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Relationships between high-molecular weight subunits of glutenin proteins and bread-making quality of the major moroccan-grown common wheats

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ملخص

في هذا البحث توصلنا إلى ترقيم جزئيات بروتين الجلوتنين لثمان أنواع من القمح الطري المغربي. و تم أيضا تحديد جودتها من خلال اختبارات ديولوجية وفيزيوكيمائية. و قد تمكن ضبط بعض و الروابط بين وجود بعض الجزئيات الجلوتنينية و حسن أو رادئة الجودة. و تؤكد النتائج و جود علاقة قوية بين الجزئيات و بعض الصفات النوعية لعينات القمح (قيمة الترسيب، قيمة الترسيب المحددة، مدة الخلط المناسب، ثبات العجينة، إضعاف العجينة، الإنتفاخ الألفيوجرافي، و مطاطية العجينة). فوجود الجزئيات 5 و10 يدل على حسن الصفات التكنولوجية للقمح في حين يدل تواجد الوحدتين 2 و 12على رداءة قوة الدقيق و ليونته. فيما يخص الوحدتين او2 فلم نسجل أية علاقة تذكر بينها و بين خصائص الجودة. أما فيما يتعلق بالوحدتين الجلوتنينيتين 17و18 فهي متصلة إيجابيا و بشكل ملموس مع مدة الخلط المناسب، قوة العجينة، الإنتفاخ الألفيوجرافي و مطاطية العجينة. الشيئ الذي لا يسمح للخلاصة للقول على أن قيمة الجودة مرتبطة بشكل و ثيق مع القوة الإجمالية للدقيق .

الكلمات المفتاحية : القمح الطري - صفات الخبيز- الجودة عوامل الترابط - الرحلا ن الكهربائي- الجلوتنين

Résumé

La numérotation des sous-unités gluténines à haut poids moléculaires a été réalisée sur huit principales variétés marocaines de blé tendre. Les sous-unités 2, 5, 10 et 12 sont significativement corrélées avec la valeur de sédimentation spécifique, le temps de développement, la stabilité de la pate, l'indice de tolérance au pétrissage, l'indice de gonflement et l'extensibilité de la pâte. Les sous-unités 5 et 10 ont été positivement corrélées à la qualité alors que les sous-unités 2 et 12 sont plutôt des marqueurs de la mauvaise qualité des blés. Aucune relation entre la qualité et les sous-unités 1 et 2* n'a été trouvée. Les sous-unités 17 et 18 sont positivement et significativement corrélées avec le temps de développement, la force alvéographique, l'indice de gonflement et l'extensibilité de la pâte. Il en est de même pour la sous-unité 8 et l'indice de sédimentation. La sous-unité 7 a été étroitement et négativement liée aux mêmes paramètres que ceux reliés aux sous-unités 17 et 18. La note de cuisson n'est reliée positivement qu'avec l'indice de gonflement et l'extensibilité de la pâte qui sont des indicateurs d'une faible qualité.

Mots clés : Blé tendre - Qualité boulangère - Corrélations - Electrophorèse - Gluténines

Summary

The numbering of high-molecular weight glutenin subunits for eight moroccan common wheats was accomplished. Their bread-making quality was determined via some physico-chemical and rheological tests. It was found that subunits 2, 5, 10, and 12 were significantly correlated with: specific sedimentation value, peak time, dough stability, mixing tolerance index, swelling index, and dough extensibility. Subunits 5 and 10 correlated well with good quality whereas glutenin subun..s 2 and 12 were indicators of poor quality. No relationship of subunits 1 and 2* to quality was noticed. The glutenin subunits numbered 17 and 18 were positively and significantly correlated with: peak time, alveograph strength, swelling index, and dough extensibility. A negative and significant relationship was found between subunit 8 and sedimentation value whereas subunit 7 correlated negatively and significantly with: peak time, alveograph strength, swelling index and dough extensibility. Finally, the baking score correlated positively with the swelling index and dough extensibility which are indicators of rather weak doughs.

Key words: Common wheats - Bread-making quality - Correlations - Electrophoresis - Glutenins

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INTRODUCTION

About one-half of the storage proteins in wheat is classified as glutenins. The high-molecular weight (HMW) subunits of these proteins account for about 10 % of storage proteins. Varieties of common wheats contain three to five different HMW Glutenin subunits. These polypeptides are encoded by three small gene families located on chromosomes 1A, 1B and 1D (1).

