

# Water-Soluble Pentosans of Wheat Flour. II. Characterization of Pentosans and Glycoproteins from Wheat Flour and Dough Mixed under Various Conditions<sup>1</sup>

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## ABSTRACT

Fractionation of water-soluble pentosans from doughs on a column of DEAE-cellulose indicated that treating the doughs with bromate and iodate reduced the amount of carbohydrate in fraction I, and increased the quantity of carbohydrate in fractions II to V (assumed to be glycoproteins) which contained most of the pentosan protein. In the absence of oxidizing agents, treatments that broke dough down during mixing showed an opposite effect. Pentosans from bromated and iodated dough mixed to optimum development (farinograph peak time) showed a marked increase in absorbance (280 nm.) of protein components obtained during gel filtration. Infrared (IR) amide II absorption, a secondary amide band of protein, shifted from 1,518  $\text{cm}^{-1}$  in pentosans extracted from flour to 1,538  $\text{cm}^{-1}$  in pentosans from doughs. The shift indicated a conformational change from the extended  $\beta$ -configuration (1,518  $\text{cm}^{-1}$ ) to the  $\alpha$ -configuration of helical coil in the glycoprotein molecules. A short wavelength shift in protein absorbance (280.5 to 278 nm.) and in the IR amide II band, accompanied by a large increase in intensity, further indicated that the hydrogen-bonding capacity of pentosans was markedly increased when flour constituents were mixed into dough. The increase in ultraviolet absorbance probably resulted from aggregation of structures containing pentosans, proteins, and/or glycoproteins. Pentosans and glycoproteins apparently act as a bridge between protein and carbohydrate components. Oxidizing agents appear to play an important role in maintaining the bridge.

Several reports have been published concerning the influence of water-soluble pentosans on water-binding capacity and consistency of dough (1,2,3). Kuendig et al. (4,5) and Neukom et al. (6,7) did detailed studies of the glycoproteins associated with water-soluble pentosans. Viscosity properties of water-soluble pentosans and molecular weights of individual fractions isolated from chromatography on DEAE-cellulose were previously reported (8). Much of the work on water-soluble pentosans has been done with pentosans extracted from wheat flour. Wall (9) has suggested that pentosans and glycoproteins are present as transitional compounds permitting physical association and even chemical bonding between carbohydrates and proteins. To better understand the reactivity of pentosans and associated glycoproteins (4,5,6) with oxidizing agents, we used water-soluble pentosans from wheat flour-water doughs. To demonstrate the changes brought about in glycoprotein components of water-soluble pentosans during dough mixing, we studied the samples by anion-exchange chromatography on DEAE-cellulose, gel filtration, and ultraviolet and infrared spectroscopy.

## MATERIALS AND METHODS

Wheat flour-water doughs from a typical hard red winter wheat flour (Scout-69), 12.2% protein (14% moisture basis), were prepared as previously described (8). The water-soluble pentosans were isolated and purified from the flour and

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lyophilized doughs according to the procedure of Cawley (10) and Kuendig et al. (4), as previously described (8).

The previous procedure (8) was used to fractionate the  $\alpha$ -amylase-treated (purified) pentosans (400 mg.) on a column of DEAE-cellulose and for gel filtration of purified pentosans and DEAE-cellulose fractions (15 mg.) on a column of Bio-Gel P-150. During gel filtration, protein components were located in effluent fractions by automatically scanning the effluent at 280 nm. (Uvicord, LKB Instrument Co., Sweden).

#### Ultraviolet and Infrared Spectra of Purified ( $\alpha$ -Amylase-Treated) Pentosans

Ultraviolet spectra (240 to 370 nm.) were obtained using a Beckman D.U. spectrophotometer using 0.25% (w./v. in 0.1M NaCl) solutions of lyophilized sample with 0.1M NaCl as reference.

Infrared spectra of purified pentosans were obtained using a Perkin-Elmer infrared grating spectrophotometer (Model 421) and the technique (with slight modification) developed by Stinson and O'Donnel (11) with optically pure potassium bromide (KBr). Discs were prepared by grinding 3 mg. sample with 300 mg. KBr (Lot No. 9221, Matheson Coleman and Bell Co., Norwood, Ohio) and pressing the mixture in a die. The die was connected to a vacuum pump to evacuate air and to remove moisture. The mixture was then compressed (using a hydraulic press with gauge) to a transparent disc about 1 mm. thick under pressure of 9,000 lb. per sq. in. for 30 min.

