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Research Article

Application of Angkak (Red Yeast Rice) Extract as Natural Red Colorant in Making of Low Fat Fruity Probiotic Yoghurt

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Abstract

Angkak or red fermented rice is a product produced from the fermentation of rice substrates by *Monascus* spp. For centuries, angkak has been consumed extensively in Asia as a natural food coloring of fish, Chinese cheese, red wine and sausages. Angkak has been used as dietary supplement because of its chemical compound that give advantages to human's health. Despite many benefits of angkak as a pigment, there are still limited direct application in food products processed for human consumption such as yoghurt. Based on this reason, angkak extract was formulated to make low fat fruity yoghurt which was acceptable to consumers preferences. The results showed that until 30% concentration of angkak extract, there is no effect to the growth of lactic acid bacteria. The most favourite of low fat fruity yoghurt is yoghurt with 2.5% concentration of angkak extract and 20% of added fresh fruit. The chemical analysis results show that low fat fruity yoghurt contains 4.07 pH, 14.9 oBrix total soluble solid content and 1.26% titratable acid. The physical analysis result show that low fat fruity yogurt has 2500 mPas viscosity. The proximate analysis results show that low fat fruity yoghurt contains 85.59% water, 0.73% ash, 3.12% protein, 0.11% fat, and 10.45% carbohydrates. The microbial analysis results show that low fat fruity yoghurt contains 9.65 log CFU/ml total lactic acid bacteria and < 0.47 log MPN/ml. This study showed that most of the analyses carried out on low fat fruity yogurt are within the standard recommendation.

Keywords: *Angkak; Fruity; Yoghurt; Functional Food; Low Fat; Monascus spp*

Introduction

Angkak (red yeast rice) is a fermentation product mainly produced from *Monascus purpureus* [1]. They produced heat-stable pigments during cultivation [2] and can be used in a wide pH range. During fermentation process, this yeast synthesized pigments as primary metabolite known as monascin and ankaflavin (yellow), monascorubin and rubropunctatin (orange), and monascorubramine and rubropunctamine (red) which reported to have antimicrobial activity against pathogen [3]. It also produced secondary metabolite called monacolin K (lovastatin) that can inhibit cholesterol biosynthesis in patients with hypercholesterolemia [4]. It also posses as anti-cancer, anti-inflammatory, and anti-diabetic [5]. For centuries angkak has been consumed extensively in Asia as a natural food coloring of fish, Chinese cheese, red wine and sausages [6].

Coloring substances have been used to enhance the appeal of foods since the beginning of recorded history [7]. Food synthetic colorants (the most interesting group of food additives) used in the food industry is a common practice because the color of a product is important for its attractiveness to the consumer [8]. However, their use range and dosage are restricted strictly around the world as it is reported that some synthethic colorants from azo dyes lead to detriment the health of children [9], even the worse case, development of

cancer [10]. At present there is awareness among consumers about the health effect of synthetic food dyes in food system. Incited by this increasing demands of consumers, food industry try to empower themselves in order to make a final product which are not only delightful, nutritive, attractive, and high sensorial quality products but also safe to be consumed such as the use of more natural ingredients (natural pigment for coloring foods) in the foodstuffs formulation [11].

Despite many benefits of angkak as a pigment, there are still limited direct application in food products processed for human consumption such as yoghurt. Based on this reason, angkak extract was formulated to make low fat fruity yoghurt acceptable to consumers preferences. This study also determines the effect of angkak extract to lactic acid bacteria and nutrition facts of low fat fruity yoghurt.

Materials and Methods

Preparation of angkak extract

Angkak were finely ground into powder using an electric mill. The powder then was extracted using water as a solvent with powder and water ratio 1: 10 (w/v) and placed in a rotary shaker at 300 rpm for 1 hour. The extract was filtered using a filter cloth to obtained the filtrate [12]. Then the filtrate were being used for reconstituting skim milk powder.

Antimicrobial activity of angkak extract

Antimicrobial activity of angkak extract was performed using agar well diffusion method. The extract for this testing was filter sterilized using Millipore with pore size 0.2 µm. On a petri dish filled with MRSA which was inoculated with lactic acid bacteria culture for making yoghurt, the holes were made in five different location and each hole was filled with angkak extract on different concentrations: 0, 5, 10, 20 and 30% (w/v). The plate was then incubated for 24h at 37°C. Antibacterial activity was determined through the zone of bacterial growth inhibition around the disk.

