## RECENT DATA ON THE BIOCHEMICAL BASIS OF DURUM WHEAT QUALITY (\*)

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#### I. INTRODUCTION

DURUM WHEAT (TRITICUM DURUM DESF.) IS THE RAW MATERIAL OF CHOICE FOR THE MANUFACTURE OF PASTA PRODUCTS BECAUSE OF THE SUPERIOR RHEOLOGICAL PROPERTIES OF DURUM WHEAT PASTA DOUGHS AND THE IDEALLY SUITED COLOR AND COOKING QUALITY OF DURUM WHEAT PASTA. UNLIKE COMMON WHEAT, AND IMPORTANT PART OF WHICH CAN BE USED FOR ANIMAL FEED, THE MAJOR OPENING OF DURUM WHEAT IS PASTA FOR HUMAN FOOD. SINCE PASTA, AT LEAST IN COUNTRIES SUCH AS FRANCE AND ITALY, MUST BE MANUFACTURED FROM PURE DURUM WHEAT SEMOLINA, IT IS ESPECIAL-LY IMPORTANT THAT QUALITY OF DURUM WHEAT MEETS DEMANDS OF SEMOLINA AND PASTA-MAKING INDUSTRIES.

DURUM WHEAT QUALITY GENERALLY INCLUDES ALL CHARACTERISTICS OF DURUM WHEAT AND MORE ESPECIALLY :

- THE SEMOLINA YIELD, I.E. THE WEIGHT OF SEMOLINA OF A GIVEN PURITY THAT CAN BE PROCESSED ON WHEAT BASIS.

- THE ABILITY OF SEMOLINA TO BE PROCESSED INTO PASTA WHICH IS BRIGHT YELLOW IN COLOR AND WHICH, WHEN COOKED, RESISTS DESINTE-GRATION AND RETAINS A FIRM STRUCTURE.

(\*) PRESENTED AT SECOND FLAVOR CONFERENCE ON THE QUALITY OF FOODS AND BEVERAGES, ATHENS, 20-24 JULY 1981. IT IS PRIMARILY THE SECOND GROUP OF CHARACTERISTICS THAT WE ARE DEALING WITH TO DAY SINCE IT IS THE MOST CLOSELY RELATED TO BIOCHEMICAL COMPOSITION OF SEMOLINA AND SINCE POOR COOKING QUA-LITY VARIETIES RECENTLY RAISED CONCERNS IS SOME COUNTRIES, SO THAT THE PRODUCTION OF HIGH COOKING QUALITY VARIETIES IS PRESENTLY A MAJOR OBJECTIVE OF DURUM WHEAT BREEDERS.

IN OPPOSITION TO PASTA COLOR QUESTION, WHICH IS RELATIVELY WELL UNDERSTOOD, EXPLAINING VARIETAL DIFFERENCES IN COOKING QUALITY IN TERMS OF SIMPLE DIFFERENCES IN BIOCHEMICAL COMPOSITION IS AN OLD RESEARCH OBJECTIVE THAT HAS REMAINED UNFULFILLED AND CONSIDERA-BLE WORK IS STILL NEEDED IN THIS FIELD.

Accordingly, we would like to underline our recent progress CONCERNING THE BIOCHEMICAL BASIS OF DURUM WHEAT COOKING QUALITY.

At present, fast and small scale methods for direct estimation of the cooking quality of durum wheats are of course available. Some consist in processing grains into semolina and pasta disks or spaghetti, cooking and appreciating their characteristics through the use of some kind of rheological apparatus. However, cooking quality assessment at the breeding stage is a more critical problem, since cooking quality scores of durum wheat samples are highly influenced by growing conditions. This is shown on this slide in which I have gathered the cooking quality scores (10 : excellent ; 0 : very poor) of 24 durum wheat samples consisting in 3 varieties grown in 8 different locations.

