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The influence of sprouting and resistance to sprouting on the alpha-amylase content and baking quality of Dutch wheat and rye

by

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In the countries of North-western Europe the quality of wheat and rye is seriously damaged in some years by the occurrence of sprouting, due to much rain at the time of harvest.

Much attention has already been given to this phenomenon, bringing to light the fact that there are great varietal differences in susceptibility to sprouting. Thus there are wheat varieties which exhibit great resistance to sprouting. The grains of these varieties are not yet capable of germinating when the corn is ripe for harvest, normal germinating power only appearing after a period of ripening. In other varieties the power of germination is already present at the time of harvest, so that in the case of damp weather during the harvest, much loss can be suffered by germination of the grains in the ear.

The existence of these varietal differences provides the growers with the opportunity of combining, by selection, the property of resistance to sprouting with other desired properties. For this a scientific basis is necessary with an increased insight into germination, the factors influencing it and the cause of the occurrence of such distinct varietal differences in susceptibility to sprouting.

In connection with the foregoing, it is important to consider the methods for determining the degree of damage by sprouting with its influence on the baking quality, and the methods for determining the resistance to sprouting. In this address I propose to give an idea of some research work which has been carried out by our Institute concerning these methods.

The poor baking quality of wheat and rye damaged by sprouting is caused chiefly by the presence of the enzyme alpha-amylase in a ten to a hundred times higher concentration than in grain not damaged by sprouting.

In these high concentrations this enzyme, formed during germination, causes a far-reaching degradation of starch to dextrins during the process of baking.

The determination of the degree of sprout damage can be carried out in many ways. I mention a few possible ones here:

1. Determination of the alpha-amylase content.

2. Determination of the starch breakdown after autolysis of the milled grain at temperatures which also occur during the process of baking.

3. Determination of the degree of germ development.

In our Institute the determination of the alpha-amylase content receives much careful attention. In 1947 our former co-worker Miss Hoskam published a colorimetric method (1), advancing on the work of Sandstedt et al. (2). With this method erythrodextrin, obtained through the action of beta-amylase on starch, is used as substrate, the blue colour produced with iodine being used as a measure for the starch breakdown. The alpha-amylase activity is computed as the amount of erythrodextrin in mg, converted per minute (at 30° C) by the alpha-amylase present in 1 g. flour (Hoskam units).

Next, following up the work of the Swedisch investigators Molin (3) and Hagberg (4), we developed a method based on the autolysis of the flour at 70-75° C, and the viscosimetric determination of the starch breakdown. With this method the micro-plastometer of Höppler (fig. 1) is used. I will mention a few particulars here as this method has not yet been published.

