International Food Research Journal 26(3): 811-817 (June 2019)

Journal homepage: *http://www.ifrj.upm.edu.my*

Jambolão extracts as synthetic additive substitutes in fresh chicken sausage during cold storage

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Article history

Received: 16 September, 2017
Received in revised form: 25 November, 2018
Accepted: 15 January, 2019

Keywords

natural antioxidant
hydroethanolic extract
lipid oxidation

Abstract

The World Health Organization (WHO) recently included processed meats on the carcinogenic potential products list, mainly because of some synthetic additives used on these products. Besides, consumers have started to look for a healthier and preservative-free diet, worrying about additives that are included in food products. These reports demand urgent development of healthier meat products with natural additives as the best alternative. Therefore, the aim of the present work was to develop and study the stability of a new chicken fresh sausage formulation through the use of Jambolão pulp and peel extract (JPPE) instead of the synthetic additives normally used. Two experiments were performed: (1) extract characterisation (colour, pH, total soluble solids content, phenolic compounds and antioxidant activity by DPPH• radical capture method), and (2) fresh chicken sausage characterisation in the different concentrations (positive control – 0.25% sodium nitrite, negative control - no additives and addition of 2% and 4% of Jambolão extract) on the centesimal composition and stability evaluation of sausage through colour analysis (L^*, a^*, b^*), pH, water activity and lipid oxidation (TBARS) during 12 days cold storage period. Hydroethanolic extract presented better antioxidant activity (EC_{50} = 12.15 $mg.mL^{-1}$) than aqueous extract (EC_{50} = 23.40 $mg.mL^{-1}$). Extract addition did not change the main quality parameters of chicken fresh sausage, but on the other hand accelerated its lipids oxidation. Minor amounts of Jambolão extract should be tested.

Introduction

Sausage production increases the shelf life of meat products in a fast, practical, nutritious and economic way. Fresh sausages are the most consumed meat products in several countries, mainly due to their affordable cost. However, high fat content, the nature of raw materials, and the absence of heat treatment render fresh sausages vulnerable to oxidative processes thereby requiring the addition of synthetic additives, such as antioxidants, in their formulations.

Antioxidants are important to prevent or delay oxidative processes which is one of the main problems observed in meat products, causing changes in sensory parameters, loss of nutritional value and free radicals formation. However, the use of synthetic antioxidants has been questioned since studies have shown that there is an association between the consumption of processed meats and the increased risk of certain types of cancer (Larsson *et al*., 2006; Larsson and Wolk, 2012). Nitrites and nitrates used as preservatives in processed meat products contribute towards oxidative processes prevention; but throughout the storage period they produce the potentially carcinogenic nitrosamines that may be responsible for the association of processed meats with cancer (Larsson *et al*., 2006; Pegg and Shahidi, 2006) and the inclusion of processed meats on the list of carcinogenic potential products by the World Health Organization. Therefore, the use of natural additives to inhibit or delay oxidation processes becomes a viable alternative for meat products (Dimitrios, 2006).

Jambolão (*Syzygium cumini* Lam.) is a native fruit from Indonesia, grown in many countries and known for its high antioxidant activity and rich in

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anthocyanins. Anthocyanins are natural substance that aroused interest due to its nutritional and therapeutic effects. Studies involving Jambolão fruits have been carried out with different extraction methods and significant amounts of antioxidants such as phenolic compounds, anthocyanins and tannins have been found (Luzia and Jorge, 2009; Veber *et al*., 2015). However, practical studies involving the use of Jambolão extracts in meat products are still lacking.

In this sense, and considering that the demand for "natural" foods which are free of synthetic additives and have health benefits has significantly increased, the objective of the present work was to develop and study the stability of a new chicken fresh sausage formulation through the use of Jambolão pulp and peel extract (JPPE) instead of the synthetic additives normally used.