IT IS CLEAR THAT BREEDERS CANNOT GET ENTIRE SATISFACTION FROM THESE METHODS SINCE THEY ARE HARDLY ABLE TO ACCOUNT FOR THE RESPECTIVE INFLUENCE OF THE GENOTYPE AND OF THE ENVIRONMENT EXCEPT IN MULTIPLYING THE NUMBER OF FIELD TESTS.

THEREFORE, I THINK IT FRUITFUL TO CLEARLY DISTINGUISH BETWEEN - BREEDING TESTS THAT MUST GIVE AN ACCOUNT OF THE VARIETAL INTRINSIC QUALITY OF GENOTYPES AND

- COMMERCIAL TESTS (INCLUDING MICRO COOKING TESTS) THAT

MUST ASSESS THE COMMERCIAL QUALITY OF SAMPLES I.E. THE RESULT OF INTERACTIONS BETWEEN INTRINSIC QUALITY AND GROWING CONDITIONS OF THE PLANT.

IT TURNS OUT THAT MOST OF THE TESTS THAT ARE USED BY BREE-DERS DERIVE FROM METHODS WHICH WERE ORIGINALLY DEVELOPED TO EVALUA-TE COMMERCIAL QUALITY AND, UNTIL RECENTLY, ONLY FEW STUDIES HAVE BEEN DEVOTED TO THE DEVELOPMENT OF METHODS ALLOWING A DIRECT ASSE-SSMENT OF INTRINSIC COOKING QUALITY OF THE GENOTYPES. WE THINK FURTHERMORE THAT SUCH METHODS ARE REQUIRED TO HAVE THE FOLLOWING CHARACTERISTICS :

- INDEPENDANCE OF THE RESULTS WITH REGARD TO THE AGRONOMI-CAL RECORD OF THE SAMPLE

- HIGH CORRELATION WITH THE VARIETAL RANKING THAT WOULD HAVE RESULTED FROM CONVENTIONAL EXPERIMENTS

- POTENTIAL FOR ANALYZING A LARGE SERIES AND A SMALL AMOUNT OF MATERIAL.

WE THINK THAT RECENT PROGRESS IN THE KNOWLEDGE ON THE BIO-CHEMICAL COMPOSITION OF DURUM WHEAT KERNEL AND ITS GENETIC CONTROL OPEN NEW FIELDS OF INVESTIGATION IN VIEW TO UNDERSTAND THE BIOCHE-MICAL BASIS OF COOKING QUALITY AND TO DEVELOP BIOCHEMICAL TESTS WHICH PERFECTLY MEET THE ABOVE-MENTIONED CHARACTERISTICS.

For example, it is largely accepted that cooking quality of durum wheat is associated with the quantity and the quality of its proteins, particularly of its gluten proteins. As the quantity of proteins is influenced to a large extent by environmental factors and the quality only is heritable, we shall underline hereunder the possibilities of developing breeding tests for high intrinsic cooking quality based on gluten quality (i.e. viscoelastic properties) and composition of the two main gluten fractions : gliadins AND glutenins.

## I - RELATIONSHIP BETWEEN VISCOELASTIC PROPERTIES OF GLUTEN AND COOKING QUALITY OF DURUM WHEAT VARIETIES

At first, the viscoelastic properties of gluten were determined by means of a viscoelastograph. This apparatus allowed to follow the strain of a solid in terms of applied stress and of time the gluten disk was put on the sample plate ; a constant load was applied for 40 seconds and then removed ; the time dependence of the thickness variation of the gluten disk was simultaneously scanned before and after loading off.

THE ABSOLUTE ELASTIC GLUTEN RECOVERY (E2 - E1) WAS INFERED FROM THE VALUE OF THE PARAMETERS : E1 (THICKNESS IMMEDIATLY BEFORE LOADING OFF) AND E2 (FINAL THICKNESS, 20 SECONDS AFTER LOADING OFF).

