In vitro digestion assays using dynamic models for essential minerals in Brazilian goat cheeses

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Abstract

Goat cheeses have important nutritional properties, with an emphasis on proteins, lipids (high digestibility) and essential minerals. This study analyzes the bioavailability of Ca, Mg and Zn in Brazilian cheeses using an *in vitro* dynamic digestion method. Two self-produced fresh cheeses, cow and goat *Minas frescal* cheese, and two commercial matured goat cheeses, *Blue* and *Pyramid*, were analyzed. Brazilian goat cheeses are potential sources of essential minerals (Ca, Mg and Zn). Variations of 103 - 598 mg/100 g for Ca, 13.62 - 41.64 mg/100 g for Mg and 9.79 - 13.23 mg/100 g for Zn were observed in the studied samples. The pH concentration, enzyme performance and protein and lipid content of Brazilian cheeses affected the solubility of essential minerals in the intestinal fraction. The percentages of minerals found in the permeate stream, equivalent to absorption of Ca and Zn, were lower in *Minas frescal* goat cheese than *Minas frescal* cow cheese, whereas that of Mg was higher. *Pyramid* and *Minas frescal* goat cheeses had the higher values of Mg and Zn bioavailability, respectively. This study supports, for the first time, the usefulness of the dynamic simulation of the human gastrointestinal tract for the study of mineral bioavailability in cheeses.

Key-words: food analysis; Brazilian cheeses; mineral bioaccessibility; dynamic model.

1. Introduction

The demand for goat cheese is related to its high digestibility and low-calorie supply when compared to cow's cheese, which is less digestible, and rich in cholesterol and other types of lipids (Haenlein & Anke, 2011). Goat cheese consumption is also associated with health maintenance and chronic disease prevention (Bergillos-Meca et al., 2015; Moreira et al., 2019). Brazil is the main producer of goat milk in the South American continent, with a reported 25.3 million L/year produced (IBGE, 2018) and with an expected increase of 50% by 2030 (Cabral et al., 2020; Pulina et al., 2018). Different types of goat cheeses are currently available for consumption in Brazil, and have a high commercial value (Rohenkohl et al., 2011).

Goat cheeses have high concentrations of proteins, calcium and other minerals, and significantly contribute to the recommended daily intake of these elements (Cabral et al., 2008; Khouzam, Pohl & Lobinski, 2011). The main minerals reported in the composition of goat cheeses are calcium (Ca), magnesium (Mg) and zinc (Zn) (Moreira et al., 2019). Minerals are essential for the proper functioning of an organism, having important organic functions (Cámara et al., 2005); and therefore, deficiencies in some essential minerals are still a public health concern.

The use of gastrointestinal simulators to reproduce the *in vitro* behavior of nutrients through the gastrointestinal tract is relatively recent, however, they can approximately predict the mineral's transit through the digestive tract (Godoy et al., 2020). There are some important differences between static and dynamic simulators. Static gastrointestinal simulators generally use a single set of initial conditions for each phase of digestion and do not consider the evolution of parameters over time, nor the dynamic conditions that food experiences in the digestive system (Dupont & Mackie, 2015; Thuenemann, 2015). However, as digestion is a dynamic process, factors such as

pH changes, peristaltic movements, gastric emptying, continuous changes and secretion flow rates make dynamic models more similar to the *in vivo* conditions of the human digestive system (Sensoy, 2021). Consequently, research using different dynamic models for *in vitro* digestion have been developed in the last decade; to better mimic the physiological conditions of the human digestive tract (González et al., 2019; Hur et al., 2011; Marzorati et al., 2013; Rivas-Montoya et al., 2016; Verhoeckx et al., 2015).

The use of dynamic models has advanced the understanding of several important aspects, such as the metabolism of nutrients, the behavior of minerals in the digestion process and the effect of interactions between the food matrix and intestinal microbiota; achieving important results in human health and nutrition research (Terpend et al., 2013).

According to Godoy et al. (2020), computer-controlled dynamic models are capable of efficiently reproducing the physiological conditions of the human gastrointestinal tract, and may be used to study the bioaccessibility and bioavailability of minerals. Therefore, the objective of this work was to analyze the mineral behavior of different Brazilian goat cheeses along the digestive tract, through *in vitro* experiments utilizing a membrane bioreactor system that mimicks the human gastrointestinal tract. A cow cheese was also analyzed for comparative purposes. The results obtained may serve as a complementary and/or prior study to more complex and expensive human interaction studies. To the best of the authors' knowledge, this is the first study that uses a dynamic gastrointestinal simulator to analyze the mineral bioaccessibility and bioavailability of cheeses throughout a dynamic digestion process.

2. Material and Methods

2.1. Chemical and reagents

The salivary simulated liquid (SSF) and the gastric simulated liquid (GSF), corresponding to the oral and gastric phases of the digestion, respectively, were prepared using the following reagents: potassium chloride (KCl, 99.5% purity) supplied by Merck, sodium chloride (NaCl, 99% purity), sodium hydrogen carbonate anhydrous ($NaHCO_3$, 99.5% purity) from Sigma-Aldrich, potassium dihydrogen phosphate anhydrous (KH_2PO_4, 99% purity), ammonium carbonate ($(NH_4)_2CO_3$, 30.0% purity) supplied by Merck, magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$, 98% purity), hydrochloric acid (HCl, 37% purity), and calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$, 99% purity) purchased from Panreac. The SSF and GSF compositions were obtained according to Brodkorb et al. (2019).

Different enzymes and salts were needed to reproduce the different phases of human digestion (oral phase, gastric phase, duodenal phase and intestinal absorption phase). The enzymes and salts used in this study were supplied by Sigma-Aldrich and were as follows: alpha-amylase from human saliva (Lot: SLCD1111), pepsin extracted from porcine gastric mucosa (Lot: BCCC1803), lipase obtained from porcine pancreas (Lot: SLBH6427V), pancreatin extracted from porcine pancreas (Lot: SLBT4919), trypsin obtained from bovine pancreas (Lot: SLCB2341), bile salts supplied by Sigma-Aldrich. The use of phospholipids (Lipoid P45) purchased from Lipoid GmbH, and ultrapure water (18.2 MΩcm-1, Milli-Q Plus system, Millipore Bedford, MA, USA) was also necessary.

