Food Science and Quality Management
ISSN 2224-6088 (Paper) ISSN 2225-0557 (Online)
Vol.53, 2016

Temperature and Baking Duration Changes the Physicochemical Properties, Dietary Fiber Content and InVitro Calcium Bioavailability of Spirulina platensis

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Abstract
Spirulina platensis is considered as a good source of calcium as it has high calcium content. Calcium in *spirulina* is found attached to sulphated polysaccharide with ionic bond. Fortification of bakery products with *spirulina* as a functional food have been developed. Processing food using baking method was favoured and preferred by consumers. During baking, calcium and other minerals may change its form due to the interaction with other components in food resulting to the change of its solubility. Consequently, it can affect the bioavailability of calcium. This study aims to determine the changes of physicochemical properties during baking and the calcium bioavailaibility during processing in a baking system simulation models. *S. platensis* was added with deionized water (1: 1.5 w/v) forming the mixture with cookies dough-like consistency. Dough was stamped, and heated in various temperature (120, 150 and 180 °C) and duration (10, 20 and 30 minutes). The results showed that temperature and duration of baking affect the physicochemical properties of the product, which decreased the moisture content, calcium solubility, water holding capacity (WHC) and oil holding capacity (OHC) (p <0.05) as well as the retention of the minerals calcium, magnesium and phosphorus in the product (p <0.05). The solubility of calcium during baking were 3.24 to 10.21 mg Ca /100 ml, wherein the calcium that could pass through the dialysis membrane ranged from 0.04 to 0.71 mgCa/100 ml. *Invitro* calcium bioavailability of *S. platensis* was influenced by the temperature and baking duration.
Keywords: *Spirulina platensis*, Calcium, Bioavailability, Temperature, Baking, Dietary fiber

1. Introduction

Spirulina is a blue-green microalgae (Cyanophyceae) and easy to be cultivated. *Spirulina platensis* was reported for its potential source of calcium. The calcium content in *S. platensis*, ranging from 700 to1000 mg per 100 g, is much higher compare to vegetables and milk or yogurt (Tietze, 2004). Consumption of 4 g of spirulina per day is recommended by FAO to get most of the daily calcium requirement (Habib, 2008).

Calcium in *S. platensis* is found embeded with sulfated polysaccharides and referred as Ca-Spirulan (Ca-SP). Ca-Spirulan is a sulfated polysaccharide chelated with Ca ions, and mainly composed of rhamnosa and fructose, with the molecular weight of 3 x 10^5 (Lee *et al*., 2001). Lee *et al*. (2000) explained that ionic calcium bound to sulfate groups. Ca-SP undergo an ionization when dissolved in distilled water and passed through ion exchange columns. Ca-SP hydrolysis using sulfuric acid caused a polysaccharide degradation resulting to the production of netral and acidic polysaccharide. Furthermore, as described by Lee *et al*. (2001), polysaccharides can undergo depolymerization in pH 5.5 buffer solution. Acid-soluble polysaccharides consist mainly of glucans (Shekharam *et al*., 1989).

Nowadays *spirulina* is mainly consumed as a health supplement. However, with the shifting of lifestyles, the consumption of functional food is preferred rather than supplements due to the physiological effects of medication-like feeling from supplement intake. The production of functional food is a fusion of science and art as it combine the scientifically proven beneficial content of materials and the art of food formulation. Therefore, the term of tailor-made recipe or food designer is known in the field of food science (Rajasekaran and Kalaivani, 2013). Adding *spirulina* to foods become a trend nowadays especially in bakery products. The addition of Spirulina to the level of 0,5-3 grams per serving size is practiced as an ingredient in the bakery products (Anonim, 2011). The addition of beneficial materials to the food products can cause the enhancement or reduction of favourable components in food due to the processing effect

Baking is one type of food processing method and applied mainly for bakery products (bread, biscuits and cookies). Bakery product is popular for consumers because it offers a distinct sensory characteristics. Unique flavor and color were developed as the crust formation occured during baking (Matz, 1972). In addition, baking can extend the product shelf-life. Baking is a dry cooking process in high temperatures that can reach 110-240°C (Fellows, 2000). Several studies of *S.platensis* addition into various bakery products, especially cookies and flakes, revealed that the products are favored by the consumers (Lelana *et al*., 2012).

