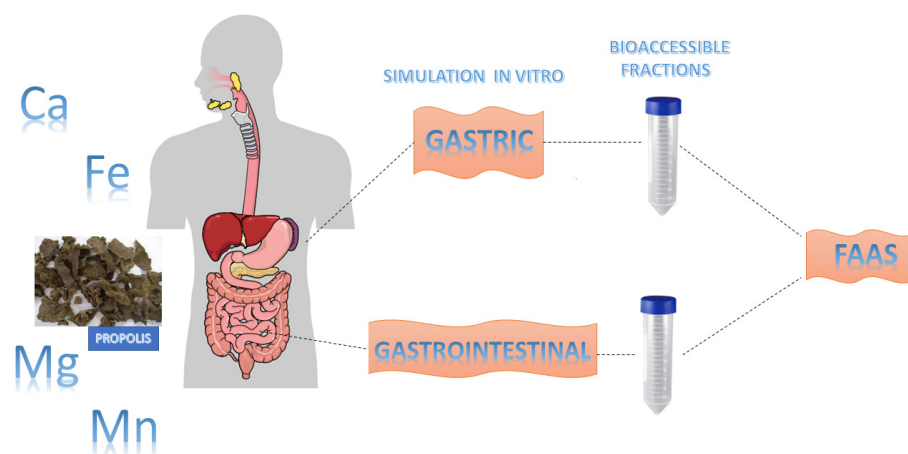


ARTICLE

Bioaccessibility of Ca, Fe, Mg, and Mn in Brazilian Propolis

Lana P. B. Pereira^{id}, Robson Carlos M. Brito^{id}, Kelly das Graças Fernandes Dantas*^{id}✉

Faculdade de Química, Universidade Federal do Pará, Rua Augusto Corrêa, nº 1, Guamá, Belém, 66075-110, PA, Brazil



The objective of this study was to determine and evaluate the bioaccessibility of Ca, Fe, Mg, and Mn in *Apis mellifera* and *Melipona* propolis samples. Flame atomic absorption spectrometry was used for the determination of total Ca, Fe, Mg, and Mn in digested propolis, and of the bioaccessible fractions. The levels of Ca, Fe, Mg, and Mn obtained after digestion ranged from 423.43 – 4368.42, 3.85 – 814.92, 139.02

– 1170.68, and 14.28 – 67.29 mg kg⁻¹, respectively. The bioaccessible contents after simulated gastric digestion of Ca, Fe, Mg, and Mn ranged from 17.62 – 63.35, 0.12 – 1.64, 26.06 – 86.34, and 12.08 – 83.05%, respectively. In the simulation of the gastrointestinal tract, the concentrations ranged from 3.05 – 73.08% for Ca, 0.63 – 2.45% for Fe, 0.16 – 43.26% for Mg, and 1.47 – 8.45% for Mn. In general, the simulation showed higher bioaccessible contents after gastric, compared to gastrointestinal digestion.

Keywords: propolis, inorganic elements, gastric digestion, bioaccessibility

INTRODUCTION

An increasing demand for healthy lifestyle habits has been observed, as well as for the consumption of food with functional properties. In this context, apiculture products have aroused the interest of both consumers and researchers, thanks to their chemical composition. Propolis, in particular, stands out for its therapeutic properties (antimicrobial, anti-inflammatory, healing, and anesthetic) that can be used in the pharmaceutical industry and food.¹

Propolis is a generic term used to describe the complex mixture of softwood, gummy and balsamic substances collected by bees from buds, flowers, and plant exudates, to which the bees add salivary

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secretions, wax, and pollen to produce the final material. It is also called “Bee-glue” which is a natural resin substance present in bee hives, and used by Honey bees as a cementing material to close open spaces in their hives.^{2,3}

The beneficial use of propolis has prompted an investigation of its chemical composition because propolis contains polyphenols (flavonoids, phenolic acids, and esters), phenolic aldehydes, and others.^{2,3} The flavonoids are affected by the source and ecological botanical environment where the bee lives.² In propolis can be found micro and macro elements like Mn, Fe, Si, Mg, Se, Ca, Na and, vitamins B1, B2, B6, C, and E.^{3,4}

Bioaccessibility has been defined as the fraction of a component that is released from the matrix in the gastrointestinal tract, making it available for intestinal absorption, it can be measured via several experimental models, such as in vitro digestion.⁵ On the other hand, no study has reported on the bioaccessibility of inorganic elements in propolis samples. The bioaccessible fractions show more accurately the contribution after consumption of these inorganic elements present in food.

