Comparison of Pentosans Extracted from Conventional and Continuous Bread[1]

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ABSTRACT

Total water-solubles and pentosan preparations were extracted from the crumb and crust of conventional and continuous produced bread at 1, 4, 7, and 9 days from the date of baking. More total water-solubles and crude pentosans were isolated from the continuous baked bread than from the conventional bread. The amount of extractable carbohydrate material in the crude pentosan preparations decreased as the bread aged. Amylase treatment of the crude pentosans resulted in similar recoveries for both bread types at the different days of extraction and was similar to the flour recovery product. DEAE-cellulose column chromatography of the amylase-treated pentosans resulted in five fractions of which fraction 1 was essentially a pure pentosan and was recovered in highest yield. Intrinsic viscosity values obtained from the crumb of the conventional baked bread for fraction 1 at the different days were all similar but slightly lower than values obtained from the crumb of the continuous bread. Fraction 1 of the original flour pentosans had an intrinsic viscosity value that was lower than any of the bread crumb values. The ratio of component sugars, arabinose:xylose for fraction 1, was similar for the crumb and crust of the conventional bread pentosans at the different days of extraction; however, the ratio was slightly lower than for the same fraction of the continuous bread pentosans.

In recent years, the pentosans of wheat flour have been of extreme interest to several different groups of workers. The pentosans which are found in the water-soluble and in the "squeegee" or "sludge" fraction of wheat flour have been examined for their chemical composition, properties, and basic structure (1-5). In addition, the effects of pentosans on dough and bread properties have been undertaken including two recent studies (6,7).

Little information is available in the literature concerning directly the pentosans found in bread. Gilles et al. (8) studied the constitution of water-soluble polysaccharides derived from bread crumb. These workers reported that the pentosan in the water-soluble polysaccharide of bread crumb possesses a highly branched structure that was structurally similar to the pentosan in the original flour.

The present study was undertaken with several purposes in mind. The primary objective was to determine if any differences existed in the pentosans isolated from the crumb and crust of continuous or conventional produced bread when compared to pentosans extracted from the original flour. These two types of bread differ considerably in their method of production. Conventional bread relies on bulk fermentation, whereas continuous produced bread is dependent on a rapid mechanical development of the dough. The action of the enzymes present in yeast on flour constituents during dough fermentation may well result in a marked difference in the pentosan composition and structure extracted from conventional bread as compared to pentosans from bread in which the dough is

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subjected to mechanical development. Another objective was to ascertain whether differences were present between the pentosans isolated from the crumb and crust of the two types of bread. Finally, the effect of aging on bread pentosans was investigated.

MATERIALS AND METHODS

Flour Sample

A composite lot of 1969 Miag milled flour was used for the pentosan isolation and to produce the conventional and continuous bread. Flour analysis on a 14.0% moisture basis was as follows: 14.0% protein, 0.40% ash, 62.2% farinograph absorption, and farinograph peak time of 6.0 min.

Bread Samples

The following formulas and procedures were used to produce conventional and continuous bread:

a) *Conventional Bread.* The formula with percentages of ingredients based on flour weight is shown below:

Flour (14.0% m.b.)	5,000 g.
Sugar	5.0%
Salt	2.0%
Milk	2.0%
Shortening	3.0%
Yeast food	0.5%
Yeast	3.0%
Water	62.0%

A 20-qt. Hobart mixer (Hobart Mfg. Co., Troy, Ohio) was used to mix the dough. Mixing time was determined by the appearance and feel of the dough, with an indication of the mixing time also obtained from the farinogram.

After mixing the dough was fermented for 3 hr. under controlled temperature (30°C.) and relative humidity (80%). The dough was divided into 1-lb. loaves, rounded into balls, and allowed to rest for 15 min. The bread was moulded, placed in bread pans, and proofed for 55 min. at 30°C. and 85% relative humidity, and then baked for 25 min. at 230°C.

b) *Continuous Bread.* A Wallace & Tiernan Laboratory model continuous baking unit (Baker Process Co., Belleville, N.J.) was used to make the continuous bread (9). The baking formula, with percentage of ingredients based on flour weight, is given below:

Flour	5,000 g.
Sugar	8.0%
Salt	2.25%
Milk	2.0%
Shortening	3.25%
Yeast food	0.5%
Yeast	2.75%
Water	66.0%
Potassium bromate	60 p.p.m.
Potassium iodate	12 p.p.m.

