



Acta Scientiarum. Animal Sciences

ISSN: 1806-2636

ISSN: 1807-8672

Editora da Universidade Estadual de Maringá - EDUEM

Pereira, Queila Dias; Kwiatkowski, Angela; Munhoz, Cláudia Leite; Porto, Ricardo Santos; Costa, Celso Soares; Curvo, Lucimar Rodrigues Vieira; Diemer, Odair
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Acta Scientiarum. Animal Sciences, vol. 44, e53516, 2022
Editora da Universidade Estadual de Maringá - EDUEM

DOI: <https://doi.org/10.4025/actascianimsci.v44i1.53516>

Available in: <https://www.redalyc.org/articulo.oa?id=303171455002>

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Pantanal's fish native meatballs has the nutritional values increased with the use of pequi in its recipe

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ABSTRACT: The study aimed to evaluate whether the addition of pequi increases the nutritional value of meatballs made with piranha and pacu fillets. Piranhas were obtained from the colony of fishermen; pacu with the fish farmer and the pequis were collected in nature. Fish were filleted and triturated, and the resulting masses were used to prepare 4 types of meatballs: piranha fillet with 2.0% pequi pulp, piranha fillet without pequi pulp, pacu fillet with 2.0% pequi pulp, and pacu fillet without pequi pulp. After preparation, acceptance, purchase intention and frequency of consumption were evaluated with untrained tasters. Chemical composition parameters evaluated were crude protein, lipids, ash, moisture, carotenoids and antioxidant activity. Results of acceptability for all formulations were similar. The chemical composition of piranha meatballs presented differences ($p < 0.05$) for lipids and carotenoids. Pacu meatballs showed no difference ($p > 0.05$) for moisture, carotenoids, and antioxidant activity. The addition of pequi did not alter the organoleptic characteristics, however increased the nutritional values, therefore, it can add value to the product to be marketed and be more nutritionally attractive to the consumer.

Keywords: aquaculture and foods; artisanal fisheries; food acceptability; *Piaractus mesopotamicus*; *Pygocentrus nattereri*; technology of fish.

Received on May 4, 2020.
Accepted on September 11, 2020.

Introduction

Consumption of fish from large rivers is understood as an ecosystem service by promoting the health and well-being of local and global populations, with high economic value (Lima, Carvalho, Nunes, Angelini, & Doria, 2020). Therefore, it is common to use fish native to the Upper Paraguay Basin in Brazil; among fish species used for human consumption, two belonging to the family Characidae were used in this study, piranha (*Pygocentrus nattereri* Kener, 1858) and pacu (*Piaractus mesopotamicus* Holmberg, 1887).

Despite the low commercial value of piranha, it presents relative abundance in the fishing of the Mato Grosso do Sul State (Brazil) (Catella, Campos, & Albuquerque, 2015). This species presents good musculature, large number in fisheries, wide distribution, protein of high biological value, its use is feasible for human consumption (Barros, Mateus, Braum, & Bonaldo, 2010; Reenamole, & George D'Cruz, 2015; Moraes, Cárdenas, & Malta, 2019).

Pacu, with high cost, is one of the most caught fish in the Paraguay basin, especially due to its sport fishing (Santos et al., 2018; Curvo et al., 2020). In addition, it is largely used in fish farming in Brazil, especially in the Midwest region, it is also well appreciated by many consumers from North Africa (Diemer, Boscolo, Signor, Klein, & Feiden, 2014; Valladão, Gallani, & Pilarski, 2016).

Pequi (*Caryocar brasiliense*) is a typical fruit of the Brazilian Cerrado, it has high nutritional value and offers high potential for sustainable use in Brazil. The main properties of pequi fruit include benefits for human health, as it is rich in vitamins and essential oils (Machado, Mello, & Hubinger, 2013; Santana, Kurozawa, Oliveira, & Park, 2013; Guedes, Antoniassi, & Faria-Machado, 2017) and still have no genotoxic or clastogenic effects (Traesel et al., 2017).

