

Development of freeze-dried products with frog meat for special diets: microbiological and sensory traits¹

Desenvolvimento de produtos liofilizados com carne de rã para dietas especiais: características microbiológicas e sensoriais

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ABSTRACT - Frog meat has high nutritional value and can be used by children with food intolerances and by the elderly to prevent osteoporosis and arterial hypertension. In this study, we developed different instant-soup preparation methods using seasoned and shredded frog meat with vegetables ready for consumption after rehydration; as well as preparation methods for shredded, seasoned and dehydrated frog meat for use in the preparation of soups. Four preparation methods were considered in the sensory tests: M1 - Freeze-drying of the soup after preparation with seasoned frog back meat plus vegetables, M2 - Freeze-drying of the soup after preparation with seasoned frog leg meat plus vegetables; M3 - Soup prepared with freeze-dried seasoned frog back meat plus vegetables; and M4 - Soup prepared with freeze-dried seasoned frog leg meat plus vegetables. The freeze-dried powdered soups and the freeze-dried seasoned meats used in the preparation of the soups were subjected to the following microbiological analyses: count of thermotolerant coliforms and *Bacillus cereus*; identification of coagulase-positive *Staphylococcus*; and isolation and identification of *Salmonella* spp. The results of microbiological analyses showed that all samples met the standards established by the National Health Surveillance Agency of Brazil for frog meat, dried food fish and powder mixtures for instant products. The results of the acceptance test revealed that all soups were accepted in terms of overall impression and purchase intention, although M3 was statistically the least accepted ($P < 0.05$). The paired-comparison test did not indicate a preferred sample ($P > 0.05$). The availability of quality semi-prepared products will facilitate and expand frog meat consumption and marketing, promoting both the growth of the industry and the production sector and rendering frog farming more attractive and competitive.

Key words: Frog meat. Food quality. Food Technology. Instant soups. Special diet.

RESUMO - A carne de rã apresenta alto valor nutricional, e pode ser utilizada por crianças que apresentem intolerância alimentar e idosos na prevenção da osteoporose e hipertensão. Neste estudo foram desenvolvidas diferentes métodos de preparo de sopas instantâneas usando carne de rã temperada e desfiada acrescida de legumes, prontas para consumo, após reidratação e também desenvolvidos métodos de preparo da carne de rã, desfiada, temperada e desidratada para utilização no preparo de sopas. Na realização dos testes sensoriais foram considerados quatro métodos de preparo: M1 - Liofilização da sopa após preparo com carne do dorso da rã temperada e legumes, M2 - Liofilização da sopa após preparo com carne da coxa da rã temperada e legumes; M3 - Sopa preparada com carne temperada e liofilizada do dorso da rã e adição de legumes; e M4 - Sopa preparada com carne temperada e liofilizada da coxa da rã e adição de legumes. As sopas liofilizadas em pó e as carnes temperadas e liofilizadas utilizadas no preparo das sopas foram submetidas a análises microbiológicas: contagem de coliformes termotolerantes e *Bacillus cereus*; identificação de *Staphylococcus* coagulase positiva; isolamento e identificação de *Salmonella* spp. Os resultados das análises microbiológicas evidenciaram que todas as amostras atenderam o estabelecidos pela Agência Nacional de Vigilância Sanitária do Brasil para carne de rã, pescado seco e mistura em pó para produtos instantâneos. Os resultados do teste de aceitação indicaram que todas as sopas foram aceitas em relação a impressão global e intenção de consumo, apesar da M3 ter sido estatisticamente a menos aceita ($P < 0,05$). O teste de comparação pareada não indicou uma amostra preferida ($P > 0,05$). A disponibilidade de produtos semi-prontos de qualidade facilitará e ampliará o consumo promovendo o crescimento da indústria e do setor produtivo, tomando a rãicultura mais atraente e competitiva.

Palavras-chave: Carne de rã. Qualidade do alimento. Tecnologia de alimentos. Sopas instantâneas. Dietas especiais.

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INTRODUCTION

According to a report published by the Food and Agriculture Organization of the United Nations (2018), the world food fish consumption is estimated to reach 21.5 kg per capita in 2030. In the FAO report (2018), frogs are included in the food fish group as “other aquatic animals” with a production of 96,000 t.

Frog meat consumption has increased, and its fans are attracted not only by its taste, but, mainly, by its nutritional properties (PAIXÃO; BRESSAN, 2009; SEIXAS FILHO *et al.*, 2020). The use of frog meat is recommended in diets aimed at fighting cholesterol, obesity and high blood pressure; for the treatment of gastrointestinal disorders mainly for children allergic to other animal proteins (MELLO *et al.*, 2006; OLIVEIRA *et al.*, 2013; RODRIGUES *et al.*, 2014).