Many studies have been conducted on the bioaccessibility of inorganic elements in different samples. Khouzam, Pohl & Lobinski (2011),⁵ determined the bioaccessibility of essential micronutrients in samples of cheese, bread, fruit and vegetables by DF-SP-ICP-MS. Peixoto, Mazon & Cadore (2013),⁶ studied the bioaccessibility of metals in samples of chocolate milk by ICP-OES and GFAAS. Kulkarni, Acharya & Rajurkar (2007),⁷ evaluated the bioaccessibility of some essential elements in samples of grass of wheat by INAA. Dutta, Maharia, Acharya & Reddy (2014),⁸ estimated the bioaccessibility of trace elements in plants by INAA and ICP-MS. Bossu, Menezes & Nogueira (2020)⁹ determined the bioaccessibility of zinc, calcium and phosphorus in milk by ICP OES. Jacobs et al. (2021)¹⁰ studied the bioaccessibility of metals in samples of grape skins by MIP OES. Alves, Nunes & Dantas (2017),¹¹ evaluated the bioaccessibility of copper, iron and manganese in amazonian fruits by GF AAS. Mingroni et al. (2019)¹² estimated the bioaccessibility of calcium, copper and manganese in fresh and dried fruits by FAAS.

The goal this study was evaluate the bioaccessibility of calcium, iron, magnesium, and manganese in *Apis mellifera* and *Melipona* propolis samples by flame atomic absorption spectrometry (FAAS).

MATERIALS AND METHODS

Instrumentation

A flame atomic absorption spectrometer (model Thermo iCE 3300, Cambridge, UK) was used for determination of Ca, Fe, Mg, and Mn in digested propolis samples, and bioaccessible fractions. The wavelength, and hollow-cathode lamp current used for Ca, Fe, Mg, and Mn were 422.7, 248.4, 285.2, and 279.5 nm, and 5, 6, 4, and 5 mA, respectively.

A cryogenic mill (Model 6770, SPEX CertPrep) was used to grind the samples.

A microwave oven (Start E, Milestone, Sorisole, Italy) was used to digest the samples.

A thermostatic bath (Model Q226M, Quimis, Brazil) was used in the gastric and gastrointestinal tract simulation tests.

A centrifuge (Quimis, São Paulo, Brazil) was used to separate the bioaccessible fractions.

Reagents, solutions, and samples

All reagents used were of analytical grade. All dilutions and solution preparations were performed with ultrapure water from a Synergy-UV water purification system (Millipore, Bedford, MA, USA).

The Ca, Fe, Mg, and Mn reference solutions were prepared at a concentration of 1000 mg L⁻¹ (Sigma, USA).

Lanthanum oxide (Spectron, Brazil) and concentrated hydrochloric acid (Isofar, Brazil) were used to prepare the solution of lanthanum chloride (1%, m v⁻¹) for determination of Ca and Mg in samples, and bioaccessible fractions by FAAS.

Microwave digestion of the samples used 30% (w w⁻¹) H₂O₂ (Impex, Brazil), and 14.0 mol L⁻¹ HNO₃ (Sigma, Brazil).

Sodium chloride and hydrochloric acid (both from Synth, São Paulo, Brazil), sodium bicarbonate (CRQ, Brazil), pepsin (Sigma-Aldrich, cat. no. P7012, São Paulo, Brazil), pancreatin (Sigma-Aldrich, cat. no. P1750, São Paulo, Brazil), and bile salts (Sigma-Aldrich, cat. no. 48305, St. Louis, MO, USA) were used in the simulated gastric, and gastrointestinal digestion.

The samples were obtained in different locations from Pará State, where seven samples of bee species *Apis mellifera* (PNT: Nova Timboteua city, PCo: Colares city, POu: Ourém city, PCP: Capitão Poço city, PBr: Bragança city, PVi: Vigia city and PSA: Santo Antônio do Tauá city), one sample of bee species *Scaptotrigona* sp (PBel 2: Belterra city) and one sample of bee *Friseomellita varia* (PBel 1: Belterra city) were collected.