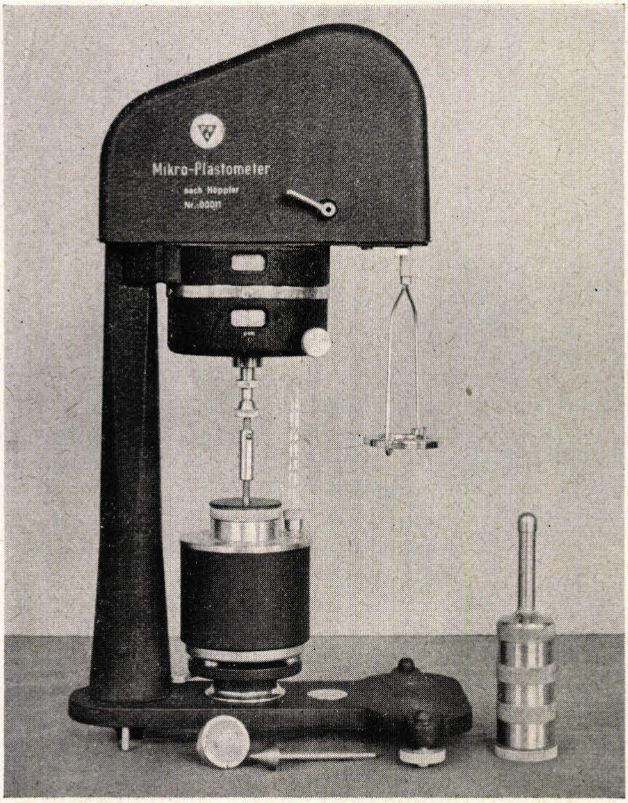

Fig. 1 Micro-plastometer according to Höppler

5 g. flour are mixed with 5 ml. buffer solution (10 g Ca acetate, 10 g. Na acetate and 1 ml. glacial acetic acid per litre, pH 5.7). About 1.5 g. of this mixture is put into a receptacle of the micro-plastometer. Four of these receptacles are placed in a metal case with a closely fitting screw-lid, then immersed for 10 minutes in a water-bath of 75° C and afterwards for 15 minutes in a water-bath of 30° C. The plasticity is now measured in arbitrary units. I cannot here enter into details of this measuring.

A definite connection exists between this plasticity and the alpha-amylase content (fig. 2). The data for this figure have been taken from the investigation of wheat samples in which artificial sprouting of varying degree had been produced. I shall refer to this investigation later. This figure shows that a comparatively low content of alpha-amylase already causes a very great reduction of the plasticity, but a very high content is necessary to bring the plasticity practically to zero. A simple rectilinear relation exists between the logarithms of plasticity and alpha-amylase content. This is also reproduced in figure 2. It is remarkable that this connection is present over such a large stretch. The alpha-amylase content varies here from 0.5 tot 200!

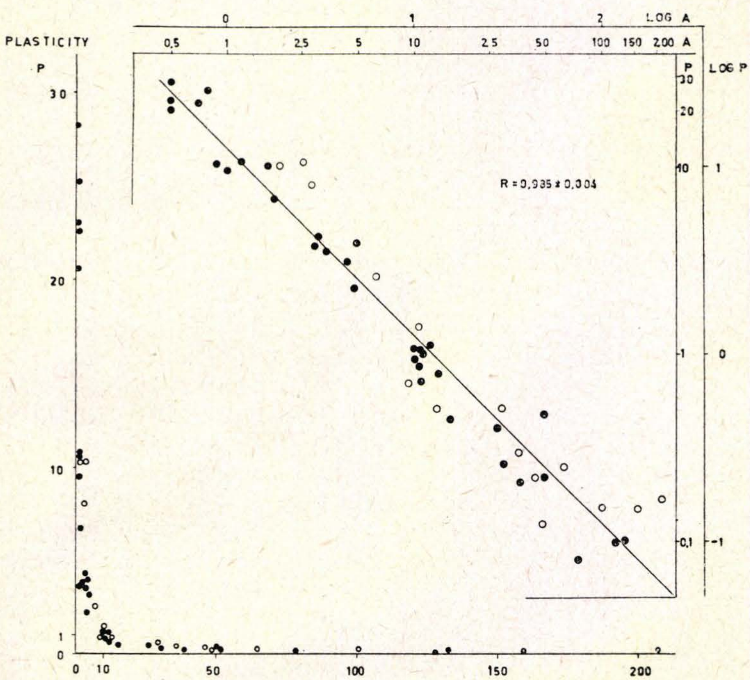

Fig. 2 Correlation between alpha-amylase content (A) and plasticity (P) and between log A en log P in artificially sprouted wheat

The degree of sprouting can also be established by observation of the degree of germ development in the unmilled grain. For this purpose we have worked out a method whereby four stages of development are recorded, viz.:

0. Germ dormant.

1. Swelling of the germ (the thin skin over te germ is hereby lifted up, remaining in this position even when the germ afterwards shrinks through desiccation).

2. The beginning of shoot and root formation. The sum of the length of shoot and main root is, however, not yet greater than the length of the grain.

3. Advanced germination.

At least 100 grains are examined with a binocular microscope and sorted according to these stages. Should one find from the 100 grains N_1 at stage 1, N_2 at stage 2 and N_3 at stage 3, then the value S as a measure for visible sprouting can be calculated as follows:

$$S = 10 \sqrt{\frac{N_1 + 3\,N_2 + 6\,N_3}{6}}$$

The coefficients are so chosen that S can vary from 0 to 100.

