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ROLE OF LOW MOLECULAR WEIGHT GLUTENINS IN
DETERMINING COOKING QUALITY OF PASTA PRODUCTS**

It is clearly established that gluten proteins controlled the cooking quality of durum wheat pasta products as well as the baking quality of common wheat flour.

Figure 1

LOW MOLECULAR WEIGHT GLUTENINS :
LARGE PROTEIN AGGREGATES WHICH
YIELD UPON REDUCTION SUBUNITS WITH
APPARENT MW OF 12,000 TO 60,000

Among gluten proteins low molecular weight glutenins (LMWG) are those proteins which correspond to large protein aggregates and yield upon reduction subunits with apparent molecular weight of 12,000 to 60,000.

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Figure 2

GLUTEN PROTEINS

TYPE	MW (K)	LOCI	PROPERTIES
GLIADINS	25-70	GLI-1**;GLI-2	MONOMERIC
HMW GLUT.	65-130*	GLU-1	POLYMERIC
LMW GLUT.	12-60*	GLI-1	AGGREGATIVE

*AFTER REDUCTION

**OMEGA AND GAMMA GLIADINS

They are different from the high molecular weight glutenins (HMWG) by the smaller size of their subunits and because they are genetically controlled by loci located on the short arms of chromosomes 1 while HMWG have several loci located on the long arm of groupe 1 chromosomes. They are polymeric and aggregative proteins while gliadins are monomeric materials.

Figure 3

PROTEINS	SDS PAGE SUBUNITS OF TOTAL PROTEINS							
	281	566	622	750	806	872	901	928
	-316			-776	-847			-1071
HMWG	—————							
LMWG	—————							
VLMWG	—————							
α GLIAD	—————							
β GLIAD	—————							
γ GLIAD	—————							
ω GLIAD	—————							
ALB-GLOB	—————							
MW ($\times 10^3$)	110	68	62.5	51.5	44.5	43	41.5	35

However they are not easily identified by SDS polyacrylamide gel electrophoresis because of the occurrence of protein fractions belonging to gamma gliadin and omega gliadin proteins in the LMW glutenin subunit zone. Nevertheless by combining differences in solubility, ion exchange chromatography and SDS-PAGE mobilities, it is possible to identify LMWG subunits specifically and to appreciate their contribution to the total protein pool.

Before explaining how LMWG could have a major role in determining the cooking quality of pasta let us recall three well known findings on pasta cooking quality, on the relationship occurring between gamma gliadins and cooking quality, and on the genetic control of gamma gliadins and LMWG :

a)

Figure 4

- PASTA COOKING PROPERTIES
- 1 - MATTER LOSSES, SWELLING
 - 2 - VISCOELASTICITY, FIRMNESS
 - 3 - CONDITIONS OF SURFACE

Pasta cooking quality is related to three main parameters : matter losses in boiling water and water absorption (or swelling) during cooking ; viscoelastic behaviour and firmness after cooking ; pasta desaggregation or condition of surface of cooked pasta.

It is now well documented that firmness and condition of surface are two independant parameters.

b)

Figure 5

- PASTA COOKING QUALITY
- 1 MATTER LOSSES, SWELLING
 - 2 VISCOELASTICITY : PROTEIN CONTENT
 γ 45/LMWG²
 - 3 CONDITIONS OF SURFACE

It is also well documented that the cooked pasta viscoelasticity is not only correlated to the protein content but also genetically determined by the gamma 42/45 group of loci : glutens extracted from durum wheat varieties with the gamma 45 allele are characterized by their high firmness and their high viscoelasticity ; the following cooked pasta are rated as good or very good in firmness.

c)

Figure 6

ALLELIC TYPES OF GLI-B1 LOCUS

ALLELE	GLIADINS	LMW GLUTENINS
42	γ -42 ω -33-35-38	LMW-1 (4)
45	γ -45 ω -35	LMW-2 (3)

LMWG are under the genetic control of the GLI-B1 group of loci, which has two group of allelic types : gamma 42 and gamma 45. LMWG subunits (a quadruplet) are coded by the gamma 42 group of loci ; LMWG subunits (a triplet) are coded by the gamma 45 group of loci.

