DISTRIBUTION OF THIAMIN (VITAMIN B_1) AND FAT IN WHEAT GRAIN.

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In our former investigations concerning the thiamin of cereals (7, 8) we have treated the thiamin contents of wheat, rye, barley, and oats, and those of their different milling and baking products. Special attention has been paid to the question how thiamin is distributed between the different parts of wheat grain.

One of the results is that we found the old idea according to which the thiamin of wheat grain would mostly be accumulated in germ and bran to hold good only as far as the germ is concerned. It appeared that the thiamin content of the outer layers of bran tissue is low, distinctly lower than that of the grain on an average.

Another interesting observation concerned the relation between the contents of thiamin and fat. In the investigation of a flour series formed by mixing flour streams of a commercial mill in an order decided by the rising ash content it appeared that not only the thiamin content but also the fat content increased fairly regularly with the increasing of the ash content. In other milling products, as shorts, bran and germ preparations the relation of the fat and thiamin contents was not equally regular, but it was notably more fixed than the relation of the ash and thiamin contents. No evident correlation could be stated between the protein and thiamin contents.

In the experiments mentioned above we used the wellknown yeast fermentation method of Schultz, Atkin and Frey for the determination of thiamin. This method is very sensitive, although not very convenient. As the thiochrome method of Jansen, according to several recent investigations, gives results accordant with those obtained by animal experiments, we have adopted this method in the experiments the present paper deals with. The purpose of the experiments was to explain further the distribution of thiamin in wheat grain and the mutual relations of the thiamin, fat, and ash contents in the different parts of grain. Special attention has been paid to the thiamin content of bran tissues.

Methods of Analysis.

On developing a method suitable for our purposes to determine thiamin we made among others experiments in which the same sample was tested by using both the yeast fermentation method and the thiochrome method. The former was used virtually in the form developed by HEYNS (5, 7); and the latter in the form explained below. It appears in the examples presented in Table I that in some cases the yeast fermentation method will give very much higher results than the thiochrome method. No attempt was made to find out the reason of these differences (cf. 6).

Table I. Comparison between Fermentation and Thiochrome Methods.

Wheat product	Thiamin, µg/g (dry basis)	
	Fermentation method	Thiochrome method
Whole meal	6.4	3.5
5th break flour	7.6	4.1
Germ, separated by hand	51.0	35.0
Commercial germ preparation »Ebe»	22.0	13.5

After much experimenting to find out which of the many modifications of the thiochrome method would best suit our purposes we adopted the following procedure. An extract from the wheat product to be treated was prepared by boiling it with a 15-fold amount 0.1 N H_2SO_4 for 3 minutes. The pH of the extract was brought to 4.5 by adding 1/6 of the volume of 1.2 mol. sodium acetate (4). 100 mg diastase and 50 mg pepsin per 1 g of the sample under investigation was added to free the thiamin possibly bound in cocarboxylase and proteins (2, 3). The mixture was incubated at 50° C. for 2 hours or at 35° C. over night, and after that such an amount of 0.1 N H_2SO_4 was added that the volume was 20 ml per 1 g of the sample. The mixture was boiled up, then cooled down to room temperature and filtered. Thiamin was determined in the usual way in the filtrate by oxidizing it to thiochrome in an alkaline solution, and thiochrome was taken into butanol. Fluorescense was measured with Pulfrich photometer (Zeiss) by using filter K 7. The control test was treated in the same way, but without the ferricyanide oxidation. On using the filter K 7 the extract need not be purified with zeolite or butanol. Purifying takes time and part of thiamin is easily lost when using zeolite. As a control solution in the photometer we used sulphate of quinine. The use of Pulfrich photometer demands a good deal of training because the adjusting has to be done very quickly as the influence of the ultraviolet light makes the strength of fluorescence to decrease rather rapidly.

Crude fat was determined by extraction the samples, which had been preliminarily dried, with ether in the Twisselmann's apparatus.

All the thiamin, fat, and ash contents are given calculated on dry basis. The small amounts of sand contained in some samples are excluded from the final figures for ash.

Germ.

It is generally known that the germ of wheat kernel contains plenty of vitamin B 1. Althoug the weight of the germ, is very small, 3 percent of the weight of the grain at most, it is not impossible that the germ contains a great part of the whole amount of thiamin in wheat grain. In a commercial mill the separation of the germs is far from complete. Most part of germs gets into other products and even in best case only about 1/4 into the germ preparation proper. On the other hand, the commercial germ preparations contain considerable amount tissues from the other parts of the grain, small parts from both the endosperm and bran.

