NOTE

A Method for Detecting Adulteration in Durum Wheat Pasta by Polyacrylamide Gel Electrophoresis

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ABSTRACT

A method was devised to detect nondurum wheat gliadin proteins in pasta using aluminum lactate polyacrylamide electrophoresis at pH 3.1. Pastas were prepared from various mixtures of durum and hard red winter wheat flours, and gliadins were extracted with 70% ethanol. Electrophoretic zymograms of pasta extracts matched those of the corresponding flour extracts. Durum wheat extracts lack certain slow moving gliadin protein bands present in other flours. Pasta adulterated with as little as 5% hard red winter wheat flour could be detected with this method.

The best pasta is traditionally made with 100% semolina or durum wheat flour because these flours have low amounts of certain proteins (glutens) that make pasta sticky when cooked. Durum flour also imparts an appealing yellow color.

The U.S. government routinely buys 100% durum wheat pasta products for military and civilian agencies. To assure that the pasta is 100% durum, the government uses a color test based on the presence of the yellow carotenoid pigments in the pasta (AACC 1983, GSA 1975, USDA 1982). However, this test is flawed because the standards are too low, and consequently, pasta with significant levels (up to nearly 100%) of nondurum wheat has sufficient pigmentation to pass the government test and is purchased as 100% durum. Also, wheat cultivars vary in pigment content, further confounding the test. Because durum wheat flour is more expensive than other flours, there is economic incentive to adulterate pasta with cheaper flour.

Lookhart et al (1982) recently laid the groundwork for this new test for pasta. They tested the 88 most commonly grown U.S. wheat cultivars, including durums, and found that durums produced very different electrophoregrams than did other wheats. They also found that all nondurum cultivars tested had at least one distinct protein band, which migrates significantly slower than does the slowest durum protein band. Our adaptation of their system shows that identical results can be obtained from ground pasta and wheat flour. Thus, we can detect pasta adulterated with as little as 5% hard red winter (HRW) wheat flour by electrophoresis. This procedure combines relatively quick and easy extraction, gel preparation, and staining procedure with good sensitivity, which may make it preferable to other tests devised to date.

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MATERIALS AND METHODS

Reagents

Acrylamide and L-ascorbic acid (sodium salt) were obtained from Sigma Chemical Company; Coomassie Brilliant Blue G-250 was obtained from Serva, Inc.; N,N^1-methylene-bisacrylamide was obtained from Eastman Chemical Co.; aluminum lactate was obtained from K & K Laboratories; ferrous sulfate and trichloroacetic acid (TCA) were obtained from Fisher Scientific; lactic acid (85%), ethanol (95% nondenatured), and glycerine were obtained from J. T. Baker; and hydrogen peroxide (30%) was obtained from Matheson.

Preparation of Pasta and Color Test

Various mixtures of pure durum and HRW wheat flour were prepared (Table I): 10 g of flour was added to 8 ml of deionized water, mixed, and divided into thin strips on weighing paper for air-drying. After two days, the dry pasta was ground in a Wiley mill and extracted with saturated butyl alcohol for carotenoid pigments, as per AACC Method 14-50 (1983). Pigment concentration was determined on a Perkin-Elmer Coleman 44 spectrophotometer at 450 nm, using a standard curve prepared with β-carotene. The standard curve includes several concentrations of β-carotene between 1.3 and 6.2 ppm (maximum error 5%).

Table I
Carotenoid Pigment Content of Pastas at Varying Levels of Hard Red Winter Wheat

Percent of HRW Wheat Flour	Carotenoid Pigment (ppm)[a]
0	6.0
5	5.7
10	5.6
20	5.3
50	4.1
100	2.3

[a] Federal standard for pasta requires at least 2.5 ppm.

Gliadin Extraction

The methods of extraction and electrophoresis were adapted from Lookhart et al (1982). Wheat seed or pasta was ground in a Wiley mill, and 0.25 g of the resulting flour was extracted with 1.25 ml of 70% ethanol at room temperature for 1 hr, followed by centrifugation at 5,875 × *g* for 10 min. The supernatant was decanted into tubes containing 12 drops of glycerine, one drop of methyl green, and a glass bead to facilitate mixing with a vortex mixer. Smaller quantities and volumes can be used with equal results.

Electrophoresis Apparatus

The electrophoresis apparatus was a Bio-Rad protean dual vertical slab gel electrophoresis cell with 12 × 16-cm siliconized glass plates, 3-mm spacers, and 15 well combs. The power supply was an ISCO model 492.

