

**Biosorption, localization and bioavailability of zinc in *Spirulina platensis* culture**<sup>1,\*</sup>Jatupornpongchai, A., <sup>2</sup>Phoopat, N., <sup>3</sup>Limpirat, W. and <sup>1</sup>Ningsanond, S.<sup>1</sup>*School of Food Technology, Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima 30000, Thailand*<sup>2</sup>*Institute of Food Research and Product Development, Kasetsart University, Bangkok 10900, Thailand*<sup>3</sup>*Synchrotron Light Research Institute. Nakhon Ratchasima 30000, Thailand***Article history:**

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A study of *Spirulina platensis* taking up Zn was conducted in modified culture media with various Zn salts. *Spirulina platensis* cultured for 7 days in modified Zarrouk's medium 1.6  $\mu\text{mol Zn}$  of  $\text{ZnSO}_4$  (MZS) contained the highest Zn content. The MZS were  $69.55 \pm 2.70$   $\mu\text{g/g}$  dry weight for Zn concentration,  $63.11 \pm 1.40$  %db for protein content,  $0.46 \text{ day}^{-1}$  for specific growth rate ( $\mu$ ),  $0.55 \text{ g/L.day}$  dry weight cell for productivity ( $P_x$ ), and 1.50 days for doubling time ( $t_d$ ). The majority of Zn deposit was in the cytoplasm which was 2.7-fold higher than that in the cell walls. Fourier transform infrared (FTIR) spectra showed the decreasing of peak intensity for the amide groups after Zn uptake which indicated their important role in the binding of Zn. The simulated digestion in gastric and intestinal tracks of *S. platensis* cells cultured in MZS cells was the accessibility of  $55.20 \pm 0.57$  and  $63.55 \pm 0.21\%$ , respectively. After sequentially gastric and intestinal digestion, the Zn availability was  $34.63 \pm 0.95\%$ .

**1. Introduction**

*Spirulina (Spirulina platensis)* is a filamentous cyanobacterium, known as micro blue-green algae. It has been used as food supplements and extracted functional components or nutraceuticals for forming health products (Jin *et al.*, 2020). *Spirulina* contains high amino acids, polyunsaturated fatty acids, vitamins and pigments having a proximate composition of 60-70% proteins, 12-16% carbohydrates, 9-14% lipid and 4-9% minerals, and low Zn content (Campanella *et al.*, 1999; Gershwin and Belay, 2008). *Spirulina platensis* and other Cyanobacteria cells consist of complex structures having unique cellular functional groups such as carboxyl, hydroxyl, sulphate, amines and other negatively charged groups which can bind metals (Şeker *et al.*, 2008). In addition, it can be used in nutraceuticals, biomedical research, food and cosmetics (Mary Leema *et al.*, 2010; Jeamton *et al.*, 2011). More than 70% of *S. platensis* has been commercialized mainly as health food. It is cultivated in several countries such as the USA, China, Japan, Taiwan and Thailand. Zinc (Zn) is an essential trace mineral for various biochemical functions in humans, plants, and animals (Habib *et al.*, 2008).

Zn is also consistent with >300 enzymes (Zastrow and Pecoraco, 2014). Zn binding proteins have been

categorized as metalloproteins which are intracellular proteins in living cells. Therefore, Zn plays a preventive role against free radical formation to protect biological structures from damage resulting in correcting immune functions, enhancing growth in children, preventing neurological diseases such as Alzheimer's disease and Parkinson's disease and preventing prostate cancer (Franklin and Costello, 2007; Haase *et al.*, 2008; Leuci *et al.*, 2020). International Zinc Nutrition Consultative Group (IZiNCG) suggests a daily intake dosage of supplemental zinc for adult men and women with 10-13 and 8-9 mg/day, respectively (Holtz *et al.*, 2004). However, 32% of the world population had a problem with zinc deficiency, especially in developing countries such as Africa, and south-central Asia (Black *et al.*, 2008). Hence, taking supplemental Zn is common practice for solving that problem. The addition of zinc in food or supplemental zinc products has to consider the bioavailability and solubility of zinc which it is depending on Zn sources (Allen, 1998).

