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Additional Information



***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.

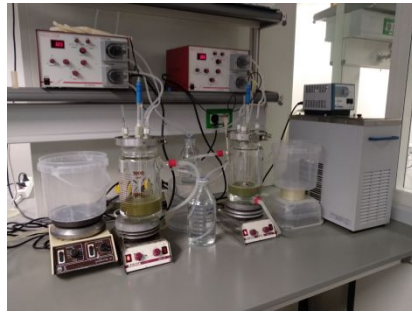
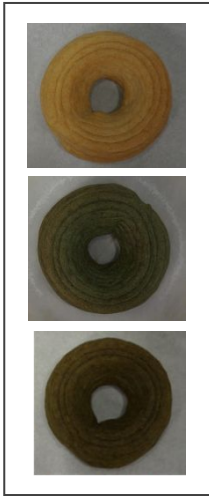
The combination of the exceptional nutritional value of microalgae with colouring and therapeutical properties, associated with an increase demand of natural products, make microalgae worth exploring for utilization in the future in feed, food, cosmetic and pharmaceutical industries, with recognized 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

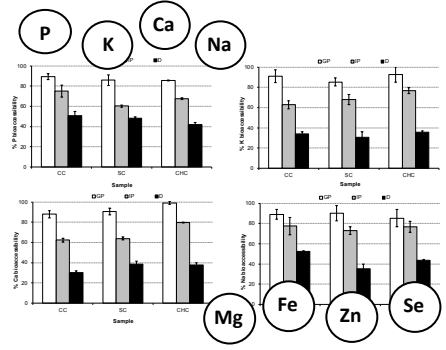
A handwritten signature in black ink, appearing to read 'J. Martínez Monzó', with a long horizontal flourish extending to the right.

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Cookies enriched
with Spirulina and Chlorella



In vitro digestion



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, bioaccessibility, cookies

29 **1. Introduction**

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

57 reported as highly prevalent nutritional problems around the world, affecting mostly
58 developing countries ranking 9 and 11, respectively, in the list of the major risk factors
59 for global burden of disease. Iron deficiency has been related to health and productivity
60 of adults and to impairment of cognitive development in infants and young children.
61 Zinc deficiency may lead to retarded skeletal development and immunodeficiency
62 disorders.^{7,8} Interventions targeting mineral deficiencies include dietary variation and /
63 or supplementation. However, the enrichment of food with a naturally high mineral
64 content matrix, such as microalgae, can be useful in avoiding the use of
65 supplementation. Microalgae have biologically active constituents such as pigments,
66 fatty acids, vitamins, and minerals, among others.⁹ Nowadays, there are numerous
67 commercial applications for microalgae in food and animal feed. For example, in food,
68 microalgae can enhance the nutritional value of pasta,¹⁰ cookies,¹¹ and breadsticks.¹²
69 In addition, mineral absorption by the human body is a key item for study, because
70 there are anti-nutritional factors responsible for lowering the bioaccessibility of these
71 minerals.¹³ The observed effects of the temperature-time combinations of heat
72 treatment will influence the levels of the anti-nutritional factors and bioaccessibility of
73 minerals. During heat treatment, minerals are not destroyed owing to their heat
74 stability. Depending on the heat treatment, the endogenous (and bacterial) enzymes
75 will be, in most cases, inactivated. This implies that endogenous enzymes such as
76 phytase, cellulase, and pectinase will not contribute further to the improvement of the
77 mineral bioaccessibility. However, heat treatments will reduce the content of the
78 antinutritional factors such as phytic acid, tannins, and phenolic compounds, up to
79 40%, as reported for legumes or pulses.^{14,15}

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

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

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

97

98 **2. Material and methods**

99

100 **2.1. Raw materials**

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

104

105 **2.2. Dough formulation and cookies preparation**

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

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

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

121

122 **2.3. *In vitro* digestion**

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

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

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

140

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 °C, and ash was gravimetrically quantified. The residue of incineration was extracted with HCl (hydrochloric acid) (50% v/v) and HNO₃ (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

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

173

174 **3. Results and Discussion**

175 Water and ash content (mean values and standard deviations) for studied cookies are
176 shown in Table 1. Lower values of water content were observed in samples with
177 Spirulina compared to the rest and higher Spirulina concentrations resulted in lower
178 water content in cookies. However, ash content in Spirulina cookies was significantly (p
179 < 0.05) higher than Chlorella and control cookies. Ash content of S1.5C and S2C were
180 high compared to all samples.

