Influence Of Heating And Freezing On The Survival Of *Yersinia Enterocolitica* In Ground Beef

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ABSTRACT

The behavior of *Yersinia enterocolitica* O:3 to different temperatures have been studied. The tests have been carried out on an artificial contaminated minced meat with *Yersinia enterocolitica* O: 3. Following the contamination sources represented by frozen and cooked minced meat. We have considered appropriate to carry out some investigations that revealed the chemical changes in minced meat during 13 weeks of storage at freezing temperature and the optimum growing and preserving temperatures of *Yersinia enterocolitica*, their life period at low temperatures or at high temperatures. The analysis of the results obtained has demonstrated that *Yersinia enterocolitica* O:3 was not completely destroyed by freezing temperatures at –18 in maximum 13 weeks, but destroyed relatively quickly at 70°C and 80°C in maximum 10 and 3 minutes respectively.

INTRODUCTION

The current interest posed by the Yersinia species is determined by the fact that these bacterial species generate a series of morbid entities which come more and more frequent to the attention of bacteriologists, epidemiologists and surgeons. Numerous studies have been published regarding the infections caused by these bacteria during the latest years, and their searches on this theme developed. During the last ten years of the last millennium, numerous researchers drew the attention on the fact that the bacteria species of Yersinia are frequently involved in the food borne diseases with acute diarrheic syndrome in humans. Currently, although many Yersinia species have been discovered and taxonomically classified, three species are considered as the main pathogens for humans: Yersinia pestis, Yersinia pseudotuberculosis and *Yersinia enterocolitica (1). Yersinia enterocolitica* can be transmitted by contaminated water and food and has been isolated from meat, meat products *(2).* The ability of *Y. enterocolitica* to multiply at low temperatures can account for the increase in food borne infections caused by these organisms as a result of the wide diffusion of refrigerated products. This enables long survival of these bacteria in cold water or refrigerated food. It has been observed that they can survive in cooled water for six months *(3).* Fresh meat products are commonly marketed at refrigerated temperatures (2–5°C). However, many undesirable changes of the products can occur during refrigeration due to microbial growth and lipid oxidation, which give rise to quality reduction, meat spoilage, and economic loss. Lipid oxidation is a major cause of quality deterioration in meat and its cooked products. Ground meat tends to become rancid and brown more rapidly, due to pigment and lipid oxidation. An oxidative reaction in muscle foods leads to degradation of lipid and proteins, resulting in deterioration of flavor, texture and nutritive value, and is considered as one of the major problems in the development of new convenient meat products and processes *(4).* Lipid oxidation is often responsible for quality loss via formation of rancid flavor and is affected by the duration and temperature of storage of meat *(5).* So, the aim of the present study is to determine the effect of heating and freezing on survival of *Yersinia enterocolitica* and the quality of ground beef by determination the changes in pH, Thiobarbituric acid reactive substances (TBARS) and the total volatile basic nitrogen (TVBN).

MATERIAL AND METHODS

Preparation of samples

Whole sirloins were purchased aseptically in sterile polyethylene bags from a local retailer. Excess fat was trimmed with sanitized knife. Sirloin tips were placed on a rack, covered and roasted. Roasted sirloin tips were then cooled to 5 °C in a refrigerator. Roasted beef was sliced with sanitized knife, minced by a sterile glass blender jar, divided into 25 g and placed in sterile Petri dishes and immediately refrigerated *(6)*.

Cultures

Virulent lyophilized *Yersinia enterocolitica* (serovar. O3) were obtained from Department of Serology Unit, Animal Health Research Institute (AHRI), Dokki, Egypt. The organism was activated by inoculation of tryptic soy broth supplemented with 0.6% yeast extract (Difco Detroit, MI, USA) and incubation for 24 h at 25°C. Cells from the broth culture were harvested by centrifugation at 10 000 × g for 10 min at 4 ± 1°C. The cell pellets were washed twice in 0.1 M sterile phosphate buffered saline (PBS) then serial dilutions were made in PBS and triplicate 0.1 ml volumes of the dilutions were surface plated on Yersinia selective agar (CIN agar) with Yersinia antimicrobic supplement. The colonies were counted after incubation at 25°C for 24-84h. The cultures were inoculated in ground beef sample to reach the final inoculums levels 10^5 CFU/g raw ground beef *(7)*.

Treatment

A.Thermal study

The inoculated raw ground beef samples were allotted to three different temperatures (70, 80 and 100 °C) and six different dwell times (10, 20,30,40,50 and 60 min.). Portions (25 g each) of the inoculated grounded beef were separately placed into a sterile blender with approximately 225ml of sterile (PBS) to make dilution 1:10 and blended for 2 min. The mixture was aseptically transferred to a round sterile flask. The flasks containing the samples were secured into a water bath which maintained at the target study temperature. A zero time sample was taken immediately. The sample slurry remained in motion and completely immersed during the heat treatment. Three triplicate samples were removed at designed time (0, 10, 20,30,40,50 and 60 min.) and placed into sterile tube. The samples were rapidly cooled in an ice bath. After cooling, 1ml portion were transferred to 9 ml sterile diluents (PBS) and mixed thoroughly. The resulting 1:10 dilution was further serially diluted *(8)*.