The demand for healthy food increases every day, and the substantiation of nutritional quality of fish stimulates its consumption (Cardoso, Lourenço, Costa, Gonçalves, & Nunes, 2013). Estiasih, Trowulan, and Rukmi (2017) stated that several studies show that enrichment of food products has a positive effect on health, particularly protection against cardiovascular diseases, cancer and improves the development and function of the brain.

Among the various products available on the market, meatballs stand out for being a product with high nutritional content, easy to prepare and affordable (Silva et al., 2011). According to Mirzal, Rinidar, Razali, Sugito, and Nurliana (2016), meatballs are very popular among people of different backgrounds and ages. Thus, the study aimed to assess whether the addition of pequi increases the nutritional value of meatballs made with piranha and pacu fillets.

Material and methods

This research was carried out in the city of Coxim, state of Mato Grosso do Sul, Brazil. This city is known as the “National City of Fish or Gateway of Pantanal”. Located in the northern portion of the Pantanal Hydrographic Basin, between the geographical coordinates 18°30'25" S and 54°45'36" W, with an area of 6,409,224 km² and an estimated population of 33,543 inhabitants (5.02 hab km⁻²), in 2019. With a focus on fishing tourism, where professional fishermen and amateurs from around the world are attracted by rivers that have a large number of fish, such as Coxim, Jauru, Taquari, among others in the Upper Paraguay basin, which forms the Pantanal.

Research approach

This research uses a qualitative and quantitative approach, experimental and analytical characteristic with field work and laboratory tests, according to determination of Guidelines of the National Council for Animal Experimentation Control, Ministry of Science, Technology and Innovation (CONCEA/MCTI) (Brasil, 2019), Dutcosky (2011), Medeiros, Kwiatkowski, Clemente, and Costa (2011), Peuckert, Viera, Hecktheuer, Marques, and Rosa (2010), Cortez Netto et al. (2010), Instituto Adolfo Lutz (2008), Silva and Queiroz (2006) and Mensor et al. (2001).

Importantly, there was no way of manipulating live specimens or euthanizing them. All individuals were acquired dead. Piranhas came from artisanal fishing, acquired in the colony of artisanal fishermen Z-2 Rondon Pacheco located in the city of Coxim, state of Mato Grosso do Sul, Brazil.

Experimental design (data processing and analysis)

The study was conducted from August 2018 to December of 2019. Samples of pequi were collected manually in the rural area of the city of Coxim, state of Mato Grosso do Sul, Brazil. Only the naturally fallen fruits were collected and those suitable for consumption were selected. The shell cuts were made with care not to reach the core (pulped part) with the use of stainless steel knives. The shell were washed in chlorinated water and subjected to a whitening process in boiling water for 5 minutes. Subsequently, it was performed the pulping and triturated in a household blender until obtaining the pequi pulp, which was added to the meatballs.

Piranhas and pacus were gutted, washed in chlorinated water and the fillets were taken without skin. Fillets were crushed in a grinder to obtain a dough, which passed through a wash, that was performed with cold water mixed with sodium bicarbonate (two grams of sodium bicarbonate for each liter of water). The dough was filtered to remove excess water and meatballs were prepared, two formulations using the piranha fillet with (T1) or without (T2) 2.0% pequi pulp, and two others using the pacu fillet with (T3) or without (T4) pequi (Table 1).

Table 1. Recipe of meatballs made with file of piranha and pacu.

Ingredients	Without pequi (g)	With pequi (g)
Fillet of piranha (T1 and T2 or pacu (T3 and T4)	85.0	85.0
Water	2.5	2.5
Breadcrumbs	6.0	6.0
Dehydrated onion	0.6	0.6
Egg	2.0	-
Textured soybean protein	2.1	2.1
Salt	1.2	1.2
Dehydrated parsley	0.6	0.6
Pequi pulp	-	2.0
Total	100.0	100.0

Ingredients were homogenized, rolled as meatballs and baked in the oven at 200°C for approximately 35 minutes. After roasting, samples were subjected to sensory acceptance tests.

For evaluations, first, a Free and Informed Consent Term was delivered, explaining to the tasters the purpose of the research and requesting an agreement to participate in the acceptance test of meatballs, wherefore, all volunteers have agreed to participate in the tests.

