MACARONI BROWNNESS[1]

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ABSTRACT

Brownness in macaroni has been attributed to a Maillard-type reaction, to an enzymatic reaction, or to bran contamination. However, brownness arising from a varietal characteristic of durum wheat has been found to be due to a water-soluble protein. This component is readily extracted with water, producing a reddish-brown solution, and exhibits an absorption maximum at 400 mμ. It is a basic protein as shown by the amino acid composition, by its behavior during electrophoresis, and by ion-exchange column chromatography. Analysis suggests that the component is associated with copper.

Traditionally, color is one of the most important considerations in assessing durum wheat quality. Since the desirable color for the processed product is clear bright yellow, factors which discolor it are undesirable. One such factor, a varietal one, is that which imparts a brownish tinge. This is found in macaroni processed from some durum types grown outside North America and from certain older varieties of durum grown at one time in North America, as well as from varieties of hard red spring wheat.

Numerous reports have been published on both enzymatic and nonenzymatic browning of food, and the reactions involved have been elucidated in many cases. Few investigations have been reported, however, on the origins of brownness in pasta products. Harris and co-workers (1,2,3) reported that semolina milled from durum wheat damaged by sprout, immaturity, or blight gave rise to brownness in macaroni. The brownness in these cases was not a varietal characteristic, since undamaged wheat of the same variety was unaffected. Menger (4) suggested that nonenzymatic browning in wheat pastes could easily result from condensation processes involving soluble carbohydrates.

Brownness in macaroni no doubt can arise from a Maillard-type reaction, from an enzymatic reaction, or from bran contamination. There is yet another type of browning reaction which involves soluble proteins: in the presence of a small amount of copper these proteins react with certain reducing agents to produce brown-colored compounds (5). Results from the present study indicate that it is this type of reaction which is responsible for inherent brownness in certain varieties of durum wheat.

Materials and Methods

Semolinas used for this study were milled from the following wheats: Taganrog, an Argentine durum which produces brownish macaroni; Mindum, the standard Canadian variety; Golden Ball and Pelissier, varieties which produce brownish macaroni; Stewart 63, a variety of high-grade Canadian

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amber durum; 2 CWAD, a grade of high-grade Canadian amber durum; and 3 Manitoba Northern, a grade of hard red spring wheat producing brownish macaroni.

The wheats were milled on an Allis-Chalmers laboratory mill in conjunction with a small-scale purifier to yield approximately 55% semolina. The major comparisons were between Taganrog and Mindum.

Semolinas were extracted with distilled water (1:2 w./w.) in a Waring Blendor under an atmosphere of nitrogen for 5 min. and centrifuged at 6,000 $\times$ g for 10 min. The extracts were lyophilized in some experiments; where macaroni was made, the residue was lyophilized as well.

Macaroni was processed by the micro macaroni method (6). For color measurement, macaroni was ground and sieved and the fraction passing a 40-mesh and retained on an 80-mesh was used. Dominant wave length, purity, and brightness were determined by the Ten Selected Ordinates Method (7) in a Beckman DU spectrophotometer with a reflectance attachment.

The dominant wave length is the wave length of the pure spectrum color which, in combination with a tungsten lamp source, produces the color. A change of 1 mμ at 576 mμ is detectable even by the eye; 576 mμ is yellow; 577 mμ amber-yellow; 575 mμ lemon-yellow. Brightness is a measure of the amount of light reflected by the sample relative to the amount reflected by a near-perfect white surface. Purity is essentially related to the pigment content. Brownness in macaroni due to variety is characterized by a low brightness value and a dominant wave length in the 577-mμ region.

The method described by Smithies (8) was used for starch-gel electrophoresis analysis; 0.017M aluminum lactate at pH 3.5; stain, Aniline Blue Black. In a few experiments 0.02M phosphate buffer at pH 8.0 was used.

Separation of the colored component was effected by chromatography on a carboxymethyl cellulose (CMC) column. The column and the water extract were suitably equilibrated with malonate buffer at pH 5.7 (9) and the extract was passed through. In some cases the colored band was mechanically separated, in others the component was eluted by use of a salt gradient as described by Clayton and Bushuk (9). The limit solution was 0.8M in sodium ion. The brown component was eluted with a salt concentration between 0.65 and 0.70M. A Vanguard Ultraviolet Analyzer equipped with 0.66 cm. quartz and with the diffraction grating set at 280 mμ was used to estimate protein in the column effluent.