Freezing study

One kg of raw ground beef was purchased and examined for *Yersinia enterocolitica* .First set of grounded beef samples (25 g each) was aseptically and separately challenged by 10^5 CFU of *Y. enterocolitica* serotype O:3 per gram raw grounded beef using automatic pipette and reground to distribute the inoculums . Second set of samples (25 g each) was uninoculated and used as negative controls. Each sample was packaged in a sterile plastic bag and stored at -18 °C for up to 2 months. Triplicate samples were plated immediately (0 time) and then at seven day intervals for 2 months to enumerate *Y. enterocolitica (8)*.

Microbiological analysis

The procedures were applied according to the FDA-Bacteriological Analytical Manual *(9)*. Samples were serially (10-fold) diluted in sterile phosphate buffer (pH 7.0). Subsequently, 1 ml aliquots of each sample were pouring plated in or spread plated onto selective agar media in duplicates. Enrichment procedure was used when no colonies were detected in the lowest dilution by direct plating methods. For the enrichment procedure, a 25 ml sample was enriched in 225 ml of the selective enrichment broth. Enrichment and plating media were incubated at 25°C for 24 h. When growth did not appear on selective plating media, the incubation was prolonged for further 24 or 48 h.

Chemical analysis

Thiobarbituric acid reactive substances (TBARS) were determined by distillation *(10, 11)*. TBARS is a measure of rancidity which corresponds to the development of off-flavors in meat products. The total volatile basic nitrogen (TVBN) and pH were determined *(12)*.

RESULTS

Table 1. Effect of thermal treatment on survival of *Y. enterocolitica* Serovar. O: 3 (10^5 CFU/g) in Raw Ground Beef

Elapsed time (minutes)	Temperature °C					
	50	55	60	65	70	80
Zero	1×10^5	1×10^5	1×10^5	1×10^5	1×10^5	1×10^5
3	1×10^5	8×10^4	33×10^3	3×10^3	2×10^2	0.0
7	1×10^5	8×10^4	12×10^3	7×10^2	1×10^2	0.0
10	1×10^5	7×10^4	6×10^3	5×10^2	0.0	0.0

Table 2. Effect of freezing at -18 °C on changes in biochemical parameters and survival of *Y. enterocolitica* serovar O: 3 (10^5 CFU/g) in raw ground beef

Storage time (week)	pH	TVBN (mg/100g)	TBARS (mg/kg)	Mean log CFU/g±S.E.
0	5.71	14	0.40	1×10^5
1	5.65	14	0.40	1×10^5
2	5.84	19.40	0.14	1×10^5
3	5.80	23.80	1.60	1×10^5
4	5.70	23.80	1.60	97×10^3
5	5.85	19.04	1.20	85×10^3
6	5.47	15.68	1.10	81×10^3
7	5.66	12.60	0.70	77×10^3
8	5.78	19.60	0.12	7×10^4
9	5.61	14.56	0.08	6×10^4
10	5.48	12.56	0.24	5×10^4
11	5.49	12.40	0.34	5×10^3
12	5.87	12.30	0.34	1×10^3
13	5.87	12.30	0.34	2×10^2

TVBN: The total volatile basic nitrogen-TBARS: Thiobarbituric acid reactive substances

DISCUSSION

Thermal treatment of *Y. enterocolitica* O: 3 in raw ground beef:

The thermal treatment of *Y. enterocolitica* O: 3 was determined in raw ground beef inoculated with 10^5 CFU/g *Y. enterocolitica* O: 3 at 50, 55, 60, 65, 70 and 80 °C for periods ranged from 0 to 10 minutes

. Results obtained in Table 1 showed that the resistance at high temperatures is very low, practically at 70 and 80 °C the strain being destroyed in maximum 10 and 3 minutes, respectively. These results are in line with those previously obtained that have studied the effect of temperature on survival of *Yersinia enterocolitica* ***(1, 13)***. The organism was susceptible to heat and is destroyed by pasteurization at 71.8°C for 18 second ***(14)***. However, if the initial level of *Y. enterocolitica* is very high, complete destruction may not occur during pasteurilization. Sublethal injury of *Y. enterocolitica* may occur when the cells are treated at 47 °C for 12-70 min ***(15)***.

Effect of freezing at -18 °C on chemical analysis and *Y. enterocolitica* O: 3 in raw ground beef:

It was obviously that in Table 2 the pH values remained approximately constant during storage period and ranged between 5.47 and 5.87, possibly due to the influence of different events, such as acidification by lactic acid bacteria and production of volatile bases ***(16)***.