We have earlier shown that most part of the thiamin of wheat kernel is accumulated in the end nearest the germ (7). When a few thousand grains were cut off very near the germ so that the germ ends weighed 13.4 to 18.3 per cent of the total weight, it appeared that the thiamin content of these pieces was 13 to 16 times as great as that of the bigger parts and they contained 67 to 77 per cent of the total quantity of thiamin in grain.

To study more closely the thiamin content of the germ and the relation of the thiamin and fat contents of the germ with the corresponding contents of the whole grain we made the following experiment. The germs were separated from a few thousand kernels with the rounded tip of a tool. The microscopic investigation showed that the preparation obtained in this way contained very little of other tissues besides germs, while in many cases it could be statet that there was some amount of germ tissue left in the body of grain. It could not be stated how great a part of the embryo, especially of the scutellum, on an average was left unseparated, but concluding from the fact that the weight of the picked germs was more than 1.5 per cent of the whole grain, the main part of the embryo must have been separated. The results of the tests are given in Table II.

Table II. Contents of Ash, Thiamin, and Fat in Wheat Grain and Separated Embryo (Dry basis).

Sample	Yield, %	Ash, %	Thiamin		Fat	
			Content, μg/g	Per cent of total	Content, %	Per cent of total
Summer Wheat:						
Kernel	(100)	1.83	3.87	100	1.97	100
Embryo	1.56	5.61	35.1	14.2	18.80	14.9
Winter Wheat:						
Kernel	(100)	2.03	3.50	100	2.00	100
Embryo	1.71	5.67	35.0	17.1	15.80	13.5

It can be concluded from the figures in Table II that although about 15 per cent of the thiamin of wheat kernel was removed with the separated germs the main part of it, however, remaiend untouched in the kernel. It is possible that the germ contains a substantial part of the whole amount of thiamin in wheat grain. The thiamin content of the separated germs was approximately ten times as great as the average thiamin content of wheat grains, and about 40 to 50 times greater than that of those parts of grains that are poorest in thiamin (see below).

The embryo of wheat contains very much lipoids, as is well known. In these experiments the crude fat content of the germs separated by hand was found to be 16 to 19 per cent (Table II), which is about 8—10 times higher than the fat content of wheat grain as a whole. This being the case, the relation of the thiamin and fat contents in the separated germs was almost the same as in whole grain, a little greater in germs, however. The results do not give any support to the thought that it would be possible to reduce the fat content of wheat grains by simple mechanic means by taking off the germs or the main part of them without causing a material decrease in the thiamin content of the grains.

The ash content of the germs separated by hand was nearly 6 per cent (Table II) or about the same as that of ordinary bran from a commercial mill. The ash content of germs is not more than about 3 times as great as that of whole grains, and it would not be as easy to conclude the thiamin content of the germs by it as by the fat content, even if the increase of the thiamin content in ordinary flour generally corresponds to the increase of the ash content. It will be shown later on that, on the other hand, the thiamin content of the outer layers of bran tissue is very much lower than could be expected on the ground of their relatively high ash content.

Bran.

The thiamin content in bran from a commercial mill is often somewhat lower than in such products that contain more tissues from under the bran coats. It can be anticipated even by this that the thiamin content of bran tissue is not quite as great as was earlier supposed. Of course the supposition is possible, too, that on washing wheat part of the thiamin of the bran tissue could be lost; we have earlier shown (8), however, that this process cannot have any noteworthy influence on the thiamin content of bran. In spite of the fact that the thiamin content of bran from a commercial mill is not as great as that of some other milling products, it is regularly greater that the average content of the grain, so that the separation of bran from milling products meant for human nourishment reduces the thiamin content of food.

In an earlier experiment (7) we could separate from the surface of the wheat grain a small amount of material in which the thiamin content was about three quarters of the content of whole grain. It was due to the inadequacy of our method that parts of the protruding embryos were got into the bran preparation, and so there was cause to suppose that the thiamin content of separated bran tissues was considerably low.

To get as precise a conception as possible of the thiamin content of bran tissue we constructed a small simple scouring machine, able to take 1—2 kilos grains at a time. With this apparatus it was possible to take off a few percents from the outer layers of bran without separating considerable amount of other tissues of grain with them. Results of a typical scouring experiment have been given in Table III.