## RESULTS AND DISCUSSION

### DEAE-Cellulose Chromatography

All samples yielded five main peaks upon chromatography on DEAE-

TABLE I. YIELDS OF LYOPHILIZED FRACTIONS OF WHEAT FLOUR ELUTED FROM A COLUMN OF DEAE-CELLULOSE

Pentosan Source	Fraction No.					
	I	IIa	IIb	IIIa+IIIb	IV	V
Flour (Scout-69)	16 <sup>1</sup>	3	22	21	20	17
Plain dough (P.T. <sup>2</sup> )	18	4	25	16	16	16
Plain dough (D.T. <sup>3</sup> )	28	2	21	19	16	15
Bromated <sup>4</sup> dough (P.T.)	19	4	22	17	16	22
Bromated dough (D.T.)	16	...	25	18	22	19
Iodated <sup>4</sup> dough (P.T.)	15	...	29	15	17	24
Iodated dough (D.T.)	14	4	23	15	23	21
Plain dough (P.T., rested <sup>5</sup> )	23	2	24	15	15	18
Plain dough (D.T., rested)	28	3	23	16	15	14
Bromated dough (P.T., rested)	9	...	28	15	17	30
Bromated dough (D.T., rested)	11	...	28	13	21	28
Dough (1 $\mu$ eq. NEMI <sup>6</sup> /g. flour, P.T.)	26	1	21	18	19	15
Dough (1 $\mu$ eq. cysteine-HCl/g. flour, P.T.)	24	2	19	22	13	19

<sup>1</sup>Percent yield of fraction based on material recovered from column.

<sup>2</sup>P.T. = mixed to peak time.

<sup>3</sup>D.T. = mixed to departure time.

<sup>4</sup>Bromated and iodated: 1  $\mu$ eq. oxidant/g. flour.

<sup>5</sup>Rested = dough rested in fermentation cabinet at 30°C. and 95% r.h. for 3.0 hr.

<sup>6</sup>NEMI = *N*-ethylmaleimide.

cellulose. Some treatments yielded subdivisions of peaks II and V into two components (II a,b and V a,b) based on carbohydrate content. Subdivision of peak II into two components agreed with observations reported by D'Appolonia *et al.* (12). The results of column chromatography are shown in Figs. 1 and 2.

Changes in composition or concentration of the eluting solvent are denoted by arrows in Figs. 1 and 2. Recoveries of material applied to the column ranged from 77 to 95% (average 86%). The yields of lyophilized DEAE-cellulose fractions are given in Table I. The percentage distribution of carbohydrates

TABLE II. DISTRIBUTION OF CARBOHYDRATES<sup>1</sup> IN FRACTIONS ELUTED FROM A COLUMN OF DEAE-CELLULOSE<sup>2</sup>

Pentosan Source	Fraction No.					
	I	Ila	Ilb	IIla+IIlb	IV	V
Flour	20	4	32	17	16	10
Plain dough (P.T.)	24	4	33	13	15	10
Plain dough (D.T.)	30	2	27	16	14	11
Bromated dough (P.T.)	28	4	34	13	10	11
Bromated dough (D.T.)	24	...	29	13	20	14
Iodated dough (P.T.)	21	...	38	12	11	18
Iodated dough (D.T.)	19	4	29	11	20	19
Plain dough (P.T., rested)	31	2	40	10	10	7
Plain dough (D.T., rested)	35	6	28	11	10	9
Bromated dough (P.T., rested)	12	...	40	12	12	24
Bromated dough (D.T., rested)	18	...	31	12	18	20
Dough (1 $\mu$ eq. NEMI/g. flour, P.T.)	28	1	30	17	13	11
Dough (1 $\mu$ eq. cysteine-HCl/g. flour, P.T.)	26	3	24	24	9	12

<sup>1</sup>Based on the phenol-sulfuric acid colorimetric assay (480 nm.) of lyophilized fractions and the % yields of individual fractions.

<sup>2</sup>Abbreviations as in Table I.

TABLE III. DISTRIBUTION OF PROTEINS<sup>1</sup> IN FRACTIONS ELUTED FROM A COLUMN OF DEAE-CELLULOSE<sup>2</sup>

Pentosan Source	Fraction No.					
	I	IIa+IIb	IIIa	IIIb	IV	V
Flour	2	4	17	19	33	26
Plain dough (P.T.)	2	5	14	8	33	38
Plain dough (D.T.)	trace	2	14	5	40	38
Bromated dough (P.T.)	2	6	12	10	24	46
Bromated dough (D.T.)	2	4	12	12	35	35
Iodated dough (P.T.)	1	7	12	8	30	42
Iodated dough (D.T.)	2	5	13	4	29	48
Plain dough (P.T., rested)	4	4	12	9	28	43
Plain dough (D.T., rested)	2	4	8	7	30	48
Bromated dough (P.T., rested)	1	3	11	6	34	45
Bromated dough (D.T., rested)	1	5	9	5	30	50
Dough (1 $\mu$ eq. NEMI/g. flour, P.T.)	...	2	14	4	40	39
Dough (1 $\mu$ eq. cysteine-HCl/g. flour, P.T.)	...	1	13	10	20	47

<sup>1</sup>Based on Lowry colorimetric analysis (750 nm.) of lyophilized fractions and the % yields.

<sup>2</sup>Abbreviations as in Table I.

