Determination of Common Wheat Content in Pasta Products

P. FEILLET and K. KOBREHEL, Laboratorie de Technologie des Blés Durs et du Riz, Institut National de la Recherche Agronomique, 34060 Montpellier Cedex, France

ABSTRACT

A new method is proposed to determine the amount of common wheat flour in pasta products. It is based upon the dosage of a specific polyphenoloxidase only present in *Triticum vulgare*. Water extract of ground pasta is fractionated by polyacrylamide gel electrophoresis in *tris*-buffer; polyphenoloxidase content is measured by densitometry after specific staining. Genetic and agronomic origin of wheats, extraction rate, drying temperature up to 70°C., or presence of eggs do not modify the results.

The quality of pasta products processed from durum wheat semolina is most often superior to pasta in which common wheat (*Triticum vulgare*) semolina is

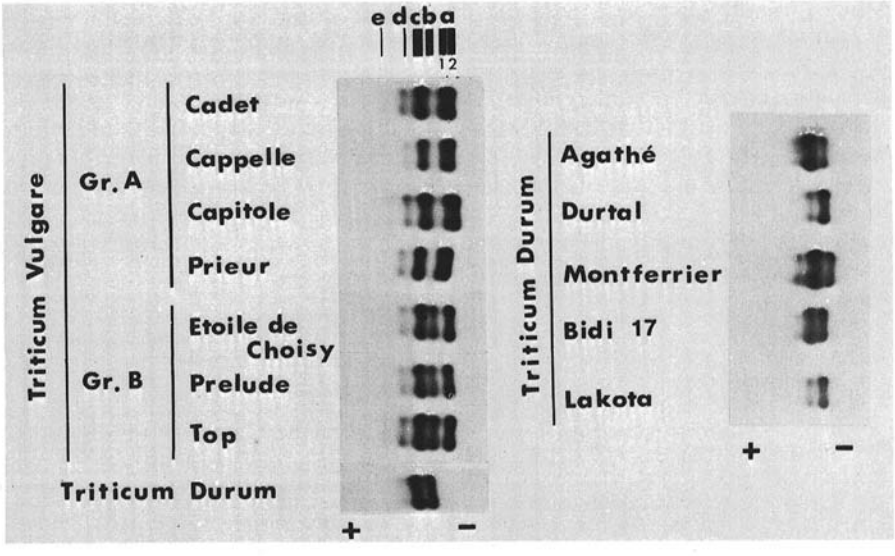

Fig. I. Polyphenoloxidase composition of several varieties of common and durum wheats as detected by 8 p. 100 polyacrylamide gel electrophoresis (*tris*-buffer, pH 8.6, 7 v. per cm.,210 min., staining by catechol).

incorporated. For this reason, in some countries like France and Italy, the use of common wheat for the confection of pasta products is an adulteration.

So far, many methods have been proposed to detect the presence of *T. vulgare* in these products; most of them derive from Matveef's method (1), but, as stressed by Fruchard et al. (2), they lack specificiety for many common wheats which contain approximately as much sitosterol palmitate as the durum wheats (3).

The test of Brogioni and Franconi (4), based on the infrared spectroscopic detection of complex lipids characteristic of common wheat, did not bring the expected solution.

Several authors (5–8) have proposed a method to determine the amount of common wheat in pasta products by estimating specific soluble proteins (9–11). Unfortunately, all these methods are either not sensitive enough or time-consuming and tedious, and comparative tests carried out in specialized laboratories of several countries do not give concordant results. Recently we have tried to bring improvements in this line (12), yet the problem remains largely unsolved.

Therefore, investigations have been conducted with the use of specific enzymes to detect the presence of common wheat in pasta products. In this paper a new method is proposed, measuring specific water-soluble polyphenoloxidase activity after slab polyacrylamide gel electrophoresis in basic buffer.

MATERIALS AND METHODS

Materials

Seventeen durum and 48 common wheats of known variety from different French growing areas were analyzed. Three of them (Agathe, Durtal, and Mandon varieties) are durum wheats obtained by cross-breeding of common and durum wheats.

Milling

Except where otherwise stated, a Brabender Quadrumat Junior mill fitted for semolina processing with a laboratory-made purifier attachment (13) was used. Extraction rate was in the range of 45 to 55%.

Pasta Processing

Dough obtained from 100 g. semolina kneaded with 30 ml. water was sheeted, pressed through a die, and dried in the room atmosphere. In some cases, drying was carried out at 50°, 70°, or 90°C. for 24 hr. Egg noodles were also made (14).

Polyphenoloxidase Extraction

One gram of semolina or ground pasta was mixed with 20 ml. deionized water. After standing 1 hr. at room temperature, the solution was centrifuged at 5,000 × g and the supernatant saved.