Analyses were conducted at the Laboratory of Sensory Analysis of the Federal Institute of Education, Science and Technology of Mato Grosso do Sul, *Campus Coxim* (IFMS) and at the Praça da Concha Acústica, located in the downtown of the city of Coxim, being, therefore, people without training, of both sexes, aged 18 to 50 years. The test was applied to 60 tasters for Piranha meatballs and 60 for pacu meatballs, totaling 120 untrained tasters (who analyzed both formulations, that is, 120 meatballs with the addition of pequi and 120 without the addition).

To this end, acceptance test was applied according to Dutcosky (2011), evaluating the attributes: general appearance, color, flavor, and aroma, by means of a 9-point structured hedonic scale (1 = Dislike Very Much; 9 = Like Very Much).

Purchase intention was evaluated using a 5-point scale (1 = Absolutely Would Buy; 2 = Probably Would Buy; 3 = Maybe; 4 = Probably Would not Buy; 5 = Definitely Would not Buy). The frequency of consumption was assessed using a 4-point scale (1 = Daily; 2 = Twice to three times a week; 3 = Once to twice a month; 4 = Once to twice a semester).

For acceptability, we used the Acceptability Index (AI), according to the equation: $AI (\%) = (\text{average of all answers}/9) \times 100$. It was used as a parameter for the Acceptability Index the value of $\geq 70\%$ (on a scale of 0 to 100%), following the recommendation of Peuckert et al. (2010).

Chemical composition of meatballs was analyzed in triplicate and the samples were selected at random. Analysis of moisture, ash and lipids were carried out according to the standards of the Instituto Adolfo Lutz (2008). In summary, lipids were extracted by the Soxhlet method, taking the hexane as solvent; the moisture, for weight loss after heating in chamber at 105°C, to constant weight; ash by incineration in muffle furnace at 600°C. Crude protein was analyzed by the Kjeldahl method, as recommended by Silva and Queiroz (2006). As for total carotenoids, the methodology of Medeiros et al. (2011) was followed. Total antioxidant activity was evaluated by the method of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging in accordance with Mensor et al. (2001).

Statistical analysis

Results were tested by Analysis of Variance (ANOVA), and if there were significant differences at 5%, Tukey's test and tests for normality and homoscedasticity were applied in the free software R Core Team (2017), version 2.15 using Agricolae (Mendiburu, 2019) and Plotly (Sievert, 2018) packages in R Core Team (2017).

Results and discussion

In relation to the mean results of meatballs prepared with piranha filet for acceptability, purchase intent and frequency of consumption, it was not observed significant differences ($p > 0.05$) between the two formulations, and the evaluators stated that moderately liked for the attributes of acceptance and once to twice a month about the frequency of consumption (Table 2).

Table 2. Average results of the evaluation of acceptability, purchase intent and frequency of consumption of meatballs with piranha filet.

Attributes of acceptability	Without pequi	With pequi	CV (%) *	P
General appearance	7.69	7.64	16.20	0.82
Aroma	7.62	7.71	15.32	0.69
Color	7.13	7.33	23.29	0.53
Taste	7.40	7.34	18.40	0.83
Purchase intent	2.05	2.14	47.41	0.66
Frequency of consumption	2.07	2.76	37.09	0.65

*Coefficient of variation. Average values did not differ ($p > 0.05$) by ANOVA.

According to Verbeke and Vackier (2005), regular consumption of fish is part of a healthy diet, which is one of the main reasons for consumption, but the information should be in line with sensorial aspects that will influence the supply and demand (Verbeke & Vackier, 2005).

The acceptance rate was 82.9% for the formulation without pequi and 83.4% with pequi, thus, both formulations of Piranha meatballs were well accepted. Results of similar acceptance were reported by Borges,

Passos, Stedefeldt, and De Rosso (2011) with an index of 89.4% acceptance for meatballs made with meat of *Menticirrhus americanus* in the evaluation of school nutrition of the Baixada Santista. Comparing the acceptability obtained with other products prepared from fish meat, such as breaded pacu (*Piaractus mesopotamicus*), tilapia (*Oreochromis niloticus*) and jundiá (*Rhamdia quelen*), it can be observed a good acceptance, with no sensory differences between the types of fish (Cortez Netto et al., 2010). Corroborating the results of this study and demonstrating encouraging conditions in the search for new products and technologies that promote the consumption of fish.

