INVESTIGATIONS ON THE BAKING QUALITY OF DIPLOID AND TETRAPLOID RYE 1)

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SUMMARY

In an investigation into the baking quality of samples of rye from Dutch experimental fields, the activity of the amylolytic enzymes has been measured directly and indirectly by determining the alpha-amylase content and the maltose figure, by amylographic investigations and by baking tests.

The investigation comprised 48 samples of diploid rye from crop 1948, from 8 varieties and 6 experimental fields, and samples of diploid and tetraploid rye from the crop 1948—1951. Large inter-field differences were found, whereas the varietal differences were small.

Compared with the diploid rye the tetraploid rye was higher in protein content, and an indication was got that this rye was less susceptible to loss of quality in consequence of sprouting.

Introduction

Breeding work in Western Europe for improving rye is predominantly directed to increasing yielding ability and regularity of production. Disease resistance, cold tolerance, resistance to sprouting and straw characters such as height, flexibility and stiffness, are of essential importance in this connexion. On the other hand very little attention is given to improving the quality of the grain, in spite of the fact that a large part — in certain countries the largest part — of the rye crop is used for human consumption. This applies in the first place to the Scandinavian countries and to various districts in Germany, where rye bread takes a foremost place in the diet. In the Netherlands also, where in the southern and eastern provinces rye bread is consumed with relish, the question of baking quality cannot be shelved.

Due consideration of the quality problem as an essential part of a breeding programme, is however, only possible with sufficient knowledge of the anatomy, the chemical and the technological properties of the rye kernel, which are of capital importance as regards the baking quality. Although science in this sphere is only in the initial stage, investigations in Western Europe in the last decades have made important contributions to a better insight in the problem. Great importance must be attached to the work of the Norwegian investigator Schulerud (1939), whose extensive experiments led him to stress the significance of starch and the starch-splitting enzymes for the quality of bread. A correct balance between the degree of gelatinization and amylolysis is a requisite for the formation of an elastic, regular and porous bread crumb. German investigations (Müntzing, 1951, Scholz, 1939, 1940), carried out with the amylograph of Brabender (1937), pointed in the same direction.

Proceeding from this point, Aust and Ossent (1941), examining German rye varieties, ascertained that the strains with a high protein content were characterized by a medium to low maltose-forming capacity. They attributed

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special importance to the last-named value as a measure of the resistance of the grain to sprouting. Pelshenke (1941) and later Hintzer (1946) tested a number of varieties of different origin, making it possible to draw conclusions as to the measure in which diverse properties connected with the grain quality are characteristic of the variety. Both authors conclude that the breakdown of starch taking place during the process of baking, by which the baking quality is in a great measure determined, is predominantly influenced by the conditions of growth, though there are indications that the alpha-amylase content is to a certain extent subject to the influence of variety. Furthermore, Pelshenke calls attention to the glucose content and the maltase activity connected therewith, which appear to be typical varietal characters, little influenced by the environment.

Although it is obvious from the foregoing that already certain more or less detailed conceptions of the baking quality of rye have been formed, it is likewise plain that continued research is indispensable in order to verify present opinions and increase fundamental knowledge. The investigation of samples of eight Netherlands varieties of rye, each taken from six districts, with which the first part of this article deals, must also be considered in this light.

Concerning the agricultural history of tetraploid rye we can be brief, simply referring to a few surveys (Bremer-Reinders, 1950, Müntzing, 1951, p. 7–9, Wagner, 1951). It is sufficient to mention that before the second World War, experiments were carried out on certain plants in different countries, concerning the influence of the doubling of chromosomes on properties, agriculturally important. In Sweden especially, due to the application of colchicine, important results were obtained in the case of some plants with a low number of chromosomes, such as rye, red clover and sugar beet. This colchicine treatment also found favour in Germany and since 1946 similar investigations are being carried out in the Netherlands at the Institute of Agricultural Plant Breeding at Wageningen.

