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Physicochemical, Microbiological and Organoleptic Properties of Cowpeas (*Vigna unguiculata*) Yoghurt with the Addition of Gotu Kola Leaf (*Centella asiatica* (L.) Urban) Extract

(Sifat Fizikokimia, Mikrobiologi dan Organoleptik Yogurt Kacang Panjang (*Vigna unguiculata*) dengan Tambahan Ekstrak Daun Pegaga (*Centella asiatica* (L.) Urban))

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ABSTRACT

Cowpea yogurt is a functional food product made from cowpeas and gotu kola leaves. This mixture of cowpeas and gotu kola leaves is expected to increase its functional value i.e., protein and antioxidant activity. This research aims to analyze the physicochemical, microbiological, and organoleptic properties of cowpeas yogurt with the addition of gotu kola leaf extract. The study used a Completely Randomized Design (CRD) with one factor, with the ratio of cowpea's milk: gotu kola leaf extract, namely F1 (98%: 2%), F2 (96%: 4%), F3 (94%: 6%), K2 (100%: 0%), and K1 (100% pure milk). Testing parameters include viscosity, pH value, (moisture, ash, fat, protein, carbohydrate) content, antioxidant activity, total lactic acid bacteria, and organoleptic (color, aroma, taste, texture). The results showed that the highest pH and viscosity values in K2 were 3.77 and 419.59 cP; the highest moisture content in F3 was 85.04%; the highest ash, fat, and carbohydrate content in K2 were 0.62%, 1.87%, and 10.21%; the highest protein content in F1 was 3.25%; the highest antioxidant activity in F3 with an IC_{50} value of 87.008 ppm; the total lactic acid bacteria in K2 met the minimum SNI requirements of 10^7 CFU/mL; and K2 showed the most favorable overall organoleptic test. This study shows that the addition of gotu kola leaf extract affects on the physicochemical, microbiological, and organoleptic properties of cowpea yogurt.

Keywords: Antioxidant; cowpeas; gotu kola leaf; probiotic; yoghurt

ABSTRAK

Yogurt kacang panjang ialah produk makanan berfungsi yang diperbuat daripada kacang panjang dan daun pegaga. Campuran kacang panjang dan daun pegaga ini dijangka dapat meningkatkan nilai fungsinya iaitu aktiviti protein dan antioksidan. Penyelidikan ini bertujuan untuk menganalisis sifat fizikokimia, mikrobiologi dan organoleptik yogurt kacang panjang dengan penambahan ekstrak daun pegaga. Kajian menggunakan Reka Bentuk Rawak Lengkap (CRD) dengan satu faktor, dengan nisbah susu kacang panjang: ekstrak daun pegaga iaitu F1 (98%: 2%), F2 (96%: 4%), F3 (94%: 6%), K2 (100%: 0%) dan K1 (100% susu tulen). Parameter ujian termasuk kandungan kelikatan, nilai pH, kandungan (kelembapan, abu, lemak, protein, karbohidrat), aktiviti antioksidan, jumlah bakteria asid laktik dan organoleptik (warna, aroma, rasa, tekstur). Keputusan menunjukkan bahawa nilai pH dan kelikatan tertinggi dalam K2 ialah 3.77 dan 419.59 cP; kandungan lembapan tertinggi dalam F3 ialah 85.04%; kandungan abu, lemak dan karbohidrat tertinggi dalam K2 adalah 0.62%, 1.87% dan 10.21%; kandungan protein tertinggi dalam F1 ialah 3.25%; aktiviti antioksidan tertinggi dalam F3 dengan nilai IC_{50} 87.008 ppm; jumlah bakteria asid laktik dalam K2 memenuhi keperluan SNI minimum 10^7 CFU/mL; dan K2 menunjukkan ujian organoleptik keseluruhan yang paling baik. Kajian ini menunjukkan bahawa penambahan ekstrak daun pegagan memberi kesan kepada sifat fizikokimia, mikrobiologi dan organoleptik yogurt kacang lembu.

Kata kunci: Antioksidan; daun gotu kola; kacang lembu; probiotik; yogurt

Introduction

Degenerative diseases are non-communicable and chronic diseases indicated by the deterioration of cell and organ function in the body (Handajani, Roosihermiatie & Maryani 2010). One of the causes of the emergence of degenerative diseases is the high-fat content and lack of fiber intake in the body. Both of these have the potential to cause the formation of free radicals. One effort that can be made to prevent the presence of free radicals in the body is to eat foods high in antioxidants and fiber. Some foods such as fruits, vegetables, and legumes can be used as a source of natural antioxidants (Carlsen et al. 2010).

Cowpeas are one type of nuts with low-fat content, high protein content, have the potential to be a source of minerals and vitamins, and rich in nutraceutical compounds such as dietary fiber (Ariviani, Sholihin & Nastiti 2021). Cowpeas also contain phenolic substances (antioxidants) (Cai, Hettiarachchy & Jalaluddin 2003). The fiber in legumes is usually in the form of soluble fiber which is a type of pectin or gum carbohydrate. This fiber has a metabolic effect on the body, which can provide protection to the health of the digestive tract, especially the small intestine and colon. The *soluble* fiber in cowpeas can also act as a prebiotic (Kusharto 2006). Cowpeas also contain water-insoluble dietary fiber in the form of cellulose and hemicellulose which are widely found in seed coats and parts of the peanut endosperm (USDA 2016). In previous studies, cowpeas milk fermented by *Lacticaseibacillus rhamnosus* Yoba showed good in nutritional value, high antioxidant activity and sensory acceptance (Chawafambira, Jombo & Mkungunugwa 2022), Other studies have shown that the nutritional quality of cowpea milk can be achieved through fermentation by *Lactobacillusacidophilus, Bifidobacterium* spp, and *Streptococcus thermophilus* (Aduol et al. 2020). This study is more interesting than previous studies due to the addition of gotu kola leaves which can increase its functional value such as antioxidant activity.

Gotu kola plant is one of the plants that contain antioxidants. Zainol et al. (2008) showed that gotu kola contains several active ingredients with strong antioxidant levels. Triterpenoid saponins are one such active ingredient. Triterpenoid saponins contain several other active ingredients such as asiaticoside, centelloside, madecosside, glocoside, and others. In addition to active ingredients, gotu kola also contains minerals such as calcium, magnesium, potassium, iron, and phosphorus (Sutardi 2016).

Consumption of food and beverages must be considered in human health, especially in the digestive system. Food products that contain probiotics can be used as an option because they contain good bacteria (lactic acid bacteria). Lactic acid bacteria produce antimicrobial substances that act as natural antibiotics to fight pathogenic bacteria, help maintain digestive health, prevent various diseases, and improve digestibility in lactose-sensitive individuals. Lactic acid bacteria are classified as probiotics because they are antimicrobial, tolerant to stomach acid, and safe to use. Lactic acid bacteria that are classified as probiotics must have the ability to produce antimicrobial substances that can suppress the growth of enteric bacteria that are pathogenic. These substances include organic acids, hydrogen peroxide, diacetyl, and bacteriocins (Melia et al. 2017; Weerathilake et al. 2014).