Metal ions absorption in algae has been reported to be active and passive processes depending on the type of metal ions and different cell functional groups. The binding mechanisms are electrostatic interaction, ion exchange and ion complexation (Li *et al.*, 2003; Chen *et*

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al., 2007). Fourier transform infrared (FTIR) spectroscopy is used for metal analyses in biological sources, e.g., Cyanobacteria, yeast and bacteria and plants (Ferreira *et al.*, 2011; Liu *et al.*, 2013). However, very little information is available on Zn absorption of the *S. platensis* cells. Therefore, the objective of this study was to bio-adsorb Zn in *S. platensis* with various sources of zinc salts and determined the location of the Zn deposit in the cell and kinetic growth of *S. platensis*. As well, in vitro accessibility and availability of organic Zn in cultivated *S. platensis* were studied.

## 2. Materials and methods

### 2.1 Chemicals and materials

Zinc chloride ( $ZnCl_2$ ), Zinc nitrate ( $Zn(NO_3)_2$ ) and Zinc sulfate ( $ZnSO_4$ ) were purchased from Ajax Finechem (Scoresby, VIC, Australia). Sodium hydrogen carbonate ( $NaHCO_3$ ) and standard Zinc was purchased from Fisher Scientific Co. (Fair Lawn, NJ, USA). Nitric acid ( $HNO_3$ ) and Hydrochloric acid (HCl) were purchased from Merck KGaA (Darmstadt, Germany). Perchloric acid ( $HClO_4$ ) was purchased from Mallinckrodt Baker Inc. (Phillipsburg, NJ, USA). Pepsin and bile extract were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Pancreatin was purchased from Acros Organics (Fair Lawn, NJ, USA). A commercial *S. platensis* sample (SPC) was purchased from The Royal Chitralada Project, Bangkok, Thailand.

### 2.2 Spirulina culture and preparation

The *S. platensis* IFRPD 1208 strain was obtained from the algae laboratory, Institute of Food Research and Product Development, Kasetsart University, Bangkok. The starter was prepared in Zarrouk's medium, pH  $8.5 \pm 0.2$  (ZM) with an optical density (OD) of 0.2 at 560 nm. The *S. platensis* was cultured in a ZM medium using the indoor 200 mL culture tubes (containing 150 mL of medium) and modified Zarrouk's media with different zinc salts such as Zinc sulfate (MZS), Zinc nitrate (MZN) and zinc chloride (MZC) at concentrations of 0.4, 0.8 and 1.6  $\mu\text{mol}$  of Zn. The culture tubes were incubated at  $30 \pm 2^\circ\text{C}$  with 12 Klux light intensity and operating control with the light/dark cycle of 16/8 hrs and 2 L/min of  $CO_2$  flow rate. The culture was done in 7 days with OD monitoring using a spectrophotometer (Libra S22, Biochrom, Cambridge, UK) at 560 nm during cultivation. *Spirulina platensis* cells were separated using a nylon mesh with 35  $\mu\text{m}$  filter pore size (Nylon mesh 140-T, Swiss Nybolt, Hebei, China), washed in 20 mL distilled water 3 times in order to remove a medium from the cells (Duangsee *et al.*, 2009), and dried using a freeze dryer (Heto FD8, Holten AS, Allerod, Denmark). Dried spirulina samples were packed

into the  $7 \times 10$  cm zip lock low-density polyethylene bag (Zipper bag, Aro, Samutsakorn, Thailand), and kept in the desiccator at  $25^\circ\text{C}$  until use (not longer than 2 months).

The study of the cell's functional groups, Zn digestibility and Zn availability samples were investigated using the 8 days of cultivated *S. platensis* in both Zarrouk's medium and in modified Zarrouk's medium in the same manner as previously mentioned.