(Table II) and proteins (Table III) were calculated from standard curves by duplicate determinations of lyophilized DEAE-cellulose fractions. The amount of fraction I, a pure arabinoxylan (4,6,13) was considerably larger in pentosans from doughs than in pentosans from flour. Plain dough mixed to departure time gave the largest increase in fraction I compared to flour (Table I). However, overmixing (departure time) plain dough with added bromate dramatically reduced the yield of fraction I. Fraction II (glycoprotein II) is responsible for the characteristic gelling reaction of water-soluble pentosans (6,8). Fractions I and II of pentosans from all samples contained low amounts of protein (up to 2% in fractions I and II, to 4.5% in fraction II), which closely agree with values reported by D'Appolonia et al. (12).

Fraction IV of pentosans increased much more in bromated, overmixed dough than in plain, overmixed dough (Tables I and II). Adding bromate also substantially increased protein in fraction V. The percentage distribution of carbohydrate and protein (Tables II and III, respectively) among the various chromatographic fractions indicated that fraction II (a+b) contained 29 to 38% of the total carbohydrate in purified pentosans. Fraction II (a+b) of bromated

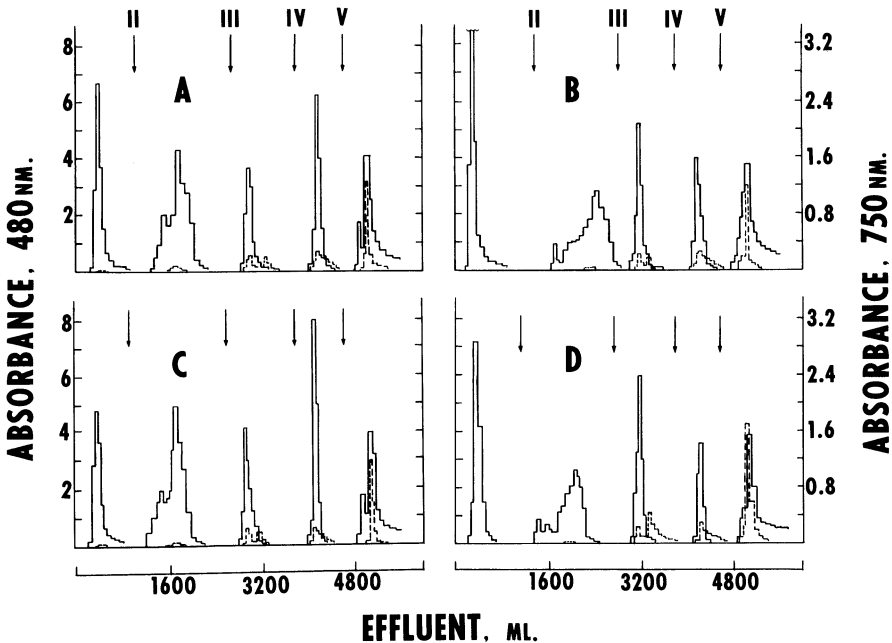


Fig. 1. Fractionation of  $\alpha$ -amylase-treated pentosans (400 mg.) from flour (Scout-69 wheat) and doughs on a column (3.5  $\times$  45 cm.) of DEAE-cellulose. Curves are an average from two fractionations. Solid line: carbohydrates (phenol-sulfuric acid method, 480 nm., absorbance  $\times 10^{-1}$ ); dashed line: proteins (Lowry procedure, 750 nm.). Components eluted with: I, water; II, 0.0025M; III, 0.025M; IV, 0.125M sodium tetraborate; and V, 0.5N NaOH. A: Iodated (1  $\mu$ eq. oxidant/g. flour) dough, mixed to peak time. B: Dough (1  $\mu$ eq. NEMI/g. flour), mixed to peak time. C: Iodated dough, mixed to departure time. D: Dough (1  $\mu$ eq. cysteine-HCl/g. flour), mixed to peak time.

dough mixed to optimum (peak time) contained relatively more carbohydrate and protein. In addition, more proteins were in fraction V of bromated dough (Table III) than in control dough.

Figure 1 shows the chromatographic patterns of pentosans from doughs mixed with potassium iodate, cysteine-HCl, and *N*-ethylmaleimide (NEMI). Adding iodate (Fig. 1, A,C) caused changes similar to those in bromated doughs, but the most pronounced changes were in fractions II and V of iodated doughs (Fig. 1, A,C). The yield (Table I) of fraction I from pentosans of iodated dough was relatively low compared with the doughs treated with cysteine-HCl and NEMI (Fig. 1, D and B, respectively, and Table I). Adding NEMI greatly increased pentosans in fraction I (Fig. 1, B) as in overmixed, plain dough (Table I). Fraction II of the iodated dough (Table III) mixed to optimum (Fig. 1, A) contained significantly more proteins than fraction II from other treatments did (Fig. 1).