The chemical composition of Piranha meatballs presented differences ($p < 0.05$) in the analysis of lipids and carotenoids, but for the other parameters there was no significant difference ($p > 0.05$) (Table 3).

Table 3. Average results of the chemical composition of meatballs made with Piranha fillet.

Nutritional composition	Formulation		CV (%) [*]	P
	Without pequi	With pequi		
Moisture (%)	74.3	78.96	10.90	0.53
Protein (%)	26.52	26.64	4.69	0.91
Lipids (%)	6.27	7.95	6.17	0.009 ^{*1}
Ash (%)	3.62	3.43	3.81	0.16
Carotenoids (mg 100 g ⁻¹)	1.4	3.8	11.21	0.0005 [*]
Antioxidant activity (%)	9.93	9.94	0.15	0.609

^{*}Coefficient of variation. Average values did not differ ($p > 0.05$) by ANOVA.

Differences in lipids can be attributed to the large amount of lipids present in pequi. According to Lima et al. (2007), the four major components of pequi almond are lipids (51.51%), proteins (25.27%), carbohydrates (8.33%) and dietary fiber (2.2%), and as in the pulp as the almond of pequi, it can be observed the predominance of unsaturated fatty acids with 61.35 and 52.17%, respectively.

Oliveira et al. (2006) studied pequi pulps from Montes Claros, Minas Gerais State, Brazil, and found a content of carotenoids of 11.34 mg 100 g⁻¹, higher than in this present study, nevertheless, in this research, the contents were evaluated in Piranha meatballs, i.e., with the inclusion of other ingredients. On the other hand, comparing Piranha meatballs with and without pequi, it can be noted an increase of 63% in the level of carotenoids, therefore, the addition of pequi pulp increases the concentrations of carotenoids and, thus, the addition of pequi pulp can be an alternative to potentiate the nutritional value of meatballs.

Pequi fruit contains various carotenoids, including b-carotene, lycopene, z-carotene, cryptoflavin, b-cryptoxanthin, antheraxanthin, zeaxanthin, mutato-xanthin, violaxanthin, lutein, and neoxanthin (Azevedo-Meleiro & Rodriguez-Amaya, 2004). Carotenoids have some fundamental functions for human health (Uenojo, Maróstica Junior, & Pastore, 2007). Pequi fruit presents lipids with a composition of monounsaturated fatty acids (MUFA), especially oleic, and in carotenoids, which have been associated with protection against cardiovascular diseases, increasing cardiac relaxation and contractility (Oliveira et al., 2017).

A research of Leão, Franca, Oliveira, Bastos, and Coimbra (2017) on pequi pulp reports the presence of antioxidant compounds with high antioxidant potential, which were found to be exceptionally higher than those of fruits and by-products reported in the literature.

Regarding pacu meatball, the average results for acceptability, purchase intent and frequency of consumption showed no significant differences ($p > 0.05$) between formulations and the tasters declared that they liked moderately for the acceptability attributes, would probably buy with the intention of buying and consume once or twice a month in the frequency of consumption (Table 4). According to Cayot (2007); (Nadricka, Millet, & Verlegh, 2020), in addition to the demand for safe food, there is always a need to eat foods that taste good, as consumer behavior and perception of food organic quality are important in terms of consumption. Sensory quality shows a positive in consumer choice and acceptance of food products (Imm, Lee, & Lee, 2011; Horvat, Granato, Fogliano, & Luning, 2019; Gębski, Jezewska-Zychowicz, Szlachciuk, & Kosicka-Gębska, 2019; Hagmann & Siegrist, 2020).

