Advance Journal of Food Science and Technology 14(5): 131-137, 2018
DOI:10.19026/ajfst.14.5910
ISSN: 2042-4868; e-ISSN: 2042-4876

Submitted: April 3, 2017 Accepted: June 3, 2017 Published: August 25, 2018

Research Article

Development of New Product "Cocoa Spirulina as Functional Food"

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Abstract: The aim of this research was to study nutrition fact of new product "Cocoa Spirulina" as functional product. This nutrition fact can be used as a reference in its application to the food industry. Cocoa Spirulina formulation used is the ratio of spirulina and cocoa powder in a row; it is put 2: 10; 1.5: 10; and 2: 10. The Data of the water content are 5.8-6:07%. The Ash content is 4:18 to 4:51%. The Protein content of Cocoa Spirulina is smaller when compared to unfermented spirulina powder, 0.66-2.28%. The fat content is 8.94-11.66%. While the antioxidant power is 1:19-1,223 mmol/g, Total Phenolic Content (TPC) are 12.81-13:32%. Phenolic and flavonoid compound are 8.47-28.47%, chlorogenic acid (11.86-18.53%), syringic acid (5.45 to 6.49%) and epicatechin (1.67-2.98%). This product is presented using cold water and the best formulation is the second formulation. This is because the second formulation, nutritional and functional properties contained therein is completing (all detected), as well as in terms of taste is also still acceptable.

Keywords: Coconut cream, cocoa powder, cocoa spirulina, nutrition fact

INTRODUCTION

Currently, public awareness of the importance of healthy living is increasing. The demand for food has shifted from previous demands. The basic consideration of today's consumers in choosing food not just to get fed, nutrient content and delicacy, but also the effects to the health of the body which are called functional foods (Goldberg, 1994).

One of the many results of recent studies proves that the cocoa plant (Theobroma cacao), namely the cocoa beans as raw material for processed products of chocolate, contains many bioactive compounds. One of them is the flavonoid compounds that are beneficial to our health (functional food).

At this time, plantations and the cocoa industries in Indonesia mostly sell or export only in the form of cocoa beans. Although products processed from chocholate have slightly developed in Indonesia, went bankrupt due to the low image of the form of cocoa beans and processed. Besides, cocoa consumption in Indonesia is still very low. Therefore, it is necessary to diversify the processed products of chocolate. So the cocoa industry in Indonesia can regain its footing and improve the country's economy.

Processed product diversification of chocolate as well as innovative products that can be developed is Cocoa Spirulina beverages. Cocoa Spirulina is a beverage made by blending between cocoa powder and spirulina powder that has been fermented before. The purpose of the fermentation spirulina is to reduce the fishy flavor. And the addition of coconut cream honey to improve the flavor. Use of spirulina powder is to obtain health function. This is because Spirulina is a blue-green algae that has gained considerable popularity in the healthy food industry (Sasson, 1997). It is also an important source of a variety of bioactive compounds, including phenol, with different physiological effects (toxic or curative) against human health. Spirulina acts as an antioxidant, antimicrobial and antiviral activities that are important. Spirulina contains vitamin B1 (thiamine), B2 (riboflavin), B3 (niacin), B6 (pyridoxine), B9 (folic acid), vitamin C, vitamin D, vitamin A and vitamin E (Babadzhanov *et al*., 2004) and it contains more complete nutrition and it can be easily absorbed by the body so that digestion and may boost immunity (Khomsan, 2008).

In addition, the Cocoa Spirulina also used cocoa powder mixed with Dutch system. This is because while it contains polyphenols, it also contains vitamins B complex (Steinberg *et al*., 2003). Polyphenols in cocoa are subgroup phytochemicals including flavonoids (Waterhouse *et al*., 1996). More than 4,000 flavonoids have been identified and there may still be many more yet to be discovered. Flavonoids have several functions, among others: anti-inflammatory, anti-carcinogenic, anti-microbial and anti-allergic. They are also powerful antioxidant that helps protect cells