Total determination

The samples were first ground in a cryogenic mill. An amount of each sample was weighed (0.25 g, $n = 3$) into digestion flasks. A volume of 4.0 mL of 14.0 mol L⁻¹ HNO₃, and 4.0 mL of 30% w/w H₂O₂ were added, and the samples were digested in the microwave oven cavity. The heating program was performed in three steps: the first step (ramp) was performed for 10 min at 200 °C (800 W); the second step (hold) was performed for 20 min at 200 °C (800 W), and the third step cooled the system through forced ventilation for a period of 50 min. The solutions obtained after digestion were transferred to volumetric flasks and diluted to 14 mL with ultrapure water, and digested Ca, Fe, Mg, and Mn were determined by FAAS.

Bioaccessible fractions determination

The procedure was performed in two stages, one for determining gastric digestion and the other for gastrointestinal digestion. The procedure of gastric digestion and gastrointestinal digestion used in this study was adapted from Khouzam et al. (2011),⁵ Kulkarni et al. (2007),⁷ Stelmach, Pohl & Szymczycha-Madeja (2014),¹³ and Moreda-Piñeiro et al. (2012),¹⁴ as the simulation process conditions being most suitable for propolis samples.

Simulated gastric digestion

The in vitro gastric digestion process was performed in triplicate ($n = 3$), where 1.0 g of each sample was weighed into volumetric flask, and then 10 mL gastric fluid (10 g L⁻¹ of pepsin in 0.15 mol L⁻¹ NaCl with pH adjusted to 2.5 with 2 mol L⁻¹ HCl) was added. The solution was stirred for 1 min. The volumetric flasks were placed in a thermostatic bath at 37 °C for 4 h with constant agitation. After agitation, the flasks were placed in an ice bath for 30 min, and then centrifuged for 20 min at 4000 rpm. The supernatants were filtered using 0.45 μ membrane cellulose filters (Millipore, Bedford, MA, USA), and then acidified with HNO₃ for a final acid concentration of 5.0% (v/v). The solutions obtained were analyzed by FAAS.

Simulated gastrointestinal digestion

The gastrointestinal digestion procedure was performed after the gastric digestion. The solutions obtained after the gastric digestion procedure were adjusted to pH 7.0 with 0.1 mol L⁻¹ NaHCO₃, and then 10 mL of intestinal fluid (0.5 g pancreatin and 0.25 g of bile salts were made up to a final volume of 100 mL with 0.1 mol L⁻¹ NaHCO₃) was added. The solutions were agitated for 1 min and placed in a thermostatic bath at 37 °C for 4 h with constant agitation. After agitation, the volumetric flasks were placed in an ice bath for 30 min and then centrifuged for 20 min at 4000 rpm. The supernatants were filtered using 0.45 μ membrane cellulose filters (Millipore, Bedford, MA, USA), and then acidified with HNO₃ for a final acid concentration of 5.0% (v/v). The solutions obtained were analyzed by FAAS.

Evaluation of the methods

The evaluation of measurements by FAAS was evaluated by the method of addition and recovery, where known concentrations of Ca (0.5, 1.0, 1.5, and 2.0 mg L⁻¹), Fe (3.0, 5.0, 7.0, and 9.0 mg L⁻¹), Mg (0.1, 0.3, 0.5, and 0.7 mg L⁻¹), and Mn (0.5, 1.5, 2.5, and 3.5 mg L⁻¹) were added to four digested samples and four

bioaccessible fractions for further analysis by FAAS. The certified reference material (GBW 07604, Poplar leaves) ($n = 3$) was also used for evaluating the measures by FAAS.

The efficiency of the sample preparation procedure was evaluated by adding known concentrations of Ca, Fe, Mg, and Mn to the samples for further microwave digestion and analysis by FAAS.

RESULTS AND DISCUSSION

Evaluation of sample preparation and analysis procedures

The accuracy of the measurements by FAAS was evaluated by the method of addition and recovery of analyte. The digested and bioaccessible fractions were spiked with four different concentration levels, and the resulting solutions analyzed by FAAS. Recoveries found for Ca, Fe, Mg, and Mn were 107.4 – 119.4%, 86.7 – 116.5%, 80.0 – 106.3%, and 89.6 – 114.3%, respectively. The results obtained of the certified reference material (GBW 07604 – Poplar leaves) indicated that the concentrations of elements determined by FAAS method are in agreement with the certified value (Ca: 110.0%; Fe: 87%; Mg: 95%; Mn: 90%) at a 95% confidence level (Student's t test; $t_{\text{critical}} = 4.30$).

