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Evaluation of single-kernel density of scab-damaged winter wheat**

A. Grabowski[1], *R. Siuda*[1]*, *L. Lenc*[2], *and S. Grundas*[3]

[1]Institute of Mathematics and Physics, University of Technology and Life Sciences, Al. Prof. Kaliskiego 7, 85-789 Bydgoszcz, Poland
[2]Chair of Phytopathology and Molecular Mycology, University of Technology and Life Sciences, Ks. Kordeckiego 20, 85-225 Bydgoszcz, Poland
[3]Institute of Agrophysics, Polish Academy of Sciences, Doświadczalna 4, 20-290 Lublin, Poland

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A b s t r a c t. Measurements of single-kernel mass and volume made on healthy (control) and scab-damaged samples of grain of three winter wheat varieties never resulted in lower values of mean single-kernel density for scab-damaged grain. This finding, contrary to common opinion, can be explained as being a result of the comparable magnitude of relative decrease (due to infestation) of two features (mass and volume) that define single-kernel density. The discrepancy between results presented in this paper (kernel volume was determined with an air pycnometer) and the results in some other reports (liquid pycnometers used) can result from the different methods applied for kernel volume measurements: when a liquid medium is used the surface tension effect tends to overestimate the volume, especially for scabby kernels that are known to be shrivelled *ie* possessing voids and pores at the surface that the liquid cannot penetrate. As a consequence kernel density of scabby kernels can be significantly underestimated.

K e y w o r d s: wheat, scab, single-kernel density

INTRODUCTION

Fusarium spp. invading grain is known to cause a grain disease called fusarium head blight or scab. Scab-damaged kernels differ from sound kernels in many respects. First of all, they differ in chemical composition, also due to contamination with mycotoxins, which results in a lowering of grain quality (Bechtel *et al.,* 1985; Boyaciouglu and Hettiarachchy, 1995; Seitz and Bechtel, 1985; Wąsowicz, 1991). Changes in chemical composition are accompanied by changes in physical properties, such as mass, volume, density, kernel colour, hardness, *etc*. These changes can influence grain processing parameters dependent on physical traits (Dobraszczyk *et al*., 2002; Dziki and Laskowski, 2004, 2005) and some procedures proposed for improvement grain quality (Siemens and Jones, 2008). Furthermore, changes in physical properties can also be a basis for the development of different approaches for rapid and cost-effective methods both for grading grain quality and for improving the quality of grain by removal of damaged kernels (Dowell *et al*., 2006; Pearson *et al*., 2007). Huff (1980) and Huff and Hagler (1982, 1985) used buoyancy of grain in liquids of various densities for the separation of less dense (more buoyant) fractions of grain. The buoyant fractions were much more contaminated with mycotoxins than the samples of grain before separation. Tkachuk *et al*. (1991) showed a distinct negative correlation between kernel density and the intensity of visible symptoms of scab in wheat. Mycological and chemical examinations of the samples investigated by Tkachuk *et al*. (1991) proved that the higher the level of infestation with *Fusarium* and contamination with deoxynivalenol, the lower was the density of wheat kernels. Our own preliminary evaluations of SK (single-kernel) density of control and scabby grain for two varieties (cultivated in the years 2004 and 2005), based on measurements of SK mass and volume, showed greater mean SK density for scabby grain (Siuda and Sadowski, 2005). Although SK volume measurements came from a simple homemade air micropycnometer, careful analysis of the instrument properties revealed no reasons for erroneous systematic underestimation of smaller volumes (Siuda and Rozwadowski, 2006). Therefore, a more advanced air micropycnometer was developed and constructed, and the measurements were repeated on three varieties of winter wheat cultivated and harvested in the year 2006. The obtained results showed

*Corresponding author's e-mail: siuda@utp.edu.pl
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again that mean SK density of the control (healthy) grain again did not exceed the density of the scabby grain samples. Similar results ware also obtained in the year 2007. It has to be stressed, however, that several definitions for SK density are in use (Chang, 1988). In the present report SK density is the ratio of SK mass to SK volume, the latter as determined with air pycnometer *ie* as the volume that can include possible internal voids inaccessible for air.