2.2. Samples

Samples of goat and cow *Minas frescal* type cheeses were obtained from a pilot plant (FEA-UNICAMP, Brazil). Cheese was produced by processing goat and cow milk samples (*in natura* form) that were acquired directly from two producers located in Rio Claro-SP and Amparo-SP, Brazil, on three different days. The milk samples were heat treated by slow pasteurization (65 °C/30 min). After this process, the milk samples were cooled to 4 °C and stored in a cold chamber (4 ± 1 °C) until cheese processing. The efficiency of pasteurization was evaluated by the alkaline phosphatase (AOAC, 2006 - Method 979.13) and peroxidase enzyme activity (Lanara, 1981).

The goat and cow *Minas frescal* cheeses were prepared according to Diamantino et al. (2014), with modifications. The milk was heated to 35 °C, with 250 ppm of calcium chloride 50%, previously activated (30 °C/8 h) lactic culture (1.5%, v/v) consisting of *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* (R704 - Chr. Hansen, Hoersholm, Denmark), and a coagulant (CHY-MAX Powder Extra NB, Chr. Hansen, Hoersholm, Denmark) in sufficient quantity to coagulate the milk in 35 min. The gel was cut, with the aid of horizontal and vertical liras, into cubes of 1.5 to 2.0 cm. After resting for 5 min, slow stirring was performed for 30 min. The curd was then kept at rest for 10 min and partial draining of the curd was started. A saline solution (1.3% NaCl in relation to the volume of milk) at 35 °C was added to the mass, followed by stirring and another rest period (10 min). The curd was placed in plastic molds and successive turnings were performed after 15, 30 and 45 min. The cheeses were fermented for 4 h at room temperature and stored in a cold chamber (4 ± 1 °C). After 24 h of refrigerated storage, the cheeses were removed from the plastic molds and their pH was measured to assure that the fermentation was adequate.

In addition to the fresh self-made cheeses (*Minas frescal*), commercial goat cheeses, *Blue* goat cheese and *Pyramid* goat cheese, were also purchased directly from a producer in Amparo-SP (Brazil), from three distinct batches. The *Blue* goat cheese is a Brazilian cured cheese inspired by the *Blue* Stilton English cheese, so called since it has veins of the fungus *Penicillium roqueforti*. The *Pyramid* goat cheese is lactic-fermented and takes approximately 24 days to mature with a charcoal coating, until its flowery bark is completed with white molds of the *Penicillium candidum* type.

The fresh (*Minas frescal* goat and cow cheese) and commercial (*Blue* goat cheese and *Pyramid* goat cheese) samples were freeze-dried at -40 °C for 48 h (lyophilizer model LS3000, Terroni, Brazil), ground (mill model A11 Basic, IKA, China), vacuum packed and transported to the Department of Physiology and Biochemistry of Animal Nutrition, CSIC (Granada, Spain), where they were kept under refrigeration (4 ± 1 °C) until laboratory analyses were performed.

2.3. Mineral and nutrient composition analysis of cheese

Moisture (method 934.01) and total ash content (method 942.05) were determined using official methods (AOAC, 2000). Fat content was extracted with chloroform:methanol (2:1) and quantified by Soxhlet (AOAC, 2000). Total nitrogen was analyzed according to the Dumas procedure using LECO Truspec CN equipment (LECO Corporation, St. Joseph, MI, USA). Protein content was calculated using the factor of 6.38 (for milk and dairy products). All analyses were performed in triplicate.

Aliquots of ground freeze-dried cheeses and fractions obtained during the *in vitro* digestion process were wet mineralized by the addition of concentrated HNO_3:$HClO_4$ (1:4) and heating to high temperatures (180 - 220 °C) (Block Digestor Selecta S-509; J. P. Selecta, Barcelona, Spain); and analysis of Ca, Mg and Zn were carried out by flame-

125 atomic-absorption spectroscopy (FAAS) in a Perkin-Elmer Analyst 700
126 Spectrophotometer (Norwalk, CT, USA). Blank samples were included in order to
127 decrease or eliminate the interferences between different samples and chemicals used.
128 Standard solutions were prepared from Tritisol (Merck, Darmstadt, Germany) and
129 lanthanum chloride (0.3%) was added to the samples and standards for Ca and Mg
130 measurements, to avoid interferences. Certified external standards (European
131 Commission, Reference Materials Unit, Geel, Belgium) were used to test the accuracy of
132 the method: skimmed milk powder (ERM-BD150) for Ca and Mg and lyophilized brown
133 bread (BCR 191) for Zn. The measured values were always within the certified ranges.
134

135 **2.4. Use of the dynamic model to estimate the bioaccessibility of essential minerals**

136 The Gastrointestinal Tract Simulating Membrane Bioreactor (GITSMB)
137 (hereinafter called SimuGIT) (Rivas-Montoya et al., 2016), was used in the present assay.
138 It consists of a continuous stirred-tank reactor (CSTR) connected in series with a
139 continuous plug-flow tubular reactor (PFTR) and equipped with a tubular ceramic
140 microfiltration (MF) membrane module (Figure 1).

141 Gastric digestion in the stomach is simulated with a CSTR, a universal benchtop
142 controller for stirred and rocking motion systems supplied by Braun Biotech International
143 (Biostat B model). It consists of an autoclavable borosilicate glass culture vessel (2 L)
144 equipped with a propeller agitator (180 W, Rushton model) and a proportional integral
145 derivative (PID) unit control system for temperature, pressure and pH. In the CSTR the
146 stirring rate was 100 rpm. The CSTR is heated (or cooled if necessary) by a heating jacket
147 containing a fluid (water) connected to a thermostatic bath. The temperature is measured
148 by a digital Pt-100 sensor with an accuracy of ± 0.1 °C. The CSTR is also connected to
149 automated peristaltic pumps (Eyela, model MP-3) to gradually feed different

150 physiological fluids, such as HCl and/or $NaHCO_3$ (1 M), during the GIT simulation.
151 These reagents are used in the pH control loop, in which a pH electrode is used (Hamilton,
152 model Easyferm Plus K8).