Culture preparation

Three bacterial for yoghurt making (*Streptococcus thermophilus*, *Lactobacillus bulgaricus*, and *Bifidobacterium bifidum*) obtained from laboratory of food microbiology SEAFAST Center, Bogor Agricultural University. Microorganism cultures were maintained in de Man Rogosa Sharpe Agar (MRSA) and deMan Rogosa Sharpe Broth (MRSB) at 37°C until use. For stock culture, about 5% pure culture was added to 10% skim milk and incubated at 37°C for 24h. For starter culture, 5% of stock culture were taken and putted to 12% skim milk and incubated at 37°C for 24h.

Yoghurt preparation

SNF (solids nonfat) of milk was first set to 12% (w/v) with skim milk powder, reconstituted with angkak extract added 2% of maize powder and then heated on hotplate (with magnetic stirrer) at 90°C till dissolve well. Then milk was cooled to 43°C and inoculated with 5% of each starter culture (ratio culture 1:1:1), then place on a 50 ml sterile cup. The cup was incubated at 37°C for 24h.

Sensory evaluation

A preliminary trial on angkak extract concentration (1.0, 2.5, 5.0, 7.5, 8.0, 9.0, 10%) was conducted in order to determine the formulation of angkak extract to skim milk powder. The three concentration (2.5, 5.0 and 7.5%) were chosen and undergone sensory evaluation. The best acceptance yoghurt were again formulated with different amount of strawberry sliced (10% to 30%) and the sensory evaluation was undergone again to know the impact of fruit addition to consumer preferences. The sensory evaluation of yoghurts was performed by consumer acceptance test according to American Standard Testing Method [12]. Around 70 panelist evaluated the yoghurt based on the color, aroma, taste, texture and overall impression of the product, using a 7-point hedonic scale (1-dislike very much; 2-dislike moderately; 3-dislike slightly; 4-Neither like nor dislike; 5-like slightly; 6-like moderately; 7-liked very much).

Physicochemical and microbial analysis of yoghurt

pH of the yoghurt samples was measured using a pH meter (Thermo Electron Corp. USA), total soluble solid using handheld optical refractometer (Atago), titratable acidity, proximate analyses (moisture, ash, fat (soxhlet method), protein (Kjedahl method) were performed according AOAC method [13] and total carbohydrate content was measure using by difference method. For microbial analysis, total lactic acid bacteria in yoghurt was measured using pour plate method and total coliform as indicator of hygiene was measured using Most Probable Number (MPN) method [14].

Statistical Analysis

The results of proximate composition analysis, physicochemical and microbiological analyses of yoghurt samples were obtained from two independent experiment in duplicate. Data from sensory evaluation were analyzed using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). For rating hedonic test, Duncan's Multiple Range test (DMRT) was applied for mean comparison when one-way analysis of variance (ANOVA) showed significant differences at the 95% confidence level.

Results and Discussion

As it is reported that angkak extract has antimicrobial effect, this research performed the antimicrobial test to determine if angkak extract inhibit the growth of lactic acid bacteria.

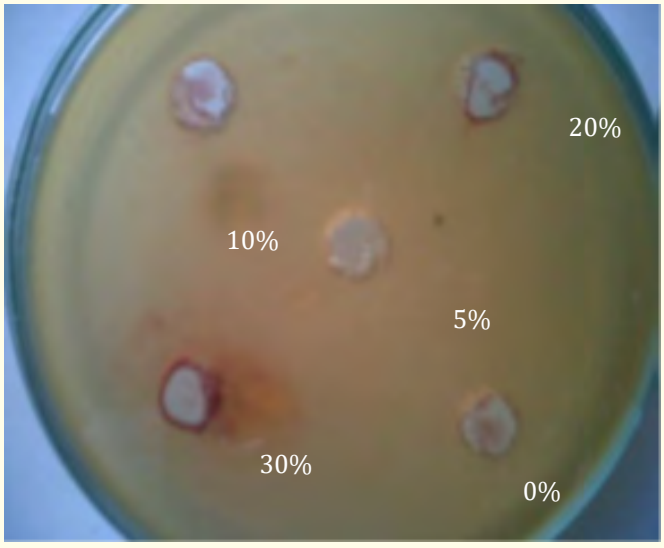
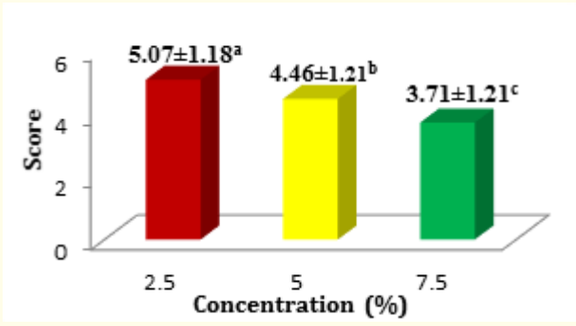


