Effect of different cooking treatments on the residual level of sulphites in shrimps

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Abstract

Sulphiting agents (or sulphites) are a class of food additives identified in Europe by codes E220-E228. Their addition in crustaceans is permitted with specific legal limits for avoiding the so-called "black-spot" that is a defect that compromises the marketability of these products. High levels of ingested sulphites may cause pseudo-allergenic reactions in susceptible people. Moreover, they can exercise mutagenic and citotoxic effects other that destroy some vitamins such as thiamine, folic acid, nicotinamide and pyridoxal. The residual level of sulphites in crustaceans can be considerably affected by the specific method of cooking. In this study, 5 traditional procedures of cooking – grilling, oven, frying, steaming and stewed cooking – were compared to verify their effect on the residual concentration of sulphites in shrimp samples. The analytical determination was carried out using a fully validated and accredited analytical method by ion chromatography with conductivity detection. The results demonstrated that cooking leads to the decrease of sulphites levels in the products, with the highest percentage of reduction (55.3%) obtained by steaming and the lowest using oven (13.9%). The results of this study confirm that the specific method of cooking should be taken into account during "total diet studies" and risk assessment for appraising the effective number of sulphites ingested from crustaceans consumption.

Introduction

Crustaceans are food products widely consumed worldwide, especially in Japan, Australia and United States (Usuki, 2001; Gillet, 2008). Among these, shrimps are considered the most important product on the market. This type of seafood is particularly vulnerable, and the spoilage reactions are rapid, compromising product shelf-life that is in every way short, not exceeding 7 days, when stored in ice (Yamagata and Low, 1995; Goncalves *et al*., 2003; Mejlholm *et al*., 2005).

The most important commercial problem of shrimp deterioration is the appearance on the product of melanosis (also known as "Black-Spot"). This defect consists of dark spots on the carapace, due to the contact with ice, which decrease the organoleptic quality of the product, considerably, compromising marketability (Otwell *et al*., 2008; Smaldone *et al*., 2011; D'Amore *et al*., 2020). This important defect and the extension of product shelf-life is usually obtained by adding a food additive belonging to the "sulphiting agents" category, identified in Europe by codes E220 - E228. The addition of these additives in shrimps is permitted by the European Legislation, and specific limits have been set (European Commission, 2008) (Table 1). However, it is well-known that high levels of ingested sulphites may cause several toxic effects in humans, such as peudo-allergenic reactions in susceptible people, mutagenicity and cytotoxicityn (Stamati *et al*., 1992; Iammarino *et al*., 2012). Moreover, the nutritional quality of food is notably compromised, since these additives can destroy some vitamins such as thiamine, folic acid, nicotinamide and pyridoxal. The no observed adverse effect level (NOAEL) of sulphiting agents, expressed as SO_2, was set at 72 mg SO_2/kg bw/day, while the acceptable daily intake (ADI) for humans is equal to 0.7 mg SO_2/kg bw/day (JECFA, 1999; Vandevijvere *et al*., 2010; Iammarino *et al*., 2017).

The treatment of sulphites addition in crustaceans is usually made by product immersion in 1.25% (w/v) sodium metabisulphite solution in water for utmost 3 min. In the case of shrimps, approximately 500 specimens are treated with about 30-gallon batch of sodium metabisulphite (Virgilio Omar, 1998). As described, the sulphuring treatment of shrimps is characterized by high variability of the residual amount of sulphurous anhydride in the product, representing a food safety risk. Moreover, the actual intake of this additive from the diet is heavily influenced by the cooking technique used before consumption. It is well-known that some types of cooking can modify the composition of food. For instance, many studies deepened the formation of toxic compounds in foods, such as acrylamide in fried potatoes, nitrosamines in meats, etc. (Xu and An, 2016; Iammarino, 2020). Other than producing toxic compounds, cooking may also exercise "positive" effect in food, such as food additives removal. Under this point of view, the literature is scarce, since also the "Total Diet Studies", which evaluate the variation of food composition after cooking, developed their attention more on food contaminant residues than food additives (D'Amato *et al*., 2013).

This study is collocated in this context. It represents the first study about the actual intake of sulphites from shrimps' consumption, after different types of cooking: griddled, stewed cooking, frying, steaming and oven. The analysis was carried out by using a fully validated and accredited analytical method by ion chromatography with suppressed conductivity detection.

