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In vitro bioaccessibility of minerals from microalgae-enriched cookies

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Dr. Professor Laura Bravo,
Food & Function

Dear Professor Laura Bravo
We are sending you the manuscript "In vitro bioaccessibility of minerals from microalgae-enriched cookies", for revision as a research paper and if you consider publication in the Food & Function journal.
Microalgae are able to enhance the nutritional content of conventional food and feed preparation and hence to positively affect humans and animal health due to their original chemical composition, namely high protein content, with balanced amino acids pattern, carotenoids, fatty acids, minerals, vitamins, polysaccharides, sterols, phycobilins and other biologically active compounds, more efficiently than traditional crops.
The combination of the exceptional nutritional value of microalgae with colouring and therapeutical properties, associated with an increase demand of natural products, make microalgae worth exploring for utilization in the future in feed, food, cosmetic and pharmaceutical industries, with recognized advantages comparing with the traditional ingredients. Food safety regulations for human consumption are the main constraint for the biotechnological exploitation of microalgae resources, but successful cases of approval as a novel food in the last years broadens perspectives. In this paper the use of microalgae in breadsticks formulation has been used to reinforce the mineral content in iron and selenium.

Yours sincerely

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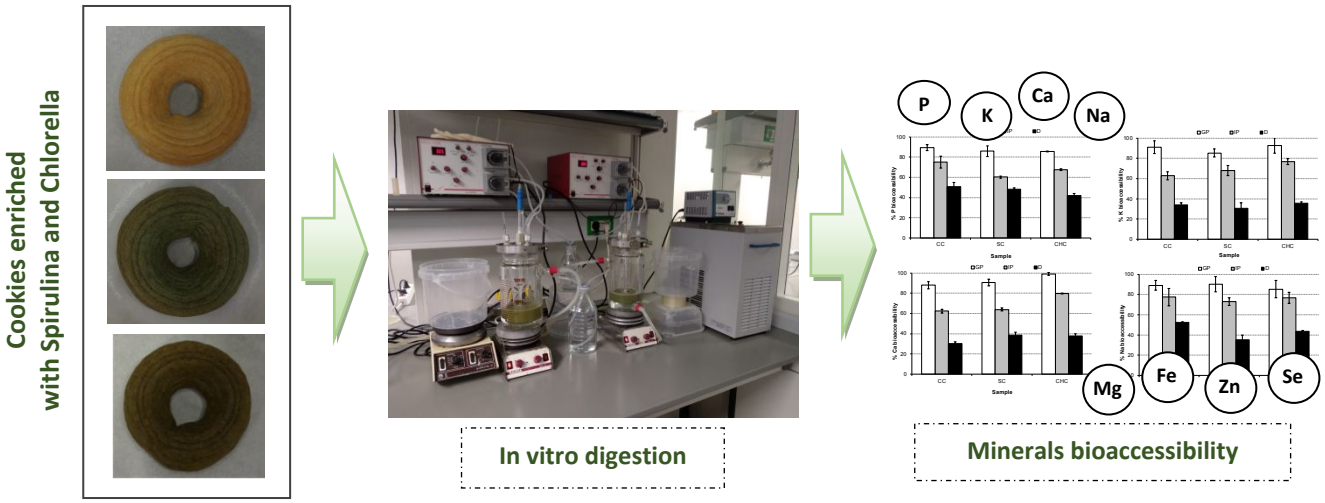
Cookies enriched
with Spirulina and Chlorella
In vitro digestion
P
K
Ca
Na
Mg
Fe
Zn
Se
Minerals bioaccessibility

In vitro bioaccessibility of minerals from microalgae-enriched cookies

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Abstract

Microalgae have several biologically active constituents such as pigments, fatty acids, vitamins, and minerals, among others. Nowadays, there are numerous commercial applications for microalgae in food and animal feed. Minerals have many functions in the human body, from structural to metabolic function; as mineral absorption by the human body is important, its study is also key because of anti-nutritional factors responsible for lowering the bioaccessibility of these minerals. The aim of this work was to evaluate the mineral bioaccessibility in cookies, enriched with *Arthrospira platensis* (Spirulina) and *Chlorella vulgaris*, using *in vitro* static systems that simulate digestive processes. Using microalgae as an ingredient to enrich cookies with minerals was a good alternative because cookies presented a higher content in minerals compared to control samples. When the microalgae concentration in formulation increased (within studied range), higher P, Se, Na, and Mg amounts were observed in cookies. Cookies enrichment with 1.5 or 2% Chlorella or Spirulina are foods classed as “high in selenium”. Incorporating *A. platensis* and *C. vulgaris* in cookie formulations, therefore, allowed greater accessibility of P, K, Ca, Mg, Fe, Zn, and Se for absorption in the body, compared with control cookies.

