Comparison of biochemical and functional properties of various cysteine rich low *Mr* wheat proteins

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In addition to gluten proteins, flour contains 15 to 25 % by weight of water-extractable, non-gluten proteins. In wholemeal flour, the proportion is even higher and a substantial amount of the flour proteins may be non-gluten proteins.

Low Mr proteins differ from gluten proteins in extractability, lipid affinity and amino acid composition.

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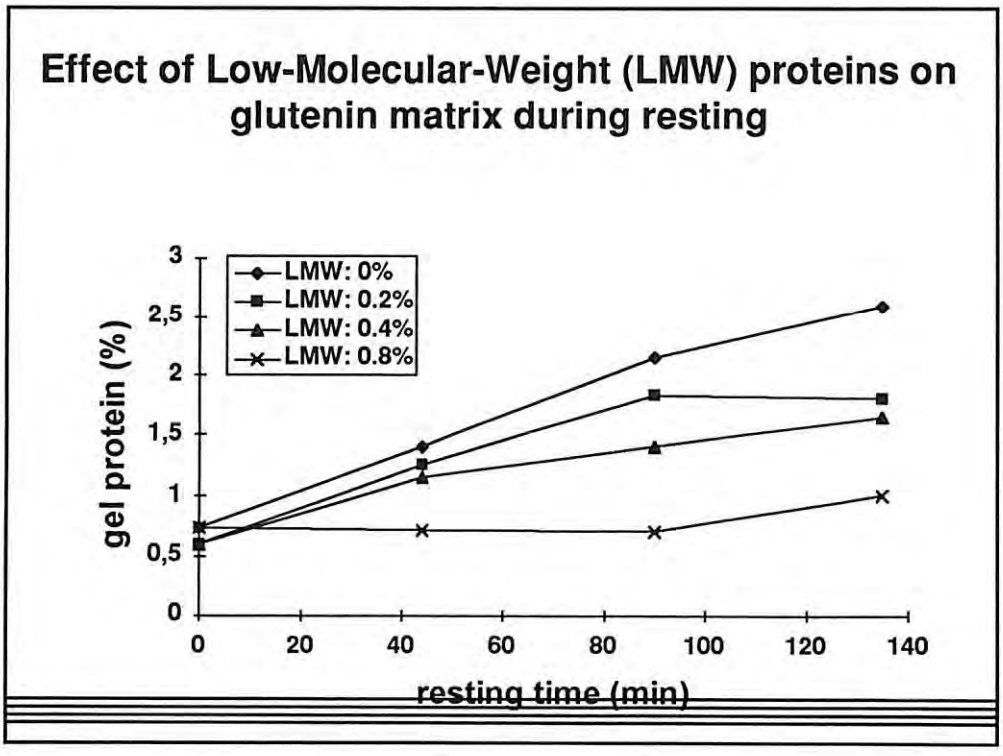
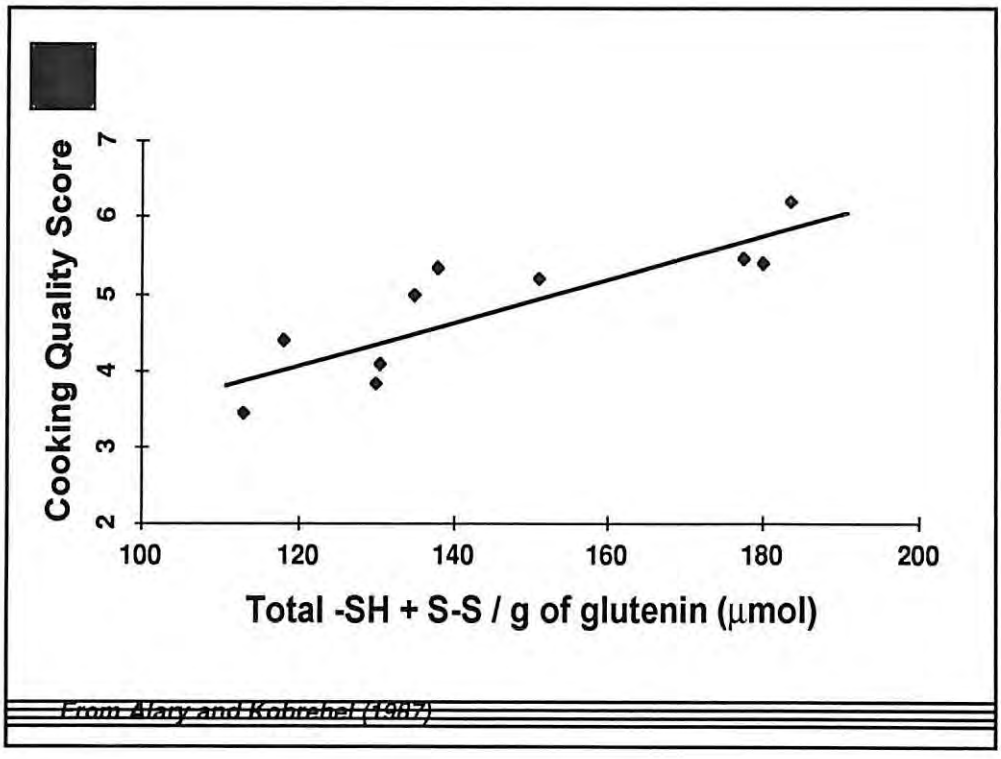