A caveat should be mentioned, however: frog meat has elevated production costs and is poorly available in the market. Once the benefits of its consumption are widely publicized and easy-to-prepare options presented, consumption will be intensified by the increased demand.

Like other food fish, frog meat is vulnerable to deterioration, especially due to its pH being close to neutrality and its large amounts of water available for the growth of microorganisms, high nutritional content and high unsaturated fatty acid content (BARONE *et al.*, 2017; SOARES; GONÇALVES, 2012).

The Brazilian legislation (BRASIL, 2001) provides for restrictions on the presence of some human pathogenic bacteria that may be found in food fish. Those addressed in the legislation pertaining to products derived from chilled or frozen fish are: coliforms at 45 °C - tolerance of 10²; coagulase-positive staphylococci - tolerance of 5 × 10²; and *Salmonella* spp. - absence in 25 g of sample. Additionally, for the dried food fish and powder mixtures for instant products, the count of *Bacillus cereus* should be zero.

A study led by Weichert *et al.* (2007) on frog meat consumption showed that, in 45% of points of sale, the reason for buying frog meat is linked to its functional and beneficial health use. For 86% of the interviewed consumers, the taste, texture and quality of the meat are the reasons for buying it, whereas 63% of them complained about the price of the product. Oliveira *et al.* (2017) conducted a survey on the knowledge about the benefits of frog meat with health professionals involving doctors and nutritionists. Among the respondents, 53% did not know the benefits of frog meat, whereas 29% believed in the potential of using it in the diet of people with food allergy problems and 14% considered this meat a great option for low-sodium diets.

According to Sabrá (2015), when a patient with food allergies starts a dietary treatment, he or she will be deprived of the main protein sources of the usual diet, which increases the risk of malnutrition. The diet must be managed by supplementing these nutrients with the introduction of unusual proteins, where frog meat is the first choice.

In this study, we developed freeze-dried soups of frog meat and vegetables and seasoned, freeze-dried frog meat aiming to facilitate preparation and storage, especially for consumers who need low-calorie, hypoallergenic and highly digestible diets, and also aiming at the utilization of different cuts and value-adding by the food agribusiness.

MATERIAL AND METHODS

The frog meat used in the present study were acquired from an establishment under State inspection and stored in a freezer at -18 °C. At the Food Laboratory of the Augusto Motta University Center (Unisuam), the thawed frog carcasses underwent a blanching and deboning process following the methodology used by Lindener Junior *et al.* (2013). The separate cuts (legs and back) were mixed with vegetables (yam and carrot), salt, dried spices (garlic, onion, turmeric, ginger, paprika, thyme, rosemary, basil) and water. After the cooking process, the soups corresponding to preparation methods M1 and M2 were cooled and frozen at -40 °C for 3 h and then transferred to the LS 3000 Bf freeze-drier, where they remained 24 h. To prepare the soups that corresponded to methods M3 and M4, the blanched and deboned frog back and leg meats seasoned with salt, turmeric, ginger, paprika, thyme, rosemary and basil were frozen separately at -40 °C for 3 h and then transferred to the LS 3000 B freeze-drier, where they remained for a period of 24 h. After freeze-drying, the seasoned back and leg meats were used in the preparation of the soups (M3 and M4). In the preparation of these soups, the separately seasoned and freeze-dried frog back and leg meats were mixed with fresh vegetables (yam and carrot), salt and dried seasonings (garlic, onion, turmeric, ginger, paprika, thyme, rosemary, basil), with water heated to 100 °C.

Samples of soup and freeze-dried seasoned frog meat were sent to the Microbiology Laboratory of the State Center for Food Quality Research of the Agricultural Research Corporation of Rio de Janeiro State (PESAGRO) for bacteriological analysis, following the recommendations of ANVISA (BRASIL, 2001) for fresh frog meat, dried food fish and powder mixtures for instant preparation. Thermotolerant coliforms and *Bacillus cereus* were counted; coagulase-positive *Staphylococcus* were identified; and *Salmonella* spp.

were isolated and identified, following the methodology proposed by American Public Health Association (2015) and Brasil (2003).

The project was previously submitted for approval by the Human Research Ethics Committee at Unisuam (CAAE 60904916.1.0000.5235). After learning the content and objectives of the survey, all the tasters filled out the consent form (*Termo de Consentimento Livre e Esclarecido*, TLCE). The acceptance test was carried out with 50 elderly people aged 60 to 85 years, in individual booths, at the Sensory Analysis Laboratory of Unisuam. The degree of acceptance was determined using a structured 7-point hedonic scale, and the taster expressed their attitude towards consumption in the same form (MINIM, 2013).

