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Microencapsulated jabuticaba (*Myrciaria cauliflora*) extract added to fresh sausage as natural dye with antioxidant and antimicrobial activity

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ABSTRACT

The aim was to evaluate the addition of microencapsulated jabuticaba extract (MJE) to fresh sausage as natural dye with antioxidant and antimicrobial activity. Fresh sausages without dye, with cochineal carmine and with addition of 2% and 4% MJE were evaluated for chemical, microbiological and sensory properties during 15 days of refrigerated storage. TBARS values were lower ($P < 0.05$) throughout the storage period in sausages with 2% and 4% MJE (below 0.1 mg of malondialdehyde/kg sample) than in control and carmine treatments (from 0.3 to 0.6 mg of malondialdehyde/kg sample). T2% and T4% also showed lower microbial counts on storage days 4 and 15 for APCs. The addition of 4% MJE negatively influenced ($P < 0.05$) sensory color, texture and overall acceptance attributes. On the other hand, T2% presented similar ($P > 0.05$) sensory acceptance to control and carmine treatments in most of the attributes evaluated except for a decrease in color. Thus, addition of 2% MJE to fresh sausage can be considered as a natural pigment ingredient.

1. Introduction

In Brazil, about 89% of pork is consumed as processed products such as fresh sausages, cooked ham and bologna. Consumption of these meat products is growing, mainly due to the convenience and easy preparation. However, consumers have been linking the consumption of meat products to the increase of obesity, cancer and cardiovascular disorders and are increasingly seeking for safe, nutritious, healthy, convenient and tasty food (ABPA, 2015; Lemos, 2015). Besides, during the processing and storage of fresh sausages, one of the biggest problems is discoloration and lipid oxidation, which causes a decrease in food quality, adversely affecting the color, flavor and texture of the product (Mielnik, Sem, Egelandsdal, & Skrede, 2008). Thus, one of the meat industry's main challenges is to replace synthetic preservatives and antioxidants with natural ones (Almeida et al., 2015; Pham, Willians, Kin, Xiong, & Schilling, 2014; Qi, Huang, Huang, Wang, & College, 2015; Stefanello et al., 2015).

Fresh sausages, and most meat products, undergo some degree of processing before reaching the consumer, and sometimes manufacturers

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have to replace the color lost during processing and storage. Color decisively influences the consumer's preference for food products. Consequently, this has led the food industry to develop and use different synthetic and natural dyes in foods (Barros & Stringheta, 2006; Bridle & Timberlake, 1997).

The number of allowed synthetic additives has been reduced because of their toxicity, which in turn has gradually increased the demand for natural dyes. Among natural dyes, the most commonly used in the food industry are annatto, curcumin, betalains, cochineal carmine and anthocyanins extracts (Silva, Constant, Figueiredo, & Moura, 2010a). Currently, the use of natural products as colorants as well as antioxidants and antimicrobials has increased.

Jabuticaba peel could represent a viable alternative for providing red color to food as well as having antioxidant and antimicrobial activity. Jabuticaba is a native fruit from Brazil with higher productivity in the Southeast. From a nutritional point of view, it is of interest because of its rich composition of niacin, iron and anthocyanins (Donadio, 2000).

Anthocyanins are water soluble vegetable pigments, responsible for the color of dark fruit, that belong to the class of phenolic compounds known as flavonoids. These compounds may present antioxidant, anti-inflammatory, antimutagenic, chemoprotective and antimicrobial activities. Alezandro, Granato, and Genovese (2013a) evaluated daily intake of jabuticaba for 40 days in rats and assessed the oxidative stress related to diabetes. Observed benefits included an improved lipid profile and reduced oxidative stress. Besides reducing water consumption and energy intake, jabuticaba reduced total cholesterol levels and triacylglycerol, increased antioxidant capacity of plasma and decreased lipid peroxidation in plasma and brain tissue. According to Reynertson et al. (2006), some flavonoid compounds extracted from jabuticaba presented an antiproliferative effect against HT29 and HCT116 colon cell lines. Leite et al. (2011) verified the consumption of lyophilized jabuticaba peel in rat diets and observed an increase of antioxidant potential in the plasma of those animals. According to Lima, Correa, Saczk, Martins, and Castilho (2011), anthocyanins from jabuticaba are more concentrated in the peel, which can be considered a residue from the processing of jabuticaba pulp, jams and liqueurs. The high concentration of bioactive anthocyanins in the fruit peels makes them a potential natural ingredient with antioxidant and antimicrobial activities.

However, the stability of these pigments when extracted from fruits and vegetables may be affected by exposure to oxygen, sunlight, metal ions, pH alterations, enzymes and temperature fluctuations. Among the current technological alternatives, spray drying may be used to microencapsulate pigments and avoid loss of bioactivity (Castañeda-Ovando, Pachecho-Hernadez, Paez-Hernadez, Rodriguez, & Galan-Vidal, 2009; Fang & Bhandari, 2010).

