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Detection of central nervous system tissue in meat and meat products with a newly developed immunoassay selective for Myelin proteolipid protein

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Abstract

Bovine spongiform encephalopathy (BSE) is most likely transmitted by the consumption of central nervous system (CNS) tissue of infected animals. In this study, an immunochemical assay for CNS in meat and meat products was developed using an antibody against Myelin proteolipid protein (PLP), which is very specifically expressed in the CNS. Solvent extraction of CNS-contaminated meat yielded a highly enriched PLP fraction. Subsequent Western blot analysis specifically detected the PLP band at 29 kDa. The detection limit for unprocessed CNS in raw meat was less than 0.025% and the quantification limit was calculated to be 0.049%. The PLP epitope was relatively stable during storage at 5 °C or –21 °C and during heating at 75 °C and 95 °C. Amounts of 0.1% CNS could be reliably detected in cooked bologna type sausage, cooked liver sausage and fermented sausage. Thus, the new assay allows highly specific and sensitive determination of CNS contaminations in meat and meat products.

Keywords: Bovine spongiform encephalopathy; Central nervous system; Myelin proteolipid protein; Solvent extraction; Western blot

1. Introduction

Variant Creutzfeldt-Jakob disease (vCJD) in humans is most likely transmitted by consumption of infectious tissues from animals with transmissible spongiform encephalopathy (TSE) (Hill et al., 1997; Will et al., 1996). Cattle, infected by bovine spongiform encephalopathy (BSE), are most relevant for human nutrition in this regard (Dormont, 2002a). The infectious agent, most likely the pathological isoform of the prion protein (PrP^{sc}) (Collins, Lawson, & Masters, 2004) is mainly located in the central nervous system (CNS) of infected animals (Dormont, 2002b). As a consequence, the presence of CNS tissue in beef and beef products is banned in many countries, such as USA, Canada, the countries of the EC, or Switzerland[1]. In some

Abbreviations: vCJD, variant Creutzfeld-Jakob disease; CNS, central nervous system; TSE, transmissible spongiform encephalopathy; BSE, bovine spongiform encephalopathy; PrP^{sc}, prion protein; GFAP, glial fibrillary acidic protein; NSE, neuron specific enolase; PLP, proteolipid protein; SDS–PAGE, sodium dodecyl sulphate–polyacrylamide gel electrophoresis; TBS, tris buffered saline.

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[1] Federal Register/Vol. 69, No. 7, January 12, 2004; Meat Hygiene Directive 2003-18 (Amended), July 24 2003; annex XI of regulation (EC) No. 999/2001 in connection with regulation (EC) No. 270/2002; Art 181 Tierseuchenverordnung, SR 916.401.

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countries, such as Germany, the use of CNS tissue in meat products is generally not accepted (Heits, 2003).

In the last years, various assays to detect CNS tissues in meat products have been developed. Determination of cholesterol concentrations was suggested as a screening method to test the presence of brain in meat products. However, this assay shows low sensitivity and low specificity, because other legal food ingredients, such as yolk or liver, also contain high cholesterol concentrations (Lücker, Eigenbrodt, Wenisch, Leiser, & Bülte, 2000). Niederer and Bollhalder developed an alternative approach, which is based on the fatty acid profile, analyzed by gas-chromatography–mass spectrometry (Niederer & Bollhalder, 2001; Noti, Biedermann-Brem, Biedermann, & Grob, 2002). Immunochemical assays use in principle glial fibrillary acidic protein (GFAP) (Schmidt, Hossner, Yemm, Gould, & O'Callaghan, 1999) and neuron-specific enolase (NSE) (Lücker et al., 1999) as CNS specific target proteins. Furthermore, it was suggested that the assessment of GFAP mRNA by reverse transcription-polymerase chain reaction could be used to detect CNS contaminations in meat (Nowak, Mueffling, Kuefen, Ganseforth, & Seyboldt, 2005).