The breakdown in dough structure caused by adding NEMI and cysteine-HCl (14,15) might be responsible for changes in water-soluble pentosans observed

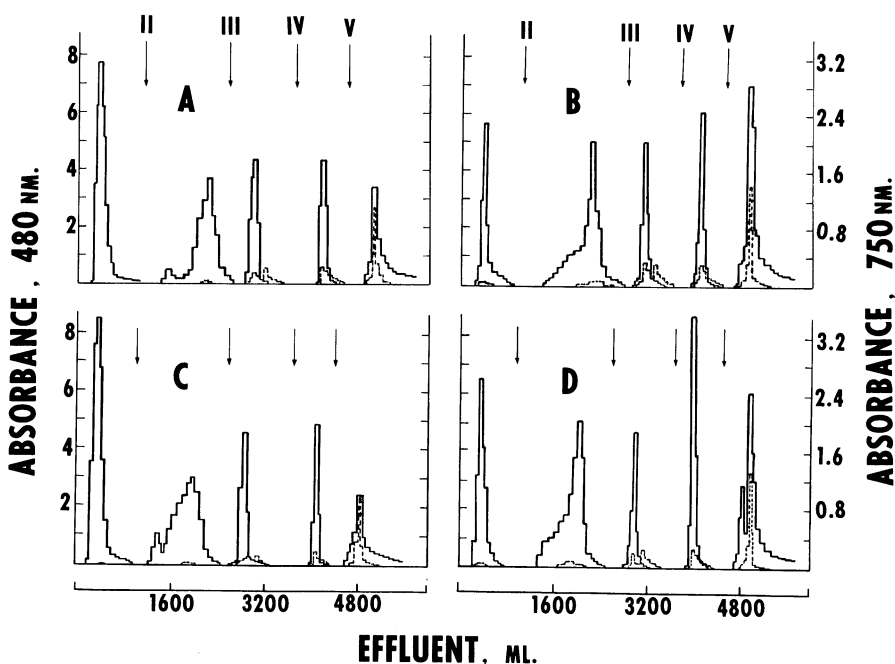


Fig. 2. Fractionation of  $\alpha$ -amylase-treated pentosans (400 mg.) from rested doughs on a column (3.5  $\times$  45 cm.) of DEAE-cellulose. Curves are an average from two fractionations. Solid line: carbohydrates (phenol-sulfuric acid method (480 nm.), absorbance  $\times 10^{-1}$ ); dashed line: proteins (Lowry procedure, 750 nm.). Components eluted with: I, water; II, 0.0025M; III, 0.025M; IV, 0.125M sodium tetraborate; and V, 0.5N NaOH. A: Plain dough mixed to peak time; rested. B: Bromated (1  $\mu$ eq. oxidant/g. flour) dough mixed to peak time; rested. C: Plain dough mixed to departure time; rested. D: Bromated dough mixed to departure time; rested. Rested refers to dough rested in fermentation cabinet at 30°C. and 95% r.h. for 3.0 hr.

during DEAE-cellulose fractionation; a large increase in fraction I (Fig. 1, B,D), similar to their behavior from plain, overmixed (mixed to departure time) doughs. The increase in the carbohydrate component of fraction I, a pure arabinoxylan (4,6) containing a little protein contaminant, suggested that structural breakdown of dough, caused after overmixing and by treatments with NEMI and cysteine-HCl, also caused a decrease in association between carbohydrate and protein components of water-soluble pentosans. A large increase in the protein component of fraction V (Fig. 1, D), when doughs were treated with cysteine-HCl, was not accompanied by a corresponding increase in the carbohydrate component, which supports the above observation.

To allow bromate some time to react, doughs were rested at 30° C. and 95% r.h. for 3.0 hr. Resting the doughs caused dramatic changes in the chromatographic patterns of water-soluble pentosans (Fig. 2). Figure 2 (A and C) and Table I show that fraction I increased when plain doughs mixed to peak time were rested; but fraction I was markedly reduced by the presence of bromate in doughs (Fig. 2, B,D). Subfraction IIa was absent in pentosans from bromated doughs (Fig. 2, B,D). In addition, fraction II, eluted by 0.0025M  $\text{Na}_2\text{B}_4\text{O}_7$ , and fraction V, eluted by 0.5N NaOH, of pentosans from bromated doughs increased more than from plain rested doughs (Fig. 2, A,C and Table I). Resting of doughs changed the distribution of carbohydrates (Table II) and proteins (Table III). Fractions II, IV, and V of plain, rested doughs contained 53 to 59% of the carbohydrates and 76 to 81% of the proteins in water-soluble pentosans, compared with 71 to 76% and 81 to 84%, respectively, from bromated, rested doughs. In addition, carbohydrates in fraction I (Table II) were reduced to one-half when bromate was present in dough, whereas the carbohydrates in fraction V of bromated doughs were 2.3 to 3 times that in fraction V of plain, rested doughs. Treating with bromate increased the carbohydrate components of the fractions that contained higher amounts of proteins (i.e., fractions IV and V).

In general, except for bromated, nonrested dough mixed to peak time (Table II), adding oxidizing agents (bromate and iodate) to dough significantly increased fraction II, a major contributor to the viscosity and gelling properties of water-soluble pentosans (8). Treating overmixed doughs with oxidizing agents (Fig. 1, C and Fig. 2, D) substantially decreased the amount of fraction I, and caused a large increase in fraction IV containing much of proteins from purified pentosans. The opposite effect was observed with plain overmixed doughs (Table II and Fig. 2, C), and doughs treated with NEMI (Fig. 1, B) or cysteine-HCl (Fig. 1, D).

