

Influence of cell history on the subsequent inactivation of *Campylobacter jejuni* during cold storage under modified atmosphere



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ARTICLE INFO

Keywords:

Foodborne pathogen
Slaughter process
Stresses
Food safety
Strain variability

ABSTRACT

Worldwide, *Campylobacter* infections are the main cause of human bacterial enteritis and broiler meat is considered as the most important source of human campylobacteriosis. Some mitigation strategies have been focused on reduction of *Campylobacter* at the slaughtering steps. This study aimed to determine the influence of consecutive stresses inspired by slaughtering steps on the subsequent inactivation of *Campylobacter jejuni* during cold storage under different modified atmospheres. Using a full experimental design, three strains of *C. jejuni* of poultry origin were submitted to consecutive heat (46°, 50° or 54 °C for 4 min) and cold (−4° or 3 °C for 2 h) stresses by plunging cultures into baths at appropriate temperatures. Cultures were then stored at 6 °C during seven days under modified atmospheres (70% O₂/30% CO₂ or 50% CO₂/50% N₂). Inactivation of *C. jejuni* induced by cold storage was shown to depend significantly ($P < 0.0001$) upon the heat stress previously applied. It was shown to be the highest under the atmosphere enriched in oxygen, after application of 54 °C. Strain inactivation variability was also quantified. These results show that consecutive stresses influence further inactivation of *C. jejuni* during storage and consequently the contamination level at consumer's plate.

1. Introduction

Since 2005, *Campylobacter* has been the main cause of bacterial enteritis worldwide in humans (EFSA and EDC, 2018). Despite being largely under-estimated, the number of reported confirmed cases of *Campylobacteriosis* was 246,158 in 2017 with an EU notification rate of 64.8 per 100,000 population (EFSA and EDC, 2018). Infection with *C. jejuni* can lead to chronic sequelae, such as Guillain-Barré syndrome (GBS), characterized by the damage of the peripheral nervous system leading to a reversible neuromuscular paralysis (Nyati and Nyati, 2013).

The main reservoirs for *C. jejuni* are avian species and farmed poultry (Young et al., 2007). In food, the occurrence of *Campylobacter* remains high in broiler meat (EFSA and EDC, 2018) and it is considered to be the most important single source of human *Campylobacteriosis*. In 2017, 37.4% of the 13,445 samples of fresh broiler meat (single or batch, aggregated data from all sampling stages) were found to be *Campylobacter* positive (EFSA and EDC, 2018).

Several microbiological risk assessment studies have been performed to assess the potential effects of control measures on prevention/reduction of *Campylobacter* concentration in broiler meat production. Some studies focused more on pre-slaughter stages associated with broiler rearing (Hermans et al., 2011; Lin, 2009) including the use of

probiotics (Saint-Cyr et al., 2017) or vaccine (Meunier et al., 2017). Another effective strategy to reduce the number of campylobacteriosis cases may reside in limiting the entry of chicken carcasses highly contaminated by *Campylobacter* into the market. In this context, the European Commission has just set a microbiological process hygiene criterion for *Campylobacter* in broiler carcasses (European Commission, 2017). A limit of 1000 CFU g^{−1} on carcasses after chilling has been defined. The EFSA (European Food Safety Authority) determined that more than 50% human risk reduction could be achieved if broiler carcasses complied with this new microbiological criteria of 1000 CFU g^{−1} (European Commission, 2017). For food producers, the compliance with this hygienic criterion requires improvements in slaughter hygiene to limit cross-contamination but also investigation of new control measures which may favor *Campylobacter* inactivation following slaughter. Focusing on the susceptibility of the bacteria to the processing steps may represent one way to tackle *Campylobacter* risk.

C. jejuni is able to survive to slaughterhouse environments and during poultry processing. In the poultry farm-to-fork chain, several steps may be stressful for bacteria regarding its growth requirements (microaerophilily and thermotolerance), such as scalding, chilling and storage. Scalding consists in immersing chicken carcasses into a hot water bath, which may induce heat stress to *C. jejuni*. At the end of the slaughter process, the chilling step enables quick refrigeration of