Recently, proteolipid protein (PLP) was introduced as a highly specific marker protein for CNS tissues (Sandmeier et al., 2006). PLP is the most abundant Myelin protein of the CNS accounting for 50–55% of the Myelin proteins (Eng, Chao, Gerstl, Pratt, & Tavaststjerna, 1968; Lees & Brostoff, 1984). Due to its hydrophobicity, it can be easily extracted from brain by extraction with organic solvents (Folch, 1942).

The aim of this study was to develop a new Western blot assay with improved specificity compared to the established methods and improved sensitivity for raw and processed meat. For that purpose, a polyclonal antiserum against PLP was used.

2. Materials and methods

2.1. Chemicals and technical equipment

All solvents (Fisher scientific, Schwerte, Germany) and chemicals (Fluka, Buchs, Switzerland) were of analytical reagent grade quality. Deionized water for buffer solutions was distilled before use. Anti-PLP rabbit serum was provided by the Institute of Biochemistry, Emil-Fischer-Centre, University of Erlangen, Germany, commercially available monoclonal mouse anti-PLP antibodies were purchased from Biotrend, Köln, Germany. The CY5-conjugated monoclonal goat-anti-rabbit IgG and goat-anti-mouse IgG antibodies were obtained from Dianova, Hamburg, Germany. The low molecular mass marker used in all experiments was purchased from Sigma, Taufkirchen, Germany, and culture media from Merck VWR, Darmstadt, Germany.

Homogenisation of sample material was carried out with a Waring blender (Waring Laboratory, Torrington, USA) or a T18 basic Ultra Turrax (Janke & Kunkel, Staufen, Germany). Electrophoretical separation of proteins was performed in a PROTEAN 3 minicell (BioRad, Hercules, USA); the blotting device was obtained from Biometra, Göttingen, Germany. For the fluorescence measurement of Western blots a STORM 860 Phosphoimager (Molecular Dynamics, Sunnyvale, USA) was used.

2.2. Samples and sample treatment

Minced porcine meat, mixtures of minced porcine and bovine meat (1:1) and minced lamb were purchased from local butcher shops. All other sample tissues and sausages were provided by the Federal Research Centre for Nutrition and Food, Kulmbach, Germany (BFEL). Samples containing different amounts of central nervous tissue were thoroughly blended in a Waring blender.

For storage experiments, 0.6% bovine spinal cord tissue was added to a mixture of minced pork and beef and thoroughly blended. Samples from this mixture were stored for different time periods at 5 °C in a refrigerator or at −21 °C in a freezer.

Heating experiments were also performed with mixed minced meat (pork and beef) samples containing 3.5% bovine spinal cord tissue. Samples of the thoroughly blended mixtures were kept at 75 °C or 95 °C in a water bath for different time intervals.

Three different types of standardized sausages were produced at the Institute of Microbiology and Toxicology at the BFEL according to the German guidelines for the preparation of meat products (Heits, 2003): a cooked bologna type sausage ("Schinkenwurst"), a cooked liver sausage ("Leberwurst"), and a fermented sausage ("Teewurst"). Several different batches of each sausage type were prepared containing 0, 0.1, 0.3, 0.5, and 2% bovine spinal cord, which was added prior to processing. Two batches of cooked bologna type sausage and cooked liver sausage and three batches of the fermented sausage were prepared for each concentration. Furthermore, a cooked bologna type sausage containing 10% porcine brain was prepared.

For the production of "Schinkenwurst", cooled raw materials were processed to a meat emulsion, filled in cans and the sealed cans were heated in a cooker for 30 min at 75 °C. The ingredients of "Leberwurst" were heated at 90 °C and then processed to a meat emulsion, which was filled in cans. The sealed cans were again heated at 80 °C for 1 h. The raw materials of "Teewurst" were processed in a cutter without heating and filled in artificial casings. The sausages were stored for 4 d in a cooling chamber at 14 °C and 85% humidity.

