

STUDIES ON CHEMICAL MECHANISM OF RHEOLOGICAL CHANGES DURING DOUGH FERMENTATION

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CHAPTER III

EFFECTS OF PURIFIED LIPOXYGENASE ISOZYMES ON FLOUR DOUGH 2)5)

1. Introduction

The effects of lipoxygenase on the rheological properties of wheat flour dough have been examined. Daniels et al. (1970) proposed that soy lipoxygenase mediated the oxidation of the sulfhydryl group (SH) and consequent structural changes in the dough protein. Also, Frazier et al. (1977) investigated the effects of the soy lipoxygenase enzyme on the mechanical development of wheat flour dough. They suggested that the added soy lipoxygenase reacts with the lipids of dough to increase mixing tolerance. Using a mixograph, Hosney et al. (1980) investigated the mechanism which lipoxygenase increases mixing tolerance. To show the role of lipoxygenase during breadmaking, however, these investigators used soy flour or partially purified soy lipoxygenase. No one studied effect of endogenous wheat lipoxygenase on glutenin and dough properties.

In a previous CHAPTER II, it showed purification and enzymatic characterization of wheat lipoxygenase. The objective of this present chapter was to determine the role of wheat lipoxygenase on the dough during fermentation, using the purified lipoxygenase.

2. Materials and Methods

A. Wheat Flour

The flour used was an unbleached commercial blend as described in "Materials and Methods" of CHAPTER I .

B. Preparation of the Flour Treated with Purified Lipxygenase Isozymes

Each purified lipxygenase isozyme (5,000 units) in 400 ml of 50 mM acetate buffer (pH 5.5) with several drops of toluene was incubated and shaken with the wheat bread flour (500 g) at 40 °C for 4 hr. After incubation, the slurry was frozen immediately, lyophilized, and ground by impact mill (Retsch Ultra Centrifugal Mill with the 0.5-mm filter). The control flour was treated by the same method but without enzyme. These flours were used for the mixograph studies, ethanol fractionation of their glutenin subunits, and determining SH groups, S-S bonds, and foaming activity.

C. Operation of Mixograph

Mixograph studies were carried out by the same methods as described in "Materials and Methods" of CHAPTER I .

D. Determination of Foaming Activity

The foaming activity for the flours treated with or without lipxygenase was measured by the same method as described in

"Materials and Methods" of CHAPTER I .

E. Bread-Making Test

Using 2,000 g of bread flour and adding 36,000 units of purified lipoxygenase isozymes, breads were baked by the method of Nagao et al. (1981).

F. Determination of Sulfhydryl (SH) group and disulfide (S-S) bond contents

Sulfhydryl (SH) and disulfide (S-S) contents of the flour were determined by amperometric titration using silver nitrate by the method developed by Sokol et al. (1959) and modified by Tsen and Anderson (1963). The result is expressed as micromole per gram of flour protein.

G. Fractionation of Constituent Subunits from Glutenin

The constituent subunits of glutenin from the treated flours were fractionated by the same methods as described in the "Materials and Methods" of CHAPTER I .

3. Results and Discussion

A. Mixograms of Flours Treated with Purified Lipxygenase Isozymes

As shown in Fig. 20, mixograms of the flours treated with a lipxygenase isozyme (particularly L-3), peak intensity exhibited less resistance and shorter dough development time (peak time) than the control flour; showing maximum intensity of 3.0 (L-1), 3.2 (L-2), and 2.8 (L-3) to 3.6 (control), and also peak time 2.8 min, 3.6 min, and 3.4 min to 5.6 min, respectively. In the CHAPTER I, it demonstrated that as fermentation time was increased, the fermented dough showed less resistance to extension and a shorter peak time which was concomitant with the increase in lipxygenase activity during fermentation. The purified lipxygenase caused a similar mixogram performance on the dough. Particularly, adding the L-3 isozyme to the flour affected the dough to change rheological properties. The performance is similar to that of fast-acting oxidants such as potassium iodate. It suggested that the L-3 isozyme plays significant role to change dough rheology during fermentation.

Comparing the soybean lipxygenase (Hoseney et al. 1980) which allowed to increase dough mixing tolerance, wheat lipxygenase caused differential physical properties on the dough. Veldink et al. (1977) demonstrated that soybean lipxygenase attacks unsaturated triglycerides, whereas wheat lipxygenase mainly reacts with free fatty acid and

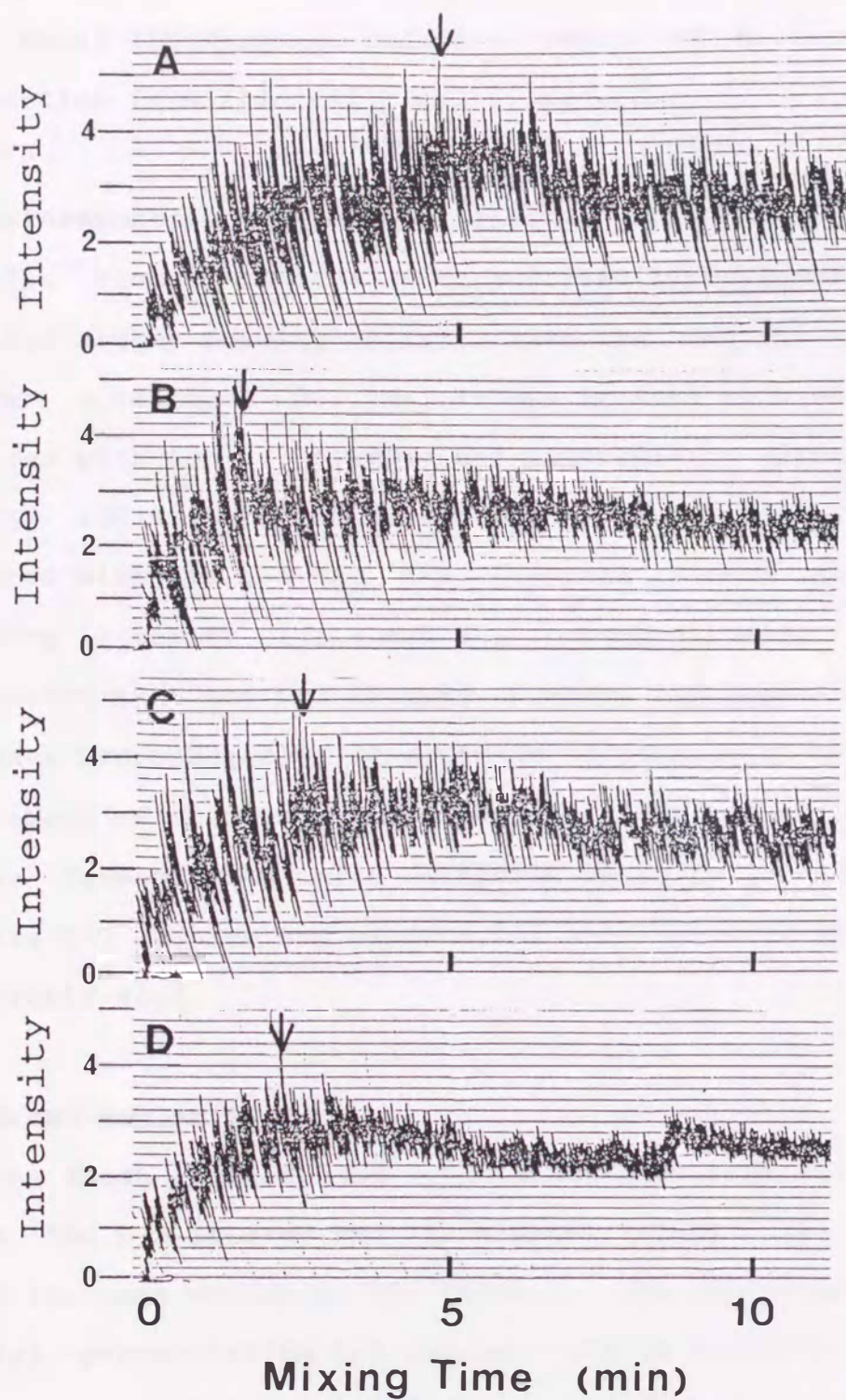


Fig. 20. Mixograms showing effects of purified wheat germ lipoxxygenase isozymes on supplemented flour.

A: control flour without enzyme supplement.
 B, C, and D: flours supplemented with isozymes L-1,
 L-2, and L-3, respectively.
 Arrows indicate peak of mixing development.

monoglycerides. That is possible reason why the flour treated with wheat lipoxygenase isozymes exhibited different mixograph properties from flour with soy lipoxygenase.

B. Determination of Foaming Activity

The flours treated with purified lipoxygenase isozymes exhibited higher foaming activity than the control flour without enzyme treatment. Of the flours treated with enzyme, the one treated with the L-3 isozyme had particularly higher foaming activity (53.4) than that of the control flour (42.9). The flours treated with the L-1 and L-2 isozymes showed slightly higher foaming activity (44.4 and 46.7, respectively). These results suggested that the L-3 isozyme of wheat lipoxygenase affects the surface hydrophobicity of glutenin to decrease, by which the enhancement of foaming activity occurred. It was very important because fermentation also affected dough to increase foaming activity L-3 isozyme can oxidize not only linoleic acid, but also linolenic acid.

C. Bread-making Test

As shown in Table X II, the bread made from the flour treated with the L-3 isozyme had the highest volume. The others had almost the same volume as the control. The improvement of bread-making properties by L-3 isozyme related positively with the enhancement of foaming activity of the flours treated with the lipoxygenase L-3 isozymes. Payne et al. (1981) also suggested the

positive relationship between foaming activity and bread-making quality. These results indicate that lipoxygenase isozyme plays a significant role in bread-making and its intensity depends on each isozyme's activity against linolenic acid. Consequently, the L-3 isozyme especially influences bread-making quality.

Table X II . Effect of Purified Wheat Germ Lipoxygenase Isozymes on Baking Performance

Flour Dough	Loaf Volume (ml)	Relative Loaf Volume
Control	1,800	100
Treated with Isozyme		
L-1	1,840	102
L-2	1,860	103
L-3	1,980*	110

Values are the average of two replications.

* Highly significant difference ($P < 0.001$) between the marked value and others.

D. Effect of Lipoxygenase on SH Group and S-S Bond Content in the Doughs

The flours supplemented with each purified lipoxygenase isozyme exhibited lower content of SH groups and higher content of S-S bonds than the control flour (Table X III). An increase in S-S content of the treated flour apparently is caused by lipoxygenase-mediated oxidation of SH groups in flour protein, though the SH content did not differ among the flours treated with the three main lipoxygenase isozymes. Some investigators (Graveland et al. 1977, Nicolas and Drapron 1983, Galliard 1987)

have suggested same manner which SH groups of protein are oxidized by adding soy bean lipoxygenase. During fermentation wheat lipoxygenase actually mediates to oxidate SH group of protein (Nagao et al. 1981).

Table X III . Effect of Wheat Germ lipoxygenase Isozymes on Sulfhydryl (SH) and Disulfide (S-S) Contents in Flours Treated with or without Lipoxygenase Isozymes

Flour treated with LPG Isozyme	SH (μ eq/g of protein)	S-S (μ eq/g of protein)	SH/S-S (%)
L-1	15.5	106.8	14.5
L-2	15.6	110.1	14.2
L-3	15.4	106.6	14.5
Without isozyme	16.5*	101.9*	16.1*

Values are the average of two replications.

* Significant difference ($P < 0.01$) between the marked value and others.