A first effect of low Mr proteins was shown by addition to flour. During resting of the dough, the amount of gel protein increases flour is mixed <u>without</u> LMW protein as the glutenin proteins are cross-linking to larger aggregates. But, the low Mr proteins are able to decrease the rate of gel protein reassembly during resting. For instance, addition of 0.2 % of LMW protein, that is only 2 % of the protein present prevents the complete reformation of gel protein. Increased additions have more profound effects on the glutenin matrix. Addition of 0.8 % prevents any cross-linking of glutenin proteins (at least in the case of cv. Rektor: this slide).

As suggested earlier, low Mr proteins probably act as end group blocker. During mixing, some S-S bonds are broken down and low Mr proteins can react with the thiol groups. Thus reassembly is retarded or even prevented by low Mr proteins.



Another effect was demonstrated in the case of durum wheat pasta by Kobrehel and Alary. A low Mr, cysteine-rich, fraction was isolated using low concentrations of soap (Na tetradecanoate) and formerly called durum wheat sulfur-rich glutenin or DSG.

The slide illustrates the correlation between pasta cooking quality and total -SH plus S-S content of the DSG fraction.

So, low Mr proteins are important for their proportion in flour. They may be also important in quality.

We therefore set out to compare biochemical and funtional properties of various cysteine rich low Mr wheat proteins

In a first part we'll report a comparison with literature data

In a second part, we'll report some recent results based on molecular mass and immuno affinity.

Comparison of amino acid composition
(correlation in %; groups of proteins averaged)

	S	Lig.	CM	LTP	P3-1	P3-2
α-amyl. inhib.	79	85	71	54	90	97
S-fraction		86	80	39	82	77
Ligolin			73	52	87	82
CM				57	83	67
LTP					56	43
P3-1 (< 17)						90

First part: comparison with literature data

In this slide, we show a comparison based on amino acid composition.