2.3. Inoculation of sausage samples

Different species of Lactobacilli (*Weissella brevis, Weissella viridescens* and *Leuconostoc mesenteroides*), Enterobacteriaceae (*Enterobacter* spp., *Proteus vulgaris*, *Serratia marcescens* and *Hafnia* spp.), and mould (*Penicillium nordicum*) from the culture collection of the BFEL were used for

inoculation of cooked bologna type sausage. Lactobacilli and Enterobacteriaceae grew overnight (14–16 h) at 30 °C in MRS broth (Merck, Darmstadt, Germany) or standard I broth (Merck, Darmstadt, Germany), respectively. In addition, a spore suspension was prepared with *P. nordicum* and 0.1% peptone water. Slices of cooked bologna type sausage containing 2% bovine spinal cord and slices of sausage without CNS tissue as negative control were inoculated with the overnight cultures (10^6–10^8 bacteria/mL) and the spore suspension (approx. 10^8 spores/mL). The samples were incubated for 3 d at 30 °C or 7 d at 25 °C. Some slices of sausage were inoculated with a mixture of all the above described microbes and incubated for 7 d at 30 °C. After incubation, samples were frozen and kept at −21 °C until extraction.

2.4. PLP extraction

A quantity of 2.5–5 g of the homogenized sample material was extracted three times with at least 4 mL *n*-hexane per gram of sample material by repeated blending (Ultra-Turrax) and mixing. The suspensions were filtered and the filtrates unified. The solvent was removed using a rotary evaporator at 80–100 mbar. The temperature of the water bath did not exceed 30 °C. In order to remove lipids, the residue was resuspended in 30 mL diethyl ether and centrifuged at 3500 rpm for 10 min at 8 °C. The supernatant was discarded. The removal of the lipids was repeated once.

The resulting off-white pellet was dried and stored at −21°C.

2.5. SDS–PAGE and Western blotting

For sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS–PAGE) and Western blotting, 5 μg/μL of the PLP extract was dissolved in Laemmli sample buffer (Laemmli, 1970). After a denaturation step in a water bath at 75 °C for 25 min, electrophoresis was performed with 15% polyacrylamide gel at 140 V for 75 min in SDS–PAGE buffer containing 25 mM tris-(hydroxymethyl)-amino methane (Tris Base), 200 mM glycine and 0.1% SDS. Proteins were transferred to a nitrocellulose membrane (Protran BA 85, Schleicher & Schuell, Dassel, Germany) by electro blotting at 150 mA for 45 min using 25 mM Tris Base and 150 mM glycine in 10% methanol as transfer buffer.

To verify successful protein transfer, the membrane was stained with Ponceau solution (0.2% Ponceau S in 3% trichloroacetic acid) and washed with TBS (tris buffered saline; 50 mM Tris Base and 150 mM NaCl, adjusted to pH 7.4 with 35% HCl) in order to remove the dye. Afterwards, the blot was incubated with blocking solution (TBS pH 8.0 containing 5% skim milk powder and 0.5% Triton X-100) for 45 min. For specific staining of PLP, the membrane was incubated in polyclonal anti-PLP rabbit serum diluted 1:250 in dilution buffer for 18 h and afterwards washed again with TBS. The incubation with diluted secondary antibody (1:500) was performed for 2 h. Antibody dilution may vary, using different batches of polyclonal antibody. After another washing step (TBS), the fluorescence on the membrane was read.

All antibodies and antisera were diluted in TBS pH 8.0 containing 3% bovine serum albumin and 0.5% Triton X-100.

Western blot analysis with the commercially available monoclonal anti-PLP-antibody was performed the same way, except the dilution of the primary antibody, which was 1:100 instead of 1:250.

As a negative control, a duplicate of each Western blot was treated identically, with the exception that in the first incubation step pure dilution buffer was used instead of the PLP-antibody. In some cases, proteins on the polyacrylamide gels were stained, using a silver stain kit ("silver stain plus" BioRad, Hercules, USA) according to the manufacturer's instructions.

All experiments were performed at least in duplicate [$n = 2$ (Fig. 1, Figs. 2B, 4B, 5, 6B), $n = 3$ (Fig. 3), $n = 4$ (Fig. 2A), $n = 5$ (Fig. 4), and $n > 10$ (Fig. 6A)].