Baking can cause changes in physicochemical properties, as well as loss of the nutrients in foods. This is

mainly related to the temperature, duration of heating as well as pH (Fellows, 2000; Latunde-Dada Neale, 1986). Colin-Herion *et al*. (2009) reported that processing of apple to apple sauce increased the soluble dietary fiber (SDF). Thed and Philipps (1995) revealed during domestic cooking of potato, microwave-heating and deep frying increase insoluble dietary fiber (IDF) and resistant starch, while boiling and baking had less effect further SDF was not affected by cooking. In legumes, Chitra *et al*. (1996) reported that auoclaving and roasting increased IDF, while fermentation reduced. Extrusion cooking caused increased IDF in Phoenix flour (Vasanthan *et al*., 2000). Latunde-Dada and Neale (1986) reported that the availability of mineral may change during baking. The minerals may undergo modification in conformation during or after processing, or interact with other components, resulting to the formation of mineral complex in a varied molecular weight. Subsequently, this alteration affects mineral solubility in a food system (Santoso *et al*., 2006).

Watzke (1998) revealed that the mineral content in food is not a primary parameter for assessing the quality of the food as its bioavailability is more important. Bioavailability of calcium is an indicator of the ability of calcium fraction in food to be absorbed and functioned in the body. Some factors that affect the efficiency of calcium absorption are solubility, acidity, the presence of other mineral with a valence of 2^+, fiber and fat in the diet. Calcium is absorbed easily in the ionic form, the higher the solubility of calcium salts, the easier the calcium to be absorbed. The interaction mechanism between the mineral-fibers can occur through physical adsorption, ion exchange and complex formation (Torre *et al*., 1995).

Minerals in a soluble forms is predicted to be easier to be absorbed, however not all dissolved minerals are absorbable (Clydesdale, 1988). Mineral bioavailability can be cheaply, easily and quickly determined by using *invitro* methods (Roig *et al*., 1999; Miller *et al*., 1981). Thus, to determine the potential of *S. platensis* as a source of calcium in the baked products, it is necessary to understand the change in physicochemical properties and calcium bioavailaibility of calcium in *Spirulina* during processing in a baking system simulation models.

2. Material and Methods

2.1 Apparatus

Atomic Absorption Spectrophotometer AAS (Perkin Elmer), spectrophotometer (Genesys 20 Thermospectronic, USA), oven (Eyela Windy Oven WFO-601 SD), sentrifuge (Kokusan H-26 F, Japan), an analytical balance (Denver Instrument Company, AA-200, USA), water bath shaker (Polyscience dual action shaker, USA).

2.2 Material and regents

Spirulina platensis obtained from PT Transpangan Spirulindo, Jepara, Indonesia, with the content of moisture, protein, fat, ash and carbohydrate by difference was 6,18%, 63,45%, 0,59%, 7,55% and 28,41%, respectively. The digestive enzymes and biliary salts were purchased from Sigma Chemical Co. The working solutions of these enzymes were prepared immediately prior to use. The pepsin solution was obtained by dissolving 1.6 g of pepsin in 10 ml of HCl (0,1 M). The solution of pancreatin and biliary salts was prepared by dissolving 0.2 g of pancreatin and 1.25 g biliary extract in 50 ml of 0,1 M $NaHCO_3$. The dialysis membranes with a pore size (MWCO) of 6000-8000 Da were soaked in deionized water for 1 hour before used. Calcium and magnesium standard solution were prepared immediately prior to use by diluting the standard in deionized water to the concentration of 1000 mg/Liter. All regents used were of analytic grade and deionized water was used through all the experiment step. All glassware and polyethylene materials were washed in a laboratory dish washer, rinsed with deionized water, soaked in HNO_3 (10%) overnight and then rinse repeatedly three times with deionized water.

2.3 Semi-solid dough of S. platensis

Semi-solid dough of *S.platensis* was prepared by the following procedure: 50 grams dry biomasss of *S.platensis* was added with 75 ml of deionized water, mixed until the consistency of cookies dough was achieved. The dough was put into a cookies mold, stamped into a size of 1 x 3 cm above the 5 x 5 cm pan, and baked in the oven with varied temperature (120, 150, 180°C). Samples were taken at intervals of 10, 20 and 30 minute. Semi-solid dough was made in 2 batches for each treatments. Control was a semi-solid dough without heating.

2.4 Moisture content, Water Holdng Capacity, Solubility and Oil Holding Capacity

Moisture content was analyzed using thermogravimetric (AOAC, 1995). Water holding capacity and solubility (water absorption index) were determined using the method by Onyango (2004). Samples were sieved through a 500µm siever. A 2.0 g$\pm$0.005 g sample was placed in a tared centrifuge tube and 20 ml distilled water added. After standing for 15 min (with intermittent shaking every 5 min), the sample was centrifuged at 4000 rpm for 15 min. The supernatant was decanted into a tared aluminium pan and weight gain in the gel was noted. Water absorption index (WAI) was calculated as [(weight gain of the gel)/(dry weight)]. The supernatant was evaporated to dryness at 105°C until reaching constant weight. Water solubility index (WSI) was determined as [(weight of dried supernatant)/(weight of dry sample)x100]. Oil holding capacity (OHC) was determined by mixing sample (0.5 g) with palm oil (7 ml) for 1 h, then centrifuging at 2000 x g for 5 min. After decantation, the sample was weighed

and OHC was calculated as the percentage of oil trapped by the sampel (Subagio, 2006).

