International Food Research Journal 24(1): 367-371 (February 2017)
Journal homepage: *http://www.ifrj.upm.edu.my*

Study on chemical, physical, microbiological and sensory of yoghurt enriched by *Spirulina platensis*

[1*]Agustini, T.W., [2]Soetrisnanto, D. and [1]Ma'ruf, W.F.

[1]*Fisheries Department, Faculty of Fisheries and Marine Science, Diponegoro University*
Jl. Prof. H. Soedarto, S.H, Tembalang campus, Semarang, 50275 Indonesia
[2]*Department of Chemical Engineering, Faculty of Engineering, Diponegoro University*
Jl. Prof. H. Soedarto, S.H, Tembalang, Semarang, 50275 Indonesia

Article history

Received: 5 October 2015
Received in revised form: 10 March 2016
Accepted: 23 March 2016

Keywords

Enriched yoghurt
Spirulina platensis
Chemical
Physical
Microbiological parameters
Sensory

Abstract

Yoghurt is a fermented food product which is commonly consumed by people and its consumption increase by year. This increasing would results in significant development on diversification of yoghurt due to its benefit for human health. The addition of *Spirulina platensis* into yoghurt can increase the quality of yoghurt especially on nutritional and functional properties of the product. Therefore, this study was aimed to determine the best concentration of *S. platensis* to yoghurt product, to observe the characteristics of chemical, physical, microbiological and hedonic of enriched yoghurt. The materials used were yoghurt added with different concentration of *S. platensis* (0.5%; 0.75%; 1%; 2% and 3%). Based on the results, the best treatment of *S. platensis* addition was 1% that can be accepted by panelist based on hedonic test. The best treatment of 1% *S. platensis* was then compared to control (0% *S. platensis*) and observed for proximate analysis, viscosity, pH, total lactic acid, and total lactic acid bacteria of the product. Enriched yoghurt with 1% addition of *S. platensis* has significant different ($p<0.05$) to control on protein, viscosity and total lactic acid bacteria, but not significant different ($p>0.05$) on water content, ash, carbohydrate, fat, total lactic acid, pH, hedonic and the product comply with Indonesian National Standard.

Introduction

Functional food become an important issue recently and people awareness increase in consuming functional food which is supporting for their health. In modern era, food is not requested as energy and nutritional resources but also contribute for immune system for the body causing by nutritional depletion, apart from increase antibody system. (Roberfroid, 2000; Merad, 2003). The concept of functional food also referred to as 'nutracueticals or pharmaceutical food' has been recognized to achieve optimal health and can possibly reduce the risk of diseases (Mollet and Rowland, 2002).

Yoghurt is considered as one of functional food and can be used to improve digestive system of human body. Consumption of yoghurt increase by year and there are many variation of yoghurt produced with different flavor and characteristic. Some research have been conducted on yoghurt related to its processing technique (Fangary *et al*.,1999; Kumar and Mishra, 2004; Afonso *et al.*, 2008; Shoji *et al*., 2013; Ribeiro *et al*., 2014) and its quality (Bouteille *et al*., 2013; Oliveira *et al*., 2015) but no one has been found regarding to utilization of spirulina for enriching its nutritional value. Nowadays, inclusion of some food materials which has high nutritional benefit such as *Spirulina platensis* is becoming popular. As food material which has high protein and micronutrient, spirulina is not only used as single cell protein, but can also be used as functional food (Li and Qi, 1997). Generally, *Spirulina* is utilized as healthy food for human (Becker, 1994) and is produced in the form of capsule, juice, or tablet. The benefit of consuming Spirulina is that it contain phycosianin which is potential to prevent the leukemia cell growth (Liu *et al*., 2000).

Many foods can be supplemented by *Spirulina* to increase their nutritional value. Some food such as noodle, cake, biscuit, can be supplemented by *Spirulina* to increase their nutritional value (Henrikson, 1989). He further stated that *Spirulina* can be consumed for 10 g/day for maintaining health, not only for kids but also for elderly. This study was purposed to find out the best concentration of *S. platensis* added into yoghurt which considered to acceptance by people and also to observe the characteristics of chemical, physical, microbiological and hedonic of enriched yoghurt.

