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A Feasibility Study of the Use of a Modified Maes Protein Extraction Process and Chromosome Substitution Lines for Bread Wheat Quality Identification

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A Feasibility Study of the Use of a Modified Maes Protein Extraction Process and Chromosome Substitution Lines for Bread Wheat Quality Identification*

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Although bread-baking quality has been extensively studied, our knowledge of the flour fractions or components that specifically influence baking quality is limited. However, new techniques and genetic materials are currently available that should provide better clues to unlocking the secrets of flour quality. On the one hand, the development of such techniques as moving boundary electrophoresis, starch-gel electrophoresis and air classification has provided laboratory research aids. On the other hand, such genetic tools as isogenic lines, monosomic techniques and chromosome substitution provide raw materials more amenable to baking quality research.

In the research reported here, the Maes continuous extraction process as modified by MATTERN *et al.* (1968b) for fractionation of hard red winter wheat flours has been utilized in a wheat quality study employing chromosome substitution lines. MATTERN *et al.* (1968b) have shown that the 40% isopropyl alcohol, 2% NaCl, 3.85% lactic acid, and 0.1% potassium hydroxide solvent sequence, when passed through a column of wheat flour mixed with sand, would provide for 94-99% recovery of the wheat flour protein without protein degradation. This extraction process would thus provide for a satisfactory partitioning of the wheat flour protein into alcohol solubles (gliadin), 2% NaCl solubles, acid solubles and alkali solubles (primarily glutenin). The relative proportions of these components could thus be a possible clue to quality if raw materials were available that were largely similar genetically but differed in major quality characteristics. Such materials in the form of chromosome substitution lines

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are available at the Nebraska Agricultural Experiment Station and have been described by MORRIS *et al.* (1966, 1968).

Individual chromosomes of the strong-quality hard red winter wheat variety 'Cheyenne' have been transferred into the soft red spring variety 'Chinese Spring'. The lines were studied after the 4th backcross to Chinese Spring had been completed. According to MORRIS *et al.* (1966, 1968), major factors for the strong dough-mixing characteristics of Cheyenne are located on chromosomes 4B, 7B and 5D. Bread-baking characteristics such as loaf volume, crust appearance, and bread grain and texture, were largely influenced by factors located on 1B, 4B, 7B and possibly 1A, with lesser effects contributed by factors located on 4D, 5D, and 7D. WELSH *et al.* (1968) studied bread-baking quality of the same recipient variety, Chinese Spring, with substituted chromosomes from the hard red spring wheat varieties 'Thatcher', 'Hope' and 'Timstein'. They reported that only chromosome 2A of Thatcher had any individually great effect and that consisted of increased loaf volume and baking absorption. Of Hope, the authors stated that 11 different chromosomes influenced some quality aspect but that chromosome 2B had the greatest effect of any single chromosome substituted. For Timstein, they reported quality differences in the case of 16 different chromosome substitutions with the most important effects coming from substituted chromosomes 3B and 6B. The rather conspicuous absence of important factors for bread-baking quality in the D genome is of considerable interest. Earlier WELSH and HEHN (1964) reported that certain aneuploid 1D stocks show marked quality changes. Weak dough strength was noted by SCHMIDT *et al.* (1966) and MORRIS and SCHMIDT (1964) in 1D monosomics of hard red winter wheats studied. Interestingly, BOYD and LEE (1967) found this to be the major effect contributed by the D genome in a study of electrophoretic patterns of gluten proteins from hexaploid 'Canthatch', the tetraploid derivative of Canthatch and 3 reconstituted hexaploids derived from the tetraploid Canthatch and 3 varieties of *Aegilops squarrosa* L.

EASTIN *et al.* (1967) reported marked differences in starch-gel electrophoretic patterns of classically prepared gliadin proteins of the Cheyenne-Chinese Spring substitution lines, but they were not able to associate these with any clear-cut flour quality factors.