THE ELASTIC RECOVERY WAS DETERMINED FOR A LARGE NUMBER OF SAMPLES OF VERY DIFFERENT VARIETAL AND AGRONOMICAL ORIGINES. FROM THE EXAMINATION OF ALL THE DATA, WE REACHED THE FOLLOWING CONCLU-SIONS :

- WITHIN ALL SAMPLES, ABSOLUTE RECOVERY VALUES RANGED FROM 0.3 TO 2.1 MM

- IN A GIVEN VARIETY, ABSOLUTE ELASTIC RECOVERY VARIED WITHIN NARROW LIMITS AROUND AND AVERAGE VALUE WHICH WAS AS MUCH LOW AS THE WHEAT PROTEIN CONTENT WAS HIGH

- LOWER WAS THIS AVERAGE VALUE, MORE IMPORTANT WERE THE FLUCTUATIONS

- A VARIETY. COULD BE CHARACTERIZED BY THE AVERAGE VALUE OF ITS GLUTEN VISCOELASTICITY

- THE 122 VARIETIES THAT WERE ANALYZED WERE DISTRIBUTED INTO TWO BASICALLY EQUAL CLASSES AROUND THE MEAN VALUES 0.6 AND 1.8 MM

- THE VISCOELASTIC PROPERTIES OF COOKED GLUTEN DISKS ARE THEREFORE MAINLY GENETIC DEPENDENT,

WELL KNOWN VARIETIES WITH EITHER A HIGH OR A LOW COOKING QUALITY WERE CHECKED FOR THEIR GLUTEN VISCOELASTIC PROPERTIES. It could be noticed that all the varieties known for their good cooking quality had a gluten, the absolute elastic recovery of which was higher than 1.6. At the contrary, glutens from Low or medium cooking quality varieties have an absolute elastic recovery lower than 1.0 (excepted Lakota, that can be regarded as a medium cooking quality variety).

THEREFORE, HIGHER IS THE ELASTIC RECOVERY VALUE, HIGHER IS THE INSTRINSIC COOKING QUALITY.

# II - SEARCH FOR A RELATIONSHIP BETWEEN GLIADIN ELECTROPHORETIC PATTERNS AND VISCOELASTIC PROPERTIES OF GLUTEN

122 DURUM WHEAT VARIETIES OF DIFFERENT GENETICAL ORIGINES WERE EXAMINED.

After extraction by 70 % ethanol, gliadins were fractionated in polyacrylamide gel according to the electrophoretic technique of BUSHUK and ZILLMAN. Components mobilities were established by reference to a standard <u>51</u> band, in agreement with the common wheat gliadin nomenclature.

By giving a special attention to the gamma gliadin region, durum wheats were classified into two main groups.

- group 45 was characterized by presence of a strong band 45 and absence of band in the 38 - 42 region

- group 42 was characterized by absence of band  $\underline{45}$  and presence of a strong band  $\underline{42}$ .

68 varieties out of 122 belonged to the  $\underline{45}$  type, 50 to the 42 type and 4 to neither type.

ONE OF THE MOST INTERESTING RESULTS OF THIS STUDY WAS THE OCCURENCE OF A VERY CLOSE CORRESPONDANCE BETWEEN THE ELECTROPHORE-TIC TYPE OF THE DURUM VARIETIES AND THEIR VISCOELASTIC PROPERTIES.

- 61 out of the 68 varieties (90 %) above classified in the 45 gliadin type had a gluten, the elastic recovery of which is over 1.2 mm

- 49 out of the 50 varieties (98 %) above classified in the  $\underline{42}$  gliadin type had a gluten, the elastic recovery of which is lower than 1.2 mm.

THEREFORE, THERE IS AN AGREEMENT BETWEEN THE GLIADIN ELEC-TROPHORETIC PATTERN COMPOSITION AND THE ABSOLUTE ELASTIC RECOVERY OF GLUTEN.