153 In Figure 1, the diagram of the dynamic digestion model used in the study is
154 shown. It is worth noting that the samples of fresh and matured cheeses were only
155 introduced to the CSTR after the simulation of the oral phase. Therefore, during the oral
156 phase, the fresh and matured cheeses were mixed with simulated salivary fluids and the
157 corresponding enzymes for 2 minutes, a 10 mL aliquot was removed for mineralization
158 with subsequent analysis of essential minerals, and the entire resulting cake was
159 introduced into the CSTR to simulate the stomach gastric phase.

160

161 **Insert Figure 1**.

162

163 The CSTR works with the impulsion and return pumps so that by modifying the
164 flow rates with the PID control system, it is possible to regulate the pressure inside the
165 hydraulic circuits, as well as the filtration rate of the product. The operating pressure can
166 be adjusted accurately ($P_{setpoint}$ ± 10 mmHg) with a spring-loaded pressure-regulating
167 valve (SS-R4512MM-SP model, Swagelok) and monitored by a digital pressure gauge
168 (Endress+Hauser, model Ceraphant PTC31).

169 The PFTR consists of a stainless-steel cylindrical tube (supplied by
170 Prozesstechnik GmbH, Basel, Switzerland) equipped with a single channel
171 microfiltration ceramic membrane supplied by Atech Innovations GmbH. The MF
172 membrane used is constructed of an α-Al_2O_3 active surface with a mean pore diameter
173 equal to 0.05 µm, and the dimensions are 1000 mm length, 6 mm duct diameter, and 2 ±

0.5 mm thickness. The permeate was registered by a precision electronic mass balance with USB connectivity (Sartorius, model Quintix 5102, accuracy equal to 10 mg).

2.5. SimuGIT Conditions

Oral Phase and Gastric Phase

The oral phase was carried out by mixing 25 g of each type of cheese with 17.5 mL of SSF, 1.25 mL of alpha-amylase solution (300 - 1500 U/mg protein), 125 µL of $CaCl_2 \cdot 2H_2O$ of 0.3 M concentration and 6.125 mL of ultrapure water. The ratio of final saliva fluid to food preparation was 1:1. The oral phase was stirred for 2 min and the pH was adjusted to 7.0. After the oral phase, a sample was taken (10 mL) for further analysis and the rest proceeded to the next phase.

The trial was carried out at a temperature of 37.5 ± 1 °C, for the duration of the process, using a bath that keeps the reactor jacket warm. The reactor agitation speed was set at 100 rpm to reproduce stomach motility.

The gastric phase started with the introduction of 800 mL of GSF into the reactor and dropping the pH to 3.0, as an empty stomach was simulated before adding the food. Once the GSF was at 37.5 ± 1 °C, 40 g of the food mixture and SSF were added to the reactor tank. Sequentially, 10 mL solution of pepsin (599 U/mg) and 50 mg of phospholipid (Lipoid P45) were added, and the pH was adjusted to 3.0 through controlled dosing of 6 M HCl. The gastric phase developed in 30 min, with a 10 mL aliquot being removed from the CSTR. This aliquot was mineralized with subsequent assessment of essential mineral concentration by FAAS.

Duodenum Phase

The pH of the GSF was raised to 6.5 with the introduction of 1 M $NaHCO_3$ at a rate of 4.5 mL/min. This pH simulates the action of pancreatic juices on the food being digested. Then, 10 mL of pancreatic lipase solution (100 - 400 U/mg protein), bile salts (for a final concentration of 5 mM), 10 mL of pancreatin solution (10%) and 1 mL of trypsin solution (≥ 7500 BAEE U/mg solid, 50 mg/test) were added. For all samples, 10 mL aliquots were collected after 10 min at the end of the duodenal phase.

Intestinal Absorption Phase

The simulation of intestinal absorption was performed by pumping the fluids from the CSTR into the PFTR, where filtration through the 0.05 µm membrane occurs; based on the tests performed previously (Abad et al., 2019; González et al., 2019). The data of the trials were recorded by the control system connected to the computer, allowing for the programming, control and supervision of all the elements of the simulator.

The overpressure limit of the system was set at 50 mmHg. Once the circuit was primed and the fluids began to permeate, the intestinal absorption phase was considered to have begun, and lasted for 180 min. Samples of 10 mL were taken at 30, 60, 90, 120, 150 and 180 min from both, permeate and retentate streams.

2.6. Data and statistical analysis

The levels of Ca, Mg and Zn were determined in quadruplicate from the aliquots after carrying out the oral, gastric, duodenal and intestinal absorption phases by FAAS described in item 2.3. The equations referring to the bioaccessibility, intestinal absorption and bioavailability percentages were calculated as follows:

$$Bioaccessibility\ (\%) = \frac{Soluble\ mineral\ in\ X\ phase}{Total\ mineral\ in\ cheese} \cdot 100 \quad \text{Ec. [1]}$$

$$Absorption\ (\%) = \frac{Absorbed\ mineral\ through\ the\ membrane}{Soluble\ mineral\ in\ gastric\ phase} \cdot 100 \text{ Ec. [2]}$$

$$Bioavailability\ (\%) = \frac{Absorbed\ mineral\ through\ the\ membrane}{Total\ mineral\ in\ cheese} \cdot 100 \text{ Ec. [3]}$$

The results obtained were evaluated by Analysis of Variance (ANOVA), Tukey's test (95% confidence) and coefficient of variation (CV); using an extension of Microsoft Office Excel (version 2013) and Statgraphics Centurion XVI.II (Statistical Graphics Corporation, USA).

