Silver precipitation: a new and easy to use method for measuring of wheat kernel surface area

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Summary
Morphology, shape and surface area of wheat kernel are important factors for the characterisation of winter wheat (*Triticum aestivum*) varieties in the milling industry. The surface area of kernel, due to the lack of a simple and reliable method, is not measured, but usually calculated from the length and diameter of kernel. Silver precipitation, a new original method has been developed for the measurement of kernel surface area. This method is suitable for measuring the surface of cereal seed in case of individual kernel or more kernels together. According to the silver precipitation method, metal silver was precipitated on the kernel surface. After this step the precipitated silver was dissolved from the kernel and the silver concentration in the solution was measured by inductively coupled plasma optical emission spectrometry. The concentration of silver in the solution is in close correlation with the surface area of kernel. The method was calibrated to the direct surface measurement of individual kernels and was compared to the calculated surface results.

Index words: surface, wheat, kernel, silver precipitation, ICP-OES

Introduction
Kernel morphology and texture influence the value of winter wheat. Knowing the surface area and the thickness of bran the volume of bran can be calculated. Generally digital image analysis (DIA) is used to measure kernel length, width, perimeter on grain samples [Kwok 1984, Marshall *et al.* 1984, Kwok 1989]. Sometimes, the surface area was calculated on the basis of two or three dimensional pictures for one kernel, but it is not applicable for more kernels simultaneously. For the calculation of surface area, different methods are applied [Kwok 1990]. The most simple area calculation is the calculation of ellipsoid surface with two axes:
The ratio of major and minor axes of ellipsoid is characterized by the eccentricity (e_1):

$$e_1 = \sqrt{1 - \frac{b^2}{a^2}}, \; (1)$$

where „*a*" and „*b*" is the half of the major and minor axes. The surface of the ellipsoid can be calculated by the following equation:

$$A = 2b^2\pi + \frac{2ab\pi ArcSin(e_1)}{e}. (2)$$

The calculation of three axes ellipsoid according to Dieckmann [Dieckmann 2003] gives an estimation for the surface with less than 2.1% error applying the following equations:

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$$e_2 = \frac{ArcCos\left(\frac{c}{a}\right)}{\sqrt{1-\frac{c^2}{a^2}}} \qquad (3)$$

$$A = 2\pi\left(c^2 + abe_2 + \frac{b^2-c^2}{3ab}e_2^{\,3}\left(c^2 - \frac{a^2}{2} + \frac{a^4b^2+3a^4c^2-12a^2c^4+8b^2c^4}{40a^2b^2}e_2^{\,2}\right)\right) \qquad (4)$$

In most cases the equation (2) is applied for the estimation of the surface of the kernel because the two dimensional measurement can be carried out easily by image analyzing systems. None of these calculation methods takes into consideration the difference between the shape of wheat kernel and ellipsoid.

More precise surface and volume results can be obtained by parametric modeling of wheat grain morphology according to Mabille's method [Mabille & Abecassis 2003]. This model takes into consideration the shape of kernel and calculates with the depth of crease as well.

Our aim was to develop a simple, reliable and robust method for direct surface measurement where the number of kernels is not a limiting factor. Furthermore we wanted to measure the specific surface area (surface in mm^2 divided by mass in mg), and its relationship with the mass of kernel. The specific area of kernel could be a parameter that is applicable for numeric description of the shape. In the literature there was no available method for direct measurement, therefore we had to apply a new approach for solving the problem.

Materials and methods

Silver precipitation method for surface measurement of wheat kernel

According to the developed method metal silver was precipitated on the surface of the kernel in some steps. The precipitated silver was dissolved from the surface and the measured silver concentration of the solution is directly linked to the surface of the kernel. After calibration and validation the surface can be calculated from the measured concentration of silver in the solution.

The steps of silver precipitation, dissolvation and measurement are as follows:

1. The air dry wheat kernels (10-12% water content) were placed to 2 % (m/m) solution of ascorbic acid (Merck, extra pure, special grade C) for 5 minutes.

2. After removing the kernels from the solution they were placed on a filter paper at room temperature for 5 minutes. The kernels were not dried, only the drops of solution were eliminated from the surface.

3. The kernels were placed into a 0.1 M silver amine ($Ag(NH_3)_2^+$) solution and were shaken gently for one minute. The 0.1 M silver amine solution was prepared from $AgNO_3$ (Merck, Darmstadt, Germany) and concentrated NH_3 solution (Merck, Darmstadt, Germany). 1.70 g solid silver nitrate was dissolved in 80 ml deionized water and concentrated NH_3 solution was added in drops to the solution until the dark brown precipitated silver hydroxide totally dissolved. The solution was diluted to 100 ml with deionised water. In the silver-amine the surface of wheat kernels became black immediately that indicated the precipitation of metal silver.

