

uncertainties. These are related, in particular, 1) to our limited knowledge on the pathogenesis of TSE in semi-resistant or resistant genotypes, 2) to the actual meaning of “resistance” in relation to the possible existence of silent carrier states, 3) to the possible emergence, under the selective pressure of the breeding programs, of TSE strains which have no longer their genetic target in the susceptible genotypes, but in those now considered as resistant.

All the above uncertainties could affect the success of the program but has also great impact for diagnosis and surveillance. This underlines the need of a careful monitoring of the breeding programs which still represent only a component of the health strategies for the control and prevention of animal TSE.

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## S10.5

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### HIGH PRESSURE/TEMPERATURE INACTIVATION OF TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES INFECTIVITY

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Bovine spongiform encephalopathy (BSE) contamination of the human food chain most likely resulted from nervous system tissue in mechanically recovered meat used in the manufacture of processed meats. The widespread occurrence of BSE, coupled with uncertainties about the rigorous implementation of precautionary measures, underline the need for a processing step that would ensure the absence of infectivity in processed meat products.

We have previously shown that the application of several short pulses of high pressure (690-1200 MPa) to hot dog paste ‘spiked’ with 263K scrapie-infected brain can reduce the level of infectivity by  $10^3$  to  $10^6$  mean lethal doses ( $LD_{50}$ ) per gram of tissue (Brown P. *et al.*, PNAS 100: 6093–6097, 2003). By using the same scrapie strain, we have recently explored a larger range of pressure/temperature/pulse combinations, and a variety of processed meat products (e.g., hamburger, pureed baby food, pâté, meat broth, and pet food) as substrates. We have also studied high pressure inactivation of other strains of transmissible spongiform encephalopathies, (BSE; mouse adapted BSE, mBSE; mouse adapted vCJD, mvCJD).

Brain samples from BSE, mBSE, mvCJD and 263K affected animals were treated at 600MPa/130°C/5’ and then assayed by western blotting to measure the

amount of residual PrPres. In order to get an accurate estimate of PrPres concentration, each sample was analysed using a two-fold dilution series. This experimental setting allowed us to demonstrate a reduction in the content of PrPres in all treated samples in comparison with their untreated counterparts: mvCJD and 263K showed the highest reduction (32-fold, about 1.5 log), whereas BSE and mBSE showed only a 4 to 8 fold reduction respectively.

In the second part of this study, 263K-spiked hot-dog aliquots were subjected to a range of different combinations of pressure, temperature and length of treatment. Results from western blot analysis of these samples showed that an exposure as short as 1-minute yields 1 log reduction of PrPres, and that the maximal extent of PrPres clearance (1.5 log) is obtained after 3 minutes, with no increase after further exposure. Keeping the time and temperature constant, progressive destruction of PrPres was observed with pressure increases from 600 to 1000 MPa; keeping time and pressure constant, a similar outcome was obtained when the temperature was increased incrementally from 115 to 134°C.

The results of our study indicate that sensitivity to high pressure treatment is strain-specific, with the maximal effect observed in brain samples from animals infected with the 263K and mvCJD inocula. This is not surprising when considering that TSEs infectious strains can be easily distinguished on the basis of their pathological, biochemical, and physical properties, such as the resistance to inactivation following autoclaving or dry heat processing (Somerville R.A. *et al.*, J. Biol. Chem. 277: 11084-11089, 2002). The small reduction in PrPres concentration in cattle and mouse BSE is in accordance with data obtained by other authors using hydrated autoclaving and confirm that this strain is particularly resistant to inactivation procedures (Taylor D. *et al.*, J. Gen. Virol. 83: 3199-3204, 2002). These results, while awaiting support by ongoing infectivity bioassay studies, suggest that the BSE agent requires a different combination of conditions to achieve satisfactory decontamination. We have previously observed that pressures of 1200 MPa result in loss of  $10^6$  mean lethal doses per gram of 263K scrapie-infected brain tissue, however, the use of harsh conditions of treatment in an industrial setting may encounter technical constraints and has to be weighed against the possible alteration of the food product. The definition of the process of PrPres clearance as the sum of pressure, temperature and time shows how to deal with this limitation: the same PrPres clearance can be obtained at two different pressures simply adjusting the peak temperature or, to a lesser extent, the time of treatment. These data (always subject to bioassay confirmation) will provide a useful background to develop inactivation protocols specifically designed for each food product yielding the desired combination of quality and safety.