

Quality of Macronutrient of Cow's Milk with Addition of Soybean Oil and Phycocyanin Extract as Functional Food**Angela Nitia Nefasa^{1*}, Eudia Christina Wulandari², Marcelinus Christwardana³, H. Hadiyanto⁴**^{1,2}Study Program of Animal Husbandry Peternakan, Faculty of Animal Husbandry, Universitas of Boyolali, Boyolali, 57315, Indonesia.³Study Program of Chemical Engineering, Institut Teknologi Indonesia, Tangerang Selatan, 15314, Indonesia⁴Department of Chemical Engineering, Faculty of Engineering, Diponegoro University, Semarang, 50275, IndonesiaEmail: nnitia19@gmail.com; angelanitia@uby.ac.id**ABSTRACT**

The purpose of this study was to determine the further effect of soybean oil and phycocyanin extract against the quality of macronutrient (fat, protein, lactose) and also Solid Non-Fat (SNF) in cow's milk. Two different substances, i.e., soybean oil and different amount of phycocyanin extract was added into the milk. The result showed the increase of protein content gradually from 3.49 ± 0.230 to 3.88 ± 0.010 when phycocyanin was added. The inverse with the decreased fat content from the control sample, but after the addition of phycocyanin extract, it was increased gradually 6.15 ± 0.210 to 6.35 ± 0.21 . SNF has increased from 10.61 ± 0.014 to 10.87 ± 0.007 , while the decreasing level of lactose has been shown from 5.74 ± 0.021 to 5.62 ± 0.028 due to addition of phycocyanin. In conclusion, the addition of phycocyanin extract can increase the protein and SNF content of milk. This phycocyanin extract also acts as an antioxidant agent so that it can reduce the oxidation process, which might be caused by the addition of soybean oil. Soybean oil might be used as a food additive to improve the fat content of milk but must be accompanied by an antioxidant agent to prevent the oxidation process.

Keywords: cow's milk; fat; functional food; phycocyanin; soybean oil

INTRODUCTION

So far, milk and milk-based product have been known to play a role in the health sector. Functional food derivate from milk and its product has bee widely reviewed ((Boland, 2010; Bhat and Bhat, 2011; Gasmala et al., 2017). In recent years, many studies learn about the relationship between cow's milk with cardiovascular (Rice, 2014; Dehgan et al., 2018; Foncheta et al., 2019). Cardiovascular disease is one of the dangerous diseases that caused the highest number of deaths worldwide, not only in a developed country but also in developing countries (WHO, 2017), so it needs to be stopped. One way to reduce the risk of cardiovascular disease is to consume food that has a balanced ratio of omega-6 and omega-3 for about 4:1 (Simopoulus, 2006). However, the ratio of omega-6 and omega-3 of cow's milk is only between 1,6:1 (Haug et al., 2007). It is clearly seen that the ratio of the omega-6 content of milk is very lacking. Nefasa et al. (2014) were reported that soybean oil could increase the omega-6 of cow's milk in the manufacture of functional food products to prevent cardiovascular disease. Discussion of cow's milk as a functional food, in general, can not be separated from the discussion of its macronutrient content. Generally, it is known that macronutrients are the essential nutrients needed by the body in large quantities. Each macronutrient (carbohydrate, protein, fat) has a unique set of properties that affect health, but all of them are sources of energy, and the balance of energy determines of weight (Carreiro, 2016). Variations in macronutrient composition can cause weight gain, decrease, or remain constant (Leibel et al., 1992). Reported by Akil and Ahmad (2011) that obesity (overweight) can actually cause the risk of cardiovascular disease. Therefore, it is necessary to pay attention to macronutrient intake to reduce the risk of cardiovascular disease.

In this study, functional food products derived from cow's milk have been made as samples by considering the balance of the omega-6 and omega-3 ratios. The functional food is made from cow's milk with the addition of soybean oil and phycocyanin extract. Then the milk through the pasteurization process. Soybean oil was added to increase omega-6 cow's milk because soybean oil contains omega-6 with a high level of around 62.1 g / 100 ml (Haug et al., 2007). In contrast, phycocyanin extract was added to reduce the risk of oxidation in the product. This is because phycocyanin extract is a source of antioxidants extracted from microalgae (*Spirulina* sp.) (Hadiyanto et al., 2018). However, the main topic of this study is the quality of macronutrients.

