Characterization of Red Beans Yogurt (Phaseolus vulgaris L) with addition of dates (Phoenix dactylifera)

Characterization of Red Beans Yogurt (Phaseolus vulgaris L) with addition of dates (Phoenix dactylifera)

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Abstract. Yogurt is one of the probiotic products that is commonly consumed and widely developed across the world. This experiment aims to determine the effects of red beans, dates, and starter culture addition on a certain ratio of full cream milk powder and characterized by Indonesian National Standard also known as SNI number 2981 :2009. This experiment was conducted with the ratio of red beans to dates (v/v) were 40:60, 50:50, and 60:50 and the starter cultures (%v/v) were 5, 15, and 25. This experiment method consisted of making red beans juice, dates juice and fermentation. The results of the experiment showed that the protein content (%) of the ratio of red beans to dates (v/v) and starter cultures (%v/v) were 40:60 and 5, 50:50 and 5,60:40 and 5; 40:60 and 15, 50:50 and 15, 60:40 and 15; 40:60 and 25; 50:50 and 25, 60:40 and 25 were 3.455, 3.63, 3.335, 2.805, 2.965, 2.915, 2.305, 3.10, and 3.15. While the fat content (%) were 2.22, 2.015, 1.55, 1.58, 1.515, 1.28, 1.27, 2.20, and 1.655; and the ash content (%) were 0.795, 0.88, 0.855, 0.835, 0.855, 0.91, 0.78, 0.82, and 0.89. The protein content that was not in accordance with the SNI was only found in yogurt with a ratio of red beans to dates 60:40 (v/v) and starter cultures of 5 (%v/v). Yogurt made from red beans, dates, and addition of full cream milk powder has the potential to become a healthy drink with a new taste.

1. Introduction

Yogurt is a probiotic drink made from fermented milk and is quite popular in the world by involving lactic acid bacteria [1] so that it is beneficial for health and contains nutrients in it [2]. Lactic acid bacteria is a type of probiotic bacteria [3] which breaks down lactose in milk into lactic acid which can lower the pH (acid) so that pathogenic bacteria cannot grow in yogurt [4]. In addition, the breakdown of protein in milk or as proteolysis is the role of lactose in order to produce peptides and amin o acids that contributes to bacterial growth and metabolic growth so as to create the taste of fermented products [5].

Some microorganisms, such as lactic acid bacteria, can multiply well in milk because of the lactose content which is used as a carbon source and fuel for the growth of microorganisms [6]. Lactose is the main source of carbohydrates and sugars that provides energy value in cow's milk [7]. Apart from being in liquid form, lactose is also found in full cream milk powder [8], which also helps the fermentation process due to lactose being the source of carbohydrates [9], and hydrolyzed by the enzyme β-galactosidase to produce galactose and glucose monosaccharides in order to lower the pH and create a sour taste of yogurt [10].

Vegetative products, such as fruits, vegetables, and beans are ideal substrates for the growth of probiotic bacteria [11] that includes red beans (Phaseolus vulgaris L.) [12,13] dates (Phoenix dactylifera) [14,15] and Terminalia catappa Linn seed [16]. Peanuts production in Indonesia, including red beans, reaches 613,365 tons/year and the average annual increase is 1% [17]. Vegetative products not only could be used as an alternative raw material for making yogurt, but also, like dates, could be used as an alternative for sweeteners [18] and nutrition [19].

Research on yogurt from plant-based with the addition of full cream milk is coconut [20], Mangosteen [21] cinnamon [22], soya bean [23], black soybean [24]. So that the quality of yogurt is guaranteed and suitable for consumption, it must be in accordance to the Indonesian National Standard (SNI) number 2981:2009. This study aims to determine the effects of adding red beans and dates with full cream milk on yogurt production and finding out the proximate according to SNI 2009.

2. Materials and Methods

2.1. Materials

Dried red beans, Deglet Noor dates (Palm'Fruit), starter culture (Biokul) and full cream milk powder (Dancow) were obtained from local market (Indonesia). Chloroform (p.a, Sigma Aldrich), methanol (p.a, Sigma Aldrich), Selenium (Sigma Aldrich), H2SO4 (p.a, Sigma Aldrich), NaOH (p.a, Sigma Aldrich), H3BO3 (p.a, Sigma Aldrich), HCl (p.a, Sigma Aldrich).

2.2. Making red beans juice

Five hundred g of dried red beans were washed and soaked for ±12 hours in 1 L water, then boiled for ½ hour. Red beans and water are blended in a ratio of 1:2 (v/v) and filtered [25]

2.3. Making dates juice.

Three hundred g of dates flesh and water are blended at a ratio of 1:3 (w/v) and filtered [26].

