IODOMETRIC METHOD OF THE DETERMINATION OF THE EXPOSED DAMAGE SURFACE OF CEREAL GRAIN

A. Miś[1], S. Grundas[1], Y. Takeda[2], S. Hizukuri[2]

[1] Institute of Agrophysics, Polish Academy of Sciences, Doświadczalna 4, 20-236 Lublin, Poland
[2] Department of Biochemical Science and Technology, Faculty of Agriculture, Kagoshima University
21-24 Korimoto-1, Kagoshima 890, Japan

A b s t r a c t. In this paper, a method for determining the area of the exposed damage surface of grain endosperm by means of the amperometric measurement of iodine adsorption has been presented.

According to the amperometric method, iodine is liberated by titrating standard potassium iodate into an acid solution containing potassium iodide and starch. In the place of starch specimen, the grain sample of a small amount was used. The elaborated method is based on a strict separation of a total adsorption of iodine by grain sample on its following parts: the grain coat adsorption and the exposed damage surface adsorption considering the coefficient of iodine adsorption by unit surface of damage endosperm. The separation of the total adsorption was possible owing the use of a test damage surface with measured area and a different soaking time of grain sample.

Basing on this method three kinds of barley grain originating from various growth regions were evaluated. It was found that the total adsorption of iodine by tested grain is determined in a different degree by both features of coat and endosperm in relation to the origin of the grain. This fact indicates that only the surface area of the exposed damage can constitute an objective and accurate measure for this kind of mechanical damage of cereal grain.

K e y w o r d s: cereal grain, mechanical damage, iodometry, area of exposed grain endosperm surface

INTRODUCTION

The mechanical damage is characterized as the disturbance of the tissue continuity of grain, that is of various form, from the attrition of the grain coat and its crack going to inside of the grain endosperm, through the deforma-tion and breakage to the complete crumbliness of grain [5].

For all these forms of damage, the joint feature is an exposed damage surface of grain endosperm. Therefore it seems that an area of the exposed damage surface is a proper measure of the amount of this kind of damage to grain sample.

Aiming at the estimation of this parameter, the amperometric method of determining iodine sorption by starch [3] was adopted. According to the amperometric method, iodine is liberated by titrating standard potassium iodate into an acid solution containing potassium iodide and starch. In the place of starch specimen, the grain sample of a small amount was used. This replacement does not disturb the principle of the amperometric method because the damage surface of grain also contains mainly starch components and in the case of the exposed damage of grain endosperm, iodine can be very easily adsorbed by its surface.

It is obvious that the liberated iodine du-ring titration is adsorbed with a different in-tensity both by the exposed damage surface and the grain coat as well as by grains of vari-ous origin and variety. Among existing meth-ods worked out by Mc Ginty [4], Chowdhury and Buchele [1], and Christenbury and Bu-chele [2] and based on the estimation of total

sorption of different solutions by grain as a measure of amount of mechanical damage, there is not one that dissolved these methodological difficulties. Therefore the mentioned methods give total measurement values from which do not result real amounts of mechanical damage.

The proposed method of the determination of the area of the exposed damage surface allows to eliminate this obstacle by the use of a proper procedure and by addition of a test damage surface to grain sample. The principle of the method consist in the separation of the total iodine adsorption by grain sample on its following parts: the grain coat adsorption and the exposed damage surface adsorption considering the coefficient of iodine adsorption by damage surface. On this basis the area of the exposed damage surface to grain sample can be exactly calculated and accepted as a measure of the amount of this kind of grain damage.

MATERIALS AND METHODS

The studies were conducted on 3 lots of barley grain (*Hordeum sativum Jess*) differing as to the place of growth. A Canadian and Australian barley were obtained from a commercial feed shop in Kagoshima, whereas Japanese one came from the Experimental Station of the Kagoshima University from the harvest of 1993.

Before the studies all the grain samples were cleaned off any foreign material and dust and the fraction with thickness below 2.0 mm was separated. The moisture of the studied samples was determined by standard method (130 °C for 1 h). The weight of a one grain for the samples was calculated from the weight of 1000 whole grains taken randomly.