The aim of the study is to provide detailed evidence that the mean single-kernel density can be approximately the same in both healthy and scab-damaged kernels. Furthermore, we also want to explain why this finding may only seemingly contradict the results reported in the some other papers (Huff, 1980; Huff and Hagler, 1982, 1985; Martin *et al.,* 1998).

MATERIALS AND METHODS

Two lots of three varieties (Satyna, Tonacja, Trend) of winter wheat (*Triticum aestivum* L. *emend*) grain were obtained from two plots cultivated in the year 2006. One lot of each variety was harvested from a plot where the crop grew in natural conditions, while the other one was harvested from a plot where inoculation of the heads with a suspension of *F. culmorum* conidia (concentration 1 10^6 cm^{-3}, intensity of the sprinkling 0.09 dcm^3 of the suspension per m^2) was carried out during the flowering stage. Grain from the plots where inoculation was not applied will be referred to as control, whereas that from the inoculated plot as scabby or damaged. The control grain had no visual symptoms of infestation. After harvesting and threshing, lots of both control and damaged grain were dried in air at room temperature, then stored in the same conditions for about two months and the measurements were then carried out within three weeks, subsequently for samples taken from both control and damaged lots of particular varieties.

From the lots of control and damaged grain, samples of 377 to 430 kernels were taken randomly. For each kernel were measured both mass and volume and SK density was calculated as the ratio of SK mass to SK volume. SK masses were taken from kernel examinations made one by one with an SKCS 4100 (Single Kernel Characterization System, Perten Instruments, Springfield, IL) device that allowed measurement of mass at position 0.1 mg. SK volumes were measured with an homemade air micropycnometer prior to the SKCS measurements.

The principle of SK volume measurement is based on measurements of air pressure in the micropycnometer chamber (with pressure sensor HPXA 6115A, Semiconductors, US) before and after its expansion, and when the chamber is either empty or containing a kernel. Knowledge of the four pressures permitted determination of the volume of the SK on the basis of the law describing the polytropic process:

$$pV^k = \text{const}, \tag{1}$$

where: p and V stand for the pressure and volume of a constant amount of gas, respectively, while k is an exponent of the polytropic process. Test measurements showed that if the time between the expansion and pressure readouts was set as at least 0.7 s, the process could be assumed as an isothermal one. Denoting measured pressures as p with subscript 1 or 2 for the values before and after expansion, respectively, and superscript 0 for an empty chamber, while without superscript for the chamber with a kernel, Eq. (1) allows us to write two equations:

$$p_1^0 Vc = p_2^0 (V_c + V_a),$$

and

$$p_1 (V_c - V_k) = p_2 (V_c + V_a - V_k), \tag{2}$$

where: $V_c = 297.8 \pm 5.4$ mm^3, $V_a = 255.4 \pm 4.6$ mm^3 and V_k denote the volume of the chamber, the additional volume due to expansion and the volume of the kernel, respectively. The above equations lead to the formula:

$$V_k = V_c \left(1 - \frac{(p_1^0 / p_2^0) - 1}{p_1 / p_2 - 1} \right), \tag{3}$$

that enables one to determine kernel volume based on measurements of relevant pressures. Calibration of the pycnometer was made with six steel balls of volumes ranging from 7.92 ± 0.08 to 48.84 ± 0.21 mm^3 determined on the basis of precisely measured diameters. Coefficient of determination for the relationship between volumes measured with the pycnometer and calculated volumes was 0.9995. The pycnometer gave no possibility for using gas other than atmospheric air. The measurement of kernel volume starts with putting a kernel by hand into the measuring chamber, and the computer that controls the chamber expansion process, pressure measurements and data acquisition is then activated. Prior to the measurement of every twenty kernels, a measurement of pressure with an empty chamber is made and used in the volume determination of these twenty kernels. The cycle for one kernel lasts *ca*. 5 s, however, the necessary hand operations elongate this time and practical throughput of this half-automated instrument is *ca*. 120 kernels per hour.