Figure 7

GENETIC CONTROL OF GLUTENINS

GLUTENINS	LOCI	ALLELIC TYPES
LOW MW	GLI-B1	2 (42/45)
HIGH MW	GLU-A1	5
	GLU-B1	8

Much more HMWG allelic type (5 x 8) have been identified :
in durum wheat.

It is not easy to explain the differences in the physical properties of gluten extracted from 42 or 45 type durum wheats on the only basis of the presence of the 42 or 45 gamma gliadins. This for two reasons : the physicochemical properties of these two proteins are very like the same ; their contents do not excess 2-3 % of the total pasta proteins. LMWG, which are genetically linked to gamma 42 and gamma 45 gliadins could be more responsible for the differences between 42 and 45 type durum wheat gluten.

Figure 8

LMWG ALLELIC TYPES AND GLUTEN RECOVERY

GLIAD	GLIAD	LMWG	N	GLUTEN RECOVERY
42	42	42	n ₁	LOW
45	45	45	n ₁	HIGH
42	45	45	1*	HIGH

*PROVIDED BY N. POGNA

The discovery of a variety which is $\gamma 42/\omega$ 45/LMWG 45 type and of which the gluten recovery is high brings the proof that $\gamma 42$ is only a genetic marker.

Figure 9

VARIETAL DIFFERENCES IN LMW GLUTENINS

- PHYSICOCHEMICAL, FUNCTIONAL ?
 - QUANTITATIVE : % GLUTEN PROTEINS ?
-

Then one more question arises : are the differences between gluteins due to differences in the quantity of LMWG or in their physicochemical and functional properties or to both parameters. Let us try to answer this question.

I. LMWG CONTENT

The amount of LMWG in semolina was first determined by combining solvent extraction, ion exchange chromatography and SDS-PAGE.

Figure 10

PROTEIN COMPOSITION OF DURUM WHEAT

PROTEINS	CALVINOR (42)	AGATHE (45)
SALT SOLUBLE	23.5	19.2
GLIADINS	33.4*	22.2**
GLUTENINS	33.5	49.5
INSOLUBLE	9.6	9.1
* γ -42 : 3.9	** γ -45 : 2.6	

Two varieties were examined : CALVINOR (type 42) and AGATHE (type 45). We first confirmed the higher amount of glutenins in good quality durum wheat and a lower content in gliadins, but only minor differences in γ 42 and γ 45 gamma gliadins (at least in absolute value).

Figure 11

GLUTENIN COMPOSITION OF DURUM WHEAT

GLUTENINS	CALVINOR (42)	AGATHE (45)
HIGH MW	10.2	11.5
LOW MW	15.1	27.7
VERY LOW MW	8.2	10.3
PER CENT TOTAL PROTEINS		

The contributions of the different types of glutenins - in which we have included VLMWG - to the total glutenin content are 10.2 and 11.5 (HMWG), 15.1 and 27.7 (LMWG), 8.2 and 10.3 (VLMWG) for gamma 42 and gamma 45 type durum wheat respectively. These data certainly give no more than indications on the content in these glutenins families.

