ELECTROPHORETIC COMPOSITION AND INTRINSIC VISCOSITY OF GLUTENS FROM DIFFERENT VARIETIES OF WHEAT[1]

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ABSTRACT

The protein compositions of hard and soft wheat flours were compared by electrophoresis of the gluten and water-soluble constituents fractionated from selected samples of the defatted flours. Although the amount of total protein was higher in the hard wheat than in the soft wheat flours, the amount of water-solubles, as recovered from gluten washing, was essentially the same. Hard wheat flours contained more of the electrophoretic components alpha, gamma, and omega but the same amounts of beta and fast components. A comparison based on gluten rather than on the flour was also made. When defined as the sum of the alpha, beta, gamma, and omega components, the gluten proteins of the hard wheat flours contained more alpha, less beta, and identical amounts of gamma and omega components. The reported differences were small but statistically significant. Purified gluten fractions from the hard wheats exhibited the greater intrinsic viscosities. This difference probably reflects the higher alpha content in the hard wheat class.

Variations in composition of proteins from different varieties of wheat have been investigated by many cereal chemists (3). Chemical analyses and physical-chemical methods have been employed. One of the more important physical techniques for the characterization of protein mixtures is moving-boundary electrophoresis. Wheat gliadin electrophoresis studies were reported by Schwert, Putnam, and Briggs (6) in 1944. A review of wheat protein electrophoresis has recently been made by Abbott (1).

In 1948 Laws and France (5) described electrophoresis studies on four hard red winter wheat glutens. This comparative study showed no significant difference in the gluten protein of several good and poor quality flours. Unlike their investigation, the aim of this work was to find whether significant electrophoretic compositional differ-

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ences exist between hard and soft wheat gluten proteins.

The lack of good buffer systems for gluten electrophoresis has been a major handicap in using this analytical tool for such investigations. Recently, Jones, Taylor, and Senti (4) described a buffer system that produced symmetrical patterns in the ascending and descending limbs. This advance now offers a better opportunity for detecting possible differences in the composition of wheat proteins.

Materials and Methods

The basic materials used in this study were nine samples of hard red winter wheat flour representing three different varieties grown at three locations, and eight varieties of soft wheat flour. The hard wheat varieties were Concho, Ponca, and CI 12871; they were grown at Manhattan, Powhattan, and Hutchinson, Kansas. The soft wheat class was represented by American Banner, Thorne, Clarkan, Trumbull, Kawvale, Fairfield, Blackhawk, and Wabash, all grown at Wooster, Ohio. All flours were Buhler-milled, straight grade flours and were from the 1957 crop. The protein range of hard wheat flours was 10.8 through 14.9% and that of the soft flours, 9.0 through 10.5%. Duplicate fractionations and analyses were made on some of the flour samples.

The method of separating the gluten and water-soluble fractions from the wheat flours differed in minor respects from that recently described by Jones and co-workers (4). Starch and other nongluten materials were washed from 25 g. of butanol-defatted flour by kneading the dough ball between two stirring rods in multiple 10-ml. aliquots of 0.1% sodium chloride solutions. All solutions and containers were chilled in an ice water bath. The wash solution containing the water-soluble materials was decanted into centrifuge tubes and centrifuged (2,000 × G). After the supernatant solutions were collected, the remaining starch cake was slurried with additional sodium chloride solution to assure dissolution of any remaining solubles. After a second centrifugation, the supernatant was combined with the first, and the starch remaining was discarded. The combined water-soluble fraction was lyophilized.

The ball of washed gluten was dispersed at about 4% protein concentration in 0.01*N* acetic acid in a Waring Blendor[3]. The dispersion was centrifuged at 21,000 × G. The clear supernatant solution was heated to 98°–100°C., cooled quickly, and then lyophilized. Protein was determined by the Kjeldahl procedure (N × 5.7) for all the isolated gluten and soluble fractions, as well as for all the defatted

[3] Reference to specific equipment or organizations does not necessarily constitute endorsement by the U. S. Department of Agriculture.

flour samples.