2.5 Dietary fiber

A gravimetric-enzymatic method insoluble (IDF) and soluble dietary fiber (SDF) were determined in the duplicate samples. A 1.0 g±0.005 g sample is added 25 ml water, gelatinization by boiling 15 min in the presence of (0.1 ml) a heat-stable α-amylase, incubation with pepsin at acid pH 2 for 1 h at 40ºC, and incubation with pancreatin at neutral pH for 1 h at 40 ºC. IDF is filtered off with celite as the filter aid. The residue was washed 2x10 ml ethanol 90% and 2x10 ml acetone. SDF is precipitated from the filtrate with 4 volumes of ethanol and recovered by filtration in the same way as IDF. SDF residue was washed 2x10 ml ethanol 78%, 2x10 ml ethanol 95% and 2x10 ml acetone. Residues were dried and weighed. All the dried residues are corrected for protein, ash and blank for final calculation of SDF and IDF values. Total dietary fiber was calculated (TDF=IDF+SDF) (Asp *et al*., 1983).

2.6. Wet Ashing

Mineral content (Ca, Mg, P) was prepared using wet ashing method. Two until five grams sampel was weighed, 10 ml of HNO_3:H_2SO_4 (1:1) was added, and the mixture was incubated overnight. Then 25 ml deionized water was added, heated untill the solution was clear. After cooling, residue was disolved in 25 ml HCl, and added with deionized water to 50 ml.

2.7. Mineral determination

Calcium and magnesium were measured by flame atomic absorption spectroscopy (FAAS) at λ= 422,7 nm (for Ca) and λ= 285,2 nm (for Mg). Phosphorus was determined by spectrophotometry at λ=440 nm.

2.8. Invitro Calcium bioavailability

Calcium bioavailability was determined according to Miller *et al*. (1981) and Roig *et al*. (1999). The procedure as follows: weighed the sample equivalent to 2 grams of protein, added with deionized water to 100 g, and the pH was adjusted to reach pH 2 using HCl 6 N. Twenty-gram aliquot was put into 2 tubes, the first tubes was used fortitratable acidity and the second tube for bioavailability test. Each tube was added with 1 ml pepsin, sealed and incubated for 2 hours at a temperature of 37°C then freezed for one night.

Titratable acidity was determined on a 20 g aliquot of the pepsin digest, thawed and to which 5 ml of pancreatin-bile extract mixture was added. Titratable was defined as the equivalents of KOH (0,5 N) required to titrate the combined pepsin digest pancreatin-bile extract mixture to reach pH 7,5 (tube 1).

Bioavailability test was determined on another tube (tube 2). The aliquot was thawed. Dialysis bag containing an amount of $NaHCO_3$ equivalent to the titratable acidity measured previously was placed into the tube and incubated in a 37°C shaking water bath until the pH reached about 5 (approximately 30 min). Pancreatin-bile extract mixture (5 ml) was added and the incubation was continued for an additional 2 hours. At the end of incubation period, the dialysis bag was removed and rinsed with deionized water. The volume of dialysate was measured and the mineral content of dialysate was analyzed (D). Soluble calcium as a product of in vitro gastrointestinal digestion which remained in the non-dialysate fraction was collected by centrifugation at 3000 rpm at 4°C for 1 hour. The sediment was discarded and the calcium content of supernatan was measured (Snd). The soluble calcium was calculated (D + Snd).

3. Results And Discussion

3.1 Changes in moisture content, solubility, Water holding Capacity (WHC) and Oil Binding Capacity (OHC)

The moisture content of the product decreased (p<0.05) after the baking process. The initial moisture content in dough was 55% and subsequently decreased after baking as the product underwent drying process that reduce its moisture content. The magnitude of moisture content loss was in parallel with the duration of baking and the degree of baking temperature. Figure 1 shows that moisture content of the product baked for 10 minutes (2 to 37.9%) was higher than 30 minutes (2-7%). The water content of dried food products is 7% or lower. The higher loss of moisture content in a higher baking temperature was also observed. The loss of water content in product heated at a temperature of 180 °C was faster than the other two temperature treatments. The higher temperatures lead to the faster evaporation rate. The drying process occurred during baking causes physical change, making the product become dry and easily brittle which implies a decrease in its ability to dissolve in water and reduction of WHC (p<0.05).