Yoghurt is a popular fermented milk product obtained by fermenting milk with microorganisms such as *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. bulgaricus. Yoghurt has many health benefits due to its high-quality protein, vitamin, mineral, and calcium content. In addition, its health benefits are increased by the presence of good bacteria, called probiotic bacteria (Benmeziane et al. 2021; Zare et al. 2012). Yoghurt has been reported to boost the immune system and prevent disease due to its probiotic bacterial component. Probiotic growth can be stimulated by fortifying yoghurt using ingredients such as plant extracts to increase its health benefits (Mohamed Ahmed et al. 2021; Sarker & Siddiqui 2023).

Yoghurt is generally made from animal milk. However, animal milk contains lactose and does not contain fiber. Therefore, yoghurt made from animal milk is not good for consumption by people with lactose intolerance. According to Aswal, Kalra and Rout (2013), vegetable milk can be used as an alternative basic ingredient for making yoghurt because it does not contain lactose, low fat, and contains fiber. Plant milk has a positive effect because it is rich in antioxidant activity and fatty acids that can reduce the risk of cardiovascular disease, cancer, and diabetes. Nowadays, people also tend to choose to change their animal-based diet to a plant-based diet which includes cereals, nuts, seeds, vegetables, and fruit for several reasons such as the desire for a healthy lifestyle and environmental awareness. Excessive consumption of animal foods (products) can lead to cardiovascular disease and increased cholesterol. Cereals, legumes, whole grains, and nuts contain dietary fiber, vitamins, minerals, and antioxidants so these foods are classified as functional and nutraceutical foods (Aydar, Tutuncu & Ozcelik 2020; Sebastiani et al. 2019).

Based on this idea, the author was interested in making plant-based yoghurt products from cowpeas with the addition of gotu kola leaf extract which aims to produce yoghurt products high in protein and fiber, low in fat and lactose, and rich in antioxidants. This product was expected to be an alternative food for people with degenerative diseases and lactose intolerance. This study aims to analyze physicochemical, microbiological, and organoleptic properties cowpeas yoghurt with the addition of gotu kola leaf extract.

Materials and Methods

The ingredients used in this study were cowpeas from Gunung Kidul Yogyakarta, gotu kola leaves from Samigaluh Kulon Progo Yogyakarta, and others ingridients such as skim milk powder (Indoprima), plain yoghurt (Biokul), sugar (Gulaku), aquadest, fresh milk from Krapyak Yogyakarta, chemicals such as ethanol 70%, HCl 25%, hexane solvent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), methanol PA, vitamin C, physiological solution (NaCl 85%), filter paper media (MRS-A), Na_2SO_4, $CuSO_4$, Selenium/TiO_2, H_2SO_4, NaOH, $Na_2S_2O_3$, H_3BO_3, Mr-BCG indicator, and 0.02 N HCl standard solution Purchased from Sigma Aldrich USA.

Gotu kola leaf extraction was carried out by the methods of Nina and Liani (2014) and Rahayu et al. (2021) with modifications. Gotu kola leaves were separated from the stem and roots, then thoroughly washed using running water. Gotu kola leaves were dried in a cabinet dryer for six hours at a temperature of 500 ºC and dried gotu kola leaves were ground in a grinder. Gotu kola leaf powder was macerated for 24 h using 70% ethanol solvent (1 g powder: 10 mL ethanol) % w/v. Next, the maceration extract was filtered using Whatman filter paper no. 1 and evaporated using a rotary evaporator (T: 60 ºC; rotation: 125 rpm; pressure: 100Mbar) until a thick extract was obtained.

Cowpea yoghurt was made using the method of Uwa (2019) with modifications. The making of cowpea yoghurt begins with a mixture of pasteurized (T: 80 ºC; t: 10 min) cowpea milk, skim milk, and sugar. Next, cowpea milk was cooled to a temperature of ± 40 ºC and added starter (6 g) while stirring until evenly distributed. In this study, the bacterial starter used was 'Biokul' plain yoghurt. 'Biokul' plain starter contains bacteria including *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus*, and Bifidobacterium. Cowpea milk was put into several containers (100 mL) and added gotu kola leaf extract according to a predetermined formulation with a ratio of cowpea milk and gotu kola leaf extract, namely F1 (98%: 2%), F2 (96%: 4%), F3 (94%: 6%), K2 (100%: 0%), and K1 (100% pure milk). The yoghurt sample was put into an incubator to ferment for ±12 h with a temperature of 37 ºC. The proximate (moisture, fat, ash, protein, carbohydrate) analyses were executed using standard methods prescribe by the Association of Official Analytical Chemist (Horwitz & Latimer Jr. 2005) with some modifications.

The physical property of yoghurt analyzed in this study is viscosity. Viscosity testing was done by putting 250 mL of sample into a glass beaker. The viscosity value of the sample was measured using a brookfield viscometer with spindle number 2 and a speed of 60 rpm. The spindle was inserted into the sample until the boundary mark. The dial needle has waited until it shows a stable number, then the resulting number was recorded (Molyneux 2004). The pH values of samples were measured using a digital pH-meter. Antioxidant activity was analyzed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method at a wavelength of 516 nm with a UV-Vis spectrophotometer. The calculation of free radical damping activity was calculated using the formula:

$$\text{RSA (\%)} : \frac{(\text{control absorbance} - \text{sample absorbance})}{\text{control absorbance}} \times 100\%$$

Microbiological analysis was executed using the method of Fardiaz (1993). Total Lactic Acid Bacteria (LAB) testing of yoghurt used an incubation time of 48 h at 37 *°C*. The minimum limit for the total number of lactic acid bacteria was 10^7 CFU/mL (BSN 2009). The calculation of the total lactic acid bacteria can be calculated using the formula:

$$\text{Total BAL (CFU/mL)} = \text{Number of colonies } \times \frac{1}{\text{FP}}$$

where FP is the dilution factor.

Organoleptic analysis of yoghurt was carried out by 30 semi-trained panelists, namely students of Food Technology Universitas Ahmad Dahlan. The parameters assessed include aroma, color, taste, and texture. The assessment uses 5 scales of favorability levels, namely 1 (strongly dislike), 2 (dislike), 3 (somewhat like), 4 (like), and 5 (very like). Data from the analysis has been written as the mean ± standard deviation. The research data was processed using Statistical Program for Social Science (SPSS) software version 26 with statistical analysis of oneway ANOVA Test (Analysis of Variances). ANOVA serves to determine the presence or absence of the effect of adding gotu kola leaf extract on the physico-chemical, microbiological, and organoleptic properties of cowpeas yoghurt. If the data obtained were significantly different, then proceed with the Duncan Multiple Range Test (DMRT) analysis at a significance level of 5%.