3. Results and discussion

3.1. Chemical composition

For the nutritional composition of the cheeses study, the evaluation of the major components was carried out. The moisture, ash, lipid and protein content found in *Minas frescal* goat cheese (mean values ± SE) were 55.6 ± 0.1%, 3.84 ± 0.1%, 19.7 ± 0.05% and 19.7 ± 0.04%, respectively. While the average contents of the same components evaluated in the *Minas frescal* cow, *Blue* goat cheese and *Pyramid* goat cheese were of 56.6 ± 0.01%, 3.78 ± 0.03%, 21.1 ± 0.03% and 18.3 ± 0.02%; 55.9 ± 0.03%, 2.58 ± 0.03%, 19.6 ± 0.1% and 21.0 ± 0.04%; 55.6 ± 0.03%, 1.83 ± 0.03%, 22.9 ± 0.1% and 19.5 ± 0.1%, respectively. Statistical differences ($P < 0.05$) were observed between cheeses for all analyzed chemical parameters.

All cheeses analyzed in this study had a moisture content above 55%, and therefore, they may be classified as cheeses with high moisture content; considering the

guidelines of decrees n° 146/96 and n° 352/97 that regulate the technical terms of identity and quality of Brazilian cheeses (Brazil, 1996; Brazil, 1997, respectively).

It is noteworthy that the *Minas frescal* goat and cow cheeses had the highest percentage of ash when compared to matured cheeses (*Blue* goat cheese and *Pyramid* goat cheese). Da Silva et al. (2017), analyzed the behavior of *Minas frescal* cheese under refrigerated storage conditions for 28 days and reported mean ash values of 3.46 ± 0.5%, close to the range reported in the present study. As the fat percentages reported by Brazilian cheeses were less than 24.9%, they were classified as low-fat cheeses (10 - 24.9%) (Brazil, 1996). Similar results were reported by Marques et al. (2020), who found maximum fat percentages of 20.6% in fresh cheese samples obtained from pasteurized and unpasteurized milk.

All protein values shown by Brazilian cheeses in the current study are higher than those observed by Resende et al. (2020), who reported mean protein values of 15.9 ± 1.5% in samples of artisanal *Minas frescal* cheeses. Among the solid components of milk, proteins are important, both from a nutritional and technological point of view. Caseins are responsible for the structure of the cheese and for capturing other constituents; which makes the casein-fat relationship very important for the sensory characteristics of the product and for controlling losses through whey (Fernandes et al., 2013). Resende et al. (2020) reported that the cheese making steps, including the type of salting, the maturation time and the amount of rennet added to the dough, can cause greater proteolysis, resulting in a reduction of protein content.

Although differences in the composition of *Minas frescal* cheeses were observed, similar results for moisture content (51.1 to 68%), ash (2.6 to 3.4%), lipids (21.0 to 34.9%) and proteins (13.5 to 18.6%) have already been reported in other studies (Cunha, Viotto & Viotto, 2006; Da Silva et al., 2017; Fritzen-Freire et al., 2010; De Jesus et al.,

2020; Resende et al., 2020; Ribeiro, Simões & Jurkiewicz, 2009). Santos et al. (2017) analyzed several traditional *Minas frescal* cheeses and reported average moisture values ranging from 39.0 to 54.1%; fats from 23.0 to 35.5% and proteins from 20.3 to 29.3%; values similar to those presented in this study. Therefore, the results of the chemical composition of Brazilian cheeses described in this study are all within the nutritional values/standards established by Brazilian legislation, and are consistent with other works published in the literature.

The mineral content (mg/100 g) and the bioaccessibility percentages estimated by the solubility of essential minerals (Ca, Mg and Zn) of Brazilian cheeses, after *in vitro* digestion simulation of the different phases of the dynamic model, are depicted in Table 1.

As expected, Ca was the predominant mineral, followed by Mg and Zn. There were some variations in content however, ranging from 103 – 598 mg/100 g for Ca, 13.62 - 41.64 mg/100 g for Mg and 9.79 - 13.23 mg/100 g of Zn in the studied samples. Thus, Brazilian goat cheeses are potential sources of essential minerals (Ca, Mg and Zn) for the human diet. In addition, two types of goat cheese studied had higher Ca contents when compared to cow's cheese.

Insert Table 1.

3.2. Mineral bioaccessibility

The essential minerals (Ca, Mg and Zn) are mostly absorbed in the upper part of the intestine (duodenum), although partial absorption in the colon, especially at lower pH, cannot be discounted (Bohn et al., 2018; Scholz-Ahrens et al., 2007). Thus, the assessment of solubility percentages in the oral, gastric, and intestinal phases are

important for understanding the process/mechanism of digestion of these elements in the studied matrices. It is known if solubilization is a prerequisite for minerals to be available; this depends on the presence of complexing compounds, concentration of the mineral and the pH (Bohn et al., 2018). Therefore, the solubility percentages obtained after the simulation of the digestion phases, called "bioaccessible fraction", may indicate the possibility of absorption for these nutrients at the different stages of the gastrointestinal digestion (Ec. 1). In Table 1 shows that *Minas frescal* goat cheese had the highest soluble percentages for Zn in the oral, gastric and intestinal phases, and *Pyramid* goat cheese had the highest percentages of solubility for the minerals Ca and Mg in these three stages of digestion when compared as at other cheese samples. Significant differences among cheeses were observed in mineral solubility at all the digestion steps ($P < 0.05$).

Unlike organic micronutrients (vitamins) and phytochemicals, minerals do not undergo significant metabolism during the gastrointestinal digestion phases (oral, gastric and intestinal). However, oxidation/reduction may occur, influencing the solubility of these elements.