The accuracy of the sample preparation was also evaluated. The recoveries obtained for Ca, Fe, Mg, and Mn were 94.9 – 103.9%, 88.6 – 108.8%, 83.3 – 97.4%, and 107.4 – 118.5%, respectively.

Table I shows the figures of merit obtained in the determination of Ca, Fe, Mg, and Mn in digested and bioaccessible fractions of propolis by FAAS.

Table I. Figures of merit

Parameters	Ca	Fe	Mg	Mn
LOD (mg kg ⁻¹)	3.82	0.29	0.09	0.56
LOQ (mg kg ⁻¹)	12.74	0.98	0.32	1.88
C _o	0.3137	0.0567	0.9976	0.9976
R ²	0.9998	0.9961	0.9976	0.9976

LOD: Detection limit; LOQ: Quantification limit; C_o: Characteristic concentration; R²: Correlation coefficient.

Total and bioaccessible contents

Table II presents the total and bioaccessible fraction concentrations of Ca, Fe, Mg, and Mn in propolis by FAAS.

Table II. Total and bioaccessible contents (mg kg⁻¹) in propolis

Samples	Ca (mg kg ⁻¹)			Fe (mg kg ⁻¹)			Mg (mg kg ⁻¹)			Mn (mg kg ⁻¹)		
	Total	DG ^a	DGI ^b	Total	DG ^a	DGI ^b	Total	DG ^a	DGI ^b	Total	DG ^a	DGI ^b
PBe1	423.43 ± 26.23	268.25 ± 7.86 (63.35)	309.43 ± 13.17 (73.08)	167.65 ± 17.91	2.76 ± 0.004 (1.64)	1.68 ± 0.01 (1)	139.02 ± 4.15	120.03 ± 1.01 (86.34)	43.25 ± 1.41 (31.11)	39.4 ± 0.76	8.84 ± 0.06 (22.44)	1.19 ± 0.13 (3.02)
PBe2	4368.42 ± 139.78	2286.65 ± 4.28 (52.34)	1407.06 ± 50.56 (32.21)	366.66 ± 3.48	1.41 ± 0.01 (0.38)	9.00 ± 0.44 (2.45)	823.27 ± 7.02	537.66 ± 6.94 (65.31)	356.19 ± 1.68 (43.26)	52.41 ± 1.40	13.91 ± 0.28 (26.54)	1.61 ± 0.06 (3.07)
PNT	2081.87 ± 18.71	650.37 ± 17.77 (31.24)	239.25 ± 50.56 (11.49)	280.91 ± 10.31	< LOD	2.15 ± 0.20 (0.76)	1040.06 ± 30.69	475.44 ± 5.66 (45.71)	269.68 ± 1.68 (25.93)	48.26 ± 1.96	40.08 ± 1.17 (83.05)	4.08 ± 0.26 (8.45)
PBr	2262.89 ± 100.28	398.87 ± 36.15 (17.62)	69.06 ± 10.16 (3.05)	714.92 ± 83.51	0.89 ± 0.01 (0.12)	4.54 ± 0.08 (0.63)	539.49 ± 49.20	253.87 ± 6.18 (47.06)	73.68 ± 2.74 (13.66)	63.92 ± 3.80	7.77 ± 0.24 (12.15)	1.06 ± 0.05 (1.66)
PCo	1902.11 ± 75.33	489.72 ± 1.02 (25.75)	311.56 ± 21.30 (16.38)	247.66 ± 20.15	1.84 ± 0.12 (0.74)	4.15 ± 0.19 (1.67)	428.75 ± 24.05	116.5 ± 8.48 (27.17)	33.75 ± 1.06 (7.87)	67.29 ± 1.40	15.37 ± 0.10 (22.84)	1.69 ± 0.08 (2.51)
POu	2711.75 ± 43.13	1136.34 ± 43.88 (41.90)	1638.12 ± 20.50 (60.4)	814.57 ± 27.87	5.83 ± 0.46 (0.71)	6.20 ± 0.15 (0.76)	616.49 ± 7.42	452.22 ± 3.40 (73.35)	136.25 ± 0.53 (22.10)	50.58 ± 0.49	12.32 ± 0.11 (24.36)	0.81 ± 0.06 (1.60)
PSA	1747.48 ± 43.16	759.63 ± 29.69 (43.47)	64.06 ± 8.22 (3.66)	< LOD	< LOD	< LOD	368.41 ± 9.80	253.31 ± 12.64 (68.76)	30.87 ± 0.11 (8.37)	28.57 ± 0.25	3.45 ± 0.09 (12.08)	0.42 ± 0.02 (1.47)
PCP	2973.60 ± 27.71	611.84 ± 20.90 (20.57)	328.54 ± 38.90 (11.04)	47.61 ± 0.57	< LOD	< LOD	1170.68 ± 57.81	305.06 ± 0.97 (26.06)	8.00 ± 1.50 (0.68)	14.28 ± 0.12	7.40 ± 0.11 (51.82)	0.37 ± 0.02 (2.59)
PVi	1358.56 ± 60.18	692.97 ± 8.35 (51)	197.62 ± 22.98 (14.54)	3.85 ± 0.57	< LOD	< LOD	392.84 ± 0.99	188.34 ± 6.05 (47.94)	0.63 ± 0.01 (0.16)	50.37 ± 0.35	7.91 ± 0.06 (15.70)	1.28 ± 0.05 (2.54)

