Method for prevention of Campylobacter cross-contamination of poultry carcasses at water cooling

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Abstract. Campylobacteriosis is one of the main causes of food poisoning with insufficient thermal treatment of poultry meat products. Raw poultry meat may be largely contaminated by campylobacteriae, including pathogenic strains. In this regard, the authors developed the method to prevent cross-contamination of the surface of carcasses at water cooling. The study concerns the antimicrobial properties of a technological processing aid containing: 15±2% peracetic acid, 18±2% hydrogen peroxide (TPA) during 25 minutes of exposure. C. jejuni was used as a test culture. Sampling, microbiological, organoleptic and physicochemical studies were carried out according to standard procedures. It was found that the use of 0.03% TPA solutions for cooling carcasses allows inactivating C. jejuni in a cooling medium and preventing cross-contamination of carcasses. TPA solutions of 0.1% concentration reduce QMA&OAMO, prevent cross-contamination and inactivate C. jejuni on the surface of poultry carcasses. In terms of physicochemical and organoleptic parameters, broiler chicken carcasses cooled in 0.1% TPA solution meet the requirements of GOST 31962-2013. Compared to control samples the treated carcasses are characterized by the change in the color of the carcass surface and internal fat – the color becomes paler.

1. Introduction
One of the important problems is food toxicoinfections, among which campylobacteriosis remains the least studied disease. Campylobacteriosis is one of the four leading causes of diarrheal disease in the world [1].

In the European Union, about 8.0-15.7% of broiler chicken carcasses are contaminated with Salmonella and 75.8% with Campylobacter. Many publications on the problem of pathogens in raw poultry products provide data on carcass surface contamination. In this regard, it is important to have the same data for domestic products – for comparability of estimates of microbial contamination in modern conditions [4].

According to Rospotrebnadzor (over 2010 and 2016), poultry meat obtained from domestic agricultural producers and taken after cooling or after packaging was contaminated with campylobacteriae in 33% of studies [3].

Of all raw poultry products, including carcasses of broiler chickens, turkeys, quail, as well as semi-finished products, campylobacteriae were identified in 45% of cases. Campylobacter bacteria in
poultry reach 90%, and therefore poultry meat is considered the main source of food campylobacteriosis. The mechanism of survival and subsequent cross-contamination of Campylobacter bacteria is not sufficiently studied and requires additional research to reduce the risks of food diseases associated with the consumption of poultry meat, since it has a large specific weight in the food patterns of the population [2].

The introduction of modern technologies that reduce the intensity of campylobacteriae contamination of various poultry products is necessary to reduce the number of cases of food toxicoinfections and bacterial poisoning [1].

In view of the above, the purpose of the study was to develop a method for preventing cross-contamination of poultry carcasses with Campylobacter bacteria at water cooling.

2. Materials and Methods

The study was carried out in the Laboratory of Sanitary and Hygienic Assessment of Raw Materials and Products of the All-Russian Scientific Research Institute of Poultry Processing Industry. The study utilized a technological processing aid based on peracetic acid (hereinafter TPA) containing: 15±2% peracetic acid and 18±2% hydrogen peroxide.

The sampling was carried out according to GOST 31467-2012 [5]. The Campylobacter bacteria were identified according to GOST ISO 10272-1-2013 [10]. The Bolton broth and Columbia blood agar were used for inoculation. Organoleptic and physicochemical studies were carried out according to GOST 31470-2012 [6]. The number of mesophilic aerobic and optional-anaerobic microorganisms (QMA&OAMO) was determined according to GOST 7702.2.1-2017 [9].

The disinfectant activity of TPA solutions was detected according to P 4.2.3676-20 [8]. C. jejuni was used as the test culture.

TPA solutions were prepared in concentrations from 0.005; 0.001; 0.01; 0.09; 0.45 and 0.9% of peracetic acid at 500 ml solution per test object using tap water.

Batiste test objects contaminated with C. jejuni were placed in TPA working solutions. After 25 minutes, 2 test objects were taken out and after double washing in water (5 min each), they were seeded on Bolton broth.

To establish the modes of TPA solutions, a cooling bath was modeled for the prevention of cross-contamination of the surface of carcasses with campylobacteriae. The surface of the broiler chicken carcasses was contaminated with C. jejuni test culture. For this purpose, 0.1 ml of a daily culture was added in a container with cold tap water at the rate of 2.0 liters per carcass, and the carcasses were placed into it for 5 minutes. The TPA was then added to certain concentrations and kept for 25 minutes. Water was planted from control and test containers, as well as washes were made from the carcasses of broiler chickens.

3. Results and Discussion

Table 1 shows the results of the studies on disinfectant activity of TPA solutions with respect to C. jejuni in experiments with batiste test objects. The table shows that 0.001, 0.005% TPA solutions do not inactivate C. jejuni on batiste test objects: after 24 hours of incubation, homogeneous opacity and sediment were noted in Bolton broth (Fig. 1a). After subsequent transfer to Columbia blood agar and incubation for 24-48 hours, the growth typical for campylobacteriae was noted: in the form of flat, moist mucous, grayish, small colonies resembling “condensate drops” (Fig. 1b). It was found that 0.01, 0.09, 0.45 and 0.9% TPA solutions provide the inactivation of C. jejuni during 25 minutes of exposure in the experiments with batiste test objects.
Table 1. TPA disinfectant activity with respect to C. Jejuni

| Control | TPA solutions, % |
|---------|-----------------|
|         | 0.001 | 0.005 | 0.01  | 0.09  | 0.45  | 0.9   |
| +       | +     | +     | -     | -     | -     | -     |

Note: “+” – C. jejuni growth; “-” – no C. jejuni growth.

