SLIDE 1

The Low-Molecular-Weight Glutenin Composition of French Bread Wheats and its Effect on Dough Properties.

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It is a great pleasure for me to be with you this morning and discuss some problems about low-molecular-weight glutenin composition especially among French bread wheats.

Because storage proteins have a major influence on grain quality, many investigations have been carried out on relationships between electrophoretic patterns and the quality potential of the wheat genotypes.

It is only in the last ten or twelve years that it has been possible to correlate the presence of specific protein components of wheat with rheological properties of dough.

SLIDE 2

In the beginning of the 80's, we know that Payne and coworkers, were the first to demonstrate associations between specific high-molecular-weight subunits of glutenin and baking strength. These correlations were more or less confirmed and extended by several groups in France, Netherlands, Australia, Canada, USA, Spain, etc.

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Texte oral

In addition, further studies were carried out in order to understand the basis of the relationship between HMW subunits such as '5+10', '7+9', '2*' and higher baking strength, covering protein purification, cloning the genes encoding most of these HMW subunits and trying to correlate functionality with specific features in the amino acid sequence (β-turns, number and location of the cysteine residues).

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In contrast, as recently pointed out by Gupta and coworkers, **much less is known about the association of specific low-molecular-weight subunits of glutenin with bread wheat quality, even though LMW subunits also form disulfide-bonded aggregates and make up a larger proportion of the gluten proteins than HMW subunits.**

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Accordingly, it became essential to investigate these LMW subunits, all the more so because, **in durum wheats, LMW fractions were shown to strongly affect gluten properties. While, in earliest studies by Damidaux and coworkers (1978) a higher firmness or viscoelasticity was found associated with the presence of the electrophoretic component γ-gliadin 45, it was recently demonstrated that the direct causal agents of gluten viscoelasticity were in fact LMW subunits of glutenin, γ-gliadins simply acting as genetic markers of the whole *Gli-B1* locus. In addition, it was found that (i) allelic variation at HMW loci played a considerably less important role than that of LMW and that (ii) simple differences in the amount of LMW glutenin types (without involving structural differences) might be the cause of differences in pasta quality.**

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The main aim of the present investigation was to assess the possible relationship of LMW subunits with dough properties among bread wheats and its consequences in the stimulation of breeding and development of cultivars capable of satisfying more especially the requirements of French and South-Western Europe baking industries.

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Let me recall that in these countries, dough quality assessment is largely based on Chopin Alveographic curves.

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So, we started by investigating the high molecular weight allelic types from a set of more than 100 French bread wheat cultivars and we investigated the mean value of the quality attributes of their dough as measured by Chopin Alveograph, i.e. *W*, *P*, *G* and *P/L* parameters and also their baking score to find out if such or such type of pattern would impart such or such type of curve.

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Here we see the result of the first attempt of separation of cultivars, based on HMW alleles, to explain their difference in *W* index.

Unfortunately, only two different groupings were separated by the computer, based on HMW '6+8' or '7' (lowest *W* scores), and on '7+8' or '7+9' (highest *W* scores), respectively. It means that the allelic variation of HMW subunits has a limited value for predicting dough properties among French bread wheat genotypes. Surprisingly, *Glu-D* alleles '5+10' or '2+12' were not found as variables explaining differences in *W* index among French wheats. Also, a number of major discrepancies were observed between the score based on HMW composition and dough strength or baking score.

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Here we see that the same allelic type (and the same theoretical score) may contain very good (class B1) or premium (A) wheats as well as wheat unsuitable for baking purposes (D2).

Actually, this should not be surprising if we remember that earliest studies on HMW subunits were developed by Brittish groups in view to improve the quality level of their doughs that were traditionnally characterized by low tenacity and excessive extensibility. They worked on HMW subunits that were considered as determining dough tenacity. This is O.K., but it was perhaps a mistake that many research groups in the world rushed to do the same while their own genetic pools and environmental conditions contributed sometimes to yield wheats having different or opposite deficiencies !

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In the case of French bread wheats, the requirements for bread-making are clearly different than for English or American bread: so long as dough strength has a sufficient level, the most critical parameter and perhaps the best indicator of French bread-making ability is dough extensibility. So, an investigation of other proteins, and especially LMW subunits of glutenin - that are still the least characterized fractions of gluten - was necessary.

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But you know that, in contrast to gliadins and HMW subunits of glutenin, that are easily resolved in 1D systems, LMW subunits have proved much more difficult to analyze in a 1D (SDS-PAGE) system because of their overlapping mobilities with the gliadins. Recently, a considerable step forward to overcome the overlap with gliadins was provided by Gupta and Shepherd through the development of a two-step one-dimensional SDS-PAGE.

In the present study we are proposing an alternative procedure, based on a new sequential extraction, that proved quite efficient in giving a clear-cut separation between reduced glutenin polypeptides and gliadin monomers, that probably requests less skill than that of Gupta, and that enables a routine3characterization of LMW subunits (as well as HMW) by regular SDS-PAGE.

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In this procedure, flour is selectively extracted by 70 % ethanol, but after a treatment by a Tris-HCl buffer containing 3 % Triton X-114, a detergent used in the studies of membranes or of lipid-transfer proteins by Didier Marion. Gliadin monomers can be precipitated from the ethanol supernatant while glutenin subunits are obtained by reducing the ethanol insoluble fraction and simply analyzed by SDS-PAGE.

SLIDE 14:

Here we see the SDS-PAGE patterns of the three fractions

If we look carefully, it shows that there is very limited overlapping between the three fractions, including between the B-type LMW subunits and γ-gliadins. The basis of this improvement has not been clarified yet.

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Based on this procedure, it becomes possible to screen large series of genotypes for glutenin subunits, including both HMW and LMW, without any significant contamination by gliadins.

This slide shows an example of SDS-PAGE analysis of variation in B-type LMW glutenin subunits from a set of French wheat cultivars.

SLIDE 16

Now we look at the schemes of the 9 main types of B LMW subunits that we could identify so far among a set of 53 French cultivars, with indications of the mean values and standard deviations of *W*, *G* and *P/L* attributes of dough quality (expressed as % of the score of the standard cv. Capitole).