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Materials and methods

The following chemicals were used: sodium sulfite (≥98%), fructose (> 99%) and sodium carbonate anhydrous (>99.5%) were supplied by Merck KGaA (Stenheim, Germany); carbonate-free sodium hydroxide (50%, w/w) was purchased from J.T. Baker (Deventer, Netherlands). The solvent used for preparing the eluents, stabilizing solution and standard solutions was ultrapure water (minimal resistance 18.2 MΩ-cm), supplied by Milli-Q RG unit from Millipore (Bedford, MA, USA). Prior to use, the mobile phase was degassed with nitrogen. The working standard solutions at concentrations 1.0, 2.5, 5.0, 10.0 and 20.0 mg L^{-1} were prepared by diluting the sulphites standard solution at a concentration of 1000 mg L^{-1} using the stabilizing solution.

Three kg of shrimps of 41-50 pieces/kg size, with sulphiting agents declared on the label (E223 – sodium metabisulphite) were collected from a local market, subdivided into 6 aliquots (1 kg the first and 400 g the others) and stored at -18°C in laboratory until analysis. The first aliquot was analyzed six times in order to ascertain the homogeneity of food additive in the whole sample. The other aliquots were analyzed three times (2 repetition for each test), each aliquot after cooking the product using the following procedures: griddled (200 °C, 5-6 min), stewed cooking (100 °C, 10 min), frying (180 °C, 5 min), steaming (100 °C, 10 min) and oven (180 °C, 20 min). Each procedure was based on the usual domestic procedures, and they were made obtaining proper level of cooking of each sample.

After cooling, the edible part of shrimps was collected and analyzed using a fully validated and accredited analytical method by ion chromatography with suppressed conductivity detection (Iammarino *et al*., 2013).

For sample extraction, a stabilizing solution was used. This StS was composed of 50 mM NaOH and 10 mM fructose both in ultrapure water and it was used for both standard preparation and sample extraction. The particular composition of this mixture allows retarding the oxidation of sulphite to sulphate and removing some matrix interfering compounds.

4 g of sample (only edible part) homogenized by blade homogenizer were weighed and 40 mL of StS were added. The sample was mixed for 30 min in horizontal shaker. The obtained mixture was centrifuged at 250xg at room temperature and the supernatant was filtered on paper (Whatman No. 40, Springfield Mill, UK). Finally, 2 mL of filtrate were filtered again using Anotop 10 LC, 0.2-μm, 10 mm filters (Whatman, Springfield Mill, UK) and injected in the HPIC system.

A Dionex DX500 chromatographic system (Dionex Corporation, Sunnyvale, CA, USA) composed of a quaternary gradient pump (model GP50), a Rheodyne injection valve with a 25-mL injection loop (model RH9125, Cotati, CA, USA), an electrochemical detector with an automatic temperature compensation (model ED40 set to conductivity mode) and an anion self-regenerating suppressor (model ASRS II, 4 mm) set to 50 mA, was used for all analytical determination. The chromatographic separations were accomplished using the IonPac® AS9-HC ion-exchange column (250x4 mm i.d., particle size 9 μm; Dionex Corporation, Sunnyvale, CA, USA) coupled to gradient elution based on two solutions: 8mM Na_2CO_3 and 2.3 mM NaOH (A) and 24mM Na_2CO_3 (B). The gradient program started with isocratic step for 15 min at 100%A, a gradient step to 50%A in 1 min, and then isocratic for 4 min. Finally, the system was re-equilibrated at 100% A for 20 min. (flowrate: 1.0 mL min^{-1}, total run time: 40 min). The reservoir bottles (DX500 2L bottles; Dionex) were pressurized with pure nitrogen (~0.8 MPa) and the system was interfaced via proprietary network chromatographic software (PeakNet™, Dionex Corporation, Sunnyvale, CA, USA) to a personal computer for instrumentation control, data acquisition and processing.

This analytical method was submitted to full validation procedure, according to Thompson harmonized validation guidelines (Thompson *et al*., 2002), and in agreement with Regulation No. 2017/625/EU and Decision No. 657/2002/EC (European Commission, 2002; European Parliament/Council, 20147). The analytical procedure was accredited by ACCREDIA, the Italian Organism for laboratory accreditation, since 2009. It is regularly submitted to proficiency test round (at least 1 per year) and checked by internal control chart every 6 months. This method is applied for official control of meats and seafood at the Chemistry Department of Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata (Foggia, Italy) and it was used for developing another comprehensive monitoring of sulphite levels in shrimps marketed in Italy (Iammarino et al., 2014). All significant validation parameters (*i.e.*, linearity sensitivity, selectivity, accuracy, robustness and measurement uncertainty) were evaluated and the most important analytical performances which characterize this method are resumed in Table 2. In Figure 1, a chromatogram example of a standard solution of sulphites is shown.