The broth, which contained 60.0% of water based on the flour weight plus the sugar, salt, milk, yeast food, and yeast, was allowed to ferment for 2.5 hr. This material was then transferred to the mixing bowl of a Hobart mixer and the remaining water added, plus the flour, liquid shortening, and oxidation solution. Only sufficient mixing to incorporate the ingredients thoroughly was allowed at this stage. The premixed dough was transferred to the continuous unit where it was subjected to mechanical development. The mixing speed in the developer for the particular flour used was 160 r.p.m. The extruded doughs in the pans were proofed under controlled temperature and relative humidity conditions for 55 min., then baked for 20 min. at 230°C.

Differences in formulation between the two types of bread were essential because of the differences in processing.

Moisture Determination

Moisture was determined by the AACC procedure (10) on the crumb and crust of the two types of bread the same day of baking and again after 4, 7, and 9 days of baking.

Isolation of Total Water-Solubles

The total water-solubles were isolated from the crumb or crust of the bread or the original flour by mixing 300 g. of the material with 1,200 ml. of distilled water in a Waring Blendor for 3 min. at low speed. The slurry was centrifuged and the supernatant shell-frozen and freeze-dried.

Isolation of Crude Pentosans

Crude pentosans were isolated from the crumb, crust, or flour by the method employed for the isolation of total water-solubles with the addition of the following steps. The supernatant was heated to 92°C. for 4 min. followed by centrifugation. It was then filtered through filter paper and dialyzed for 3 days against distilled water.

Amylase Treatment of Crude Pentosans

The crude pentosans isolated from the crumb and crust of the bread which had been stored for different days as well as the flour pentosans were treated with α-amylase 2x crystallized (Nutritional Biochemical Corp., Cleveland, Ohio) to remove soluble starch. The procedure used has been described previously (5).

DEAE-Cellulose Column Fractionation

The amylase-treated pentosans from the crumb, crust, and flour were subjected to DEAE-cellulose column chromatography, with five fractions obtained as described previously (5).

Sugar Analysis in DEAE-Cellulose Fractions

The sugars present in the fractions obtained from the different pentosan samples were detected by paper and gas-liquid chromatography. A portion of each fraction was hydrolyzed with 1N H_2SO_4 for 4 hr. in a boiling-water bath. The hydrolyzed material was neutralized with barium carbonate and spotted on a paper chromatogram (5). The same sample was then evaporated to dryness,

reduced with sodium borohydride, and acetylated with acetic anhydride and pyridine.

Boric acid resulting from the reduction was removed by passing the reduction product through a column containing Dowex 50 X 12 H+ to remove the sodium ions. The effluent and washings were concentrated, methanol added, and the solution heated to 50°C. for 2 min. and again concentrated. The methanol treatment which removed the boric acid was repeated twice more.

Acetylation was done in sealed acylation tubes. The time required for acetylation also was investigated. A study was made in which a sample of hydrolyzed pentosans which had been reduced and passed through a Dowex 50 X 12 H+ column and then treated with methanol was acetylated for 1, 2, 3, 4, and 5 hr. The acetylated material was then analyzed for component sugars on the gas chromatograph. The data obtained indicated that the results for the 1-hr. acetylation were the same as those at the other periods. Consequently, a 1-hr. acetylation period was selected for analysis of the different samples.

Intrinsic Viscosity and Optical Rotation

Intrinsic viscosity and specific optical rotation of the different fractions were measured by dissolving a portion of each in 0.5N NaOH solution. An Ubbelohde viscometer was used for the viscosity measurements and a Galileo polarimeter for the optical rotation determinations.

Molecular Weight

Molecular-weight measurements on fraction 1 of the fractionated pentosans were determined with a Melabs Recording Osmometer (model CSM-2, Melabs, Inc., Palo Alto, Calif.).

RESULTS AND DISCUSSION

Bread Samples

The crumb and crust of the continuous produced bread contained 14.6% protein (N X 5.7) on a dry basis; the conventional crumb and crust contained 15.5% protein (N X 5.7) on a dry basis.

