UTILIZATION OF DNA COMET ASSAY AND HALF EMBRYO TEST TO IDENTIFY IRRADIATED LENTIL

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Legumes make an important contribution to human nutrition on a worldwide basis. Insect infestation cause extensive damage to stored grains. Over the last few decades some countries adopted food irradiation as a safe food process. Radiation's processing on foods improves hygienic quality and extends their shelf life. The use of radiation treatment to reduce the microbial population and thereby extend the shelf life in legumes has been reported in many papers. Irradiation has been shown to be an effective pest control method for these commodities and a good alternative to prohibited methyl bromide. Radiation disinfestation can facilitate trade in foods that often harbor insect pests of quarantine importance. Although the wholesomeness of irradiated food is no longer a question there is a need for irradiation control in the international trade of foods, in order to enhance the consumer confidence in the regulation. As a screening methods to identify irradiated lentils, processed by e-beam as a food treatment to disinfestation, the DNA Comet Assay and Half Embryo tests were performed. The methodologies used in this work are based upon biological changes that occur in Brazilian lentils. The samples were irradiated in an electron beam accelerator facility of Radiation Dynamics Inc., USA (E= 1,5 MeV, I=25mA). The irradiation doses were 0,7; 1,4 and 3,0 kGy at dry conditions. The thickness of samples was less than 0,5 cm. A sensitive technique to detect DNA fragmentation is the microgel electrophoresis of single cells or nuclei, also called "comet assay". Since the large molecule of DNA is an easy target for ionizing radiation, changes in DNA offer potential as a detection method. It is restricted to foods that have not been subjected to heat or other treatments, which also cause DNA fragmentation. Lentil samples were crushed with a mortar and pestle and was transferred to 3ml ice-cold PBS. This suspension was stirred for 5 minutes and filtered. 100µl cell suspension was mixed with 500µl of low-melting agarose (0,8% in PBS). 100µl of this mixture was spread on pre-coated slides. The slides were immersed in lysis buffer (0,045M TBE, pH 8.4, containing 2,5% SDS) for 15 minutes. Electrophoresis was carried out using the same TBE buffer, but devoid of SDS, at a potential of 2V/cm for 2 minutes. Silver staining was carried out for 1 hour following fixing. Duplicate measurements for each sample were carried out and 100 cells were counted for each dose level. The migration patterns of DNA was evaluated with a standard microscope. Germination tests were carried out in the irradiated and non-irradiated dry lentils, which allows observing characteristically variations on the shoots and roots. The half-embryo test is based on the inhibition of shooting in seeds or grains due to irradiation. It is characterized by its easy detection and sensivity. The shoots and roots were observed during 5 days of culturing period under specified conditions. The difference observed in this variety was analyzed only after irradiation treatment at room temperature. Irradiated half-embryo showed markedly reduced root grows and almost totally retarded shoot elongation. Differences between irradiated and non-irradiated half-embryo could be observed.