181

182 **3.1. Mineral content of cookies**

183 Figure 2 shows P (a), K (b), Ca (c), and Na (d) content of each formulation (SC and
184 CHC) regarding 0 - 2% of microalgae. In Figure 2a greater microalgae concentrations
185 in formulation cookies resulted with more P content in samples. There were significant
186 ($p < 0.05$) differences between samples with Chlorella and Spirulina in the 1 and 1.5%
187 concentrations. Nevertheless, increasing microalgae concentration to 2% in cookies,
188 saw no significant ($p > 0.05$) differences between them. The greatest differences were
189 seen in K content, regarding the control, were observed in cookies with 1.5% or 2% of
190 microalgae. Potassium values (Figure 2b) in CHC were like the control, except for the
191 2% concentration cookies; greater than the others. However, SC showed different K
192 content at different Spirulina concentrations, without a definite trend. Calcium content
193 (Figure 2c) did not show significant ($p > 0.05$) differences with added Chlorella,
194 independent of the concentration assay. However, Spirulina formulation incorporation
195 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

224 significant relation to microalgae concentration was presented by P (0.8374, $p < 0.05$),
225 followed by Se (0.8127, $p < 0.05$), Na (0.7427, $p < 0.05$), and Mg (0.7262, $p < 0.05$).
226 When microalgae concentrations increased within the studied range, higher K, Se, Na,
227 and Mg content were observed in cookies.
228 According to the regulation no. 1924/2006 of the European Parliament and of the
229 Council of 20 December 2006 on nutrition and health claims made in foods²¹ cookies
230 enriched with 1.5 or 2% of Chlorella or Spirulina are a food “high in selenium”,
231 considering that the intake of Se requires concentrations in plasma of 55 μg per day for
232 both men and women.²² Although addition of microalgae increased P, K, Ca, Fe, Mg,
233 and Zn content in cookies, none of them reached the levels of claims.

234

235 **3.2. *In vitro* digestibility (IVD) and mineral bioaccessibility**

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

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

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

251 in gastric (GP), intestinal (IP), and final digestion (D). Bioaccessibility was calculated
252 using equation (1) proposed by Khouzam, Pohl, and Lobinski²⁵ and Sahuquillo *et al.*²⁶,

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

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

257 Figures 4 and 5 show mean values and standard deviations of mineral bioaccessibility
258 percentages, relative to the total quantity present in cookies of each formulation.
259 Mineral bioaccessibility in GP ranged from 80 to 100%, IP from 60 to 90%, and D 16 –
260 70%, depending on the mineral quantified. Magnesium bioaccessibility was higher than
261 the other studied minerals, notably in IP and D. Vitali *et al.*²⁷ observed this trend in
262 whole grain tea biscuits, showing values of Mg bioaccessibility \approx 75%. In contrast, Se
263 bioaccessibility was the lowest among studied minerals at D, as food composition
264 affects the ability of enzymes to breakdown solubilised Se. The selenium not
265 solubilised after D might be present in form of undigestible Se-containing
266 polysaccharides, as was observed by Bhatia *et al.*²⁸ These authors indicated that the
267 formation of Se-containing polysaccharides explain the low Se bioaccessibility found in
268 mushrooms. Furthermore, Fe and Zn showed an antagonistic effect on Se
269 absorption.^{29,30} The antagonistic effect for Zn occurred between natural forms of Zn and
270 Se at concentrations potentially encountered in wheat grain.³¹

