EN 13783 - English version

Foodstuffs — Detection of irradiated food using Direct Epifluorescent Filter Technique/Aerobic Plate Count (DEFT/APC) — Screening method

1 Scope

This European Standard specifies a microbiological screening method for the detection of irradiation treatment of herbs and spices, using the combined direct epifluorescent filter technique (DEFT) and aerobic plate count (APC). The DEFT/APC technique is not radiation specific, therefore, it is recommended to confirm positive results using a standardised method (e.g. EN 1788, prEN 13751) to specifically prove an irradiation treatment of the suspected food.

The method has been successfully tested in interlaboratory tests with herbs and spices [1] to [5].

2 Principle

The method is based on the comparison of the APC with the count obtained using DEFT. The APC gives the number of viable microorganisms in the sample after a possible irradiation and the DEFT count indicates the total number of microorganisms, including non-viable cells, present in the sample. The difference between the DEFT count and the APC count in spices treated with doses of 5 kGy to 10 kGy is generally about or above 3 to 4 log units. Similar differences between DEFT and APC counts can be induced by other treatments of the foods leading to death of microorganisms, e. g. heat, thus positive results shall be confirmed.

A known volume of sample is filtered through a membrane filter at reduced pressure in order to concentrate the microorganisms on the filter. The microorganisms are stained with a fluorochrome, acridine orange (AO), resulting in an orange and orange-yellow fluorescence under illumination with blue light at 450 nm to 490 nm. These microorganisms are counted using an epifluorescence microscope to give the DEFT count. However, microorganisms which were non-viable before irradiation show green fluorescence and are not counted. In parallel, the APC is determined from a second portion of the same test sample [6] to [10].

3 Limitations

A limitation of the method is encountered when there are too few microbes in the sample (APC < 10^3 cfu/g). If fumigation or heat treatment has been used for decontamination, the DEFT/APC-difference of counts can be similar to the difference of counts obtained after irradiation. However, the use of fumigation can be detected.

Some spices such as cloves, cinnamon, garlic and mustards contain inhibitory components with an anti-microbial activity which may lead to decreasing APC (false positive result).

4 Validation

The combined DEFT/APC method has been applied to herbs and spices, [2], [3]. When samples of allspice, peppers and cardamom were irradiated with a dose of at least 10 kGy, the differences between DEFT and APC were greater than 6 log units for irradiated allspice and white pepper, greater than 4,5 log units for black pepper, and greater than 7 log units for cardamom [2]. When spices such as peppers, paprika, cardamom, cinnamon and ginger and herbs such as thyme, marjoram, basil and oregano were analyzed after irradiation with doses of 5 kGy and 10 kGy, differences between DEFT and APC varied between 3,9 and 6,8 log units and between 5,7 and 7,5 log units, respectively [3].

A BCR collaborative study [1] was conducted including 192 samples of whole allspice, whole and powdered black peppers, whole white pepper, paprika powder, cut basil, cut marjoram, and crushed cardamom unirradiated or irradiated with doses of 5 kGy and 10 kGy and analyzed by eight laboratories. The average values of the differences between DEFT and APC in samples irradiated with doses of 5 kGy and 10 kGy were 5,1 and 6,1 log units, respectively. For all irradiated samples analyzed, the differences between DEFT and APC count generally increased to at least 3,5 log units, whereas the difference in the case of unirradiated spices was insignificant. The reproducibility relative standard deviations for the differences were 12,3 %, 19,9 % and 20,7 % with the doses of 10 kGy and

5 kGy and for unirradiated samples, respectively, indicating acceptable variabilities among laboratories.

The probability of judging an unirradiated sample as irradiated (false positive result) using the limit of 4,0 log units is low [1]. On the other hand, the limit of 4,0 log units will sometimes give false negative result. This has been demonstrated for samples of basil [1] and [11].