Doctors in the kitchen: standardization of a home cooking method for *Chamelea gallina* and *Mytilus galloprovincialis* to eliminate microbiological risk

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Abstract

It is known that the consumption of edible lamellibranch mollusks (ELMs), undercooked or raw, is a vehicle for the oral transmission of viral and bacterial pathogens. The ELMs most frequently fished/cultivated and consumed in Italy are *Mytilus galloprovincialis* and *Chamelea gallina.* The aim of this project was to standardize the cooking conditions of *M. galloprovincialis* and *C. gallina,* reproducible in a domestic environment, to obtain food in which the microbiological risk was eliminated but, at the same time, preserved the original organoleptic characteristics. The results showed that for *C. gallina* and *M. galloprovincialis*, cooking for 2 minutes after opening all shells, according to a method suitable for home application, is sufficient to eliminate the microbiological risk.

Introduction

Edible lamellibranch mollusks (ELMs) are filter-feeding organisms that bioaccumulate microorganisms and toxic substances present in the marine environment, thus representing a significant biological and chemical risk for those who consume them (Lattos *et al*., 2021). Their consumption has increased globally in recent decades (Summa *et al*., 2022; Santos *et al*., 2023). *Mytilus galloprovincialis* and *Chamelea gallina* are respectively the most widespread mussel and clam species in Italy, especially in the Adriatic Sea, the first in natural banks and in farms and the second in natural banks exclusively (European Commission, 2021 and 2022) In Italy, it is very common to eat ELMs seared in a pan and then partially cooked with the belief that the opening of the shell is an indication that the mollusks have been properly cooked and therefore healthy. In some Italian regions, ELMs are even eaten raw by adding a splash of lemon on the raw pulp with the mistaken belief that acidification is sufficient to eliminate the microbiological risk. In recent years, alternative cooking methods for ELMs have become widespread, which allow them to increase their shelf life while maintaining sensory characteristics that overlap with those of the fresh product, in particular sous vide cooking (Russo *et al*., 2023). Regulation CE/2073 2005 (European Commission, 2005) identifies *Escherichia coli* and *Salmonella* as food safety criteria for ELMs released for sale. Even if *Salmonella* is not a natural inhabitant of the aquatic environment, several *Salmonella* serovars are widely distributed in the marine environment with a high prevalence in bivalves (Rubini *et al.,* 2018). Moreover, clinically relevant serovars of *Salmonella* can survive in seawater and within bivalves for significant periods of time after just one exposure event (Morrison *et al.,* 2011). Between *Vibrios*, natural inhabitants of the aquatic environment, we reported, in the Adriatic Sea prevalence of *Vibrio parahaemolyticus* and *Vibrio cholerae* non O1 non O139 in ELMs by about 25% and 7%, respectively (Ottaviani *et al.,* 2009; 2010a). Moreover, the prevalence of TDH-related hemolysin-positive strains of *V. parahaemolyticus* was higher than that reported in other European and non-European countries (Ottaviani *et al*., 2013). Still, disease cases due to *V. parahaemolyticus* and non O1 non O139 *V. cholerae*, with ELMs or seawater from the Adriatic Sea as the source of infection, have been reported (Ottaviani *et al*., 2010b; Ottaviani *et al*., 2013; Ottaviani, Medici *et al*., 2018). Current purification systems to be applied on ELMs in the post-harvest phase, if significantly reduce the levels of *E. coli*, are not as effective against *Salmonella* and *Vibrio* (Croci *et al.,* 2002; Barile *et al*., 2009). Therefore, to minimize the risk for the final consumer, information on the correct preparation of this food is essential. In this regard, the European Food Safety Authority has repeatedly suggested that shellfish producers indicate on the label that the product "must be consumed after cooking", without, however, providing specific indications on the times and temperatures to be used (EFSA Panel BIOHAZ, 2015). This is also supported by Regulation EC/178 2002 (European Commission, 2002), which underlines the importance of introducing a specific label on foods as a strategic measure to contain the risk. Regulation EC/1169 2011 (European Commission, 2011) also recommends that food information should be accurate and easily understandable for the consumer. Regarding containment measures through cooking for viral contamination of ELMs, the available data are discordant. A cooking that ensures that the internal temperature of the mollusk flesh is maintained at 90°C for a minimum of 1.5 minutes is recommended as safe virucidal treatment by current EU regulations (FAO and WHO, 2012). Sow *et al.* (2011) indicated 3 minutes of cooking as a suitable virucidal treatment. The most stringent cooking conditions are instead reported by Pascoli *et al.* (2016), who pointed out the need to cook the ELMs for at least 2 minutes after opening the shells to eliminate the viral risk. To the best of our knowledge, only one study has investigated containment measures by cooking of bacterial contamination of ELMs, on species other than *M. galloprovincialis* and *C. gallina,* and using bacterial strains not isolated from the marine environment for experimental contamination (Boffo *et al.,* 2018). Boffo *et al*. (2018) reported that the time required to open all the shells was sufficient to eliminate the bacteriological risk. It has been shown that the loss of organoleptic quality of ELMs is directly related to cooking times (Ovissipour *et al*., 2013). Considering the scenario described so far, it is essential to research widely distributed commercial species, such as *M. galloprovincialis* and *C. gallina*, cooking conditions suitable for domestic use, capable of eliminating the microbiological risk, and at the same time preserving their organoleptic quality as much as possible. This project therefore pursues the following objectives: i) evaluate whether the most restrictive cooking times reported in the literature (Pascoli *et al*., 2016) for eliminating viruses are also effective for bacteria; ii) standardize the cooking conditions easily reproducible in a domestic environment to eliminate the microbiological risk; iii) define the cooking recipe to be indicated on the label for the preparation of healthy and tasty *M. galloprovincialis* and *C. gallina*.