Gel Electrophoresis

Twenty-four grams of Cyanogum 41 was dissolved in 300 ml. *tris*-HCl buffer, pH 8.6 (6.05 g. *tris*-buffer and 40.5 ml. 0.2M HCl diluted to 1,000 ml.). After filtration, 1 ml. DMAPN (β-dimethylaminopropionitril) and 0.3 g. ammonium persulfate were added. After polymerization, the gel was equilibrated against *tris*-HCl buffer for 16 hr. Fifty microliters water extract was applied to the slot of the gel. Electrophoresis was performed for 210 min. at 7 v. per cm. with tap water circulating through cooling plates on both sides of the gel.

Polyphenoloxidase Staining

The interior part of the sliced gel was soaked in 5% aluminium lactate solution for 10 min., washed with water, and placed 5 or 6 hr. into catechol solution (2.5 g. catechol, 1.95 g. *tris*-hydroxymethyl aminomethane, 0.20 g. ethylenediaminetetraacetate, and 0.15 g. boric acid diluted with 1,000 ml. water). After staining, the gel was stored in 3% acetic acid.

Common Wheat Content Estimation

The relative concentration of the most basic polyphenoloxidase band *a* (Fig. 1),

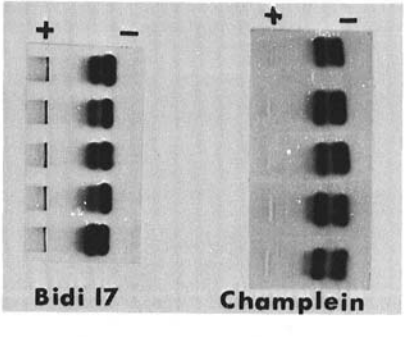

Fig. 2. Polyphenoloxidase composition of Bidi 17 (durum wheat) and Champlein (common wheat) from six growing areas. Conditions as for Fig. 1.

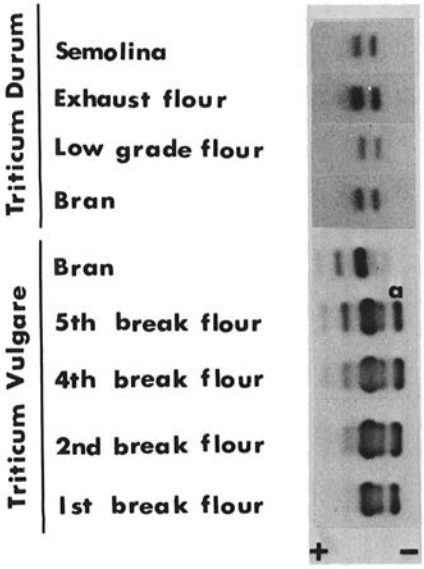

Fig. 3. Polyphenoloxidase composition of several flour streams from durum and common wheats. Conditions as for Fig. 1.

which is specific for common wheat, is determined by densitometry (Vitatron densitometer, without filter). The common wheat content of the sample is calculated with reference to the calibration curve obtained from macaroni samples with definite content of common wheat. Results are expressed in grams common wheat per 100 g. macaroni.

The common wheat content can also be estimated by a visual comparison between standard macaronis and unknown samples.

RESULTS AND DISCUSSION

The comparison of the electrophoretic patterns show an important difference between the polyphenoloxidase composition of wheats (Fig. 1). The common wheat, characterized by band *a*, can be divided into two groups. In group A, band *a* is composed of two fractions a_1 and a_2; band *b* is absent. In group B, band *b* is present but not band a_2.[1]

The electrophoretic patterns of durum wheats are different each from the other but none has band *a*. According to Kobrehel and Gautier (15), band *a* seems due to genome D present in common wheats and absent in durum wheats.

The number and the intensity of the electrophoretic patterns are not modified by the growing conditions (Fig. 2).

As shown Fig. 3, the fraction *a* is evenly distributed in different break flours

[1]Group A varieties: Agriss, Aida, Aronde, Atou, Cadet, Cama, Capta, Capitole, Cappelle, Champlein, Diplomat, Flindor, Frülgold, Halist, Hardi, Helima, Joss, Justin, Kolibri, Kroupring, Peguy, Prieur, Rex, Robert, Sirius, Splendeur; group B varieties: Amiral, Athys, Bicop, Boulo, Cesar, Etoile de Choisy, Clairon, Felix, Gaillard, Goya, Julhar, Kluber, Marly, Marzotto, Moisson, Prelude, Rescue, Solo, Thatcher, Top, Toro.