Spectra of various colored complexes were obtained on a Unicam SP 700 recording spectrophotometer. Analysis for iron was carried out with 1:10 phenanthroline (10). Copper was determined by the method of Stark and Dawson (11) with oxalyldihydrazide, acetaldehyde, and ammonia. For the determination of these metallic ions, the samples were ashed and extracted with concentrated HCl in some cases; in others, the samples were digested with concentrated nitric and sulfuric acids.

Total nitrogen content of the colored component was determined by the micro-Kjeldahl method. Ammonia was collected in 4% boric acid and titrated

with dilute hydrochloric acid; bromcresol green indicator was used.

The Lowry test (12) and amino acid analysis (Beckman-Spinco Model 120 amino acid analyzer) were carried out on the component. Hydrolysis of the sample for amino acid analysis was carried out as described by Tkachuk and Tipples (13).

The effect of 350 p.p.m. ascorbic acid on the color of macaroni was also studied. Absorption spectra of an aqueous extract and water-saturated butanol extract of macaroni treated with ascorbic acid were obtained.

Results and Discussion

Effect of Water-Soluble Fraction on Color of Macaroni. It was noted that water extracts of semolina which produced brownish macaroni were invariably reddish brown, whereas extracts of semolina which yielded good macaroni were generally very pale yellow. The effect of removal of the water-soluble fraction on the color of macaroni was therefore studied. The freeze-dried solid residue from an aqueous extraction was processed into macaroni and the color determined. Results are presented in Table I.

TABLE I
EFFECT OF REMOVAL OF WATER-SOLUBLE FRACTION ON COLOR OF MACARONI

	Mindum		Taganrog	
	Control	Water-Solubles Removed	Control	Water-Solubles Removed
Purity, %	27.1	25.2	23.2	24.2
Brightness, %	68.2	69.1	63.0	67.7
Dominant wave length, mμ	576.0	575.5	576.8	575.8

Macaroni processed from Mindum has a good color, as shown by a high value for brightness and a dominant wave length of 576.0 mμ, and is not substantially affected by the removal of water-solubles. Macaroni from Taganrog, on the other hand, is brownish, as indicated by the lower brightness and the higher dominant wave length. Removal of the water-soluble fraction from Taganrog improved its macaroni color markedly, as shown by the increase in brightness and a decrease of 1 mμ in the dominant wave length.

Isolation of Colored Component. The colored component was easily separated by chromatography on a CMC column. A distinct reddish-brown band separated at the top of the column when extracts of Taganrog, Golden Ball, Pelissier, and 3 Manitoba Northern were run. Extracts of high-grade Canadian amber durum (e.g. Mindum, Stewart 63, and 2 CWAD) produced only a very slightly colored band.

These bands were isolated and examined further by starch-gel electrophoresis, with 0.017*M* aluminum lactate at pH 3.5. The colored component migrates as a reddish-brown band on the gel and stains with the protein dye. The electrophoretic behavior was similar with 0.02*M* phosphate buffer, pH 8.0,

the component migrating to the cathode. It was determined by paper chromatography that phenols (other than tyrosine) are not associated with this component.

Since bran contamination can also give rise to brownness in macaroni, water extracts of bran-contaminated samples of flour were also studied. A brown component is isolated from an extract of bran by chromatography on a CMC column, but this component exhibits a different electrophoretic mobility from that of the brown component from Taganrog semolina.

Absorption Spectrum of Colored Component. Absorption spectra of solutions of the following four colored compounds were determined: the colored polymer produced by mixing arabinose with glycine (a product of a Maillard-type reaction); a colored product formed when *p*-cresol is mixed with tyrosinase (enzymatic browning reaction); an extract of bran; and the colored component isolated by chromatography on a CMC column. These are presented in Fig. 1, A. The component causing brownness in macaroni, i.e., the component from Taganrog isolated on CMC, shows an absorption maximum at 400 mμ, as well as a typical protein absorption maximum at 280 mμ. The other three brown compounds do not show definite maxima in the visible range.