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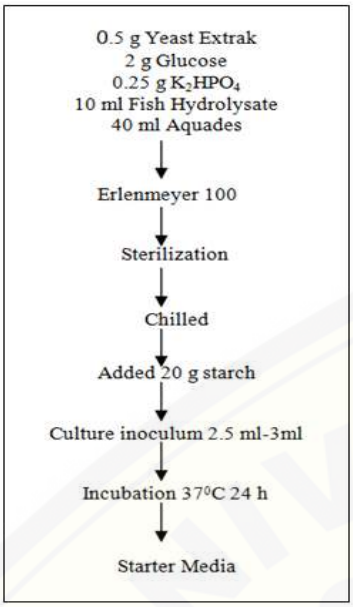

Fig. 1: Flowchart of preparation of microbial starter

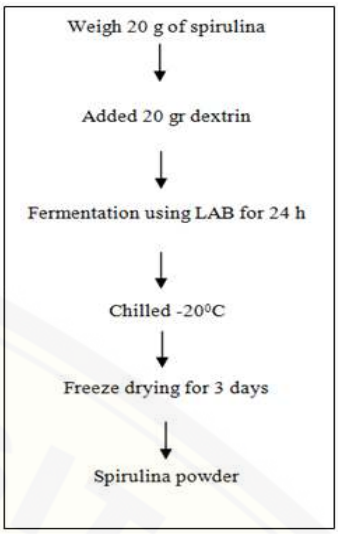

Fig. 2: Flowchart of preparation of powder cream

from damage caused by the excess of free radicals. Many cocoa contains flavonoids, especially epicatechin and catechin (Bearden *et al*., 2000).

Cocoa Spirulina is a functional beverage that is beneficial to health. Cocoa Spirulina research is expected to produce innovative new products and open up new opportunities in the diversification of food. Furthermore, the results of this study are also expected to have the opportunity to be developed by the food industry as a commercial enterprise that is beneficial to health and the whole society.

MATERIALS AND METHODS

Time and place of research: This research was conducted in laboratorium of food processing technology, laboratorium of Biochemistry University of Jember and laboratorium of antioxidant University of Nation Malaysia. Time of research start from January until December 2016.

Materials: The base material used in this study is instant Van Houten cocoa powder, spirulina powder, honey, coconut, sugar, water, distilled water, cultures of lactic acid bacteria *L bulgaricus*, *S. thermophillus* and *L. plantarum*. While the chemicals used are hydrolyzate anchovies, yeast extract, K2HPO¬4, protamex hydrolysis, glucose, dextrin, ether, ethanol, HCL, NaOH, reagent nelson, arsenomolibdat, CaCO3, Pb Acetate, Na oxalate, H2SO4, K2SO4, benzene, MeOH, HCl, H3PO4, phosphate buffer, acetonitril (ACN), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu, Na2CO3, standard B complex vitamins (*thiamine*, *riboflavin*, *niacin*, *pyridoxine*), standard phenolic and flavonoid control (*protocatechuic acid*, *catechin*, *syringic acid*, *chlorogenic acid*, *epicatechin*, *hydroxinamic acid and rutin*), a standard for Total Phenolic Content (TPC) is a *gallic acid*.

Methods: Making the starter (Fig. 1): Starter was made by growing three types of lactic acid bacteria that *L bulgaricus*, *S. thermophillus* and *L. plantarum*. The medium used is yeast extract 0.5 g, 0.2 g of glucose, 0.25 g K2HPO4, 10 mL hydrolyzate anchovies, 40 mL of distilled water and 20 g of starch. Material is sterilized by autoclaving, except starch was sterilized by radiation (UV). Then the result was cooled to produce starter media. Furthermore, pure cultures of bacteria use inoculated and incubated at room temperature 37°C for 24 h.

Spirulina powder manufacture (Fig. 2): Twenty grams of spirulina were mixed with 20 g of dextrin were fermented using lactic acid bacteria. A total 300 mL of distilled water was added to the mixture. Then kept in freezer (-20°C) for 24 h and freeze dried for three days to produce spirulina powder.

Manufacture of coconut cream (Fig. 3): Cream is made from a mixture of coconut and dextrin. 50 g coconut filler is added in the form of dextrin with a ratio of 20% by weight of coconut. Then it is blended until homogeneous and white. It is refrigerated for a day, put freeze drying for 2 days until result cream powder.

Formulation of Cocoa Spirulina (Fig. 4): Three formulations of cocoa spirulina were developed using different ratio of cocoa powder, spirulina, cream and sugar. The three different mixtures were mixed manually. The different fermentations are shown in Fig. 4.