The moisture content of the crumb from both bread types decreased as the bread aged, while moisture in the crust increased.

Total Water-Solubles Recovered

Table I shows data for the total water-solubles isolated from the crumb and crust of the two types of bread. More total water-solubles were isolated from the continuous crumb and crust than from the conventional bread. This result would be due undoubtedly to the different process involved in continuous baking and the lack of bulk fermentation. The different formulas used in the two types of baking also would contribute to such results. The amount of water-solubles extracted from the continuous crumb increased during aging; however, the protein content of the water-solubles remained constant. This would indicate that more protein material was extracted from the crumb as the bread aged.

Crude and Amylase-Treated Pentosans

Data for the amount of crude and amylase-treated pentosans extracted from the crumb and crust of the conventional and continuous bread crumb are given in Table II.

TABLE I. DATA FOR WATER-SOLUBLES EXTRACTED FROM CONVENTIONAL AND CONTINUOUS PRODUCED BREAD

Day	Crumb		Crust	
	Solubles %	Protein[a] %	Solubles %	Protein[a] %
	Conventional Bread			
1	7.9	8.5	6.5	7.4
4	6.4	9.2	6.5	7.8
7	8.0	9.0	6.7	8.6
9	8.5	8.8	...	...
	Continuous Bread			
1	11.2	7.3	10.4	6.2
4	11.5	7.3	8.6	5.9
7	12.1	7.2	10.4	6.1
9	12.4	7.3	12.3	6.1

[a]Expressed on a dry basis.

The amount of crude pentosans isolated from the continuous bread crumb was greater than that extracted from the conventional bread crumb. The total amount of protein material in the crude pentosans increased from day 1 to day 9. The amount of crude pentosans extracted from the bread crumb decreased as the bread aged. Subtracting the protein material from the percent recovery resulted in a decreased yield of essentially carbohydrate material as the bread aged. This decrease in extracted carbohydrate material may, in part, be due to a retrogradation of the amylopectin which would result in a lower amount of extractable carbohydrate material.

TABLE II. DATA FOR CRUDE AND AMYLASE-TREATED PENTOSANS FROM CONVENTIONAL AND CONTINUOUS BREAD

Day	Crude Pentosans				Amylase-Treated Pentosans			
	Crumb		Crust		Crumb		Crust	
	Recovery %	Protein[a] %	Recovery %	Protein[a] %	Recovery %	Protein[a] %	Recovery %	Protein[a] %
	Conventional Bread							
1	1.6	12.9	2.2	9.1	0.68	9.8	0.72	10.2
4	1.9	11.9	2.5	9.6	0.71	4.8	0.77	5.2
7	1.1	25.0	2.1	13.5	0.70	6.5	0.84	5.1
9	1.1	25.4	...	...	0.69	5.0	...	...
	Continuous Bread							
1	2.7	11.3	2.4	8.7	0.76	17.5	0.65	15.3
4	2.5	11.9	2.6	11.5	0.89	21.3	0.80	14.5
7	2.1	16.6	2.1	11.3	0.76	22.2	0.73	19.9
9	2.4	17.5	2.5	10.3	0.88	21.4	0.76	19.0

[a]Expressed on a dry basis.

The protein content of the amylase-treated pentosans from the continuous crumb or crust was in all cases higher than from the conventional crumb or crust. For the amylase-treated pentosans, if the protein material extracted is subtracted from the yield, the recovery of remaining product which would be predominantly pentosan material was similar in all cases. Isolation of crude pentosans from the original flour followed by α-amylase treatment produced a recovery value similar to those shown in Table II.

The component sugars present in the amylase-treated pentosans after hydrolysis were arabinose, xylose, and galactose.

DEAE-Cellulose Fractionation

Table III shows the recovery of the amylase-treated pentosans for the five DEAE-cellulose fractions for days 1 and 7. Analyses for days 4 and 9 also were conducted with similar data obtained. Fraction 1, which was essentially the pure pentosan fraction, was recovered in highest yield in all cases. It was not possible to show any progressive differences in the recovery of the five fractions for the different days of extraction. The total percent recovery for the conventional crumb pentosan fractionations was higher than those obtained for the continuous crumb pentosans.