E. Fractionation of Glutenin Subunits from the Flours Treated with Lipoxygenase Isozymes

The total protein of soluble-glutenin from flours (100 g) treated with lipoxygenase isozymes (L-1, L-2, and L-3) and without enzyme (control) was 680.7, 691.2, 531.1, and 1745.3 mg, respectively. Comparing the control flour, the flour treated with lipoxygenase isozymes apparently contained a little amounts of soluble-glutenin. This is the same manner as fermentation caused decrease in glutenin fraction (Table I). Among these flours treated with lipoxygenase isozymes, the flour treated with L-3 lipoxygenase isozyme showed lower amount of soluble-glutenin than other flours. The constituent subunits of soluble-glutenin from

the flours treated with three purified lipoxygenase isozymes and without enzyme were fractionated into three fractions (AF-1, AF-2, and AF-3 as shown in Fig. 1) by the method of Danno et al. (1978) (Table X IV). The total recovery of proteins in the fractions were 96.1, 94.3, 97.8, and 92.9% on L-1, L-2, and L-3 isozymes and control, respectively. These results showed almost the same recovery of extraction by this procedure. Comparing the control flour, all flours treated with lipoxygenase isozymes showed significant decreases in the amount of the AF-1 fraction (agregative subunit) and the AF-3 fraction (low molecular weight subunit), but only a slight decrease in the amount of the AF-2 fraction (high molecular weight subunit). These results suggested that lipoxygenase caused the decomposition of aggregative subunit by same manner as fermentation did, as shown in the previous CHAPTER III . Furthermore, according to these decomposition of aggregative subunit in glutenin, pentosans bound with aggregative subunit are released and affect on the rheological properties of dough.

Table X IV . Effects of Lipoxygenase Isozymes on Proportion of Ethanol Fractionated Polypeptides

Lipoxygenase Isozyme	Fractionated Protein			Total Protein (mg)
	AF-1 (mg)	AF-2 (mg)	AF-3 (mg)	
Control	752.3 (46.4)*	246.5 (15.2)	622.6 (38.4)	1621.4 (100)
L-1	232.0 (35.5)	189.2 (28.9)	233.0 (35.6)	654.2 (100)
L-2	215.8 (33.1)	174.2 (26.7)	271.8 (41.7)	651.8 (100)
L-3	174.0 (33.5)	154.4 (29.7)	191.0 (36.8)	519.4 (100)

Values are the average of two replications.

* () ; Percent of total protein

4. Summary

All flours treated with lipoxygenase isozymes showed shorter dough development time (peak time) and less resistance after peak time on mixogram, and lower SH contents of protein than the control flour treated without lipoxygenase. Comparing flours supplemented with or without the purified lipoxygenase isozymes, the flour treated with the L-3 isozyme especially exhibited the highest foaming activity and greatest influence on bread-making quality. These results suggested that the L-3 isozyme induced chemical changes during dough fermentation.

Futhermore, from those flours the constituent subunits of their glutenin were fractionated. Comparing the control flour treated without lipoxygenase, the flour treated with lipoxygenase exhibited a lower ratio of aggregative subunit and low molecular weight subunit in soluble-glutenin. These results showed that lipoxygenase, especially L-3 isozyme, caused similar decomposition of aggregative subunit as fermentation did.

CHAPTER IV

EFFECT OF PURIFIED PENTOSANS ON DOUGH RHEOLOGY 3)4)6)

1. Introduction

In the CHAPTER I , it suggested that aggregative subunit of glutenin contained pentosans as a major carbohydrates components and these pentosans were released during fermentation to induce rheological changes of fermented dough.

Wheat pentosan has a structure chracterized by a β -(1,4)-xylan backbone with branching α -L-arabinofuranosyl groups (Brillouet et al. 1982, Brillouet and Joseleau 1987). However, it was difficult to separate them into pentosans having different properties and different structure.

A number of investigators separated wheat bran into classes of various polysaccharides according to their different solubilities in some solvents (Schweitzer and Wursch 1979, Anderson and Clydesdale 1980, Ring and Selvendran 1980, Brillouet et al. 1982). However, these methods were not enough to purify the pentosans because of their aggregative characteristics.

In this study, to determine the properties of pentosans constituting aggregative subunit of glutenin and the effect of these pentosans on the fermentation, pentosans were purified from water soluble hemicellulose (WSH) of wheat bran and then characterized. Finally, effect of these purified pentosans on the dough properties is investigated.

2. Materials and Methods

A. Wheat Bran

Wheat bran from hard spring wheat was provided by Nisshin Flour Milling Co., Ltd.. Protein content was 14.5%, moisture 14.8%, and ash 4.35% which determined by AACC approved methods (1983).

B. Wheat Flour

The flour used was unbleached commercial blend as described in "Materials and Methods" of CHAPTER I .

C. Reagents

Cellulase Onozuka RS was purchased from Yakult Co., Ltd. (Tokyo). This enzyme is a macerating enzyme derived by Trichoderma viride and contains at least 16,000 units per gram of filter paper decomposing activity (Tomiya et al. 1968). The enzyme also contained β -1,4-xylanase activity (4,300 units per gram of crude enzyme). Other reagents used were analytical grade.

D. Preparation of Water-soluble Hemicellulose (WSH) from Wheat Bran

Soluble hemicellulose was prepared by the method in Figure 21. This preparation method was developed by Shiiba et al. (1992). Details of the individual steps are as follows.

Step 1. To remove soluble protein and starch, wheat bran (2

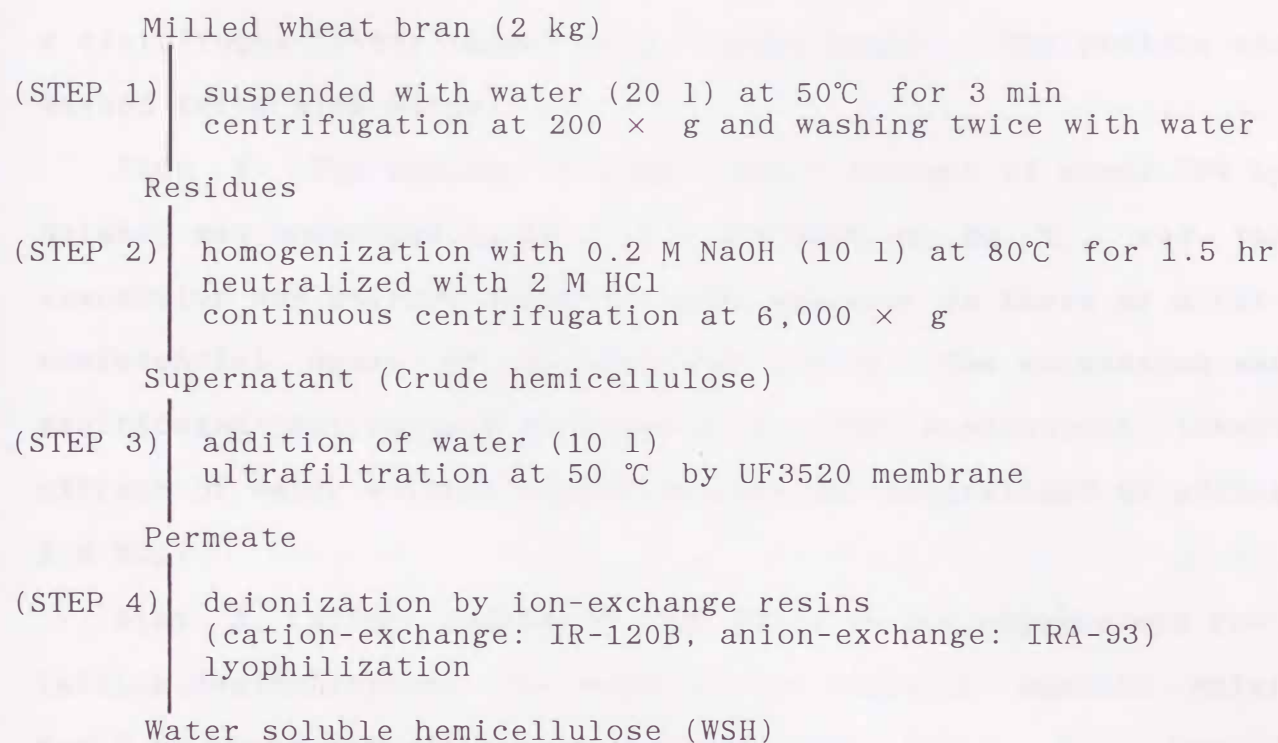


Fig. 21. The scheme for isolation and purification of water soluble hemicellulose (WSH) from wheat bran.

kg) was suspended with 20 l of water at 50 °C and the suspension was vigorously stirred by an agitator (model Super F, Nisshin Engineering) at circumferential speed of 25 m/sec for 3 min. After agitation, solid matter was separated from the solution by a centrifugal filter (model O-20, Tanabe Tekko). The residue was washed twice with water.

Step 2. The residue obtained (water content of about 50% by weight) was suspended in 10 l of 0.2 M NaOH at 80 °C, and the suspension was stirred using the same agitator as above at a circumferential speed of 20 m/sec for 1.5 hr. The suspension was centrifuged continuously at $6,000 \times g$. The supernatant (crude extract of water soluble hemicellulose) was neutralized by adding 2 M HCl.

Step 3. After adding 10 l of water to the supernatant containing hemicellulose, the solution was dialyzed against water for 3 hr by an ultrafiltration system (model RUW-2, Nitto Denko) equipped with a polysulfonic membrane UF-3520 (model P-18, total membrane area of 0.76 m^2 , inner diameter of 11.5 mm, Nitto Denko) having a molecular weight cutoff of 20 kDa. The flow rate of solution and pressure against membrane were 13 l/min and 8 kg/cm^2 , respectively. During dialysis, the flux was maintained at $20 \text{ l/hr} \cdot \text{m}^2$ and the temperature of the solution was maintained at 50 °C by electric heater controlled with a thermostat. After dialysis, the solution was concentrated by almost 5 l by the same system.

Step 4. The solution concentrated was deionized by ion-

exchange system with each 500 ml of cation and anion exchange resins (model IR-120B and IRA-93, respectively, Organo, Tokyo). The flow rate of the solution was 125 ml/min. The retentate obtained was immediately lyophilized, ground and sieved by impact mill (ultracentrifugal mill with the 0.5 mm filter, Retsch) to obtain carbohydrate isolates, WSH.

E. Hydrolysis of the Fractionated Hemicellulose

To carbohydrate of fractionated sample (10 mg), 5 ml of 2 M trifluoroacetic acid (TFA) was added. After a steady stream of nitrogen was bubbled into the sample for 60 sec, the samples were immediately sealed and placed in an oven at 105 °C for 2 hr. The hydrolyzed solution was cooled and centrifuged at $3,000 \times g$ for 10 min. Two ml of supernatant was evaporated at 50 °C to remove TFA on a rotary vacuum evaporator. The dried matter was dissolved with 0.8 ml of distilled water.

F. Analysis of Sugar and Protein Composition in Pentosan Fractions

A portion of the hydrolyzed carbohydrate was filtered through 0.45 μm of pore size membrane and the filtrates (20 μl) were directly analyzed by HPLC at 80 °C (Hitachi model 655A-12) equipped with solvent-delivery system controlled by a model L-5000LC controller, a model 655A-40 automatic sample injector, and refractometer (Shodex RI SE-61, Showa Denko). The column packed with 10 μm particle size of silica (Shodex KS-801P 200 mm $\times$ 3

mm i.d., Showa Denko) was used. Data were recorded and processed with a Hitachi Chromato Integrator D-2000. Super-purified water produced by Mill-Q Labo (Millipore) was used as the solvent at a flow rate of 0.7 ml/min over 20 min. The solvent was degassed on line by a degasser model 546B (GL-Science, Tokyo). Glucose, xylose, and arabinose concentrations were estimated from standard curve of the quantified peak area. Crude protein in pentosans was determined by the micro Kjeldahl method (AACC approved method 1983, protein: N $\times$ 5.7).

G. Determination of Uronic Acids

Uronic acids were determined by the meta-phenylphenol method using glucuronic acid as a standard (Blumenkranz and Asboe-Hansen 1973).

H. Determination of Phytic Acids

Phytic acids were determined by the method of Tangendjaja et al. (1980). The HPLC system used was described above. A reverse phase C-18 column (300 mm $\times$ 4 mm i.d.) was obtained from GL-Science (Tokyo). Pure phytic acid was purchased from Wako (Tokyo).