Figure 1, B, presents the absorption spectra of aqueous extracts of semolina

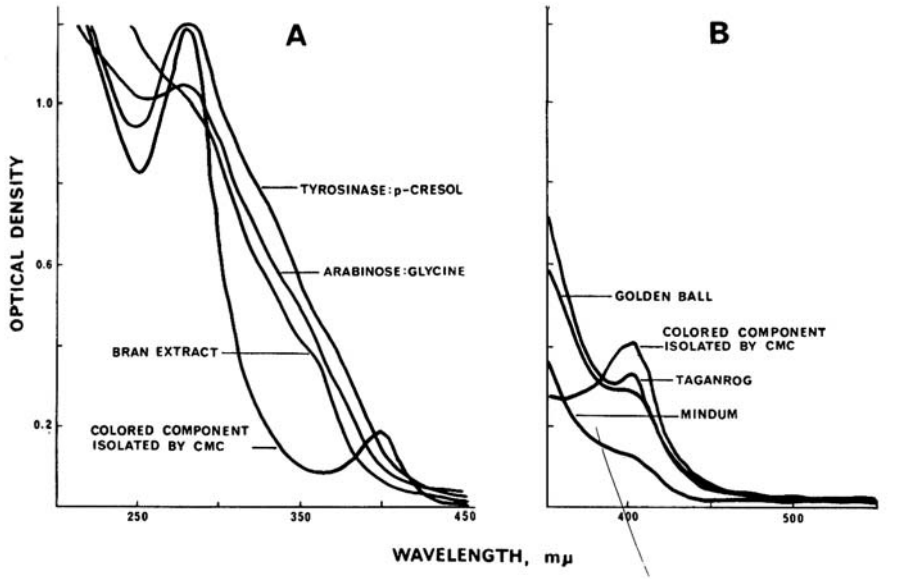

Fig. 1. A, absorption spectra of solutions of: arabinose:glycine (Maillard-type reaction); tyrosinase:*p*-cresol (enzymatic reaction); and extract of bran; and colored component isolated on CM cellulose. B, absorption spectra of aqueous extracts of semolinas from Taganrog, Golden Ball, and Mindum; and of colored component isolated on CM cellulose.

as well as the spectrum of the colored component isolated on CMC. In an extract of a semolina which produces satisfactory macaroni, such as the 2 CWAD grade of Canadian durum, or a variety such as Mindum, the absorption maximum at 400 mμ is barely detectable. On the other hand, in an extract of Taganrog or of Golden Ball, a variety poor in macaroni-making quality, as well as in the sample isolated on CMC, there is a distinct maximum at 400 mμ.

Since cytochrome C is reddish brown in solution as well as in the crystalline form, a comparison was made of the spectrum of cytochrome C with that of the colored component. Cytochrome C has several maxima in the visible range, none of which corresponded to the maximum of the brown component. Furthermore, analyses for iron in all samples studied showed very little, if any, difference in the iron content.

Analysis for Copper. Thompson reported that a copper-protein complex was involved in certain browning reactions (5). A mixture of bovine serum albumin with hydroquinone or ascorbic acid in the presence of copper oxide produced a brown-colored solution on standing, the absorption spectrum of which was very similar to that of the isolated colored component from Taganrog. Analyses for copper were carried out on samples of semolina or flour, and on freeze-dried water-soluble extracts. The method (11) is very sensitive, capable of detecting 0.1 γ of copper in the presence of proteins. Results are given in Table II.

TABLE II
ANALYSIS FOR COPPER IN SEMOLINA AND FREEZE-DRIED EXTRACTS

	Copper
	μg./g. sample
Semolina	
Mindum	1.1
Stewart 63	0.9
Pelissier	2.3
Golden Ball	1.6
Taganrog	2.1
Flour, HRS wheat	2.2
	mg./g. sample
Freeze-dried extracts	
Colored component isolated by CMC	0.391
Water-soluble fraction from Taganrog	0.131
Water-soluble fraction from Mindum	0.071

It can be seen that semolinas which produce brownish macaroni—Pelissier, Golden Ball, Taganrog, and HRS wheat—have more copper than Mindum or Stewart 63 semolina. In the freeze-dried samples, too, the copper content is higher in Taganrog than in Mindum. The component isolated by chromatography on CMC is highest in copper content.