Keywords: minerals, spirulina, chlorella, bioaccesibility, cookies

1. Introduction

Minerals have many functions in the human body. Sodium (Na) and potassium (K) are present as salts in body fluids, having the physiological function of maintaining osmotic pressure. Minerals are part of the tissues' structure; for example, calcium and phosphorus (P) in the bones are key functional components of the skeleton. In addition, they are important in metabolic functions, such as muscle function, nerve stimulation, enzymatic and hormonal activities, and oxygen transport. Magnesium (Mg) is an essential mineral which is found in bones and human tissues.[1] Iron (Fe), an essential element for almost all living organisms, participates in a wide variety of metabolic processes. In the human body, Fe mainly exists in complex forms bound to protein (haemoprotein) such as haem compounds (haemoglobin and myoglobin), haem enzymes, and non-haem compounds (flavin-iron enzymes, transferrin, and ferritin).[2] The body requires Fe for the synthesis of its oxygen transport proteins, in particular haemoglobin and myoglobin, and for the formation of haem enzymes along with other iron-containing enzymes, involved in electron transfer and oxidation reductions.[2,3] Zinc (Zn) is essential for a normal growth and development of the human body, because it plays an important role in gene expression, regulation of cellular growth, and differentiation,[4] beside to development of the immune response. Zinc has a recognised action on over 300 enzymes implied in the metabolism of nucleic acids, carbohydrates, and proteins; participating as a cofactor.[5] Selenium (Se) is another element, which is an essential trace mineral of fundamental importance to human health. As a constituent of selenoproteins, Se has structural and enzymatic roles, best known as an antioxidant and catalyst, producing active thyroid hormones. Selenium is needed for a functioning immune system and appears to be a key nutrient in counteracting the development of virulence and inhibiting HIV progression to AIDS; it is also required for sperm motility and may reduce the risk of miscarriage. Deficiency in Se has been linked to adverse mood states, while selenium is presented, both, as an antioxidant and anti-inflammatory agent.[6] Among mineral insufficiencies, deficiencies in Fe and Zn are

reported as highly prevalent nutritional problems around the world, affecting mostly developing countries ranking 9 and 11, respectively, in the list of the major risk factors for global burden of disease. Iron deficiency has been related to health and productivity of adults and to impairment of cognitive development in infants and young children. Zinc deficiency may lead to retarded skeletal development and immunodeficiency disorders.[7,8] Interventions targeting mineral deficiencies include dietary variation and / or supplementation. However, the enrichment of food with a naturally high mineral content matrix, such as microalgae, can be useful in avoiding the use of supplementation. Microalgae have biologically active constituents such as pigments, fatty acids, vitamins, and minerals, among others.[9] Nowadays, there are numerous commercial applications for microalgae in food and animal feed. For example, in food, microalgae can enhance the nutritional value of pasta,[10] cookies,[11] and breadsticks.[12] In addition, mineral absorption by the human body is a key item for study, because there are anti-nutritional factors responsible for lowering the bioaccessibility of these minerals.[13] The observed effects of the temperature-time combinations of heat treatment will influence the levels of the anti-nutritional factors and bioaccessibility of minerals. During heat treatment, minerals are not destroyed owing to their heat stability. Depending on the heat treatment, the endogenous (and bacterial) enzymes will be, in most cases, inactivated. This implies that endogenous enzymes such as phytase, cellulase, and pectinase will not contribute further to the improvement of the mineral bioaccessibility. However, heat treatments will reduce the content of the antinutritional factors such as phytic acid, tannins, and phenolic compounds, up to 40%, as reported for legumes or pulses.[14,15]

Bioavailability is a term used to describe the proportion of a nutrient in foods that can be used for normal bodily functions. Many quantification techniques have been proposed for bioavailability; the most reliable methods for bioavailability studies are *in vivo* measurement of absorption in humans, with or without using a labelling technique.[16] Still, human *in vivo* studies are time-consuming, high-priced, complex, and

produce variable results. *In vitro* methods are being extensively used at present since these are quick, safe, and do not have the ethical restrictions of *in vivo* methods. *In vitro* methods either simulate the digestion and absorption processes (for bioavailability) or only the digestion process (for bioaccessibility), while the concentration of a nutrient, in some type of final extract, is the response measured.[17] The *in vitro* method proposed by the COST INFOGEST network is a general standardised and practical static digestion method based on relevant conditions that can be applied for various purposes.[18] The objective of this consortium was to harmonise *in vitro* static systems that simulate digestive processes by defining key parameters and conditions.

Therefore, the aim of this work was to evaluate the mineral bioaccessibility in cookies enriched with Spirulina and Chlorella.

2. Material and methods

2.1. Raw materials

Commercial wheat pastry flour, salt, granulated sugar, and butter were purchased from a local supermarket (Alcampo, Valencia, Spain). Freeze-dried *Arthrospira platensis* (Spirulina) and *Chlorella vulgaris* were supplied by AlgaEnergy (S.A., Madrid, Spain).

2.2. Dough formulation and cookies preparation

Three kinds of cookie doughs were formulated; with Spirulina, Chlorella, and a control sample without microalgae. Water (25%), butter (18%), granulated sugar (13%), and salt (0.2%) were the basic ingredients. Spirulina dough and Chlorella dough contain binomial microalgae-wheat pastry flour combinations at different levels of concentrations: 0.5 - 43.3%; 1.0 - 42.8%; 1.5 - 42.3%, and 2.0 - 41.8%.

Butter and sugar were manually mixed until a fluffy texture was achieved. Salt, microalgae, and wheat pastry flour were gradually added into the formulation and

mixed with a dough hook in a food processor (Kenwood chef classic, KM400/99 plus, Kenwood Corporation, Tokyo, Japan), kneading for 5 min at a low speed and ambient temperature. After mixing, cookies were shaped into cylinders, were frozen at -18 °C for 90 min in a fast freezing blast chiller (SINCOLD, A.T.O. SRL, Treviso, Italy), then were baked at 140 °C for 55 min on a stainless steel plate covered with baking paper, in a steamer oven (Convotherm OES 6.06 mini CC, Convotherm Elektrogeräte GMBH, Eglfing, Germany). Baked cookies were named: Control Cookie (CC), Spirulina Cookie (SC), and Chlorella Cookie (CHC).

2.3. *In vitro* digestion

Sample *in vitro* digestibility was assessed by the standardised static *in vitro* digestion method suitable for food (COST INFOGEST network) proposed by Minekus *et al*.[18] The *in vitro* digestion protocol is summarised in Figure 1, where four steps have been followed: oral phase, mixing the sample and simulate salivary fluid (SSF) (1:1) with amylase at pH 7 for 2 min; gastric phase, mixing the oral bolus and simulate gastric fluid (SGF) (1:1) with pepsin at pH 3 for 2 h; intestinal phase, mixing the gastric chyme and simulate intestinal fluid (SIF) (1:1) with enzymes at pH 7 for 2 h; and filtration, centrifuging at 4500 rpm for 30 min and then filtering through a 1 μm glass-fibre membrane.

