Stability of chocolate bars fortified with nanocapsules carotenoid of *Spirulina platensis*

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Abstract. A chocolate bar and products is a universal product, suitable as a vehicle for fortified spirulina to enhance premium nutrition. This study was conducted to determine the stability of milk and dark chocolate fortified nanocapsule carotenoids of *Spirulina platensis*. The base of chocolate formula was designed by community industry of chocolate bar in Kulon Progo, Yogyakarta. Two types of chocolate (milk and dark) are fortified with nanocapsules carotenoid of Spirulina. The composition of chocolate paste: cocoa butter is 27.5:25 (milk) and 58:24.5 (dark), nanocapsule was added amount 0.372%. Fortified and control product were tested on 80 untrained panelists. The results showed that fortified chocolate did not show differences in aroma, taste, and texture with control. The dominant profile of aroma and chocolate flavor between fortified products and controls in the milk or dark chocolate was detected similar components but the intensity is slightly different. Fortified chocolate with nanocapsules spirulina showed the flat bloom development was lower than control. The equation of regression of milk chocolate stated linear regression curve \( y = 1.5655 + 27.611 \) and dark chocolate \( y = 1.2713 x + 26.559 \). Fat blooming reaches the limit of the white index number (> 31.5) in milk chocolate appear at the 6th month of storage and the dark one start at the 9th. The results showed dark chocolate has a shelf life 1.5 times longer than milk.

1. Introduction

*Spirulina platensis* is a microalga of the group Cyanobacteria, has been consumed for thousands of years by the Aztecs and nations in Africa as food, and has been designated as a material that is Generally Recognizes As Safe (GRAS) since 1981 by the FDA, no toxicity was found in administration with dosage of 24g / kg body weight for 12 weeks (Hutadilok-Towatana et al. (2010)[1]. Spirulina is generally consumed as a health supplement and producing various active ingredients important for health. Several studies related to the benefits of *S. platensis* as a supplement that is as an anti-viral source as anti-hyperlipidemic (Nah et al., 2012) [2], anti-diabetic (Gupta et al., 2010) [3], and have good mineral bioavailability of Fe, calcium. In addition spirulina is also a source of protein, fatty acids, antioxidants, a high source of carotenoids (4000mg / kg) (Henrikson, 2000)[4].

In Indonesia, there are no commercial food products that contain *S. platensis*, whereas in some countries *S. platensis* has been added to food for various purposes. The disadvantages of adding spirulina dry biomass are mainly the presence of fishy odor and bitter after taste. A fishy odor is mainly due to high mineral content. Aguero et al. (2003)[5] identified 54 volatile compounds in *S.*
platensis, and found 23 volatile carbonyl compounds, including heptanal and some aromatic ketones which caused off-flavor. To overcome the off flavor, one way is to extract the active ingredients then the ingredients are applied in the food system. Research has succeeded in extracting S. platensis carotenoid compounds and coating the active ingredients with various coatings with the spray dry method so that the powder is nanocapsules in size and can eliminate the specific aroma. The application of spirulina nanocapsules in the food system can be used as a way to improve the nutritional quality of food. The addition of active ingredients into chocolate products is currently being done, the addition of fatty acids EPA and DHA (Toker et al. 2018)[6], the addition of dried fruits such as raisins and cranberries (Cagindi & Otles., 2009)[7], pectin (Fajri et al. 2017 )[8], prebiotics and probiotics (Konar et al. 2016)[6], so that the application of carotenoid nanocapsules as bioactive components in chocolate is very potential.

Chocolate is a derivative product from cocoa beans which is liked by many people because of its distinctive nature, which is solid at room temperature and melts at body temperature. Chocolate is a multi-component system consisting of cocoa powder and sucrose (total solids of 65–75%) in a continuous fat phase, primarily composed of cocoa butter. The directives 2000/36 / EC, relating to cocoa and chocolate products intended for human consumption define "Dark chocolate", as the product obtained from cocoa products and sugars, containing not less than 35% of total dry cocoa solids, including not less than 18% cocoa butter and not less than 14% of dry non-fat cocoa solids. "Milk chocolate" is defined as the product obtained from cocoa products, sugar, and milk or milk products, with not less than 25% of total dry cocoa solids; not less than 14% dry milk solids (obtained by partly or totally dehydrated full cream milk, semi-or full-skimmed milk, cream, or from partly or completely dehydrated cream, butter or milk fat); not less than 2.5% dry non-fat cocoa solids; not less than 3.5% milkfat and not less than 25% total fat (cocoa butter and milk fat) (European Council, 2000). The type of chocolate the main factor affecting matrix degradation index during digestion. The different ingredients and their proportions that conform to the food matrix, like fat, emulsifiers, and crystalline lactose, have a significant impact on the structure and consequently their matrix degradation during digestion, deterioriation during storage (Lonchampt & Hartel, 2004)[9]. Dark chocolate is a food product that has the highest free anti-oxidant index with a value of 13,120 ORAC (a unit of absorbance capacity of a product against free radicals), followed by milk chocolate in position two with a value of 6,740 ORAC. This value is considered to be very high because blueberries, which are the fruit with the highest antioxidants, only have an ORAC value of 2.5. Dark chocolate has lower calories compared to milk chocolate (Kelishadi, 2005)[10]. However, Detournay (2016) revealed that Indonesian chocolate consumption is very low, around 300 g/capita/year, in Malaysia by 600 grams and in European countries 8-12 kg. Increased chocolate consumption can be initiated through extensification of chocolate production.

