

## Optimization of bread dough preparation and staining methods in epi-fluorescence light microscope (EFLM) observations

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Studying dough microstructure is considered as a useful and effective tool to understand the effect of different process parameters on dough and the final product characteristics. In this study, sample preparation and staining protocols were established to study the dough micro-structure with epi-fluorescence light microscope (EFLM). For this purpose, dough sample preparation and staining conditions with respect to the type of staining dye, solvent, dye concentration, staining time and the type of filter used for EFLM were studied. The results showed that sodium Fluorescein (1% w/v) and Rhodamine B (0.1% w/v) in 2-methoxyethanol solvent give the best staining results. The double staining technique used enabled us to simultaneously and successfully observe the starch granules and protein network in the dough. The resting time after the addition of the dye had an impact on the quality of microscopic images. Among different resting times investigated, 60 min time gave the best results. Among the different EFLM filters, the MWBV2 filter for 420 nm spectrum gave the best results. Using image processing software and the specialized plugins the quality of EFLM images was improved. When a suitable protocol and methodology was established, the microstructure of dough was studied under different mixing regimes (under-mixing, optimal mixing and over-mixing). Overall, the results indicated that EFLM can be successfully used for studying the dough microstructure and this technique gives comparable results to well-known CSLM technique.

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