Figure 12

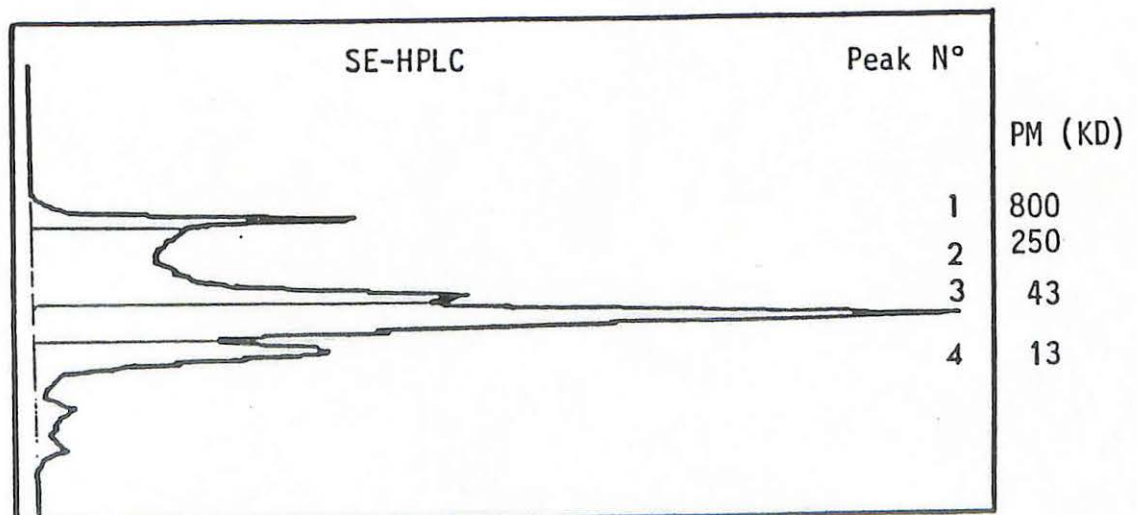
GLUTENIN COMPOSITION OF DURUM WHEAT

GLUTENINS	CALVINOR (42)	AGATHE (45)
HIGH MW	30.5	23.2
LOW MW	45.1	56.0
VERY LOW MW	24.4	20.8
PER CENT TOTAL GLUTENINS		

Nevertheless, expressed in percent of total glutenin, they demonstrate that the strong viscoelasticity of the AGATHE gluten is simultaneous to a high percentage of LMWG in the glutenin fraction. Conversely, both HMWG and VLMWG are in higher concentration in the glutenin of 42 type durum wheat.

Our second approach was to extract proteins with a SDS-phosphate buffer (that we have shown to be able to extract mainly LMWG, VLMWG and salt soluble proteins) and to fractionate the solubles by size exclusion chromatography.

Figure 13



SE HPLC OF SDS-PHOSPHATE BUFFER EXTRACT

Four peaks were identified with molecular weight ranging from 800 KD to 13 KD. It can be postulated that both peaks 1 and 2 are mainly constituted by aggregated low molecular weight glutenins but this has still to be proved.

Assuming that this hypothesis is true it is quite satisfactory to quote the following results.

Figure 14

CORRELATION BETWEEN HPLC PEAKS
SURFACE AND GLUTEN PROPERTIES

HPLC PEAKS	GLUTEN*	
	FIRMNESS	RECOVERY
P1	0.81	0.88
P2	0.75	0.82
P3	- 0.85	- 0.86

*HEAT SHAPED

Having compared 24 durum wheat samples (6 varieties, 4 locations) we found a highly significant correlation between the contents in P1, P2, (P1 + P2) peaks and the gluten* firmness and the gluten* elastic recovery.

* Heat shaped.