In the Province of Yogyakarta there is a village with a group of women who process chocolate with the concept of planting and processing and marketing local chocolate products grown in Kali Bawang, Kulon Progo Regency. This phenomenon is one of the concepts that support SDG’s related to sustainable consumption and production. It was further revealed by Detournay (2016), that the chocolate industry can be developed from the home industry, if managed properly, an increase in production capacity of 5kg / day to 150 kg/day within 5 years, up to 300 kg/day 10 years, with an increase in the workforce from 7 to 80 and 163. This can be used as an effort to increase chocolate consumption in Indonesia and improve the economic communities.

In the home industry, product quality control is an important thing that must be done. Chocolate products are especially popular because of their sensory properties. Chocolate lovers seek for functional chocolate that offers clinically proven physical or emotional health benefits. However, consumers’ attitudes towards functional foods do not depend only on their perceived health, but also on
the sensory quality, price, and convenience, as any conventional product[11]. Also, the bioactive compound added to the functional food matrix must maintain its original chemical structure and consequent functionality during the entire shelf-life period. The quality of chocolate is influenced by the quality of the cocoa used. During processing, the chocolate composition, in terms of type and amount of each ingredient, plays an important role in obtaining a high-quality product. During storage, chocolate surfaces turn grayish and are known as fat bloom, inducing considerable color changes, namely lightness, nuance, and saturation color. The turning gray of chocolate principally appears as the result of errors during defined phases of the technological production processes, such as tempering, forming, cooling or as a consequence of extremely long storage. Changes in chocolate surface color can be sensory evaluated using visualization techniques or through colorimetric or spectrophotometric methods. The Whiteness Index (WI) can be used as one parameter to determine the color quality characteristics, which are related to conditions during the production process and formulated ingredients (Papadakis et al. 2000)[12].

This study was done to investigate the stability of milk and dark chocolate fortified nanocapsule carotenoids of Spirulina platensis. The result can be applied to develop local chocolate bar industry to increase community income and overcome poverty.

2. Material and methods

2.1. Spirulina chocolate production

Fermented cocoa beans were obtained from cocoa farmers in Kulon Progo Regency, the processing production of chocolate was done at Pawon Gendhis Women Farmers Group, Banjarsari, Kulon Progo Regency, Yogyakarta. The beans were roasted for 15 minutes at 80 °C, cooled and stripped of the epidermis, ground to a paste. The cocoa paste is mixed with pre-melted cocoa butter, this mixing is carried out for 12 hours at 40 °C in a bowl mill. The ratio of cocoa mass: cocoa butter for milk products (25: 27.5) and dark (40:31). Spirulina milk and dark chocolate formulations are presented in Table 1. Milk powder, sugar, baking soda, and vanilla flavoring are added in the 90th minute, every 90 minutes stirring the mixture is done with the method of turning it back to avoid clot in the end product. Furthermore, lecithin was added into chocolate and continued for conching for 4 hours at 37°C. The next process is tempering, raising the temperature to 55°C and lowering it to 27°C, then raising it again to 30°C (milk), 35 °C (dark), carotenoid nanocapsules from Spirulina platensis added 0.372%. The specifcation of nanocapsules carotenoid of Spirulina were respectively: 3.63% moisture content, 22.3 mg / g carotenoids, and particle size  777.4 nm. Nanocapsules was produced by the product the Fish Processing Technology Laboratory, Department of Fisheries, Gadjah Mada University. Then the step is for molded and cooled, packed with aluminum foil, stored at 10 °C until testing will take. The analysis was carried out at intervals of observations every 90 days, for 360 days.

| Ingredients         | Milk Chocolate (g/batch) | Dark Chocolate (g/batch) |
|---------------------|--------------------------|--------------------------|
| Fermented cacao beans | 750                      | 1,200                    |
| Cocoa butter        | 825                      | 900                      |
| Granulated Sugar    | 675                      | 555                      |
| Milk Powder         | 750                      | 345                      |
| Vanilla flavor      | 9                        | 9                        |
| Baking soda         | 3                        | 3                        |
| Lecithin            | 9                        | 9                        |

*1 batch= 3kgs
2.2. Determination of fat, water, and carotenoid content
Determination of fat and water content used AOAC methods (AOAC 2005)[13]. Carotenoid was tested by using spectrophotometric methods (Cagampang & Rodriguez 1980)[14]. The β-carotene content of the sample was separated using a separating funnel with a mixture of petroleum ether and acetone (1:1) solvent, the ether phase obtained was added with sodium sulfite, and assessed at λ 450 nm.

2.3. Determination of the melting temperature of chocolate bars
The melting temperature was determined using Thermocouple (specifications). A cube of chocolate bar (2.5 x 2.5 x 1 cm) placed on a watch plate D=5 cm, pierced with a sensor as deep as 0.5 cm, and placed on a glass beaker it contained 100 ml demineralized water, heated by adjusted temperature on 55°C an increase in temperature at 1°C per minute, the last chocolate droplet melted is its melting temperature. The test was repeated 5 times.

