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Bioaccessibility of Ca, Cu, Fe, Mg, Zn, and crude protein in beef, pork and chicken after thermal processing

Eveline A. Menezes[a], Aline F. Oliveira[b,c], Celia J. França[d], Gilberto B. Souza[b], Ana Rita A. Nogueira[b,*]

[a] Instituto de Ciências Exatas e da Natureza, Universidade da Integração Internacional da Lusofonia Afro-Brasileira, Unidade Acadêmica dos Palmares Rodovia CE 060, km 51, 62785-000 Acarape, CE, Brazil
[b] Embrapa Pecuária Sudeste, Rodovia Washington Luiz, Km 234, C.P. 339, 13560-970 São Carlos, SP, Brazil
[c] Group of Applied Instrumental Analysis, Departamento de Química, Universidade Federal de São Carlos, Rodovia Washington Luiz, Km 235, C.P.676, 13565-905 São Carlos, SP, Brazil
[d] Faculdade ASSER, Campus Rio Claro, 13500-200 Rio Claro, SP, Brazil

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ABSTRACT

The bioaccessibility of Ca, Cu, Fe, Mg, Zn, and crude protein was evaluated after submitting beef, pork, and chicken to five different thermal treatments. The bioaccessibility of crude protein and metals were simulated by using *in vitro* enzymatic digestion with a gastric fluid solution and dialysability approach. Inductively coupled plasma optical spectrometry was used to quantify the dialyzable fraction and the total mineral content after microwave-assisted digestion. Graphite furnace atomic absorption spectrometry quantified Cu in chicken dialyzable fraction. The increase of temperature and heat exposure period decreased the protein bioaccessibility. Considering the total and dialyzable fraction, beef is an important source of Cu, Fe, Mg, and Zn to the human diet. The results of Fourier-transform infrared spectroscopy indicated physical changes in the treated samples related to protein denaturation, which was probably responsible for the decreased bioaccessibility of minerals and protein, mainly at higher temperatures.

1. Introduction

Meat contains valuable nutrients for human health (FAO, 2014). It is a primary source of protein, vitamins, and minerals in human nutrition, particularly for the trace minerals selenium, zinc, and heme-iron, from myoglobin and hemoglobin (Cabrera, Ramos, Saadoun, & Brito, 2010). Meat and meat products are essential components of diets in developed countries and their consumption has generally been associated with good health and prosperity in those countries.

Despite the predominant consumption of beef in certain developed nations, such as Canada and Australia (Baghurst, Record, & Leppard, 2000), pork is the most widely consumed meat in developed countries, representing over 50% of the total meat consumed. Unlike beef, most pork is consumed in further processed form (ham, sausages), with fresh pork normally consumed in much smaller quantities. In developed countries, the average amount of meat consumed each day, including pork, beef, veal, and mutton, is approximately 110 g (FAO, 2009). Chicken is considered a healthier diet choice since it has lower fat content as well as higher proportion of polyunsaturated fatty acids (PUFA) compared to other meat types (Riovanto, Marchi, Cassandro, & Penasa, 2012).

The trace mineral content of meat varies depending on the breed, rearing, diet, cut and carcass processing (Cabrera et al., 2010; Purchas, Simcock, Knight, & Wilkinson, 2003). Meat is generally cooked before consumption, to increase palatability, digestibility, and safety (Alfaia, Lopes, & Prates, 2013; Bognár, 1998; Tornberg, 2005). Goran, Tudoreanu, Rotaru, and Crivineanu (2016) reported that physical, chemical and sensory properties change during cooking, resulting in weight loss, modifications of water-holding capacity, texture changes due to protein and fat denaturation, water and color loss (or enhancement), and aroma release.

The mineral and vitamin loss after thermal treatment decreases the nutritional value of the product. The loss of minerals in cooked food varies depending on the mineral, and the cooking process employed and is significantly smaller after roasting compared to boiling the same type of food (Bognár, 1998). Microwave cooking has gained considerable importance as a convenient, energy and time-saving method, but this cooking method can cause changes in moisture, proteins, carbohydrates, lipids, minerals, and vitamins, and result in higher moisture

* Corresponding author.
E-mail address: ana.nogueira@embrapa.br (A.R.A. Nogueira).