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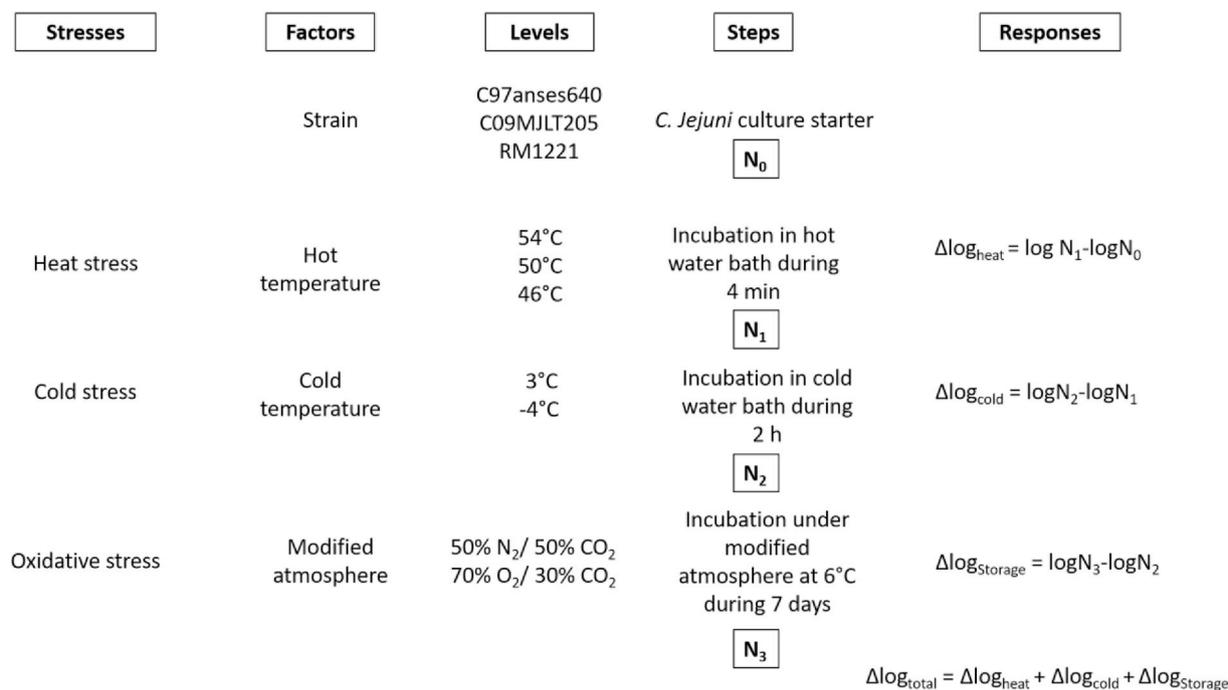


Fig. 1. Global scheme of experimental procedure. The different factors are represented with their associated levels. After each step, the inactivation (viable bacterial population N lost) was calculated ($\Delta\log$).

chicken carcasses, which is necessary to lower the growth rate of pathogenic and spoilage microorganisms (James et al., 2006), is supposed to generate cold stress to *C. jejuni*. At last, chicken cuts are generally conditioned under modified atmosphere and stored under chilled temperature until consumption. Depending on the producer, the gas mix of the package may contain high concentrations of oxygen or not. This latter step induces both cold and possibly oxidative stresses. However, if the influence of single stresses on *Campylobacter* behavior is well-described, the effect of stresses occurring sequentially still needs to be more deeply understood in order to point out conditions of slaughter steps that favor *Campylobacter* inactivation during poultry products storage.

Indeed, the exposure to a stress may condition the bacterial behavior following the exposure to another stress. Increased resistance has been for example already reported in *Bacillus* (den Besten et al., 2010; Desriac et al., 2013). For instance, after being subjected to mild acid or oxidative stress, *C. jejuni* appeared to be more resistant to a subsequent lethal stress (Murphy et al. 2003a, 2003b; Oh et al., 2015). Such cross-protection response from one stress to another has also been reported, like genes involved in heat shock response in *C. jejuni* NCTC 11168 which were upregulated in response to acid stress (Reid et al., 2008b). Additionally, starved *C. jejuni* cells were able to better withstand heat stress (Klančnik et al., 2009).

Besides, it is well established that strains have different behavior when subjected to stress (Chan et al., 2001; Cools et al., 2003; Newell et al., 2001) and this is highly relevant for poultry product in the sense that poultry meat is naturally contaminated by different strains of *C. jejuni* and often more than one species (Colles et al., 2003; Johnsen et al., 2006; Rivoal et al., 2005).

In the current study, several strains of *C. jejuni* were submitted to stresses inspired from those encountered during slaughter, and subsequently incubated under modified atmosphere. The effect of cell history *i.e.* the effect of hot and cold temperature applied following successive heat and cold stresses, on the subsequent inactivation of *C. jejuni* strains during chilled storage under various modified atmospheres, was then quantitatively analysed.

2. Materials and methods

2.1. Bacterial strains and culture conditions

Experiments were performed on three *C. jejuni* strains isolated from poultry products. *C. jejuni* C09MJLT205 was isolated from chicken legs distributed at the market level (Guyard-Nicodeme et al., 2013). *C. jejuni* RM1221 was isolated by Fouts et al. (2005). The strain *C. jejuni* C97Anses640 was isolated from a poultry product (Guyard-Nicodeme et al., 2016). All strains were stored at -80°C in brain heart infusion (BHI, Biomérieux, Marcy l'Etoile, France) supplemented with 20% (v/v) glycerol. *C. jejuni* strains were routinely cultured on Karmali (Oxoid, Dardilly, France) agar plates at 42°C for 48–72 h under microaerophilic conditions (5% O₂, 10% CO₂, and 85% N₂). Then, one or two colonies were transferred into 20 mL of Mueller-Hinton broth (MH, Oxoid, Dardilly, France) and incubated for 20 h under the same conditions under agitation (90 rpm). A 1/100th dilution was transferred into a fresh MH broth and incubated under the same conditions for 18 h, so that the culture reached stationary phase. Finally, a 1/10th dilution was carried out to obtain what we called the 'starter culture' further used for challenge-test experiments.

To enumerate viable *C. jejuni* cells, suspensions were serially tenfold diluted and surface plated on Columbia sheep blood agar plates (Biomérieux, Marcy l'Etoile, France) and incubated for 48 h at 42°C under microaerophilic conditions using a SPIRAL plater (EasySpiral Interscience, Saint Nom, France).