The three loci responsible for their synthesis (namely: Glu-A1, Glu-B1 and Glu-D1) exhibit extensive allelic variation which is partly responsible for the differences in bread-making quality among cultivars (2).

The HMW glutenin subunits are best resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). This technique is used to separate the subunits so they can be numbered in specific ways (3;4;5). Several reports showed that HMW subunits of glutenin proteins correlate well with desirable grain-quality attributes (6;7;8;9;10;11).

PAYNE *et al.* (12)assigned quality scores to each of the commonly occurring subunits. The scores of each of the subunits were summed to create a Glu-1 quality score for each variety. The scores seem to give useful correlations with some quality parameters (7).

The present study aims to establish possible correlations between some bread-making quality tests and HMW glutenin subunits electrophoregrams of eight major Moroccan-grown common wheats. The numbering was achieved using the procedure of PAYNE & LAWRENCE (3) and some French wheat standards, of known HMW glutenin composition.

MATERIALS AND METHODS

Wheat samples

Wheat samples were supplied by the breeding service of the "Institut National de la Recherche Agronomique", Rabat, Morocco. They constitute the main wheat cultivars listed in the official national catalogue. They are collected at Marchouch agronomic research station, crop year: 1986. French wheat standards were supplied by Dr. BRANLARD, Clermont-Ferrand, I.N.R.A., France. The moroccan cultivars used are reported in table I. The french samples reported in table II are those that figure in the photograph (see recto better characterize glutenin subunits of moroccan wheats).

Table I. List of the moroccan wheat varieties analyzed

Wheat varieties	Year of prelease
Sais (1615)	1985
Potam	1975
Tegyey 32 (5/70-32)	1976
Tegyey 9 (5/70-9)	1977
Nesma (149)	1973
Marchouch 8	1984
Jouda (1646)	1984
Marchouch 9 (Sibara)	1985

Table II. Some french wheat standards used in the study (only those listed in figure 3)

Wheat varieties	HMW Glutenin Subunits formulea
Prinqual	2 , 2* , 17/18 , 12
Top	3 , 7 , 8 , 12
Rex	1 , 5 , 7 , 9 , 10
Champlein	4 , 7 , 8 , 12
Disponent	1 , 5 , 6 , 8 , 10

Moisture determination

This determination analysis was carried out according to the AFNOR (Association Française de Normalisation) method NF - VO3 - 707. Five g of flour were oven-dried for 1.5 hr. at 130-133°C. The weight loss was quantified and expressed as moisture content of the sample (13).

Protein determination

The method used was the AFNOR method NF VO3-050. It is a Kjeldahl procedure in which proteins were quantified by multiplying the nitrogen content by 5.7 (13).

ZELENY test

The ZELENY test consists of measuring the volume of swollen or agglomerated flour particles in dilute lactic acid solution (14). A.F.NOR. method NF-VO3-704 was followed to determine the sedimentation value (13). Flours with very low extraction rate were used. Flour (3.20±0.05 g) was introduced into a graduated cylinder and 50 ml of a bromophenol blue solution was added and shaken with a ZELENY shaker for five minutes. Then, 25 ml of a solution composed of lactic acid and propanol (180 ml lactic acid mixed with 200 ml propanol diluted to 1 liter with distilled water) was added. The cylinder was again shaken for 5 min and placed on a horizontal support for 5 min. The suspended material decanted and its volume was read (in milliliters) as a sedimentation value. The values below 18 are indicative of lines with poor quality. Above 38 milliliters, flours are qualified as very strong and should be blended with flours of poorer quality. In between these two values, wheat is of good to excellent breadmaking quality (15).

Farinograph test

The farinograph records the resistance of dough to mixing as a function of time. The Brabender Farinograph with the small (50 g) mixing bowl was used. The procedure was carried out according to the A.A.C.C. method 54 - 21 (16).

Alveograph test

The instrument continuously records air pressure in an inflated dough bubble until it breaks. The parameters measured from the curves are: tenacity (P), swelling index (G), and work of deformation or strength (W). The technique described in A.F.NOR method NF VO3 - 710 was used (13).