^aGastric digestion; ^bGastrointestinal digestion; The values between parentheses are the percentages of the values of bioaccessibility.

Determination of the total element concentrations in propolis

Nutrients such as vitamins and minerals even in small amounts are essential for the body. Ca, Fe, Mg, and Mn are important in the body development, and they are present in the teeth, bones, and some play a fundamental role as a structural part in many enzymes.¹⁵

The total content of Ca, Fe, Mg, and Mn in propolis samples is presented in Table II. The results obtained showed high concentrations of essential elements in the samples.

The levels of Ca obtained in digested propolis samples ranged from 423.4 – 4368.4 mg kg⁻¹. The samples from Belterra city presented different values of Ca. Lower levels of Ca were found in PBel 1 compared to those in PBel 2. The Ca concentrations found in propolis by Finger et al. (2014)¹⁶ were lower (1660 mg kg⁻¹) compared to the values obtained for most of the samples studied.

The levels of Fe (648.2 – 852.4 mg kg⁻¹) found in propolis from Argentina by Cantarelli et al. (2011),¹⁷ and level of Fe (49.17 mg kg⁻¹) found in propolis by Matuszewska et al (2021)¹⁸ were similar to the values obtained in this study.

Mg content in propolis ranged from 139.0 to 1170.7 mg kg⁻¹. PCP presented the highest levels of Mg in all samples. The lowest concentrations were found in PBel 1. This study obtained concentrations of Mg similar to the values (328.0 – 971 mg kg⁻¹) obtained by Bonvehí & Bermejo (2013)¹⁹ in propolis from Southern Spain.

The Mn concentrations found in samples ranged from 14.3 to 67.3 mg kg⁻¹. PCo presented the highest concentrations of Mn among the studied samples while PCP presented the lowest. Matuszewska et al. (2021)¹⁸ found the concentration of Mn (7.2 mg kg⁻¹) in propolis samples were lower compared to the values obtained for most of the samples studied.

Bioaccessibility of elements in propolis samples

The total element concentration is very important but it is not sufficient to evaluate the risk or benefit related to the intake of elements in food. Bioaccessibility tests can be estimated the fractions released into the gastrointestinal tract during digestion²⁰. The results of bioaccessibility in propolis are shown in Table I.

The Ca contents obtained in the bioaccessible fractions of propolis ranged between 17.6 and 63.4% for simulated gastric digestion, and between 3.1 and 73.1% for simulation gastrointestinal digestion, respectively. The highest level of bioaccessible Ca was found in PBel 1 (propolis of stingless bees). Machado, Silva, Fanchiotti & Costa (2001)²¹ reported that 30% of ingested Ca is absorbed by the body. Most samples presented bioaccessible levels during gastrointestinal simulation below that absorbed by the body.

The levels of Fe found in the gastric and gastrointestinal fractions ranged from 0.1 to 1.6% and 0.63 to 2.5%, respectively. PBel 1 presented the highest levels of bioaccessible Fe in simulated gastric digestion (1.6%) while PBel 2 showed the highest contents of bioaccessible Fe in simulated gastrointestinal digestion (2.5%). Higher levels of bioaccessible iron were obtained in the gastrointestinal digestion of most samples studied. All samples showed bioaccessible levels below the iron absorption level. According to Germano & Canniatti-Brazaca (2002)²² the iron absorption is 10%.