The statistical analysis was developed in order to evaluate di difference of sulphite concentration among raw sample and treated with different types of cooking procedures. In this regard, the one-way ANOVA and the t-test were used for comparison, with a confident interval of 95% ($p<0.05$).

Results and discussion

Six replicated analysis were carried out in order to verify the homogeneity of sodium metabisulphite in the matrix. The obtained result was 121.9 mg kg^{-1} (expressed as sulphurous anhydride) with a standard deviation of 1.5 mg kg^{-1} which confirmed the homogenous distribution of food additive in the sample.

In Figure 2, a graphical elaboration of the mean values of sulphites (expressed as

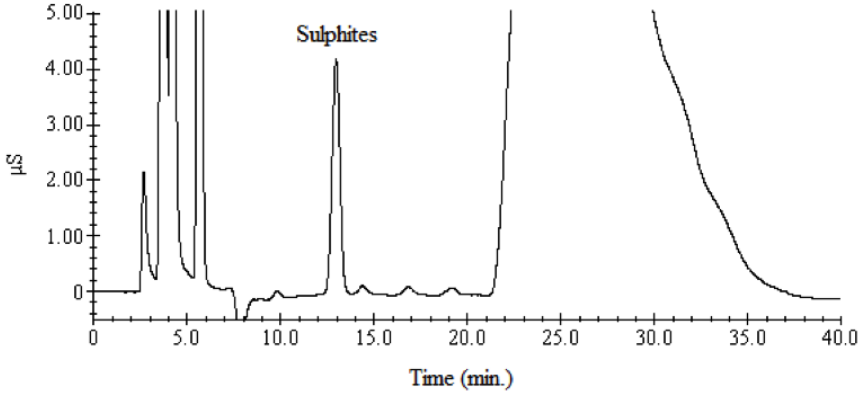

Figure 1. Chromatogram of a standard solution of sulphites at a concentration of 10.0 mg L^{-1}.

sulphurous anhydride), obtained by analyzing this shrimp sample both before and after different types of cooking treatment, is shown. The one-way ANOVA confirmed the significant difference between sulphite concentration detected in the raw sample and that quantified in the same sample after cooking, confirming a decrease. More in depth, the concentrations quantified in samples treated by steaming, stewed cooking and griddled, were equal to 54.5±4.2, 87.5±2.7 and 80.8±3.1 mg kg^{-1}, respectively, resulted significantly lower that the raw material ($p<0.05$).

A decrease of the initial number of sulphites was also verified treating the shrimps by frying (95.0±3.6 mg kg^{-1}) and oven (105.0±3.9 mg kg^{-1}). However, this decrease resulted as not statistically significant ($p<0.05$).

As the final result, the highest decrease of sulphite amount was obtained by treating the shrimps by steaming, with a percentage of reduction equal to 55.3%; while the lowest decrease was registered by cooking in the oven, with a percentage of reduction of 13.9% (Figure 3). In Figure 4, some chromatogram examples of the shrimp sample analyzed both before and after different cooking treatments are shown.

These results are substantially in accordance with other studies focused on the modification of sulphite levels in food after heat treatment. Martìnez-Alvarez *et al*. (2009) reported that sulphites are leached out from shrimp meat during cooking, accumulating in the water, so much so that the water is usually reused for cooking shrimps, given the high quantity of residual sulphites. This significant decrease of sulphites concentration after cooking was also veri-

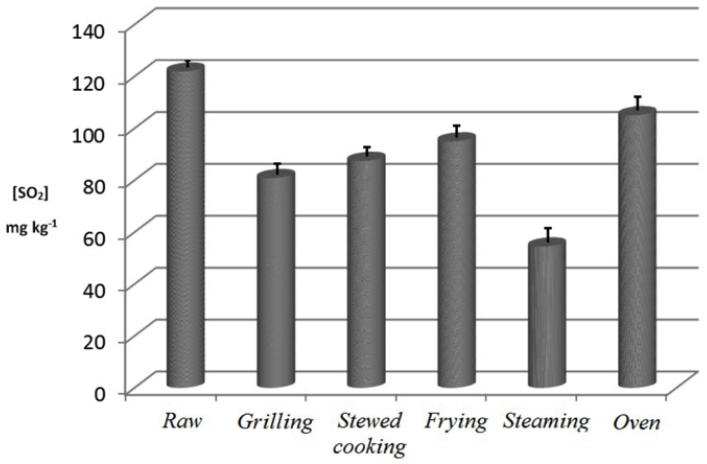

Figure 2. Mean sulphurous anhydride concentrations detected in raw and cooked shrimps samples (5 different treatments)

