

## The Effect of Different Doses of Calcium Supplementation on Iron Availability from *Spirulina Platensis* by an *in Vitro* Digestion/Caco-2 Cell Culture Model

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### Introduction

Iron deficiency anemia is a serious widespread issue throughout the world (1) and affect approximately 20 % of the world population, despite the fact that the average daily diet contains iron far in excess of the amount needed for metabolic purposes. This is mostly due to the low availability of food iron. Beside the low iron availability from food, interaction between iron and other nutrients such as calcium, zinc and copper were also shown to lower the iron availability (2,3). Because of these reasons, various approaches have been used in improving the iron intake and absorption in human. One of the alternatives is the use of *Spirulina spp.*, a microalgae that is already popular in many Asian countries as a food supplement. Its represent one of the richest protein sources of plant origin (60-70%) and is a good source of vitamins and minerals (4). The simple cultivation technology and the good quality of their protein, as well as the absence of any toxic side effects, favor their large-scale production. The aim of this study was to examine the effect of different doses of calcium on iron availability from cultured *Spirulina platensis*, and ferrous sulphate as a reference.

### Materials and Methods

*In vitro* digestion/Caco-2 cell culture system (5) was used to measure iron availability. Samples were incubated with 55.5 kBq <sup>59</sup>Fe overnight before being subjected to digestion with pepsin followed by pancreatin-bile extract. All conditions were maintained to mimic the enzymatic process and pH of the intestinal lumen. The digests was later passed through a dialysis bag (12,000 molecular weight cut-off) and then layered onto the Caco-2 cells that were grown on bicameral inserts for an incubation period of one hour. Radioactivity of the contents from the upper

chambers, lower chambers and cells were determined by using gamma counter. The total iron uptake for each meal was calculated based on the percentage of radioiron taken up and transported by Caco-2 cells and the amount iron originally present in the upper chamber.

### Results and Discussion

A comparison of iron availability from the various samples was made. Significant decrease was found in iron dialysate from Fe and SP in present of calcium supplementation compared with FE and SP alone ( $P < 0.05$ ). However, among the different doses of calcium in both FE and SP groups there were no significant difference. As for the total iron uptake, there was significant difference between the mean of FE and the rest of the groups. However, among spirulina groups (SP and SP + calcium) there is no significant difference in the total iron uptake. There is also no significant difference in iron uptake among groups with different doses of calcium for both FE and SP. Many studies documented the inhibitory effects of calcium on iron absorption in human. Although several of the studies are conflicting, overall the evidence indicates that calcium present at levels common in meals and from ingestion of supplements inhibits the absorption of iron. In the present study, the ratio of calcium to iron were 26.7: 1, 53.3:1 and 106.7:1 respectively. These ratios have been shown to inhibit iron absorption in humans (4). In the present of calcium supplementation, iron from FeSO<sub>4</sub> is significantly reduced compare to SP, which don't seem to be inhibited by the present of added calcium. This result indicates that the iron from spirulina is more available compared to FeSO<sub>4</sub> eventhough the total iron uptake from FeSO<sub>4</sub> is more compared to spirulina.

This study is consistent with other studies done in rats (6,7).

### Conclusions

We conclude that spirulina is a good source of iron in the present of different doses of calcium. However, there is a need for further study in human is needed to confirm this.

### Benefits from the study

This study showed that *Spirulina platensis* have the potential to be cultured locally and commercialized as the health benefits of it especially as iron availability is good.

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**Project Publications in Refereed Journals**

None.

**Project Publications in Conference Proceedings**

None.

**Graduate Research**

Loh Su Peng (On going). Nutritional Biochemistry [PhD]. Universiti Putra Malaysia