4. The kernels were washed in deionised water and wiped off on filter paper for 1-2 minutes.

5. Either individual kernels or a certain mass (e.g. 1.00 g) of kernels were placed to a test tube and 1 ml concentrated HNO_3 was added for dissolving the metal silver. The black colour disappeared from the surface within 10 seconds and the kernels became golden brown.

6. 9 ml deionised water was added to the solution for dilution.

7. After mixing, the silver concentration of this solution was determined with a Perkin Elmer Optima 3300 DV inductively coupled plasma optical emission spectrometer (ICP-OES) on 328.068 nm wavelength. The diluted nitric acid solution contains dissolved organic matters in a very low concentration therefore it has no interfering effect on the ICP measurement of silver.

The silver precipitation in case of one kernel results 5-30 mg/L silver concentration in the diluted solution (step 6.), which is much higher than the silver detection limit (0.001 mg/L) of ICP-OES instrument. Therefore, the repeatability of silver measurement is excellent, it is below 0.2 %. The estimation of reproducibility of silver precipitation method has limitations, because each wheat kernel has a unique surface and the method is not reproducible on the same kernel. The total surface of 1.00 -1.00g of wheat kernel (25-35 kernels together) has smaller deviation than the individual kernels. The relative standard deviations of 1 g kernel measurements were between 2-4 %, which include the inhomogeneity of samples as well. The application of silver precipitation method itself, depends on the number of samples, requires 15-30 minutes. The ICP-OES measurement of one sample takes 1 minute, 200-300 sample can be measured daily.

The picture of wheat kernels in the different phase of silver precipitation surface measurement method is presented on Fig. 1. The size and the volume of kernel might have been changed somewhat by the effect of solutions. Because of the relatively short time periods of the first steps of silver precipitation method, the size of the black, silver coated kernel is almost the same as the original one. By the effect of nitric acid solution water can infiltrate into the deeper region of kernel, which is swollen, and sometimes the surface is cracked. This has no interfering effect on the measured surface values, but silver precipitation can not be repeated on the same kernel.

1. The original, air dry wheat kernel

2: The kernel with the precipitated silver layer on the surface

3: The kernel after dissolving the silver from its surface with nitric acid.

Figure 1. The same wheat kernel in different phases of silver precipitation surface measurement method.

Parameters and sample preparation for the scanning electron microscopy pictures
The structure and homogenous thickness of precipitated silver layer were crucial questions in developing the method. That is why a scanning electron microscope (AMRAY-1830I) with Energy Dispersive X-Ray Microanalysis interface (EDAX Inc.) was applied for the investigation of the precipitated silver on the wheat kernel. The silver-coated, black kernel was dried, cross cut and fixed in cold polymerized polymethyl-metacrylate (PMMA), polished and gold-coated by magnetron spattering deposition.

Results and discussions

Evaluation of the scanning electron microscopy pictures
The energy dispersive X-ray microanalysis made the identification of silver on the wheat and the estimation of thickness and homogeneity of precipitated silver layer possible. The scanning electron microscopy pictures (Fig. 2.) show a 20-25 μm thickness of silver layer. Silver was not detectable neither inside the kernel nor in the aleuron layer, but in the bran. There was no detectable inhomogeneity in the silver precipitation layer according to the energy dispersive X-ray microanalysis. The applied silver precipitation method resulted a homogeneous and well charcterised silver layer, therefore a correlation between the silver content and wheat kernel surface area was supposed.

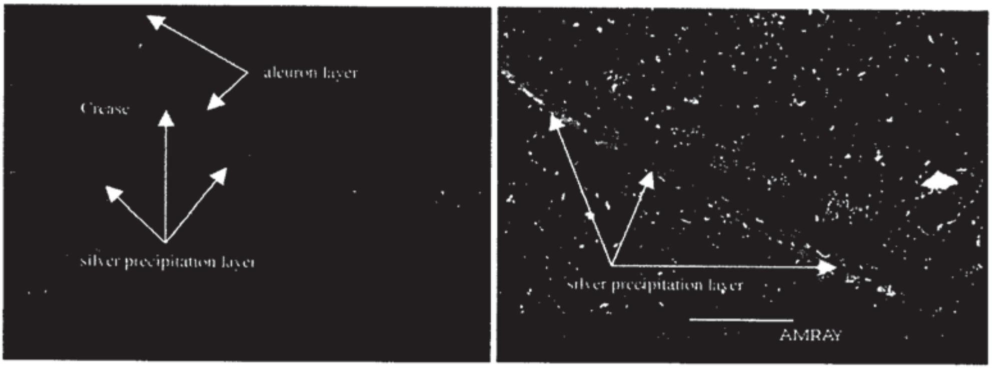

Figure 2. Scanning electron microscopic pictures of silver in the plane section of wheat kernel after silver precipitation.