Materials and Methods

Edible lamellibranch mollusks used for experiments

Lupine clam (*C. gallina*) and Mussel (*M. galloprovincialis*) of commercial size, *i.e.*, 3-5 and 5-8 cm per mollusk, respectively, both supplied by the purification company ELMs Co.Pe.Mo (Ancona, Italy), were used.

Preliminary tests to define the characteristics of the cooking pan

1 kg of *C. gallina* was chosen as the quantity on which to carry out the experiment because it corresponds to the weight of the commercial size. Regarding the type of material chosen for the pans to be tested, we decided on aluminum ones with a non-stick bottom, as they allow for excellent heat distribution across the entire cooking surface and avoid local overheating and burning. Regarding the shape and size of the pans to be tested, we opted for those commonly found in a domestic kitchen and used for cooking ELMs, that is, slightly flared round pans with 3 different upper internal diameters of 26, 28, 30 cm, a height of 5 cm, and a thickness of 5 mm. The mollusks were poured into the pans, covered with a glass lid of the same size as the pan, and placed on a 7 cm diameter gas burner (3 kW power) at intermediate heat of a four-burner gas cooker and cooked for 2.5 minutes. The cooking time was measured with the aid of a stopwatch. After 2.5 minutes, the cooking stopped, and the number of organisms with open shells was counted for each pan compared to the total number of organisms. This preliminary test was repeated twice. The two independent tests returned consistent results. In the 26 cm pan, after 2.5 minutes, approximately 30% of the mollusks showed open shells, while in both the 28 and 30 cm pans, this percentage rose to approximately 50%. Since the 28 cm pan is more common than the 30 cm one in a home kitchen, it was decided to carry out the subsequent cooking tests with a pan of this size.

Preliminary test to evaluate the time required for all the shells to open

This information was essential to calibrate the cooking times of experimentally contaminated ELMs once all the shells were opened. 1 kg of *C. gallina* and 1 kg of *M. galloprovincialis* on a 28 cm pan were cooked for the minimum time required for all the shells to open. The cooking time was measured with the aid of a stopwatch. The temperature was monitored during cooking using a probe thermometer (Delta Ohm, Padua, Italy) inserted into the pan between the mollusks, taking care not to let it encounter the bottom. Moreover, to monitor the internal temperature of the mollusk flesh, a second probe thermometer was inserted inside the body of a single test mollusk, trying not to break the adductor muscles and thus keeping the animal semi-closed and the liquid inside. The probe sensors were calibrated to +/- 0, 1°C. This preliminary test was repeated twice.

Bacterial strains used for edible lamellibranch mollusk contamination

All strains have been isolated from *C. gallina* or *M. galloprovincialis* (Table 1). *V. parahaemolyticus and V. cholerae* non O1 non O139 fresh enrichments were prepared from a stock culture grown on 3% NaCl Luria-Bertani broth (LB Difco; Becton, Dickinson and Company, Milan, Italy) until prey reached an OD_{600} of 0.20 (~1.8×10^8 CFU per mL) (Ottaviani *et al.,* 2020). *Salmonella* and *E. coli* fresh enrichments were prepared from a stock culture grown on Brean Heart Infusion broth (BHI Difco; Becton, Dickinson and Company, Italy) until prey reached an OD_{600} of 0.50 (~5×10^8 CFU per mL) (Ottaviani *et al*., 2017; Pieralisi *et al*., 2024).

