A COMPARATIVE STUDY OF THE MONOLAYERS OF VARIOUS CEREAL PROTEINS AND OF WHEAT GLUTENS OF DIFFERING CHARACTERISTICS

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Summary

Surface films of the proteins extracted by anhydrous acid chloroethanol from strong wheat, weak wheat, rye, barley, oat, defatted oat, and carob germ flour were examined by monolayer techniques at the air/water and the oil/water interface. All proteins gave "ptygmatic" films showing very little differences in the II–A isotherms. They were clearly differentiated in their surface rheological characteristics. These studies indicated that the limiting surface viscosity at infinite time and the rate constant of the surface viscosity would be the most likely characteristics to differentiate between gluten films from different wheat flours. A comparative study of five different wheat glutens spread from dispersion in aqueous acid chloroethanol showed significant differences in the limiting surface viscosities but differences in the rate constants were not significant statistically.

I. INTRODUCTION

In two previous papers (Tschoegl and Alexander 1960*a*, 1960*b*) monolayer studies at the air/water (A/W) and the oil/water (O/W) interface were reported on films of wheat gluten, the elastic coherent protein complex obtained by washing out the starch and water-solubles from a wheat flour dough. The present paper extends these basic studies to a comparative study of monolayers of different cereal proteins and of different wheat glutens. The first part was carried out chiefly to obtain pointers which could be followed up in comparing the proteins from wheat flours of different baking quality and handling properties. Such differences in wheat flours are reflected, on an exaggerated scale, in similar differences among cereals. Of the commoner cereals only wheat and rye may be regarded as bread cereals. From barley and oats it is not ordinarily possible to obtain an aerated loaf of bread. The protein of the germ of the carob bean, *Ceratonia siliqua*, was included in this study since it is the only known non-wheat plant protein capable of forming a coherent gluten.

It is hoped that these investigations will eventually contribute to our understanding, in terms of gluten structure, of the reasons for differences in the baking quality and handling properties of different wheat flours. A possible practical application would be in the field of wheat breeding. Very small samples of test material are sufficient for examination by monolayer techniques. If a reliable correlation can be found between film properties and flour quality, the baking characteristics of a new wheat strain could be predicted from quite small samples.

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II. Materials and Methods

Table 1 shows the analytical and physical testing data of the flours used. The procedures by which these flours were obtained and the analytical methods employed are described elsewhere (Tschoegl 1961). Defatted oat flour was prepared by extracting the oat flour twice for 5 hr with petroleum ether (boiling range 40–60°C).

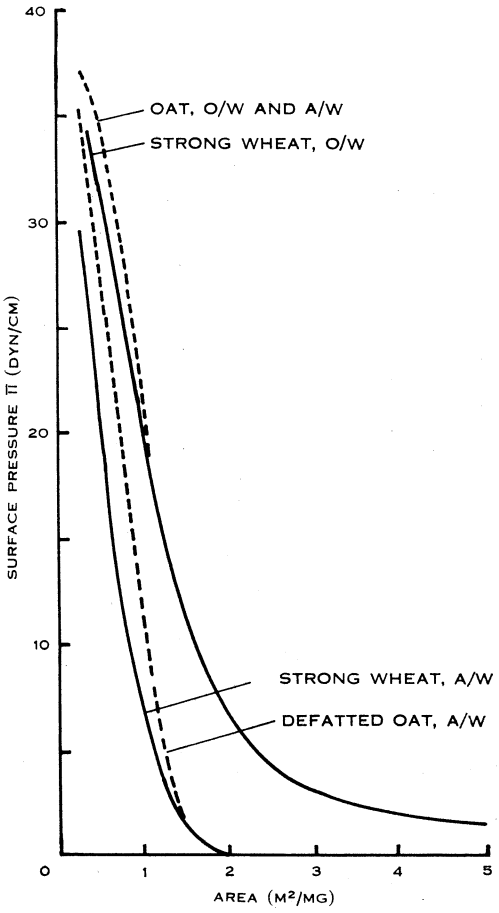

Fig. 1.—Surface pressure–area isotherms at pH 6·8 and $\mu = 0{\cdot}1$.

The wheat flours were chosen according to "strength" (baking quality) and "balance" (extensibility of the dough) as determined on the Chopin alveograph. The strong, medium, and weak flours were "well-balanced", while the harsh, medium, and extensible flours were all of medium strength.

