

Two Metal Ions Improve Brightness in Wheat-Dough Products and Affect Aqueous Dispersion of Gluten¹

KESWARA RAO VADLAMANI² and PAUL A. SEIB^{2,3}

ABSTRACT

Cereal Chem. 74(3):318–325

Zinc and aluminum ions at 0.05% of wheat flour, dry basis (7.4 and 18.5 mmol/100 g, respectively), improved the brightness of raw and dried spaghetti and salt and alkaline noodles. They also retarded bacteria and yeast and mold growth in salt noodles held at 25°C for two days as determined by total plate counts. Neither metal ion caused a change in noodle cooking quality, but they imparted a slight aftertaste in cooked noodles. Wheat flour dough mixed with 0.05% zinc or 0.025% aluminum ion (fwb), when kneaded in aqueous 0.1% calcium chloride, gave gluten

with increased brightness. Zinc and aluminum ions appear to complex with enzymic browning chromophores in wheat dough and gluten and change their spectral properties. Zinc and aluminum ions affected the dispersion of gluten in water at pH ~5.0 and facilitated its spray-drying, but they were not detrimental to baking quality. Citric and tartaric acids at 5 mmol/100 g of gluten (db) gave wet gluten with pH ~4.5, which improved its brightness and water dispersibility.

Enzymic browning is recognized more widely in cut fruits and vegetables than in wheat-based foods. Yet noodles (Kruger et al 1992, Baik et al 1995, Rao and Seib 1996); pasta products (Kobrehel et al 1972); vital wheat gluten (Kim et al 1991); and refrigerated doughs such as biscuits and rolls undergo enzymic browning during processing and storage. The problem is acute in stored raw noodles and pasta, both of which are valued for their fresh flavor. Noodles and pasta usually are made from expensive low-extraction flours because bran contains a high concentration of polyphenol oxidase (PPO) (Marsh and Galliard 1986, Hatcher and Kruger 1993, Baik et al 1994), the enzyme that catalyzes browning.

Enzymic browning is one cause of the darkening of vital wheat gluten (Kim et al 1991). The dark color of gluten may be undesirable in certain gluten-supplemented foods such as rolls, restructured fish and meats (Sharma and Seltzer 1977), and vegetarian analogs (Muroi et al 1970, Tanaka et al 1976).

Polyphenol oxidase has a copper(II) prosthetic group and is inhibited by chelating and by reducing agents (Mayer and Harel 1979, Whitaker 1994). Other metal ions, such as magnesium(II) and calcium(II), might also displace copper(II). A dip in 2% calcium chloride and 1% zinc chloride solutions was reported to be effective in reducing enzymic browning in apricots, peaches, and pears during storage at 0–2°C (Bolin and Huxsoll 1989). Sapers et al (1994) reported that a dip in aqueous solution containing sodium erythorbate and zinc chloride effectively controlled the enzymic browning in mushrooms.

The objectives of this investigation were to: 1) determine the effects of zinc and aluminum ions on brightness of wheat-based products, and 2) evaluate their effects on final product quality. In carrying out those objectives, it was discovered that zinc and aluminum ions formed aqueous dispersions of gluten at pH ~5.0

MATERIALS AND METHODS

Materials

Commercial milling products were a hard red winter (HRW) wheat flour with 10.6% protein and 0.47% ash; a commercial

pastry flour with 8.7% protein and 0.40% ash; and a durum semolina with 13.0% protein and 0.75% ash on a 14.0% moisture basis (mb). Commercial vital wheat gluten samples included three from the United States and one each from Mexico, Australia, Finland, Sweden, Netherlands, and France. Most experiments with commercial gluten were done on sample no. 3 (Manildra Milling Co.), which had 68.0% protein and 0.60% ash.

All chemicals were reagent grade, and water was distilled. Zinc and aluminum salts were obtained from Fisher Scientific Co. (Fair Lawn, NJ), except aluminum lactate (K&K Laboratories, Plainview, NY); sodium aluminum sulfate (SAS) (General Chemical Corp., Parsippany, NJ); sodium aluminum phosphate, acidic (SALP) (Rhone-Poulenc Basic Chemical Co., Shelton, CT); and zinc bromide (Aldrich, Milwaukee, WI). Dihydroxyphenylalanine (DOPA) and polyphenol oxidase (PPO, mushroom) were obtained from Sigma Chemical Co. (St. Louis, MO). Some salts were added in hydrated form but the levels are reported on an anhydrous basis.

General Methods

Protein ($N \times 5.7$), moisture, and ash in wheat flour and gluten samples were determined according to AACC Methods 46-11, 44-15A, and 08-11, respectively (AACC 1995). Analytical data for flour are reported on a 14.0% mb. The pHs of dough and wet gluten were measured by a special surface pH electrode (model 1001, Sentron, Federal Way, WA) as described by Miller et al (1994). Mixograms were run on a 10-g mixograph (AACC Method 54-60), and zinc and aluminum levels in products were determined by atomic absorption spectroscopy after dry ashing (AOAC 1975). Dried noodle doughs and gluten samples were ground on a mill (1093 Cyclotech, Tecator Inc., Sweden) to pass through a wire-mesh screen with openings of 0.5 mm. Particle size distribution of gluten samples was measured using a Horiba centrifugal automatic particle size analyzer (CAPA-300, Horiba Ltd., Kyoto, Japan) by dispersing gluten (~0.1 g) in absolute ethanol (20 mL). Standard particles (Duke Scientific Corp., Palo Alto, CA) with mean diameters and standard deviations of 10.9 ± 1.2 , 31.9 ± 2.2 , and 50.9 ± 3.0 μm gave experimentally determined values of 9.0 ± 0.2 , 35 ± 0.3 , and 55.3 ± 2.0 μm , respectively.

The color of noodle dough sheets was monitored for 24 hr using a chromameter (CR-210, Minolta Corp., Ramsey, NJ) calibrated at $L^* = 98.7$. Three readings (L^* , a^* , and b^*) were taken for each noodle dough sheet on duplicate samples at 0, 2, 4, and 24 hr. A separate area of the product, each ~50 mm in diameter, was used for each measurement. The colors of raw and dried spaghetti

¹Contribution No. 96-551-J from the Kansas Agricultural Experiment Station, Manhattan, KS 66506.

²Graduate research assistant and professor, respectively, Department of Grain Science and Industry, Kansas State University, Manhattan, KS 66506.

³Corresponding author. E-mail: pas@wheat.ksu.edu

strands (~20 laid side-by-side) were measured on duplicate samples. The powdered gluten was packed carefully into a sample holder using a spatula. Three measurements were made by rotating the holder 120° using a ~50 mm diameter sensor.

