

Microbiological evaluation and concentration of vitamin A, iron and zinc in preparations of sururu mussels (*Mytella falcata*)

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Abstract

This study aimed to assess the nutritional quality and security of a meal based on sururu, with or without coconut milk, and its micronutrients profile. The degree of contamination by heavy metals (As, Hg, Pb and Cd) was assessed in samples of sururu *in natura*, from the Mundaú-Manguaba estuarine complex (in the city of Maceio, state of Alagoas, Brazil). Assessment was conducted in accordance with the method described by the Association of Official Analytical Chemists (AOAC, 2005), as well as with the hygiene and sanitary conditions of sururu meals prepared with or without coconut milk, accounting for thermotolerants coliforms, coagulase-positive *Staphylococcus* and *Salmonella sp.*, recommended by the Brazilian Health Ministry, the Agriculture Ministry (2006), AOAC (2005) and the American Public Health Association (APHA, 2004). Micronutrient profile was assessed according to the methodology of AOAC (2005). Only As (0.63 mg) and Pb (0.34 mg), in a range allowed by the legislation, were found in our samples. No microbiological contamination was found. Micronutrient assessment showed that sururu meals had higher Fe content than bovine meat (sururu without coconut milk, 17.56 mg, and sururu with coconut milk, 13.8 mg), and that sururu is a good source of zinc (sururu without coconut milk, 0.97 mg, and sururu with coconut milk, 1.22 mg), and some vitamin A (sururu without coconut milk, 3.13 mg, and sururu with coconut milk, 4.73 mg). Results indicate that there was no chemical or microbiological contamination in these samples. Sururu meals are source of Fe and Zn and contain vitamin A, thus, they might be an effective option in the recovery of nutritional deficiencies.

Keywords: Sururu. Coconut Milk. Heavy Metals. Food Safety. Food Hygiene. Micronutrients.

Introduction

The mussel mollusk is amply consumed in the Brazilian Northeast, mainly in Alagoas. Predominantly found in the Mundaú-Manguaba Lagoon Estuarine Complex (MMLEC) (Complexo Estuarino Lagunar Mundaú-Manguaba (CELMM)), it is considered the most important mollusk from the state's economic point of view, since many families survive on the fishing and selling of this food.¹ It is well accepted in the cuisine, easy to find and capture, and has been explored *through handmade methods* both for subsistence consumption and for the consumer market. Mussel preparations are connected to the Alagoan culture itself and are found rooted in the eating habits of the region,¹ above all else when used together with coconut milk.

Brazil is considered one of the great producers of coconut, 85% of its production being concentrated in the Northeast, in addition to being the biggest producer of industrialized coconut milk worldwide, which is amply utilized in the most diverse Brazilian culinary dishes. Coconut is a food rich in fat, carbohydrates, proteins, and vitamins (A, B1, B2, B5, and C).² In addition to this, its composition presents mineral salts, especially potassium, sodium, phosphorus, chlorine, and magnesium. As such, the preparation of mussels with coconut milk added increases the availability of proteins, polyphenols, vitamins, and minerals.²

On the other hand, there are no studies about the microbiological quality of the mussel preparations with and without coconut milk. What is said in respect to chemical contamination by heavy metals, makes it necessary to carry out more studies on this food so amply utilized. It is known that the prolonged exposure to toxic agents, as is the case with heavy metals, can cause damage to health when ingested in a continuous manner and in levels above what is permitted by legislation. Such is the case with mercury, which can lead to poisoning of the central nervous system, and lead, which can cause a condition known as saturnism. Yet cadmium and arsenic can cause cancers.³ Moreover, it is not just the salubrity of the fishing environment that determines the sanitary hygienic quality of the final product, but also the after stages of fishing, such as is the case with handling, cooking, and storage of the food.⁴ These are determinants in the contamination of fish, that present a nutritional composition very accommodating to the proliferation of debilitating microorganisms and pathogens.⁵

Based on the above, the objective of the present article was to study the quality and the microbiologic safety of the preparation of mussels with and without coconut milk, and its micronutrient profile.