Gel Preparation

Gels were prepared, as per Lookhart et al (1982) with some modifications: 18.6 ml of stock A, 11.2 ml of stock B, and 16 ml of stock C (Table II) were combined with ascorbic acid and brought to 80 ml with deionized water. This produced a 7% gel, which we found preferable to the 6% gel used by Lookhart et al (1982). The gel solution was placed in the freezer for 1 hr before the gels were poured. The hydrogen peroxide catalyst was added to the gel solution, stirred, and quickly poured into the slab former. The gel forms in less than 1 min, and gels can be run immediately or stored overnight in the refrigerator.

Two liters of tank buffer were prepared (Table II); 600 ml of this buffer was poured into the top chamber, and the remainder was diluted to 4 L for the bottom chamber. Stock solutions can be refrigerated and stored for up to one month.

Electrophoresis

Each well contained a layer of 20 μl of extract. The electrophoresis was run toward the negative pole at 300 V constant for 4 hr. Tap water at 18° C was used as the coolant. The methyl green front and a few fast moving proteins pass completely off the gel during the run.

Staining and Photography

After electrophoresis, the gels were carefully removed from the plates and put in 100 ml of 12.5% TCA for 15 min. Siliconized glass plates greatly facilitate gel removal. Then, 7 ml of 1% aqueous Coomassie Blue G-250 was added. Bands appeared within 1 hr, but the gels were allowed to stain overnight, to increase band intensity. Gels were then stored in 100 ml of 10% TCA. Transfer to 7% acetic acid caused the bands to intensify slightly, but these faded with long-term storage. The gels were put in plastic bags with a small quantity of storage solution, heat-sealed, and then placed on a light box and photographed, using Kodak technical pan film 2415.

Table II
Recipes for Gel and Tank Buffer Solutions

Gel Solution[a]	80 ml
Acrylamide	5.6 g (7%)
N,N[1]-methylene-bisacrylamide	0.28 g
L-ascorbic acid (Na salt)	0.08 g
Fe SO_4	1.6 mg
Aluminum lactate	0.2 g
Lactic acid	0.36 ml
Catalyst	
3% hydrogen peroxide	0.4 ml
Tank buffer, total volume	2,000 ml
Aluminum lactate	2.5 g
Lactic acid	3.7–3.1 pH
Stocks	
A	
Total volume	100 ml
Acrylamide	30 g
N,N[1]-methylene-bisacrylamide	1.5 g
B	
1 m*M* $FeSO_4$	0.152 g/l
C	
Total volume	100 ml
Aluminum lactate	1.25 g
Lactic acid	0.45–3.1 pH

[a]For one gel.

RESULTS AND DISCUSSION

Figure I shows an electrophoresis gel of six commercial pasta extracts and representative durum and HRW wheat cultivars. The pastas were chosen so that three of them (rows E,F,H,I,N, and O) had relatively high color scores and three (rows C,D,J,K,L, and M) had low color scores. The pastas with low scores showed protein bands characteristic of farina and not found in semolina. Although these low scoring pastas appeared to be adulterated, they passed the government color score test. On the other hand, the pastas with high color scores had no noticeable nondurum protein bands, confirming that they were apparently unadulterated.

Figure II shows zymograms of our homemade pasta prepared with mixed durum and HRW wheat flours. Nondurum protein bands are clearly present in the pasta extract that contains only 10% HRW wheat pasta, and faint nondurum bands can also be seen in the 5% HRW wheat extract. Test sensitivity might be increased by other staining methods. Pure durum and HRW wheat pasta and flour extracts are shown for comparison. We did not note any differences between flour extracts and extracts from pastas prepared from these flours.

Table I shows the carotenoid pigment extracted from these mixed pastas. The relationship between HRW wheat content and carotenoid pigment was linear, as expected. Pasta from 100% HRW wheat had 2.3 ppm carotenoid pigment, just 0.2 ppm below the government standard for 100% durum pasta.

Jones et al (1982) showed that electrophoregrams of durum wheats are clearly different from those of other wheat classes. None of the cultivars they tested had slow moving protein bands (less than 18 units), whereas all nondurum wheats had at least one slow moving band. They noted that durum wheats do not have the D genome, which is known to control synthesis of some gliadins (Konzak 1977). We tested 22 common durum cultivars and confirmed the absence of these slow moving bands; the results will be published elsewhere.