Fig. 3: Flowchart of preparation of coconut cream

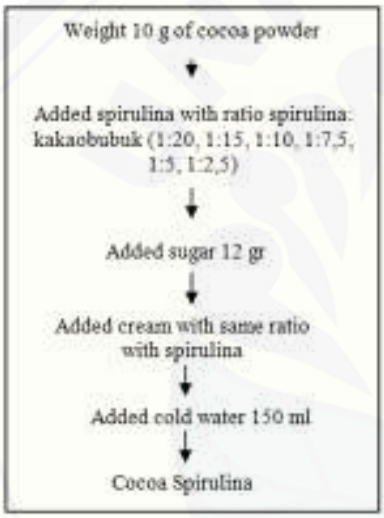

Fig. 4: Formulation of cocoa spirulina

Analysis of chemical composition: The protein hydrolyzate powder was analyzed in triplicates and then the mean value was recorded. Protein, ash, fat and moisture content were determinated according to the method of AOAC (2000). Determination of the antioxidant capacity use of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) (Subagio and Morita, 2001).

Sensory analysis: A Hedonic scale of 1-5 with twenty-trained panelists were employed to Evaluate the sensory characteristics of fish sauce and savory salt on appearance, color, aroma, taste, texture, mouth feel and overall acceptability. The 5-point hedonic scale was rated as 5 = extremely like, 4 = like, 3 = moderately, 2 = dislike, 1 = strongly dislike. The individual scores were averaged and analyzed.

Analysis of the vitamin B complex (thiamine, riboflavin, niacin and pyridoxine): The sample powder (1 g) was mixed with 6 mL of 0.1 M HCl for the determination of vitamin B1, B2 and B3, while for B6, 1 g of sample was mixed with 6 mL 0.06 N HCl and homogeneous. Then autoclaved (Sakura Neoclave 3022) at 1210C for 30 min. The samples were then stored at room temperature and centrifuged (Hermle Z 323 K) at 10,000 rpm for 15 min. Supernatant was removed and filtered through a membrane filter WhatmanNillon (0.2 m). Determination of vitamin B complex is done with Shimadzu HPLC series. The column used is a reverse phase C18 column. Mobile phase consist of 0.04 M phosphate buffer (pump A), ACN (pump B) and 0.02 M phosphate buffer (pump C) as depicted Eckert (2004). Eluent flow rate of 1.0 mL/min and 20 mL injection volume.

Analysis of phenolic and flavonoid: Samples (1 g) were mixed with 50 mL of MeOH using a homogenizer for 10 min and Mixtured centrifuged (Hermle Z 323 K) at 4,000 rpm for 5 min. The residue was re-extracted twice with the same procedure, until there was no color in the supernatant. Supernatant obtained was combined and filtered through a filter paper. Supernatant was removed and filtered through a membrane filter WhatmanNillon (0.2 m) before applying to HPLC. Determination of vitamin B complex was done by Shimadzu HPLC series. The column used is a reverse phase C18 column. Mobile phase was a mixture of methanol: distilled water (2.5: 97.5, v/v) at pH 3 with H3PO4 to pump A and a mixture of methanol: distilled water (50: 50, v/v) at pH 3 with H3PO4 to B. pumps the eluent flow rate at 1 mL/min and 10 mL injection volume.

RESULTS AND DISCUSSION

Sensory analysis: A test was conducted to determine the best product formulations according to the preference panel. A testing conducted on Cocoa Spirulina, including color, aroma and taste. This study uses 30 panelists. A test of the resulting formulations with ratio spirulina, cocoa powder, cream and sugar is presented in Table 1. The maximum limit of the addition of spirulina powder still acceptable according to the panelists is 2 g, because more than 2 g are put, fishy taste becomes very prominent. While the minimum limit is 1 g, because if it is less than 1 g of the flavor of spirulina does not feel at all worry about the effect of spirulina on the body, it is also less than the maximum.

Cocoa steeping water is used in Spirulina, namely the use of cold or hot water. This is because if warm or hot water, spirulina powder does not dissolve completely. Spirulina contains a lot of protein, so that when reconstituted with hot water will cause coagulation of protein denaturation and decrease the solubility (Wu *et al*., 1999).