Material and methods

Extracts preparation and physicochemical measurements

Jambolão fruits were harvested at Instituto Federal do Triângulo Mineiro - Campus Uberaba from January to May 2016, washed under running water, sanitised with sodium hypochlorite solution (2.5%) for 15 min and then frozen. To prepare the powder, the frozen fruits were thawed, the seeds were removed, and the remaining portion (pulp and peel) was then freeze-dried (-54°C) for 4 d in a benchtop freeze drier (L101, Liotop, Brazil). After freeze-drying, the material was ground in a refrigerator mill and sieved (48 mesh) for particle size uniformity. The powder obtained was placed in plastic bottles and stored in a freezer until extract preparation.

Extraction was performed following the methodology described by Adámez *et al*. (2012) with modifications. Extracts were prepared at 1:10 (w/v) with distilled water (aqueous extract) and ethanol 50% (v/v) (hydroethanolic extract). In both cases, the extraction process was performed in an ultra-thermostat water bath (MA184/BX, Marconi, Brazil) at 45°C for 3 h. Extracts were filtered through filter paper Whatman #1 (Whatman International Ltd., UK) and stored in a refrigerator under dark conditions until analyses.

For pH measurements, a portable pH meter (T-1000, Tekna) was used. Colour of the extracts was determined using a portable colorimeter (MiniScan EZ, HunterLab, USA) with 10° observation angle, illuminant D65, and the CIE Lab colour scale. The soluble solid content (°BX) of the extracts was measured with a manual refractometer (2WAJ, Abbe). All measurements were done in triplicates.

Preliminary tests were performed in order to determine the stabilisation time between JPPE and DPPH$^{\bullet}$ (2,2-diphenyl-1-picryl-hydrazil) radical (60 min) and the best dilutions (40 to 400 µL.mL^{-1}) for the aqueous and (20 to 200 µL.mL^{-1}) hydroethanolic extracts. Absorbances were read at 515 nm every 5 min in spectrophotometer (UV–VIS lambda 35, Perkin Elmer, USA).

Antioxidant activity of extracts

The DPPH$^{\bullet}$ radical capture test described by Brand-Williams *et al*. (1995) and Rufino *et al*. (2007) was used to evaluate the antioxidant activity of extracts. Ten dilutions of extracts were made using 99% ethanol in triplicate in order to plot the standard curve and calculate the inhibition of absorption. Absorbance readings were taken after the reaction time of the Jambolão extracts with the DPPH$^{\bullet}$ radical (60 min) using a spectrophotometer (UV–VIS lambda 35, Perkin Elmer, USA) at 515 nm. The EC_{50} calculation was used to obtain results. EC_{50} is the required amount of antioxidant to reduce the DPPH$^{\bullet}$ radical absorbance by 50%.

Total phenolic content of extracts

The total phenolic content was analysed according to Singleton *et al*. (1999) using the Folin-Ciocalteau reagent and gallic acid as the standard for the calibration curve. Extracts were diluted 100 times to perform the analyses. 500 µL of sample (extract or gallic acid) were mixed in a test tube with distilled water (4 mL), Folin-Ciocalteau reagent (500 µL) and saturated sodium carbonate solution (500 µL). The samples, prepared in triplicate, were placed into a water bath at 37°C for 30 min. The absorbance at 750 nm was determined using a spectrophotometer (UV-380G, Gehaka, Brazil), and the total phenolic content was calculated using a standard curve of gallic acid. The results were expressed as mg gallic acid equivalent.g^{-1} extract. The best extract (hydroethanolic or aqueous) to be used in the production of fresh chicken sausage was chosen from the total phenolic content and the antioxidant activity results.

Production and stability of fresh chicken sausage formulations

Chicken breast meat used for the fresh sausage production was purchased from a butcher's shop located in Uberaba, MG – Brazil, two days before manufacturing and kept refrigerated (± 2°C). All manufacturing, packaging and storing procedures were carried out at the meat processing plant of

Instituto Federal do Triangulo Mineiro – Campus Uberaba.