Other methods using electrophoresis of soluble proteins in pasta have been devised (Resmini 1968, Garcia-Faure et al 1969, Feillet and Kobrehel 1972). Most of them have been unsatisfactory because they lacked sensitivity, were too time consuming and

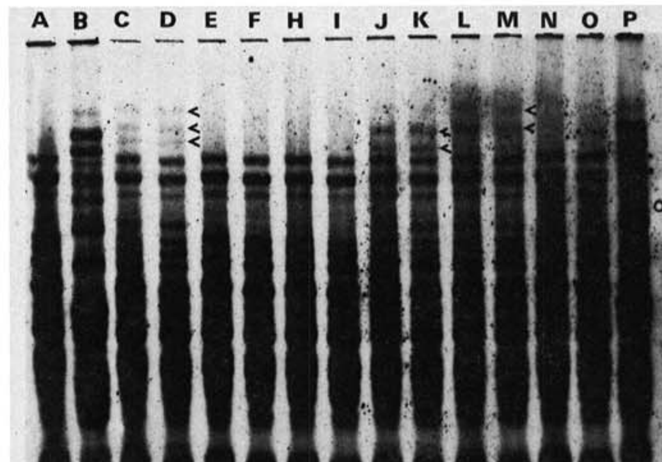

Fig. 1. Electrophoresis gel of six commercial pasta extracts and representative durum and hard red winter extracts: A, durum wheat extract; B and P, hard red winter wheat extract; C,D,J,K,L, and M, extracts of pastas with low color scores. C and D, J and K, and L and M are replicates. Note the slow moving, nondurum protein bands in these gels (arrows). E,F,H,I,N, and O represent extracts of pastas with high color scores. These extracts contained no detectable nondurum protein bands. E and F, H and I, and N and O are replicates.

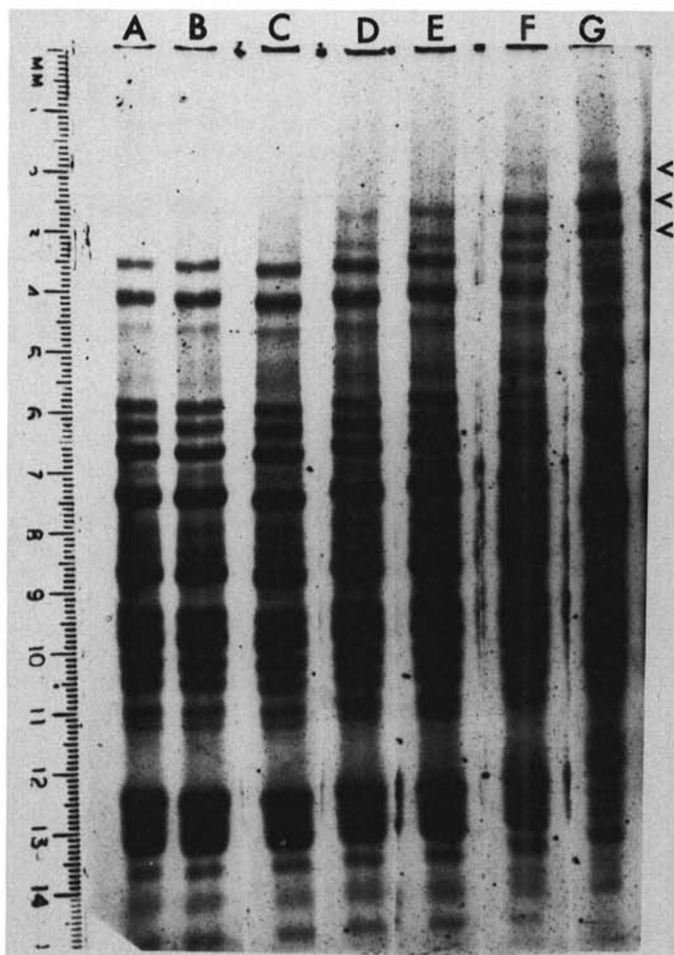

Fig. 2. Electrophoresis gel of homemade pasta extracts: A, 100% durum wheat flour extract; B, 100% durum pasta extract; C,D,E,F, and G, 5, 10, 20, 50, and 100% hard red winter wheat pasta extracts, respectively. Note the slow moving, nondurum protein bands in C,D,E,F, and G (arrows).

tedious, or were difficult to repeat. Feillet and Kobrehel (1974) measured water-soluble polyphenoloxidase bands following electrophoresis, and this method appears to have great promise. Methods that measure the presence or absence of saturated steryl esters have also been devised (Hsieh et al 1980, 1981; and Gilles and Youngs 1964).

Some errors and misinterpretations are possible, based on a number of factors, including variety, environment, milling fraction of the wheat, such additives as eggs or colorings, or heat used in the drying process. All of the above tests are susceptible to one or more of these complicating factors. Our method is no exception, because different varieties have different banding patterns. However, gliadin banding patterns have been shown to be reliable and stable within a given variety of wheat. Also, the general protein staining procedure used does not require that active enzymes be present. Thus, errors due to heat inactivation or age of the product are unlikely.

Our method combines quick extraction, electrophoresis, and staining procedures with good sensitivity. If the variety of the contaminating wheat is known, an accurate quantitative determination should be possible. Thus, this procedure is a different but equally powerful means of detecting adulteration in pasta.

Evidence from this study suggests that certain government agencies should reevaluate the purchase standards for pasta products based on the carotenoid color score test.

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