Studies on Vitality of Commercial Gluten. I. Physical, Chemical and Technological Characteristics[1]

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ABSTRACT

Twenty-seven samples of commercial vital gluten were subjected to various physical, chemical, and technological tests in an attempt to develop a rapid, simple test of vitality for breadmaking purposes. Coefficients of correlation were calculated between test parameters and vitality as measured by the baking test. Of the compositional parameters, sodium content and the ratio of bound lipid to free lipid were significantly positively correlated to vitality of gluten. Of the physical characteristics, force required to stretch a sample of wet gluten was highly significantly correlated with vitality.

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Total world wheat gluten production for 1987–1988 was 240,000 metric tons; most of the production was used by the baking industry (International Wheat Gluten Association, *private communication*). For this application, the gluten must retain as much of its natural viscoelastic properties as possible. Such commercial gluten is generally referred to as "vital" gluten. During processing, some of the vitality may be destroyed by suboptimal processing conditions, especially too high a temperature in the drying stage (Hyldon 1964).

Vitality of gluten is generally assessed by its ability to increase the volume and improve the crumb texture of bread baked from a standard flour fortified with the gluten. Baking tests are time-consuming: accordingly they are not generally suitable for process control.

Several predictive tests of gluten vitality, including water absorption (method 38-20, AACC 1983), extensibility of hydrated gluten (B. W. Morrison, Ogilvie Mills, Montreal, *personal communication*,), and dough mixing properties of blends of gluten, starch, and water solubles (Booth and Timms 1978) or of gluten and starch (Stenvert et al 1981) have been investigated, but all have been only partially successful. It is acknowledged by the gluten industry that a rapid, simple and accurate test of gluten vitality would be extremely useful. Additionally, there is a need of fundamental information on the biochemical, chemical, and physical nature of gluten vitality.

This paper reports results of various chemical and physical tests on 27 samples of commercial vital gluten from international sources in the context of further attempts to develop a practical test of gluten vitality.

MATERIALS AND METHODS

Gluten Samples

Twenty-seven samples of commercial gluten were obtained from international sources through the International Wheat Gluten Association (IWGA). All were used in the preliminary study. Subsequently, on the basis of the preliminary test results, eight samples were selected for further study to represent the full range of vitality covered by the 27 samples.

For comparative purposes, two additional gluten samples were prepared in the laboratory from two widely different Canadian bread wheat cultivars, Katepwa (KP) and HY320 (HY). The grain of the two cultivars was milled into flour on the Buhler experimental mill. Gluten was prepared from the two flours as follows. Flour was made into a dough with water (60% absorption for HY and 65% for KP) in a GRL dough mixer (Hlynka and Anderson 1955) for 2 min. After resting for 30 min, the dough was washed gently in running tap water until the wash water appeared clear. The resulting wet gluten was cut into small pieces and freeze-dried. The dried glutens were crushed by hand and ground in a coffee grinder to a particle size of less than 149 μ m.

Devitalized gluten was prepared by heating dry commercial vital gluten (sample 26) in a closed container at 110°C for 20 hr.

Analytical Methods

Moisture content was determined by an IWGA standard method (IWGA 1983). Ash and protein (N × 5.7) contents were determined using AACC approved methods 08-01 and 46-12, respectively (AACC 1983). Starch content was determined by the method of Aman and Hesselman (1984). Total pentosan content was determined by the method of Cerning and Guilbot (1973). Lipid content was determined by extracting gluten with *n*-hexane followed by extraction with water-saturated *n*-butanol according to Bekes et al (1983). Sodium content was determined with the Perkin Elmer 403 atomic absorption spectrophotometer equipped with a hollow cathode sodium lamp, according to the method described in manufacturer's manual with minor modification. Particle size was determined by sifting 20 g of dry gluten through two U.S. standard sieves, nos. 100 (149-μm openings) and 200 (75-μm openings), for 10 min on a Ro-tap sieve shaker. Average value of duplicate measurements is reported. The rate of hydration and water absorption were measured according to IWGA standard method 102 (IWGA 1983). The gluten stretching test was conducted according to the technique of Matsuo (1978). Wet gluten content was determined according to ICC standard method 137 (1986) using the Glutomatic 2100 (Falling Number AB,

