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Note

Elimination of measurement error in light microscopy investigations of starch granules geometry**

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A b s t r a c t. The authors propose a method for the elimination of dust particles from analysis of geometry of potato starch granules. The form and size differences between potato starch granules and dust particles permitted the application of suitable filters. The effectiveness of the method was verified through comparisons of particle size distributions of potato starch granules without dust particles and with deposited dust particles after the application of the algorithm/filter. At the same time, determination was made of the dynamics of dust deposition in the laboratory (*ca.* 450 particles cm^{-2} h^{-1}).

K e y w o r d s: light microscopy, image analysis, particle geometry, starch, dust

INTRODUCTION

Analysis of images of the shape and size of particles finds an increasing application in many areas of agrophysics (Emadzadeh *et al.*, 2010; Esehaghbeygi *et al.*, 2010; Khojastehnazhand *et al.,* 2009) among others in the study of the morphology of granules of starch of various origin. Most of those studies are conducted with the use of microscopy (Gallant *et al.*, 1997) including scanning electron microscopy, SEM (Landillon *et al.*, 2008; Stasiak *et al.*, 2011), atomic force microscopy (Baldwin *et al.*, 1998), and light microscopy (Wilson *et al.*, 2006).

Measurements and analyzes realized with the help of light microscopy can be conducted both in a water medium (or another solvent) and in the dry state. In the case of dry measurements there is a real risk that the specimen will be contaminated with depositing dust particles. This constitutes an additional source of uncertainty that, depending on the type of particles examined, may cause a significant distortion of results.

The aim of the study was to develop a method permitting the elimination of measurement error caused by dust deposition in examination of the shape and size of starch granules with the method of light microscopy.

MATERIAL AND METHODS

The measurements were conducted on commercially available potato starch, using a microscope type Morphologi G3, Malvern (UK). The microscope was provided with software permitting the analysis of shape, size and particle size distribution (PSD).

The most important element of the procedure of measurement under a microscope (without referring to the phase of sampling and, if required, prior preparation of the specimen) is the placement of the specimen on the slide glass in such a way that the particles studied do not touch one another. At the same time it is highly important that the field of view of the microscope covers as many particles as possible, as the greater the number of particles analyzed the more representative the result. That stage of the research procedure was realized with the help of the SDU (sample dispersion unit) (Morphologi G3 User Manual 2008). In the measurement described the applied pressure was 0.14 MPa, and the time of settlement was 3 min. Both of the values were determined experimentally.

The first phase of the study was characterization of potato starch granules. The scan covered a circle of 1cm in diameter. The scan area was selected experimentally, so that on the one hand the number of particles within the scan area was large enough (representative measurement), and on the other that the time of measurement could be as short as possible (to reduce the effect of settlement of dust particles

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during the scanning itself). The measurements were conducted using a 20x objective (which corresponded to an actual magnification rate of 987 times). The objective provided rates of magnification high enough to allow limitation of uncertainty resulting from image analysis, and at the same time the duration of scanning with the objective was relatively short and amounted to *ca*. 80 min. The measurements were made in 12 replications (scans). The time elapsed between the first and the last scan was approximately 38 h (*ca*. 1.5 day).

The second phase of the study was characterization of dust particles and determination of the dynamics of settlement of those particles on the microscope slide glass. A clean slide glass was left uncovered for 24 h. Dust particles that settled on the glass during that time permitted focusing. The duration of slide glass exposure prior to measurement is not critical, as the dynamics of increase in the number of dust particles on the glass is referenced to the values of the first measurement.

The scanning of dust particles was conducted in three series: the first series lasted for 25 h, the second for 147 h, and the third for 132 h. After the first 25 h series of scanning a difference was noted in the dynamics of dust settlement during a day, therefore the subsequent times of scanning in the next two series were extended to *ca*. 6 days. The slide glass remained placed in the microscope all the time, which permitted the deposition of dust. The scan area and the duration of a single scan were identical as in the case of measurements conducted on starch granules.

The analysis of the shape and size of the particles was performed on the basis of parameters available in the microscope software (Morphologi G3 User Manual 2008) – size parameters: circle equivalent diameter (d_{CE}), area, length and width of particle, and non-dimensional parameters of shape: aspect ratio, circularity, convexity, and solidity.

RESULTS AND DISCUSION

The registered images of potato starch granules were similar to those reported in earlier publications (Stasiak *et al.*, 2009; 2011; Singh and Kaur, 2009). The PSD of starch granules, as described by the parameter d_{CE}, is presented in Fig. 1 (line No. 1), and Table 1 presents the mean values of parameters describing the morphology of potato starch granules calculated on the basis of image analysis. Also in this case the results obtained were in conformance with literature data (Baldwin *et al.*, 1998; Stasiak *et al.*, 2011). However, it was noted that in the successive replications of the measurement (successive scans) the size distribution of the same starch granules on the microscope slide glass shifted towards smaller values (Fig. 1 line No. 2, Figs 2 and 3). The cause of those changes was dust settlement.