So, the testing will be conducted on the quality of the cow's milk macronutrient as a functional food. The content of macronutrients included fat, protein, and lactose content. SNF (Solid Non-Fat) content will also be tested. The purpose of this study was to determine the further effects of soybean oil and phycocyanin extracts addition on the content of macronutrients (fat, protein, lactose) and also SNF in cow's milk. The benefit of this research is to provide additional information about cow's milk macronutrients as functional food products, especially to reduce the risk of cardiovascular disease.

METHODS

Material

Fresh milk is the main material for this study. It is obtained from the dairy farm at the Faculty of Animal Husbandry and Agriculture - Diponegoro University. Other materials such as soybean oil were obtained from a supermarket in Semarang; phycocyanin extract was obtained from the Center of Biomass and Renewable Energy - Diponegoro University. Sulfuric acid, amyl alcohol, potassium sulfate, copper (II) sulfate, sodium hydroxide, hydrochloric acid solution, all the material were purchased from Sigma Aldrich (St. Louis, USA).

Procedure for Making Pasteurized Milk

In this study, there was six milk sample, and each weight of cow's milk was 200 grams. Soybean oil was prepared 0,45% and phycocyanin extract 0%, 0.5%, 1.0%, 1.5%, 2% of total raw milk weight as shown in Table 1. Sample with no food additive was also prepared as a control. Next step, soybean oil was added for each sample also for phycocyanin extract and mixed. When it was done, the sample was ready to be pasteurized. The method of pasteurized milk using the low-temperature long time (LTLT) at temperature 63 °C for 30 minutes by using a water-bath DLAB DWB 20-P (Beijing, China). After the pasteurized process, the sample needs to be cooled for 5 minutes. Next, the sample was transferred to a bottle for laboratory analysis. The remaining unused sample will be stored at 4 °C.

Table 1. Composition of milk samples

Sample	Cow's Milk (g)	Soybean Milk (%)	Phycocyanin Extract (%)
T0	200	0	0
T1	200	0.45	0
T2	200	0.45	0.5
T3	200	0.45	1
T4	200	0.45	1.5
T5	200	0.45	2

Measurement of fat content

Measurement of fat content was adapted from FSSAI (2012) using the Gerber and Gerber Tube methods. First, 10 ml of sulfuric acid was added to the Gerber tube. Next, immediately add 10.75 ml of milk and 1 ml of amyl alcohol then the tube is closed tightly and shaken firmly. After that, the tubes were centrifuged at 1200 rpm for 5 minutes and the tubes were placed in a waterbath with a temperature of about 40°C. The result is that the milk fat will be separated, and the amount of fat is determined by looking at the scale on the Gerber tube.

Measurement of protein, lactose, and SNF

In this study, the protein content of pasteurized milk was measured by the Micro-Kjeldahl technique. This technique and its calculations are adapted from Kumar et al., (2007). The first step the sample was prepared about 10 ml, heated at 38 °C to 40 °C, and cooled to room temperature. Then the sample was transferred to a Kjeldahl flask. While waiting for the sample to cool, prepare a mixed reagent to convert the organic nitrogen to ammonium sulfate. Sulfuric acid, potassium sulfate, and copper (II) sulfate are used as reagents. Then, the reagent is added to the sample, mixed well, and boiled. After that, remove the ammonia using sodium hydroxide. When ammonia is released, it must be followed by distillation, collection, and titration with hydrochloric acid solution. In the end, the nitrogen content was calculated from the volume of the hydrochloric acid solution used at the time of the titration, then multiplied by 6.38 in order to get the crude protein content:

$$\text{crude protein (\%)} = 6,38 \times \frac{1.4007 (V_2 - V_1)N}{W} \times DF \frac{100}{10} \quad (1)$$

where, V_1 is the volume of the standard solution of hydrochloric acid used in the test blank; V_2 is the volume of the standard hydrochloric acid volumetric solution used in distillation; W is the weight of the sample taken for analysis (in grams); N is the normality of the standard volumetric solution of hydrochloric acid; DF is a dilution factor. Meanwhile, to measure SNF and lactose content of pasteurized milk using Lactoscan (Lactoscan, Bulgaria).