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TABLE I
Range of Moisture, Protein, Ash, Wet Gluten Content, and Falling Number of Gluten-Flour Blends[a]

Parameter	Commercial			Laboratory		
	Range	Mean[b]	CV, %	KP	HY	DG
5:95 Blends						
Moisture, %	12.1–12.3	12.2	0.73	12.0	12.0	11.9
Protein, %	13.0–13.5	13.3	1.2	13.2	13.3	13.3
Ash, %	0.48–0.50	0.49	1.7	0.45	0.47	0.49
Wet gluten, %	28.8–31.4	30.0	3.8	31.5	30.2	28.0
Falling number	334–367	345	3.5	362	342	350
7:93 Blends						
Moisture, %	12.0–12.3	12.2	0.98	12.0	12.0	11.9
Protein, %	14.3–14.9	14.7	1.41	14.5	14.7	14.7
Ash, %	0.47–0.49	0.48	1.74	0.44	0.46	0.48
Wet gluten, %	30.0–32.9	31.2	3.8	33.1	31.6	28.7
Falling number	324–352	336	2.76	347	345	350

[a]All values are expressed on dry matter basis.
[b]$n = 8$.

TABLE II
Range of Farinogram Parameters of Gluten-Flour Blends

Parameter[a]	Range	Mean[b]	CV, %	Base Flour
5:95 Blends				
WA, %	53.1–54.2	53.8	0.42	50.5
DDT, min	1.5–2.5	2.02	13.0	1.3
Stability, min	5.0–10.3	6.8	19.6	1.8
MTI, BU	30–65	47.5	17.0	70
7:93 Blends				
WA, %	53.8–55.3	54.3	0.81	...
DDT, min	1.9–3.5	2.5	15.7	...
Stability, min	5.5–12.2	8.1	20.0	...
MTI, BU	15–55	38.1	21.1	...

[a]WA = water absorption (%, 14% mb), DDT = dough development time (min), Stability = dough stability (min), MTI = mixing tolerance index (Brabender units).
[b]$n = 26$.

TABLE III
Range of Moisture, Ash, Protein, and Starch Contents of 27 Commercial Glutens, and Loaf Volumes of Breads from Gluten-Flour Blends (5:95)

Parameter	Range	Mean[a]	CV, %
Moisture, %	5.5–9.4	7.45	15.0
Ash, %	0.59–1.2	0.83	18.4
Protein, %	73.2–81.8	77.60	2.8
Starch, %	2.7–15.0	10.70	30.4
Loaf volume,[b] cm^3			
Straight dough procedure	515–615	566.5	5.3
Remix procedure	520–600	561.0	4.6

[a]$n = 27$.
[b]Loaf volume of 100% base flour = 370 cm^3.

Stockholm, Sweden).

The Zeleny sedimentation test was carried out according to the AACC approved method 56-60 (AACC 1983) except that the sample size was reduced from 3.2 to 0.75 g. The sodium dodecyl sulfate (SDS) sedimentation volume was determined by the method described by McDermott (1985).

α-Amylase activity was determined by the method of Campbell (1980) as modified by Kruger and Tipples (1981). Exoprotease activity was determined using the method of Bushuk et al (1971) as modified by Macri (1985). Endoprotease activity was determined by the method of Preston et al (1978).

Amino acid compositions were determined on an LKB 4151 Alpha Plus automatic amino acid analyzer. Samples were hydrolyzed in 6*N* HCl for 24 hr at 110°C under vacuum.

Baking Procedure

Flours obtained by blending a commercial soft wheat flour (8.5% protein, 14% mb) and gluten were first checked for water absorption, dough development time, dough stability, and mixing tolerance index on the Brabender Farinograph equipped with 50-g bowl, according to the constant flour weight procedure (method 54-21, AACC 1983). Four replicates of each sample were baked according to the AACC straight dough baking formula and procedure (method 10-10, AACC 1983) and the GRL Remix formula and procedure (Irvine and McMullan 1960).