Calibration of the method
Determination of the function between the measured silver concentration and the surface area is an important part of the method development. Since there is a lack of wheat surface standards and standard method, the measured silver concentration values were compared to the results of direct surface measurements. The direct surface measurement was a labor-intensive, difficult procedure. After the last step of silver precipitation method, which was the silver dissolving step, we got a yellow kernel. This kernel was placed in water for one day long. The kernel became soft and water saturated. The bran was cut with a sharp scalpel, removed with lab tweezers and placed to the flat glass of a HP Scanjet 5550 scanner. The flatted bran was scanned with 1200 dpi resolution. The area of bran on the scanned picture was evaluated and the surface was calculated with the Adobe Photoshop 7.0 software.
The correlation between the surface and the silver concentration can be described by equation 5:

$$A = 4.07 * C_{Ag10} = 4.07 * \frac{m_{Ag}}{0.01} \qquad R^2 = 0,9864 \qquad (5),$$

where C_{Ag10} is the measured silver concentration in mg/L unit in the 10 ml solution, m_{Ag} is the mass of silver in mg and A is the surface area in mm^2.

Surface values obtained by the silver precipitation method were compared to the surface values calculated by different methods. 20 wheat kernels of the MV 21 wheat variety were selected for comparison. The length, width and thickness of kernels were measured. The results of calculations according to equation 2, 4 and Mabille's method are compared on Fig. 3. The Mabille's method shows the closest correlation with the measured surface values. The ratio of measured and calculated surface was not independent from the size of kernel. In case of small kernels the calculations more often overestimate the surface significantly due to the difference in the theoretical and real shape of kernel (Fig. 4.).

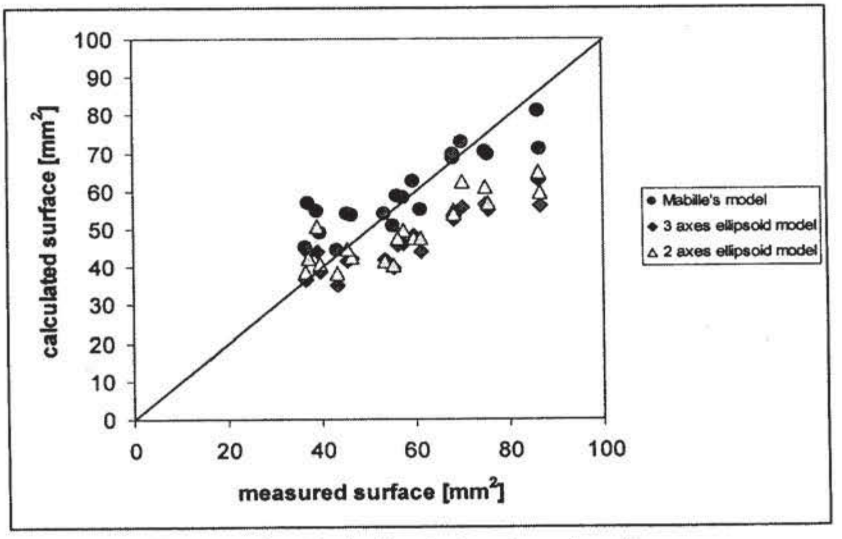

Figure 3. Comparison of measured and calculated wheat kernel surface areas.

Effect of concentration of applied solutions on the silver precipitation method

The sensitivity of the silver precipitation method to the concentration of ascorbic acid and silver amine solution is important because it determines the robustness of the method. The concentration of ascorbic acid was changed in the 0.5-8 % (m/m) range, the silver-amine concentration was changed in the 0.025-0.4 M range. Because the method is not reproducible on the same kernel, the precipitated silver on 1.00 g sample of kernels was measured in three replications with one set of solution. The obtained results are presented on Fig 5-6.

The concentration of ascorbic acid has significant effect on the mass of precipitated silver, therefore the ascorbic acid solution must be prepared accurately. 1 % deviation in the concentration of ascorbic acid solution results in approximately 1 % error in the calculated kernel surface area. The method is less sensitive to deviation of the concentration of silver-amine solution if the concentration is equal or higher than 0.1 M. 1 % deviation in the concentration of silver-amine solution results in approximately 0.1 % error in the calculated kernel surface area.