2.4. Making yogurt from red beans and dates.

The method is modified from [25] where there are 2 variables, starter cultures (5%, 15%, 25% v/v) and red beans:dates ratio (v/v) (40:60, 50:50, 60:40) which resulted in 9 sample variants as presented in Table 1.

Table 1. Variable variation of yogurt

Starter Culture (v/v)	Red beans: Dates Ratio (v/v)
5%	40:60
	50:50
	60: 40
15%	40:60
	50:50
	60: 40
25%	40:60
	50:50
	60: 40

Red beans:dates solution are divided into 3 part, 40:60, 50:50, and 60:40, then 6% (b/v) of full cream milk powder was added to each part and stirred until homogenous. The solution was pasteurized at 80°C and allowed to stand until 40°C then added 5%, 15%, 25% (v/v) starter cultures to each solution. The solution was fermented for 8 hours at room temperature. Yogurt was then stored in the refrigerator for 18 days before characterization (fat, protein, and ash content).

2.5. Determination of protein content.

Protein level determination was carried out using the Kjeldahl method [27]. Two grams of the sample were put into a Kjeldahl tube, then 1 gram of selenium and 2 mL H2SO4 were added, then the KjelDigester was pre-heated until the temperature reached 420oC [28]. The Kjeldahl tube containing the sample was attached to the KjelDigester and scrubber unit, then the digestion was turned on at 420oC for 1 hour. After that, the tube rack was removed and left to reach room temperature. Fifty mL of distilled water and 10 mL of 40% (w/v) NaOH were added to the Kjeldahl tube containing the sample from the destruction in the distillation unit. The distillate was accommodated in 2% (w/v) H3BO3 then titrated with HCl solution (0.01400 mol/L). The end point of the titration was determined with a potentiometer (ZDJ-4B). Protein content (%) was calculated according to equation (1)

$$Protein\ content\ (\%) = \frac{(V_p - V_b) x N x 1.4007 x Fk}{Weight\ of\ sample\ (g)} \quad (1)$$

Description:
Vp = HCl volume required for sample titration (mL)
Vb = HCl volume required for blank titration (mL)
N = Normality of HCL solution
Fk = Protein conversion factor (6.25)

2.6. Determination of fat content.

Determination of fat content was carried out gravimetrically based on a modified method Folch, Lees and Sloane-Stanley [29]. Mix 40 ml chloroform/methanol (2:1, v/v) with 2 mL of sample. Thoroughly homogenized the mixture and centrifuged at 1509 g for 10 min. The clear homogenate was transferred to a separating funnel. Subsequently, 7.8 mL of water was mixed with the homogenate and allowed to stand until phase separation was observed. The proportion of water to homogenate was 2:10 (v/v) to ensure that no interfacial fluff was formed in the biphasic system obtained. The lipid layer (lower layer) was collected. The aqueous layer (top layer) was rinsed with chloroform/methanol mixture (2:1, v/v) and was allowed to stand for ±15 min [30,31] until phase separation. The ratio between the aqueous layer and the rinsing solvent was around 1:1 (v/v) to prevent interfacial fluff. The lipid layer was collected and combined with the previous collection. The combined lipid fraction was then evaporated to dryness in a rotary evaporator (KNF RC600) and dried to constant weight under vacuum and the lipid content determined gravimetrically. Fat content is calculated according to equation (2)

$$Fat\ content\ (\%) = \frac{(C-A)}{B}\ x\ 100\% \quad (2)$$

Description:
A = Weight of empty flask (g)
B = Weight of sample (g)
C = Fixed weight of flask + sample after heating (g)

2.7. Determination of ash content.

Ash content was obtained using gravimetric test method [32]. Five gram of sample was heated in a muffle furnace at a temperature of 100 °C for 1 hour then the temperature was raised to 200 °C for 2 hours and finally the temperature was raised to 550 °C for 5 hours. The sample was cooled in a desiccator and then weighed until it got a constant weight. Ash content (%) was calculated according to equation (3)

$$Ash\ content\ (\%) = \frac{(C-A)}{B}\ x\ 100\% \quad (3)$$

Description:
A = Weight of empty cup (g)
B = Weight of sample (g)
C = Fixed weight of cup + sample after heating (g)

3. Results and Discussion

Proximate testing was carried out to determine the protein content, fat content, and ash content in red beans yogurt with the addition of dates.