Statistical Analyses

Data were analyzed by standard statistical methods. Where required, the Student *t* test was used to compare values of means. Linear regression and correlation analyses between analytical data and vitality were executed on the University of Manitoba's Amdahl 5870 computer using the Statistical Analysis System (SAS 1985) program package for REG (linear regression) and ANOVA (analysis of variance).

RESULTS AND DISCUSSION

Breadmaking Quality

The glutens were blended with commercial soft wheat flour at two different levels, 5 and 7%. The reason for choosing these two levels was to bring the protein content of the composite flours up to approximately 11.5 and 12.5% (14% mb): the two protein levels approximate those of commercial bread flours. Some researchers have used starch (Stenvert et al 1981) or starch and water solubles of wheat flour (Booth and Timms 1978) as base materials for blending with gluten to determine its quality for breadmaking. For practical reasons, it was decided to use flour rather than starch in the present study, since this is how gluten is used in the baking industry.

Gluten-flour blends were first analyzed for moisture, protein, ash, wet gluten content, and falling number (Table I). For all parameters except wet gluten content, the value for the blend was the same as the value calculated from the composition. For commercial glutens, 5% addition increased wet gluten content by 1.8–4.4 percentage units, whereas 7% addition increased it by 3.0–5.9 percentage units. Addition of denatured gluten at 5 and 7% increased wet gluten content by 1.0 and 1.7 percentage units, whereas laboratory-prepared glutens increased wet gluten content to 4.5 and 6.1 percentage units for KP and 3.2 and 4.6 percentage units for HY. The increase in wet gluten content was not directly related to the amount of added gluten, indicating variations in vitality of glutens. These differences can be attributed to either the wheat type from which the gluten was obtained or to changes brought about by processing conditions, or both.

Addition of gluten affected the farinograph parameters according to its vitality (Table II). Water absorption increased by 0.5 percentage units for addition of 5% denatured gluten and by 3.8 percentage units for vital KP gluten. For commercial samples, the increase was 2.6–3.7 percentage units. At 7% gluten addition, water absorption increased by 1.0–4.5 percentage units. Dough development time and stability value increased, whereas mixing tolerance index decreased with the addition of gluten.

In preliminary experiments, the baking performance of gluten-flour blends (5:95) was tested by two different baking procedures. The two methods gave approximately the same loaf volumes. However, the AACC method gave slightly larger differences among gluten samples (Table III). Accordingly, this procedure was selected for the remainder of the study.

Four different controls were used in the baking experiments: 1) 100% base flour (soft white spring), 2) base flour fortified with each of the two laboratory-prepared glutens (KP and HY), 3) base flour (8.5% protein, 14% mb) fortified with a high-quality bread flour (13.7% protein, 14% mb) to approximately the same average protein content as that of the gluten-flour blends, and 4) base flour supplemented with devitalized gluten.

On the basis of the two sets of baking results, eight samples were selected for further study to cover the range of vitality represented by the 27 commercial glutens.

A loaf volume of 370 cm^3 was obtained from 100% base flour. At the 5% level of addition of vital gluten, a marked improvement of baking quality was observed: the actual increase of loaf volume varied with gluten sample (presumably because of differences in vitality). When the level of gluten addition was increased to 7%, a further improvement in loaf volume was obtained (Fig. 1).

The laboratory-prepared glutens (KP and HY) gave the best results with the 5:95 blend but only the KP gluten gave the best results for the 7:93 blend. Two commercial glutens (samples 5 and 27) were close to the KP gluten at the 7% level of addition.

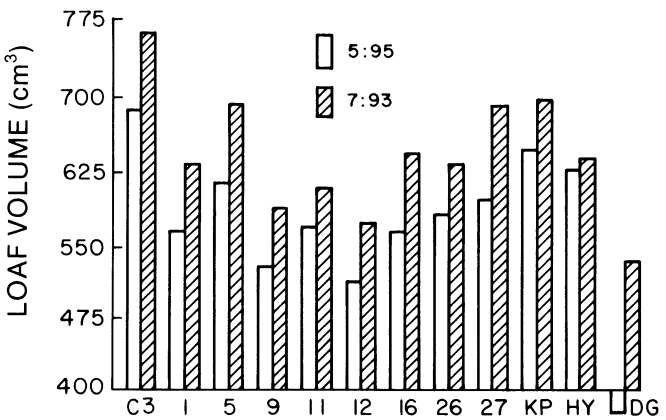

Fig. 1. Loaf volumes of breads from two (5:95 and 7:93) gluten-flour blends and one flour-flour blend (C3). See test for identity of samples.