SDS-PAGE procedure

• Protein extraction

The extraction procedure used is similar to the one described by WRIGLEY *et al.* (17). The extraction solution contained (100 ml): 4 g of SDS (sodium dodecyl sulfate), 1.5 g of Tris (tris-hydroxy-methyl-aminomethane), 10 ml glycerol, and a very small amount (≈50 mg) of bromophenol blue. These chemicals were dissolved in about 50 to 70 ml of distilled water and the pH was adjusted to 6.8 with a freshly prepared HCl solution (≈ 6 N). Then the final volume was brought up to 100 ml with water. Extraction was done by mixing 100 mg of ground meal with 800 µl of the extraction solution in polypropylene microcentrifuge tubes. The tubes were let to stand for about 16 hours at room temperature (20°C), heated at 90 - 100°C for 5 minutes, and then centrifuged for 20 min at 2 200 x g. The supernatants were collected for electrophoresis.

• Electrophoresis procedure

Electrophoresis of total protein was run on a 10% acrylamide gel as described by PAYNE & COWORKERS (1;18). The procedure was based on that first described by LAEMMLI (19). The numbering system used to characterize glutenin subunits, was the one described by PAYNE & LAWRENCE (3). To distinguish between 2 and 2* subunits, 7% gels were used (20).

Statistical analysis

The data were analyzed by standard statistical methods. 1 and 0 were used respectively to indicate presence and absence of a given glutenin subunit. These binary variables allowed execution of correlation analyses between HMW glutenin subunits and quality tests. Significance of correlation coefficients were calculated at 95 and 99% confidence limits using the F-test (21; 22).

RESULTS AND DISCUSSIONS

General quality appraisal decisions on wheat quality were made from ZELENY, Alveograph, and Farinograph tests. The determination of weak or of strong wheats, using the farinograph results, was made possible by the AACC Farinograph Handbook (23). The grading according to ZELENY and Alveograph tests were determined according to MAUZE *et al.* (15). The results of these tests are reported in tables III and IV. Alveograph and Farinograph diagrams are reported in figures 1 and 2. HMW glutenin subunit formulas of these cultivars are reported in table V. The baking scores reported in tables VI and VII were calculated, from glutenin subunit formulas, according to PAYNE *et al.* (12). Positive and negative contributions of each individual (or pairs of) HMW subunit(s) of glutenin, was evaluated according to their allelism and to a compilation of numerous literature data. The overall evaluation of wheat varieties via the quality tests and electrophoresis of glutenin proteins is shown in tables VIII and IX. To ascerain some of the literature data used to associate HMW glutenins with quality, correlation coefficients are reported in tables X and XI.

In general, the cultivars Nesma, Jouda, Potam, Saïs, Marchouch 8 and Marchouch 9 were of good quality with respect to the quality tests performed. However, Tegyey 32 and Tegyey 9 have rather poor quality, as shown by their electrophoretic patterns and quality data. The similarity between Tegyey 32 and Tegyey 9 is not surprising because the two varieties have similar pedigrees and are, genetically, closely related.

As far as HMW glutenin subunit formulas are concerned, we identified 7 different patterns (figure 3): 2*, 5, 7, 8, 10 (Nesma); 1, 5, 10, 17, 18 (Jouda); 2*, 5, 7, 10 (Potam); 2, 7, 8, 12 (Tegyey 32); 1, 5, 7, 8, 10 (Saïs); 2*, 5, 10, 17, 18 (Marchouch 8 and Marchouch 9) and 2, 7, 12 (Tegyey 9). The varieties Marchouch 8 and Marchouch 9 have similar HMW glutenin patterns because they are genetically closely related. The baking scores determined according to PAYNE *et al.* (1987) showed that all cultivars have good quality scores except Tegyey 9 and Tegyey 32 (baking scores below 8). Hence an overall agreement between the two procedures of quality evaluation was observed (tables VIII and IX), which means that in fact we may be able to predict quality from HMW glutenin subunit formulas (using one or a half of a grain) without having to run objective tests that are sample- and time-consuming. Discussions on correlations between individual and pairs of glutenin subunits are presented hereafter.

Table III. Quality criteria of moroccan common wheats

Common Wheat Varieties	Flour Protein %M.S.	ZELENY test		Alveograph test				
		S.V. cm3	S.S.V. cm3/%prot	W 1000 ergs	G cm3	P mm	L mm	P/L
Nesma 149	14.20	27	1.90	260	16.5	125	54	2.31
Jouda 1646	13.55	43	3.17	400	19.0	153	74	2.07
Potam	12.15	39	3.21	210	18.0	89	64	1.39
Tegyey 32 (5/70-32)	12.90	19	0.68	105	15.0	68	46	1.48
Saïs (1615)	10.67	30	2.81	175	18.0	73	68	1.07
Marchouch 9	13.17	34	2.58	370	19.5	138	76	1.82
Marchouch 8	15.10	41	2.72	280	19.0	119	73	1.63
Tegyey 9 (5/70-9)	12.70	27	2.13	270	14.0	151	41	3.68