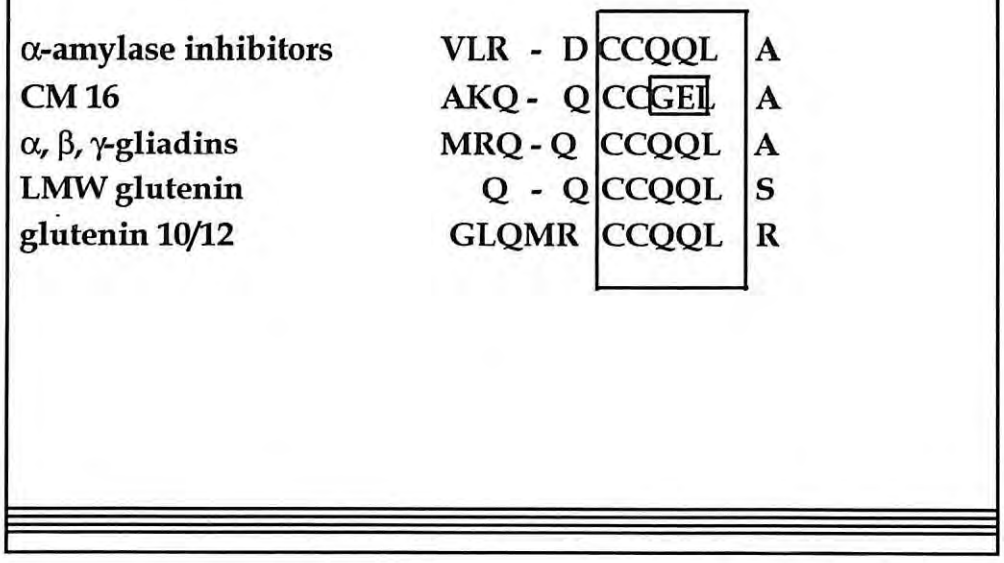
A correlation between the % of amino acid, (groups of proteins averaged) was calculated. Some homology exists between the various groups. In contrast, very high correlations are found only between P3 subfractions and α-amylase inhibitors. Relatively high similarity exists among ligolin, S-fraction and P3-1. On the other hand, there is less similarity between CM and P3-2, while LTP doesn't show any strong correlation.

Sequence homology of low *Mr* wheat proteins
(homology in %)

	CM2	CM3	CM16	CM17	0.53	0.19	0.28
CM1	86	39	41	37	34	31	33
CM2		50	45	37	38	34	37
CM3			45	37	41	41	30
CM16				78	27	27	27
CM17					26	26	30
0.53						94	58
0.19							56

Amino acid sequences also demonstrate very clear similarities. For instance, there is a high % of homology betweenCM1 and CM2, CM16 and CM17 and among inhibitors 0.19, 0.28 and 0.53, especially between 0.19 and 0.53. In contrast, a very low homology appears between any of the CM fractions and any of the inhibitors (the lowest homologies (< 30 %) being observed between CM16-CM17 and the inhibitors)

Comparison of the most homologous amino acid sequence of various wheat proteins



Data bases of protein sequences (PIR), that include as many as 45,000 polypeptides, showed that the only types of proteins to have some homology with α-amylase inhibitors were the following: CM, α–, β–, γ-gliadins, LMW glutenins and HMW glutenins 10 and 12.

This allowed to identify a conserved region, as indicated in the slide, excepted for the GE residues of the CM 16.

Consensus pattern ('signature') of α-amylase inhibitors and CM proteins

[N-term]

$X_{(3-6)}\,C\,X_{(10)}\,L\,X_{(2)}\,C\,R\,X_{(5)}\,Q\,(X)\,C...$

Where:

C = cysteine
L = leucine
Q = glutamine
R = arginine
X = any amino acid

The same results coming from the data base programme allowed to identify a consensus pattern (or 'signature') of α–amylase inhibitors and CM-proteins, consisting of conserved cys, leu, gln and arg residues.

Comparison of α-amylase inhibition

Inhibitor	origin of α-amylase			
	Bacillus subtilis	*Aspergillus oryzae*	human saliva	yellow mealworm
0.19	–	–	+	+
0.28	–	–	+	+
0.39	–	–	–	+
0.53			+	
CM		–	–	–
P3	–	–	+	

One of the most typical functional properties of low Mr wheat proteins is their ability to inhibit α-amylases (and also proteases).

A comparison of α-amylase inhibition by various low Mr proteins is shown on this table. Data from the literature indicate that 0.19 and 0.28 inhibit both human saliva and yellow mealworm α-amylases, the latter only being inhibated by the protein 0.39. Low Mr proteins of the fraction P3 also inhibit the human saliva α-amylase, whereas no inhibition was found for subunits of CM proteins.

Hence, on the basis of α-amylase inhibition, P3 could be homologous *e.g.* to fractions 0.19 or 0.28, but not to CM-proteins.