TABLE IV
Relationship Between Vitality (Loaf Volume of 5:95 Gluten-Flour Blend) and Analytical and Technological Parameters of Gluten

Parameter	*r*	*n*
Ash	0.01	27
Protein	0.26	27
Starch	0.15	27
Pentosan	0.40	8
Free lipid (FL)	−0.52	8
Bound lipid (BL)	0.03	8
Total lipid	−0.23	8
BL:FL	0.73**[a]	8
Sodium	0.79**	8
Particle size (μm)		
>150	−0.12	27
75–150	−0.20	27
<75	0.24	27
Water absorption	0.02	27
Hydration time	−0.58*	27
Stretching force	0.73**	26
SDS sed. vol.		
Unsifted	0.01	8
Sifted	0.35	8
α-Amylase	0.59	8
Exoproteolytic activity	0.02	8
Endoproteolytic	0.53	8

[a] *, ** Significant at 95 and 99%, respectively.

As expected from the known differences in the quality of the flours used to prepare KP and HY glutens, KP gluten performed better than HY gluten. However the difference was not as great as expected from the known difference in quality of the two wheats. The increases in loaf volume obtained by the addition of the two laboratory-prepared glutens were lower than that obtained by fortification with high-quality, high-protein flour indicating that a small amount of "baking quality" is lost even when the gluten is prepared under the most gentle laboratory conditions.

Composition

The 27 gluten samples showed a range of moisture, ash, protein, and starch contents (Table III). The coefficients of correlation between vitality and compositional parameters are presented in Table IV.

Moisture content is important in relation to the cost of the gluten to the user. It is also related to the stability of the gluten during storage (Wooton et al 1982). Because of the hygroscopic nature of vital gluten, moisture content was checked from time to time during the course of this study. While some variation was observed, the content remained under 10% for all the samples.

The amount of starch present in gluten depends upon the efficiency of separation during processing and perhaps on the type of flour used (e.g., soft or hard wheat). To standardize for protein content, gluten is sometimes mixed with flour by the manufacturer. No relationship was found between starch content (or protein content, which is indirectly related to starch content) and vitality.

Pentosan content (Table V) of gluten samples was lower than that found in flour (Holas and Tipples 1978); some of the soluble

TABLE V
Pentosan, Free Lipid (FL), Bound Lipid (BL), Total Lipid (TL), BL:FL Ratio, and Sodium Contents of Gluten Samples

Sample	Pentosan (%)	FL (%)	BL (%)	TL (%)	BL:FL	Sodium (ppm)
01	1.18	0.75	6.20	6.95	8.27	168
05	1.17	0.51	4.80	5.31	9.41	1,950
09	0.95	1.80	6.50	8.30	3.61	192
11	0.71	1.75	6.90	8.65	3.94	92
12	0.68	0.95	4.80	5.75	5.05	60
16	0.60	0.90	6.84	7.74	7.60	60
26	1.11	0.90	6.65	7.55	7.39	825
27	0.78	0.76	5.90	6.66	7.76	2,150
KP	0.86	0.52	7.50	8.02	14.40	110
HY	0.77	0.54	7.10	7.64	13.10	154

