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Research Article

Nitrite Levels Before and after Washing in Salted Fish

Abstract

Background: Gastric adenocarcinoma is the fourth most common malignancy worldwide and is globally the second leading cause of cancer-related deaths each year. Salted foods and exposure to *H. pylori* infection during the infancy have been considered the most important environment risk factors for gastric cancer. Despite increased access to electric energy, allowing the use of refrigerators to better conserve foods, many people keep consuming salted food, by washing them before eating with the intention of reducing salted flavor and also supposing to decrease or avoid risk of diseases. The aim of this study was to investigate the efficacy of reducing nitrites levels in salted fish by washing the fish before consuming, to find if washing salted food should be an efficient measure to reduce, or even avoid, the nitrites related gastric cancer risk.

Methods: Nine paired samples of fresh and salted fish and 20 additional samples of salted fish were taken for analyses. The nine paired samples were used to comparison of nitrites levels between salted and fresh fish, and the 20 additional salted fish samples for analyses of the effect of washing the fish in reducing nitrites levels. To simulate the usual procedure of living salted fish submerged in fresh water before consumption, we kept the salted samples under water during different periods of time and checked the nitrites levels at each "washing time". For every experiment a p value of 0.05 was considered, and t-student tests were performed.

Results: The washing procedure did not reduce significantly the nitrites levels in salted fish, even after long periods of immersion ($p=0.807$), and the levels of nitrites in washed salted fish remained much higher than that of the fresh samples, maintaining the consumers exposed to nitrites, known carcinogens related to gastric cancer, and giving an equivocal and hazard feeling of protection to the population.

Conclusion: Salted fish has higher concentration of nitrites compared to fresh. Washing, or living salted fish under water, does not provide significant decrease of the nitrites levels.

Background

Gastric adenocarcinoma is the fourth most common malignancy worldwide and is globally the second leading cause of cancer-related deaths each year [1,2].

The death rate from gastric cancer has gradually declined over the last several decades in the United States and worldwide, indicating that environmental factors (e.g. diet) play a critical role in the etiology of this malignancy [3].

However, in undeveloped regions, incidence and mortality remain very high [4] and opportunities for research on cancer prevention include investigating specific circumstances of exposure to known carcinogens [5].

Salted foods and exposure to *H. pylori* infection during the infancy have been considered the most important environment risk factors for gastric cancer, and several new meta-analyses support the carcinogenic effect of high salt intake in the context of *Helicobacter pylori* infection [6,7].

Despite increased access to electric energy, allowing the use of refrigerators to better conserve foods, many people keep consuming salted food. Cultural habits and lack of information regarding the hazards of salting foods seem to be responsible for the high intake

of these types of food. Even having available fresh foods, and refrigerators at home, many people, usually prefer to consume salted food.

Nevertheless, before eating salted food, people wash the food with the intention of reducing salted flavor and also supposing to decrease or avoid risk of diseases related to salted food, as is the case of gastric cancer.

With the aiming of investigate the efficacy of reducing nitrites levels in salted fish by washing the fish before consuming, we decided to conduce a high controlled experiment, simulating the washing procedure, and compared the nitrites levels of salted fish before and after washing the fish, to find if washing salted food should be an efficient measure to reduce, or even avoid, the nitrites related gastric cancer risk.

Material and Methods

Samples description

Samples from both fresh and salted Pirarucu, a traditional Amazonian fish, were obtained at Ver-o-Peso market, the most famous fish market in Belém, the capital of Pará state, in Brazil.

Nine paired samples of fresh and salted fish and 20 additional samples of salted fish were taken to Neurochemistry Lab, at Federal

University of Pará, and analyzed in several manners as described below.

The 29 samples of salted food have been salted 8 days before, as usual, taking the proportion of 1kg (2.2lbs) of salt to each 4kg (8.8lbs) of fish. The nine samples of fresh fish were taken for analyses at the same day that the fish were caught.

The nine paired samples were used to comparison of nitrites levels between salted and fresh fish, and the 20 additional salted fish's samples for analyses of the effect of washing the fish in reducing nitrites levels.

Assay of nitrite level

Samples were initially diluted 1:2 in PBS (phosphate buffer saline) and then 500µl of each diluted sample were mixed with the same volume of Griess reagent (0.1% naphthylethylen + 1% sulfanilamide in 5% phosphoric acid). The nitrites levels were analyzed using a spectrophotometer with a wave length of 540nm, measured and referred to a standard curve of known concentrations of Sodium nitrite [8].

Washing procedure

To simulate the usual procedure of living salted fish submersed in fresh water before consumption, we kept the salted samples under water during different periods of time and check the nitrites levels at each "washing time".

We established four washing protocols: 15 minutes, 60 minutes, 360 minutes and 720 minutes of "washing time". The 20 salted samples were divided in four groups of five samples, and each group was analyzed before and after washing, according to the protocols.

Statistical analyses

The nitrites levels of the nine paired samples were compared taking the average levels of nitrites of each group (salted and fresh fish).

The nitrites levels of each salted sample group submitted to the washing protocols were measured before and after each defined washing time and the results were compared.

For every experiment a p value of 0.05 was considered, and t-student tests were performed using SPSS 17.0 pack (SPSS Ins. Chicago, IL, USA).

Results

The nitrites levels (µM) of the nine fresh fish samples, used for the first experiment of comparing salted and fresh fish, varied from 4.54 to 8.49 (mean 6.32, SD 1.40), and from 16.40 to 31.67 (mean 24.76, SD 6.15) in the nine salted fish. The nitrites levels of salted fish were superior to that of the fresh fish, and these results were statistically significant (p<0.05) (Figure 1).