Several factors may have affected the solubility percentages of Brazilian cheese samples during the three phases (oral, gastric and intestinal) of the dynamic digestion process. Among them, we can mention pH concentration, enzyme performance and even the protein and lipid content of the analyzed samples. In the oral phase, a higher pH generally limits the availability of divalent minerals, since solubility decreases with a higher pH (> 7), forming insoluble oxides/hydroxides (Bohn et al., 2018). Bohn et al. (2018), also reported that changes in pH can help to release compounds or elements from the matrix through hydrolysis reactions, while different concentrations of enzymes help in the degradation of the matrix and in the release of essential compounds or elements. On the other hand, Wang et al. (2019) reported that at the beginning of the gastric phase

pepsin has a low performance related to high pH (> 6), while its activity gradually increases as the pH of the samples decreases, reaching maximum values of enzymatic performance at the end of gastric digestion. The protein and lipid content of cheese samples may also have affected the solubility percentages of essential minerals. For Ca, the literature reports that proteins may increase the absorption of this mineral. According to Lorieau et al. (2018), protein intake is known to stimulate the release of acid in the stomach and acidify the gastrointestinal contents, which in turn increases the absorption of Ca. Lorieau et al. (2018), also reported that the fatty acids released in the gastrointestinal digestion stages can acidify the stomach, contributing to increased solubility percentages for essential minerals. Therefore, this behavior may have been more accentuated in goat cheese samples, since these samples have in their composition greater amounts of short and medium chain fatty acids when compared to cheeses made with cow milk (Haenlein & Anke, 2011). These factors may explain the relatively low solubility of some essential elements in the intestinal fraction, despite this being the main absorption site for these nutrients.

3.3. Mineral bioavailability

The percentages of Ca, Mg and Zn absorbed from the bioaccessible fraction by the *in vitro* dynamic digestion model for Brazilian cheese samples are shown in Table 2 (Ec. 2). Values were calculated that considered the amount of soluble mineral absorbed by diffusion through the membrane during the intestinal absorption, for each of the analyzed samples. As expected, we found that the percentages of availability of essential minerals (Ca, Mg and Zn) in Brazilian cheeses increased with digestion time, with maximum values reached at 180 min.

When comparing only the samples of *Minas frescal* goat and cow cheeses, we observed that after 180 min of digestion, the absorption percentages for Ca and Zn in *Minas frescal* goat cheese were lower than those reported by *Minas frescal* cow cheese. For Ca, we observed that *Minas frescal* cow cheese had a percentage of 16.5 ± 0.2%, double of the *Minas frescal* goat cheese, with 8.30 ± 0.02%. Although goat milk and its derivatives have an average composition similar to cow's milk and dairy products, in terms of protein, fat and lactose, differences in amino acid composition, the secondary structures of milk proteins and smaller fat globules (related to the presence of short and medium chain fatty acids) may have affected the absorption of essential minerals in these cheese samples (Clark & Mora García, 2017; Haenlein & Anke, 2011; Hodgkinson et al., 2018; Khouzam, Pohl & Lobinski, 2011).

Insert Table 2.

Although the protein content of goat and cow's milk are similar, differences in the quality and structure of caseins may have affected the bioavailability percentages of essential minerals. In cheese technology, the casein found in cow milk is responsible for forming a firmer clot when compared to goat milk; therefore, during the gastrointestinal digestion phases, the cow cheese, being more rigid, was digested more slowly by the enzymes; and this rigidity may have affected the bioavailability percentages of essential minerals in the *Minas frescal* cow cheese sample (Walstra, Woulter & Geurts, 2006).

Although several researchers have reported that goat milk and its dairy products have smaller fat globules when compared to cow's milk and dairy products, which is related to the presence of short and medium chain fatty acids and allows for better intestinal absorption of its nutrients (Clark & Mora García, 2017; Hodgkinson et al.,

2018); the lipid content and structure presented by the *Minas frescal* goat and cow cheese samples did not seem to affect the results of the dynamic model. On the other hand, *Minas frescal* cow cheese had the highest fat content, as well as the highest absorption percentage for Ca and Zn, when compared to Minas frescal goat cheese. This is likely due to the fact that complete digestion of the samples was achieved due to; peristaltic movements, temperature, pH control, the correct enzymatic balance, which simulated the intestinal conditions as close as possible to those found in humans.

Although *Minas frescal* goat cheese has a significantly higher calcium content when compared to *Minas frescal* cow cheese, the absorption percentage at the end of dynamic digestion was significantly lower (Table 2; Ec. 2). During the digestion simulation process, the calcium in *Minas frescal* goat cheese may have joined with other compounds, mainly milk proteins (caseins), forming more complex molecules capable of reducing the fractions available for absorption; since the formation of these molecules made it impossible for calcium to pass through the microfiltration membrane.

Comparing among goat cheese samples, we observed that *Minas frescal* goat cheese presented the highest percentages of absorption for all minerals analyzed in this study, when compared to mature cheese samples (*Blue* goat cheese and *Pyramid* goat cheese). Therefore, the absorption percentages of essential minerals were not influenced by changes occurring during maturation (relative humidity and storage temperature, as well as their fluctuations) of the samples. According to Cichosz, Aljewicz & Nalepa (2014), cured cheeses have good nutrient availability, lower water activity and high fat content (as observed in this study), which in combination with protein density makes the matrix more solid. This, in turn, provides a better buffering capacity and lower oxygen content, which can provide greater protection for microbial cells during the passage from

396 the stomach to the intestine. Thus, the ripening conditions do not positively affect the
397 bioavailability percentages in the matured cheese samples evaluated in this study.

398 Figure 2 shows the percentage values of bioavailability (Ec. 3) for Ca, Mg and Zn
399 after *in vitro* digestion, estimated by the dynamic model for Brazilian cheese samples.
400 The bioavailability of the minerals was calculated considering the percentage of mineral
401 absorbed, in relation to the initial amount of each of the minerals in the sample and taking
402 into account differences in solubility during the digestive process.

403

404 **Insert Figure 2.**

405

406 At the beginning of the intestinal absorption phase, it was noted that small
407 percentages of Ca (Figure 2A), Mg (Figure 2B) and Zn (Figure 2C) from the initial total
408 content were available and passed through the microfiltration membrane (0.05 µm).
409 Statistical differences ($P < 0.05$) were observed between the results obtained for mineral
410 concentrations in the samples, and during each digestion time; for all elements analyzed
411 in this study. However, to avoid overlaps, only statistical differences obtained for the 180
412 min sampling time were represented in Figure 2. As expected, during the intestinal phase,
413 there is a gradual increase in the absorption percentages for all analyzed minerals, with
414 maximum values obtained at 180 min. Thus, the highest mineral absorption percentage
415 in *Minas frescal* cow cheese (9.85 ± 0.1%) was for Ca, *Pyramid* goat cheese (32.8 ± 0.1%)
416 was for Mg and *Minas frescal* goat (1.08 ± 0.002%) was for Zn.