Adding oxidizing agents to dough appears to significantly change proportions of the various DEAE-cellulose fractions. The oxidizing agents might intensify the association between carbohydrates and proteins as indicated by a large increase in proteins and carbohydrates in the fractions (II to V) known to be glycoproteins (4,7). That suggests conformational change and molecular reorganization in water-soluble pentosans treated with oxidizing agents.

#### Gel Filtration of Purified Pentosans from Flour (Scout-69) and Doughs

Figure 3 shows elution patterns of carbohydrate and protein components of purified pentosans. Protein elution was recorded by double scale expansion to increase instrumental sensitivity with relatively low amounts of proteins in 15-mg. samples of pentosans. Treating doughs with oxidizing agents markedly

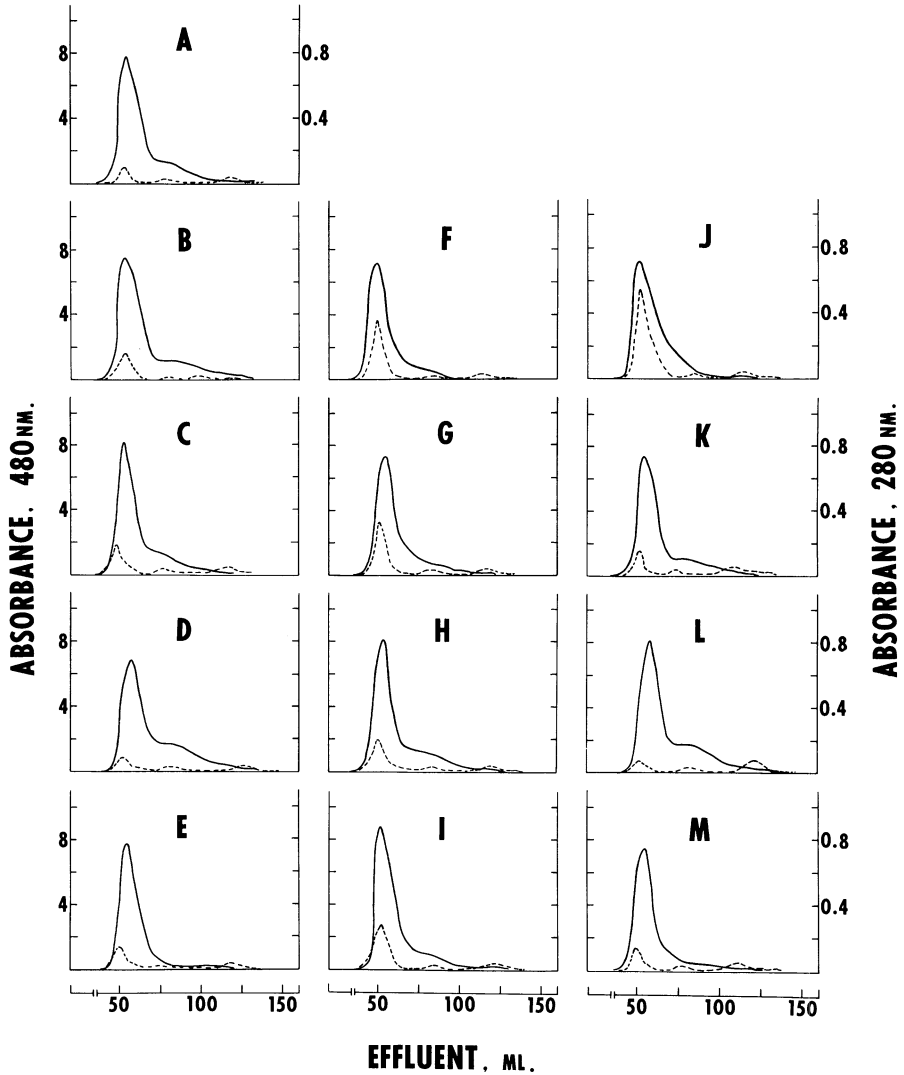


Fig. 3. Gel filtration of  $\alpha$ -amylase-treated pentosans (15 mg.) from flour (Scout-69 wheat) and doughs through a column ( $2.5 \times 40$  cm.) of Bio-Gel P-150 (100 to 200 mesh). Components were eluted with 0.10M NaCl (18 ml./hr). Solid line: carbohydrates (phenol-sulfuric acid procedure, 480 nm., absorbance  $\times 10^{-1}$ ); dashed line: proteins (280 nm., double scale expansion). A: Flour (Scout-69 wheat). B: Dough mixed to peak time. C: Dough mixed to peak time; rested. D: Dough mixed to departure time. E: Dough mixed to departure time. F: Dough bromated ( $\mu\text{eq. oxidant/g. flour}$ ) and mixed to peak time. G: Bromated dough mixed to peak time; rested. H: Bromated dough mixed to departure time. I: Bromated dough mixed to departure time; rested. J: Dough iodated ( $\mu\text{eq. oxidant/g. flour}$ ) and mixed to peak time. K: Iodated dough mixed to departure time. L: Dough ( $1 \mu\text{eq. cysteine-HCl/g. flour}$ ), mixed to peak time. M: Dough ( $1 \mu\text{eq. NEMI/g. flour}$ ).

increased the absorbance of protein components (Fig. 3). Our previous work (8) reported a large increase in the protein content of pentosans obtained from doughs treated with NEMI and cysteine-HCl. Figure 3 (L,M) shows that this increase in total quantity of protein was not accompanied by an increase in UV-absorbance.