The levels bioaccessible of Mg found in the gastric and gastrointestinal simulations ranged from 26.1 to 86.3% and from 0.2 to 43.3%, respectively. PBel 1 showed the highest levels of bioaccessible Mg in the gastric simulation (86.3%), and PBel 2 presented the higher levels of bioaccessible Mg in the gastrointestinal simulation. All samples presented bioaccessible levels below the value of absorption of Mg in gastrointestinal simulation, which according to Monteiro & Vannucchi (2010)²³ is 45% of the total ingested.

The bioaccessible Mn contents found in the simulated gastric, and gastrointestinal digestions ranged from 12.1 to 83.1%, and from 1.5 to 8.5%, respectively. The highest concentrations of Mn available for absorption were found in PNT. According Fairweather-Tait (1992),²⁴ the daily absorption of Mn by the body is 3 – 4% of the total ingested. In the gastrointestinal simulation, most of the samples presented levels similar to the percentage of Mn absorbed per day.

The results shown that in most of the samples, the bioaccessible fraction was higher in the gastric phase than in the gastrointestinal phase (Figure 1).

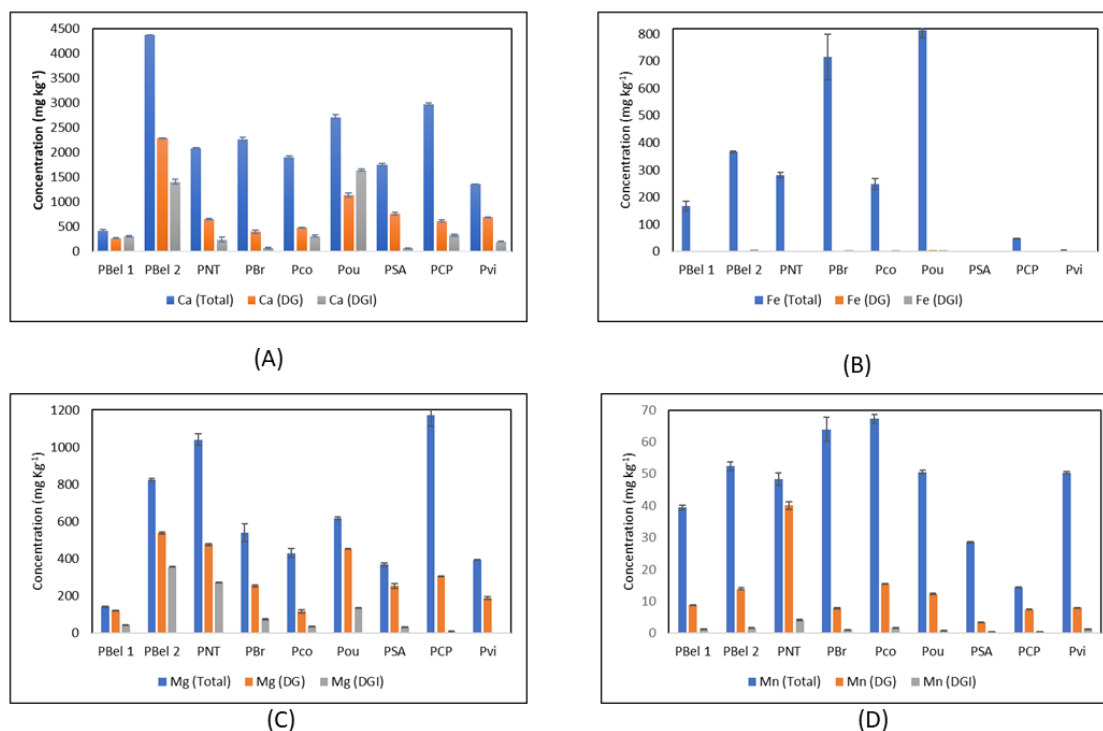


Figure 1. Total and bioaccessible contents (mg kg⁻¹) of calcium (A), iron (B), magnesium (C), and manganese (D) in propolis.