3.1. Effect of the Ratio of Red Beans, Dates, and Starter Culture on Protein Content

The effect of the ratio of red beans and dates as well as starter culture on protein content is presented in Figure 1. The protein content (%) in the ratio of red beans to dates 40:60 (v/v) with starter cultures (%v/v) as much as 5, 15, and 25, respectively, were 3,455, 3.63, and 3,335%. While the protein content (%) with the ratio of red beans to dates 50:50 (v/v) with starter cultures (%v/v) of 5, 15, and 25 were 2.805, 2.965, and 2.915%. Protein content (%) with the ratio of red beans to dates 60:40 (v/v) and starter cultures (%v/v) of 5, 15, and 25 were 2.305, 3.10, and 3.15%.

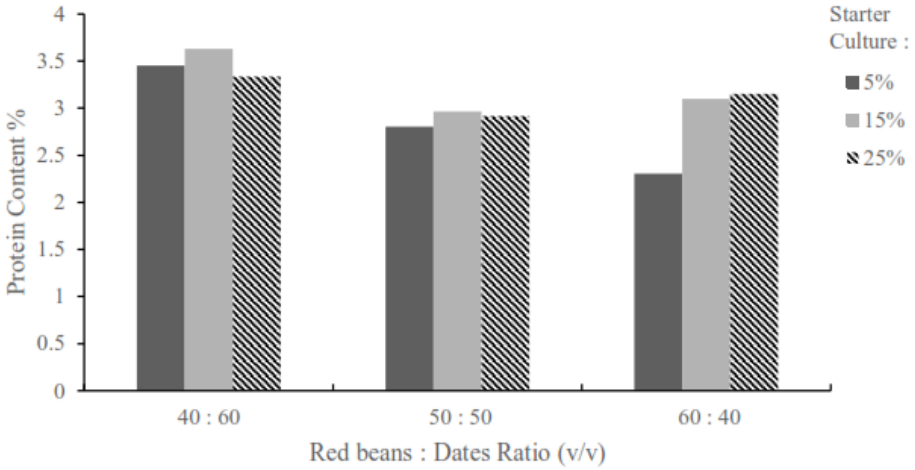

Figure 1. Yogurt protein content of various red beans:dates ratios in starter cultures

The increase in protein content was suspected to be caused by the amount of nutrients contained in dates as an energy source for the growth of starter cultures and red beans which provided vitamins, minerals, and growth factors for starter cultures [33–35]. The ratio of red beans to dates 40:60 (v/v) with a starter culture of 15% (v/v) had the highest protein content because we suspected it was due to having the highest content of dates so that the nutrients for the growth of the starter cultures were also the highest and the number of starter cultures were neither exceeded nor insufficient than the amount of nutrients available [36]. Meanwhile, the protein content in the ratio of red beans to dates 40:60 (v/v) with 5% (v/v) and 25% (v/v) starter cultures was lower than 15% (v/v) starter cultures, it was suspected that the available amount of nutrients either exceeded or were insufficient in the amount needed to stimulate the growth of starter cultures as much as 5% (v/v) or 25% (v/v) [37]. The same reason applied to the 50:50 (v/v) ratio of red beans to dates. Unfortunately, the protein contents for that ratio and content of the starter cultures were lower than the 40:60 (v/v) ratio variable. We suspected this was due to fewer dates were added so that the amount of energy source for the growth of starter cultures was also reduced even though there was an addition of red beans so that the protein content for that variable was not reduced too much [38,39]. Protein content in the ratio of red beans to dates 60:40 (v/v) was between the ratio of 40:60 (v/v) and 50:50 (v/v). This was suspected that although there was a reduction in dates, the addition of red beans caused the protein content of a ratio of 60:40 (v/v) with starter culture levels of 5% (v/v), 15% (v/v), and 25% (v/v) had no significant decrease [12]. Variable ratio of 60:40 (v/v) and 5% (v/v) starter culture content had a protein content that was not in accordance with SNI. This was suspected that it was due to the dates juice contained in these variables was very little to provide nutrients as an energy source for the growth of starter cultures when compared to other variables [40] and the number of starter culture contained in the yogurt were only 5% (v/v) so that there were not enough starter cultures to increase the protein content for that variable to reach the SNI [41]. The comparison of yogurt protein content in this study with SNI is presented in Table 2.

Table 2. Comparison between experimental protein on yogurt and SNI

Red Beans : Dates Ratio (v/v)	Starter Culture (% v/v)	Yogurt Protein Content (%)	
		Average Experimental Results	SNI
40:60	5	3.63	Min 2.7
	15	3.335	
	25	2.805	
50:50	5	2.965	
	15	2.915	
	25	2.305	
60:40	5	3.1	
	15	3.15	
	25		

3.2. Effect of the Ratio of Red Beans, Dates, And Starter Culture On Fat Content

The results of the determination of fat content are shown in Figure 2 where the ratio between red beans to dates 40:60 (v/v) with a starter culture of 5% (v/v) has a fat content of 2.220%, then it became 2.015% when the starter cultures were 15% (v/v) and decreased to 1.550% when the starter cultures were 25% (v/v). This also occurred for the ratio of 50:50 (v/v) and the amount of the starter cultures as much as 5, 15 and 25% (v/v) where there was a decreased in fat content of 1.58, 1.51 and 1.28%.