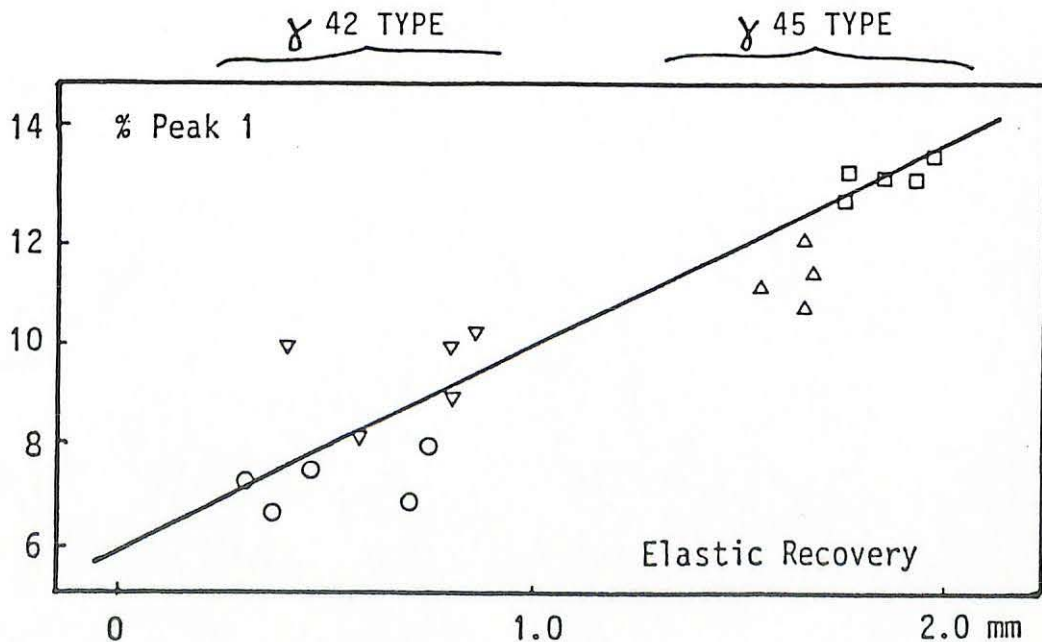
Figure 15

VARIANCE IN HPLC PEAKS SURFACE

HPLC PEAKS	% OF VARIABILITY		F TEST	
	VARIETY	LOCATION	VARIETY	LOCATION
P1	91.0	1.4	**	NS
P2	91.6	4.1	**	NS
P3	93.8	1.2	**	NS

Furthermore, analysis of variance has shown that the variability in P1, P2 and P3 contents is almost exclusively genetically dependant while the location has no effect.

Figure 16



RELATIONSHIP BETWEEN PEAK 1 SE HPLC OF SDS-PHOSPHATE BUFFER SOLUBLE PROTEINS AND GLUTEN ELASTIC RECOVERY

This relation is more visible by plotting the amount of P1 (in % of total SDS-phosphate buffer soluble proteins) versus the gluten elastic recovery.

On the basis of the content in P1, two groups of durum wheat are constituted which overlap the 42-type and 45-type durum wheat groups.

II. FUNCTIONAL PROPERTIES OF LMWG

Because temperature is an important parameter of pasta technology either during the drying operation which are more and more frequently performed above 70°C or during cooking, pasta being left in boiling water for about 10 minutes (depending of the shape), we concentrated our investigations on the behavior of pasta proteins under well defined hydrothermic treatment.

The first finding is that the formation of disulfide bonds is mainly responsible (but not only) for the insolubilization of pasta proteins during heat treatments.

Figure 17

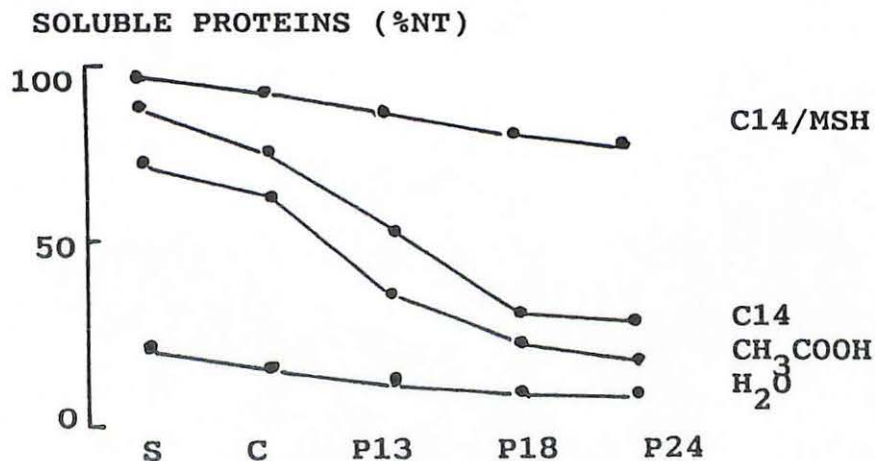
EFFECT OF HEAT TREATMENT AT 30 %
MOISTURE CONTENT ON PASTA PROTEIN SOLUBILITY

TREATMENT		SEQUENTIAL EXTRACTION			
MIN.	T°C	MSH	SDS	SDS	MSH
CONTROL		51	52	100	1
30	120	42	58	43	67 (?)
120	120	20	68	31	57

MSH : 1 % MERCAPTO ETHANOL
SDS : 0.5 % SDS

Pasta with 30 % moisture content were left at 120°C for 30 and 120 minutes. By comparison to the control a sharp decrease in SDS solubility was observed after heat treatment ; then the "insoluble SDS" proteins were extracted in presence of mercapto ethanol (at least if the treatment is not too long) ; when using mercaptoethanol in the first solvent, the decrease in solubility was not so important.