2.4. Determined of fat bloom
The fat blooming analysis was performed using the Spectro-colorimetry method, measuring L*, a* and b*. L* (brightness), value 0 = black and 100 = white with chromameter (Minolta CR400). The parameter a* is for the color (-) green to (+) red and the parameter b* indicates the color (-) blue to (+) yellow and the numbers a or b equal to 0 indicate a neutral/gray color. The amount of fat bloom that is formed is indicated by WI measured using calculations (Sonwai and Rousseau, 2006)[15]:

\[
WI=100-\left(100-L^*\right)^2 + (a^*)^2 + (b^*)^2 \right)^{1/2}
\]

2.5. Visualization of fat bloom
Visualization of fat bloom was taken by Samsung Galaxy S8 (milk) cellphone camera, white black - auto, focus - auto, shutter speed - auto, frame 5: 9, focus - 1.7, ISO - auto, and Poco phone F1 (dark) white black - auto, focus - auto, shutter speed - auto, ISO – 100, frame 16:9, anti-banding 50 Hz.

2.6. Hedonic test
The hedonic test was conducted to determine the response of consumer acceptance of chocolate products (without and with the fortification of the carotenoid spirulina nanocapsules) tested on 70 adults (aged 16-50 years). The level of preference of the panelists on the product was assessed with a score (1: very dislike, 2: dislike, 3: somewhat like, 4: like 5: very like) (Meilgard et al., 2007)[16].

2.7. Trained panels
Trained panelists were prepared to conduct Quantitative Data Analysis testing. Trained panelists were selected from 30 prospective panelists using the triangle method to determine one sample that was different from the three samples provided and performed 7 replications. Panelists with more than 70% correct answers will graduate at an advanced stage. The selection process was carried out in 3 stages. Furthermore, the selected panelists selected to conduct training on a dark and milk chocolate, as well as related criteria for the same perception.

2.8. Quantitative descriptive analysis (QDA) testing
QDA testing is carried out by trained panelists. Panelists did profiling by describing chocolate bar samples based on color, texture, and describing in more detail about the aroma profile, taste and after taste of the product. Panelists write profiles with examples of products that are similar to the results of each panelist’s description. The profiling stage is continued with the Focus Group Discussion stage. At the focus group, discussion products are provided according to references from panelists. The panelists sampled all the ingredients that had been prepared including the sample instructions based on the results of the profiling test and discussed together to determine the type of dominant aroma and taste.
that was detected as an attribute. The next step is a scalar test (line length of 10 cm), trained panelists describe the taste and aroma of the sample by measuring the attributes on each attribute line (Meilgard et al., 2007) [16].

2.9. Determined of melting time product in the mouth
Trained panelists determined chocolate bar weighing + 5 grams. When chocolate is placed in the oral cavity expressed as 0 minutes, the first time chocolate feels melted in the mouth the count is stopped and recorded the length of time using a stopwatch.

2.10. Determined the chocolate quality according to the ISO standards
Panelists trained in evaluating the quality of chocolate according to the categories listed in the ISO standard for chocolate products and carried out according to the scoring method described by Popov-Raljić Laličić-Petronijević (2009) [17].

2.11. Analysis data
Data were analyzed by analysis of variance, at a 95% confidence level. Follow-up tests for parameters that show significant differences using the Dunnet test or Duncan Multiple Range Test (DMRT).

3. Results and discussion

3.1. Performance of dark and milk chocolate spirulina
The addition of the carotenoid nanocapsule Spirulina platensis shows a specific brown color for both types of chocolate (dark and milk). The appearance and color of the chocolate bar can be seen in figure 1 and table 2. In table 2, it shows dark chocolate has a darker color (lower L-value), whereas between milk chocolate without or with the addition of spirulina is no different.

Figure 1. The appearance of (a) dark chocolate, (b) dark chocolate spirulina, (c) milk chocolate, and (d) milk chocolate spirulina

| Table 2. The color of products dark and milk chocolate spirulina (based on L, a, b) |
|---------------------------------|-----|----------------|-----|----------------|
| Color attribute                | Dark chocolate | Milk Chocolate |
|                                | Without | With Spirulina | Without | With Spirulina |
| L                               | 31.53±0.67AA | 29.44±0.59AB  | 33.27±0.54BC | 33.2±0.04BC   |
| a                               | 12.34±0.05AA | 11.69±0.28AB  | 16.5±0.16BC | 16.66±0.32BC  |
| b                               | 8.65±0.09AB  | 7.54±0.29AB   | 9.13±0.04AC | 13.26±0.04AD  |

Note: Numbers followed by letters indicate that comparisons are made for each attribute (line) at a significance level of 95%. Numbers followed by lowercase letters show a comparison between chocolate without addition vs spirulina chocolate on the same type of chocolate, capital letters show a comparison between all treatments. Different letters mark off the differences.
3.2. The rate of chocolate spirulina melts in the mouth
Table 3. shows the variation of melting temperature of the 2 types of chocolate ranging from 32.27 to 34.17°C, dark chocolate has lower melting temperature than milk chocolate and there is no difference between chocolate without addition and chocolate with the addition of spirulina. Another variation is seen that the speed of chocolate melting in the mouth is almost similar in the dark group. Chocolate milk without the nanocapsules of spirulina has the longest melting time.