To further investigate the possible relationship between these types of patterns and the variation of the quality attributes, various statistical methods were investigated.

In the next slides, I am going to illustrate the results obtained by segmentation analysis, a method, based on *t* test of Student, that suggests groupings of patterns with significantly different mean values of each quality attribute considered as the explained variable.

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For instance, taking here into account both LMW and HMW subunits to explain *dough strength (W* index), much more classes can be distinguished than when using HMW only. This slide shows that different levels of baking strength can be significantly distinguished, based first on HMW types, second on LMW types (each HMW type is separated into 2 classes explained by LMW types), one of the LMW classes being further separated by *Glu-D1* allelic types.

SLIDE 18: Segmentation Analysis Taking into Account HMW Subunits (Explained Variable: *G* from Alveograph)

When considering *G* index, i.e. dough extensibility, 2 + 2 classes can be distinguished on the basis of HMW subunits only.

SLIDE 19: Segmentation Analysis Taking into Account both LMW and HMW Subunits (Explained Variable: *G* from Alveograph)

More classes are distinguished taking into account both HMW and LMW subunits. But, in this case, the first level of separation (the most significant) involves the LMW patterns. Each class is further splitted at a more secondary level by *Glu-B1* HMW subunits. Also, the association of LMW types n° 4, 5, or 7 and HMW allele '7+8' seems to impart the highest scores of extensibility (105 % of that of the standard cv. Capitole). So, there is a cumulative effect, but LMW subunits are the ones that better explain the variation in dough extensibility.

Now let's move on the major conclusions of this study.

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a) Within a set of wheats with identical HMW patterns, specific LMW subunits are associated to significantly higher (e.g. LMW type 7) or lower (e.g. types LMW 1 or 9) levels of baking strength. **The variability in LMW types may contribute to explain the variability in** baking strength.

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b) **Among French bread wheats, in contrast with other types of wheats, and also because of the specificity of South-Western European bread-making and possibly of environmental conditions), the variation of LMW subunits seems to be of almost equivalent importance as that of HMW subunits in determining baking quality. Accordingly, it can be recommended to breeders to take into account both types of subunits. At present, the association of HMW '7+8' or '7+9' with LMW types '7' or '4' seems to correspond to the highest potential of baking strength.**

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c) **When focusing on the attribute *G* (extensibility), the reliability of the prediction is much higher than for baking strength. Because dough extensibility is presently the most difficult attribute to control, I should say that new wheat genotypes should be selected first on the basis of their LMW pattern, using possibly HMW as additional markers.**

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In addition, **the relationship between LMW and extensibility is confirmed. Whether this relationship results from a different molecular weight distribution that is induced by LMW subunits, or from their higher sulfur content (a good supply of -SH and S-S being required to allow an ordely slipping - extensibility - of molecules), needs to be addressed in future work.**

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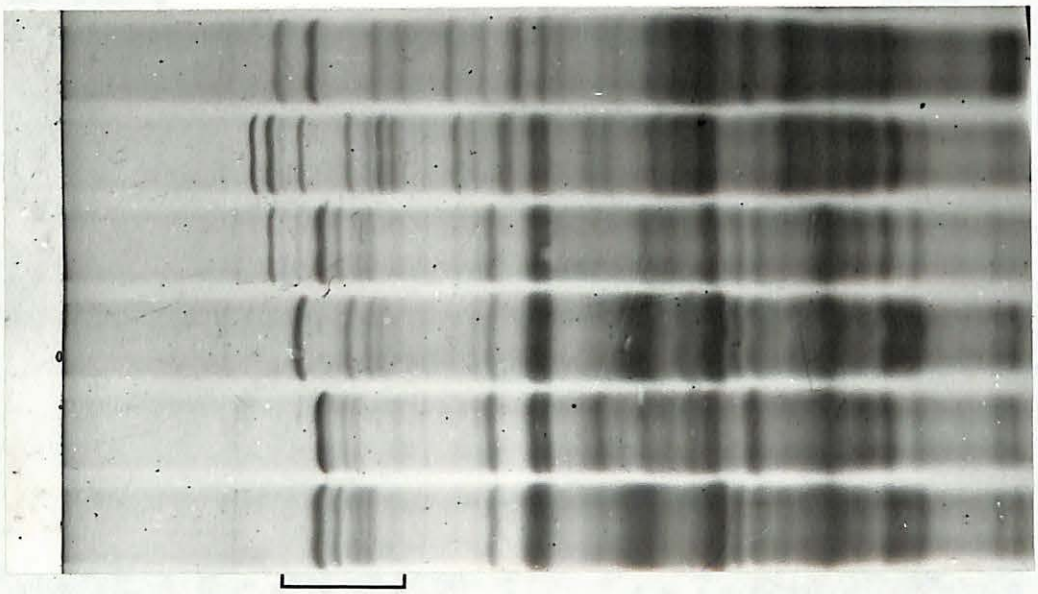
d) Finally, the effect of LMW on baking quality might, at least partially, result from the quantity of protein produced by the different alleles (e.g. LMW class # 1, the only one that contains one single B band, is a grouping of extremely poor cultivars). However, it cannot be ruled out that one allele might appear more effective either in strengthening a dough or in improving extensibility. As a consequence, our present work is aimed at purifying the main LMW subunits to achieve a better understanding of their mechanism of aggregation in wheat doughs.

