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Bioaccessibility Assessment of Cu, Fe, K, Mg, P, and Zn in Thermally Treated Lamb Meat

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A bioaccessibility test with raw and cooked lamb meat samples was performed. The evaluated cooking devices were grill, microwave oven, air fryer, pressure cooker, and electric oven. Physicochemical parameters and the total mass fraction of Cu, Fe, K, Mg, P, and Zn were determined in raw and cooked samples by inductively coupled plasma optical emission spectrometry (ICP OES). The trueness was evaluated using certified reference materials, with recoveries from 87 to 101%. The pressure cooking presented the major changes, including the highest values of internal temperature, loss of inorganic elements after cooking, and the lowest values of moisture and analyte mass fractions. An *in vitro* gastrointestinal simulation was performed, and the method was validated by an addition and recovery test, in which the trueness varied from 87 to 115%. The bioaccessibility ranged between 28-56, 4-19, 68-76, 41-54, 48-57, and 1-21% for Cu, Fe, K, Mg, P, and Zn, respectively. The cooking methods promoted changes in the meat samples, thus affecting the bioaccessibility of the nutrients. Based on the recommended dietary intake (RDI) calculation, lamb meat can be considered a good Fe, P, and Zn source.

Keywords: bioaccessibility, lamb meat, ICP OES, RDA, sample preparation

Introduction

According to the Food and Agriculture Organization of the United Nations (FAO), meat is a valuable livestock product from a nutritional perspective due to its contents of vitamins, proteins and inorganic elements.[1] Population and income growth are leading to increased meat consumption, and consequently, the diversity of the meat consumed. Lamb meat still accounts for a small part of global production, although its consumption has grown in recent years.[2,3]

The thermal processing of meat, such as grilling, frying, and roasting, promotes physicochemical changes in its composition, affecting the final quality of the product for several reasons, such as loss of vitamins, inorganic elements, moisture and protein content.[4-6] Furthermore, thermal processing can modify the color, flavor and appearance. These changes promote the formation of volatile compounds that directly influence consumer acceptance, besides improving digestibility, ensuring microbiological safety, and improving the organoleptic properties of the food.[7,8] Different approaches can be used for cooking, only a few of which were evaluated in this study. Grilling is a form of cooking that involves dry heat applied to the surface of food, primarily through thermal radiation. Heat transfer when using a grill occurs by direct conduction. In contrast, the microwaves produced by a magnetron in the microwave oven cavity are absorbed by polar molecules, generally water present in the food, generating the conduction and rotation of these molecules and producing thermal energy.[9] Air frying is an alternative method of frying where the heating is caused by a hot air source that results in a uniform temperature distribution throughout the food.[10] In pressure cooking, the meat is cooked immersed in water under high pressure to obtain more tender meat. In the electric oven, the food is cooked through the combination of conduction, convection, and radiation using dry heat, where the food is placed on a pan or grid to improve heat circulation.[8]

The human body has daily inorganic element needs for different purposes. Trace elements such as copper and zinc play an essential role in coenzymes, proteins, and lipids that build up the muscles, organs, blood cells, and some soft tissues. Magnesium is important for neuromuscular

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transmission.[11] Iron is present in blood myoglobin and hemoglobin, responsible for transporting oxygen in the body.[12] Phosphorus promotes rigidity of the teeth and skeletal structure.[13] Potassium balances the liquids inside and outside the cells, participates in muscle contraction and relaxation and maintains blood pressure.[11]

Chemical analysis is the starting point to determine the nutritional value of food, but the actual ingestion of nutrients depends on the body's ability to process them. Bioavailability is the amount of nutrients ingested that is absorbed and available for the various physiological functions of the organism, which is directly related to the food matrix, absorption, and transport by intestinal cells.[14] Bioaccessibility is defined as the quantity of nutrients from the food matrix available for absorption in the gastrointestinal tract.[15,16] This depends on the analytes' release from the food matrix and the gastrointestinal conditions.[14]

Studies applying *in vitro* gastrointestinal simulation to determine the bioaccessibility of minerals in different matrices are available in the literature. Most researchers have evaluated the pH and temperature and simulated the enzymatic activity of the stomach and intestinal juices under controlled conditions, achieving high trueness and reproducibility.[14-18] The results obtained from *in vitro* tests have been comparable to *in vivo* ones, indicating its feasibility, avoiding *in vivo* tests, which are much more expensive, besides potentially involving ethically questionable processes.[19,20]

In this context, this study evaluated the Cu, Fe, Mg, K, P and Zn bioaccessibility using an *in vitro* gastrointestinal digestion model with samples of lamb meat raw and submitted to five different cooking methods. The experiment allowed estimating the availability of the abovementioned minerals after typical food preparation processes. The recommended dietary intake (RDI) was also assessed based on the total mass fraction.