Figure 3 shows the relation between S and the alpha-amylase content, based on the same investigation as the former figure. Here the amylase content has again been set out on a logarithmic scale and then a more or less rectilinear relation with S is shown. The correlation, however, is less strong here than in the former figure (the correlation-coefficient is 0.926 ± 0.022 against 0.985 ± 0.004 in the former case). At a certain value of S very varying alpha-amylase contents can occur. We must, however, remember that the samples examined were very heterogeneous, some coming from white wheat, which is susceptible to sprouting, some from red, more sprout-resistent wheat. I shall return to this later.

In figure 3 the average percentages of strongly germinated grains at certain S values, are also given. It appears that at an S value of 50 almost 10 per cent of such grains occur. Thus the higher S values are related to very extreme cases. On the other hand, the S value still gives a good differentiation in the region of slight sprout damage, where the percentage of strongly germinated grains is no longer a good criterion. This region of slight sprout damage, where the alpha-amylase content lies between 1 and 10, is of great importance for practical purposes, because here the dividing-line lies between the wheats whose baking quality has not suffered through sprouting and those with a definitely deteriorated baking quality (resulting from too high an alpha-amylase content).

In an investigation of wheat samples from Dutch experimental fields, baking tests were carried out and stickiness of bread crumb was estimated by hand, using a scale of points from 0 to 4. A significant correlation ($r = 0.75 \pm 0.10$) was found between these figures for stickiness and the

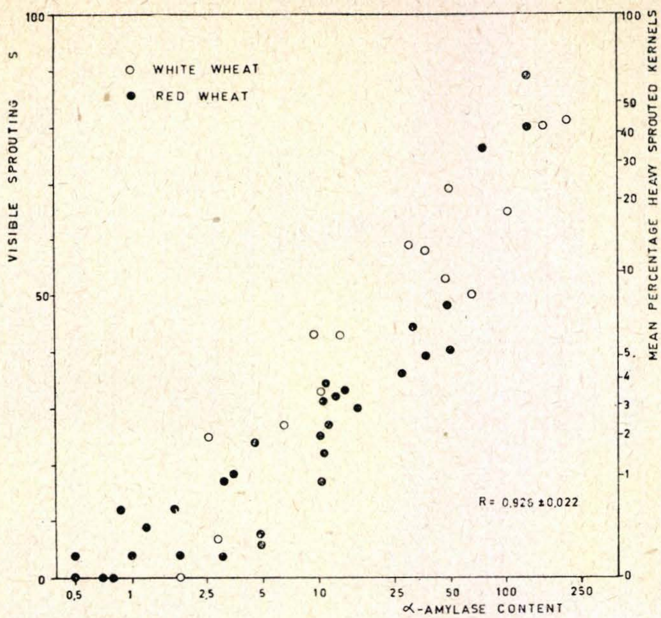

Fig. 3 Correlation between alpha-amylase content and visible sprouting in artificially sprouted wheat

alpha-amylase content (fig. 4), whereas the correlation of alpha-amylase and bread volume was not significant (r = 0.17 ± 0.15).

Fig. 4 also shows the relation between alpha-amylase content and stickiness based on an earlier investigation on samples of rye. It is evident that this relation is not very different for either wheat or rye. In the case of both, the greatest increase of stickiness occurs when the alpha-amylase content rises from about 1 to 10. This is the region already mentioned, where the division lies between undamaged baking quality and strongly diminished baking quality due to sprouting. This figure clearly shows that the judging of the diminished baking quality on the basis of the alpha-amylase content can be carried out with wheat and rye in a more or less similar way.

For practical purposes it is important to find out how this evil of sprout damage can be combated. One of the most important ways is the cultivation of varieties which are as resistent as possible to sprouting. It is known that great differences exist in this respect. On the whole, white wheats are not very resistant, whereas red wheats as a rule show a great degree of resistance.