417 The stomach and duodenum conditions were simulated by a CSTR (Figure 1),
418 where the variables of agitation, temperature, reactor level, kinetic pH adjustment, with
419 addition of HCl simulating gastric juices, subsequent addition of $NaHCO_3$, simulating
420 pancreatic juices and alkaline secretion as well as the dosage of pepsin, pancreatic lipase

and bile juices were precisely controlled. Controlling these variables was essential so that the stomach and duodenum conditions were correctly used by the human gastrointestinal simulator. The temperature was monitored throughout the duration of the tests, with no significant changes being observed, remaining constant around 37.5 °C. On the other hand, the PID control system (Figure 1), through the action of the peristaltic impulse and return pumps, maintained the system pressure at 50 mmHg during the digestion tests. According to Kim et al. (2005) and Hasler (2006), a pressure of 50 mmHg reliably simulates the actual physiological pressure of the gut within the human body. Correct control of these variables indicates that the dynamic gastrointestinal simulator worked correctly, as the temperature and pressure variables remained constant during digestion for all cheese samples analyzed in this study. The control of all these variables was observed in recent research that also used dynamic models for in vitro digestion in several matrices (Alminger et al., 2014; González et al., 2019; Verhoeckx et al, 2015).

4. Conclusions

The studied cheeses presented a nutritional value within the composition established by Brazilian legislation, being excellent sources of essential minerals.

Analyzing the solubility percentages in the three stages of gastrointestinal digestion (oral, gastric and intestinal), we observed that the *Pyramid* goat cheese presented the highest percentages of solubility for Ca and Mg; while for Zn the *Minas frescal* goat cheese stood out among the other cheese samples.

At 180 min of digestion, the absorption percentages of Ca and Zn presented by *Minas frescal* goat cheese were lower than those reported for *Minas frescal* cow cheese. On the other hand, the absorption percentages of essential minerals were not influenced

by the changes that occurred during the maturation of the samples, since fresh cheeses had higher bioaccessibility values than matured cheeses.

The present study shows, for the first time, the usefulness of the dynamic simulation of the human gastrointestinal tract for the study of mineral bioaccessibility and bioavailability in cheeses.

Author Contribution

José Teixeira: Methodology, Formal analysis, Writing - original draft. Juliana Pallone: Resources, Conceptualization, Writing - original draft, Supervision. Isabel Seiquer: Methodology, Formal Analysis, Investigation, Writing - original draft. José Morales-González: Methodology, Formal Analysis. José Vellido-Pérez: Methodology, Formal Analysis. Antonio Martinez-Ferez: Conceptualization, Investigation, Writing - original draft, Supervision.

Funding

The authors would like to thank the São Paulo Research Foundation (FAPESP) for the scholarship grant of PhD student José Teixeira, and for the research internship abroad (regular scholarship n° 2018/08864-8 and BEPE - n° 2019/13600-2). Juliana Azevedo Lima Pallone would like to thank the financial support of Brazil (FAPESP 2018/09759-3). The Coordination for the Improvement of Higher Education Personnel (CAPES) (Financial Code 001).

Declarations

Informed Consent: Not applicable.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Professor Dra. Mirna Lucia Gigante and the PhD student Debora Parra Batista for collaboration in the processing of *Minas frescal* cheeses in the pilot plant.

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634 **Figure captions**

635 **Figure 1.** Flow diagram of the dynamic *in vitro* Gastrointestinal Tract Simulating
636 Membrane Bioreactor (SimuGIT).

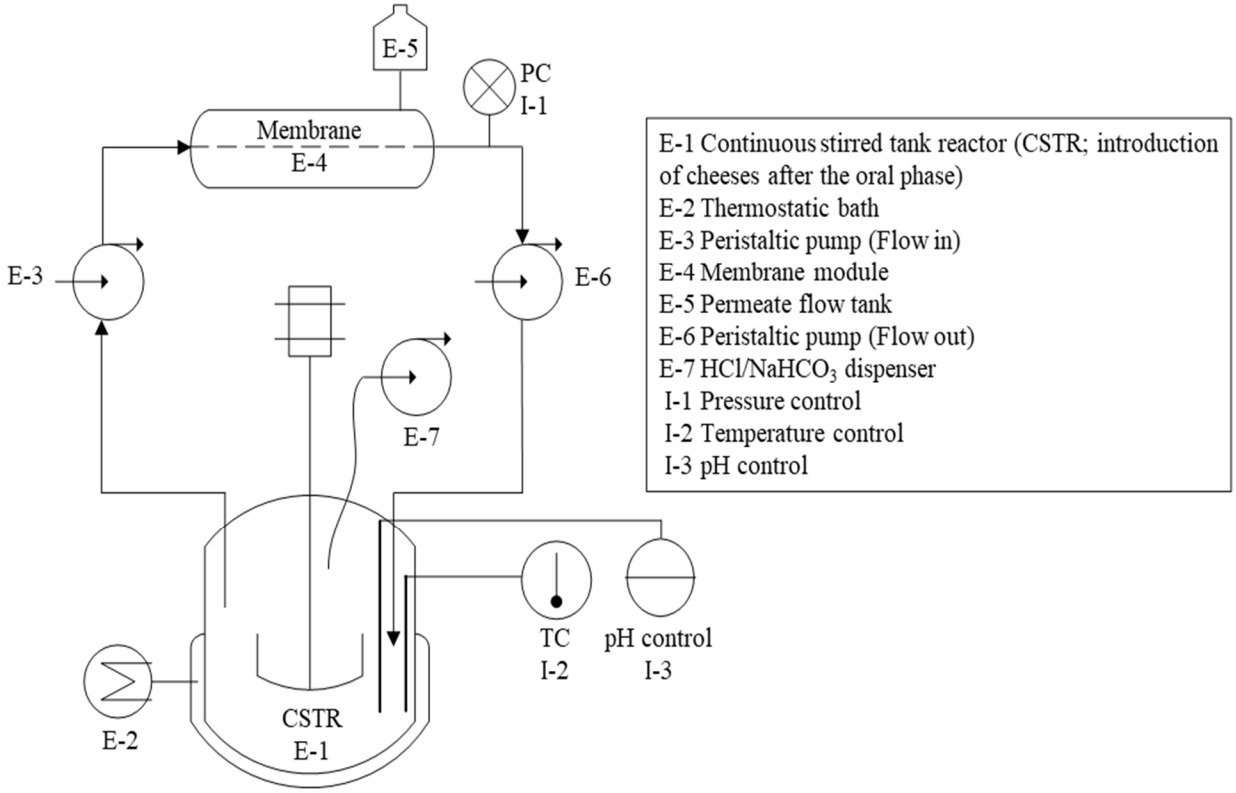
637 **Figure 2.** Bioavailability of Ca (A), Mg (B) and Zn (C) during the *in vitro* digestion of
638 Brazilian cheeses. Bioavailability was calculated as the percentage of mineral absorbed
639 from the initial quantity in the digested sample. Different letters indicate significant
640 differences between samples for Ca, Mg or Zn ($P < 0.05$), ANOVA + LSD test (only
641 statistical differences at 180 min are depicted, to avoid overlapping).