Figure 1: *Antimicrobial activity of angkak extract.*

The result (Figure 1) showed that angkak extract didn't showed any inhibition activity, no inhibition zone were formed around each hole. The extracts didn't exhibit inhibition against lactic acid bacteria used for yoghurt preparation. So far, no previous report on antimicrobial activity of angkak extract to lactic acid bacteria used for yoghurt making. Only one research reported *Monascus* extract didn't affect the growth of *Lactobacillus* in meat products [15]. From preliminary trial, three remaining concentration (2.5, 5.0, and 7.5%) of angkak extract were selected and analyzed using sensory evaluation to determine the preferable concentration.

Figure 2: *Yoghurt with different angkak concentration.*

From sensory evaluation, there were no difference between samples for texture and aroma. However there were significant difference between samples for color, taste and overall. From the Duncan test (Figure 3), yoghurt with 7.5% extract (score 3.7) was slightly disliked because it made the yoghurt appearance too dark. Panelist mentioned that the redness was uncommon to be found in product like yoghurt. Yoghurt with 2.5% extract was slightly liked by panelist (score 5.1). This showed that color affected the preference of yoghurt.



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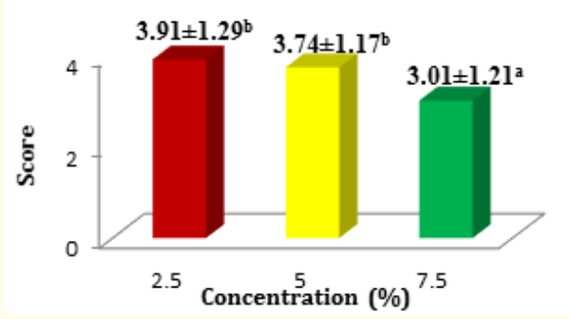

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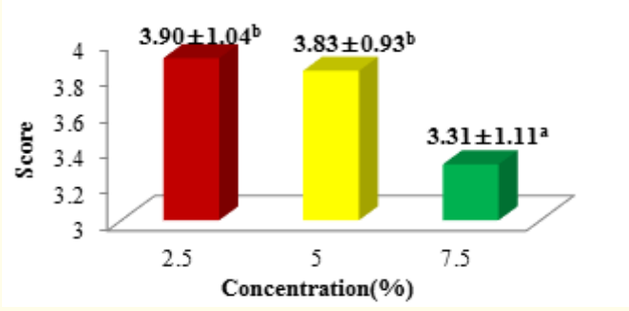

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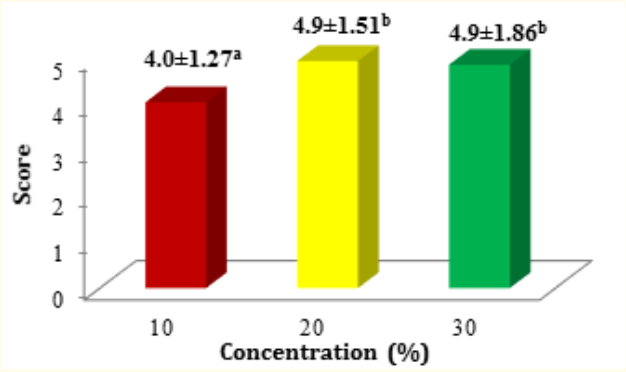
Figure 3: *Result of rating hedonic of angkak extract to (a) color, (b) taste, and (c) overall (n=70). Mean values with different superscript significantly different ($p < 0.05$).*



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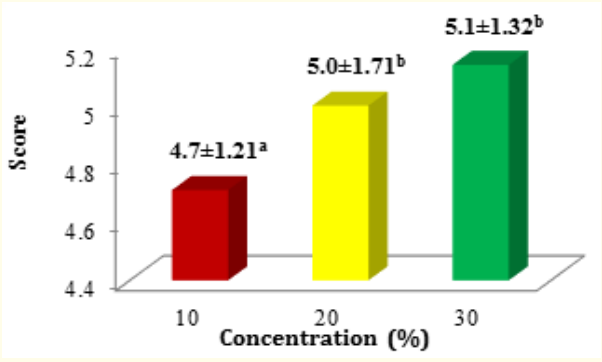