The dynamics of increase in the number of dust particles on the slide glass during successive measurements is presented in Fig. 4.

The dynamics of increase in the number of dust particles on the slide glass was different on various days (Fig. 4). This could have results from differences in air humidity or in the time of the measurement day (the daily activity of employees increases the concentration of dust in the air). In view of that variability, the calculation of the mean increase in the number of dust particles during an hour may be burdened with a high uncertainty. Nevertheless, the results of all the measurement series were averaged and it was calculated that in our laboratory dust settles at an average rate of *ca*. 450 particles cm^{-2} h^{-1}. That information, with all its inaccuracy, is significant. It permits the duration of scanning to be planned *eg* through the determination of the size of the scan area or through the selection of the rate of magnification so as to minimise the effect of the process of dust settlement on the specimen. It is obvious that the dynamics of settlement of dust particles may vary significantly in various laboratories.

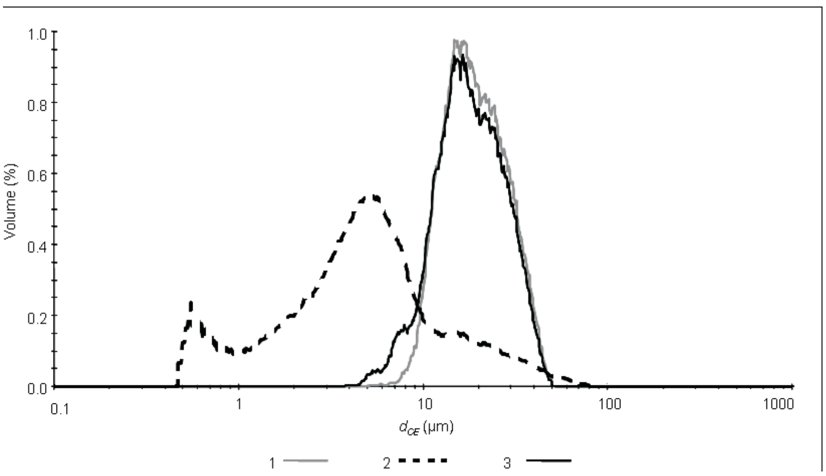

Fig. 1. PSD of potato starch: 1 – first measurement – without dust particles; 2 – measurement carried out about 38 h after the first one (influence of dust particles can be observed); 3 – the same measurement as in 2, but after the software procedure of elimination of dust particles.

T a b l e 1. Mean values of parameters describing the size and shape of starch granules and dust particles

Parameters	d_{CE} (μm)	Area (μm^2)	Length (μm)	Width (μm)	Aspect ratio	Circularity	Convexity	Solidity
				Starch				
Mean	9.89	179.61	11.58	9.15	0.80	0.78	0.87	0.93
SD*	2.05	68.21	2.28	1.84	0.00	0.03	0.03	0.01
CV**	0.21	0.38	0.20	0.20	0.00	0.04	0.03	0.01
				Dust				
Mean	3.17	12.56	4.10	2.93	0.77	0.89	0.97	0.96
SD*	0.40	3.11	0.50	0.40	0.03	0.03	0.02	0.01
CV**	0.13	0.25	0.12	0.14	0.04	0.03	0.02	0.01

*standard deviation, **coefficient of variation.

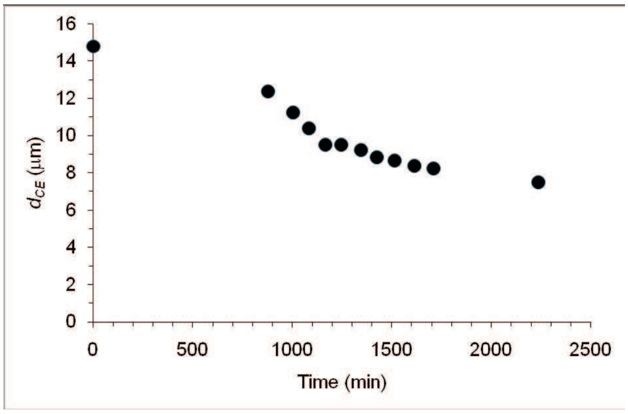

Fig. 2. Dynamics of changes in d_{CE} during successive replications of scanning of potato starch. The time of the successive measurements is counted in relation to the first measurement.