The test (artificial) damage surface of the grain was made by transversely cutting a single grain throughout its middle by means of a sharp blade. Only a whole grain without visible losses of its parts, was taken to the cutting. The obtained test damage surface of the grain was flat without any cavities or cracks.

The test damage surface per gram of the cut grain for all kinds of barley grain was determined on the basis of the measurement of the surface area of the test damage with utilization of a xerographic technique and using the following procedure. The 50 whole grains were cut and halves of the grains were put their cut side on the plexiglass bottom of the square cassette. The regular arrangement of the grain halves on the cassette prevented adjoining each other. The cassette with grain halves was put on a screen of a xerocopier (Fuji Xerox type Able 1301) and made a xerocopy at 400 % of the magnification. Then the calculated weight ratio of the paper with test damage surface pictures to the paper with picture of whole cassette was multiplied by a real surface area of the cassette and divided by the weight of the cut grain sample for obtaining the area of the test damage surface per gram of cut grain. Measurements of this parameter were made in two repetitions.

The calculated area of the test damage surface was utilized at using different weight additions of the cut grain to the samples as the equivalence of a definite area of the exposed damage surface. The procedure of using these additions was as follows. From 10 g grain sample only the whole grains were taken to the cutting and such their number that their surface after cutting was the equivalence of the test damage surface required. After the cutting, the cut grains were put back into the same sample from which they were taken. The use of this procedure secured every time the same grain coat surface and the definite increment of the exposed damage surface of the tested grain.

By means of amperometric method [3] modified by Takeda *et al.* [6], the total iodine adsorption by tested grain sample - TA was determined using a below procedure. A special beaker (diameter 5.5 cm and hight 6.5 cm) was connected to a water bath at 25 °C and placed on a ground of magnetic stirrer (Type IS-3G). Inside of the beaker there was a stirrer arm (length 5.0 cm) rotated at a constant rate (300 rpm). Into the beaker with the rotating stirrer arm were poured 20 ml of 1M KOH solution and then

a 10 g grain sample weighed with accuracy to the one grain weight and simultantly a time of a beginning of grain soaking in KOH solution was caught. The purpose of the soaking was dissolving starch on the damage surface of endosperm, therefore a real surface of iodine adsorption was multiplied in relation to the damage surface.

After achieving of required time, the soaking process was stopped by neutralization of KOH solution adding 60 ml water solution containing 25 ml of 1M HCl. Then two platinum electrodes (length 2.5 cm each) were put into the solution and placed near the middle of the beaker. This place position of the electrodes and their streamlined shape were sufficiently a good protection before disturbances caused by occasional contact with grain. The Pt electrodes were connected with a pen recorder (Type 3056), and on a graph paper, moving 2 cm/min, a voltage of electric current appearing on the electrodes was written. The recorder and electrode sensitivity were set at 5 μV and 0.5 mV per centimeter of the graph paper, respectively.

Then 5 ml of 0.4M KI solution was added and a KIO_3 pipette connected with a peristaltic pump (Type 2132 LKB) was put into the solution. The flow rate of the pump was adjusted to 0.1 ml KIO_3/min and its value every time before the measurements was exactly determined by a test flow during 5 min. To the titration of the solution containing the grain sample, 1.6 mM KIO_3 was used.

Exactly after 2 min from the soaking finish, the pump and recorder were simultantly started. The process of titration was finished when the recorder pen reached the '10' position of the graph paper as the equivalence of 100 μV of appeared electrode current. From the graph paper, a total titration time was with accuracy 0.05min (1mm on the paper) read out as a time distance between the beginning point of titration and the finish point appeared by an intersection of the titration curve and the equivalent line of 100 μV of the electrode current (Fig. 1).