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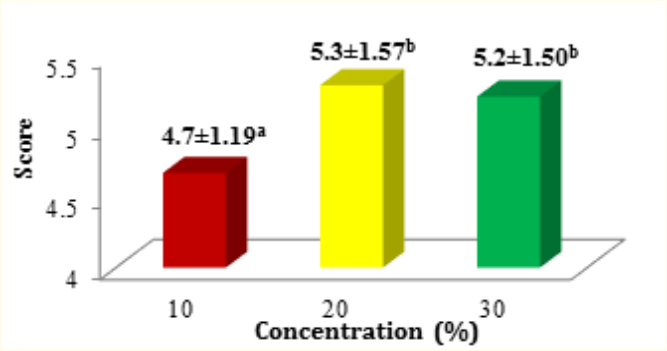

(c)

Figure 4: *Result of rating hedonic of strawberry addition (a) taste, (b) texture, and (c) overall (n=70). Mean values with different superscript are significantly different (p < 0.05).*

Color was the first attribute which determine whether a product is accepted or rejected [16]. The taste of yoghurt were difference between 2.5%, 5% with 7.5% where the yoghurt with 7.5% was slightly disliked (score 3.0) and significantly difference to 2.5 and 5%. Overall acceptance, there were significantly difference between samples. Yoghurt with 7.5% got the lowest score (3.0). Even though yoghurt with 2,5% and 5% concentration gave better score and significantly difference to yoghurt with 7.5% concentration, the acceptance of yoghurt was still low probably caused by the bitter taste from the extract.

The result showed that yoghurt with 2.5% of angkak concentration was the most preferable concentration. However, the score obtained was still low from the acceptance threshold. In order to increase the acceptance, the yoghurt was added with strawberry coated with sugar. Not only intended to reduce the acidity of yoghurt [16], the addition of strawberry also intended for masking the bitter taste from angkak. The strawberry with different concentration (10, 20, and 30%), which was already blanched, were aseptically putted to yoghurt. From sensory evaluation result (Figure 4), addition of 20% and 30% of strawberry increase the taste, texture, and overall acceptance of yoghurt. No significance difference between samples for color and aroma of the yoghurt. As there is no significant difference between addition 20% and 30% of strawberry, yoghurt with 20% strawberry were chosen, considering economical factor. The best formula chosen for making low fat fruity yoghurt found was with 2.5% angkak extract and 20% strawberry. The chemical composition of low fat fruity yoghurt shown in Table 1.

Chemical compositions	Amount (%)
Moisture	85.59
Ash	0.73
Protein	3.12
Fat	0.11
Carbohydrate	10.45
Total acid	1.26

Table 1: *Chemical composition of the best formula of low fat fruity yogurt.*

The chemical composition of low fat fruity yoghurt was successfully meet the Indonesian National Standard (INS) for low fat yoghurt product [17]. The fat content found in this study also can be categorized as free fat yoghurt because fat content less than 0.5%. The low fat content in this product was caused by natural properties of skim milk where fat was removed partially from whole milk. The viscosity, total solid content, and pH of this product was 2500 cp, 14.9°Brix, and pH value 4.07. Maize powder act as stabilizer in yogurt, not only for improving the texture and increasing total solid content and but also preventing syneresis. As low pH value caused the formation of more coagulant, the total soluble solid will be increased, and the observed viscosity will be higher [18]. Tamime and Robinson [19] mentioned that usually yoghurt has total solid range between 14-16 oBrix, result in this study still in the recommended range. The yoghurt had the total lactic acid bacteria of 9.6 log CFU/ml, where it meets INS for low fat yoghurt which required minimum 7 log CFU/ml [17]. According to Tamime and Robinson [19], probiotic beverage is required to contain ≥ 6 log CFU/ml of total lactic acid bacteria. Therefore, low fat fruity yogurt can be categorized as probiotic yoghurt. The result from total coliform test showed that low fat fruity yoghurt had a total koliform ≤ 0.48 log MPN/ml, where maximum coliform can be tolerated found in yogurt is 1 log MPN/ml [17]. The non presence of coliforms in the samples indicated that there was no post pasteurization contamination at one or more stages during processing [19].

Conclusion

The present study showed that angkak extract can be applied to make yoghurt where the extract did not inhibit the growth of lactic acid bacteria. The concentration of angkak and strawberry used for making yoghurt were positively influence the acceptance of yoghurt. The utilization of angkak extract for making yoghurt represented important economic and technological alternative for development of new dairy products. For the future research, identification of main phytochemical and amount of compounds responsible for the color in yoghurt must be conducted in order to increase the value of the yoghurt.

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