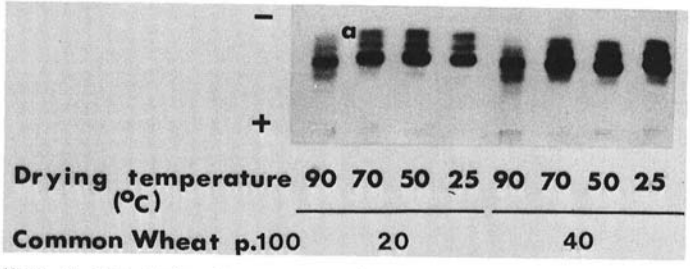

Fig. 4. Effect of the drying temperature of pasta products on the polyphenoloxidase electrophoretic patterns. Conditions as for Fig. 1.

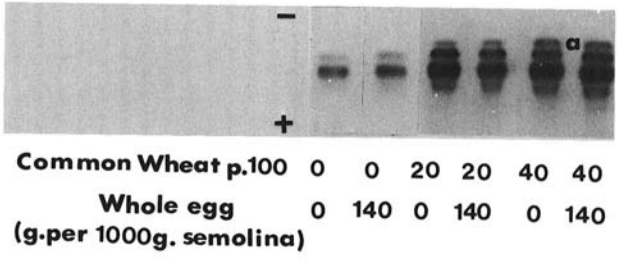

Fig. 5. Effect of the egg content in pasta products on the polyphenoloxidase electrophoretic patterns. Conditions as for Fig. 1.

obtained by milling common wheat on a semi-industrial laboratory mill (16). It is present at low concentration in common wheat bran, probably by flour contaminations, and absent from all the products of durum wheat.

It is important to note that the electrophoretic patterns are not modified when macaronis are dried at temperatures as high as 70°C. At 90°C., bands are fainter but still visible (Fig. 4).

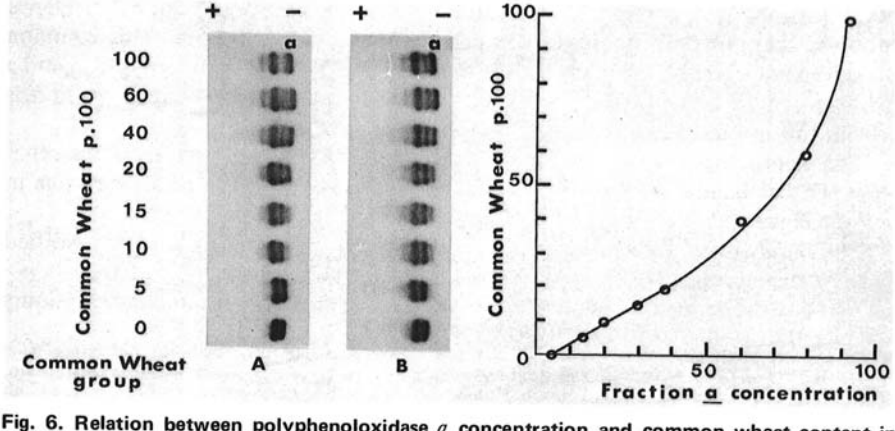

Fig. 6. Relation between polyphenoloxidase *a* concentration and common wheat content in pasta products. Conditions as for Fig. 1.

The presence of eggs in noodles does not modify the electrophoretic patterns (Fig. 5).

As shown in Fig. 6, the content of the polyphenoloxidase *a* is proportional to the percentage of common wheat in pasta products when it does not exceed 50%. The group of common wheat variety (A or B) has no effect on the results.

Looking at the modification of polyphenoloxidase during germination, it was found that no qualitative or quantitative changes occur in the electrophoretic patterns of sprout-damaged wheats.

Three macaroni samples with a known amount of common wheat were analyzed with this method. Results tabulated below show that the mean values are close to the theoretical value. Standard deviations range from 4.9 to 1.3.

	Common wheat content (p. 100 macaroni)		
No. of Analysis	*Theoretical Value*	*Mean Value*	*Std. Dev.*
6	40	39.8	4.9
6	20	19.5	2.3
6	3	2.0	1.3

In conclusion, the results show that specific common wheat polyphenoloxidase is easily detectable by electrophoresis. The composition of this enzyme is not modified by the growing conditions or by the purity of semolina. The drying temperature, to some extent, and the presence of eggs, do not disturb the analysis.

As a consequence, the method described for determining the common wheat content in pasta products has high specificity, while its application is general; however, the presence of bran is not detectable by this method. It is sensitive enough to detect an amount of 3% of common wheat in pasta. Results can be obtained within 10 hr.; simultaneous analysis can be performed.

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