The *in vitro* digestibility (IVD) (%) was calculated as the difference between the initial mass and the undigested mass (after correcting for the blank assay, B) divided by the initial mass and multiplied by 100 according to Batista *et al*.[11] Analyses were repeated in triplicate.

Cookie samples at high level (2%) of microalgae (SC, CHC) and CC were subjected to *in vitro* digestion, gastric (GP), intestinal (IP), and *in vitro* digestion (D) samples were collected according Minekus *et al.*[18], and samples were freeze-dried with use of a protease inhibitor when it was necessary.

2.4. Analysis

2.4.1. Water content

Water content (x_w) (g water/100 g sample) was determined by vacuum oven drying at 105 °C until constant weight.[19] Cookies were analysed in triplicate.

2.4.2. Ash and mineral content

Total ash content was determined following method 930.05 of AOAC procedures.[19] A sample of 500 mg was incinerated at high pressure in a microwave oven (Muffle P Selecta Mod.367PE) for 24 h at 550 ºC, and ash was gravimetrically quantified. The residue of incineration was extracted with HCl (hydrochloric acid) (50% v/v) and HNO_3 (nitric acid) (50% v/v) and made up to an appropriate volume with distilled water.[20] Minerals were measured using standard solutions for calibration purposes. The multimineral determination was performed by using an inductively coupled plasma optical emission spectrometer (700 Series ICP-OES; Agilent Technologies, Santa Clara, United States), with an axial viewing and a charge-coupled device detector. The instrumental parameters used for the multi-element determination were with a radiofrequency generator of 40 MHz, a power of 1 kW, plasma gas flow rate of 15 $Lmin^{-1}$, auxiliary gas flow rate of 1.5 $Lmin^{-1}$, and nebuliser gas (One Neb 2) pressure of 200 kPa. The elements and the analytical spectral lines (nm) used were, P (214.914), K (766.491), Ca (317.933), Na (589.592), Mg (285.213), Fe (238.204), Zn (213.857), Cu (327.395), Mn (259.372), and Se (196.026). Mineral composition (macro- and micro-elements) were expressed as mg/100 g. Samples were analysed in triplicate for cookies and for gastric phase, intestinal phase, and final digested samples (Figure 1).

2.5. Statistical analysis

Analysis of variance (ANOVA) simple or multifactor, with a confidence level of 95% ($p < 0.05$), using Statgraphics (Centurion XVII Software, version 17.2.04) was applied to

evaluate the differences among cookies samples, the effect of microalgae concentration, and the type of microalgae. Furthermore, a correlation analysis among microalgae concentration, formulation, and P, K, Ca, Na, Mg, Fe, Zn, Cu, Mn, and Se content in the cookies, with a 95% significance level, was carried out (Statgraphics Centurion XVII).

3. Results and Discussion

Water and ash content (mean values and standard deviations) for studied cookies are shown in Table 1. Lower values of water content were observed in samples with Spirulina compared to the rest and higher Spirulina concentrations resulted in lower water content in cookies. However, ash content in Spirulina cookies was significantly ($p < 0.05$) higher than Chlorella and control cookies. Ash content of S1.5C and S2C were high compared to all samples.

3.1. Mineral content of cookies

Figure 2 shows P (a), K (b), Ca (c), and Na (d) content of each formulation (SC and CHC) regarding 0 - 2% of microalgae. In Figure 2a greater microalgae concentrations in formulation cookies resulted with more P content in samples. There were significant ($p < 0.05$) differences between samples with Chlorella and Spirulina in the 1 and 1.5% concentrations. Nevertheless, increasing microalgae concentration to 2% in cookies, saw no significant ($p > 0.05$) differences between them. The greatest differences were seen in K content, regarding the control, were observed in cookies with 1.5% or 2% of microalgae. Potassium values (Figure 2b) in CHC were like the control, except for the 2% concentration cookies; greater than the others. However, SC showed different K content at different Spirulina concentrations, without a definite trend. Calcium content (Figure 2c) did not show significant ($p > 0.05$) differences with added Chlorella, independent of the concentration assay. However, Spirulina formulation incorporation significantly increased ($p < 0.05$) Ca content. Here it was only the type of microalgae

196 added that presented a significant change ($p < 0.05$) in Ca content of samples when an
197 ANOVA multifactor was applied to the results; microalgae concentration was not
198 significant within the studied range (0.5 - 2%). The increase of Spirulina from 0.5 to
199 1.5% and Chlorella from 0.5 to 1% did not show significant ($p > 0.05$) differences
200 regarding the control cookie when measuring the Na content (Figure 2d). Nevertheless,
201 the use of 2% of microalgae in cookies increased the Na content by 25%.
202 Figure 3 shows Mg (a), Fe (b), Zn (c), and Se (d) content for each formulation (SC and
203 CHC) regarding 0 - 2% of microalgae addition. Microalgae incorporation in the cookie
204 formulation provoked an increase in Mg (Figure 3a), which was statistically significant
205 ($p < 0.05$) at 1% of Chlorella and, 0.5 and 1.5% of Spirulina addition, after addition of
206 1.5% microalgae there was no further effect on mineral content. Furthermore, the
207 addition of microalgae resulted in a significant increase ($p < 0.05$) in Fe and Se,
208 observed in Figures 3b and 3d, respectively, after 0.5% Spirulina and 1.5% Chlorella
209 addition. There were significant differences ($p < 0.05$) between of Spirulina and
210 Chlorella addition when Fe was evaluated but there were no significant differences ($p >$
211 0.05) in Se content. Moreover, addition of 2% microalgae did not increase the content
212 of these microminerals in cookies compared with the 1.5% addition. Likewise, authors
213 have presented that breadsticks enriched with Chlorella and Spirulina showed that Fe
214 and Se content was significantly higher than the control.[12] Figure 3c shows Zn content
215 of each cookie; the control shows no Zn content, however the addition of microalgae
216 provoked an increase of this mineral. With SC showing higher Zn content than CHC at
217 1.5 and 2% concentrations. Copper and manganese were not detected in any sample.
218 We found that the use of microalgae as an ingredient to enrich cookies with functional
219 minerals was a good alternative because in this study cookies presented with a higher
220 content of minerals. Cookies with Spirulina would be the better choice, as these
221 samples showed the highest mineral levels. Pearson's statistical correlation analysis
222 established correlations among microalgae concentration in formulations and P, K, Ca,
223 Na, Mg, Fe, Zn, Cu, Mn, and Se content in cookies. The results showed that the most