*Corresponding author.
Email: *tagustini@yahoo.com*

Materials and Methods

Materials used was *Spirulina platensis* in the form of powder and purchased directly from the cultivation area at Sukoharjo, Central Java, Indonesia. *S. platensis* powder was produced from fresh spirulina and was dried directly by using mechanical oven at temperature of 40°C for 10 hours to get final water content below 10%. This spirulina powder was further used as fortification substance into yoghurt. There are two steps of study, i.e: 1). Preliminary study : formulation of yoghurt was conducted by adding *S. platensis* powder at different concentration of : 0.5%, 0.75%, 1%, 2% and 3%. Analysis of the product was conducted based on sensory test (Hedonic test). 2). Primary study: The best treatment resulted from Preliminary study which was : 1% spirulina (yoghurt A), was further used in Primary study and compared to 0% spirulina (yoghurt B).

Preparation of product

Methods used to prepare yoghurt : fresh cow milk was heated at 80°C for 15 minutes (Pasteurization) and was cooled to achieve temperature of 43°C. *S. platensis* powder was added in different concentration of 0% and 1.0%, and homogenized. Starter of *Lactobacillus bulgaricus, Streptococcus thermophillus* and *Lactobacillus acidophilus* were added with ratio of 1:1:1 as much as 5%. Let the mixture for 6 hours to be fermented and 1% flavoring agent, 5% gelatin and 10% sucrose were finally added and stirred thoroughly. Homogenized mixture was then cooled at refrigerated temperature before subjected for further analysis. (Venice *et al*., 2013 with modification)

This study used experimental laboratories method with paired T-test for the Primary study by comparing the best treatment of yoghurt (1% *S. platensis* concentration – yoghurt A) to control treatment (0% *S. platensis* concentration – yoghurt B). The experiment was conducted in triplicate. The products were subject to some parameters test of proximate analysis i.e : fat, protein, carbohydrate, ash, and water content (AOAC, 2007), viscosity and pH (AOAC, 2007), total lactic acid (National Standardization Agency – SNI 2981:2009), total lactic acid bacteria (Feldsine *et al.*, 2007) and hedonic test (National Standardization Agency, 2009). The data obtained were then analyzed statistically for distribution, normality and then comparison by using t-Test for obtaining the characteristic of enriched yoghurt based on nutritional and some functional properties.

Results and Discussion

Preliminary study

Yoghurt was made by adding *S. platensis* powder with different concentration of 0.5%; 0.75%; 1%; 2%; and 3%. This study was designed to observe the highest concentration of *S. platensis* powder in producing enriched yoghurt which is acceptable by panelist based on hedonic test. As stated by Guldas and Irkin (2010) that high *Spirulina* addition into food product will affect to sensory and unpleasant taste and flavor of the product.

Hedonic test

Hedonic test was done by assessing the product based on appearance, flavor, taste and consistency. Based on hedonic test, enriched yoghurt added with 1% *Spirulina* powder showed the best result of 7.13± 0.94. This value was considered as the highest concentration of *Spirulina* added into the yoghurt while maintain the product from hedonic test point of view (Score more than limit border for hedonic test). Enriched yoghurt with 1% *Spirulina* has characteristic of more acceptable flavor, better appearance comparing to that of 2% and 3% *Spirulina* addition. This concentration of 1% *Spirulina* powder (yoghurt A) would then be used for Primary study and compared to product without *Spirulina* addition (0% spirulina – yoghurt B).

Primary study

Proximate analysis of yoghurt

Proximate analysis of yoghurt A was compared to yoghurt B. Table 1 shows the different in proximate analysis between these two products. Based on T-test, there is no significant different on water, fat, ash and carbohydrate content between the samples. Whereas for protein content is influenced by treatment. According to Askar and Sugiarto (2005), water content of some comercial yoghurts in the range of 71% – 90%. This variation of water content is due to native condition of raw material (fresh milk) in which they have different initial water content and also method of processing. Increase water content of yoghurt will consequently reduce viscosity which results in changing texture to become watery. In addition (Christwardana *et al.*, 2013), said that due to small portion of *S. platensis* added in the yoghurt (1%) and also less water content of dried *S. platensis* powder (3-6%), so that there is no significant different between two samples treatment.

Based on National Standardized Agency (2009), ash content of yoghurt has maximum value of 1.0%.