Preparation of Salt and Alkaline Noodle Doughs

Wheat flour (100 g, 14% mb) was mixed slowly in a 4 qt (4 L) Hobart mixer fitted with a cake paddle, while a salt solution was added over a period of 1 min. The salt solution contained water (34 mL), sodium chloride (2 g), and a second salt: either zinc chloride (100 mg), zinc sulfate (100 mg), zinc acetate (100 mg), calcium chloride (100 mg), aluminum sulfate (320 mg), aluminum chloride (240 mg), or aluminum lactate (540 mg). Two other salts, sodium aluminum phosphate, acidic SALP (590 mg) and sodium aluminum sulfate SAS (450 mg), were blended in powder form with flour before adding salt solution (2 g of sodium chloride in 34 mL of water). A blank noodle dough also was prepared without a second salt. The dry and liquid ingredients for each batch were mixed for 1 min at speed 1 and for 4 min at speed 2. The crumbly dough was pressed into a sheet 5.5 mm thick on a noodle machine (Ohtake Mfg. Co., Tokyo, Japan). The resultant thick dough piece was sealed in a polyethylene bag and allowed to rest for 20 min. Thereupon, the thickness of the noodle dough sheet was reduced to 1.5 mm in five steps. Individual dough sheets (200 × 200 mm) were cut from the resultant sheet, sealed in polyethylene bags (250 × 300 mm), and stored at 25°C.

Alkaline noodle dough sheets were prepared in the same manner except for a minor change in the mixing step. Wheat flour (100 g) was blended in the mixer, while a solution of sodium and potassium carbonates (0.9 and 0.1 g, respectively, in 20 mL of water) was added over a period of 20 sec at mixing speed 1. Immediately thereafter, a second salt solution containing 14 mL of water and either zinc chloride (100 mg), zinc sulfate (100 mg), zinc acetate (100 mg), calcium chloride (100 mg), aluminum sulfate (320 mg), aluminum chloride (240 mg), or aluminum lactate (540 mg) was added over a period of 20 sec during continued mixing at speed 1. SALP (590 mg) and SAS (450 mg) were blended again in powder form with flour before adding the carbonate-salt solution. After an additional 20 sec of mixing at speed 1, mixing was continued for an additional 4 min at speed 2. A blank with only carbonate salts also was prepared. The alkaline noodle doughs (pH ~10.5) were formed and sheeted. Samples (200 × 200 mm) of raw dough sheets were stored in sealed polyethylene bags at 25°C.

In another experiment, salt noodle doughs were adjusted to pH ~5.0 with mineral acids. The other ingredients besides HRW wheat flour (100 g) and sodium chloride (2 g) were: water (34 mL) (blank sample), 0.02M hydrochloric acid (34 mL), or 0.01M sulfuric acid (34 mL). Salt noodle dough sheets were cut from the long dough sheet, sealed in polyethylene bags, and stored at 25°C.

Microbial Count of Raw Salt Noodles

Raw salt noodle dough sheets were prepared with 0.1% zinc chloride or 0.32% aluminum sulfate. The blank sample of salt noodle dough contained additional sodium chloride (127 mg) in the formula. Each noodle dough sheet was cut into three pieces, and individual pieces were sealed in polyethylene bags and stored at 25°C. Noodle dough sheets were sampled before storage or at one and two days of storage. Ethanol-swabbed packages were opened aseptically, and samples (11 g) were removed by cutting triangular wedges. The samples were homogenized for 2 min in 99 mL of 0.1% sterile peptone water (Difco Laboratories, Detroit, MI) in stomacher bags, which are sterile polyethylene bags used for mincing samples. Aliquots (0.1 mL) of serial dilutions in 0.1% peptone water were prepared. Total bacterial and yeast and mold counts were performed by the pour plate method (Mayor and Moberg 1992). The plates were incubated for 24–48 hr at 35 ± 1°C for total bacterial counts and for five days at 25°C for yeast

and mold counts. The total bacterial counts were expressed as CFU/g (colony forming units/g) of raw noodles, and yeast and molds as number per gram. The experiments were performed in duplicate.

Cooked Noodles

Salt and alkaline noodles (1.5 mm wide) were prepared by cutting from dough sheets (1.1 mm thick) containing zinc chloride (100 mg/100 g of flour) or aluminum sulfate (320 mg/100 g of flour). Blank noodles containing neither zinc chloride nor aluminum sulfate also were prepared. The freshly prepared noodles were cooked in gently boiling distilled water (500 mL). Optimum cooking time and cooking loss were measured as described by Rho et al (1988). Tensile breaking force and elongation tests were performed according to Guan and Seib (1994b) using a texture analyzer (TA-XT2, Stable Micro Systems, Haslemere, Surrey, England). Sensory panel evaluation was done by triangular taste tests (ASTM 1968) by five untrained panelists. All experiments were replicated twice.

Preparation of Spaghetti

Fresh and dried spaghetti were prepared with and without zinc chloride. Durum semolina (770 g, db) was placed in the 4-L mixer fitted with a flat paddle agitator. Water (230 mL), with or without zinc chloride (770 mg), was added over a 2-min period to the semolina while the mixer was running at speed 1. Thereafter, the mixer was run for another 3 min at speed 2. The mixing bowl was covered with a damp cloth, and the crumbly dough allowed to rest at room temperature for 15 min. Spaghetti was made according to Guan and Seib (1994a) using a small pasta press (Demaco model S-25, DeFrancisci Machine Co., Brooklyn, NY) fitted with a spaghetti die with 84 circular, Teflon-lined holes, 1.8 mm diameter (D. Maldari & Sons Inc., Brooklyn, NY).

Gluten Isolation

Hard red winter (HRW) wheat flour (100 g, 14.0% mb) was added to the bowl of a pin mixer containing a solution (60 mL) of aluminum ion (0.025% or 9.3 mmol/100 g of flour) as either aluminum sulfate, aluminum chloride, aluminum lactate, or sodium aluminum sulfate or a solution (60 mL) of zinc ion (0.05% or 7.4 mmol/100 g of flour) as either zinc chloride, zinc sulfate, zinc acetate, or zinc bromide. The ingredients were mixed into a dough that was placed in 0.1% aqueous calcium chloride. The mixture was allowed to rest for 15–20 min. Starch was washed from the dough by hand with water (200 mL) containing 0.1% calcium chloride. The mixture was poured over a U.S. 32 wire mesh, and the overs were washed again (3 × 200 mL) until most of the starch was washed away from the gluten. Finally, the gluten ball was washed with water (2 × 100 mL) and then mixed with water (100 mL) at high speed in a Waring blender for ~15–30 sec. After the stability of the suspended gluten was noted visually, the mixture was freeze-dried.

In other experiments, 0.2M hydrochloric acid or 0.1M sulfuric acid (60 mL) was mixed with flour (100 g, 14% mb) into doughs. Gluten was isolated from the resulting doughs by washing with 0.1% aqueous calcium chloride, followed by water, and then freeze-drying. Isolated gluten contained a minimum of 80% protein on a dry basis.

Spray-Drying of Gluten

Gluten was isolated from a HRW wheat flour (500 g, 14.0% mb) dough containing zinc chloride (500 mg, 7.4 mmol/100 g) as described above. The gluten was suspended in distilled water (500 mL) by blending vigorously in a Waring blender to produce a suspension with 10–15% gluten solids. The suspension was spray-dried in a fluid-bed granulator/coater (laboratory model 100, Applied Chemical Technologies Inc., Florence, AL) with air inlet and outlet temperatures of 93 and 70°C, respectively, and a slow flow rate of 1 mL/min.