significant relation to microalgae concentration was presented by P (0.8374, $p < 0.05$), followed by Se (0.8127, $p < 0.05$), Na (0.7427, $p < 0.05$), and Mg (0.7262, $p < 0.05$). When microalgae concentrations increased within the studied range, higher K, Se, Na, and Mg content were observed in cookies.

According to the regulation no. 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made in foods[21] cookies enriched with 1.5 or 2% of Chlorella or Spirulina are a food “high in selenium”, considering that the intake of Se requires concentrations in plasma of 55 µg per day for both men and women.[22] Although addition of microalgae increased P, K, Ca, Fe, Mg, and Zn content in cookies, none of them reached the levels of claims.

3.2. *In vitro* digestibility (IVD) and mineral bioaccessibility

The IVD analysis reproduces the chemical-enzymatic catalysis that occurs in the proximal tract of the monogastric digestive system.[23]

The IVD differences between the initial mass and the undigested mass results were similar among the three samples. Mean values and standard deviations, in parentheses, were 72% (3), 73% (2), and 74% (4) for CC, CHC, and SC, respectively. No significant difference for IVD between the microalgae cookies and control was observed. Other authors who used a different static *in vitro* digestion method for cookies observed this trend.[11]

This study used the term bioaccessibility referring to the fraction of mineral that was released from the examined cookies during *in vitro* digestion becoming accessible for absorption. Bioaccessibility should be distinguished from the term bioavailability, which is the fraction of nutrients or food components that have been efficiently *in vivo* digested, assimilated and then absorbed in the body.[24] Consequently, it could be concluded that bioaccessibility of the studied minerals is a prerequisite for their bioavailability. Total concentrations of P, K, Ca, Na, Mg, Fe, Zn, and Se were quantified

in gastric (GP), intestinal (IP), and final digestion (D). Bioaccessibility was calculated using equation (1) proposed by Khouzam, Pohlb, and Lobinski[25] and Sahuquillo *et al.*[26],

$$Bioaccessibility = \left(\frac{A}{B}\right) \times 100 \quad (1)$$

where A is the concentration of the element in the bioaccessible fraction following gastric, intestinal, and completed simulated (final) digestion; B is the concentration of the element in the sample before digestion. The minerals present in tap water and the reagents were also analysed and corrected in the final bioaccessible fraction.

Figures 4 and 5 show mean values and standard deviations of mineral bioaccessibility percentages, relative to the total quantity present in cookies of each formulation. Mineral biaccessibility in GP ranged from 80 to 100%, IP from 60 to 90%, and D 16 – 70%, depending on the mineral quantified. Magnesium bioaccessibility was higher than the other studied minerals, notably in IP and D. Vitali *et al.*[27] observed this trend in whole grain tea biscuits, showing values of Mg bioaccessibility ≈ 75%. In contrast, Se bioaccessibility was the lowest among studied minerals at D, as food composition affects the ability of enzymes to breakdown solubilised Se. The selenium not solubilised after D might be present in form of undigestible Se-containing polysaccharides, as was observed by Bhatia *et al*.[28] These authors indicated that the formation of Se-containing polysaccharides explain the low Se bioaccessibility found in mushrooms. Furthermore, Fe and Zn showed an antagonistic effect on Se absorption.[29,30] The antagonistic effect for Zn occurred between natural forms of Zn and Se at concentrations potentially encountered in wheat grain.[31]

Phosphorus, potassium, and magnesium bioaccessibility (Figures 4a, 4b, and 5a, respectively) were similar for the three cookies, in each digestion phase. However, Ca and Na bioaccessibility (Figures 4c and 4d, respectively) presented slight differences among samples. In GP and IP, Ca bioaccessibility of CHC was higher than the other cookies, but at D, Ca bioaccessibility percentages of SC and CHC did not show significant differences ($p > 0.05$). At D, Na bioaccessibility percentages of SC and CHC

were significantly (p < 0.05) lower than the Na bioaccessibility percentage of CC. Lower bioaccessibility of Na could be because of the competition of other monovalent competing ions such as K,[32] since K content of SC and CHC were double than in CC (Figure 2b). Iron is one of the most studied elements for its bioavailability using *in vivo* and *in vitro* methods.[33-36] In this study, SC and CHC showed a significantly (p < 0.05) higher Fe bioaccessibility percentage (Figure 5b) than CC at the end of gastrointestinal digestionD. Iron bioaccessibility, like Zn (Figure 5c) were similar for SC and CHC and were without significant differences (p > 0.05). Figure 5d shows Se bioaccessibility percentage in studied cookies at D of SC was lower than CHC, probably because of the higher Fe content of SC (Figure 3b), and its antagonistic effect on Se absorption.[29,30]