Experimental

Reagents and solutions

Deionized water (resistivity of 18.2 MΩ cm at 25 °C) obtained from a Milli-Q water purification system (Millipore, Bedford, USA) was used to prepare all analytical solutions. All the glassware and polypropylene flasks used were decontaminated in 10% (v v^{-1}) nitric acid for 24 h and rinsed with deionized water. Nitric acid (Synth, Diadema, Brazil) was previously purified using a sub-boiling acid distiller (Model BSB-939-IR, Distillacid, Berghof, Berlin, Germany). It was used combined with hydrogen peroxide 30% m m^{-1} (Sigma-Aldrich, Hamburg,

Germany) for sample digestion. Calibration standards and solutions for standard addition experiments were prepared by diluting a single-element analytical grade stock solutions of Cu, Fe, K, Mg, P, and Zn (Fluka, Buchs St. Gallen, Switzerland) after successive dilutions with deionized water. Pepsin, bile salts, and pancreatin (Sigma-Aldrich, USA), sodium bicarbonate (Synth, Diadema, Brazil), hydrochloric acid (Merck, Darmstadt, Germany), and sodium hydroxide (Synth, Diadema, Brazil) were used in the bioaccessibility tests.

Instrumental

An inductively coupled plasma optical emission spectrometer (ICP OES) (Agilent Technologies, Model 5110, Synchronous Vertical Dual View, Mulgrave, Australia) equipped with a sea spray nebulizer, double-pass cyclonic spray chamber, and peristaltic pump was used for all elemental determinations. The instrumental parameters for the analytical measurements were 1.2 kW of applied radiofrequency power, 12.0 L min^{-1} plasma gas flow rate, 1.0 L min^{-1} auxiliary gas flow rate, 0.7 L min^{-1} nebulizer gas flow rate, 12 rpm peristaltic pump, 15 s stabilization time, and 3 s integration time. The axial viewing position was used for Cu, Fe, P and Zn, while radial viewing was used for K and Mg. The monitored wavelengths were 324.754 (I), 259.940 (II), 766.491 (I), 280.270 (II), 213.618 (I), and 213.857 (I) nm for Cu, Fe, K, Mg, P, and Zn, respectively, where (I) stands for atomic and (II) for ionic lines. Argon, with purity of 99.999% (White Martins-Praxair, Sertãozinho, Brazil) was used for plasma generation, sample nebulization and plasma auxiliary gas.

The samples were freeze-dried (Model EC, MicroModulyo, New York, USA) for 48 h and cryogenically milled (MA775, Marconi, Piracicaba, Brazil) using a program of 30 s on interspersed with 30 s off intervals. A pHmeter (Model pHS3-BW, BEL, Monza, Italy), a centrifuge with capacity for 15 and 50 mL polypropylene tubes (Excelsa II 206 BL, FANEM, São Paulo, Brazil), and a heating device (Dubnoff bath, NT232 Nova Técnica, São Paulo, Brazil) stabilized at 37 °C with continuous auto stirring were used during the *in vitro* bioaccessibility experiments.

For total mass fraction determination, the samples were acid decomposed in a single reaction chamber (SRC) microwave oven (UltraWave™, Milestone, Sorisole, Italy) pressurized with nitrogen (99.9%) (White Martins-Praxair, Sertãozinho, Brazil). The bioaccessible fraction was acid decomposed in a 12-position carrousel cavity microwave oven (Multiwave GO, Anton Paar, Graz, Austria), and ICP OES was carried out for quantification of elements.

For the cooking processes, a domestic microwave oven (Eletrolux, 31L MEF41, Manaus, Brazil), a domestic air fryer (Philco, Manaus, Brazil), a grill (NKS, Rio de Janeiro, Brazil) and a pressure cooker (Mondial, 4L PE-09, Manaus, Brazil) were used.

Samples

Fresh lamb loin cuts were acquired from a local market (São Carlos, SP, Brazil). Samples from six different lamb loins were cut into ca. 2.5 cm cubes, randomly mixed, and divided into groups for control (raw) and for culinary treatments. All the experiments were performed in triplicate.

The certified reference materials (CRMs) bovine liver (NIST 1577c) and bovine muscle powder (NIST 8414), from National Institute of Standards and Technology (Gaithersburg, MD, USA), were used to validate the total mass fraction determination by calculating the trueness and to guarantee metrological traceability.

