**Production of flour made from bullfrog’s meat and bone**

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**Abstract**

Bullfrog meat marketed in Brazil has been gaining ground among the population due to its soft flavor, low calorie content, and high protein content. It is recommended for treating gastrointestinal and allergic diseases. This study aims to maximize the use of bullfrog carcass by applying a heating technology that enables the use of bones in gastronomy, which contain high calcium bioavailability, and represent about 40% of the product's total weight. A meat-and-bone flour called Low Cost Alternative Food has been developed, which, in addition to being functional and rich in protein and calcium, presents similar flavor to that of unmixed bullfrog meat, allowing food enrichment. Dehydration of the skeleton was carried out in an oven, and the remaining material was crushed and then vacuum packed, all in compliance with the standards for good manufacturing practices and under good sanitary conditions, making it microbiologically fit for human consumption for a total of 60 days while stored under refrigeration. Results showed a composition of 25% mineral matter, 60% protein which presented a 90% digestibility rate, only 12% fat, and 2% carbohydrates. This composition can compensate for calcium deficiency and malnutrition due to the quality of its protein.

**Keywords:** frog meat; diet; functional food; gastronomy; good manufacturing practices.

**Practical Application:** A low cost functional food made from meat and bony parts of Lithobates catesbeianus.

### 1 Introduction

Promotion of healthy eating begins by facing the economic difficulties which the country undergoes. It is now increasingly difficult to purchase food that is proper for daily consumption; which is why a balanced diet is currently one of the greatest challenges in our daily lives. This scenario is directly connected to the need for environmental protection, since parts of the food product which are not fit for consumption end up becoming pollutant material. Therefore, we must harness all that each food product can provide as nutrition source.

Meat, in general, is not regarded as a primary source for calcium, but a few studies on frog meat have been reporting the presence of high calcium content in this particular food. Such bioavailability is similar to that of the calcium present in milk and dairy - of about 20 to 30% - indicating that frog meat can be proposed as an alternative way to fight the deficiency of this mineral, also preventing osteoporosis, arterial hypertension, cancers of the colon and stomach, among other diseases.

On top of that, traits of the frog meat which are captured by human senses, such as color, smell, flavor and texture, characterize it, due to the presence of ten amino acids essential to humans, as an excellent food to be used as alternative therapy (Nóbrega et al., 2007).

Despite the difficulties in feeding the frogs for optimum performance, since their nutrient levels are obtained through the consumption of other animals, mainly fishes and birds, resulting in high operational cost for its farming and affecting retail price (Seixas et al., 1998a, b, 2008, 2010), bullfrog Lithobates catesbeianus farms in Brazil, notably in the Southeastern Region, are going through continuous, however irregular, expansion. On the one hand, medicinal use of frog meat, combined or not with its culinary values (flavor, biological value, and meat texture) fueled this expansion. On the other hand, high production costs, inferred from lack of zootechnical knowledge, result in high retail price, thus making production and sales circuit unstable. Nevertheless, frog farming lodges great potential in aquaculture, and can be used as instrument for local development (Nascimento et al., 2013).

Incorrect nutritional management is the main cause for problems in frog farming. Absence of a commercial feed designed to attend the specific nutritional demands of frogs leads to the use of fish feed, compromising proper development.

Mello (2009) observed that frog farming technologies developed in Brazil have been employed by countless Latin American countries, which have been adjusting Brazilian farming systems to attend the various local climate conditions. Development of farming methods has resulted in a considerable increase in the amount of frog farmers, especially in South America.

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Protein contents of bullfrog meat are similar to that of other lean meats, being 16 to 19% crude protein (CP). It also has high digestibility, surpassing the standards proposed by the United Nations Food and Agriculture Organization (FAO) and by the World Health Organization (WHO) (Food and Agriculture Organization, 1985) for children and adults in all essential amino acids, being, however, slightly deficient in sulfur-containing amino acids, such as leucine and valine, as already reported by Sabra et al. (1986) and in the nineties by Rancé et al. (1999).

Regarding lipid content, bullfrog meat shows a rate of 0.6 to 0.7% - with cholesterol values worthy of special attention, at about 40 mg/100g, a low rate when compared to other commercial meat, such as bovine (120 to 200 mg/100g), pork (100 to 300 mg/100g) and poultry (100 to 150 mg/100g). It also contributes with calcium (16 to 20 mg/100g), iron (1 mg/100g) and niacin (2.7 mg/100g); still, there is little study about the nutritional value of frog meat, and its dietary applications in preventing or even treating specific diseases (Oliveira, 2007).