Table 3. The temperature of melting chocolate and the length of chocolate melt in the mouth.

| Chocolate       | Melting Temperature (°C) | The length of melting time in the mouth (min) |
|-----------------|--------------------------|---------------------------------------------|
| Milk            | 32.83±0.35a              | 5.28±1.36a                                  |
| Milk Spirulina  | 32.27±0.15a              | 4.25±1.18c                                  |
| Dark            | 34.17±0.40b              | 4.50±1.58b                                  |
| Dark Spirulina  | 34.00±0.52b              | 4.50±1.54b                                  |

Note: Numbers followed by different letters show significant differences in the same column

3.3. The content of fat, water, energy, and beta carotene
The fat content, energy of spirulina chocolate has fulfilled SNI 7934: 2014 standard (chocolate and chocolate products) and USDA. table 4. shows that the addition of Spirulina carotenoid nanocapsules was proven to increase the spirulina chocolate carotenoid content (p <0.05).

Table 4. Fat, water, energy and β-carotene content of spirulina chocolate

| Description          | Without | %RDA | Fortified | %RDA | USDA | SNI |
|----------------------|---------|------|-----------|------|------|-----|
| **Milk Spirulina**   |         |      |           |      |      |     |
| Lipid (%)            | 35.24±1.13 | 38.73 | 37.49±0.32 | 41.19 | 34.21 | >31 |
| Carbohydrate (%)     | 52.42±0.57 | 13.98 | 51.27±1.15 | 13.67 | 55.26 | -   |
| Energy (cal/100g)    | 537.88±7.65 | 19.74 | 553.18±15.3 | 20.30 | 579  | -   |
| β-carotene (μg/100g) | 564.76±6.05a | 15.69 | 812.67±31.06b | 22.57 | -    | -   |
| **Dark Spirulina**   |         |      |           |      |      |     |
| Lipid (%)            | 27.38±0.05 | 40.87 | 28.20±0.06 | 42.09 | 42.63 | >31 |
| Carbohydrate (%)     | 60.57±0.06 | 22.02 | 7.05±0.15  | 20.75 | 46.36 | -   |
| Energy (cal/100g)    | 509.67±0.18 | 25.48 | 510.34±1.4 | 25.52 | 598  | -   |
| β-carotene (μg/100g) | 859±14.14a | 23.86 | 5.619±74.95b | 156.08 | -    | -   |

Note: Numbers followed by different letters show significant differences in the same line

Consumer preferences for spirulina chocolate showed that panelist preferences ranged to close to a score of 4 (score 3; somewhat like, score 4; like) on various attributes (table 5). The addition of spirulina did not affect the aroma (p>0.05). Overall, panelists revealed that the addition of nanocapsules to dark chocolate increased the level of panelist preference (p<0.05).

Table 5. Consumer preferences for dark and milk chocolate spirulina products

| Chocolate          | The score of Costumer Preference using Hedonic Test | Appearance | Texture | Aroma | Taste | Overall |
|--------------------|---------------------------------------------------|------------|---------|-------|-------|---------|
| Dark Chocolate     |                                                   | 3.42±1.17A | 3.45±1.06A | 3.76±0.9A | 3.27±1.1A | 3.48±0.3A |
| Dark Spirulina     |                                                   | 3.67±1.11B | 3.82±0.81B | 3.73±0.91A | 3.76±1.06B | 3.75±0.6B |
| Milk Chocolate     |                                                   | 3.59±1.44B | 4.06±1.74C | 3.86±1.67B | 4.03±1.63C | 3.88±0.28B |
| Milk Spirulina     |                                                   | 3.76±0.18B | 4.15±0.26C | 3.78±0.02B | 4.03±0.35C | 3.93±0.40B |
Note: Numbers followed by letters indicate that comparisons are made for each attribute (line) at a significance level of 95%. Numbers followed by lowercase letters show a comparison between chocolate without addition vs spirulina chocolate on the same type of chocolate, capital letters show a comparison between all treatments. Different letters mark off the differences.

3.4. Profile and intensity of aroma and taste attributes of dark and milk chocolate bars
Chocolate aroma profile did not differ between spirulina chocolate compared to chocolate brown without spirulina. The aroma and taste profile of the QDA test results can be seen in Tables 6 and 7. The aroma and taste profile of Dark chocolate (3 types) is less than milk chocolate (7 types). However, the intensity of the aroma and taste were found between chocolate without the addition of chocolate compared with the addition of spirulina did not differ (p> 0.05). The authentic taste of chocolate identified in both dark and milk products is a bitter taste.

