Original scientific paper

Development and characterization of low fat cooked yacare (*Caiman yacare*) meat sausages

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A b s t r a c t: The limited consumption of yacare (Caiman yacare) is due to cultural and economic factors, beyond a limited availability of products based on this meat. Here cooked sausages were developed from yacare meat shavings and fat substitutes (inulin and soy protein), and characterized. Moisture ranged from 63.90% (T1) to 59.89% (T3), a decrease with the increase in the inulin content (T1 had the lowest, T3 the highest inulin content). The protein content decreased from 27.67 (T1) to 25.32% (T3). The highest lipid content was 5.36% (T2) and the lowest 1.69% (T3). The ash content ranged from 4.50 to 4.62%. The highest luminosity value was obtained for T2 (59.69) and the lowest for T3 (57.24). The highest average shear force (18.01 N) was obtained for T3. Good sensory characteristics were obtained for all treatments, with acceptability indexes varying from 68.67 to 87.11%. However, the highest purchase intention was declared by 72% of panelists who certainly or probably would purchase T1.

Keywords: yacare meat, sausage, inulin, soy protein.

Introduction

Considered a reptile with high population density, the yacare (Caiman yacare) lives in different aquatic environments, between salt pans, freshwater lagoons, perennial and temporary rivers, and swamps, the proportions and stability of which vary from region to region (*Campos et al.*, 2010). The breeding of yacare, if well managed, can contribute to the economic and ecological evolution of a region by the production of an alternative source of proteins from animals intensively adapted to the natural conditions of that specific environment (*Carreira & Sabbag*, 2015).

Yacare meat is considered a rich source of proteins of high digestibility and biological value. It contains insignificant amounts of cholesterol and great technological potential for the elaboration of derived products. The meat processing, in addition to the noble cuts, generates shavings that are sold as baits. The preparation of meat products with this waste is an option to develop food products with high added value and obtain further economic gains (*Romanelli et al.*, 2002). Among a range of potential meat products that can be obtained from the

processing of yacare, cooked sausage can be underlined as an innovative technological option because this product is not found on the market.

The addition of pork fat is allowed in the production of cooked sausages. Due to the low fat content of yacare meat, fat replacers would be required in cooked yacare meat sausages. Literature reports several ingredients that can act as substitutes for animal fat in meat products (*Colmenero et al.*, 2012; *Cavenaghi-Altemio et al.*, 2013). Different product categories can be utilized as fat replacers: non-meat proteins (milk and soy proteins), base carbohydrates (carrageenan, starches and fibers such as inulin) and mixtures of ingredients (*Yashini et al.*, 2019). These fat substitutes are commonly used by the sausage meat industry, mainly for their ability to form gels, contributing consistency to the final product.

Textured soy protein is obtained industrially through the extrusion of defatted white soy bran. It is an essential ingredient in the preparation of some meat products and can be added in amounts up to 20% without changing the flavor (*Masson & Gelins-ki*, 2014). Soy proteins are the main functional components of some meat product technologies. Several

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authors mention that soy in meat products improves texture and emulsifying capacity, intensifies its appearance, firmness, juiciness, sliceability, and cooking efficiency, and reduces the formulation cost (*Xiong*, 2005; *Youssef & Barbut*, 2011).

Inulin is a natural storage oligosaccharide from several plants, including chicory, dahlia, and Jerusalem artichoke (*Barclay et al.*, 2010). After it is extracted and dried, inulin is identified as a white, hygroscopic powder, with a neutral odor and flavor, which can be included in foods without changing the appearance, viscosity and flavor of the formulations (*Franck*, 2002). It is characterized by its low caloric value and the formation of opaque gels in high concentrations, when mixed with water or other aqueous liquid, forming a compound similar to fat. In this sense, inulin has been used industrially in foods as a fat replacer to reduce the caloric value, promote water retention, and enrich foods with fiber (*Franck*, 2002; *García et al.*, 2006).