Table 1. Proximate analysis of yoghurt A (1% *Spirulina*) and yoghurt B (0% *Spirulina*)

Parameter	Yoghurt A	Yoghurt B
Water (%)	81.545±0.51	81.191±0.51
Protein (%)	3.551±0.85	2.288±0.035**
Fat (%)	0.704±0.595	1.112±0.141
Ash (%)	0.113±0.006	0.105±0.007
Carbohydrate (%)	14.804±0.197	15.293±0.646

Therefore both samples of yoghurt comply with Indonesian National Standar. Ash content of yoghurt resulted from this study is relativelly lower than commercial yoghurt in the range of 0.29% - 0.80% (Askar and Sugiarto, 2005). It is expected that ash content of enriched yoghurt is higher than those of commercial one, as we know that *Spirulina* contain high ash content and Fe for 10.66% and 527.75 ppm, respectively (Agustini *et al*., 2015). However, in this study we found that there is no significant different between yoghurt A and yoghurt B and this is due to small amount of spirulina added to yoghurt A. Ash content of yoghurt A and B are 0.113±0.006% and 0.115±0.007%, respectively and both have complied with Indonesian National Standard (maximum value of ash : 1 %).

Yoghurt A showed significant different on protein content comparing to yoghurt B. This perfomance elucidated that adding *Spirulina* powder can improve the nutritional value especially on protein of the yoghurt. *S. platensis* powder used in this study has protein content of 67.18% (Agustini *et al.*, 2015) and other researcher obtained for protein content of *S. platensis* in the range of 56% - 62%. This high content of protein can obviously increase the total protein when adding to food product even at low concentration (Christwardana *et al*., 2013). Fermentation process taking place during yoghurt production may also influence protein increase. Protein contained in the samples (yoghurt A : 3.551% and yoghurt B: 2.288%) seems to be comparable than Indonesian National Standard (2.7% protein - NSA, 2009). However, comparing with other comercial yoghurt which have protein content in the range of 2.279% - 2.933% (Ihsanudin, 2014) and 0.95% - 3.42% (Askar and Sugiarto, 2005), yoghurts resulted from this study are comparable.

Fat content of yoghurt produced in this study showed that there is no significant different between them ($p<0.05$). Quality standar of yoghurt for fat content is minimum 3%, low fat yoghurt : 0.6% - 2.9% and non fat yoghurt maximum 0.5%. Therefore, yoghurt produced fom this study is considered as low fat yoghurt.

Based on T-test there is no significant different on carbohydrate between yoghurt A and yoghurt B. Yoghurt A has carbohydrate content of 14.804%; yoghurt B has carbohydrate of 15.292%. From other research there have been stated that Romulo (2012) produced yoghurt with carbohydrate content of 10.45%, whereas Hartoto (2003), performed yoghurt with carbohydrate content 9.30%. Yoghurt A (with 1% added *S. platensis* powder) performed higher in carbohydrate compared to yoghurt B.

Table 2. Total lactic acid, viscosity, pH and total lactic acid bacteria of yoghurt A (1% *Spirulina*) and yoghurt B (0% *Spirulina*)

Parameters	Yoghurt A	Yoghurt B
Total lactic acid	1.558±0.032	1.376±0.261
Viscosity (cPs)	358.920±44.296	192.627±13.317**
pH	4.707±0.045	5.070±0.047
Total lactic acid bacteria (col/g x 10^9)	1.693±0.021	1.347±0.034**

Analysis of Lactic acid, pH, Viscosity, Total Lactic acid bacteria of yoghurt

Total lactic acid, viscosity, pH and total lactic acid bacteria of yoghurt obtained from this study are presented on Table 2. Based on Table 2 and t-test, total lactic acid resulted from the samples have no significant different ($P > 0.05$). This confirms that adding of *S. platensis* powder has no effect on total lactic acid of yoghurt. According to Askar and Sugiarto (2005), acid containing on yoghurt is the major product typically resulted from yoghurt. This acid resulted from fermentation process of carbohydrate inside the milk (lactose) by lactic acid bacteria to produce lactic acid. This is confirmed by lower pH resulted from enriched yoghurt A meaning that total lactic acid of yoghurt A higher than yoghurt B. Bacteria of lactic acid utilized lactose as energy and carbon sources for their growth. According to National Standardized Agency (2009), quality standard of total lactic acid of yoghurt is 0.5% – 2.0%. Askar and Sugiarto (2005), was found that total lactic acid on commercial yoghurt which has the value of 0.73% to 1.92%. This is obviously performed that total lactic acid of yoghurt added *S. platensis* powder comparable to that of commercial product.

