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Accumulation et formation des agrégats protéiques dans le grain de blé tendre (*Triticum aestivum* L.) en développement

Larbi Rhazi

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HAL Id: tel-04309336

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HABILITATION A DIRIGER DES RECHERCHES

Présentée par

Larbi RHAZI

Chargé de Recherche

Unité de Recherche Transformations & Agro-Ressources

(UP 2018.C103)

Accumulation et formation des agrégats protéiques dans le grain de blé tendre (*Triticum aestivum* L.) en développement

Ecole doctorale en Sciences, Technologie et Santé

Université Picardie Jules Verne

Soutenue le 17 Septembre 2019 devant le jury composé de:

Dr. Marie-Odile BANCAL	Rapporteur
Dr. Jean-Michel CHARDIGNY	Rapporteur
Dr. Gilles CHARMET	Rapporteur
Pr. Michèle BOITEL	Examineur
Pr. Emmanuel PETIT	Examineur
Dr. Thierry AUSSENAC	Directeur de l'HDR

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Curriculum vitae

Agro-Resource Sciences

EDUCATION AND QUALIFICATIONS

- **2003: PhD** in Agro-resource sciences, option reactivity, National Polytechnic Institute of Toulouse (ENSIACET).

Thesis title: "Formation of glutenin polymer in bread wheat grain (*Triticum aestivum* L.)".

Defended July 4th, 2003.

Supervisor: Dr Thierry AUSSENAC.

<i>Chair:</i>	F. Silvestre, INP Toulouse, ENSIACET
<i>Reviewer:</i>	B. Launay, INP Lorraine, ENSIA
<i>Reviewer:</i>	E. Triboi, INRA Clermont-Ferrand
<i>Examiner:</i>	R. Cazalis, ESA Purpan
<i>Examiner:</i>	T. Aussenac, ISA Beauvais

- **1999: Diploma of Advanced studies (DEA):** Agro-resource sciences, option reactivity, National Polytechnic Institute of Toulouse (ENSIACET).

Dissertation title: "Valorization of Sunflower Cake Proteins". Laboratory of Agro-Industrial Chemistry, ENSIACET and laboratory of Agro-Physiology, ESAP.

Supervisors: Dr. Françoise SILVESTRE and Dr. Thierry AUSSENAC.

- **1998: Master I** in Biology, option Biochemistry (University Hassan II, Faculty of Sciences and Technology, Mohammedia, Morocco).

Supervisor: Dr. Saïd ELANTRI.

- **1996: DEUG (Associate Degree)** in Life and Earth Sciences (University Hassan II of Sciences and Techniques of Mohammedia, Morocco).

Supervisor: Dr. Saïd ELANTRI.

SKILLS

- Conduction and supervision research; analysis data toward project direction goals and milestones.
- Writing technical reports and creating presentations.
- Strong organizational, multi-tasking, and problem solving skills.
- Excellent oral and written communication skills, including giving presentations to all levels clients and students.
- Writing and presenting reports.
- Writing scientific publications.
- Scientific supervision.
- Research project development
- Self motivated and driven to exceed goals.
- Working effectively with minimal direction or feedback and willingly making decisions independently.
- Scientific project management (FUI, FSOV, CASDAR, ANR, région...)
- Management and supervision of staff (Student engineers, Postdoctoral fellows, PhD students, technicians, trainees ...)
- Research platform and service delivery management.
- Management and maintenance of analytical and molecular devices.
- Time management
- Screening and Marker analysis for plant breeding programs.
- Designing and implementing of research, study, consulting and training programs.
- Techniques of molecular biology, plant biotechnology and improvement.
- Development of analytical, biochemical and molecular protocols.
- Establishment and management of plant experimentation.
- Enzymatic and electrophoretic techniques.

- Chromatographic techniques.
- Asymmetrical flow field flow fractionation (AFFFF) technique.
- *In Situ* Molecular Marking Techniques
- Handling of radioactive materials.

PROFESSIONAL EXPERIENCE

RESEARCH

- **2010-Present:** Research associate, Project Manager.
- **2006-Present:** Project Manager.
- **2006-2007:** Research assistant.
- **2006-2010:** Manager of Analytical Chemistry and Molecular Biology platform (UniLaSalle).
- **November 2003-March 2006:** Manager of the Biolub program at the Enzymatic and Cell Engineering Laboratory at Compiègne University of Technology (UTC) (in collaboration with Biogemma, Limagrain, Total).
- **July-November 2003:** Post-doctoral studies in the laboratory of Agro-physiology (ESAP).