Results and Discussion

This study showed that yogurt product from cowpeas combination with gotu kola leaves has good functional value according to physical and chemical properties. Physical and chemical properties of cowpeas yogurt can be seen at Tables 1, 2, and 3.

Table 1 shows the viscosity test results of yoghurt samples between 418.84 cP and 483.60 cP. Purwantiningsih et al. (2022) shows that fermented products that lead to yoghurt have a viscosity value of around 8.28-13.00 cP. Thus, the viscosity results of all yoghurt samples were classified as having a high viscosity value. Factors that can affect yoghurt viscosity include the use of milk type, protein content, and total solids contained in milk (Edy, Fitri & Retno 2021). K2, F1, F2, and F3 used cowpea's milk as raw material in making yoghurt. The high viscosity value in the sample can be caused by the starch content in the cowpeas. Directorate of Nutrition of the Ministry of Health of the Republic of Indonesia (2009) stated that cowpea contains starch content of approximately 50.5%-67%. This high starch content has the potential to cause an increase in the viscosity value of cowpea yoghurt samples. The addition of skim milk to each yoghurt sample also affects the viscosity value produced. The addition of skim milk can affect the viscosity of yoghurt because there was an increase in protein levels and an acidic atmosphere below the isoelectric point of milk protein (Sugianto et al. 2020). In an acidic atmosphere, proteins can clump together and form gels (Yulian, Yoyok & Sri Mulyani 2014). The viscosity of yoghurt was also influenced by the pasteurization process when making cowpea's milk. The pasteurization process with high temperatures (80 °C) and a long time can increase the solids, so that the water content in milk will be bound by starch granules that cause swelling (Satria 2018).

Table 2 shows the results of testing the pH values of yoghurt samples ranging from 3.69 to 4.01. The National Standardization Agency of Indonesia (2009) stated that the quality requirements for yoghurt pH values range from 3.8 to 4.5. This indicates that the pH value of K2, F1, F2, and F3 do not meet the SNI requirements. K2, F1, F2, and F3 exhibit a decrease in pH value along with the addition of gotu kola leaf extract. Gibson et al. (2010) suggest that a source of energy to support probiotic growth during fermentation can be obtained by adding prebiotics. Prebiotics can be derived from natural sources such as herbal plants. Gotu kola leaf extract can be used as a prebiotic due to its functional properties such as fiber and carbohydrates containing sugar groups, especially glucose (Kusuma 2020). Hidayat, Kusrahayu and Mulyani (2013) shows that lactic acid bacteria (LAB) will use available carbohydrates during the fermentation process to produce lactic acid and organic acids, resulting in a decrease in pH value. The decrease in pH value was also influenced by the type of starter used.

TABLE 1. Results of the physical properties analysis of cowpeas yoghurt with the addition of gotu kola leaf extract

Sample	Viscosity value (cP)
K1	483,60±9,64^{b}
K2	419,50±0,95^{a}
F1	419,04±0,78^{a}
F2	418,84±0,17^{a}
F3	418,95±0,18^{a}

Different letter notations show significant differences between formulations ($p<0.05$) in the same column. KI = yoghurt with 100% of pure milk; K2 = yoghurt with 100% of cowpea's milk; F1 = yoghurt with 98% of cowpea's milk and gotu kola leaf's extract variance of 2%; F2 = yoghurt with 96% of cowpea's milk and gotu kola leaf's extract variance of 4%; F3 = yoghurt with 94% of cowpea's milk and gotu kola leaf's extract variance of 6%.

TABLE 2. Results of the chemical properties analysis of cowpeas yoghurt with the addition of gotu kola leaf extract

Sample	pH value	Moisture content (%)	Ash content (%)	Protein content (%)	Fat content (%)	Carbohydrate content (%)
K1	4.01±0.08^{c}	79,78± 0,28^{a}	0,91± 0,14^{c}	3,40± 0,06^{d}	1,13± 0,06^{a}	14,79± 0,30^{b}
K2	3.77±0.02^{b}	84,35± 0,74^{b}	0,62± 0,03^{b}	2,95± 0,05^{a}	1,87± 0,07^{e}	10,21± 0,72^{a}
F1	3.72±0.01^{ab}	84,74± 0,14^{b}	0,55± 0,08^{a}	3,25± 0.03^{c}	1,62± 0,00^{d}	9,84± 0,17^{a}
F2	3.70±0.03^{ab}	84,87± 0,29^{b}	0,57± 0,01^{ab}	3,19± 0,06^{c}	1,51± 0,04^{c}	9,90± 0,29^{a}
F3	3.69±0.02^{a}	85,04± 0,07^{b}	0,59± 0,03^{ab}	3,07± 0,04^{b}	1,39± 0,01^{b}	9,90± 0,09^{a}

Different letter notations show significant differences between formulations (p<0.05) in the same column. KI = yoghurt with 100% of pure milk; K2 = yoghurt with 100% of cowpea's milk; F1 = yoghurt with 98% of cowpea's milk and gotu kola leaf's extract variance of 2%; F2 = yoghurt with 96% of cowpea's milk and gotu kola leaf's extract variance of 4%; F3 = yoghurt with 94% of cowpea's milk and gotu kola leaf's extract variance of 6%

Purwantiningsih et al. (2022) shows that the addition of a starter to yoghurt samples can increase the production of lactic acid and organic acids, resulting in a more acidic environment when more bacterial types were used.

Table 2 shows the results of testing the moisture content of yoghurt samples ranging from 79.78% to 85.04%. The National Standardization Agency of Indonesia (2009) stated that yoghurt products with plant-based protein (legumes) should have moisture content between 83% and 85%. This indicates that the moisture content of K2, F1, and F2 meets the SNI requirements, while the moisture content of F3 does not meet the SNI requirements. K2, F1, F2, and F3 show an increase in yoghurt's moisture content as more as gotu kola leaf extract was added. Nur, Devi and Hidayati (2017) shows that fresh gotu kola leaf contains approximately 85.46% water. The high moisture content in gotu kola leaf results an increase of the moisture content of cowpeas yoghurt as more as extract was added in the treatment. The moisture content of yoghurt samples can also be influenced by the type of bacterial starter used. In this study, Biokul plain was used as the starter, which contains four types of lactic acid bacteria (LAB). The more bacterial types contained the more nutrients needed by LAB during the fermentation process. Water is one of the nutrients required for the growth of LAB (Chaudhary & Pankaj 2018).