2.2. Experimental procedure

C. jejuni strains were submitted to stresses inspired from conditions encountered during slaughter chicken process. Slaughter is associated with several steps considered as stressful for *C. jejuni*. These steps are scalding, chilling, and are followed by cold storage under modified atmosphere. Scalding consists in immersing chicken carcasses into hot water bath. Then, chicken carcasses are submitted to a quick refrigeration during the chilling step. At last, after cutting carcasses in pieces, chicken cuts are conditioned under modified atmosphere and

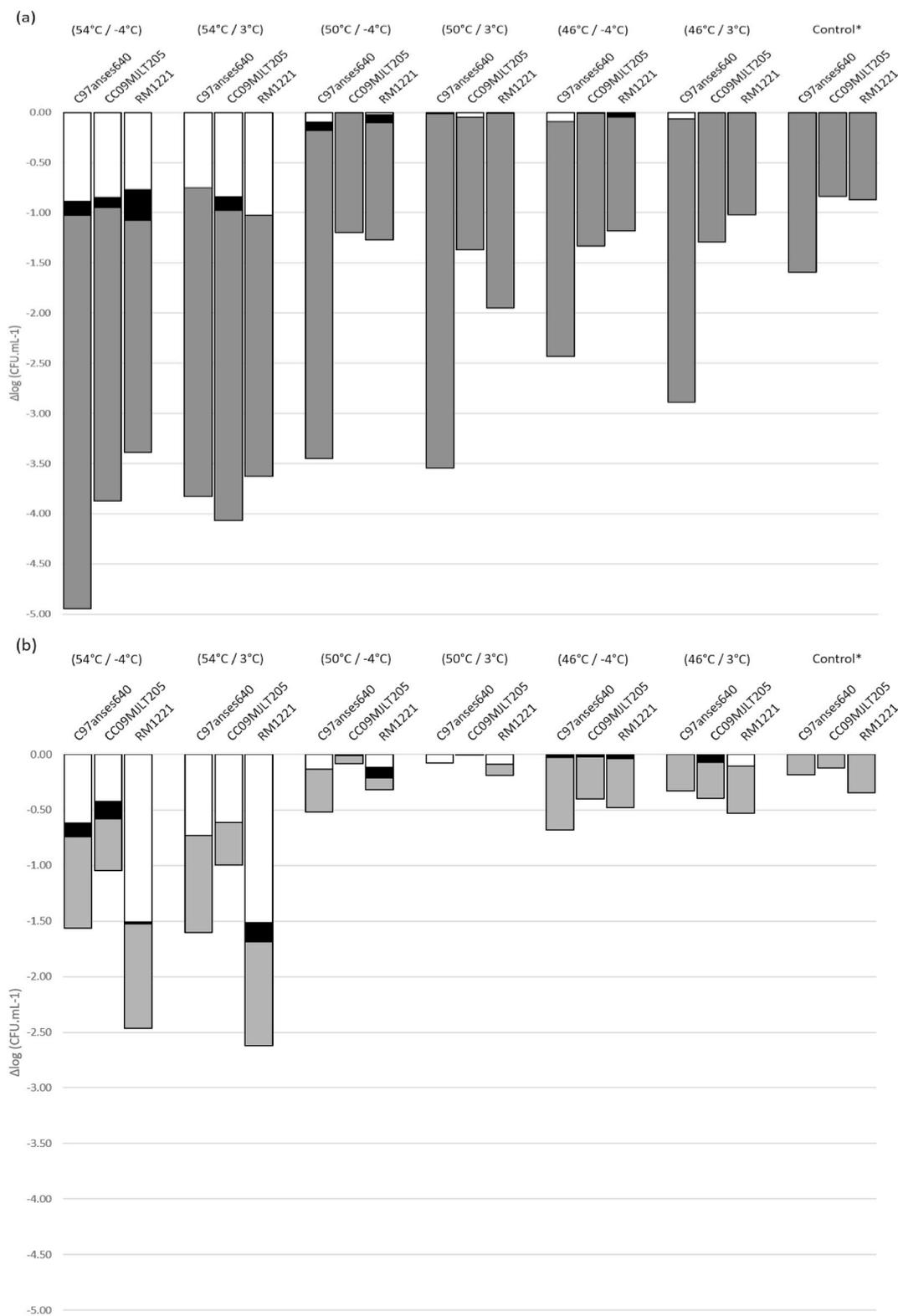


Fig. 2. Contribution of factors (temperature applied during hot and cold bath, strain and gas composition of the atmosphere) on the inactivation of *C. jejuni* induced by each step of the experimental procedure (hot bath in white, cold bath in black, storage at 6 °C in grey) under atmosphere 70% O₂/30% CO₂ (a) and atmosphere 50% N₂/50% CO₂ (b).

stored under chilled temperature.

The three previously mentioned stresses were reproduced as closely as possible in the laboratory, and named as heat stress, cold stress and storage stress, respectively. The first two stresses, i.e. heat and cold stresses were assumed to influence the future behavior of *C. jejuni*

during chilled storage under modified atmosphere. As such, they were considered as participating to *C. jejuni* adaptive history (Fig. 1).

Ten mL of starter culture with an initial concentration of 8 log₁₀ CFU mL⁻¹ from each strain were transferred into a glass tube and submitted to consecutive heat, cold temperature and storage at 6 °C

under modified atmospheres according to a full experimental design.