Conventional SDS-PAGE



HMW subunits

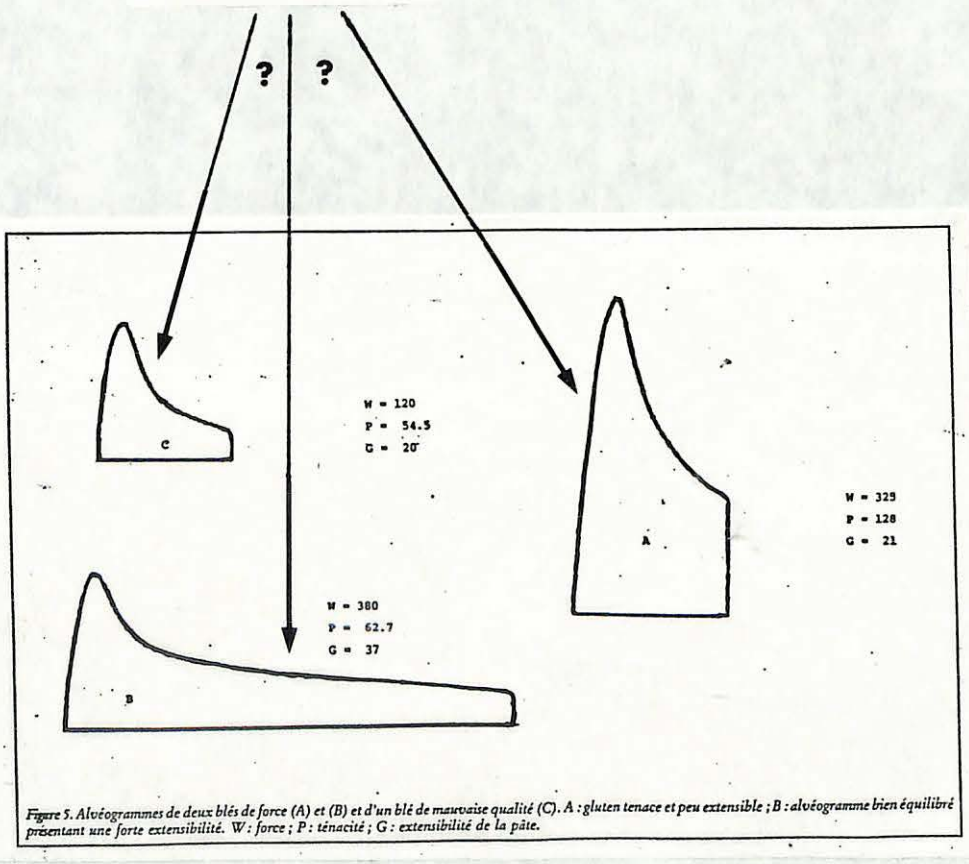
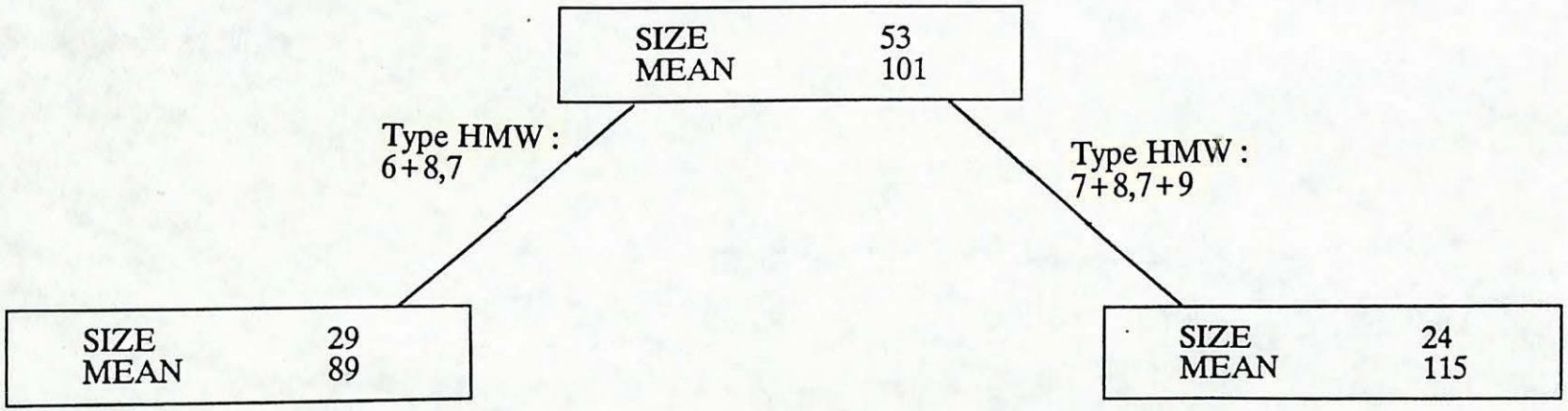


Figure 5. Alvéogrammes de deux blés de force (A) et (B) et d'un blé de mauvaise qualité (C). A : gluten tenace et peu extensible ; B : alvéogramme bien équilibré présentant une forte extensibilité. W : force ; P : ténacité ; G : extensibilité de la pâte.

Different types of Alveographe curves



First attempt of separation of significantly different classes of SDS-PAGE patterns considering (Explained variable: *W* index, explanatory variables: allelic types of HMW subunits of glutenin.

Some major discrepancies in the relationship between allelic variation at HMW loci, baking strength and class of French baking

Cultivar	HMW Allelic Type	HMW Score	Baking Strength	Baking Class
Darius	2-7-12	4	8	A
Carlos	"	4	8	B2
Rotonde	"	4	3	D2
Drakkar	2-6-8-12	3	5	B1
Thésée	"	3	6.5	B2
Appolo	"	3	3	D2
Magister	"	3	2.5	D2
Delfi	5-7-8-10	10	7	B1
Fidel	"	10	5	B2
Bosco	"	10	4.5	D2

Specifications for Typical French Bread-Making

- Medium protein content (10.5 - 12.0 %)
- Relatively high baking strength (W index, from Alveograph) but with a satisfactory balance between *P* (resistance to dough deformation) and *G* (dough extensibility).
- *G* index being sometimes a better indicator of French bread-making ability than W.