I. SE(Size-Exclusion)-HPLC

In order to estimate molecular-weight distribution of polysaccharide chains in arabinoxylans, the SE-HPLC was used. The Waters Ultrahydrogel 1000 column (300 mm $\times$ 7.8 mm i.d.) used in

these experiments has a separation range of 10-1,000 kDa for polysaccharides. Molecular weight of polysaccharide was estimated from retention time of sample peak. Pullulan as molecular weight marker was P-800 (853 kDa), P-400 (380 kDa), P-200 (186 kDa), P-100 (100 kDa), P-50 (48 kDa), P-20 (23.7 kDa), P-10 (12.2 kDa), and P-5 (5.8 kDa). Super-purified water was used as the solvent. Carbohydrate was eluted at a flow rate of 0.5 ml/min. After being filtered through 0.45 μ m of pore membrane size, 100 μ l of 3% carbohydrate sample solution was applied on the column. The effluent was collected in 0.5-ml fractions, and the total carbohydrate in the fractions was determined by measuring the absorbance at 480 nm by the phenol-sulfuric acid method of Dubois et al. (1956).

J. Purification of Pentosans from WSH by Chromatography

A DEAE-Sepharose CL-6B column (Pharmacia, 300 mm $\times$ 100 mm i.d.) was equilibrated with 20 mM tris-HCl buffer (pH 8.5). After dialysis against the same buffer overnight at 4 $^{\circ}$ C, the WSH solution (15 ml of 1% WSH) was applied on the DEAE-Sepharose column. Elution was done first with 150 ml of the same buffer and then with a linear gradient of 0 - 0.5 M NaCl in the same buffer at a flow rate of 0.6 ml/min. The effluent was collected in 5-ml fractions and was continuously monitored by absorption at 280 nm. Also the total carbohydrate in the fractions was determined by measuring the absorbance at 480 nm by the phenol-sulphuric acid method of Dubois et al. (1956). After the arabinoxylan fractions

were pooled and dialyzed against 100 mM tris-HCl buffer (pH 8.5) overnight at 4 °C, the dialyzate was concentrated to about 20 ml using an ultrafiltration system (Asahipak C5P, Asahi Kasei Kogyo Co., Tokyo) with a membrane (molecular weight cutoff of 13 kDa).

The pentosan concentrate from the DEAE-Sepharose CL-6B column was loaded on a Sephacryl S-200 column (Pharmacia, 1,000 mm × 30 mm i.d.) which was equilibrated previously with 100 mM tris-HCl buffer (pH8.5). After elution by same buffer at a flow rate of 0.4 ml/min, the effluent was collected in 5-ml fractions, and the total carbohydrate in the fractions was determined by measuring the absorbance at 480 nm by the phenol-sulfuric acid method of Dubois et al. (1956). Arabinoxylan fractions were pooled and dialyzed against distilled water overnight at 4 °C; the solution was frozen immediately and lyophilized. These purified arabinoxylans were used for the subsequent experiments.

K. Endo-1,4- β -D-xylanase Treatment of Pentosans

The endo-1,4- β -D-xylanase (EC 3.2.1.8.) used in this study was purified from Cellulase Onozuka RS (Yakult Co., Tokyo) by the modified method of Stuttgen and Sahm (1982). The xylanase (64 units) obtained was used to hydrolyze WSH or pentosan isolates (1 ml contained 30 mg of total carbohydrates) with 100 mM acetate buffer (pH 5.5). After incubation (40 °C, 60 min), 0.5 ml of 2 M NaOH was added to the reaction mixture and the solution was immediately heated (95 °C, 10 min) to inactivate xylanase. After filtration with 0.45 μ m membrane, the solution was fractionated

by SE-HPLC.

L. Methylation Analysis of Pentosans

Pentosans (5-mg samples) were subjected to micro-scale methylation by a modified method of Hakomori (1964), and the methylated product was extracted with chloroform. The extract was washed several times with water and concentrated to a syrup by evaporation. The methylated samples were hydrolyzed in 1 ml of 90% formic acid at 100 °C for 1 hr in a sealed test tube, and the hydrolysate was evaporated to obtain a dried sample. Furthermore, the hydrolysate sample was hydrolyzed in 1 ml of 1 M TFA at 121 °C for 1 hr in a sealed test tube, and neutralized by the complete evaporation of TFA. After dissolution with 1 ml of water, the methylated sugars in the solution were hydrogenated with sodium borohydride and then acetylated with 2 ml of a mixture of pyridine and acetic anhydride (1:1). The resulting alditol acetate was examined on a gas chromatography (GC) equipped with a FID by the method of Kusakabe et al. (1977). The stainless steel column (1,000 mm × 3 mm i.d.) was packed with 3% ethylene succinate cyanoethyl silicon copolymer medium on Gas Chrom Q (100-120 mesh) (purchased from GL-Science), and the column temperature was maintained at 168 °C. The nitrogen gas was used as carrier gas at a flow rate of 50 ml/min.

M. Operation of Mixograph

Mixograph was carried out by the same methods as described in

3. Results and Discussion

3-1. PURIFICATION AND CHARACTERIZATION OF TWO PENTOSANS FROM WSH

A. Purification of Pentosans from WSH on Chromatography

The WSH obtained was applied to the DEAE-Sepharose CL-6B column and separated into two major carbohydrate components as shown in Fig. 22: i.e., one of them (AX-1) passed through the column and the other component (AX-2) was eluted with a linear gradient from 0.1 - 0.3 M NaCl. The total carbohydrate corresponding to AX-2 was equivalent to almost 60% of the soluble hemicellulose applied, while proteinaceous material was mainly coeluted with the carbohydrate material in AX-2, little proteinaceous material was detected in AX-1.

The two fractions were further purified by a Sephacryl S-200 column. The elution profiles as shown in Fig. 23 revealed that AX-2 had a little wider distribution in molecular weight than that of AX-1, but both had almost same elution time. In addition, the elution profile of the proteinaceous portion contained in AX-2 showed a pattern almost associated with carbohydrate. It is speculated from these results that arabinoxylan chain in AX-2 was linked to the peptide moiety by the same sugar-amino acid linkage that was found first between arabinogalactan and a peptide moiety, which is a glycosidic link involving the hydroxy group of hydroxyproline (Strahm et al. 1981).

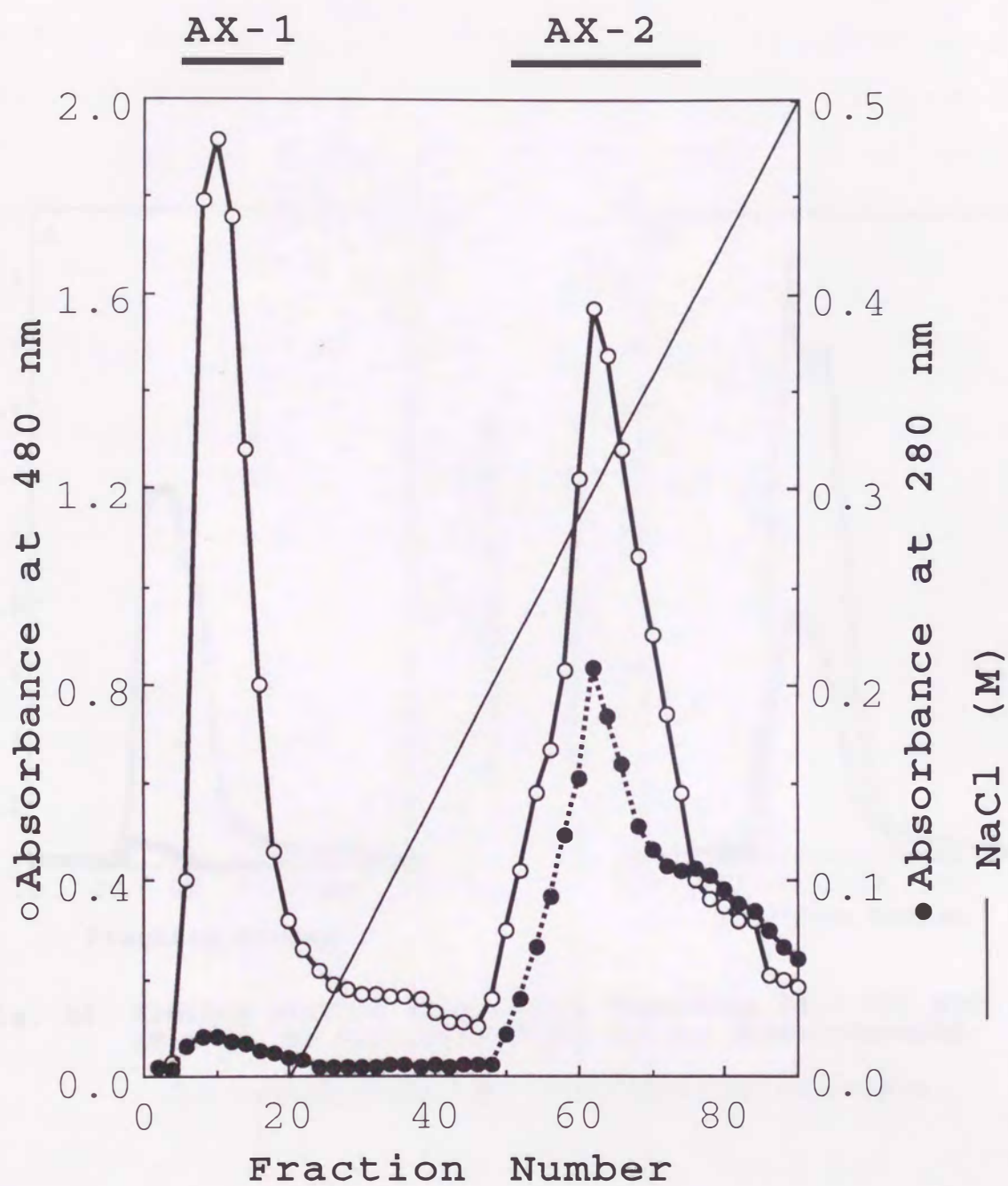


Fig. 22. Separation of pentosans from WSH by DEAE-Sephadex CL-6B column chromatography.

○ : carbohydrate, ● : proteinaceous materials.
 Pentosan fractions are denoted as AX-1 and AX-2.

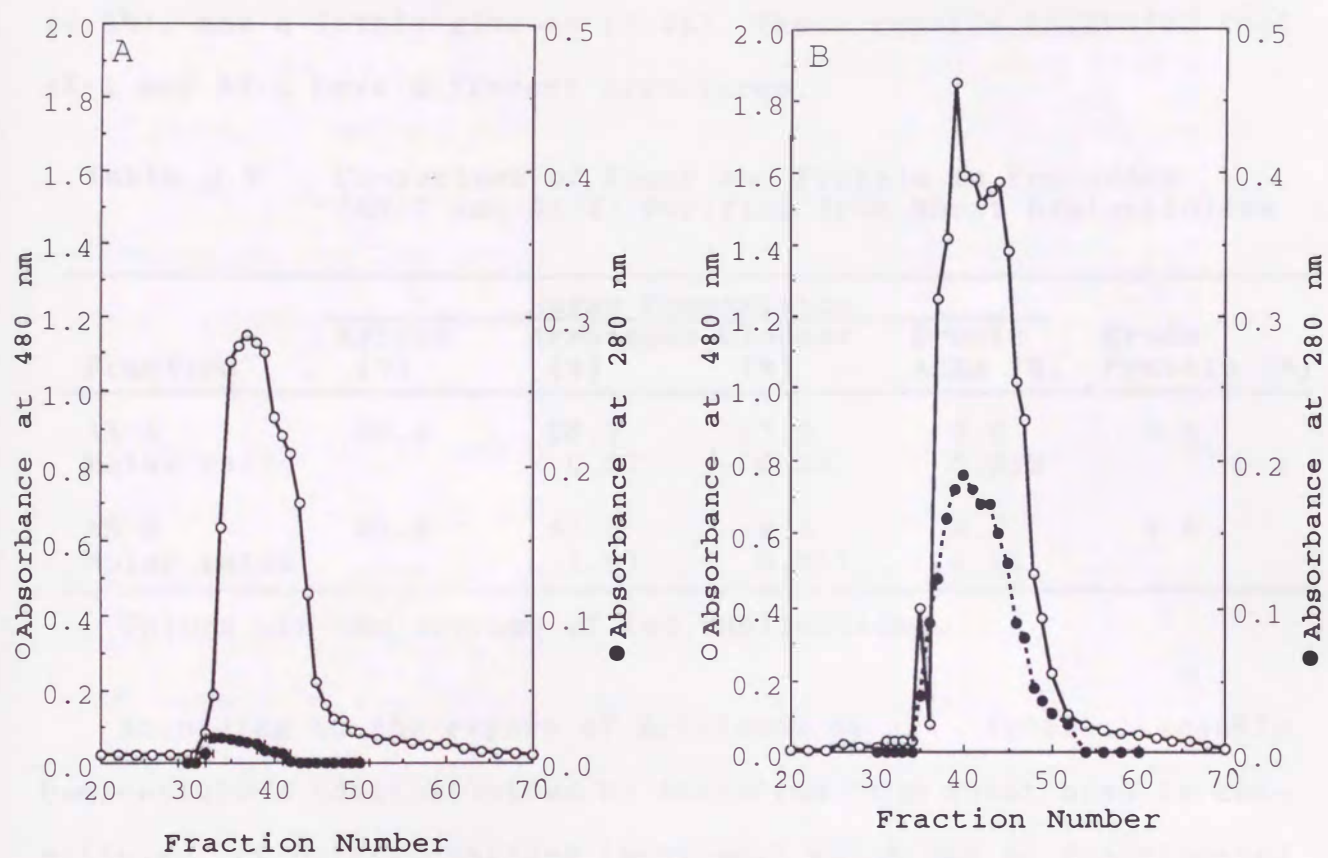


Fig. 23. Elution profile of pentosan fractions AX-1 (A) and AX-2 (B) by Sephacryl S-200 column chromatography.