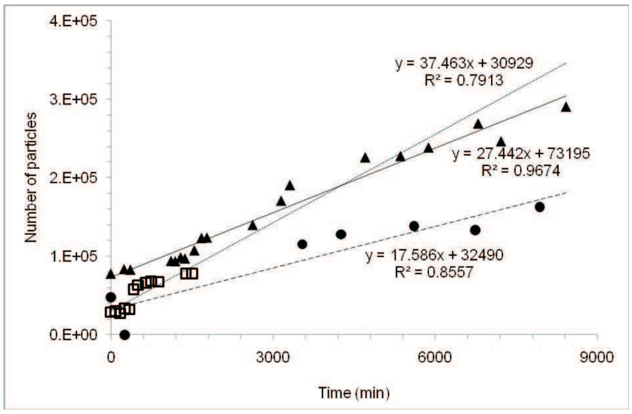

Fig. 4. Dynamics of increase in the number of dust particles on the scanned area of 3.14 cm^2 in 3 measurement series denoted with the symbols ▲ ● □.

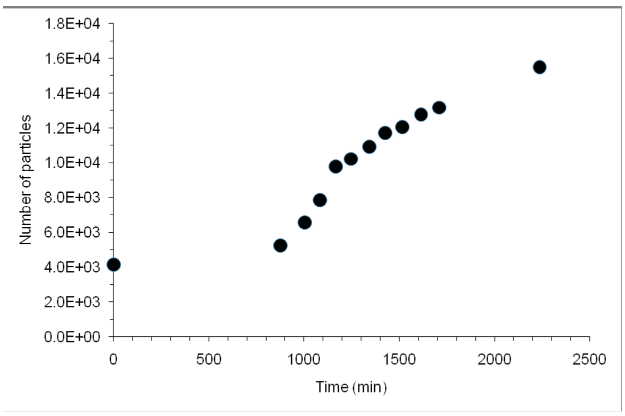

Fig. 3. Dynamics of changes in the number of particles during the successive replications of scanning of potato starch granules. The time of the successive measurements is counted in relation to the first measurement.

Therefore, every laboratory that does not have a possibility of air filtering should perform an analysis of the dynamics of settlement of their own dust.

An example of dust particle size distribution in our laboratory is given in Fig. 5. Table 1 presents the mean values of parameters describing the size and shape of dust particles. As can be seen from Fig. 5 and Table 1 the mean size of the dust particles is of the order of several microns. The important information is that the maximum size of the dust particles did not exceed 30 μm in length and 20 μm in width, which corresponds to *ca*. 20 μm d_{CE}. The values of practically all parameters of shape presented in Table 1 (circularity, convexity, solidity and aspect ratio) are characterized by very low variability (coefficients of variation up to 0.04). A greater variability was noted for the parameters of size (coefficients of variation up to 0.25).

Taking into account the differences in the size and shape of starch granules and dust particles (Table 1), an algorithm was developed for the elimination of the effect of the

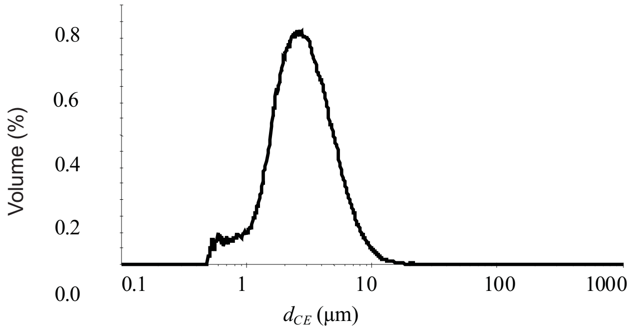

Fig. 5. Example of dust particle size distribution.

phenomenon of dust particles settlement on the results obtained. As the final measurement of starch granules was the most dust-contaminated, that measurement was selected for the assessment of the functionality of the algorithm.

The following filter, that could be defined within the software of Morphologi G3, was applied – elimination of all particles for which:

– d_{CE} was lower than 4 microns,

– parameter circularity was lower than 0.85,

– parameter solidity was lower than 0.985.

The effect of the filtering procedure could be accepted as satisfactory because the distribution of the remaining particles was very close to the distribution of starch granules from the first measurement *ie* in the situation when no dust had yet settled on the specimen studied (Fig. 1, lines Nos 1 and 3).

CONCLUSIONS

1. The primary source of the lack of repeatability of results of analyzes of the geometry of the studied particles was the settlement of dust on uncovered specimen under the microscope.

2. In cases where the shape and/or size of the particles under study differ significantly from the shape and/or size of dust particles the effect of dust settlement can be eliminated through the application of software filters.

3. In measurements of starch granules an effective filter reducing the effect of dust settlement can eliminate particles for which $d_{CE} < 4$ μm, circularity < 0.85 and solidity < 0.985.

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