The paired-comparison test was carried out to assess whether, after reconstitution, the soup ready for consumption (M2) would exhibit any sensory disadvantage in relation to the soup prepared with fresh vegetables (M4). The same 50 tasters who participated in the acceptance test chose the preferred sample in individual booths, in the sensory analysis laboratory, where they were served: a freeze-dried soup of frog legs and vegetables (M2) reconstituted with water heated to 100 °C; and a soup prepared with freeze-dried frog leg meat (M4) plus fresh vegetables. Both samples were coded with three-digit numbers and introduced at the same time to the judges, who were warned that a choice was necessary, that is, they should indicate their preferred sample, following the methodology proposed by Minim (2013).

RESULTS AND DISCUSSION

According to RDC no. 12, which establishes the Sanitary Microbiological Standards for Food in Brazil (BRASIL, 2001), all samples were within the recommended microbiological limits for frog meat, dried food fish products and powder mixtures for instant products. For the samples of freeze-dried soup (M1 and M2) and freeze-dried frog meat used in the preparation of the soups (M3 and M4), the count of thermotolerant coliforms was less than 0.3. MPN.g⁻¹. *Salmonella* and *Bacillus*

cereus were not detected, and all samples were negative for coagulase-positive *Staphylococcus* (Table 1).

The coliform group comprises enteric bacteria and encompasses the genera *Escherichia*, *Enterobacter*, *Klebsiella* and *Citrobacter*. The coliform index expresses hygienic conditions. Infected handlers, handlers with inadequate personal hygiene and/or water contaminated with human fecal matter are likely sources of food contamination (MEDEIROS *et al.*, 2017). All products were within the recommended standards for thermotolerant coliforms, with counts below 0.3/g, indicating the good quality of the raw material and the adoption of Good Manufacturing Practices in the preparation of the products.

Traditionally, staphylococci are divided into two categories: coagulase-positive and coagulase-negative. This ability to coagulate plasma is an important marker of pathogenicity (SILVA *et al.*, 2015). In all samples analyzed in this study, no presence of coagulase-positive *Staphylococcus* was observed.

Species of the genus *Salmonella* are important because they cause food infections (DADIÉ *et al.*, 2017). The Brazilian legislation determines the absence of this microorganism in 25 g of sample. *Bacillus cereus* strains, on the other hand, are known to cause food poisoning and the current legislation recommends that analyses of this microorganism be carried out on partially prepared food fish products and powder mixtures for instant preparations (BRASIL, 2001). In all the samples analyzed in this study, there was no growth of these microorganisms. According to Soares and Gonçalves (2012), due to the large amount of water present in fish, it degrades very quickly. Drying is a method that aims to prolong the shelf life of a food based on water removal, which inhibits the activity of some enzymes as well as microbial development. When food is preserved by drying, its moisture content is reduced to a point where deterioration and the deteriorating microorganisms in it are inhibited (JAY, 2005).

Freeze-drying also allows a faster reconstitution because of the channels formed by the ice crystals, which leave spaces for the liquid to penetrate after the heating process (WANG *et al.*, 2013). The low pressure and temperature during water removal make it possible to

Table 1 - Results of microbiological analyses of the freeze-dried soups and meat

Sample	Thermotolerant coliforms (MPN/g)	Coagulase-positive <i>Staphylococcus</i> (CFU/g)	<i>Salmonella</i> spp. (25 g)	<i>B. cereus</i> (CFU/g)
Freeze-dried soup with frog back meat	< 0.3	Negative	Absent	Absent
Freeze-dried soup with frog leg meat	< 0.3	Negative	Absent	Absent
Freeze-dried frog back meat	< 0.3	Negative	Absent	Absent
Freeze-dried frog leg meat	< 0.3	Negative	Absent	Absent

maintain the nutritional quality of the food and to preserve it, since freeze-dried products have low water activity, which inhibits bacterial growth (CIURZYŃSKA; LENART, 2013; HATANO, 2013; YAMAGUCHI *et al.*, 2017.).

All soups received an average score greater than five points for all attributes on the 7-point hedonic scale (1-I dislike extremely, 2- I dislike very much, 3 -I dislike, 4- neither like nor dislike, 5- I Like 6- I liked very much 7- I liked extremely), meaning they were sensorially accepted in terms of appearance, flavor, texture and overall impression (Table 2). The percentage of tasters who stated that they liked the product extremely, for preparation methods M1, M2, M3 and M4, was 58%, 44%, 26% and 66% respectively, whereas 22%, 34%, 28% and 18% stated they liked it very much. Although the soup prepared using freeze-dried seasoned back meat (M3) was statistically less accepted than the others ($p < 0.05$), it was also well-rated.