Table 6. The dominant profile and intensity of aroma attributes in dark and milk chocolate spirulina using Quantitative Data Analysis

| Aroma          | Standard Aroma        | Chocolate Control | Chocolate Fortified Spirulina |
|----------------|----------------------|-------------------|------------------------------|
| Dark Chocolate | 6.74 ± 1.91^a        | 6.63 ± 2.07^a     |
| Sweet         | 5.50 ± 1.08^a        | 5.77 ± 0.58^a     |
| Milk          | 4.87 ± 0.62^a        | 5.29 ± 0.45^a     |
| Rehydrated milk | 6.02 ± 1.83^a      | 5.79 ± 1.53^a     |
| Burnt cocoa   | 4.35 ± 2.57^a        | 4.85 ± 1.70^a     |
| Sauted cacao  | 4.16 ± 2.52^a        | 4.62 ± 2.49^a     |
| Fermented cocoa beans | 1.65 ± 1.02^a | 2.24 ± 1.45^a     |
| Cacao paste   | 4.69 ± 2.43^a        | 5.27 ± 2.34^a     |
| Cacao butter  | 4.95 ± 2.26^a        | 6.06 ± 2.60^a     |
| Fine granulated sugar | 5.27 ± 1.37^a | 5.33 ± 2.44^a     |

Note: Numbers followed by different letters show significant differences in the same line

Table 7. The dominant profile and intensity of taste attributes in dark and milk chocolate spirulina using Quantitative Data Analysis

| Aroma          | Taste Standards        | Chocolate Control | Chocolate Fortified Spirulina |
|----------------|-----------------------|-------------------|------------------------------|
| Dark Bitter    | 7.47 ± 1.46^a         | 7.51 ± 0.96^a     |
| Sweet         | 4.63 ± 0.68^a         | 5.51 ± 0.85^a     |
| Sour          | 5.03 ± 0.63^a         | 5.21 ± 0.33^a     |
| Rehydrated milk | 6.63 ± 0.76^a      | 6.78 ± 1.46^a     |
| Burnt cocoa   | 3.40 ± 2.66^a         | 3.36 ± 2.47^a     |
| Sauted cacao  | 2.91 ± 2.18^a         | 2.88 ± 2.29^a     |
| Fermented cocoa beans | 3.22 ± 2.67^a | 3.60 ± 3.29^a     |
| Cacao butter  | 4.02 ± 2.84^a         | 3.94 ± 2.22^a     |
| Fine granulated sugar | 7.32 ± 2.06^a | 7.32 ± 1.95^a     |
| Bitter        | 7.47 ± 1.46^a         | 7.51 ± 0.96^a     |

Note: Numbers followed by different letters show significant differences in the same line
3.5. Discoloration of milk chocolate and dark chocolate during storage

Changes in the color of chocolate products during storage (Table 8). The longer storage time increases the L value, while there is almost no change in the a and b color parameters in dark and milk chocolate.

**Table 8.** Color changes (L, a and b) of spirulina chocolate products for 360 days storage

| Storage (days) | Without Spirulina | With Spirulina |
|---------------|-------------------|----------------|
|               | L     | a    | b    | L     | A    | b    |
| **Dark Chocolate** |       |      |      |       |      |      |
| 0             | 31.53 | 12.34| 8.65 | 29.44 | 11.69| 7.54 |
| 90            | 30.10 | 11.88| 7.77 | 31.59 | 12.69| 8.37 |
| 180           | 32.11 | 12.64| 8.37 | 32.10 | 12.57| 8.03 |
| 270           | 34.41 | 11.41| 8.61 | 33.35 | 11.89| 8.82 |
| 360           | 35.33 | 12.86| 8.40 | 34.63 | 12.36| 8.69 |
| **Milk Chocolate** |       |      |      |       |      |      |
| 0             | 33.27 | 16.50| 9.13 | 33.20 | 16.66| 13.26|
| 90            | 32.63 | 12.59| 7.49 | 31.84 | 13.01| 8.14 |
| 180           | 32.82 | 11.94| 8.21 | 32.91 | 11.75| 8.17 |
| 270           | 36.65 | 13.38| 8.62 | 35.80 | 12.58| 7.52 |
| 360           | -     | -    | -    | -     | -    | -    |

- : the product was damaged before the 360th day of testing

3.6. The rate of fat blooming spirulina chocolate

The development of fat bloom milk and dark chocolate spirulina formation during storage is determined by measuring the white index. The white index rate shows an increase during storage, the rate of damage between chocolate without spirulina is faster than fortified chocolate. The speed of milk and dark chocolate damage is presented in figure 2.

![Figure 2](image-url)

**Figure 2.** Rate of formation of fat blooming on spirulina chocolate; (a) milk chocolate; (b) dark spirulina

Visualization of the chocolate bar for 270 days of storage shows that the development is increasingly widespread and can be detected by vision. Figure 3 shows the process of developing fat blooming in the products. Formation of fat blooming on milk chocolate looks more intensive than dark chocolate.
Figure 3. The appearance of dark chocolate spirulina day (a) 0, (b) 90th, (c) 180th, (d) 270th, milk chocolate spirulina day (e) 0th, (f) 90th, (g) 180th, (h) 270th.

The results of observations of appearance, texture, and odor by trained panelists are presented in table 9. The assessment results show the composition of chocolate (dark and milk), as well as differences in storage duration, affect the parameters of observation (appearance, texture, and aroma). There is an interaction between types of chocolate with long storage, while carotenoid nanocapsules do not affect the product.