The acceptance rate was 81.5% for the formulation without pequi and 80.5% with pequi, therefore, the two formulations of pacu meatballs were well accepted, with results close to those found with Piranha meatballs, indicating that the meat of Piranha can be used for aggregation of value. Rodrigues, Sousa, Santos, and Neto (2016) reported that approximately 85% of the tasters would recommend meatballs of panga (*Pangasius hypophthalmus*), demonstrating the wide acceptability of meatballs based on fish, corroborating the results of this research.

Table 4. Average results of the evaluation of acceptability, purchase intent and frequency of consumption of meatballs with pacu fillet.

Attributes of acceptance	Formulations		CV (%) [*]	P
	Without pequi	With pequi		
General appearance	7.08	7.19	18.15	0.668
Aroma	7.41	7.17	21.35	0.406
Color	7.15	7.20	16.66	0.816
Taste	7.69	7.41	19.86	0.324
Purchase intent	2.17	2.44	40.25	0.113
Frequency of consumption	2.79	2.93	28.38	0.362

^{*}Coefficient of variation. Average values did not differ ($p > 0.05$) by ANOVA.

Data contribute to contextualize the importance of seeking different ways for fish processing, and thus ensure that fish meat can be consumed by a larger number of people, since fish is one of the healthiest meats. The ingestion of fish brings benefits to human health, as it contains vitamins, minerals and essential fatty acids that reduce the risk of heart diseases; and preparation method is essential for the maintenance of the benefits of this food, and it is highly recommended by physicians and nutritionists (Scherr, Gagliardi, Miname, & Santos, 2015).

The results of the chemical composition for pacu meatballs showed no difference ($p > 0.05$) for moisture, carotenoids and antioxidant activity. However, the remaining parameters (crude protein, lipid, and ash) differed ($p < 0.05$) (Table 5).

Table 5. Average results of the chemical composition of meatballs prepared with pacu fillet.

Nutritional composition	Formulation		CV (%) [*]	P
	Without pequi	With pequi		
Moisture (%)	74.94	75.94	1.45	0.19
Protein (%)	6.93	7.55	3.79	0.04 [*]
Lipids (%)	15.57	16.88	1.88	0.006 [*]
Ash (%)	1.47	1.69	1.75	0.0006 [*]
Carotenoids (mg 100 g ⁻¹)	29.14	30.65	2.96	0.105
Antioxidant activity (%)	9.97	9.95	0.32	0.445

^{*}Coefficient of variation. ^{*}Average values differ $p < 0.05$) by ANOVA.

Differences in protein, lipid and ash should be in function of the chemical composition of fish, agreeing with Lima, Mujica, and Lima (2012), who reported that chemical composition of a fish is extremely variable and depends on factors related to the species, environment, season of the year, quality and quantity of food consumed, stage of sexual maturity, age and part of the body examined.

Levels of carotenoids in pacu meatballs were high in comparison with Piranha meatballs, the difference may be because pacu derived from farming and Piranha from fishing. According to Mesquita, Teixeira, and Servulo (2017), fish bred in captivity are given artificial diets (feed), in which carotenoids are incorporated to intensify meat color.

Fish species have antioxidant compounds to protect lipids and other compounds that contain double bonds, against damage caused by reactive oxygen species, which can be amino acids, peptides, ascorbic acid, carotenoids and phenolic compounds. These compounds belong to various chemical groups and make use of their antioxidant effects via different modes of action (Jooyandeh & Aberoumand, 2011; Špitalniak-Bajerska, Szumny, Kucharska, & Kupczyński, 2018; Zhang, Zhao, Qiu, & Chi, 2019). Both samples of meatballs, with and without pequi pulp, show about 10% antioxidant activity, which should be from fish, since pequi pulp in a small concentration did not differentiate in relation to its presence or absence in the composition.

Studies in animal models have shown that both have antioxidant and anti-inflammatory properties; they act as a protector against oxidative damage in Deoxyribonucleic acid (DNA) and other macromolecules, prevent lipid peroxidation in blood and secondary mutagenic effects (Grisolia, 2017; Volkoff, Estevan Sabioni, Coutinho, & Cyrino, 2017). This property can be important in fish products for people due many cellular disorders.