Sample preparation and cooking

The cooking procedure was based on that proposed by Menezes *et al.*,[6] with some modifications. The samples were divided into portions of 100 g in the following categories: (*i*) raw, uncooked without any manipulation; (*ii*) grill, grilled for 3 min on each side in the preheated grill at 200 °C for 90 s; (*iii*) microwave oven, samples placed on a plastic film at 820 W for 1.5 min; (*iv*) air fryer, cooked at 180 °C for 10 min; (*v*) pressure cooker, in which the samples were placed in a pressure pan along with 810 mL of water; and (*vi*) electric oven, where the meat was placed in a stainless steel pan and cooked for 8 min until reaching an internal temperature of 76 °C. The water used for pressure cooking was previously analyzed, and the content of the evaluated analytes was negligible. No seasoning or oil was added in any of the cooking procedures. After heat treatments, the samples were weighed and freeze-dried.

Physicochemical parameters

The temperature inside of the samples was measured after the cooking procedure using a digital thermometer. Weight loss was calculated from the difference between the initial and final weight of the raw and thermally processed samples. The moisture was calculated from the masses before and after freeze-drying the raw and thermally processed samples.

Part of the samples were used for total mass fraction analysis and submitted to bioaccessibility testing, in which the bioaccessible fractions were determined.

Analytical methods

Elements determination

The total mass fraction was quantified as a guideline for the bioaccessible fraction. The sample decomposition consisted of weighing 100 mg of freeze-dried samples and CRMs, in triplicate and adding 8.0 mL of HNO_3 (4.2 mol L^{-1}) plus 2.0 mL of H_2O_2 (30% m m^{-1}) to the samples. The analytical blank was prepared in the same way without the sample. Volumes of 150 mL of water and 5 mL of concentrated HNO_3 were placed into the microwave SRC chamber and pressurized with $N_{2(g)}$ to 40 bar. The samples were microwave-treated according to the following heating program: (*i*) 2.5 min ramp to 140 °C, (*ii*) 2.5 min hold at 140 °C, (*iii*) 2.5 min ramp to 180 °C, (*iv*) 2.5 min hold at 180 °C, (*v*) 10 min ramp to reach 220 °C, and (*vi*) 10 min hold at 220 °C. After cooling to room temperature, the samples were transferred to polypropylene tubes, and the volume was completed to 15 mL with deionized water.

In vitro enzymatic digestion test and its quantification

The *in vitro* digestion process was based on García-Sartal *et al.*,[15] with some modifications. The procedure consisted of weighing ca. 500 mg of each of the freeze-dried samples in glass flasks, in triplicate, addition of 10 mL of water, and adjusting the pH to 2 with a 6 mol L^{-1} HCl solution under stirring for 10 min. Then 5 mL of gastric solution (6% (m v^{-1}) pepsin in 0.1 mol L^{-1} HCl) was added to the samples, and the flasks were placed in a heating device at 37 °C for 2 h under constant stirring at 200 rpm. The samples were then placed in an ice bath for 15 min to stop the enzymatic activity, and the pH of the solution was adjusted to 7 with a 6 mol L^{-1} NaOH solution. An aliquot of 5 mL of the intestinal solution (0.4% (m v^{-1}) pancreatin in addition to 2.5% (m v^{-1}) bile salts dissolved in 0.1 mol L^{-1} $NaHCO_3$) was added to the samples. The solution was returned to the heating device for an additional 2 h at 37 °C and 200 rpm. After that, the samples were again placed for 15 min in an ice bath and transferred to polypropylene tubes. The mixture was centrifuged for 10 min at 3600 rpm to obtain the bioaccessible fraction (see Figure 1). The solution was stored at −20 °C until decomposition and quantification. The analytical blank was submitted to the same procedure without the sample.