Solutions for electrophoresis were prepared by dissolving known weights of the gluten and water-soluble fractions in 0.017*M* aluminum lactate-lactic acid buffer, pH 3.2, conductivity 600 to 610 micromhos at 1°C. The aluminum lactate was a recrystallized preparation of commercial aluminum lactate. Further recrystallization did not alter the conductivity. Average solution concentrations used were 0.5% for the gluten and 0.4% for the water-solubles. Dialysis in a large volume of buffer stored in a refrigerator preceded electrophoresis. Dilution was determined by weighing the dialysis bags before and after dialysis. The gluten solutions were then filtered through coarse fritted funnels and stored in the cold in tightly stoppered bottles; the water-soluble solutions were not filtered but were centrifuged for 10 minutes in a Sorvall laboratory centrifuge.

Electrophoresis runs were carried out at 1°C. in a Spinco Model H electrophoresis apparatus equipped with a cylindrical-schlieren lens system and a Rayleigh interference system. Gluten solutions were run for 130 minutes and the water-solubles for 90 minutes, both at 8.8 v. per cm. Photographs of the moving boundary were taken at regular intervals. The relative concentration of each component was determined from the relative number of Rayleigh fringes lying below the peak in the schlieren diagram. Fringes in the delta and epsilon boundaries were ignored in this calculation.

Total protein concentration was calculated from the total number of fringes in the gluten fractions and from the fringes corresponding to the moving boundaries in the water-soluble fractions, using a specific refractive increment of 0.00188, in units of 100 ml. per g. With a 2.5-cm. thick cell, 172 fringes corresponded to 1% gluten concentration. The protein in the original weighed sample of fraction was calculated from the concentration in solution after correction for dilution during dialysis.

Viscosity measurements were made only on the gluten fraction using the same solution as for electrophoresis. Flow times of the original solution and of three dilutions were determined in Ostwald viscometers at 24.9°C., constant within 0.01°C. The values of intrinsic viscosity were then derived from a plot of $(\eta_{rel}-1)/c$, with c as the concentration and η_{rel} the ratio of outflow times of solution to solvent. The concentration of the undiluted gluten solution was determined by differential refractometer measurements.