New Sequential Extraction

0.5 g ground seeds or flour

↓

15 ml Tris-HCl buffer (100 mM Tris, 6 mM EDTA, 100 mM KCl, 0».5 g **Triton X 114**, pH 7.8)
(1 hour at 4° C, supernatant discarded)

↓

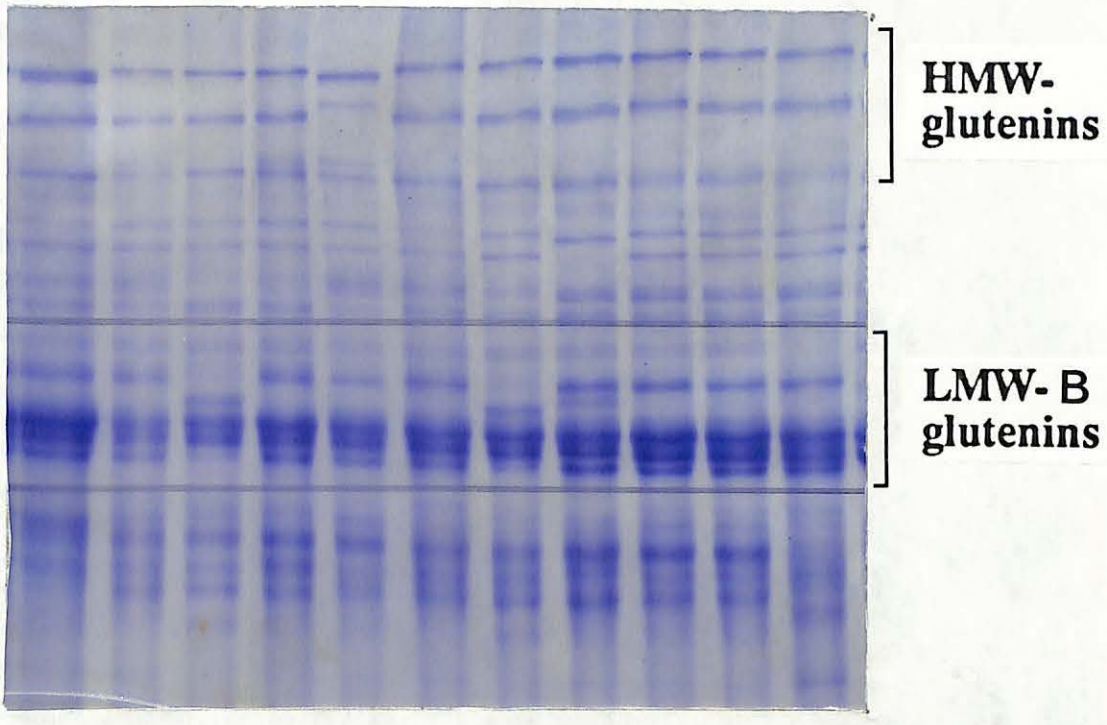
Residue ---> resuspended in 8 ml ethanol 70 %
(2 hours at 4° C)

↓

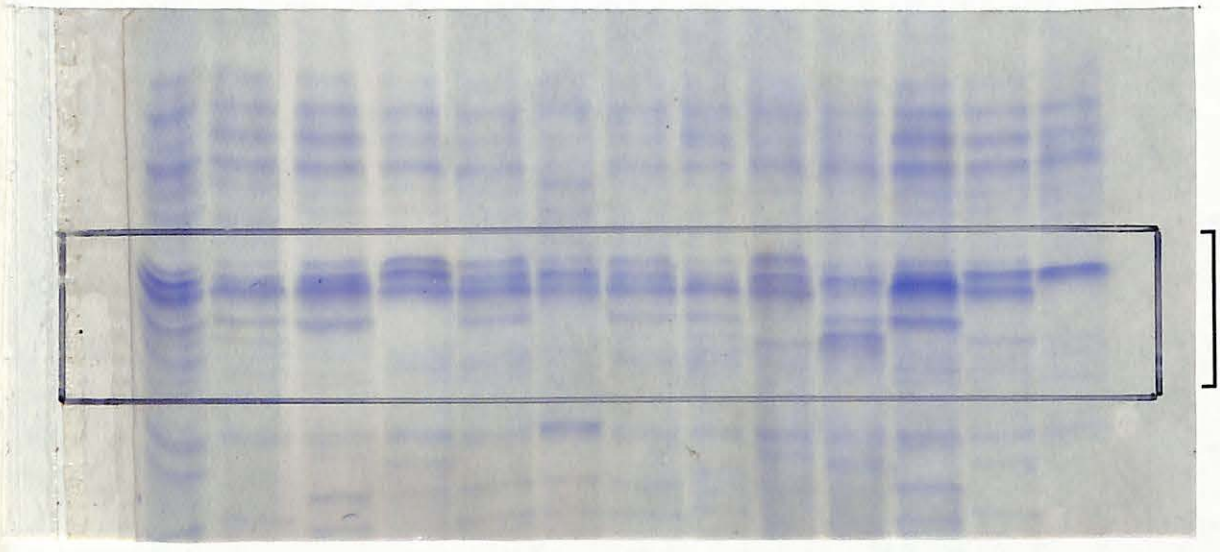
Supernatant ---> acetone precipitation
---> **gliadin monomers** ---> SDS-PAGE

↓

Residue ---> resuspended in 5 ml reducing agent
(1M Tris-HCl, 5.6 % SDS, 5 % mercaptoethanol, pH 6.8)
---> **glutenin subunits**
---> 5 µl loaded on SDS-PAGE gel.



Screening for variation in B-type LMW glutenin subunits by SDS-PAGE from a set of French wheat cultivars.



Screening for variation in B-type LMW glutenin subunits by SDS-PAGE from a set of French wheat cultivars.