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

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

288 Table 2 shows mean values (and standard deviations) of mineral content (mg/100
289 g_{digested cookies}) in samples after gastrointestinal digestion. Minerals, P, K, Ca, Mg, Fe, Zn,
290 and Se have content in digested SC and CHC that were significantly higher ($p < 0.05$)
291 than CC. Incorporation of Spirulina in cookie formulations allows higher accessibility of
292 calcium, iron, and zinc content for absorption in the body compared to cookies with
293 Chlorella. However, incorporation of Chlorella in cookie formulations allows for higher
294 accessibility of potassium and selenium content for absorption in the body compared to
295 Spirulina cookies. Furthermore, Na content in digested SC was significantly lower ($p <$
296 0.05) than in CC and CHC. This can be a positive aspect to prevent hypertension and
297 reduce blood pressure since the relation of Na serum concentration with blood
298 pressure.³⁷

299

300 **4. Conclusions**

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

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

307

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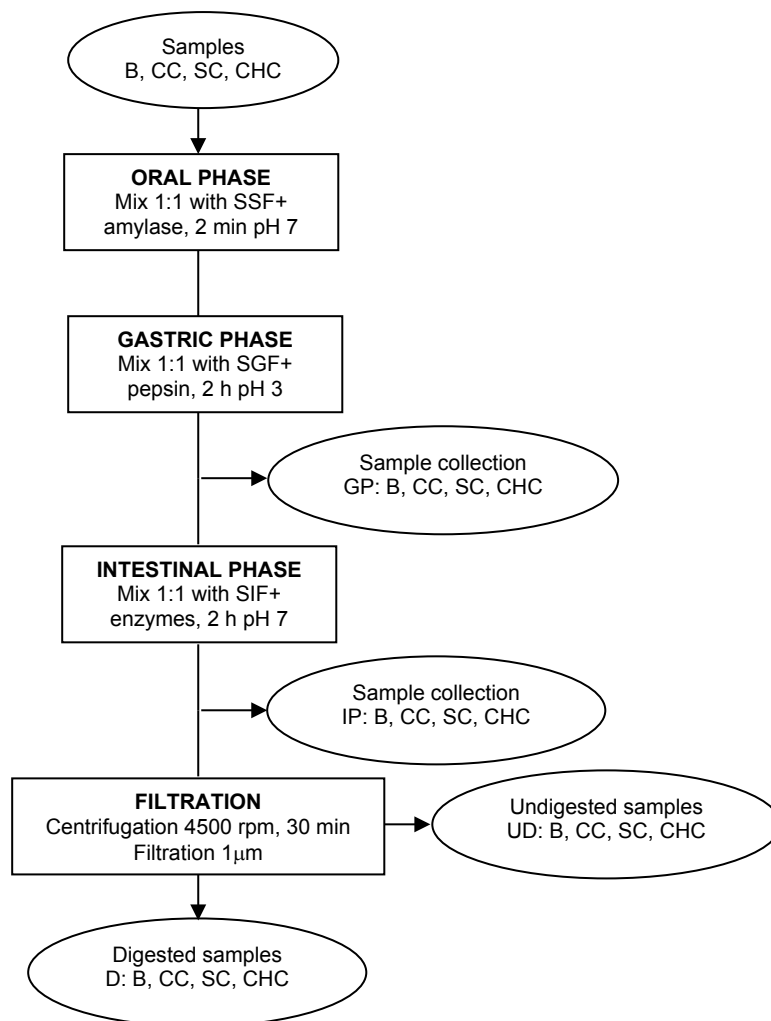


Figure 1. Flow diagram of simulated in vitro digestion method. SSF, SGF, and SIF are Simulated Salivary Fluid, Simulated Gastric Fluid, and Simulated Intestinal Fluid, respectively. B, CC, SC, and CHC are Blank, Control Cookie, Spirulina Cookie, and Chlorella Cookie, respectively.