The solubility of product sharply decreased after 10 minutes baking compared to the solubility of dough (Figure 2). Statistical analysis showed that degree of temperature (p<0.05) and duration of baking (p<0.05) significantly affect solubility. The reduction of Water Holding Capacity (WHC) after baking process was also observed. WHC is a capability of protein matrix to absorb and hold the water to be remained in the matrix including bound water, hydrodynamic water, capillaries water and physically entraped water.

This is presumable because the proteins undergo Maillard reactions that lead to the decrease of water

binding capability. Sequier *et al*. (2010) mentioned that at temperatures above 100°C, milk protein can undergo Mailard reactions. The temperature used in this study ranged at 120-180°C, and *Spirulina platensis* contained high amounts of protein so that the Maillard reaction may occur during the baking process. Zanhi and Jideani (2012) explained that intrinsic factors that influence water binding proteins including amino acid composition, protein conformation and properties of hydrophilicity/polarity. Adeleke and Odedeji (2010) stated that WHC also influenced by the high affinity of water caused by the reduction of water content. This implies that the lower moisture content of the product consequently increase its water holding capacity. This is observed in the sample baked at high temperature (180°C) possess a lower moisture content and higher WHC than that baked at lower temperature.

Prior to the baking process, *S. platensis* was able to bind fat in 5ml/g (control). Naturally, *Spirulina* contains a protein that is dominated by hydrophobic amino acids (Cohen, 1997), so it has a good ability to bind fat. However, after undergoing a process of baking, the ability to bind fat was decline, in line with the length of heating duration (p<0.05). Conformational change of protein is possibly occur during the baking process resulting to the alteration of open-configuration of hydrophobic protein which consequently reduce its hydrophobicity and decrease its ability to bind fat (Kinsela, 1976).

3.2 Mineral (Ca, Mg, P) retention after baking process

The results of this study indicate that retention of mineral Ca, Mg and P decreased during the baking process. The higher of the temperature and the longer duration of heating causes a greater loss. The temperature and duration of baking affect the retention of the mineral content in the product (p<0.05). This is especially apparent in the mineral calcium (Figure 5.a.). Sediaoetama (1993) states that the reduction of mineral content in the food is obviously noticed resulting from the damage of nutrients contained in the food during processing. The damage during processing possibly caused by pH, oxygen, light and heat or a combination thereof. Moreover, Purwaningsih *et al*. (2011) and Dewi (2010) proves that the processing of conch and clam by steaming is better than boiling and baking as it retain mineral content of raw material. Nurjanah *et al*. (2015) reveal that in the phenomenon of an increase in the mineral content of the product after heating, the mineral strongly bound to the material, and the vice versa. Calcium retention of the product in this study is low, conceivably because of a weak bond of calcium binding to the matrix, so it is easily influenced by the baking process. Lee *et al*. (2000) explain that the calcium in the *S.platensis* is bound to the sulfate group of sulphated polysaccharide with ionic bound.

Food processing using high temperature leads to the evaporation of water in food. The higher the temperature used, the more the molecules of water emergee from food to the surface resulting to a loss of water soluble nutrient (Winarno, 2008). Minerals, such as Ca, P, Fe, K, Cu and Zn, are likely released from food matrix as the release of water during the heat process. Yoshie *et al*. (1999) explains that the types of minerals possess a vary bonding pattern. Zn is easily lost during processing than Mg. The results of this study showed that calcium is more easily influenced than phosphorus and magnesium during processing. The mineral retention of the product are as follows P> Mg> Ca, respectively.

3.3 Changes in dietary fiber content of food after baking

Baking process affect dietary fiber contained in food products. The results of this study show that the total dietary fiber content in *S. platensis* after the baking process ranged from 13.77 to 22.27%. Changes in each fractions of dietary fiber is presented in Table 1. However, the tendency of change can not be considered only from the total dietary fiber. After baking, the proportion of insoluble dietary fiber increased sharply with the increasing of temperature (p<0.05). The proportion of insoluble dietary fiber to total dietary fiber (IDF/TDF) ranged from 20.15 to 111.54 times. Naturally, *S. platensis* was mainly dominated by the insoluble dietary fiber. The proportion of insoluble dietary fibers in the control (dough) was approximately 20.15 time higher than soluble dietary fiber, after baking at temperatures of 120, 150 and 180°C, the proportion increased for 39.59; 67.87 and 92.79 time, respectively.