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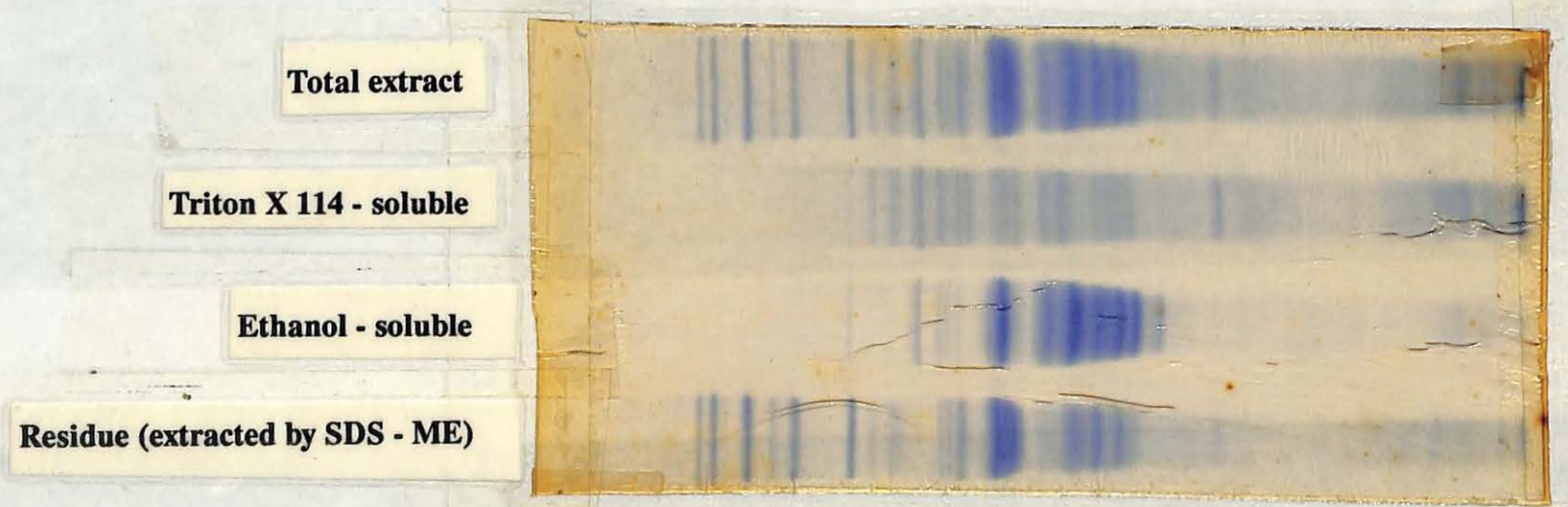
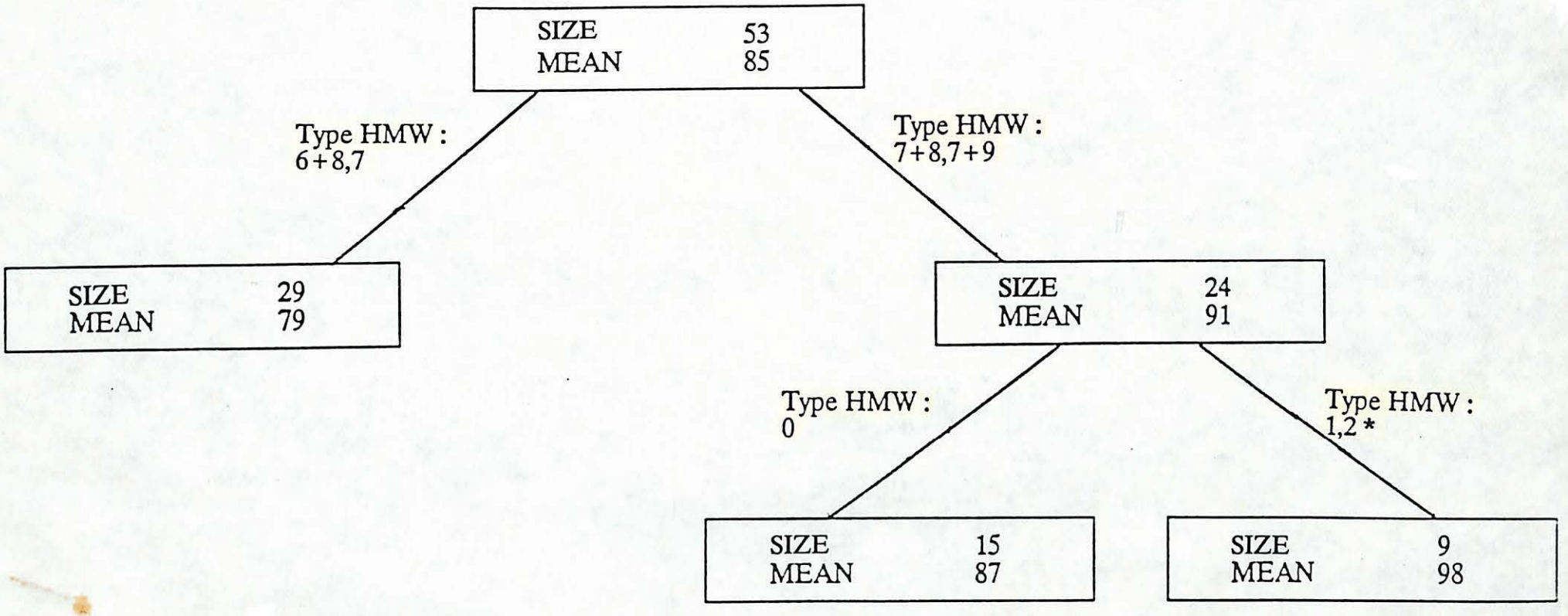
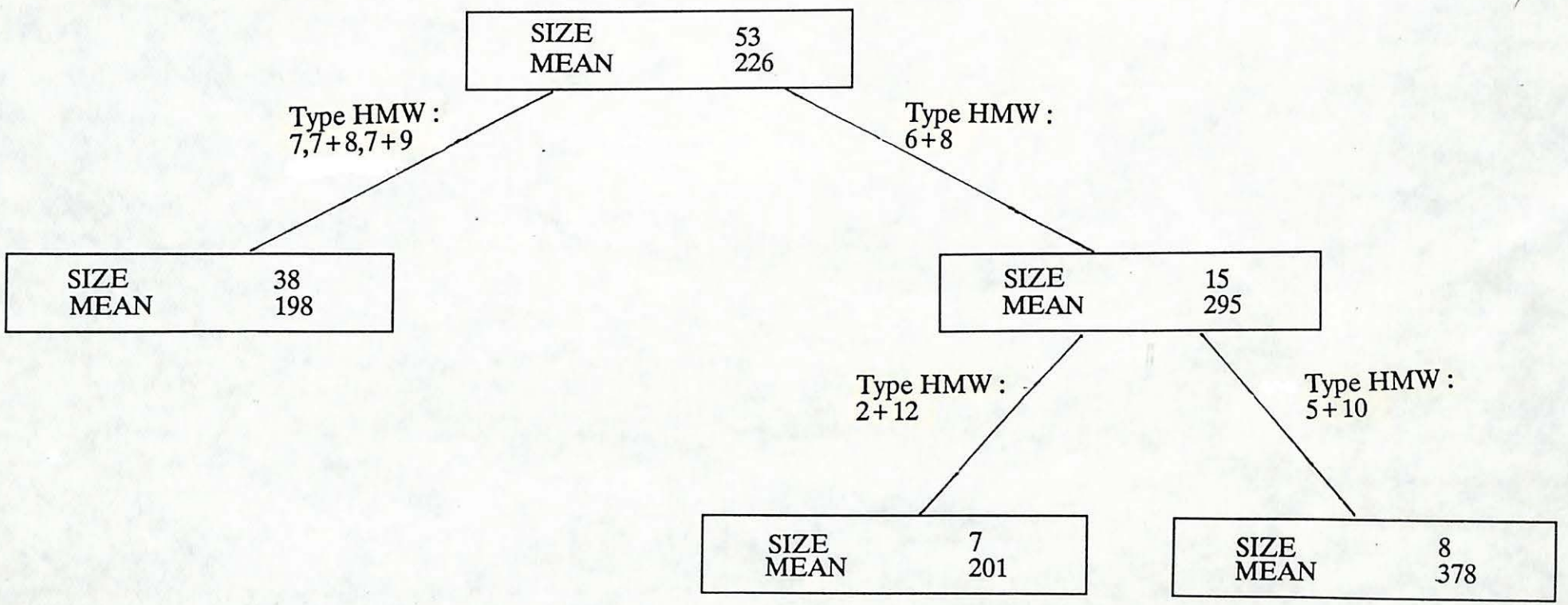
SDS-PAGE of the three types of protein fractions obtained by sequential extraction