Table 2 shows the results of testing the ash content of yoghurt samples, ranging from 0.55% to 0.91%. The National Standardization Agency of Indonesia (2009) stated that the quality requirement for ash content in yoghurt is a maximum of 1%. This indicates that the ash content of all yoghurt samples meets the SNI requirements. The ash content reflects the amount of minerals present in a food substance. The ash content of K2, F1, F2, and F3 was influenced by the relatively high mineral content in cowpeas yoghurt. Dlamini (2016) reported that in 100 g of cowpeas, there were approximately 110 mg of calcium, 8.3 mg of iron, 184 mg of magnesium, 424 mg of phosphorus, 1112 mg of potassium, and 16.2 mg of sodium. The increase in the ash content in F1, F2, and F3 with the addition of more extract can be attributed to the mineral content in gotu kola. Sutardi (2016) shows that many minerals such as iron, phosphorus, potassium, magnesium, calcium, and sodium can be found in gotu kola plants. Fresh gotu kola contains about 6.064% ash (Nur, Devi & Hidayati 2017). The ash content of yoghurt samples can also be affected by the fermentation process carried out by the bacterial starter. Lactic acid bacteria produce lactic acid from the breakdown of lactose into glucose and generate minerals like magnesium as by-products of the fermentation process. Naila Maziya, Avliya Quratul and Nanang (2020) stated that fermented soy (soygurt) can increase the mineral content it contains, particularly calcium, magnesium, and zinc as by-products.

Table 2 displays the results of testing the protein content of yoghurt samples, ranging from 2.95% to 3.40%. The National Standardization Agency of Indonesia (2009) stated that the quality requirement for yoghurt is a minimum protein content of 2.7%. The protein content of yoghurt can be determined by the quantity of ingredients added (Nugroho et al. 2023). The higher protein content in the ingredients, the more it can increase the protein content of the yoghurt. F1, F2, and F3 show a decrease in protein content as more as gotu kola leaf extract was added because the amount of cowpea's milk used was reduced. According to Poedjiadi (2006), cowpeas contain relatively high protein content, about 24.4 g per 100 g. Gotu kola plants contain 2.4 g of protein per 100 g (Joshi & Chaturvedi 2013). This indicates that cowpea's protein content was higher than that of gotu kola. Thus, F3 has the lowest protein content which is 3.07%, because it used the least cowpea's milk. The protein content of yoghurt samples can also be influenced by the protein content in each of their additional ingredients and the bacterial starter used. The addition of skim milk to all samples can increase the protein content of yoghurt, as skim milk contains approximately 3.7% protein in 100 grams (Ramadhan 2016). Biokul plain starter contains four types of lactic acid bacteria (LAB), namely *Lactobacillus bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus*, and Bifidobacterium. The more types of lactic acid bacteria (LAB) it contains, the higher the protein content because the cellular components of LAB also contain protein (Yeyen, Mahargono & Nurdeana 2016). K2 has the lowest protein content which is 2.95%. The process of making cowpeas yoghurt involves several rounds of heating (using high temperatures) on its main ingredients, such as boiling cowpeas and pasteurizing cowpea's milk. According to Sumiati (2010), the use of high temperatures can cause protein denaturation, leading to the formation of coagulation that reduces solubility or the ability to dissolve.

Table 2 shows the results of testing the fat content of yoghurt samples between 1.13% and 1.87%. The National Standardization Agency of Indonesia (2009) states that the fat content for yoghurt is a maximum of 3.3%; low-fat yoghurt (YRL) is 0.6% to 2.9%; and non-fat yoghurt (YTL) is <0.5%. This shows that all yoghurt samples belong to low-fat yoghurt (YRL). The fat content in yoghurt was strongly influenced by the fat content of the main raw materials and mixed ingredients when making yoghurt (Weerathilake et al. 2014). K2, F1, F2, and F3 showed a decrease in fat content along with the addition of extracts because the amount of cowpea's milk used would be less. According to Poedjiadi (2006), the fat content in cowpea is 1.40 g in 100 g. Gotu kola contains about 0.27% fat in its 100 g (Wawan et al. 2019). This indicates that the fat content in cowpea is higher than the fat content in gotu kola. Thus, F3 produced the lowest fat content of 1.39% because cowpea's milk was used the least.

Table 2 shows the results of testing the carbohydrate content of yoghurt samples between 9.84% and 10.21%. Analysis of carbohydrate levels using the AOAC (2005) by difference method, so that the carbohydrate levels depend on the reducing factor. F1, F2, and F3 showed an increase in carbohydrate levels along with the increasing number of extracts added. Natalini, Edy Djauhari and Putri Karina (2009) stated that fresh gotu kola plant contains about 6.9 g of carbohydrates in its 100 g. F3 had the highest carbohydrate content because gotu kola extract was added the most. K2 produced the highest carbohydrate content compared to F1, F2, and F3, which was 10.21%. According to Poedjiadi (2006), cowpeas contain about 59.10 g of carbohydrates in its 100 g. Thus, K2 had the highest carbohydrate content because it used 100% cowpea's milk in its sample. The carbohydrate levels of yoghurt samples can also be affected by the bacterial starter used. Biokul plain starter contains 4 types of lactic acid bacteria (LAB). According to Chaudhary and Pankaj (2018), LAB utilizes sugar (carbohydrates) as a nutrient for growth.

Table 3 shows the results of testing the antioxidant activity of yoghurt samples with IC_{50} values ranging from 87.01 ppm to 204.33 ppm and Activity Antioxidant Index (AAI) values ranging from 0.98 to 2.30. According to Syaifuddin (2015), the lower the IC_{50} value, the stronger the antioxidant activity. The AAI value is a method used to standardize antioxidant test results based on the DPPH method. The AAI value was used to classify the antioxidant properties of the test samples (Vasić et al. 2012). The differences in the antioxidant activity values of yoghurt samples were due to the combination of various antioxidants present in the overall ingredients (Samichah & Syauqy 2014). K2, F1, F2, and F3 show an increase in antioxidant activity as more gotu kola leaf extract was added. Yogeswaran et al. (2016) stated that gotu kola contains triterpenoids, which were active compounds with strong antioxidant properties. According to Nina, Rizna Triana and Faiza (2014), methanol extracts of gotu kola leaves contain a total triterpenoid content of 245.04 mg BE/g of the extract. Natural antioxidant compounds commonly found in vegetables or green leaves were chlorophyll. Gotu kola leaves have a chlorophyll content of 831.5 mg/kg (Nurdin et al. 2009).

Another ingredient used in yoghurt production such as cowpeas can also influence the antioxidant activity of the samples. Cowpeas were believed to have fairly strong antioxidant content. According to Yulistian et al. (2015), cowpea's seed extract in 80% acetone contains a total phenolic content of 8,081.4 mg GAE/g, a total flavonoid content of 33,308.3 mg QE/g, and an antioxidant activity of 25,973.3 mg TE/g. The antioxidant activity of yoghurt can also be influenced by the fermentation process carried out by lactic acid bacteria. Af'idah and Trimulyono (2019) stated that an increase in lactic acid levels during the fermentation process can trigger increased antioxidant activity. This study showed that yogurt product from cowpeas combination with gotu kola leaves has an influence on the microbiological property. Microbiological property of cowpeas yogurt can be seen at Table 4.