Thus, this work aimed to develop cooked sausages containing yacare (*Caiman yacare*) meat residues with added fat replacers (texturized soy protein and inulin), and characterize the obtained products through chemical, physical, microbiological, and sensory analyses.

Material and methods

Yacare (Caiman yacare) meat

Yacare (*Caiman yacare*) meat shavings were donated by Caimasul Ltda (Corumbá, MS, Brazil). They were transported to the Laboratory of Food Technology, Federal University of Grande Dourados, Dourados, MS, Brazil, under refrigerated conditions. Meat shavings were stored up to two weeks under freezing until processing.

Cooked sausages obtained from yacare meat

For the preparation of the cooked sausages, the meat shavings were milled in a grinder with a 12 mm disc (Weg, Jaraguá do Sul, SC, Brazil) at 1.5°C. Then, the ingredients of the formulations were added, according to the three treatments listed in Table 1, and the sausage batters were manually homogenized for 10 min at 4°C. Subsequently, the sausage batters were stuffed into natural bovine casing in horseshoe format. Next, the sausages were submerged in liquid smoke for 1 min. (Figure 1). Then they were cooked until the internal temperature reached 72°C, when they received a thermal shock with cold water below 5°C. After

Table 1. Formulations utilized for the cooked yacare (*Caiman yacare*) meat sausages

Inquadiant	Formulation treatment (g/100g)		
Ingredient	T1	T2	Т3
Yacare meat shavings	83.94	82.94	81.94
Inulin	1.00	2.00	3.00
Cold water	8.095	8.095	8.095
Textured soy protein	2.50	2.50	2.50
Refined sodium chloride	2.00	2.00	2.00
Spices	1.10	1.10	1.10
Fat emulsifier	0.50	0.50	0.50
Sugar	0.40	0.40	0.40
Smoke aroma	0.40	0.40	0.40
Ascorbic acid	0.05	0.05	0.05
Sodium nitrite	0.015	0.015	0.015

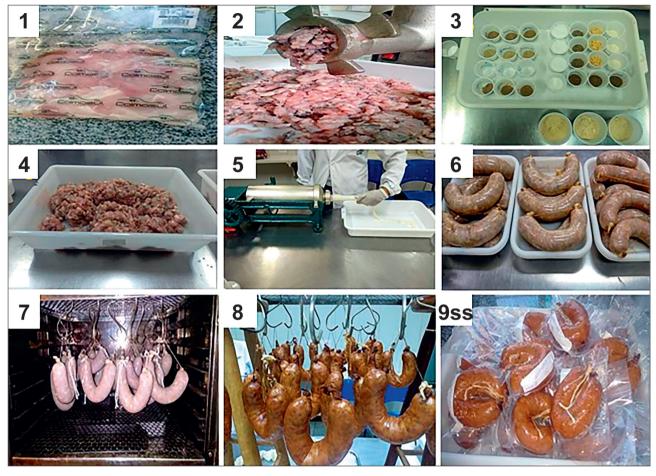


Figure 1. Development of cooked sausages of yacare (*Caiman yacare*) meat shavings. (1) Raw material; (2) Grinding; (3) Weighing of the ingredients; (4) Homogenization; (5) Stuffing the meat batter into casing; (6) After submersion in liquid smoke; (7) Cooking; (8) Drying; (9) Vacuum packaging

cooling, they passed through a varnish bath and drying at room temperature (28–30°C). The cooked sausages were identified as T1, T2, and T3, according to their formulation, vacuum packed, and then stored at 4°C for further analysis. Additives and condiments were supplied by Cavenaghi Eireli (Dourados, MS, Brazil).