Experimental contamination of edible

lamellibranch mollusks

It was carried out in tanks of an experimental aquarium (Adriatic Sea International, Rome, Italy), each filled with 39.6 L of water at 3% salinity, maintained at 14°C, and constantly aerated. ELMs were evenly distributed in the tanks and allowed to acclimatize for 24 hours, then the contamination was performed with the four bacterial strains taken individually and with the mix *V. parahaemolyticus* + *Salmonella*. For each experimental contamination, 11 commercial packs of 1 kg of ELMs were used, one of which was immediately analyzed for natural contamination. The remaining 10 were placed in the tank (about 0.05 L of water for each *C. gallina* and about 0.20 L for each *M. galloprovincialis*). After 24 hours of acclimatization, the artificial contamination of the ELMs for each target bacterium was carried out by inoculating 400 mL of the fresh enrichments. Table 2 shows the concentrations of the target bacteria used for contamination and the expected concentrations inside ELMs after 3 hours of exposure, as previously standardized (Ottaviani *et al*., 2017; Ottaviani, Chierichetti *et al*., 2018; Ottaviani *et al*., 2020; Pieralisi *et al*., 2024) (Table 2). Then, the samples were collected aseptically and taken to the laboratory for cooking tests and bacteriological analysis.

Cooking tests

For each experimental contamination, the cooking tests were repeated 3 times, and each bacterial analysis was carried out in triplicate (n=9). One pack was analyzed to enumerate the target bacterium (or mix) and verify the level of contamination reached. The remaining 9 were cooked in 3 independent experiments, testing for each experiment 3 cooking times. The cooking times chosen for *C. gallina* were 3 minutes, corresponding to the total opening of the shells, 5 minutes, taking as a reference 2 minutes after the opening of the shells as the time necessary for the elimination of the viruses (Pascoli *et al,* 2016), and 6 minutes in case the elimination of bacteria requires longer times than that of viruses. Following the same criteria, the cooking times chosen for *M. galloprovincialis* were 3.5, 5.5, and 6 minutes. The temperature was monitored during cooking using a probe thermometer (Delta Ohm, Padua, Italy) inserted into the pan between the mollusks, taking care not to let it encounter the bottom. The probe sensors were calibrated to ±0, 1°C. At the end of the heat treatment, the ELMs were immediately chilled with ice and water to avoid further heating before bacteriological analyses.

Bacteriological analysis

The methods used for analysis of ELMs pre- and post-contamination and post-cooking, respectively, are indicated in Table 3. For the microbiological analysis of ELMs before and after contamination, to count the target bacteria, we used standardized quantitative methods (Ottaviani *et al*., 2017; Ottaviani *et al*., 2020; Ottaviani *et al*., 2024). For the post-cooking analyses, to determine the absence or extremely low values of the target bacteria, we used standard qualitative methods for *Salmonell*a spp., *V. parahaemolyticus*, *V. cholerae* (ISO 6579-1, 2017; ISO 21872-1, 2017) (ISO, 2017a and 2017b) quantitative method by the most probable number (MPN) for *E. coli* (ISO 16649-3) (ISO, 2015).

Results and Discussion

Preliminary test to evaluate the time required for all the shells to open

The two independent tests returned consistent results. Temperature trends among mollusks and within the test mollusk showed overlapping patterns. For both *C. gallina* and *M. galloprovincialis,* during cooking, the temperature reached 90°C after 140 sec. For *C. gallina*, the shells began to open after 150 seconds when the temperature reached 95°C. After 165 s, the temperature reached 100°C, and after 180 seconds (3 minutes), all individuals were open. For *M. galloprovincialis*, the shells began to open after 165 seconds when the temperature reached 95°C. After 180 seconds, the temperature reached 100°C, and after 210 seconds (3.5 minutes), all individuals were open. The slightly longer opening times of mussels compared to clams could be due to their larger size, but further investigations would be necessary to confirm this hypothesis.

Experimental contamination of edible lamelli-

Table 1. Target bacteria used for the experiments.

Strain	Origin	Toxin genes
Escherichia coli	*Chamelea gallina* from the Adriatic Sea, Italy	/
Salmonella enterica subsp enterica serovar Napoli	*Chamelea gallina* from the Adriatic Sea, Italy	/
Vibrio parahaemolyticus	*Mytilus galloprovincialis* from the Adriatic Sea, Italy	*tdh-; trh-*
Vibrio cholerae non O1 non O139	*Mytilus galloprovincialis* from the Adriatic Sea, Ancona, Italy	*ctxA-; stn/sto-*

tdh, thermostable direct hemolysin gene; *trh*, TDH-related hemolysin gene; *ctxA,* cholera toxin gene; *stn/sto*, heat stable enterotoxin gene.