Results

Kjeldahl analyses of the defatted flour samples are summarized in

TABLE 1

FRACTIONATION AND ELECTROPHORETIC COMPOSITION OF FLOURS AND INTRINSIC VISCOSITY OF GLUTEN FRACTIONS

VARIETY	WEIGHT PERCENTAGE FOUND IN FLOUR OF 14% MOISTURE													(C) INTRINSIC VISCOSITY
	(A) PROTEIN BY KJELDAHL IN:			(B) ELECTROPHORETIC COMPONENTS FOUND										
	Flour	Gluten	Water Solubles	f_D[a]	f_A	Alpha		Beta		Gamma		Omega		
						D	A	D	A	D	A	D	A	
Hard:														
Concho M[b]	10.84	8.08	1.04	1.28	1.17	4.79	4.34	1.43	1.40	0.87	1.36	0.11	0.23	0.33
Concho H-1[c]	11.52	8.68	1.08	0.98	0.87	4.73	4.31	1.06	1.36	1.06	1.29	0.23	.19	.39
Concho H-2	11.52	8.80	1.04	1.17	1.10	5.45	4.99	1.17	1.51	1.44	1.55	0.15	.23	.35
Concho P	13.54	10.32	1.12	1.32	1.13	5.71	5.18	1.93	2.08	1.02	1.25	0.19	.45	.37
CI-12871-M	11.45	8.40	1.08	1.18	1.06	5.04	4.47	0.72	1.55	1.63	1.36	0.15	.19	.38
CI-12871-H-1	11.95	9.32	1.00	1.13	1.09	5.99	5.16	1.58	1.85	1.24	1.51	0	.30	.34
CI-12871-H-2	12.06	9.44	1.00	1.09	1.02	5.84	5.32	1.17	1.43	1.58	1.96	0.30	.30	.32
CI-12871-P	14.75	11.12	1.16	1.25	1.17	7.05	6.00	1.28	1.96	2.19	2.19	0	.42	.35
Ponca M-1	11.92	8.68	1.12	1.25	1.14	5.18	4.65	1.14	1.48	1.40	1.51	0.19	.23	.31
Ponca M-2	11.37	8.32	1.08	1.29	1.25	5.14	4.58	0.91	1.40	1.51	1.55	0.19	.19	.34
Ponca H-1	11.62	8.60	1.04	1.44	1.21	5.21	4.76	1.32	1.62	1.36	1.55	0.11	.30	.35
Ponca H-2	11.75	8.64	1.08	1.25	1.13	5.29	4.72	1.25	1.59	1.32	1.51	0.26	.34	.33
Ponca P	14.85	11.24	1.28	1.36	1.25	6.98	6.30	1.81	2.00	1.58	2.15	0.38	0.38	0.36
Soft:														
Am. Banner-1	8.87	5.96	1.08	1.19	1.11	3.42	3.11	1.04	1.23	0.88	1.08	0.19	0.19	0.35
Am. Banner-2	9.25	6.52	1.12	1.11	1.04	3.46	3.11	1.04	1.27	0.81	1.08	0.23	.23	.28
Thorne	9.05	6.92	1.08	1.31	0.96	4.16	3.96	1.08	1.35	1.04	1.19	0.23	.23	.30
Clarkan-1	10.01	7.64	1.04	1.23	1.15	4.52	4.02	1.38	1.65	1.07	1.34	0.19	.23	.35
Clarkan-2	9.80	7.88	1.04	1.19	1.11	4.14	3.71	1.34	1.57	1.00	1.23	0.15	.15	.31
Trumbull	9.27	7.24	1.04	0.88	0.88	4.68	4.15	1.11	1.46	1.15	1.34	0.12	.15	.31
Kawvale	10.04	7.48	1.08	1.19	1.15	4.46	3.92	1.12	1.31	1.23	1.38	0	.23	.30
Fairfield	9.33	6.80	1.08	1.20	1.12	3.94	3.59	1.04	1.20	1.00	1.20	0.19	.15	.30
Blackhawk-1	10.46	8.12	1.16	1.34	1.22	4.21	3.79	1.15	1.49	1.26	1.38	0.19	.19	.29
Blackhawk-2	10.48	8.16	1.12	1.42	1.26	4.44	4.05	0.99	1.49	1.61	1.57	0.19	.19	.30
Wabash	9.40	7.04	1.08	1.34	1.15	3.82	3.55	1.15	1.49	0.99	1.03	0.19	0.23	0.28

[a] The letters D and A refer to descending and ascending limbs of the electrophoresis cell.
[b] The letters M, H, and P refer to wheat grown at Manhattan, Hutchinson, or Powhattan, Kansas.
[c] The numbers (1) and (2) refer to analyses on duplicate fractionations of the same flour sample.

Table I(A). Recovery of nitrogen in the gluten and water-soluble fractions is also given. About 10 to 20% of the nitrogen was lost in fractionation. Experiments on a Ponca flour indicated that about one-third of this unaccounted-for protein was in the discarded starch, and about two-thirds was acid-insoluble material which did not go into solution in acetic acid.

Figure 1 shows typical electrophoresis patterns of a hard and soft wheat gluten and the corresponding water-soluble fraction. Only ascending limbs are shown because in the aluminum lactate buffer the limbs are nearly identical.

Five peaks, designated alpha, beta, gamma, omega, and delta, appear in the gluten electrophoresis patterns. Other workers have reported that two alpha peaks (4) were resolved in moving-boundary electrophoresis of Ponca gluten in other buffer systems. Also, four beta peaks (8) have been resolved in the starch gel electrophoresis of Ponca gluten. However, in the moving-boundary system using aluminum lactate buffer, the alpha components yield only one peak. In one case, mentioned below, two beta peaks were observed. The components are treated as single species units in this work. The delta peak represents the salt boundary. The material preceding the main gluten