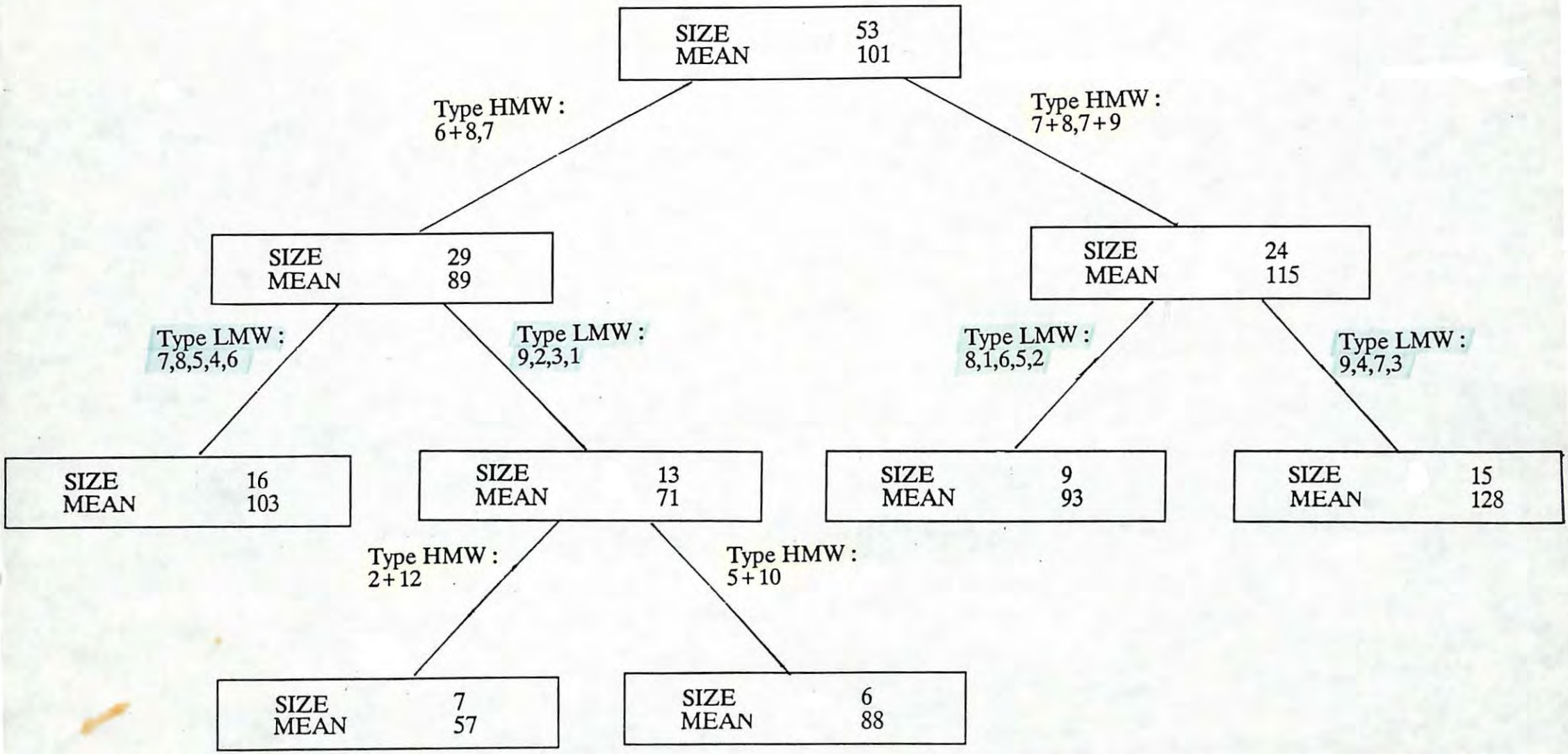




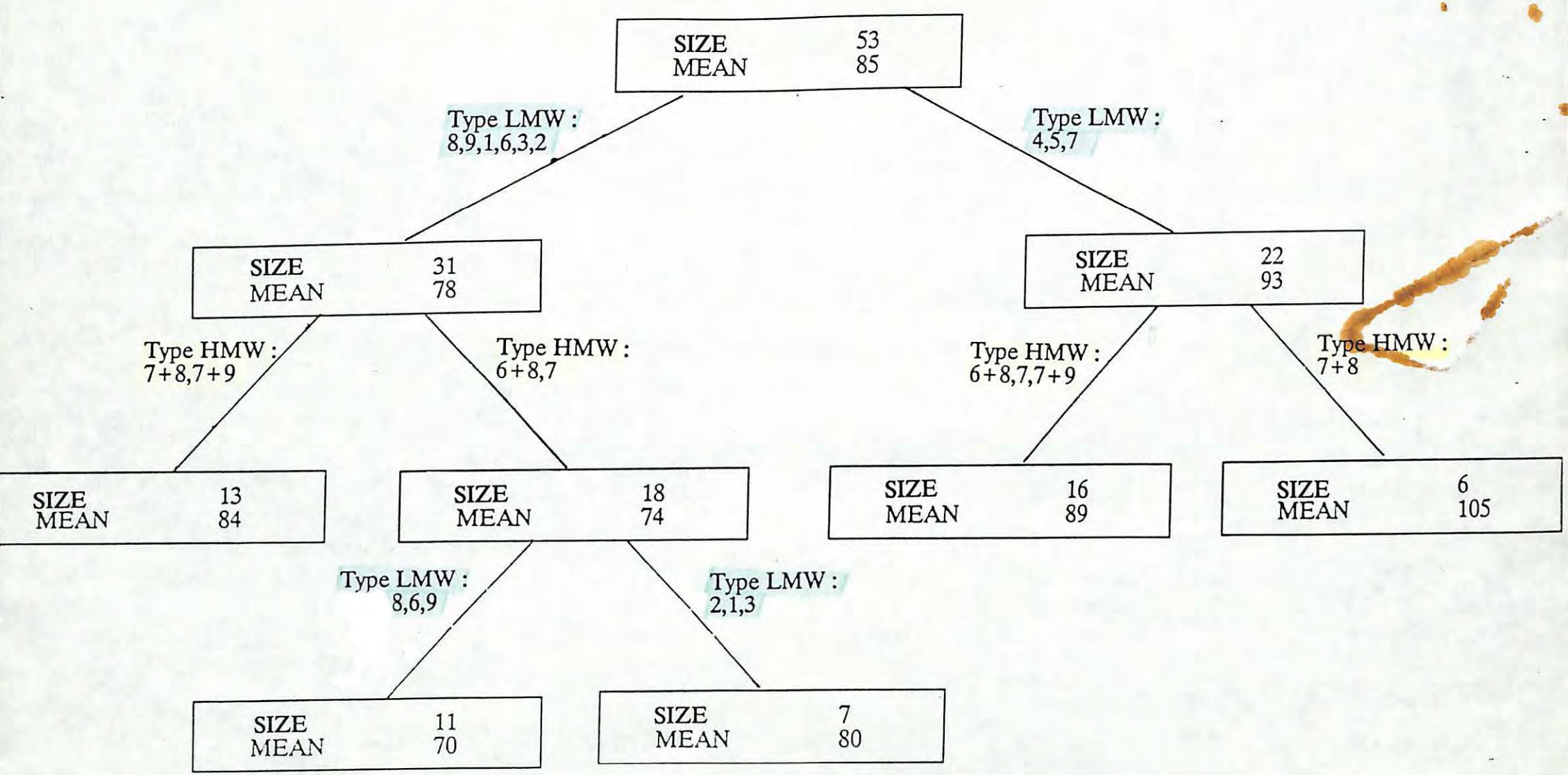
SLIDE 16: Segmentation Analysis Taking into Account HMW Subunits (Explained Variable: *G* from Alveograph)