Heat stress consisted in immersing *C. jejuni* cultures in hot water baths at 46°, 50° or 54 °C for 4 min. 54 °C was chosen as the maximal observed temperature of scalding baths in French chicken slaughterhouses, 46 °C, the maximum growth temperature (Hazeleger et al., 1998) of *C. jejuni* and 50 °C, an intermediate temperature.

After exposure to heat stress, tubes were immediately cooled for 5 min in a water bath at 22 °C, corresponding to the conditions of ambient temperature occurring during the 5 min between scalding and chilling steps in slaughterhouse.

Cold stress was applied immediately after cooling by plunging the previous cultures into cold water with ethylene glycol at -4° or 3 °C for 2 h. The temperature of 3 °C was chosen because it represents the targeted core temperature of poultry carcasses during the chilling step and -4 °C is the minimal surface temperature measured during different visits performed in several slaughterhouses. Strains were exposed to cold stress for 2 h similarly as chicken carcasses during the chilling step.

At last, cultures were stored at 6 °C for seven days under the modified atmospheres (70% O₂/30% CO₂ or 50% N₂/50% CO₂) commonly used for the packaging of poultry cuts. These two atmospheres were generated by filling jars with the *ad hoc* gas mixture (Air Liquide, Carquefou, France). Cultures were placed in bottles with porous silicone caps (Hirschmann Laborgeräte GmbH & Co. KG, Eberstadt, Allemagne) enabling gas exchange. The temperature of 6 °C was chosen according to the average temperature of consumer refrigerators (Laguerre et al., 2001).

During experiments, the temperature applied during heat and cold stress was recorded using a temperature probe Kistock KTT220 (KIMO, Montpon, France) placed in a control tube. The gas composition (% O₂ and % CO₂) of the jar atmosphere was measured using the Oxybaby M + device (WITT-GASETECHNIK GmbH & Co KG, Witten, Germany). It was performed just after filling with gas mixture and regularly over time to check the stability of the gas composition.

After each step, viable counts of *C. jejuni* were enumerated to determine the viability loss resulting from the application of each step, *i.e.* $\Delta\log_{10}$. A total of 152 (3 strains x 4 repetitions x 12 experimental conditions + 8 controls) experiments were performed.

2.3. Statistical analysis

Four independent replicates were carried out and statistical analysis was performed on $\Delta\log_{10}$ transformed values. An ANOVA ($\alpha = 5\%$) was performed using the software XLSTAT (version 19.03.45028), an Addin of the Microsoft Excel software.

3. Results

3.1. Cumulative effect of subsequent heat, cold and storage stresses on inactivation of *C. jejuni* ($\Delta\log_{total}$)

From the starter culture with an initial concentration of 8 \log_{10} CFU mL⁻¹, the mean cumulative *C. jejuni* inactivation, due to the consecutive heat, cold and storage stresses, varied from 0.02 to 4.9 \log_{10} CFU mL⁻¹ as function of strains and atmosphere conditions. Detailed results are available in [Supplementary Material Table S1](#).

During experiments, controls were performed and submitted only to the cold storage under modified atmosphere. The inactivation induced by the only storage step varied from 0.9 to 1.69 \log_{10} CFU mL⁻¹ with the 70%O₂/30% CO₂ atmosphere and from 0.1 to 0.3 \log_{10} CFU mL⁻¹ with the 50% N₂/50% CO₂ atmosphere.

For the bacterial samples undergoing all steps, the storage step with the 70%O₂/30% CO₂ atmosphere, had the main impact on *C. jejuni* inactivation, the bacterial population decrease varied from 1.0 to 3.9 \log_{10} CFU mL⁻¹ (Fig. 2a). In addition, the highest inactivation during storage was reached after the application of the highest temperature during heat stress. Heat only produced an inactivation following

application of 54 °C with a mean inactivation between 0.8 and 0.9 \log_{10} CFU mL⁻¹, whereas the inactivation was negligible when 50° and 46 °C were applied. In our conditions, cold temperature had also a negligible impact on the *C. jejuni* inactivation, indeed a maximum of 0.3 \log_{10} CFU mL⁻¹ reduction was achieved. The highest cumulative inactivation was obtained for the C97anses640 strain. This strain appeared to be less resistant than C09MJLT205 and RM1221 strains, especially during storage. For instance, the inactivation of strain C97anses640 at 50 °C/-4 °C was 3.3 \log_{10} CFU mL⁻¹ (mean value) compared to 1.2 \log_{10} CFU mL⁻¹ for both C09MJLT205 and RM1221 strains (Fig. 2a).

Using the 50% N₂/50% CO₂ atmosphere, the cumulative inactivation of *C. jejuni* was mainly attributable to the storage step and varied from 0.1 to 0.9 \log_{10} CFU mL⁻¹ (Fig. 2b). However, especially at 54 °C, the heat stress contributed equally with the cold storage to the cumulative inactivation, except for the strain RM1221. Indeed, for the latter, the inactivation in the hot water bath was higher than during the storage step, *i.e.* mean values of 1.5 and 0.9 \log_{10} CFU mL⁻¹, respectively.

3.2. Effect of heat/cold temperature, modified atmosphere, strains and interactions on subsequent inactivation during storage affected by cell history ($\Delta\log_{storage}$)

When subjected to mild stress, bacteria may become more resistant to another stress applied subsequently. In this study, we tested if the application of successive hot and cold temperature enhanced the resistance of *C. jejuni* during storage. In other words, we studied the effect of cell adaptive story on the subsequent behavior of *C. jejuni*.