Table IV gives the protein content of the five DEAE-cellulose fractions obtained for the pentosans extracted and fractionated at the different days. Fraction 1 contained the lowest amount of protein, with similar values obtained for the crumb and crust of the two types of bread. Fraction 2 showed slightly higher protein contents in the conventional fractionated pentosans and higher values in the same fraction of the continuous bread pentosans from day 4 on. The protein contents for fraction 3 were all considerably higher in the crumb and crust of the continuous fractionated pentosans than in the same fraction for the conventional bread pentosans. This same observation was noted with fractions 4 and 5. The reason for this was that the protein contents of the amylase-treated unfractionated pentosans extracted from the continuous bread were considerably higher than those from the conventional bread.

TABLE III. DEAE-CELLULOSE FRACTIONATION OF AMYLASE-TREATED PENTOSANS

Fraction	Conventional		Continuous	
	Crumb %	Crust %	Crumb %	Crust %
	Day 1			
F_1	39.7	38.8	42.1	38.7
F_2	18.3	19.9	9.1	18.5
F_3	16.7	11.1	18.2	9.5
F_4	15.9	16.9	19.2	20.7
F_5	9.5	13.3	11.4	12.6
	Day 7			
F_1	41.1	48.1	39.2	37.0
F_2	17.1	15.0	14.7	16.7
F_3	11.1	8.4	19.4	16.7
F_4	17.9	20.4	19.4	18.5
F_5	12.8	8.0	7.4	11.1

TABLE IV. PROTEIN CONTENTS[a] OF DEAE-CELLULOSE PENTOSAN FRACTIONS

Day	Fraction	Conventional		Continuous	
		Crumb %	Crust %	Crumb %	Crust %
1	1	2.5	1.2	1.1	0.8
	2	5.3	4.3	3.7	2.9
	3	15.5	9.7	24.0	11.6
	4	13.6	19.1	30.8	21.8
	5	21.3	14.3	24.2	22.8
4	1	1.7	1.8	1.7	2.1
	2	3.0	3.1	8.7	6.5
	3	8.3	6.1	18.3	18.0
	4	9.3	14.2	25.1	24.2
	5	7.3	7.7	31.6	27.2
7	1	0.9	1.6	1.5	1.3
	2	2.7	2.1	12.2	6.4
	3	8.9	4.3	23.0	19.9
	4	15.3	16.2	29.0	24.9
	5	14.4	16.0	30.6	28.5
9	1	1.1	...	2.5	1.0
	2	2.9	...	7.9	5.9
	3	13.3	...	19.9	15.7
	4	13.2	...	23.2	25.2
	5	9.8	...	22.9	27.9

[a]Expressed on a dry basis.

The intrinsic viscosity values for DEAE-cellulose pentosan fraction 1 at the different days of extraction are shown in Table V. The values for the conventional crumb at the different days were all similar and were slightly lower than the values obtained for the continuous crumb. With the exception of day 1, the intrinsic viscosities for the crust of the conventional and continuous bread were lower than the corresponding crumb. The intrinsic viscosity value for fraction 1 of the original flour pentosans was 3.0, which was lower than the values obtained for fraction 1 of the crumb pentosans.

A decrease in intrinsic viscosity was noted from fraction 1 to fraction 4 for all DEAE-cellulose pentosan fractions studied. Fraction 4, in all cases, gave the lowest intrinsic viscosity value.

TABLE V. INTRINSIC VISCOSITY VALUES FOR DEAE-CELLULOSE PENTOSAN FRACTION 1

Day	Conventional		Continuous	
	Crumb η	Crust η	Crumb η	Crust η
1	3.55	3.20	3.80	3.85
4	3.35	2.60	3.85	2.50
7	3.50	2.90	3.75	2.80
9	3.50	...	3.80	2.80

The highest negative optical rotation for the different fractions from the different pentosan samples was obtained with fraction 1. The remaining fractions had lower negative optical rotations with a progressive decrease occurring from fraction 1 to fraction 4.