This was probably because of the gastric phase the low pH of the gastric fluid can increase the solubility of metal and precipitation can occur in the gastrointestinal phase.²⁰ Furthermore, the elevated presence of compounds such as phytates, oxalates, fibers, and other compounds can reduce the bioaccessibility of elements during gastrointestinal digestion because increases the likelihood of such compounds undergoing complexation reactions with elements present in the samples.^{12, 20}

CONCLUSIONS

The bioaccessibility levels of Ca, Fe, Mg, and Mn found in propolis by gastric and gastrointestinal simulation showed that these inorganic elements are available for absorption in the body. The study of the bioaccessibility of inorganic constituents in propolis is an important contribution to the development of studies on this product, in addition to achieving a better chemical characterization of *Apis mellifera* and *Melipona* propolis samples.

Conflicts of interest

The authors declare that they have no conflict of interest.

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REFERENCES

- (1) Mello, B. C. B. S.; Petrus, J. C. C.; Humbinger, M. D. Desempenho do processo de concentração de extratos de própolis por nanofiltração. *Food Sci. Technol.* **2010**, *30* (1), 166-172. <https://doi.org/10.1590/S0101-20612010000100025>
- (2) Almuhayawi, M. S. Propolis as a novel antibacterial agent. *Saudi J. Biol. Sci.* **2020**, *27*, 3079–3086. <https://doi.org/10.1016/j.sjbs.2020.09.016>
- (3) Anjum, S. I.; Ullah, A.; Khan, K. A.; Attaullah, M.; Khan, H.; Ali, H.; Bashir, M. A.; Tahir, M.; Ansari, M. J.; Ghramh, H. A.; Adgaba, N.; Dash, C. K. Composition and functional properties of propolis (bee glue): A review. *Saudi J. Biol. Sci.* **2019**, *26*, 1695 – 1703. <https://doi.org/10.1016/j.sjbs.2018.08.013>
- (4) Przybyłek, I.; Karpinski, T. M. Antibacterial Properties of Propolis. *Molecules* **2019**, *24*, 1-17. <https://doi.org/10.3390/molecules24112047>
- (5) Khouzam, R. B.; Pohl, P.; Lobinski, R. Bioaccessibility of essential elements from White cheese, bread, fruit and vegetables. *Talanta* **2011**, *86*, 425-428. <https://doi.org/10.1016/j.talanta.2011.08.049>
- (6) Peixoto, R. R. A.; Mazon, E. A. M.; Cadore, S. Estimation of the Bioaccessibility of Matallic Elements in chocolate drink poder using an in vitro digestion method and spectrometric techniques. *J. Braz. Chem. Soc.* **2013**, *24*, 884-890. <https://doi.org/10.5935/0103-5053.20130111>
- (7) Kulkarni, S. D.; Acharya, R.; Rajurkar, N. S.; Reddy, A. V. R. Evaluation of bioaccessibility of some essential elements from wheatgrass (*Triticum aestivum* L.) by in vitro digestion method. *Food Chem.* **2007**, *103*, 681–688. <https://doi.org/10.1016/j.foodchem.2006.07.057>
- (8) Dutta, R. K.; Maharia, R. S.; Acharya, R.; Reddy, A. V. R. Analysis of bioaccessible concentration of trace elements in plant based edible materials by INAA and ICPMS methods. *J. Radioanal. Nucl. Chem.* **2014**, *300*, 185-189. <https://doi.org/10.1007/s10967-014-3013-5>
- (9) Bossu, C. M.; Menezes, E. A.; Nogueira, A. R. A. Bioacessibilidade de zinco, cálcio e fósforo em extrato de soja e amostras de leite bovino, caprino e ovino. *Quim. Nova* **2020**, *43* (6), 718-722. <http://dx.doi.org/10.21577/0100-4042.20170544>
- (10) Jacobs, B.; Bonemann, D. H.; Pereira, C. C.; De Souza, A. O.; Luckow, A. C. B.; Lisboa, M. T.; Ribeiro, A. S.; Cadore, S.; Nunes, A. M. Avaliação da concentração total e da fração bioacessível de metais em amostras de casca de uva de cultivares tannat e cabernet sauvignon por MIP OES. *Quim. Nova* **2021**, *44* (5), 547-552. <http://dx.doi.org/10.21577/0100-4042.20170709>
- (11) Alves, B. S. F.; Nunes, P. O.; Dantas, K. G. F. Evaluation of in vitro Bioaccessibility of Copper, Iron and Manganese in Amazonian Fruits. *Rev. Virtual Quim.* **2017**, *9* (6), 2288-2298. <http://dx.doi.org/10.21577/1984-6835.20170136>
- (12) Mingroni, T. T.; Hamada, J.; Xavier, A. D. S.; Cavalcante, C.; Do Nascimento, A. N. In vitro Evaluation of Ca, Cu, and Mg Bioaccessibility in Fresh and Dried Fruits. *J. Braz. Chem. Soc.* **2019**, *30* (1), 108–115. <http://dx.doi.org/10.21577/0103-5053.20180159>
- (13) Stelmach, E.; Pohl, P.; Szymczycha-Madeja, A. Evaluation of the Bioaccessability of Ca, Fe, Mg and Mn in Ground Coffee Infusions by in vitro Gastrointestinal Digestion. *J. Braz. Chem. Soc.* **2014**, *25*, 1993-1999. <https://doi.org/10.5935/0103-5053.20140183>
- (14) Moreda-Piñero, J.; Moreda-Piñero, A.; Romaris-Horta, V.; Dominguez-Gonzales, R.; Alonso-Rodriguez, E.; López-Mahía, P.; Muniategui-Lorenzo, S.; Prada-Rodriguez, D.; Bermejo-Barrera, P. Trace metals in marine foodstuff: Bioavailability estimation and effect of major food constituents. *Food Chem.* **2012**, *134*, 339-345. <https://doi.org/10.1016/j.foodchem.2012.02.165>
- (15) Gharibzahedi, S. M. T.; Jafari, S. M. The importance of minerals in human nutrition: Bioavailability, food fortification, processing effects and nanoencapsulation. *Trends in Food Sci. & Technol.* **2017**, *62*, 119-132. <https://doi.org/10.1016/j.tifs.2017.02.017>
- (16) Finger, D.; Kelte Filho, I.; Torres, Y. R.; Quináia, S. P. Propolis as an indicator of environmental contamination by metals. *Bull. Environ. Contam. Toxicol.* **2014**, *92*, 259-264. <https://doi.org/10.1007/s00128-014-1199-4>