Chemical analysis

Proximate composition

Moisture, crude protein, and crude ash contents of the cooked sausages were determined in triplicate according to the methods described by *AOAC* (2012). Moisture was determined by the oven drying method at 105°C until constant weight (method 950.46B), protein by the Kjeldahl method (method 928.08) and ash by the muffle oven technique (method 920.153). The lipid content was obtained in triplicate by the extraction method with cold organic solvent (*Bligh & Dyer*, 1959). The carbohydrate content was estimated by difference.

pH of the cooked sausages was measured in triplicate using a digital pH meter (Instrutherm model pH-2000, São Paulo, Brazil) by mixing 25 g of the sample and 10 ml of distilled water, according to the method described elsewhere (*Spitzer & Werner*, 2002).

Water activity

Water activity of the cooked sausages was determined in triplicate in a hygrometer (Aqualab, São José dos Campos, SP, Brazil) at 25°C with 1 g of sample.

Physical analysis

Instrumental color

The color [CIE L*(lightness), a* (redness), b* (yellowness)] of the cooked sausages was evaluated using a colorimeter (Minolta Chroma Meter CR 410), with measurements standardized with respect to the white calibration plate (*Jiménez & Gutiérrez*,

2001). The analysis was performed in triplicate in the internal part of the sausages.

Shear force

Texture analysis of the cooked sausages was carried out using a texture analyzer Model TAXT-plus (Stable Micro Systems, Surrey, England) calibrated with a standard weight of 5 kg. Products kept at 2°C were equilibrated at room temperature (28–30°C) before analysis. Samples of 15×15×115 cm were cut, placed in the texture analyzer and submitted to a cutting/shearing test (speed of 1.0 mm/s, distance of 30 mm) using a Warner-Bratzler shear blade (1 mm thick) to determine the shear force (N), which indicated the firmness of the sample. A minimum of 10 replicates of each treatment were analyzed (*Kang & Chen*, 2014).

Microbiological analysis

Microbiological analyses of the cooked sausages were performed for thermo-tolerant coliforms at 45°C, coagulase positive *Staphylococcus aureus* (CPS), and *Salmonella* sp. in accordance with the methodology described elsewhere (*USDA/FSIS*, 1998).

Sensory analysis

Sensory analyses of the cooked sausages were conducted by 50 trained panelists ranging in age from 20 to 51 years. A nine-point hedonic scale (9=like extremely; 1=dislike extremely) was used for evaluation of the attributes color, odor, texture and taste. The treatments were heated in microwave ovens for 5 s, then they were cut transversely 2 mm thick, and served in disposable containers, coded with three-digit random numbers. Overall acceptability was evaluated in terms of purchase intention using a 5-point scale, where 5 = certainly would purchase,

4 = probably would purchase, 3 = perhaps would purchase/perhaps would not purchase, 2 = probably would not purchase and 1 = certainly would not purchase, which was expressed as the percentage of total score (*Cavenaghi-Altemio et al.*, 2018). The acceptability index (AI) was calculated according to the following equation: AI = (average of the attributed grades/maximum attributed grade) x 100. The sample was considered acceptable if the AI was greater than 70% (*Stone & Sidel*, 1993).

Statistical analysis

Statistical results were evaluated through analysis of variance (ANOVA) and the Tukey's test for comparison of means, at a level of 5% of significance, using the statistical software Statistica 7.0. The sensory attributes and the purchase intention results were analyzed in percentages.

Results and Discussion

Chemical analysis

Proximate composition

The proximate compositions carried out for the cooked sausages prepared with yacare (*Caiman yacare*) meat shavings according to treatments T1 (1% inulin), T2 (2% inulin) and T3 (3% inulin) are shown in Table 2.

A significant difference (p<0.05) in moisture was observed between treatments, ranging from 63.90% for T1 to 59.89% for T3, showing the moisture decreased with the increase in the inulin content (Table 2). This can be explained due to the high water binding capacity of the inulin (*Rashid et al.*, 2018). Moreover, the addition of oligosaccharides in foods acts as a moisture reducer, limiting the water available in foods and preparations (*Gomes et al.*, 2007).