○ : carbohydrate, ● : proteinaceous materials.

B. Analysis of Purified Pentosans

As shown in table X V , some differences in composition were observed between AX-1 and AX-2. The main components of isolated pentosan AX-1 were xylose, arabinose, and glucose, whereas pentosan AX-2 was mainly comprised xylose and arabinose. AX-2 also contained uronic acid (4.7%), proteinaceous materials (4.8%), and a little glucose (2.4%). These results suggested that AX-1 and AX-2 have different structures.

Table X V . Comparison of Sugar and Protein in Pentosans (AX-1 and AX-2) Purified from Wheat Hemicellulose

Fraction	Sugar Composition				Crude Protein (%)
	Xylose (%)	Arabinose (%)	Glucose (%)	Uronic Acid (%)	
AX-1	50.4	28.7	17.3	0.6	0.8
Molar ratio	1	0.57	0.34	0.012	
AX-2	42.4	45.5	2.4	4.7	4.8
Molar ratio	1	1.07	0.057	0.11	

Values are the average of two replications.

According to the report of Brillouet et al. (1982), soluble hemicellulose (hemicellulose B) extracted from wheat bran is constituted of polysaccharides (pentosan) which can be fractionated by ethanol precipitation in the range 60-90% ethanol, consisted mainly of xylose and arabinose with a molar ratio of 1:1.09-1.14 and also contained 5.4-5.7% uronic acid. Also some investigators (Medcalf et al. 1968, D'Appolonia and MacArther 1975, and Lineback et al. 1977) suggested that pentosan from wheat flour consists of a xylan with highly branched arabinose. Comparing

those results, AX-2 corresponds to those pentosans. On the other hand, Cole (1969) reported as a similar pentosan as AX-1 that flour contained short chained xylan with a few arabinose like AX-1 which influenced dough rheology.

The gel filtration chromatography profiles of the original WSH and two pentosans purified on SE-HPLC are shown in Fig. 24 (A, B, and C line, respectively). The polysaccharides from the two arabinoxylans (AX-1 and AX-2) were eluted in a single and symmetric peak between the void volume (V_o) and total bed volume (V_t). The molecular weight distribution of both purified arabinoxylans was estimated by SE-HPLC from a calibration plot of elution times versus the molecular weight of pullulans. It showed that AX-1 and AX-2 had almost same peak with the symmetric molecular weight distribution which corresponded to molecular weight of around 300-350 kDa of pullulan. These results demonstrated a reason why the obtained WSH showed a single and symmetric peak on SE-HPLC (Fig. 24A line).

As shown in Fig. 24A (dotted line), almost 40% of the original WSH was decomposed by endo-1,4,- β -D-xylanase, but the rest of WSH resisted against that enzymatic reaction. Enzymatic hydrolysis of AX-1 arabinoxylan, catalyzed by endo-1,4,- β -D-xylanase, caused the change in molecular weight distribution to a lower level that corresponded to a molecular weight lower than 10 kDa as shown in Fig. 24B (dotted line). However, even by same enzyme treatment the molecular weight distribution of AX-2 did not change as shown in Fig. 24C. These results suggested that

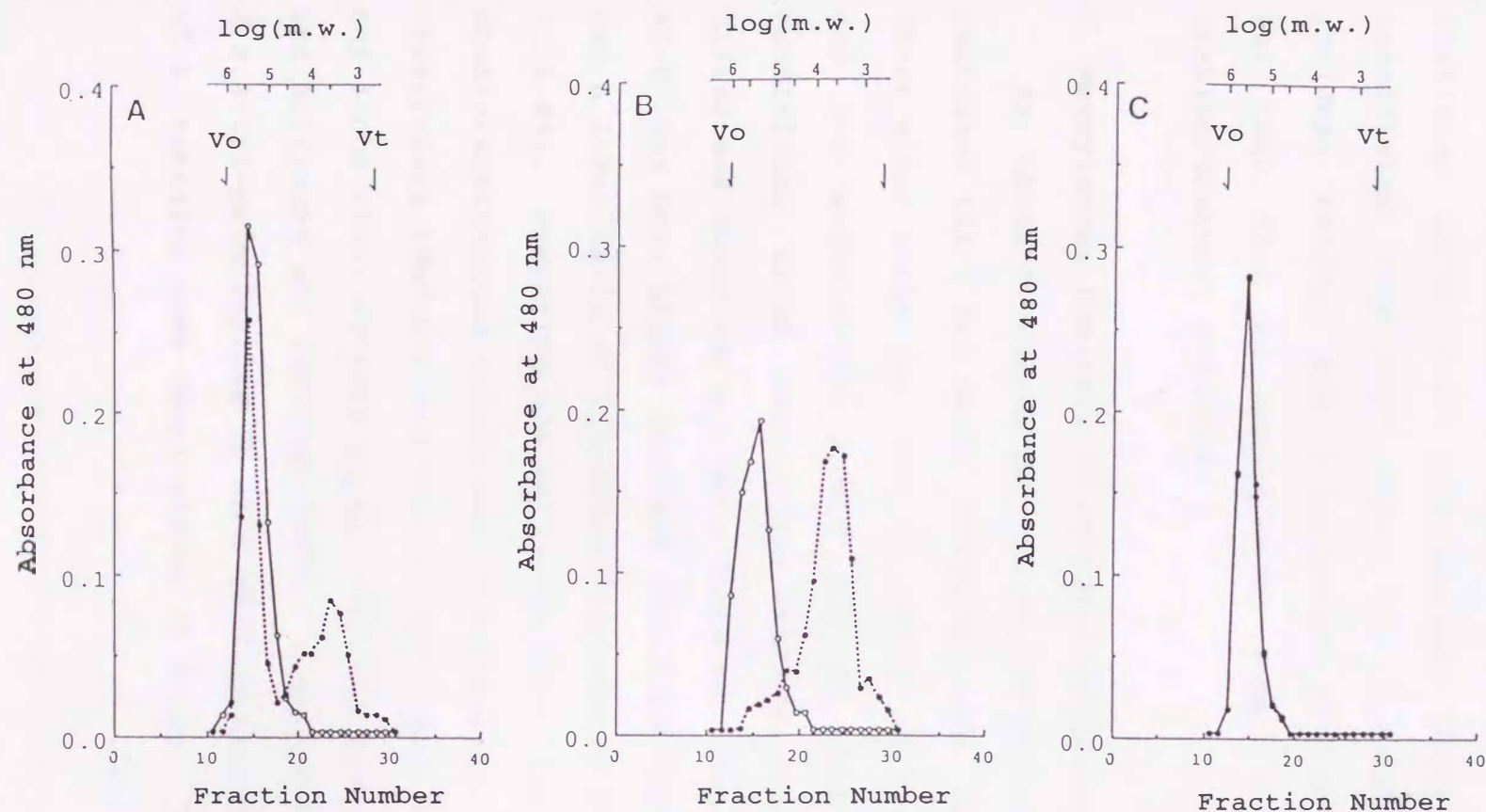


Fig. 24. Elution profile of purified wheat pentosans (○) and the enzymatic hydrolysed pentosans (●) by SE-HPLC on an Ultrahydrogel 1000 column.

A: water soluble hemicellulose (WSH), B: pentosan AX-1, C: pentosan AX-2.

Vo and Vt show the void volume and total bed volume for this column, respectively.

The logarithmic molecular weights (upper axis) were estimated from pullulan standards.

xylanase does not catalyze for AX-2 because of its unique structure. According to Kusakabe et al. (1983), xylanase specifically works for the xylose residues that are devoid of branches of L-arabinofuranose residues or 2-O- β -D-xylopyranosyl-L-arabinose units in the arabinoxylan. This suggests that AX-1 is constituted from main chain of 1,4-linked- β -D-xylopyranose residues having only a few amount of L-arabinofuranosyl branch but that AX-2 is constituted from a xylan with many arabinofuranosyl branches.

C. Methylation Analysis of Purified pentosans

As shown in Figure 25, GC on the methylated sugars from two pentosans (AX-1 and AX-2) revealed some derivatives. Although three minor peaks (No. 4, 7, and 8) were not identified and 2- and 3-O-methylxylose could not be separated under these conditions, seven derivatives from the two pentosans were identified and their amounts were compared. Table X VI showed that AX-2 has more highly branched structure than AX-1, because AX-2 had a lower ratio of 2,3-dimethylxylose to 2- or 3-methylxylose (1:1.88), comparing the ratio for AX-1 (1:0.59). The presence of double-substituted xylose was also detected, as reported by many researchers (Medcalf and Gilles 1968, Woolard et al. 1976, Bacic and Stone 1981, Shibuya et al. 1983 and Shibuya and Iwasaki 1985, and Brillouet and Joselean 1987). A higher proportion (5.5%) of 2,3,4-tri-methylxylose in AX-2 than in AX-1 (0.6%) suggested that AX-2 contains some short chains of xylan. Thus, it showed that