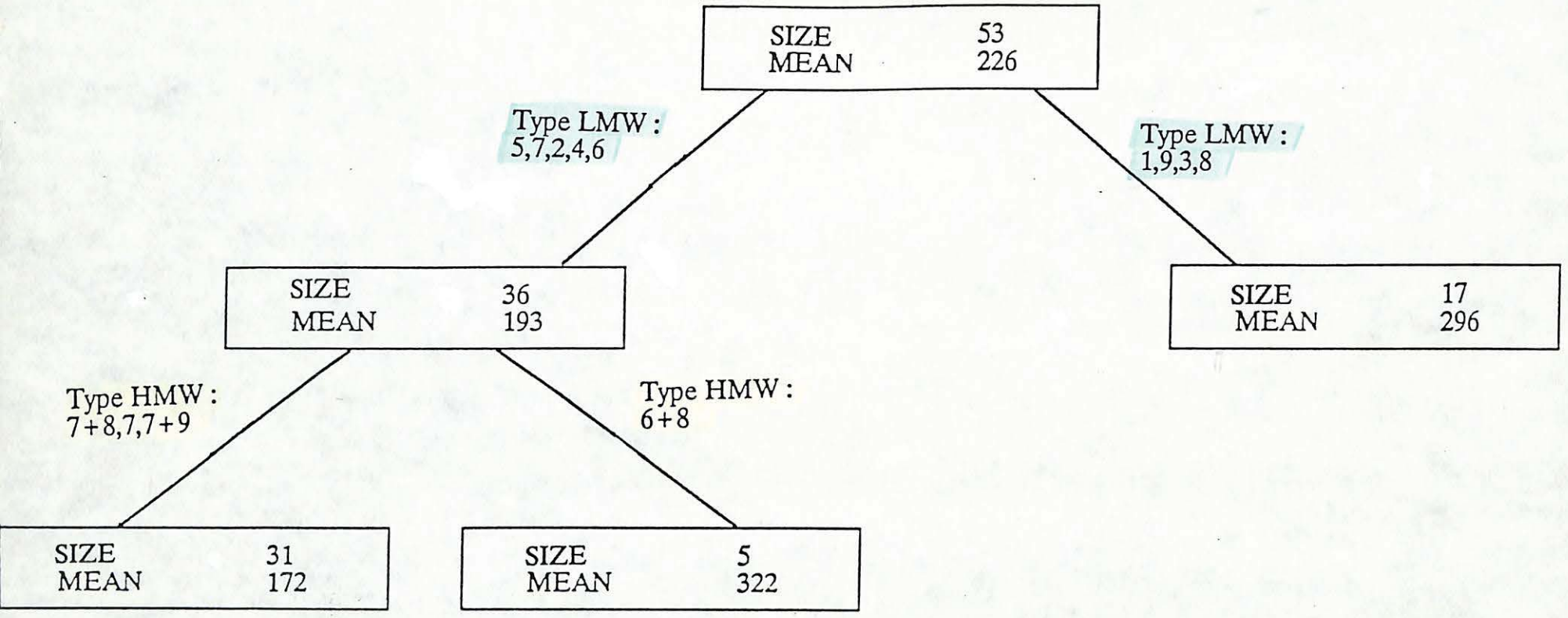
SLIDE 17: Segmentation Analysis Taking into Account HMW Subunits (Explained Variable: *P/L* from Alveograph)