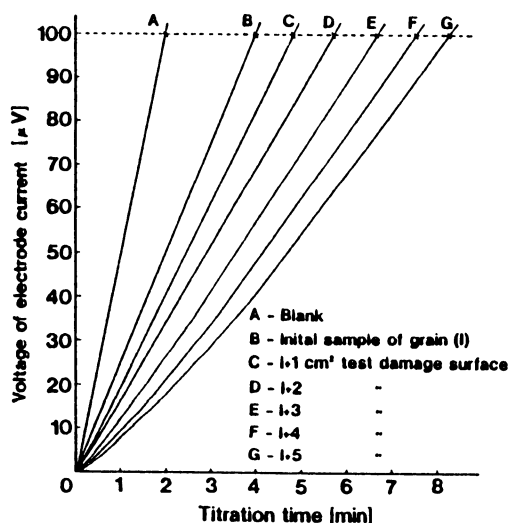The total iodine adsorption by grain sample - TA was calculated by subtraction of the total

Fig. 1. Titration curves for Canadian barley at 3 minutes of grain soaking.

titration time for reference solution (blank) from the one obtained for solution with the grain sample and multiplication by real flow rate value. The TA value was expressed in millilitres of 1.6 mM KIO_3/10 g of grain sample and noted with accuracy 0.001 ml.

On the basis of the above procedure for determining the TA value, a two part experiment was conducted. In its first part aiming at the elaboration of a method of the determination of the area of the exposed damage surface of grain, one kind of tested grain, the Canadian barley, was studied using two experimental factors: a time of the grain soaking and an amount of the damage surface of grain endosperm. The 5 levels of soaking time: 1, 2, 3, 4 and 5 min and 6 levels of damage surface area: an initial grain sample and the ones containing 1, 2, 3, 4 and 5 cm^2 of additional test damage surface were used. For all these combinations, the measurements of the TA value were made in two repetitions.

Aiming at the presentation of the practical use of the proposed method at the estimation of the damage surface area to barley grain of various kinds, the second part of this experiment was conducted. All kinds of barley grain were estimated using for each

one 4 experimental combinations: soaking time (1 and 3 min) x damage surface area (initial sample and the one containing 3 cm^2 of additional test damage surface). For these 4 combinations, the measurements of the TA value were made in 3 repetitions.

RESULTS AND DISCUSSION

The general characteristics of studied barley grain are presented in Table 1. The values of such parameters as moisture content, weight of a single grain and test damage surface of one cut grain showed a significant differentiation between the studied kinds of barley. For all mentioned parameters, the same ranking of the kinds of barley was observed.

However, values of another parameters

mechanical damage on the total adsorption of iodine by grain. Mean values of the total iodine adsorption - TA for Canadian barley in relation to the soaking time of the grain in 1 M KOH solution are shown in Fig. 2. Increasing soaking time was always causing the increase of TA values, as a result of dissolving more amount of starch on a damage surface of grain endosperm. This connection described a linear relation although a longer soaking time (5 min) caused visible deviations. The slope of the plot line was increasing with the increase of damage surface area to grain. The lengthened lines of the plot gave one intersection point. The ordinate of this point was equal with the adsorption of iodine by the grain coat of tested sample - CA (Fig. 2).

T a b l e 1. Characteristics of studied barley grain

Grain features	Unit	Kinds of barley grain		
		Canadian	Australian	Japanese
Moisture content	%	12.0	12.6	13.0
Weight of one grain	g	0.0353	0.0408	0.0434
Test damage surface per gram of cut grain with standard deviation	cm^2/g	3.112 ±0.019	3.226 ±0.030	3.255 ±0.007
Test damage surface of one cut grain	cm^2	0.110	0.132	0.141
Cut grain weight per 3 cm^2 of test damage surface	$g/3\ cm^2$	0.9640	0.9299	0.9217

such as the test damage surface per gram of cut grain and the cut grain weight per 3 cm^2 of the test damage surface were less differentiated and maximum differences between the kinds were equal with one grain value of these parameters. It can indicate that the ratio of a surface area of the transverse section of grain to the grain weight is constant and its value can be involved with botanical species of grain. Therefore for practical use, it would be enough cutting weighed grain sample without the additional determination of the area of test damage surface. This fact can make the presented method still more useful.