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losses compared to conventional methods (Cross & Fung, 1982).

Some researchers have focused on the total content of nutrients present in foods (Ramos, Cabrera, & Saadoun, 2012). However, regarding nutrition, measurement of the total nutrients present does not provide the information necessary for an accurate evaluation of the effects. Bioaccessibility testing is needed to determine if the minerals reported are released from the food matrix into the gastrointestinal tract, making them accessible for intestinal absorption (Cardoso, Afonso, Lourenço, Costa, & Nunes, 2015; Fernández-García, Carvajal-Lérida, & Pérez-Gálvez, 2009; Moreda-Piñeiro et al., 2011).

There is little information on the effects of thermal treatment on bioaccessibility of nutrients to our knowledge. Depending on factors such as meat type and processing, the constituents can be more or less bioaccessible. (Afonso et al., 2015; Cardoso et al., 2015).

The bioaccessibility of nutrients in foods can be estimated using *in vivo* and/or *in vitro* methods (Afonso et al., 2015; Lima et al., 2014; Peixoto, Devesa, Vélez, Cervera, & Cadore, 2016; Rebellato, Pacheco, Prado, & Pallone, 2015). The combination of these methods provides information that can help interpret the results. *In vitro* methods can be applied using a system of simulated gastrointestinal digestion with pepsin in the gastric phase, and a mixture of pancreatin and bile salts in the intestinal tract phase. The analyte diffuses through a semipermeable membrane in the intestinal phase, which is used to measure the dialyzable fraction of the compound (Miller, Schricker, Rasmussen, & Van Campen, 1981). The present investigation was carried out to determine the bioaccessibility of Ca, Cu, Fe, Mg, and Zn through *in vitro* simulation with raw and thermally processed samples of beef, chicken and pork. Moreover, the digestibility of proteins after submitting the samples to the different cooking processes was determined, and thermally processed samples were characterized using infrared spectroscopy.

2. Material and methods

2.1. Reagents and materials

All solutions were prepared with deionized water (Milli-Q™ system, Millipore, Bedford, MA, USA). All glassware was previously decontaminated with 10% (v v^{-1}) HNO_3 for 24 h and rinsed with water. The analytical curve solutions were prepared from 1000 mg l^{-1} stock solutions of Ca, Cu, Fe, Mg, and Zn (Tritisol, Merck). Nitric acid (Merck, Darmstadt, Germany) was previously purified using a sub-boiling distillation system (Berghof, Eningen, Germany) and 30% m v^{-1} hydrogen peroxide (Labsynth, Diadema, SP, Brazil) was used to digest the samples. The digestive enzymes pancreatin, pepsin, and bile salts were obtained from Sigma-Aldrich (St. Louis, MO, USA), and membranes of 10 to 12 kDa, 33 × 21 mm and 25 Å porosity (Cial, Brazil) were used in the dialysis process. For crude protein determination and digestibility tests, sulfuric acid and sodium hydroxide were used (Merck, Germany). Copper sulfate, potassium sulfate, trichloroacetic acid, and bromo cresol green and methyl red indicator solutions were acquired from Synth (Rio de Janeiro, Brazil). For Fourier-transform infrared spectroscopy (FTIR) analyses, KBr (Carlo Erba, Italy) pellets were prepared.