Table 2 shows mean values (and standard deviations) of mineral content (mg/100 $g_{digested\ cookies}$) in samples after gastrointestinal digestion. Minerals, P, K, Ca, Mg, Fe, Zn, and Se have content in digested SC and CHC that were significantly higher (p < 0.05) than CC. Incorporation of Spirulina in cookie formulations allows higher accessibility of calcium, iron, and zinc content for absorption in the body compared to cookies with Chlorella. However, incorporation of Chlorella in cookie formulations allows for higher accessibility of potassium and selenium content for absorption in the body compared to Spirulina cookies. Furthermore, Na content in digested SC was significantly lower (p < 0.05) than in CC and CHC. This can be a positive aspect to prevent hypertension and reduce blood pressure since the relation of Na serum concentration with blood pressure.[37]

4. Conclusions

Using microalgae as an ingredient to enrich cookies with functional mineral content was a good alternative, because they presented a greater content of minerals compared to control cookies. Cookies enriched with 1.5 or 2% of Chlorella or Spirulina are foods classed as “high in selenium”. Spirulina and Chlorella incorporation in cookie

formulations allowed for greater accessibility of P, K, Ca, Mg, Fe, Zn, and Se content for absorption in the body than control cookies.

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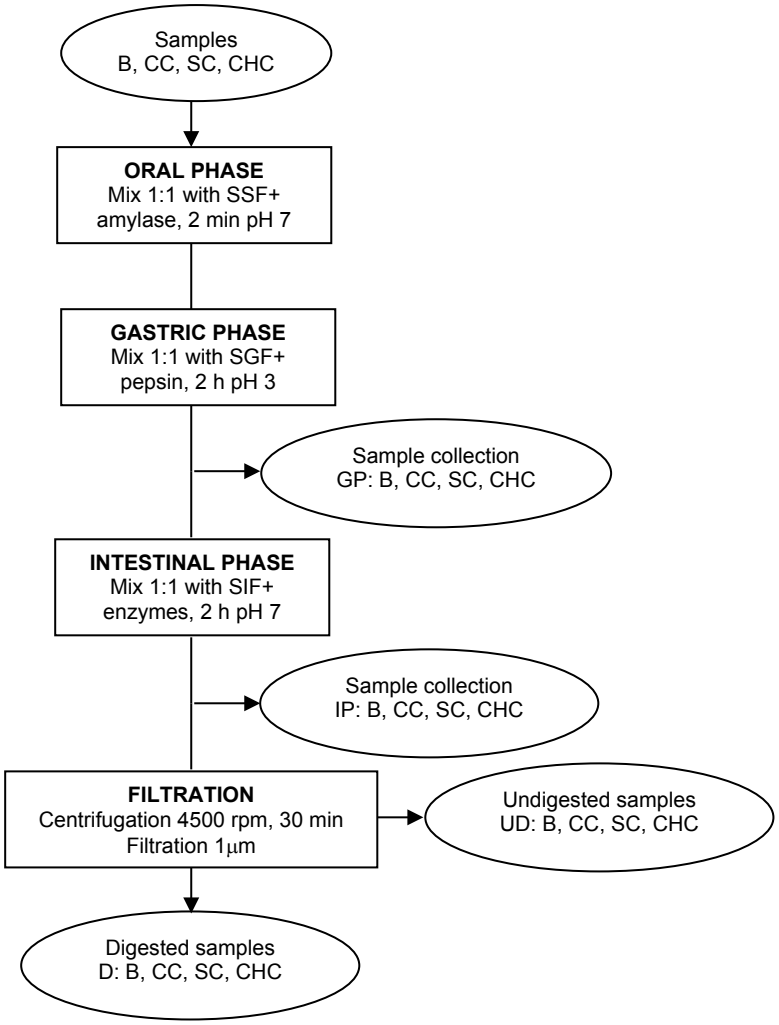

Figure 1. Flow diagram of simulated in vitro digestion method. SSF, SGF, and SIF are Simulated Salivary Fluid, Simulated Gastric Fluid, and Simulated Intestinal Fluid, respectively. B, CC, SC, and CHC are Blank, Control Cookie, Spirulina Cookie, and Chlorella Cookie, respectively.