Additionally, spirulina that has previously been treated fermentation using lactic acid bacteria will form acid flavor. Protein with addition of acid or heating will

Table 1: Formulation Cocoa Spirulina with different ratios of cocoa powder, spirulina, cream and sugar

	Composition			
Formulation	Cocoa powder (g)	Spirulina powder (g)	Cream (g)	Sugar (g)
1	10	2	2	12
2	10	1.5	1.5	12
3	10	1	1	12

Formulation selected from A sensory test, includes three categories: color, taste and smell

Table 2: Proximat analysis of cocoa spirulina

	Percentation (%)			
Formulation	Water content	Ash content	Protein	Fat
1	6.07±0.148	4.51±0.24	2.27±0.36	11.65±0.17
2	5.926±0.105	4.22±0.02	1.45±0.16	9.97±0.13
3	5.808±0.021	4.17±0.065	0.65±0.05	8.94±0.16

Data are presented as the proximate analysis method AOAC (2000), average (means) ± Standard Deviation (SD), repetition (n = 3)

cause coagulation. In the iso-electric pH (pH specific solution typically ranges from 4 to 4.5 wherein the protein has the same positive and negative charge, so mutually neutralize) the solubility of the protein is decreased or settled. At temperatures above 60°C, protein solubility decreases (coagulation) because at high temperatures, it increases the kinetic energy of protein molecules, causing vibrations strong enough to damage the structure of the bond or the secondary, tertiary and quaternary that causes coagulation (Wu *et al*., 1999).

Chemical analysis: The results showed Cocoa Spirulina components are heavily influenced by the amount of material composition and multiple treatment ingredients in the product (Table 2).

Cocoa Spirulina protein content is very small, whereas according to Belay *et al*. (1993), spirulina can be considered as a source of protein because it contains more than 60% protein on a dry weight. This is due to fermentation treatment on spirulina. During the fermentation process solving content occurs-the content of the material to be more modest. While the addition of coconut cream did not significantly affect the protein content.coconut cream contains very little protein. Cocoa fat content in Spirulina is also high, ranging from 8.94-11.65%. This is because the product consists of cocoa CocoaSpirulina powder, spirulina and creams; these materials have a large enough fat content. Based on data from USDA nutrition fact (2010), coconut cream has a fat content of 17.5%, cocoa powder Dutch system spirulina 13:14% and 7.6%.

The antioxidant activity: Results from this study showed that the antioxidant activity of Spirulina Cocoa decreased with decrease in the amount of spirulina, which is 1:23 mmol/g until 1:19 mmol/g (Fig. 5).

Cocoa content of antioxidants in Spirulina is still relatively low. In fact, spirulina has great potential antioxidants such as phycocyanobilin (PCB) (McCarty, 2007). The process of fermentation using lactic acid bacteria to reduce the fishy flavor in spirulina can also

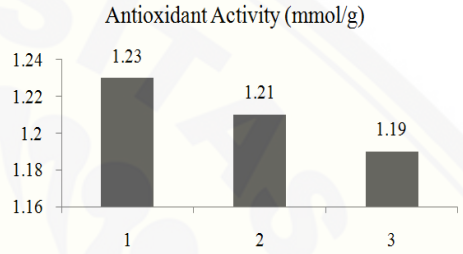

Fig. 5: Antioxidant activity of cocoa spirulina; Data are presented as an antioxidant activity, average and repetition (n = 3)

increase the amount of antioxidants in it. This is due to the antioxidant properties of ethanol extract 80% of the protein-rich product obtained by fermentation of lactic acid fermentation showed that the product can be regarded as a powerful antioxidant (Pyo *et al*., 2004). Antioxidant content is low because the amount of spirulina are used in the formulation of a maximum of 2 g, thereby causing an increase in the amount of antioxidants not too significant. Besides Dutch cocoa powder is used also causes antioxidant activity and total phenolic lower than other powdered cocoa. According to Miller (2008) alkalinization which is used to reduce the acidity and raise the pH of cocoa, such as Dutch cocoa, it reduces the levels of procyanidin (Miller, 2008) although cocoa powder is still above 10% of flavanol-containing foods when compared to foods listed in the USDA Database procyanidin.