Because absorbance of proteins in the 250 to 300 nm. region is due to aromatic amino acids (tryptophan, tyrosine, and phenylalanine), some change involving aromatic amino acids apparently occurs that changes the intensities (absorbance) and elution volume. UV absorption maxima of proteins shift to shorter wavelengths and decrease in intensity when secondary and tertiary structures of the molecules are disrupted (16,17). The increase in absorbance (at 280 nm.) by protein or glycoprotein components of water-soluble pentosans observed on treating with oxidizing agents could result from changes in the chromophores of aromatic amino acids. An increase in absorbance intensity could result from aggregation of structures containing proteins or glycoproteins closely associated with pentosans, i.e., strengthening of secondary and tertiary structures. It also has been suggested (18) that an increase in UV absorbance occurs whenever aggregation of protein molecules increases the amount of light lost by Rayleigh scattering. Pentosans from bromated and iodated doughs mixed to optimum (peak time) had the most pronounced changes in absorbance (Fig. 3) and elution volumes, indicating a requirement for optimum work for maximum association (aggregation) of soluble polysaccharides and proteins aided by oxidizing agents. The results from DEAE-cellulose chromatography and gel filtration support the suggestion of Udy (19) that adding bromate increases interactions between soluble polysaccharides and proteins.

#### **Ultraviolet Spectra of Proteins and Glycoproteins Associated with Purified Water-Soluble Pentosans**

To permit better understanding of the glycoproteins (5,6,7) of water-soluble pentosans and to gain additional support for the results obtained during gel filtration, UV spectra of purified pentosans from flour and doughs were obtained.

Figure 4 also shows that pentosans extracted from plain dough (Fig. 4-2) had higher absorption intensity (at 278 nm.) than flour pentosans. Treating dough with bromate (Fig. 4-3) and iodate (Fig. 4-4) further increased absorption intensity at 278 nm. over that of pentosans extracted from flour (Fig. 4-1) or plain dough. As reported earlier (8), protein content in the pentosan preparations (Fig. 4) from flour, plain and oxidized doughs were 16.4, 15.6, and 22.0%, respectively. This indicates that differences in analytical composition or proportion of components may not account for the differences in UV absorbance. The increased intensity of absorption due to protein upon oxidation likely results from aggregation of pentosan components containing glycoproteins. The UV spectra of purified pentosans (Fig. 4) support earlier results from gel filtration (Fig. 3) in which bromated and iodated dough had increased absorbance at 280 nm. due to protein.

Pentosans from doughs (Fig. 4-2, 4-3, 4-4) also shifted in maximum for protein absorption from 280.5 nm. in flour pentosans to 277.5 nm. in dough pentosans (Fig. 4) perhaps from a change in protein structure caused by transferring the chromophoric groups from a hydrophobic environment in the protein matrix to



an aqueous environment of lower refractive index (20). Hydrogen bonding also causes a bathochromic shift in UV absorbance when the solute is a hydrogen donor (17). Pentosans (soluble and insoluble) are effective hydrogen-bonding agents (1,21). Hydrogen bonding is aided also by tyrosyl hydroxyl groups of protein (16).

Additional absorbance maximum at 320 nm. reported by Kuendig *et al.* (5) and assigned to ferulic acid ester groupings (22) was present in Fig. 4 with slightly lower intensity. Disappearance of the 320 nm. peak during gel formation suggested that ferulic acid ester groups were implicated in the mechanism of oxidative gelation, probably giving rise to intermolecular cross-linking upon mild oxidation (22, 23).