The sausage meat matrix was composed of 90% chicken breast, 10% pork backfat and other ingredients as shown in Table 1. The raw materials were grinded on 8 mm discs, blended with ingredients for 5 min and stuffed into natural pork casings with 30 mm diameter by using manual filler. Sausages were then tied in 10 cm intervals, packed in polyethylene plastic bags (without vacuum) and stored under refrigeration (± 2°C) and under dark conditions for 12 d. Three replicates of each treatment were produced and two sausage pieces (10 cm) were assigned for each five storage periods (0, 3, 6, 9 and 12 days), totalling 60 experimental units. Extract concentrations were chosen based on the conditions used by Baldin *et al*. (2016).

Table 1. Formulations used for fresh chicken sausages preparation.

Raw material	Treatment			
	PC	NC	EX2	EX4
Chicken breast (%)	90	90	90	90
Pork backfat (%)	10	10	10	10
Ingredients*				
Water (%)	5.0	5.0	5.0	5.0
Commercial condiment** (%)	4.0	4.0	4.0	4.0
Sodium tripolyphosphate (%)	0.25	0.25	0.25	0.25
Curing salt (99% NaCl + 1% $NaNO_3$) (%)	0.25	-	-	-
Jambolão extract (%)	-	-	2.0	4.0

PC = Positive Control: fresh sausage with 0.25% curing salt; NC = Negative Control: fresh sausage without antioxidant; EX2 = fresh sausage with 2% Jambolão extract; EX4 = fresh sausage with 4% Jambolão extract. *Based on raw material quantity (chicken breast + pork backfat); **Commercial chemical free blend composed of NaCl (98%) and natural herbs and spices.

The colour analysis was carried out using a portable colorimeter (CR 400, Konica Minolta, Japan) with 10° observation angle, illuminant D65 by measuring *L** (lightness), *a** (red intensity), and *b** (yellow intensity) scale values of the CIE system. Results were taken as an average of five measurements at different points of each sample. The pH was determined in triplicate using a pH meter (T-1000, Tekna). Water activity (aW) analysis was carried out using an electronic water activity meter (4TE Aqua Lab, USA) at 25°C in triplicate.

The moisture, mineral residue (ash) and protein percentage were determined using AOAC protocols (AOAC, 2005). Briefly, samples were dried in air circulating oven at 105°C for 12 h for moisture content determinations. Afterwards, the remaining material was placed in a muffle furnace at 550°C for 24 h for the determination of ash content. The crude protein content was determined by Kjeldahl method with conversion factor 6.25 to convert total nitrogen into crude protein. The lipid determination was carried out using the Butirometer method (Terra and Brum, 1988) in which the organic matter with the exception of lipids was carbonised by sulfuric acid. The lipid content was separated by centrifugation with the aid of amyl alcohol and directly measured from the butirometer scale.

The lipid oxidation was assessed by the determination of thiobarbituric acid reactive substances (TBARS) with malondialdehyde (MDA) as the main lipid peroxidation product according to the rapid, wet method described by AMSA (2012). Briefly, samples (1 g) were weighed in polypropylene centrifuge tubes, added with thiobarbituric acid solution (5 mL), incubated for 10 min in a water bath at 100°C, cooled under running tap water and centrifuged (5,000 g at 4°C) for 10 min. Following centrifugation, a portion of the supernatant was carefully pipetted to a spectrophotometer cuvette and absorbance readings were carried out using a spectrophotometer at 532 nm. The TBARS results (mg MDA.kg^{-1}) were obtained by multiplying absorbance readings by 2.77.

Statistical analysis

The obtained data were analysed as a Completely Randomised Design (DIC) using two treatments (Aqueous and Hydroethanolic extracts) with three repetitions. ANOVA and Tukey's test were performed using a 5% significance level. In order to evaluate the stability of sausage formulations over the storage period, data of colour, pH and water activity were analysed as 4 × 5 completely randomised factorial design (4 treatments and 5 storage times). Data from chemical composition were analysed as a 4 x 3 factorial design (4 treatments and 3 storage times). All results were obtained from three repetitions. ANOVA and regression analyses were performed using a 5% significance level. All statistical analysis were carried out using R software (R Core Team, 2013).