These results suggest that copper is associated with the colored component. Extraction of semolina with a $10^{-3}M$ solution of ethylenediaminetetraacetate,

pH 5.1, does not affect the color of the extract, indicating that copper is firmly complexed.

Total nitrogen content of the colored component isolated by column chromatography was 11.7 mg./100 mg. sample. With the factor 5.7, this corresponds to 66.7% protein. By the Lowry test, the sample was calculated to contain about 70% protein. Bovine serum albumin was used for the calibration.

Results of the amino acid analysis are presented in Table III.

TABLE III
AMINO ACID ANALYSIS OF THE COLORED COMPONENT ISOLATED BY CMC

AMINO ACID	Protein	Protein	AMINO ACID	Protein	Protein
	μM/g.	mg./g.		μM/g.	mg./g.
Tryptophan	14	2.6	Glycine	676	38.6
Lysine	332	42.5	Alanine	658	46.8
Histidine	167	23.0	Cystine	321	33.1
Ammonia	1,056	16.9	Valine	498	49.4
Arginine	404	63.1	Methionine	107	14.0
Aspartic acid	635	73.1	Isoleucine	277	31.3
Threonine	347	35.1	Leucine	549	62.1
Serine	448	39.0	Tyrosine	203	33.1
Glutamic acid	1,118	144.4	Phenylalanine	239	35.2
Proline	581	56.4			

When the results are compared with those reported for wheat flour by Tkachuk (14), the principal difference shows up in the proportion of basic and acidic amino acids. Ammonia arises from the deamination of glutamine and asparagine and from the partial destruction of serine and threonine. As an approximation, the rate of destruction of threonine and serine as given by Tkachuk (14) would account for 142 μM of ammonia, leaving 914 μM due to glutamine and asparagine. Subtracting this figure from the sum of glutamic and aspartic acids (1,118 + 635) leaves a total of 839 μM of glutamic and aspartic acids. The total of lysine, histidine, and arginine is 903 μM, giving an excess of 64 μM of cationic groups.

It can be concluded on the basis of the amino acid analysis that the colored component is a basic protein. This is substantiated by its behavior during chromatography on CMC. The fact that it is eluted with a fairly high concentration of salts indicates an excess of basic amino acids. Its electrophoretic behavior at pH 8.0 further gives proof of its basic nature.

Effect of Ascorbic Acid. It has been reported that ascorbic acid, in fairly high concentrations (350 p.p.m.), reduces pigment loss but causes browning in macaroni (15). This was confirmed in the present study. The slight discoloration is reflected in a decrease of 2% in brightness. There is also a slight increase in the absorption maximum at 400 mμ of an aqueous extract from macaroni treated with ascorbic acid. Menger (16) reported that ascorbic acid reduced pigment loss but had little effect on browning; in the presence of lecithin, however, ascorbic acid stimulated browning. She also reported that

an orange-red pigment, extractable with water-saturated butanol, was formed upon addition of ascorbic acid.

The absorption spectrum of a water-saturated butanol extract of ascorbic acid-treated macaroni showed the same number of peaks as that from untreated macaroni. The peaks in the extract treated with ascorbic acid were slightly higher. If new pigments are formed by reaction of ascorbic acid, they are not detectable by absorption spectrophotometry. There is an indication that a brownish pigment is extractable with water as shown by a slight increase in absorption at 400 mμ.

Since ascorbic acid also acts as a flour improver (17), the reactions involving ascorbic acid may be competitive. Thus the undesirable browning of macaroni may occur only in high concentrations of ascorbic acid with the possible interaction of a copper-protein complex.

Results of this study offer a method of predicting brownness in macaroni.

Analysis for copper might give an indication of brownness, but the method requires careful preparation of samples. A direct determination on an aqueous extract is not sufficiently sensitive to be useful. The sample must be ashed or digested with acid.

On the other hand, measuring the absorbance at 400 mμ of an aqueous extract of semolina offers a simple method of predicting the degree of brownness. This was found for a number of semolina samples examined. In a study of new varieties of durum wheat, this simple test might be applied to determine whether the processed product will be brownish.

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