Table 2. Proximate composition of	the cooked yacare	(Caiman yacare) meat sausages
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Determination (%)	T1	T2	Т3
Moisture	$63.90^a \pm 0.30$	$61.26^{b}\!\pm0.40$	$59.89^{\circ} \pm 0.38$
Protein	$27.68^a \pm 0.52$	$26.10^{\mathrm{a}} \pm 0.82$	$25.33^{\mathrm{a}} \pm 0.83$
Lipids	$3.34^\text{b} \pm 0.24$	$5.36^a \pm 0.67$	$1.69^{\text{c}} \pm 0.16$
Ash	$4.50^{\mathrm{a}} \pm 0.03$	$4.62^{\rm a}\!\pm 0.12$	$4.58^a \pm 0.07$
Carbohydrates	0.58	2.66	8.51

Legend: Means with the same letter in the same row do not differ statistically at 5% (P>0.05). Treatments (T1, T2, and T3) according to Table 1.

The moisture content in yacare meat decreases with the animal age and body portion. For example, average values of 77.18% of moisture were reported for yacare back meat in animals aged 14 months, which was higher than values obtained for tail meat and animals aged 26 months (*Vicente Neto et al.*, 2007). This value is higher than those found in the present study mainly because of the water-absorbing ingredients *e.g.* texturized protein soy and inulin that were added to the cooked sausages.

The protein content decreased from 27.67 (T1) to 25.32% (T3), without significant difference (p>0.05) between the samples (Table 2). The Brazilian Technical Regulation on Sausage Identity and Quality establishes that the minimum protein content for cooked sausages must be 14% (*MAPA*, 2000). Protein values ranging from 18.39 to 19.44% were reported elsewhere for the yacare meat (*Romanelli et al.*, 2002). These differences may be related to the type of breeding, sex, and the age of the animal (*Vicente Neto et al.*, 2007; *Fernandes et al.*, 2017).

The lipid content was 3.34% for T1, 5.36% for T2, and 1.69% for T3, with a significant difference between all treatments (p<0.05) (Table 2). Lipid contents around 3.94% were obtained elsewhere for vacare carcasses (Fernandes et al., 2015). Despite the difference between treatments, the lipid content of the cooked sausages was quite similar to that obtained for yacare meat. Lipid concentration can vary depending on the sex and age of the animal, and the meat cut. The tail, for example, has muscles that execute more excessive physical activities due to locomotion in aquatic environments, and the tail also contains energy reserves that accumulate in the muscular tissues in the form of fat that is available for use when there is food shortage (Vicente Neto et al., 2007).

The ash content ranged from 4.50 to 4.62%, without a significant difference (p>0.05) between treatments (Table 2). Ash contents of 1.00-1.05 and 0.70-0.95% were reported for yacare meat by Romanelli et al. (2002) and Vicente Neto et al. (2007), respectively. The ash content is equivalent to the mineral material present in the product and is influenced by the raw material (Oliveira Filho et al., 2012). Thus, the high ash contents of the sausages (Table 2) could be explained by the addition of condiments and salts with inorganic residues (Nascimento et al., 2007; Cavenaghi-Altemio et al., 2013), which did not vary for the different formulations. In accordance with this, literature reports ash contents of 3.92 and 8.21% for unsmoked and hot smoked sausages, respectively (Fernandes et al., 2013).

Carbohydrates were obtained by difference, and amounted to 0.58, 2.65, and 8.51% for T1, T2, and T3, respectively. The contribution of carbohydrates from the yacare tissue is minimum (0.07%) (Fernandes et al., 2015). Thus, variations in carbohydrate contents are also related to the sausage composition, so T1 contained the lowest amount of carbohydrate, which was likely due to it having the lowest inulin content (1%), while more carbohydrate was found in T2 (2% inulin) and T3 (3% inulin), explained by the addition of greater amounts of oligosaccharide inulin, *i.e.*, the more inulin added, the greater the carbohydrate content.