Figure 1. Growth of campylobacteria in media: (a) – growth of campylobacteria in Bolton broth; (b) – growth of campylobacteria on blood agar.

Table 2. Study of TPA antimicrobial properties on cooling medium microflora at carcasses cooling

| Indicator                  | Cooling medium                  | Tap water (control) | TPA solutions, % |
|----------------------------|---------------------------------|---------------------|-----------------|
|                            |                                 |                    | 0.01 | 0.03 | 0.05 | 0.1 | 0.2 |
| C. jejuni, CFU/cm³         |                                 | (2.8±0.13)·10³     | (1.2±0.05)·10³ | N/D | N/D | N/D | N/D |
| QMA&OAMO, CFU/cm³          |                                 | (1.7±0.3)·10⁴     | 50   | <10  | <10  | <10  | <10 |

Note: N/D – not detected.

Table 2 shows the results of TPA studies on water microflora while cooling carcasses which surface is contaminated with C. jejuni.

Table 2 shows that 0.01% solutions of the agent do not ensure the inactivation of C. jejuni, its content was (1.2±0.05)·10³ CFU/cm³. The use of 0.03, 0.05, 0.1 and 0.2% TPA solutions provides for the inactivation of C. jejuni in water after 25 minute exposure. The use of TPA for cooling 0.01% solutions at 25 minute exposure reduces QMA&OAMO to 50 CFU/cm³, and the use of 0.03% solutions – to single colonies.

Table 3 shows the results of TPA studies on surface microflora of carcasses contaminated with C. jejuni. TPA solutions of 0.01% concentration reduce QMA&OAMO from (4.6±0.22)·10⁶ to (1.3±0.06)·10⁵ CFU/cm³, C. jejuni content from (2.7±0.12)·10⁵ CFU/cm³ to (2.2±0.01)·10⁵ CFU/cm³. TPA solutions of 0.03 and 0.05% concentration reduce QMA&OAMO to single colonies in 1 cm³, but
do not ensure the inactivation of C. jejuni, its content was \((1.8\pm0.08)\times10^3\) and \((1.6\pm0.07)\times10^3\) CFU/cm\(^3\), respectively.

The solutions of 0.1-0.2% concentration reduce QMA&OAMO to single colonies in 1 cm\(^3\) and ensure the inactivation of C. jejuni.

**Table 3.** Studies of TPA antimicrobial properties on surface microflora of carcasses contaminated with C. jejuni

| Indicator                  | Tap water (control) | TPA solutions, % |
|----------------------------|---------------------|------------------|
|                            |                     | 0.01  | 0.03  | 0.05  | 0.1   | 0.2   |
| C. jejuni, CFU/cm\(^3\)    | \((2.7\pm0.12)\times10^3\) | \((2.2\pm0.01)\times10^3\) | \((1.8\pm0.08)\times10^3\) | \((1.6\pm0.07)\times10^3\) | N/D   | N/D   |
| QMA&OAMO, CFU/cm\(^3\)     | \((4.6\pm0.22)\times10^6\) | \((1.3\pm0.06)\times10^3\) | <10    | <10    | <10    | <10    |

Next, the effect of cooling carcasses in 0.1% TPA solution on meat quality was studied. The physical and chemical studies of meat, as well as the organoleptic assessment of broth (transparency, aroma) prepared from poultry meat and cooled in the usual way and in TPA solutions, did not establish reliable differences. Poultry meat met the requirements of GOST 31962-2013 [7]. However, compared to the control samples, the treated carcasses did not show a slight change in the color of the surface of carcasses and internal fat – their color becomes paler.

Table 4 shows physical and chemical parameters of carcasses cooled in tap water and in 0.1% TPA solution. The table shows that the content of volatile fatty acids in carcasses cooled in 0.2% TPA solution was 2.44±0.56 mg KOH, in carcasses cooled in the usual way (control) – 2.43±0.56 mg KOH. The peroxide number of fat in carcasses cooled in the TPA solution was 0.44±0.03 mg KOH/g, and in the control – 0.1±0.01 mmol O\(_2\)/kg. The acid number of fat in the carcasses cooled in the TPA solution was 0.44±0.03 mg KOH/g, and cooled in the usual way – 0.43±0.03 mg KOH/g.

**Table 4.** Physical and chemical parameters of carcasses cooled in tap water and in 0.1% TPA solution

| Indicator                  | Tap water | 0.1% TPA solution |
|----------------------------|-----------|-------------------|
| Volatile fatty acids (mg KOH) | 2.43±0.56 | 2.44±0.56         |
| Fat peroxide number, mmol O\(_2\)/kg | 0.1±0.01  | 0.2±0.02          |
| Fat acidity value (mg KOH/g)  | 0.43±0.03 | 0.44±0.03         |

**4. Conclusion**

The tested TPA has a pronounced antimicrobial effect on campylobacteria. The study with batiste test objects revealed that 0.01% TPA solutions ensure the inactivation of C. jejuni at 25 minutes of exposure. The use of 0.03% TPA solutions for cooling carcasses provides for inactivation of C. jejuni after 25 minutes exposure in the cooling medium. The cooling of carcasses in 0.1% TPA solutions reduces QMA&OAMO to single colonies in 1 cm\(^3\) and ensures the inactivation of C. jejuni on their surface.

According to physicochemical and organoleptic parameters, the carcasses of broiler chickens cooled in a 0.1% TPA solution at exposure for 25 minutes meet the requirements of GOST 31962-
2013 [7]. Compared to the control samples, the treated carcasses changed the color of the surface of the carcasses and internal fat – the color becomes paler.

References
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