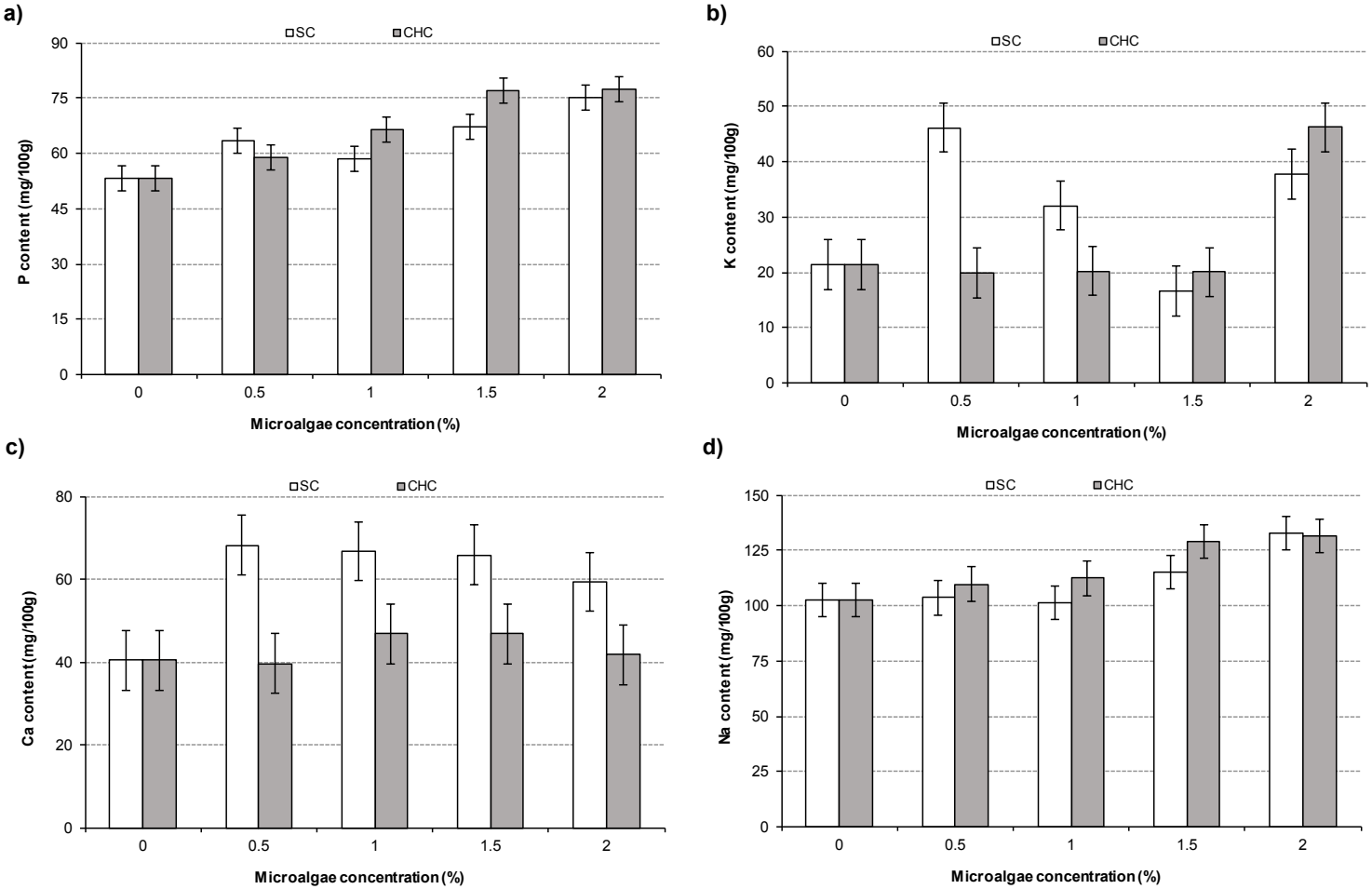

Figure 2. Mean values and Least Significant Difference (LSD) intervals of P (a), K (b), Ca (c), and Na (d) content of each formulation (SC and CHC).

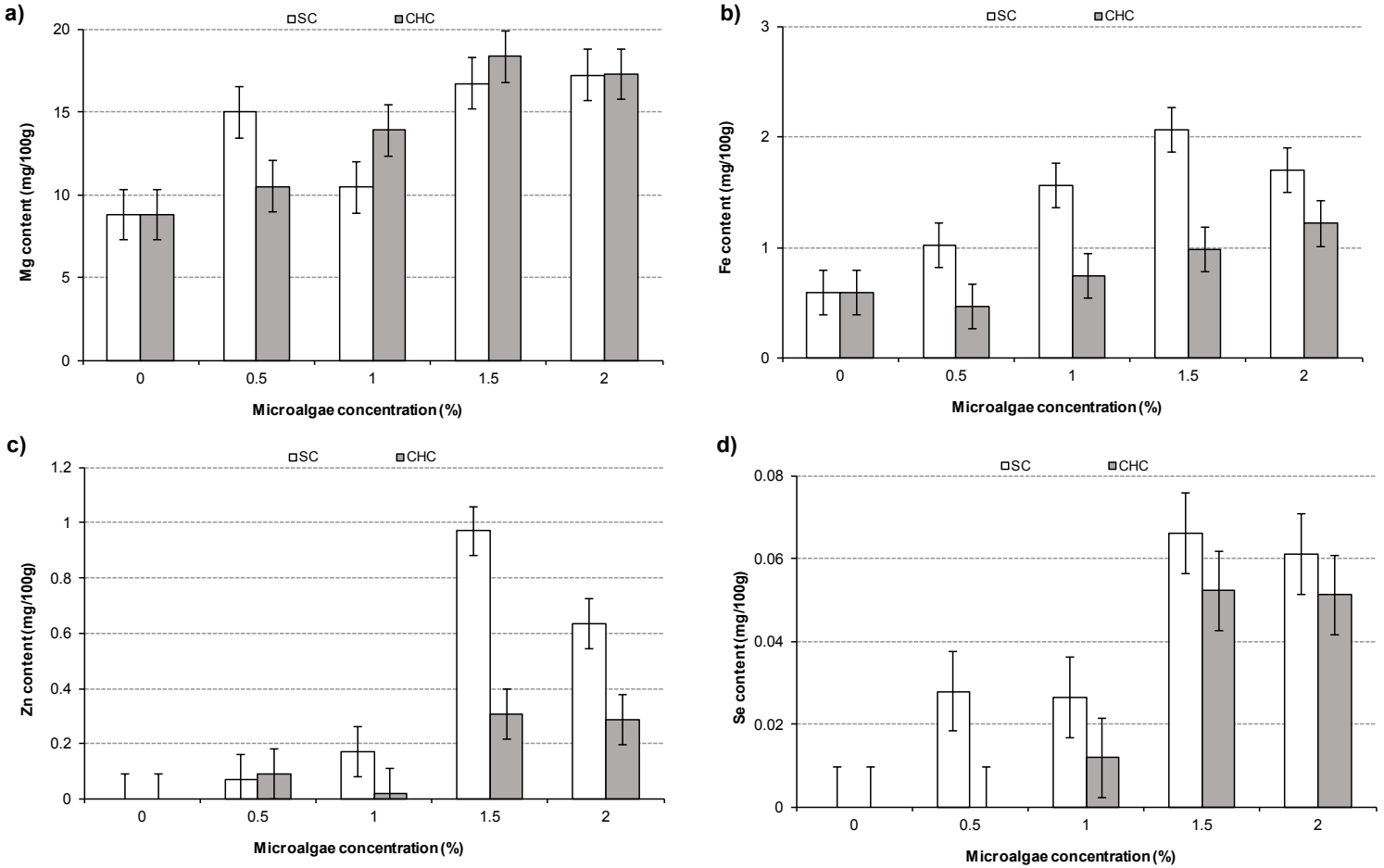

Figure 3. Mean values and Least Significant Difference (LSD) intervals of Mg (a), Fe (b), Zn (c), and Se (d) content of each formulation (SC and CHC).