### 2.3 Growth measurement

Protein content (%dry basis) was calculated from nitrogen in the samples using the modified Kjeldahl analysis method of González López *et al.* (2010). Biomass (g dry weight/L) was determined according to method 930.04 of AOAC (1995). Doubling time ( $t_d$ ) expressed as days required for the cell growth, cell productivity ( $P_x$ ) expressed as g/L per day and specific growth rate ( $\mu$ ) to double, were calculated by following equations:

$$\text{Doubling time } (t_d, \text{day}) = \frac{\ln 2}{\mu}$$

Where  $\mu$  = Specific growth rate ( $\text{day}^{-1}$ )

$$\text{Cell productivity } (P_x, \text{g/L.day}) = \frac{(X_i - X_0)}{t_i}$$

Where  $X_0$  = initial biomass density (g dry weight /L),  $X_i$  = biomass density at time  $i$  (mg/L dry weight), and  $t_i$  = time interval between  $X_0$  and  $X_i$  (day)

$$\text{Specific growth rate } (\mu, \text{day}^{-1}) = \frac{\ln X_i - \ln X_0}{t_i - t_0}$$

Where  $X_0$  = initial biomass density (mg dry weight/L),  $X_i$  = biomass density at time  $i$  (mg dry weight/L),  $t_0$  = initial time (day) and  $t_i$  = time  $i$  (day)

### 2.4 Determination of Zn

The 100 mg of dried *S. platensis* cells were digested in a 7 mL acid mixture (65%  $HNO_3$ : 85%  $HClO_4$  = 5:2, v/v) using the 50 mL Erlenmeyer flask until a clear solution was observed. The digested solution was transferred to a 25 mL volumetric flask to adjust the volume with deionized water. Zn content was determined according to the method of 999.11 AOAC (2000) using the atomic absorption spectrometry; AAS (Perkin Elmer AA spectrometer model PinAAcle 900F, MA, USA)

### 2.5 Isolation of cell fractions

Cell fractions were prepared according to the modification method of Gan *et al.* (2004). Briefly, the dried cell sample was suspended at 10% (w/v) in 0.1 M Phosphate buffer solution pH 7.0 with 0.5% lysozyme and incubated at  $35^\circ\text{C}$  for 4 hrs. The mixed cell solutions

were centrifuged at 10000×g for 15 mins. This procedure was repeated 4 times. Supernatant and pellets were determined a Zn concentration using AAS as above mention. Suspension in supernatant represented a cytoplasm fraction while the pellets were a cell wall fraction.

## 2.6 Determination of cell functional groups

Functional groups of the cell were investigated using the Attenuated Total Reflectance Fourier Transformed Infrared (ATR-FTIR) Spectrometer (Bruker Tenser 27 GmbH, Germany). The FTIR spectra were recorded in the region of 4000-700 cm<sup>-1</sup>, the acquisition of 64 scans and the resolution of 4 cm<sup>-1</sup>. The OPUS 7.0 software (Bruker, GmbH, Germany) was used for the intensity and frequency identification.

## 2.7 Zn accessibility

Dried ZM (SPZ) and 1.6 μmol MZS (SPM) were used the determination of Zn accessibility. The commercial *S. platensis* sample (SPC) was used for control. Accessibility of Zn was done using two sequential steps: 1) gastric digestion simulation and 2) intestinal digestion simulation.

## 2.8 Gastric digestion simulation

The 500 mg of lyophilized *S. platensis* samples were transferred into a 50 mL polypropylene tube, then 20 mL of deionized water was added, and pH was adjusted to 2.0 with 6 M HCl. Subsequently, 315 U/mL pepsin was added to the sample before incubation at 37°C in a shaking bath with 150 rpm shaking for 2 hrs. Then the samples were placed into the ice-water bath to terminate the enzyme reaction, centrifuged with 13000×g at 4°C for 15 mins. The supernatant was collected for the determination of Zn content and Zn digestion in intestinal simulation (McDougall et al., 2005).