Organic Acid and Zinc Chloride Treatment of Commercial Gluten

Commercial gluten (10 g, db) was suspended in 2.5, 5.0, and 10 mM of aqueous citric acid or sodium citrate, tartaric acid, acetic acid, or zinc chloride solution (100 mL). Each suspension was mixed with a magnetic stirring bar for 10 min followed by high-speed shearing for 1 min (Ultra Turrax mixer, Tekmar Co.). After the pH of the suspension was recorded, it was centrifuged at $1,000 \times g$ for 10 min, and the supernatant collected. The sediment was resuspended in distilled water (100 mL) and centrifuged again. The combined supernatants and the sediments were freeze-dried immediately. The weights of the fractions were recorded, after which they were ground together, and measured for brightness. In another experiment, gluten (10 g, db) and zinc chloride (50 mg) in water (20 mL) was kneaded together in a 10-g mixogram bowl (National Mfg.Co., Lincoln, NE) for 4 min. The sample was freeze-dried, ground, and brightness determined.

Gluten Quality

Hydration capacity and gluten index was determined according to Perten (1989), and gluten bake tests (5 g, db) were done according to Czuchajowska et al (1990). The force required to overcome the adhesiveness of gluten was measured. Gluten (1 g, db) was hand-mixed into a homogenous wet mass with 1.75 mL of distilled water. The sample was pressed carefully into a sample holder well (13 mm diameter, 10 mm deep). Using a TA-XT2 texture analyzer, the wet gluten was compressed with a cylindrical probe (10 mm diameter) at a speed of 1.0 mm/sec and to a maximum force of 0.5 N. The probe was retracted immediately, and the maximum force required to overcome the adhesiveness of gluten to the probe surface was taken from the negative peak. The measurements were performed on duplicate samples.

For breadbaking, the protein level in a commercial pastry flour (8.7% protein) was increased to 11.7% by adding one each of two gluten samples: a commercial gluten and a gluten isolated from a dough mixed with or without 7.4 mmol of zinc ion/100 g of flour. A blank dough was mixed without adding gluten. A straight-dough, pup-loaf baking procedure was used (AACC Method 10-10B) with the formula: flour (100 g, 14.0% mb), sucrose (6 g), nonfat dry milk (4 g), shortening (3 g), and instant dry yeast (2 g). Doughs were fermented for 90 min, proofed for 36 min at 30°C, and baked for 24 min at 218°C. Loaf volume was measured immediately after baking, and bread crumb was evaluated 1 hr after baking.

Pigments Extracted from Noodle Doughs and Gluten Containing Zinc and Aluminum Ions

Salt noodle doughs were prepared from HRW wheat flour (100 g), water (34 mL), and sodium chloride (2 g), with zinc chloride (100 mg,

7.4 mmol/100 g) or aluminum chloride (240 mg, 18.5 mmol/100 g). For the blank, salt noodle dough contained an additional 127 mg (22 mmol sodium ion/100 g) of sodium chloride. The mixed noodle doughs were divided into two portions. One portion was freeze-dried immediately, and the other portion was stored for 24 hr at 25°C in a sealed polyethylene bag and then freeze-dried. Pigments were extracted from a sample (10 g, 14.0% mb) with water-saturated butanol (50 mL) for 1 hr at room temperature (AACC method 14-50). The extract was filtered and evaporated to dryness <50°C using a rotary evaporator. The residue was mixed with water-saturated butanol (10 mL), and the mixture was filtered. The absorbance of the clear extract was recorded every 10 nm against a solvent blank in a 350–600 nm range on a single beam spectrophotometer (Spectronic 601, Milton Roy Co., Rochester, NY). Gluten also was isolated by water-washing of dough that contained flour (100 g) and zinc chloride (100 mg) or aluminum chloride (120 mg). The freeze-dried ground gluten (10 g) was extracted with water-saturated butanol (50 mL), and absorption spectra for those extracts were recorded. The free lutein content in a sample was calculated as described by Johnston et al (1980) from the absorbance at 449 nm. All experiments were replicated twice.

Reaction of Enzymic Browning Products with Zinc and Aluminum Ions

Aqueous 10 mM DOPA (1.5 mL) in a solution of PPO (0.5 mL, 0.12 mg/mL) was incubated at ambient temperature for 20 min. Water (18 mL) was added, and the absorbance of the mixture was recorded at 220–700 nm. The effects of zinc and aluminum ions were determined by adding 1.5 mL of a salt solution (15 mM) to the PPO reaction mixture. Salt solutions were added either before or after incubation, and the resultant mixtures were made to volume (20 mL) before recording the spectra.

Statistical Analysis

Analysis of variance (ANOVA) was performed using a completely randomized design with subsampling according to the general linear model procedure (SAS 1990).

RESULTS AND DISCUSSION

Color of Noodle Doughs Containing Zinc and Aluminum Ions

All concentrations of salts and metal ions are weight %, based on flour (db) unless otherwise stated. The color changes of salt noodle doughs stored for up to 24 hr at room temperature are reported in Table I. The changes for raw alkaline noodle doughs were similar and are not given. Brightness (L^*) values decreased continuously with time for treated and blank noodle doughs.

TABLE I
Brightness (L^*) and Yellowness (b^*) of Salt Noodle Doughs Containing Added Salts^a and Stored at 25°C

Storage Time (hr)	Blank	CaCl ₂ (0.1%)	ZnCl ₂ (0.1%)	ZnSO ₄ (0.1%)	Zn(OAc) ₂ (0.1%)	Al ₂ (SO ₄) ₃ (0.32%)	AlCl ₃ (0.24%)	Al (Lac) ₃ (0.54%)	SALP (0.59%)	SAS (0.45%)
L^* Value ^b										
0	80.5	80.4	82.8	81.4	80.4	84.1	83.5	82.0	81.0	81.0
2	76.1	76.5	79.4	78.0	75.9	80.0	79.9	78.0	77.3	77.4
4	75.0	75.3	78.6	76.9	74.9	79.0	78.9	76.9	76.0	76.6
24	70.5	71.4	75.0	72.4	70.4	75.8	75.6	73.5	73.0	73.7
b^* Value ^c										
0	18.3	18.3	16.0	16.1	13.8	14.2	14.8	16.0	16.9	16.8
2	21.7	21.7	18.8	19.5	16.2	18.1	19.8	19.8	19.8	20.2
4	22.2	22.3	19.0	20.4	16.4	18.8	18.4	19.5	19.5	20.2
24	20.7	21.2	20.3	21.5	17.7	18.9	18.9	19.3	19.3	18.9

^a Abbreviations: Ac = acetyl, Lac = lactoyl, SALP = sodium aluminum phosphate, acidic, SAS = sodium aluminum sulfate.

^b Higher L^* value indicates a brighter product. All L^* values at 2–24 hr, except for CaCl₂ and Zn(OAc)₂, are statistically different from the blank at $P = 0.05$.

^c Higher b^* value indicates more yellowness. All b^* values at 2–24 hr, except for CaCl₂, and at 24 hr for ZnCl₂ and ZnSO₄, are statistically different from the blank at $P = 0.05$.

Among zinc salts tested at the 0.1% level, zinc chloride was more effective than zinc sulfate in preserving brightness, whereas zinc acetate was ineffective. Calcium chloride was ineffective at the 0.1% level, indicating that the metal ions in aluminum and zinc chlorides were the active components. Among aluminum salts (all tested at 0.05%), aluminum ion, aluminum sulfate, and aluminum chloride were equally effective, whereas aluminum lactate, sodium aluminum phosphate, and sodium aluminum sulfate (SAS) were less effective (Table I).