A 5 mL aliquot of the bioaccessible fraction was microwave-assisted acid digested with 3 mL of 7 mol L^{-1} of HNO_3 and 2.0 mL of 30% (m m^{-1}) of H_2O_2. The heating program consisted of heating for 10 min to 180 °C, and 25 min hold at 180 °C. After cooling to room temperature, the samples were transferred to polypropylene tubes and the volume was completed to 15 mL with deionized water.
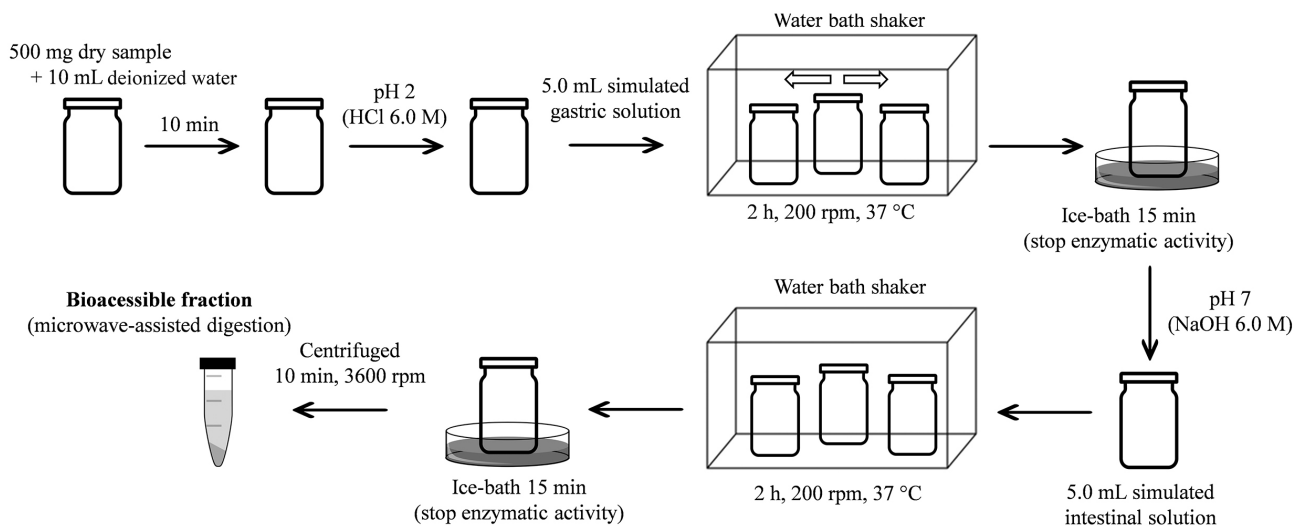
Figure 1. Representative scheme of the *in vitro* bioaccessibility test.

The trueness of the *in vitro* digestion test was evaluated by an addition and recovery test of spiked samples. For that, additional flasks (in triplicate) were employed and submitted the same steps since the beginning of *in vitro* digestion procedure. Fractions of 500 mg of each freeze-dried sample received two levels of each spiked element, corresponding to approximately 50 and 100% of the mass fraction of the bioaccessible fraction of the raw lamb meat. The added spiked values were defined after the bioaccessibility experiment, with different levels for each of the studied analytes: Cu (0.8 and 1.5 mg kg^{-1}), Fe (4 and 9 mg kg^{-1}), K (0.5 and 1 mg kg^{-1}), Mg (260 and 510 mg kg^{-1}), P (0.2 and 0.58 mg kg^{-1}), and Zn (6 and 16 mg kg^{-1}).

Results and Discussion

Physicochemical parameters

Physicochemical parameters can indicate tendencies for each of the cooking processes and/or evaluated elements. In this way, the samples' internal temperature, weight loss, and water content were evaluated. All the assessed physicochemical parameters are presented in Table 1.

According to the results shown in Table 1, there was a statistically significant variation of the internal temperature of the meat for the different thermal processes, ranging from 63 to 87 °C. The data were evaluated by the Tukey's test.[21]

Lower temperatures, statistically similar, were observed for meat cooked in the grill and electric oven, while pressure cooking provided the highest temperatures. The temperature was clearly associated with the weight loss caused by the thermal process. Indeed, the grilling and electric oven cooking caused lower weight loss than other procedures.

Table 1. Internal temperature, weight, and moisture of the samples before and after thermal processing (n = 3)

Thermal process	Temperature / °C	Weight loss / %	Moisture / %
Raw	–	–	73.4 ± 0.2[a]
Grill	63 ± 1[a]	14.8 ± 0.1[a]	65.9 ± 0.5[b]
Microwave oven	81 ± 1[b]	38.1 ± 0.1[b]	57.6 ± 0.4[b]
Air fryer	76 ± 1[c]	31.6 ± 0.1[b]	61.2 ± 0.7[b]
Pressure cooker	87 ± 1[b]	48.2 ± 0.1[c]	55.9 ± 0.6[b]
Electric oven	65 ± 1[a]	25.8 ± 0.1[b]	63.1 ± 0.3[b]

Results are expressed as 95% confidence intervals of three authentic repetitions. Means in the same column with different letters are significantly different (Tukey's test, $p < 0.05$).

Thus, the higher the meat's internal temperature, the greater the loss of elements during cooking. However, the weight loss percentage is also related to the cooking method, temperature exposure period, type of meat used, and the heating rate.[9] Weight loss is a relevant parameter, resulting mainly from water loss and consequently causing loss of soluble salts, proteins, phosphates, and aromatic compounds.[22] The weight loss results were in agreement with the literature.[5,23-25] Similar results were obtained by Ferreira *et al.*,[26] in which the authors obtained weight loss of roasted and grilled chicken meat of 17.3 and 13.1%, and water loss of 67.7 and 68.8%, respectively. The lowest percentage of water was observed for the meat prepared by pressure cooking (55.9 ± 0.6%), and the highest for grilled meat (65.9 ± 0.5%). Only the results for raw meat moisture were statistically different from the results for the cooking processes (Table 1).