Amounts of cysteine in low *Mr* proteins

Protein	cysteine	free thiol
Purothionin	8	–
0.19	10	0
0.28	10	0
0.39	10	0
0.53	9	0/1
CM1, CM2	10	–
CM16, CM17	10	–
P3	8-10	0

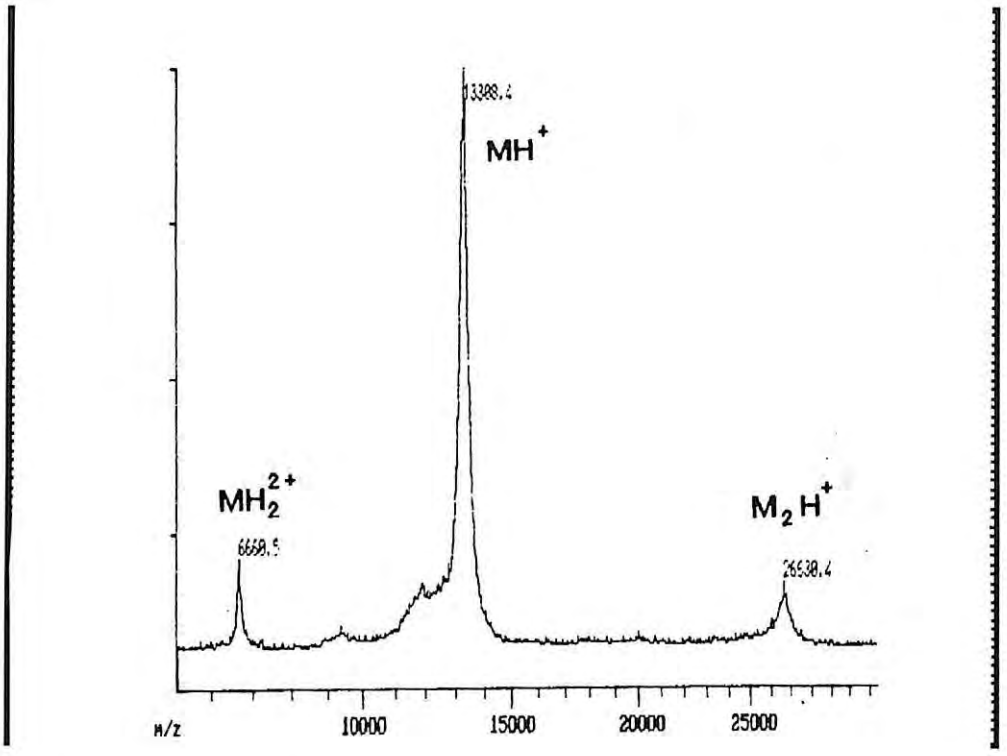
– = not determined

This slide shows tre amount of cysteine residues in various low Mr proteins.

First af all, it is apparent that these fractions contain very high amounts of cysteine (7-9 % is very high for cereal proteins).

Also, some homology between the various members of the low Mr proteins appears from the number of cysteine residues. Except for inhibitor 0.53 in which there is still some doubt (9 or 10 cysteines ?), all the other proteins have an even number of cysteine (8 or 10) and are likely to have no free thiol. [Of course, the S-S bonds are also likely to be disrupted, to give reactive thiols, capable to react with other thiols from other proteins].