Yellowness (b^*) increased with storage time up to 4 hr for all noodle doughs and then remained constant. All salt noodle doughs containing zinc and aluminum salts were less yellow when compared to the blank, and aluminum salts were more effective than zinc salts. Calcium chloride had no effect on the yellowness of noodle dough.

Zinc or aluminum salts in noodle doughs increased the acidity to pH ~5.0. Adjustment of a salt noodle dough from pH 6.0 to 5.0 with either aqueous hydrochloric or sulfuric acid resulted in only a slight improvement in noodle dough brightness (Table II). Addition of zinc or aluminum salt to the formula gave the brightest noodle doughs. These results indicate that zinc or aluminum salts improve salt noodle dough brightness by a mechanism other than reducing dough pH.

Kruger et al (1992) and Baik et al (1995) reported a positive correlation between the rate of change in brightness in noodle dough and the level of PPO. Adding zinc or aluminum salts to a

TABLE II
Brightness (L^* Value)^a of Salt Noodle Doughs^b Stored at 25°C with pH Adjusted by Mineral Acids or Zinc Salts

Storage Time (hr)	Blank	HCl	H ₂ SO ₄	ZnCl ₂	ZnSO ₄
0	82.0	81.8	82.4	84.0	84.1
4	75.7	76.2	76.6	79.7	79.7
24	70.6	71.3	72.4	76.1	76.2

^a All L^* values at 24 hr are statistically different from the blank at $P = 0.05$.
^b All doughs had pH 5.1–5.2 except the blank at 6.0. Reagent added per 100 g (14.0% mb) of flour were: HCl, 0.02M (34 mL); H₂SO₄, 0.01M (34 mL); ZnCl₂ (100 mg); and ZnSO₄ (210 mg).

TABLE III
Brightness (L^* Value) of Raw and Dried Spaghetti Containing Zinc Ion

Spaghetti	Storage Time (hr) ^a	Blank	Treated ^b
Raw	0	68.0	71.9
	2	67.5	69.7
	4	66.6	69.2
	24	64.9 ^{a,c}	67.7 ^b
		60.4 ^a	64.6 ^b
After drying			

^a Stored at 25°C.
^b Treated sample contained 0.1% zinc chloride (based on semolina).
^c Values in a row with different letters are significantly different at $P = 0.05$.

TABLE IV
Cooking and Textural Characteristics of Salt and Alkaline Noodles Containing Zinc and Aluminum Ions^a

Product	Cooking Time (min)	Cooking Loss (%)	Breaking Force (N)	Elongation (%)
Salt noodles				
Blank	4.0	4.8 ^{a,b}	0.42 ^a	143 ^a
Zn ⁺⁺	4.3	5.7 ^b	0.46 ^b	144 ^a
Al ⁺⁺⁺	4.1	4.9 ^a	0.41 ^a	154 ^b
Alkaline noodles				
Blank	7.0	8.6 ^c	0.54 ^c	127 ^c
Zn ⁺⁺	7.5	8.8 ^c	0.54 ^c	129 ^c
Al ⁺⁺⁺	7.3	8.9 ^c	0.59 ^d	132 ^d

^a Zinc and aluminum ions were added at 0.05% flour weight basis.
^b Values in a column with different letters are significantly different at $P = 0.05$.

noodle dough increased the initial brightness (L^*) values, but the rate of change of brightness with time was approximately the same for all noodle doughs (Table I). Thus, Zn⁺⁺ and Al⁺⁺⁺ ions do not appear to reduce the rate of browning.

The effect of zinc ion on the brightness (L^*) of raw and dried spaghetti is shown in Table III. Inclusion of zinc chloride at 0.1% based on durum semolina improved the brightness (L^*) of both raw and dried spaghetti.

Microbiological Quality of Raw Salt Noodles

Total counts of bacteria and counts of yeasts and molds for raw salt noodle doughs stored at room temperature are shown in Fig. 1. During the two days of storage, the total bacterial counts in the blank sample increased exponentially (Fig. 1A). In contrast, the noodle dough containing 0.05% aluminum ion had a reduced bacterial count, and that with 0.05% zinc ion was intermediate.

The yeast and mold counts for the salt noodle dough declined for some unknown reason after one day of storage at room temperature (Fig. 1B). After two days of storage, aluminum ion at 0.05% inhibited yeast and mold growth, and zinc ion (0.05%) was somewhat less effective. These data indicate that aluminum ion especially may be beneficial in preserving raw salt noodles.

Cooked Noodle Quality

Analysis of the salt noodles before and after cooking showed a 100% retention of zinc (0.05%) and aluminum (0.05%) ions in

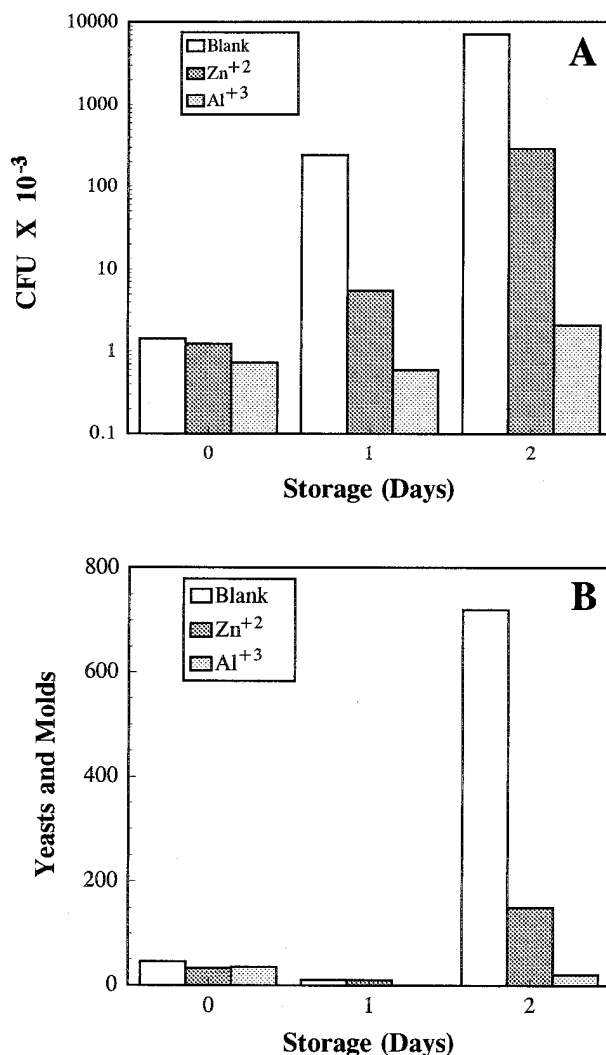


Fig. 1. Total bacterial count (A) and yeast and mold count (B) of stored (25°C) raw salt-noodle doughs containing 0.05% zinc ion and 0.05% aluminum ion based on dry flour weight.

cooked noodles. However, overcooking caused 2 and 16% losses of zinc and aluminum ions, respectively, in cooking and wash waters. The recommended dietary allowances (RDA) for adult women and men are 12 and 15 mg of zinc, respectively (RDA 1989). There is no recommended dietary intake for aluminum ion, although it is generally recognized as safe (CFR 1995). Consumption of 100 g (db) of zinc-supplemented noodles would provide about three times the RDA. The prudence of adding zinc or aluminum ions should be examined (Kendrick et al 1992, Prasad et al 1993, Armstrong and Maresh 1996, Biesel 1996, Pennington and Schoen 1996).