Based on t-test, there is significant different on viscosity of yoghurt between two treatments. Increase viscosity of yoghurt A is due to higher number of lactic acid bacteria performed by adding *S. platensis* powder. Thus increase in lactic acid bacteria has brought about increase in lactose destruction into lactic acid and this result in increase in viscosity. According to Wahyudi and Samsundari (2008), formation of lactic acid by lactic acid bacteria

Tabel 3. Hedonic test of yoghurt enriched by *S. platensis* powder

Parameter	Spirulina addition	
	Yoghurt A	Yoghurt B
Appearance	8.07±1.46	7.20±1.21
Flavor	7.80±1,24	6.87±1,38
Taste	7.93±1.36	7.07±1.34
Consistency	8.33±1.21	7.53±1.28

during processing of yoghurt can increase total acid, so that there is casein coagulation which result in gel-like forming (semi solid) and increasing viscosity of yoghurt.

Total Lactic acid bacteria (LAB) resulted from yoghurt A and B can be seen on Table 2. Based on t-test, the number of lactic acid bacteria from both samples showed significant different ($p < 0.05$). Addition of *S. platensis* powder on yoghurt has revealed in increasing number and accelerate growth of lactic acid bacteria. Nutrition content of *S. platensis* can be utilized by the bacteria for their growth. Increase of *S. platensis* on yoghurt showed positif result in improving survival rate of lactic acid bacteria present in yoghurt (Fadei *et al*., 2013). The growth and survival rate of lactic acid bacteria are much better and this phenomenon can be described that percentage of nitrogen from free amino acid, pepton deliberated from *S. platensis* will be used as nutritional source by lactic acid bacteria. In addition, some commercial yoghurt has total lactic acid bacteria of 1.485×10^9; and yoghurt A : 1.693×10^9. The result showed that yoghurt A has higher lactic acid bacteria compared to yoghurt B and commercial product.

pH of yoghurt B decreased slower comparing to yoghurt A. There is no significant different on pH of the samples. Addition of 1% spirulina in the yoghurt tend to decrease pH of yoghurt sharply because there is more number of lactic acid bacteria present in the product. This phenomenon was due to available nutrition providing by *S. platensis* powder, so that the growth of lactic acid bacteria was accelerated. This condition gave more possibility for fermentation process to proceed and lactose degraded into lactic acid which consequently decreased the pH.

Hedonic test

Hedonic test is considered as subjective method for quality analysis of food in which it was conducted by 30 panelist. The result showed that yoghurt A (with 1% *S. platensis*) more preferable than yoghurt B (without *S. platensis*) especially for appearance and consistency. However, based on statistical analysis, no significant difference was observed for the attribute appearance, flavor, taste, consistency and overall impression of both samples. As stated by Ribeiro *et al.* (2014), who found that yoghurt seems to be stable for sensory attribute even during storage at refrigerator for 35 days. In this study the samples was observed directly on finished product after processing, and hence no effect of spirulina addition was observed on the finished product.

Conclusion

Based on the study on application of *S. platensis* into yoghurt, it can be concluded that the best treatment to produce enriched yoghurt was by addition of 1% *S. platensis* which acceptable by panelist, and enriched yoghurt with 1% addition of *S. platensis* has significant different to control (yoghurt B) on protein, viscosity and total lactic acid bacteria ($p<0.05$), but not significant different on water content, ash, carbohydrate, fat, total lactic acid, pH, hedonic ($p>0.05$) and the product comply with the standard (Indonesian National Standard).

Acknowledgements

We are ackowledged and thank to Directorate General of Higher Education for this honorable grant through National Competitive Grant of MP3EI, fiscal year 2013-2014 and participation of CV. Neo Algae in contributing *Spirulina* and cooperation for this work.

References

Afonso, I. M., Cruz, P., Maia, J. M. and Melo, L. F. 2008. Simplified numerical simulation to obtain heat transfer correlation for stirred yoghurt in a plate heat exchanger. Food and Bioproducts Processing 86: 296-303.

Agustini, T. W., Suzery, M., Sutrisnanto, D., Ma'ruf, W. M. and Hadiyanto. 2015. Comparative study of bioactive compounds extracted from fresh and dried Spirulina sp. Procedia Environmental Science 23: 282-289.

Askar, S. and Sugiarto. 2005. Chemical and organoleptic test of yoghurt quality. In Hidayati, N. (Eds). Proceeding of National Technical Meeting on Agricultural Functional. 13-14 September 2005. Bogor.

Association of Official Analitycal Chemist (AOAC). 2007. Official Method of Analysis of the Association of Official Analytical of Chemist. Arlington: The Association of Official Analitycal Chemist, Inc.

Becker, E. W. 1994. Microalgae: biotechnology and microbiology, Cambridge: Cambridge University

Press

Bouteille, R., Cordelle, S., Laval, C., Tournier, C., Lecanu, B. and Schlich, P. 2013. Sensory exploration of the fresh sensation in palin yoghurt and yoghurt-like products. Food Quality and Preference 30: 282–292.