TEACHING ACTIVITIES:

- **2006-Present:** Main topics on Plant Biotechnology, Plant breeding and improvement, Plant Biology, Plant histology, Molecular biology (UniLaSalle).
- **1999-2003:** Teaching assistant for biochemistry and food sciences lab work at Ecole Supérieur d'Agriculture de Purpan (ESAP, engineering school)
- **1999-2003:** Teaching assistant in applied statistics on quality at Ecole Supérieur d'Agriculture de Purpan (ESAP, engineering school).
- **2001-2003:** Teaching assistant for Plant physiology lab work at Ecole Supérieur d'Agriculture de Purpan (ESAP, engineering school).

SUPERVISION

- Supervising and training to different levels (BTS/ HND, Master I, Master II) at the Agro-Physiology laboratory (ESAP).
- Supervising and training of students (Master I, Master II, PhD) at the Enzymatic and Cell Engineering Laboratory, Compiègne University of Technology (UTC).
- Supervising and training of students (BTS/HND, Master I, Master II, PhD, Post-Doctoral fellows), UniLaSalle.

SCIENTIFIC PUBLICATIONS

Research activities as well as collaborations with academic and industrial institutions, allowed me to contribute to the redaction of many diverse scientific productions in several scientific fields such as biochemistry, chemistry, biotechnology, molecular biology, physiology. I worked on prokaryotes such as bacteria and yeast and eukaryotes such as plants and humans.

All my scientific productions were tightly related to my real involvement in research activities. In addition, no publication was due to my plateforme responsibilities.

Finally, publications (article, oral and poster communications) are referenced in order to be cited in this document of HDR.

STATISTIC:

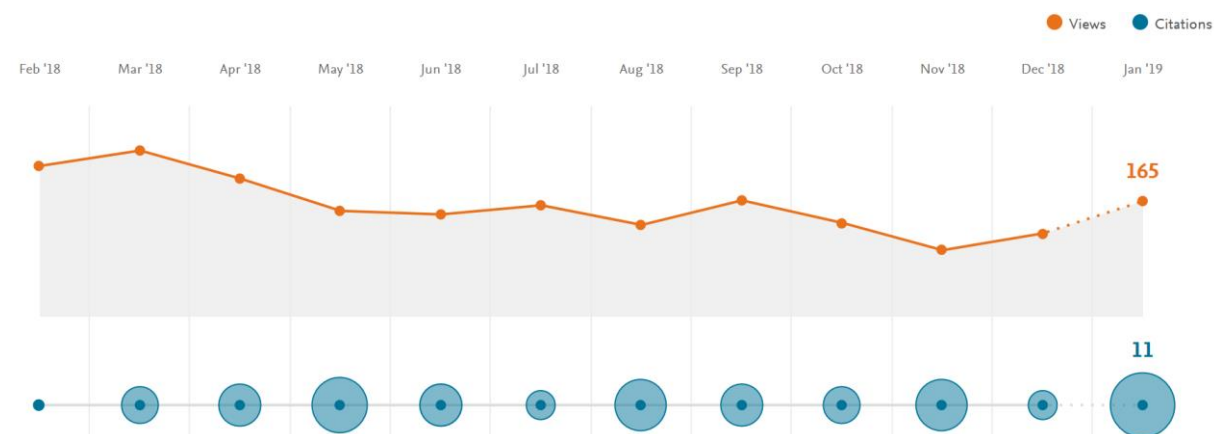


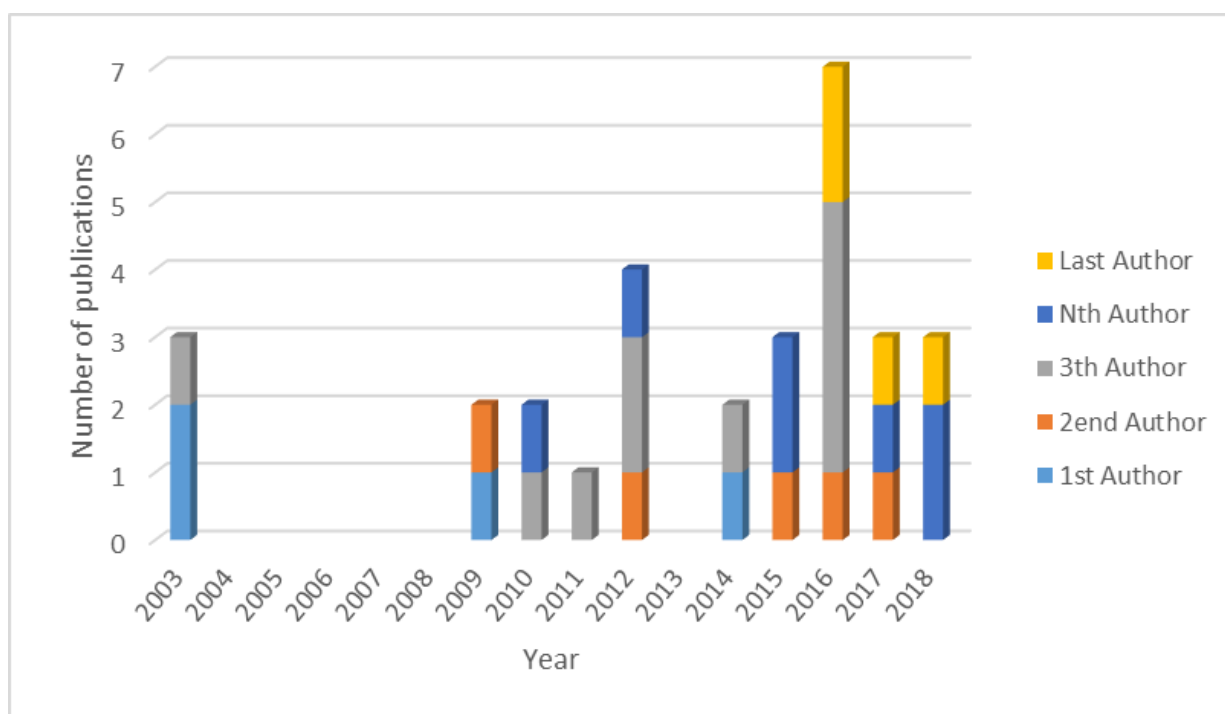
Statistic data from my Mendeley profile for January 2019.

Performance timeline



Evolution of views and citations in the last 12 Months





Review of publications of international peer reviewed journals by year and author position (Nth author: from 4th to penultimate position).

ARTICLES:

- A1.** Effect of in situ gluten-chitosan interlocked self-assembled supramolecular architecture on rheological properties and functionality of reduced celiac-toxicity wheat flour. Ribeiro M., Picascia S, **Rhazi L.**, Gianfrani C, Carrillo J.M., Rodriguez-Quijano M., Branlard G, Nunes F. M. *Food Hydrocolloids*, Volume 90, May 2019, Pages 266-275.
- A2.** In situ gluten-chitosan interlocked self-assembled supramolecular architecture reduces T-cell-mediated immune response to gluten in celiac disease. Ribeiro M., Picascia S, **Rhazi L.**, Gianfrani C, Guerreiro A.C.L., Domingues P., Carrillo J.M., Rodriguez-Quijano M., Branlard G, Igrejas G., Nunes F. M. *Molecular nutrition and Food Research*. 2018 DOI: 10.1002/mnfr.201800646
- A3.** Storage Proteins Accumulation and Aggregation in Developing Wheat Grains. Aussenac T. and **Rhazi L.** *Global Wheat Production*. <http://dx.doi.org/10.5772/intechopen.75182>, (2018).
- A4.** Use of a combination of in vitro models to investigate the impact of chlorpyrifos and inulin on the intestinal microbiota and the permeability of the intestinal mucosa. Réquilé M., González Alvarez DO., Delanaud S., **Rhazi L.**, Bach V., Depeint F. and Khorsi-Cauet H. *Environ Science and Pollution Research*. 25(23):22529–22540, (2018).
- A5.** Use of qNMR for speciation of flaxseeds (*Linum usitatissimum*) and quantification of cyanogenic glycosides. Roulard R., Molinie R., Fontaine J.-X., Jamali A., Cailleu D.,