Results and discussion

Jambolão pulp and peel extracts characterisation

Table 2 shows the results for pH, total soluble solid content (°Brix), colour parameters (*L**, *a**, *b**), and DPPH• radical and total phenolic assays of JPPE. Results from the antioxidant activity by means of DPPH• radical assay, expressed as the necessary sample amount to diminish 50% DPPH• radical

Table 2. Mean values of pH, total soluble solid content (°Brix), colour parameters (*L*, a*, b**), DPPH• radical and total phenolic assays of Jambolão pulp and peel extract (JPPE).

Extracts	EC_{50} (mg. mL^{-1})	Total phenolics (mg.100 g^{-1})	pH	°Brix	*L**	*a**	*b**
Hydroethanolic	12.15 ± 0.36[b]	254.32 ± 0.31[a]	4.32 ± 0.03[a]	22.27 ± 0.10[a]	17.27 ± 1.92[b]	24.67 ± 3.32[b]	8.69 ± 1.80[a]
Aqueous	23.40 ± 0.54[a]	79.43 ± 0.30[b]	3.55 ± 0.04[b]	6.38 ± 0.22[b]	33.65 ± 2.65[a]	43.66 ± 0.40[a]	8.23 ± 1.67[a]
VC (%)	2.57	18.20	0.98	1.18	9.08	6.92	20.52

Means followed by the same lower case letters in a column do not differ significantly ($p < 0.05$). VC = variation coefficient; EC_{50} = antioxidant concentration required to diminish 50% initial DPPH• absorbance.

absorbance (EC_{50}), were 12.15 mg.mL^{-1} and 23.40 mg.mL^{-1} for hydroethanolic and aqueous extracts, respectively. Literature about JPPE is scarce, and thus, results were compared to Jambolão extracts produced with different plant parts. Veber *et al*. (2015) reported EC_{50} 56.09 ± 0.05 and 2.27 ± 0.09 mg.mL^{-1} for 30 min infusion at 80°C, and also 23.07 ± 0.39 and 3.75 ± 0.23 mg.mL^{-1} for 50% hydroethanolic Jambolão leaf and green fruit extracts, respectively. On the other hand, Luzia and Jorge (2009) found higher EC_{50} values (118.66 × 10^{-3} mg.mL^{-1}) for Jambolão seeds extracted in ethanol for 30 min at room temperature.

Regarding total phenolic content, hydroethanolic JPPE mean value was 3-fold higher (254.32 mg.100 g^{-1} gallic acid equivalent) than aqueous JPPE (79.43 mg.100 g^{-1} gallic acid equivalent). Kuskoski *et al*. (2006) found 194.7 mg.100 g^{-1} and 229.6 mg.100 g^{-1} gallic acid equivalent, for methanolic and ethanolic Jambolão extracts, respectively. Ethanolic extracts of green Jambolão pulp assessed by Brandão *et al*. (2011) showed 671.37 ± 2.62 mg.100 g^{-1} gallic acid equivalent, even higher than reported by Veber *et al*. (2015) using 50% hydroethanolic extracts (109.17 mg gallic acid equivalent. 100 g^{-1}) and ripen fruit extract (208.30 ± 2.24 mg.100 g^{-1} gallic acid equivalent). According to Veber *et al*. (2015) and Vizzotto and Pereira (2011), aqueous extracts may hinder total phenolic compound results because of the high level of impurities (organic acids, sugars, soluble proteins), resulting from the use of solvent. The differences concerning results of antioxidant activity and phenolic compounds of extracts could be due not only to different extraction methods, but also to influences from the habitat from which the fruits were harvested (Oliveira *et al*., 2009; Brandão *et al*., 2011; Veber *et al*., 2015). In the present work, hydroethanolic JPPE had better results than aqueous JPPE for antioxidant and total phenolic compounds, and for this reason, these extracts were chosen for the fresh chicken sausage production.

With regard to pH and soluble solid content of JPPE, significant differences between the two tested extracts have been found, with hydroethanolic JPPE showing higher values (4.32 and 22.27, respectively). According to Migliato *et al*. (2011), hydroethanolic extraction provides higher °Brix than aqueous, which is in agreement to our results. CIE *L** and *a** colour parameters of aqueous extracts showed higher ($p < 0.05$) luminosity and redness, while *b** (yellowness) did not differ between extracts.