During the cooking process, denaturation of myofibrillar proteins and collagen contraction occur, resulting in loss of water.[24] The results obtained from grilling and electric

oven processes presented lower loss of water, which is explained by the formation of a crust on the surface of the meat that reduces the loss of liquids (water and/or fat). The amount of water retained is essential because it promotes intramuscular tissue solubilization and tenderization, which is a critical parameter for consumer acceptance.[23,24]

Total mass fraction

The total mass fraction of the analytes was determined to calculate the bioaccessible fraction. The samples were acid digested, as previously described, for analysis. The limits of detection (LOD) and limits of quantification (LOQ) were calculated according to Thomsen,[27] considering the background equivalent concentration (BEC), the signal-to-background ratio (SBR), and relative standard deviation (RSD) of 10 consecutive measurements of the blank solution. The LOQ is defined as 3.3-fold the LOD. All the LODs values were calculated based on the respective BEC values for each monitored wavelength, considering all dilutions. The obtained values for LOD and LOQ (mg kg^{-1}) were 0.43 and 1.4 for Cu, 0.48 and 1.6 for Fe, 9.1 and 30 for K, 0.27 and 0.91 for Mg, 0.78 and 2.6 for P, and 0.16 and 0.53 for Zn for LOD and LOQ, respectively. The trueness of the method, evaluated by CRMs of the bovine liver and bovine muscle, presented recoveries varying from 87 to 101%, in good agreement, according to the Taverniers *et al.*[28] and the analytical method validation and quality assurance. The total mass fractions of the thermally processed samples and CRMs and respective recoveries are presented in Table 2. Considering the evaluated elements, the analytes in raw lamb meat had the following decreasing order of concentration: K > P > Mg > Zn > Fe > Cu. The same pattern was observed for the meat cooked by each method.

Purchas *et al.*[29] observed that the mass fraction of Na and K decreased after lamb meat cooked by braising, roasting, and frying. Gerber *et al.*[4] observed that the amount of Ca, Na, K, Mg, and P decreased after thermal processing of beef, pork, and veal samples, concluding that time, medium, and cooking temperature influence the loss of inorganic elements. The authors also observed that when cooking was done with water, the losses were more pronounced. Similar results were obtained in the present study for K, Mg, and P, as shown in Table 2. The opposite was observed for Zn in samples prepared by microwave and Cu prepared by microwave, grilling and pressure cooking. According to the statistical test, the mass fraction of Zn and Cu increased compared to the raw samples and the other cooking procedures. Goran *et al.*[30] evaluated beefsteak and pork chops under different thermal preparation methods. They also observed an increase of Zn concentration in thermally processed beef samples, which was related to the increase of the insoluble Zn fraction from denatured proteins. This behavior was substantial for beefsteak but not for pork chops. Iron concentration in the raw, grilled, and microwaved samples presented similar values but were greater (statistically different) than the other cooking procedures.

Table 2. Determined values (mean ± SD, n = 3) for CRM (bovine liver, NIST 1577c and bovine muscle, NIST 8414) and the respective trueness. Mass fraction (mean ± SD, n = 3), and the RDI[a] of raw and thermally processed lamb meat samples

CRM	Determined value (trueness / %)					
	Cu / (mg kg^{-1})	Fe / (mg kg^{-1})	K / (g 100 g^{-1})	Mg / (mg kg^{-1})	P / (g 100 g^{-1})	Zn / (mg kg^{-1})
NIST 1577c	253.2 ± 3.2 (92)	186.3 ± 2.8 (94)	1.008 ± 0.010 (99)	918.0 ± 14.2 (96)	1.097 ± 0.009 (93)	168.2 ± 1.9 (93)
NIST 8414	2.46 ± 0.05 (87)	64.3 ± 1.5 (90)	1.537 ± 0.010 (101)	600.5 ± 6.4 (97)	0.761 ± 0.006 (91)	134.8 ± 0.4 (95)
Sample processing	Mass fraction					
	Cu / (mg kg^{-1})	Fe / (mg kg^{-1})	K / (g 100 g^{-1})	Mg / (mg kg^{-1})	P / (g 100 g^{-1})	Zn / (mg kg^{-1})
Raw	2.7 ± 0.1[a]	59 ± 1[a]	1.3 ± 0.1[a]	913 ± 47[a]	0.75 ± 0.04[a]	68 ± 2[a]
RDI / %	30	74	28	23	107	62
Grill	2.9 ± 0.2[b]	56 ± 4[a]	1.1 ± 0.1[ac]	847 ± 50[b]	0.66 ± 0.02[b]	75 ± 4[bc]
RDI / %	32	70	23	21	94	68
Microwave oven	3.1 ± 0.3[b]	58 ± 3[a]	0.92 ± 0.02[b]	835 ± 52[b]	0.63 ± 0.02[b]	96 ± 7[d]
RDI / %	34	73	20	21	90	87
Air fryer	2.6 ± 0.1[a]	50 ± 2[b]	0.99 ± 0.02[a]	763 ± 16[c]	0.63 ± 0.01[b]	71 ± 3[b]
RDI / %	29	63	21	19	90	65
Pressure cooker	2.9 ± 0.1[b]	52 ± 2[b]	0.38 ± 0.01[d]	510 ± 21[d]	0.42 ± 0.02[d]	78 ± 2[c]
RDI / %	32	65	8	13	60	71
Electric oven	2.6 ± 0.1[a]	50 ± 2[b]	0.94 ± 0.04[bc]	750 ± 22[c]	0.60 ± 0.03[b]	79 ± 2[c]
RDI / %	29	63	20	19	86	72