2.6. Evaluation and quantification of Western blot readouts

The PLP bands in Western blots were quantified using the ImageQuant 5.0 Software (Molecular Dynamics, Sunnyvale, USA). At a height corresponding to 29 kDa, a rectangular area in the size of the band was defined in every lane and the average fluorescence intensity of these areas was calculated (λ_{exc} 635 nm; λ_{em} 650 nm long path). For background correction, the fluorescence value of the negative control was subtracted.

The detection limit and quantification limit was calculated according to the ACS guidelines, which recommend that the detection limit is located at least 3σ and the quantification limit at least 10σ above the gross blank signal (ACS Committee on Environmental Improvement, 1980). Similar recommendations are given by the IUPAC (Thompson, Ellison, & Wood, 2002) and the EU (see Decision 2002/657/EG) (EC, 2002). In the latter document, the detection limit is called CCalfa and the quantification limit CCbeta. Whereas ACS does not give recommendations on the number of repetitions to determine the value and SD of the

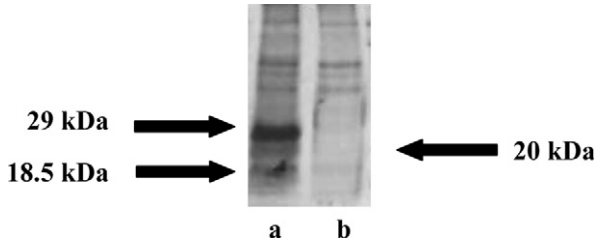

Fig. 1. Specific enrichment of PLP by solvent extraction. Samples were extracted with *n*-hexane and run on a 12% PAA-gel by electrophoresis. Protein bands were visualized by silver staining. (a) Minced porcine muscle containing 10% porcine brain tissue, (b) minced porcine muscle.

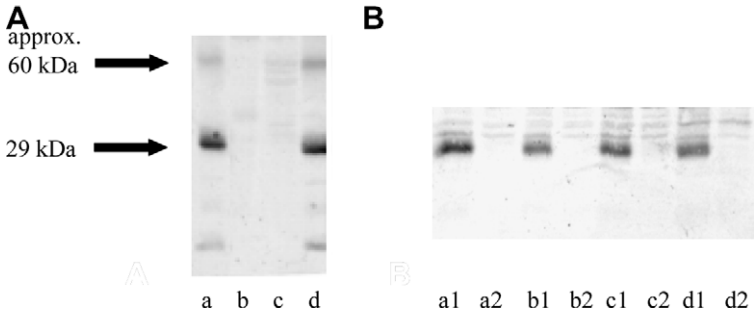

Fig. 2. Western blot analysis of a PLP extract. Samples were extracted with *n*-hexane, run on a 15% PAA-gel and blotted on a nitrocellulose membrane. PLP was detected by incubation with a polyclonal rabbit-anti-PLP-antiserum and a CY5-labelled fluorescent secondary antibody. Panel A, Tissue specific expression of PLP (a) porcine brain tissue, (b) bovine peripheral nerve, (c) minced meat (pork and beef 1:1), (d) bovine spinal cord. Panel B, Detection of 0.05% bovine spinal cord in different meat matrices (a1) pork, (b1) beef, (c1) pork and beef 1:1, (d1) lamb; the lanes a2, b2, c2 and d2 show the corresponding CNS-free meat matrices.

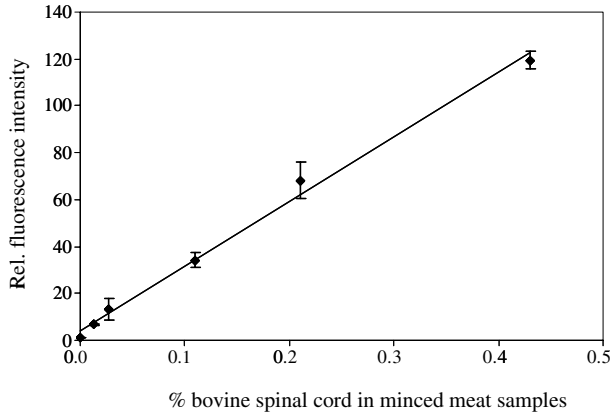

Fig. 3. Calibration curve of the PLP assay. Samples of minced porcine and bovine muscle containing different amounts of bovine spinal cord tissue were extracted with *n*-hexane and analyzed by Western blot. Polyclonal PLP antiserum was used as primary antibody, and a CY5 conjugate as a secondary antibody. $Y = 274.8x + 4.30$; $R^2 = 0.9942$. The mean of three independent standard curves ± SD is shown.