Statistical Analysis

In this study, the research experiment used was a completely randomized design (CRD) with five treatments and three replications. Analysis of variance. If the ANOVA test results are significant, it will be followed by Duncan's Multiple Range Test. Then, the analysis was continued with the Pearson Correlation Test

RESULT AND DISCUSSIONS

Fat content

The fat content of milk in the sample was 6.32% (T0), 6.4% (T1), 6.15% (T2), 6.35% (T3), 6.3% (T4), respectively. , 35% (T5). Based on the variant test, this data is not significant ($p > 0.05$). According to Haug et al, (2007) the average fat content of cow's milk is 3.3 g/100 ml. Bylund (2003) added that the minimum and maximum limit for the fat content of cow's milk is 3.0 g/ml to 6.0 g/ml. In general, this indicates that the fat content of pasteurized milk products with the addition of soybean oil and phycocyanin extract is classified as a good standard. However, the results obtained from testing the fat content showed a slightly high amount compared to the maximum limit of normal fat content in cow's milk. This increase in fat content was found in the control sample (T0). The high fat content in cow's milk is influenced by several factors including race/genetics, environment/management, health/physiology, and feed

nutrition factors (National Research Council, 1988). Fat contributes to the taste of milk, comfort when the product is in the mouth (taste in mouth) and the visual attributes of milk, and is also associated with the effect of creaminess (Carthy et al., 2017). The high fat content in milk causes a milky taste like creamy aroma, butter aroma, vanilla flavor, and causes a layer texture in the mouth, and higher viscosity (Richardson-Harman et al., 2000). From a visual perspective, an increase in fat content causes a whiter color to appear in milk (Phillips et al., 1995). When compared with the T0 sample, the fat content in the T1, T2, T3, T4 samples tends to decrease. The decrease in fat content in the milk sample is thought to be due to the soybean oil oxidation process. Soybean oil contains high levels of omega-6 around 62.1 g/100 g (Haug et al., 2007). Omega-6 is a polyunsaturated fatty acid (PUFA) which has two or more double bonds and in omega-6 the double bond is at the sixth carbon from the end of the chain (Ander et al, 2003). Pignitter and Somoza (2012) state that high concentrations of polyunsaturated fatty acids (PUFAs) make vegetable oils more susceptible to oxidation, resulting in loss of PUFAs due to the formation of volatile and non-volatile lipid oxidation products which are fast and volatile. Apart from the addition of soybean oil, the decrease in fat content in milk may also be influenced by the total loss of PUFAs that are naturally found in milk. According to Haug et al., (2007) cow's milk contains linoleic acid (LA) as the main PUFA (1.2 g/l) and alpha-linolenic acid (0.75 g/l). So far, the oxidation process that occurs due to the influence of processing technology which causes a reduction in the fat content of milk is being studied. Dunkley (1982) states that there are no technological problems limiting the possibility of fat reduction in milk processing. However, in Shanmugam et al, (2012) it is said that the non-thermal processing method with the sonication method can reduce the size of the casein and fat molecules in skim milk. The link between reducing the size of fat molecules and reducing the amount of fat content needs to be studied further. However, the oxidation process was slightly reduced when the sample (T1 to T4) was given additional phycocyanin extract. These samples showed a gradual increase in fat content from 6.15% to 6.35%. This increase indicates that phycocyanin can function as an antioxidant agent, so that the oxidation process can be inhibited. Phycocyanin is a source of antioxidants and natural dyes extracted from microalgae (*Spirulina* sp.) (Hadiyanto et al., 2018). Phycocyanin captures oxygen radicals because it contains an open tetraphyrol chain that can bind peroxy radicals by donating a hydrogen atom attached to the 10th C atom of a tetraphic molecule (Romay et al., 2003).

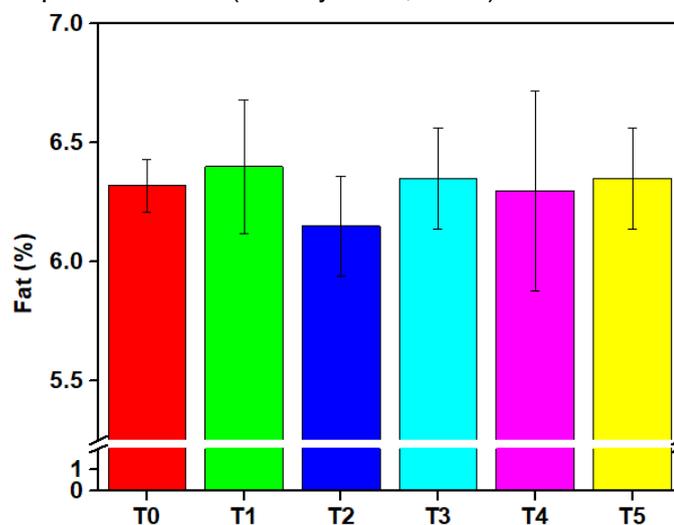


Figure 1. The effect of adding phycocyanin extract on fat content in cow's milk containing soybean oil