The method of SCHMIDT (5) has found favour in the investigation of resistance to sprouting in wheat varieties. According to this method bundles of ears are brought into damp surroundings and then, at regular times, the degree of germination is estimated, using a certain scale of points. In the case of this estimation the first beginning of sprouting

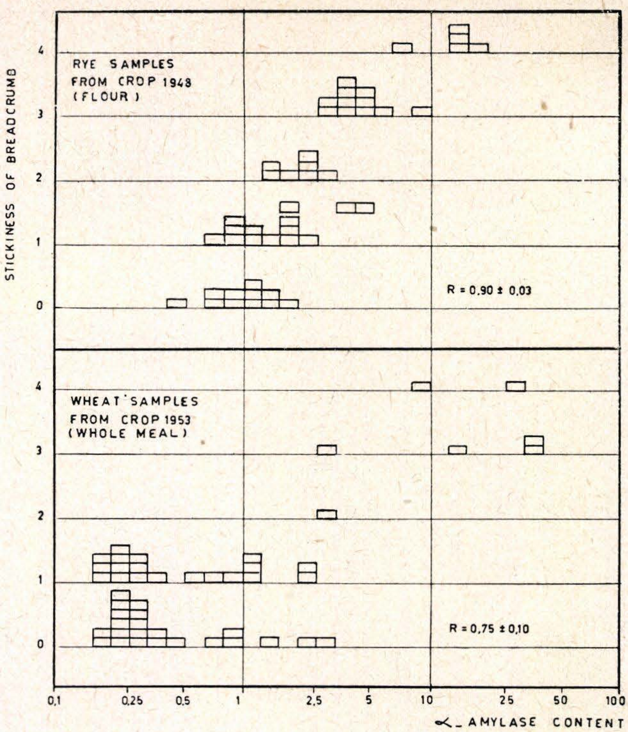

Fig. 4 Correlation between alpha-amylase content and stickiness of bread crumb in samples of rye flour from 1948 crop (upper part) and in samples of whole wheat meal from 1953 crop (lower part)

is not noticed, as the grains are not taken from the ears and the germs are thus only visible when they have reached a certain length. This rather rough method is useful for the comparison of a few varieties who can be investigated together. However, little thought has been given to modifying this method in such a way that it can be carried out in controlled circumstances. Perhaps it is also desirable to substitute a finer method for the rough estimation of the degree of germination.

In this connection I mention a few results here of an investigation in which bundles of ears were kept some time in damp surroundings, according to the principle of the method of SCHMIDT, but in which, at the same time, an investigation of the grains was made according to the methods just described. The data for figures 2 and 3 concerning the relation between alpha-amylase content, plasticity and visible sprouting were derived from this investigation. As far as the treatment of the ears was concerned the investigation was carried out by the Institute of Agricultural Plant Breeding at Wageningen, while the investigation of the grains was carried out by our Institute.

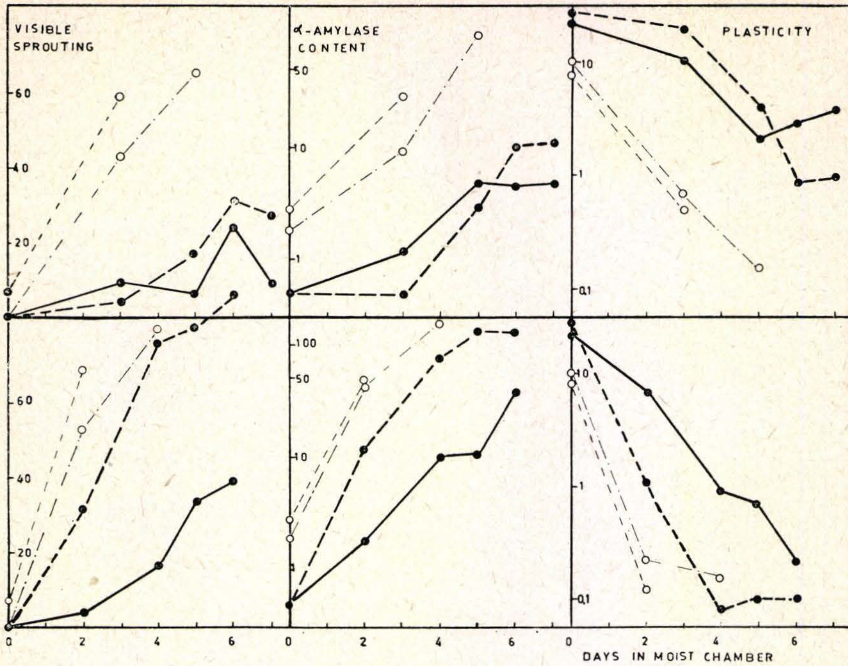

Fig. 5 Correlation between number of days in moist chamber and visible sprouting in S units (see text), alpha-amylase content in Hoskam units and plasticity in arbitrary units in 4 wheat varieties. Upper half: immediately after harvest. Lower half: 15 days after harvest

o— — — — —o Minister	White wheat varieties	
o— - — - —o Staring		
●— — — —● Heine's VII	Red wheat varieties	
●————● Mado		