Wheat was hard and smallgrained (Manitoba No. 2). All impurities were carefully removed from it, but it was not washed.

Table III. Scouring Series of Wheat.

Scouring No.	Yield, per cent of kernel weight	Removed material contained (dry basis)			
		Ash, %	Thiamin, μg/g	Fat, %	Fiber, %
0	Original wheat	1.81	3.8	2.18	3.1
1	0.4 (0—0.4)	3.06	0.9	1.82	25.2
2	0.8 (0.4—1.2)	2.84	1.2	2.14	24.7
3	0.7 (1.2—1.9)	3.03	1.6	3.19	25.4
4	1.2 (1.9—3.1)	3.93	3.3	5.26	19.9
5	1.2 (3.1—4.3)	4.89	4.3	6.67	15.5
6	1.1 (4.3—5.4)	5.15	5.4	6.80	8.7
7	1.7 (5.4—7.1)	5.05	6.3	6.45	10.9
8	2.0 (7.1—9.1)	4.65	5.9	6.04	—
9	1.8 (9.1—10.9)	4.31	6.3	5.55	7.7
10	3.7 (10.9—14.6)	3.84	6.8	5.07	—
11	2.7 (14.6—17.3)	3.48	7.1	4.62	—
12	2.8 (17.3—20.1)	3.18	8.2	4.43	—
13	4.9 (20.1—25.0)	2.77	8.3	3.74	4.1
14	5.0 (25.0—30.0)	2.40	6.7	3.52	—
15	5.0 (30.0—35.0)	2.04	5.7	2.94	—
16	5.4 (35.0—40.4)	1.77	5.2	2.49	—
17	5.2 (40.4—45.6)	1.56	4.0	2.28	—
18	4.4 (45.6—50.0)	1.48	3.2	2.05	—
19	50.0 (Rest)	0.88	1.5	1.09	0.6

It appears in Table III that the separation of all parts of wheat grain from each other by scouring was not nearly complete, not even as good as it is in a commercial mill. So it is possible in a commercial mill to get an extraction of nearly 80 per cent with an ash content of 0.88 per cent while in the present case the corresponding yield was only 50 per cent (see Table III, N:o 19). Similarly the ash content of some products of a commercial mill may be greater than that of the product N:o 6 in our experiment. But in one respect this procedure is better by far than the ordinary commercial milling process: it was possible to separate a part of the bran tissue in a very pure form. The microscopic investigation showed that at the first scouring (Nos 1 & 2) scarcely anything else besides outer layers of bran (pericarp) was removed from the grain. On further scouring, however, small parts of the protruding germs begin to come off, while part of even the outer layers of bran sticks on in the grain. Especially the groove of the grain remains untouched until a rather late phase. Even at the fourth scouring the wearing off of the germs could be clearly stated. It has also had a remarkable influence on the results of analysis in Table III, especially on the contents of thiamin and fat.

On the ground of the figures given in Table III it can be stated that the thiamin content of the outer bran tissues of wheat is very low, only about one quarter of the average content of grain, or about as low as that of the whitest patent flour containing mainly inner part of the endosperm. The removing of pericarp, then, does not by any means reduce the thiamin content of wheat, as was earlier supposed; on the contrary, it increases it. The experiment shows also that it is possible by technically simple means to remove a remarkable amount (at least 6 to 7 per cent of the weight of grain) of the outer layers of grain richest in fiber without lessening the thiamin content. This appears in the figure 1, too, that shows part of the results of the experiment.

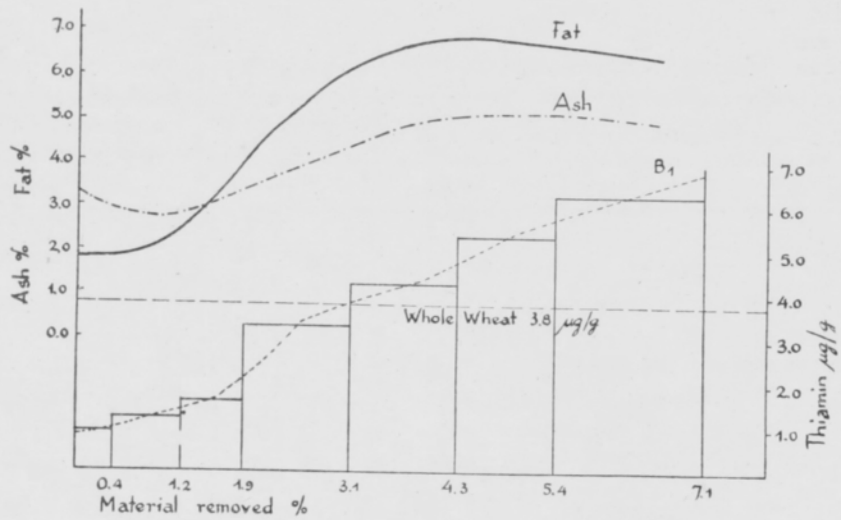

Fig. 1. Effect of scouring on wheat.