Tetraploid rye is a very vigorous plant, with a broad thick leaf and big ears. Compared with normal diploid rye, the volume of the kernel is about 50 per cent greater, the germination capacity also being greater. On the other hand, the fertility is considerably less although important progress has already been made in this respect by systematic breeding. According to Müntzing (1951, p. 63–65) the best Swedish tetraploid strains equal normal diploid rye, as far as yielding ability is concerned. This is also the case with Netherlands tetraploid material in the experience of Bremer and Bremer-Reinders. It is to be expected that this favourable development will continue on further breeding, and that before long tetraploid rye will be used on a practical scale in other Western European countries besides Germany, where tetraploid Petkuser has been on their market since the autumn of 1951.

Data concerning the value of this rye for the preparation of rye bread are as yet fairly scarce. A tentative baking investigation with two Swedish tetraploid strains is mentioned by Jung (1948). Both proved to have a distinctly higher protein content than the diploid comparison material, baking tests with the tetraploid rye resulting in about 10 per cent greater bread volume. A more extensive investigation of the same strains is described by Müntzing (1951, p. 7—9). Protein, ash and crude fibre content of the tetraploid strains were distinctly higher than those of the diploid. The bread properties of the former, such as volume, internal texture and colour of the crumb, were better. A like

superiority of tetraploid strains attributed by Müntzing to the relatively high protein content, was apparent in the preparation of "Knäckebröd", so well-liked in Sweden. In this respect the prospects of this rye can be called very promising.

In view of the certainly not unfavourable picture that the Swedish investigations have produced regarding the baking properties of tetraploid rye, the question arises as to what the strains cultivated in the Netherlands exhibit in this respect. At the request of the Institute of Agricultural Plant Breeding, samples of every harvest since 1948 have been analysed and their baking qualities tested in the laboratory of the Cereals Department of the Central Institute for Nutrition Research T.N.O. The second part of this article describes the results obtained hereby, and in a discussion of the collective results, a comparison is made between the different data for tetraploid rye and the corresponding values for normal rye.

A small portion of the grain from each sample was reserved for the determination of a few analytically important data, while part of the rest was milled to flour and part to meal. In the southern provinces of the Netherlands the latter is finely milled, in the eastern provinces a more coarsely milled rye meal is made. Both are used for the preparation of a brown rye bread which forms part of the daily diet (Koopmans, 1951). In the experiments the finely milled meal was used. With the flour a white bread was baked, which is normally not made in the Netherlands, but which in Denmark, Norway and various districts in Germany, is a popular food. This type of baking test is very attractive, since differences in quality are more clearly revealed by the better developed shape and texture, than in the case of brown rye bread, thus making a more precise evaluation possible.

Finally it must be mentioned that in the foregoing nothing has been said about assessing the value of the grain from the point of view of milling technology. Such an evaluation has been intentionally omitted, although here also important differences exist in connexion with the size shape, fullness and hardness of the kernel and grittiness of the flour (SCHMIDT und SCHOLZ, 1938). The requirements in this respect, are, however, not yet clearly defined and, moreover, often differ with the district. Furthermore, the methods of investigation in this sphere have not yet been sufficiently developed. Systematic research in this field also is necessary, if it is to be possible, in the long run, to provide the breeders with directions regarding the milling properties of their selections.

METHODS OF INVESTIGATION

Hectolitre weight and 1000 kernel weight were determined in the usual way, the protein content according to Kjeldahl, using a conversion factor of 5.83.

The alpha-amylase content was determined according to Hoskam (1947). This method is based on the determination of the colour appearing when iodine is added to a solution of erythrodextrin, after this has been exposed to the action of an aqueous extract of meal or flour at 30° C.