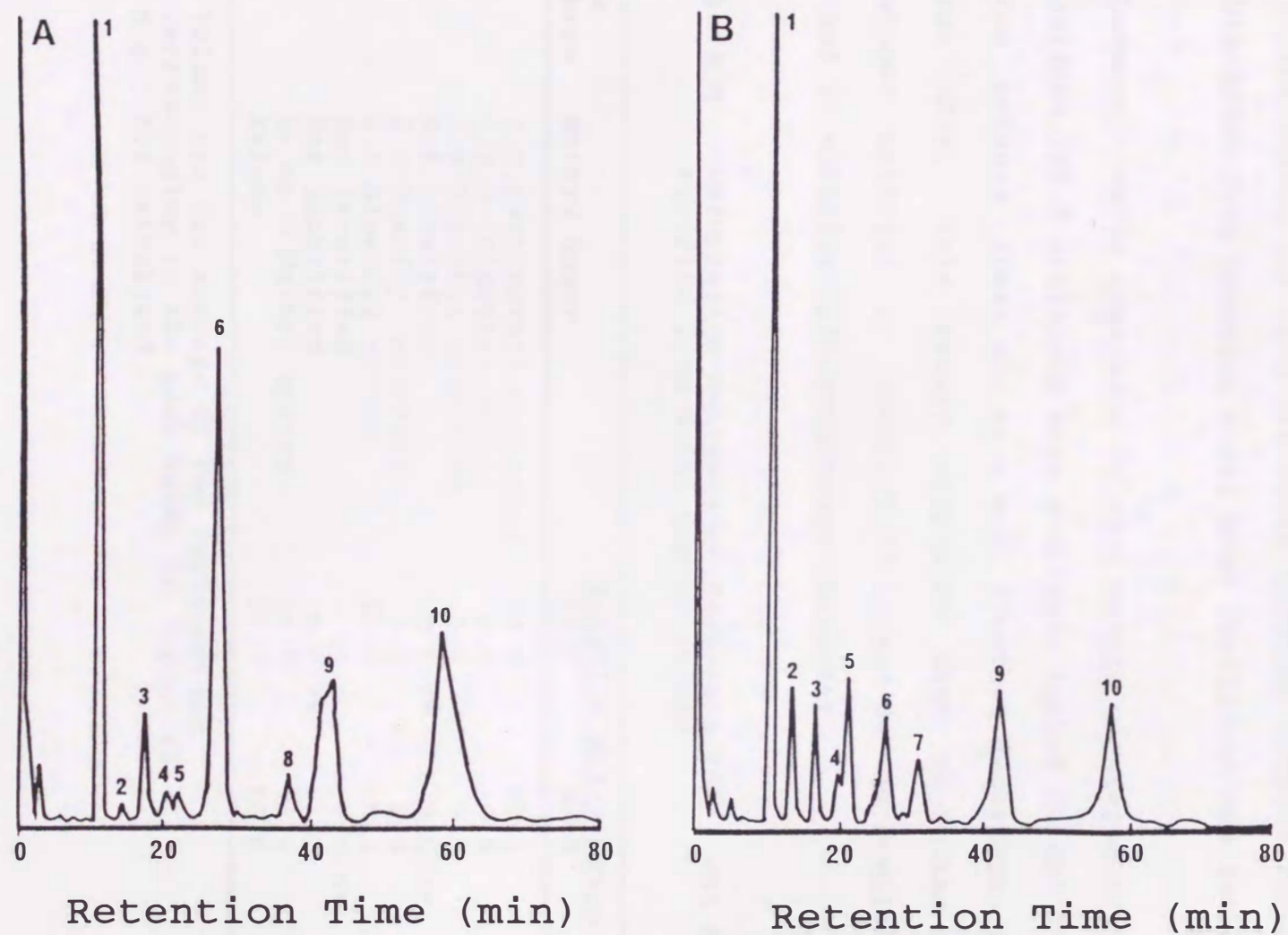


Fig. 25. Separation of methylated sugars from the purified wheat pentosans (A: AX-1, B: AX-2) by GC.

37% of the 1,4-linked- β -D-xylopyranosyl residues in AX-1 are unsubstituted, 22% are singly branched at the 2- or 3-position, and 40% are doubly branched at the 2- or 3-position. On the other hand, AX-2 is constituted of 21% of unsubstituted, 39% single substituted, and 40% of double-substituted xylopyranosyl residues. The values for AX-2 are close to other results reported for arabinoxylan from beeswing wheat bran (Brillouet and Joselean 1987).

Futhermore, while arabinose in AX-1 mainly consisted of terminal residues, AX-2 contained some arabinose linked to the 2- or 3-position because these are much more dimethyl arabinoses from AX-2 than AX-1. This result suggested that AX-2 has the proteinaceous material or uronic acid linked to the arabinosyl residue and it exhibits cation-exchange character.

Table X VI . Methylation Analysis of Pentosans (AX-1 and AX-2) Purified from Wheat Hemicellulose

Peak Number*	Methyl Sugar	Relative Molar Ratio	
		AX-1	AX-2
1	2,3,5-Trimethyl arabinose	24.5	27.1
2	2,3,4-Trimethyl xylose	0.6	5.5
3	3,5-Dimethyl arabinose	3.5	5.6
4	Not identified	n.c.**	n.c.**
5	2,5-Dimethyl arabinose	1.0	9.3
6	2,3-Dimethyl xylose	25.0	8.1
7	Not identified	-	n.c.**
8	Not identified	n.c.**	-
9	2- or 3-Methyl xylose	14.7	15.2
10	Xylose	27.0	15.6

Values are the average of two replications.

* Corresponding to the peak shown in Figure 25.

** n.c.; not calculated.

3-2. RHEOLOGICAL PROPERTIES OF FLOURS WITH PURIFIED PENTOSANS

As shown in Figure 26, comparing control flour (without pentosan), mixogram of the flour with pentosan exhibited less mixing tolerance. In particular, the flour containing the AX-2 affected pronouncedly. Even adding only 0.2% of AX-2 to the flour was enough to change mixing properties of dough: i.e., showing 5.5 of maximum intensity for the flour adding 0.2% of AX-2 to 6.5 for control, respectively. Consequently, AX-2 having a more branched structure than AX-1 caused decrease in resistance of dough. That mixograph result was close to the mixogram of the fermented dough (as shown in Fig. 2) showing less resistance to extension than nonfermented dough. Due to the decomposition of glutenin aggregative subunit during fermentation, the pentosan was probably released and caused to change dough properties. Of these two species (AX-1 and AX-2) of wheat pentosan, the pentosan AX-2 having highly branched arabinose particularly affects rheological changes of the fermented dough. These results reflect on much amounts of arabinose containing in glutenin aggregative subunits as shown in Table V. Thus, the pentosans released by the decomposition of structure in glutenin aggregative subunit reaches to almost 0.2% concentration to flour dough, which is sufficient to change dough rheology.

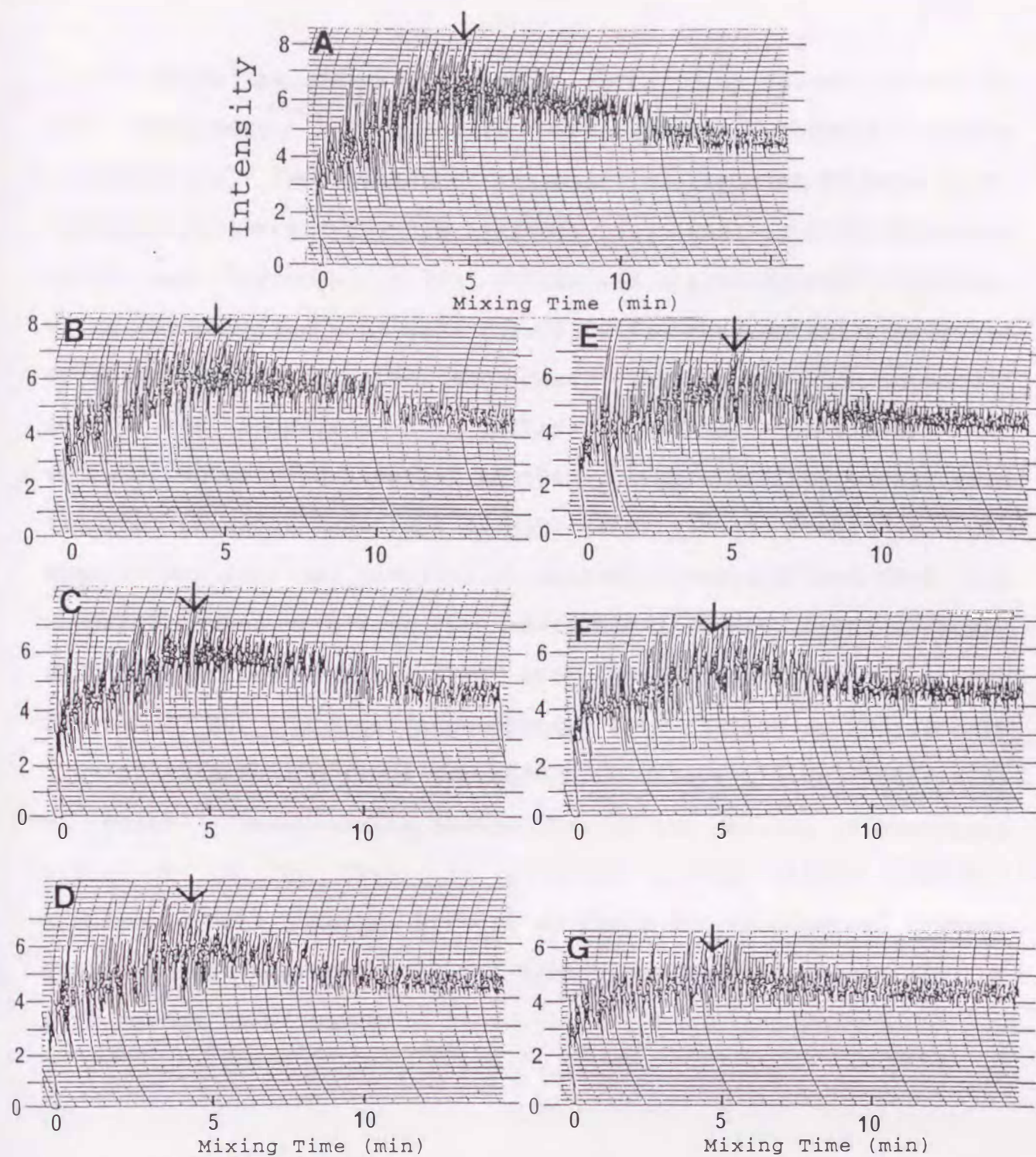


Fig. 26. Mixograms showing effects of wheat pentosans on supplemented flour.

A: control flour without pentosan supplement.

B, C, and D: flours supplemented with pentosan AX-1 0.2, 0.5, and 1.0%, respectively.

E, F, and G: flours supplemented with pentosan AX-2 0.2, 0.5, and 1.0%, respectively.

Arrows indicate peak of mixing development.

4. Summary

To study the effect of pentosan released by decomposition of the structure in glutenin aggregative subunit during fermentation, two species of pentosan (AX-1 and AX-2) were fractionated from wheat soluble hemicellulose (WSH) by DEAE-Sepharose CL-6B and Sephacryl S-200 column and characterized. Further, using the purified pentosans effect on the rheological properties of dough was investigated. AX-1 passed through the DEAE-Sepharose column was comprised principally of xylose, arabinose, and glucose, while AX-2 adsorbed on this column contained uronic acid and proteinaceous materials as well as xylose and arabinose. Enzymatic analysis and methylation analysis resulted that AX-2 had a more branched structure than AX-1. The mixograph studies showed that the flour supplemented with AX-2 exhibited less mixing tolerance than the flour supplemented with AX-1. Even adding only 0.2% of AX-2 to the flour was enough to change mixing properties of dough. These results showed that of two species of pentosans contained in the flour, a pentosan having highly branched arabinose particularly affects on the dough rheological changes during fermentation because the fermented dough showed similar performance on mixogram.

CHAPTER V

CONCLUDING REMARKS

Since mixograph studies showed that organic acids produced by fermentation are not enough to change the dough rheology, it is suggested that factors other than acidity played a major role in chemical changes during fermentation. The fermentation caused a change in protein solubility; there was a large decrease in acetic-acid-soluble proteins and an increase in 70% ethanol-soluble proteins. However, these phenomena were not caused by a change in molecular weight of the polypeptides constituting gluten, because SDS-PAGE and gel filtration on Sephacryl S-300 showed no detectable change in molecular weights of proteins in nonfermented and fermented dough, and also proteolytic activity in the fermented dough was low. On the other hand, the results of RP-HPLC analysis and measurement of surface hydrophobicity of glutenin showed that the conformation of glutenin changed during fermentation which is related to increasing foaming activity, while conformation of gliadins (70% ethanol soluble protein) did not change.

These results suggested that the conformational change of glutenin occurs as a result of decrease in surface hydrophobicity of glutenin without changing the molecular weights. To investigate materials causing the conformational change of glutenin and decrease in its surface hydrophobicity, attention was focused on lipids containing glutenin (particularly, glutenin aggregative

subunit). In fact, the results of determination of lipids extracted from doughs having different fermentation times showed that the ratio of free unsaturated fatty acids (linoleic and linolenic acid) to total lipids extracted from dough decreased during fermentation. Therefore, lipoxygenase was determined. As the fermentation time increased, lipoxygenase activity increased linearly. In proportion to increase in lipoxygenase activity during fermentation, hydrophobicity of glutenin decreased and foaming activity of the fermented dough increased.