[a]RDI: recommended dietary intake for men between 19 and 70 years old. Results are expressed as 95% confidence intervals of three authentic repetitions. Means within the same column with different letters are significantly different (Tukey's test, $p < 0.05$). CRM: certified reference material.

To evaluate the loss of inorganic elements by pressure cooking, the cooking water was analyzed, and the mass fractions of analytes were 2.11 ± 0.02 mg kg^{-1}, 0.400 ± 0.001 g 100 g^{-1}, 170.6 ± 0.5 mg kg^{-1}, 0.141 ± 0.001 g 100 g^{-1}, 0.57 ± 0.04 mg kg^{-1} for Fe, K, Mg, P, and Zn, respectively. The Cu mass fraction was lower than LOQ (< 1.4 mg kg^{-1}). According to the results, approximately 4% of Fe, 31% of K, 19% of Mg, 19% of P, and 1% of Zn leached when cooked in the pressure pan. The results agreed with physicochemical parameters. The meat cooked in the pressure pan showed the most significant loss from cooking, which means that part of the analyzed elements leached to the cooking medium (water). The analyte loss can be linked to the presence of sugars, amino acids, and proteins reduced by Maillard reactions (MR) during heating. These reactions are characterized by the formation of hundreds of different substances associated with the acceptance of the processed product. Otherwise, only the early-stage products (measured as furosine) of the MR predominate in grilled and fried meat cooked with an internal temperature below 90 °C.[31] This temperature is similar to that obtained in the present study, with a maximum of 87 °C. According to the Food Safety and Inspection Service of the U.S. Department of Agriculture (USDA-FSIS),[32] lamb meat cooking by home methods such as grilling and frying with internal temperatures between 63 and 71 °C results in meat with low MR generation, pleasant color, and good safety.

Reference daily intake (RDI)

According to the American Institute of Medicine,[33] the reference daily intake (RDI) is the daily food intake sufficient to meet the nutritional needs of the majority of healthy individuals in a population. In turn, the recommended dietary allowances (RDA) for men between 19 and 70 years are 900 µg, 8 mg, 400-420 mg, 700 mg, and 11 mg for Cu, Fe, Mg, P, and Zn, respectively. For women, these values are 900 µg, 18 mg (19-50 years)-8 mg (51+ years), 310 mg (19-30 years)-320 mg (31+ years), 700 mg, and 8 mg of Cu, Fe, Mg, P, and Zn, respectively. For K, there is only a daily intake recommendation of 4.7 g for men and women from 19 to 70 years old.[33] Brazilian regulations propose similar values. Resolution 269 from the National Sanitary Surveillance Agency (ANVISA) recommends daily intake values for adults (19-50 years old) of 900 µg, 14 mg, 260 mg, 700 mg, and 7 mg of Cu, Fe, Mg, P, and Zn, respectively.[34] The value for K is not reported in this resolution.

The percentages of RDI were calculated using RDA values from the Institute of Medicine and the obtained total amounts from each cooking procedure, assuming an intake of 100 g of lamb meat. The results are presented in Table 2. The RDI value represents the daily amount that an individual can safely consume. For example, a man who eats 100 g of lamb meat cooked in an electric oven is consuming 29, 63, 20, 19, 86, and 72% of Cu, Fe, K, Mg, P, and Zn of the respective RDI.

According to the percentages obtained from RDI, it can be stated that lamb loin is a source of Fe, P, and Zn. The RDI information of the bioaccessible fraction of raw and/or thermally processed food is considered necessary for public health since the deficiency or excess can cause positive or negative effects on human health.