- (17) Cantarelli, M. A.; Camiña, J. M.; Pettenati, E. M.; Marchevsky, E. J.; Pellerano, R. G. Trace mineral content of Argentinean raw propolis by neutron activation analysis (NAA): Assessment of geographical provenance by chemometrics. *LWT-Food Science Technol.* **2011**, *44*, 256-260. <https://doi.org/10.1016/j.lwt.2010.06.031>
- (18) Matuszewska, E.; Klupczynska, A.; Maciołek, K.; Kokot, Z. J.; Matysiak, J. Multielemental Analysis of Bee Pollen, Propolis, and Royal Jelly Collected in West-Central Poland. *Molecules* **2021**, *26*, 1-18. <https://doi.org/10.3390/molecules26092415>
- (19) Bonvehí, J. S.; Bermejo, F. J. O. Element content of propolis collected from different areas of South Spain. *Environ. Monit. Assess.* **2013**, *185*, 6035-6047. <https://doi.org/10.1007/s10661-012-3004-3>
- (20) Souza, L. A.; Souza, T. L.; Santana, F. B.; Araujo, R. G. O.; Teixeira, L. S. G.; Santos, D. C. M. B.; Korn, M. G. Determination and in vitro bioaccessibility evaluation of Ca, Cu, Fe, K, Mg, Mn, Mo, Na, P and Zn in linseed and sesame. *Microchem. J.* **2018**, *137*, 8-14. <https://doi.org/10.1016/j.microc.2017.09.010>
- (21) Machado, D. F.; Silva, R.; Fanchiotti, F. E.; Costa, N. M. B. Probióticos, prebióticos e simbióticos e seus efeitos na biodisponibilidade de cálcio. *Nutrire Rev. Soc. Bras. Aliment. Nutr.* **2001**, *22*, 73-83.
- (22) Germano, R. M. A.; Canniatti-Brazaca, S. G. Importância do ferro em nutrição humana. *Rev. Soc. Bras. Alim.* **2002**, *24*, 85-104.
- (23) Monteiro, T. H.; Vannuchi, H. Funções plenamente reconhecidas de nutrientes: Magnésio, Vol16. International Life Sciences Institute (ILSI) Brasil, São Paulo, 2010.
- (24) Fairweather-Tati, S. J. Bioavailability of trace elements. *Food Chem.* **1992**, *43*, 213-217. <https://doi.org/10.1079/NRR19960016>