# Detection of Flour or Farina from Triticum aestivum in Macaroni, by Starch-Gel Electrophoresis of Water-Soluble Proteins

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**ABSTRACT**

A water-soluble protein component, A, has been found which appears without exception at a higher concentration in *Triticum aestivum* than in *T. durum*. Quantitation of A is achieved by referring the height of densitogram peak A to the height of peak B, which corresponds to a second component that is present in all varieties studied from both species. Extreme values of peak height ratio (PHR) in both species are tentatively established. On the basis of these values, the maximum and minimum possible contents of *T. aestivum* in a mixture are expressed as a function of PHR. All the varieties of *T. aestivum* studied are detected when they contribute more than 60% to the mixture. In over 90% of all possible binary combinations between varieties of *T. aestivum* and *T. durum*, the maximum proportion of undetected *T. aestivum* has been 30%.

Bread wheat (*Triticum aestivum*) yields milling products that are, as a rule, not suitable for macaroni manufacture. Paste products of optimum quality require semolina from *T. durum* as raw material. When blends of hard or soft wheat and durum wheat are used the quality is reduced.

Biochemical differences have been sought between the two wheat species that could afford the estimation of the percentage of *T. aestivum* endosperm products in macaroni (1 to 6). The exact amount of bread-wheat flour or farina in an unknown mixture cannot be calculated as a function of any given biochemical characteristic due to intraspecific variability. Once the extremes for a biochemical interspecific difference have been established in the two wheat species, calculation of the maximum and minimum possible content of *T. aestivum* in the unknown mixture can be based on the measurement of such characteristic. Only the minimum can be deduced from the proposed chemical differences—sitosteryl palmitate and petroleum ether lipoprotein content—because some varieties of *T. aestivum* give values included in the *T. durum* range (4,6). This means that some varieties go undetected when a single test is used. Fortunately, all varieties low in sitosteryl palmitate found at this laboratory have a high content of petroleum ether lipoprotein, and vice-versa (4).

The conclusion to be drawn from the above considerations is that a good estimation of *T. aestivum* flour or farina in macaroni requires the concurrent use of several tests.

Potential interspecific differences have been selected by preliminary screening of composite samples, and then tested in individual varieties. Thus, a water-soluble protein has been found which appears without exception at a higher concentration in *T. aestivum* than in *T. durum*. A simplified method is proposed for the quantitation of this protein.

TABLE I. PEAK HEIGHT RATIOS OF VARIETIES OF TRITICUM AESTIVUM AND T. DURUM

| T. aestivum | | T. aestivum | | T. durum | |
|---|---|---|---|---|---|
| North and South American varieties | | | | | |
| Sinvalocho | 2.4 | Bledsoe | 2.0 | Durum A[a] | 1.2 |
| Atlas 66 | 2.3 | Concho | 2.0 | Durum B[a] | 1.1 |
| Cheyenne | 2.3 | Thatcher | 2.0 | Leeds | 1.0 |
| Comanche | 2.3 | Timiglia | 2.0 | Wells | 1.0 |
| Klein Cometa | 2.3 | HRW-A[a] | 1.9 | Golden Ball | 0.8 |
| Marquis | 2.2 | Manitou | 1.9 | Lakota | 0.8 |
| Mida | 2.2 | Rescue | 1.9 | Mindum | 0.8 |
| Selkirk | 2.2 | Bison | 1.8 | DT-191 | 0.7 |
| Aniversario | 2.1 | HRW-B[a] | 1.8 | Stewart 63 | 0.7 |
| Lee | 2.1 | Lucero | 1.8 | Pelissier | 0.6 |
| Wichita | 2.1 | | | | |
| European varieties | | | | | |
| Amyntas | 2.4 | Roma | 2.1 | Hybrido-D | 1.2 |
| Lusitano | 2.4 | Virgilio | 2.1 | Claro Fino | 1.2 |
| P. Gemelli | 2.4 | Blanquillo | 2.0 | Limnos | 1.2 |
| Restauracao | 2.4 | F. Aurora | 2.0 | S. Capelli | 1.1 |
| Grano Tenero[a] | 2.4 | Impeto | 2.0 | Cappelli | 1.1 |
| Navarro-122 | 2.3 | Mara | 2.0 | Amarelejo | 1.0 |
| Pirana | 2.3 | Mentana | 2.0 | Jerez-36 | 1.0 |
| Strampelli | 2.3 | Mexicano 1481 | 2.0 | Raspinegro | 1.0 |
| 38230-24[b] | 2.3 | Mucaba | 2.0 | Electra | 0.9 |
| Candeal | 2.2 | Rojo | 2.0 | Patrizio-6 | 0.9 |
| Dr. Mazet | 2.2 | Chaimite | 1.9 | 63849-A6[b] | 0.9 |
| Montnegre | 2.2 | Estrella | 1.9 | Alaga | 0.8 |
| Navarro-105 | 2.2 | Libero | 1.9 | Oviachic | 0.8 |
| Pane-247 | 2.2 | Mocho | 1.9 | Capelti-8 | 0.7 |
| Pella | 2.2 | R. Arroniz | 1.9 | Farto | 0.7 |
| Rex | 2.2 | Tavares | 1.9 | Methoni | 0.7 |
| Rieti | 2.2 | Cabezorro | 1.8 | Recio | 0.7 |
| Aragon-03 | 2.1 | Chamorro | 1.8 | Andalucia | 0.6 |
| Ariana | 2.1 | Pane-2 | 1.8 | Ledesma | 0.5 |
| Hibrido-J1 | 2.1 | Dimas | 1.8 | | |
| Niki | 2.1 | Generoso | 1.7 | | |