The basic importance at evaluation of mechanical damage by means of the amperomtric method has the determination of the effect of the time soaking and of the amount of

The effect of amount of damage surface on the total iodine adsorption - TA for Canadian barley is shown in Fig. 3. The increase of the area of added test damage surface to grain sample was causing the linear rise of the total iodine adsorption. The adding of 3 cm^2 of the test damage surface at 1 and 3 min of soaking gave the increase of TA values equal with 0.155 and 0.266 ml, respectively. The intersection point of lengthened plot lines allowed to determine both the CA and the area of exposed damage surface - AD value for studied grain (Fig. 3).

Apart from the graphical way of determination of CA and AD values, demonstrated in Figs 2 and 3, there is more unfailing one per calculation that is described below. On the basis of the experimental data presented in
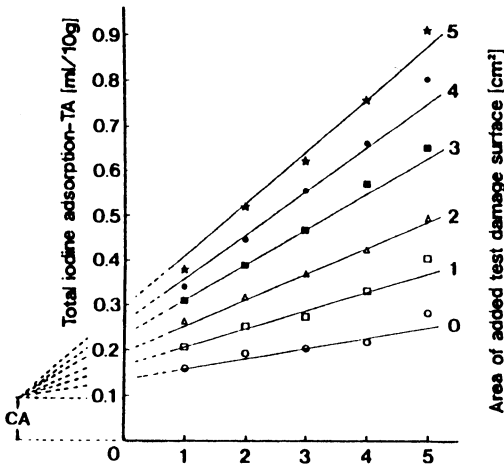
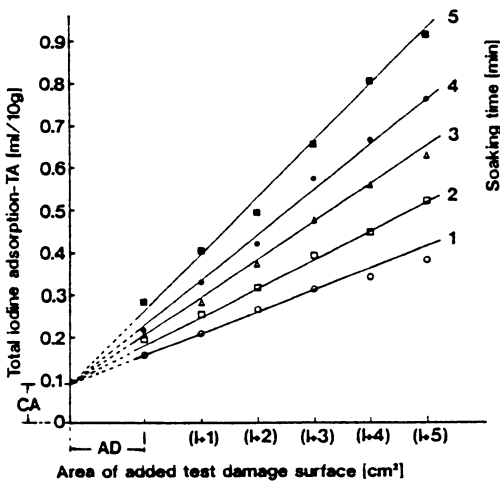**Fig. 2.** Effect of the soaking time of grain on the total iodine adsorption - TA for Canadian barley.

Fig. 3. Relationship between the area of test damage surface added into initial sample (I) and the total iodine adsorption - TA for Canadian barley.

Figs 2 and 3 showing that the grain coat adsorption - CA is constant and does not depend on time of grain soaking and of the amount of the damage surface area of grain - AD and the relationship between AD values and the total iodine adsorption of grain sample - TA is of a linear character and accepting every area with the same damage surface, regardless of its localization and origin, adsorbs the same amount

of iodine, the TA value can be written in the form of an equation:

$$TA = CA + (C \cdot AD) \quad [ml/10g] \qquad (1)$$

where C - coefficient of iodine adsorption by damage surface of grain. The TA value expresses the total amount of iodine, which is adsorbed by 10 g of grain sample, both by its grain coat and exposed damage surface.

The C and CA can be named as auxiliary parameters because their values are constant for a given grain regardless of extent of the area of damage surface to the grain. To educing formulas for calculating the auxiliary parameters of the Eq. (1), TA values of 4 experimental combinations: the soaking time x the damage surface area were taken. They were as follows:

a - TA value for initial grain sample soaked for 1 min;

b - TA value for initial grain sample soaked for 3 min;

c - TA value for initial grain sample containing 3 cm^2 of additional test damage surface and soaked for 1 min;

d - TA value for initial grain sample containing 3 cm^2 of additional test damage surface and soaked for 3 min.

The coefficient of iodine adsorption by damage surface of grain sample - C was calculated using a formula:

$$C = (d - b) \, 3^{-1} \quad [ml/cm_2] \qquad (2)$$

The C value means the amount of iodine expressed in ml of 1.6 mM KIO3, which is adsorbed by 1 cm^2 of exposed damage surface of the grain sample.

