



## Fatty acid profile and bacteriological quality of caiman meat subjected to high hydrostatic pressure



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### ABSTRACT

Our objective was to examine the effects of high hydrostatic pressure (HHP) on physico-chemical properties and bacteriological quality of caiman (*Caiman crocodilus yacare*) meat. Caiman tail meat was cut into 25 g chunks, individually vacuum packaged, and allocated to four treatments: non-treated (NT; not subjected to HHP), and samples subjected to HHP at 200 MPa (P2), 300 MPa (P3), and 400 MPa (P4). Physico-chemical properties, fatty acid profile, and bacteriological quality were evaluated. All HHP treatments (P2, P3, and P4) demonstrated lower ( $P < 0.05$ ) microbial loads than NT. HHP decreased ( $P < 0.05$ ) n-3 polyunsaturated fatty acid content and hypocholesterolemic/hypercholesterolemic ratio. However, HHP increased ( $P < 0.05$ ) n-6/n-3 ratio as well as indices of atherogenicity and thrombogenicity, which are critical indicators for the risk of cardiac diseases. The results suggest that while HHP improves bacteriological safety, it can compromise the fatty acid profile and nutritive value of caiman meat, which is rich in polyunsaturated fatty acids.

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### 1. Introduction

Non-traditional and exotic meat animals contribute to global food security by providing high quality animal proteins critical to human nutrition (Hoffman & Cawthorn, 2012). The popularity of exotic meats, as caiman (*Caiman crocodilus yacare*) meat, is increasing due to their valuable source of animal proteins of high biological value (Romanelli, Caseri, & LopesFilho, 2002) and lower lipid content which is low in saturated fatty acids (SFA), high in polyunsaturated fatty acids (PUFA) and n-3 fatty acids, and has a desirable PUFA/SFA ratio than the meats from conventional livestock (Hoffman & Cawthorn, 2013; Vicente-Neto et al., 2010).

The growing consumer demand for high quality meat products has stimulated the industry to develop novel preservation technologies (Aubourg, Tabilo-Munizaga, Reyes, Rodríguez, & Pérez-Won, 2010). High hydrostatic pressure (HHP) is a non-thermal

preservation technology, wherein foods are subjected to pressure at or above 100 MPa using liquids as pressure transmitter (Clariana, Guerrero, Sárraga, & Garcia-Regueiro, 2012). The HHP treatment improves food safety by inactivating microorganisms and extending the shelf life (Vaudagna et al., 2012). Furthermore, HHP can be also applied to pre-packaged foods, thus decreasing the risk of recontamination (Toepfl, Mathys, Heinz, & Knorr, 2006).

Depending on the level of pressure and duration of exposure, HHP can cause physico-chemical changes in food matrix, such as proteolysis, lipolysis, and alteration of fatty acid composition (Clariana et al., 2012; He et al., 2012; McArdle, Marcos, Kerry, & Mullen, 2011; Wang et al., 2013). These HHP-induced physico-chemical changes can influence nutritive value (McArdle, Marcos, Kerry, & Mullen, 2010; Yagiz et al., 2009) of muscle foods. Previous studies reported that HHP improved microbial quality and induced changes in the physico-chemical properties of fresh beef (McArdle et al., 2010, 2011) and chicken (Bolumar, Andersen, & Orlie, 2011). However, the impacts of HHP on bacteriological quality and physico-chemical properties of PUFA-rich fresh caiman meat have not been examined. Therefore, the aim of the present study was to

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evaluate the effects of HHP on physico-chemical properties, fatty acid profile, and bacteriological quality of caiman meat.

## 2. Materials and methods

### 2.1. Caiman meat

Using the tail meat from four caiman (*Caiman crocodilus yacare*) carcasses (30-month old, farm-raised) in each trial, the experiment was repeated five times ( $n = 5$ ). For each trial, the tail cuts of caiman carcasses were procured from a federally-inspected slaughterhouse at Caceres, Mato Grosso, Brazil. The cuts were deboned, and tail meat (500 g) from each carcass was individually vacuum-packaged, frozen, and transported in dry-ice to Universidade Federal Fluminense, where they were thawed at 4 °C overnight. Thawed meat was cut into 25 g chunks, individually vacuum packaged, and the packages were randomly allocated to four pressure treatments.

### 2.2. High hydrostatic pressure treatment

High pressure processing was accomplished at the Embrapa Agroindustria de Alimentos (Rio de Janeiro, Brazil). Based on previous research (Canto et al., 2012), 4 treatments were used in this study: non-treated control (NT; vacuum-packaged but not subjected to HHP), and samples subjected to 200 MPa (P2), 300 MPa (P3), and 400 MPa (P4) HHP. The equipment used for HHP treatment was a Stansted Fluid Power pressurizer (model S-FL-850-9-W, Essex, United Kingdom). A solution of ethanol:water (7:3 volume ratio) was used as the pressure-transmitting liquid. Vacuum-packaged meat was subjected to HHP for 10 min at 20 °C. Meat pH, proximate composition, and fatty acid profile were evaluated on samples immediately after HHP treatment, whereas bacteriological quality was examined at specific time points during refrigerated storage of HHP-treated vacuum packaged meat.