Methodology

Characterization of the collection site

To process the analyses of the study, the sururu mussel (*Mytella falcata*) was collected in the largest area of natural cultivation in the municipalities of Maceió and Coqueiro Seco, along the lakes Mundaú and Manguaba, which extend the middle coast of the State of Alagoas and are part of the estuarine system named Mundaú/Manguaba Estuarine Lagoon Complex (MMLEC) (Complexo Estuarino Lagunar Mundaú-Manguaba (CELM)). It situates itself between the latitudes of 9.4° to 8.4° S, and longitudes of 35.4° and 36.2° W.

Raw materials and Methods

To carry out the research on heavy metals, nine samples were collected *in loco*, belonging to three different points in the MMLEC. The samples *in natura* were packed in polyethylene compartments properly sealed, frozen, and sent directly to the Institute of Food Technology (IFT)(Instituto de Tecnologia de Alimentos (ITAL)), located in Campinas-SP, where the heavy metal analyses were carried out - arsenik (As), lead (Pb), mercury (Hg), and cadmium (Cd), in accordance with the method of the *Association of Official Analytical Chemists* (AOAC).⁶ The results of the analyses were compared to the chemical contaminant standards of the National Division of Sanitary Vigilance (Divisão Nacional de Vigilância Sanitária).⁷

To evaluate the microbiological quality of the mussel preparations, the Ministry of Health Sampling Plan, National Agency of Sanitary Vigilance (Plano de Amostragem do Ministério da Saúde, Agência Nacional de Vigilância Sanitária - ANVISA).⁸ Ten samples were produced, five being mussel with coconut milk (MWCM) and five being mussel without coconut milk (MSCM). After collection, the samples were codified, properly packed, and immediately sent to the Laboratory of Food Control and Quality at the Federal University of Alagoas (Laboratório de Controle e Qualidade de Alimentos da Universidade Federal de Alagoas), for analysis. Counts of Thermotolerant coliforms, coagulase-positive *Staphylococcus* and *Salmonella* sp. research were carried out, in accordance with the methodology described by the *American Public Health Association* (APHA)⁹ and Ministry of Agriculture and Food Supply (Ministério da Agricultura, Pecuária e Abastecimento (DAS)).¹⁰

Nine samples were analyzed for the study of micronutrients (iron, zinc, and vitamin A), three being in mussels *in natura*, three in mussels with coconut milk, and three in mussels without coconut milk. All of the samples were codified and packed in polyethylene compartments properly

sealed, frozen and sent to the IFT, where chemical analyses were carried out according to the methodology of the *Association of Official Analytical Chemists (AOAC)*.⁶

Statistical Analyses

For variables of the study that met the assumptions of normality (Lilliefors test) and homogeneity of residual variances (Leven test), parametric statistics were carried out (*post-hoc* of *t-Student* and *Tukey-HSD test*) preceded by analysis of variance - ANOVA (by the F test). For the variables that did not present normality and homoscedasticity of residuals, the nonparametric Mann-Whitney test was applied. In both cases, a value of 5% for probability of experimental error was adopted.

Results

Chemical evaluation – heavy metals

In table 1, the medium concentrations of heavy metals found in the samples of analysed mussels are represented. Among the researched metals, the presence of As and Pb was detected, though within the limits recommended by the prevailing legislation,⁷ not having statistical differences ($p>0.05$) between the medium values of the referred metals in relation to the location of mussel collection. The presence of Cd and Hg was also not evident in the samples.

Table 1. Average concentrations of arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg) in samples collected at the beginning and ending of locations: Dique Estrada and the village of Cadóz - Coqueiro Seco, and final averages of all samples. Maceió-AL, 2009.

	Reference values ⁷ (mg/kg)	Average beginning values of Dique Estrada (mg/kg)	Average ending values of Dique Estrada (mg/kg)	Average values of Cadóz (mg/kg)	Final average (mg/kg)
As	1	0.544	0.93	0.42	0.63
Cd	1	Nd	nd	nd	nd
Pb	2	0.31	0.286	0.43	0.34
Hg	0.5	Nd	nd	nd	nd

Microbiological evaluation

The results of the microbiological analyses are described on Table 2. All of the evaluated samples of mussel preparations with and without coconut milk presented themselves within the microbiological standards recommended by the prevailing legislation,⁸ which establishes as a maximum limit 5×10^3 NMP/g of thermotolerant coliforms; 10^3 UFC/g *coagulase (+)*/g *Staphylococcus* and absence of *Salmonella sp.*, for cooked, industrialized, refrigerated, or frozen bivalve mollusks.