The influence of the different factors and their interaction on inactivation of *C. jejuni* during cold storage was analysed by ANOVA. The ANOVA highlighted that the temperature of the cold bath did not influence significantly the inactivation during storage. Therefore, a new ANOVA was performed without considering cold stress. The model was significant ($p < 0.0001$) as indicated in [Table 1](#). The ANOVA showed that there were significant effects of strain, temperature applied during heat stress, and packaging atmosphere. During the cold storage, strains C09MJLT205 and RM1221 exhibited similar inactivation around 1.1 \log_{10} CFU mL⁻¹. In contrast, the strain C97anses640 appeared to be less resistant to storage than the two other strains with a mean inactivation of 1.8 \log_{10} CFU mL⁻¹ (Fig. 3a).

Regarding heat stress, the highest temperature of 54 °C induced the highest inactivation associated with storage. Indeed, the mortality reached a mean of 1.9 \log_{10} CFU mL⁻¹ compared to that obtained at 46° and 50 °C (1 \log_{10} CFU mL⁻¹) (Fig. 3b).

Inactivation of *C. jejuni* during storage was more than five times higher under the modified atmosphere 70% O₂/30% CO₂ than under the atmosphere 50% N₂/50% CO₂ ($p < 0.0001$), with a mean inactivation of 2.2 \log_{10} CFU mL⁻¹ and 0.4 \log_{10} CFU mL⁻¹, respectively (Fig. 3c).

The ANOVA showed significant interactions between strains and atmosphere, temperature applied during heat stress and atmosphere.

While the mean inactivation resulting from storage under the

Table 1
Results from ANOVA of the influence of the different factors and their interaction on inactivation of *C. jejuni* during cold storage.

| Source | DF | Sum Sq | Mean Sq | F value | P |
|-------------------|-----|---------|---------|---------|----------|
| Model | 9 | 160.941 | 17.882 | 35.661 | < 0.0001 |
| Error | 124 | 62.181 | 0.501 | | |
| Total | 133 | 223.122 | | | |
| Heat | 2 | 19.254 | 9.627 | 19.198 | < 0.0001 |
| Atmosphere | 1 | 110.907 | 110.907 | 221.169 | < 0.0001 |
| Strain | 2 | 16.725 | 8.363 | 16.677 | < 0.0001 |
| Heat*Atmosphere | 2 | 6.419 | 3.210 | 6.401 | 0.002 |
| Atmosphere*Strain | 2 | 12.806 | 6.403 | 12.768 | < 0.0001 |