## III - SEARCH FOR A RELATIONSHIP BETWEEN HIGH MOLECULAR WEIGHT GLUTE-NIN ELECTROPHORETIC PATTERNS AND VISCOELASTICITY OF GLUTEN

80 DURUM WHEAT VARIETIES WERE EXAMINED.

After extraction from wheat kernels and reduction using sodium dodecyl sulfate-mercapto ethanol-tris buffer, protein were fractionated in vertical S.D.S. - polyacrylamide gels according to PAYNE and coworkers. Special attention was given in high molecular weight glutenin type subunits which consist in a A region (m.w. : 85 000 - 110 000) and in a B region (m.v. : 62 000 - 70 000)

PROTEIN POLYMORPHISM WAS LARGER IN THE A REGION (18 TYPES) THAN IN THE B REGION (8 TYPES). THE MAJOR TYPES ARE PRESENTED ON THE SLIDE.

In general, the major types (we referred to as a 3-10-13 and A 6-11) of A subunits and also B subunits N° 3, 4, 5 were not found associated with cooking quality potential since they occured

IN BOTH HIGH AND POOR INTRINSIC COOKING QUALITY VARIETIES. HOWEVER, A RELATIONSHIP WAS NOTICED BETWEEN SOME UNUSUAL TYPES AND INTRINSIC COOKING QUALITY. FOR INSTANCE, ALL VARIETIES HAVING PATTERNS WITH SUBUNITS A1, OR A2, OR A5-A8 HAD POOR INTRINSIC COOKING QUALITY, INCLUDING VARIETIES (ISA 1) BELONGING TO THE GLIADIN TYPE 45. IN THE SAME WAY, ALL VARIETIES HAVING PATTERNS WITH BOTH A3 - A6 SUBU-NITS HAD EXCELLENT INTRINSIC COOKING QUALITY.

Concerning the major types of B region, the distribution showed a relationship with intrinsic cooking quality which is in agreement with the gliadin 45/42 distribution : B2 component seemed strongly linked to 45 gliadin band whereas B1 seemed more associated to 42 gliadin band. Such a result could be explained considering the exact nature of the different high molecular weight subunits. We think that B 3, 4, 5 subunits are typical alcohol insoluble glutenins, like A region high molecular weights are, whereas B1 or B2 actually agrees with some omega gliadin bands which look closely linked to 42 or 45 components.

#### CONCLUSION

THE DISCOVERY OF AN EXCELLENT AGREEMENT BETWEEN THE GLIA-DIN ELECTROPHORETIC PATTERNS OF THE DURUM WHEAT VARIETIES AND THEIR GLUTEN VISCOELASTIC PROPERTIES CAN BE REGARDED AS AN APPRE-CIABLE PROGRESS IN THE UNDERSTANDING OF THE BIOCHEMICAL SUPPORT OF TECHNOLOGICAL QUALITY AND THE USEFULNESS OF POLYACRYLAMIDE GEL ELECTROPHORESIS IN BREEDING PROGRAMS IS DEMONSTRATED.

IN THIS PURPOSE, AND IN ADDITION TO PASTA COLOR AND PROTEIN CONTENT DETERMINATIONS, WE WOULD SUGGEST USING THE FOLLOWING BREE-DING DIAGRAM : - Screening (as soon as the F3 generation, and even of half F2 kernels), for the presence of the electrophoretic component 45.

- Screening from the F5 generation (on 45 type lines) for high elastic recovery of gluten (higher than 1.5 mm).

- CONFIRMATION (AT THE LATTER BREEDING STAGES) OF THE COOKING QUALITY OF LINES BY CHECKING THROUGH MICRO AND PILOT COOKING TESTS AND EVALUATION OF THE EFFECTS OF THE GROWING CONDITIONS,

THE VARIETAL CLASSIFICATION BASED ON GLIADINS IS CORROBO-RATED AND EVEN FURTHER SPLITTED UP BY THE STUDY OF SOME HIGH MOLE-CULAR WEIGHT FRACTIONS BUT, IN GENERAL, THE ASSOCIATION BETWEEN ELECTROPHORETIC COMPOSITION AND INTRINSIC LOOKING QUALITY IS STRON-GER IN GLIADINS THAN IN HIGH MOLECULAR WEIGHT GLUTENIN SUBUNITS.