TABLE 3. Results of the antioxidant activity analysis of cowpeas yoghurt with the addition of gotu kola leaf extract

Sample	IC_{50} (ppm)	AAI (Activity antioxidant index)
K1	204.33±0.68^{f}	0.98±0.00^{a}
K2	152.47±0.36^{e}	1.31±0.01^{b}
F1	148.33±3.30^{d}	1.35±0.03^{b}
F2	114.65±1.22^{c}	1.74±0.02^{c}
F3	87.01±0.38^{b}	2.30±0.01^{d}
Vitamin C	7.57±0.052^{a}	26.42±0.18^{e}

Different letter notations show significant differences between formulations (p<0.05) in the same column. KI = yoghurt with 100% of pure milk; K2 = yoghurt with 100% of cowpea's milk; F1 = yoghurt with 98% of cowpea's milk and gotu kola leaf's extract variance of 2%; F2 = yoghurt with 96% of cowpea's milk and gotu kola leaf's extract variance of 4%; F3 = yoghurt with 94% of cowpea's milk and gotu kola leaf's extract variance of 6%

TABLE 4. Microbiological analysis results of cowpeas yoghurt with the addition of gotu kola leaf extract

Sample	Total LAB (CFU/mL)
K1	$2,11 \times 10^{7}$
K2	$2,05 \times 10^{7}$
F1	5×10^{6}
F2	6×10^{6}
F3	6×10^{5}

Different letter notations show significant differences between formulations (p<0.05) in the same column. KI = yoghurt with 100% of pure milk; K2 = yoghurt with 100% of cowpea's milk; F1 = yoghurt with 98% of cowpea's milk and gotu kola leaf's extract variance of 2%; F2 = yoghurt with 96% of cowpea's milk and gotu kola leaf's extract variance of 4%; F3 = yoghurt with 94% of cowpea's milk and gotu kola leaf's extract variance of 6%

Table 4 shows the results of testing the total lactic acid bacteria (LAB) in yoghurt samples ranging from 6×10^5 to 2.11×10^7. The National Standardization Agency of Indonesia (2009) stated that the quality requirement for total lactic acid bacteria (LAB) in yoghurt is a minimum of 10^7. This indicated that the total LAB in K1 and K2 meet the SNI requirements, while F1, F2, and F3 did not meet the SNI requirements. K1 has the highest total LAB compared to the other samples because the type of milk used (pure milk) contains lactose (carbohydrate) which is the most suitable nutrient source for the growth of LAB. Muhamad Raihan, Wendry Setiyadi and Roostita (2020) stated that during the fermentation process, lactose in milk was broken down into lactic acid and organic acid by the starter bacteria. The number of lactic acid bacteria will multiply and grow faster with an increase in the lactose content of the milk, resulting in a greater overall production of lactic acid. K2, F1, F2, and F3 produce fewer total LAB compared to K1 because legumes (the primary ingredient in cowpea's milk) contain sugars called raffinose and stachyose, not lactose.

The reduction in total lactic acid bacteria (LAB) in yoghurt was also influenced by other factors, such as the dilution used during bacterial inoculation, temperature, and the bacteria's optimum pH. In this study, the dilution used was the last three dilutions (10^{-4}, 10^{-5}, and 10^{-6}). The higher the dilution of a suspension, the more it can result in a reduction in the number of bacteria present. The addition of gotu kola leaf extract to F1, F2, and F3 can affect the environment to become more acidic during the fermentation process. Acidic conditions lead to the accumulation of H^+ ions inside the cells, causing an electrolyte imbalance in bacterial cells. This makes bacteria try to expel H^+ ions, and indirectly, bacteria will release a large amount of ATP, leading to cell death (Archer 2004). Therefore, the bacterial suspension in yoghurt samples may have already been damaged or even died before the fermentation process was completed. This study showed that yogurt product from cowpeas combination with gotu kola leaves has an influence on the organoleptic properties. Organoleptic properties of cowpeas yogurt can be seen in Table 5.

Color is a sensory attribute that can be used to assess the quality of food products and can attract the attention of consumers. Table 5 shows that the highest level of preference for the color of yoghurt was found in K1 at 4.43 ± 0.73^c, which means that the panelists like the color of K1, which is white. The lowest level of preference for the color of yoghurt was found in F3 at 3.60 ± 1.04^a, indicating that panelists like the color of F3, which is off-white to yellowish. The National Standardization Agency of Indonesia (2009) stated that a good yoghurt should have a white color. Therefore, K1 meets the SNI requirements because the color of the yoghurt was milky white. F3 produces off-white to yellowish color because it contains the highest addition of 6% gotu kola leaf extract. The change in color was due to the presence of pigments (chlorophyll) found in gotu kola leaves (Miftah 2014).

The aroma of a product plays a significant role in determining consumer acceptance. Table 5 shows that the highest level of preference for the aroma of yoghurt was found in K1 at 4.00 ± 0.87^b, which means that panelists like the aroma of K1, which has the characteristic sour milk aroma. The lowest level of preference for the aroma of yoghurt was found in F2 at 2.73 ± 0.87^a, indicating that panelists did not like the aroma of F2, which has a strong leafy aroma. The analysis results indicated that the more gotu kola leaf extract was added, the lower the panelists' preference for the aroma of yoghurt. Sutardi (2016) stated that gotu kola contains bitter substances (vellarine) and tannin. The presence of these substances

TABLE 5. Results of the organoleptic properties analysis of cowpeas yoghurt with the addition of gotu kola leaf extract

Sample	Color	Aroma	Taste	Aftertaste (Bitterness)	Texture	Overall
K1	4.43 ± 0.73^c	4.00 ± 0.87^b	4.43 ± 0.63^c	4.20 ± 0.76^d	4.23 ± 0.68^b	4.33 ± 0.66^a
K2	4.07 ± 0.64^{bc}	3.67 ± 0.66^b	3.70 ± 0.79^b	3.77 ± 0.77^c	3.63 ± 0.96^a	3.50 ± 0.86^b
F1	3.83 ± 0.70^{ab}	2.93 ± 0.98^a	3.03 ± 1.00^a	3.23 ± 0.86^b	3.40 ± 0.81^a	3.03 ± 0.67^c
F2	3.70 ± 0.92^{ab}	2.73 ± 0.87^a	3.00 ± 0.91^a	3.03 ± 0.85^b	3.40 ± 0.86^a	2.63 ± 0.62^d
F3	3.60 ± 1.04^a	2.73 ± 1.05^a	2.77 ± 0.90^a	2.60 ± 0.97^a	3.47 ± 0.90^a	1.97 ± 0.67^e