Babadzhanov *et al*. (2004) mentioned that *S. platensis* contains hemicellulose and pectin, as well as glucan (Shekharam *et al*.,1989). Cleary *et al*. (2007) state that during the baking of bread, β-glucan from barley did not undergo molecular degradation. IDF proportion to SDF increased in this study presumably due to the interaction between the proteins with sugar to produce Maillard reaction resulting to the formation of melanoidin, a brown polymeric nitrogen, at the final phase (Friedman, 1996). Frying or baking have a greater impact on the formation of Maillard Reaction Product (MRP) than boiling (Chao *et al*., 2009), Delgando-Andrade *et al*. (2010) adds culinary treatment and raw materials also influence MRP content. The occurrence of melanoidin is likely to cause a high quantities of the IDF concentration. In the analysis of dietary fiber, melanoidin is indigestible by proteases and is insoluble so it is detected as insoluble fiber.

Some researchers revealed that the high fiber content affects the bioavailability of minerals. However, if the fiber is digestible by the colonic microbiota and produce short chain fatty acids, consequently it will increase the absorption of calcium. Rasmussen *et al*. (2009) stated that consumption *S. platensis* in the diet of mice improve

growth of gastrointestinal microbiota.

3.4 Bioavailability of Calcium

The results show that the mineral content of samples decreased after baking (Figure 5). The amount of soluble calcium decreased after the baking process (Table 2), not all soluble calcium were able to pass through the dialysis membrane. The calcium content of dialysate was in the range of 0,04-0,71mg /100ml, whereas total soluble calcium ranges from 3.28 to 10.92%. Bronner and Pansu (2002) revealed that the amount of calcium that is able to be absorbed by the body is up to half to two-thirds of the amount consumed. The mineral content in food is just one of the initial parameters for assessing the quality of the food and its bioavailability is more important (Watzke, 1998).

Bioavailability of calcium is an indicator of the ability of the minerals calcium to be absorbed by the body. In vitro testing of bioavailability assumes that the soluble minerals can be absorbed by the body. It is further mentioned that the minerals are soluble and can pass through the dialysis membrane, this assumption is used to predict *invivo* bioavailability of mineral (Roig *et al*., 1999, Miller *et al*., 1981). The decrease of calcium content in the dialysate affected by temperature and duration of heating (p <0.05). Increase of the temperature during processing causes the amount of calcium that is capable of passing through dialysis membrane decreased. The indications of mineral solubility reduction in the bioavailability test has been detected from solubility and WHC, which dropped after the baking process (Figure 2 & 3). The decline in the ability of the product to dissolve after the baking process implicated in the decline of calcium that can be dissolved and dialyzed during *invitro* bioavailability. This condition can be possible due to interaction between minerals with proteins in the product. The protein content of *S. platensis* reach up to 60% and is suspected during the heating run into denaturation. Interactions between proteins and minerals during processing can cause mineral becomes difficult to dissolve, it is mainly in materials with a high protein content. The minerals in food may changes in form during or after processing, or it can also interact with other components. This is resulting in an increase or decrease of its solubility (Santoso *et al*. 2006). Sequier et al (2010) adds a negative effect on calcium solubility was observed after invitro digestion of over heated milk (116°C, 16 min, 3 times) compared with ultra high temperatue (UHT) milk (150°C, 6 s).

The results show that the largest portion of calcium content is in the fraction of non dialysate (Snd). Snd indicated the amount of soluble calcium that failed to pass through the dialysis membrane, which ranged 3,28-10,92 mg Ca/100 ml. This condition causes percentage of calcium content in the dialysate only ranged from 0.72 to 6.5%. During the heating process minerals undergo hydrolysis reaction through the following mechanism:

$[M\ (H_2O)_6\ n] \leftrightarrow [M(H_2O)_5\ (OH)]^{(n-1)} + H$, M is mineral

(H x L) as protein reacted to mineral following this mechanism:

$H \times L + M^{++} \leftrightarrow ML^{+} \times L^{+}$ (L=Ligan).

Temperature of food processing can change the pH, leads to the imperfect hydrolysis process. Mineral ions that are almost identical in nature will interact between each other and binds to the protein resulting to the formation of mineral-protein complex called a protonic ligand complex. The formation of this ligand causes the reduction of mineral solubility in water (Darmono, 1995). In addition to the denaturation of proteins, the high content of insoluble fiber may also interact with the minerals contained in the product. According to Frolich (1995), the cooking process lead to the reorganization of dietary fiber components that affect the availability of minerals. Reorganization of dietary fiber components include changes in ionization of -OH and -COOH groups, as well as the stability of the bond and breakdown of the bond between monomers or polymers. Pectin glycosidic bond hydrolyzed at pH 4 and pH 7. After the baking process complete, the proportion of insoluble fiber increased sharply (Table 1). The Ca in *S. platensis* is bound to sulfated polysaccharide with ionic bond, therefore supposedly along the ionization process calcium should be dissolved properly. *Invitro* test of bioavailability begins with the adjustment of pH to reach the value of 2 and followed by the digestion with pepsin. In these conditions, it is suspected that the ionic bond of calcium in easily to be ionized and it improves its solubility.

