized in a shaking water bath at 40^{o}C, 100 rpm for 1 hr. The extract was then filtered using vacuum pump and the filtrate was pasteurized at 70^{o}C for 30 mins. Finally, the MFDS extract was stored in refrigerator at 4±1°C before usage.

2.5 Strawberry puree preparation

Ripe strawberry was washed, then cut in small pieces before pureed by blender for 1.5 mins. Strawberry puree was pasteurized at 72^{o}C for 15 mins, stored in a freezer at -18°C and was thawed before usage.

2.6 Production of Monascus fermented durian seed yogurt

Yogurt was produced according to Pimentel *et al*. (2017) with some modifications. A mixture consists of full cream UHT milk, 10% (w/v) refined sugar, and 2% (w/v) skimmed milk powder were pasteurized at 90±1^{o}C for 5 mins. The mixture's temperature was then lowered to 60±1^{o}C and 0.5% (w/v) gelatine powder and 7.5% (v/v) MFDS extract were added. Subsequently, the milk mixture was then divided into five containers according to the formulation and when the mixture's temperature reached 43±1^{o}C, each mixture was inoculated with 0.5% (b/v) commercial culture starter and strawberry puree while 5 concentration levels were added (0%, 5%, 10%, 15%, 20%) (w/v). The mixture was stirred until all components are evenly mixed. Subsequently, the yogurt mixture was poured into sterile plastic cups and allowed to ferment at 43±2°C for 6 hrs. Finally, each MFDS Yogurt was then stored in a refrigerator at 4±1°C for 16 hrs and it is assumed as day 0.

2.7 Analysis method

2.7.1 Total lactic acid bacteria

The total lactic acid bacteria assay was performed using MRS (De man, Rogosa, and Sharpe) agar through the pour plate method. The 0.5 mL sample was mixed with 4.5 mL of sterile 0.1% peptone water solution and

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homogenized, to make a dilution of 10^{-1}. Then, the same dilution method was carried out until a dilution of 10^{-10} has been achieved. Samples of 1 mL were taken from 10^{-5} until 10^{-10} dilution series and placed onto sterile Petri dishes, followed by addition of the MRS agar (50°C). Homogenization was done by shifting the Petri dish to form a figure eight. The media was left to solidify. Finally, the Petri dish was incubated at 37°C for 48 hrs. The bacteria colonies were counted with a colony counter based on the appearance of clear zones with round shape white colonies (Wijaya *et al*., 2012).

2.7.2 Physical analysis

The pH analysis was performed by using SI Analytics Lab 855 pH meter. The pH meter was calibrated with standard buffer solution of pH 4.0 and pH 9.0. The electrode was then rinsed with distilled water and dried. A sample of 5 mL was diluted to 50 mL with distilled water then homogenized. The electrode was immersed into the sample until stable readings were obtained (Dhineskumar and Ramasamy, 2016). pH analysis was performed in triplicates.

Total acids analysis was determined by acid-base titration method. Ten millilitre of samples was diluted to 100 mL with distilled water. Ten millilitres of the diluted sample was poured into an Erlenmeyer and three drops of 1% phenolphthalein indicator were added. The sample was then titrated with a standardized 0.1 N NaOH solution, giving it a stable pink colour (Widagdha and Nisa, 2015). Titratable acidity analysis was performed in triplicates. Total acids were calculated with the formula:

$$\frac{Vol.NaOH \times N\,NaOH \times 90 \times dilution\ factor}{Vol.sample \times 1000} \times 100\%$$

Syneresis in MFDS yogurt with the addition of strawberry puree was determined by the whey separation method. The sample was weighed first to determine the initial weight. Then the whey that comes out of the sample through a pipette was removed, final weight of the sample was weighed (Wijaya *et al*., 2012). This test was carried out with two replications and was carried out on days 0 and 7. Syneresis was calculated with the following formula:

$$\frac{Initial\ weight - Final\ weight}{sample\ weight} \times 100\%$$

Colour analysis sample was carried out using CR-10 Konika Minolta Color Reader. The sample was prepared inside the transparent 25 mL plastic cup (D= 5.5 cm) and measured with colour reader. The results were expressed as L (lightness; 0 = black, 100 = white), a* (-a = greenness, +a = redness), b* (-b = blueness, +b = yellowness), C (chroma; 1-100), and °h (hue; 0° - 360°) (XRite, 2015). The test was conducted in triplicates.

2.7.3 Sensory evaluation

Sensory evaluation of all yogurt samples were carried out by the hedonic test (preferred test). This test was performed by 50 untrained panellists to assess the panellists' preference for colour, taste and mouthfeel for all yogurt samples. Each sample was filled inside 25 mL plastic cup and encoded with three different numbers. Panellists were then asked to fill out the questionnaire using a seven-point hedonic scale, i.e., 1 = dislike very much, 2 = rather dislike, 3 = dislike slightly, 4 = neutral, 5 = rather like, 6 = like moderately, 7 = like very much.