Fraction 17
mass: 13,313.7 +/- 5.7 Da



Another comparison has been based on mass determination

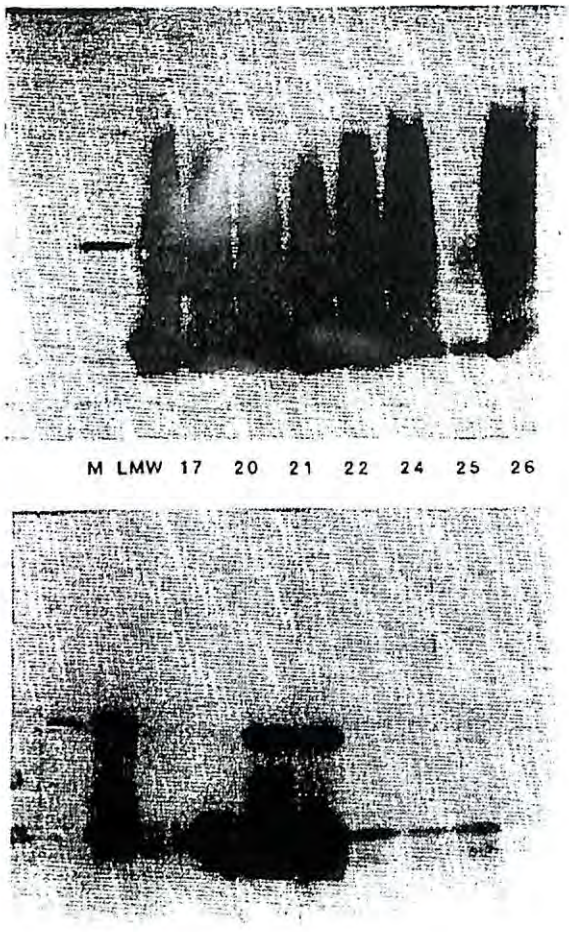
This was in fact a matrix assisted laser desorption mass spectrometry. The mass found for the fraction examined on this slide (subfraction 17 of P3) was 13,317.7 ± 5.7 Da, a very accurate value. You may also notice that a dimer is likely to be present on the mass pattern.

Comparison molecular masses as determined by amino acid or mass spectrometry

		α-amylase inhibitor			
		0.19	0.28	0.39	0.53
Peak	mass	13337	13156+	13056	13185
17	13309	+	+		
18	13246		+		
19	13219		+		
21	13170		+		+
23	13051			+	

Because the determinations by mass spectrometry could be very accurate, some homologies can be reported when comparing with the mass determinations derived from amino acid composition. For instance, between subfraction 17 and inhibitor 0.19, or between subfraction 21 and inhibitor 0.53 (The homology is less clear for inhibitor 0.28 due to some overlapping in the molecular masses).

Unreduced



This figure shows some differences in immunoaffinity among the various RP-HPLC subfractions (17, 20-22, 24-26) of P3 for antibodies prepared from the whole P3 fraction, considering both reduced and unreduced proteins.

There are some striking differences in the affinity for polyclonal antibodies between the fractions. Most peak interact strongly, while others (25) do not interact, suggesting large structural or compositional differences between e.g. fractions 17 or 20, 21-22 and 24-26. It is likely that these various proteins may or not resemble to each other in a immunochemical sense. Also, it can be seen that dimers and tetramers are present especially in the unreduced state

Immunoaffinity comparison between low Mr proteins

Antibody \ Proteins	LTP	CM3	CM16	0.19	0.28	P3
LTP	++	-	-	-	-	-
CM16	-	-	+	-	-	-
0.19	-	-	-	++	+	++
0.28	-	-	-	+	++	+