Table 9. Results of Factorial Analysis of Variance of Chocolate

|                     | Chocolate composition | Storage time | Interaction (Chocolate composition *storage time) |
|---------------------|-----------------------|--------------|---------------------------------------------------|
|                     | F         | p  | F      | p  | F         | p   |
| Appearance          | 437.556   | 0.000 | 87.024  | 0.000 | 80.444     | 0.000 |
| Texture             |           |     |        |     |            |       |
| Structure, break & firmness | 274.749 | 0.000 | 179.553 | 0.000 | 68.839     | 0.000 |
| Chewiness           | 96.711    | 0.000 | 26.605  | 0.000 | 11.447     | 0.000 |
| Aroma               |           |     |        |     |            |       |
| Odor                | 47.032    | 0.000 | 83.333  | 0.000 | 12.280     | 0.000 |
| Taste               | 203.294   | 0.000 | 188.353 | 0.000 | 36.941     | 0.000 |

The deterioration process on chocolate has categorized by Popov-Raljić Laličić-Petronijević (2009). The change in quality during storage time can be seen in figure 4. Based on quality category, the dark chocolate had still in good quality at the end of storage, but the milk one the quality was broken into unacceptable.
Figure 4. The rate of declining quality category of spirulina chocolate during 270 days storage. Quality category was determined in dependence of scores spans; products, which were evaluate with (less than 2.5: unsatifsactory, unacceptable; 2.5–3.5: good quality products; 3.5–4.5: very good quality and 4.5–5: excellent products (Popov-Raljić Laličić-Petronijević, 2009).

3.7. Discussion
The appearance of chocolate without addition and with the addition of the nanocapsule spirulina shows a distinctive brown color (figure 1). Table 2 shows that there is a color difference between dark and milk. Dark chocolate is slightly darker than milk chocolate. Statistically the color of milk chocolate without addition is not different from fortification chocolate, while the addition of dark chocolate causes a brighter color, due to the nanocapsule powder that is added to light green. The constituent material can affect the microstructure of chocolate and has implications for color and melting behavior. Melting behavior in this study showed that dark chocolate has a higher melting temperature than dark chocolate (table 3), the addition of nanocapsules did not affect changes in melting temperature (p> 0.05). Indarti et al (2013) [18] explained that chocolate quality is good if it has a melting temperature above 24 °C with consideration that quality is maintained during the distribution and storage process. Popov-Raljić & Laličić-Petronijević (2009) [17] explained that generally the melting point of chocolate ranges from 32–34°C, so that the melting temperature of spirulina chocolate meets these requirements. The speed of melted chocolate in the mouth on milk chocolate without addition is likely due to the presence of sucrose content which causes low melting speed. Reported by Halim et al. (2016) [19] that the melting point of sugar is at high temperatures (>200°C), resulting in increased melting time in the mouth, whereas the addition of nanocapsules causes agerat changes in the matrix resulting in decreased melting time. The composition of solids in dark chocolate is greater than milk chocolate so that the addition of nanocapsules does not cause a change in melting time. However, lower melting time for dark chocolate is possible because of the lower sugar composition (table 1). Dark chocolate in this study contains a amount milk, the minimum solid content of dark chocolate is 35% and cocoa fat is not less than 18%. Milk powder in dark chocolate is permitted, but does not exceed 18% because chocolate containing more than 18% milk can be categorized as milk chocolate (CODEX STAN 87-1991). Furthermore, differences in melting behavior can be caused by the temperature of the tempering process used differently. Stages of tempering dark chocolate are carried out at 35 °C, while milk chocolate is at 30 °C. Tempering is a technique of controlled pre-crystallisation employed to induce the most stable solid form of cocoa butter, a polymorphic fat in finished chocolates. Temperatures promotes crystallization of triacylglycerols (TAGs) in cocoa butter.
to effect good setting characteristics, foam stability, molding properties, product snap, contraction, gloss and shelf-life characteristics. Afoakwa et al. (2008) [20] states that fat crystallization behavior during tempering play vital roles in defining the structure, mechanical properties and appearance of products. Wide variations in mechanical properties and appearance occur in products from different particle sizes and temper regimes. Particle size is inversely related to texture and color, with the greatest effects noted with hardness, stickiness and lightness.

Spirulina chocolate from this research was included in the category of original chocolate. The real chocolate is called the term couverture, which means it is made from crushed, mixed and thawed cocoa beans and butter, which produces pure chocolate in a liquid form. Spirulina chocolate products contain fat in accordance with SNI 7934: 2014 and USDA, carbohydrate and energy content in accordance with USDA, which is approximately 538-553 cal/100 grams, so that spirulina chocolate can be used as an alternative snack (table 4). Serving size of 1 oz chocolate dish (26.8 grams), will produce about 144-148 calories of energy and is a snack with additional benefits because it contains carotenoids. The addition of nanocapsules increased the chocolate carotenoid content which was very large both in milk and dark chocolate (p <0.05). The dominant aroma and taste profiling results in both dark and milk chocolate did not show an off-flavor component. This shows that the addition of spirulina nanocapsules does not affect the aroma and taste of chocolate. The type of aroma and taste profile that is recognized predominantly by panelists in milk chocolate is more varied than dark chocolate, which is related to the ingredients and composition formulated in chocolate. The bitter taste is a characteristic of dark chocolate type chocolate is also found in the dark chocolate flavor profile, even in milk chocolate.

The Detournay statement (2016) explains that there are three main varieties of cocoa (Forastero, Criollo and Trinitario, 90% of the world’s cocoa production is from the Forastero type, including the type cultivated in Indonesia. The uniqueness of Indonesian cocoa beans is the taste of cocoa beans which is different from island to island. Chocolate from Sulawesi smells of fruit and is very acidic, while cocoa beans from Java have a bitter taste. The raw material for making dark chocolate and milk in this study comes from the village of Kalibawang which is on the island of Java, thus having a characteristic namely bitter chocolate beans and can be detected in chocolate milk products, with a composition of milk and sugar greater than the type of dark.