In order to confirm these hypothesis, three subunits constituting soluble-glutenin were fractionated from the fermented dough having various fermentation times and these constituents were compared. These results showed that the ratio of aggregative subunit (abbr. GAS) and low molecular weight subunit (abbr. LMWS) in soluble glutenin decreased during fermentation, by which the surface hydrophobicity of glutenin decreases. The HPLC analysis and SDS-PAGE of glutenin subunits also showed that hydrophilic polypeptides were released from GAS. Thus, these results confirmed that the decomposition of the structure in GAS occurs during fermentation, because soluble glutenin containing LMWS decreased during fermentation and it was related to the increasing 70% ethanol soluble fraction (gliadins). In fact, LMWS and gliadins (α , β , γ -gliadin) consist of almost the same polypeptides coded by the same region of the same chromosome (Bietz and Wall 1980, Burnouf and Bietz 1984).

Decomposition of GAS stimulated the release and activation of lipoxygenase because this enzyme is bound with the hydrophobic site in the lipid-binding glutenin, as reported by Frazier et al. (1981), and the activated lipoxygenase stimulated decomposition of GAS. There is a positive feedback cycle between them. That is why during fermentation lipoxygenase activity and GAS decomposition increased concomitantly.

Also the decomposition of GAS caused release of 80% of pentosans bound with the aggregative subunit and the total amount of pentosans released reaches to almost 0.2% of the flour which change in the dough rheological properties during fermentation.

Futhermore, in order to reveal these chemical changes during fermentation, lipoxygenase and pentosans are purified and characterized, and then using these purified materials, the effects on the glutenin constituents and dough rheology are examined.

By means of ion-exchange and gel-filtration chromatography, three major isozymes (L-1, L-2, and L-3) and one minor isozyme (L-a) of lipoxygenase were isolated from wheat germ. Of these isozymes, the L-3 isozyme was characterized by different enzymatic properties from others, though all isozymes have almost same molecular weight (110 kDa). Particularly, the only L-3 isozyme oxidized linolenic acid with high activity, while the L-1 and L-2 isozymes showed weak or no activity. From these results, it was predicted that each isozyme has different effects on wheat

flour or dough.

All flours treated with lipoxygenase isozymes showed shorter dough development time and less resistance after peak time on mixogram, and lower SH contents of protein than the control flour treated without lipoxygenase. Comparing flours with or without each of three major lipoxygenase isozymes, the flour treated with the L-3 isozyme exhibited the highest foaming activity and the greatest influence on bread-making quality.

These results show that L-3 wheat lipoxygenase isozyme particularly induced chemical changes during dough fermentation.

Futhermore, these flours were used to fractionate constituent subunits of their glutenin. Compared to the control flour treated without lipoxygenase, the flour treated with lipoxygenase exhibited a lower ratio of GAS and LMWS in soluble-glutenin. This result showed that lipoxygenase, especially L-3 isozyme, caused a similar decomposition of GAS as fermentation. According to the decomposition of GAS, almost 80% of pentosans bound with GAS is also released.

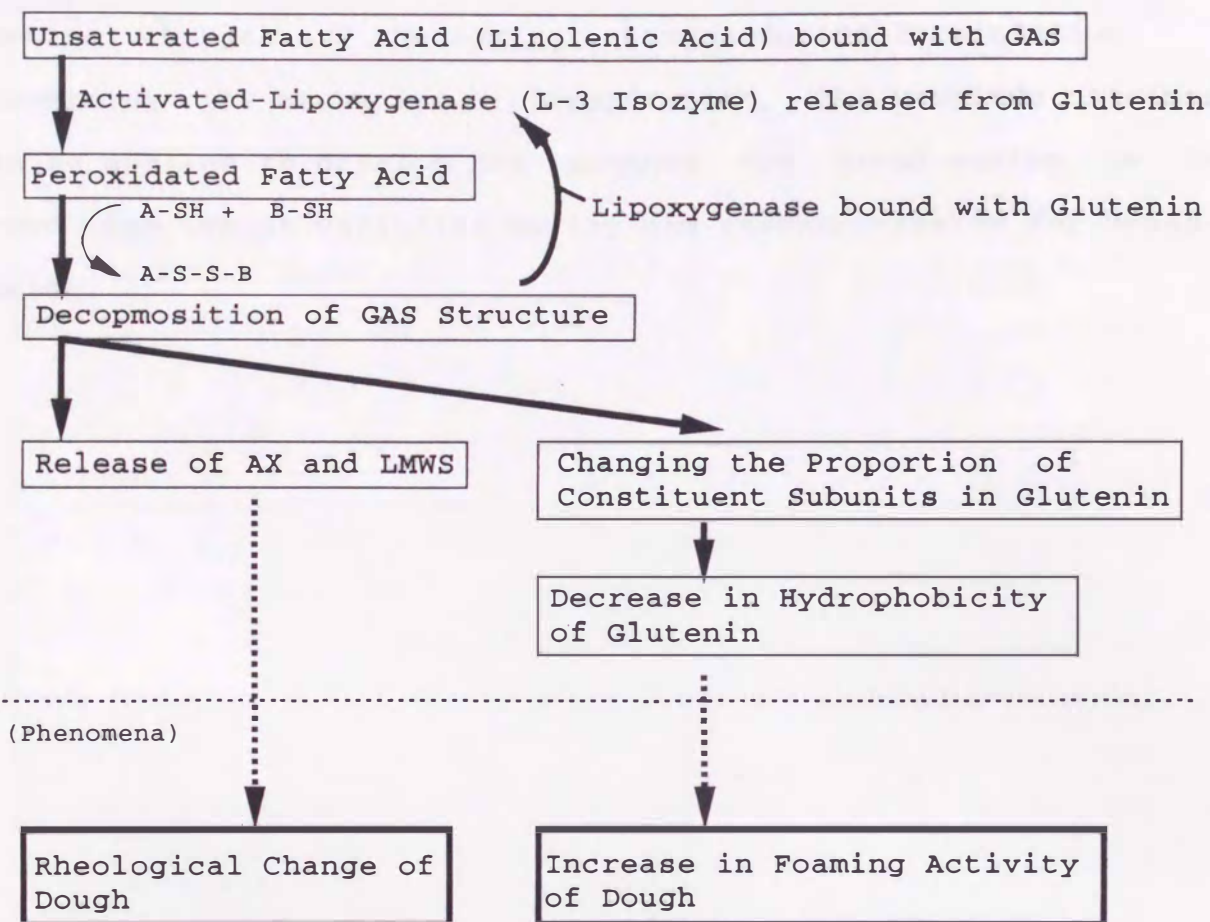
As pentosans contained in wheat flour, two types of water soluble pentosans have been found. In this study, these pentosans were separated and purified by the newly developed methods using DEAE-Sepharose and gel-filtration from water soluble hemicellulose of wheat bran. Furthermore the effect of pentosans on the dough properties was investigated. Of two types of pentosans separated, the AX-2 pentosan was characterized as arabinoxylan

with more branched arabinoxylan than the AX-1 pentosan, and also AX-2 contained more glucuronic acids and proteins, besides carbohydrates, than AX-1. The mixograph studies showed that the flour supplemented with AX-2 exhibited less mixing tolerance than the flour supplemented with AX-1. Even adding only 0.2% of AX-2 to the flour was enough to change mixing properties of dough. Of the two types of pentosans in wheat flour, only pentosan having highly branched arabinose affects the rheological changes in dough during fermentation. That reflects on the large amount of arabinose contained in GAS.

These results eventually provided the scheme of chemical changes during fermentation, as shown in Fig. 27.

During mixing flour and water, a part of lipoxygenase bound with hydrophobic site of glutenin is released and activated. The lipoxygenase, particularly the L-3 isozyme, catalyzed an oxidation of unsaturated fatty acids such as linoleic acid or linolenic acid contained within GAS. The peroxidated fatty acid and the following radical reaction caused a glutenin conformational change and reacted with a sulfhydryl group (SH) in GAS to oxidate it. As a result, GAS is decomposed. The decomposition of GAS causes release of LMWS and pentosans from the glutenin. The released LMWS having gliadine-like characteristics and the released pentosans which are characterized as arabinoxylan branched with large amount of arabinose, affected the dough rheology to exhibit shorter dough development time and less

(Chemical Changes during Fermentation)



GAS: glutenin aggregative subunit
 A-SH, B-SH: polypeptides having sulfhydryl group
 A-S-S-B: polypeptides having disulfide
 AX: pentosan (arabinoxylan) with highly branched arabinose
 LMWS: low molecular weight subunit

Fig. 27. The scheme of chemical changes during fermentation.

mixing tolerance on mixogram.

Consequently, this study revealed the relationship between chemical changes and rheological changes during fermentation. Concerning the mechanism of fermentation, the knowledge obtained can be applied to develop new methods for bread-making or to breed new wheat varieties having new characteristics for bread-making.

Abschließende Bemerkungen

Da Mixographstudien zeigten, daß aus Fermentation gewonnene organische Säuren die Rheologie des Teiges nicht ausreichend verändern, liegt es nahe, daß andere Faktoren eine Hauptrolle in den chemischen Reaktionen während der Fermentation spielen. Die Fermentation bewirkte eine Änderung der Proteinlöslichkeit. Der Anteil der in Essigsäure löslichen Proteine ist stark gesunken, während der der in 70%igem Ethanol löslichen Proteine stieg. Dieses Phänomen wurde nicht durch eine Änderung des Molekulargewichts des polypeptitbildenden Glutens bewirkt, da SDS-PAGE und Gelfiltration mit Sephacryl S-300 keinen meßbaren Unterschied im Molekulargewicht der Proteine in nichtfermentiertem und fermentiertem Teig zeigten und die proteolytische Aktivität im fermentierten Teig niedrig war. Andererseits zeigten die Ergebnisse der RP-HPLC-Analyse und die Messung der Oberflächenhydrophobie von Glutenin, daß sich die Konformation von Glutenin während der Fermentation ändert, was mit steigender Schäumungsaktivität einhergeht, während sich die Konformation von Gliadin (in 70%igem Ethanol lösl. Protein) nicht verändert.

Diese Ergebnisse deuten darauf hin, daß die Änderung der Konformation von Glutenin als Resultat der sinkenden Oberflächenhydrophobie ohne Änderung des Molekulargewichts auftritt. Zur Untersuchung von Material, das eine Konformationsänderung von Glutenin und Abnahme der Oberflächenhydrophobie hervorruft, konzentrierte sich die Aufmerksamkeit auf gluteninhaltige Lipide (insbesondere aggregative Fraktionen). Tatsächlich zeigten die Analysen aus Teig extrahierter Lipide nach unterschiedlicher

Fermentationsdauer, daß der Anteil an ungesättigten Fettsäuren (Linol-und Linolinsäure) im Verhältnis zur Gesamtmenge der extrahierten Lipide abnahm. Mit steigender Fermentationsdauer stieg der Gehalt an Lipoxxygenase linear. Gleichzeitig mit dem Anstieg der Lipogenasemenge während der Fermentation sank die Hydrophobie des Glutenins, die Schäumungsaktivität des fermentierten Teiges stieg.

Um unsere Hypothese zu unterstützen wurden drei lösliches Glutenin bildende Untereinheiten mit unterschiedlicher Fermentationsdauer aus dem Teig fraktioniert. Der Vergleich der drei Fraktionen zeigte, daß der Anteil der aggregativen Fraktion (Abk. GAS) und der der niedrigmolekulargewichtigen Fraktion (Abk. MWS) in löslichem Glutenin während der Fermentierung stieg, die Hydrophobie des Glutenins gleichzeitig abnahm. SDS-PAGE und HPLC-Analyse der Gluteninfraktionen zeigten außerdem, daß hydrophile Polypeptide von der GAS freigesetzt wurden. Somit bestätigen diese Ergebnisse, daß die Zersetzung der Struktur der GAS während der Fermentation auftritt, da LMWS-haltiges Glutenin während der Fermentation abnahm, was mit der Abnahme des Gehaltes der in 70%igem Ethanol lösliche Fraktion (Gliadin) in Verbindung stand. Tatsächlich bestehen LMWS und Gliadin nahezu aus den gleichen Polypeptiden, codiert in der gleichen Region des gleichen Chromosoms (Biez und Wall 1980, Burnouf und Bietz 1984).