Water activity and pH

The water activity was 0.960 for T1, 0.951 for T2, and 0.954 for T3, so there was a significant difference between all treatments (p<0.05) (Table 3). The water activity results confirmed that cooked sausages are considered high water activity foods,

Table 3. Water activity, pH, instrumental color, and shear force of the cooked yacare (*Caiman yacare*) meat sausages

Determination	T1	T2	Т3
Water activity	$0.960^a \pm 0.000$	$0.951^{\rm b}\!\pm0.000$	$0.954^c \pm 0.001$
pН	$5.61a\pm0.02$	$5.53a \pm 0.09$	$5.60a \pm 0.04$
L*	$58.52a.b \pm 1.05$	$59.69a \pm 1.28$	$57.24b\pm1.37$
a*	$6.08^{\text{a}}\pm1.34$	$4.93^{\mathrm{b}}\pm1.04$	$4.93^\text{b} \pm 0.88$
b*	11.7^9 a ± 1.39	$10.5^{7a} \pm 1.49$	$10.2^9 a \pm 0.95$
Shear force (N)	$14.33b\pm1.07$	$15.33b \pm 0.93$	$18.01a \pm 0.63$

Legend: Means with the same letter in the same row do not differ statistically at 5% (P>0.05). L*: lightness); a*: redness, b*: yellowness. Treatments (T1, T2, and T3) according to Table 1.

favoring the growth of microorganisms (*de Alcanta-ra et al.*, 2012). However, there are conditions that guarantee the safety of these products such as the addition of preservatives, *e.g.* sodium nitrite, and storage under refrigeration temperature.

pH values ranged between 5.53 to 5.61, with no significant difference (p>0.05) between treatments (Table 3). These pH values were similar to those obtained for yacare meat, which reached pH values of 5.5–5.7 at 36–48 h after slaughter (*Taboga et al.*, 2003; *Vicente Neto et al.*, 2007).

Physical analysis

Instrumental color

Instrumental color was determined for the parameters luminosity (L*), chroma a*, and chroma b* (Table 3). The highest L* value was obtained for T2 (59.69) and the lowest for T3 (57.24). These treatments differed from each other (p<0.05). However, neither T2 nor T3 differed from T1 (p>0.05).

Rodrigues et al. (2007) reported L* values for yacare meat ranging from 54.01 to 56.02. The obtained values were slightly superior to these values (Table 3). However, it must be considered that other ingredients contribute not only to the luminosity but all color parameters. Another aspect that must be considered is that small differences in the size of the meat fragments used to elaborate the sausages could have occurred due to manual homogenization of the meat batter. In relation to inulin, it has luminous characteristics close to those of fat, which means inulin's ability to reflect light is similar to that of fat (Menegas et al., 2013). Therefore, inulin should not affect drastically the luminosity when it is used as a fat replacer (Menegas et al., 2013).

Low values of chroma a* (redness) ranging from 4.93 to 6.08 were obtained for the three treatments, without significant difference between them (p>0.05) demonstrating that the sausages had a light color (Table 3) because the higher the value of chroma a*, the redder the color of the meat evaluated

(*Trindade et al. 2005*). Regarding chroma b* (yellowness), the values ranged from 10.29 (T3) to 11.79 (T1). However, there were significant differences observed between the treatments (p> 0.05) (Table 3).

Shear force

The instrumental texture was evaluated in terms of shear force (Table 3). Results showed that the highest average shear force (18.01 N) was obtained for T3 (3% inulin), differing (p> 0.05) from T1 (14.33 N) and T2 (15.33 N), the values of which did not differ from each other (p>0.05).

Thus, the increase in the inulin content in the T1-T3 series positively influenced the shear force. This fact could be related to the gel strength of inulin, as it depends mainly on inulin concentration (*Mensink et al.*, 2015). This same tendency of increasing hardness by replacing fat with different concentrations of fiber was observed in other sausage meat products (*Selgas et al.*, 2005).