Addition of 0.05% zinc and aluminum ions to salt noodles increased the cooking time by 8 and 3%, respectively, and cooking losses by 19 and 2% (Table IV). Zinc ion increased the strength of the cooked salt noodles by ~10%, whereas aluminum ion increased elongation by ~10%. On the other hand, zinc and aluminum ions had little effect on the cooking and textural properties of alkaline noodles (Table IV). Sensory analyses by five untrained panelists using triangular tests demonstrated that 0.05% zinc or aluminum ion in salt noodles could be detected. However, all the panelists agreed that the difference in flavor was not objectionable.

Gluten Color

Gluten isolated from a dough containing zinc or aluminum salts had improved brightness (L^*) and reduced yellowness (b^*) upon drying (Table V). Zinc and aluminum ions improved the brightness of gluten by ~3 L^* units and reduced the yellowness by ~5–7 b^* units. Addition of zinc or aluminum salts reduced dough pH to ~5.0. Adjustment of dough to pH 5.0 by adding dilute mineral acids before gluten isolation resulted in only a minor improvement in gluten color (Table VI).

Turbulent mixing of commercial gluten in 10 parts of 2.5–10.0 mM zinc chloride, where the level of zinc ion was 0.16–0.64% based on gluten weight, followed by decanting of excess liquid phase and freeze-drying the sediment, did not improve the brightness of gluten (Fig. 2A), even though the concentration of zinc in the freeze-dried gluten was 0.16 g/100 g or almost the same as when gluten was isolated from a wheat flour dough mixed with

TABLE V
Zinc and Aluminum Salts and Their Effects on Brightness (L^*) and Yellowness (b^*) of Gluten

Additive ^a (% fwb)	L^* Value	ΔL^*	b^* Value	Δb^*
Blank	86.4	...	13.2	...
ZnCl ₂ (0.1)	89.8	+3.4	8.5	-4.7
ZnSO ₄ (0.21)	89.7	+3.3	8.4	-4.8
ZnBr ₂ (0.3)	90.1	+3.7	8.0	-5.2
Zn(OAc) ₂ (0.16)	89.6	+3.2	7.7	-5.5
Al ₂ (SO ₄) ₃ (0.16)	89.5	+3.1	6.5	-6.7
AlCl ₃ (0.12)	89.9	+3.5	6.7	-6.5
Al(Lac) ₃ (0.27)	89.9	+3.5	6.3	-6.9
SAS (0.45)	89.9	+3.4	6.8	-6.8

^a Abbreviations: Ac = acetyl, Lac = lactoyl, SAS = sodium aluminum sulfate, fwb = flour weight basis.

TABLE VI
Comparison of Various Mineral Acids and Zinc Salts on Dry Gluten Brightness (L^*) and Yellowness (b^*)

Treatment	Dough pH	Gluten pH	L^* Value ^a	b^* Value
Blank	6.0	5.9	86.5a ^a	13.2a
HCl	5.0	5.0	88.6b	10.5b
H ₂ SO ₄	5.0	5.0	88.4b	10.3b
ZnCl ₂ (0.1%)	5.2	5.2	89.8c	8.5c
ZnSO ₄ (0.21%)	5.3	5.3	89.7c	8.4c
ZnBr ₂ (0.3%)	5.2	5.2	90.1c	8.0c
Zn(OAc) ₂ (0.16%)	5.5	5.5	89.6c	7.7c

^a Values in the same column with different letters are significantly different at $P = 0.05$.

0.05% zinc ion. Apparently, zinc ion did not penetrate into the gluten and react with the pigments, whereas it did in the dough. In support of that hypothesis, kneading the commercial sample of

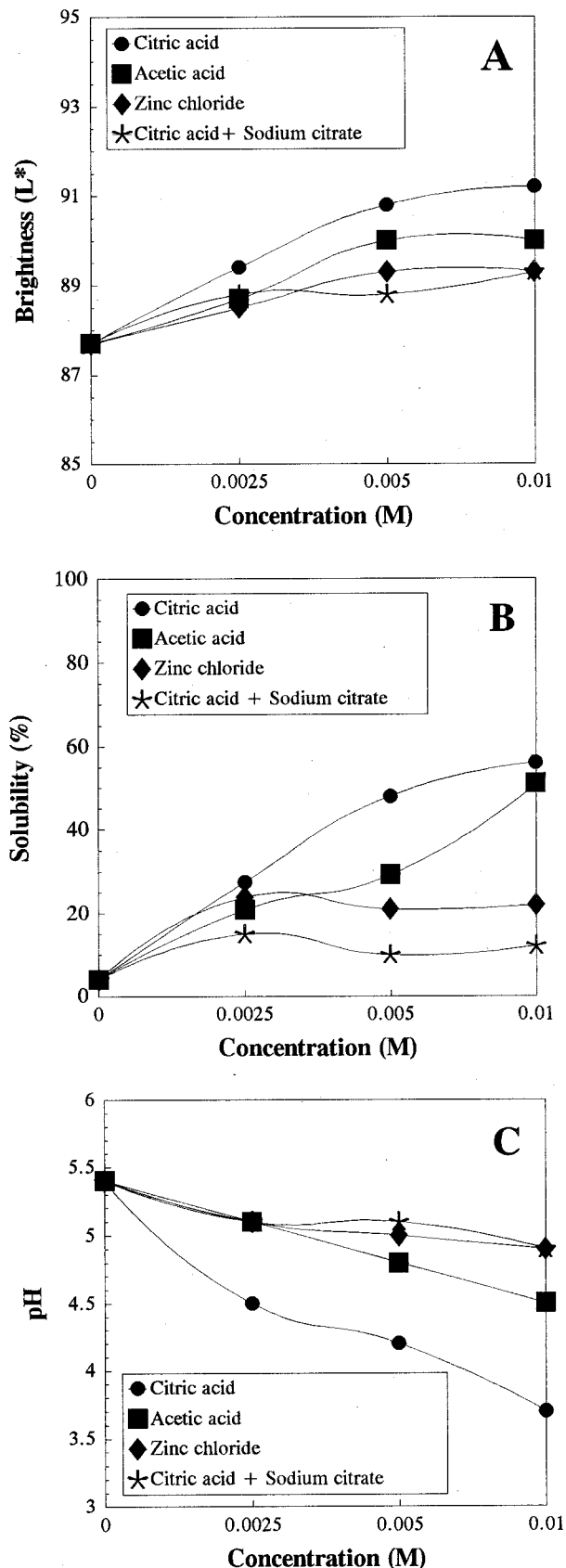


Fig. 2. Brightness (A), solubility (B), and pH (C) of commercial gluten (one part) after mixing with 10 parts of water containing an additive. Gluten was drained, freeze-dried, and ground.

gluten with two parts of water containing 3.9 mM zinc ion (0.25% based on gluten) to form a cohesive mass and then freeze-drying did improve brightness of the ground gluten from L^* of 86.7 to 89.9.