blank, IUPAC recommends six and the EU twenty repetitions. Thus, the following method was applied to determine the detection and quantification limits:

Twelve blank values (samples of porcine peripheral nerves or skeletal muscle) were prepared and analyzed by Western blot, and the fluorescence was measured as described before. The detection limit was calculated as $y_{blank} + 3.3 \times SD_{blank}$ and the quantification limit as $y_{blank} + 10 \times SD_{blank}$.

With this definition of the detection limit, the probability of a false negative result is always 50% (Mocak, Bond, Mitchell, & Scollary, 1997). Therefore, the detection limit was also experimentally determined. For this purpose, bovine spinal cord was added to minced meat in eight different concentrations close to the calculated detection limit (between 0.013% and 0.56%). The CNS content of each sample was determined 5–7 times (as indicated) and the number of positive results for each concentration was recorded.

For the determination of the calibration curve, bovine spinal cord was added to minced porcine and bovine muscle at six different concentrations between 0% and 0.5% (0%, 0.013%, 0.027%, 0.11%, 0.21%, and 0.43%). PLP was determined as described before and the percentage of CNS in the meat matrix was plotted against the relative fluorescence intensity. The standard curve is the mean of three independent experiments.

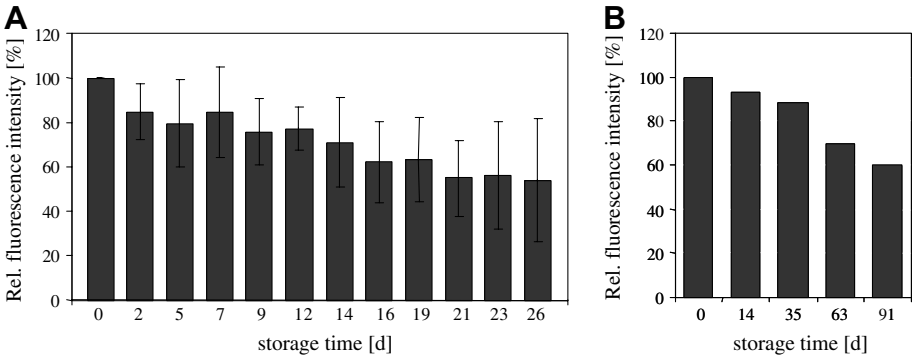

Fig. 4. Stability of the PLP epitope during storage. Samples of minced pork and beef (1:1) containing 0.6% brain tissue were stored at 5 °C (A) or −21 °C (B), respectively, for several time intervals between 2 d and 13 weeks. The PLP extract was then analyzed by Western blot. For detection, the polyclonal PLP antibody and a CY5 conjugated secondary antibody were used. The chart displays the fluorescence signal intensities of the PLP band in % related to the intensity of an aliquot which was analyzed without storage.

3. Results and discussion

Myelin proteolipid protein (PLP) is a novel marker for the immunochemical detection of central nervous system tissues (CNS) (Sandmeier et al., 2006). In this study, an immunochemical method (Western blot) using PLP as a marker protein for CNS contaminations in raw and processed meat was developed.