RESULTS AND DISCUSSION

SKCS measurements provided values for SK mass, width, moisture and hardness index. From these parameters only mass was taken for SK density determination. Influence of fusariosis on remaining SK traits is currently elaborated and will be published separately. Inspection of raw data on SK density obtained directly from measurements showed that a number (not numerous) of items were obviously incorrectly recorded. Therefore, data from each sample of grain were systematically tested for the presence of outliers based on statistical properties of the series of SK density values.

For each series the first quartile, Q_1, and the third quartile, Q_3, were determined and the data outside the range $[Q_1\text{-}t(Q_3\text{-}Q_1); Q_3\text{+}t(Q_3\text{-}Q_1)]$ removed, where the value of factor, t, equal to 3, is recommended for discarding extreme outliers, while t = 1.5 for the so called middle outliers (Tukey, 1977). Outlier removal was performed twice, once with each of both of the above values. In most cases the number of outliers was a dozen or so. The resulted values of SK density for all samples and applied procedures for outliers removal, as well as for raw data, are listed in Table 1.

The series of data obtained after discarding the middle outliers was taken for further analysis, since the properties of such data can be expected to be more regular. Some statistical parameters characterising the results of measurements selected in a such way are listed in Table 2. Additionally, plots of cumulative distribution functions were made in order to get a better insight into the properties of data series. Fig. 1 present such plots for only one variety, since for the remaining two the plots were qualitatively similar.

The accuracy of directly measured SK mass and volume can be characterised by standard combined uncertainties $u_C(m)$ and $u_C(V)$, respectively, taking into account standard uncertainties of type A and type B according to the formula (Anonymous, 1993):

$$u_C(x) = \sqrt{u_A^2(x) + u_B^2(x)}, \quad (4)$$

where: x stands for m or V. As the readout for mass was at position 0.1mg, the standard uncertainty of type B for mass measurements can be assumed to be equal to $u_B(m) = \pm 0.1/\sqrt{3} = \pm 0.058$ mg. Evaluation of type A uncertainty would need a series of measurements to be made for the same mass (single kernel). This was not feasible with the SKCS 4100, since the instrument crushed the examined kernel and no repetitions of the mass measurements were possible. Therefore, standard combined uncertainty for mass has to be temporarily assumed equal to $u_B(m)$ and as such it is most likely underestimated. Eight reference volumes were measured in 15 repetitions in order to get information on $u_C(V)$. The results showed that relative standard uncertainty, $u_C(V)/V$, never exceeded 0.02. As SK density is defined as the ratio of SK mass to SK volume, the standard uncertainty of density, $u(d)$, can be expressed by:

$$\frac{u(d)}{d} = \sqrt{\left(\frac{u_C(m)^2}{m}\right) + \left(\frac{u_C(V)^2}{V}\right)}. \quad (5)$$

Table 1. Maximum, mean and minimum values for SK density (g cm^{-3}) in all series of measurements for raw data and after removal of two types of outliers

Variety	Category	Type of outliers removed								
		None (raw data)			Extreme			Middle		
		max	mean	min	max	mean	min	max	mean	min
Satyna	Control	2.020	1.435	0.886	1.643	1.436	1.286	1.561	1.430	1.311
	Damaged	2.571	1.442	0.824	1.761	1.436	1.070	1.639	1.439	1.233
Tonacja	Control	2.222	1.454	0.923	1.677	1.449	1.207	1.590	1.449	1.308
	Damaged	3.483	1.483	0.704	1.868	1.462	1.039	1.705	1.455	1.223
Trend	Control	2.379	1.448	0.872	1.593	1.444	1.276	1.562	1.443	1.322
	Damaged	5.369	1.476	0.171	1.768	1.447	1.129	1.625	1.450	1.268

Table 2. Mean values and standard deviations for SK mass, volume and density obtained from measurements made on samples of control and damaged grain of three varieties. Middle outliers were removed from the data prior to calculations. The number of kernels for which data were left after outliers removal is shown in column three