Different letter notations show significant differences between formulations (p<0.05) in the same column. KI = yoghurt with 100% of pure milk; K2 = yoghurt with 100% of cowpea's milk; F1 = yoghurt with 98% of cowpea's milk and gotu kola leaf's extract variance of 2%; F2 = yoghurt with 96% of cowpea's milk and gotu kola leaf's extract variance of 4%; F3 = yoghurt with 94% of cowpea's milk and gotu kola leaf's extract variance of 6%

indirectly affects the aroma of F2. In addition to being influenced by the leafy aroma, the sample's aroma was also affected by the beany aroma of cowpeas. According to Ekafitri and Isworo (2014), a common issue in processing peanuts into peanut milk was the development of a beany odor caused by the presence of lipoxygenase enzymes in peanut seeds. The National Standardization Agency of Indonesia (2009) states that good yoghurt should have a characteristic aroma. Therefore, K1 meets the SNI requirements because it has the characteristic sour milk aroma, which is typical of yoghurt in general.

Taste is a sensory parameter assessed by the sense of taste and is highly important in determining consumer acceptance. Table 5 shows that the highest level of preference for the taste of yoghurt was found in K1 at 4.43±0.63[c], which means that panelists like the taste of K1, which has a slightly sour (sweet) taste. The lowest level of preference for the taste of yoghurt was found in F3 at 2.77±0.90[a], indicating that panelists did not like the taste of F3, which has a slightly sour (slightly sweet) taste. The analysis results indicated that the more gotu kola leaf extract was added, the lower the panelists' preference for the taste of yoghurt. The addition of 6% gotu kola leaf extract to F3 resulted in a very bitter taste and a sour (astringent) taste, leading to a dislike of the taste of yoghurt by the panelists. According to Sutardi (2016), the presence of vallerine and tannin in gotu kola can lead to a bitter-astringent taste. The National Standardization Agency of Indonesia (2009) stated that good yoghurt should have a characteristic sour taste. Therefore, K1 and K2 meet the SNI requirements because they have the characteristic sour (slightly sweet) taste of yoghurt that aligns with panelist preferences.

Table 5 shows that the highest level of preference for the aftertaste (bitterness) of yoghurt was found in K1 at 4.20±0.76[d], which means that panelists like the aftertaste of K1, and the aftertaste of the sample was not bitter. The lowest level of preference for the aftertaste of yoghurt was found in F3 at 2.60±0.97[a], indicating that panelists do not like the aftertaste of F3, and the aftertaste of the sample was very bitter. The analysis results indicated that the more gotu kola leaf extracts were added, the lower the panelists' preference for the aftertaste of yoghurt. F3 had the highest addition of gotu kola leaf extract, which was 6%. The more extract added to the sample, the stronger the aftertaste (bitterness) produced. Sutardi (2016) stated that the bitterness in gotu kola was caused by the presence of bitter substances like vallerine and tannin, which contain concentrated acid. Both compounds make the aftertaste of F3 undesirable and less acceptable to the panelists.

Texture is a sensory parameter that can be evaluated by the sense of touch (taste). Table 5 shows that the highest level of preference for the texture of yoghurt was found in K1 at 4.23±0.68[b], meaning that panelists like the texture of K1, and the texture of the sample was very thick. The lowest level of preference for the texture of yoghurt was found in F1 at 3.40±0.81[a], indicating that panelists were satisfied with the texture of F3, and the texture of the sample was thick. In this study, the majority of the yoghurt produced has a soft and homogenous (thick) texture. Therefore, all yoghurt samples have a homogenous texture.

The overall assessment was a combination of what was seen, what was touched, and what was felt (Riski, Faizah & Raswen 2016). Table 5 shows that the highest level of preference for the overall yoghurt was found in K1 at 4.33±0.66[a], indicating that panelists like the overall K1. The lowest level of preference for the overall yoghurt was found in F3 at 1.97±0.67[e], which means that panelists strongly dislike the overall F3. The analysis results indicate that K1 was most preferred by the panelists because its color, aroma, taste, and texture resemble the criteria of yoghurt available in the market. On the other hand, F3 was the least preferred because the aroma, taste, and strong bitter aftertaste from gotu kola leaves reduce the overall rating of the yoghurt sample.

Conclusions

The addition of gotu kola leaf extract significantly affects the physicochemical (viscosity, pH value, moisture content, ash content, protein content, fat content, carbohydrate content, and antioxidant activity), microbiological (total lactic acid bacteria), and organoleptic properties of cowpeas yoghurt.

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REFERENCES

Aduol, K.O., Onyango, A.N. & Imathiu, S.M. 2020. Proximate, microbial and sensory characteristics of cowpea milk fermented with probiotic starter cultures. *European Journal of Agriculture and Food Sciences* 2(4): 1-7.

Af'idah, F. & Trimulyono, G. 2019. Uji aktivitas antioksidan dan kadar asam laktat yoghurt tempe kedelai (*Glycine max*) dan yoghurt tempe kacang hijau (*Vigna radiata*). *LenteraBio* 8(1): 17-24.

Archer, D.L. 2004. Freezing: An underutilized food microbiology. *International Journal of Food Microbiology* 90(2): 127-138.

Ariviani, S., Sholihin, N.H. & Nastiti, G.P. 2021. Pengembangan tepung kecambah kacang tunggak (*Vigna unguiculata*) sebagai sereal fungsional kaya serat pangan dan berpotensi antioksidan. *Jurnal Teknologi Hasil Pertanian* 14(2): 84.

Aswal, A., Kalra, M. & Rout, A. 2013. Preparation and evaluation of polyherbal cosmetic cream. *Der Pharmacia Lettre* 5(1): 83-88.

Aydar, E.F., Tutuncu, S. & Ozcelik, B. 2020. Plant-based milk substitutes: Bioactive compounds, conventional, and novel processes, bioavaibility studies, and health effects. *Journal of Functional Foods* 70: 103975.

Benmeziane, F., Raigar, R.K., Ayat, N.E.H., Aoufi, D., Djermoune-Arkoub, L. & Chala, A. 2021. Lentil (*Lens culinaris*) flour addition to yoghurt: Impact on physicochemical, microbiological and sensory attributes during refrigeration storage and microstructure changes. *LWT* 140: 110793.

BSN. 2009. *SNI 2981: 2009*. Jakarta: Badan Standardisasi Nasional.

Cai, R., Hettiarachchy, N.S. & Jalaluddin, M. 2003. High-performance liquid chromatography determination of phenolic constituents in 17 varietes of cowpea. *J. Agric. Food Chem.* 51(6): 1623-1627.