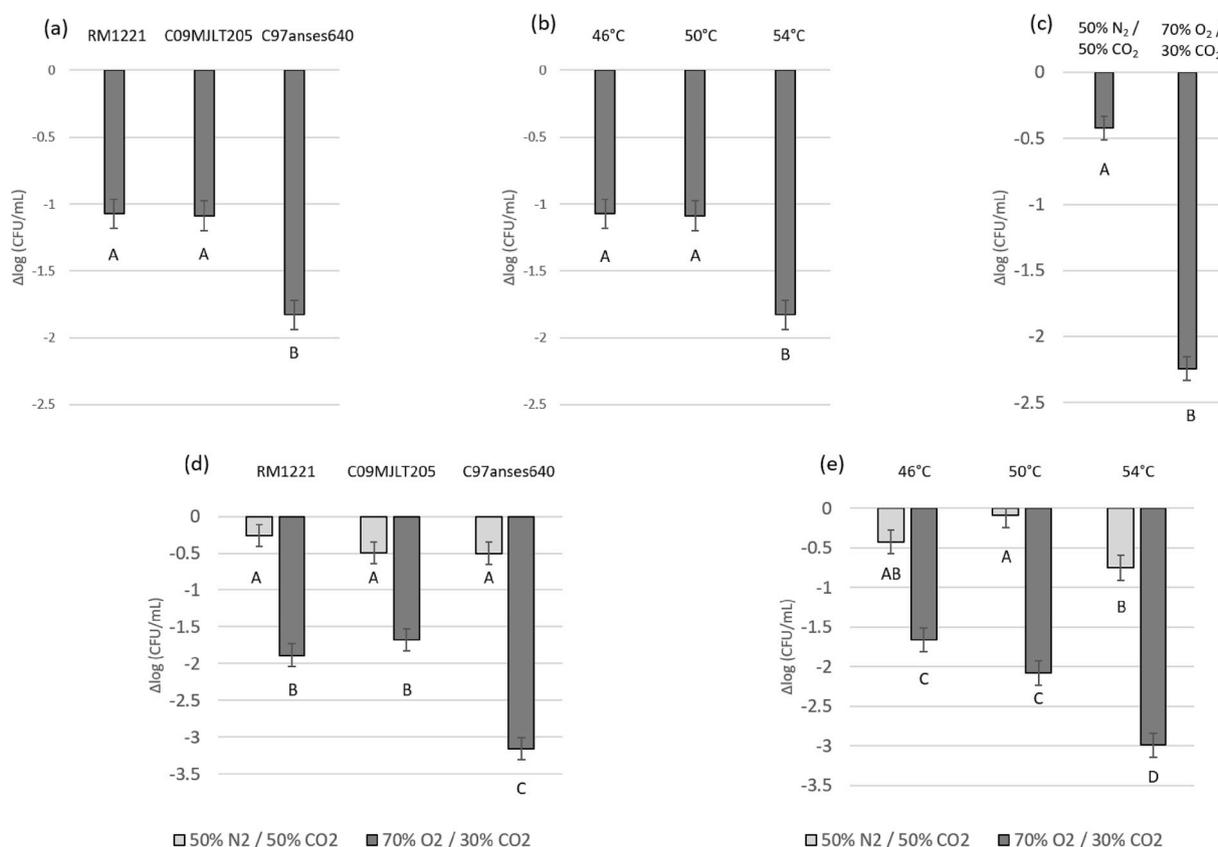


Fig. 3. Effect of factors and interactions on inactivation during storage at 6 °C under modified atmosphere: strain effect (a), mean effect of temperature applied during heat stress all strains combined (b), mean effect of the gas atmosphere composition all strains combined (c), interaction between strains and atmosphere (d) and mean interaction between temperature of heat stress and atmosphere all strains combined (e). A, B, C Different capital letters indicate significant difference according to the Least Significant test ($P < 0.05$) in inactivation.

atmosphere 50% N₂/50% CO₂ was low and similar no matter the strain used, (between 0.3 and 0.5 log₁₀ CFU mL⁻¹), the inactivation was largely higher under 70% O₂/30% CO₂ and different between strains ($p < 0.0001$). Indeed, strain C97anses640 appeared to be less resistant than the strains C09MJLT205 and RM1221. The mean inactivation of strain C97anses640 was 3.2 log₁₀ CFU mL⁻¹ compared to that of C09MJLT205 and RM1221 strains, i.e. 1.9 and 1.7 log₁₀ CFU mL⁻¹, respectively (Fig. 3d). Besides, this study pointed out that similar inactivation during heat treatment does not necessarily lead to similar inactivation during storage. Indeed, under 70% O₂/30% CO₂ atmosphere, the inactivation during heat stress was similar for each strain but higher for the strain C97anses640 during the storage. Thus, this result highlights the importance of the cell history, and the difficulty to predict future behavior while considering only a previous phenotypic response to a specific stress.

A significant interaction between atmosphere and heat stress was characterized by a similar mean inactivation at 46° and 50 °C under the atmosphere 70% O₂/30% CO₂, (1.7 and 2.1 log₁₀ CFU mL⁻¹, respectively), compared to that obtained at 54 °C, i.e. 3.0 log₁₀ CFU mL⁻¹ (Fig. 3e). Under the atmosphere 50% N₂/50% CO₂, the inactivation following application of 54 °C was also the highest and was 0.8 log₁₀ CFU mL⁻¹. In contrast, the inactivation resulting from application of 46 °C was slightly higher than that obtained following application of 50 °C, i.e. 0.4 and 0.1 log₁₀ CFU mL⁻¹ respectively.

4. Discussion

The objective of this study was to assess the effect of the cell history on the subsequent survival of three *C. jejuni* strains. Stressful steps from slaughterhouses were identified and then reproduced in laboratory.

The first step was to immerse the bacteria in hot water bath at temperature ranging from 46° to 54 °C. The inactivation during this step varied between 0 and 1.5 log₁₀ CFU mL⁻¹ according to strain and temperature. From heat inactivation, it was possible to calculate the decimal reduction time or D-value. These D-values corresponds to the time necessary to kill 90% of an initial population or decrease the initial population by one log. The D-value is a common metric and enables to compare more easily results from different studies. In our experiments, heat stress was applied during 4 min. We measured that 1 min was the time necessary to reach the temperature desired. Thus, once reached, the set temperature was maintained during 3 min. For a selected temperature, D-values were calculated by dividing the 3-min constant temperature application by $\Delta\log$ lost following application of the heat stress.

All collected D-values from different studies in the literature were gathered in Table 2. We can see an important variability between results of decimal reduction times for *C. jejuni*, mainly at 50 °C. It appears that variability was higher at 50 °C than at 55 °C, which was also mentioned by McCarthy et al. (2018). In our study, *C. jejuni* was submitted to heat stress in suspension rather than on chicken muscle like in the real scalding step. However, the high variability of collected D-values could not enable to visualize a difference of heat resistance between meat matrices and laboratory media, as it could have been expected. Indeed, it has been shown that the heat resistance was higher for *Salmonella* when attached to muscle as opposed to free cells (Humphrey et al., 2007). *C. jejuni* cells could also be better recovered from 5-min boiling if they were previously attached to chicken muscle as opposed to carrots (de Jong et al., 2012). D_{50°C}-values varied from 3.5 to 39 min which corresponds to an inactivation from 0.08 to 0.9 log₁₀ in 3 min. D_{55°C}-values varied between 1.5 and 2.8 min and were

Table 2
D-values (min) of *C. jejuni* collected from different studies.