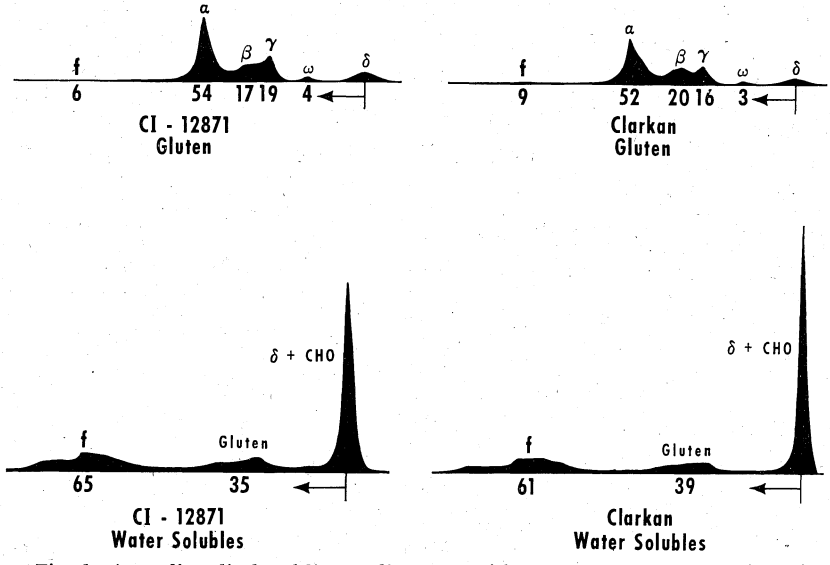

Fig. 1. Ascending limb schlieren diagrams with component concentrations for hard wheat (CI-12871-P) gluten and water-solubles and for soft wheat (Clarkan-1) gluten and water-solubles. Components are designated as α (alpha), β (beta), γ (gamma), ω (omega), and f (fast). The position of the initial boundary and immobile material is δ (delta).

protein group is designated as *f*, indicating fast moving. The value given under each peak (Fig. 1) is the percentage of component calculated from the ascending pattern.

The pattern of the water-solubles shows three main regions. The fastest moving component labeled *f* is evidently heterogeneous material, which probably contains the albumin and globulin components. The middle section is the gluten area, so labeled because of similar mobilities to isolated gluten. The large stationary peak is a composite of the salt boundary and soluble carbohydrate material. This large peak is not included in the calculation of protein concentration.

Relative concentrations of components measured somewhat differently in the two limbs of the electrophoresis cell. In Table I(B) are given the total amounts of each component in the flours. These figures are the sums of the protein contained in the gluten and water-soluble fractions. The totals calculated in ascending and descending limbs are given separately.

In the typical electrophoresis analysis given in Fig. 1, the proportionation among the components in each fraction is shown. The total concentration of protein found in the gluten solution corresponded to 10.9% of the weight of 14%-moisture flour for CI 12871-P, and 7.6% of the flour for Clarkan-1. These values agree with the Kjeldahl analyses, 11.1% and 7.6%, respectively, in Table I(A).

The total concentration of moving material in the water-soluble fractions represented 0.83 and 0.80% of the flour for CI 12871-P and Clarkan-1, respectively. The corresponding Kjeldahl analyses, 1.6 and 1.04%, respectively, were greater because some of the nitrogen in this fraction is dialyzable.

From the total protein accounted for by electrophoresis of each fraction and the proportion of each component in each fraction, the calculated total percentage amount of each component is shown in Table I(B). The mobility of each component was the same in all samples and in both classes within experimental error. The mobilities expressed in units of 10^{-5} cm.^{2} per volt-second are:

Component	*Ascending Limb*	*Descending Limb*
Alpha	5.58	5.26
Beta	3.95	3.79
Gamma	3.26	3.07
Omega	1.94	1.40

These values are somewhat lower than those of Jones *et al.* (4). The difference lies in the concentration of aluminum lactate, which was somewhat lower in their work, as shown by the conductivity of the buffers.

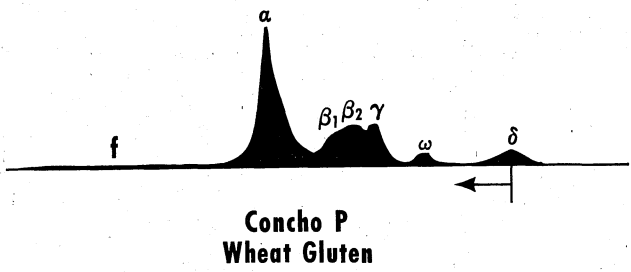

Fig. 2. Ascending limb schlieren diagram of hard wheat (Concho P) gluten showing a double beta peak (β_1 and β_2).

Figure 2 shows the ascending limb for electrophoresis of hard wheat Concho P gluten. This gluten clearly demonstrates a double beta peak. Although splitting of the beta peak was not unusual after 2 to 3 hours' electrophoresis, this is the only gluten from the 17 surveyed that showed the double beta peak throughout the run. The Concho P variety had the highest content of beta in flour and in gluten.