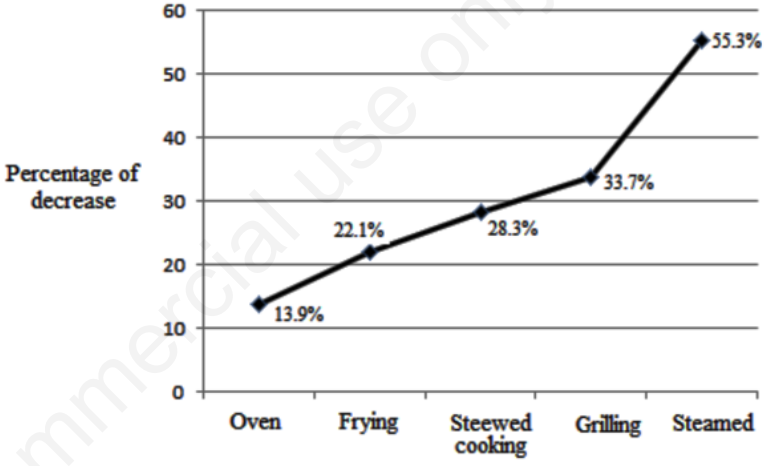

Figure 3. Mean percentage of decrease of sulphurous anhydride concentrations detected after 5 types of cooking treatment of shrimps sample.

Table 1. Food preservatives admitted by Regulation No. 1333/2008/EC in unprocessed molluscs and crustaceans.

Food additives	Max level (mg kg^{-1})	Restrictions/exceptions	Notes
Sulphur dioxide - sulphites E220 – E228	150	Only fresh, frozen and deep-frozen crustaceans and cephalopods; crustaceans of the *Penaeidae*, *Solenoceridae* and *Aristaeidae* family up to 80 units per kg	Maximum levels are expressed as SO_2 related to the total quantity, available from all sources, an SO_2 content of not more than 10 mg/kg or 10 mg/L is not considered to be present. Maximum limits in edible parts.
	200	Only crustaceans of the *Penaeidae, Solenoceridae* and *Aristaeidae* family between 80 and 120 units per kg	
	300	Only crustaceans of the *Penaeidae, Solenoceridae* and *Aristaeidae* family over 120 units per kg	

Table 2. Analytical method validation parameters.

Analyte	LOD (mg kg^{-1})	LOQ (mg kg^{-1})	Fortification level (mg kg^{-1})	Mean recovery % (n=6)	RSDr % (n=6)	Expanded measurement uncertainty (k=2)	Accreditation	Robustness (Matrix)
Sulphites	2.7	8.2	10	92.1	5.5	15.8%	Yes	Seafood
			40	88.4	5.8			Meat
			80	85.2	5.0			Fruit and vegetables

RSDr, Relative standard deviation.

fied by Peña-Egido *et al*. (2005) which analyzed burger samples after grilling.

Sulphiting agents' intake in the diet is due to several food and beverage types in which they are permitted by the legislation, *i.e.*, peeled, cut, shredded, frozen, dried, canned and bottled fruit and vegetables, jam, jellies and marmalades, processed potato products, products, wine, beer, flavoured drinks and others. Thus, the risk assessment must take into account the sum of all these intakes and this means that each amount has to be very low to avoid exceeding the ADI.

Under this point of view, regarding risk assessment, the reference data related to Italian food consumption were found in the INRAN-SCAI 2005-06 report (Leclercq *et al*., 2009) and they were considered for evaluation. Moreover, the indications reported in the official European Food Safety Authority document (European Food Safety Authority, 2012) were considered for ADI studies, taking as reference 12 and 70 kg as the bodyweight of children/adolescents and adults/elderly, respectively. The mean consumption of crustaceans reported in the INRAN-SCAI 2005-06 report is equal to 3.9 g die^{-1}. Thus, after calculation based on the results of the present study, the percentage of ADI reached taking into account the raw product was equal to 5.7% and 1.0% for children/adolescents and adults/elderly, respectively. These percentages considerably decrease if the sulphurous anhydride concentration detected after cooking by steaming is considered. These ADI percentages, which may be considered as the effective intake after food preparation (cooking) were corresponding to 2.5% and 0.4% for children/adolescents and adults/elderly, respectively. These last values are reassuring within the risk assessment; however, the type of cooking influences the ADI percentages, significantly. Indeed, if cooking by oven is taken into account, the ADI percentages are not significantly different from those obtained for the raw product, being equal to 4.9% and 0.9% for children/adolescents and adults/elderly, respectively.