Commercial gluten mixed in 10 parts of 2.5–10.0 mM citric acid gave a maximum increase of 3 L^* units; acetic acid was less effective. The order of the additives for improving the brightness was the same as their order for lowering gluten pH (Fig. 2C) and solubilizing gluten (Fig. 2B). Citrate buffer (2.5–10.0 mM) gave no improvement in color or solubilization of gluten (Fig. 2A and 2B), in disagreement with the claim of improved dispersibility of gluten with sodium citrate (Johansson 1979).

It is well known that the particle size of a powdered material affects its appearance. The median particle size of all the gluten samples prepared in this investigation varied from 2.8 to 4.3 μm , with ~80% of particles under 10 μm size. These differences in the particle sizes of the gluten samples, however, did not correlate with differences in L^* values (*data not shown*). Kim et al (1991) reported that slight differences in particle size of gluten samples did not affect L^* values.

Model Studies of Color Change by Zinc and Aluminum Ions

Wheat flour contains a yellow xanthophyll called lutein (Lepage and Sims 1968) and a colorless (pH 6) flavone called tricetin (Anderson and Perkin 1931) as well as other phenolics, some of which are likely polymeric (Maga and Lorenz 1974, Sosulski 1982). Gluten isolated from wheat flour by dough washing is known to contain pigments and products (phenolics) of enzymic browning, that are extractable by aqueous alcohol at 25°C (Kim et al 1991). Water-saturated butanol extracts of untreated gluten (blank) showed three absorbance peaks between 385–495 nm in its visible spectrum (Fig. 3), but an extract of gluten isolated from a wheat flour dough containing 0.025% aluminum ion (9.2 mmol/100 g of flour) showed no absorption peaks. An extract of gluten isolated from a dough mixed with zinc ion gave peaks of reduced intensity or reduced background. The absorbance of enzymically synthesized dopaquinone (browning product) also was depressed when aluminum and zinc ions were added to a final concentration of 1 mM (Fig. 4). The absorption peak at 280 nm in Fig. 4 is DOPA and the broad peaks at 320 and 480 nm are browning products, the latter being dopaquinone (Chen et al 1991).

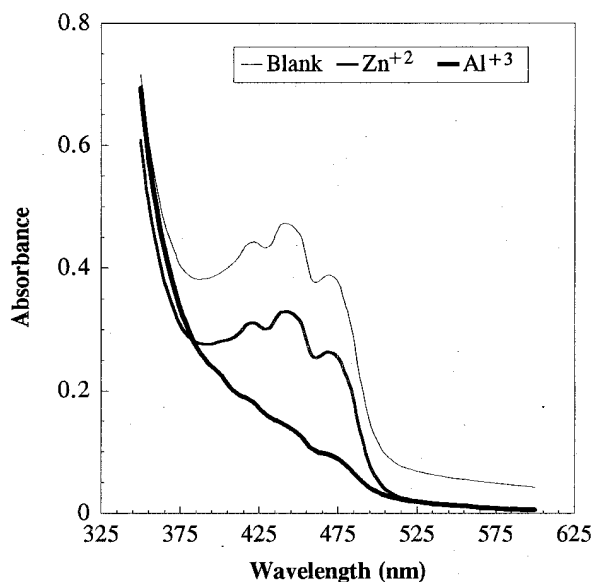


Fig. 3. Absorption spectra of dopaquinone upon addition of aluminum or zinc ion: dopaquinone produced from the action of mushroom polyphenol oxidase on DL-dihydroxyphenylalanine; dopaquinone after making to 1 mM with aluminum ion; and dopaquinone after making to 1 mM with zinc ion.

Attempts to demonstrate color changes on extracts of flour or aged noodle doughs were not successful because of the low levels of browning pigments. However, the data from the gluten extracts and the model-browning experiment suggest that zinc and aluminum ions interact with browning phenolics to decrease their absorbance of visible light, which is translated into an increase in brightness (L^*). Other polyphenolics in foods, such as anthocyanins, are known to form various anthocyanin-metal complexes that change colors and intensities (Markakis 1974, Coffey et al 1981).

Water Dispersibility of Gluten Promoted by Zinc and Aluminum Ions

Dilute acid (Lusena 1950) or dilute base (McConnell 1955) usually is used to disperse gluten. As the concentration of zinc or aluminum ion was increased in a wheat dough, the strength of the dough decreased, and the gluten isolated from the weakened dough had improved dispersibility. A minimum of 0.1% zinc chloride (0.05% zinc ion) or 0.16% aluminum sulfate (0.025% aluminum ion) in the dough was needed to obtain gluten that dispersed in water at pH ~5.0. Addition of zinc chloride (0.1%) or aluminum sulfate (0.16%) “softened” the dough and caused the gluten to be extensible and sticky, which made the isolation of gluten difficult. However, inclusion of 0.1% calcium chloride in the dough-kneading water countered the weakening effect of those salts during gluten isolation and gave an elastic nonsticky gluten (Fig. 5).

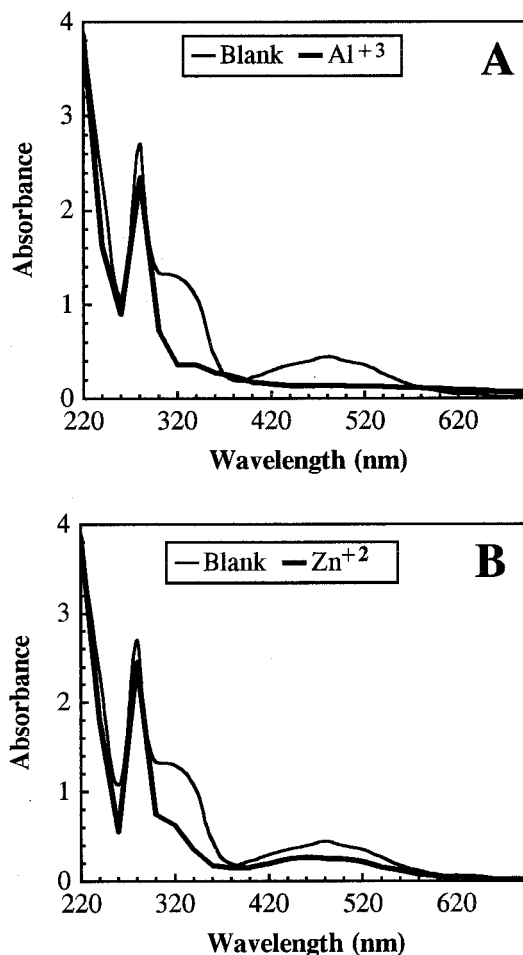


Fig. 4. Visible absorption spectra of water-saturated butanol extracts of gluten samples. Gluten samples were isolated from the commercial flour dough containing 0.05% zinc (A) and 0.025% aluminum (B) ion (based on dry flour weight) by the dough-washing procedure.