S.V: Sedimentation value; S.S.V.: Spescific sedimentation value; Strength (or work of deformation); G: Swelling index; P : Tenacity; L : Extensibility

Table IV. Quality criteria of moroccan common wheats

Common wheat varieties	Flour Protein (% m.s)	Farinograph test					
		W.A. 14%m.b.	A.T. (min)	P.T. (min)	T/S (min)	D.T. (min)	M.T.I. U.B.
Nesma 149	14.20	56.78	2.50	4.50	6.50	9.00	40
Jouda 164	13.55	59.26	3.25	7.00	9.25	12.50	30
Potam	12.15	46.11	1.75	5.50	11.00	12.75	30
Tegyey 32 (5/70-32)	12.90	49.81	1.25	2.00	1.50	2.75	85
Saïs (1615)	10.67	48.67	1.00	3.00	6.00	7.00	65
Marchouch 9	13.17	55.49	4.50	8.00	10.00	14.50	30
Marchouch 8	15.10	62.52	3.50	5.25	6.00	9.50	50
Tegyey 9 (5/70-9)	12.70	63.97	1.75	2.50	3.50	5.25	70

W.A.: Water absorption; A.T: Arrival time; P.T.: Peak time; T/S: Tolerance or stability; D.T: Departure time; M.T.I.: Mixing tolerance index

Table V. High molecular weight glutenin subunits of the eight moroccan-grown common wheat varieties

Varieties	1	2	2*	5	7	8	10	12	17	18
Nesma (149)	-	-	+	+	+	+	+	-	-	-
Jouda (1646)	+	-	-	+	-	-	+	-	+	+
Potam	-	-	+	+	+	-	+	-	-	-
Tegyey 32 (5/70-32)	-	+	-	-	+	+	-	+	-	-
Saïs (1615)	+	-	-	+	+	+	+	-	-	-
Marchouch 9	-	-	+	+	-	-	+	-	+	+
Marchouch 8	-	-	+	+	-	-	+	-	+	+
Tegyey 9 (5/70-9)	-	+	-	-	+	-	-	+	-	-

Bands 1 and 2* were not significantly correlated with any of the quality attributes (table X). These results agree with those reported by NG & BUSHUK (6), but do not support the data obtained by BRANLARD & DARDEVET (11) or those noted by PAYNE and coworkers (7;12) where these subunits were assigned important baking scores. It is however difficult to precisely ascertain that the 1 and 2* bands have no effect on the quality because the number of cultivars analysed is not that important.

Subunits 2 and 12 were significantly correlated negatively to stability (T/S), sedimentation value (S.V.), specific (S.V.), peak time (P.T.), swelling index (G) and extensibility (L), and positively to mixing time index (M.T.I.) (table 10). The correlations obtained for the subunits 5 and 10 are the exact opposite (in sign) of those noted for bands 2 and 12. The rheological parameters correlated positively with subunits 2 and 12 are negatively related to quality, whereas those that are positively associated to them are indicators of poor quality. These results for subunits 5 plus 10 and 2 plus 12, add further evidence to that already accumulated (6;7;8;9;11;12) that these subunits play key roles in relation to their functional properties.

The coexistence of the bands 7 and 8 is normally associated with good quality as evaluated by SDS-sedimentation values (12). Our results do not statistically support that because subunit 8 is only associated significantly with ZELENY test, whereas subunit 7 was negatively and significantly correlated with peak time (P.T.), strength (W), (G) and (L) (table XI). These results do not agree with those of NG & BUSHUK (6).