Knowledge of the amino acid composition of wheat proteins has also been limited. Recently with the use of automatic ion-exchange chromatographic analysis, data are being accumulated rapidly. TKACHUK (1966) reported little variation for amino acid composition among a hard red spring, a white winter, a red winter and a durum wheat. He stated that any significant variation could be masked since the apparatus is accurate only to about 3 or 4%, and further, that those proteins with characteristic patterns do not occur in large amounts in wheat flours. However, MATTERN *et al.* (1968a) reported that sufficient amino acid variation was present among high-protein lines derived from the crosses of 'Atlas 66' with 'Wichita' and 'Comanche' to warrant selection for lines with better amino acid balance. The purpose of this research was to determine the feasibility

of using a flour protein extraction process in conjunction with chromosome substitution lines for studying bread wheat quality. Since the lines we tested were only in the fourth backcross, any conclusions we draw must be tentative.

MATERIALS AND METHODS

The Cheyenne x Chinese Spring[3] substitution lines along with the two parental varieties were grown under irrigation at Aberdeen, Idaho, over a period from 1963-1965. Not all of the lines were grown in the same year. However, the parental lines and other check lines were grown each year. Comparisons of mixing curve results for each year showed no significant year effects. There were protein content differences between years. The recipient variety, Chinese Spring, produces flour with a short mixing time, very little mixing tolerance, and very poor loaf volume and loaf characteristics. In contrast, the donor variety, Cheyenne, has a moderately long mixing time, good mixing tolerance and adequate or satisfactory loaf volume and loaf characteristics.

Protein content was determined by the Kjeldahl method. Mixograms of 30 grams of flour milled from various stocks were recorded at 25 C with the mixograph. Loaves were baked from either 100 g or 25 g of flour using the AACC test baking procedure. Oxidation requirements were determined from three preliminary bake tests. Absorption was adjusted to optimum by an experienced operator.

The protein fractionation procedure was the same as the modified Maes method described by MATTERN *et al.* (1968b) except that smaller amounts of flour were used. In this study one-gram flour samples were mixed with 2 g of pumice and 25 g of sand and the mixture placed in a glass column of 1.9 cm diameter and 19 cm long. The solvent sequence was 40% isopropyl alcohol (V/V), 2.0% sodium chloride (W/V), 3.85% lactic acid (W/V), and 0.1% potassium hydroxide (W/V). Volumes of solvents collected were 60, 75, 60 and 100 ml, respectively. Representative percentages of the total flour protein contained in these volumes are shown in Figure 1.

In the hydrolysis for amino acid analyses, the weight of sample given by MATTERN *et al.* (1968b) for protein fractions was increased to 0.1 g. (dry weight basis) for ground wheat samples. The remainder of the hydrolysis procedure remained unchanged. Amino acid analyses were made by ion exchange column chromatography using a Beckman-Spinco Amino Acid Analyzer Model 120C.

RESULTS AND CONCLUSIONS

Protein Composition of Chromosome Substitution Lines

The 21 Cheyenne x Chinese Spring[3] substitution lines were grown at Aberdeen, Idaho, in two groups. Four lines, 2A (XIII), 2B (II), 2D and 7A were not grown until 1965 while the remaining 17 had been grown in 1963. Protein composition data for the 4 lines and parents are shown in Table 1, and for the

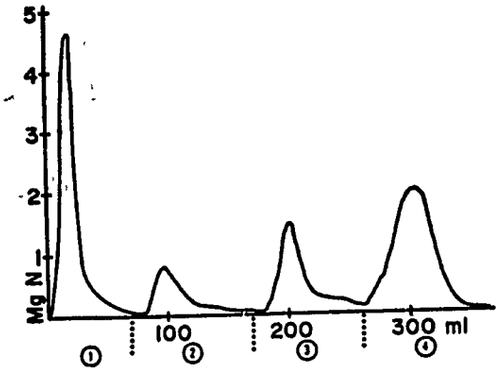

FIG. 1. Protein fractions from 1 g. of flour using a four solvent system.
(1) 40% Isopropyl alcohol.
(2) 2% NaCl.
(3) 3.85% Lactic acid.
(4) 0.1% KOH.

17 lines in Table 2. Check substitution lines 3D and 4B from the 1963 set were included in 1965. Loaf and mixing characteristics for these checks as well as the parent varieties were similar for the two years. The two protein fractions of most interest in Tables 1 and 2 are the alcohol solubles (gliadin) and alkali solubles (glutenin).

TABLE 1. Protein composition of 4 Cheyenne x Chinese Spring[5] chromosome substitution lines expressed as percent of total recovery. Grown at Aberdeen, Idaho, 1963.