[a]Commercial blends.

[b]Greek varieties.

## MATERIALS AND METHODS

### Samples

Samples of flour from 63 varieties of *T. aestivum* and 29 of *T. durum* were used in this study. These were grown either commercially or experimentally in Spain and other European, North American, and South American countries (1967 crop) (see Table I).

Macaroni was obtained from three varieties of *T. aestivum* and three of *T. durum* in our pilot plant.

**Milling Fractions**

One sample of *T. aestivum* and one of *T. durum*, 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 remilled fractions: flour, bran, and shorts.

**Method**

The samples, 10 g. of flour or ground paste, were slurried with 10 ml. of deionized water and allowed to stand overnight in the refrigerator (3°C.). After centrifugation for 30 min. at 10,000 X g in a Sorvall Superspeed centrifuge, up to 3 ml. of clear supernatant was obtained. These extracts were directly fractionated by urea starch-gel electrophoresis in aluminum lactate buffer, pH 3.2, according to Woychik et al. (7). A piece of filter paper (10 mm. by 3 mm., Whatman No. 3) was soaked in the extract and inserted in the gel slot.

After electrophoresis, gels were stained with nigrosine solution (0.05% in acetic acid:water 1:1 v./v.) for 21 hr., washed with tap water, and destained with 80% ethanol for 5 hr. The gels were transferred to tap water prior to densitometry. With the use of gels 3 mm. thick and staining with the lower side up, sharp patterns were obtained. Reflectance densitometry was performed in a Chromoscan densitometer (Joyce and Loebel).

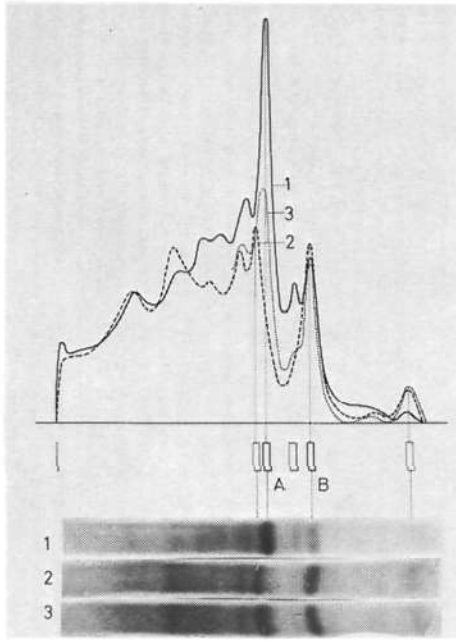


**Fig. 1. Starch-gel electrophoretic patterns of water extracts: 1, *T. aestivum*; 2, *T. durum*; 3, *70% T. durum + 30% T. aestivum*.**

When an unknown sample was to be analyzed, its water extract was run in duplicate, together with duplicates of the water extracts from a known variety of *T. aestivum* used as reference, and from a mixture of 70% of the unknown and 30% of the *T. aestivum* reference.