Table 2. Microbiological evaluation of the preparations of mussels with coconut milk and mussels without coconut milk. Maceió-AL, 2009.

Samples	Variables		
	coliforms at 45°C (NMP/g)	<i>coagulase (+)</i> <i>Staphylococcus</i> (UFC/g)	<i>Salmonella sp.</i>
MWCM*	<3 ^a	<10 ^a	Absence
MSCM**	<3 ^a	<10 ^a	Absence

Equal letters on the same line indicate the absence of significant difference ($p > 0.05$) by the non-parametric Mann-Whitney test.

*MWCM -> Mussels with coconut milk

**MSCM -> Mussels without coconut milk

Evaluation of micronutrients (vit. A, Fe, and Zn)

The analysis of each researched micronutrient is shown in table 3. Vitamin A presented higher levels in the mussel samples *in natura*, followed by the mussels with coconut milk and the mussels without coconut milk. Iron was the micronutrient found in the largest quantity, such that the mussels *in natura* presented the greatest concentrations of this micronutrient. The preparation of mussels without coconut milk presented an iron level significantly higher (17.5 mg) to that found in the mussels with coconut milk (13.8 mg).

Table 3. Concentrations of micronutrients in mussels *in natura*, mussels with coconut milk and mussels without coconut milk (average \pm standard deviation). Maceió-AL, 2009.

Variable	Mussels <i>in natura</i>	MWCM*	MSCM**
Vit. A ($\mu\text{g}/\text{kg}$)	8.17 \pm 0.408 ^a	4.73 \pm 0.711 ^b	3.13 \pm 0.146 ^c
Fe (mg/kg)	22.36 \pm 1.504 ^a	13.80 \pm 0.519 ^b	17.56 \pm 1.059 ^c
Zn (mg/kg)	1.89 \pm 0.585 ^a	1.22 \pm 0.208 ^b	0.97 \pm 0.543 ^c

Different letters on the same line indicate significant difference ($p < 0.05$) by the test

Post Hoc Tukey (HSD).

*MWCM -> Mussels with coconut milk

**MSCM -> Mussels without coconut milk

Zinc was the trace element that presented the fewest losses after cooking (42%), being the preparation of mussels with coconut milk, which presented the highest concentrations of this mineral.

Discussion

The results of the present study showed, in general terms, that heavy metal contamination in mussels was not detected at the sites researched. These results are different from other studies carried out in the Southeast Region of the country, particularly in the study by Machado *et al.*,¹¹ that found levels of contamination for Cd, Pb, and Hg (0.11; 0.08; and 0.2 mg/kg) in *Crassostrea brasiliiana* mangrove oysters from the Cananeia-SP estuary.

In the present study not one of the analysed samples of sururu showed contamination by Hg, different from the study conducted by D'amato *et al.*,¹² in research carried out on the Fluminense-RJ Lowlands with the *Perna perna* mussel, which verified the presence of Hg with values that varied from 0.03 to 0.06 mg/Kg.

Pereira *et al.*¹³ determined the levels of Pb, Cd, Hg, Cu, and Zn in bivalves (*Crassostrea brasiliiana*, *Perna perna* e *Mytella falcata*), in the Santista-SP Lowlands. The authors found contamination in the samples evaluated for Cd (0.091 mg/kg) and Hg (0.023 mg/kg), but values less than those of the present study for Pb (0.2 mg/kg), as we found a concentration of 0.34mg/kg of this metal in our samples.

On the other hand, a study carried out seven years before by the CETESB,¹⁴ also in the Santista-SP Lowlands, found levels of contamination of Pb, Cd, and Hg in bivalve samples similar to those of the present research, indicating that the contamination found in these studies may be recent.

As for food preparations for consumption, various researchers are incisive in revealing cross contamination due to hygienic failures on behalf of handlers, utensils, equipment, and surfaces in the contamination of the final product.^{15,16} Another important aspect relates to the disregard for thermal treatment of the mollusks, for it is well known that temperature and cooking time are fundamentally important factors in the control of sanitary conditions of the final product.