Thus Cocoa Spirulina can still be categorized as beverage that contain antioxidants. Phenolic compounds in cocoa and spirulina is an important antioxidant, since the high redox potential. They act as a reducing agent, hydrogen donor singlet quenchers, oxygen and metal chelating agents (Tsao and Deng, 2004). Related health effects such as antibacterial phenolic compounds (Ezoubeiri *et al*., 2005), antimutagenic (Pedreschi and Cisneros-Zevallos, 2007), anticarcinogenic (Kähkönen and Heinonen, 2003), antithrombotic and vasodilatory activity (Cook and Samman, 1996) have been reported. The beneficial effect was directly related to the antioxidant properties.

Table 3: Phenolic and flavonoid content in cocoa spirulina

	Formulation (mg/100 g)		
Componen	1	2	3
Protocatechuic acid	0.639	0.783	0.764
Catechin	8.47	19.357	28.476
Syringic acid	5.45	6.996	Not detected
Chlorogenic acid	11.866	14.941	18.53
Epicatechin	2.121	2.374	2.988
Hydroxinamic acid	0.382	0.128	0.109
Rutin	0.594	0.556	0.455

Data are presented as phenolic and flavonoid compounds using HPLC, at a wavelength of 280 nm

Table 4: Results vitamin B complex on cocoa spirulina

	Componen vitamin B kompleks (mg/100 gr)			
Formulations	Thiamine	Riboflavin	Niacin	Pyridoxine
1	45.75	192.67	19.5	94.18
2	43.58	158.76	19.06	85.61
3	41.8	137.392	Not detected	70.921
Spirulina fermented	50.78	250.88	28.6	138.32

Data are presented as vitamin B complex by using HPLC at a wavelength of 210 nm for the determination of vitamin B1 (thiamine), B2 (riboflavin) and B3 (niacin). Determination of vitamin B6 with a wavelength at 290 nm

In addition, the potential health benefits of spirulina phenolic compounds have been extensively studied. In humans, spirulina proved to be a powerful antioxidant that may prevent oxidative damage to biomolecular like DNA, lipids and proteins that play a role of chronic diseases such as cancer (Droge, 2002). The antioxidant activity of the extract of spirulina was recently studied by several authors by Troloc Equivalent Antioxidant Capacity (TEAC) and other methods (Li *et al*., 2007). DPPH scavenging activity of spirulina extract is in positive correlation with increased levels of phenols in the extract. Thus, the antioxidant properties of spirulina extract are associated with a high phenolic content (Halliwell and Gutteridge, 1989).

Total phenolic and flavonoid content: In the research content of phenolic and flavonoid used method of High Performance Liquid Chromatography (HPLC). The results showed that in Cocoa spirulinahas many phenolic compounds and flavonoids such as protocatechuic acid, catechin, epicatechin, syringic acid, choloregenic acid, hidroxinamic acid and rutin. The largest component in the sample is catechin which is 28.5 mg/100 g in the third formulation. Phenolic and flavonoid content results are shown in Table 3.

From Table 3, it can be seen that Cocoa Spirulina contains many phenolic compounds and flavonoids, particularly catechin, chlorogenic acid and syringic acid and epicatechin. At first, cocoa known to contain some polyphenols, such as catechins, epicatechin and quercetin (Kim and Keeney, 1983; Bonvehi and Coll, 1997; Osakabe *et al*., 1998; Sanbongi *et al*., 1998), but it also contains chlorogenic acid (Kim and Keeney, 1983). the phenolic and flavonoid content of cocoa other spirulina as protocatechuic acid acid, syringic acid, hydrocinamic acid, rutin and chlorogenic acid is most likely derived from spirulina. All these compounds have been reported to identify phenol in spirulina (Onofrejova *et al*., 2009).

Vitamin B complex: B vitamins are eight water-soluble vitamins that play an important role in cell metabolism. Vitamin B once considered to be a single vitamin, referred to as vitamin B (much as people refer to vitamin C or vitamin D). Later research showed that they are chemically distinct vitamins that often coexist in the same foods. In general, supplements containing all eight are referred to as vitamin B complex. Each B vitamin supplement called by the specific name of each vitamin, as B1 (thiamine), B2 (riboflavin), B3 (niacin), B6 (pyridoxine) and others (Woolston, 2008). In this study, vitamin B complex in Cocoa Spirulina determined using HPLC. The results showed that the amount of vitamin B Cocoa Spirulina higher than spirulina powder. Results of the amount of vitamin B complex cocoa spirulina are shown in Table 4. In the table, for formulation 3 content of niacin was not detected because the expected minimum limit HPLC instrument is 19 mg/100 g.