2.7.4 Statistical analysis

The experiments data were processed with IBM SPSS Statistics 19 (IBM, Armonk, NY, USA). This study was conducted with five replications and analysed by Analysis of Variance (ANOVA), in randomized block design (RBD) with the confidence level of 95%. If significant differences were found, Duncan's Multiple Range Test (DMRT) would be carried out at 5% significance level. P-value less than 0.05 ($P < 0.05$) was accepted as statistically significant.

3. Results and discussion

3.1 Total lactic acid bacteria and pH

MFDS yogurt with different strawberry puree concentrations had a significant effect on total LAB ($P<0.05$). The total LAB in MFDS yogurt with different concentrations of strawberry puree ranged from Log 9.1480 to 10.1878 CFU/mL (Table 1). Overall, all treatments with different concentrations of strawberry puree were included in the category of yogurt quality

Table 1. pH, Total lactic acid, total LAB, and syneresis of MFDS yogurt combined with strawberry puree at different concentrations

Strawberry Puree Concentration	pH	Total Acids (%)	Total LAB (log CFU/mL)	Syneresis (%)	
				Day 0	Day 7
0%	4.475±0.013^{c}	0.74±0.0488^{a}	9.1480±0.181^{a}	0.27±0.144^{a}	1.12±0.043^{a}
5%	4.462±0.006^{c}	0.83±0.0293^{b}	9.4346±0.311^{ab}	1.59±0.105^{b}	1.76±0.036^{ab}
10%	4.449±0.005^{c}	0.97±0.0174^{c}	9.5167±0.676^{abc}	1.71±0.029^{b}	2.37±0.257^{b}
15%	4.384±0.005^{b}	1.05±0.0150^{d}	9.8584±0.723^{bc}	16.45±0.276^{c}	18.73±0.797^{c}
20%	4.287±0.017^{a}	1.17±0.0700^{e}	10.1878±0.256^{c}	19.76±0.703^{d}	22.52±1.424^{d}

Values are presented as mean±SD. Values with different superscripts within the same column are significantly different ($p<0.05$).

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requirements, namely there is a minimum total LAB log of 7 CFU/mL. According to Chandan and O'Rell (2006), LAB can utilize simple sugars from fruit such as glucose and fructose as a carbon source. Glucose, fructose, and sucrose are found in strawberry. In addition to simple sugars, LAB growth is also affected by several micronutrient components such as vitamins, minerals, and dietary fibre. Pantothenic acid (Vitamin B5), riboflavin (Vitamin B2), niacin (Vitamin B3) are a group of vitamins essential for the growth of most LAB (Foucaud *et al*., 1997; Letort and Julliard, 2001; Wegkamp *et al*., 2010). Increased concentrations of strawberry puree, in addition to providing additional substrate for LAB can also have a negative impact due to the accumulation of organic acids derived from the metabolism of LAB or after the addition of strawberry puree. Homofermentative LAB produces metabolites in the form of lactic acid as the main product (90%), as well as other metabolites such as CO_2 and formic acid in small amounts (Pimentel *et al*., 2017). Citric acid is the dominant organic acid found in strawberry, which is around 88% of the total organic acids in ripe strawberry, while malic acid is the second most abundant acid component after citric acid (Asao and Asaduzzaman, 2019). The pH value of the MFDS yogurt ranged from 4.475 to 4.287 (Table 1). The pH of MFDS yogurt control was not significantly different from the pH of MFDS yogurt with added 5% and 10% strawberry puree. This could be due to the buffering capacity of organic acids and phenolic compounds found in strawberry puree. According to Jeong *et al*. (2018), organic acids and phenolic compounds can provide buffering capacity in yogurt. The pH values in the 15% and 20% treatments were significantly different due to the buffering capacity of the yogurt which was unable to withstand a decrease in pH.

3.2 Total acid

Homofermentative LAB utilizes a substrate in the form of simple sugars such as lactose in milk or glucose to be converted to lactic acid as its main metabolite product. The addition of strawberry puree can cause an increase in the total acid of MFDS yogurt significantly ($P<0.05$). Table 1 shows that yogurt has a total acid range of 0.50-2.00%. The increase in total acid besides the presence of lactic acid can also be due to the presence of organic acids from strawberry puree which is counted as total lactic acid. This can be explained on the basis that strawberry fruit has a dominant acid in the form of citric acid, therefore, strawberry puree increases the amount of initial organic acid in yogurt.