The formula of calculation of the CA parameter was educed according to the below meaning:

$$(d - c) - (b - a) = \Delta \qquad (3)$$

The Δ increment is caused only by additional 3 cm^2 of the test damage surface because the grain coat adsorption was a constant and the same regardless of the amount of damage surface to grain. From proportion:

$\Delta \qquad \sim \quad$ 3 cm^2 of test damage surface

and

(b - a) ~ X cm^2 of initial damage surface, X can be calculated:

$$X = 3(b - a)\, \Delta^{-1} = 3(\frac{d - c}{b - a} - 1)^{-1} \quad (4)$$

Then one can put the contents of the X in the place of AD parameter into the below formula obtained by transformation of Eq. (1):

$$CA = TA - (C \cdot AD) \quad (5)$$

After replacement of C by the contents of Eq. (2) and TA by the b value, a final form of this formula for calculating the grain coat adsorption - CA was as follows:

$$CA = b - (d - b)\,(\frac{d - c}{b - a} - 1)^{-1} \quad [\text{ml}/10\ \text{g}] \quad (6)$$

The CA value means the amount of iodine expressed in ml of 1.6 mM KIO$_3$, which is adsorbed by the grain coat of 10 g grain sample.

Having calculated values of the CA and C, the basic parameter that is the area of exposed damage surface - AD can be very simply determined by means of the below formula obtained by transformation of Eq. (1):

$$AD = \frac{TA - CA}{C} \quad [\text{cm}^2/10\ \text{g}] \quad (7)$$

The AD value means the area of the exposed damage surface of grain endosperm expressed in cm^2 per 10 g grain sample.

For calculating the AD, one can take the TA values determined for grain sample soaked for 3 min. This period of soaking time allows to obtain enough high sensitivity of the amperometric titration and a good repeatability of the AD measurements. If this period is shorter (e.g., 1 min), the sensitivity of the amperometric method is lower. However, if the soaking period is too long (e.g., 5 min), it causes higher increases of the TA rather than linear (Fig. 2).

One should confirm that it is possible to calculate the AD according to formula (4) in which the X is the synonym of the AD parameter. But acceptance of this way of calculating the AD would require every time (e.g. at successive stages of a mechanical destruction of the same grain) measuring TA values for all

4 experimental combinations and which would make the way too tedious and time-consuming. Furthermore, the repeatability of AD measurements would be insufficient to accept the way because the TA measurements for the 4 combinations are always loaded some errors (i.a., $\bar{a} \pm \sigma_a$, $\bar{b} \pm \sigma_b$, $\bar{c} \pm \sigma_c$ and $\bar{d} \pm \sigma_d$) and such form of the formula (4) is conductive to an effect so-called summation and multiplication of the unit errors (σ_a, σ_b, σ_c and σ_d).

To eliminate the negative factors, we propose to accept a procedure for determining the AD parameter as follows. First to calculate the auxiliary parameters C and CA using formulas (2) and (6), respectively. Then basing on average values of C and CA and taking TA values measured for the grain soaked 3 min to calculate the AD parameter according to formula (7).

On basis of the procedure of determination of the area of exposed damage surface of grain described above, the total iodine adsorption - TA at the 4 experimental combinations was determined for 3 kinds of studied barley grain. The results of these measurements are presented in Table 2. Analyzing the obtained TA values for the particular kinds of barley grain, a large differentiation of them was observed. The Japanese barley characterized the lowest TA values for all the experimental combinations and the Canadian barley had the highest ones.

The dispersion of TA values within 3 replications, evaluated by the standard deviation, did not exceed 0.009 ml/10 g and which constituted a negligible fraction of the measured value. Because it indicates a sufficient precision of the amperometric method, it can be useful for determining the adsorption of iodine by grain.

In Table 3, the mean values of all characteristic parameters for Eq. (1) are listed. The values of the C, CA and AD parameters were calculated according to the formulas (2), (6) and (7), respectively. Among the parameters, the coefficient of iodine adsorption by damage surface - C was the weakest differentiation. Its mean values for Canadian, Australian and Japanese barley were 0.089, 0.079

T a b l e 2. Mean values of total iodine adsorption - TA for 4 experimental combinations (a, b, c and d) with standard deviation [ml/10 g]

Kinds of barley grain	Total iodine adsorption			
	a	b	c	d*
Canadian	0.159 ±0.005	0.206 ±0.006	0.314 ±0.002	0.472 ±0.005
Australian	0.128 ±0.007	0.182 ±0.009	0.281 ±0.008	0.418 ±0.006
Japanese	0.049 ±0.002	0.057 ±0.004	0.147 ±0.004	0.225 ±0.008

* Explanation in text.

