DETECTION OF TISSUES OF CENTRAL NERVOUS SYSTEM IN CANNED MEAT PRODUCTS WITH THE RIDASCREEN RISK MATERIAL-TEST

Stahnke, Christian, Weber, Herbert

University of Applied Sciences, Faculty of Food Technology, Luxemburger Str. 10, D-13353 Berlin, Germany

Background

British authorities announced on March 20, 1996 that a connection between BSE and the new variant of the Creuzfeldt Jakob disease (CJI) could not be excluded. The responsible prions could be contained in the proteins of risk material and trigger illness in humans with the new variant of the CJI. Risk materials are brain, spinal cord, tonsils and skulls with eyes of cattle, sheep and goats over 12 months old, the spleen of sheep and goats of all age groups, and as of January 1, 2001 the entire intestine of cattle of all age groups as well. The European Union adopted preventive measures in response to the current level of scientific knowledge. These include the prohibition of using meat and bone meal in animal fodder (2000/766/EG) and the prohibition of processing specific risk material in foods (2000/418/EG).

The RIDASCREEN® Risk material from R-Biopharm was examined for its applicability as an early recognition test for risk material in foods. It is a sandwich-enzyme immunoassay for the semiquantitative analysis of risk material in meat and meat products, which rapidly and sensitively determines the presence of glial fibrillary acidic protein (GFAP) in spinal cord and brain by means of ELISA testing. Thus risk can be demonstrated by detecting the presence of brain and spinal cord (CNS risk material) of cattle, calf, sheep, goat or pig in meat products. However, whereas the incidence of brain and spinal cord substance in the meat and meat products can be detected, no statement can be made about the origin of BSE [2, 3].

Objectives

The aim of this study was to determine the suitability of the RIDASCREEN Risk Material Test in canned meat products.

Methods

The basis of the test is the antigen-antibody reaction. Risk material is detected by the determination of glial fibrillary acid protein (GFAP), a cellular marker, which can be found in very high concentrations in CNS tissue. The wells of the microtiter strips are coated with specific antibodies against GFAP. If the sample is contaminated with risk material, the glial fibrillary acidic proteins will bind to the specific capture antibodies. The bound GFAP is detected by an antibody peroxidase-conjugate directed against GFAP (enzyme conjugate). Any unbound enzyme conjugate is then removed in a washing step. Chromogen/substrate is added to the wells, bound enzyme conjugate converts the stained red chromogen into a blue product. The addition of the stop reagent leads to a color change from blue to yellow. The measurement is made photometrically at 450nm. The absorbance is proportional to the concentration of risk material in the sample [1].

The applicability was checked with differently treated pork sausage. Cattle brain was added to one portion of the pork sausage, and cattle spinal cord to the other, in different proportions. These portions were then processed as preserves heated to F-values of 10 and as preserves to be kept under refrigeration, in order to assess and clarify the effects of different manufacturing processes and storage conditions on the usefulness of the test. Preserves heated to F-values of 10 were freshly produced and autoclaved, however at 2 different temperatures and times. There were two groups of preserves to be kept under refrigeration: one freshly manufactured, and one exceeding the expiration date (expiration of minimum shelf life) [2].

Results and Discussion

The RIDASCREEN® Risk test determined that all the positive samples of preserves heated to F-values of 10 by sterilization at 121 °C for 10 min also yielded positive results. In this case the test is very well suited for detecting CNS in meat and meat products. With a longer sterilization lasting for hours at a temperature of 110 °C (same F-value of 10), the defined added quantities of risk material could not be detected with each sample. This can be attributed to the denaturation of the proteins and therefore also the glial fibrillary acidic protein. In order that the Ridascreen® Risk-material test can be effectively applied for reliable evaluations, care must be taken that preserves heated to F-values of 10 are produced and sterilized at certain temperatures and times. Here sterilizations with high temperatures and short times are better suited for CNS detection than sterilizations at lower temperatures and times.

In the analysis of preserves to be kept under refrigeration, the added quantities of risk material could be detected after pasteurization. Furthermore it was determined that with products exceeding their expiration date, the demonstrability of the existing CNS was lowered, although slight quantities (0.2% cattle brain) could still be detected. The tests of preserves heated to an F-value of 10 and preserves to be kept under refrigeration demonstrated that spinal cord substance can be far more easily detected than cattle brain. This is due to the higher proportion of glial fibrillary acidic protein found in spinal cord tissue. The Ridascreen® Risk material-test can be applied for analysis and determination of the incidence of risk material. It is suited for use in preserves to be kept under refrigeration and very well suited for preserves heated to F-values of 10.

Conclusion

Applicability:	For all meat products; very good at higher temperatures and shorter times; only conditionally at low temperatures and longer time
Demonstrability:	Very good when cattle spinal cord is added; good with the addition of cattle brain; very good with freshly manufactured samples; only conditionally with samples exceeding their expiration date; only a quantitative statement possible concerning the origin of the CNS

References

[1] Instructions for use, Ridascreen® Risk Material Test

[2] *Diplom* thesis by Christian Stahnke / TFH Berlin, Germany, Mai 2002

[3] Schmidt G.R.; Hossner, K.L.; Yemm, R.S.; Gould, D.H.; Callaghan J.P.: An Enzyme-linked Immunosorbent Assay for Glial Fibrillary Acidic Protein as an Indicator of the Presence of Brain or Spinal Cord in Meat. J. Food Protect. 62, 394-397 (1988)

Results

Table 1 Overview of the measured values and results of preserves heated to F-values of 10 at 121 °C / 50min

Sample number	Added portion of risk material	Determined portion of risk material after sterilization	Recovery rate
A	0.5 % cattle brain	0.20%	40 %
B	0.5 % spinal cord	0.70%	140 %
C	1.0 % cattle brain	0.30%	30 %
D	1.0 % spinal cord	2.00%	200 %
E	2.0 % cattle brain	0.70%	35 %
F	2.0 % spinal cord	> 2.0 %	> 100 %
G	0.0 % no CNS	0.00%	100 %

Table 2 Overview of the measured values and results of preserves heated to F-values of 10 at 110 °C / 275min

Sample number	Added portion of risk material	Determined portion of risk material after sterilization	Recovery rate
AX	0.5 % cattle brain	0.00 %	0 %
BX	0.5 % spinal cord	0.10 %	20 %
CX	1.0 % cattle brain	0.00 %	0 %
DX	1.0 % spinal cord	0.15 %	15 %
EX	2.0 % cattle brain	0.05 %	2.50 %
FX	2.0 % spinal cord	0.21 %	10.50 %
GX	0.0 % no CNS	0.00 %	100 %

Table 3 Overview of the measured values and results of preserves to be kept under refrigeration, exceeding expiration date

Sample number	Added portion of risk material	Determined portion of risk material after pasteurization	Recovery rate
0.10%	0.1 % spinal cord	0.23%	230%
0.20%	0.2 % cattle brain	0.10%	50%
1.00%	1.0 % cattle brain	0.35%	35%
2.00%	2.0 % cattle brain	0.50%	25%
0.00%	0.0 % no CNS	0.00%	100%

Table 4 Overview of the measured values and results of freshly manufactured preserves to be kept under refrigeration

Sample number	Added portion of risk material	Determined portion of risk material after pasteurization	Recovery rate
A	0.1 % spinal cord	0.58%	580%
B	0.2 % spinal cord	1.10%	550%
C	0.0 % no CNS	0.00%	100%
E	0.5 % cattle brain	0.65%	130%
F	0.2 % cattle brain	0.27%	135%