Carlsen, M.H., Halvorsen, B.L., Holte, K., Bøhn, S.K., Dragland, S., Sampson, L., Willey, C., Senoo, H., Umezono, Y., Sanada, C., Barikmo, I., Berhe, N., Willett, W.C., Phillips, K.M., Jacobs, D.R. & Blomhoff, R. 2010. The total antioxidant content of more than 3100 foods, beverages, spices, herbs and supplements used worldwide. *Nutrition Journal* 9(3): 1-11.

Chaudhary, S. & Pankaj, A. 2018. Dates and diabetes. *Journal of Social Health and Diabetes* 6(2): 109-110.

Chawafambira, A., Jombo, T.Z. & Mkungunugwa, T. 2022. Effect of *Lacticaseibacillus rhamnosus* yoba fermentation on physicochemical properties, amino acids, and antioxidant activity of cowpea-peanut milk. *Journal of Food Quality* 2022: 3192061.

Direktorat Gizi Depkes RI. 2009. *Daftar Komposisi Zat Gizi Pangan Indonesia*. Jakarta: Departemen Kesehatan RI.

Dlamini, N.P. 2016. Sensory and nutritional quality of an extruded sorghum and cowpea blend as a complementary food for school age children. Degree Dissertation. Faculty of Natural and Agricultural Sciences. University of Pretoria (Unpublished).

Edy Permadi, Fitri Suciati & Retno Budi Lestari. 2021. Kualitas yoghurt susu kambing PE dengan suplementasi ekstrak buah lakum terhadap viskositas, total asam dan total padatan terlarut. *Jurnal Sains Peternakan* 9(1): 40-47.

Ekafitri, R. & Isworo, R. 2014. Pemanfaatan kacang-kacangan sebagai bahan baku sumber protein untuk pangan darurat. *Jurnal PANGAN* 23(2): 134-145.

Fardiaz, S. 1993. *Analisis Mirobiologi Pangan.* Jakarta: Raja Grafindo Persada.

Gibson, G.R., Scott, K.P., Rastall, R.A. & Tuohy, K.M. 2010. Dietary prebiotics: Current status and new definition. *Food Science and Technology Bulletin: Functional Foods* 7(1): 1-19.

Handajani, A., Roosihermiatie, B. & Maryani, H. 2010. Faktor-faktor yang berhubungan dengan pola kematian pada penyakit degeneratif di Indonesia. *Buletin Penelitian Sistem Kesehatan* 13(1): 42-53.

Hidayat, I.R., Kusrahayu & Mulyani, S. 2013. Total bakteri asam laktat, nilai pH, dan sifat organoleptik *drink yoghurt* dari susu sapi yang diperkaya dengan ekstrak buah mangga. *Animal Agriculture Journal* 2(1): 160-167.

Horwitz, W. & Latimer Jr. G.W. 2005. *Official Methods of Analysis of AOAC International* 18th ed. USA: AOAC International.

Ivan Sugianto, Dwi Kristiastuti Suwardiah, Niken Purwidiani, & Asrul Bahar. 2020. Pengaruh penambahan susu skim dan yoghurt plain terhadap sifat organoleptik yoghurt sari jagung. *JTB* 9(2): 829-837.

Joshi, K. & Chaturvedi, P. 2013. Therapeutic efficiency of *Centella asiatica* (L.) Urb. an underutilized green leafy vegetable: An overview. *International Journal of Pharma and Bio Sciences* 4(1): 135-149.

Natalini Nova Kristina, Edy Djauhari Kusumah & Putri Karina Lailani. 2009. Analisis fitokimia dan penampilan polapita protein tanaman pegagan (*Centella asiatica*) hasil konservasi *in vitro. Bul. Littro* 20(1): 11-20.

Kusharto, C.M. 2006. Serat makanan dan peranannya bagi kesehatan. *Jurnal Gizi dan Pangan* 1(2): 45-54.

Kusuma, Y.D. 2020. Pemberian ekstrak daun pegagan dengan prebiotik *Lactobacillus acidophilus* terhadap persentase lemak abdominal dan karkas itik hibrida. Surabaya: Universitas Airlangga (Unpublished).

Mohamed Ahmed, I.A., Alqah, H.A.S., Saleh, A., Al-Juhaimi, F.Y., Babiker, E.E., Ghafoor, K., Hassan, A.B., Osman, M.A. & Fickak, A. 2021. Physicochemical quality attributes and antioxidant properties of set-type yoghurt fortified with argel (*Solenostemma argel* hayne) leaf extract. *LWT* 137: 110389.

Melia, S., Purwati, E., Yuherman, Jaswandi, Aritonang, S.N. & Silaen, M. 2017. Characterization of the antimicrobial activity of lactic acid bacteria isolated from buffalo milk in West Sumatera (Indonesia) against *Listeria monocytogenes*. *Pakistan Journal of Nutrition* 16(8): 645-650.

Miftah Nur Azizah 2014. Kadar betakaroten dan organoleptik minuman daun pegagan hijau instan dengan penambahan konsentrasi gula pasir yang berbeda. Thesis. Universitas Muhammadiyah Surakarta (Unpublished).

Molyneux, P. 2004. The use of the stable radical diphenyl picryl hydrazyl (DPPH) for estimating antioxidant activity. *Journal of Science and Technology* 26(2): 211-219.

Muhamad Raihan Aufa, Wendry Setiyadi Putranto & Roostita L Balia. 2020. Pengaruh penambahan konsentrasi jus jambu biji merah (*Psidium guajava* L.) terhadap kadar asam laktat, Vitamin C, dan akseptabilitas set yoghurt. *Jurnal Teknologi Hasil Peternakan* 1(1): 8-16.

Naila Maziya Labiba, Avliya Quratul Marjan & Nanang Nasrullah. 2020. Pengembangan soyghurt (yoghurt susu kacang kedelai) sebagai minuman probiotik tinggi isoflavon. *IAGIKMI & Universitas Airlangga* 4(3): 244-249.

Nina Artanti, Rizna Triana Dewi & Faiza Maryani. 2014. Pengaruh lokasi dan pelarut pengekstraksi terhadap kandungan fitokimia dan aktivitas antioksidan ekstrak pegagan (*Centella asiatica* L. Urb). *Jurnal Kimia Terapan Indonesia* 16(2): 88-92.

Nina Salamah & Liani Farahana. 2014. Uji aktivitas antioksidan ekstrak etanol herba pegagan (*Centella asiatica* (L.) Urb) dengan metode fosfomolibdat. *Pharmaciana* 4(1): 23-30.

Nugroho, M.R., Wanniatie, V., Qisthon, A. & Septinova, D. 2023. Sifat fisik dan total bakteri asam laktat (BAL) yoghurt dengan bahan baku susu sapi yang berbeda. *Jurnal Riset dan Inovasi Peternakan* 7(2): 279-286.