On examining the high figures of the fiber content of the outer bran tissue (Table III) the idea presents itself that the physiological value of these tissues for man cannot be specially high, nor is their influence on the baking quality of flour advantageous. It may be mentioned here that in analysis the fiber content of the grains after removing (altogether) 7.1 per cent of material from them was found to be 1.7 per cent (calculating by the Table III, 2.0 per cent) which is only about 55 (65) per cent of the content of the whole kernel.

On following the mutual relations of the ash, thiamin, and fat contents in Table III it is seen that they are not congruent with each other in every phase. The most conspicuous feature is perhaps the comparatively high ash content of the outer layers of bran contra a very low thiamin and rather low fat content. The relation of the thiamin content to the fat content seems to be smaller in bran tissues than in other parts of the kernel.

Commercial Flour

In Table IV there are represented ash, fat, and thiamin contents of the flour streams of a commercial mill. The mill works according to an English five-break system, but by war time orders a great part of the sieves have been changed into coarser ones so that the ash content of many flour streams is higher than in normal times. The wheat mixture contained 55 per cent of Manitoba No. 2, 25 per cent of Russian, 10 per cent of Finnish summer wheat, and 10 per cent of Swedish winter wheat.

Table IV. Contents of Ash, Thiamin, and Fat of a Series of Mill Streams.

Wheat product	Ash (dry basis) %	Thiamin (dry basis) μg/g	Fat (dry basis) %
Wheat	1.80	3.8	2.00
1st Bk	0.78	1.3	1.32
2nd Bk	0.60	1.1	1.26
3rd Bk	0.64	1.4	1.42
4th Bk	1.39	2.8	2.62
5th Bk	3.19	5.5	4.34
X	0.98	5.7	2.22
Y	1.33	3.8	2.42
BM 1	0.69	1.2	1.41
BM 2	1.61	5.2	2.82
BM 3	4.17	8.6	5.38
»Manna» (middlings)	0.53	0.9	0.92
A	0.61	1.7	1.23
B	0.58	1.4	1.16
C	0.55	1.6	1.21
B 2	0.70	3.5	1.65
D	0.56	1.6	1.36
E	1.28	6.6	2.39
F	1.35	6.5	2.77
G	1.22	4.3	2.58
H	1.64	7.1	3.35
J	2.35	11.2	4.36
K	1.91	9.4	3.69
L	1.41	3.4	2.32
M	3.06	7.6	4.75
CO	3.32	11.8	5.02
Plats	0.92	3.3	1.90
Germ. conc.	3.62	13.5	7.58
Shorts	5.44	9.4	6.70
Bran	6.12	5.8	5.63

It appears in the table that in many cases the ash, thiamin, and fat contents agree with each other very well, but there are exceptions, too. Especially the thiamin content of bran is considerably lower than could be expected on the ground of the high ash content. The results support the rule given by Abdon and Laurell in their exhaustive study (1) that »In each series of mill products (break and reduction flour, and bran) a higher fat contents corresponds to a higher vitamin content. When the fat content is the same, the reduction flour has the highest vitamin content, the bran the lowest and the break flour shows a value lying in between.»

In many European countries the control of the grinding of wheat is almost solely based on the ash content. Even before the war there was the method in many mills to keep the quality of different types of flour constant by frequent ash analyses. During the war the maximum, often also the minimum of the ash content in the allowed types of flour has been fixed by decree (in Finland the ash content of wheat flour has mostly been limited to 0.95 to 1.05 per cent,corresponding to an extraction of about 82 per cent). If the maximum of the ash content is fixed, it is in the miller's interest to try to gain as high an extraction as possible without exceeding the limit. This is done by mixing the flour streams according to the ascending ash till the ash content of the mixture has risen to the desired point. Table V presents a flour series formed in this way from millstreams of a big mill.

Table V. Series of Flours Formed by Mixing Mill Streams according to the Rising Ash Content.

