THE STEROLS OF RIPENING WHEAT

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The information on the steroids of the wheat grain that has accumulated up to the present time is limited to a small number of papers and has no systematic nature [1, 2]. The authors of the present communication are the first to have made an attempt at a systematic study of the change in the composition of the sterols in the lipids of wheat of the "Mironovskaya 808" variety in five stages of the development of the plant and four stages of post-harvest ripening.

It can be seen from Table 1 that the total amount of steroids in the wheat lipids in the period of ripening from the stage of milky ripeness to the end of storage varies between 2 and 3%. At the first study point (the end of the formation of the grain), the biosynthesis of the sterols is apparently not so intensive and the total amount of sterols is 1.5%.

By mass-spectrometric analysis performed as described by Brooks [3], the following sterols were found in wheat grain: β-sitosterol, stigmasterol, ergosterol, campesterol, brassicasterol, lanosterol, and 24-methylene-24,25-dihydrolanosterol. The ratio of the sterols changes according to the stage of ripening (Fig. 1).

Table 2 gives information on the changes in the relative amounts of the sterols in their mixture in all nine stages of the investigation obtained by comparing the relative intensities of the molecular ions in the corresponding mass spectra. The amount of β-sitosterol falls sharply from 40 to 30% (stage of the beginning of waxy ripeness) and then rises again, while the content of brassicasterol rises to 25-30%. In this period, brassicasterol is possibly the main sterol of the plant, and in the other stages this role is played by β-sitosterol, the amount of which is considerably greater than that of the other sterols, as reported by other

TABLE 1. Change in the Total Content of Sterols in Wheat Lipids during the Stages of Ripening (% on the weight of the lipids)

Expt. No.	Ripening					Post-harvest ripening			
	1	2	3	4	5	6	7	8	9
1	1,5	2,2	2,9	2,0	3,1	2,5	2,9	2,7	2,9
2	2,4	2,4	2,7	2,5	2,8	2,7	2,5	2,6	2,7

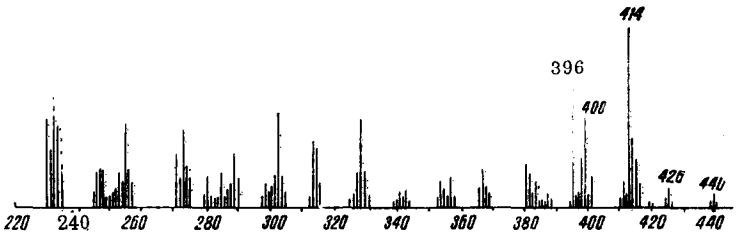

Fig. 1. Mass spectrum of the mixture of sterols isolated at the stage of the beginning of grain formation (1st stage).

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TABLE 2. Relative Amounts of Sterols in Their Mixture (percentages on the sum of the molecular peaks)

Sterols	M	Stage								
		1	2	3	4	5	6	7	8	9
β-Sitosterol	414	40	22	13	16	38	40	43	45	50
Brassicasterol	398	4	7	25	34	7	6	5	3	2
Campesterol	400	17	17	18	18	19	20	21	22	20
Lanosterol	426	5	5	6	6	7	6	5	4	3
Stigmasterol	412	6	6	6	6	7	7	7	8	8
Ergosterol	396	26	21	20	19	20	19	19	18	17
24-Methylene-24, 25-dihydrolanosterol	440	2	2	2	1	2	2	—	—	—

authors [2]. The replacement of one main sterol by another during the development of the plant is an interesting phenomenon. It is likely that at the "beginning of waxy ripeness" stage brassicasterol is mainly present in the cell membranes, the features of the structure of its molecule (as compared with β-sitosterol) consisting in the presence of an additional double bond in the side chain. Because of this, brassicasterol can form π-complexes with biological materials which are capable of affecting the total metabolism in the plant cell. It is characteristic that it is just in these stages that the intensive accumulation of protein takes place [4].