Discussion

The results reported in Table I show that there were no gross qualitative differences among the various flours investigated, either in kind of electrophoretic components present or even in relative amounts of components. Analysis of variance was applied to determine significant sources of variation in the data.

These analyses are subject to the usual uncertainties of electrophoresis. The different peaks were only partially resolved, and the results in the two different limbs of the electrophoresis cell were not identical. Further, the concentrations as measured across the boundaries of any individual component may be considerably biased compared to the true concentrations of component (2). In this study, however, the various samples were fractionated and analyzed under identical conditions, and any bias not taken into account by the statistical procedure should affect all analyses to about the same extent.

Fractionation. The mean values of nitrogen analyses of the fractions are summarized for each class in Table II. The probabilities, P, are shown for significance of the variance between classes, compared to that of samples within a class, and also for significance of the variance between samples in a class, compared to that between duplicate fractionations.

The hard wheat flours contained the higher percentage total protein and gluten, showing that the samples were normal in terms of

TABLE II

PERCENTAGE DISTRIBUTION OF NITROGEN (AS PROTEIN) IN FRACTIONS ISOLATED FROM HARD AND SOFT WHEAT FLOURS (14% MOISTURE) AND ANALYSIS OF VARIANCE

MATERIAL	MEANS, % OF FLOUR		P, %	
	Hard	Soft	Between Classes *vs.* Samples in Class	Between Samples in Class *vs.* Duplicates
Total protein	12.24	9.63	>99.95	>99.95
Gluten	9.20	7.25	>99.95	>99.95
Water-soluble	1.09	1.08	NS[a]	98
Unaccounted	1.95	1.30	>99.95	97.5

[a] NS denotes a probability (P) value of less than 90%.

protein content.

The water-soluble fraction was less variable among samples, however, and no significant difference was found between these fractions in the two classes of flour. The relation of the *f* material in this fraction to the gluten components is discussed under electrophoretic composition.

Finally, the amount of unaccounted nitrogen was greater in the hard wheat flours than in the soft. This result may indicate a difference in protein composition since the unaccounted nitrogen is also a larger fraction of the total nitrogen in hard wheats. There is some evidence in other work that this material is a different protein or, perhaps, an insoluble alpha component since, in one case examined in detail, the unaccounted material was largely acid-insoluble.

Electrophoretic Composition. The mean amounts of each component found in the flours are summarized for the two classes in Table III. The P values, for comparison of variances between classes with those of samples in a class, were high for all components except *f*, which appears to be relatively nonvariant. This material represents two-thirds of the water-soluble fraction (Fig. 1), which was relatively nonvariant compared to the other fractions. The different amino acid composition of the water-soluble fraction, as determined by Woychik

TABLE III

MEAN PERCENTAGES OF ELECTROPHORETIC COMPONENTS IN FLOURS (14% MOISTURE) AND ANALYSIS OF VARIANCE

COMPONENT	MEANS, % OF FLOUR		P, %	
	Hard	Soft	Between Classes *vs.* Samples in Class	Between Samples in Class *vs.* Duplicates
f	1.18	1.16	NS[a]	NS
Alpha	5.27	3.92	99.95	98
Beta	1.46	1.27	90	93
Gamma	1.50	1.18	98	95
Omega	0.23	0.18	95	NS

[a] NS denotes a probability (P) value of less than 90%.

TABLE IV

MEAN PERCENTAGE OF ELECTROPHORESIS COMPONENTS IN GLUTEN PROTEIN ($\alpha + \beta + \gamma + \omega$)

COMPONENT	MEANS, % OF GLUTEN		P, %	
	Wheat Flours		Between Classes *vs.* Samples in Class	Between Samples in Class vs. Duplicates
	Hard	Soft		
Alpha	62.34	59.77	>99.95	>99.95
Beta	17.26	19.47	97.5	NS
Gamma	17.68	17.81	NS[a]	NS
Omega	2.72	2.85	NS	NS

[a] NS denotes a probability (P) value of less than 90%.

et al. (7), is additional evidence that the *f* component is distinct from the gluten components.