642

643 **Table captions**

644 **Table 1**. Bioaccessibility of Ca, Mg and Zn in Brazilian cheeses after the different phases
645 of the dynamic model of *in vitro* digestion.

646 **Table 2**. Percentage of Ca, Mg and Zn absorbed from the bioaccessible fraction during
647 the *in vitro* digestion of Brazilian cheeses.



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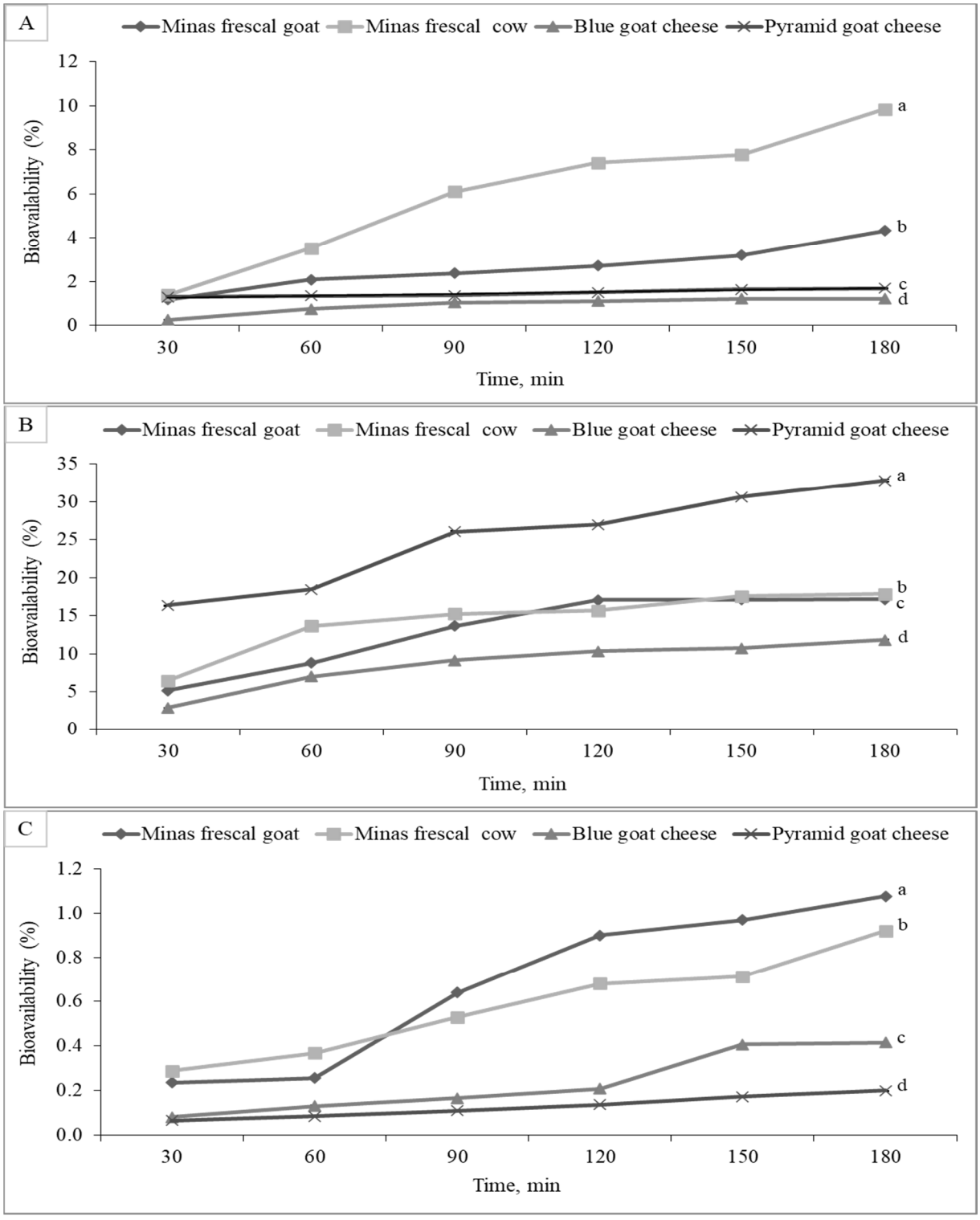

Figure 2. Bioavailability of Ca (A), Mg (B) and Zn (C) during the *in vitro* digestion of Brazilian cheeses. Bioavailability was calculated as the percentage of mineral absorbed from the initial quantity in the digested sample. Different letters indicate significant differences between samples for Ca, Mg or Zn ($P < 0.05$), ANOVA + LSD test (only statistical differences at 180 min are depicted, to avoid overlapping).