Figure 5 gives a survey of the results. The investigation was concerned with four varieties of wheat, viz. two white varieties and two red ones. Immediately after the harvest, a portion of the ears was made into bundles and brought into a damp space. The upper half of the figure relates to this. Another portion was kept dry for 15 days and only then brought into damp surroundings. The results of this are given in the lower half of the figure. Samples were taken for investigation as soon as the bundles were brought into the damp space and again after differing numbers of days, as given in the figure. These samples were dried and threshed, afterwards being investigated according to the methods described. It can be seen from the figure that during the stay in the damp space visible sprouting and alpha-amylase content increases, while plasticity after gelatinization, measured with the microplastometer, decreases. In the first series (upper half), however, there is evidence of a marked difference between the white and the red wheats, due to the difference in the resistance to sprouting. After remaining five days in the damp space, the white wheats are in a very advanced stage of germination, whereas the red ones only just show a beginning of germination.

In the second series (lower half), the situation proves to be different insomuch that one of the red wheats, the variety Heine's VII, having lost much of its resistance, now shows more similarity with the white wheats. In the period of 15 days between the harvest and being brought into the damp space, this wheat has ripened sufficiently to have a normal germinating power. The other red wheat, the variety Mado, still shows a satisfactory resistance here. Apparently the normal germinating power of this variety is only reached after a still longer period of ripening.

This investigation proves that the described methods are really of use for studying resistance to sprouting and the length of the ripening periods of wheat varieties. The advantage of these methods is that even after a very short stay in the damp space, whereby only just the beginning of germination can occur, the differences are already manifest. The figure shows that the stay in the damp place can be limited to about two days without any objection. This makes it possible to investigate more material in a rather small damp room, which sooner justifies the equipment of an installation for keeping temperature, relative humidity and the like, constant.

The described investigations are incorporated in a greater scheme, of which it is the aim to find measures to combat the loss of quality suffered by wheat and rye through sprout damage. The problem of the resistance to sprouting is very complicated. Still little is known with certainty of the nature of the connection with the colour of the wheat. Of the mechanism of germination and the part played by growth substances and enzymes also not much is known, so I cannot otherwise end than by remarking that there is still a great region here lying open for investigation.

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SUMMARY

In the countries of North-western Europe the quality of wheat and rye is seriously damaged some years by the occurrence of sprouting, due to much rain at the time of harvest. The poor baking quality of these

grains is principally due to the presence of the enzyme alpha-amylase in a concentration ten to a hundred times greater than in grain not damaged by sprouting. In these high concentrations this enzyme, formed during germination, causes during the process of baking a strong breakdown of starch to dextrins, resulting in stickiness of the bread crumb.

Some experience was gained concerning the application of three methods for determining the degree of sprout damage, viz.:

1. Determination of the alpha-amylase content according to the method of Hoskam (1).

2. Viscosimetric determination of the starch breakdown after autolysis of the milled grain at 70 – 75° C. Measuring of the plasticity after gelatinization, with the micro-plastometer according to Höppler.

3. Morphological determination of the degree of development of the germs in the unmilled grain.

In the investigation of samples of wheat in which sprouting to different degrees has been artificially produced, the results obtained by these methods showed a definite correlation (see fig. 2 and 3).

In the investigation of samples of wheat and rye, in which baking tests were taken, a definite correlation was found between the alpha-amylase content and stickiness of the bread crumb (see fig. 4).

It is evident that the methods named can also be used in connection with the determination of the resistance to sprouting in wheat varieties. In such an investigation of four wheat varieties, viz. two white wheats and two red ones, the difference in resistance to sprouting was clearly brought to light (see fig. 5).