

P.17.- The Development of Rapid Immunoassays for Specified Risk Material through the Use of Bioinformatics

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A novel approach to the development of immunotests for Specified Risk Material (SRM) in cooked meat products is being investigated in which protein sequence databases are being interrogated to identify the sequence of tissue-specific proteins using in silico protease digestion. Short peptide sequences within these tissue specific proteins will be identified which are unique to each protein. These peptides will be created through in silico protease digestion of the target proteins. The peptides will be synthesised and used to raise monoclonal antibodies for use in immunoassays. The assays will be carried out by first digestion of the cooked meat samples with the protease identified in the in silico studies followed by evaluation of the proteolytic digests using the antibodies incorporated into lateral flow devices. To date the bioinformatic studies have identified twelve peptide sequences in nine proteins that are theoretically specific to four SRM tissues, brain, eye, ileum and tonsil (lymphoid). These peptides have been synthesized and are being used to raise monoclonal antibodies. Four of the peptide sequences are theoretically specific to ruminant species and two specific to bovine therefore the final test kits may provide both tissue and species specificity. A complementary approach is simultaneously being investigated, in which monoclonal antibodies are raised against bovine SRM protease digests that have been affinity purified using polyclonal antibodies raised against avian brain and bovine muscle digests. For this part of the study, enzyme digestion procedures have been developed that rapidly solubilise 60-95% of the protein in cooked meat samples, the resultant peptides having a mean molecular mass of approximately 5kDa. More rigorous digestion procedures are being developed for the exposure of the peptide sequences identified as specific to the target tissues. Ultimately these will be used in conjunction with the monoclonal antibodies raised to the synthetic peptides, to provide a test kit for SRM in cooked meat products. The optimized assays will be validated in an interlaboratory trial, using cooked meat containing known amounts of SRM and subsequently used in a European survey of cooked meat products. This research project is being supported by the EU 5th Framework 'Competitive and Sustainable Growth' Programme (1998-2002) and by the UK Food Standards Agency. Additional information can be obtained from the project website (www.srmtest.info)

Keywords

Specified Risk Material, Bioinformatics, Lateral flow devices, Proteomics, Proteolytic digestion