Drago *et al*. (2005) add that the combination of pH 2 and pepsin digestion provides a higher mineral solubility (Zn, Mg and Ca) in milk than pH 3.5. However, when the complex of mineral-fiber is formed, this condition may leads to the reduction of solubility. In this study, hemicellulose is suspected to bind with calcium forming a complex and affect the bioavailability of calcium. Yuanita (2005) explained that in the long bean, specific binding between Fe with hemicellulose and lignin through the hydroxyl groups, carboxyl, carbonyl oxygen, and β-glycosidic is observed. Sipos *et al*. (1995) revealed that the binding of mineral with polysaccharides involving the deproteinized-OH group or glycosidic oxygen is more stable than the type of the bond containing -COOH groups or through the carbonyl oxygen or ether.

The interaction of dietary fiber and minerals is thought to cause the molecular size increases. Thus, if it occurs in this study, it is possible that the fractions restrained from passing through the membrane dialysis bag. Minerals (Ca) content in the dialysate was low (Table 2). Roig *et al*. (1999) explained that using dialysis bags with a larger pore size (10000-12000 Da) can increase the calcium milk content in the dialysate compared with research

conducted by Reykdal and Lee (1991), which used a 6000-8000 Da. It shows that after heating process mineral interactions with other molecules can increase molecular size. This statement is reinforced by Santoso *et al*. (2006) that after heating the mineral can be in various size fractions. Research on macroalgae showed that after boiling in water, minerals can be divided into three fractions, low (<10000Da), medium (10000-200000), and high (> 200000 Da). After the heating process, calcium in the high molecular weight (> 200000) fraction will increase, while the calcium that is in the low molecular weight fraction will decrease. This phenomenon is believed to occur also in this study so that the complex is not able to pass through the dialysis membrane (6000-8000Da).

4. Conclusion

Temperature and duration of heating implicated in the reduction of solubility, water binding capability (WHC) and oil holding capacity (OHC) and enhance the proportion of insoluble fiber to insoluble fiber as well as decrease the rention of the minerals Ca, Mg, P in the product (p<0.05). Calcium *invitro* bioavailability revealed that not all the soluble calcium can pass through the membrane. The solubility of Ca during baking were 3.24 to 10.21 mgCa/100ml, wherein the Ca that could pass through the dialysis membrane ranged from 0.04 to 0.71 mgCa/100ml. Changes of the calcium solubility and calcium in-vitro bioavailability during baking are due to because the interaction of calcium with protein and or fiber causing the increase of molecular size and was influenced by temperature and baking duration. Higher temperatures and longer heat exposure lead to the reduction of calcium bioavailability. Thus, temperature and baking duration are the important factor to be considered in baking process, in order to maintain the bioavailability of calcium from *Spirulina platensis*.

Acknowledgment

The author would like to thank the Institute for Research and Community Service Universitas Gadjah Mada for funding research through grant Young Lectures No. LPPM-UGM/1128/LIT/2014.