Determination of the kernel mass and surface function

If two objects with different volumes have the same shape the larger one has smaller surface area - volume ratio. It results that the volume - surface area function is nonlinear.

If the density of sample is independent from the size, the surface area - mass function must be nonlinear as well.

The surface of more than 100 kernels of winter wheat MV 21 variety was measured for the determination of individual kernel mass and surface function.

Surprisingly a linear correlation was obtained between the kernel mass and surface (Fig. 7.). Therefore, in case of one variety, the specific surface area, what is the surface area divided by the mass, is independent from the size of kernel (Fig. 8.).

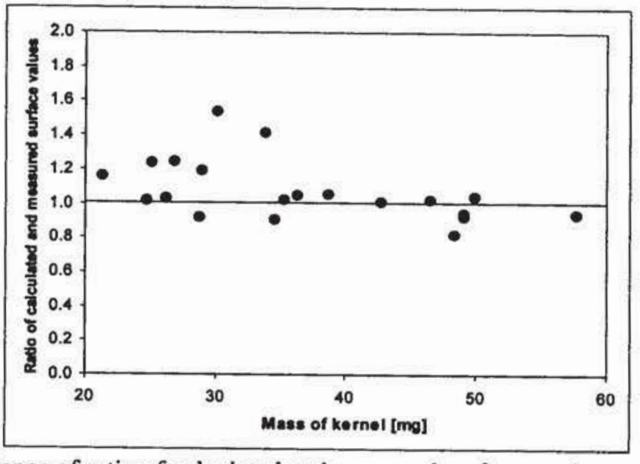

Figure 4. Dependence of ratio of calculated and measured surface on the mass of kernels. The origin and variety of kernels are the same.

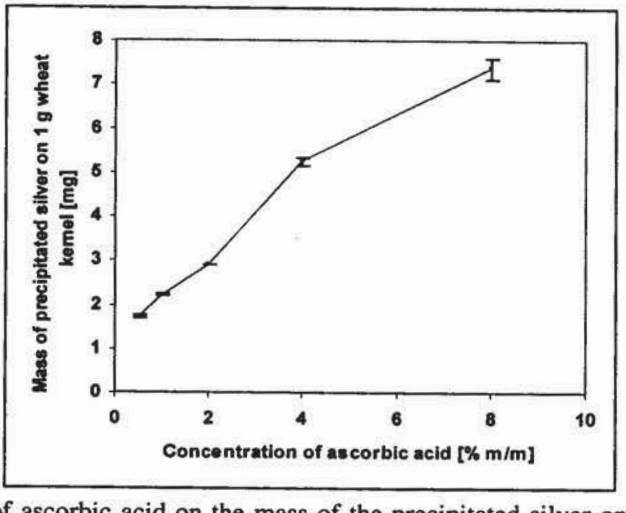

Figure 5. Effect of ascorbic acid on the mass of the precipitated silver on 1 g wheat kernel sample. The silver-amine concentration was 0.1 M.

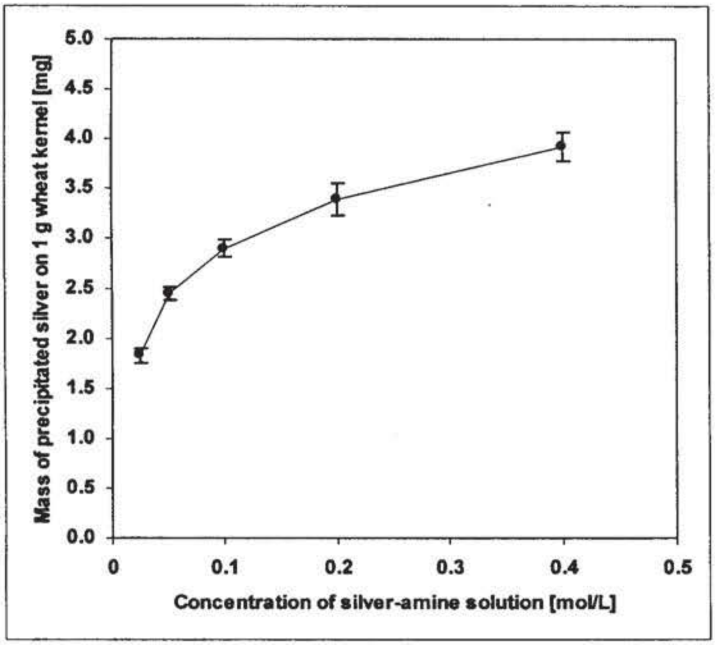

Figure 6. Effect of silver amine concentration on the mass of the precipitated silver on 1 g wheat kernel sample. The ascorbic acid concentration was 2 % (m/m).