Christwardana, M., Nur, M. A. and Hadiyanto. 2013. *Spirulina platensis*: Its potential for functional food. Journal of Applied Food Technology 2(1): 1-4.

Fadei, V., Mohammad-Alasti, F. and Khosravi-Darani. K. 2013. Influence of *Spirulina platensis* powder on the starter culture viability in probiotic yoghurt containing spinach during cold storage. European Journal of Experimental Biotechnology 3(3): 389–393.

Fangary, Y. S., Barigou, M. and Seville, J. P. K. 1999. Simulation of yoghurt flow and prediction of its end-of-process properties using rheological measurement. Trans IChemE 77(C): 35-39.

Feldsine, P., Abeyta, C. and Andrew, W. H. 2007. International methods committee guidelines for validation of qualitative and quantitative food microbiological official methods of analysis. Journal of Association of Official Analitycal Chemist International 85(5): 1187-1200.

Guldas, M. and Irkin, R. 2010. Influence of *Spirulina platensis* powder on the microflora of yoghurt and acidophilus milk. Mljekarstvo 60(4): 237–243.

Ihsanuddin, M. H. 2014. The effect of *Spirulina platensis* powder addition on yoghurt quality. Semarang, Indonesia : Diponegoro University, Bachelor thesis.

Hartoto, M. 2003. Production of Symbiotic yoghurt using Mixed culture *(S. thermophylus, Bifidobacterium bifidum, Lactobacillus casei)*. Indonesia: Bogor Agricultural University, Bachelor thesis.

Henrikson, R. 1989. Earth Food Spirulina (Eds 6). Essential fatty acids and phytonutrients, Hawaii: Ronore Enterprises. Inc.

Kumar, P. and Mishra, H. N. 2004. Yoghurt powder – A review process technology, storage and utilization. Trans IChemE 82(C2): 133-142.

Li, D. M. and Qi, Y. Z. 1997. Spirulina industry in China: Present status and Future prospects. Journal of Applied Phycology 9: 25–28.

Liu, Y., Xu, L., Cheng, N., Lin, L. and Zang, C. 2000. Inhibitory effect of pycosianin from *Spirulina platensis* on the growth of human leukemia K562 cells. Journal of Applied Phycology 12: 125 –130.

Menrad, K. 2003. Market and marketing of functional food in Europe. Journal of Food Engineering 56: 181-188.

Mollet, B. and Rowland, I. 2002. Functional foods: At the frontier between food and pharmacy. Current opinion in Biotechnology 13: 483-485.

National Standardization Agency. 2009. Indonesian National Standard 2981-2009. Yoghurt.

Oliveira, A., Alexandre, E. M. C., Coelho, M., Lopes, C., Almeida, M. and Pintado, D. P. F. 2015. Incorporation of strawberries preparation in yoghurt: Impact on phytochemicals and milk protein. Food Chemistry 171: 370–378.

Ribeiro, M. C., Chaves, K. S., Gebara, C., Infante, F. N. S., Grosso, C. R. F., and Gigante, M. L. 2014. Effect of microencapsulation of Lactobacillus acidophilus LA-5 on physicochemical, sensory and microbiological characteristics of stirred probiotic yoghurt. Food Research International 66: 424–443.

Roberfroid, M. B. 2000a. A European consensus of scientific concepts of functional foods. Nutrition 16: 689-691.

Roberfroid, M.B. 2000b. Concept and strategy of functional food science: the European perspective. American Journal of Clinical Nutrition 71: 1660S–1664S.

Romulo, A. 2012. Study on application of angkak extract for low fat yoghurt as functional food. Bogor, Indonesia: Bogor Agricultural University, Banchelor thesis.

Shoji, A. S., Oliveira, A. C., Balieiro, J. C. C., Freitas, O., Thomazini, M., Heinemann, R. J. B., Okuro, P. K., and Favaro-Trindade, C. S. 2013. Viability of *L. acidophilus* microcapsules and their application to buffalo milk yoghurt. Food and Bioproduct Processing 91: 83–88.

Venice, C. I., Bergamini, C. V., Zalazar, C. A. and Perotti, M. C. 2013. Effect of Lactose Hydrolysis during manufacture and storage of drinkable yoghurt. Journal Food and Nutrition Disorder 2(5):1-7. doi:10.4172/2324-93225

Wahyudi, A. and Samsundari, S. 2008. Healthy with Fermented Milk, Malang: UMM Press.