Table 2. Concentration of the target bacteria.

Target bacteria	Concentration of the inoculum	Expected concentration inside ELMs after 3 hours esposition
Escherichia coli	5×10^8 CFU/mL	$105/10^6$ CFU/mL
Salmonella enterica subsp enterica serovar Napoli	5×10^8 CFU/mL	$105/10^6$ CFU/mL
Vibrio cholerae non O1 non O139	1×10^8 CFU/mL	$104/10^5$ CFU/mL
Vibrio parahaemolyticus	1×10^8 CFU/mL	$104/10^5$ CFU/mL

ELMs, edible lamellibranch mollusks; CFU/mL, colony-forming units per mL.

branch mollusks

After 3 hours of bioaccumulation with the target bacteria, the quantitative analyses returned the results reported in Table 4.

Cooking tests and bacteriological analysis

The temperature trends during the cooking of ELMs are shown in Figure 1. For *C. gallina*, cooking for 3 minutes, *i.e.*, for the time required for the shells to open, determined the absence in 25 g of *V. parahemolytyicus*, *V. cholerae* non O1 non O139*, Salmonella,* mix *Salmonella* + *V. parahaemolyticus,* and values <18 MPN/100 g for *E. coli*. in the three independent cooking tests and in the replicates for each test. These results were confirmed as expected at 5 and 6 minutes. For *M. galloprovincialis*, cooking for 3.5 minutes, *i.e*., when all shells were open, gave negative results in 25 g for *V. parahaemolyticus* and *Salmonella* and values < 18 MPN/100 g for *E. coli* in the 3 independent cooking tests and replicates for each test. However, for mix *Salmonella* + *V. parahaemolyticus* and *V. cholerae* non O1 non O139, 2 of the 3 cooking tests gave positive results in 25 g in all 3 replicates. Cooking for 5.5 and 6 minutes always determined the absence in 25 g of *V. parahaemolyticus*, *V. cholerae* non O1 non O139*, Salmonella,* mix *Salmonella* + *V. parahaemolyticus,* and values <18 MPN/100 g for *E. coli.* For *C. gallina*, 3 minutes total cooking time, although sufficient to eliminate the bacteriological risk, was not sufficient for viruses, which would require at least 2 minutes of cooking from the opening of the shells to be eliminated (Pascoli *et al*., 2016). For *M. galloprovincialis*, the results obtained demonstrate that the minimum time required for the opening of the shells was not sufficient to eliminate the bacteriological risk. By extending the cooking time for a further 2 minutes, all target bacteria were eliminated, and, according to the literature, this time also allows the elimination of viruses. Therefore, for both *C. gallina* and *M. galloprovincialis*, 2 minutes of cooking after opening all the shells under standardized conditions is suitable to eliminate microbiological risk (Figure 1). In experimental cooking tests, using ice, we immediately blocked the effect of high temperatures on bacterial loads after the set time. In the kitchen, after cooking, the ELMs must necessarily be left to cool for a few minutes at room temperature before being shelled and consumed because the temperature is not compatible with immediate handling and eating. This means that the high temperatures in the body of the mollusk are maintained longer as a further guarantee for the consumer. The bacterial strains used for the experimental contamination had been previously isolated from the same species of ELMs in the Adriatic Sea, and this certainly adds value to our study, where a situation as similar as possible to what could occur naturally was recreated. The concentrations of *V. cholerae* non O1 non O139 and *V. parahaemolyticus* used for experimental contamination are undoubtedly much higher than those naturally present in seawater and consequently inside mollusks (Ottaviani *et al.,* 2020). Such high concentrations were deliberately selected to reproduce a hypothetical situation of high bacteriological risk in which a natural contamination of the ELMs was combined with incorrect storage/handling at any level of the post-harvest food chain up to the final consumer. Furthermore, the Centers for Disease Control and Prevention stated that 10^5 CFU/g corresponds to the average infectious dose for *V. parahaemolyticus* (CDC, 2006). Unlike vibrios, the presence of *Salmonella* is sporadic in the marine environment (Morrison *et al*., 2011; Rubini *et al.,* 2018); however, in choosing the concentration of this target bacterium, we used the same criterion as for vibrios. Finally, to experimentally reproduce the worst risk, we contaminated the ELMs with both marine (*V. parahaemolyticus*) and fecal (*Salmonella*) bacteria at high concentrations. Cooking is recommended to improve the sanitary quality of ELMs, but a balance is often struck to retain the organoleptic quality of these organisms. Our primary objective was to provide the consumer with useful information to obtain food free from microbiological risk. However, to maintain an organoleptic quality as similar as possible to that of the raw product, cooking times longer than 6 minutes were not tested. The comparison between the organoleptic quality of the mollusks before and after cooking was not carried out since the cooking times tested were already the shortest to obtain healthy food. However, *C. gallina* and *M. galloprovincialis* visually maintained a hydrated pulp after all cooking times tested. We have shown that the minimum time required to open all the shells is not always sufficient to inactivate

Table 3. Analytical methods used.