Figure 18

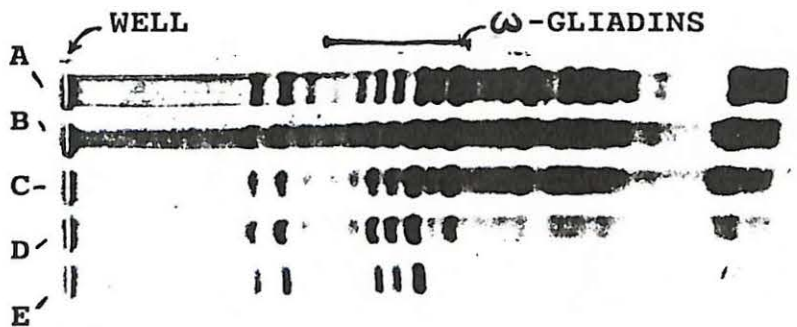


EFFECTS OF HEAT TREATMENT (2HRS-90°C)
AT 13 % (P13), 18 % (P18) AND 24 % (P24)
MOISTURE CONTENT. S : SEMOLINA ;
PC : CONTROL PASTA

In another experiment pasta with 24 %, 18 % and 12 % moisture content were left during 2 hours at 90°C. Stronger was the heat treatment (i.e. higher the humidity), larger was the lost of solubility in sodium myristate, a hydrophobic disrupting agent. Simultaneously, most of the residual proteins were soluble with mercaptoethanol.

PAGE allows to identify more accurately the proteins which aggregate during heat treatments.

Figure 19

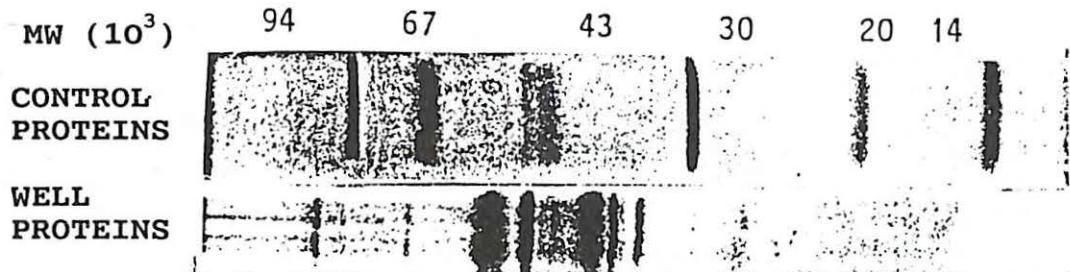


PAGE (PH=3.2) OF CHLORO-2-ETHANOL SOLUBLE PROTEINS
 A : SEMOLINA ; B : PASTA DRYED AT 55°C ;
 C, D AND E : PASTA LEFT FOR 2 HRS AT 90°C AT 13 %, 18 % AND 24 % MOISTURE CONTENT

Beside the well known heat resistance of omega gliadins, which have a very low content in sulphur, it is most valuable to outline the disappearance of the streaks and of the "well" proteins (still staying in the sample wells after electrophoresis) after heat treatment.

To identify the "well" proteins we sliced out the first millimeter of the gel after the well, of which we dissolved the proteins with a mercaptoethanol-phosphate buffer.

Figure 20

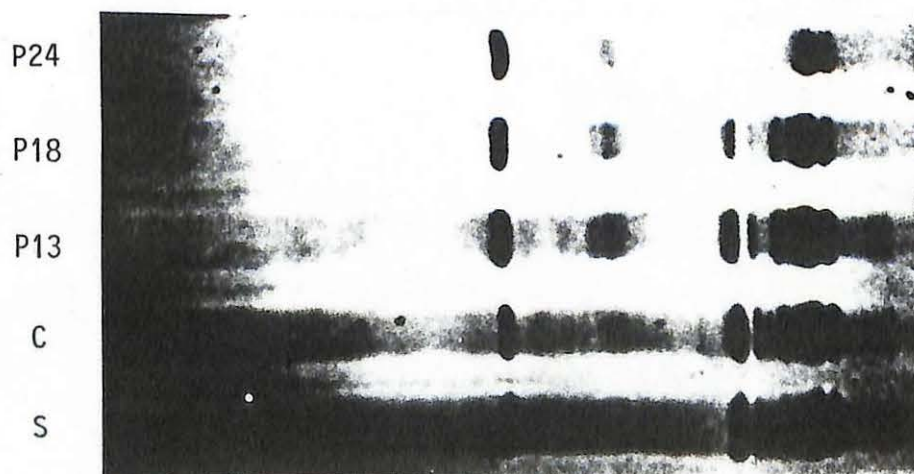


SDS-PAGE OF REDUCED "WELL PROTEINS"
(STAYING IN THE WELL IN PAGE pH = 3.2)