| Lab medium | pH | Medium | Strain | D _{50°C} (min) | D _{55°C} (min) | Reference | | |
|------------|-------------------|------------------------|------------------------|-------------------------|-------------------------------|-------------------------------|-----------------|----------------------------|
| Lab medium | 7.2 | Mueller-hinton broth | C97anses640 | No inactivation | 2.5 ^a | This study | | |
| | | | C09MJLT205 | No inactivation | 2.8 ^a | | | |
| | | | RM1221 | No inactivation | 1.5 ^a | | | |
| | 7.4 | Heart infusion broth | AR6 | 36 | 5.3 | Nguyen et al. (2006) | | |
| | | | L51 | 39 | 4.6 | | | |
| | ND | 1% peptone solution | H-840 | ND | 0.6 | Blankenship and Craven (1982) | | |
| | | | composite of 5 strains | ND | 1.1 | | | |
| | Food matrix | ND | Physiological saline | 1503 | ND | 1.14 | Sörqvist (1989) | |
| | | | | 6.8 | Skim milk | FRI-CF3 | | 5.4 |
| | | FRI-CF6 | 4.7 | 1 | | | | |
| FRI-CF8 | | 3.5 | 0.9 | | | | | |
| FRI-CF12 | | 4.4 | 1 | | | | | |
| FRI-CF16 | | 5.1 | 0.9 | | | | | |
| 7.2 | | Milk | 24791 | 5.7 | | ND | Waterman (1982) | |
| 16000 | | | ND | ND | | | | |
| 21033 | | | 7.2 | ND | | | | |
| 172589 | | | ND | ND | | | | |
| 16509 | 7.3 | | 1.1 | | | | | |
| ND | Chicken carcasses | 5388 | 36 | ND | Blankenship and Craven (1982) | | | |
| | | H-840 | ND | 2.1 | | | | |
| 8 | Chicken carcasses | composite of 5 strains | ND | 2.3 | Osiriphun et al. (2012) | | | |
| | | Endogeneous flora | ND | 0.8 ^a | | | | |
| 6 | Lamb meat | FRI-CF8 | 5.9 | 1 | Koidis and Doyle (1983) | | | |
| | | FRI-CF31P | 11.2 | 1.2 | | | | |
| | | FRI-CF401S | 13.2 | ND | | | | |
| | | FRI-CF402S | 11.1 | ND | | | | |
| | | FRI-CF403S | 8.96 | ND | | | | |
| | | FRI-CF404S | 13.3 | 1.23 | | | | |
| | | ND | Chicken carcasses | Endogeneous flora | | ND | 0.9 | Berrang and Dickens (2000) |
| | | ND | Chicken carcasses | Endogeneous flora | | ND | 1.4 | |

ND: Not Determined.

^a D_{55°C}-values were calculated using the formula $\log D_{55^\circ\text{C}} = \log D_{54^\circ\text{C}} - (T_{55^\circ\text{C}} - T_{54^\circ\text{C}})/Z$. Z = 4.99 °C was estimated by calculating the mean Z reported from different studies (Blankenship and Craven, 1982; Doyle and Roman, 1981; Sörqvist, 1989; Waterman, 1982)

found in the range of what was observed in previous studies (mean D_{55°C} of 1.5 ± 1.3 min). In light of the variation illustrated in Table 2, heat strain sensitivity, experimental method, and variables such as medium may greatly influence the inactivation of *C. jejuni*. In our study, for 46 °C and 50 °C, no inactivation has been observed. Indeed, we considered that inactivation was significant if Δlog was greater than 0.5 log₁₀ CFU.mL⁻¹ i.e. the experimental error due to microbial enumeration (Pujol et al., 2012). These results are in accordance with the very high D-values found by Nguyen et al. (2006).

To our knowledge the effect of cold temperature applied for a short period (i.e. two or several hours) on *C. jejuni* has not yet been reported. In this study, application of a cold temperature during 2 h had no significant effect on the survival of *C. jejuni*. However, these results have to be interpreted with caution, especially regarding possible extrapolation with the chilling step of the slaughter process. Unlike our experimental conditions, the cold temperature applied during the chilling step is generally associated with desiccation due to ventilation. In laboratory, such conditions could not be mimicked. Nevertheless, the potential influence of desiccation might induce microbial inactivation. For instance, Zoz et al. (2016) have highlighted the impact of relative humidity on survival of *Listeria monocytogenes*. Likewise, a study led by Rivoal et al. (2016), investigated the relation between temperature, chilling time and air velocity on the inactivation of eight strains of *C. jejuni* by the use of a miniaturized chilling room prototype. This work highlighted the significant effect of the duration of the chilling step on the inactivation of *C. jejuni*. A significant interaction between the temperature and the air flow during the process was also pointed out by the authors. However, it is important to reinforce here that carcasses contaminated with more than 3 log CFU.g⁻¹ of *Campylobacter* could not be significantly decontaminated during the chilling step in slaughtering house.

