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Reduction of norovirus in meat products

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Introduction

Norovirus is one of the major causes of human gastrointestinal disease. The classical route of infection is fecal – oral, and in case of poor hygiene, foods can be contaminated. Foodborne outbreaks of norovirus are usually transmitted through contaminated water, shellfish, soft berries, salads and other vegetables. Only in rare cases, meat products have been involved in outbreaks, but the risk for contaminating "ready-to-eat" meat products by infected workers surely exist.

Objective

The objective of the study was to investigate the heat inactivation of noroviruses on frankfurters inoculated with noroviruses before heat treatment, either on the surface of the sausage or into the meat batter before stuffing.

Methods

VIRUSES
Norovirus Geno Group II were obtained from a human stool sample. Dilutions of the viruses were made in Peptone Saline Diluent (PSD) (0.85 % NaCl with 0.1 % Peptone). For heat treatment experiments in PSD, an initial concentration of approx. 10^7 viruses/ml (estimated from RT-PCR Ct values) was used. The surfaces of frankfurters were inoculated by immersing into the norovirus suspension for 30 minutes. The frankfurters were allowed to dry before heat treatment. For the pilot plant testing raw frankfurters were inoculated with approx. 5,000 viruses (3.7 log)/cm^2 on the surface, or approx. 1,000 viruses (3.0 log)/g to the meat batter.

RECOVERY OF VIRUSES FROM SURFACE OF FRANKFURTERS
Viruses were recovered from the surface samples by vigorous mixing of 1 cm^2 of the casing in 5 ml PSD (IKA minishaker MS2, 2100 rev. x $min.^{-1}$) for 1 minute followed by 5 minutes rest and repeated mixing for 1 min. The casing was removed and the liquid sample was centrifuged for 15 min. at 3,000 g. 100 µl of the supernatant was used for RNAse treatment.

RECOVERY OF VIRUSES FROM THE CENTER OF FRANKFURTERS
Viruses from the center of frankfurters were recovered by stomaching 3 g of sausage in 4 ml of RLT lysis buffer (Qiagen) added 1 % 14.3 M 2-mercaptoethanol for 1 minute at normal speed in a Seward 80 stomacher. Then 3 ml of the RLT/2-mercaptoethanol buffer was added and the mixture stomached for 1 minute at high speed. After 1 minutes rest, this step was repeated twice. 1.5 to 2.0 ml of the mixture was centrifuged at 10,000 g for 20 minutes to separate fat and meat particles and 800 µl of the aqueous phase was centrifuged at 10,000 g for 20 minutes. 100 µl of the aqueous phase was used for RNAse treatment.

RNASE TREATMENT
The 100 µl samples were added 1 µl Promega RNAse One and 11 µl 10x RNAse reaction buffer. This mixture was incubated for 15 minutes at 37 °C. 28 µl of PSD were added to a total volume of 140 µl. The RNAse treatment was used to eliminate RNA from virus with heat damaged capsid (*Topping et al.* 2011, J. Virological Methods 156: 89-95).

RNA EXTRACTION
RNA from the 140 ml RNAse treated samples were purified by QIAmp Viral RNA Mini Kit (Qiagen) according to the manufacturers manual.

REVERSE TRANSCRIPTASE PCR
The amount of noroviruses in the samples was analyzed with a real time reverse transcriptase PCR on a Stratagene Mx P3005 PCR machine, using Stratagene Brilliant II QRT-PCR 1-step Master Mix Kit. The primers, probes and internal amplification control RNA (IAC) and the PCR procedure is described in the SOP Vital 018 from the EU Vital project (www.eurovital.org). The number of viruses was calculated from the number of PCR cycles needed for the amplification curve to exceed the baseline (Ct). Optimal amplification was assumed and 1 log reduction (10 x) is accordingly equal to a 3.32 higher Ct value.

Results

Laboratory scale testing
The reduction of norovirus GG II in PSD after heat treatment for 5 minutes at different temperatures or at 80°C for different time intervals is shown in Figure 1 and 2 respectively.

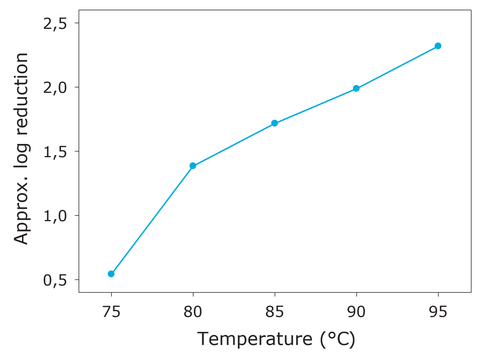

FIGURE 1. Noroviruses GG II suspended in peptone/saline diluent heated for 5 minutes at different temperatures.

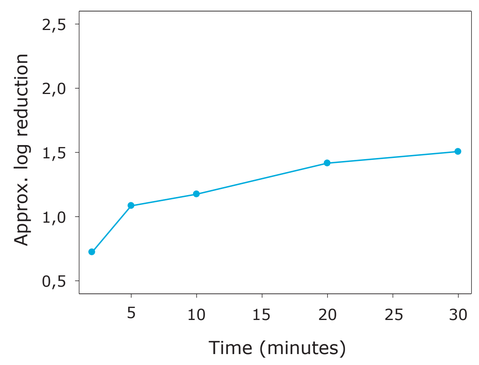

FIGURE 2. Noroviruses GG II suspended i peptone/saline diluent heated to 80 °C for 2, 5, 10, 20 or 30 minutes.

When the noroviruses were inoculated on the surface of frankfurters and heated to 80°C for 10 or 20 minutes an almost similar reduction of noroviruses was obtained on frankfurters as in PSD. After 10 min. the reduction was 1.2 log in PSD and 1.3 log on the frankfurters, and after 20 min. the reduction was 1.4 log and 1.4 log respectively. After 30 min. heat treatment at 80°C noroviruses was only detected in 2 out of 6 frankfurters, although the inoculated frankfurters (controls) all had a Ct value around 34 corresponding to approx. 2,000 viruses (3.3 log)/cm^2.

Pilot Plant testing
Four sausages were not heat-treated and used as inoculation controls. Eight sausages were treated with a traditional heat/smoke-treatment: Warming at 50°C for 15 min., drying at 60 °C for 20 min., smoke at 60°C for 13 min., cooking at 78°C for 20 min. and drying at 50°C for 2 min. No viruses were detected on the heat/smoke treated frankfurters. The IAC was amplified indicating that the heat/smoke treatment did not inhibit the RT-PCR (Table 1).

In frankfurters inoculated with noroviruses in the meat batter before stuffing and heat/smoke-treatment, viruses was still detectable after the heat/smoke- treatment. Even a heat treatment to a core temperature of 85 °C did not eliminate the noroviruses. The noroviruses may be protected by the proteins or fat in the frankfurters (Table 1).

Inoculation	Core				Surface
Temperature of frankfurter	58 °C	68 °C	75 °C	85 °C	Heat/smoke (78 °C)
Ct value	36.1	37.5	33.7	35.3	No Ct

TABLE 1. Average Ct values(n=8) from reverse transcriptase real time PCR of samples from core or surface inoculated frankfurters which were heated to different temperatures. The difference in Ct values at the different temperatures is probably caused by insufficient recovery of noroviruses from the coagulated meat/fat matrix.

Conclusion

A standard heat/smoke process for frankfurters eliminated noroviruses on the surface. However, when the noroviruses were inoculated into the meat batter, the noroviruses was detected in the center of the frankfurter, even after heat treatment to a core temperature of 85 °C.