Nur, A.A.K., Devi, M. & Hidayati, L. 2017. Pengaruh penambahan pegagan (*Centella asiatica* (L.) Urban) terhadap daya terima dan mutu kerupuk. *Jurnal Aplikasi Teknologi Pangan* 6(3): 109-114.

Nurdin, Kusharto, C.M., Tanziha, I. & Januwati, M. 2009. Kandungan klorofil berbagai jenis daun tanaman dan Cu-turunan klorofil serta karakteristik fisiko-kimianya. *Jurnal Gizi Pangan* 4(1): 13-19.

Poedjiadi, A. 2006. *Dasar-Dasar Biokimia 2006*. Jakarta: UI-Press.

Rahayu, Ni Komang Triana, I Dewa Gede Mayun Permana & G.A. Kadek Diah Puspawati. 2021. Pengaruh waktu maserasi terhadap aktivitas antioksidan ekstrak daun pegagan (*Centella asiatica* (L.) Urban). *Itepa: Jurnal Ilmu Dan Teknologi Pangan* 10(2): 163-171.

Ramadhan, F. 2016. Pengaruh konsentrasi susu skim dan suhu fermentasi terhadap karakteristik yoghurt kacang koro (*Canavalia ensiformis* L). Skripsi. Bandung : Program Studi Teknologi Pangan, Universitas Pasundan (Unpublished).

Rut Asih Setiawati & Maria Marina Herawati. 2018. Pengaruh Penambahan Sari Buah Jeruk Siam dalam Pemanfaatan Kacang Gude (*Cajanus cajan*) sebagai yoghurt. *Prosiding Konser Karya Ilmiah Peluang dan Tantangan Pembangunan Pertanian Berkelanjutan di Era Global dan Digital*, 13 September, Fakultas Pertanian dan Bisnis, Universiti Kristen Satyawacana, Salatiga.

Sarker, A. & Siddiqui, R.A. 2023. Effects of ultrasonic processing on the quality properties of fortified yoghurt. *Ultrasonics Sono Chemistry* 98: 106533.

Sebastiani, G., Barbero, A.N., Borrás-Novell, C., Casanova, M.A., Aldecoa-Bilbao, V., Andreu-Fernández, V. & García-Algar, O. 2019. The effects of vegetarian and vegan diet during pregnancy on the health of mothers and offspring. *Nutrients* 11(3): 557.

Sumiati, T. 2010. Pengaruh pengolahan terhadap mutu cerna protein ikan mujair (*Tilapia mossambica*). Bogor: Institut Pertanian Bogor.

Sutardi, S. 2016. Kandungan bahan aktif tanaman pegagan dan khasiatnya untuk meningkatkan sistem imun tubuh. *Jurnal Litbang Pertanian* 35(3): 121-130.

Syaifuddin. 2015. Uji aktivitas antioksidan bayam merah (*Alternanthera amoena* Voss.) segar dan rebus dengan Metode DPPH (1,1-Diphenyl-2-Picylhydrazyl). Semarang: Universitas Islam Negeri Walisongo.

Samichah, S. & Syauqy, A. 2014. Aktivitas antioksidan dan penerimaan organoleptik yoghurt sari wortel (*Daucus carrota* L). *Journal of Nutrition College* 3(4): 501-508.

Riski Tanjung, Faizah Hamzah & Raswen Efendi. 2016. Lama fermentasi terhadap mutu teh Daun Sirsak (*Annona muricata* L.). *JOM Faperta UR* 3(2).

Theresia I. Purwantiningsih, Maria Adlofina B. Bria & Kristoforus W. Kia. 2022. Kadar protein dan lemak yoghurt yang terbuat dari jenis dan jumlah kultur yang berbeda. *Journal of Tropical Animal Science and Technology* 4(1): 66-73.

U.S. Department of Agriculture (USDA). 2016. *Basic Report: Nutrient Data for: 16060, Cowpeas, Catjang, Mature Seeds, Raw*. Washington D.C.

Uwa, E.R. 2019. Uji kandungan protein total, asam total dan kesukaan yoghurt canglo (Kacang Tolo) dengan variasi berat daun pandan (*Pandanus amaryllifolius* Roxb.). Yogyakarta: Universitas Sanata Dharma (Unpublished).

Vasić, S.M., Stefanović, O.D., Ličina, B.Z., Radojević, I.D. & Čomić, L.R. 2012. Biological activities of extracts from cultivated Granadilla Passiflora alata. *EXCLI Journal* 11: 208-218.

Wawan Agustina, Feni Khoerunisa, Taufik Rahman & Lia Ratnawati. 2019. Karakterisasi dan pengujian aktivitas antimikroba minuman probiotik antanan (*Centella asiatica* (L.)). *Jurnal Riset Teknologi Industri* 13(1): 88.

Weerathilake, W.A.D.V., Rasika, D.M.D., Ruwanmali, J.K.U. Ruwanmali & Munasinghe M.A.D.D. 2014. The evolution, processing, varieties and health benefits of yoghurt. *International Journal of Scientific and Research Publications* 4(4): 1-10.

Yeyen Prestyaning Wanita, Mahargono Kobarsih, & Nurdeana Cahyaningrum. 2016. Sifat fisik yoghurt sawo (*Achras zapota*). In *Seminar Nasional Inovasi Teknologi Pertanian*. Banjarbaru.

Yogeswaran Lokanathan, Norazzila Omar, Nur Nabilah Ahmad Puzi, Aminuddin Saim & Ruszymah Hj. Idrus. 2016. Recent updates in neuroprotective and neuroregenerative potential of *Centella asiatica. Malaysian Journal of Medical Sciences* 23(1): 4-14.

Yulian Candra Setianto, Yoyok Budi Pramono & Sri Mulyani. 2014. Nilai pH, viskositas, dan tekstur *yoghurt drink* dengan penambahan ekstrak salak pondoh (*Salacca zalacca*). *Jurnal Aplikasi Teknologi Pangan* 3(3): 110-113.

Yulistian, Dhoni Prielananta, Edi Priyo Utomo, Siti Mariyah Ulfa & Eriyanto Yusnawan. 2015. Studi pengaruh jenis pelarut terhadap hasil isolasi dan kadar senyawa fenolik dalam biji kacang tunggak (*Vigna unguiculata* (L.) Walp) sebagai antioksidan. *Kimia Student Journal* 1(1): 819-825.

Zainol, M.K., Abd-Hamid, A., Yusof, S. & Muse, R. 2008. Antioxidative Activity and total phenolic compounds of leaf, root and petiole of four accessions of *Centella asiatica* (L.) Urban. *Food Chemistry* 81(4): 575-581.

Zare, F., Champagne, C.P., Simpson, B.K., Orsat, V. & Boye, J.I. 2012. Effect of the addition of pulse ingredients to milk on acid production by probiotic and yoghurt starter cultures. *LWT–Food Science and Technology* 45: 155-160.

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