

ESTIMATION OF *TRITICUM AESTIVUM* IN PASTA FLOURS: INTERSPECIFIC LIMITS FOR SITOSTERYL PALMITATE CONTENT

By R. GARCIA-FAURE, F. GARCIA-OLMEDO and J. M. VALLEJO-ACEVEDO

Sitosteryl palmitate (SP) content of flour was shown to be not significantly affected by normal variations in milling yield. Since the distribution of fat in a wheat kernel does not follow the same pattern as does SP, it is preferable to consider SP content on the basis of DM content of the flour. A survey of 46 *Triticum aestivum* and 24 *T. durum* flours showed that the latter contained SP, but its level did not exceed 1.5 mg/100 g. *T. aestivum* varieties show a two-peak distribution, with the maxima at approximately 4 mg/100 g and at 12 mg/100 g, respectively. Three *T. aestivum* flours were within the *T. durum* range and three others were close to it. Limits for sitosteryl palmitate content in *T. aestivum* and *T. durum* were tentatively established at 16.5 mg/100 g and 1.5 mg/100 g respectively. Based on these limits, a method is proposed for the estimation of the minimum amount of *T. aestivum* in a mixture.

Introduction

Detection and measurement of *Triticum aestivum* endosperm, flour or semolina, in pasta products have become of interest from the point of view of quality and market control. Among the physical and chemical differences between *T. aestivum* and *T. durum* endosperms proposed, sitosteryl palmitate content seems to be the most general and significant.

Although cholesterol-like substances had been previously reported in wheat flour,^{1,2} Walde & Mangels³ were the first to observe that a precipitate that did not appear in acetone extracts of *T. durum* was formed at 0° in acetone extracts of *T. aestivum*. The precipitate was tentatively identified as a sterol ester,³ and confirmed as a mixture of sterol palmitates by Spielman.⁴

Matweef,⁵ following Walde & Mangels, checked the occurrence of sitosteryl palmitate in a number of *T. aestivum* and *T. durum* varieties and proposed its gravimetric or colorimetric determination as a means of quantification of *T. aestivum* products in macaroni. Some improvements of Matweef's procedure have been suggested by different authors.⁶⁻⁸ The remaining problem appeared to be poor recovery of the products. Gilles & Young⁹ reported a t.l.c. estimation of sitosteryl palmitate, and finally an accurate method was developed in this laboratory.¹⁰

In the present paper a survey of sitosteryl palmitate (SP) content in a wide number of varieties, as well as in milling fractions, is reported, and the minimum proportion of *T. aestivum* in a mixture is tentatively established as a function of the SP level.

Experimental

Wheat varieties

Forty six *T. aestivum* varieties and 24 *T. durum* varieties were used in this study. These were of diverse origin but were grown either commercially or experimentally in Spain (Crops of 1965 and 1966), with the exception of six *T. durum* samples grown in the U.S.A.

Three varieties, Magdalena and Aragón O3 (*T. aestivum*), and Híbrido-D (*T. durum*), were employed in the fractionation experiment.

Milling fractions

Samples of 2 kg each were normally milled in a Buhler experimental mill, to give three break flours and three reduction flours plus bran and shorts. Bran and shorts were pooled and run through the mill again to give three re-milled fractions: flour, bran and shorts.

Analytical methods

Sitosteryl palmitate content was determined essentially as described previously:¹⁰ 1 g of *T. aestivum* or 3 g *T. durum* product was extracted with diethyl ether in a Soxhlet apparatus. The extract was then fractionated by preparative scale t.l.c. on a 5% AgNO₃ silica gel layer using carbon tetrachloride for development. The lipid was applied as a band 3 cm long. Sitosteryl palmitate was detected under u.v. light (sodium fluoresceinate spray) as the strongest of two bands appearing between the application line and the solvent front. The fainter one was tentatively identified as sitostanyl palmitate and had the higher *R_f*. An SP standard might be run when this procedure is first applied. The adsorbent zone containing SP was transferred with suction to a small column, and this substance was eluted with chloroform until 3 ml were collected. Evaluation was carried out by Tchugaeff colour reactions: 1 ml zinc chloride reagent (melted ZnCl₂, 40 g, in glacial acetic acid, 150 ml) and 1 ml acetyl chloride were added to the eluate, the mixture was heated at 65° for 15 min and read in the colorimeter at 525 nm. Sitosteryl palmitate synthesised in the laboratory was used as a standard. Alternatively, cholesterol palmitate (Fluka A. G. purum) can be used with due correction for the difference in molecular weight.

Sitosteryl levels as low as 1 mg/100 g can be measured with good reproducibility (variation coefficient, $100 \frac{S}{\bar{X}} = 4.7\%$).

Ash by the I.C.C. method¹¹ and fat by the A.A.C.C. method¹² were determined in all milling fractions.

Results and Discussion

The dependence of SP content on milling yield and fat content has been studied in connexion with the setting of tentative limits for SP level in the endosperm of *T. aestivum* and *T. durum*.