In the present study, the thermotolerant coliform and coagulase (+) *S.* counts were in accordance with the microbiological standards, suggesting that the storage, handling, and thermal treatment applied to the preparations of mussels with and without coconut milk were considered satisfactory in terms of the sanitary hygienic aspects. Similar data were obtained by Cordeiro *et al.*¹⁶ with *Perna perna* mussel samples, submitted to the same cooking process.

The cooking, however, also exerts a negative function over the micronutrients, due to the losses in the cooking water. In the case of zinc and iron, this phenomenon can be explained by the presence of complex soluble composites that generally are lost to the cooking water.¹⁷

When the levels of micronutrients researched in mussels are compared with those found in the food composition tables of ground beef,¹⁸ it is observed that the total iron level of the mussels is higher than the meat in both preparations. *Adding to the fact that, according to Cabrera *et al.*,¹⁹ the particular form of Fe chemical substance found in mussels, in general, is similar to that found in meat products, and therefore, presents high bioavailability. As such, the consumption of this mollusk may be of interest in the treatment of anemia, for being considered an alternative source of iron.

Consolidated data from the Panamerican Organization of Health (Organização Panamericana da Saúde) and specific studies carried out in some states (Paraíba, Pernambuco and São Paulo) are in agreement over the evidence that 40 to 50% of children less than five years old are carriers of anemia.²⁰ Ferreira *et al. study.*²¹ found 96.4% of children with anemia, residing 60 months in shanty towns on the margins of Maceió.

Similar works were carried out with oysters, mussels, and bivalve mollusks in sand. A study of mussel species, also bivalve (oyster), showed that they are an abundant source of high bioavailability zinc, probably due to its chemical form and stability in the mussel.²²⁻²⁴ These data were also shown in the present study, after the cooking process.

The good bioavailability of zinc in oysters was also proven by Caetano *et al.*,²⁴ in carrying out a biological test with laboratory animals that consumed diets absent of zinc and enriched with oysters. It was observed that the concentrations of this mineral were greater in the femur of the animals who fed on this bivalve mollusk.

In turn, Pedrosa & Cozzolino,²² after analysing the chemical composition and the mineral content, including Fe and Zn, in raw and cooked mussels (*Anomalocardia brasiliiana*) from Natal-RN, observed similar results to the present research study - that is, a significant loss of Zn level post-cooking, leaving the cooked mussel with 2.99 mg. However, in the present study, even with the post-cooking losses, the mussel preparations were considered sources of zinc, as they presented more than 15% of the RDI (Recommended Daily Intake - reference of 100g of solids intake for children from 1-7 years old).²⁵

The mussel preparations with and without coconut milk presented low levels of vitamin A in relation to the RDI, which advocates that the average intake of children ages 1-7 years old is of 425 μg .²⁶

In Brazil, despite the change in the state of children's nutrition and its evident advances, the problem of poor nutrition still persists, especially in determined subgroups of the North and Northeast.²⁷ The findings of the present study are important and indicate that the consumption of mussels is a viable alternative for the prevention and combat of subnutrition, mainly in locations where this mollusk is easy to access and well appreciated by the low income population.

Associated with protein-energy malnutrition, the shortage of vitamins and minerals, especially the deficiency of iron in lactating women and women of reproductive age, and of vitamin A in pre-schoolers, was verified.²⁷ The shortage of iron compromises early childhood development, since this mineral appears to be involved in the immunological and cognitive function.²⁸ In turn, vitamin A performs an important role in terms of vision, upward growth (through the stimulation of the nocturnal secretion of the growth hormone), in the development of bones (through its effect on protein synthesis and bone cell differentiation), and of epithelial tissue (basal cell differentiation in mucosal epithelial cells).²⁹

Associated with these shortages, there is the deficiency of zinc, important mineral for growth and development, because it acts in the regulation of the growth hormone (GH) and growth factor similar to insulin (IGF-1),² in addition to interfering with mitogenic hormones, acting on cell proliferation, and other important enzymatic functions.³⁰

Conclusion

It was found that the analysed mussel preparations with and without coconut milk were in satisfactory microbiological and chemical condition. Such preparations can be consumed as an alternative food in diets of children in poverty conditions and with nutritional problems, as they are seen to be sources of iron and zinc in quantities considered satisfactory.

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