CCXXIX. DISTRIBUTION OF SUGAR AND NITROGENOUS SUBSTANCES IN WHEAT GRAIN¹.

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THE problem of the distribution of sugar and nitrogenous substances in wheat grain is of great practical and theoretical interest. A solution of this problem would provide the miller and baker with a number of indications concerning the composition of the different products of milled grain, enabling them to carry out the technological process upon more rational lines. The existing data on this subject are scanty.

Schulze and Frankfurt [1894] found that the germ of the wheat grain contains up to 25 % of sucrose on a dry weight basis. Apparently on the strength of this finding many subsequent investigators have considered that all the sugar of the wheat grain is concentrated in the germ. Having learned from a number of detailed analyses of wheat [Kiesel and Kretovitch, 1933] that in the whole ripe wheat grain the principal sugar consists of sucrose and that monosaccharides are almost absent, we have attempted to find out whether sucrose occurs in the endosperm as well as in the germ, and if so, whether the greater part of it is located in the outer or inner layers of the endosperm; also whether it can be found in the cells of the aleurone layer.

As regards nitrogenous substances we were interested principally in the question as to what portions are present in the cells of the aleurone layer, as it is known that flour from the latter fails to yield gluten.

We could not find in the literature data of direct analyses of the aleurone layer of wheat grain, although in the work of Teller and Teller [1932] on the nitrogenous substances of wheat bran it is indicated that the "epicarp" of the wheat grain contains little gliadin.

EXPERIMENTAL.

In order to determine the distribution of sugar between the germ and the other parts of the grain, a portion of the grain containing the germ was cut off from the rest and each part was analysed separately. As these analyses have shown (see below) that the portion of the grain without the germ² contained considerably more sucrose than the germ, it was desirable to obtain still more accurate determination of sugar distribution between the inner and outer layers of the endosperm and of the aleurone layer.

¹ The present investigation has been carried out in co-operation with S. S. Tanagos.

² Under the term "germ" we understand the portion of the grain called so by the millers, viz. the sprout.

For this purpose the grain was divided by a scalpel into three parts. First, the part containing the germ was cut off, and then the rest was divided into one part containing the inner kernel of the endosperm and another consisting of the bran coat with the aleurone layer and the outer layers of the endosperm.

Simultaneously with the analyses of the different parts of the grain an analysis of the whole grain was made as a check.

Material used for the investigation consisted of the grain "Cooperatorka" of the 1931 crop from the Crimea. The analyses were carried out according to the method of Kiesel [1930]; in the aqueous solution obtained after extraction of the finely ground material by 82 % alcohol and evaporation of the latter *in vacuo* and after clearing of the solution by lead acetate the following determinations were made: (1) *reducing sugars*, (2) *sucrose*—by the difference between the sugar determination obtained after 5 minutes' hydrolysis by 2 % HCl at 70° and (1), and (3) *resistant polysaccharides*—by the difference between the sugar determination after 24 hours' hydrolysis by 1 % HCl at 70° and (2). All determinations were made according to the Bertrand method and calculated on a dry weight basis.

A summary of the results of analysis of the grain divided into two parts—one containing the germ and the other without it—and of the control analysis of the whole grain is given in Table I.

Table I. *Sugar as % of dry weight of different portions of wheat grain.*

Material investigated ...	Part containing germ; 22.24 % of weight of grain	Part without germ; 77.76 % of weight of grain	Whole grain (control)
Reducing sugars	0.21	0.0	0.0
Sucrose	2.18	1.45	1.67
Resistant polysaccharides	0.0	0.0	0.0

By control computation of the data of the first two columns on the whole grain basis the following was obtained:

Reducing sugars	0.04 %
Sucrose $1.13 + 0.49 =$	1.62 %
Total	1.66 %

Results of the investigation of the grain divided into three parts are given in Table II.

Table II. *Sugar as % of dry weight of different parts of wheat grain.*

Material investigated ...	B Part without germ; 80.94 % of weight of grain		
	A Part containing germ; 19.06 % of weight of grain	Outer layers of endosperm, with the bran coat and aleurone layer; 29.73 % of weight of "B"	
			Inner layers of endosperm; 70.27 % of weight of "B"
Reducing sugars	0.0	0.0	0.0
Sucrose	2.96	2.58	0.88
Resistant polysaccharides	0.01	0.0	0.0

Control computation of these figures on the whole grain basis shows the following ratio of the absolute quantities of sugar in the various parts of the

grain: 0.56 % (part containing germ) + 0.62 % (outer endosperm layer) + 0.50 (inner endosperm layer) = 1.68 %.