Variety	Category	No. of kernels	Mass (mg)	Volume (mm^3)	Density (g cm^{-3})
Satyna	Control	380	49.1±9.0	34.4±6.5	1.430±0.046
	Damaged	360	39.0±13.1	27.1±9.0	1.439±0.077
Tonacja	Control	369	51.4±7.8	35.5±5.5	1.449±0.052
	Damaged	339	38.0±11.7	26.2±8.3	1.455±0.090
Trend	Control	414	46.7±8.7	32.4±6.2	1.443±0.044
	Damaged	349	36.9±11.4	25.4±7.8	1.450±0.069

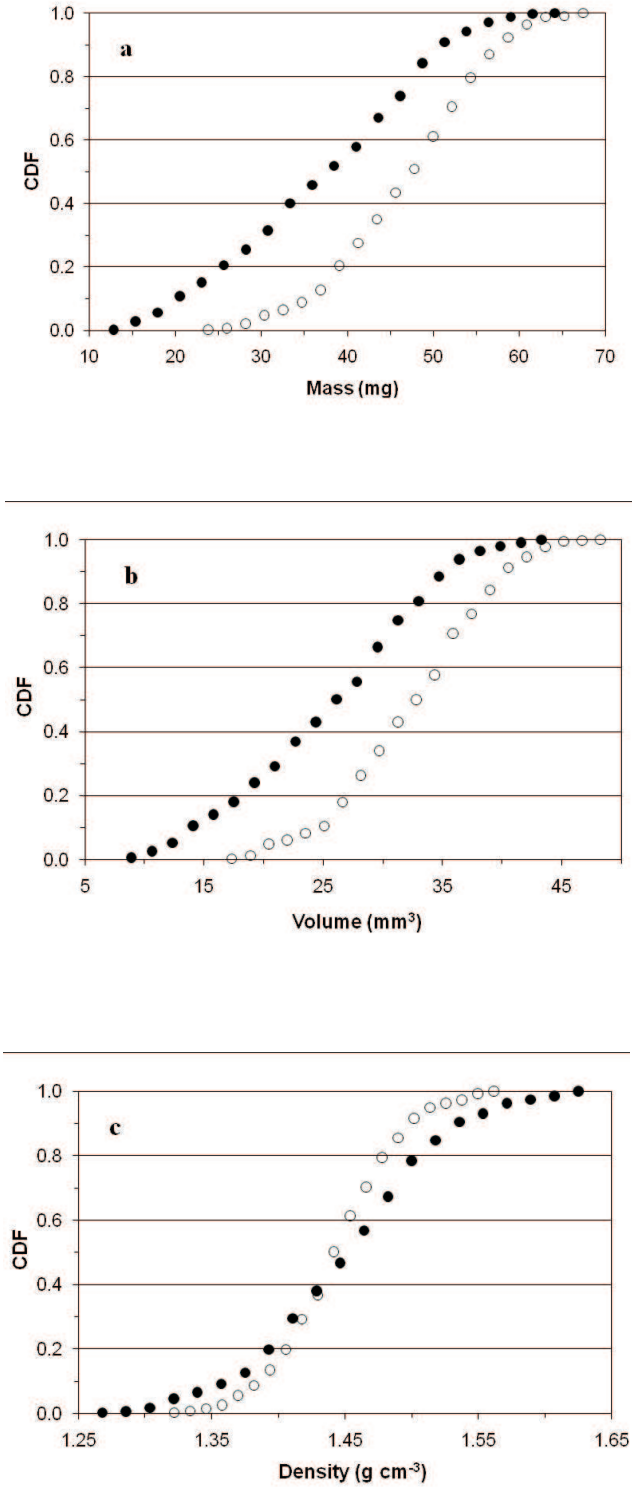

Fig. 1. Cumulative distribution functions (CDF) for single kernel: a – mass, b – volume, and c – density distributions for cv. Trend; ○ control grain, ● damaged grain.