Characterisation and stability of fresh chicken sausage with JPPE

No significant differences were found for moisture, crude protein and lipid content of fresh chicken sausages. Ash content, however, differed among treatments (Table 3). Positive control presented higher ash content than the other treatments (3.32 g.100 g^{-1}) possibly because of the presence of nitrite in this formulation (Nascimento *et al*., 2012).

Table 3. Mean values of ash, moisture, crude protein, lipid, pH and colour parameters (*L*, a*, b**) of different fresh chicken sausage formulations.

Treatments	Ash (g.100 g^{-1})	Moisture (g.100 g^{-1})	Protein (g.100 g^{-1})	Lipids (g.100 g^{-1})	pH	a_w	*L**	*a**	*b**	TBARS (mg MDA.kg^{-1})
PC	3.32 ± 0.36[a]	69.10 ± 1.32	19.24 ± 1.30	5.99 ± 1.07	5.66 ± 0.11	0.97 ± 0.01[b]	56.82 ± 2.25	3.16 ± 0.50	13.66 ± 1.25[a]	0.42 ± 0.11[c]
NC	2.96 ± 0.11[b]					0.98 ± 0.00[a]			12.95 ± 1.32[a]	0.57 ± 0.12[b]
EX2	2.87 ± 0.04[b]					0.98 ± 0.00[a]			13.00 ± 1.13[a]	0.64 ± 0.13[b]
EX4	2.87 ± 0.07[b]					0.98 ± 0.00[a]			11.45 ± 1.04[b]	0.76 ± 0.21[a]

PC = Positive Control: fresh sausage with 0.25% curing salt; NC = Negative Control: fresh sausage without antioxidant; EX2 = fresh sausage with 2% Jambolão extract; EX4 = fresh sausage with 4% Jambolão extract. Means followed by the same lower case letters in a column do not differ significantly ($p < 0.05$).

Proximate composition of fresh chicken sausages is in agreement with Padrão de Identidade e Qualidade da linguiça frescal (BRASIL, 2000), that stablishes maximum values of 70% and 30% for moisture and lipids, respectively, and minimum 12% for protein. Results concerning sausages' pH and *L** (luminosity) did not show difference in neither of the tested effects (treatment, storage time, and their interaction), as depicted in Table 3. Water activity (a_w) of positive control was significantly lower ($p < 0.01$) than the other treatments, which can be explained by the higher amount of NaCl (Honikel, 2010) from the curing salt composition (99% NaCl + 1% $NaNO_3$).

*a** showed significant difference between storage times ($p < 0.01$) and *b** between treatments and storage times ($p < 0.01$). *b** however did not differ among positive control, negative control and 2% JPPE addition, whereas 4% JPPE addition showed the lowest value (11.45), certainly because of the high inclusion of hydroethanolic JPPE which has purple colour since *b** scale ranges from blue (-60) to yellow (+60). The redness intensity of samples decreased throughout time (3.78 to 2.87), regardless of the treatment (Figure 1). This could probably be due to myoglobin oxidation in the chicken sausages. When this situation happens, an increase in the concentration of the oxidised form of this protein, metmyoglobin, is observed (Silva, 2014; Vargas, 2015). *b** values increased over time (Figure 1). As the samples' redness faded, the yellow intensity increased, regardless of the treatments. Changes in *b** values during storage time (12.22 to 13.72) can be described by the intensity of the oxidative process, which tends to increase the yellow colour of products because of rancidity (García-Esteban *et al*., 2004).