Microbiological analysis

Microbiological evaluations of the cooked yacare (*Caiman yacare*) meat sausages for coliforms at 45°C, coagulase positive Staphylococci (CPS) and *Salmonella* sp. were carried out in order to confirm the microbiological safety of the sausages consumed by panelists during sensory analysis. The results of these determinations are shown in Table 4. Results showed that all sausages met the standards of Brazilian legislation for meat sausages (*ANVISA*, 2001). Thus, sensory analysis was performed for sensory attributes, acceptability indexes and purchase intention.

Sensory analysis

The means and standard deviations for the sensory attributes of appearance aroma, color, taste, texture, and overall impression of the cooked sausages by the acceptance tests are expressed in Table 5.

Table 4. Microbiological analyses of the cooked yacare (*Caiman yacare*) meat sausages

Microbiological analyses	T1	Т2	Т3
Coliforms at 45°C	$< 1.0^{\circ} \times 102 \text{ CFU/g}$	$< 1.0^{\circ} \times 102 \text{ CFU/g}$	< 1.0°×102 CFU/g
CPS	$< 1.0^{\circ} \times 103 \text{ CFU/g}$	$< 1.0^{\circ} \times 103 \text{ CFU/g}$	$< 1.0^{\circ} \times 103 \text{ CFU/g}$
Salmonella sp.	Absence in 25 g	Absence in 25 g	Absence in 25 g

Legend: CFU: colony forming units; CPS: coagulase positive Staphylococcus. Treatments (T1, T2, and T3) according to Table 1.

Attribute	T1	T2	Т3
Appearance	$6.30^a \pm 1.84 \ (70.00)$	$6.36^a \pm 1.71 \ (70.67)$	$6.60^{a} \pm 1.82 (73.33)$
Aroma	$7.52^a \pm 1.23 \ (83.56)$	$7.32^a \pm 1.38 \ (81.33)$	$7.08^a \pm 1.47 \ (78.67)$
Color	$6.18^a \pm 1.90 \ (68.67)$	$6.20^{\rm a} \pm 1.90 \ (68.89)$	$6.60^a \pm 1.71 \ (73.33)$
Taste	$7.84^a \pm 1.11 \ (87.11)$	$7.52^{a,b} \pm 1.39 \ (83.56$	$7.06b \pm 1.95 \ (78.44)$
Texture	$7.08^{a} \pm 1.50 \ (78.67)$	$7.04^a \pm 1.38 \ (83.56)$	$6.44^a \pm 1.41 \ (78.44)$
Overall impression	$7.28^a \pm 1.37 \ (80.89)$	$6.98^{a} \pm 1.36 (77.56)$	$6.84^{a} \pm 1.87 (76.00)$

Table 5. Sensory analysis of the cooked yacare (Caiman yacare) meat sausages

Legend: Means with the same letter in the same row do not differ statistically at 5% (P>0.05). Values in parenthesis are the acceptability index (%). Treatments (T1, T2, and T3) according to Table 1.

The average scores for the appearance and color ranged from "I did not like it" to "I liked it moderately" in all treatments. The same was observed for the texture of T3 and the overall impression of T2 and T3. For the aroma and flavor, the average scores ranged from "I liked it moderately" to "I liked it very much" in all treatments. The same was observed for the texture of T2 and T3 and for the overall impression of T1 (Table 5).

The increase in shear force for T3 (3% inulin) was not noticed by the panelists in the acceptance test for the texture attribute, as there was no significant difference (p>0.05) between treatments for this attribute (Table 5). The properties of inulin related to the texture could be improved by adding other

ingredients such as gums in the formulations until the desired effect on the product is achieved (*da Silveira et al.*, 2015).

Similar results were reported for different yacare sausage treatments (*Fernandes et al.*, 2013). However, that study revealed significant decreases in some sensory attributes of hot smoked sausages due to excessive dehydration and the highest salt concentration (*Fernandes et al.*, 2013).

The acceptability indexes ranged from 70.00 to 87.11% across all the sensory attributes, except for the color of T1 and T2, which attained 68.67 and 68.89%, respectively (Table 5). When the acceptability index is equal to or greater than 70%, the product is considered accepted (*Stone & Sidel*, 2004).

