SURVIVAL OF *E. coli* O157:H7 IN CHINESE-STYLE SAUSAGE DURING THE DRYING PROCESS

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Background

Escherichia coli O157:H7 is a gram-negative, facultative anaerobe which has been identified as the causative agent of hemorrhagic colitis, a condition that manifests itself by severe abdominal cramps and bloody diarrhea (Riley *et al.*, 1983). Since the first recognition of this pathogen in 1982, several outbreaks of hemorrhagic colitis in the United States, Canada, and the United Kingdom have been caused by this organism (Doyle, 1991).

Foods of animal origin seem to be the primary sources of *E. coli* O157:H7 infection. The organism has been isolated from swine and sheep (Doyle and Schoeni, 1987), cattle (Borczyk *et al.*, 1987) and chickens (Beesy *et al.*, 1985). Retail meats from these animal have also reported to harbor *E. coli* O157:H7 (Doyle and Schoeni, 1987).

Pork is the main ingredient of Chinese-style sausage. It is made from ground meat with larger size. During the manufacture of Chinese-style sausage, it is cured and dried. the possible presence of *E. coli* O157:H7 in raw meat used for the manufacture of Chinese-style sausage may present a potential health hazard. Therefore, understanding the fate of this pathogen during the manufacture of Chinese-style sausage is indispensable before appropriate safety measures can be taken.

Objectives

The purpose of this study was to determine the fate of *E. coli* O157:H7 during the drying, a step in the manufacturing process, of Chinese-style sausage. Beside, the effect of curing agent on the survival characteristics of *E. coli* O157:H7 was also identified.

Methods

Preparation of inoculum

Four strains of *E. coli* O157:H7 including 933 (beef isolate), A8993-C32 (human isolate), MF6707 (veal isolate) and 18731A (veal isolate) were used as test organisms in this study. They were all obtained from Food Safety Inspection Service, Department of Agriculture, USA. After two successive activation of the test organism in Tryptic soy broth (TSB) at 37°C for 24-h,a loopful activated culture was then inoculated into 10 ml TSB and incubated at 37°C for 18 hr. Equal volume of cultures of each strain were then combined to serve as the inoculum.

Preparation of sausage

The sausage was prepared according to the recommended procedures (Anonymous, 1984). Fresh pork was obtained from a local processing plant. It was first tested to ensure the absence of *E. coli* O157:H7.

In preparing the sausage, the fat and lean portions of pork were initially ground separately through a chopper fitted with a 1.0 cm plate. Ground meat containing about three parts lean to one part fat were thoroughly mixed with the curing ingredient shown in Table 1 in a mixer for ca. 3 min. After curing at refrigeration temperatures (5-7°C), the sausage batter was inoculated with *E. coli* O157:H7 at a level of ca. 10^6/g then stuffed into edible collagen casing (Naturin-Werk Berker & Co., Germany) and sectioned by hand. Sausages were then dried in a 50°C air-blast drier for 6 hr. In addition, meats without curing agents while inoculated with *E. coli* O157:H7 were also prepared and served as the control samples.

Microbiological analysis as well as pH and temperature determinations

Samples were assayed for the presence of *E. coli* according to the methods described by Okrend *et al.* (1990) and Abdul-Raouf *et al.* (1993). For enumeration of *E. coli* O157:H7, a 25 g meat samples was first homogenized with 225 ml sterile 0.1% peptone water. Serial dilutions were made with Butterfield's phosphate diluent. One tenth of a ml of each dilution was spread on MacConkey sorbitol agar with 0.2 g/l MUG and incubated at 35°C for 24 hr. Typical colonies were picked and confirmed as *E. coli* O157:H7 with biochemical tests of IMViC reaction and lysine decarboxylase production as well as serological test with O157 and H7 antisera (Difco). Aerobic plate counts (APC) were determined by procedures described in the Bacteriological Analytical Manual (Food and Drug Administration, 1984).

The pH of samples were analyzed by blending the samples with equal amount of distilled water and determine the pH with a digital pH meter. Temperature of the sausage during drying was recorded with a temperature recorder (UR180 recorder, Yokogawa Electronic Co., Japan).