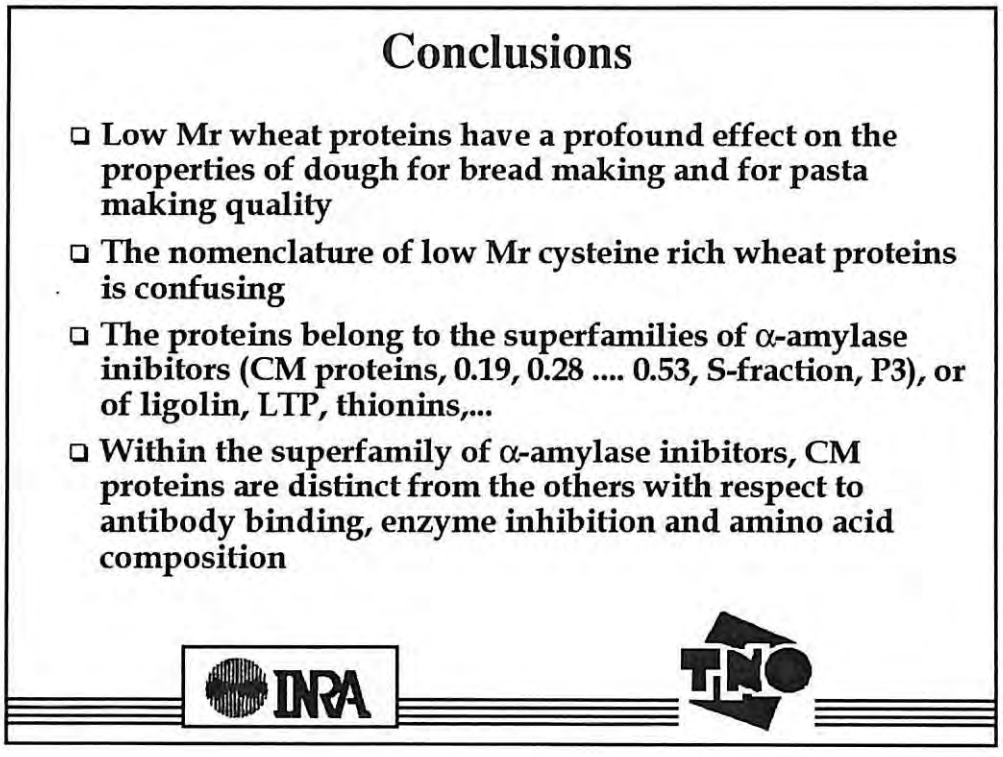
Similar experiments using antibodies raised against various low Mr proteins showed strong reactions between LTP with itself, or CM 16 with itself, which is OK. But a reaction was observed between P3 and the two α-amylase inhibitors, in contrast with CM 16 that showed no reaction with any of the inhibitors.

Immunoaffinity comparison

Proteins / Antibody	P3 fractions 14 - 17	P3 fractions 22 - 26	CM16	0.19	0.28
CM16	-	-	+	-	-
0.19	++	-	-	++	+
0.28	-	++	-	+	++

To check for possible cross-reactions between P3 and the two inhibitors, the immunoaffinity comparison was investigated considering RP-HPLC subfractions 14-17 and 22-26 of fraction P3.

And here, it is clear that subfraction 14-17 is homologous of inhibitor 0.19, that subfraction 22-26 is homologous of inhibitor 0.28, whereas the CM fraction doesn't react with the antibody raised against the α–amylase inhibitors.



In conclusion,

- Low Mr wheat proteins have a profound effect on the properties of dough for bread making and for pasta making.

- The nomenclature of low Mr cysteine rich wheat proteins is confusing.

- These proteins belong to the superfamilies of α-amylase inibitors (CM proteins, 0.19, 0.28 .... 0.53, S-fraction, P3), or of ligolin, LTP, thionins, ...

- But, the most original conclusion is the following: based on amino acid composition, amino acid sequence, α–amylase inhibition, molecular mass and immuno affinity, all the results support the conclusion that, within the superfamily of α-amylase inhibitors, CM proteins are distinct from the others.

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2. 17 Proteins
3. 02 Baking

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Cysteine rich low M_r (M_r 7000 - 15000) wheat proteins, e.g. Chloroform-Methanol (CM) extractable proteins, Lipid Transfer Proteins (LTP), purothionins, ligolin and albumins (0.19, 0.28 and 0.53), are reported to be important for breadmaking and pasta making quality. A comparison is made between these proteins by biochemical (SDS-PAGE, IEF, immunoblotting, RP-HPLC, SH/SS-reactivity, enzyme inhibition) and physico-chemical (mass spectrometry, surface tension) methods and by comparing amino-acid sequence information. Most of the proteins, except LPT, belong to the superfamily of wheat alpha-amylase inhibitiors (CM, albumins, ligolin), whose molecular masses vary between 12,727±119 and 13,309±6 Da as determined by mass spectrometry, or to the superfamily of viscotoxins (purothionin). By immunoblotting differences are observed between CM proteins, LTP and albumins. Also, within the group of albumins differences exist in antibody binding.

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