BIOMONITORING BY COMBINATION OF IMMUNOMAGNETIC SEPARATION AND DIRECT EPIFLUORESCENCE FILTER TECHNIQUE

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INTRODUCTION: Traditional microbiological methods have proved to be slow and insensitive in controlling the quality and microbiological safety of perishable products. There is a need for more rapid, sensitive and specific methods that will facilitate product release and identify hygiene and safety problems more rapidly so that corrective action can be taken. Several new methods that use a variety of different principles have been developed (overview see [1, 2]). These include the direct epifluorescence filter technique (DEFT) [3-6].

DEFT is performed by homogenisation of samples. This procedure is supported by a Stomacher device followed, if demanded, by enzymatic disintegration of the homogenate (see below). The sample is further processed by prefiltration through a 5µm pore nylon filter and passage of the filtrate through a 0.2µm pore black polycarbonate filter to collect and concentrate microorganisms. These are subsequently stained with fluorochrome and prepared for following examination and counting by epi-fluorescence microscopy.

Depending on the components of the food samples analyzed, a disadvantage of DEFT is that one may get microscopic images which cannot be evaluated because particles of the food sample can be collected on the filter along with the microorganisms. The prefiltration through a nylon filter with pores smaller than 5µm can prevent this occurring but one cannot be sure that all microorganisms pass the prefiltration module. Thus, the number of detectable bugs in the epifluorescence microscope will not map reality. We set out to isolate microorganisms from these heterogeneous suspensions by combination of DEFT and immunomagnetic separation reducing levels of background particles. Magnetic particle-based technology [7] offers distinct advantages for the rapid processing of large numbers of cells.

MATERIALS AND METHODS: Dried apricots and figs were used in the experiments. 10g of fruits were mixed with 90mL of phosphate buffered solution (PBS) in a ´400´ Closure Bag (Seward) and homogenised in a Stomacher device (Seward Lab System) for 10 minutes.

Uncoated paramagnetic, polysterene, Tosyl-activated (450T) beads (4.5 µm) (DYNAL) and/or enzyme mixtures were added. Beads were resuspended and washed in appropriate buffer and stored at 4°C before use. A wide number of enzyme preparations (Novo Nordisk; Erbslöh; Röhm) with corresponding activities was tested.

Disassembly of the structure of fruit particles was followed by measuring the decrease of particle size with a Helos laser diffraction system (Sympatec).

RESULTS: Mixtures of cellulases, hemicellulases and pectinases were used to disintegrate fruit particles remaining after homogenizing.

By help of those enzymes the amount of small particles in the suspension increased after treatment (Fig. 1).

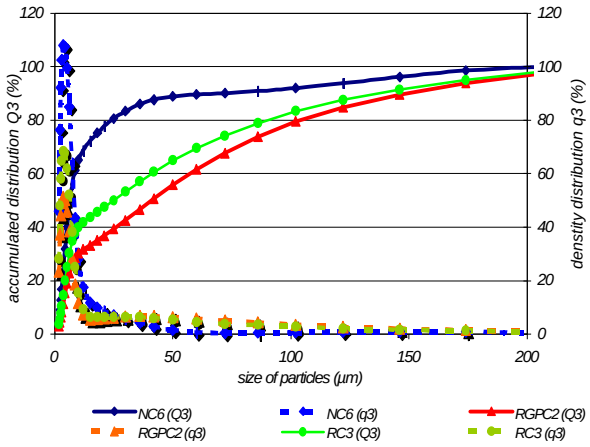

Fig. 1: Particle size in a fig probe after treatment with cellulase preparations.

Best results were achieved with the following enzyme mixtures.

Table 1. Optimum quantity of enzymes.

	fig	apricot
amylase	0.5 % RA5	0.5 % RA5
pectinase	5.0 % EP2	5.0 % NGP3
cellulase	2.0 % NC6	4.0 % RGC4
other supplements	none	2.0 % NGP2

Experiments concerning recovery rate of magnetic beads from fruit pulp are documented in Fig. 2.

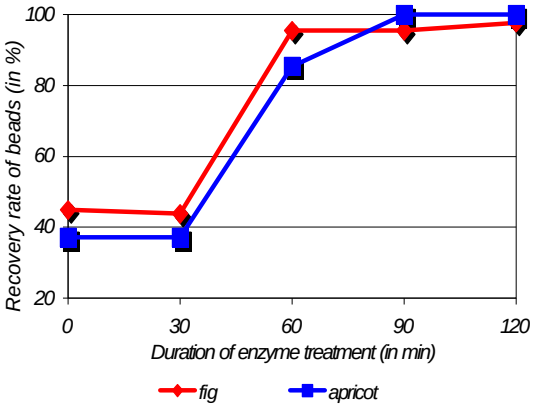

Fig. 2: Recovery rate of magnetic beads after enzymatic treatment.

It was clearly shown that beads cannot be isolated completely from the probes without sufficient enzyme treatment. Only after incubation for a period of between 60 and 90 minutes at 45°C could more than 95% of beads be recovered from the fruit pulp by magnetic processing.

Enzyme treatment studies and studies with magnetic beads in fruit pulps gave confidence that the combination of DEFT and Magnetic particle-based technology can lead to sensitive, specific and rapid methods, which can be directly applied to food samples.

An aim of further experiments is to evaluate the coating of the beads with related antibodies, or other highly specific ligands such as lectins, against relevant microorganisms. The results will be shown at the conference.

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