The maltose figure and the pre-existing maltose were determined according to Rumsey-van der Lee (Pelshenke, 1938). The maltose figure expresses the number of grams reducing sugars, calculated as maltose, present in 100 g meal or flour, if an aqueous suspension hereof is subjected to autolysis for 1 hour at 27° C. The pre-existing maltose gives a similar figure for the amount of

reducing sugars originally present. Subtraction of the pre-existing maltose from

the maltose number gives the corrected maltose figure.

The amylogram gives a graph of the course of the viscosity if an aqueous suspension of flour is heated to about 95° C, the temperature being raised at a rate of 1½° C per minute. The so-called amylograph of Brabender is used for this purpose.

The following data were derived from the amylogram:

a the temperature at which the viscosity begins to increase,

b the temperature at which the viscosity reaches a maximum value,

c the value of the maximum viscosity,

d the percentage decrease of the viscosity 5 minutes after the maximum.

The last two values especially may be considered as a criterion for the correct water-binding capacity of the flour.

Finally, baking tests were carried out, in this case baking tests with rye

flour (white pan bread) and with rye meal (brown oven plate bread).

The formula for the baking test with rye flour was as follows: 100 parts (by weight) of flour, 2 parts of yeast, 2 parts of salt and 61 parts of water were mixed for 5 minutes, followed by a first proof of 15 minutes (dough temperature 26 to 27° C). The dough was divided into pieces for loaves of 600 g and moulded for pan bread. After a final proof of 45 minutes, baking for about 45 minutes followed at an oven temperature of about 220° C.

In the case of the baking test with rye meal, the following formula was used: 100 parts (by weight) of meal, 1 part of yeast, 1 part of salt and 48 parts of water were mixed for 10 minutes, followed by a first proof of 50 minutes (dough temperature 26 to 27° C). The dough was divided into pieces for loaves of 650 g and moulded as oven plate loaves. After a final proof of 45 minutes, baking of about 120 minutes followed at an oven temperature

decreasing from about 210° to about 150° C.

The loaves were judged one day after being baked. Attention was paid to such properties as volume, shapeliness, colour and bloom of the crust, as well as colour and texture of the crumb. Great differences were especially noticeable in the degree of stickiness of the crumb. This quality was judged in two ways, viz. by external examination and by measurements. The external examination was expressed as a score number (higher according to the better quality of the bread, thus less sticky). The measurements were carried out with the panimeter (Hintzer, 1951). A cylinder cut out of the bread is pressed together by this instrument with gradually increasing force. Then the pressure is released and observation is made as to what percentage of the original height the bread cylinder recovers. A slight measure of recovery is an indication of sticky bread.

The content of soluble substance in the bread crumb was determined by extracting a certain amount of finely crumbled bread with water. After filtra-

tion the extract was evaporated and the dried residue weighed.

Investigation of normal (diploid) Rye from the harvest of 1948

1 Material and plan of experiments

Investigation was made of 48 samples of 8 rye varieties from 6 interprovincial experiment fields. The following varieties were used in the investigation:

- I Petkuser (a German variety),
- II Brandt's Marien (a German variety),
- III Kungsråg (a Swedish variety),
- IV Vlaams kortstro (a Belgium variety),
- V-VIII Four selections from Netherlands breeders.

The experiment fields were divided over the country as follows:

- A Borgercompagnie. South-Groningen (Reclaimed peat sub-soils),
- B North-east Polder (Marsh silt soils),
- C Raalte. West-Overijssel (Sand),
- D Beesel. Northern South-Limburg (Sand),
- E Oosterhout. West-Brabant (Sand),
- F Eede. Zealand-Flanders (Sand).

The investigation of the samples embraced the determination of the content of protein and alpha-amylase and of the maltose figure, investigation with the amylograph and baking tests with flour and meal. The results were evaluated statistically, variance, covariance and correlation calculations being carried out. The following properties were involved in this operation:

- 1 Protein content,
- 2 Alpha-amylase content (log),
- 3 Corrected maltose figure,
- 4 Amylogram; maximum viscosity (in Brabender units),
- 5 Amylogram; temperature of the viscosity maximum,
- 6 Amylogram; percentage decrease of the viscosity within 5 minutes after the maximum has been reached,
- 7 White rye bread; panimeter value (log, i.e. log (100 % recovery); cf. (2)),
- 8 White rye bread; % soluble substance in the crumb,
- 9 White rye bread; stickiness, external examination,
- 10 Brown rye bread; stickiness, external examination.