3.1. Selective extraction of PLP with organic solvents

Because of its highly lipophilic character, PLP is soluble in organic solvents. Therefore, it can easily be separated from the matrix of meat or meat products by solvent extraction. Based on the procedure for the preparation of brain cephalin by Folch (Folch, 1942), a simple method was developed, comprising an extraction step using *n*-hexane followed by the removal of lipids with diethyl ether. Most of the meat proteins could be removed by this extraction step. SDS–PAGE, followed by silver staining, showed that Myelin PLP with a molecular weight of 29 kDa was by far the most abundant protein in the lipophilic protein extract of a meat sample containing 10% porcine brain tissue (Fig. 1). In the negative control, which consisted only of minced meat, hardly any protein bands could be detected. The two other bands, which specifically appeared below the PLP band in samples containing CNS tissue, could be assigned to an isoform of PLP called DM-20 (Nave, Lai, Bloom, & Milner, 1987) at 20 kDa and to Myelin basic protein (MBP) at 18.5 kDa (Bates et al., 2000). PLP and DM-20 were identified by immunochemical reactions, whereas MBP was identified by tryptic in-gel digestion and subsequent matrix-assisted laser desorption ionisation-time of flight-mass spectrometric (MALDI-TOF MS) analysis (Bizzozero, Malkoski, Mobarak, Bixler, & Evans, 2002; Seeber et al., 2004).

3.2. PLP analyses by Western blot using a commercially available monoclonal anti-PLP antibody

Western blot analysis of PLP in meat products was performed using a commercially available monoclonal anti-PLP antibody raised against the amino acids 272–276, which are the C-terminus of the protein. Besides PLP, the antibody also recognizes two bands which could be assigned to a dimer and to the PLP isomer DM-20 (data not shown). Western blot analyses of meat and meat products using the monoclonal anti-PLP antibody had several disadvantages: Considerable batch-to-batch variations of the antibody were observed, hampering the reproducibility of the assay. Furthermore, relatively high antibody concentrations must be applied. Finally, the stability of the epitope during heating and sausage preparation proved to be insufficient: Even concentrations as high as 10% CNS tissue (porcine brain) in cooked bologna type sausages were not detectable (data not shown).

3.3. Development of a Western blot protocol using a newly developed polyclonal anti-PLP antibody

Because of the problems with the commercial monoclonal anti-PLP antibody, a polyclonal antiserum was used, which had been raised against the peptide *CGKGLSATVTGGQKGRGSR*. This sequence is identical to the amino acids 109–129 of Myelin proteolipid protein. The antiserum recognizes PLP with high specificity and affinity.

The polyclonal anti-PLP antibody was now used to develop a Western blot protocol for a selective detection of CNS tissues in meat products. Skim milk powder proved to be the most efficient blocking reagent. Optimal antibody dilutions were determined to be 1:250 for the anti-PLP antiserum and 1:500 for the secondary antibody. Antibody binding was visualized by the fluorescence labelled secondary antibody using a fluorescence reader. Fig. 2A shows a typical fluorescence readout image of a Western blot analysis carried out with tissue samples from porcine brain, bovine spinal cord, bovine peripheral nerve and minced meat from porcine and bovine skeletal muscle. A distinct band at about 29 kDa was visible in the samples containing CNS tissue (lanes a, d), whereas it was absent in minced meat which served as negative control (lane c) as well as in peripheral nerve tissue (lane b). Apart from a very low background signal, neither unspecific bands nor cross-reactions can be detected. In some samples containing CNS tissues, weak bands at approximately 60 kDa and 12 kDa are visible, which most probably result from dimerization and fragmentation of PLP during sample treatment and meat processing.

The species origin of the meat matrix did not noticeably influence the detectability of CNS tissue (Fig. 2B). Therefore, minced pork and a mixture of minced pork and beef were used for the further experiments.

3.4. Quantification of CNS contaminations in unprocessed meat products

For the validation of the newly developed assay, a calibration curve was generated with samples of a mixture of porcine and bovine minced skeletal muscle containing between 0.013 and 0.43% bovine spinal cord tissue (Fig. 3). The R^2 value of the calibration curve was 0.9942 and the linear range was in good accordance with the quantification limit of 0.049% CNS tissue in unprocessed meat, which was calculated according to the ACS guideline (ACS Committee on Environmental Improvement, 1980). The detection limit of the assay was calculated according to the ACS guideline as 0.015% CNS in unprocessed meat. In order to evaluate the theoretical detection limit, minced meat was spiked with different levels of porcine brain tissue and analyzed with the PLP Western blot. At a supplementation level of 0.015%, CNS was mostly ($n = 4/7$) and at 0.025%, always reliably detected in minced pork and beef ($n = 5/5$).