There were more of the remaining components, alpha, beta, gamma, and omega, in the hard wheat flours. This consequence is largely caused by the greater protein content of hard wheat flours since these components represent most of the protein.

Calculation of component concentration on the basis of gluten (Table IV) provides a better comparison than one on the basis of flour because the direct effect of variation in total gluten protein can thereby be eliminated. The gluten proteins were assumed to be the sums of alpha, beta, gamma, and omega components. Apparently, there is significantly more alpha in hard wheat gluten proteins and less beta. Table IV shows clearly that the gluten proteins do not vary proportionately with the total gluten protein.

The P values, for comparison of variance between samples within the classes with variance between duplicates, are also given in Table IV. These figures show that alpha, especially, was more variable than was ascribable to duplication of fractionated analyses. Evidently glutens are quite definitely variable in composition within a class, too. The analytical method is precise enough to demonstrate this variability between samples even though the variability of gluten composition is relatively small.

Although proteins in the flour samples investigated are significantly variable in composition, there is still some uncertainty in ascribing the source of observed variability to the two classes of wheat. Variability of gluten composition can be the composite of several sources which cannot be differentiated by the statistical procedure. In principle, sources of variance among the flour samples are those between classes, between varieties within a class, and between conditions under which the wheat was grown and handled.

Because samples from the two classes of wheat were grown at two

locations, the variance observed between classes is a composite of the three sources. The variance between the soft wheat samples, all grown at one station, is a composite of two sources: varieties and growing conditions. The samples of hard wheats, three varieties each grown at three locations, can ideally be used to distinguish variance between varieties from variance between growing conditions. When this test was made, neither source was significant for any component of gluten. Therefore, because the variance between the hard wheat samples due to location was not significant and because the variance between classes was relatively large for the alpha content of gluten, it can be tentatively concluded that the two classes of wheat have glutens of different composition.

The soft wheat flours contained distinctly less protein than did the hard wheat flours. Because of this difference, it might be postulated that the greater alpha content of the glutens of the hard wheat flour was a function of protein content rather than class of wheat. Information on this point was obtained by determining the coefficient of correlation for α content in gluten with protein content in the flour. This correlation was not significant (P was about 80%), nor were any of the other gluten components correlated with protein content of flour. Thus, based on this tentative evidence, the class of wheat is a more significant source of variation in gluten composition than is protein content of flour.

Another point is the estimation of the possible range in alpha content of glutens of various flours. The observed range was 5% of alpha. Most of this range is attributable to differences among wheat samples because 5% was significantly greater than the variation between duplicates. Furthermore, if the unaccounted material lost in fractionation was also partly alpha, as suggested, then the range of gluten protein composition may be greater than it appears from Table IV since a greater proportion of protein was lost from the hard wheat samples. In any case, enough variation in the alpha component has been found to suggest that some of the variation in gluten properties may result from variability in gluten protein composition. This idea is strengthened by intrinsic viscosity measurements.

Intrinsic Viscosities. In other works to be published[4], the alpha component had a higher intrinsic viscosity than the other major components. The intrinsic viscosity of gluten in aluminum lactate is thus largely dependent on the alpha protein.

Average intrinsic viscosity of the hard wheat glutens was 13% higher than that of the soft wheats, and the two classes were signifi-

[4] Taylor, N. W., Cluskey, J. E., and Senti, F. R., unpublished data.

TABLE V

INTRINSIC VISCOSITIES OF GLUTENS AND ANALYSIS OF VARIANCE

MEANS, 100 ML/G		P, %	
Wheat Flours		Between Classes *vs.* Samples in Class	Between Samples in Class *vs.* Duplicates
Hard	Soft		
0.35	0.31	99.8	NS[a]

[a] NS denotes a probability (P) value of less than 90%.

cantly different (Table V). Thus, the higher intrinsic viscosities of the hard wheat flours correlate with their higher alpha content. The glutens of the two classes are definitely different in both protein composition and properties, although this difference is a small one.

Although intrinsic viscosities were 13% greater in the hard wheat glutens, the average alpha content was greater by only 7% of the mean. This fact suggests that the relatively minor variation in components may cause marked changes in physical properties. At the same time, it is not suggested that the main source of variability in gluten properties is protein composition; instead, protein composition is one possible, and rather likely, source of variability.

Acknowledgments

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