## RESULTS AND DISCUSSION

Typical electrophoretic patterns of water-soluble proteins from flours of the two species of wheat are shown in Fig. 1. Among the faster-moving components, band A is more intense in the sample of *T. aestivum* than in that of *T. durum*. Since this is the only difference that appears consistently in all varieties studied from both species, the interspecific differentiation can be based on it.

To avoid the use of internal standards in estimating component A, we have chosen another component as reference, band B, which is consistently present in both species. Peak height has been found more reproducible than peak area, probably owing to the partial overlap of another band close to band A. For this reason, the ratio between peak heights (PHR) of bands A and B has been selected as the numerical expression of this biochemical character. The determination of PHR is reproducible (variation coefficient, $(S/\overline{X}) < 0.10$) and is not significantly affected by changes in the water:flour ratios from 0.7 to 1.5 (v./w.), so that flour moisture level need not be taken into account.

In some *T. durum* flours with low PHR, peak A is not clearly resolved and PHR is more accurately estimated by extrapolation. This can be done because a linear relationship exists between PHR and percentage of *T. aestivum* (Fig. 2). For this reason it is recommended that in routine analysis of unknown samples, duplicates of their water-extracts be run together with duplicates of a known variety of *T. aestivum* and of a mixture of this variety with the unknown sample (30:70 w./w.).

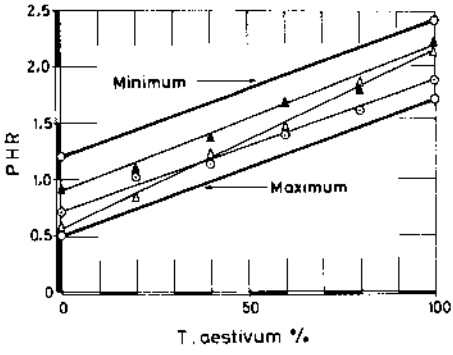


**Fig. 2 (left). PHR values of three series of *T. durum*-T. *aestivum* mixtures and limits of possible *T. aestivum* content of a mixture as a function of PHR.**

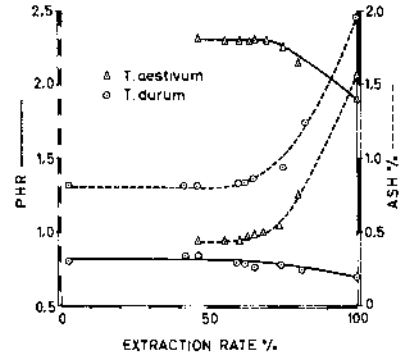


**Fig. 3 (right). Variation of PHR with milling extraction rate in one variety of *T. durum* and one of *T. aestivum*.**

The addition of 30% of *T. aestivum* flour clearly resolves peak A in the case of *T. durum* with the lowest PHR.

Variation of PHR with milling extraction rate has been investigated in one variety of *T. durum* and one of *T. aestivum* (Fig. 3). Normal variations of extraction rate do not affect PHR in either one, although a significant decrease is observed for extraction rates above 75% (approximately).

For a given sample, PHR of flour does not differ significantly from that of the ground macaroni.

Results from a survey of PHR values from varieties of *T. durum* and *T. aestivum* grown in American and European countries are presented in Table I.

Values obtained for *T. aestivum* ranged from 1.7 to 2.4, and the range for *T. durum* was from 0.5 to 1.2. From these data, the PHR of an unknown mixture being given, its maximum and minimum possible content of *T. aestivum* can be calculated as shown graphically in Fig. 2.

For the PHR range of 1.2 to 1.7, the difference between the maximum and minimum is about 60%. Although this is far from ideal, it still offers advantages over the methods based on sitosteryl palmitate (6) or petroleum ether protein (4), because an upper limit to the possible *T. aestivum* content of the mixture can be based on PHR, which is not the case with the other methods. All varieties of *T. aestivum* are detected when they contribute more than 60% to the mixture. In mixtures involving varieties of *T. durum* with PHR values higher than 0.8 and of *T. aestivum* with values higher than 1.9 (over 90% of all possible binary combinations), the maximum amount of undetected *T. aestivum* is 30%.

Since low-PHR varieties of *T. aestivum* might have high sitosteryl palmitate or petroleum ether protein, and vice-versa, it is obvious that the concurrent use of the three tests will narrow the degree of uncertainty in estimating *T. aestivum* in a mixture by a single test.

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