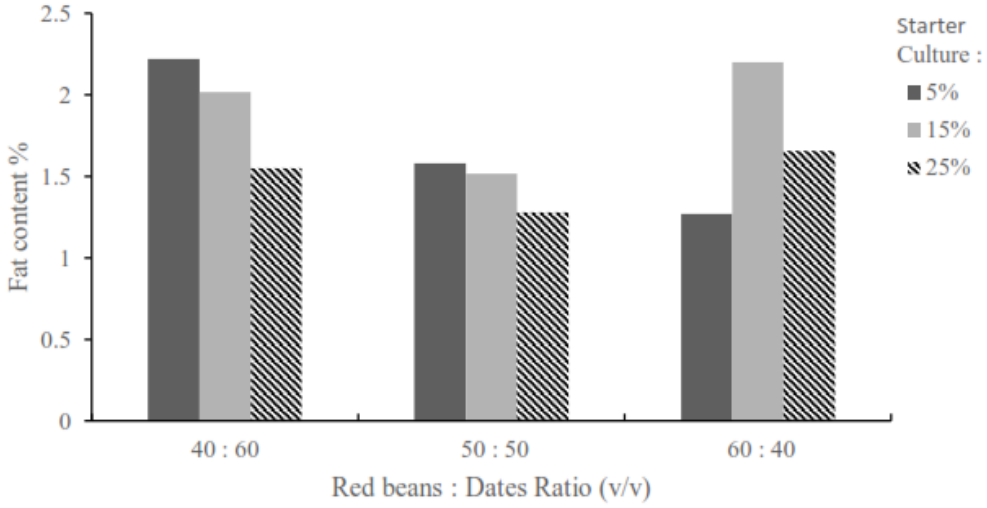

Figure 2. Yogurt fat content on various red beans:dates ratios in starter culture

According to [42], it was suspected the decrease in fat content was caused by the lipolysis reaction by the starter culture so that the fat content decreased along with the increase of starter cultures contained in these variables however for the ratio of red beans:dates 60:40 (v/v) with starter cultures of 5, 15 and 25% (v/v) resulted in a fat content of 1.27, 2.20, 1.65% . According to [42], it was suspected that the decrease in fat content was caused by the lipolysis reaction by the starter cultures affecting the decrease in the fat content along with the increase of the starter cultures contained in the variables. However, for the ratio of red beans to dates 60:40 (v/v) with the starter cultures of 5, 15 and 25% (v/v) resulted in a fat content of 1.27, 2.20, 1.65% which was suspected it was due to the amount of red beans added for that ratio [43], but since there was date palm juice in the yogurt, the fat content for each variable at the

ratio of 60:04 had a fat content that was not constant [44].The comparison of fat content in this study with SNI is presented in Table 3.

Table 3. Comparison between experimental fat content on yogurt and SNI

Red Beans : Dates Ratio (v/v)	Starter Culture (% v/v)	Yogurt Fat Content (%)	
		Average Experimental Results	SNI
40:60	5	2.22	0.6 - 2.9
	15	2.015	
	25	1.55	
50:50	5	1.58	
	15	1.515	
	25	1.28	
60:40	5	1.27	
	15	2.2	
	25	1.655	

3.3. Effect of the Ratio of Red Beans, Dates, And Starter Culture on Ash Content

The effect of the ratio of red beans and dates and starter culture on ash content is presented in Figure 3. The ash content (%) with the ratio of red beans to dates 40:60 (v/v) and starter cultures of 5, 15, and 25 (% v/v) were 0.795, 0.88, and 0.855%. Meanwhile, the ash content (%) with the ratio of 50:50 (v/v) red beans to dates and 5, 15, and 25 (% v/v) amount of the starter cultures were 0.835, 0.855, and 0.91%. Meanwhile, the ash content (%) with the ratio of red beans to dates 60:40 (v/v) and starter cultures of 5, 15, and 25 (% v/v) were 0.78, 0.82, and 0.89%.