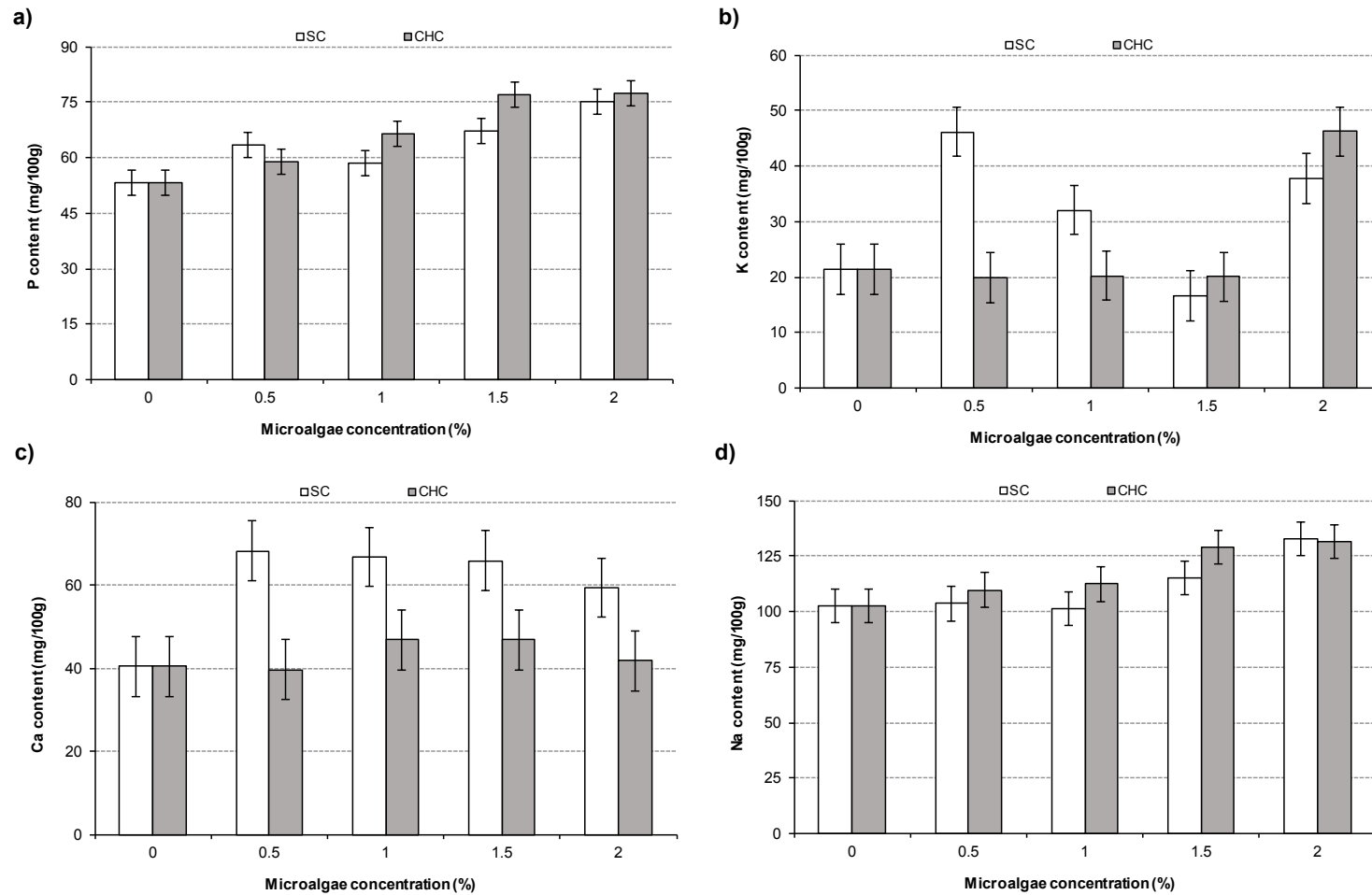


Figure 2. Mean values and Least Significant Difference (LSD) intervals of P (a), K (b), Ca (c), and Na (d) content of each formulation (SC and CHC).