Table VI gives the number average molecular weights obtained for DEAE-cellulose fraction 1 of the different pentosan samples. It was difficult to draw any direct conclusions from the molecular-weight results. The number average molecular weight for fraction 1 of the original flour pentosans was 70,000.

The ratio of component sugars, arabinose:xylose in fraction 1, of the different pentosan samples fractionated is presented in Table VII. As already mentioned, fraction 1 was essentially a pure pentosan fraction containing only small amounts of protein and only arabinose and xylose as component sugars after acid hydrolysis. In general, the ratio of component sugars for this fraction was similar for the conventional crumb and crust at the different days of extraction. Similar values, likewise, were obtained among the crumb and crust of the continuous bread pentosans for fraction 1. The ratio of component sugars, however, for the continuous bread pentosans for this fraction did appear to be slightly higher than the values for the conventional bread pentosans. A lower ratio of arabinose:xylose would mean that there were fewer xylose sugar units per arabinose unit than would be the case with a higher ratio. A similar ratio of arabinose:xylose to that obtained in fraction 1 of the continuous bread pentosans was found in fraction 1 of the flour pentosans.

TABLE VI. MOLECULAR-WEIGHT VALUES FOR DEAE-CELLULOSE PENTOSAN FRACTION 1

Day	Conventional		Continuous	
	Crumb	Crust	Crumb	Crust
1	108,000	...	70,000	93,000
4	98,000	100,000	98,000	62,000
7	83,000	91,000	85,000	69,000
9	90,000	...	87,000	100,000

TABLE VII. RATIO OF COMPONENT SUGARS (ARABINOSE:XYLOSE) IN DEAE-CELLULOSE PENTOSAN FRACTION 1

Day	Conventional		Continuous	
	Crumb	Crust	Crumb	Crust
1	1:1.70	1:1.73	1:1.74	1:1.78
4	1:1.59	1:1.75	1:1.79	1:1.85
7	1:1.68	1:1.61	1:1.76	1:1.82
9	1:1.64	...	1:1.77	1:1.75

TABLE VIII. RATIO OF COMPONENT SUGARS IN DAY 4 PENTOSAN FRACTIONS

Fraction	Crumb Arab:Xyl:Gal	Crust Arab:Xyl:Gal
	Conventional	
1	1:1.59:0	1:1.73:0
2	1:1.31:0	1:1.56:0
3	1:0.46:0.70	1:0.72:0.63
4	1:0.22:1.14	1:0.23:1.13
5	1:1.35:0.24	1:1.52:0.12
	Continuous	
1	1:1.79:0	1:1.85:0
2	1:1.44:0	1:1.55:0
3	1:0.40:1.03	1:0.54:0.49
4	1:0.42:1.10	1:0.24:0.95
5	1:0.86:0	1:1.64:0

Table VIII shows representative data for the component sugars found in the five DEAE-cellulose pentosan fractions for one particular fractionation in this case, day 4. Fractions 1 and 2 in all cases contained only arabinose and xylose as component sugars. Fractions 3 and 4, in addition to containing arabinose and xylose, contained also galactose, with fraction 4 containing only small amounts of xylose. Fraction 5, in some cases, contained small amounts of galactose in addition to arabinose and xylose but also contained glucose. No value is shown for glucose, however, as it was believed that this glucose was derived from the DEAE-cellulose after elution with the 0.4N NaOH.

In summary, the results of this study have indicated that there does not appear to be any major change in the pentosans of wheat flour when processed into either conventional or continuous bread. The extraction of water-soluble material from conventional and continuous bread did show that there is a difference in the amount and kind of extractable material. After α-amylase treatment, however, the recovery of carbohydrate material was similar to that recovered from the original flour. This would indicate that the differences noted in water-soluble extractable material between the continuous and conventional bread and at the different days of extraction were not due to pentosan material.

No noticeable differences were obtained in the fractionation pattern of the different pentosan samples.

Small differences were observed between the intrinsic viscosity values of fraction 1 of the conventional and continuous crumb pentosans. Also, differences in intrinsic viscosity between the crumb and crust were noted. Only very small changes resulted in the ratio of component sugars, arabinose:xylose of fraction 1, of the continuous and conventional crumb pentosans.

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