TVBN was found to be within the maximum level 20 mg N/100 g minced meat ***(17)***, with the exception of 3rd and 4th week of storage period. The increase of TVBN may originated from a combination of microbial activity and autolytic deamination of amino acids ***(18)***.

TBA reactive substance values had slightly increased, highlighting a peak after 3rd week of storage, which ranged to 1.6 mg malonaldehyde / 100g minced meat, decreased to 0.078 mg malonaldehyde / 100g minced meat on 9th week and stabilized at 0.34 mg malonaldehyde / 100g minced meat from 11th until 13th week. The stabilization of TBA values indicates that malonaldehyde formation had diminished and it might had changed into other compounds. Previous studies had stated that malonaldehyde and other short-chain carbon products of lipid oxidation were not stable for a long time because they are oxidized to organic alcohols and acids, which were not determined by the TBA test ***(19)***. Therefore, the stabilization should be interpreted as a dynamic equilibrium in which malonaldehyde production and its degradation products play a similar role.

Yersinia enterocolitica was subjected to freezing at -18 °C for 13 weeks with initial count of 10^5 CFU/g to determine the susceptibility of the strain to freeze injury. Results obtained in Table 2 showed that *Y. enterocolitica* decreased from 10^5 to 2×10^2CFU/g after 13 weeks of frozen at -18°C. It has been proved that *Yersinia enterocolitica* decreased at temperatures -18 °C after 20 days because *Y. enterocolitica* O:3 is sensitive to freezing more in solid food products compared to liquid food products. Thus, *Y. enterocolitica* was encountered in milk frozen at -18^0C at values of 10^6cells/ml after 20 days, and 10^5cells/ml after 30 days ***(1)***. At –18 °C the number of *Y. enterocolitica* cells is reduced with 2 logarithms in bovine meat and only with one logarithm in pig meat after 20 and 30 days. In poultry, at -18^0C, after 20 and 30 days the bacterial population density reduction was low on the surface of the poultry meat samples (the reduction was of 2 logarithms). The number of *Y. enterocolitica* cells maintained constant or decreased with 1-2 logarithms at -12^0C and -4^0C in analysed meat samples. *Y. enterocolitica* multiplied at refrigeration temperatures (0^0C and 2-4^0C); the multiplication was more rapid on raw bovine meat (2 logarithms) compared to the raw pig meat and raw poultry meat respectively, products for which it was noticed the rise of the cells number with maximum 1 logarithm, or even standing. Most *Y. enterocolitica* cells will be killed or injured when being stored during frozen storage at -20 °C ***(20)***. When ground beef inoculated with *Y. enterocolitica*

was stored at -20C for 30 days, approximately 83% of the inoculated cells were destroyed and 24% of the survivors were sub lethally injured *(15)*. In phosphate buffer, freezing at -18°C for 1 h followed by ambient thawing resulted in 7 % cell inactivation (killing) and 55 % cell injury *(21)*. Although *Y. enterocolitica* can grow at temperatures as low as0°C, the organism grows much more slowly as temperatures drop below 5°C *(22)*. Yersinia withstands freezing and can survive in frozen foods for extended periods even after repeated freezing and thawing *(14)*.

CONCLUSION

1. High temperature, 70 and 80 °C, ensure rapid destroying of *Y. enterocolitica* in maximum 10 and 3 minutes respectively.
2. Freezing temperature (-18 °C) applied for maximum 13 weeks do not destroy *Y. enterocolitica*.
3. *Yersinia enterocolitica* counts were not strictly correlated with chemical changes of treated minced meat samples.

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الملخص العربى

تأثير التسخين والتجميد على معيشة ميكروب اليرسينيا انتيروكوليتيكا فى اللحم البقرى المفروم

حنان مصطفى طه اللاوندى

وحدة صحة الأغذية – معهد بحوث صحة الحيوان – مصر

تم دراسة وجود اليرسينيا انتيروكوليتيكا O:3 فى درجات حرارة مختلفة. ولتحقيق ذلك فقد اجريت الاختبارات على لحم بقرى مفروم ملوث معمليا باليرسينيا انتيروكوليتيكا O:3 مع ملاحظة التغيرات الكيميائية فى اللحم البقرى خلال فترة التخزين فى درجة حرارة التجمد خلال ١٣ اسبوعا وتأثير درجة حرارة التجمد (-١٨) ودراجات الحرارة العالية على الميكروب .

اثبتت الدراسة أن ميكروب اليرسينيا انتيروكوليتيكا O:3 لم يتم تدميرها بالكامل عند درجة حرارة -١٨ درجة مئوية خلال ١٣ اسبوعا من الحفظ فى حين تم تدميرها بالكامل وبشكل سريع عند درجة حرارة ٧٠ و ٨٠ درجة مئوية لمدة ١٠ و٣ دقائق على التوالى.