The total amount of vitamin B that is very large in Cocoa Spirulina is probably caused by several things. Before it is mixed with cocoa powder, spirulina fermented using lactic acid bacteria. In the process of fermentation, the lactic acid bacteria will produce vitamins. During the fermentation process, the amount of vitamin B will increase (Soomro *et al*., 2002). Vitamin B 12, increased to 33 times during the fermentation, riboflavin rose about 8 to 47 times, pyridoxine 4 to 14 times, niacin 2-5 times, 2 to 3 times biotin, folic acid 4 to 5 times and acid pantothenat doubled (Astawan, 1991). Vitamin B 6 and B 12 is a type of vitamin B complex which has the most striking rise. The type and quality of spirulina also affect the amount of vitamin B. type spirulinaspirulina used are of

high quality and are also produced in the factory Tangerang, Indonesia. Mills usually use a machine to dry fast drying machine, which loses little nutritional composition, can be guaranteed purity and would not accept two times less pollution. So this causes fermentation spirulina contains vitamins B is higher than the usual powdered spirulina.

In addition, the use of cocoa powder with the Dutch system also affects an increasing number of vitamin B Cocoa Spirulina. Dutch cocoa powder is a moderate source of vitamin B-group such as thiamin, riboflavin, niacin, B6 and folic acid are essential to increase the production of energy from food (Steinberg *et al*., 2003). So, Cocoa Spirulina is also a health drink rich in vitamin B.

CONCLUSION

Cocoa Spirulina is a functional food, which has nutritional and functional properties of the product details as following: Cocoa Spirulina contains very little protein is 0.66% to 2.28%, but it contains a lot of fat in the range of 8.94% to 11.65% and the B complex vitamins (thiamine, riboflavin, niacin and piridoxine). Antioxidants contained in Spirulina is Cocoa flavonoids and phenolic components are especially chatechin 8.47-28.47%, chlorogenic acid (11.86-18.53%) and syringic acid (5:45 to 6:49%) and epichatechin (1.67-2.98%). Cocoa product details Spirulina is in the form of powder but presented using cold water, with the best formulation is the second formulation.

ACKNOWLEDGMENT

We would like to thanks to the Directorate of Higher Education Ministry of Education and Culture Republic of Indonesia through the Best Scholarship, join with the University Nations of Malaysia specially Faculty of Science and Technology.

REFERENCES

AOAC, 2000. Proximat Analysis. Gaithersbug, pp: 1058-1059.

Astawan, W., 1991. Vegetable Food Processing Technology is Appropriate. Pressindo Academic, Jakarta, Indonesia.

Babadzhanov, A.S., N. Abdusamatova, F.M. Yusupova, N. Faizullaeva, L.G. Mezhlumyan and M.K. Malikova, 2004. Chemical composition of spirulina platensis cultivated in Uzbekistan. Chem. Nat. Comp., 40(3): 276-279.

Bearden, M.M., D.A. Pearson, D. Rein, K.A. Chevaux, D.R. Carpenter, C.L. Keen and H. Schmitz, 2000. Potential Cardiovascular Health Benefits of Procyanidins Present in Chocolate and Cocoa. In: Parliament, T.H., C.T. Ho and P. Schieberle (Eds.), Caffeinated Beverages: Health Benefits, Physiological Effects and Chemistry. American Chemical Society, Washington, DC, pp: 177-186.

Belay, A., Y. Ota, K. Miyakawa and H. Shimamatsu, 1993. Current knowledge on potential health benefits of *Spirulina*. J. Appl. Phycol., 5(2): 235-241.

Bonvehi, J.S. and F.V. Coll, 1997. Evaluation of bitterness and astringency of polyphenolic compounds in cocoa powder. Food Chem., 60(3): 365-370.

Cook, N.C. and S. Samman, 1996. Flavonoids-chemistry, metabolism, cardioprotective effects and dietary sources. J. Nutr. Biochem., 7(2): 66-76.

Droge, W., 2002. Free radicals in the physiological control of cell function. Physiol. Rev., 82(1): 47-95.

Eckert, V., 2004. Analytical HPLC Application: Separation of B - Vitamines + C. Merck KGA, Darmstadt, Germany.