Table I summarises the values obtained for ash, fat, and SP content of flour, re-milled flour, shorts, and bran in three wheat varieties, one *T. durum* and two *T. aestivum*. In both species, lower SP levels seem to be present in the outer parts of the kernel (pericarp and seed coats), compared with the endosperm. Although a greater proportion of this substance (2 to 3-fold) has been found in hand-dissected germ, it is not high enough to affect markedly the SP content of bran and short (Table I), where germ is mainly included as a minor component.

In Fig. 1, nine milling fractions from each of the above varieties have been arranged in order of ash content from low to high, and the average values for ash and SP content have been plotted against milling yield.

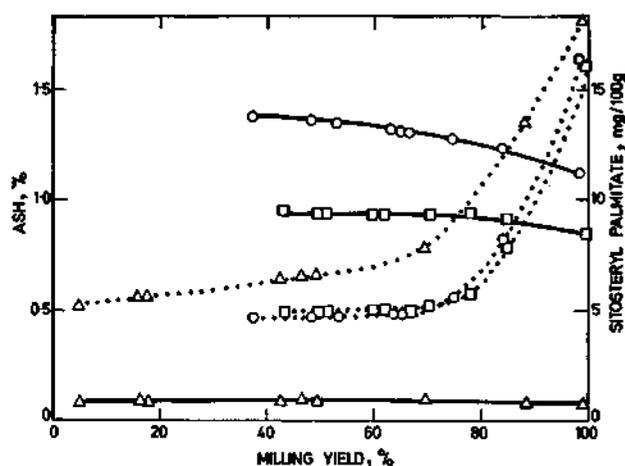


FIG. 1. Sitosteryl palmitate and ash versus milling yield in two *T. aestivum* varieties (Magdalena, Aragón O3) and one *T. durum* (Híbrido-D)

○ Magdalena □ Aragón-O3 △ Híbrido-D
..... Ash — Sitosteryl palmitate

As the milling yield increases greater amounts of particles from the outer layers of the endosperm and from bran are incorporated into flour. Variations in the content of a particular substance in flour due to variations in milling yield will be more noticeable with greater differences in its level relative to the endosperm and the other fractions. Data in Fig. 1 show a fairly even distribution of SP from the inner to the outer layers of the endosperm. A variation of milling yield between 75% and 80%, which implies a sharp increment in ash, does not significantly change SP content. The total variation intervals for SP amounted to 21% of the flour values in Magdalena, 12% in Aragón O3, and 10% in Híbrido-D. Even so, variability due to maximum changes in milling yield is considerably smaller than intervarietal difference within species, as will be seen later.

In Fig. 2, the same arrangement of milling fractions of Fig. 1 has been kept, fat and SP being similarly plotted. Since fat content is greatly dependent on milling yield and its distribution in the kernel does not parallel that of SP, more

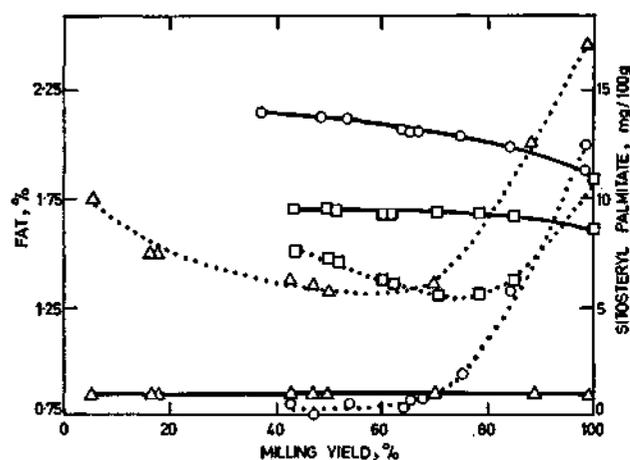


FIG. 2. Sitosteryl palmitate and fat versus milling yield in the same varieties of Fig. 1

○ Magdalena □ Aragón-O3 △ Híbrido-D
..... Fat — Sitosteryl palmitate

TABLE I

Distribution of sitosteryl palmitate in milling fractions†

Name of Product	Fraction, %			Ash, %			Fat, %			Sitosteryl palmitate mg/100 g		
	A	M	D	A	M	D	A	M	D	A	M	D
Flour*	69.9	68.0	49.6	0.57	0.55	0.72	1.23	1.05	1.33	9.4	13.1	0.9
Re-milled flour	8.0	7.9	20.2	1.01	1.14	1.12	1.45	1.85	1.45	9.1	11.3	1.0
Shorts	8.6	9.0	18.5	2.88	3.16	3.60	2.90	4.60	4.60	6.9	8.2	0.5
Bran	13.3	14.3	10.8	6.94	6.41	5.59	4.15	5.90	5.80	4.7	4.1	0.5
Whole wheat**	99.8	99.2	99.1	1.65	1.68	1.87	1.78	2.08	2.45	8.5	11.1	0.8

† All data refer to dry matter

* Pooled break and reduction flours

†† A, Aragón O3; M, Magdalena; D, Híbrido-D

** Sum of previous fractions

reproducible results will be obtained by referring SP to dry matter. In view of these results, the SP content of normally milled flours has been adopted in these studies.