Discoloration of chocolate during storage shows that prolong storage increase L-value, which has implications for increasing brightness, presumably due to the formation of white spots that are on the surface of the product. The L value at the beginning of storage for dark chocolate spirulina and milk ranges between (29-31) and (33). The rate of increasing L-value in dark chocolate and milk are similar, but milk chocolate has a higher indicating that the rate of the spot is more intensive (table 8). Something similar was reported by Popov-Ralić & Laličić-Petronijević (2009) [17] in chocolate with various types of sugar after storage for 1 year experiencing an increase in L-value. The WI is used to measure the formation of white and gray spots and the loss of shine that is typical of fat bloom. According to Bricknell & Hartel (1998) [21], these spots indicate the formation of crystals throughout the surface, which leads to the diffusion of reflected light and interferes with colorimetric properties. The speed of fat bloom can be determined by observing the white index contained in the product, the higher the white index indicates the more fat blooming that occurs in the product. According to Popov-Ralić & Laličić-Petronijević (2009) [17], white spots / fat bloom begin to be seen when the white index reaches the range 31-31.5. Based on the WI increasing velocity equation (figures 2 a and b) it can be seen that dark chocolate will reach a value of 31.5 when stored until (139 days) while dark without addition (95), the results of the equation obtained in milk chocolate found that WI was 31.5 is predicted to begin after storage for 67 days (milk) and 116 days (milk spirulina). The two chocolates use cocoa butter as a source of fat, with different percentages.
Although the amount of cacao butter used for dark chocolate is higher, the ratio of dark chocolate is more than the ratio of milk chocolate which is almost balanced, so chocolate milk will have a lower viscosity, with more micropores than dark chocolate.

A study reported that storage in ideal conditions (20 °C), chocolate bar formulations with 100% cocoa butter show good stability until storage, for 100 days no fat bloom is detected. However, if the product is subjected to fluctuating temperatures, it is found that formulations with 100% cacao butter actually experience fat bloom more quickly. Other studies on milk chocolate revealed that milk chocolate at 16 °C / 28 °C cycles and, using different colorimetric methods, only observed color changes at the surface of the chocolate and fat bloom formation after 52 days of cycling. This result indicated that fat bloom formation is not guaranteed at the chocolate surface, even if incompatible fats are used [22].

Halim et al. (2018) stated that the partial replacement of cocoa butter (4.5% replaced by coconut oil) increased storage time from 6 weeks to 8 weeks, coconut oil keep the product to fat bloom. However, an increase in WI occurs if prolong storage. It is predicted that the appearance of bloom occurs due to storage at prolonged fluctuated temperature. According to Lonchampt and Hartel (2004) [9], temperature fluctuations will increase the bloom rate even though at small variations between 24 °C and 24 °C ± 1. Therefore, it is necessary to control the temperature during storage, because fat bloom can still be formed even though intervals only vary by 1 degree. Other researchers added, the transition from the βV to the βVI polymorph generally occurs in blooming chocolate. However, the absence of a visible fat bloom does not indicate an absence of internal polymorphic transitions [21]. The typical polymorphic transitions of fat bloom may either occur naturally or be caused by external factors. For fat bloom to be visible, re-crystallization must occur; this generally results from temperature fluctuations caused by crystal formation at the surface, which interferes with light reflection, resulting in a whitish, dull appearance. For well-tempered chocolate stored at 20 °C for long periods, it is accepted that a possible βV to βVI polymorphic transition is a solid-solid transformation. Sometimes, if re-crystallization occurs only under the surface, the fat bloom, although present, will not be visible [9]. This explanation can explain the results of this research for dark chocolate in this research. Based on the prediction of WI, dark chocolate fat bloom began to appear on day 139. However, fat bloom has not been visible on day 180. However, on dark chocolate until day 180 visually there is still no change in color compared to initial day 0 (figure 3). The addition of Spirulina nanocapsules can extend the shelf life of the product. The addition of nanocapsules in the product causes an increase in mass in the formulation and may affect the microstructure of the chocolate product. The presence of pores (micro-holes) in chocolate may facilitate fat migration to the surface and fat bloom consolidation. The success of the tempering step impacts the presence of these pores; over-tempered dark chocolate showed 4% porosity, whereas appropriately tempered chocolate showed only 1%.

The relationship between the composition of chocolate compilers and storage time show interaction (table 9), meaning that the components contained in the recipe could affect the sensory properties of the product, namely appearance, structure, break, firmness, chewiness, odor, and taste. Afaokwa et al. (2009) [23] explained that the different ingredients in chocolate recipes affect the microstructural and rheological properties of the final product. In particular, different amounts of fat involve changes in the particle-particle interaction, in terms of the distance between the particles and their distribution, as well as, the solid fat and non-fat particles. Lower cocoa butter concentrations, parallel to high fraction of solid particles, such as in the dark chocolate formulation, promote particles interactions, involving higher values of rheological characteristics. On the other hand, higher cocoa butter amount, even if in presence of higher amount of non-fat particles (sugar), together with the presence of milk fat (from milk powders), reduce resistance to flow. The rate of decline in the quality of chocolate between
products added without the addition of spirulina showed no difference. This is possible because the addition of nanocapsule mass which tends to low, and the presence of lecithin which is an emulsifier. The presence of lecithin and crystalline lactose can also influence the final product properties. Dark products with more solids were found to be able to maintain the quality of chocolate during storage compared to milk chocolate (figure 4). At the end of storage dark chocolate still has very good quality, while chocolate milk is poor quality (unacceptable).

