

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 steigbild 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-CuCl₂ 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 CuCl₂ 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 ± 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*).

The steigbild method (according to WALA)

The steigbild method consists out of 3 rising phases. During the first rising phase 0.6 ml of the filtrate is allowed to rise into the steigbild filter (S&S 2043A); conditions 20 °C, 60 % rH. The next day 0.7 ml 0.25 % AgNO₃ solution is allowed to rise; during this phase the filters are covered with a glass bowl to increase the rH. After a 3 hours drying period 2.0 ml 0.25 % FeSO₄ solution is added. The next day the filters are developed by placing them in diffuse sunlight for approximately 6 hours.

Sample preparation was performed as above, that is 3 times per sample. Each sample preparation was pipetted in 2 fold replicate onto the filters, so in total 6 filters were obtained per sample.

Results biocrystallisation method

Texture analysis

In figure 1 the mean values for the variable 'Kappa' and the three ROIs are shown for the 3 wheat samples. The difference between the wheat samples Goldblume – Lux and Goldblume – Capo was significant ($F_{\text{Kappa}} = 29$ and 36 respectively; $p < 0.01$ at ROI 70). No significant difference was found between Lux and Capo. In figure 2 the mean values for the variable 'Sum Variance' and the three ROIs are shown for the 3 wheat samples. The difference between the wheat samples Goldblume – Lux and Goldblume – Capo was significant ($F_{\text{Kappa}} = 22$ and 23 respectively; $p < 0.01$ at ROI 70). Again, no significant difference was found between Lux and Capo.

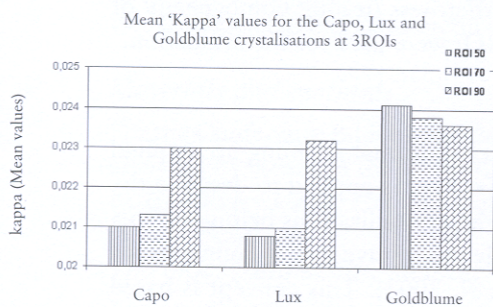


Fig. 1: Mean 'Kappa' values for the 3 wheat varieties

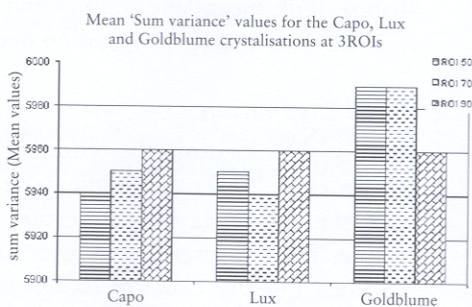


Fig. 2: Mean 'Sum variance' values for the 3 wheat varieties

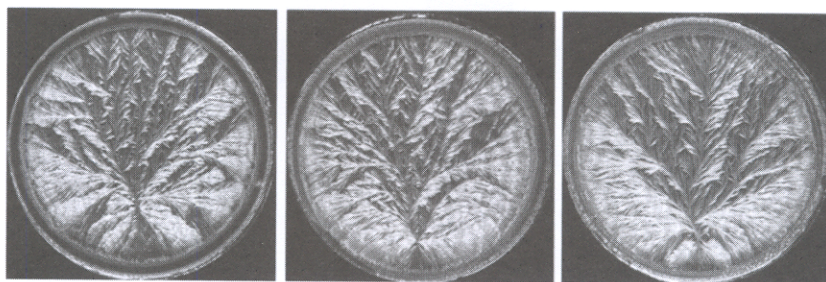
Visual evaluation

The 9 sample preparations could be grouped correctly according to the sample they originate from.

The crystallisations originating from Lux and Capo resemble each other; both exhibit substance spirals in connection with short side needles and a slightly irregular sideneedle setting due to a wide angle of ramification. The differences were mainly found in the peripheral zone; Capo is able to mold the CuCl_2 into the peripheral zone, whereas Lux displays a crystal free peripheral zone.

Goldblume contains long sideneedles, some interwoven sections and a more regular side needle setting with a smaller angle of ramification.

No growth and differentiation reference series for wheat have been made up to yet. Interpretation is based on apple and carrot reference series (Blokma *et al.* 2003). Lux and Capo reflect a combination of growth and ripening phenomena, whereas Goldblume merely shows growth phenomena. These differences could be partly due to the differences in wheat varieties.



Lux

Capo

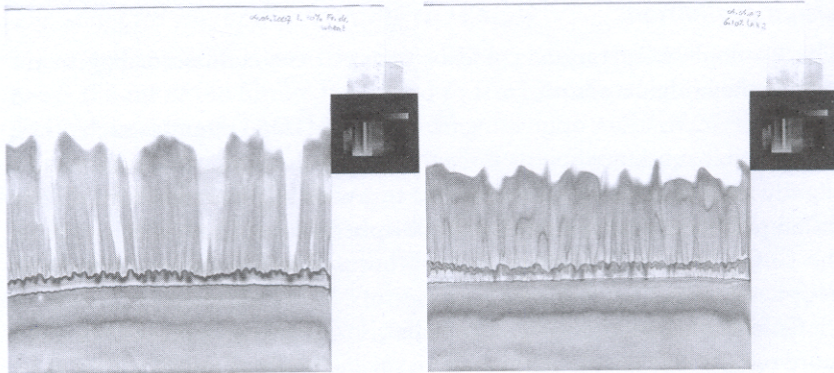
Goldblume

Results steigbild method

All individual steigbilds could be grouped correctly according to the sample they originate from.

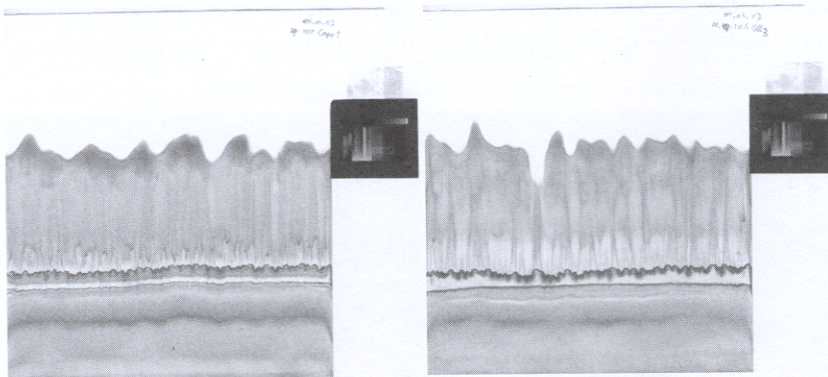
Lux and Capo resemble each other the most; distinct bowls with beards. Although Lux had a very slow rise. Lux exhibits very distinct flags arising directly from the bowls and clear drops.

Goldblume has rather blurred bowls, relating somewhat to the freeze dried standard.



Freeze dried wheat standard

Lux



Capo

Goldblume

Conclusions

The 3 wheat varieties Capo, Lux and Goldblume could be differentiated visually and by means of a computerised texture analysis. The differentiation was significant between Goldblume – Lux and Goldblume – Capo ($p < 0.01$ at ROI 70).

The visual evaluation of the crystallisations and the steigbild filters confirms the likeness between Capo and Lux. The Lux and Capo crystallisations reflect a combination of growth and ripening phenomena, whereas Goldblume merely shows growth phenomena. These differences could be partly due to the differences in wheat varieties. The steigbild filters show likeness in the bowl zone between Goldblume and the freeze dried wheat standard.

Literature

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