## 2.9 Intestinal digestion simulation

The 5 mL of intestinal solution (4% pancreatin and 2.5% bile salts dissolved in 0.1 M NaHCO<sub>3</sub>) was added to a 50 mL tube containing a digested sample from the simulated gastric digestion. The pH of the mixture was adjusted to 7.5 using the 1 M NaHCO<sub>3</sub>. Then the sample was incubated in a shaking bath with 150 rpm shaking at 37°C and for 2 hrs. Enzymatic activity was stopped by transferring the tube into an ice-water bath. The sample was centrifuged with 13000×g at 4°C for 15 mins and collected as a supernatant for Zn content determination (Gil-Izquierdo et al., 2003).

Accessibility of Zn after the digestion simulation in gastric and intestinal tracts, expressed as a percentage of dry basis (%db), was calculated from the following:

$$\text{Accessibility of Zn (\%db)} = \frac{\text{Zn in the supernatant after each simulated digestion step}}{\text{Total Zn in the sample}} \times 100$$

## 2.10 Zn availability

The availability of organic zinc in the sample was conducted in two sequential steps starting with gastric digestion and then intestinal digestion as previously explained. The final sample was then used for Zn availability. Dialysis tubing (molecular weight cut-off of 10-14 kDa; Fisher Scientific Co., Pittsburgh, PA, USA) filled with sufficient NaHCO<sub>3</sub> solution (pH 7.5) was then placed into the polypropylene tube, before parafilm sealing and capping. Subsequently, the tube was incubated in a shaking bath at 37°C and 150 rpm for 2 hrs. The enzymatic activity was stopped in an ice-water bath. The solution outside the dialysis tubing contained Zn that would remain in the gastrointestinal tract. The solution in the dialysis tubing would represent available Zn to be absorbed and transported to serum (Moreda-Piñeiro et al., 2012).

Zn availability after the sequential digestion simulation expressed as %db, was calculated from the following:

$$\text{Accessibility of Zn (\%db)} = \frac{\text{Zn in the solution inside the dialysis tubing}}{\text{Total Zn in the sample}} \times 100$$

For Zn content determination, 5 mL supernatant outside or inside the dialysis tubing was transferred to a 50 mL flask, followed by the addition of 15 mL mixed HNO<sub>3</sub>:HClO<sub>4</sub> (5:2) solution before the sample was hydrolyzed to obtain 25 mL clear solution before AAS measurement in the same manner as described previously.

## 2.11 Statistical analysis

All of the analyses were performed in triplicate. The data was analyzed statistically by using a statistical software program (SPSS version 16.0, SPSS, Chicago, IL, USA). In order to verify the significance of different effects among treatments, One-way analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) were performed with significance at  $p \leq 0.05$ .

## 3. Results and discussion

### 3.1 Growth and Zn uptake

Biomass of *S. platensis* cultured in ZM, MZS, MZN and MZC media at concentrations of 0.4, 0.8, and 1.6 μmol Zn for 7 days are shown in Figure 1A. The *S. platensis* cultured in MZS (1.6 μmol Zn) had the highest biomass of 4.03±0.01 g/L dry weight, Zn of 69.55 μg/g dry weight and protein concentration of 63.11% compared with MZC and MZN. Normally, Zn in the media is not the main growth factor for *S. platensis*

cultivation, but nitrogen and carbon sources are the main factors. The growth of *S. platensis* depends on the nutrients of the medium, especially the balance between carbon and nitrogen. If nitrogen and carbon are not assimilated in the cell, the growth rate could not reach its maximum (Gordillo *et al.*, 1998; Rodrigues *et al.*, 2011). Zn is an essential mineral for cell growth and ZnSO<sub>4</sub> in the medium at all concentrations was the best salt form to significantly increase Zn content in *S. platensis* cells (Figure 1B). Results in Figure 1C showed that increasing Zn content in growth media did not give higher cell protein content. Only ZnSO<sub>4</sub> at 0.8 and 1.6 μmol of Zn provided cells with the same protein content as found in cells cultured in the standard ZM. The rest of the media fortified with various Zn salts significantly lowered protein contents in the cell. This trend was similar to that found in cell biomass. The effects of ZnSO<sub>4</sub> on Zn uptake, cell biomass and protein content of *S. platensis* cells might be due to the increase in protein-bound

sulfur, such as cysteine (Cys), methionine (Met), histidine (His) as well as prokaryotic enzyme systems (Menon and Varma, 1982; Zander *et al.*, 2011).