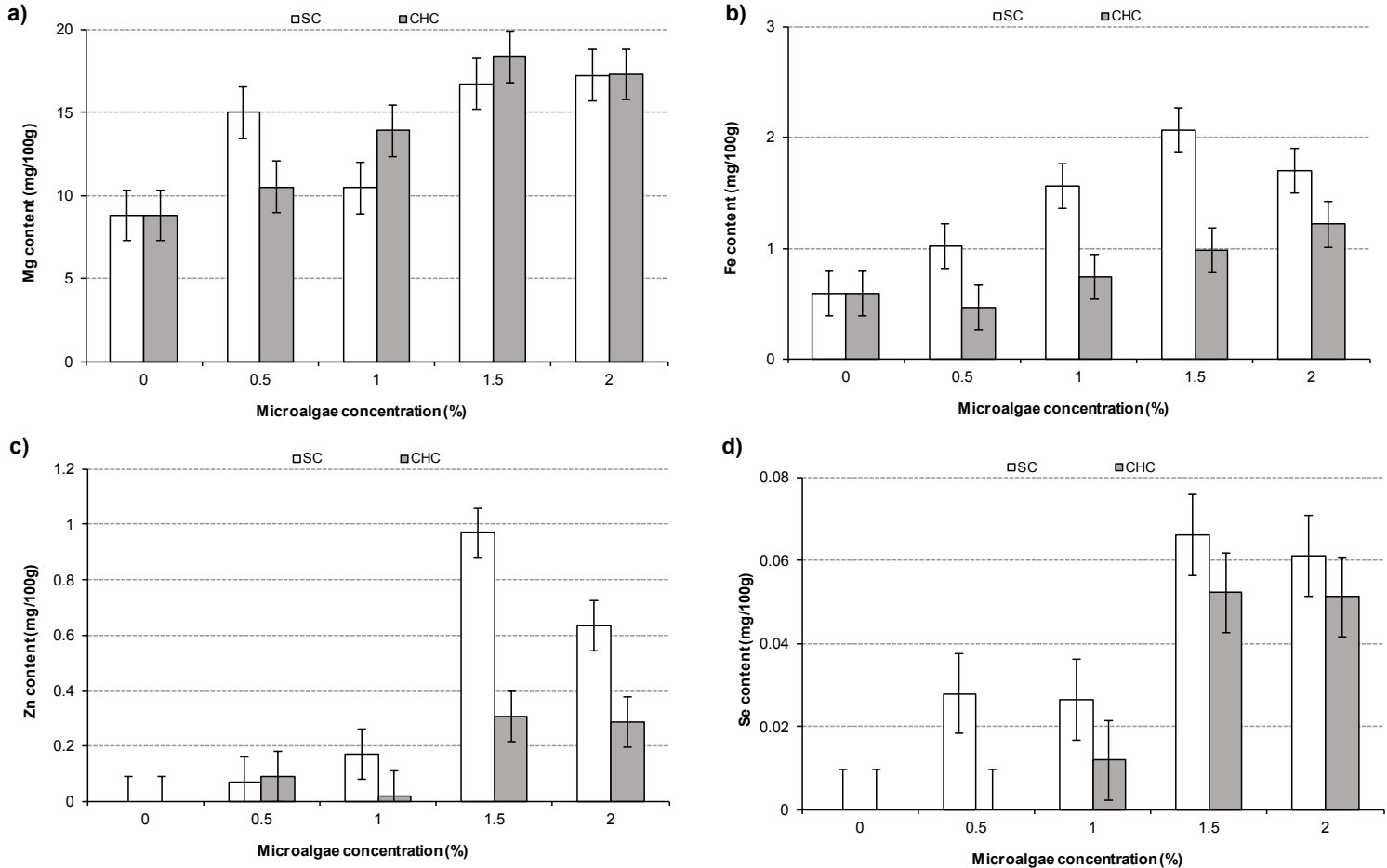


Figure 3. Mean values and Least Significant Difference (LSD) intervals of Mg (a), Fe (b), Zn (c), and Se (d) content of each formulation (SC and CHC).