Fig. 3 (a) shows the results obtained for SP content of flour in a survey of 46 *T. aestivum* and 24 *T. durum* varieties. The *T. aestivum* distribution seems to show two maxima at about 4 mg and 12 mg respectively. All *T. durum* varieties are included in the 0.1–1.5 mg/100 g of flour interval. Only 3 *T. aestivum* varieties are actually included in this interval and 3 more are included in the next interval (1.6–3.0 mg/100 g). The conclusion to be drawn from these results is that in flour SP levels above 1.5 mg/100 g indicate the presence of *T. aestivum* endosperm in pasta products. Since there are three *T. aestivum* varieties included in the *T. durum* interval, SP contents lower than 1.5 mg/100 g do not guarantee purity. For a given SP level, the minimum percentage of *T. aestivum* present can be calculated in terms of the maximum SP content found for this species. This minimum is shown in Fig. 3 (b) as a function of SP content. The higher the SP content the closer the estimated minimum will be to the true *T. aestivum* percentage of the mixture.

Although the existence of some *T. aestivum* varieties with SP values similar to those of *T. durum* implies that this difference cannot be used alone to solve the problem, the

test is useful because it allows detection of most *T. aestivum* varieties. Since other interspecific biochemical differences are bound to show similar problems, i.e., intraspecific variability and some exceptions, more than one test will probably have to be used not only for qualitative identification, but for a better quantitative estimation of the whole range of *T. aestivum* varieties. In this connexion, several other interspecific differences have been found at this laboratory, and are being confirmed at present.

Although a high number of wheat varieties were used, and these were diverse in origin, the limits proposed for this interspecific difference are only tentative and many more varieties should be tested in other countries.

It has been shown that high SP content is associated with the D genome of *T. aestivum*^{1,0} and is not due to an interaction of this genome with genomes A and B, which are present in both *T. durum* and *T. aestivum*. The distribution of the two maxima in Fig. 3 (a) suggests a simple genetic control for this biochemical characteristic. Therefore, it should be pointed out that as a result of breeding programmes new varieties can make this or other tests obsolete, so that then new interspecific differences should be available to cope with these changes.

Acknowledgments

The authors wish to acknowledge financial support (Grant FG-Sp-134) from the U.S. Department of Agriculture, Foreign Research and Technical Program Division.

Instituto Nacional de Investigaciones Agronómicas,
Avda. Puerta de Hierro s/n,
Madrid, 3, Spain

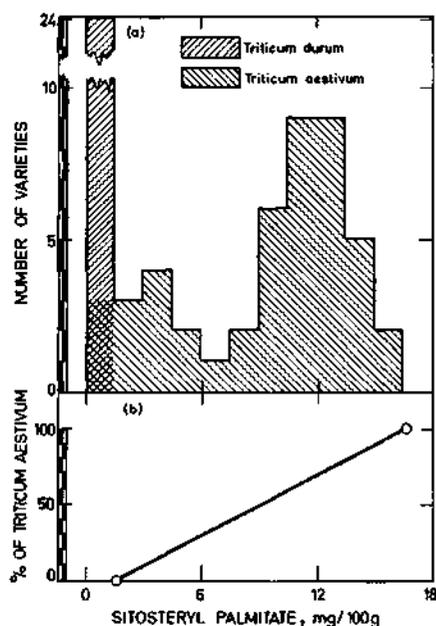


FIG. 3 (a). Distribution of sitosterol palmitate in the *T. aestivum* and *T. durum* species. (b) Minimum proportion of *T. aestivum* in a mixture versus sitosterol palmitate content

References

1. Rittenhausen, H., *J. prakt. Chem.*, 1863, **88**, 145
2. Ball, C. D., *Cereal Chem.*, 1926, **3**, 19
3. Walde, A. W., Mangels, C. E., *Cereal Chem.*, 1930, **7**, 480
4. Spielman, M. A., *Cereal Chem.*, 1933, **10**, 239
5. Matweef, M., *C.r.hebd. Séanc. Acad. Agric. Fr.*, 1952, **39**, 658
6. Alliot, R., *Annls Falsif. Fraudes*, 1957, **579**, 116
7. Zoubovsky, R., *Pâtes alimentaires Semoules et Industrie*, 1958, **77**, 4
8. García Faure, R., García Olmedo, F., Sotelo, I., Salto, M., *Bol. Inst. nac. Invest. Agron.*, 1965, **25**, 395
9. Gilles, K. A., & Young, V. L., *Cereal Chem.*, 1964, **41**, 502
10. García Olmedo, F., *Bol. Inst. nac. Invest. Agron.*, 1965, **25**, 409
11. International Association for Cereal Chemistry, *Methods* (The Association: Wien, Austria), 1960
12. American Association of Cereal Chemists, *Cereal Laboratory Methods* (7th edn.) (The Association: St. Paul, Minnesota, U.S.A.), 1962