The kinetics of *S. platensis* culture including specific growth rate ( $\mu$ , day<sup>-1</sup>), doubling time ( $t_d$ , day) and cell productivity ( $P_x$ , g/L.day) are shown in Table 1. Zarrouk's modified with 1.6 μmol Zn from ZnSO<sub>4</sub> showed significant highest growth parameters as 0.46 day<sup>-1</sup> of specific growth rate, 1.50 days of doubling time and 0.55 g dry weight/L.day of cell productivity. Sulfur is commonly assimilated with Cys and Met, which potentially limits cyanobacteria growth (Giordano *et al.*, 2005). These results support the relation between sulfur and the growth rate.

### 3.2 Location of zinc in *Spirulina platensis*

#### 3.2.1 Zn in cell fractions

*Spirulina platensis* cells were separated into two fractions: cytoplasm component in the supernatant and wall materials in pellets. The results showed that Zn concentrated in the cytoplasm in the amount of 60.50 μg/g dry weight (72.8%) and presented in wall 22.62 μg/g dry weight (27.2%). Previous research showed the uptake of Zn, Cd and Se of phytoplankton (*Phaeodactylum tricorutum*, *Prorocentrum minimum*, *Tetraselmis levis* and *Chlorella autotrophica*). These normally implicated an initial rapid surface absorption and the transport of the metal into the intracellular, respectively (Wang and Dei, 2001). The cell surface absorption incorporated complexation with algal extracellular organic compounds, e.g., extracellular polysaccharides. In addition, inorganic selenite could be transformed into organic forms through binding with proteins, lipids, polysaccharides, and other cellular components in *S. platensis* (Li *et al.*, 2003). The present results of Zn uptake in the cell, therefore, showed the same transport mechanism. The low concentration of Zn in the wall fraction compared with the cytoplasm component might be due to limited binding sites of the wall materials.

### 3.3 Functional groups of *Spirulina platensis* cells

FTIR spectra of lyophilized *S. platensis* cells were normalized to evaluate the effects of MZS media on the functional group of the cell. The FTIR spectra in the 2000-800 cm<sup>-1</sup> region were shown in Figure 2A. Band assignments are based on the references in Figure 2B. The bands at

1652 cm<sup>-1</sup> can be assigned to amide I C=O and/or C-N groups of proteins. Amide II appeared at 1541 cm<sup>-1</sup> indicating the N-H and/or C-N groups of proteins. Infrared (IR) spectra showed the complex formation of the phosphate groups with Ba<sup>2+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup>, Cu<sup>2+</sup>, Sr<sup>2+</sup>