Most of this material is composed with proteins with molecular weight ranging from 35.000 to 50.000. That say in the range of the LMWG subunits.

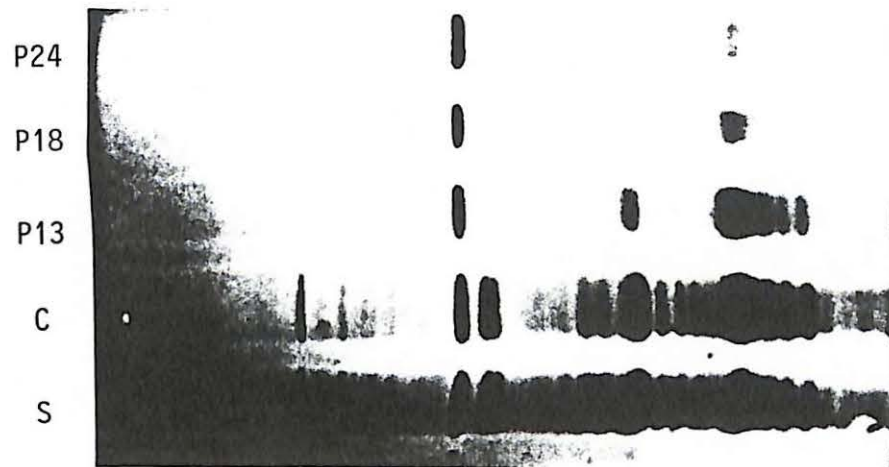
SDS-PAGE of SDS phosphate soluble proteins extracted from the same products after or not reduction lead to similar conclusions.

Figure 21



SDS-PAGE OF SDS PHOSPHATE BUFFER SOLUBLE PROTEINS. S : SEMOLINA ; C : CONTROL PASTA ; P13, P18, P24 : PASTA LEFT AT 90°C FOR 2 HRS AT 13, 18, 24 % MOISTURE

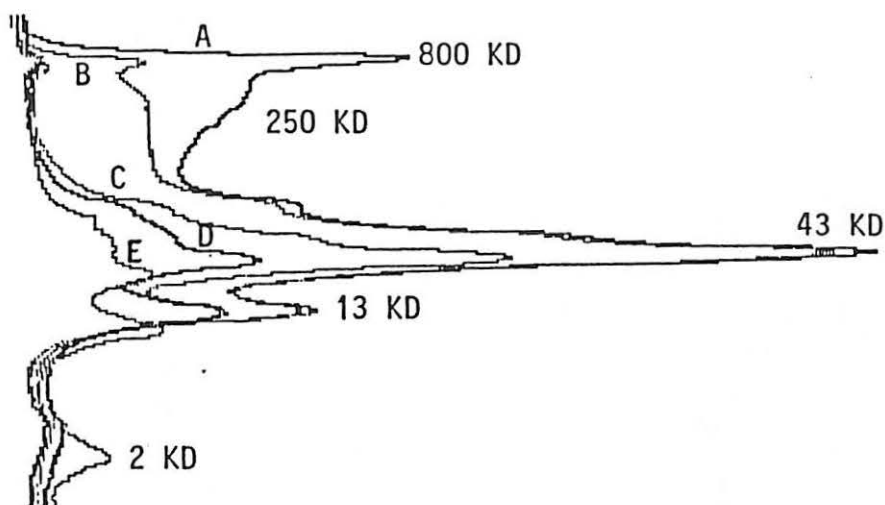
Figure 22



SDS-PAGE OF REDUCED SDS PHOSPHATE SOLUBLE
PROTEINS. S : SEMOLINA ; C : CONTROL PASTA ;
P13, P18, P24 : PASTA LEFT AT 90°C FOR 2HRS
AT 13, 18, 24 % H₂O

S.E. HPLC of the same (unreduced) extracts confirm these
results.