Material used for the investigation of the substances of the aleurone layer consisted of wheat grain, the germ and endosperm of which had been devoured by the storage weevil (*Calandra granaria* L.) by the ravages of which the inside of the grain had been cleaned out so neatly that the part left intact contained only the bran coat and the aleurone layer. Even under the microscope it was impossible to detect any particles of endosperm. Microscopic investigation showed that the cells of the aleurone layer and their contents had been left untouched by the weevil.

For the purpose of chemical investigation of the emptied grain it was subjected to a thorough grading and separation from foreign matter, ground as finely as possible and analysed for sugar content and various nitrogenous substances.

Repeated analyses have shown an absence of sugar from the empty grain and consequently from the aleurone layer.

Analyses of the nitrogenous substances contained in the empty grain, and consequently in the aleurone layer, have been carried out in the following manner.

A 10 g. air-dry aliquot of the material was extracted consecutively by water at 70–75° 4 times, 30 minutes each; by a 5 % solution of K_2SO_4 4 times, 3 hours each; by 70 % ethyl alcohol 4 times, 3 hours each; and lastly by 0.2 % solution of NaOH 4 times, 3 hours each.

Total nitrogen was determined by the Kjeldahl method in the original material, in the extracts obtained and in the residue.

Furthermore the aqueous extract was precipitated by normal lead acetate, and in the carefully washed precipitate a separate determination was made of the nitrogen of the water-soluble proteins; in the filtrate amide-N was determined by the Sachsse method and NH_3 -nitrogen by the Longy method. Results of the analyses are summarised in Table III.

Table III.

Fraction of nitrogen					% of dry weight	% of total nitrogen
Total	...	...	...	...	3.54	—
Non-protein	...	...	...	...	0.88	24.85
NH_3	...	...	...	...	0.33	9.33
Amide	...	...	...	...	0.22	6.20
N of water-soluble proteins	...	...	...	...	0.47	13.28
N dissolving in 5 % solution of K_2SO_4	...	...	...	...	0.18	5.09
N dissolving in 70 % alcohol	...	...	...	...	0.21	5.85
N dissolving in 0.2 % solution of NaOH	...	...	...	...	0.96	27.10
N insoluble in 0.2 % NaOH	...	...	...	...	0.80	22.60

SUMMARY.

The data obtained permit the following conclusions.

Sucrose is contained not only in the germ of the wheat grain but also in the endosperm, the total amount being greater in the latter than in the former.

Considering that the weight of 100 whole grains is 3.200 g. and the weight of 100 empty ones 0.371 g., i.e. that the latter amounts to 11.6 % of the weight of the former, and taking into consideration the data of Table II, it is easy to estimate that the concentration of sugar in the outer layers of the endosperm is about 4.8 times greater than in the inner layers. This fact shows that the sugar is distributed in the grain in the same manner as is gluten [Cobb, 1925].

Analyses of the grain emptied by the weevil show that there is apparently no sugar in the aleurone layer.

As regards nitrogenous substances the results obtained by us confirm the data of Teller and Teller [1932], who found that wheat bran contains little gliadin. Our results indicate that there is practically no gliadin in the cells of the aleurone layer. Thus it becomes clear why flour from the cells of the aleurone layer does not yield gluten.

As regards glutenin and albumin, they compose the principal part of the protein substances of the aleurone layer, and the aleurone grains of wheat apparently contain a very insignificant amount of globulin. It is possible that during the process of extraction a part of the globulins may have passed into the water-soluble protein fraction, as the empty grains contain 6.57 % ash of dry weight substance, but the error caused by this factor could not be very great as in a volume of 100 cc. of the extracted liquid the concentration of salt was approximately 0.6–0.7 %.

Thus the general conception of aleurone grains as deposits of globulins does not apply to the aleurone grains of wheat.

In regard to the conclusions drawn from analysis of the empty grains it might be argued that the weevil while eating the grain might have caused some changes in the substances of the aleurone layer and that therefore the picture given by our analyses is not quite true. We do not however regard this as a serious objection since the microscopic appearance of the aleurone layer of such emptied grains is perfectly normal.

In conclusion we wish to express our thanks to Prof. A. R. Kiesel for his interest in this work.

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