The dispersions used in the comparative study of cereal proteins were prepared by direct extraction of the flours with a 0·1N solution of hydrogen chloride in carefully purified anhydrous 2-chloroethanol. The dispersions used in the com-

TABLE 1

ANALYTICAL AND PHYSICAL TESTING DATA OF FLOURS

	Strong Wheat	Weak Wheat	Medium Wheat	Harsh Wheat	Extensible Wheat	Rye	Barley	Oat	Defatted Oat	Carob Germ
Protein (% dry basis)	11·71	9·92	12·50	10·60	10·12	6·52	6·15	8·20	10·52	62·2
Moisture (%)	15·0	12·8	14·4	14·6	14·0	13·4	12·2	9·2	10·8	9·3
Ash (% dry basis)	0·55	0·53	0·48	0·57	0·46	0·38	0·59	0·74	—	—
Fat (% dry basis)	1·91	2·06	1·80	1·97	1·85	1·56	1·70	8·68	2·37	9·20
Fat/protein ratio	0·16	0·21	0·14	0·19	0·18	0·24	0·28	1·06	0·23	0·15
Alveogram "strength"	84	13	48	46	35	—	—	—	—	—
Alveogram "balance"	1·23	1·10	1·42	0·75	3·56	—	—	—	—	—

parative study of wheat glutens were prepared by dispersing the vacuum-dried glutens in a 0·01N solution of hydrochloric acid in aqueous 2-chloroethanol containing 30% water. The preparation of the glutens and the dispersants is described in detail elsewhere (Tschoegl 1961).

Films were spread at the interface between air or carbon tetrachloride and various aqueous substrates (buffers of different pH and ionic strength (μ)) from an all-glass "Agla" syringe as detailed by Tschoegl and Alexander (1960*a*). Surface pressure measurements were carried out with two hanging plate surface balances, either in a Langmuir trough (A/W), or in a circular crystallizing dish (O/W). Surface viscosity and rigidity were determined using an oscillating needle torsion pendulum. Details of apparatus and methods are given in the two preceding papers (Tschoegl and Alexander 1960*a*, 1960*b*). All curves shown represent the average of at least two runs. The measurements were carried out at $25 \pm 0 \cdot 5$°C.

III. Results

Surface pressure (Π, dyn/cm)–area (A, m²/mg) curves were obtained at the A/W interface at pH 6·8 (μ 0·02 and 0·1) and at the O/W interface at pH 6·8 and 2·1 (μ 0·1) from the extracts of strong and weak wheat flour, rye, barley, oat, defatted oat, and carob germ flour.

All the extracts behaved very similarly to gluten (Tschoegl and Alexander 1960*a*) in all essentials, showing great stability, high compressibility (about 0·05 cm/dyn at about 1·1 m²/mg), hysteresis effects depending on the ionic strength of the substrate, an effect of pH on the expansion of the isotherms at areas above about 1·1 m²/mg, and little effect of ionic strength on the isotherms. All compressibility–area curves also showed, although in varying degrees, the characteristic kink between 2·5 and 3·5 m²/mg at the O/W interface.

The wheat, rye, barley, and carob germ isotherms were virtually identical at the O/W interface and are represented in Figure 1 by the strong wheat isotherm. There was a little more variation at the A/W interface where the weak wheat, rye, barley, and carob germ isotherms fell slightly to the right of the strong wheat isotherm shown in Figure 1. The oat isotherm at the O/W interface departed from the others at areas below about 1·1 m²/mg. At the A/W interface the behaviour of the oat isotherm was abnormal in that it was identical with that obtained at the O/W interface. The defatted oat flour extract, however, gave the A/W isotherm shown in Figure 1 while at the O/W interface the isotherm was identical with the undefatted oat isotherm. The undefatted oat flour had a much higher fat/protein ratio than the other samples (cf. Table 1). The softening point of the oat fat was much lower than that of the others. This oil could be expected to be spread along with the protein from the chloroethanol and the undefatted oat flour therefore appears to have been spread as a mixed film.

Since the surface viscosity (η_s, surface poise) and surface rigidity (G_s, dyn/cm) of wheat gluten had shown a marked dependence on the pH of the substrate (Tschoegl and Alexander 1960*b*) a study of this effect was made on the same extracts by measuring η_s and G_s as a function of the area at the O/W interface. These measurements were carried out at pH 6·8 and 2·1 at an ionic strength of 0·1.