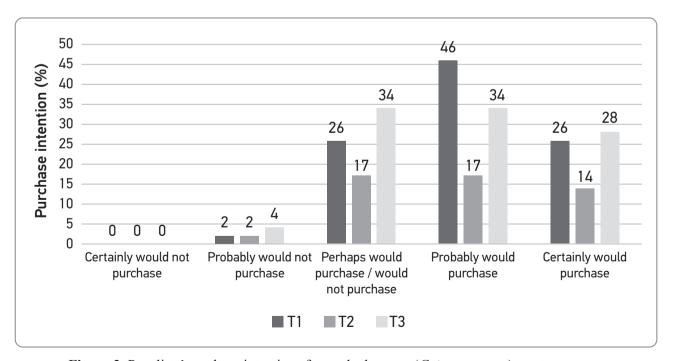


Figure 2. Panelists' purchase intentions for cooked yacare (*Caiman yacare*) meat sausages. Treatments (T1, T2, and T3) according to Table 1

Thus, T1 and T2 would not be accepted in relation to color. In comparison, an average acceptance of 78% for a smoked product prepared with yacare meat was reported, which is between the hedonic terms "I liked it moderately" and "I liked it a lot" (*Romanelli et al.*, 2002), similar to the results obtained in the current study.

Figure 2 shows the panelists' purchase intentions with regard to the cooked sausages prepared with yacare meat shavings. The summed purchase intentions "certainly and probably would buy" were 72% (T1), 62% (T2), and 64% (T3). Therefore, panelists indicated they were more likely to buy products with lower inulin content in relation to the other treatments. Other authors have achieved good results in the overall acceptability of meat products made with higher concentrations of inulin, *e.g.* 4% inulin in beef sausages (*Devereux et al.*, 2003) and

up to 7.5% inulin in bologna sausages with conventional and reduced fat content (*García et al.*, 2006).

Conclusions

Cooked sausages were successfully developed from yacare (*Caiman yacare*) meat shavings through three different treatments involving the addition of inulin as a fat replacer. All treatments presented low levels of lipids and high protein contents. T1 (1% inulin) had also the lowest carbohydrate content, which is desired for low-carbohydrate and low-fat diets. In testing, the products were considered microbiologically safe. Thus, sensory analyses indicated good sensory characteristics for all treatments. However, the purchase intention revealed the highest value of 72% of panelists certainly or probably would like to purchase T1.

Razvoj i karakterizacija nisko-masnog kuvanog punjenja od mesnih strugotina poreklom od kajmana (*Caiman iacare*)

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A p s t r a k t: Ograničena potrošnja mesa kajmana (Caiman iacare) je posledica kulturnih i ekonomskih faktora, kao i ograničene dostupnosti proizvoda na bazi ovog mesa. U ovom istraživanju je razvijeno i okarakterisano/ocenjeno kuvano punjenje (poput kobasica) od strugotina mesa kajmana i zamene masti (inulin i sojini proteini). Sadržaj vlage se kretao od 63,90% (T1) do 59,89% (T3), što ukazuje na smanjenje sa povećanjem sadržaja inulina. Sadržaj proteina je pao sa 27,67 (T1) na 25,32% (T3). Najviši sadržaj lipida bio je 5,36% (T2), a najniži 1,69% (T3). Sadržaj pepela se kretao od 4,50 do 4,62%. Najviša vrednost osvetljenosti utvrđena je kod T2 (59,69), a najniža za T3 (57,24). Najviši prosek za silu presecanja (18,01 N) dobijen je za T3. Dobijene su dobre senzorne karakteristike kod svih tretmana, sa indeksima prihvatljivosti od 68,67 do 87,11%. Međutim, najizraženija namera kupovine od 72% ispitanika koji bi sigurno želeli da kupe utvrđena je za T1.

Ključne reči: meso kajmana, kobasica, inulin, sojini protein.

Disclosure statement: No potential conflict of interest was reported by authors.

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