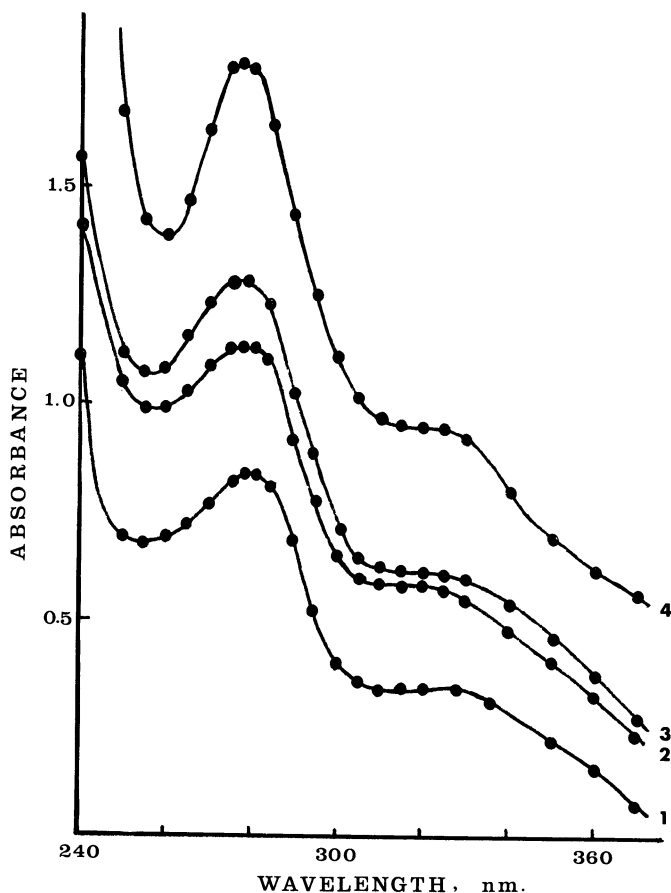


Fig. 4. Ultraviolet spectra (240 to 370 nm.) of  $\alpha$ -amylase-treated pentosans (0.25%, w./v.) from: 1) Flour (Scout-69 wheat); 2) Dough mixed to farinograph peak time; 3) Bromated dough (1  $\mu$ eq.  $\text{KBrO}_3/\text{g}$ . flour) mixed to peak time; and 4) Iodated dough (1  $\mu$ eq.  $\text{KIO}_3/\text{g}$ . flour) mixed to peak time.

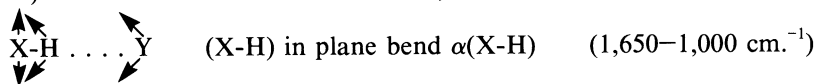
### Infrared Spectra of Purified Water-Soluble Pentosans

To study changes in water-soluble pentosans caused by mixing flour constituents into dough, we determined infrared spectra ( $4,000$  to  $500\text{ cm.}^{-1}$ ,  $2.5$  to  $20\ \mu$ ) of purified pentosans. Infrared spectra of purified pentosans from flour and doughs are shown in Fig. 5.

Several infrared bands were observed, but some were difficult to assign because their large widths caused overlapping and combined several vibrations. Relatively sharp and intense protein absorption bands (24–26) present were assigned as follows: amide I due to  $\text{C=O}$  stretching ( $1,650\text{ cm.}^{-1}$ ); amide II ( $1,538$  to  $1,518\text{ cm.}^{-1}$ ) of N-H deformation and a weak amide III of N-H band ( $1,240\text{ cm.}^{-1}$ ). The amide III band was not so clear in flour pentosans (Fig. 5, A) as in pentosans from bromated and iodated doughs. A sharp band at  $897\text{ cm.}^{-1}$  has been observed in xylans (27), and may be considered characteristic of pentosans (28).

Close examination of the amide II band ( $1,538$  to  $1,518\text{ cm.}^{-1}$ ) of the water-soluble pentosans from flour (Fig. 5, A) and doughs (Fig. 5, B to F) suggests a change in the glycoproteins (4,7) of wheat flour during the dough-making process. The amide II band of flour pentosans (Fig. 5, A) shows maximum absorbance at  $1,518\text{ cm.}^{-1}$ , but after the flour is mixed to dough the band shifts to  $1,538\text{ cm.}^{-1}$  with a marked increase in intensity of absorption, which is much more pronounced in pentosans from bromated (Fig. 5, E) and iodated (Fig. 5, F) doughs than in pentosans from flour-water dough. The infrared amide II band at  $1,520\text{ cm.}^{-1}$  has been reported to result from an extended  $\beta$ -configuration, whereas the  $\alpha$ -configuration of a helical structure absorbs at  $1,540\text{ cm.}^{-1}$  (25,26). The shift shown by amide II absorption from flour ( $1,518\text{ cm.}^{-1}$ ) to dough ( $1,538\text{ cm.}^{-1}$ ) indicates a conformational change from  $\beta$ -configuration to  $\alpha$ -configuration while flour constituents are mixed into dough.

