

Effect of sucrose addition to antioxidant activity and colour in blue pea flower (*Clitoria ternatea* L.) yoghurt

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Abstract

Blue pea flower (*Clitoria ternatea* L.) yoghurt is the result of processing milk with the addition of blue pea flower extract through Lactic acid bacteria's fermentation process viz. *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Blue pea flower (*Clitoria ternatea* L.) contains bioactive components, particularly flavonol glycosides, anthocyanins, flavones, flavonols, phenolic acids and terpenoid. This study was aimed to determine the effect of sucrose on the antioxidant activity and colour of blue pea flower yoghurt. This study used a completely randomized design with five treatments namely yogurt control = no added blue pea flower extract, and the following with 10% blue pea flower extract at different sucrose concentrations: P1 = 0% sucrose, P2 = 4% sucrose, P3 = 8% sucrose and P4 = 12% sucrose. Data analysis used the analysis of variance. The results showed that the highest antioxidant activity was P2 = 105.25 ppm. While the best colour parameter is P2 = L * 42.42, a * 5.12, b * -5.54). Based on the results of the study, the addition of sucrose 4% increased the highest antioxidant activity and colour of yoghurt extract of blue pea flowers (*Clitoria ternatea* L).

1. Introduction

Blue pea flower yoghurt results from fermented milk using the bacteria *Lactobacillus bulgaricus* and *Streptococcus thermophilus* with blue pea flower extract. Yoghurt is a drink that is quite popular around the world because it has sound health effects and has various flavours (Nurhartadi *et al.*, 2017). Yoghurt with the addition of fruit juice will increase consumer acceptance because it contains phenolic compounds and high antioxidant activity, which is useful for preventing degenerative diseases (Benozzi *et al.*, 2015; Aryana and Olson, 2017). Yoghurt processing with goat's milk is a diversified alternative to yoghurt products. Therefore, it is necessary to develop goat milk processing methods with fermentation techniques that aim to diversify and reduce "goaty flavor" the bad smell of goat milk. As consumer interest in yoghurt products increases, consumers prefer products that use natural dyes over synthetic dyes because they are healthier and have no adverse side effects. Dyes derived from plants can be used as natural alternative dyes, one of which is the butterfly pea (Ghafoor *et al.*, 2009; Yadav *et al.*, 2018; Zhang *et al.*, 2020).

Yoghurt products with blue pea flower extract can produce a natural blue to purple colour, thereby

increasing the attractiveness of consumers to consume yoghurt. Blue pea flower (*Clitoria ternatea* L.) contains natural purplish-blue pigments and anthocyanin compounds. Anthocyanins are colour pigments that produce red, purple, and blue colours. However, anthocyanin colours are strongly influenced by pH; changes in pH will change the blue pea flower's colour (Muzi Marpaung *et al.*, 2017; Lakshan *et al.*, 2019). Many studies have used natural dyes such as pandan leaves, turmeric, and dragon fruit extract as sources of natural dyes added to yoghurt. The addition of blue pea flower extract as a natural colourant for popsicles and various other food ingredients has been widely used (Baskaran *et al.*, 2019). Blue pea flower extract contains anthocyanin pigments used as an alternative to natural dyes that produce a purplish-blue colour (Escher *et al.*, 2020).

Yoghurt has long been recognized as a source of probiotics. The primary role of probiotics, in general, is to optimize digestive metabolism through the mechanism of improving the microbiota population in the digestive tract. Also, previous studies showed that anthocyanin extracts from blue pea flowers were a more attractive colour. The main compound is delphinidin glucoside (Chu *et al.*, 2016; Ibrahim *et al.*, 2019). Nurhartadi *et al.*

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(2017) reported that the sensory acceptance test of whey yoghurt cheese with 12% sucrose is most preferred. (Octaviani and Rahayuni, 2014). reported that the addition of sucrose affected the antioxidant activity of Buni fruit juice. However, the effect of sucrose on blue pea flower yoghurt is still unknown.

Therefore, research is required to study sucrose's effect on antioxidant activity and blue pea flower yoghurt colour. Research provides benefits to the development of science in food science and technology, especially food additives. This study aimed to determine the effect of sucrose on antioxidant activity and colour in yoghurt blue pea flower (*Clitoria ternatea* L.) yoghurt.