3.5. Determination of CNS contaminations in stored and processed meat

Stability of the PLP antigen was first examined in minced pork and beef (1:1), which was spiked with 0.6% CNS tissue (bovine spinal cord) and stored at 5 °C or −21 °C for several days. Fig. 4 shows the results of the storage experiments: At 5 °C, the fluorescence signal of the PLP band was barely diminished up to a storage period of 14 d. After 18 d, the signal was still more than half of the original fluorescence intensity. Even after 26 d, more than one third of the PLP was still detectable. At −21 °C, the PLP epitope was more stable. After 13 weeks, nearly two thirds of the original signal were still visible.

Heating experiments with minced meat containing 3.5% CNS tissue showed the stability of the PLP epitope at elevated temperatures. Even after a period of 180 min at 75 °C, the PLP signal was not seriously affected. Treatment at 95 °C caused a stronger decrease of the signal and a nearly complete disappearance of the PLP band after 180 min (Fig. 5).

Several tests were also performed with sausage samples which were supplemented with 0, 0.1, 0.3, 0.5, and 2% of CNS tissue prior to preparation. Three different types of standardized sausages were produced according to the German guidelines for the preparation of meat products (Heits, 2003): a fermented sausage (“Teewurst”), a cooked liver sausage (“Leberwurst”), and a cooked bologna type sausage (“Schinkenwurst”).

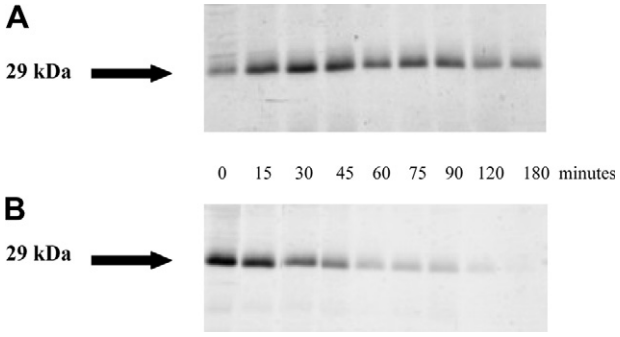

Fig. 5. Stability of the PLP epitope during heating. Aliquots of a meat sample containing 3.5% bovine spinal cord tissue were kept at 75 °C (A) or 95 °C (B) between 15 and 180 min and then analyzed by Western blot.

CNS was detectable in all sausage types with a detection limit of 0.1% as compared to a detection limit of 0.015% for unprocessed meat. Fig. 6A shows typical fluorescence readouts of the Western blot analysis of sausage samples containing 0.5% or 0.1% CNS tissue. A distinct band at 29 kDa is visible very clearly in every lane, while differences in the intensity of the non-specific background signal appeared depending on the type of sausage: The most intensive background signal appears in fermented sausage samples (Fig. 6A, lane a), which have not been heated during the production. Heating the “Teewurst” samples for 15 min at 80 °C before extraction reduced the non-specific background signal (data not shown). The background signal, however, did not interfere with the detection of PLP at or above the indicated detection limit. The use of nitrite pickling salt did not interfere with the assay.

3.6. Microbiological contaminations

Some tests were also carried out with sausage samples contaminated with several types of bacteria and mould, which usually cause meat spoilage. Incubation with these microbes did not influence the traceability of PLP (Fig. 6B). Western blot analyses of contaminated and not contaminated samples showed the characteristic PLP band only in the samples which contained CNS tissue (2% bovine spinal cord). Microbial contaminations of the samples cause the appearance of some bands at higher molecular masses. These bands, however, do not interfere with the test results.