Fidelis (2004) has evaluated the protein quality of frog meat in three presentations: boneless frog meat (BLF); bone-in frog meat (BIF) and mechanically separated meat (MSM), raw and dehydrated or dehydrated and cooked, following protein efficiency rate (PER), net protein ratio (NPR) and true digestibility methods. Results obtained for PER and NPR values were superior (p < 0.05) to that of casein standards. All presentations showed digestibility levels above 90%, attesting to high nutritional values.

According to Lima & Agostinho (1998), approximately 55% of the living animal's weight can be harnessed for commercial purposes after slaughter. All other product, such as back, deboned or chopped, is poorly exploited.

Bullfrog's liver, which corresponds to 5% of its living weight, can be used for human consumption as pâtés or special mixes, even though the practice is more common abroad (Ramos, 2000; Lima, 2008).

Studying carcass characteristics is of much significance when aiming to evaluate the quality of a system's final product (Costa et al., 2002; Restle & Vaz, 2003; Pacheco et al., 2005; Mello et al., 2006). The yield of carcass and commercial cuts and the carcass weight are relevant information for cold room directors when evaluating product value and operational costs.

Frog meat has been quoted as having optimal sensory characteristics, being rich in high biological value protein, mineral salts and vitamins, and for its low cholesterol and lipids content, therefore having low caloric value (Peluzio et al., 1995; Ramos, et al., 2004, 2005).

For this reason, the present study had as its object the broader usage of bullfrog carcass, elaborating menus which use the entirety of the carcass, including a farinaceous Low Cost Alternative Food (LCAF) which utilizes bone parts, engendering gastronomy and therapeutic recipes.

2 Materials and methods

Experiments were carried out in the Gastronomy Lab at Augusto Motta University Center (UNISUAM). Microbial analysis were conducted at the State Center for Food Quality Research (CEPQA), within the Agricultural Research Company of the State of Janeiro (PESAGRO-RIO), and centesimal analysis were conducted at the Nutrition Institute, Laboratory in the Federal University of Rio de Janeiro (UFRJ).

This research was submitted to the Committee for Ethics and Research at Augusto Motta University Center, constituted under the resolution 196/96 of the National Health Board, under the register number 101711/2016, and duly registered at the National Committee for Research Ethics (CONEP), through Plataforma Brasil.

A total of 67 kilograms of frog meat were utilized during the development of the experiments for broadening the usage of bullfrog carcass. Carcasses were obtained from a state inspected slaughterhouse, where animals were slaughtered, packaged, and transported in accordance with current legislation. Carcasses were, then, defrosted and deboned.

Protocol, built through a sequence of operations handling bullfrog carcass (Figure 1A-I) until obtaining a skeleton without musculature, aimed to produce a flour from frog meat and bone (Figure 2A-F) to be used as food enrichment, mainly for calcium, increasing the mineral's availability in traditional recipes and allowing complete use of the frog carcass.

Measuring the weight was one of the procedures in the control process of the carcasses' productivity percentage. The whole carcass weight was evaluated, followed by a second evaluation on the now deboned carcass, in order to verify to which extent the skeleton contributes to the total weight of the frog meat sold in retail.

According to Basilio et al. (2003), a large amount of fish production is disregarded as waste, accounting for significant problems in the industry, since they are of difficult disposal and undergo quick deterioration when not properly handled, interfering in production costs and efficiency, on top of generating pollution. Thus, despite frog farming production chain still not allowing for industrialization of its meat, due to strangulation problems during production, resulting from the lack of handling protocols and knowledge on the species nutritional demands, we can observe a significant amount of slaughterhouse waste cast into the environment, generating pollution as well as neglecting a protein source of great interest for human nourishment.

Results for centesimal analysis of bullfrog meat and bone flour (Table 1) confirm relevance as calcium supplement.

The bullfrog meat-and-bone flour shares very similar nutritional characteristics with bullfrog meat, which has been mentioned by many authors (Peluzio et al., 1995; Ramos et al., 2004) as having optimal nutritional properties, a high biological value protein (Organização Mundial da Saúde, 1985; Corrêa, 1998) and high nutrient bioavailability. Centesimal analysis of the LCAF showed low rates of both fats and carbohydrates, while protein concentration was of approximately 60%, and minerals represented approximately ¾ of the food's composition.