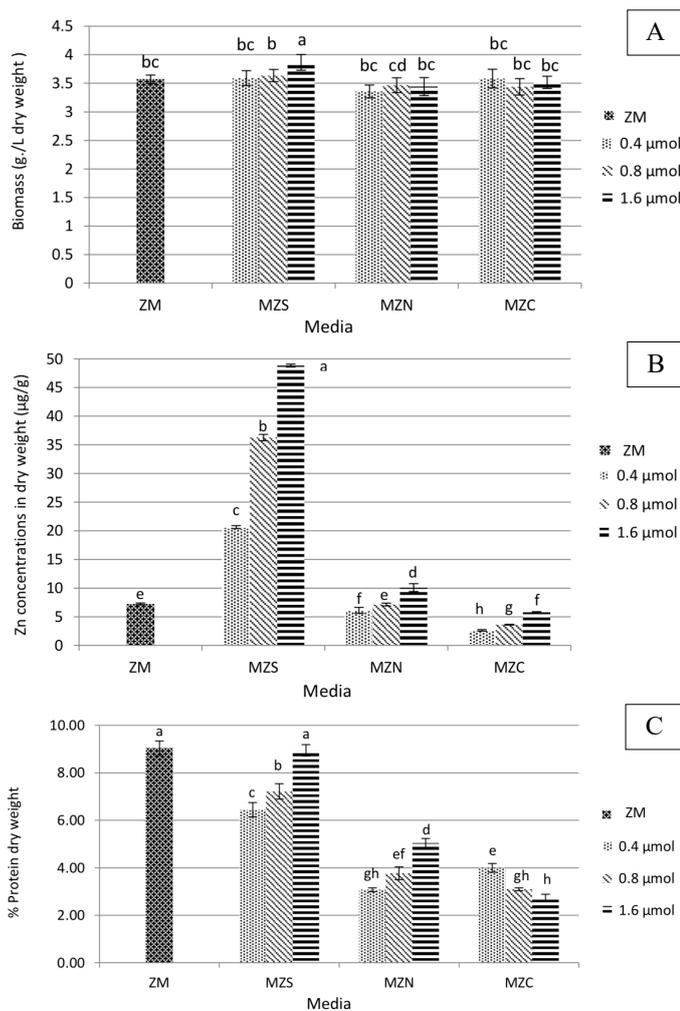


Figure 1. Biomass (A), Zn (B) and Protein (C) of *S. platensis* cultured in ZM, MZS, MZN, and MZC media. ZM = Zarrouk's medium, MZS = modified Zarrouk's medium with zinc sulfate, MZN = modified Zarrouk's medium with zinc nitrate and MZC = modified Zarrouk's medium with zinc chloride.

Values are presented as mean of triplicates  $\pm$  SD. Bars with different alphabet notations are significantly different at  $p \leq 0.05$ .

Table 1. Specific growth rate, doubling time and cell productivity of *S. platensis* cultured in ZM, MZS, MZN, and MZC media

Media	$\mu$ (day <sup>-1</sup> )	$t_d$ (day)	$P_x$ (g/L.day)
ZM	0.45±0.00 <sup>ab</sup>	1.53±0.01 <sup>bc</sup>	0.51±0.01 <sup>bcd</sup>
0.4 $\mu$ mol Zn of MZS	0.45±0.01 <sup>abc</sup>	1.54±0.05 <sup>bc</sup>	0.51±0.02 <sup>bcd</sup>
0.8 $\mu$ mol Zn of MZS	0.45±0.00 <sup>ab</sup>	1.53±0.02 <sup>bc</sup>	0.53±0.02 <sup>ab</sup>
1.6 $\mu$ mol Zn of MZS	0.46±0.01 <sup>a</sup>	1.50±0.03 <sup>c</sup>	0.55±0.02 <sup>a</sup>
0.4 $\mu$ mol Zn of MZN	0.44±0.00 <sup>bc</sup>	1.58±0.01 <sup>ab</sup>	0.48±0.02 <sup>de</sup>
0.8 $\mu$ mol Zn of MZN	0.45±0.01 <sup>abc</sup>	1.56±0.04 <sup>ab</sup>	0.49±0.01 <sup>bcd</sup>
1.6 $\mu$ mol Zn of MZN	0.44±0.01 <sup>bc</sup>	1.56±0.04 <sup>abc</sup>	0.49±0.03 <sup>bcd</sup>
0.4 $\mu$ mol Zn of MZC	0.45±0.01 <sup>abc</sup>	1.54±0.03 <sup>bc</sup>	0.51±0.02 <sup>bc</sup>
0.8 $\mu$ mol Zn of MZC	0.44±0.01 <sup>bc</sup>	1.57±0.02 <sup>ab</sup>	0.49±0.02 <sup>cd</sup>

Values are presented as mean of triplicates ± SD. Values with different superscripts within the same column are significantly different at  $p \leq 0.05$ . ZM = Zarrouk's medium, MZS = modified Zarrouk's medium with zinc sulfate, MZN = modified Zarrouk's medium with zinc nitrate, and MZC = modified Zarrouk's medium with zinc chloride.

and Zn<sup>2+</sup> ions that gave conformational change (Binder and Zschornig, 2002).