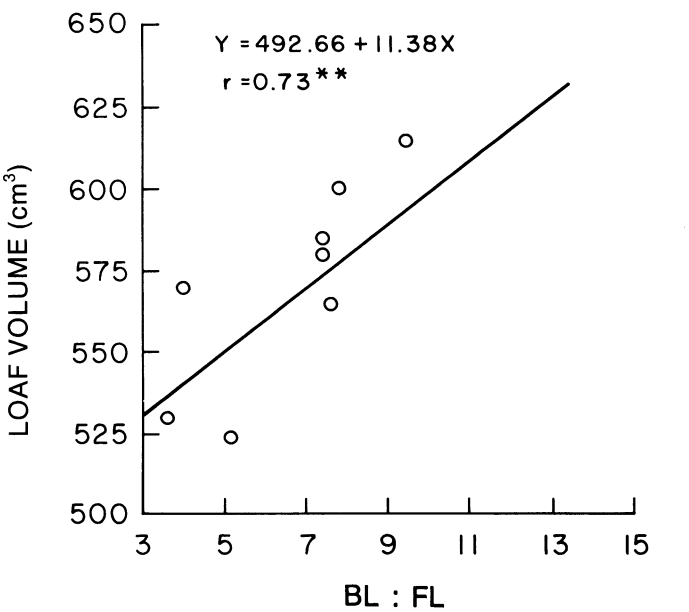

Fig. 2. Relationship between loaf volume of bread from gluten-flour blends (5:95) and the free/bound lipid ratio (**BL:FL**) of the gluten.

pentosan was removed with wash water during gluten separation. Lipid content of commercial glutens varied widely, from 5.31 to 8.65% (Table V), present mainly in bound form. The major portion of the free lipid is not extractable from isolated gluten (Chung 1986). Mecham and Weinstein (1952) reported that salt decreased lipid binding in doughs. These workers also showed lipid content of glutens washed out with salt solution to be lower than of those washed out with water. This is consistent with the observation of the effect of salt on lipid binding in doughs. Lipid contents of samples 5 and 27 were relatively low; these samples were high in sodium, suggesting that salt solution was used in their preparation. Contrary to this observation, sample 12 had low lipid and sodium contents.

No relationship was found between vitality and pentosan content or the lipid content of commercial glutens used in this study. In the case of lipids, however, the ratio of bound to free lipid (BL:FL) was highly significantly correlated with vitality (Fig. 2). The contribution of endogenous lipids to gluten vitality warrants further study.

Sodium content (Table V) appeared to contribute significantly to vitality of gluten ($r = 0.79$, $P < 0.10$). This evidence supports the use of salt by some manufacturers to improve functionality in processing. Whether this improvement is due to a direct effect of salt on gluten proteins or to the improved washing out of certain negative factors and/or retention of positive factors remains to be determined.

Amino acid compositions (not reported) were quite similar for the 10 samples analyzed. Average hydrophobicity, positive and negative charge potentials, charge ratios, frequency of charged groups, and degree of amidization were calculated from the amino acid composition data described by Bigelow (1967). Some differences were found in values of these parameters but the differences do not appear to be related to differences in vitality (Wadhawan 1988).

Data for particle size distribution, water absorption, hydration time, and stretching characteristics of wet gluten samples (Table VI) indicate that commercial glutens are highly heterogeneous. There appears to be no standardization of particle size. As reported by McDermott (1985), the correlation between the particle size distribution and vitality was not significant. However, particle size is important for compatibility with flour; finely ground glutens are preferred for uniform blending.

Variation in water absorption (Table VI) among commercial gluten samples was not related to vitality or to the particle size. However, the rate at which water was absorbed was correlated significantly to vitality ($r = -0.58$, $P < 0.05$).

The stretching test may be useful for assessing gluten vitality in a process where the raw material and the process are reasonably constant. In such cases, differences obtained in stretching values would probably represent true differences in vitality. Despite the use of widely different glutens in this study, a highly significant correlation ($r = 0.73$, $P < 0.10$) was obtained between stretching force and vitality (Fig. 3) confirming the practical utility of the test.

Sedimentation test values (Zeleny and SDS procedures, Table VII) did not distinguish between glutens of low and high vitality. The Zeleny sedimentation values of the 10 samples tested were similar (75 ml). The SDS procedure gave a wider discrimination but the correlation with loaf volume was not significant, contrary to the findings of McDermott (1985). Sample 11 gave the highest sedimentation value; this sample had the highest protein content but did not give the best performance in the bake test. In addition, McDermott (1985) reported that particle size affects the sedimentation values. Accordingly, sifted (throughs of the 149-μm sieve) gluten samples were tested for SDS sedimentation volumes (Table VII). It is not clear why the values for the throughs were higher for some samples and lower for others. When the sedimentation volume was normalized to a constant protein content, it still did not differentiate glutens according to vitality.