Ezoubeiri, A., C.A. Gadhi, N. Fdil, A. Benharref, M. Jana and M. Vanhaelen, 2005. Isolation and antimicrobial activity of two phenolic compounds from *Pulicaria odora* L. J. Ethnopharmacol., 99(2): 287-292.

Goldberg, I., 1994. Functional Foods: Designer Foods, Pharmafoods, Nutraceuticals. Chapman and Hall, New York.

Halliwell, B. and J.M. Gutteridge, 1989. Free Radicals and Other Reactive Species in Disease. In: Free Radicals in Biology and Medicine. Clarendon Press, Oxford, pp: 617-623.

Kähkönen, M.P. and M. Heinonen, 2003. Antioxidant activity of anthocyanins and their aglycons. J. Agr. Food Chem., 51(3): 628-633.

Khomsan, 2008. Certificate analysis of spirulina. Health Department, Center of Research and Development Nutrition and Food, Indonesia.

Kim, H. and P.G. Keeney, 1983. Method of analysis for (-)-epicatechin in cocoa beans by high performance liquid chromatography. J. Food Sci., 48(2): 548-551.

Li, H.B., K.W. Cheng, C.C. Wong, K.W. Fan, F. Chen and Y. Jiang, 2007. Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. Food Chem., 102(3): 771-776.

McCarty, M.F., 2007. "Iatrogenic Gilbert syndrome"--a strategy for reducing vascular and cancer risk by increasing plasma unconjugated bilirubin. Med. Hypotheses, 69(5): 974-994.

Miller, D., 2008. New Study Re-emphasizes Natural Cocoa Powder has High Antioxidant Content. (Study also Shows that Dutched Cocoas Retain Significant Levels of Antioxidants). The Hershey Company, North American.

Onofrejova, L., J. Vasícková, B. Klejdus, P. Stratil, L. Misurcova, S. Kracmar, J. Kopecky and J. Vacek, 2009. Bioactive phenols in algae: The application of pressurized-liquid and solid-phase extraction techniques. J. Pharmaceut. Biomed., 51(2): 464-470.

Osakabe, N., M. Yamagishi, C. Sanbongi, M. Natsume, T. Takizawa and T. Osawa, 1998. The antioxidative substances in cacao liquor. J. Nutr. Sci. Vitaminol., 44(2): 313-321.

Pedreschi, R. and L. Cisneros-Zevallos, 2007. Phenolic profiles of Andean purple corn (*Zea mays* L.). Food Chem., 100(3): 956-963.

Pyo, Y.H., T.C. Lee and Y.C. Lee, 2004. Effect of Lactic Acid Fermentation on Enrichment of Antioxidant Properties and Bioactive Isoflavones in Soybean. Dept. of Food Science, Center for Advanced Food Technology, Rutgers, the State Univ. of New Jersey.

Sanbongi, C., N. Osakabe, M. Natsume, T. Takizawa, S. Gomi and T. Osawa, 1998. Antioxidative polyphenols isolated from theobroma cacao. J. Agr. Food Chem., 46(2): 454-457.

Sasson, A., 1997. Microalgal Biotechnologies: Recent Developments and Prospects for Developing Countries. United Nations Educational, Scientific and Cultural Organization (UNESCO), BIOTEC Publication 1/2542, Place de Fontenoy, Paris, France, pp: 11-31.

Steinberg, F.M., M.M. Bearden and C.L. Keen, 2003. Cocoa and chocolate flavonoids: Implications for cardiovascular health. J. Am. Diet. Assoc., 103(2): 215-223.

Subagio, A. and N. Morita, 2001. No Effect of esterification with fatty acid on antioxidant activity of lutein. Food Res. Int., 34(4): 315-320.

Tsao, R. and Z. Deng, 2004. Separation procedures for naturally occurring antioxidant phytochemicals. J. Chromatogr. B, 812(1-2): 85-99.

Waterhouse, A.L., J.R. Shirley and J.L. Donovan, 1996. Antioxidants in chocolate. Lancet, 348(9030): 834.

Woolston, C., 2008. B vitamins don't boost energy drinks' power. News Paper, Los Angeles Times.

Wu, C., S. Asakawa, N. Shimizu, S. Kawasaki and Y. Yasukochi, 1999. Construction and characterization of bacterial artificial chromosome libraries from the silkworm, Bombyx mori. Mol. Gen. Genet., 261(4-5): 698-706.