Protein Content

Based on the results obtained, the protein content of milk has gradually increased. The protein content for each sample was 3.47% (T0), 3.49% (T1), 3.51% (T2), 3.55% (T3), 3.79% (T4) and 3.88% (T5). The change in the amount of protein content was statistically insignificant ($p > 0.05$). The range of protein content in milk is usually between 3.2 g/100 ml (Haug et al, 2007). So, the results show the normal range. This increase was due to the addition of phycocyanin extract. This pigment is a complex protein compound that can be extracted from blue-green algae (cyanobacteria) *Spirulina* sp., And classified as a phycobiliprotein (Markou and Nerantzis, 2013). Phycocyanin is formed by protein a and b subunits of 17,000 and 19 500 Da and carries a prosthetic group of a linear isomeric tetrapyrrole group (one chromoporous bilin) attached to subunit (a 84) and two to subunit b (b 84, b 155) (Saranraj and Sivasakthi, 2014). In several studies, phycocyanin has been added as a nutritional compound in food, especially in dairy products. In a study by Suzery et al., (2017) using *Spirulina plantesis* supplements containing phycocyanin to increase the nutritional content of yogurt products. From the results of these studies it is known that the protein and mineral content of yogurt can be increased by the addition of *Spirulina plantesis* supplements. Protein content increased from 10.30 to 10.35 gr; the highest increase was the addition of supplements by 1.0%. Meanwhile, the mineral content increased from 2.21-2.34 gr along with the addition of supplements to 1.2%. *Spirulina* sp., Itself is rich in protein (60-70%) vitamins and minerals (Saranraj and Sivasakthi, 2014). Among the various *Spirulina* species, the blue green algae *Spirulina platensis* has attracted more attention as it shows a high nutritional content characterized by a protein content of 70% and in the presence of minerals, vitamins, amino acids, essential fatty acids etc. (Campanella et al., 1999). One gram of *Spirulina* protein is equivalent to one kilogram of various kinds of vegetables. The amino acid composition of *Spirulina* protein ranks among the best in the plant world, more so than soybeans (Saranraj and Sivasakthi, 2014). In addition, *Spirulina plantesis* supplements are also used to replace stabilizers in ice cream products, and as an additional starter in several fermented milk products such as plain yogurt, probiotic yogurt, acidophilus milk (Finamore et al., 2017). On the other hand, soybean oil makes a small contribution to increasing the protein content in milk because the majority of it is triglycerides. Milk protein affects not only sensory properties, but also the physical system and functionality of milk. As part of the sensory experience, protein provides a sense of taste in the mouth, viscosity and structure for milk and its products (Drake et al, 2014). A delicious taste sensation in the mouth can occur due to the colloid protein system and also fat clots (Cadwallader, 2010). In addition, protein can change the texture of food into a gel form, if so, it can reduce taste pleasure due to inhibition of mass transfer (Jaime et al., 1993; Carr et al., 1996; Wilson and Brown 1997).

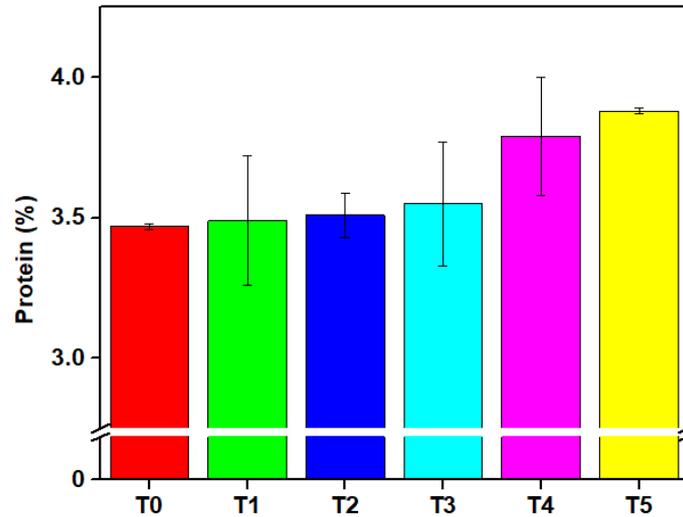


Figure 2. The effect of adding phycocyanin extract on protein content in cow's milk containing soybean oil