The added and resulting flour mixtures	Yield, per cent of total products	The flour mixture contained (dry basis)		
		Ash %	Thiamin μg/g	Fat %
Flour	63.4	0.60	1.61	1.26
Add. to 1	11.1	1.31	4.61	2.52
Flour 2	74.5	0.70	2.05	1.46
Add. to 2	4.2	2.46	8.19	4.11
Flour 3	78.7	0.80	2.59	1.61
Add. to 3	4.9	5.21	9.79	6.34
Flour 4	83.6	1.00	3.01	1.89
Add. to 4	4.0	5.44	9.40	6.70
Flour 5	87.6	1.20	3.30	2.11
Add. to 5	5.8	5.93	6.36	5.80
Flour 6	93.4	1.50	3.49	2.34
Add. to 6	6.6	6.12	5.80	5.63
»Flour« 7 .... (Whole meal)..	100.0	1.81	3.65	2.55

As it appears in Table V the ash, thiamin, and fat contents of a commercial series of flours (flours 1 to 7) follow each other considerably regularly. The fat and especially the thiamin contents of such flours (6 & 7) that contain plenty of bran remain considerably lower than could be expexted on the ground of the corresponding ash contents. This is due to the fact that the thiamin content of bran tissue is comparatively very low. This relation is in good accordance with the results of analyses given in Table III.

Discussion.

Physiologists have always recommended the use of whole meal, as this contains considerably more some important nutrients, especially vitamins of the B group than the ordinary white flour. The sale of graham meal is lessened, however, by its low baking quality, not well-liked taste, and some properties disadvantageous to digestion. The last mentioned fact is mainly caused by the grain containing some ingredients that either are wholly indigestible for man or partly digestible by means of bacteria. In this respect the experiments described above have certainly some interest, as they open the possibility to reduce the fiber contents of grains considerably without causing any or corresponding diminishing in the vitamin content, at least not in the vitamin B 1.

The relation of the thiamin content to the fat and ash contents in flours of different grades is interesting because the exact knowledge of these relations makes it possible by simple means to control the physiological value of flours. »The Conference on the Post-War Loaf» convened by the Ministry of Food of Britain recommend in their report (9) the securing of the physiological value of flour by appointing minimums for three important nutrients, vitamin B 1, nicotinic acid, and iron. This would be carried out by taking an extraction of 80 per cent at least in the mill. Considering how difficult a task it is to determine in a really reliable way thiamin and nicotinic acid it seems probable that in many cases a simpler means will have to do. One is the determination of crude fat that can be done accurately enough in any laboratory. In fact, the analysis of ash already gives a good picture of the amount of the rate of extraction and of the amount of those physiologically important ingredients as well. As it is possible, however, to add to the ash content artificially, in the same way as the amount of some vitamins for instance, the analysis of ash alone will not give sufficient security that the rate of extraction is in agreement with the orders. But the determination of the fat content gives a better security in this respect. On the ground of above experiments it seems to be comparatively certain that if a commercial flour contains a considerable amount of thiamin, for instance, but the fat content is not on a corresponding level, there is reason to suppose that all of that vitamin has not come into the flour in the »natural way», i.e. as a consequence of a high rate of extraction, but that vitamin preparation of a different origin has been added to the flour.

Summary.

The chief purpose of the experiments has been to find out the thiamin content of the different parts of wheat grain. Special attention has been paid to the relation of the thiamin and fat content in germ, in the outer bran tissues, and in flours of different grades.

On comparing the yeast fermentation method and thiochrome method with each other the former gave 45 to 85 per cent higher values for thiamin than the latter. The results presented in this paper were obtained by using the thiochrome method. All thiamin, as well as crude fat, ash, and fiber contents are given on dry basis.

70 to 80 per cent of the thiamin contained in wheat is concentrated in the germ end of the kernel.

In analysis there were obtained the following contents for the germ preparations separated in a special way: ash 5.6 & 5.7 %; thiamin 35.1 & 35.0 µg/g; fat 18.8 & 15.8 % (the corresponding figures of whole kernel; 1.8 & 2.0 %; 3.9 & 3.5 µg/g; and 2.0 & 2.0 %). The thiamin content of the germ preparation was accordingly 9 to 10 times and the fat content 8 to 10 times greater than that on whole kernel so that the relation of the thiamin and fat contents in the germ preparation was about the same

as in whole kernel. The content of ash, on the contrary, was comparatively much lower in germ.