The mean nitrites levels (µM) of the 20 salted fish samples used for the second experiment (washing procedures) before washing was 25.71 and after washing was 27.58. The mean levels of the same samples, divided in four groups of five samples according to each washing procedure are described in the Table 1.

Each group of five samples was compared before and after washing. The washing procedure didn't reduce significantly the nitrites levels in salted fish, even after long periods of immersion (p=0.807), and the levels of nitrites in washed salted fish remained much higher than that of the fresh samples (Figure 2).

Discussion

Although distal gastric cancer incidence is decreasing in rich countries, it remains as a priority health problem in many countries all over the world [7,9,10].

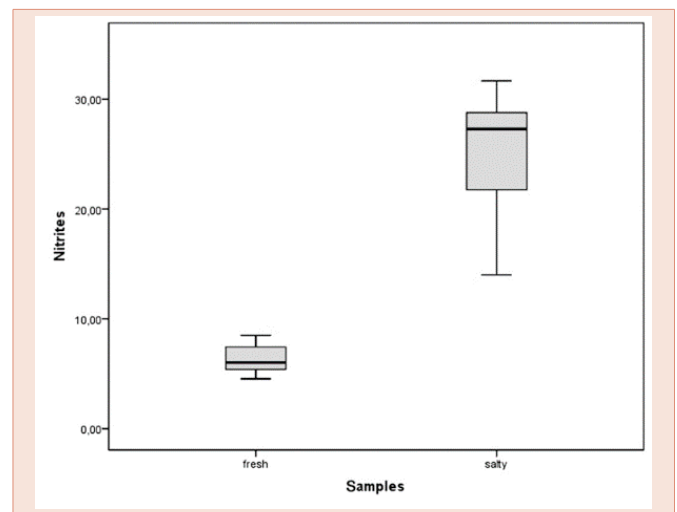


Figure 1: Comparison between nitrites levels of fresh fish and salted fish demonstrating highest levels of nitrites in salted fish (p < 0.05).

Table 1: The mean nitrites levels (µM) of the 20 salted fish samples, before and after washing.

Washing Time	Before Washing	After Washing
15 min	23.3µM	24.76µM
60 min	30.23µM	25.13µM
360 min	27.56µM	28.78µM
720 min	21.76µM	31.65µM

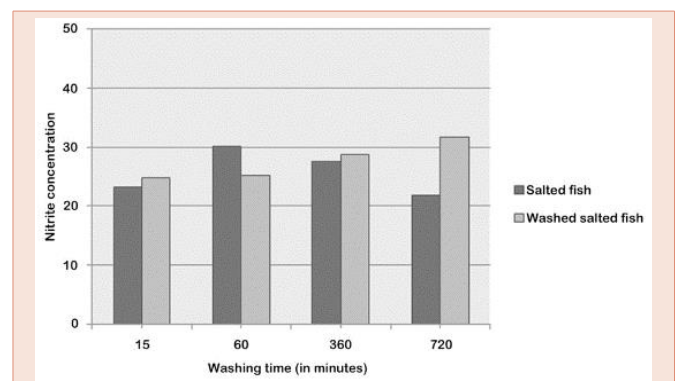


Figure 2: A parametric T test for paired samples showed that no statistically significant difference was observed among nitrites levels before and after the washing procedures (p=0.807). There were no correlations between samples before and after washing (p=0.155).

Nitrites are already known to play a crucial role in gastric cancer carcinogenesis, and alimentary habit, together with *Helicobacter pylori* exposure, remains as one of the most important contributors to the persistence of high incidence of distal gastric cancer in many regions [2,11-14].

Epidemiologic and animal experimental data supports the relation between salted food intake and gastric cancer occurrence [15-18].

In this report, we have demonstrated the difference in nitrites levels, among fresh and salted fish, frequently consumed in gastric cancer high incidence regions. Plus, we scientifically demonstrated, by simulating the food washing procedure in a controlled experiment, that, even leaving salted food under water for long periods of time (from 15 minutes to 720 minutes), the nitrites levels in salted food remain much higher than that present in fresh food.

As far as we know, we were the first to prove that the usual habit of washing salted food does not reduce the nitrites levels significantly, and does not provide protection against the risk of gastric cancer occurrence related to salted food intake.

Most risk factors for gastric cancer are already known, and some of the environment exposures should be avoided, including intake of salted food [2].

Washing salted food before eating may cause an equivocal feeling of protection against the known risk of gastric cancer, related to nitrites levels, and people should be alerted about the inefficacy of this usual procedure, as proved in our experiment.

However, besides the scientific evidence available, education is needed to modify the exposure to already known gastric cancer risk factors, looking for a reduction in incidence of this type of neoplasia in the future.

Conclusion

Salted fish has higher concentration of nitrites compared to fresh. Washing, or living salted fish under water, does not provide significant decrease of the nitrites levels.

We believe that these findings support an educational statement in the field of public health, to alert population, researchers, general practice physicians, gastroenterologists and oncologists, about the inefficacy of washing salted food as a protective attitude to avoid exposure to gastric cancer risk related to nitrite intake.

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Authors' Contributions

Conceived and designed the experiments: CBA, JLN, ASK, BMK,

NCS, GR, RB, and PPA. Performed the experiments: CBA, ASK, CAMN. Analyzed the data: CBA, JLN, GR, RB, and PPA. Wrote the paper: CBA, ASK, CAMN, GR, PPA, and RMRB. All authors read and approved the final manuscript.

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