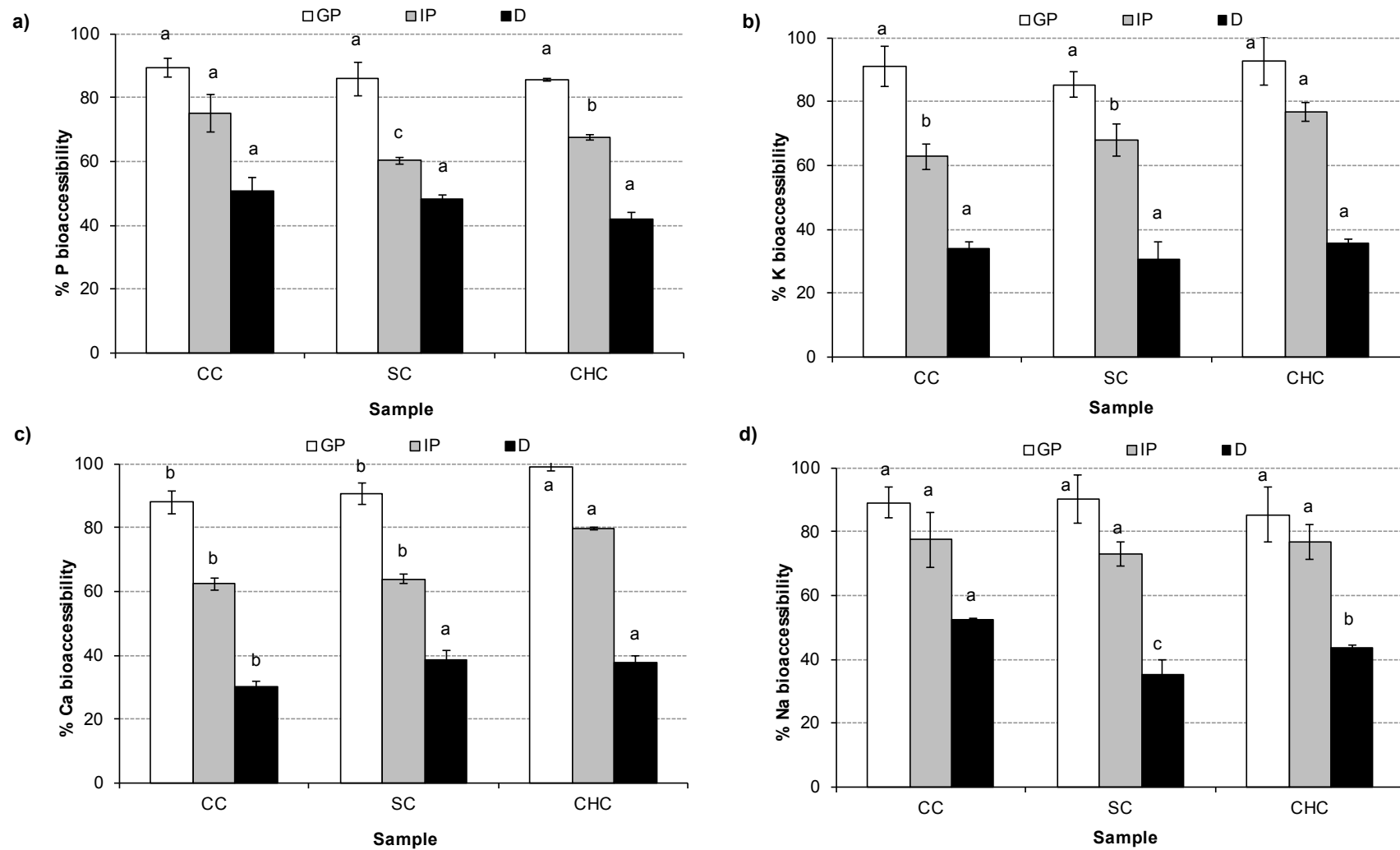


Figure 4. Mean values and standard deviation of P (a), K (b), Ca (c), and Na (d) bioaccessibility percentage relative to total quantity present in samples of each formulation (CC, SC and CHC). Letters indicate homogeneous groups established by the ANOVA ($p < 0.05$) for each *in vitro* digestion phase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