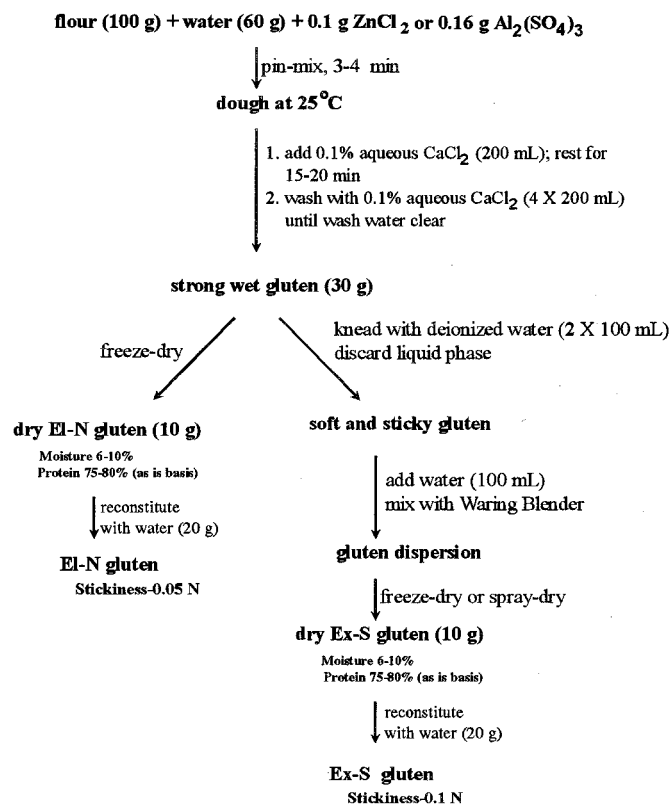


Fig. 5. Scheme to prepare an elastic nonsticky (EI-N) gluten and extensible sticky (Ex-S) gluten by the dough-washing procedure. Commercial bread flour with 10.6% protein was used.

Gluten isolated by kneading a dough containing zinc or aluminum ions in 0.1% aqueous calcium chloride would not emulsify when mixed with water. However, if the final wet gluten was washed two or three times with its weight of pure water, then the gluten dispersed readily in water. The scheme to isolate zinc or aluminum ion-dispersed gluten from flour is given in Fig. 5. All the water-soluble zinc and aluminum salts given in Table V, when added at the dough mixing step, enabled isolated gluten to be dispersed in distilled water.

The addition of zinc and aluminum salts had no effect on the yield of gluten (~10% fwb) from doughs kneaded with 0.1% calcium chloride. Gluten isolated from dough containing 0.05% zinc ion and 0.025% aluminum ion retained ~17 and 78%, respectively, of the zinc and aluminum ions.

The mechanism by which zinc and aluminum ions cause the dispersion of gluten proteins at pH ~5.0 is not known. Berg and Shi (1996) discussed the unique structural role of zinc ions in proteins. Zinc ion is not redox active and forms stable tetrahedral complexes with a variety of ligands on a protein, including sulfur from cysteine; nitrogen from histidine; and oxygen from glutamate, aspartate, and water. One might speculate that zinc ions complex with gluten and water, thereby increasing the hydration of gluten. As pointed out by George Lookhart (*personal communication*), before gel electrophoresis of proteins, aluminum lactate buffer (pH 3.1) was the solvent of choice in free-boundary electrophoresis of gliadin proteins (Jones et al 1959).

Quality of Gluten Isolated with Zinc or Aluminum Ion

The hydration capacity of extensible-sticky (Ex-S) gluten (Fig. 5) was elevated as compared to a blank sample and to commercial gluten. Its gluten index was higher than that of commercial gluten but equal to that of the blank (Table VII). When hydrated Ex-S gluten was baked, it gave a volume equal to that of the blank. However, it shrunk upon cooling, which indicated a weakened structure unable to support its weight against gravity.

TABLE VII
Comparison of Gluten Quality Parameters of Various Gluten Samples

Gluten	Hydration Capacity (g)	Gluten Index	Baked Gluten Volume (cm ³)
Commercial sample			
1	2.42a ^a	29.8a	135a
2	2.24b	24.7b	128a
3	2.14c	87.7c	97b
4	2.00d	79.0de	137a
5	2.29b	74.3e	133a
6	2.25b	83.1d	88b
No ZnCl ₂	2.24b	99.1f	120c
With ZnCl ₂	2.42a	98.2f	110c

^a Values in a column with different letters are significantly different at $P = 0.05$.

TABLE VIII
Breadbaking Performance of Various Gluten Samples

Dough	Water Absorption (%)	Mixing Time (min)	Loaf Volume (cm ³)	Crumb Grain
Pastry flour (PF) (blank)	57.0	4.0	703 ± 3a ^a	Open
PF + gluten (blank)	61.0	4.0	862 ± 16b	Open
PF + Ex-S gluten ^b	61.0	4.0	848 ± 8bc	Open
PF + Ex-S gluten + 0.1% CaCl ₂	61.0	4.0	865 ± 21b	Fine
PF + commercial gluten	61.0	4.0	835 ± 7c	Open

^a Values in column with different letters are significantly different at $P = 0.05$.

^b ExS = extensible and soft; see Fig. 5.

Zinc ion changed gluten's surface stickiness. Elastic nonsticky (EI-N) gluten had an initial stickiness value of 0.05 N, but the extensible-sticky (Ex-S) gluten had stickiness of 0.12 N (Fig. 5). An increase in zinc chloride concentration >0.1% resulted in only a minor increase in stickiness.

Breadbaking results (Table VIII) indicate that the quality of the Ex-S gluten (Fig. 5) was comparable to that of untreated and commercial gluten samples. Bread made from a pastry flour supplemented with zinc chloride-treated (Ex-S) gluten gave a slightly lower volume when compared to that of flour supplemented with untreated gluten. However, inclusion of 0.1% calcium chloride in the formula restored the loaf volume and improved the crumb grain.

CONCLUSIONS

Wheat-based foods (salt and alkaline noodles, spaghetti, and gluten) with improved brightness (L^*) and reduced yellowness (b^*) were prepared by adding low levels of zinc or aluminum ion. Apparently, zinc and aluminum ions improve brightness by reducing the absorption of light by endogenous phenolics and pigments. Zinc and aluminum ions did not stop enzymic browning. Isolating gluten in the presence of zinc and aluminum ions facilitated its dispersion in distilled water at pH ~5.0. These metal ions caused the gluten to become extensible and sticky, but did not significantly affect its baking properties. The weakening effect on gluten was overcome by adding calcium ion.