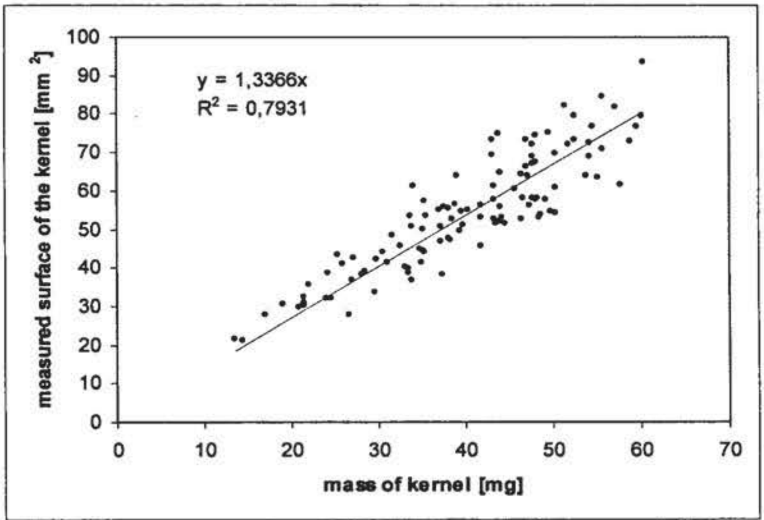

Figure 7. The measured surface area versus mass of kernel in case of MV 21 wheat variety.

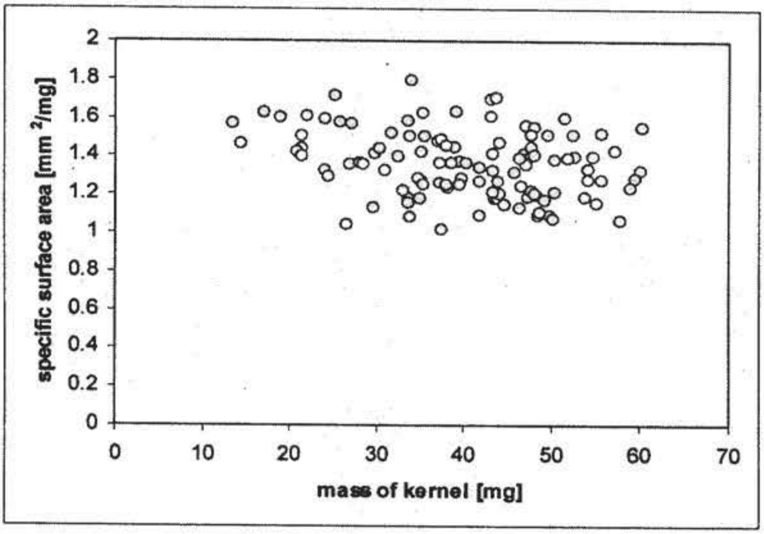

Figure 8. Correlation between the specific surface area and the mass of wheat kernel.

Conclusions

The developed silver precipitation method is suitable for measuring the surface area of wheat kernel and other seeds of cereals. It is an easy to use, not time consuming and well reproducible method. The surface area could be useful in the characterization of the shape of wheat kernel. The specific surface area was independent from the mass of individual kernels. This phenomenon was not described earlier. The small and large kernels could have the same specific surface area only if their shapes differ. The reasons of this phenomenon worth further investigations.

References

Kwok, S. (1984) Calculation and application of the anterior surface area of model human cornea. Journal of Theoretical Biology. Vol. 108. No. 2. 295-313.

Marshall, D.R. Ellison, F.W. and Mares, D.J. (1984) Effects of grain shape and size on milling yields in wheat. I. Theoretical analysis based on simple geometric models. Australian Journal of Agricultural Research. 35. (5) 619 – 630.

Kwok, S. (1989) The surface area of an ellipsoid revisited. Journal of Theoretical Biology. Vol. 139. No. 4. 573-574.

Kwok, S. (1990) Corrections. Journal of Theoretical Biology. Vol. 147. No. 1. 143.

Dieckmann (2003) URL: http://www.physik-astro.uni-bonn.de/~dieckman/SurfaceEllipsoid/SurfEll.html

Mabille, F. Abecassis, J. (2003) Parametric modeling of wheat grain morphology: a new perspective. Journal of Cereal Science. 37. 43-53.

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