Validation of *in vitro* test digestion process

The samples were spiked at two levels to evaluate the trueness of the bioaccessible fraction measurements. The standard aliquots were added from the beginning of the procedure of the *in vitro* digestion process. The standard addition was based on the determined mass fraction of the analyte in the intestinal phase for each of the monitored analytes. The concentration of the spikes added, along with the determined values quantified by ICP OES and their respective recoveries calculated from the added standard concentration, are presented in Table 3. According to the results, regardless of the spike level, the *in vitro* digestion procedure and the subsequent digestion of the bioaccessible fraction presented adequate precision and trueness, with good recoveries for all the analytes evaluated, which varied from 87 to 115%.[28]

Table 3. Recoveries for addition and recovery test (n = 3) in *in vitro* experiment with lamb meat

Analyte	Standard addition / (mg kg^{-1})	Determined value (recovery / %) / (mg kg^{-1})
Cu	2.3	2.2 ± 0.1 (96)
	3.0	3.0 ± 0.1 (100)
Fe	15	13 ± 1 (87)
	20	23 ± 1 (115)
K[a]	1.4	1.3 ± 0.1 (93)
	2.0	1.8 ± 0.1 (90)
Mg	750	741 ± 75 (99)
	1000	967 ± 126 (97)
P[a]	0.6	0.6 ± 0.1 (100)
	1.0	0.93 ± 0.01 (93)
Zn	20	21 ± 1 (105)
	30	33 ± 2 (110)

[a](g 100 g^{-1}).

Bioaccessible fraction

The mass fractions of Cu, Fe, K, Mg, P, and Zn were determined in the bioaccessible fraction obtained from the

in vitro test. Acid digestion was required to avoid spectral interferences due to the high carbon content in the solution. Grindlay *et al.*[35] reported that the presence of carbon, sulfur and/or phosphorus favors charge transfer reactions, affecting the emission signal of some elements. The LOD and LOQ values obtained from the bioaccessible fraction were, in mg kg^{-1}, 0.45 and 1.49; 1.22 and 4.07; 90 and 301; 6.0 and 20; 60 and 200; and 0.36 and 1.21 for Cu, Fe, K, Mg, P, and Zn, respectively.

The bioaccessible fractions presented in Table 4 indicate that only a proportion of the total of the elements is available to be absorbed by the organism. The results for raw meat samples presented higher bioaccessibility than the cooked samples. This can be attributed to protein denaturation during cooking and may affect the mineral bioaccessibility.[36] Denaturation of proteins by high temperatures can create additional binding sites between metal and protein, which can effectively trap the metal, making it less available for digestion.[37] Meat and fish are rich in lysine, methionine, and histidine, for which amino acids have a high affinity for metallic compounds.[38] The bioaccessible percentage of raw and cooked lamb meat samples varied from 6 to 68, 4 to 64, 2 to 73, 1 to 76, and 7 to 75% for the cooking by grill, microwave oven, air fryer, pressure cooker, and electric oven, respectively. Zinc and K were the analytes with the lowest and highest bioaccessibility, respectively, for all of the cooking procedures. Even with the lowest absolute value, probably due to leaching during the pressure cooking, K presented 76% bioaccessibility. The bioaccessibility of Cu and Fe was independent of the cooking process, and only pressure coking showed a statistical difference in the bioaccessibility of Mg and P.

In comparison with the total amount, Zn and Fe were the elements with the lowest percentage of bioaccessibility. The rates were between 1 to 21% for Zn, and between 4 to 19% for Fe. These low values may be related to the presence

of inhibitory compounds. As Etcheverry *et al.*[14] reported, the presence of Fe consumed as a supplement and high amounts of Ca in the body can inhibit the absorption of Zn.

Iron is present in meat in heme and non-heme form. Heme iron is bonded to hemoglobin and is easily absorbed by the body. However, the cooking procedures reduce the heme iron amount and consequently the bioaccessible fraction.[39] The bioaccessibility of Fe is affected by the diet since there are inhibitory compounds such as phytate and calcium in other foods that can affect this mineral absorption.[19,40] Tokalioğlu *et al.*[41] evaluated the bioaccessibility of Cr, Cu, Fe, Mg, Mn, Mo, Se, and Zn in nutritional supplements by the BARGUE method (Unified Bioaccessibility Research Group of Europe). The authors observed a decrease of Fe bioaccessibility in the intestinal fraction (3-14%) compared to the fraction of Fe in the gastric phase (55-99%). The authors reported that the pH of the gastric fluid increases the solubility of the minerals in the gastric fraction. In contrast, the pH of the intestinal fraction can lead to the precipitation of salts in the form of hydroxides, phosphates and carbonates.

Potassium was the analyte with the highest bioaccessibility, reaching 64 to 76%, with the highest bioaccessible fraction being achieved for lamb meat prepared in the pressure cooker and electric oven. It was the only element for which bioaccessibility increased after cooking.