In some cases the data themselves were not used, but their logarithms (indicated by log), as these showed a more linear relation with other data.

2 Results

Table 1 gives a survey of the field averages and the varietal averages while in figure 1 the relation between degree of elasticity (panimeter values) and some other properties is depicted graphically. It appears that the samples differ very much in baking quality. This is to be attributed chiefly to differences in the experiment fields with regard to the degree of sprouting, which is related to stronger or weaker activation of the enzymes, especially of the alpha-amylase. This phenomenon shows itself forcibly in the case of visible sprouting, but also appears even when no germination has taken place. It is this last form, also called *latent* sprouting, which characterizes the enzymic condition of the rye kernel, and by which the data 2–10 (p. 159) are also determined in a great measure, resulting in close correlations between the field averages. The varietal differences compared herewith are of little moment and are not even significant for most of the data. Neither the alpha-amylase content, nor the external examination of the stickness of white bread or dark rye bread, give any indication at all of the presence of varietal differences. In this

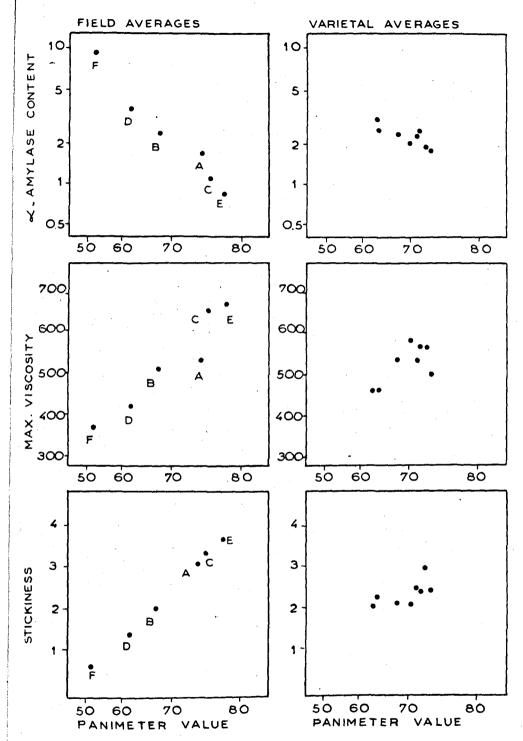


Fig. I. Relation between panimeter values and some other characters, for field averages and varietal averages.

respect, the results differ from earlier observations by Hintzer (1946), which brought to light a decided varietal influence as regards alpha-amylase. A slight indication of a varietal character is found in the properties 1, 4, 5, 6 and 8, a decided one in the corrected maltose figure and a very unmistakeable one in the degree of elasticity as determined with the panimeter. With respect to all properties the experiment field differences are very evident and greater than the varietal differences, even in the case of the panimeter values. This justifies the supposition that differences in susceptibility to sprouting of the varieties examined, were not very important, sprouting being determined to a great extent by local conditions. It can also be plainly seen in figure 1 that the varietal averages show fewer variations than the field averages.

Table 2. Correlation coefficients between various properties and alpha-amylase (No 2) and panimeter value (No 7) respectively for field averages (6 fields) and varietal averages (8 varieties) and the deviations remaining for each sample after elimination of the systematic varietal and field differences.