2. Materials and methods

2.1 Extraction of blue pea powder

The blue pea flower (*Clitoria ternatea* L.) extract (BPE) was based on the research by Nurhartadi et al. (2017), Agustine et al. (2018) and Escher et al. (2020) with a simple modification. The powder blue pea flower (*Clitoria ternatea* L.) was obtained from CV.Hasil Bumiku, Bantul Regency, special region of Yogyakarta, Indonesia. The powder blue pea flower was extracted using the maceration technique at a temperature of 60°C for 45 mins. Furthermore, the sample was filtered using a 70 mm Whatman filter paper. The extraction process was carried out with a blue pea flower ratio: distilled water at 3:1 (w/v).

2.2 Preparation of yoghurt starter

The starter of *Lactobacillus bulgaricus* (LB) and *Streptococcus thermophilus* (ST) was obtained from FTP UGM. The bacterial starter was then grown on MRS media at 30°C for 24 hrs. The method used is slightly modified (Song et al., 2016). There were five treatments in this study, namely yogurt control = no added blue pea flower extract, P1 = 10% extract of blue pea flower: 0% sucrose, P2 = 10% extract of blue pea flower: 4% sucrose, P3 = 10% extract of blue pea flower: 8% sucrose and P4 = 10% extract of blue pea flower: 12% sucrose.

2.3 Yoghurt production without blue pea flower extract addition

Yoghurt control production with the addition of *L. bulgaricus* and *S. thermophilus* was performed following the method used by (He et al., 2012; Nurhartadi et al., 2017) with modifications. Bacterial culture was added to pasteurized milk in a 1:9 and incubated at 37°C for 24 hrs.

2.4 Blue pea flower extract yoghurt with sucrose addition

The fermentation procedure for blue pea flower yoghurt (BPY) followed the research by Zhang et al. (2019) with the addition of 10% extract of blue pea flower and the addition of sucrose by 0%, 4%, 8%, and 12% (v/v). Blue pea flower extract was added to pasteurized milk and stirred until well-blended. *L. bulgaricus* and *S. thermophilus* starter with a population of 1×10^9 CFU/mL was added into the milk solution. The fermentation was carried out in an incubator at 45°C for 24 hrs.

2.5 Antioxidant activity and colour properties

The yoghurt was tested for DPPH antioxidant levels. DPPH was tested by 3.9 mL DPPH solution (DPPH concentration in ethanol 0.004 g/mL) mixed with 0.1 mL sample. The mixture was incubated for 30 mins in a dark room. After that, the samples have recorded the absorbance at a wavelength of 515 nm (Octaviani and Rahayuni, 2014). Antioxidant activity was calculated by using a standard linear equation. The standard curve used BHT. Besides, yoghurt has also been tested with an $L * a * b$ value using a CR-400 chroma meter (Igwemmar et al., 2013).

2.6 Statistical analysis

Data of DPPH antioxidant levels and colour were analyzed descriptively using ANOVA with a significance level of 5% to determine the effect of differences in the treatment of blue pea flower extract to yoghurt. Analysis continued with Duncan's test to see a significant difference. All data are processed using SPSS 16.

3. Results and discussion

3.1 Antioxidant activity

The results are shown in Table 1. It clearly showed the influence of blue pea flower extract on the antioxidant activity of yoghurt. The results showed that antioxidant activity concentration of yoghurt lactic acid control 27.33 ppm, P1 = 104.50 ppm, P2 = 105.25 ppm, P3 = 102.50 ppm and P4 = 93.03 ppm.

Table 1. DPPH scavenging antioxidant activity

Treatment	DPPH (ppm)
	Yoghurt Blue pea
Yoghurt Control	27.33 ^c
P1 (0% Sucrose)	104.50 ^a
P2 (4% Sucrose)	105.25 ^a
P3 (8% Sucrose)	102.50 ^a
P4 (12% Sucrose)	93.03 ^a

Values with different superscript are statistically different ($p \geq 0.05$)

The higher the value indicates the compound used has the potential as an antioxidant. The research data showed that blue pea flower yoghurt with the addition of sucrose showed an effect on yoghurt's antioxidant activity. Based on the data in Table 1, the antioxidant activity of P2 (4% sucrose) is the highest compared to other treatments; the higher the addition of sucrose, the antioxidant activity decreases this is due to anthocyanin damage. The higher the added sugar in the buni fruit juice decreased the antioxidant activity (Karadag *et al.*, 2009; Müller *et al.*, 2012; Chusak *et al.*, 2018). It is known that anthocyanins are substances that act as antioxidants. *Clitoria ternatea* L. exhibits antioxidant and antihyperglycemic activity. Antioxidants are substances that can reduce free radicals and oxidative stress (Birben *et al.*, 2012; Chu *et al.*, 2016; Adipogenic *et al.*, 2019).