Due to its high hydrophobicity, PLP can be extracted with organic solvents, for example with *n*-hexane. Under these conditions, most of the meat proteins are not soluble. Therefore, a simple extraction step results in a very

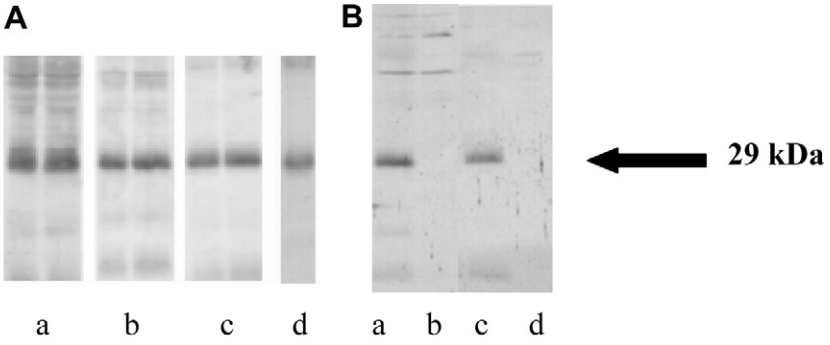

Fig. 6. (A) Detection of CNS in different sausages containing 0.5% bovine spinal cord: (a) fermented sausage “Teewurst”, (b) cooked bologna type sausage “Schinkenwurst”, (c) cooked liver sausage “Leberwurst”, (d) cooked bologna type sausage “Schinkenwurst” containing 0.1% bovine spinal cord. (B) CNS detection in sausages inoculated with a mix of microorganisms which typically cause meat spoilage. Samples were worked up as described before and analyzed by Western blot using the polyclonal anti-PLP antibody and the CY5 conjugated secondary antibody. (a) inoculated sausage containing 2% bovine spinal cord, (b) inoculated sausage without CNS tissue, (c, d) analogous to a and b, but without inoculation.

selective enrichment of the marker protein leading to lower signal/noise ratio in the immunochemical analysis as well as to increased sensitivity.

A practically selective expression of the marker protein PLP in CNS results in an excellent tissue specificity of the immunochemical assay. The tissue specificity is an important prerequisite for the application of the assay in routine food inspection (Berg, Schoen, & Westarp, 2002): False positive results due to legal food components, such as peripheral nerves or blood, would exclude the application to reinforce legal regulations. As expected, the PLP test shows high sensitivity towards CNS contaminations in unprocessed meat with a detection limit of less than 0.025% and a quantification limit of less than 0.049% CNS tissue in unprocessed meat. For a commercial NSE assay, a detection limit of 0.1% brain in lamb mince, and for a commercial GFAP ELISA, a detection level of 0.1% spinal tissue in raw lamb was reported (Hughson, Reece, Dennis, & Oehlschlager, 2003). The cut-off levels (concentration above which a positive result is reported) are 0.25% for the NSE assay and 0.2% for the GFAP ELISA, as reported by the manufacturers (Hughson et al., 2003).

Unlike the epitope of the commercial PLP-antibody, the epitope which is recognized by the new PLP-antibody was sufficiently stable during storage, heating, and sausage preparation. Thus, the detection limit of CNS in three types of sausages was below 0.1%. The detection limit for CNS in processed meat products is 0.2% for the commercial GFAP-ELISA and 0.25% for the commercial NSE Western blot assay (according to the manufacturers).

However, a distinct decline in PLP recognition during heat treatment was observed (Fig. 5). Thus, it can not be excluded that in some severely heat treated meat products, such as tropic conserves, rather heat stable and potentially infectious PrP^{Sc} contaminations are still present, although brain contaminations can not be detected anymore by the PLP Western blot.

4. Conclusion

In this study, a Western blot assay using a polyclonal anti PLP antiserum to detect CNS contamination in meat and meat products was developed. The assay showed very high tissue specificity and sensitivity. Thus, the new PLP Western blot assay can serve as a valuable method to very reliably detect CNS contaminations in meat and meat products. Based on this assay, a method can also be developed to detect CNS contaminations on surfaces which come in contact with raw meat during its preparation and handling. Furthermore, a study is currently in progress to develop a dot blot assay using the PLP antibody which will allow a considerable reduction of analysis time.

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