Protein Content

The lactose content of the milk in the sample did not experience a significant change with the addition of soybean oil and phycocyanin extract. The range of milk lactose levels in the sample was around 5.68% (T0), 5.74% (T1), 5.69% (T2), 5.69 (T3), 5.65% (T4), 5.62 % (T5). This range is still normal when referring to the writings of Haug et al, (2007), which states that the range of lactose levels in milk is 5.3 g / ml. If you look more closely at samples T1 to T4, milk lactose levels have decreased. Statistically, the reduction rate was not significant ($p > 0.05$). The decreased rate of lactose is probably due to a heat reaction. This heat reaction causes the Maillard reaction. Lactose is a reducing sugar that reacts with amino groups in the Maillard reaction (Stojanovska et al, 2017). The Maillard reaction between reducing sugars (pentose and hexose) and the primary aliphatic or secondary amines of amino acids is the starting point for the formation of an insoluble mixture of dark brown polymer pigments called melanoidins, which are dark pigments from the browning reaction when roasting, etc. (Brimer 2011). The Maillard reaction causes the lactose to condense. This condensation is followed by a series of other chemical reactions; reaction products cause changes in taste and taste, the formation of a brown color and loss of nutritional value (Berg, 1993). Another possibility is lactose isomerization. The lactose is isomerized to other sugars; can also be degraded into galactose products and into derivatives, namely glucose, and into various organic acids (Walstra, et al 2005). Lactose is also isomerized to lactulose (Berg, 1993). Theoretically, lactulose can be obtained from regrouping glucose residues into fructose molecules by two mechanisms: (1) Lobry de Bruynevan Ekenstein (formation of enolic substances from lactose and epylactose in alkaline media by transforming glucose from lactose molecules. Then converting it into fructose and producing lactulose molecules); (2) the reaction of lactose with ammonia or amines (this reaction forms lactosylamine and after hydrolysis of complex lactulose can be obtained (Aider and Halleux, 2007).

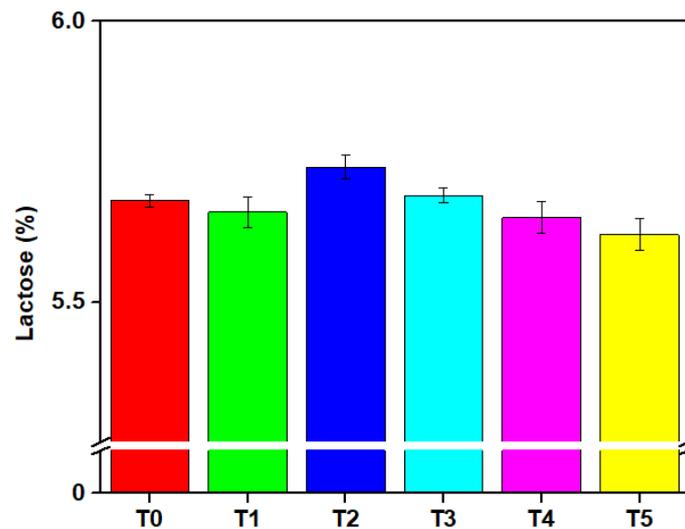


Figure 3. The effect of adding phycocyanin extract on lactose content in cow's milk containing soybean oil