The FTIR spectra band of amide I and amide II groups of cells cultured in 1.6  $\mu$ mol Zn of MZS (Figure 2A) decreased when compared with ZM. The intensity of amide I and amide II groups decreased because Zn was absorbed in cells at these functional groups of the protein. ZM showed the higher absorbance while 1.6  $\mu$ mol Zn of MZS had the lower absorbance for amide I and amide II groups. Intensity absorbance of the free amide I and amide II functional groups was decreased by Zn<sup>2+</sup>. This finding of the current study was consistent with those of Rodrigues *et al.* (2012) who performed the Zn<sup>2+</sup>, Ni<sup>2+</sup> and Pb<sup>2+</sup> onto dry *S. platensis* and *Chlorella vulgaris* using FTIR spectroscopic method. These binding would mainly be at the wall materials as reported by McLaughlin *et al.* (1981) that metal cations modulated the proteins insertion and protein binding to membranes, especially phospholipid bilayers. Therefore,

amide I and amide II played an important role in the binding of Zn<sup>2+</sup>.

### 3.4 Zn accessibility

The SPM, SPC, and SPZ were digested in simulated gastric and intestinal tracts. The SPM showed Zn accessibility of simulated gastric and intestinal tracts for 55.20±0.57 and 63.55±0.21%, respectively, which was significantly higher than the SPC and SPZ ( $p \leq 0.05$ ) (Figure 3). Zn accessibility of SPC after gastric and small intestinal digestion were 52.85±0.21 and 60.30±0.14%, respectively. This was an insignificant difference with SPZ 52.55±0.21% for gastric digestion and 60.95±0.78% for intestinal digestion. In the comparison of SPM, SPC and SPZ, the SPM showed the highest accessibility, because Zn was uptake in the SPM proper contents which is a new finding of this research. Furthermore, metal ions could be formed into metalloproteins to increase their chemical properties (Bowman *et al.*, 2016). The results indicated that the simulated intestinal digestion provided higher accessible Zn than the simulated gastric digestion. This might be due to a concerted effect of the activity of pancreatin,

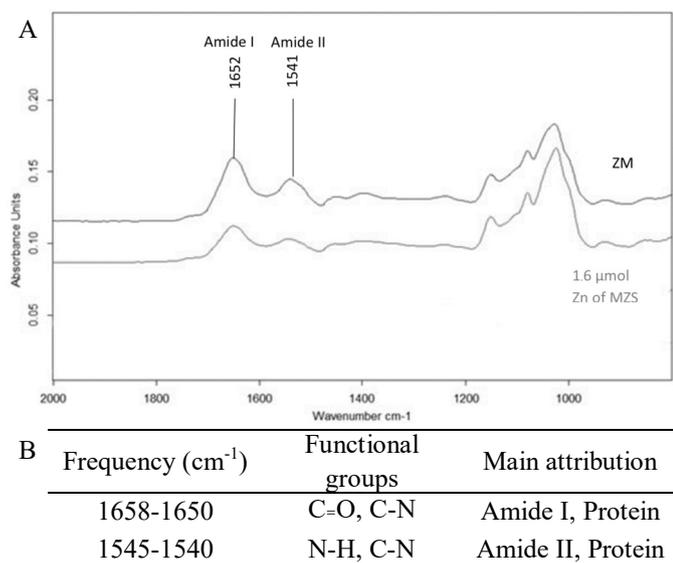


Figure 2. FTIR spectra of *S. platensis* cells cultured in ZM and 1.6  $\mu$ mol Zn of MZS media (A) and Major absorption peaks of infrared spectra of *Spirulina platensis* sample (B) (modified from Chen *et al.*, 2007; Liu *et al.*, 2013).