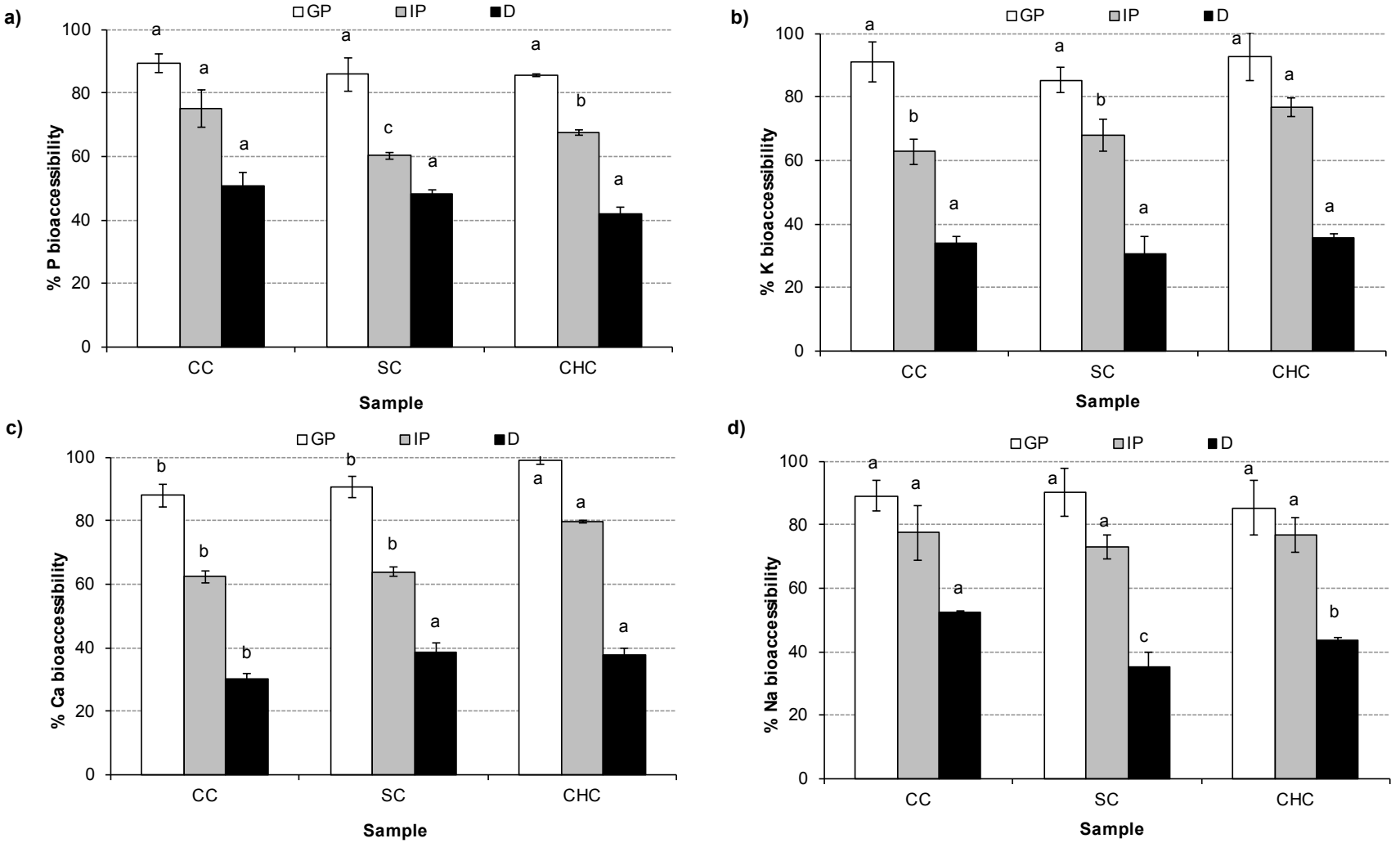

Figure 4. Mean values and standard deviation of P (a), K (b), Ca (c), and Na (d) bioaccessibility percentage relative to total quantity present in samples of each formulation (CC, SC and CHC). Letters indicate homogeneous groups established by the ANOVA ($p < 0.05$) for each *in vitro* digestion phase (GP, IP, D).