THIS RESULT, WHICH WAS CORROBORATED BY KOSMOLAK ET AL. (1980), RAISES MANY QUESTIONS ABOUT THE NATURE OF THE LINKAGE BET-WEEN THE GLIADIN ELECTROPHOREGRAMS AND THE VISCOELASTIC PROPERTIES OF GLUTEN. ARE GENES CODING FOR GLUTEN STRENGTH CLOSE TO GENES CO-DING FOR SPECIFIC GLIADIN BANDS (GENETIC MARKING) ? OR, MORE POS-SIBLY, DO GLIADIN COMPONENTS HAVE SPECIFIC CHARACTERISTICS WHICH WOULD ACT ON GLUTEN VISCOELASTICITY (CAUSE-AND-EFFECT OR FUNCTIONAL RELATIONSHIP) ? THIS LATTER HYPOTHESIS IS SUPPORTED BY RECENT FIN-DINGS OF JEANJEAN ET AL. (1980) THAT SOME GLIADIN FRACTIONS CAN PARTICIPATE TO THE FORMATION OF AN INSOLUBLE PROTEIN NETWORK WHICH GIVE HIGH VISCOELASTICITY TO THE GLUTEN UPON HEATING WHEREAS OTHERS CANNOT. IT IS ALSO SUPPORTED BY RECENT RESULTS CONCERNING THE CHE-MICAL COMPOSITION OF GLIADIN 45 - HIGHER SURFACE HYDROPHOBICITY THAN GLIADIN 42 (GODON AND POPINEAU, 1980) AND HIGH SULPHUR CONTENT COM-PARED TO OTHER GLIADINS (WRIGLEY ET AL., 1980) - WHICH ARE LIKELY TO EXPLAIN A BETTER CONTRIBUTION TO INSOLUBLE AGGREGATES AND TO VISCOELASTIC PROPERTIES OF GLUTEN, ACCORDINGLY, GLIADIN 45 COULD HAVE A UNIQUE STRUCTURE THAT ENABLES STRONG AND ELASTIC PROTEIC NETWORK TO BE FORMED DURING PASTA PROCESS AND COULD BE MORE EFFI-CIENT AT DOING THIS THAN THE OTHER GLIADINS, ON THE OTHER HAND MOST OF THE GLUTENIN SUBUNITS WITH HIGHEST MOLECULAR WEIGHT DO NOT SEEM

TO HAVE SPECIFIC PROPERTIES AND COULD MERELY ACT IN BULK AND BY THEIR RATIO TO OTHER FRACTIONS.

ON THE OTHER HAND, WE THINK THAT PASTA COOKING QUALITY CONSISTS NOT ONLY OF RHEOLOGICAL CHARACTERISTICS (FIRMNESS, ELAS-TICITY) BUT ALSO OF PHYSICAL ASPECT OF COOKED PASTA (ABSENCE OF SURFACE DETERIORATION AND OF MUSHINESS). IF THE FORMER HAS BEEN INVESTIGATED FOR A LONG TIME, THE CHEMICAL BASIS OF THE LATTER HAVE NOT BEEN REALLY EXPLORED YET AND SHOULD BE CONSIDERED APART FROM THOSE OF RHEOLOGICAL CHARACTERISTICS. IN BOTH CASES IT COULD BE ASSUMED THAT PROTEINS ALONE WILL FAIL FOR A TOTAL EXPLANATION OF THE OBSERVED PHENOMENA AND THAT NEW INSIGHTS CAN BE EXPECTED FROM OTHER CONSTITUENTS LIKE CARBOHYDRATES AND LIPIDS.

Since the latter might have together oxydations with gluten proteins (LAIGNELET, 1979, 1981), a greater attention in the future should be payed on Lipids composition and genetics as a further factor to explain Varietal differences in quality.