Hepatitis E virus is inactivated in liver pâté during heat treatment

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Introduction: Hepatitis E is a liver disease caused by infection with the virus hepatitis E virus (HEV). WHO estimates, that 20 million HEV infections are seen every year worldwide (WHO, 2020), primarily due to poor sanitation and lack of clean drinking water.

However, during the last 10 years, more than 21,000 infections caused by HEV have been registered in the EU. EFSA is concerned, since the number of infections is increasing, and because food is the primary route of HEV infections in the EU (EFSA, 2017a).

HEV can be found in liver from healthy pigs. Liver pâté is a meat spread popular in Northern and Eastern Europe. To which extent HEV is inactivated during heat treatment e.g., of liver pâtés has so far been uncertain. In the literature there is a huge discrepancy between the reported data for HEV inactivation during heat treatment. The aim of this study was to investigate the heat inactivation of HEV in Danish style liver pâté.

Materials and method:

Heat inactivation of MS2-bacteriophages

As cell culture assays to determine HEV infectivity have high detection limits and low reproducibility (EFSA, 2017b), the MS2-bacteriophage (ATCC® 15597-B1TM) was used as surrogate for HEV to estimate the number of infective virus particles by cell assay in the study.

Emulsion samples (liver/lard) of 2 g. was inoculated with MS2-phages, packed in thin PE bags and placed in water baths at 68°C, 70°C and 72°C, respectively. The emulsion was pressed as thin as possible in the bag before heating. During heating, samples were collected and placed on ice at specific times. The number of infective MS2-phages after heat treatment was determined by a plaque assay. The total amount of phage RNA after heat treatment was quantified by reverse transcriptase qPCR (RT-qPCR).

Heat inactivation of HEV

Heat treatment was conducted as described for MS2. RNA was extracted using the NucliSens Magnetic Extraction kit (bioMérieux, Marcy l'Etoile, France) and RT-qPCR was performed using the PrecisionPLUS Onestep RT-qPCR Master mix (Primerdesign Ltd, Camberley, United Kingdom) with previously published probe and primers (Dreier et al., 2005).

Results:

Heat inactivation of MS2-phages

The D-value (time to obtain a 1 log reduction) estimated at 68°C for MS2 is comparable to the D-values for HEV measured by Barnaud et al. (2012). The D-values for both studies are 2-4 minutes.

Heat inactivation of HEV

The D-values estimated from the results obtained in liver pâté in this study (7 min at 68°C and 1.4 min at 70°C) are in the same range as what can be calculated from other reports in the scientific literature. The D-values in this study are based on HEV RNA reduction, which is slower than the reduction seen in infective HEV particles, and therefore may be considered as a worst-case scenario.

Conclusions: Based on the heat inactivation studies, D-values could be estimated for both MS2 and HEV. According to the estimated D-values, a 6-log reduction of HEV is obtained after heating at 68°C for 42 minutes; at 70°C for 8.4 minutes and at 72°C for 7.2 minutes. Based on the D-values and an estimation of the concentration of HEV in a highly contaminated liver, the overall conclusion is, that liver pâté is a safe product when a 6-log reduction is obtained.

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Literature:

Barnaud E., Rogée S., Garry P, Rose N., & N. Pavio. 2012. Thermal inactivation of infectious hepatitis E virus in experimentally contaminated food. App Environ Microbiol 78(15):5153-9

Dreider J., Störmer M, & K. Kleesiek. 2005. Use of bacteriophage MS2 as an internal control in viral reverse transcription-PCR assays. J Clin Microbial 43(9):4551-7

EFSA (2017a). Hepatitis E: raw pork is main cause of infection in EU

EFSA (2017b). Public health risks associated with hepatitis E virus (HEV) as a food-borne pathogen, EFSA J doi: 10.2903/j.efsa.2017.4886

WHO (2020). Hepatitis E