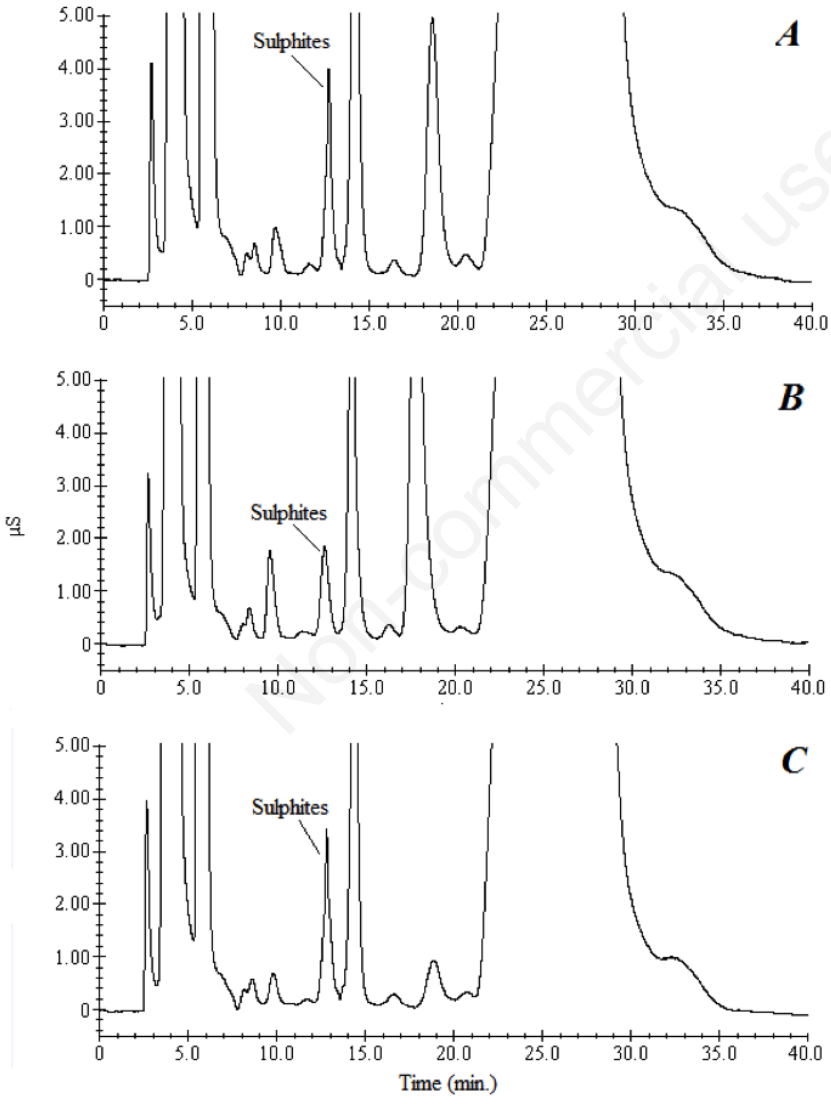

Figure 4. Chromatogram examples: raw shrimp sample (A); shrimp sample cooked by steaming ([SO_2] = 54.5 mg kg^{-1}) (B); shrimp sample cooked by oven ([SO_2] = 105.0 mg kg^{-1}) (C).

Conclusions

In this study, 5 traditional procedures of cooking: grilling, oven, frying, steaming and stewed cooking, were compared for verifying their effect on the residual concentration of sulphites in shrimp samples.

The results demonstrated that cooking leads to the decrease of sulphites levels in the products, with the highest percentage of reduction (55.3%) obtained by steaming and lowest using oven (13.9%). The results of this study confirm that specific method of cooking should be taken into account during "total diet studies" for appraising the effective number of sulphites ingested from crustaceans consumption.

Regarding risk assessment, the percentage of ADI reached taking into account the raw product was equal to 5.7% and 1.0% for children/adolescents and adults/elderly, respectively. These percentages considerably decrease if the sulphurous anhydride concentration detected after cooking by steaming is considered (2.5% and 0.4% for children/adolescents and adults/elderly, respectively). Thus, this study confirmed that the evaluation of the specific cooking type is essential in food safety studies. Indeed, taking into account the cooking by oven, the ADI percentages calculated (4.9% and 0.9% for children/adolescents and adults/elderly, respectively) resulted as significantly different from those obtained after steaming and comparable to those of the raw product.

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