Results and Discussion

When sausages were subjected to 50°C air-blasting drying, the temperature of sausage rose quickly to ca 45°C after 2 hr of drying and then increased rather slowly reaching ca. 48 °C at the end of 6-hr drying period. Changes of *E. coli* O157:H7 count, APC and pH in samples with or without curing agents during the drying period are shown in Figs. 1, 2 and 3, respectively. A slight increase of *E. coli* O157:H7 count and APC in samples without curing agents was noted during the dry period. This phenomenon is in accordance with that reported by Doyle and Schoeni (1984) who indicated that *E. coli* grew rapidly at 37-42°C and still can grew slowly at 44-45°C. The 50°C air-blasting condition did not raise the internal temperature of sausage to such extent that will cause the lethal effect on the inoculated *E. coli* O157:H7 during the drying period. Contrary to that observed in sample without curing agent, in a sausage containing

curing agents, *E. coli* O157:H7 count and APC decreased steadily as the drying period extended. For example the count of *E. coli* O157:H7 decreased from ca. 6.0×10^6/g at the beginning to ca 4.8×10^4 at the end of 6-hr during period. It is also interesting to found that *E. coli* O157:H7 in sausages is less susceptible to the drying process than *Campylobacter jejuni*. Under similar test condition, Yeh and Chou (1994) indicated that *C. jejuni* in Chinese-style sausage with an initial population of ca. 10^7 CFU/ml declined drastically during the similar drying condition and reduced to none after 6 hr of drying, regardless the presence of curing agents.

For comparison, the growth and survival of *E. coli* O157:H7 in TSB with or without curing agent were examined when they were incubated at 50°C. Under the test condition, it was found that the temperature of medium increased to 50°C in 10-12 min after the medium containing flasks were kept in the incubator. Reduction in the viable cells of *E. coli* O157:H7 was noted in both media with or without curing agent (Fig. 4). However the death rate of *E. coli* O157:H7 in medium containing curing agent is much higher than that in control medium which contained no curing agent or in sausage subject to 50°C air-blast drying as shown in Fig. 1. After 4 hr of incubation, no viable *E. coli* was detected in medium containing curing agent although it contained ca. 10^6/ml *E. coli* O157:H7 at the beginning. On the other hand, viable *E. coli* O157:H7 population of ca. 10^4/ml was detected in medium without curing agent after 6 hr of incubation (Fig 4). In addition, pH of sausage did not change significantly during the entire drying period.

Conclusions

Based on data collected in present study, we concluded that it is essentially the curing agent but not the temperature that caused the reduction of viable *E. coli* O157:H7 during the drying process of Chinese-style sausage. Under the drying condition tested, a reduction of only ca. 1.5 log CFU *E. coli* O157:H7 was noted in the sausage. If substantial amount of *E. coli* O157:H7 present in the sausage because of contaminated meat material, a large portion of this pathogen can survive the drying process and thus present a potential hazard to the consumer.

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Table 1. Curing agents for sausage.

Ingredients	g/100g meat
Sucrose	10.000
Salt	2.000
Monosodium glutamate	1.000
Soybean protein isolate	0.500
Sodium tripolyphosphate	0.200
Potassium sorbate	0.250
Sodium ascorbate	0.050
Sodium nitrite	0.012
Rice wine	1.000
Spices	
Wu-Shung powder	0.210
White pepper powder	0.125
Vanilla powder	0.125
Cinnamon powder	0.083

(Anonymous, 1984)

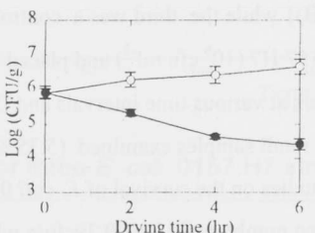

Fig. 1. Changes of *E. coli* O157:H7 count in sausage during drying period. ● ,meat with curing agents; ○ ,meat without curing agents.

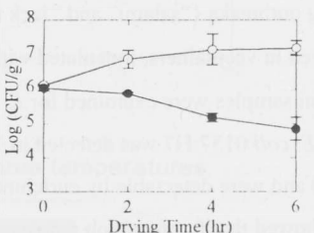

Fig. 2. Changes of APC in sausage during drying period. ● ,meat with curing agents; ○ ,meat without curing agents.

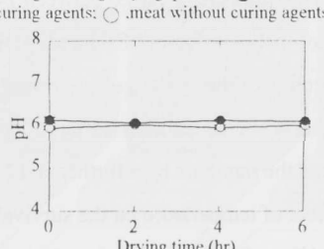

Fig. 3. Changes of pH in sausage during drying period. ● ,meat with curing agents; ○ ,meat without curing agents.

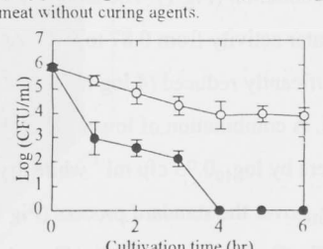

Fig. 4. Effect of curing agents on the survival of *E. coli* O157:H7 in tryptic soy broth incubated at 50 ℃. ● ,meat with curing agents; ○ ,meat without curing agents.