LITERATURE CITED

- American Association of Cereal Chemists. 1995. Approved Methods of the AACC, 9th ed. The Association: St. Paul, MN.
- ASTM. 1968. American Society for Testing and Materials Manual on Sensory Testing Methods. Special Technical Pub. No. 434, 24-67. The Society: Philadelphia, PA.
- Anderson, J. A., and Perkin, A. G. 1931. The yellow coloring matter of Khapli wheats, *Triticum dicoccum*. J. Chem. Soc. 2624-2625
- Armstrong, L. E., and Maresh, C. M. 1996. Vitamin and mineral supplements as nutritional aids to exercise performance and health. Nutr. Rev. 54:S149-S158.
- AOAC. 1975. Association of Official Analytical Chemists Methods of

- Analysis, 12th ed. The Association: Washington, DC.
- Baik, B. K., Czuchajowska, Z., and Pomeranz, Y. 1994. Comparison of polyphenol oxidase activities in wheats and flours from Australian and U. S. cultivars. *J. Cereal Sci.* 19:291-296.
- Baik, B. K., Czuchajowska, Z., and Pomeranz, Y. 1995. Discoloration of dough for Oriental noodles. *Cereal Chem.* 72:198-205.
- Beisel, W. R. 1996. Nutrition and immune function: Overview. *J. Nutr.* 126:2611S-2615S.
- Berg, J. M., and Shi, Y. 1996. The galvanization of biology: A growing appreciation for the roles of zinc. *Science* 271:1081-1085.
- Bolin, H. R., and Huxsoll, C. C. 1989. Storage stability of minimally processed fruit. *J. Food Proc. Preserv.* 13:281-292.
- Chen, J. S., Cheng-I-Wei, and Marshall, M. R. 1991. Inhibition mechanism of kojic acid on polyphenol oxidase. *J. Agric. Food Chem.* 39:1897-1901.
- Code Of Federal Regulations. 1995. Part 182. Substances generally recognized as safe. Food and Drug Administration. Human Health Services: Washington, DC.
- Coffey, D. G., Clydesdale, F. M., Francis, F. J., and Damon, R. A. Jr. 1981. Stability and complexation of cyanidin-3-glucoside and raspberry juice extract in the presence of selected cations. *J. Food Prot.* 44:516-523.
- Czuchajowska, Z. and Pomeranz, Y. 1990. Quest for a universal test of commercial gluten quality for bread making. *Cereal Foods World* 35:458-469.
- Guan, F., and Seib, P. A. 1994a. Instrumental probe and method to measure stickiness of cooked spaghetti and noodles. *Cereal Chem.* 71:330-337.
- Guan, F., and Seib, P. A. 1994b. Measuring extensibility of cooked spaghetti. (Abstr). *Cereal Foods World* 39:615.
- Hatcher, D. W., and Kruger, J. E. 1993. Distribution of polyphenol oxidase in flour mill streams of Canadian common wheat classes milled to three extraction rates. *Cereal Chem.* 70:51-55.
- Johansson, H. P. 1979. Powdered gluten composition and process for the production thereof. U.S. patent 4,150,016.
- Johnston, R. A., Quick, J. S., and Donnelly, B. J. 1980. Note on comparison of pigment extraction and reflectance colorimeter methods for evaluating semolina color. *Cereal Chem.* 57:447-448.
- Jones, R. W., Taylor, N. W., and Senti, F. R. 1959. Electrophoresis and fractionation of wheat gluten. *Arch. Biochem. Biophys.* 84:363-376.
- Kendrick, M. J., May, M. T., Plishka, M. J., and Robinson, K. D. 1992. Aluminum in biological systems. Pages 131-138 in: *Metals in Biological Systems*. M. J. Kendrick, M. T. May, M. J. Plishka, and K. D. Robinson, eds. Ellis Horwood Ltd.: West Sussex, England.
- Kim, W. S., Seib, P. A., and Chung, O. K. 1991. Origin of color in vital wheat gluten. *Cereal Foods World* 36:954-959.
- Kobrehel, K., Laignelet, B., and Feillet, P. 1972. Study of some biochemical factors of macaroni brownness (Abstr). *Cereal Sci. Today* 17:105.
- Kruger, J. E., Matsuo, R. R., and Preston, K. 1992. A comparison of methods for the prediction of Cantonese noodle color. *Can. J. Plant Sci.* 72:1021-1029.
- Lepage, M., and Sims, R. P. A. 1968. Carotenoids of wheat flour: Their identification and composition. *Cereal Chem.* 45:600-611.
- Lusena, C. V. 1950. Preparation of dried native wheat gluten. *Cereal Chem.* 27:167-178.
- Maga, J. A., and Lorenz, K. 1974. Phenolic acid composition and distribution in wheat flours and various triticale milling fractions. *Lebens. Wiss. Technol.* 7:273-278.
- Markakis, P. 1974. Anthocyanins and their stability in foods. *CRC Crit. Rev. Food Technol.* 3:437-457.
- Marsh, D. R., and Galliard, T. 1986. Measurement of PPO activity in wheat milling fractions. *J. Cereal Sci.* 4:241-248.
- Mayer, A. M., and Harel, E. 1979. Review: Polyphenol oxidases in plants. *Phytochemistry* 18:193-215.
- Mayou, J., and Moberg, L. 1992. Cereals and cereal products. Pages 995-1006 in: *Compendium of Methods for the Microbiological Examination of Foods*. C. Vanderzant, and D. F. Splittstoesser, eds. Am. Pub. Health Assoc.: Washington, DC.
- McConnell, W. B. 1955. Some characteristics of spray-dried wheat gluten. *Can. J. Tech.* 33:256-264.
- Miller, R. A., Graf, E., and Hosenev, R.C. 1994. Leavened dough pH determination by an improved method. *J. Food Sci.* 59:1086-1087, 1090.
- Muroi, C., Ogawa, G., and Inuzuka, S. 1970. Meat-like gluten product. Canada patent 848,913.
- Pennington, J. A. T., and Schoen, S. A. 1996. Total diet study: Estimated dietary intake of nutritional elements 1982-1991. *Int. J. Vit. Res.* 66:350-362.
- Perten, H. 1989. Gluten index—A rapid method for measuring wet gluten characteristics. *Milling (UK)* 182(9):38-41.
- Prasad, A., Fitzferald, J. T., Hess, J. W., Kaplan, J., Pelen, F., and Dardenne, M. 1993. Zinc deficiency in elderly patients. *Nutrition* 9:218-224.
- Rao, V. K. and Seib, P. A. 1996. Reduced browning in raw Oriental noodles by heat-moisture treatment of wheat. *Cereal Chem.* 73:88-95.
- RDA. 1989. Subcommittee on the 10th ed. of the Recommended Dietary Allowances. Food and Nutrition Board, Commission on Life Sciences, NRC-NAS: Washington, DC.
- Rho, K. L., Seib, P. A., Chung, O. K., and Deyoe, C. W. 1988. Noodles VII. Investigating the surface firmness of cooked Oriental noodles made from hard red winter wheat flours. *Cereal Chem.* 65:320-326.
- Sapers, G. M., Miller, R. L., Miller, F. C., Cooke, P. H., and Choi, S. W. 1994. Enzymatic browning control in minimally processed mushrooms. *J. Food Sci.* 59:1042-1047.
- SAS. 1990. Statistics. Vers. 6.0. The Institute, Cary, NC.
- Sharma, S. C., and Seltzer, E. 1977. Development of procedures to minimize mechanical damage in freeze-dried meat patties. *J. Food Sci.* 42:1336-1343.
- Sosulski, F., Krygier, K., and Hogge, L. 1982. Free, esterified, and insoluble-bound phenolic acids. 3. Composition of phenolic acids in cereal and potato flours. *J. Agric. Food Chem.* 30:337-340.
- Tanaka, K., Namazue, F., Ozawa, R., and Yokomizo, E. 1976. Process for the production of crab analogue meats. U.S. patent 4,000,331.
- Whitaker, J. R. 1994. Polyphenol oxidase. Pages 543-555 in: *Principles of Enzymology for the Food Science*. J. R. Whitaker, ed. Marcel Dekker: New York.

[Received July 8, 1996. Accepted February 6, 1997.]