SLIDE 18: Segmentation Analysis Taking into Account both LMW and HMW Subunits (Explained Variable: *W* from Alveograph)



SLIDE 19: Segmentation Analysis Taking into Account both LMW and HMW Subunits (Explained Variable: *G* from Alveograph)



SLIDE 20: Segmentation Analysis Taking into Account both LMW and HMW Subunits (Explained Variable: *P/L* from Alveograph)

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During the last decade, the presence of specific high-molecular-weight (HMW) subunits of glutenin has been correlated with baking quality in some countries. In France, however, this system largely failed to satisfy breeders' expectations, making it necessary to investigate other protein fractions. In this study, special attention was given to the low-molecular-weight (LMW) subunits of glutenin that are likely to influence dough properties through intermediate-size polymers. To obtain a clear-cut separation between reduced glutenin polypeptides and gliadin monomers, and enable a routine characterization of LMW subunits by 1D SDS-PAGE, flour was selectively extracted by ethanol after treatment by Triton X114, using an approach similar to that used in membrane protein studies. LMW patterns were determined in 70 French cultivars and investigated for relationships to mixing and baking properties, allowing tentative quality scores to be deduced for each LMW type. Because the most important discrepancies between HMW score and baking quality potential could be explained by differences in LMW composition, indices based on both HMW and LMW scores permitted a more accurate quality prediction. Considerable improvement was especially obtained for dough extensibility, a parameter which has been found essential in typical French baking formulas. Respective importance of structural and quantitative aspects, possible interactions between LMW and HMW compositions, and interest of a more precise description and quantification of LMW allelic types using 2D electrophoresis and image analysis were also discussed.

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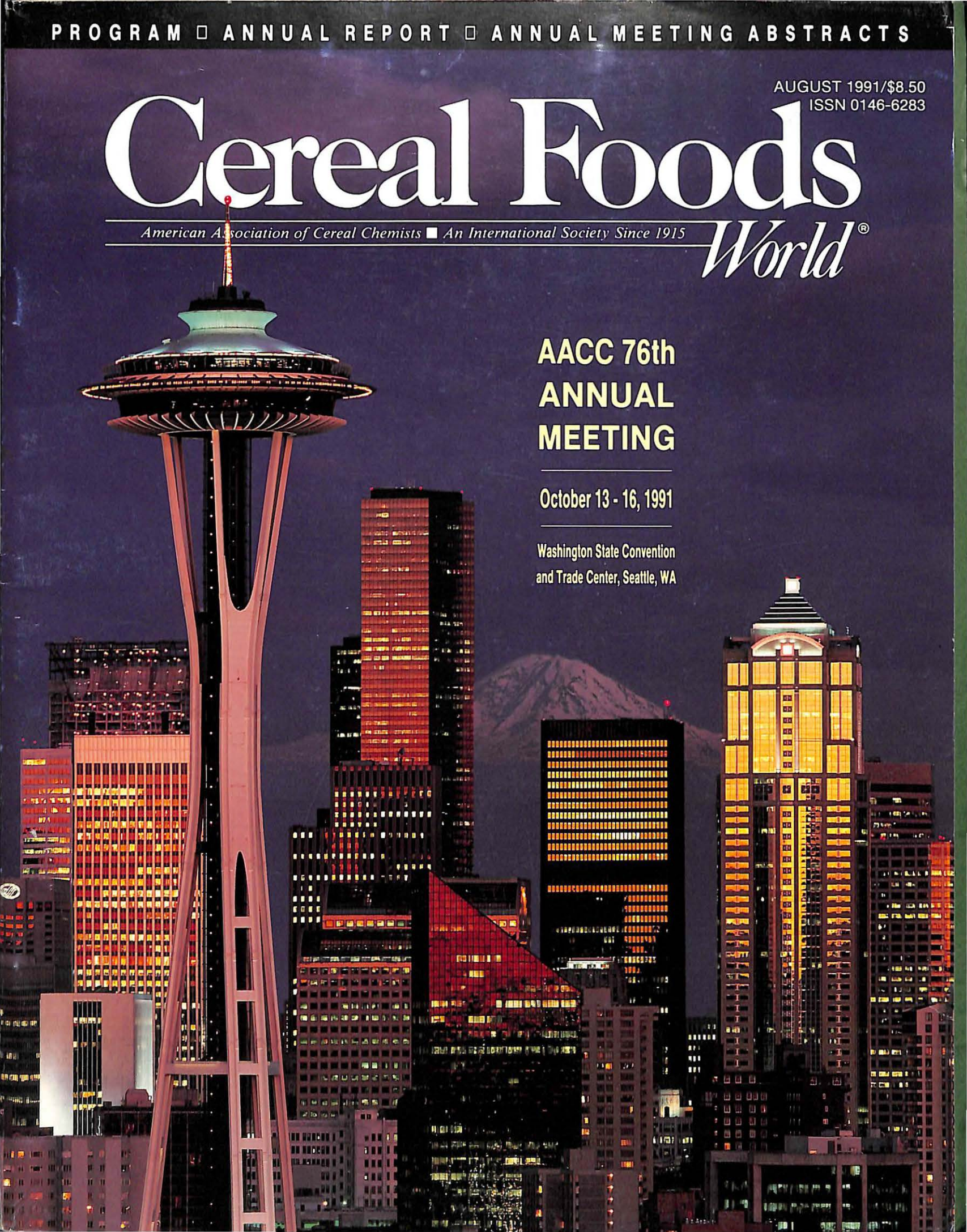

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