The product, much like bullfrog meat, already quoted in literature as a possible substitute for protein source in diets for allergic individuals, despite the lack of research regarding its use (Sabra, 2015; Martins, 1995), can serve as functional food.
Figure 1. Stages of detachment of bone parts from bullfrog carcass: longitudinal section in the medial region of the thigh (A); sagittal section in the anterior region of the thigh for bone displacement (B); restraint of thigh muscles without bone parts (C); section in the lower part of the flank, depicting the early stages of bone removal (D); final section in the flank muscle, detaching it from the carcass (E); appearance of skeleton without muscles from thigh, upper thigh and flank areas (F); detail of bullfrog carcass skeleton with long bones connected to the backbone (G); lot of 50 frog carcasses, general view (H) and detailed view (I).
Figure 2. Stages of preparation of frog meat and bone flour: arrangement of bones on trays and placement in the oven at 100 °C (A and B); removal of the trays with dehydrated bones from the oven after 9 hours (C); crushing the bones in a blender at 24,000 RPM for 10 minutes (D); obtaining the flour and packaging the product in plastic bags (E); vacuum packed flour, to avoid oxidation (F).

Table 1. Results for centesimal analysis of Low Cost Alternative Food (meat and bone flour) made from bone parts of bullfrog carcasses.

| LCAF samples (meat and bone flour) | Centesimal Analysis (%) |   |   |   |   |
|-----------------------------------|-------------------------|---|---|---|---|
|                                   | Moisture | Ash | Protein | Fat | Carbohydrates |
| 01                                | 4.76     | 23.80 | 57.66   | 11.69 | 2.09          |
| 02                                | 4.66     | 24.03 | 58.53   | 11.45 | 1.33          |
| 03                                | 4.60     | 23.81 | 57.44   | 11.59 | 2.56          |
| Average                           | 4.67     | 23.88 | 57.88   | 11.58 | 1.99          |
Regarding the product’s mineral content, similarity to meat composition suggests that its use as a primary source of calcium should be considered, based on preliminary studies which confirm the presence of high calcium rates in frog meat (Noll & Lindau, 1987; Larsen et al., 2000), indicating that it might serve as an alternative to milk, especially for individuals with allergies or lactose intolerance.

Results for microbial analysis, done in triplicate, of the Low Cost Alternative Food (LCAF) in the form of meat and bone flour, made from bullfrog (Lithobates catesbeianus) carcass, exhibited in Table 2, show that the product is within the limits demanded by current legislation for fish and its products (Brasil, 2002), indicating that the raw material was prepared under the Standards for Good Manufacturing Practices and has good hygienic-sanitary conditions, being the product fit for human consumption.

3 Conclusions

Complete usage of bullfrog carcasses’ bone parts should be feasible through a heating technique applied for maximum dehydration of the carcasses’ bone parts. The Low Cost Alternative Food (LCAF), in the form of meat and bone flour, was made in compliance with the Standards for Good Manufacturing Practices, indicating good hygienic-sanitary conditions until the 53rd day, according to shelf life testing, being the product fit for human consumption.

In addition to maximizing the usage of bullfrog carcass, LCAF can serve as an excellent functional food, since it exhibited great similarity to bullfrog meat contents, being composed of 25% mineral matter, 60% protein, with a 90% digestibility rate, only 12% lipids and 2% carbohydrates. This composition alone can compensate for calcium deficiency and malnutrition due to the quality of its protein.

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Table 2. Results for microbial analysis of Low Cost Alternative Food (LCAF) in the form of meat and bone flour made from bullfrog carcass.

| Point zero | 07 days | 15 days | 21 days | 30 days | 38 days | 45 days | 53 days |
|------------|---------|---------|---------|---------|---------|---------|---------|
| Mesophilic count | negative | negative | negative | negative | negative | 4 \times 10 \text{ UFC.g}^{-1} | negative |
| Caliform 45 °C | absent | absent | absent | absent | absent | absent | Absent in 25g |
| Salmonella sp. | Absent in 25g | Absent in 25g | Absent in 25g | Absent in 25g | Absent in 25g | Absent in 25g | Absent in 25g |
| Staphylococcus coagulase positive | absent | absent | absent | absent | absent | < 2 \times 10 \text{ UFC.g}^{-1} | < 3 \times 10 \text{ UFC.g}^{-1} |

*Staphylococcus sp - Coagulase negative.
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