Target bacteria	Methods for ELMs analysis pre and post contamination	Methods for ELMs analysis post cooking
Escherichia coli	*Plate count method (Ottaviani et al., 2017)*	*ISO 16649-3*
Salmonella enterica subsp enterica serovar Napoli	Plate count method (Pieralisi *et al.*, 2024)	ISO 6579-1
Vibrio cholerae non O1 non O139	Plate count method (Ottaviani *et al.*, 2020)	ISO 21872-1
Vibrio parahaemolyticus	Plate count method (Ottaviani *et al.*, 2020)	ISO 21872-1

ELMs, edible lamellibranch mollusks.

Table 4. Target bacteria concentration in the edible lamellibranch mollusks before cooking.

	Chamelea gallina	*Mytilus galloprovincialis*
Escherichia coli	6×10^5 CFU/g	7×10^5 CFU/g
Salmonella enterica subsp enterica serovar Napoli	8×10^5 CFU/g	5×10^5 CFU/g
Vibrio cholerae non O1 non O139	8×10^4 CFU/g	9×10^4 CFU/g
Vibrio parahaemolyticus	9×10^4 CFU/g	5×10^5 CFU/g
Vibrio parahaemolyticus + *Salmonella napoli*	$7\times10^4 + 7\times10^5$ CFU/g	$4\times10^5 + 5\times10^5$ CFU/g

CFU/g, colony-forming units per g.

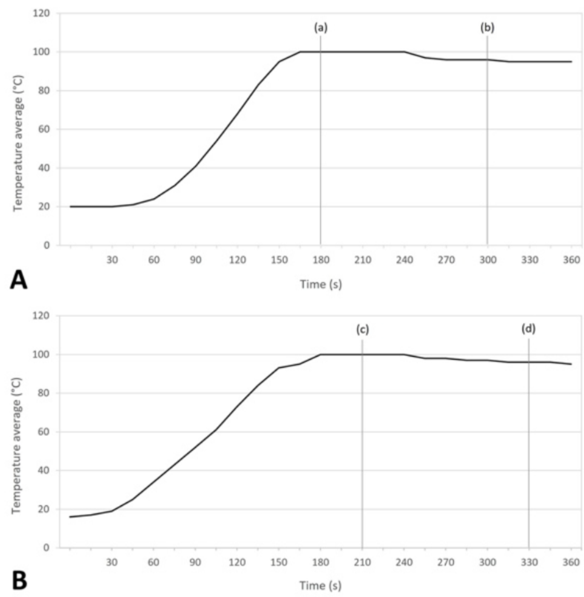

Figure 1. Observed values of time and temperature plotted on a graph during the experimental cooking tests. **A**) *Chamelea gallina*; **B**) *Mytilus galloprovincialis*. (a) Time at total valves opening and to eliminate bacteriological risk, (b) time to eliminate also virological risk; (c) time at total valves opening, (d) time to eliminate both bacteriological and virological risk.

bacteria and ensure safety. Our study also demonstrated the strategic importance of using pans of suitable size for cooking. As far as we know, this last piece of information has never been provided by previous studies.

Conclusions

This is the first study to standardize home cooking conditions for eliminating microbiological risk in *C. gallina* and *M. galloprovincialis*. Thanks to the results of this research, following Regulations EC/178 of 2002 and EC/1169 of 2011 (European Commission, 2002 and 2011), to guarantee the consumer it would be strategic to report the following on the label of commercial packages of *C. gallina* and *M. galloprovincialis:* pour 1 kg of mollusks into a non-stick aluminum pan with an internal upper diameter of at least 28 cm, cover with a lid and cook over medium heat for at least 2 minutes after opening of all the shells. The food thus treated is ready to be consumed. Our tests used a very specific approach, and this allowed us to better standardize the experiments, limiting, on the other hand, their generalizability. It is our intention soon to test other cooking methods or domestic habits in other gastronomic cultures to define the necessary strategy to eliminate the microbiological risk in different conditions.

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