### 2.3. Meat pH and proximate composition

The pH values were measured using a digital pH meter (Digimed, Sao Paulo, Brazil) after homogenizing 10 g of sample in 90 mL of distilled water (Conte-Junior, Fernández, & Mano, 2008). The moisture, protein, ash, and lipid contents were determined according to AOAC (2012).

### 2.4. Fatty acid profile

The total lipids in caiman tail meat were cold-extracted based on Bligh and Dyer (1959) modified by Conte-Junior, Soncin, Hierro, and Fernandez (2007), and fatty acid methyl esters (FAMES) were analyzed by gas chromatography equipped with flame ionization detector (Perkin Elmer, Waltham, MA, USA). The lipids were extracted from 5 g sample aliquots using a methanol:chloroform (2:1 volume ratio) mixture and fatty acid methyl esters (FAMES) were obtained through acid methylation (Chin, Liu, Storkson, Ha, & Pariza, 1992; Kishino, Ogawa, Ando, Omura, & Shimizu, 2002). FAMES (1 µL) were separated using a Carbowax/BTR column (30 m length, 0.32 mm internal diameter, and 0.25 µm particle size) (Supelco Inc., Bellefonte, PA, USA). The injector and detector temperatures were set at 260 °C and 280 °C, respectively. The initial temperature of the oven was set at 110 °C, and the temperature ramp was: increase from 150 °C to 233 °C at 40 °C/min, hold at 233 °C for 2 min, increase from 233 °C to 240 °C at 1 °C/min, and hold at 240 °C for 2 min. Helium was used as the carrier gas at a flow rate of 1.8 mL/min and 96.53 kPa. FAMES were identified by comparing the retention time of commercial standard comprising

methyl esters of 15 individual fatty acids (Supelco Inc., Bellefonte, PA, USA). Individual fatty acids were expressed as percentage of total identified FAME, and were then categorized SFA, mono-unsaturated (MUFA), and PUFA fatty acids.

The nutritional quality of lipid was assessed using three different indicators: index of atherogenicity (IA), index of thrombogenicity (IT), and hypocholesterolemic to hypercholesterolemic ratio (h/H). IA and IT were calculated according to Ulbricht and Southgate (1991) with modifications, whereas h/H was calculated according Santos-Silva, Bessa, and Santos-Silva (2002) as follows:

$$IA = (4 \times C14 : 0 + C16 : 0) / (\Sigma MUFA + \Sigma(n - 6) + \Sigma(n - 3))$$

$$IT = (C14 : 0 + C16 : 0 + C18 : 0) / (0.5 \times \Sigma MUFA + 0.5 \times \Sigma(n - 6) + 3 \times \Sigma(n - 3) + 100 \times \Sigma(n - 3) / \Sigma(n - 6))$$

$$h/H = (C18 : 1n9 + C18 : 2n6 + C20 : 4n6 + C18 : 3n3 + C20 : 5n3 + C22 : 5n3 + C22 : 6n3) / (C14 : 0 + 16 : 0)$$

### 2.5. Bacteriological analysis

Vacuum packaged samples (NT and HHP-treated) were stored at 4 °C for three months, and the bacteriological quality was evaluated on 0, 15, 30, 45, 60, 75, and 90 days of storage. Packages were opened under aseptic conditions, and the contents (25 g) were homogenized with 225 mL sterile peptone saline for 2 min in a stomacher (Stomacher 80, Seward Ltd., London, United Kingdom). The samples were serially diluted and 1 mL aliquots were inoculated into Petri dishes containing culture media (HiMedia, Mumbai, India). Total aerobic mesophilic bacteria (TAMB) and total aerobic psychrotrophic bacteria (TAPB) viable counts were enumerated by pour plating on plate count agar followed by incubation at 35 °C for 48 h and 7 °C for ten days, respectively (Erkan & Üretener, 2010). Lactic acid bacteria (LAB) counts were analyzed by pour plating with over layer plates on the Man, Rogosa, and Sharpe agar followed by incubation at 30 °C for 120 h under anaerobic conditions (Hall, Ledenbach, & Flowers, 2001). The results were expressed in log CFU/g. The presence of *Salmonella* spp. was evaluated on day 0 of storage according to the rapid method (Pignato et al., 1995), and were expressed as presence or absence in 25 g of samples.

### 2.6. Statistical analysis

The experiment was a completely randomized block design with five replicates ( $n = 5$ ). Data were analyzed using XLSTAT (Version 2012.6.08, Addinsoft, Paris, France). One-way ANOVA was performed to evaluate the influence of HHP (NT, P2, P3, and P4) on physico-chemical parameters and fatty acid profile. The experiment for bacteriological quality was a 4 × 7 factorial with four treatments (NT, P2, P3, and P4) and seven time points (0, 15, 30, 45, 60, 75, and 90 days), and was evaluated by two-way ANOVA. Tukey's test was used to determine differences among means at  $P < 0.05$  level of significance.

## 3. Results and discussion

### 3.1. Meat pH and proximate composition

HHP-treated caiman meat demonstrated greater ( $P < 0.05$ ) pH than NT samples (Table 1). Among the HHP-treated samples, P4 demonstrated the greatest pH ( $P < 0.05$ ). Our results on pH are in agreement with those of previous researchers on other meats, who observed that pressure levels greater than 200 MPa increased the

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