Substitution line	Flour protein content*	Alcohol solubles	Salt solubles	Acid solubles	Alkali solubles	Total
2A (XIII)	11.24	56.0	3.1	9.6	29.2	97.9
2B (II)	11.54	58.0	3.5	8.9	28.7	99.1
2D	10.93	55.8	2.2	8.0	29.8	95.8
7A	11.93	55.5	3.4	8.9	30.5	98.3
Cheyenne	13.85	53.0	3.6	7.3	34.4	98.3
Chinese Spring	11.52	58.8	3.6	7.8	28.6	98.8

* 14% MB.

Alcohol solubles. Since MATTERN and SANDSTEDT (1957) have shown that increasing the gliadin in wheat flour shortens the mixing time, we would expect that substitution lines with very weak dough-mixing properties would be proportionately higher in alcohol solubles than those with strong dough-mixing properties. MORRIS *et al.* (1966, 1968) reported that Cheyenne chromosomes

4B, 7B and 5D had the most influence towards stronger dough-mixing properties. Percentages of alcohol solubles for these three substitution lines tend to be on the low side but are not the lowest recorded. Further, from mixing curve photographs shown by MORRIS *et al.* (1966, 1968) we conclude that lines 6A, 6D, 1D and 3D have mixing characteristics even poorer than those of Chinese Spring. All except 3D, also, are in the high percentage alcohol solubles group. Line 1D which had a very poor mixing curve had the highest percentage of alcohol solubles. None of the lines had a percentage as low as that of the strong parent, Cheyenne. We conclude, then, that while the correlation is not absolute, there is a definite trend toward an association of high values for alcohol solubles (gliadin) with inferior dough-mixing properties.

TABLE 2. Protein composition of 17 Cheyenne x Chinese Spring[5] chromosome substitution lines expressed as percent of total recovery. Grown at Aberdeen, Idaho, 1965.

Substitution line	Flour protein content*	Alcohol solubles	Salt solubles	Acid solubles	Alkali solubles	Total
1A	12.20	56.4	3.3	7.7	29.5	96.9
3A	11.81	59.1	2.9	7.8	30.1	99.9
4A	11.42	58.8	3.3	8.0	30.0	100.1
5A	11.70	58.3	3.3	7.6	28.8	98.0
6A	11.76	60.8	2.9	8.0	27.5	99.2
1B	11.28	57.7	3.0	7.3	31.0	99.0
3B	11.28	56.6	3.2	7.6	31.0	98.4
4B	12.33	58.7	2.8	6.2	30.3	98.0
5B	12.06	59.1	2.8	6.8	28.3	97.0
6B	10.57	58.4	3.0	7.1	28.8	97.3
7B	12.91	56.7	2.7	6.5	31.7	97.9
1D	10.17	63.0	3.2	7.4	25.2	98.8
3D	12.43	59.0	1.8	6.4	27.4	94.6
4D	12.29	57.8	1.8	6.4	28.5	94.5
5D	14.44	57.2	2.2	6.6	30.2	96.2
6D	12.59	61.1	2.0	6.5	27.3	96.9
7D	10.56	60.5	2.5	6.8	27.5	97.3

* 14% MB.

Alkali solubles. The alkali solubles fraction is expected to contain the glutenins and, therefore, to influence loaf volume and other bread characteristics. MORRIS *et al.* (1966, 1968) reported that substitution lines 1B, 4B, and 7B exerted the most influence on improved bread characteristics with lesser effects from 1A, 4D, 5D, and 7D. Lines 1B, 4B, 7B, and 5D were in the high group for alkali solubles. Line 7B had the highest percentage and also baked the best loaf of bread. Percentages of alkali solubles for lines 1A, 4D, and 7D were medium to low. Line 1D which baked the poorest loaf also had 2% less alkali solubles than any other line. None of the lines had a percentage as high as that of the strong

parent, Cheyenne. Again, there is reasonably good relationship between loaf quality characteristics and percentage of alkali solubles.

Salt solubles. All lines containing a D genome chromosome from Cheyenne except 1D were low in salt solubles. Most of these lines, also, were low in total protein recovery. The reasons for both of these results are not known.