Blue pea flower has a blue colour at neutral pH and a purple colour when exposed to acids, so they have the potential to be an attractive food colouring (Müller *et al.*, 2012; Igwemmar *et al.*, 2013; Song *et al.*, 2016; Aldaw Ibrahim *et al.*, 2019). The colour is known as anthocyanin, one of the antioxidants (Müller *et al.*, 2012; Octaviani and Rahayuni, 2014; Song *et al.*, 2016; Adipogenic *et al.*, 2019; Aldaw Ibrahim *et al.*, 2019; Escher *et al.*, 2020). Antioxidants are bioactive compounds with many benefits, including anti-ageing (Birben *et al.*, 2012) to prevent degenerative diseases such as diabetes, heart disease. It also has antimicrobial properties for microbial food spoilage (Chusak *et al.*, 2018). In this study, blue pea flower extract to yoghurt is expected to increase the antioxidant activity of the blue pea flower yoghurt.

3.2 Colour

The results of the study about the effect of colour on the addition of blue pea flower extract to yoghurt are shown in Table 2. Based on Table 2 the control treatment shows the colour of yogurt ($L^* 37.35$, $a^* 2.02$, $b^* -1.32$), the addition of sucrose 0 % shows the colour of yogurt ($P1 = L^* 44.79$, $a^* 3.72$, $b^* -4.63$), the addition of sucrose 4% shows the colour of yogurt ($P2 = L^* 42.42$, $a^* 5.12$, $b^* -5.54$), the addition of sucrose 8 % shows the colour of yogurt ($P3 = L^* 42.59$, $a^* 5.04$, $b^* -5.56$) and the addition of sucrose 12 % shows the colour of yogurt ($P4 = L^* 44.49$, $a^* 4.49$, $b^* -4.92$).

The addition of sucrose extract of blue pea flowers also affects the fermented milk products' colour or the resulting yoghurt. Colour test result data or $L^* a^* b^*$ values use a chromameter. Table 2 shows that the addition of 4% sucrose increased the greenish-red content of blue pea flower yoghurt with the highest value.

Table 2. L^* , a^* , and b^* values of the Yoghurt with blue pea extract

Treatment	L, a, b value		
	L^*	a^*	b^*
Yoghurt Control	37.35 ^a	2.02 ^a	-1.32 ^b
P1 (0% Sucrose)	44.79 ^b	3.72 ^a	-4.63 ^a
P2 (4% Sucrose)	42.42 ^a	5.12 ^b	-5.54 ^a
P3 (8% Sucrose)	42.59 ^a	5.04 ^b	-5.56 ^a
P4 (12% Sucrose)	44.49 ^b	4.49 ^{ab}	-4.92 ^a

Values with different superscript are statistically different ($p \leq 0.05$)

The value of b^* shows a yellow-blue colour, with a^* negative value greater than the blue colour. The most significant blue colour is yoghurt with the addition of 4% sucrose. The value of b^* shows a^* yellow-blue colour, with a^* negative value greater than the blue colour. The most significant blue colour is yoghurt with the addition of 4% sucrose.

Blue pea flowers have a blue and purple colour when exposed to acids to be attractive food colouring. This colour is known to be anthocyanin which is one of the antioxidants. Antioxidants are bioactive compounds with many benefits, including anti-ageing, preventing degenerative diseases such as diabetes, heart disease, and others (Chusak *et al.*, 2018). Besides, it also has food-destroying antimicrobial properties. Many studies have shown that antioxidant-rich foods have an essential role in preventing various chronic diseases associated with oxidative stress. Antioxidants' mechanism in preventing oxidative stress in metabolism is very diverse, including free radical binding, inhibition of oxidative enzymes, acting as antioxidant enzyme cofactors (Karadag *et al.*, 2009).

4. Conclusion

The sucrose from various presentations affected the blue pea flowers yoghurt's antioxidant activity and the colour blue pea flowers (*Clitoria ternatea* L). The antioxidant activity and colour of yoghurt were better at presenting the addition of 4% sucrose.

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