Surprisingly, the commercial glutens contained some α-amylase and proteolytic activities (Figs. 4 and 5). α-Amylase values of glutens were similar to those of flours from sound wheat (Koksel 1986). Samples 05 and HY were higher in activity than the other samples. However, the activities of these two samples were not extreme and probably would not contribute sufficiently to the activity of a base flour so as to modify the baking potential of the gluten-flour blend.

Exoproteolytic activity, in general, was in very low in glutens compared with the exoproteolytic activity in flours (Koksel 1986). Sample 05 was considerably higher in endoproteolytic activity than the other commercial gluten samples. Laboratory-prepared glutens retained higher activity than commercial glutens. Redman (1971) obtained evidence that suggested that proteolytic activity in gluten can result from bacterial or mold contamination. It

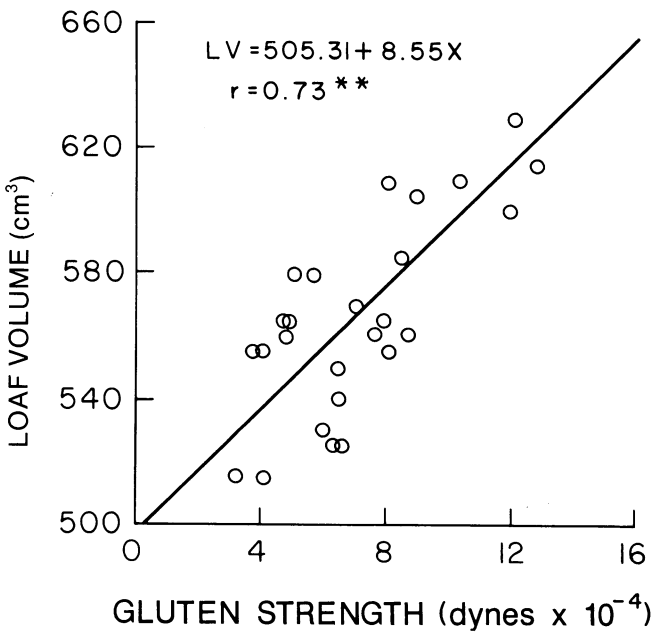

Fig. 3. Relationship between loaf volume of bread from gluten-flour blends (5:95) and strength of the gluten measured with the apparatus of Matsuo (1978).

TABLE VI
Range of Particle Size, Water Absorption, and Hydration Time of Gluten Samples

Parameter	Commercial			Laboratory	
	Range	Mean[a]	CV, %	KP	HY
Particle size					
>149 μm	2.4–35.4	16.3	69.3	0	0
75–149 μm	35.9–92.7	59.6	21.9	58.5	63.8
<75 μm	3.5–47.0	22.9	52.8	41.5	36.2
Water absorption, g/100 g	195–287	223.6	9.7	263	240
Hydration time, min	0.2–5.0	2.3	68.0	0.3	1.0

[a] $n = 27$.

TABLE VII
SDS[a] Sedimentation Volume of Unsifted and Sifted Gluten Samples

Sample	Volume (ml/g of protein)	
	Unsifted	Through 149 μm
01	88.3	34
05	100.6	150
09	111.7	105
11	156.8	146
12	76.3	97
16	86.0	104
26	65.3	80
27	84.3	125
KP	170.0	170
HY	167.0	167

[a] Sodium dodecyl sulfate.

is not known if the proteolytic activity in samples analyzed in this study are endogeneous or from microbial contamination. An extensive study would be required to clarify this point and to determine if the proteolytic activity of dry commercial gluten contributes to its functionality in breadmaking.