T a b l e 3. Mean values of determined parameters of Eq. (1) with standard deviation

Kinds of barley grain	Determined parameters		
	CA [ml/10 g]	C [ml/cm^2]	AD [cm^2/10 g]
Canadian	0.092 ±0.003	0.089 ±0.003	1.28 ±0.06
Australian	0.026 ±0.008	0.079 ±0.004	1.97 ±0.12
Japanese	0.038 ±0.006	0.056 ±0.002	0.33 ±0.07

and 0.056 ml/cm^2 of exposed damage surface, respectively.

In relation to the C values, the grain coat iodine adsorption ones - CA differentiated more the studied kinds of barley (Table 3). The Australian barley had the lowest adsorption of iodine by grain coat at mean value 0.026 ml/10g and Canadian had the highest one (0.092 ml/10 g).

The range of values of the area of exposed damage surface - the most useful parameter with point of a view of its practical utilization, was the widest among all ones (Table 3). The highest AD value was observed for Australian barley, that accounted 1.97 cm^2 of damage surface per 10 g of tested grain. The Canadian barley had a middle position with mean value 1.28 cm^2 of damage surface. The Japanese barley characterized the smallest area of exposed damage surface, that accounted 0.33 cm^2 and this value was 6 times lower than the highest one in the case of Australian barley.

Comparing rankings of the studied kinds of barley in relation to the obtained AD and TA values (Table 2 and 3), a divergence between them was stated. In spite of the lower total iodine adsorption, the Australian barley

had a distinct higher AD value than the Canadian one. This fact proves that among the TA and AD parameters only the AD one is a proper measure of amount of the mechanical damage.

The error of measurement of the C and CA parameters depends on the way of preparation and determination of the test damage surface added into tested grain and its value is decreasing when the area of added test damage surface is increasing. In the case of the C, two unit errors (σ_d and σ_b) have effects on the value of the parameter (formula 2). In this experiment, the standard deviation was low and did not exceed 0.004 ml/cm^2 knowing that the test damage surface of one cut grain adsorbed about 0.010 ml of iodine.

The higher of measurement errors was observed for CA parameter. The maximal value of the standard deviation accounted for 0.008 ml/10 g and which constituted 30 % of the mean of CA for Australian barley. Such high error of CA, among other things, results from the above mentioned the effect of summation and multiplication of the unit errors (σ_a, σ_b, σ_c and σ_d) according to the character of formula (6). At highest average of CA

(i.a., for Canadian barley), the standard deviation was lowest and reached a value equal with 3 % of the mean.

Thanks to the use of the proposed procedure of calculating the area of exposed damage surface - AD, the error of measurement of AD is minimized and depended mainly on the accuracy at measuring the TA for grain soaked for 3 min i.a. $\overline{b} \pm \sigma_b$. For the present experiment, the dispersion of obtained AD values within 3 replication, evaluated by standard deviation, was not large and placed in a range from 0.06 to 0.12 cm^2/10 g. Its maximal value was equal with an area of test damage surface of one cut grain constituting less than 0.5 % of the sample weight.

CONCLUSIONS

The suggested method allows for the evaluation of the exposed damage surface of cereal grain appeared during the distractive acting external forces on the grain structure and as a result of the biological damage.

Among the existing methods based on the evaluation of sorption of different solutions by grain as a measure of amount of the mechanical damage, only the presented method allows for a strict separation of the total adsorption on its parts caused by the grain coat and the damage surface adsorption. Using the coefficient of adsorption by damage surface, that is different for various kinds of grain, the area of exposed damage surface to a small grain sample can be determined.

The studies on the estimation of the exposed damage surface area conducted on 3 various kinds of barley grain at using the proposed method showed its usefulness as a objective and accurate method for the determination of this kind of mechanical damage of cereal grain.

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