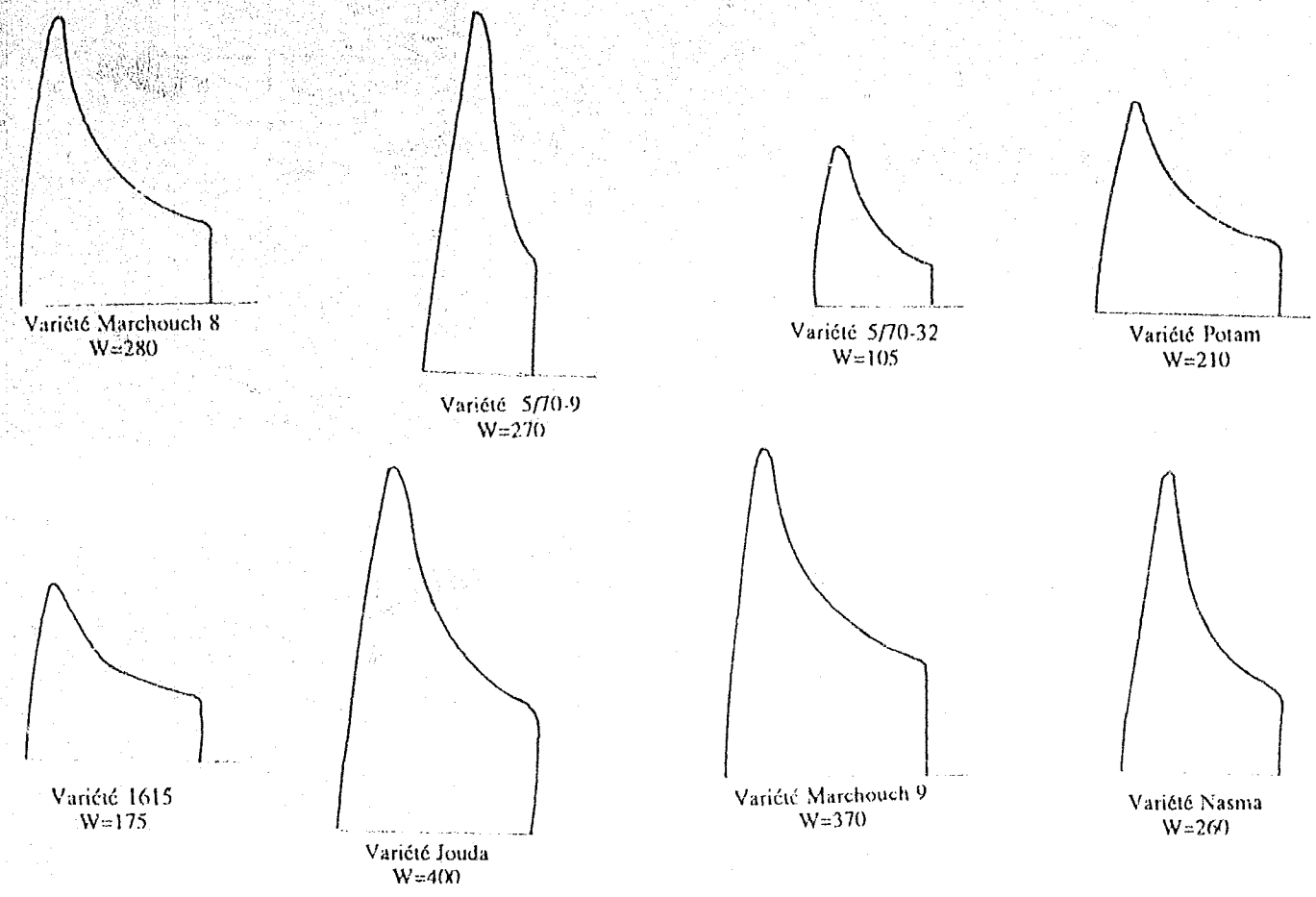
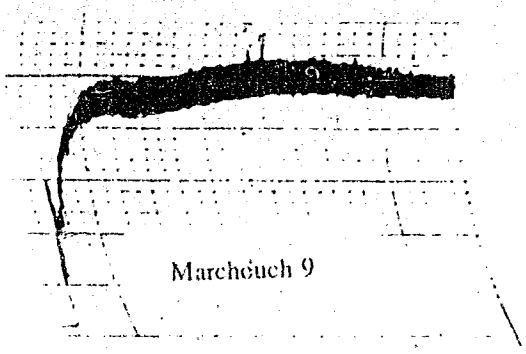


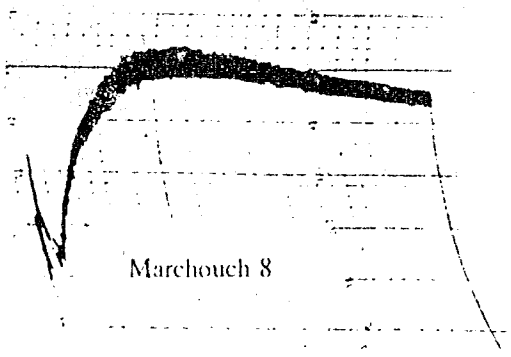
Figure 1. Alvéograms of moroccan common wheats