3.3 Syneresis

Syneresis is a shrinkage event in the gel, which causes the separation of whey (Lucey, 2004). The results showed that the higher concentration of strawberry puree added, the syneresis in yogurt increase, and increase during 7 days of storage (Table 1). Higher concentrations of strawberry puree added causes an increase in syneresis in yogurt because the protein content in milk which is the main component in the formation of curd is less. A lower pH than the isoelectric pH of casein and gelatine can affect the occurrence of syneresis in yogurt. In addition, in this study, MFDS was added in the form of an extract so that it will reduce the total soluble solids from the milk which can affect the syneresis in MFDS yogurt. Increased syneresis in yogurt during storage was caused by the rearrangement of casein in the gel network, when the arrangement occurs, the casein particles which are the constituents of the gel network are deformed and form new bonds, causes syneresis (Lucey, 2002; Serra *et al*., 2009).

3.4 Colour properties

Colour is one of the parameters that can affect consumer acceptance of a food product. An increase in the addition of strawberry puree, increases the redness (a*) value, while the lightness (L) and yellowness (b*) values decreased (Table 2). The decrease in the L value was due to the anthocyanin pigment found in strawberry puree, thereby lowering the brightness level (Andarwulan and Faradila, 2012). The intensity of the yellow colour decreased with the increasing concentration of strawberry puree. The high b* value in the control could be due to the presence of casein, fat globules, and calcium phosphate in milk in colloidal form. The yellow colour of the control yogurt can also be influenced by the presence of colour pigments in the durian seeds. According to Nugerahani *et al*. (2017) *Monascus* fermented durian seed contain yellow, orange

Table 2. Evaluation of the colour properties of MFDS yogurt combined with strawberry puree at different concentrations

Strawberry Puree Concentration	L	a*	b*	C	Hue(^{o})
0%	90.4±0.293^a	0.7±0.117^a	11.2±0.040^a	11.2±0.049^a	86.2±0.563^a
5%	85.1±0.896^a	5.4±0.194^b	8.9±0.133^b	17.3±0.110^b	65.2±0.548^b
10%	77.7±0.661^b	12.7±0.206^c	8.6±0.160^c	18.2±0.040^c	53.0±0.325^c
15%	75.1±0.840^c	18.0±0.643^d	8.3±0.242^c	18.6±0.00^d	43.4±0.190^d
20%	74.4±0.864^d	19.0±0.233^e	8.0±0.354^d	19.0±0.136^e	38.0±0.270^e

Values are presented as mean±SD. Values with different superscripts within the same column are significantly different ($p<0.05$).

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and red pigments. The more the addition of strawberry puree, the lower the b* value because the anthocyanin pigment is dominant in the strawberry puree which gives it a red colour. The addition of more and more strawberry puree also causes the chroma value to increase because the red colour of the strawberry puree can fade the intensity of the yellowish white colour of the milk. The MFDS strawberry yogurt has a °H value of 38.0 - 65.2 so it is included in the red purple category.

3.5 Sensory evaluation

The mean scores for the sensory evaluation of MFDS yogurt were presented in Table 3. The addition of strawberry puree significantly ($p<0.05$) affected all sensory parameters (colour, taste, and mouthfeel). The preference score of MFDS yogurt colour and taste was increasing as the strawberry puree concentrations increase. It happens because the increasing concentration of strawberry puree causes the colour of the MFDS yogurt to be redder, so it is more attractive. While the MFDS yogurt without the addition of strawberry puree has a pale colour. The red and attractive colour of strawberries comes from the anthocyanin pigment. Hence, the addition of strawberry puree can increase the value of preference for taste because in strawberry puree can cover both the taste and after taste of the MFDS. Bitter taste and astringency in MFDS extract are caused by the presence of phenolic compounds (Jackson, 2000; Shahidi and Naczk, 2004). The preference score of mouthfeels was declining as the strawberry puree concentrations increase. The origin of this phenomenon is referring to the presence of dietary fiber in strawberry. In addition, the higher the concentration of strawberry puree, the proportion of milk used is less so that it can affect the mouthfeel of MFDS yogurt. Nonetheless, the presence of fat in milk can give a soft mouthfeel.

Table 3. Sensory evaluation of MFDS yogurt combined with strawberry puree at different concentrations

Strawberry Puree Concentration	Taste	Colour	Mouthfeel
0%	3.54±0.820[a]	4.50±0.265[a]	6.88±0.445[d]
5%	5.10±0.735[b]	5.30±0.187[b]	6.56±0.415[d]
10%	5.80±0.485[c]	5.60±0.316[c]	5.56±0.321[c]
15%	5.92±0.295[d]	5.96±0.114[d]	2.32±0.219[b]
20%	6.48±0.415[e]	6.84±0.152[e]	1.76±0.130[a]

Values are presented as mean±SD. Values with different superscripts within the same column are significantly different ($p<0.05$).

4. Conclusion

The addition of strawberry puree on MFDS yogurt significantly affected on the microbiological properties counts, physical properties (pH, total acids, syneresis, and colour), and sensory properties (preference of colour, taste, and mouthfeel). This study highlighted that the best treatment of MSDF yogurt is the addition 10% of strawberry puree.

Conflict of interest

The authors declare no conflict of interest.

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