The presence of ergosterol in the mixture of sterols was established without doubt by mass spectrometry and by UV spectroscopy. The nature of the UV spectrum of the mixture of sterols is similar to the spectrum of pure ergosterol (λ_{max} 278.5 nm; ε = 1730). The amount of ergosterol in the mixture of sterols calculated from these figures (18.2%) is close to the results obtained by the mass-spectrometric method.

The increase in the relative amount of β-sitosterol and campesterol during the formation of the grain can be explained by the fact that they consist of the final products of biosynthesis. Since lanosterol, 24-methylene-24,25-dihydrolanosterol, and brassicasterol are those sterols from which stigmasterol, β-sitosterol, and campesterol are biosynthesized, their accumulation in the early stages and the subsequent decrease in their amount in the mixtures through metabolism is a natural phenomenon.

EXPERIMENTAL METHOD

The investigations were performed with the sterols of "Mironovskaya 808" wheat isolated in five stages of field ripening and four stages of post-harvest ripening of the grain: beginning of the formation of the grain, milky ripeness, beginning of waxy ripeness, end of waxy ripeness, full ripeness, six days' ripening in the grain, 10 days' post-harvest ripening, 20 days' post-harvest ripening, and 30 days' post-harvest ripening.

The lipids extracted from the wheat grain [5] were subjected to alkaline saponification [6], and the sterols were extracted from the unsaponifiable fraction as the complex with digitonin [7]. The digitonin complex was decomposed by heating with dimethyl sulfoxide at 100° C for 5-15 min. The sterols that precipitated when the solution cooled were extracted repeatedly with hexane, and the hexane was driven off in vacuum. The dried residue consisted of a mixture of sterols.

The total amount of sterols in each sample (see Table 1) was determined by precipitation with digitonin [5]. The mass spectra of the sterols were taken on an MKh-1306 instrument at 35° C with an ionizing voltage of 75 V. The results of a mass-spectrometric analysis performed with a mixture of sterols (lanosterol, β-sitosterol, ergosterol, and stigmasterol) of known composition showed that the errors in the determination did not exceed 2% (Table 3). The UV spectra were taken in ethanolic solution on a Specord UV-Vis instrument (c. Zeiss, Jena).

TABLE 3. Relative Contents of Sterols in a Known Mixture, %

Sterols	Actual	Found	Error
Lanosterol	30	30,4	+1,33
β-Sitosterol	30	29,5	-1,67
Ergosterol	20	20,3	+1,5
Stigmasterol	20	19,6	—2,0

SUMMARY

1. The changes in the amounts of sterols in the lipids of wheat of the variety "Mironovskaya 808" at various stages of development of plant have been studied.

2. The presence in the mixture of sterols of β-sitosterol, brassicasterol, stigmasterol, ergosterol, lanosterol, 24-methylene-24,25-dihydrolanosterol, and campesterol has been shown.

3. It has been established that the main sterol of wheat lipids is β-sitosterol; however, in the "beginning of waxy ripeness" stage the amount of this sterol falls sharply. At this stage of development of the plant the function of the main sterol is fulfilled by brassicasterol.

LITERATURE CITED

1. C. P. Berry, V. L. Youngs, and K. A. Giller, Cereal Chemistry, 45, 616 (1968).
2. F. Garcio-Olmedo, Nature (London), 220, 1144 (1968).
3. C. J. W. Brooks, Process Biochem., 2, No. 5, 27 (1967).
4. E. D. Kazakov, Seed Production with the Principles of Plant Growing [in Russian], Moscow (1973), p. 37.
5. Handbook on Methods of Investigation, Technical and Chemical Control, and the Accounting of Production in the Oils and Fats Industry [in Russian], Leningrad (1967), p. 820.
6. B. N. Tyutyunnikov, The Chemistry of Fats [in Russian], Moscow (1966), p. 606.
7. C. H. Issidorides, J. Kitagawa, and E. Mosettig, J. Org. Chem., 27, 4693 (1962).