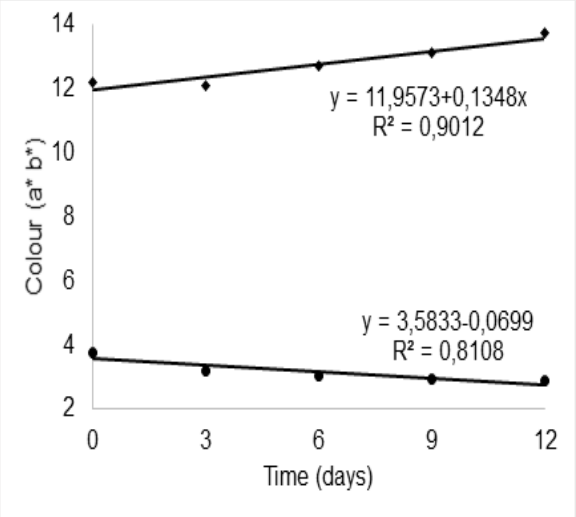

Figure 1. Evolution of colour parameters *a** (•) and *b** (♦) of fresh chicken sausages throughout storage.

Lipid oxidation by thiobarbituric acid reactive substances (TBARS) results, expressed in malonaldehyde equivalent (MDA), presented statistical difference between treatments ($p < 0.01$) and storage times ($p < 0.05$), with no interaction between effects (Table 3). According to Stefanello *et al*. (2015), it is not possible to perceive lipid oxidation odour when TBARS values are between 0.5-1.0 mg MDA.kg^{-1} meat sample, but only when the values are around 1-2 mg MDA.kg^{-1} meat sample. In the present work, lipid oxidation odour was not perceived. Nevertheless, the use of JPPE in the fresh sausages resulted in higher MDA values than control (Table 3). Similar situation has been reported by Vargas *et al*. (2016) who used pitangueira leaf extracts in beef patties, and reported that 10% extract inclusion resulted in lipid oxidation instead of protection. According to these authors, high inclusion rates of natural extracts may provide high concentration of prooxidant compounds present in natural extracts, such as pigments and metals as copper and iron. Decker (1998) also states that a balance between prooxidant and antioxidant compounds must be reached when using a multicomponent antioxidant system, as JPPE, in order to avoid undesirable results. Contrast to what has been found in the present work, Monteiro (2013), who assessed ethanolic pequi (*Caryocar brasiliense* Camb.) peel extracts (0.5, 1, and 1.5%) in chicken sausages at 4°C for 42 days, obtained TBARS values (0.421 ± 0.10 mg MDA. kg^{-1}) at the end of storage period for 1.5% extract addition, as compared to 1.375 ± 0.11 mg MDA.kg^{-1} for control. Likewise, Casagrande (2014) reported 55 and 73% MDA reduction using 2 and 4 mg.g^{-1} freeze dried ethanolic extract of grape juice pomace in fresh chicken sausage. In the present work, TBARS mean values varied from 0.5425 to 0.6283 mg MDA.kg^{-1} fresh chicken sausage throughout the 12-day storage. As expected, TBARS levels showed an increasing pattern, as depicted in Figure 2.

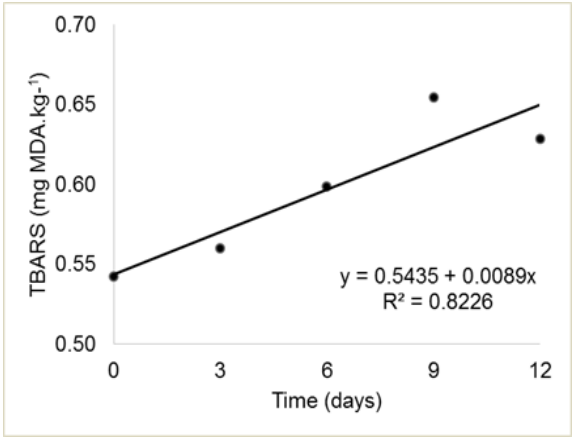

Figure 2. Evolution of TBARS of fresh chicken sausages throughout storage.

Conclusions

Jambolão hydroethanolic extracts presented better antioxidant capacity than aqueous extracts. The addition of Jambolão hydroethanolic extract did not affect moisture, protein and lipid contents, nor pH or colour parameters *L** and *a**. However, this treatment accelerated lipids oxidation. It is believed that lower concentration could exert better antioxidant effect on the fresh chicken sausages. There are very few studies on the antioxidant activity of aqueous and hydroethanolic JPPE and their applications in meat products. For this reason, further and deeper investigations on their properties are therefore recommended.

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