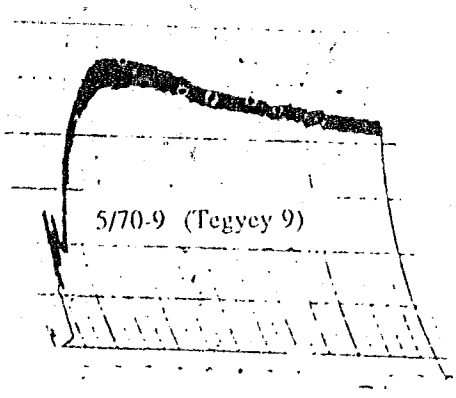
Table VI. Baking scores of wheat varieties

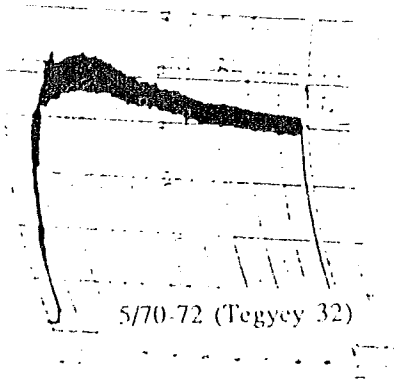
Weight varieties			Tegyey 32	Potam	Jouda	Nesma
HMW G.S.***			2-7-8-12	2*-5-7-10	1-5-10-17-18	2*-5-7-8-10
Chromo-somes	Subunits	Effect on quality	Null 7,8 2,12	2* 7 5 ,10	1 5,10 17, 18	2* 5,10 7,8,10
1A	1 2*	Positive		(+) , [3]	(+) , (3)	(+) , [3]
	Null	Negative	Null , [1]			
1B	17 , 18 13 , 16 7 , 8 6 , 8	Positive	(+) , [3]		(+), [3]	(+) , [3]
	7 20	Negative		(-) , [1]		
1D	13 , 19 2 5 , 10 3 ,12	Positive		(+) , [4]	(+) , [4]	(+) , [4]
	2 , 12 2 , 10 2.2 , 12 2,11 4 , 12	Negative	(-) , [2] B.S.** = 1+3+2=6 (Fair)	B.S.** = 3+1+4=8 (Good)	B.S.** = 3+3+4=10 (Very good)	B.S.** = 3+3+4=10 (Very good)

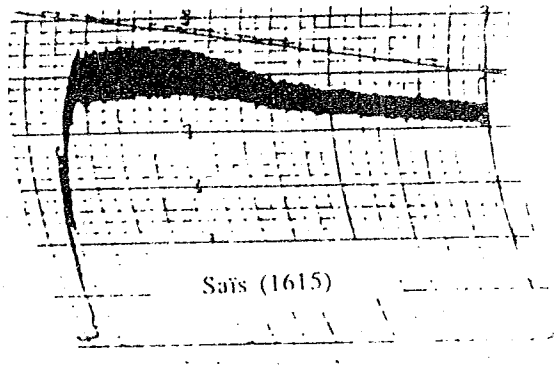
** B.S.: Baking Score
* **G.S.: Glutenin Subunits