SNF Content

Based on testing the SNF content of milk, the following results were obtained: T0 (10.55%), T1 (10.61%), T2 (10.67%), T3 (10.69%), T4 (10.78%), T5 (10.87%). According to CFDA (2020), the minimum SNF level for pasteurized milk is 8.25% and according to SNI (19-1502-1989) is 7.7%. This shows that the SNF content in pasteurized milk is relatively good. The addition of soybean oil and phycocyanin extract did not give any results ($p > 0.05$) for the amount of SNF for pasteurized milk. Cow's milk consists of 87% water and 12-13% solids consisting of 4% fat and 9% total non-fat solids, such as protein, lactose and various minerals and vitamins (Burke et al., 2018). The amount of SNF is caused by the fluctuating amount of protein, lactose, minerals, and vitamins in milk. The increase in the amount of SNF in the results obtained was mainly due to the increase in milk protein. Protein is the most variable constituent in total non-fat solids, depending on the breed of the animal, is seen as preferred by consumers and is more nutritionally important (Erb et al, 1963). Milk protein content is thought to increase due to the addition of phycocyanin extract. According to Wulandari et al, (2016), phycocyanin contains quite high protein, which is around 8 mg/ml. However, milk protein is susceptible to changes due to heating such as denaturation, but in this study the level of protein content still increased. SNF determines milk nutrition. Recently, milk with a higher SNF content such as low-fat milk and skim milk is preferred for diet programs. Also, for factories, the higher content of solid milk such as protein (casein) determines the quality of the product, especially cheese. Milk that has high protein content can increase the quality and quantity of cheese production without being given a lot of milk, thus saving production costs.

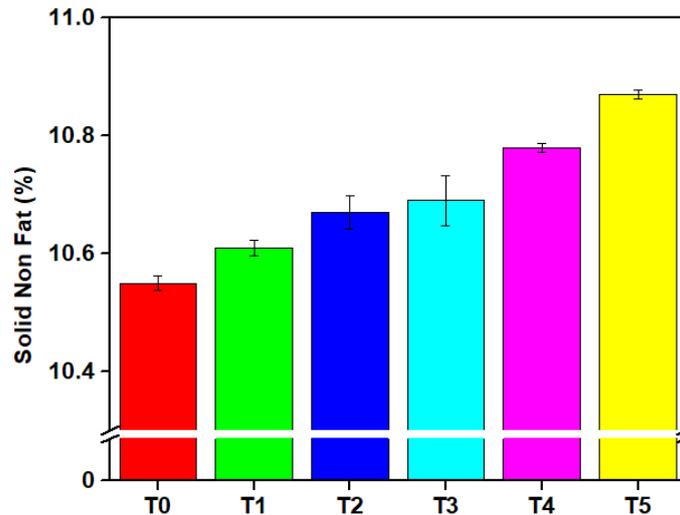


Figure 4. The effect of adding phycocyanin extract to solid-non-fat in cow's milk containing soybean oil

Correlation Between Fat, Protein, Lactose and SNF Content

In this study there are three types of correlation. The first correlation is the weak correlation between the fat and protein content of milk. The weak correlation also occurred for fat content and SNF. This correlation (weak correlation) can also state that there is no correlation or the correlation formed is insignificant. As for other content, there is another type of correlation. The second type is the strong correlation type with negative relationships. A strong correlation states that there is a correlation or there is a significant correlation. A negative relationship means that there is an opposite or inverse relationship between one content and another. If one content increases, the other will decrease, and vice versa. This type of correlation belongs to: (1) lactose and fat levels; (2) levels of lactose and protein; (3) levels of lactose and SNF. The third type is strong correlation with positive relationships. There is a positive relationship in protein levels and SNF. This positive relationship means that if the protein content increases, the SNF content will also increase and vice versa.

Table 2. Correlation test results

Macronutrient	Test	Fat	Protein	Lactose	SNF
Fat	<i>Pearson</i>	1	0,172	-0,680	1,650
	Probabilitas		0,782	0,207	7,90
Protein	<i>Pearson</i>	0,172	1	-0,780	0,973**
	Probabilitas	0,182		0,102	0,005
Lactose	<i>Pearson</i>	-6,80	-7,80	1	-693
	Probabilitas	0,207	0,120		0,195
SNF	<i>Pearson</i>	0,165	0,973**	-6,93	1
	Probabilitas	0,790	0,005	0,195	

Note: ** shows a significant correlation at the 0.01 level (probability)
 - or + shows the relationship

CONCLUSION

In conclusion, the addition of phycocyanin extract can increase the protein content of milk. There was no significant change in levels of lactose. SNF levels become affected mainly due to increased protein content. Overall, the treatment given did not significantly affect milk macronutrients and was still within the normal range. In addition, phycocyanin extract can also act as an antioxidant so that it can reduce the oxidation process that may be caused by the

addition of soybean oil. Soybean oil may be used as a food additive to increase the fat content of milk but must be accompanied by antioxidant agents to prevent the oxidation process.

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