	Duomontion	Field averages		Varietal averages		Remaining deviation	
	Properties	corr. with 2	corr. with 7	corr. with 2	corr. with 7	corr. with 2	corr. with 7
, 1	Protein content	0.47	-0.43	0.68	-0.62	0.26	-0.24
2	Alpha-amylase	_	-0.98 **)	_	-0.83 **)	_	-0.59 **)
3	Corrected maltose figure	0.83 *)	-0.84 *)	0.68	-0.62	0.70 **)	-0.41 °)
4	Max. viscosity	-0.967 **)	0.952 **)	-0.53	0.72 *)	0.73 **)	0.50 **)
5	Temperature of viscosity max	-0.960 **)	0.961 **)	-0.80 *)	0.62	-0.63 **)	0.43 **)
6	% viscosity decrease	0.94 **)	-0.93 **)	0.51	-0.71 *)	0.60 **)	-0.45 **)
7	White bread; panimeter values	0.983 **)		0.83 *)	· _	0.59 **)	_
8	id.; soluble subst.	0.994 **)	-0.97 **)	0.76 *)	-0.84 **)	0.83 **)	-0.50 **)
9	id.; stickiness ext. exam.	-0.976 **)	0.998 **)	-0.56	0.63	-0.68 **)	0.61 **)
10	Brown bread; stick- iness ext. exam	-0.952 **)	0.921 **)	-0.15	0.07	-0.36 *)	0.40 *)

^{*) =} correlation significant **) = correlation highly significant.

Table 2 gives a survey of a few results of the correlation calculations. In connexion with the grouping according to varieties and experiment fields, a distinction must be made between the correlation of varietal averages, field averages and remaining deviations. Because the number of varieties and experiment fields is not great (eight and six, resp.) only high values of the correlation coefficient are significant for the averages. In the varietal averages such high values are found in only a few cases, but many occur in the field averages. This is connected with the fact, already mentioned, that the varieties differ

very little, whilst the differences in the experiment fields are very marked. The correlations between the remaining deviations are supported by many more data, so that lower values of the correlation coefficient are already significant. For the sake of brevity, only the correlations of two of the properties were included in the table. The general picture, however, can clearly be seen. The properties 2 to 10, which are connected with the action of the amylase on the starch, are strongly correlated. Even the remaining deviations show distinct correlations.

In connexion with these correlations, the extreme values for the field averages, separately listed in Table 1, can be considered as values which on the whole coincide, indicating a relatively good and a very bad baking quality, respectively. Thus these figures provide some starting points for the classification of rye samples according to baking quality on the basis of one or more of the properties 2 to 10.

It appears from Table 2 that the protein content (No. 1) stands alone. A connexion between protein content and resistance to sprouting as was observed by Popoff (1943), was not apparent in this investigation. However, the differences in protein content are very slight here (cf. Table 1).

TETRAPLOID RYE COMPARED WITH DIPLOID RYE

1 Material

Samples of tetraploid and diploid rye were provided by the Institute of Plant Breeding at Wageningen from the harvests of 1948 to 1951 and are listed below.

Number of tetraploid samples	Number of diploid samples		
1	1		
1	2		
8	1		
11	5		
	samples 1 1		

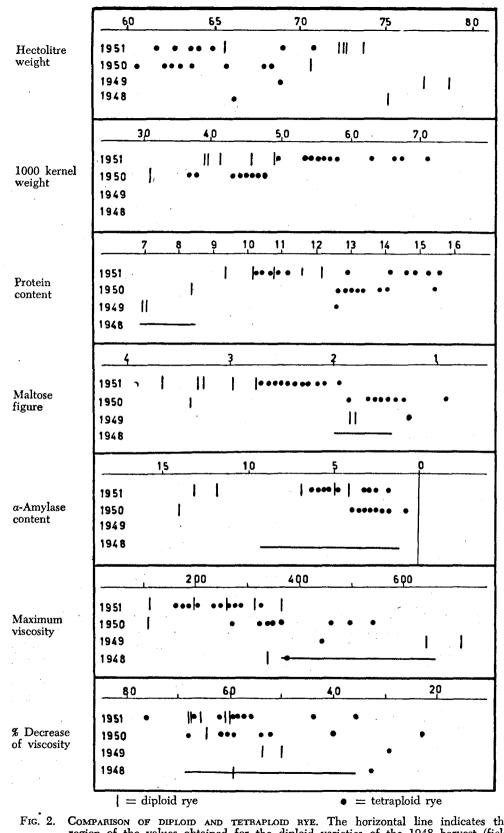
Since in these years tetraploid rye was cultivated on only a few fields the sample material was very limited. Moreover, placing these fields next to those of diploid rye was avoided, in order to prevent cross pollination. The samples of diploid rye used for comparison, therefore, had to be taken from other experiment fields. However, it was also possible for the tetraploid rye results to be compared with those of the comprehensive investigation of diploid rye samples from the harvest of 1948 (cf. Bremer-Reinders (1950)).