On the basis of these initial tests, we conclude that it is possible to associate bread-baking quality with protein composition. Certainly, the poor performance of line 1D is directly associated with its high percentage of alcohol solubles (gliadin) and low percentage of alkali solubles (glutenin). None of the lines had values very close to the strong parent. If, as reported by us earlier, the strong quality of Cheyenne is due to genes located on at least three different chromosomes, we would not expect any one line to have values equal to those for Cheyenne. Line 7B, which appears to carry the gene(s) with the strongest effects, is the closest to Cheyenne in overall performance. These data show that there is an association of bread-baking quality with protein composition, and that with refinements in techniques and more critical genetic materials this association will be increased.

Amino Acid Composition of Chromosome Substitution Lines

The amino acid composition of the chromosome substitution lines is shown in Tables 3 (A genome), 4 (B genome) and 5 (D genome). There were some variations in amino acid composition among the various lines. Most of the A- and B-genome substitution lines were low in aspartic acid when compared to the parental varieties while the D-genome lines were variable for this amino acid. In contrast, A- and B-genome lines were variable in serine content, while

TABLE 3. Amino acid composition of Cheyenne x Chinese Spring[4] chromosome substitution lines (A genome).

Amino acid	Chinese Spring	Cheyenne	1A	2A	3A	4A	5A	6A	7A
Lysine	2.60	2.60	2.48	2.60	2.60	2.49	2.55	2.44	2.52
Histidine	2.47	2.46	2.49	2.50	2.54	2.53	2.42	2.41	2.28
Ammonia	4.00	4.01	4.20	3.98	4.21	4.01	4.13	4.11	4.0[illegible]
Arginine	4.25	4.31	4.20	4.25	4.34	4.25	4.25	4.12	4.00
Aspartic Acid	5.35	5.58	4.53	4.62	4.86	4.61	4.58	4.68	4.53
Threonine	2.97	2.86	2.90	2.94	3.08	2.97	3.14	3.04	2.89
Serine	4.06	3.91	4.60	4.71	5.04	4.92	5.17	5.10	4.69
Glutamic Acid	38.25	38.83	38.17	38.00	40.50	38.20	41.60	40.60	37.90
Proline	14.13	13.85	13.60	14.00	15.20	14.42	15.40	14.90	14.06
Glycine	3.90	3.86	4.07	3.83	3.54	3.89	3.66	4.20	3.67
Alanine	3.33	3.14	3.31	3.30	3.06	3.34	3.12	3.12	3.15
Half Cystine	2.01	1.79	1.81	1.75	1.78	1.80	1.86	2.04	1.63
Valine	4.21	4.39	4.22	4.13	4.20	4.01	4.15	4.44	3.96
Methionine	1.58	1.74	1.28	1.36	1.45	1.35	1.57	1.50	1.61
Isoleucine	4.12	4.09	4.12	4.14	4.06	3.98	3.91	3.99	3.90
Leucine	7.60	7.48	7.60	7.49	7.35	7.50	7.40	7.47	7.30
Tyrosine	3.47	3.87	3.59	3.61	3.72	3.48	3.83	3.53	3.57
Phenylalanine	5.67	5.51	5.21	5.71	5.85	5.51	5.88	5.68	5.64
% Protein "as is"	12.24	14.66	12.68	11.92	12.24	11.86	11.68	12.20	12.68

Results expressed as g. of amino acid per 100 g. protein, dry weight basis.

the D-genome lines were more nearly like the parental varieties. No relationship between these values and protein quality could be established.

Substitution line 1D had the highest lysine value. In comparison with other D-genome lines, 1D was higher in salt solubles (water solubles). If a relationship between lysine content and water-soluble protein exists, then it would be expected that 1D would be higher than the other D-genome lines.

TABLE 4. Amino acid composition of Cheyenne x Chinese Spring[5] chromosome substitution lines (B genome).