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Figure and Table

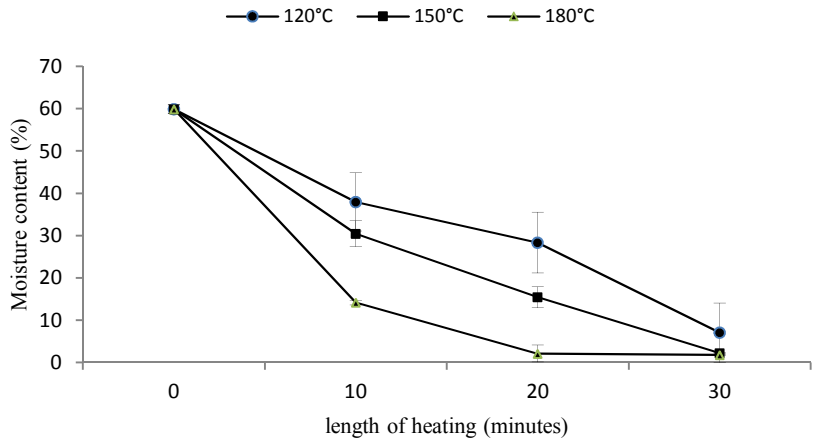

Figure 1. The decline of moisture content during baking at various temperature

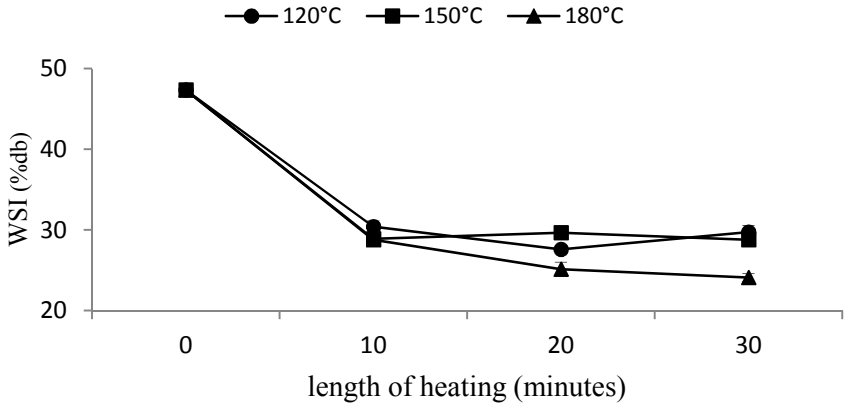

Figure 2. Solubility (WSI) during baking at various temperatures

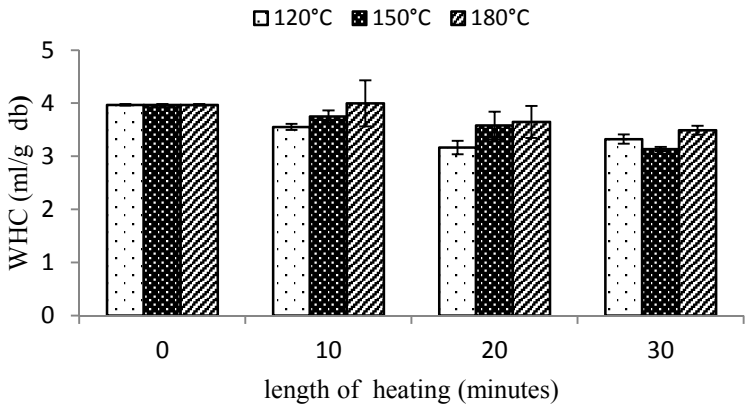

Figure 3. Water Holding Capacity of products during baking at various temperature