Figure 23



GEL PERMEATION HPLC CHROMATOGRAPHY
OF SDS-PHOSPHATE EXTRACTS-SEMOLINA : A ;
PASTA DRIED AT 55°C : B ; PASTA LEFT FOR
2 HRS AT 90°C AT 13 % (C), 18 % (D)
AND 24 % (E) MOISTURE CONTENT

Figure 24

MW(10^3)	>800	250	43	13
S	24	18	38	11
C	3	11	70	14
P13	1	0	69	22
P18	2	0	38	29
P24	0	0	37	31

SE HPLC OF SDS-PHOSPHATE SOLUBLE
PROTEINS. S : SEMOLINA ; C : CONTROL
PASTA ; P13, P18 AND P24 : PASTA LEFT
FOR 2 HRS AT 90°C AT 13, 18, 24 % MOISTURE

P1, P2 and P3 progressively disappeared from the elution curves while increases the intensity of the heat treatments. The heat sensitivity of LMWG aggregates was so confirmed.

ROLE OF VERY LOW MOLECULAR PROTEINS

The discovery by KOBREHEL and ALARY of a "durum wheat rich sulfur glutenin" (DSG) allows to go a little further.

DSG are solubilized in a purified form by low concentration of sodium myristate (3.5 mg per g. flour) from flour previously extracted by 0.5 NaCl solution and 60 % ethanol. They also solubilized with 0.01 N acetic acid but then in mixture with other proteins (mainly LMWG). Their content in SH + SH groups ranges from 140 to 180 mol/g. and is higher in good durum wheat varieties consequently these proteins are very good candidates to have a major role in pasta quality because of their ability to create disulfide linkages.

Figure 25

DURUM WHEAT SULFUR RICH GLUTENINS
· SH+SS CONTENT : 180-140 MOL/G
· SOLUBLE IN PURIFIED FORM BY C14
· SOLUBLE WITH LMWG BY CH3COOH
· MOLECULAR WEIGHT : 14,000-17,000

As a matter of fact KOBREHEL and ALARY found a highly positive correlation between the content in SS + SH group in glutenin and the condition of surface of cooked pasta ; they concluded to a functional role of DSG in preventing the desaggregation of the surface of the cooked pasta.

Figure 26

- PASTA COOKING QUALITY
- 1 MATTER LOSSES, SWELLING
 - 2 VISCOELASTICITY
 - 3 CONDITIONS OF SURFACE : DSG
(SULPHUR RICH GLUTENIN)

There is no special interest in discussing whether DSG are true glutenins (what does that mean ?) or soluble like proteins linked through hydrophobic bonds to insoluble proteins and, by consequence, insoluble.

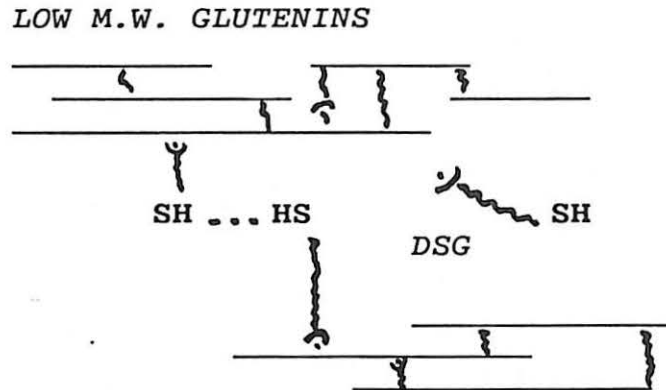
Figure 27

DURUM WHEAT SULFUR RICH GLUTENINS

DSG : LOW MOLECULAR WEIGHT MONOMERIC
PROTEINS, HIGH IN CYSTEINE, LINKED TO
LMWG BY HYDROPHOBIC BONDS, ABLE TO ENTER
INTO S-S BONDS DURING PASTA PROCESSING

Our hypothesis is that the so called DSG (low molecular weight monomeric proteins and high in cysteine) would be able to link together by disulfide bond, and then to link LMWG to which they are already linked by hydrophobic bonds.