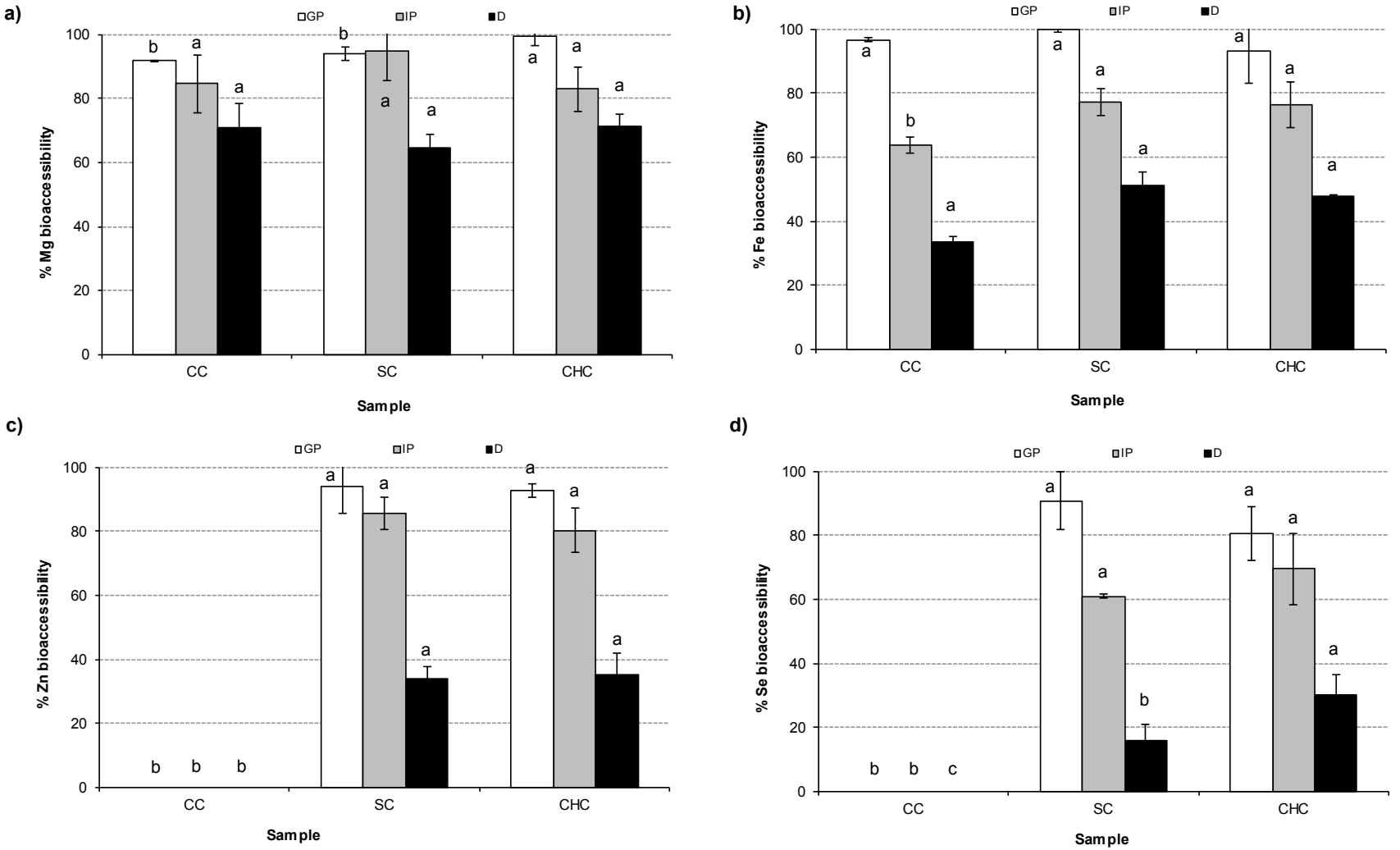

Figure 5. Mean values and standard deviation of percentage Mg (a), Fe (b), Zn (c), and Se (d) bioaccessibility relative to total quantity present in samples of each formulation (CC, SC, and CHC). Letters indicate homogeneous groups established by the ANOVA ($p < 0.05$) for each *in vitro* digestion phase (GP, IP, D).

Table 1. Mean values (and standard deviations) of water (x_w, g_w/100 g) and ash content (g/100 g) of cookies.

	x_w	Ash content
CC	5.85 (0.07)^{c}	0.3960 (0.0014)^{c}
S0.5C	3.57 (0.04)^{g}	0.586 (0.002)^{b}
S1C	5.46 (0.15)^{d}	0.589 (0.002)^{b}
S1.5C	4.80 (0.10)^{e}	0.792 (0.008)^{a}
S2C	4.23 (0.14)^{f}	0.791 (0.003)^{a}
CH0.5C	6.32 (0.09)^{a}	0.396 (0.002)^{c}
CH1C	6.02 (0.03)^{b}	0.3868 (0.0012)^{c}
CH1.5C	6.01 (0.04)^{b}	0.392 (0.005)^{c}
CH2C	5.53 (0.03)^{d}	0.391 (0.004)^{c}

The same letter in superscript within column indicates homogeneous groups established by ANOVA ($p < 0.05$).

Table 2. Mean values (and standard deviations) of mineral content (mg/100 $g_{digested\ cookies}$) in samples after gastrointestinal digestion.

Mineral	Cookies		
	CC	SC	CHC
P	27 (2)^{b}	38 (3)^{a}	33.25 (1.02)^{a}
K	7.2 (2)^{c}	11 (2)^{b}	16.1 (0.8)^{a}
Ca	13 (2)^{b}	23 (2)^{a}	15.60 (1.13)^{b}
Na	53.85 (0.24)^{a}	47 (6)^{b}	57.2 (1.3)^{a}
Mg	6.3 (0.6)^{b}	11.1 (0.7)^{a}	12.3 (0.6)^{a}
Fe	0.199 (0.009)^{c}	0.90 (0.07)^{a}	0.5826 (0.0008)^{b}
Zn	- ^{c}	0.22 (0.02)^{a}	0.101 (0.020)^{b}
Se	- ^{c}	0.010 (0.003)^{b}	0.015 (0.003)^{a}

CC: Control Cookie; SC: Spirulina Cookie; CHC: Chlorella Cookie
The same letter in superscript within rows indicates homogeneous groups established by ANOVA ($p < 0.05$).