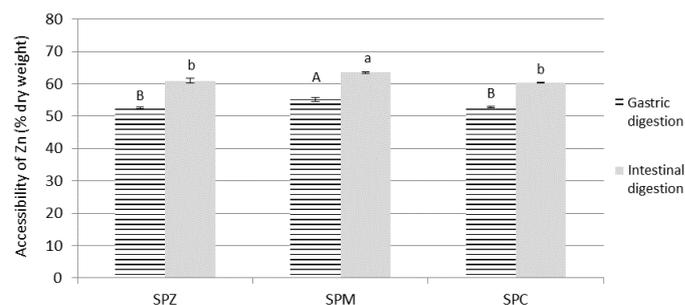


Figure 3. Zn accessibility after *S. platensis* digestion simulation. SPZ = dried ZM sample, SPM = dried MZS sample and SPC = commercial *S. platensis* sample. Capital letters and small letters indicate for each treatment. Bars with different alphabet notations are significantly different at  $p \leq 0.05$ .

containing trypsin, lipase, amylase, chymotrypsin and carboxypeptidase (Young *et al.*, 2011), and bile salts creating lipid micelles (Monsbach *et al.*, 1980). These would effectively release Zn from the matrix of *S. platensis* cells.

### 3.5 Zn availability

The sequential digestion in gastric and intestinal tracts simulation, Zn availability of SPM, SPZ and SPC were 34.63±0.95, 31.43±0.63 and 31.68±0.07%, respectively (Table 2). In the intestinal digestion simulation, Zn availability of all samples was lower than Zn accessibility. Decreasing Zn availability might be due to a lower free Zn containing. These Zn may bind with a deprotonated form of bile acid resulting in a higher molecular weight Zn complex. In addition, a short soluble peptide chain may bind Zn forming peptide-Zn. The high molecular weight of bound Zn would not be able to pass through the dialysis membrane via a passive transport mechanism (McDougall *et al.*, 2005).

Table 2. Zn availability after *S. pirulina platensis* digestion simulation

Samples	Zn content inside the tubing (µg Zn/g dry weight)	%Available Zn
SPZ	19.14±0.38 <sup>b</sup>	31.43±0.63 <sup>b</sup>
SPM	33.96±0.93 <sup>a</sup>	34.63±0.95 <sup>a</sup>
SPC	20.34±0.05 <sup>b</sup>	31.68±0.07 <sup>b</sup>

Values are presented as mean of triplicates ± SD. Values with different superscripts within the same column are significantly different at  $p \leq 0.05$ . SPZ = Spirulina sample, SPM = Spirulina sample and SPC = commercial Spirulina sample.

## 4. Conclusion

*Spirulina platensis* cultured in 1.6 µmol Zn of Zarrouk's medium modified with ZnSO<sub>4</sub> (MZS) had the highest Zn concentration, protein, biomass, specific growth rate, doubling time and cell productivity. The ZnSO<sub>4</sub> was an appropriate form for Zn bio-adsorption in *S. platensis* cells. Zn uptake was higher in the cytoplasm (72.8%) than in cell walls (27.2%). FTIR spectra analysis indicated that Zn<sup>2+</sup> would bind to amide I and amide II functional groups of the protein. Zn in *S. platensis* cells was 52-53% accessible after gastric digestion. Zn accessibility was increased to 60-64% after intestinal digestion. However, Zn availability was reduced to 31-35% as the result of bound Zn formed with bile acid and soluble proteins during simulated digestion. Further works could be studied in vitro Zn availability using cell culture-based models. Moreover, *S. platensis* could be fortified in plant-based meat which enriched Zn contents and applied in varieties of vegetarian foods.

## Conflict of interest

The authors declare no conflict of interest.

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