Figure 28



HYPOTHETIC ROLE OF DS-GLUTENIN
IN THE AGGREGATION OF LMW-GLUTENINS

This hypothesis is schemed on Figure 28.

CONCLUSION

Figure 29

HYPOTHESIS

- 1-LMWG CONTENT IS RESPONSIBLE FOR THE VISCOELASTIC PROPERTIES OF HEAT SHAPED GLUTEN IN DURUM WHEAT
- 2-LMWG AGGREGATE THROUGH HEAT TREATMENT AND THEREFORE CONTRIBUTE TO THE PASTA FIRMNESS AND VISCOELASTICITY
- 3-THE LMWG CONTENT OF DURUM WHEAT IS GENETICALLY CONTROLLED : TWO FAMILIES OF DURUM WHEAT HAVE BEEN IDENTIFIED
- 4-DSG CONTRIBUTE TO THE AGGREGATION OF LMWG, THROUGH HYDROPHOBIC AND DISULFIDE BONDS
- 5-THE LMWG-DSG BONDS ARE TIGHT ENOUGH TO PREVENT STARCH LEACHING DURING PASTA COOKING AND TO KEEP SATISFACTORY THE STATE OF SURFACE OF COOKED PASTA
- 6- γ 42 AND γ 45 GLIADIN ARE ONLY GENETIC MARKERS OF PASTA FIRMNESS

The main conclusions of this presentation are summarized on Figure 29. They are numerous but arise still more questions as listed Figure 30.

Figure 30

TO SCIENTISTS LOOKING FOR WORKS

1. TO CONFIRM DIFFERENCES IN LMWG CONTENT BETWEEN 42 AND 45 TYPE DURUM WHEAT
2. HOW THE RELATIVE AMOUNT OF LMWG IS GENETICALLY CONTROLLED ?
3. WHY DO LMWG AGGREGATE ? WHY ARE THEY SENSITIVE TO TEMPERATURE ?
4. ARE DSG LINKED TO LMWG THROUGH HYDRO PHOBIC BONDS ? ARE DSG ABLE TO CREATE -S-S- LINKAGE ?
5. TO IDENTIFY GENES CODING FOR DSG

They can be discussed with regard to the works of others :

a) The sulfhydryl and disulfide groups of wheat endosperm proteins have long been postulated to be important in determining dough properties and the baking quality of wheat flour. It has been supported that disulfide interchange catalysed by sulphhydryl groups is necessary for a good balance between elasticity and extensibility.

b) POPINEAU claimed (1984) that aggregates enriched in subunits with molecular weights over 60,000 (HMWG ?) have lower hydrophobicities than aggregates rich in 39,000-43,000 subunits (LMWG ?). He found that variation in the hydrophobic

properties of the glutenins may be larger than suggested previously.

c) He found that the not bound fractions in hydrophobic chromatography were mainly constituted of subunits with MW between 39,000-43,000 (LMWG) and that the first 0.02 M NH₄OH eluted fractions comprised subunits having molecular weights of 67,000 and 69,000 (HMW) together with subunits having MW of 14,000 (DSG??).

d) TATHAM et al. showed that heating to 80°C results in greater decreases in the secondary structure contents of the reduced LMW subunit of glutenin than of the unreduced aggregated gliadin, indicating that the latter were partially stabilized by the intact disulphide bonds.

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ABSTRACT

139 ROLE OF LOW MOLECULAR WEIGHT GLUTENIN IN DETERMINING COOKING QUALITY OF PASTA PRODUCTS,

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Low molecular weight glutenins (LMWG) are those proteins which correspond to large protein aggregates and yield upon reduction subunits with apparent MW of 12,000 to 60,000. Estimation of their content by combining sequential extraction, chromatography and electrophoresis shows that:

- a) LMWG are a major fraction of durum wheat gluten ;
- b) their content in γ -45 durum wheat type is higher (30 %) than in γ -42 durum wheat type (15 %).

LMWG are very sensitive to heat treatment and are readily denaturated during the drying step of pasta processing or during cooking. It is postulated that the strong aggregative power of those proteins confer to LMWG a central role in determining the cooking quality of pasta.