CONCLUSION

Significant correlation was obtained between vitality of commercial gluten, as measured by the bake test, and the ratio of bound to free lipid. However, it was concluded that a quality control test based on this correlation would not be practical because accurate determination of free lipid and bound lipid contents is difficult. Also, it was found that vitality was significantly positively correlated to sodium content of gluten; this correlation cannot be used as a predictor of vitality because not all manufacturers use salt (NaCl) in the separation of gluten. A highly significant correlation was obtained between force required to stretch a sample of hydrated gluten and vitality; this test would be useful for process quality control under defined and controlled processing conditions.

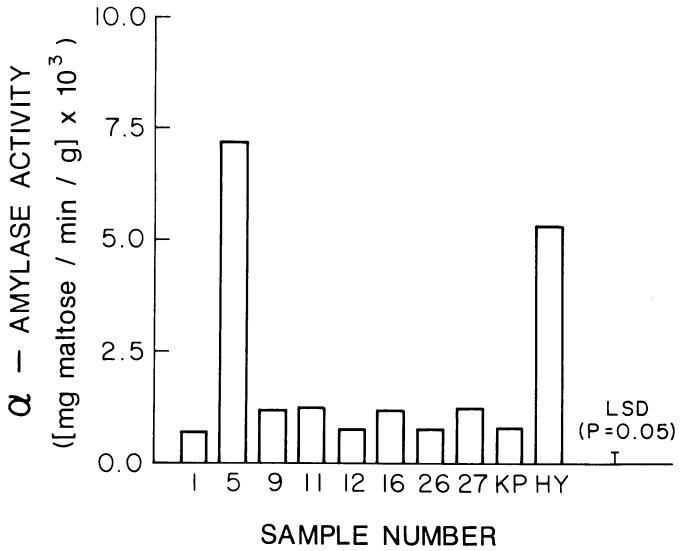

Fig. 4. α-Amylase activities of glutens. Sample characteristics are listed in Table V. (LSD = least significant difference.)

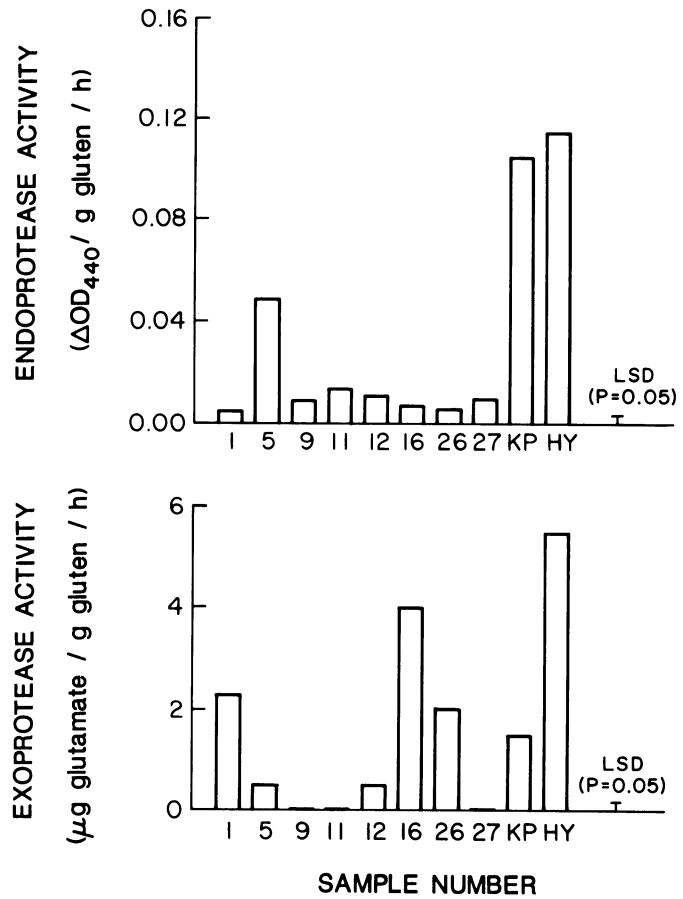

Fig. 5. Proteolytic activites of glutens. Sample characteristics are listed in Table V. (LSD = least significant difference.)

ACKNOWLEDGMENTS

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