Plots of η_s and G_s against area gave very similar pictures. Figure 2, therefore, shows plots of the absolute surface modulus ($\overline{G}_s$, dyn/cm), a combination of η_s and G_s (Tschoegl and Alexander 1960*b*), against area. In order of increasing modulus at the same area the films fall in the order: strong wheat, weak wheat, rye, barley, oat, carob. The pH of the substrate is seen to have an effect on $\overline{G}_s$ in all cases, resulting in a shift of the curves obtained at pH 2·1 towards lower areas compared with the pH 6·8 curves. The magnitude of the shift, however, varies markedly with the type of protein studied. The effect is most marked with the proteins of the bread cereals, wheat and rye, and least marked with carob germ protein. The non-bread cereals, barley and oat, occupy a middle position.

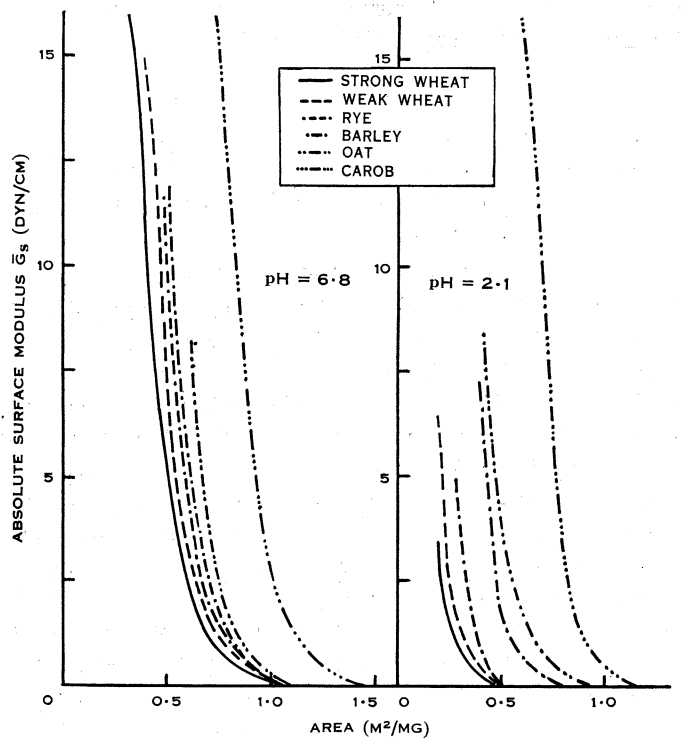

Fig. 2.—Absolute surface modulus $\overline{G}_s$ as a function of the area at pH 6·8 (left) and 2·1 (right). O/W, $\mu = 0{\cdot}1$.

It was shown previously that the surface viscosity of wheat gluten increases with time while the surface pressure remains essentially constant. If it is true that this increase reflects bond formation in the film (Tschoegl and Alexander 1960*b*) differences in the cohesiveness of the cereal "glutens" might possibly show up in this time dependence. Strong wheat and oat (defatted), representing the extreme placings of the cereals in the rank order referred to above, were therefore selected for a detailed study. The surface viscosities were measured over a range of pH values of the substrate at μ 0·02. The areas at which the films were spread were chosen to yield

comparable limiting viscosities. The experimentally determined values were fitted by the method of "internal least squares" (Hartley 1948) to the relation (Tschoegl and Alexander 1960*b*):

$$\ln (\eta_\infty - \eta_t)/\eta_\infty = -K(t - t_0),$$

where η_∞ is the limiting surface viscosity at infinite time, η_t is the instantaneous surface viscosity, K is the rate constant, t the time, and t_0 the time at which the surface viscosity is zero.

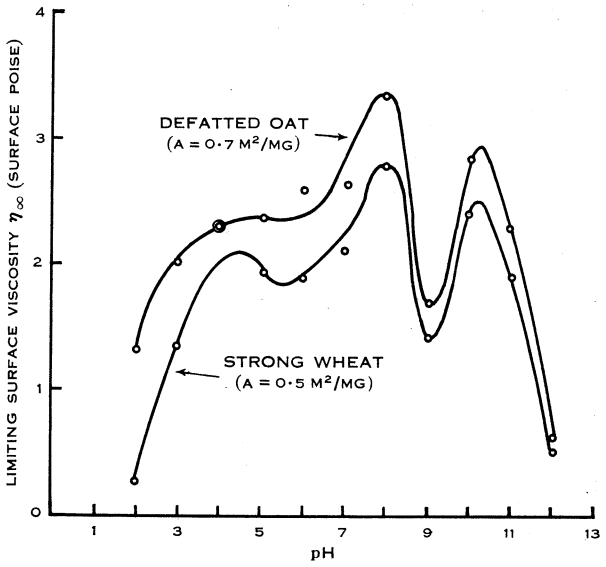

Fig. 3.—Limiting surface viscosity as a function of pH. O/W, $\mu = 0{\cdot}02$.