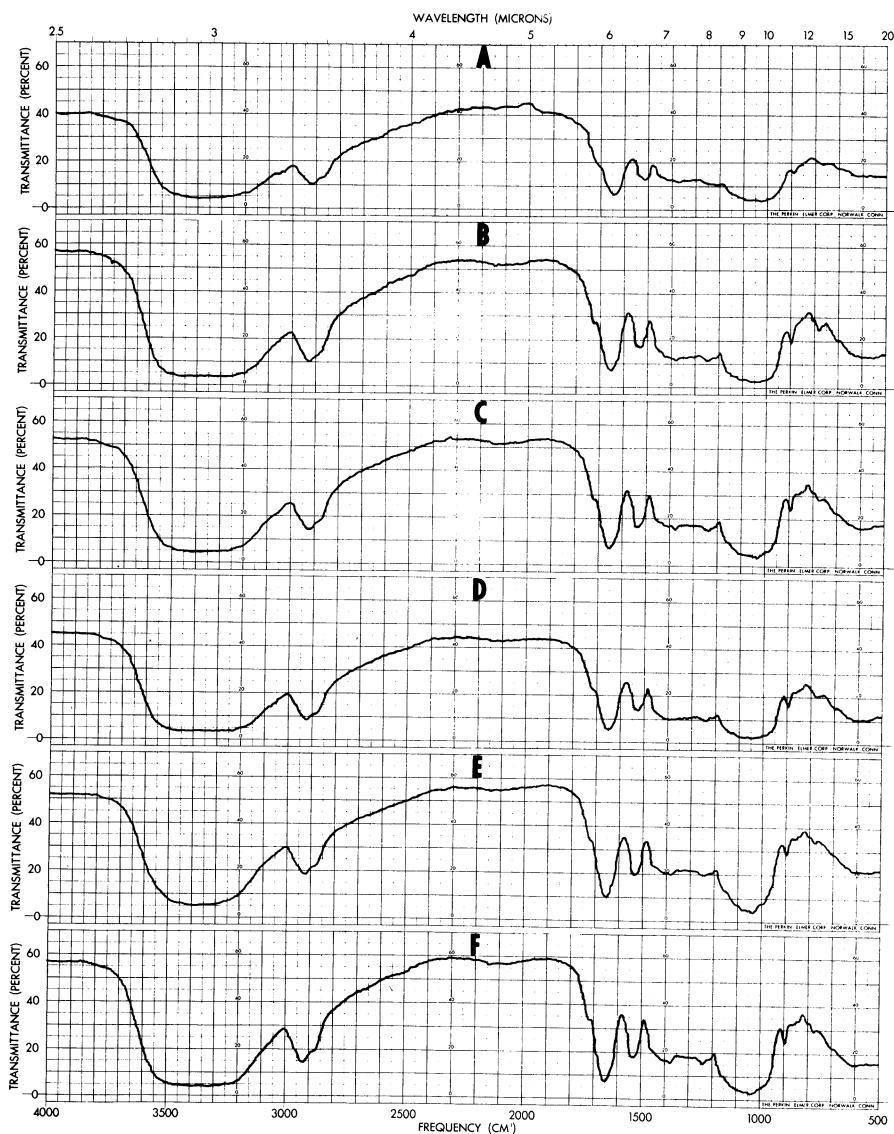
Others have observed that, on hydrogen bond formation, the N-H bending and deformation frequency (amide II, in this case) shows a hypsochromic shift (29,30). The shift is from an increase in the restoring force tending to keep the (X-H) bond directed toward the Y atom, as shown here:



The band at  $897\text{ cm.}^{-1}$ , present in xylans (27), is more pronounced in pentosans from doughs than in pentosans from flour (Fig. 5, A) which confirms the larger amounts of arabinoxylan (fraction I) obtained by chromatography of purified pentosans from doughs on DEAE-cellulose (Table I).

Our earlier studies (8) showed more water-soluble pentosans after dough mixing (about 1%) than from flour (0.65%). This could enhance the hydrogen bonding capacity of pentosans due to their increased amounts apparently present in doughs. Several workers (31–33) have shown that the hydrogen-bonding potential contributed by the amide side chains of gluten is largely responsible for protein cohesiveness. The amide groups are both acceptors and donors of hydrogen bonds, capable of interacting with other amide groups and with groups that are only acceptors or only donors. Water molecules and pentosans (21) may perform an important function by satisfying some of the hydrogen-bonding sites and facilitating interchange of hydrogen bonds among proteins and other molecular species.

The results of DEAE-cellulose chromatography, gel filtration, and UV and IR spectroscopy indicate that mixing flour into dough and treating with oxidizing agents causes a conformational change in pentosan molecules and intensifies the



**Fig. 5.** Infrared spectra of  $\alpha$ -amylase-treated pentosans using a KBr (0.30 g.) pellet (3 mg. sample). Instrument: Perkin-Elmer infrared grating spectrophotometer, Model 421. Pentosans from: A) Flour (Scout-69 wheat); B) Plain dough mixed to farinograph peak time; C) Bromated (1  $\mu$ eq.  $\text{KBrO}_3/\text{g. flour}$ ) dough mixed to peak time; D) Plain dough mixed to peak time and rested at  $30^\circ\text{C.}$ , 95% r.h. for 3 hr.; E) Bromated (1  $\mu$ eq.  $\text{KBrO}_3/\text{g. flour}$ ) dough mixed to peak time and rested at  $30^\circ\text{C.}$ , 95% r.h., for 3 hr.; and F) Iodated (1  $\mu$ eq.  $\text{KIO}_3/\text{g. flour}$ ) dough mixed to peak time.

association between carbohydrate and protein components in water-soluble pentosans. The results of IR spectroscopy also indicate that pentosans play an important role in hydrogen bonding in dough. The above results together suggest that compounds such as pentosans and glycoproteins act as a bridge between major protein and carbohydrate components of dough. In addition, the changes shown by treating doughs with oxidizing agents suggest that oxidizing agents play an important role, with pentosans and glycoproteins, in maintaining the bridge between protein and carbohydrate components during various dough stages.

#### Acknowledgment

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