Therefore, the aim of this study was to evaluate the replacement of commercial carmine cochineal dye in fresh sausage by microencapsulated jabuticaba extract as a natural dye with antioxidant and antimicrobial activity.

2. Material and methods

2.1. Production of jabuticaba residue aqueous extract and microencapsulation process

The aqueous extract of jabuticaba residue was obtained according to the procedure described by Silva et al. (2014), with modifications. Initially, the fruit was pulped to obtain its residue (peels and seeds). Extraction of the pigment from jabuticaba residue was accomplished with addition of water in a ratio of 1:3 (residue:water) (Terci & Rossi, 2002), in the absence of light and under mechanical agitation (Fisaton 713, São Paulo, Brazil) for 6 h. The fluid obtained was filtered and the crude extract was concentrated to 1/3 of its original volume using a rotary evaporator (TE-211, Tecnal, Piracicaba, Brazil) at 60 °C. After concentration, the extract was mixed with maltodextrin MOR-REX® 1910 (DE10) (Ingredion, Mogi Guaçu, Brazil) using a mechanical stirrer (Fisaton 713, São Paulo, Brazil) and microencapsulated in a spray dryer (model MSD 5.0, Labmaq, Ribeirão Preto, Brazil) with a 1.5-mm diameter atomizing nozzle at an airflow of 40 L/min. The independent variables were 150 °C inlet drying air temperature and 30 mL/min feed flowrate and the concentration of the carrier agent was 260 g of maltodextrin/kg of extract.

2.2. Total anthocyanin content

Analysis of total anthocyanin content was carried out using the method of pH difference (Giusti & Wrolstad, 2001). Absorbance of the anthocyanin was measured at 510 nm and 700 nm in a spectrophotometer (Model S-22 pound, Biochrom, Cambridge, UK) and the results were expressed in equivalent of cyanidin-3-glucoside/g extract. The molar extinction coefficient 26,900 L/cm·mol and the molecular weight 449.2 g/mol were used for cyanidin-3-glucoside (Rockenbach et al., 2011).

2.3. In vitro antioxidant capacity

2.3.1. Folin–Ciocalteu method for total phenolics

The analysis was performed according to Georgé, Brat, Alter, and Amiot (2005) with modifications. 100 μL of extract was mixed in a test tube with 500 μL of Folin–Ciocalteu reagent. After 2 min 400 μL of 7.5% sodium carbonate solution was added to the test tube and mixed. The samples, prepared in triplicate, were placed into a water bath at 50 °C for 15 min. The absorbance at 760 nm was determined using a spectrophotometer (Ultrospec 2000, Pharmacia Biotech) and phenolic total content calculated using a standard curve of gallic acid (Sigma Chemical Co., St. Louis, USA). The results were expressed as mg gallic acid equivalent/g of extract.

2.3.2. Reducing capacity of iron (FRAP)

The analysis was performed by FRAP reagent from the mixture of 300 mM sodium acetate buffer pH 3.6 with tripiridil-2,4,6-s-triazine (TPTZ Sigma-Aldrich Chemical Co., Bellefonte, PA) 10 mM in 40 mM HCl solution and ferric chloride ($FeCl_3 \cdot 6H_2O$) 20 mM in a ratio of 10:1:1, respectively. Assays were performed in triplicate and placed into water bath at 37 °C for 30 min. Results were measured using a spectrophotometer (Ultrospec 2000, Pharmacia Biotech) at 593 nm. Values were obtained using a calibration curve with a solution of 1000 mM of Trolox and expressed as μmol of Trolox equivalent/L of extract (Benzie & Strain, 1996).

2.3.3. Antioxidant activity by DPPH•

The determination of the DPPH antioxidant activity was performed according to the methodology of Brand-Williams, Cuvelier, and Berset (1995). The extraction was carried out by adding 20 mL of water to 0.01 g of sample. Aliquots of 0.1 mL of these extracts were transferred to different tubes and added by 3.9 mL of the solution of DPPH (2,2-diphenyl-1-picryl-hidrazila, Sigma-Aldrich Chemical Co., Bellefonte, PA) in methanol (6×10^{-5} M). After mixing, the tubes were left to stand under light and, after 60 min, the absorbance was measured at 515 nm. The same analysis was performed for Trolox solutions in several dilutions, making a standard curve. The results were expressed in mmol of Trolox equivalent/L of extract.

2.4. In vitro antimicrobial activity

2.4.1. Determination of antimicrobial activity of MJE

The microencapsulated jabuticaba extract (MJE) was evaluated for its antimicrobial activity. The test was carried out according to the NCCLS standard M7-A6 (NCCLS, 2003 and Martin et al., 2012), with modifications. A bacterial suspension with *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 was prepared by inoculating the microorganisms into 10 mL Brain Heart Infusion (BHI) broth