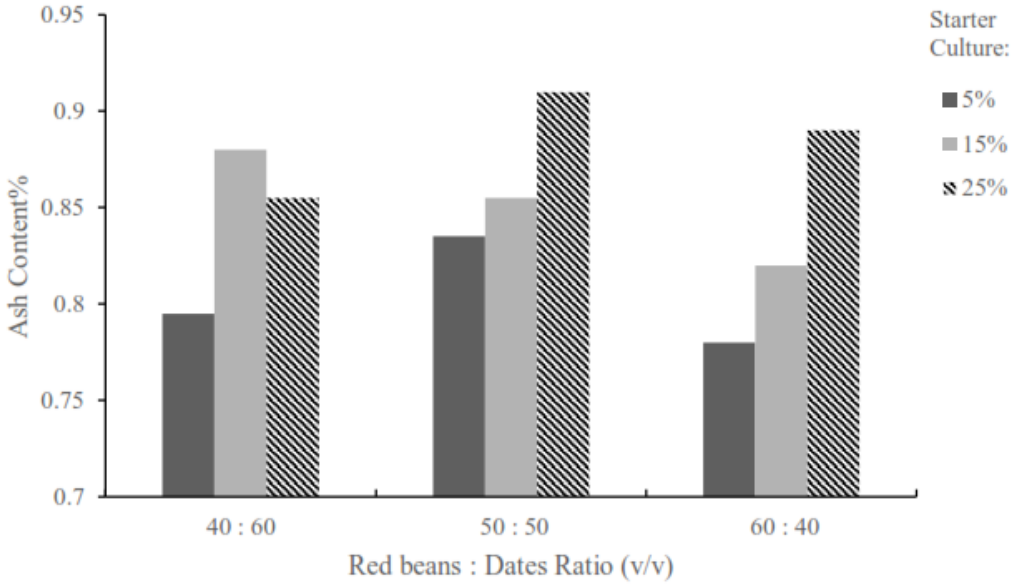

Figure 3. Yogurt ash content of various red beans:dates ratios in starter cultures

The ash content in yogurt was suspected to be caused by the metabolic activity of the starter cultures during fermentation which was influenced by the amount of energy contained in the dates juice and red beans in order to stimulate the growth of the starter culture [37,45,46]. The highest ash content for ratio of red beans to dates 40:60 (v/v) was found in starter culture content of 15% (v/v) which was suspected

to be caused by a lot of metabolic activities in the starter cultures due to the large amount of dates juice in the yogurt and the amount of starter cultures that was not less nor exceeds the amount of energy contained in the dates juice [47]. While the ash content in the ratio of red beans to dates 40:60 (v/v) with starter cultures as much as 5% (v/v) and 25% (v/v) was lower than that of a starter culture of 15% (v/v) because it was suspected that 5% (v/v) amount of starter cultures were a little amount of starter cultures to carry out metabolic activity even though there was a large amount of energy, and there was not enough of energy available to help stimulate the metabolic activity of 25% (v/v) starter culture [37]. The ash content in the ratio of red beans to dates 50:50 (v/v) increased along with the addition of starter culture content, the same goes for the ratio of red beans to dates 60:40 (v/v), and 25% (v/v) starter cultures had the highest ash content because it was suspected that there was a very high metabolic activity of starter cultures [36]. There was a decrease in ash content in the ratio of red beans to dates 60:40 (v/v) compared to the ratio of red beans to dates 50:50 (v/v) which was suspected due to a reduction in the amount of dates juice so that the amount of energy was also reduced [34]. However, the amount of decrease in this ratio was not too far from the previous ratio because it was suspected to be influenced by the content of red beans juice which provided growth factors for starter cultures to carry out metabolic activities [35].

Table 4. Comparison between experimental ash content on yogurt and SNI

Red Beans : Dates Ratio (v/v)	Starter Culture (% v/v)	Yogurt Ash Content (%)	
		Average Experimental Results	SNI
40:60	5	0.795	Max 1
	15	0.88	
	25	0.855	
50:50	5	0.835	
	15	0.855	
	25	0.91	
60:40	5	0.78	
	15	0.82	
	25	0.89	

4. Conclusion

Yogurt made from red beans and dates produced yogurt in accordance with SNI 2981:2009 with protein content ranging from 2.305-3.63%, fat content 1,27-2,22% and ash content 0.78-0.91%. Yogurt with the highest protein content of 3.63% was found in the ratio of red beans to dates 40:60 (v/v) with a starter culture content of 15% (v/v), yogurt with the highest fat content of 2.22% was found in the ratio of red beans to dates as much as 40:60 (v/v) with a starter cultures content of 5% (v/v) and yogurt with the highest ash content was found in the ratio of red beans to dates 50:50 (v/v) with a starter culture content of 25% (v/v).

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