2 Results

The most important results of the investigation are graphically depicted in figure 2, some data taken from Table 1 also being included for comparison.

The hectolitre weight of the tetraploid rye proved on the whole to be lower than that of the diploid.

The 1000 kernel weight was determined for 1950 and 1951 only. In each



COMPARISON OF DIPLOID AND TETRAPLOID RYE. The horizontal line indicates the region of the values obtained for the diploid varieties of the 1948 harvest (field averages) according to table 1. "Good extreme" is at the right hand side; "bad extreme" is at the left hand side.

separate year the tetraploid rye shows higher values than the diploid, but the

whole level in 1951 is higher than in 1950.

The protein content of the tetraploid rye reaches exceptionally high levels. Compare this with that indicated for the diploid rye harvested in 1948! In 1951 the protein contents of diploid rye were in fact also higher than in 1948, but were far exceeded by those of tetraploid rye in the same year. A higher ash content of the tetraploid rye runs parallel with this. (This connexion is not shown in figure 2).

The maltose figure for 1950 and 1951 of the tetraploid rye is on a clearly lower level (which means better as regards quality, i.e. more to the right in figure 2) than that of the diploid rye. The maltose figures of the diploid rye of 1948, however, show the same low level. It is therefore remarkable that in 1950 and 1951 a few very high maltose figures were met with solely in the

case of diploid rye.

The same is true of the alpha-amylase content and, though less distinctly,

of the percentage decrease in viscosity (amylogram).

With tetraploid rye the maximum viscosity (amylogram) is somewhat lower than is to be expected on the basis of other data. This is clearly shown, for instance, by the results of 1949. While maltose figure and decrease in viscosity indicate that sprouting of the tetraploid rye sample is less, yet the maximum viscosity of this sample proves to be lower than is the case with diploid rye of this year. The higher protein content and the correspondingly lower

starch content of tetraploid rye possibly play a part there.

This investigation also included baking tests. The baking quality proves to be chiefly determined by the measure of activation of the enzymes (sprouting), as was the case with diploid rye of 1948 (cf. 3.2). As appears from figure 2, this is stronger in 1948, 1949 and 1950 in the case of the samples of diploid rye than in that of the samples of tetraploid rye. In agreement with this, the baking tests of the samples of tetraploid rye also give better results. The difference in 1950 is very great. Figure 3 clearly shows that the samples of diploid rye give quite good bread, while one of the samples of diploid rye was so strongly attacked by sprouting that there was absolutely no sign of a

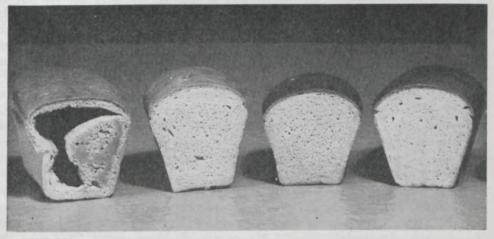


Fig. 3. Loaves from Rye flour, obtained in baking tests with diploid and tetraploid Rye of the harvest of 1950. The first loaf from the left, with heavy starch degradation, is made from diploid rye; the others are from tetraploid rye.

normal texture of the crumb. From the size of the crust it can be seen that the texture must first have been normal, but must have collapsed completely, after crust formation, owing to the increasing temperature and the accompanying stronger enzymic action.