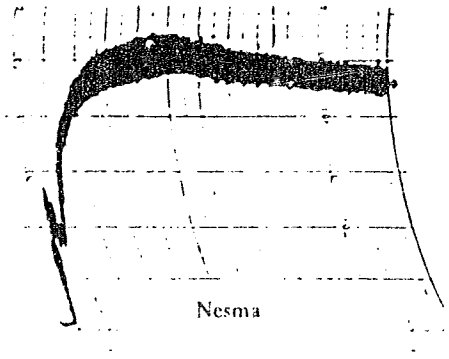


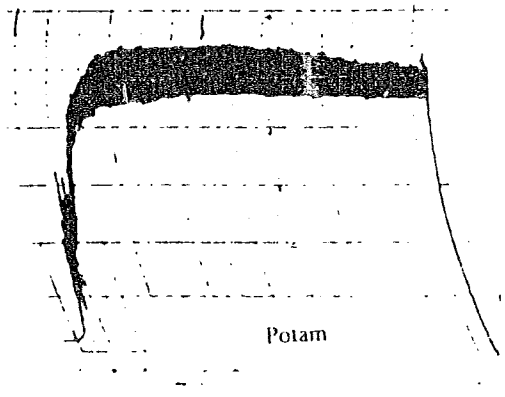












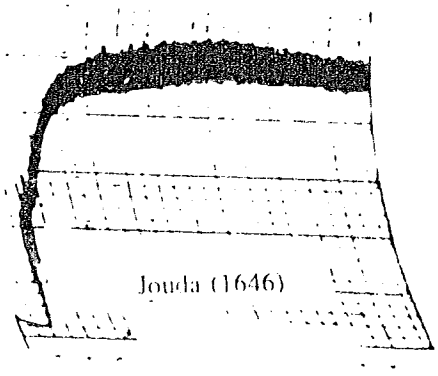


Figure 2. Farinograms of moroccan common wheats

Table VII. Baking scores of wheat varieties

Weight varieties			Tegyey 9	Marchouch 8	Marchouch 9	Sais
HMW G.S.***			2-7-12	2*-5-10-17-18	2*-5-10-17-18	1-5-7-8-10
Chromo-somes	Subunits	Effect on quality	Null 7 2,12	2* 5,10 17,18	2* 5,10 17, 18	1 7,8 5,10
1A	1					(+), (3)
	2*	Positive		(+), [3]	(+), [3]	
	Null	Negative	(-), [1]			
	17, 18			(+), [3]	(+), [3]	
	13, 16	Positive				
	7, 8					(+), [3]
1B	6, 8					
	7	Negative	(-), [1]			
	20					
	13, 19					
	2					
	5, 10	Positive		(+), [4]	(+), [4]	(+), [4]
1D	3, 12					
	2, 12		(-), [2]			
	2, 10					
	2.2, 12	Negative	B.S.** = 1+1+2=4 (Poor)	B.S.** = 3+3+4=10 (very good)	B.S.** = 3+3+4=10 (Very good)	B.S.** = 3+3+4=10 (Very good)
	2,11					
	4, 12					

** B.S.: Baking Score, ***G.S.: Glutenin Subunits

Table VIII. Correlations between glutenin subunits and common wheat quality

Common wheat varietie	Quality predicted by		
	HMW Glutenin subunits	ZELENY sedimentation test	Farinograph test
Nesma (149)	V G*	G	F
Jouda (1646)	V G	G	G
Potam	G	G	G
Tegyey 32 (5/70-32)	P**	F***	P
Sais (1615)	V G	G	F
Marchouch 9	V G	G	G
Marchouch 8	V G	G	F
Tegyey 9 (5/70-9)	P	G	P

VG*: Very good; P**: Poor; F***: Fair

Table IX. Correlation between glutenin subunits and common wheat quality

Common wheat varieties	Quality predicted by		
	HMW Glutenin subunits	Alveograph strength (W)	Alveograph swelling index (G)
Nesma (149)	V G*	G	P
Jouda (1646)	V G	G	F***
Potam	G	G	F
Tegyey 32 (5/70-32)	P**	P	P
Sais (1615)	V G	G	F
Marchouch 9	V G	G	F
Marchouch 8	V G	G	F
Tegyey 9 (5/70-9)	P	G	P

VG*: Very good; P**: Poor; F***: Fair

Tableau X. Correlation coefficients between HMW glutenin subunits and some quality parameters

Quality Parameters	1	2*	2	12	5	10
P.C.(%m.s.)	-0.44	0.48	-0.12	-0.12	0.12	0.12
S.V.	0.30	0.36	-0.71*	-0.71*	0.71*	0.71*
S.S.V.	-0.44	0.26	-0.74*	-0.74*	0.74*	0.74*
W.A.	-0.13	0.02	0.15	0.15	-0.15	-0.15
P.T.	0.08	0.54	-0.71*	-0.71*	0.71*	0.71*
T./S.	0.17	0.54	-0.80*	-0.80*	0.80*	0.80*
M.T.I.	-0.07	-0.63	0.80*	0.80*	-0.80*	-0.80*
(W)	0.18	0.23	-0.45	-0.45	0.45	0.45
(G)	0.34	0.46	-0.88**	-0.88**	0.88**	0.88**
(P)	-0.03	0.10	-0.09	-0.09	0.09	0.09
(L)	0.41	0.38	-0.85**	-0.85**	0.85**	0.85**

P.C.: Protein Content; S.V: Sedimentation value; S.S.V.: Spescific sedimentation value; W.A: Water absorption; P.T.: Peak time; T./S.: Tolerance or stability; M.T.I.: Mixing tolerance index; (W): Alveograph Strength; (G): Swelling index, (P): Tenacity; (L): Extensibility
*, **: Significant at 95 and 99 % respectively