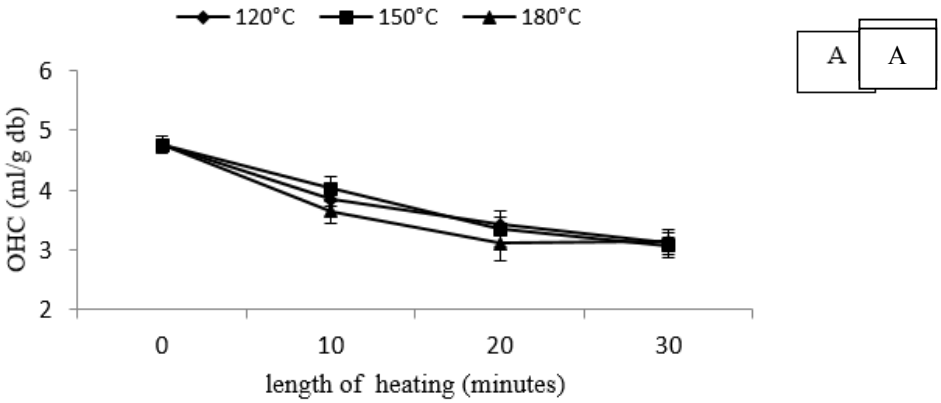

Figure 4. Oil Holding Capacity during baking at various temperature

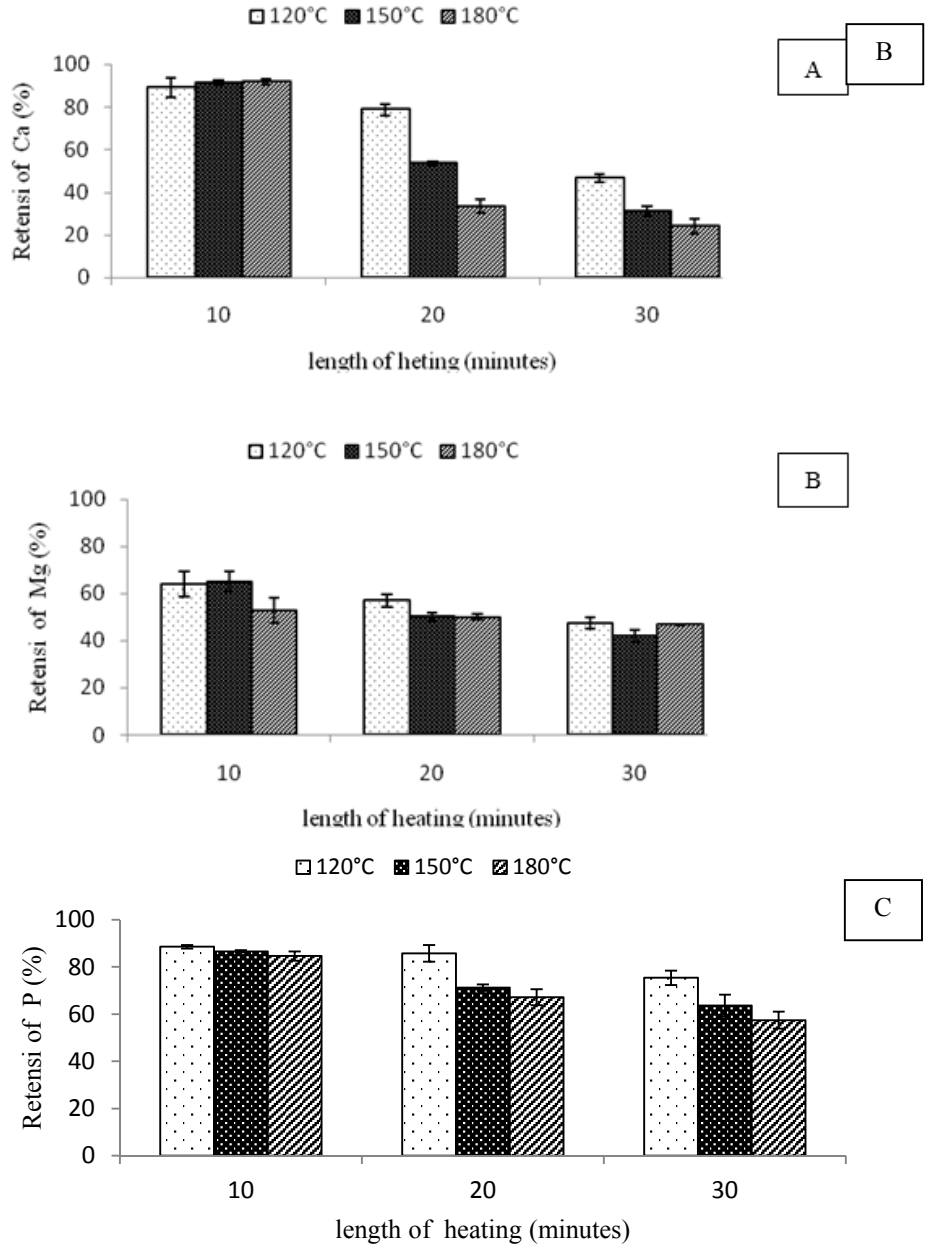

Figure 5. Retention of Ca (A), Mg (B) and P (C) after heating at a temperature of (120,150, 180°C), for (10, 20, and 30 minutes)

Table 1. Soluble Dietary Fiber (SDF), Insoluble Dietary Fiber (IDF), Total Dietary Fiber and proportion of IDF/SDF during baking

Treatment		IDF	SDF	TDF	Proportion of IDF /SDF
Temp.	Length of Duration	%			(%)
Control		18,74	0,93	19,67	20,15[a]
120°C	10'	11,47	0,29	11,76	39,55[b]
	20'	13,9	0,35	14,25	39,71[b]
	30'	21,72	0,55	22,27	39,49[b]
150°C	10'	20,94	0,57	21,51	36,74[b]
	20'	13,5	0,27	13,77	85,29[c]
	30'	14,5	0,17	14,67	81,58[c]
180°C	10'	15,5	0,19	15,69	81,58[c]
	20'	16,2	0,19	16,39	85,26[c]
	30'	14,5	0,13	14,63	111,54[d]

Values with different superscript letters in the same column indicate significant differences (p<0.05).

Table 2. Calcium dilysate, non dilysate, total soluble content and Percentage of Dialyzable Calcium after baking at various temperature and duration

Treatment		Dialysate	Soluble Non Dialysate	Total Soluble	Ca Dialyzed
Temp.	Length of	(mg Ca/100 mL)			%
Control		0,71	10,21	10,92	6,50[a]
120°C	10'	0,33	6,75	7,08	4,66[b]
	20'	0,1	4,3	4,4	2,27[c]
	30'	0,04	3,24	3,28	1,22[d]
150°C	10'	0,34	8,25	8,59	3,96[b]
	20'	0,12	6,89	7,01	1,71[d]
	30'	0,06	5,25	5,31	1,13[d]
180°C	10'	0,21	7,72	7,93	2,65[bc]
	20'	0,04	7,27	7,31	0,55[e]
	30'	0,05	6,85	6,9	0,72[e]

Values with different superscript letters in the same column indicate significant differences (p<0.05).