Figure 3 shows a plot of η_∞ against pH. This plot again reveals a maximum between about pH 7 and 8. There is a minimum around pH 9 and an indication of a minimum around pH 5·5. These curves are therefore similar to the plots of the area at 1 dyn/cm against pH, obtained from surface pressure measurements on gluten films (Tschoegl and Alexander 1960*a*). Below pH 4 the limiting surface viscosity of the strong wheat films dropped more sharply than the limiting surface viscosity of the oat films.

The plot of K against pH is shown in Figure 4. A maximum appears again between about pH 7 and 8 but there is no indication of any minima around pH 5·5 or 9. At comparable limiting viscosities the rate constants of the oat films are considerably higher than those of the wheat films.

The foregoing work on cereal protein monolayers had shown that the most fruitful approach to a comparative monolayer study of wheat glutens of differing characteristics might lie in the examination of the limiting viscosities and the rate constants near the isoelectric point. An examination of the chemical and physical changes occurring in wheat gluten on dispersion in anhydrous and aqueous acid

chloroethanol (Tschoegl 1961) had shown that partial esterification of free carboxyl groups and a slight loss of amide nitrogen occurs when gluten is dispersed in a 0·1N solution of hydrogen chloride in anhydrous chloroethanol while this does not occur in a 0·01N solution of hydrochloric acid in chloroethanol containing 30% water. The isoelectric point of gluten films spread from the latter dispersions was found to be 6·7 (Tschoegl 1961) while surface viscosity measurements on gluten films spread from anhydrous acid chloroethanol yielded an isoelectric point of 7·5 (Tschoegl and Alexander 1960*b*). Aqueous dispersions gave lower surface viscosities than anhydrous dispersions but in all other essential respects gluten films spread from these two different dispersants seemed to be identical.

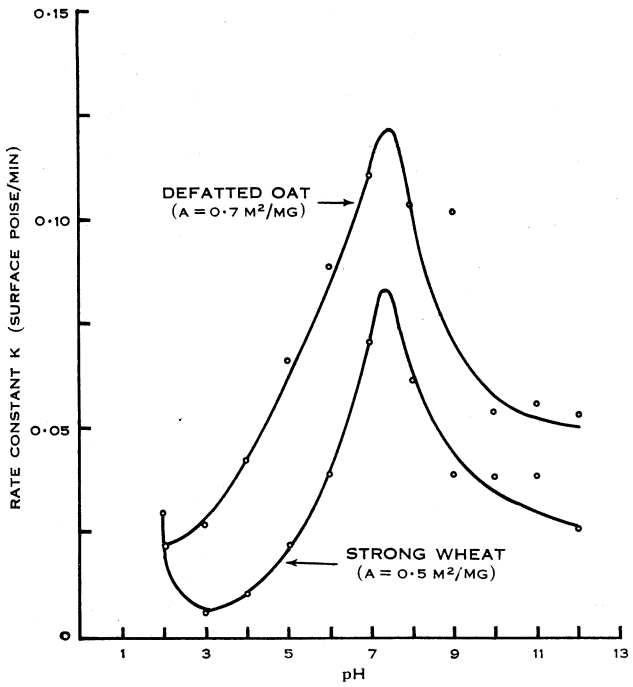

Fig. 4.—Rate constant of the surface viscosity as a function of pH. O/W, $\mu = 0{\cdot}02$.

The glutens obtained from the five different wheat flours (Table 1) were therefore spread at the O/W interface from aqueous acid chloroethanol at pH 6·4, μ 0·02, at an area of 0·4 m²/mg and the surface viscosities were measured as a function of time. Three curves were obtained from each dispersion and the limiting viscosities and rate constants were calculated individually and for the average curves. The results are shown in Tables 2 and 3.

Analysis of variance showed that the limiting viscosities were significantly different at the 1% level. The difference required for two means to be different at the 5% level was 0·33. The rate constants were not significantly different.