After the application of hot and cold temperature at the

slaughterhouse, *C. jejuni* was submitted to storage for several days under the same conditions as those found at retailers' and then at consumers' home. During storage, *C. jejuni* undergoes a combination of cold temperature and modified atmosphere. It was found in the current study that the inactivation varied from -1.0 to -3.9 log₁₀ CFU mL⁻¹ in the atmosphere 70% O₂/30% CO₂ and from 0 to -0.9 log₁₀ CFU mL⁻¹ in the atmosphere 50% N₂/50% CO₂. The inactivation of *C. jejuni* was significantly influenced by the atmosphere, with a higher effect of the atmosphere 70% O₂/30% CO₂. Indeed, *Campylobacter* are microaerophilic organisms. The presence of O₂ in high concentrations is toxic for the pathogen. For many years, studies have investigated the effect of storage during several days under aerobiosis condition or modified atmosphere. On various meat matrices, the reduction of *Campylobacter* count in aerobiosis at 4 °C varied from 0.3 to 2 log₁₀ CFU g⁻¹ (Bhaduri and Cottrell, 2004; Blankenship and Craven, 1982; Eideh and Al-Qadiri, 2011; Koidis and Doyle, 1983; Phebus et al., 1991; Vashin and Stoyanchev, 2011). In broth, similar results have also been found (Chaisowwong et al., 2012; Chan et al., 2001; Garenaux et al., 2008). However, the inactivation of *C. jejuni* was higher in our work compared to what is commonly observed in the literature. Indeed, before the storage step, bacteria were subjected to heat and cold stress which may induce a hurdle effect. Besides, one of the atmospheres used during our experiments had a concentration in O₂ much higher than in air. In other studies, it has been found that the inactivation increased with atmospheres enriched in O₂ compared to atmospheres containing only CO₂ or N₂. Boysen et al. (2007) showed that in broth, the inactivation under atmosphere 70% O₂/30% CO₂ was higher than in atmosphere 70% N₂/30% CO₂ and 100% N₂, with a reduction of 0.5–2 log₁₀ CFU mL⁻¹ and 0.3 to 0.8 log₁₀ CFU mL⁻¹ respectively.

Such results have also been found when *C. jejuni* was submitted to paraquat, a chemical oxidizing agent (Garenaux et al., 2008). After 7

days of exposition, the population was reduced by $3 \log_{10}$ CFU mL⁻¹.

In this work, thermal steps were applied successively in order to assess the impact of cell history on the subsequent inactivation of *C. jejuni* during storage. Some studies have already investigated the potential effect of cell history or adaptation of one stress to another stress. Indeed, microorganisms enduring stressful environments can protect or crossprotect themselves to survive subsequent homogeneous or heterogeneous stresses, which is called adaptive tolerance response (ATR). It has been shown that *C. jejuni* can induce an ATR after aerobic or acid stress (Gaynor et al., 2005; Jones et al., 1993; Ma et al., 2008; Martínez-Rodríguez and Mackey, 2005; Murphy et al. 2003a, 2003b; Reid et al., 2008a). On the other hand, in our study it has been shown that after successive application of heat and cold temperature, if an ATR was induced, it was insufficient to produce an adaptation effect since the inactivation was higher for stressed bacteria compared to unstressed ones.

This study was conducted on three strains isolated from poultry and more particularly on product from retail market. Tackling strain variability is not easy because the features of the strains which are associated with highest or lowest sensitivity to processing steps are not known. The choice was done in this study to select strains (i) representative of strains commonly found in poultry, and (ii) exhibiting *a priori* different behaviors regarding selected stresses (data not shown). Firstly, the representativeness of strains in poultry was sought by determining the strain genotype by MultiLocus Sequence Typing (MLST). The strain C97anses640 belonged to the Sequence Type-45 complex (ST-45), the strain C09MJLT205, to the ST-21 complex and the strain RM121, to the ST-354 complex. The ST-21 and ST-45 are the two main clonal complexes encountered in chicken (and poultry meat) worldwide. Interestingly, they are also commonly found among human isolates (Colles et al., 2003; de Haan et al., 2010; Dingle et al., 2002; Guyard-Nicodeme et al., 2015; Habib et al., 2009; Levesque et al., 2008; Ragimbeau et al., 2008; Sheppard et al., 2009). Secondly, the behavior variability between strains was determined regarding their inactivation during cold storage under modified atmosphere. High response variability between *C. jejuni* strains has also been reported in various studies (Habib et al., 2010; Ligowska et al., 2011; Vashin and Stoyanchev, 2011). These findings highlighted the difficulty and the importance to take into account strain variability in microbial inactivation studies, and, more generally in exposure assessment.

The experimental laboratory conditions applied during the present study differ from what *C. jejuni* cells really experience during the slaughtering steps because they are not attached on muscle but free in liquid suspension, and because the chilling step does not induce desiccation. Here reside some limitations of laboratory experiments. They offer the possibility of replicating standardized procedures and measures, which is necessary to obtain the proof of concept. Passed this step, a validation step in laboratory on chicken muscle will be necessary and is currently being processed.

In conclusion, to better predict *Campylobacter* contamination in poultry processing, it is essential to assess its behavior after applying not only one stress but the whole sequence of stresses encountered during the process. Indeed, cell history plays an important role and may induce some physiological responses such as ATR which influences *C. jejuni* further behavior regarding subsequent stresses. In some cases, the bacteria is able to better resist to another stress whereas sometimes, the application of successive stresses induces a hurdle effect, and in contrast, enhances bacterial inactivation. Finally, this study pointed out that there is no clear correlation between the amount of log inactivation observed in preliminary processing steps and the amount of log inactivation occurring at the latest processing steps such as storage, rendering it very difficult to predict the microbial behavior during the storage at retail and consumer's house from phenotypic information resulting from application of previous slaughtering steps.

A new generation of predictive models, including cell history information, will be necessary to progress along this route, and omics

methods might help. These new tools consider not only the bacterial behavioral response, but also the adaptation response depending on the bacterial gene expression and regulation. The investigation of predictive models including gene expression would enable to fine-tune existing exposure assessment models and consider strain-dependent physiological response.

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgements

We are grateful to the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) of Ploufragan who realized the MLST analysis.

We also especially thank the Regions Pays de La Loire and Bretagne through the "Pôle Agronomique Ouest" for their financial support in the framework of the Biomics project.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fm.2019.103263>.

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