Amino acid	Chinese Spring	Cheyenne	1B	2B	3B	4B	5B	6B	7B
Lysine	2.60	2.60	2.43	2.35	2.71	2.59	2.50	2.72	2.68
Histidine	2.47	2.46	2.31	2.22	2.25	2.58	2.48	2.50	2.69
Ammonia	4.09	4.01	3.84	3.68	3.79	4.07	4.21	4.15	4.20
Arginine	4.25	4.31	4.01	4.00	3.91	4.52	4.14	4.29	4.32
Aspartic Acid	5.35	5.58	4.94	4.76	4.80	4.45	4 38	4 08	6 01
Threonine	2.97	2.86	3.01	2 80	2.90	3.20	3 07	3.15	3.07
Serine	4.06	3.91	4.96	4.70	4 61	5.39	5.19	4 84	4.14
Glutamic Acid	38.25	38.83	37.50	37.80	37.60	41 87	40.92	30 71	40.80
Proline	14.13	13.85	14.22	14.25	14.53	15 68	14 74	14 88	14.88
Glycine	3.90	3.86	3.94	3.71	3 69	3.72	3 53	3 89	3 82
Alanine	3.33	3.14	3.27	3.21	3.24	3.10	3.17	3 21	3.19
Half Cystine	2.01	1.79	1.77	1.76	1.75	2 08	1.78	2 02	2 02
Valine	4.21	4.39	4.12	4.29	3.92	4 55	4 17	4.32	4.50
Methionine	1.58	1.74	1.52	1.37	1.50	1.51	1.02	1 54	1.65
Isoleucine	4.12	4.09	4.21	3.86	3 88	4 17	4 21	3 91	4.27
Leucine	7.60	7.48	7.86	7.49	6.76	7.83	7 66	7.48	7.77
Tyrosine	3.47	3.87	3.61	3.42	3.63	4.05	3 69	4 17	3.62
Phenylalanine	5.67	5.51	5.39	5.30	5 88	5 92	6.00	6 07	6.04
% Protein "as is"	12.24	14 66	11.74	12.25	11.62	12.30	11.96	10 54	13 32

Results expressed as g. of amino acid per 100 g. protein, dry weight basis.

TABLE 5. Amino acid composition of Cheyenne x Chinese Spring[5] chromosome substitution lines (D genome).

Amino acid	Chinese Spring	Cheyenne	1D	2D	3D	4D	5D	6D	7D
Lysine	2.60	2.60	2.98	2.76	2.57	2.49	2.57	2.42	2.72
Histidine	2.47	2.46	2.67	2.53	2 51	2.49	2.58	2.52	2.51
Ammonia	4.09	4.01	4.26	3.98	4 06	4 05	4.05	4.14	4.00
Arginine	4.25	4.31	4.50	4.35	4.11	4.14	4.17	4.10	4.29
Aspartic Acid	5.35	5.58	5.99	6.39	6.00	4.52	5 84	4.92	5.23
Threonine	2.97	2.86	3.05	2 90	2 90	3.05	2.83	2.87	3.10
Serine	4.06	3.91	4.11	3 97	3 90	4 98	3.80	4.28	4.56
Glutamic Acid	38.25	38.83	39.30	37.10	40 50	41 20	39.09	41.30	37.00
Proline	14.13	13.85	14.40	14 00	14 83	14.96	14 81	15 20	13.64
Glycine	3.90	3.86	4.07	3 69	3 65	3.62	3.62	3.59	3.93
Alanine	3.33	3.14	3.66	3 29	3 08	3.02	3.13	2 97	3 52
Half Cystine	2.01	1.79	2.12	1.94	1.97	1.75	2 21	1.75	1.86
Valine	4.21	4.39	4 63	4.34	4.26	4.21	4.38	4.26	4.45
Methionine	1,58	1.74	2.01	1 84	1.57	1 64	1.77	1.61	1.80
Isoleucine	4.12	4.09	4.56	4.12	3.96	3 94	4.14	3.99	4.27
Leucine	7.60	7.48	8.31	7.75	7.27	7.28	7.44	7.28	7.71
Tyrosine	3.47	3.87	3.84	4 20	3 81	3.76	3.66	3.61	3.27
Phenylalanine	5.67	5.51	6.39	5.73	5.89	6 02	6.15	6.00	5.55
% Protein "as is"	12.24	14.66	10.48	11 60	12.52	12.96	15.02	13.24	10.66

Results expressed as g. of amino acid per 100 g. protein, dry weight basis.

Techniques for amino acid analyses are accurate only within 3-5 per cent. Further, as TKACHUK (1966) points out, proteins known to have characteristic amino acid composition do not occur in large quantities in wheat flours. Thus, we were not able to relate the amino acid composition of these chromosome substitution lines to bread-making quality.

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