Nitrite Levels Before and after Washing in Salted Fish

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 conduct 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 Amazonic fish, were obtained at Ver-o-Peso marked, 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 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 submersed 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=0.007), 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.

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 submersed 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=0.007), 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.
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 nitrates 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 nitrates 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 nitrates 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 nitrates 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 nitrates levels of the nine paired samples were compared taking the average levels of nitrates of each group (salted and fresh fish).

The nitrates 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 nitrates 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 nitrates 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 nitrates 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 nitrates levels in salted fish, even after long periods of immersion (p=0·807), and the levels of nitrates 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].
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 fish 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, beside 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, ASK, CAMN. Analyzed the data: CBA, JLN, GR, RB, and PPA. Wrote the paper: CBA, ASK, CAMN, GR, PPA, and RM. All authors read and approved the final manuscript.

**References**

1. Liu C, Russell RM (2008) Nutrition and gastric cancer risk: an update. Nutr Rev 66: 237–249.
2. Tepes B (2009) Can gastric cancer be prevented? J Physiol Pharmacol 60: 71–77.
3. Parkin DM (2011) Cancers attributable to dietary factors in the UK in 2010. IV. Salt. Br J Cancer 105: 31–33.
4. Wang XQ, Terry PD, Yan H (2009) Review of salt consumption and stomach cancer risk; epidemiological and biological evidence. World Journal Gastroenterology 15: 2204–2213.
5. Sankaranarayanan R, Boffetta P (2010) Research on cancer prevention, detection and management in low- and medium-income countries. Ann Oncol 21: 1935–1943.
6. D’Ella L, Rossi G, Ippolito R, Cappuccio FP, Strazzullo P (2012) Habitual salt intake and risk of gastric cancer: A meta-analysis of prospective studies. Clin Nutr 31: 489–498.
7. Bornscheij J, Roekkas T, Selgrad M, Malfertheiner P (2011) Gastric cancer: clinical aspects, epidemiology and molecular background. Helicobacter 16: 45–52.
8. Green LG, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, et al. (1982) Analyses of nitrate, nitrite and [15N] nitrate in biological fluids. Ann Biochem 126: 131–138.
9. Bornscheij J, Malfertheiner P (2011) Gastric carcinogenesis. Langenbecks Arch Surg 396: 729–742.
10. Krejs GJ (2010) Gastric cancer: epidemiology and risk factors. Digestive Diseases 28: 600–603.
11. Peleteiro B, La Vecchia C, Lunet N (2012) The role of Helicobacter pylori infection in the web of gastric cancer causation. European Journal of Cancer Prevention 21: 118–125.
12. Corea P, Piazuelo MB, Camargo MC (2006) Etiopathogenesis of gastric cancer. Scandinavian Journal of Surgery 95: 218–224.
13. Liu C, Russell RM (2008) Nutrition and gastric cancer risk: an update. Nutrition Reviews 66: 237–249.
14. Tsugane S, Sazazuki S (2007) Diet and the risk of gastric cancer: review of epidemiological evidence. Gastric Cancer 10: 75–83.
15. Dias-Neto M, Pintalhao M, Ferreira M, Lunet N (2010) Salt intake and risk of gastric cancer: systematic review and meta-analysis. Nutrition Cancer 62: 133–147.
16. González CA, López-Carrillo L (2010) Helicobacter pylori, nutrition and smoking interactions: their impact in gastric carcinogenesis. Scandinavian Journal of Gastroenterology 45: 6–14.
17. Hu J, La Vecchia C, Morrison H, Negri E, Mery L, et al. (2011) Canadian Cancer Registries Epidemiology Research Group. Salt, processed meat and the risk of cancer. Eur J Cancer Prev 20: 132–139.
18. Yang WG, Chen CB, Wang ZX, Liu YP, Wen XY, et al. (2011) A case-control study on the relationship between salt intake and salty taste and risk of gastric cancer. World J Gastroenterol 17: 2049–2053.