# Biocrystallisation and Steigbild results at the Louis Bolk Instituut

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## Introduction

Holistic methods for measuring the quality of food products are developed from the point of view that living organisms contain an 'inner structure' constituting the compounds in the organism. This 'inner structure' is believed to be connected to the growth and development (i.e. farming system) of the organism. Different holistic methods are regarded to reflect this 'inner structure'.

The Louis Bolk Institute (LBI) participates within the international Triangle network (LBI, the Netherlands; BRAD, Denmark and Uni Kassel, Germany) in the standardisation and validation of the biocrystallisation method. Parallel to this work the Steigbild or Capillary dynamolysis method, a second picture forming method, is standardised too.

The standardisation of the methods entails developing standardised procedures and evaluation tools. The evaluation of crystallisations is based on computerised Image Analysis and Visual Evaluation. The Visual Evaluation is developed according to ISO norm 11035 for sensory analysis, adapted to meet the biocrystallisation pictures. In this way, 14 textural and structural morphological criteria have been developed and validated within the Triangle. The steigbild pictures are evaluated visually only.

### Materials and methods

For the Conference Bildschaffende Methoden 2007, 3 wheat varieties were analysed by means of the biocrystallisation and the steighild method. The varieties were Capo, Lux and Goldblume.

## The biocrystallisation method

The samples were cleaned by means of a 2.0 mm Retsch sieve, followed by a manual removing of damaged and broken kernels and remaining foreign particles. 100g of each sample was grinded with a centrifugal mill (Retsch ZM 100; 14.000 rpm) fitted with a 1.0 mm ring sieve. 50.0 g of the milled material was extracted in 450.0 ml deionised water (25 °C) on a horizontal shaker (Heidolph Unimax 2010) at 200 rpm for 30 min. Subsequently the extract was left to stand for 15 min. 50 ml extract was filtered over

respectively Whatman 41 and 40 paper filters. The total (dual) extraction time was set at 45 min.

In a matrix, in which different extract-CuCl<sub>2</sub> concentration ratios are used, the optimum combination of concentration ratios is assessed (i.e. the concentration ratio at which a well ramified, dendritic crystallisation structure is obtained). For the present study, the concentration ratio was set at 90 mg CuCl<sub>2</sub> and 90 mg filtrate in a total volume of 6.0 ml per plate. Sample preparation was performed 3 times per sample. Each sample preparation was pipetted in 4-fold replicate in the crystallisation chamber, so in total 12 pictures were obtained per sample.

The crystallisation chamber was calibrated at a median evaporation time of  $13 \pm 1$  hours with a freeze dried wheat meal chamber standard. All pictures were scanned and evaluated with the ACTA software (*Andersen et al.* 1999). For Visual Evaluation the criteria for conventional profiling were applied (*Huber et al.* 2007).

## Texture analysis

For the computerised analysis, the crystallisations were scanned and a circular Region Of Interest for analysis was determined (ROI 1-100 % of the crystallisation surface around the geometric centre). For each crystallisation plate a Grey-Level-Co-occurrence-Matrix (GLCM) was calculated depicting the grey-level relationship between neighbouring pixels in the ROI. 15 variables characterising this GLCM were computed (*Carstensen* 1993). Output generates the p- and F-values for the different variables relative to the ROI. Only combinations of ROIs and variables yielding data with a non-significant Shapiro-Wilk (normality testing) and Bartlett-test (testing homogeneity of variance) and a stable progression over ROI were used for the evaluation.

### Visual Evaluation

For Visual Evaluation, the criteria for conventional profiling are applied (*Huber et al.* 2007). A 'Simple descriptive test' is applied to describe the main characteristics and the gesture of the crystallisations belonging to a sample. The interpretation of the 'Simple descriptive test' is connected to the *Inner Quality Concept (Bloksma et al.* 2003). This concept is based on the universal life-processes *Growth* and *Differentiation (ripening)* and the balance or *Integration* between these processes. A crystallisation is regarded to relate to good product quality when the characteristics of both life-processes are sufficiently present in the crystallisation and are found in a balanced manner (*Integration*).