Formula (5) suggests that the absolute value of density uncertainty could increase for lighter kernels. As a consequence, one may expect to appear a similar property in the standard deviation of SK density that resulted from measurements. The knowledge of the actual dependence of the observed relative standard deviation of SK density, $s(d)/d$, versus SK volume could be helpful in settling if this is important. Figure 2 presents plots illustrating such dependence for samples of both control and damaged grain calculated within eight subintervals of equal widths (hereafter called octants) covering the whole range of SK volume for only one variety because no qualitative differences were found for the remaining two varieties. The greater values of relative standard deviation of SK density seen for damaged grain could be expected as a result of the smaller volumes of damaged kernels. For variety Trend, mean SK volumes in the extreme octants were 19.3 and 46.4 mm^3 for control samples, and 11.1 and 41.2 for damaged samples. This result in the ratios of mean SK volumes in relevant SK volume octants calculated for control and damaged samples equal to 1.74 and 1.13, respectively. One can see from Fig. 2 that these ratios are close to the ratios of relative standard deviations of SK density for damaged and control samples within relevant octants. Hence, the above evaluation allows us to assume that the observed dependency, for both categories of grain, of relative standard deviations of SK density on SK volume could be caused by changes in SK volume. This assumption is worth being verified more precisely.

The observed standard deviation of SK density, $s(d)$, can be treated as a result of the natural diversity characterising kernels in the sample, $s_n(d)$, and the uncertainty of density measurements, $u(d)$. The approximate relationship between quantities mentioned above can be written:

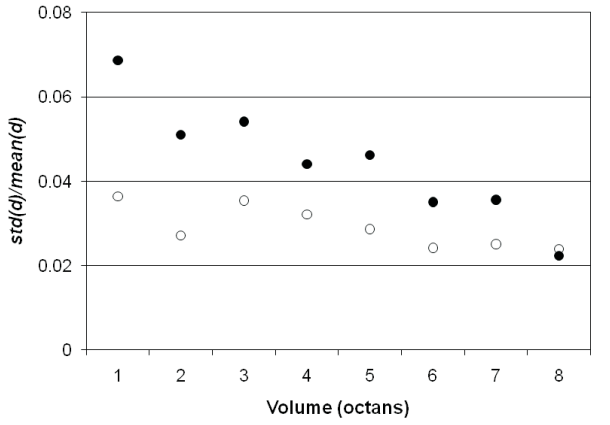

Fig. 2. Relative standard deviation of SK density *vs* SK volume for: ○ control and ● damaged samples of cv. Trend, calculated within eight equal widths subintervals (octants) covering the relevant whole ranges of SK volume.

$$s^2(d) \cong s_n^2(d) + u^2(d), \quad (6)$$

and using Eq. (5) it can be rewritten as:

$$\left(\frac{s(d)}{d}\right)^2 \cong \left(\frac{s_n(d)}{d}\right)^2 + \left(\frac{u_C(m)}{Vd}\right)^2 + \left(\frac{u_C(V)}{V}\right)^2, \quad (7)$$