2.2. Samples and sample preparation

All samples were purchased from local markets in Sao Carlos, SP, Brazil. The experiments were performed with raw beef, pork shank, and chicken breast. All meat samples were trimmed of fat, connective tissue, and skin. The raw samples were ground in a processor and homogenized, in triplicate for the different thermal processes. No seasoning was added to any sample. The thermal processes consisted of: (1) Raw (IN): no thermal processing; (2) Cooked in water (CW): samples were cooked in a stainless steel pan filled with 500 ml of cold water for 30 min; (3) Baked in microwave oven (MW): baked in a glass container covered with plastic film at 650 W power for 6 min; (4) Grilled (GR): grilled for 10 min on a preheated grill; (5) Baked in conventional oven (B1): baked in a glass container covered with foil for 45 min at 180 °C in a preheated conventional oven; and (6) Baked in conventional oven (B2): baked in a glass container covered with foil for 60 min at 180 °C in a preheated conventional oven. After cooking, the samples were ground in a processor and frozen at −20 °C. For total analysis, a part of each sample was freeze dried for 24 h (MicroModulyo, New York, USA), and cryogenically milled (MA 775, Marconi, Piracicaba, Brazil) according to the following program: 4 min of pre-freezing, and 2 min of milling, interspersed with 2 min freezing intervals. The remaining part of the samples was used for dialysis in *in vitro* tests. The samples used in the *in vitro* tests were prepared as described in section 2.6.

2.3. Instrumentation

The decomposition of samples was performed in a cavity microwave oven (Multiwave 3000, Anton Paar GmbH, Austria) equipped with temperature and pressure control and TFM™-PTFE vessels. The total content of minerals was measured with an inductively coupled plasma optical emission spectrometer (ICP OES), with radial view, a V-Groove nebulizer, and a Sturman Master spray chamber (Varian Vista RL, Mulgrave, Australia). The copper dialyzable fraction of chicken samples was measured with a graphite furnace atomic absorption spectrometer (GFAAS, GTA 100 SpectrAA-800, Varian), equipped with a transversal Zeeman corrector, using argon as the purge gas. Fourier-transform infrared measurements to evaluate structural changes in the samples subjected to thermal processing were performed in a Perkin-Elmer spectrometer (FTIR, Spectrum 1000, Perkin-Elmer, USA) and software Spectrum, version 5.3 (Perkin-Elmer, USA). The Kjeldahl crude protein was determined using a semiautomatic distiller (MA 0–36, Marconi, Brazil). The centrifugation steps were performed in an Excelsa Baby II 206-R (Fanem, Brazil) with polypropylene tubes.

2.4. Determination of total content of Ca, Cu, Fe, Mg and Zn

Certified reference materials SRM 8414 (Bovine Muscle) and SRM 1577b (Bovine Liver) from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA) were used to evaluate the accuracy of the total content determination. The microwave assisted digestions were performed as previously proposed by Gonzalez et al. (2009). The procedure consisted of weighting 250 mg of meat and reference material samples directly in the digestion flasks and adding 4 ml of 7 mol l^{-1} HNO_3 and 2 ml of 30% (m v^{-1}) H_2O_2. The digestion program was: (1) 2 min ramp up to 80 °C, (2) 3 min hold at 80 °C, (3) 4 min ramp up to 120 °C, (4) 5 min ramp up to 180 °C, (5) 5 min ramp up to 210 °C; and (6) 15 min cooling down.

After cooling, the acid digests were diluted to 10 ml with water and analyzed for measurement of Ca, Cu, Fe, Mg, and Zn by ICP OES. The instrumental parameters used for the analytical measurements were: 1.3 kW RF power, 15.0 l min^{-1} plasma gas flow rate, 1.5 l min^{-1} auxiliary gas flow rate, 0.6 l min^{-1} nebulizer gas flow rate, and 15 mm observation height. The selected lines were 396.847 nm (II) for Ca, 327.395 nm (I) for Cu, 267.716 nm (II) for Cr, 238.204 nm (II) for Fe, 280.270 nm (II) for Mg, 213.857 nm (II) for Zn (I and II are atomic and ionic lines, respectively). The limits of detection (LOD) and background equivalent concentration (BEC) were calculated according to Thompsen (2012).

2.5. Simulated in vitro gastrointestinal digestion

The digestions were performed in triplicate with simulated gastric fluid and simulated intestinal fluid; both prepared according to procedures described by Moura and Canniatti-Brazaca (2006). The simulated gastrointestinal digestion was carried out with pepsin-HCl during the gastric phase, and pancreatin and bile salts in the intestinal phase. All solutions were prepared immediately before use. The pepsin solution was obtained by dissolving 16 g of pepsin in 100 ml of 0.1 mol l^{-1} HCl.