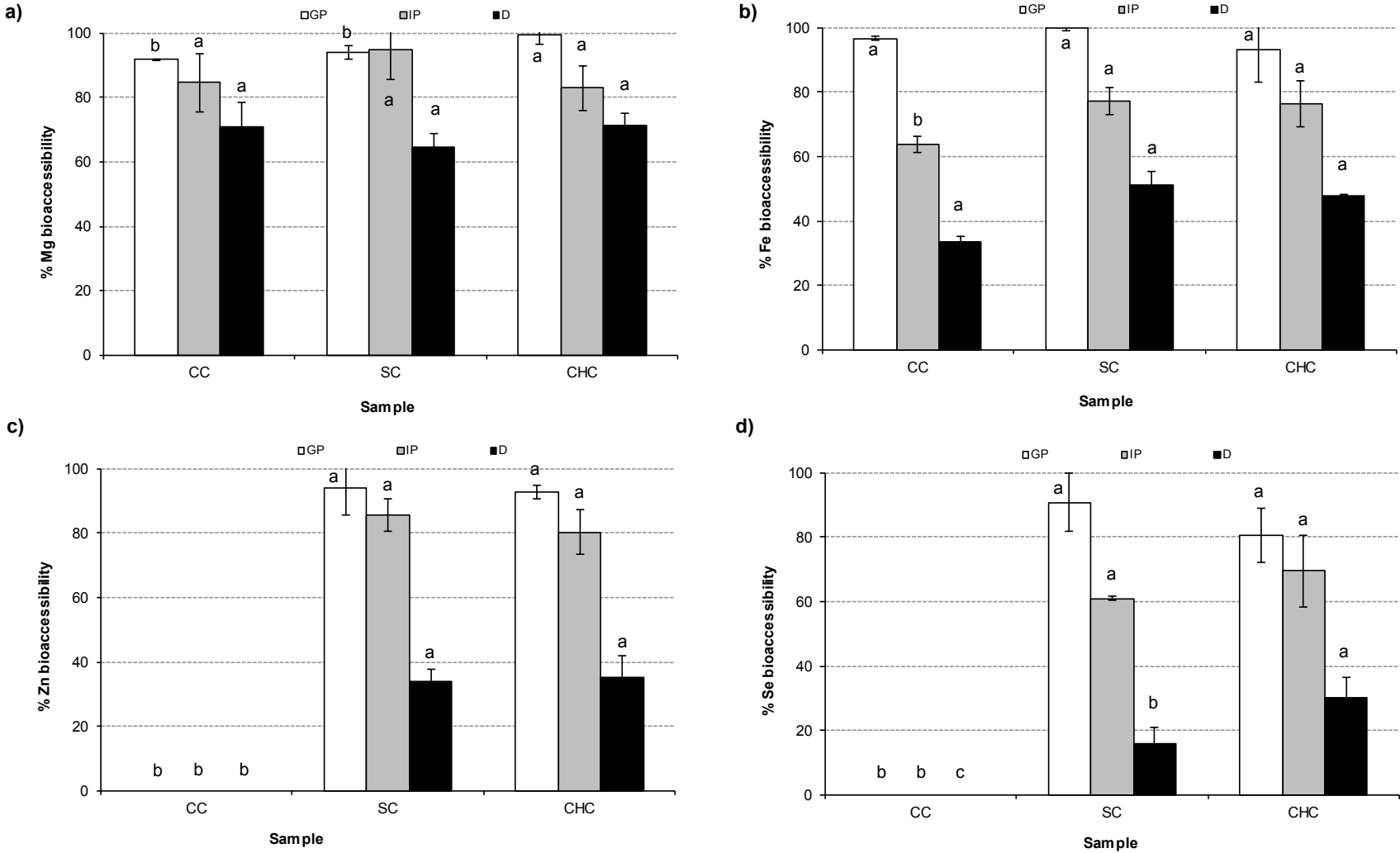


Figure 5. Mean values and standard deviation of percentage Mg (a), Fe (b), Zn (c), and Se (d) bioaccessibility relative to total quantity present in samples of each formulation (CC, SC, and CHC). Letters indicate homogeneous groups established by the ANOVA ($p < 0.05$) for each *in vitro* digestion phase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