Silva *et al.*[16] estimated the bioaccessibility of bovine liver samples after water and *sous-vide* cooking. They observed bioaccessible percentages of 62.1-95.8, 14.9-26.9, 11.2-39.5, 43.9-42.6, 31.0-43.9, and 18.0-36.3% for Ca, Cu, Fe, K, Mg and Zn, respectively. The authors concluded that cooking improves the bioaccessibility of these analytes. On the other hand, Ramos *et al.*[42] evaluated the bioaccessibility of Se, Cu, Zn, Mn, and Fe in unaged and aged meat from steers fed pasture, finding bioaccessibility percentages between 75-91, 30-45, 40-68, 55-95, 60-70% for unaged meat, and 58-80, 30-48, 40-58, 75-95, 59-70%

Table 4. Bioaccessible fraction (mean ± SD), percentage related to the total mass fraction for macro and micronutrients in raw and cooked lamb meat samples (n = 3)[a]

Sample	Mean (percentage / %)					
	Cu / (mg kg^{-1})	Fe / (mg kg^{-1})	K / (g 100 g^{-1})	Mg / (mg kg^{-1})	P / (g 100 g^{-1})	Zn / (mg kg^{-1})
Raw	1.5 ± 0.1 (56)[a]	11 ± 1 (19)[a]	0.89 ± 0.01 (68)[a]	490 ± 5 (54)[a]	0.42 ± 0.01 (56)[a]	14 ± 2 (21)[a]
Grill	0.89 ± 0.01 (30)[b]	5.4 ± 0.1 (10)[b]	0.72 ± 0.01 (68)[b]	365 ± 1 (43)[b]	0.35 ± 0.01 (53)[b]	4.1 ± 0.3 (6)[b]
Microwave oven	0.89 ± 0.12 (28)[b]	3.8 ±1.2 (7)[c]	0.59 ± 0.02 (64)[c]	341 ± 24 (41)[b]	0.31 ± 0.01 (49)[b]	4.1 ± 0.3 (4)[b]
Air fryer	1.0 ± 0.1 (39)[b]	4.2 ± 0.2 (8)[c]	0.72 ± 0.05 (73)[b]	367 ± 14 (48)[b]	0.36 ± 0.03 (57)[b]	1.5 ± 0.4 (2)[c]
Pressure cooker	0.84 ± 0.07 (29)[b]	1.9 ± 0.2 (4)[d]	0.29 ± 0.04 (76)[d]	223 ± 31 (44)[d]	0.20 ± 0.03 (48)[d]	0.6 ± 0.2 (1)[d]
Electric oven	1.0 ± 0.1 (40)[b]	4.4 ± 0.1 (9)[c]	0.71 ± 0.02 (75)[b]	359 ± 18 (48)[b]	0.35 ± 0.01 (59)[b]	5.3 ± 0.1 (7)[e]

[a]Calculated from total concentration (presented in Table 2). Results are expressed as 95% confidence intervals of three authentic repetitions. Means within the same column with different letters are significantly different (Tukey's test, $p < 0.05$).

for aged meat, respectively. Menezes *et al.*[6] evaluated the bioaccessibility of Ca, Cu, Fe, Mg, and Zn in raw and thermally processed beef, chicken and pork meat samples. The bioaccessibility percentages achieved were 12-30, 12-43, 19-28, 15-37, and 12-20% for beef; 8-22, 8-12, 8-16, 10-26, and 8-16% for chicken; and 8-27, 8-23, 8-20, 7-27 and 10-20% for pork, for Ca, Cu, Fe, Mg and Zn, respectively.

Evaluating a mineral's bioaccessibility can generate information about physiological active species in food regarding nutritional value.[17,43] Bioaccessibility depends on the food matrix, the nutritional characteristics of the food, microbial processes, food processing and preparation, and the chemical form of the food.[38]

Conclusions

Regarding the procedures evaluated in this study, lamb meat prepared by pressure cooking presented the most significant loss of analytes and bioaccessible fractions. The metrological traceability was guaranteed through the use of CRMs, achieving good trueness.

Concerning the *in vitro* digestion, not all of the total Cu, Fe, Mg, P and Zn content present in the lamb meat was released to the gastrointestinal tract. The element composition is affected by the culinary treatment, consequently affecting bioaccessibility. The lamb meat cooked in a pressure pan presented the lowest bioaccessibility, related to the loss by leaching. Although presenting relatively high bioaccessibility (ca. 76%), a RDI of only ca. 8-28% was achieved for K. The lamb meat can be considered a good source of Fe, P, and Zn, representing up to 60% RDI. Knowledge of bioaccessibility is important to choose the cooking procedure that best suits consumers' nutritional needs.

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Author Contributions

Julymar M. Higuera was responsible for investigation, performing experiments, writing original draft; Herick M. Santos for investigation, formal analysis; Aline F. de Oliveira for review and editing, investigation; Ana Rita A. Nogueira for conceptualization, supervision, funding acquisition, writing for review and editing.

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