Table 1. Bioaccessibility of Ca, Mg and Zn in Brazilian cheeses after the different phases of the dynamic model of *in vitro* digestion.

Elements	Cheeses	Mineral contents (mg/100 g)	Phases (%)		
			Oral	Gastric	Intestinal
Ca	*Minas frescal* goat	598 ± 14[a]	11.6 ± 0.01[b]	9.77 ± 0.03[c]	14.0 ± 0.1[b]
	Minas frescal cow	535 ± 9[b]	3.36 ± 0.01[d]	20.2 ± 0.1[b]	6.32 ± 0.1[d]
	Blue goat cheese	562 ± 5[b]	7.71 ± 0.02[c]	9.48 ± 0.1[d]	10.1 ± 0.1[c]
	Pyramid goat cheese	103 ± 2[c]	11.7 ± 0.03[a]	20.7 ± 0.1[a]	21.6 ± 0.2[a]
Mg	*Minas frescal* goat	41.6 ± 1.3[a]	11.1 ± 0.03[b]	7.45 ± 0.05[d]	11.3 ± 0.1[b]
	Minas frescal cow	37.6 ± 0.2[b]	1.24 ± 0.01[d]	18.4 ± 0.1[b]	3.49 ± 0.05[d]
	Blue goat cheese	32.7 ± 0.8[c]	2.82 ± 0.02[c]	16.9 ± 0.1[c]	5.07 ± 0.1[c]
	Pyramid goat cheese	13.6 ± 0.3[d]	14.1 ± 0.02[a]	21.0 ± 0.2[a]	16.5 ± 0.2[a]
Zn	*Minas frescal* goat	9.79 ± 0.2[b]	2.81 ± 0.003[a]	3.32 ± 0.01[a]	5.63 ± 0.03[a]
	Minas frescal cow	13.2 ± 0.1[a]	0.77 ± 0.01[c]	3.09 ± 0.003[b]	2.78 ± 0.01[c]
	Blue goat cheese	10.1 ± 1[b]	1.74 ± 0.01[b]	1.97 ± 0.01[c]	4.73 ± 0.01[b]
	Pyramid goat cheese	11.9 ± 0.1[a]	0.43 ± 0.003[d]	0.72 ± 0.01[d]	1.44 ± 0.01[d]

Solubility was calculated as the percentage of soluble mineral from the initial content in the digested samples. Different superscripts in the same column indicate significant differences between samples for Ca, Mg or Zn ($P < 0.05$), ANOVA + LSD test.

Table 2. Percentage of Ca, Mg and Zn absorbed from the bioaccessible fraction during the *in vitro* digestion of Brazilian cheeses.

Elements	Cheeses	Time of intestinal absorption (min)					
		30	60	90	120	150	180
Ca	*Minas frescal* goat	2.27 ± 0.003^{b}	4.03 ± 0.01^{b}	4.55 ± 0.01^{b}	5.24 ± 0.02^{b}	6.15 ± 0.02^{b}	8.30 ± 0.02^{b}
	Minas frescal cow	2.32 ± 0.002^{a}	5.90 ± 0.02^{a}	10.2 ± 0.02^{a}	12.4 ± 0.1^{a}	13.0 ± 0.04^{a}	16.5 ± 0.2^{a}
	Blue goat cheese	0.61 ± 0.005^{d}	1.75 ± 0.005^{d}	2.46 ± 0.01^{c}	2.58 ± 0.01^{c}	2.83 ± 0.01^{c}	2.84 ± 0.005^{c}
	Pyramid goat cheese	1.82 ± 0.003^{c}	1.89 ± 0.001^{c}	1.92 ± 0.001^{d}	2.12 ± 0.001^{d}	2.29 ± 0.01^{d}	2.38 ± 0.001^{d}
Mg	*Minas frescal* goat	12.7 ± 0.04^{c}	21.6 ± 0.05^{c}	33.6 ± 0.1^{a}	41.9 ± 0.1^{a}	42.1 ± 0.1^{a}	42.2 ± 0.1^{a}
	Minas frescal cow	13.2 ± 0.05^{b}	27.7 ± 0.1^{a}	30.9 ± 0.1^{c}	31.9 ± 0.2^{c}	35.7 ± 0.4^{c}	36.2 ± 0.3^{c}
	Blue goat cheese	6.02 ± 0.02^{d}	14.4 ± 0.03^{d}	18.9 ± 0.1^{d}	21.4 ± 0.1^{d}	22.2 ± 0.03^{d}	24.4 ± 0.1^{d}
	Pyramid goat cheese	19.8 ± 0.05^{a}	22.3 ± 0.04^{b}	31.5 ± 0.1^{b}	32.6 ± 0.1^{b}	37.1 ± 0.1^{b}	39.7 ± 0.2^{b}
Zn	*Minas frescal* goat	1.20 ± 0.02^{c}	1.30 ± 0.01^{c}	3.26 ± 0.01^{b}	4.58 ± 0.01^{b}	4.93 ± 0.02^{b}	5.48 ± 0.01^{b}
	Minas frescal cow	2.19 ± 0.003^{a}	2.79 ± 0.04^{a}	4.01 ± 0.01^{a}	5.15 ± 0.2^{a}	5.40 ± 0.1^{a}	6.98 ± 0.1^{a}
	Blue goat cheese	0.55 ± 0.003^{d}	0.89 ± 0.02^{d}	1.13 ± 0.01^{d}	1.41 ± 0.01^{d}	2.76 ± 0.03^{d}	2.82 ± 0.01^{d}
	Pyramid goat cheese	1.38 ± 0.006^{b}	1.78 ± 0.05^{b}	2.31 ± 0.01^{c}	2.88 ± 0.001^{c}	3.63 ± 0.05^{c}	4.20 ± 0.02^{c}

Different superscripts in the same column indicate significant differences between samples for Ca, Mg or Zn ($P < 0.05$), ANOVA + LSD test.