where: $m=Vd$ has been substituted. Eq. (7) can be applied to the data presented as plots in Fig. 2. In each octant the values of $s(d)$, d and V are known, so eight equations can be obtained from Eq. (7) with three unknown parameters common for all the equations. This gives a set of linear equations that enables us to find the unknown values of $s_n(d)$, $u_C(m)$ and $u_C(V)$. In order to obtain statistically more confident results, the range for values of SK volume was divided into different equal subintervals, ranging from 6 (sextants) to 10 (decants). Each time the relevant set of equations was solved for unknowns with the least-squares method. The resulting mean values and standard deviations for the unknowns are listed in Table 3. All parameters are noticeably greater for damaged samples, especially $s_n(d)$. This finding is not surprising since a greater dispersion of SK density in damaged material was expected. Increase of $u_C(V)$ for damaged sample was also expected because of possible influence of a more developed morphology of shrivelled infested kernels surface on pressure measurements after expansion in a micropycnometer chamber. But the small systematic (for all varieties) increase of $u_C(m)$ is more surprising. This finding suggests that the weighing device in the SKCS 4100 (under experimental conditions in the course of our measurements) was characterized by a weak dependence of the sensitivity on the measured mass. Linear fit to $u_C(m)$ versus mean SK mass (Table 2) dependence resulted in a slope equal to -0.0073 and R^2=0.53. It is worth pointing out that $u_C(m)$ values in Table 3 exceed by *ca*. one order of magnitude the evaluation of $u_B(m)$ given above. Hence, the actual accuracy of SK mass measurements was well below expectations based on the precision of the readout only. Its relative value $u_C(m)/m$ (averaged over varieties) was equal to *ca*. 0.014 and 0.020 for control and damaged samples, respectively. A similar evaluation for $u_C(V)/V$ gives 0.011 and 0.018, which results in a total experimental relative uncertainty (Eq. (5)) equal to 0.018 and 0.027 for control and damaged samples, respectively. If the calculation of $s_n(d)/d$ is based on averaged values of $s_n(d)$ and density within category, the results are 0.023 for control samples and 0.038 for damaged. These values, compared with the former ones, show that the total uncertainty due to measurements is expected to cause a narrower dispersion of SK density values when we compare it to so-called natural diversity. Nevertheless, the measurements could increase the experimental dispersion a little. Table 3 provides also comparison of $s(d)/mean(d)$, which were obtained on the basis of obtained unknowns and the right hand side of Eq. (7), with the values obtained from mean and standard deviations for SK density listed in Table 2. One can see that retrieved and experimental values are quite similar in all samples.

To conclude, the experimental relative standard deviation of SK density increased a little due to uncertainties of measurements, however, this increase should not affect mean values in the samples since both signs of experimental uncertainties can be expected to be equally probable.

Data from Table 1 show that there is no systematic influence of outliers on mean SK density in the analyzed samples, while their influence on the scatter of density values is clearly seen. Raw data contained a number of obviously false measurements that, if discarded, improved the properties of all data series independently if the extreme or middle outliers were removed. Considerable changes of mean SK mass and volume due to infestation are clearly seen from the values listed in Table 2. It can also be seen that the changes have expected signs, *ie* both features have lower means for damaged grain. Furthermore, standard deviations for damaged samples exceed distinctly standard deviations for control samples. The plots presented in Fig. 1 demonstrate, especially for damaged grain, that strong deviation from the

T a b l e 3. Values of standard deviation of single-kernel (SK) density, $s_n(d)$, uncertainties of measurements of SK mass, $u_C(m)$, and volume, $u_C(V)$, and relative standard deviation, $std(d)/\text{mean}(d)$ for all examined samples of grain. The latter quantity was determined either directly from results of measurements (Exp.) or retrieved (Retr.) from Eq. (7) using relevant values for $s_n(d)$, $u_C(m)$ and $u_C(V)$.

Variety	Category	$s_n(d)$ (g cm^{-3})	$u_C(m)$ (mg)	$u_C(V)$ (mm^3)	*Std(d)/mean (d)*	
					Exp.	Retr.
Satyna	Control	0.033±0.002	0.70±0.04	0.40±0.07	0.032	0.030
	Damaged	0.053±0.003	0.80±0.03	0.54±0.07	0.053	0.046
Tonacja	Control	0.035±0.003	0.72±0.04	0.43±0.05	0.036	0.030
	Damaged	0.066±0.004	0.79±0.03	0.49±0.05	0.062	0.053
Trend	Control	0.033±0.001	0.64±0.02	0.32±0.04	0.031	0.029
	Damaged	0.047±0.002	0.71±0.02	0.42±0.03	0.047	0.041

shape characteristic for normal distribution can be seen even visually. Contrary to plots for mass and volume, the plots for density shown in Fig. 1c resemble the shape typical for normal distribution, both for control and damaged grain.