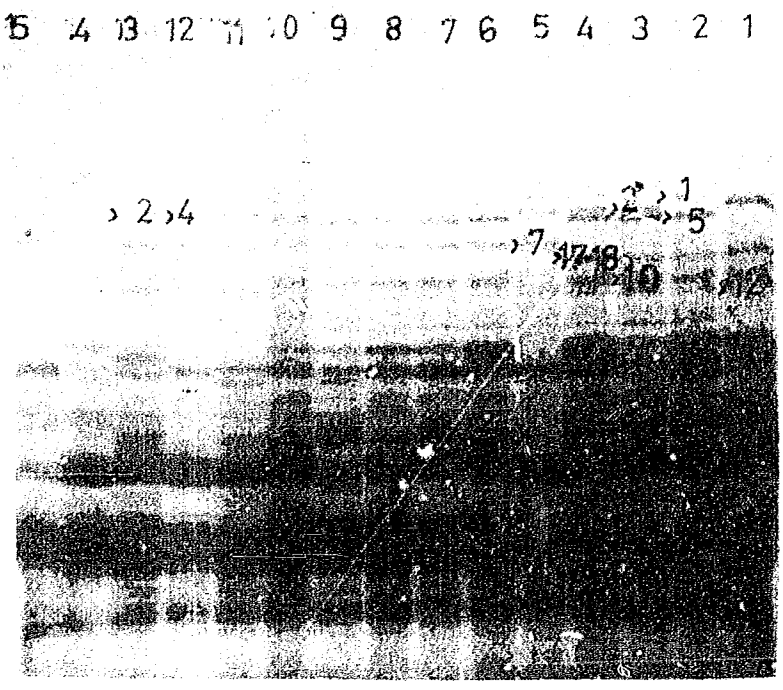


Figure 3. SDS-PAGE of eight moroccan and six french common wheats. 1- Prinqual; 2- Jouda (1646); 3- Marchouch 9 (Sibara); 4- Marchouch 8; 5- Top; 6- Nesma (149); 7- Potam; 8- Pinyte; 9- Sais (1615); 10- Tegyey 9 (5/70-9); 12- Champlein; 13- Tegyey 32 (5/70-32); 14- Prinqual; 15- Disponent.

Tableau XI. Correlation coefficients between HMW glutenin subunits and some quality parameters

Quality Parameters	7	8	17	18	Baking score
P.C. (%m.s.)	-0.55	-0.29	0.55	0.55	0.21
S.V.	-0.69	-0.72	0.69	0.69	0.55
S.S.V.	-0.42	-0.60	0.42	0.42	0.51
W.A.	-0.47	-0.45	0.47	0.47	-0.11
P.T.	-0.78	-0.60	0.78*	0.78*	0.65
T./S.	-0.43	-0.64	0.43	0.43	0.60
M.T.I.	0.52	0.52	-0.52	-0.52	-0.63
(W)	-0.78	-0.67	0.78*	0.78*	0.41
(G)	-0.74	-0.36	0.74*	0.74*	0.87**
(P)	-0.54	-0.63	0.54	0.54	0.03
(L)	-0.76	-0.37	0.76*	0.76*	0.85**

P.C.: Protein Content; S.V: Sedimentation value; S.S.V.: Spescific sedimentation value; W.A.: Water absorption; P.T.: Peak time; T./S.: Tolerance or stability; M.T.I.: Mixing tolerance index; (W): Alveograph Strength; (G): Swelling index; (P): Tenacity; (L): Extensibility

*, **: Significant at 95 and 99% respectively

A strong and significant positive relationship was found between bands 17 and 18 (table II) and some quality parameters (P.T., W, G, L). The relationsnip of these bands with peak time does not support that reported by NG and BUSHUK (6) and with the exception of the swelling index, do not support the data obtained by BRANLARD & DARDEVET (11) either.

CONCLUSION

Although the number of samples analyzed is small, strong confirmations have been found for bands 2 plus 12, and 5 plus 10, with respectively poor and good rheological properties. Concerning the baking scores, derived from HMW glutenin subunit formulas (12), no significant correlations were registered with the parameters related to good quality, unlike the results of PAYNE *et al.* (7) in which these scores were strongly associated with dough strength (W) and ZELENY test values. In fact the baking scores were only positively and significantly correlated with dough swelling index and extensibility that are indicators of poor rheological properties. Further investigations using a high number of cultivars will certainly be useful before assessing the reliability of the baking scores in predicting quality of Moroccan wheats.

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