IV. Discussion

Tschoegl and Alexander (1960*a*) ascribed the great stability and high compressibility of gluten films to the formation of "ptygmatic", or "folded", films at an interface and suggested that the required internal cohesion arose chiefly from the presence of an unusual amount of glutamine side-chains bearing the potentially

Table 2

LIMITING SURFACE VISCOSITY (SURFACE POISE) OF GLUTENS OBTAINED FROM FIVE WHEAT FLOURS

Replicate No.	Strong Wheat	Weak Wheat	Medium Wheat	Harsh Wheat	Extensible Wheat
1	1·08	1·45	1·29	1·87	1·21
2	0·81	1·42	1·26	2·31	1·82
3	0·92	1·87	1·27	2·02	1·35
Mean	0·94	1·58	1·27	2·06	1·46
S.D.	0·136	0·252	0·016	0·224	0·320
Average	0·93	1·40	1·29	2·05	1·44

strong hydrogen bond-forming amide (–$CONH_2$) end-group (Orgel 1959). Since a high content of glutamine residues is a common feature of the cereal proteins (Bourdet 1956) and the carob germ protein (Rice and Ramstad 1950), the fact that all the films investigated in this work showed the characteristics of ptygmatic films

Table 3

RATE CONSTANTS (SURFACE POISE/MIN) OF GLUTENS OBTAINED FROM FIVE WHEAT FLOURS

Replicate No.	Strong Wheat	Weak Wheat	Medium Wheat	Harsh Wheat	Extensible Wheat
1	0·0861	0·0525	0·0495	0·0328	0·0412
2	0·0563	0·0543	0·0365	0·0234	0·0288
3	0·0348	0·0568	0·0717	0·0314	0·0503
Mean	0·0590	0·0546	0·0526	0·0292	0·0401
S.D.	0·0258	0·0022	0·0178	0·0051	0·0108
Average	0·0552	0·0707	0·0436	0·0288	0·0396

lends support to this view. The II–*A* isotherms showed remarkable similarity at the O/W interface (cf. Fig. 1). The slight differences at the A/W interface could be due to differences in van der Waals cohesion. These would be expected to disappear at the O/W interface.

While surface pressure measurements thus did not clearly differentiate between cereal proteins, measurements of surface viscoelasticity produced marked differences.

At the same surface concentration (area), the absolute surface modulus increased in the rank order: strong wheat, weak wheat, rye, barley, oat, carob. Furthermore, the effect of pH on the absolute surface modulus clearly differentiated between the bread cereals and the non-bread cereals (Fig. 2). The rank order referred to above is the same in which the quality and cohesiveness of their "glutens" would place the cereal proteins (Cunningham, Geddes, and Anderson 1955). No gluten could be washed from the carob germ flour used in this study and therefore the carob germ protein also seems to fit into the trend shown by the cereal proteins. This is a little surprising as carob germ protein has been claimed by Rice and Ramstad (1950) to form a cohesive gluten. The preparative method used in this study (Tschoegl 1961) differed, however, from Rice and Ramstad's.

No satisfactory explanation can be offered at present for the differences in the influence of pH on surface viscoelasticity. Under the circumstances in which the measurements presented in Figure 2 were carried out, differences in the time dependence of the surface viscoelastic properties could be partly responsible. However, Figure 3 clearly shows the *limiting* surface viscosity at pH 2·1 of the oat protein film also to be higher than that of the strong wheat protein film.

More work would also be needed to explain the differences in the rate with which the limiting surface viscosities are reached in oat and wheat protein films. Differences in the rate constants of the five different glutens were not statistically significant. This is undoubtedly due in part to poor reproducibility.

Bulk viscosity measurements on gluten dispersions have not shown satisfactory correlation with baking strength although the sedimentation of flour suspensions in dilute lactic acid (Zeleny 1947) is said to afford a means for quick grading of wheat. Udy (1953) found that dilute acetic acid dispersions of different glutens gave different relative viscosities but he did not attempt any correlation with baking quality. While earlier experiments by Rose and Cook (1935), Harris and Johnson (1940), and Geerdes and Harris (1952) had indicated that "stronger" glutens might yield higher viscosities in sodium salicylate dispersions, Udy (1953) showed the relative viscosities of such dispersions to be independent of gluten quality.

For the five glutens examined here, the correlation coefficient between limiting surface viscosity and alveogram "strength" as a measure of baking quality was −0·566 which is not significant with only three degrees of freedom. Without the harsh gluten, however, the correlation would have been −0·991 which is significant even with only two degrees of freedom. A greater number of more precise measurements is clearly needed before correlation of film viscosity and baking quality could definitely be established or dismissed.

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