The increase in the standard deviations of measured features observed for samples taken from damaged grain (Table 2 and Figs 1) was expected because infestation, as an additional source of variability, should broaden the observed variance for the values of measured features in infested samples. Furthermore, comparison of the shapes presented in Fig. 1a, b shows a shift towards lower values and over-representation at these lower values for damaged grain. This finding is understandable in light of the well-known effects of fungal infestation, such as loss of kernel mass, shrinking and shrivelling. As a consequence, a lowering of mean values for SK mass and volume has to appear in samples of damaged grain. How these effects influence SK density depends on which one is prevailing. When mass loss prevails, the density is lowered, and when shrinking, the density is increased. Ratios of mean SK values of mass to mass and volume to volume calculated for damaged and control samples give the values 0.794, 0.739, 0.790, and 0.788, 0.738, 0.784 for the three varieties, respectively. These ratios show that losses of mass and volume due to infestation were closely comparable to each other in the investigated samples. It resulted in a much weaker change in the mean SK density.

The results of more than three hundred measurements support any of the values set in Table 2 i*e* a quite ample amount of data. Discussion on the accuracy of the measurements from the preceding subsection together with the numerous data sets give strong support for the plausibility of each of the means from Table 2. The Kruskal-Wallis non-parametric one-way analysis of variance, used for evaluation of the significance of the differences observed within varieties, classified the differences for mass and volume as significant (confidence parameter $p = 0.01$) for all varieties. In the case of SK density, the differences were significant for cvs. Satyna and Trend ($p = 0.05$), while not significant for cv. Tonacja.

The results presented above on the SK density contradict the common opinion about density of scabby kernels. Moreover, at first sight, they are inconsistent with the results reported in the cited papers (Huff, 1980; Huff and Hagler, 1982, 1985). However, this contradiction may only be apparent, since whether the kernel is buoyant or not depend on its density and the density of the liquid as well as the ability of the liquid to wet the surface of the kernel and penetrate possible pores. For kernels of smooth surface their density is a dominant factor in determining their buoyancy, while for kernels with a more developed surface morphology the surface tension of the liquid may be even more important. The role of surface tension in determination of wheat density was pointed out by (Fang and Campbell, 2000). The authors stated that wheat density, determined with gas pycnometers, gave values higher than those determined with liquid pycnometers by up to 10% (depending on the liquid). They assigned this difference to lower ability of the liquids to penetrate the pores on the kernel surface. There are also other reports which support this statement. Chang (1988) measurements (helium gas pycnometer) on healthy grain of four varieties of wheat resulted in 1.447 g cm^{-3} on average. This value is close to the ones form our studies, while (Martin *et al*., 1998) measurements with liquid pycnometer made on samples from eight wheat varieties resulted in averaged SK density 1.28 g cm^{-3} for healthy kernels and 1.08 g cm^{-3} for scab-damaged ones, both considerably lower than our and Chang results. The lower results even for healthy kernels reported by Martin *et al*. (1998) can likely be ascribed to poor wettability of kernel surfaces at the crease (anonymous Referee suggestion).

CONCLUSIONS

1. The presented results show that the samples of kernels, which are taken from lots of scabby grain of three winter wheat varieties, have a mean single-kernel density that is not lower than the control grain. This finding, which is contrary to common opinion, can be explained as being a result of the comparable magnitude of relative decrease of two features (mass and volume) defining density.

2. In experiments where an interaction of a liquid with grain takes place two hypothetical effects can be expected due to surface tension. Firstly, the limited ability of the liquid to wet the surface of the kernel can result in enhancement of a kernel buoyancy, especially of scabby kernel as its surface morphology is more developed.

3. Hence, the buoyancy of scabby kernels is connected more with the limited ability of the liquid to wet kernels surfaces than with the difference in the density of the kernel and liquid. As a consequence, removal of the buoyant fraction from a studied lot of grain makes this method still effective for the separation of more scabby grain from the lot. Secondly, liquid pycnometers overestimate kernel volume, especially for damaged grain, though it can also apply for healthy grain due to poor wettability of kernel surfaces at the crease. As a consequence the more the morphology of a kernel surface is developed the more kernel density can be underestimated when it is determined by liquid pycnometry.

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