The methods used in the preparation of the soups (M1 and M3) showed different results. There was no statistical similarity at 95% confidence for any of the studied parameters, although frog back meat was used in both methods. This finding may be associated with greater homogeneity of the fully freeze-dried product (M1), where all ingredients were rehydrated at the time of preparation. When the soup was prepared using freeze-dried seasoned frog back meat (M3), however, fresh vegetables were added at the time of preparation, and the heterogeneity of the parts that make up the back meat (meat being taken from the rib, forelegs and flank; (IDE *et al.*, 2021)) may have influenced the results. The flank is the most fibrous part of the meat on the back and may have sensorially stood out at the time of consumption, due to the different method of preparation. This fact may also have reduced the consumption intent of the M3 sample.

The present results indicate that the soup preparation methods M2 and M4, both made with frog leg meat, were

similar in appearance, flavor, overall impression and consumption intention ($p > 0.05$). The statistical difference found with respect to texture ($p < 0.05$) may be related to the method used, since fresh vegetables were added at the time of preparation (M4), resulting in a less satisfactory evaluation of the texture by consumers.

In terms of attitude (consumption intention), the same behavior was observed, that is, the soup corresponding to preparation method 3 was statistically inferior to the others ($p < 0.05$). On the other hand, on average, the tasters stated they would definitely or probably consume all the soups presented on the 5-point attitude scale: 1- Definitely would not consume. 2- Probably would not consume. 3- Probably would consume/ Probably would not consume. 4- Probably would consume. 5- Definitely would consume.

The soup formulated with a frog back and vegetables obtained a higher percentage for consumption intention, highlighting that frog back, the least noble part, can be directed to the preparation of derived products.

The paired-comparison test between the samples of freeze-dried soup of frog leg meat and vegetables reconstituted with water heated to 100 °C (M2) and soup with freeze-dried frog leg meat and fresh vegetables (M4), prepared two hours before the start of the test, indicated that a preferred sample was not chosen. In other words, there was no significant difference between the samples at the 5% level using the table proposed by Roessler *et al.* (1978). According to Marques and Costa (2015), in the freeze-drying process, the food undergoes important stages such as freezing, sublimation and desorption, which allow it to maintain its nutritional value and, during reconstitution, i.e., rehydration, it becomes similar to its original state by retaining its characteristics. According to the results obtained in the sensory analyses, dehydration by freeze-drying as used in this study did not induce sensory changes in the product after reconstitution.

Table 2 - Means* and standard deviations of the results of the acceptance test and intention to consume the dehydrated soups of frog meat and vegetables and soups prepared with dried seasoned frog meat with vegetables

Preparation method	Appearance	Flavor	Texture	Overall impression	Purchase intention
1	6.42 ± 0.10 a	6.40 ± 0.11 a	6.40 ± 0.10 a	6.26 ± 0.15 a	4.66 ± 0.10 a
2	6.14 ± 0.13 ab	6.08 ± 0.16 a	5.96 ± 0.17 ab	5.98 ± 0.18 ab	4.44 ± 0.10 ab
3	5.64 ± 0.18 b	5.40 ± 0.17 b	5.40 ± 0.21 b	5.36 ± 0.21 b	4.14 ± 0.15 b
4	6.00 ± 0.18 ab	5.86 ± 0.18 ab	5.76 ± 0.19 b	5.74 ± 0.20 ab	4.50 ± 0.13 ab
P-value	0.0063	0.0002	0.0010	0.0092	0.0313

*Means followed by different letters in the same column differ according to Tukey's test at 5% probability

CONCLUSIONS

1. All the developed products were within the recommended microbiological limits for frog meat, dried food fish and powder mixtures for instant products, according to the Microbiological Sanitary Standards for Foods established by the National Sanitary Surveillance Agency of Brazil;
2. The use of leg meat had less influence on the soup preparation method as compared with the use of back meat. The different parts that make up frog back meat may have contributed to this difference;
3. Although no products similar to those developed in this study exist in the market, they obtained good sensory acceptance, demonstrating their consumption potential, since frog meat is already recognized for its functional properties;
4. The method of preparation in which all ingredients are freeze-dried for subsequent rehydration is the best to be adopted for marketing the product, as no additional fresh vegetables are required to improve the acceptance and flavor of the instant soup;
5. The products developed with frog meat in this study can meet consumers' expectations in terms of practicality in preparation and ease of storage, contributing to increasing frog meat consumption and strengthening the frog-farming production chain.

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