The thiamin content of the outer bran tissues of wheat is very low. A small quantity of bran containing thiamin only about a quarter (0.9 µg/g) of the amount contained in whole kernel (3.8 µg/g) was separated with a scouring apparatus of simple construction. It was further possible to separate material about 7 per cent of the weight of grain from its surface without more than 7 per cent decrease in the thiamin content; while the decrease in the fiber content was greater by far (about 40 per cent). The relation crude fat/thiamin is greater in bran tissues than in whole kernel.

Analytical data are given of milling products and a series of flours of a commercial mill which was obtained by mixing mill streams in an order according to the ascending ash content.

The possibility to control the amount of thiamin in commercial flour and its physiological value on the ground of the ash and fat contents is discussed. On the ground of the fat analysis it is to some extent possible to decide whether the vitamin or mineral substances found in flour has come into it »in a natural way» i.e. as a result of a high rate of extraction, or if a remarkable part of the nutrient in question has been added into it.

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SELOSTUS.

TIAMIININ (B_1-VITAMIININ) JA RASVAN SIJAINTI VEHNÄNJYVÄSSÄ.

Kokeiden päätarkoituksena on selvittää vehnänjyvän eri osien tiamiinipitoisuutta; huomio on kiinnitetty erityisesti tiamiini- ja rasvapitoisuuden suhteeseen alkiossa, ulommissa kuorikudoksissa sekä eri asteisissa vehnäjauhoissa.

Vertailtaessa keskenään hiivakäymis- ja tiokromimenetelmiä antoi edellinen 45—85 % korkeampia tiamiiniarvoja kuin jälkimmäinen. Tässä julkaisussa esitetyt tulokset on saatu käyttämällä tiokromimenetelmää. Kaikki tiamiini-, samoinkuin raakarasva-, tuhka- ja kuitupitoisuudet ilmoitetaan kuiva-aineelle laskettuina.

70—80 % vehnänjyvän tiamiinin kokonaismäärästä on kasaantuneena alkion puoleiseen kärkeen.

Erityisellä tavalla eroitetuille, huomattavan puhtaille alkiovalmisteille (saalis 1,6 & 1,7 % jyvän painosta) saatiin analysoitaessa seuraavat pitoisuudet: tuhkaa 5,6 & 5,7 %; tiamiinia 35,1 & 35,0 μg/g; rasvaa 18,8 & 15,8 % (koko jyvän vastaavat luvut: 1,8 & 2,0 %; 3,9 & 3,5 μg/g; 2,0 & 2,0 %). Alkion tiamiinipitoisuus oli näin ollen 9—10 kertaa ja rasvapitoisuus 8—10 kertaa suurempi kuin koko jyvän, niin että tiamiini- ja rasvapitoisuuden suhde oli alkiopreparaatissa jotenkin sama kuin koko jyvässä. Alkion tuhkapitoisuus sen sijaan oli suhteellisesti paljon vähäisempi.

Vehnänjyvän ulompien kuorikudosten tiamiinipitoisuus on hyvin vähäinen. Yksinkertaisella hiontalaitteella voitiin erottaa pieni määrä kuorta, jonka tiamiinipitoisuus oli vain noin neljännes (0,9 μg/g) koko jyvän vastaavasta pitoisuudesta (3,8 μg/g). Oli edelleen mahdollista poistaa jyvän pinnasta ainesta noin 7 % jyvän painosta, ilman että tiamiinipitoisuus väheni enemmän kuin 7 %; kuitupitoisuudessa tapahtui samalla monin verroin suurempi (noin 40 %:n) väheneminen. Suhde raakarasva, tiamiini on kuorikudoksissa suurempi kuin koko jyvässä.

Esitetään analysitulokset ison myllyn tuotteista ja sarjasta jauhoja, jotka on saatu sekoittamalla juokseita kohoavan tuhkapitoisuuden mukaisessa järjestyksessä.

Käsitellään mahdollisuutta arvostella tiamiinin määrää kaupallisissa vehnäjauhoissa ja näiden fysiologista arvoa tuhka- ja rasvapitoisuuden nojalla. Rasva-analysin perusteella voidaan jossakin määrin päätellä, onko jauhoissa tavattava vitamiini- tai kivennäisaine tullut siihen »luonnollista tietä» s.o. korkean jauhosaalisprosentin seurauksena vai onko huomattava osa k.o. ravintoaineesta lisätty jauhoon.