Durch die Zersetzung der GAS wurde die Freisetzung und Aktivierung von Lipoxxygenase angeregt, da dieses Enzym mit der hydrophoben Seite an das lipidbindende Glutenin gebunden ist, wie Frazier et al (1981)

berichtet. Die aktivierte Lipogenase wiederum regt die Zersetzung der GAS an. Aufgrund dieses Kreislaufs steigen Lipoxygenasegehalt und GAS-Zersetzung proportional.

Außerdem bewirkt die Zersetzung der GAS die Freisetzung von 80% des in der aggregativen Fraktion gebundenen Pentosans. Die Gesamtmenge des freigesetzten Pentosans erreicht nahezu 0.2% des Mehls, was die rheologischen Verhältnisse im Teig während der Fermentation ändert.

Um diese chemischen Veränderungen während der Fermentation nachzuvollziehen werden Lipogenase und Pentol gereinigt und gekennzeichnet und dann benutzt, um die Auswirkungen auf Gluteninkonstellation und Teig rheologie zu untersuchen.

Mittels Ionenaustausch und Gelfiltrations chromatographie wurden drei Hauptisozyeme (L-1, L-2 und L-3) und ein Unterisozym (L-a) der Lipoxygenase aus Weizenkeimen isoliert. Von diesen Isozymen unterschied sich das L-3-Isozym durch andersartige enzymatische Eigenheiten von den anderen, obwohl alle Isozyme annähernd das gleiche Molekulargewicht (110,000) besitzen. Ausschließlich das L-3-Isozym oxidierte Linolsäure mit hoher Aktivität, während L-1 -und L-2-Isozym schwache oder keine Aktivität zeigten. Diese Ergebnisse sagen aus, daß jedes Isozym spezifische Auswirkungen auf Weizenkeime bzw. Teig hat.

Alle Mehle, die mit Lipoxygenaseisozymen behandelt wurden, zeigten kürzere Teigentwicklungsdauer, weniger Widerstandsfähigkeit nach dem Peak im Mixogram und niedrigeren SH-Gehalt der Proteine als Kontrollmehl, das nicht mit Lipoxygenase behandelt wurde. Im Vergleich von

Mehlen mit bzw. ohne jedem der drei Hauptisozyme, zeigte das Mehl, welches mit L-3-Isozym behandelt wurde die höchste Schäumungsaktivität und den größten Einfluß auf die Brotherstellungsqualität.

Diese Resultate zeigen, daß das L-3-Weizenlipogenaseisozym die chemischen Veränderungen während der Teigfermentation in besonderer Weise antreibt.

Außerdem wurden diese Mehle benutzt, um enthaltene Untereinheiten ihres Glutenins zu fraktionieren. Verglichen mit dem Kontrollmehl, welches nicht mit Lipoxygenase behandelt wurde, zeigte das Lipoxygenasebehandelte einen geringeren Gehalt an GAS und LMWS in löslichem Glutenin. Das zeigt, daß Lipoxygenase, insbesondere L-3-Isozyme, eine ähnliche Zersetzung der GAS verursachen wie Fermentation. Entsprechend der Zersetzung der GAS wird nahezu 80% des daran gebundenen Pentosans frei.

Zwei verschiedene in Weizenmehl enthaltene Arten von wasserlöslichen Pentosanen wurden gefunden. In dieser Studie wurden beide Arten durch die neu entwickelte DEAE-Sepharose und Gelfiltration von wasserlöslicher Hemicellulose aus Weizenkeimen separiert und gereinigt. Außerdem wurde der Einfluß von Pentosan auf die Teigeigenschaften untersucht. Von den beiden separierten Arten des Pentosans wurde das AX-2-Pentosan als Arabinoxylan mit mehr Verzweigungen als das AX-1-Pentosan charakterisiert. AX-2 enthält außerdem mehr neben Kohlenhydraten auch Glucuronsäure und Proteine als AX-1. Die Mixographstudien zeigten, daß das mit AX-2 sublimierte Mehl weniger Mischungstoleranz aufweist als das Mehl, welches mit AX-1 sublimiert wurde. Schon eine

Zugabe von 0.2% AX-2 zum Mehl reichte aus, um die Mischbarkeit zu verändern. Von den beiden Arten des Pentosans in Weizenmehl beeinflußt nur stark verzweigte Arabinose enthaltendes Pentosan eine Rheologieänderung des Teiges, was den hohen Gehalt an Arabinose in der GAS widerspiegelt.

Diese Ergebnisse lieferten das Schema der chemischen Veränderungen während der Fermentation, wie in Abb. 27 dargestellt.

Während dem Mischen von Mehl und Wasser wird ein Teil der an der hydrophoben Seite des Glutanins gebundenen Lipoxygenase frei und aktiviert. Die Lipoxygenase, besonders das L-3-Isozym, katalysiert eine Oxidation von ungesättigten Fettsäuren wie z.B. Linol- oder Linolinsäure, die in der GAS enthalten sind. Die peroxidierte Fettsäure und die nachfolgende Radikalreaktion bewirken eine Stellungsänderung des Glutenins, durch eine Reaktion mit der SH-Gruppe wird die GAS oxidiert und damit zersetzt. Diese Zersetzung wiederum verursacht Freiwerden von LMWS und Pentosan aus dem löslichen Glutenin. Die freiwerdende LMWS, welche gliadinartige Eigenschaften hat, und die freigewordenen Pentosane, welche charakterisiert werden als Arabinoxylan, verzweigt mit großen Mengen an Arabinose, beeinflussen die Teig rheologie und bewirken kürzere Teigentwicklungsdauer sowie geringere Mischungstoleranz im Mixogram.

Somit offenbart diese Studie das Verhältnis zwischen chemischen und rheologischen Veränderungen während der Fermentation. Bezüglich der Mechanismen der Fermentation

kann das erzielte Wissen zur Entwicklung neuer Methoden der Brotherstellung sowie zur Zucht neuer Weizensorten mit neuen Qualitäten für die Brotherstellung genutzt werden.

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REFERENCES I

- 1) SHIIBA, K., NEGISHI, Y., OKADA, K., and NAGAO, S. 1990.
Chemical changes during sponge-dough fermentation. *Cereal Chem.* 67:350.
- 2) SHIIBA, K., NEGISHI, Y., OKADA, K., and NAGAO, S. 1991.
Purification and characterization of lipxygenase isozymes from wheat germ. *Cereal Chem.* 68:115.
- 3) SHIIBA, K., IJITSU, T., HARA, H., and OKADA, K. 1992.
Preparation and characterization of water-soluble hemicellulose (arabinoxylan) from wheat bran. (in English) *Nippon Shokuhin Kogyo Gakkaishi (J. Japanese Soc. Food Sci. and Tech.)*. 39(12):1147.
- 4) SHIIBA, K., YAMADA, H., HARA, H., OKADA, K., and NAGAO, S. 1993. Purification and characterization of two arabinoxylans from wheat bran. *Cereal Chem.* 70:209.
- 5) SHIIBA, K., NEGISHI, Y., and OKADA, K. 1993.
Changes in solubility of wheat glutenin during fermentation. (in English) *Nippon Shokuhin Kogyo Gakkaishi (J. Japanese Soc. Food Sci. and Tech.)*. 40(10):727.
- 6) SHIIBA, K., YAMADA, H., HARA, H., and OKADA, K. 1994.
Preparation of cell-wall hydrolysate from wheat bran and their effect on mixing properties of dough. *Cereal Chem.* 71:279.
- 7) SHIIBA, K., NEGISHI, Y., and OKADA, K. 1995.
Determination of carbohydrates in the glutenin aggregative subunit. (in English) *Nippon Shokuhin Kogyo Gakkaishi (J. Japanese Soc. Food Sci. and Tech.)*. Research note. 42(3):207.

REFERENCES II

- AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1983. Approved Method of the AACC. Method 08-01, approved April 1961, revised October 1981; Method 44-15A, approved October 1975, revised October 1981, Method 46-12, approved October 1976, revised October 1986. The Association: St. Paul, MN.
- ANDERSON, N. E., and CLYDESDALE, F. M. 1980. An analysis of the dietary fiber content of a standard wheat bran. *J. Food Sci.* 45:336.
- BACIC, A., and STONE, B. A. 1981. Chemistry and organization of aleurone cell wall components from wheat and barley. *Aust. J. Plant Physiol.* 8:1981.
- BHATTI, T., CHAAMBERS, R. E., and CLAMP, J. R. 1970. The gas chromatographic properties of biologically important N-Acetylglucosamine derivatives, monosaccharides, disaccharides, trisaccharides, tetrasaccharides and pentosaccharides. *Biochim. Biophys. Acta.* 222:339.
- BARBER, S., BENEDITO, C., and PLANELLS, V. 1980. The sponge for breadmaking. Its influence on the rheological and fermentative characteristics of wheat flour doughs. *Rev. Agroquim. Technol. Aliment.* 20:403.
- BARRETT, F. F. 1975. Enzyme uses in milling and baking industries, In: *Enzymes in Food Processing*. (G. REED ed.), New York, U.S.A.: Academic Press., pp. 324.
- BHATTI, T., CHAMBERS, R. E., and CLAMP, J. R. 1970. The gas chromatographic properties of biologically important N-acetylglucosamine derivatives, monosaccharides, disaccharides, trisaccharides, tetrasaccharides and pentasaccharides. *Biochim. Biophys. Acta.* 222:339.
- BIETZ, J. A., and WALL, J. S. 1975. The effect of various extractions on subunit composition and associations of wheat glutenin. *Cereal Chem.* 52:145.
- BIETZ, J. A., BURNOUF, T., COBB, L. A., and WALL, J.S. 1984. Gliadin analysis by reversed-phase high-performanced liquid chromatography: Optimization of extraction condition. *Cereal Chem.* 61:124.
- BIETZ, J. A., and WALL, J. S. 1980. Identity of high molecular weight gliadin and ethanol-soluble glutenin subunits of wheat: Relation to gluten structure. *Cereal Chem.* 57:415.
- BLUMENKRANZ, N., and ASBOE-HANSEN, G. 1973. New method for quantitative determination of uronic acids. *Anal. Biochem.* 54:484.
- BRILLOUET, J. M., JOSELEAN, J. P., UTILLE, J. P., and LELIEVRE, D. 1982. Isolation, purification, and characterization of a complex heteroxylan from industrial wheat bran. *J. Agric. Food Chem.* 30:488.
- BRILLOUET, J. M., and JOSELEAN, J. P. 1987. Investigation of the structure of a heteroxylan from the outer pericarp (Beeswing bran) of wheat kernel. *Carbohydrate Research.* 159:109.

BURNOUF, T., and BIETZ, J. A. 1984. Reversed-phase high-performance liquid chromatography of reduced glutenin, a disulfide-bonded protein of wheat endosperm. *J. Chromatogr.* 299:185.

CASUTT, V., PRESTON, K. R., and KILBORN, R. H. 1984. Effects of fermentation time, inherent flour strength, and salt level on extensigraph properties of full-formula remix-to-peak processed doughs. *Cereal Chem.* 61:454.

COLE, E. W. 1969. Some physicochemical properties of a wheat flour hemicellulose in solution. *Cereal Chem.* 16:382.

D'APPOLONIA, B. L., and MacARTHUR, L. A. 1975. Comparison of starch, pentosans, and sugars of some conventional height and semidark hard red spring wheat flours. *Cereal Chem.* 52:230.

DANIELS, N.W.R., WOOD, P. S., RUSSEL-EGGITT, P.W., and COPPOCK, F. B. M. 1970. Studies on the lipids of flours. V. Effect of air on lipid binding. *J. Sci. Food Agric.* 21:377.

DANNO, G., KANAZAWA, K., and NAKANE, M. 1978. Improved fractionation of constituent polypeptides from wheat glutenin. *Agric. Biol. Chem.* 42:11.

DIEL, E., and STAN, H. J. 1978. Purification and characterization of two isozymes of lipoxygenase from soybeans. *Planta* 142:321.

DUBOIS, M., GILLES, K. A., HAMILTON, J. K., REBERS, P. A., and SMITH, F. 1956. Colorimetric method for determination of sugar and related substances. *Anal. Chem.* 28:350.

ERIKSSON, C. E., and SVENSSON, S. G. 1970. Lipoxygenase from peas, purification and properties of the enzyme. *Biochim. Biophys. Acta.* 198:449.