In general, meatballs of Piranha and pacu were well accepted by tasters and certainly would have demand in the market. In addition, they showed a chemical composition that potentiate the nutritional value; Piranha meatball showed high protein content, and pacu meatball, a higher content of carotenoids.

The addition of pequi in fish meatballs, regardless of fish species, enhances its nutritional value, therefore, it certainly makes the product more attractive for commercialization because it has an unmistakable taste and is widely used in regional recipes.

Important considerations

Global demands for healthier food and protein consumption in quantity and quality have been a major challenge for humanity today, whether from the point of view of environmental resilience and all the changes caused by large-scale production (Triches, 2020). In the same sense, areas of global interest aimed at food production to meet human needs, should be the object of everyone's concerns (Landis, 2017; Adesogan, Havelaar, McKune, Eilittäd, & Dahla, 2019; Tomas et al., 2019). In this scenario, these authors understand that the search for foods with high nutritional value and with possibilities of combining them, can be alternatives to serve the population, with regard to the local level, promoting greater food security and socio-environmental sustainability.

The biodiversity of Brazilian biomes is now threatened, requiring the search for alternative forms of consumption and proportion, increasing the portfolio of agricultural products intended for food. The importance of wetlands, such as the Pantanal biome, is highlighted, as it has a wide array of edible species, but the biocultural system has appropriated from the heritage of selecting few species, compromising the natural stock (Landis, 2017).

In the previous scope, plants and fishes in the Pantanal are distributed in an extensive fluvial plain with great economic interest on the world stage, with a view to presenting high biological diversity and exuberant natural resources (Novais et al., 2016; Curvo et al., 2020). In contrast, this place is known as a Biosphere Reserve, with its megabiodiversity threatened by the use of environmental services, mainly mineral extraction, extensive livestock, mechanized agriculture and predatory fishing (Froehlich, Cavallaro, Sabino, Suárez, & Vilela, 2017; Miranda et al., 2018; Curvo et al., 2020).

In addition to this relevant fact, it meets market trends for consumer acceptance (Reis & Schmiele, 2019). A way to improve the trade and exploitation of fish, is through processing, which can permit the expansion of the catalog of products offered, and thus, provide a greater added value (Feltes et al., 2010; Stephens et al., 2018; Van Der Weelle, Feindt, Jan van der Goot, Van Mierlo, & Van Boekel, 2019). Accordingly, Américo, Amorim, Cristo, Valle, and Salgado (2013); Stephens et al. (2018) without the knowledge of technologies, appropriate managements, fishermen get stuck in the bottleneck of marketing, limiting themselves to directly sell their fish fresh without any added value.

We highlight the need for studies that aim to understand the elaboration, chemical composition and desirable characteristics in foods, leveraging the use of innovative foods with sensory characteristics and quality, promoting health and well-being (Leão et al., 2017; Schwartz, Vandenberghé-Descamps, Sulmont-Rossé, Tournier, & Feron, 2018; Reis & Schmiele, 2019).

As pequi is a highly appreciated regional fruit, it has excellent nutritional value and its addition to fish meatballs does not cause a significant difference in organoleptic characteristics. Also, comparing *P. mesopotamicus* of good commercial value and excellent appreciation with *P. nattereri*, which presents low appreciation, little commercial value and relative abundance in catches made in the Pantanal. Therefore, the study shows that the addition of pequi in meatballs made with fillet of *P. nattereri* is an interesting alternative to improve its commercialization, increase its nutritional value and add greater commercial value to this fish so undervalued by fishermen in the Pantanal.

Conclusion

The addition of pequi did not alter the organoleptic characteristics, however it increased the levels of lipids and carotenoids in the piranha meatballs and increased the lipids and ashes in the pacu meatballs, therefore, it can commercially value the product and be more nutritionally attractive to the consumer.

Acknowledgements

The Coordination for the Improvement of Higher Education Personnel in Brazil (CAPES), the Federal Institutes of Education, Science and Technology of Mato Grosso and Mato Grosso do Sul (IFMT and IFMS), and the Catholic University Dom Bosco (UCDB).

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