It is a pity that in 1951, besides the samples of tetraploid rye, sufficient material for baking tests was only available from *one* sample of diploid rye. This was just the sample that had suffered most from sprouting, so that also in the baking tests for 1951, the tetraploid rye shows up much more favourably than the one sample of diploid rye. According to figure 2 sprouting in 1951, however, was not in the case of all diploid samples much worse than in the tetraploid, so that after all, these baking tests gave relatively little information regarding the baking quality of tetraploid in comparison with that of diploid rye.

DISCUSSION

It has already been pointed out in the introduction that the baking quality of rye in our climate depends to a great extent on whether or not an increased activity of the starch-splitting enzymes (sprouting) occurs. This activity manifests itself in many ways and in consequence a number of methods are available that, directly or indirectly, measure this activity.

The object of some methods is the measurement of an enzyme content, as, for instance, the determination of the alpha-amylase. In other methods, a determination is made of the amount of degradation products formed after a certain time of attack by the amylase, when the sample itself serves as substrate. In addition to the amylase content, the resistance of the starch to enzymic breakdown plays a role in this case. Examples of this are the determination of the maltose figure and of the content of soluble substances in the bread crumb. Also in the investigation using the amylograph, the amylase is given the opportunity of attack, the investigation in this case not being directed to the degradation products, but just to that part of the starch fraction that has not been broken down. This also applies to the determination of the stickiness of the bread, which is obtained as a result of the baking test.

In the investigation of the 48 samples of diploid rys from the harvest of 1948, great differences in enzyme activity were encountered, which can chiefly be attributed to differences between the experiment fields. With the methods of investigation used, it proved possible to establish these great differences, good agreement being achieved, as appears from the values for the correlation coefficient in Table 2.

The varietal differences were much less pronounced. That no distinct varietal differences occurred as regards the alpha-amylase content, but were met with in maltose figure and stickiness (as measured with the panimeter), could be interpreted in this sense, that in the investigated material only the nature of the starch fraction, not the formation of starch-splitting enzymes, differed from variety to variety.

In the investigation of tetraploid rye from various harvest years compared with diploid rye, the result most evident was that sundry diploid samples showed very greatly increased enzymic activity (sprouting), whilst in none of the samples of tetraploid rye did it appear to the same extent. This gives the impression that the tetraploid rye is better resistant to sprouting whether or not this be latent or visible. A conclusion, however, cannot be drawn with

certainly, because the number of samples was small and those of diploid and tetraploid rye did not come from the same experiment fields, whilst we have learnt from the investigation of diploid rye from the 1948 harvest, that the degree of damage can be very different on different fields and that slight damage is not at all unusual for diploid rye also.

It is worth while to proceed with the investigation in this direction, whereby great attention must be given to the choice of sample material. In view of the larger size of the kernel (hence also of the endosperm) and the higher protein content, it is not at all improbable that the tetraploid rye has a greater resistance to decrease of baking quality by sprouting. In this connexion, it could be thought that the starch granule receives a certain protection from amylolysis by being enveloped in protein. However, it is more probable that the explanation must be sought in the same direction as that taken by Popoff (1943), who, in the case of a high protein content accompanied by a great resistance to sprouting, attributes exceptional importance to certain growth substances. Meanwhile further investigation must teach how far the conceptions expressed here are based on reality.

The higher protein content of the tetraploid form is in itself a quality that, however, could not show itself in this investigation owing to the limited number of baking tests and the great influence that the difference in enzymic starch degradation had on the baking quality. More detailed baking with samples without abnormally increased enzyme activity, would be necessary to establish the significance of the higher protein content to the baking quality.

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