4. Conclusions
There is not different performance between without or with fortified nanocapsules. The dominant aroma and taste were richer in milk than dark chocolate. Fat blooming reaches the limit of the white index number (> 31.5) in milk chocolate appear at the 6th month of storage and the dark one start at the 9th. The results showed dark chocolate has a shelf life 1.5 times longer than milk.

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References
[1] Hutadilok-Towatana N, Reanmongkol W and Panichayupakaranant P 2010 Evaluation of the toxicity of Arthrospira (Spirulina) platensis extract J. Appl. Phycol. 22 599–605
[2] Nah W H, Koh I K, Ahn H S, Kim M J, Kang H-G, Jun J H and Gye M C 2012 Effect of Spirulina maxima on spermatogenesis and steroidogenesis in streptozotocin-induced type I diabetic male rats Food Chem. 134 173–9
[3] Gupta S, Hrishikeshvan H J and Sehajpal P K 2010 Spirulina protects against Rosiglitazone induced osteoporosis in insulin resistance rats Diabetes Res. Clin. Pract. 87 38–43
[4] Henrikson R 2010 Spirulina: World Food, How This Microalgae Transform Your Health and Our Planet (hawaii: Ronor Enterprise)
[5] Aguero J, Lora J, Estrada K, Concepcion F, Nunez A, Rodriguez A and Pino J A 2003 Volatile components of a commercial sample of the blue-green algae Spirulina platensis J. Essent. oil Res. 15 114–7
[6] Toker O S, Konar N, Palabiyik I, Pirouzian H R, Oba S, Polat D G, Poyrazoglu E S and Sagdic O 2018 Formulation of dark chocolate as a carrier to deliver eicosapentaenoic and docosahexaenoic acids: Effects on product quality Food Chem. 254 224–31
[7] Ötleş S 2009 The health benefits of chocolate enrichment with dried fruits Acta Sci. Pol. Technol. Aliment. 8 63–9
[8] Fajri F, Tamrin T and Asyik N 2018 Pengaruh penambahan pektin kulit buah kakao terhadap sifat sensorik dark chocolate J. Sains dan Teknol. Pangan 2
[9] Lonchampt P and Hartel R W 2004 Fat bloom in chocolate and compound coatings Eur. J. Lipid Sci. Technol. 106 241–74
[10] Kelishadi R 2010 Cacao to cocoa to chocolate: healthy food? ARYA Atheroscler 1
[11] Varela P and Ares G 2012 Sensory profiling, the blurred line between sensory and consumer science. A review of novel methods for product characterization Food Res. Int. 48 893–908
[12] S. P, Abdul-Malek, Kamden and K.I. Y 2000 Versatile and Inexpensive Technique for Measuring Color of Foods Food Technol 54 48–51
[13] AOAC 2005 Official Methods of Analysis. Association of Official Analytical Chemists (Washington: Benjamin Franklin Station)
[14] Cagampong G B and Rodriguez F M 1980 Methods of Analysis for Screening Crops of Appropriate Qualities (Los Banos)
[15] Sonwai S and Rousseau D 2006 Structure evolution and bloom formation in tempered cocoa
butter during long-term storage Eur. J. Lipid Sci. Technol. 108 735–45
[16] M. M. Civille G V. and Carr B T 2007 Sensory Evaluation Techniques (New York: CRC Press)
[17] Popov-Raljić J and Laličić-Petronijević J 2009 Sensory properties and color measurements of dietary chocolates with different compositions during storage for up to 360 days Sensors 9 1996–2016
[18] Indarti E, Apri N and Budijanto S 2013 Kajian pembuatan dark chocolate dengan metode tempering dan tanpa tempering J. Teknol. dan Ind. Pertan. Indones. 5
[19] Halim H S A, Selamat J, Mirhosseini S H and Hussain N 2018 Sensory preference and bloom stability of chocolate containing cocoa butter substitute from coconut oil J. Saudi Soc. Agric. Sci.
[20] Afoakwa E O, Paterson A, Fowler M and Ryan A 2008 Flavor formation and character in cocoa and chocolate: a critical review Crit. Rev. Food Sci. Nutr. 48 840–57
[21] Bricknell J and Hartel R W 1998 Relation of fat bloom in chocolate to polymorphic transition of cocoa butter J. Am. Oil Chem. Soc. 75 1609–15
[22] Aguilera J M, Michel M and Mayor G 2004 Fat migration in chocolate: diffusion or capillary flow in a particulate solid?—a hypothesis paper J. Food Sci. 69 167–74
[23] Afoakwa E O, Paterson A, Fowler M and Vieira J 2009 Fat bloom development and structure-appearance relationships during storage of under-tempered dark chocolates J. Food Eng. 91 571–81