FOLIN, O., and CIOCALTEAU, V. 1927. On tyrosine and tryptophan determination in protein. *J. Biol. Chem.* 73:627.

FRAZIER, P. J., BRIMBLECOMBE, F. A., DANIELS, N. W. R., RUSSELL-EGGITT, P. W. 1977. The effect of lipoxygenase action on the mechanical development of doughs from fat-extracted and reconstituted wheat flour. *J. Sci. Food Agric.* 28:247.

FRAZIER, P. J., DANIELS, N. W. R., and RUSSEL, E. P. W. 1981. Lipid-protein interactions during dough development. *J. Sci. Food Agric.* 32:877.

FREILICH, J., and FREY, C. N. 1939. Dough oxidation and mixing studies. II. Effects of remixing after fermentation. *Cereal Chem.* 16:495.

GALLIARD, T. 1987. Lipolytic and lipid oxidation reactions in stored wholemeal wheat flour. In: *Cereals in a European Context.* (E. HORWOOD ed.), Chichester, UK: Bournemouth. pp. 462-479.

GRAVELAND, A., MARSEILLE, J. P., and VISSER, N. H. 1977. Redox reactions in dough. *Getreide Mehl Brot.* 31:181.

- HAKOMORI, S. 1964. A rapid permethylation of glycolipid, and polysaccharide catalyzed by methylsulfinyl carbonion in dimethyl sulfoxide. *J. Biochem.* 55:205.
- HOSENEY, H., RAO, H., FAVBION, J., and SIDHU, J. S. 1980. Mixograph studies IV. The mechanism by which lipoxygenase increase mixing tolerance. *Cereal Chem.* 57:163.
- HOSENEY, R. C., and BROWN, R. A. 1983. Mixograph studies. V. Effect of pH. *Cereal Chem.* 60:124.
- HSIEH, C. C. and McDONALD, C. E. 1984. Isolation of lipoxygenase isozymes from flour of durum wheat endosperm. *Cereal Chem.* 61:392.
- HUEBNER, F. R., and WALL, J. S. 1974. Wheat glutenin subunits. I. Preparative separation by gel-filtration and ion-exchange chromatography. *Cereal Chem.* 51:228.
- HUEBNER, F. R., and BIETZ, J. A. 1985. Detection of quality differences among wheats by high-performance liquid chromatography. *J. Chromatogr.* 327:333.
- IKEZOE, K., and TIPPLES, K. H. 1968. Farinograph studies on the effect of various oxidizing agents in the sponge-and-dough system. *Cereal Sci. Today.* 13:327.
- JOHNSON, J. A., SHELLENBERGER, J. A., and SWANSON, C. O. 1946. Farinograms and mixograms as a means of evaluating flours for specific uses. *Cereal Chem.* 23:388.
- KAGEYAMA, K. 1955. Characteristics of proteinase from rice bran. *Hakko Kougakkai-shi.* 33:53.
- KATO, A., and NAKAI, S. 1980. Hydrophobicity determined by a fluorescence probe method and its correlation with surface properties of proteins. *Biochim. Biophys. Acta.* 624:13.
- KATO, A., TSUTSUI, N., MATSUDOMI, N., KOBAYASHI, K., and NAKAI, S. 1981. Effects of partial denaturation on surface properties of ovalbumin and lysozyme. *Agric. Biol. Chem.* 45:2755.
- KILBORN, R. H., and PRESTON, K. R. 1982. A modified extensigraph procedure for measuring the stretching properties of fermented doughs. *Cereal Chem.* 59:381.
- KITABATAKE, N., and DOI, E. 1982. Surface tension and foaming of protein solutions. *J. Food Sci.* 47:1218.
- KUSAKABE, I., KAMIYAMA, Y., and YASUI, T. 1977. Methyl- α -D-glucoside as a satisfactory internal standard in gas-liquid chromatographic quantitative-analysis of alditolacetates derived from Streptomyces. *Agric. Biol. Chem.* 47:2713.

- KUSAKABE, I., OHGUSHI, S., YASUI, T., and KOBAYASHI, T. 1983. Structure of the arabinoxyloligosaccharides from the hydrolytic products of corn cob arabinoxylan by a xylanase from Streptomyces. Agric. Biol. Chem. 47:2713.
- LANDIS, Q., and FREILICH, J. 1934. Fermentation response and fermentation tolerance. Cereal Chem. 11:396.
- LINEBACK, D. R., KUKUDA, N. S., and TSEN, C. C. 1977. Carbohydrate composition of water-soluble pentosans from different types of wheat flours. J. Food Sci. 42:461.
- LOWRY, O. H., ROSENBROUGH, N. J., FARR, A. L., and RANDALL, R. J. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265.
- McDONALD, C. E. 1979. Lipxygenase and lutein bleaching activity of durum wheat semolina. Cereal Chem. 56:84.
- MEDCALF, D. G., and GILLES, K. A. 1968. Comparison of chemical composition and properties between hard red spring and durum wheat endosperm pentosans. Cereal Chem. 45:550.
- MEDCALF, D. G., D'APPOLONIA, B. L., and GILES, K. A. 1968. Comparison of chemical composition and properties between hard red spring and durum wheat endosperm pentosans. Cereal Chem. 45:539.
- MILLER, B. S. and JOHNSON, J. A. 1948. High levels of alpha-amylase in baking, II. Proteolysis in straight and sponge doughs. Cereal Chem. 25:178.
- MITSUDA, H., YASUMOTO, K., and YAMAMOTO, A. 1967. Inactivation of lipxygenase by hydrogen peroxide, cysteine and some other reagents. Agric. Biol. Chem. 31:853.
- NAGAO, S., ENDO, S., and TANAKA, K. 1981. The Do-corder as a possible tool to evaluate the bread-making properties of a dough. Cereal Chem. 58:384.
- NAGAO, S., ENDO, S., and TANAKA, K. 1981. Effect of fermentation on the Do-corder and bread-making properties of a dough. Cereal Chem. 58:388.
- N'DOYE, A. S., DRAPRON, R., and NICOLAS, J. 1985. Purification and properties of lipxygenase from Niebe flour. Sci. Aliments 5:464.
- NG, P. K. W., and BUSHUK, W. 1987. Glutenin of marquis wheat as a reference for estimating molecular weights of glutenin subunits by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Cereal Chem. 64:324.
- NICOLAS, J., AUTRAN, M., and DRAPRON, R. 1982. Purification and some properties of wheat germ lipxygenase. J. Sci. Food Agric. 33:365.
- NICOLAS, J., and DRAPRON, R. 1983. Lipxygenase and some related enzymes in breadmaking. In: Lipids in Cereal Technology. (P. J. BARNERS ED.), London: Academic Press. pp. 213-236.

- PAYNE, P. I., CORFIELD, K. G., HOLT, L. M., and BLACKMAN, J. A. 1981. Correlations between the inheritance of certain high-molecular-weight subunits of glutenin and breadmaking quality in progenies of six crosses of bread wheat. *J. Sci. Food Agric.* 32:51.
- OKADA, K., NEGISHI, Y., and NAGAO, S. 1986. Studies on heavily ground flour using roller mills. I. Alternation in flour characteristics through overgrinding. *Cereal Chem.* 63:187.
- PRESTON, K. R., and KILBORN, R. H. 1982. Sponge-and-dough bread: Effects of fermentation, bromate and sponge salt upon the baking and physical dough properties of a Canadian red spring wheat. *J. Food Sci.* 47:1143.
- RING, S. G., and SELVENDRAN, R. R. 1980. Isolation and analysis of cell wall material from beeswing wheat bran. *Phytochemistry.* 19:1723.
- SCHWEITZER, T. F., and WURSCH, P. 1979. Analysis of dietary fibre. *J. Sci. Food Agric.* 30:613.
- SHASTRY, B. S., and RAGHAVENDRA, R. M. R. 1975. Studies on lipoxygenase from rice bran. *Cereal Chem.* 52:597.
- SHIBUYA, N., MISAKI, A., and IWASAKI, T. 1983. The structure of arabinoxylan and arabinoglucuronoxylan isolated from rice endosperm cell wall. *Agric. Biol. Chem.* 47:2223.
- SHIBUYA, N., and IWASAKI, T. 1985. Structure features of rice bran hemicellulose. *Phytochemistry* 24:285.
- SHIIBA, K., NEGISHI, Y., OKADA, K., and NAGAO, S. 1990. Chemical changes during sponge-dough fermentation. *Cereal Chem.* 67:350.
- SHIIBA, K., IJITSU, T., HARA, H., and OKADA, K. 1992. Preparation and characterization of water-soluble hemicellulose (arabinoxylan) from wheat bran. (in English) *Nippon Shokuhin Kogyo Gakkaishi (J. Japanese Soc. Food Sci. and Tech.)*. 39:1147.
- SHIIBA, K., YAMADA, H., HARA, H., OKADA, K., and NAGAO, S. 1993. Purification and characterization of two arabinoxylans from wheat bran. *Cereal Chem.* 70:209.
- SOKOL, H. A., DAHLE, L. K., and SCHIPKE, J. H. 1959. The oxidation of wheat flour. IV. Labile and nonlabile sulfhydryl groups. *Cereal Chem.* 40:515.
- STEVENS, F. C., BROWN, D. M., and SMITH, E. L. 1970. Some properties of soybean lipoxygenase. *Arch. Biochem. Biophys.* 136:413.
- STRAHM, A., AMADO, R., and NEUKOM, H. 1981. Hydroxyproline-galactoside as a protein-polysaccharide linkage in a water soluble arabinogalactan-peptide from wheat endosperm. *Phytochemistry* 20:1061.

- STUTTGEN, E., and SAHM, H. 1982. Purification and properties of endo-1,4- β -xylanase from Trichosporon cutaneum. European J. Appl. Microbiol. Biotechnol 15:93.
- SURREY, K. 1964. Spectrophotometric method for determination of lipoxylase activity. Plant Physiol. 30:65.
- TAKEBE, I., OTSUKI, Y., and AOKI, S. 1968. Isolation of tobacco mesophyll cells in intact and active state. Plant and Cell Physiol. 9:115.
- TANGENDJAJA, B., BUCKLE, K. A., and WOOTTON, M. 1980. Analysis of phytic acid by high-performance liquid chromatography. J. Chromatogr. 197:274.
- TOMIYA, Y., SUZUKI, H., and NISHIZAWA, K. 1968. Chromatographic patterns of cellulose components of Trichoderma viride grown on the synthetic and natural media. J. Ferment. Technol. 46:701.
- TRUONG, V. D., and MENDOZA, E. M. 1982. Purification and characterization of two lipoxylase isozymes from cowpea. J. Agric. Food Chem. 30:54.
- TSEN, C. C., and ANDERSON, J. A. 1963. Determination of sulfhydryl and disulfide groups in flour and their relation to wheat quality. Cereal Chem. 40:314.
- VELDINK, G. A., VliegNTHART, J. F. G., and BOLDINGH, J. 1977. Plant lipoxylase. In: Prog. Chem. Fats other Lipids Vol 14. London.: Pergamon Press., pp. 131.
- WALLACE, J., and WHEELER, E. L. 1979. Two lipoxylase isozymes and an activator in wheat germ. Phytochemistry 18:389.
- WALSH, D. E., YOUNG, V. L., and GILLES, K. A. 1970. Inhibition of durum wheat lipoxidase with L-ascorbic acid. Cereal Chem. 47:119.
- WEBER, K., and OSBORN, M. 1969. The reliability of molecular weight determinations by dodecyl sulfate-polyamide gel electrophoresis. J. Biol. Chem. 244:4406.
- WOOLARD, D. G., RATHBONE, N., and NOVELLIE, L. 1976. Structural studies on a glucuronoarabinoxylan from the husk of sorghum grain. Carbohydr. Res. 51:239.
- YAMAMOTO, A., YASUMOTO, K., and MITSUDA, H. 1970. Isolation of lipoxylase isozymes and comparison of their properties. Agric. Biol. Chem. 34:1169.
- ZIMMERMAN, D. C., and VICK, B. A. 1970. Hydroperoxide isomerase. A new enzyme in lipid metabolism. Plant Physiol. 46:445.