- Tavernier R., Guillot X., **Rhazi L.**, Petit E. and Mesnard F. *Analytical and Bioanalytical Chemistry* 409 (30), (2017).
- A6.** Effects of ozone treatment on the molecular properties of wheat grain proteins. Gozé P., **Rhazi L.**, Lakhal L., Jacolot P., Pauss A., Aussenac T. *Journal of Cereal Science* 75, 243-251, (2017).
- A7.** Chemical Characterization and Antioxidant Potential of *Athroisma proteiformis* Essential Oil. Rasolohery C. A., Ralaibia B. E., Ayerdi Gotor A., Merlier F., Benja R., Rakotovao M., **Rhazi L.** *The Natural Products Journal* 7, 1-8, (2017).
- A8.** Characterization of Early-Life Fecal Microbiota in Susceptible and Healthy Pigs to Post-Weaning Diarrhea. Dou S., Gadonna-Widehem P., Rome V., Hamoudi D., **Rhazi L.**, Lakhal L., Larcher T., Bahi-Jaber N., Pinon-Quintana A., Guyonvarch A., LE Huërou-Luron I., Abdennebi-Najar L. *PloS one* 12 (1), e0169851, (2017).
- A9.** The Bioavailability of Astaxanthin Is Dependent on Both the Source and the Isomeric Variants of the Molecule. Mimoun-Benarroch M., Hugot C., **Rhazi L.**, Niamba CN., Depeint F. *USAMV Bulletin* (73), 2, (2016).
- A10.** Changes in composition and function of human intestinal microbiota exposed to chlorpyrifos in oil as assessed by the SHIME® model. Reygner J., Joly Condet C., Bruneau A., Delanaud S., **Rhazi L.**, Depeint F., Abdennebi-Najar L., Bach V., Mayeur C., Khorsi-Cauet H. *International Journal of Environmental Research and Public Health* 13(11), 1088, (2016).
- A11.** Inulin Supplementation Lowered the Metabolic Defects of Prolonged Exposure to Chlorpyrifos from Gestation to Young Adult Stage in Offspring Rats. Reygner J., Lichtenberger L., Elmhiri G., Dou S., Bahi-Jaber N., **Rhazi L.**, Depeint F., Bach V., Khorsi-Cauet H., Abdennebi-Najar L.. *PLoS ONE* 11(10), e0164614, (2016).
- A12.** Innovative gliadin/glutenin and modified potato starch green composites: Chemistry, structure and functionality induced by processing. Muneer F., Andersson M., Koch K., Hedenqvist M., Gällstedt M., Plivelic T., Menzel C., **Rhazi L.**, Kuktaite R. *ACS Sustainable Chemistry & Engineering*, 4 (12), 6332–6343, (2016).
- A13.** Starch characterization after ozone treatment of wheat grains. Gozé P., **Rhazi L.**, Pauss A., Aussenac T. *Journal of Cereal Science* 70, 207-213, (2016).
- A14.** Effects of refining process on sunflower oil minor components. Ayerdi Gotor A. and **Rhazi L.** *Oilseeds and fats, Crops and Lipids*, 23 (2), D207, (2016).
- A15.** Molecular investigations of flaxseed mucilage polysaccharides. Roulard R., Petit E., Mesnard F., **Rhazi L.** *International Journal of Biological Macromolecules*, Volume 86, 840-847, (2016).

- A16.** Coping with wheat quality in a changing environment - Proteomics evidence for stress caused by environmental changes. In “Advances in Wheat Genetics: From Genome to Field. Branlard G., Lesage VS., Bancel E., Martre P., Méleard B., **Rhazi L.** Proceedings of the 12th International Wheat Genetics Symposium” (Y.Ogihara, S. Takumi, H. Handa Eds), (2015) Yokohama, Japan ISBN: 978-4-431-55674-9, 255-264, (2015).
- A17.** Environmental and varietal impact on linseed composition and on oil unidirectional expression process. Savoie R., Lazouk M-A., Van-Hecke E., Roulard R., Tavernier R., Guillot X., **Rhazi L.**, Petit E., Mesnard F., Thomasset B. Oilseeds and fats, Crops and Lipids (2015), DOI: 10.1051/ocl/2015016, (2015).
- A18.** Molecular characterization of storage proteins for selected durum wheat varieties grown in different environments Daaloul Bouacha O., **Rhazi L.**, Aussenac T., Rezgui S., Nouaigui S. Journal of Cereal Science 61, 97-104, (2015).
- A19.** Molecular characterization of three transgenic high molecular weight glutenin subunit events in winter wheat. **Rhazi L.**, Graybosch R. A., Aussenac T. Journal of Cereal Science 60, 631-638, (2014).
- A20.** Comparative Quality Analysis of Gluten Strength and the Relationship with High Molecular Weight Glutenin Subunits of 6 Tunisian Durum Wheat Genotypes. Trad H., Ayed S., **Rhazi L.**, Slim A., Teixeira da Silva J. A., Hellal R., Sghaier M., and Amara H. S. Food Sci. Biotechnol. 23(5), 1363-1370, (2014).
- A21.** A laboratory protocol for determining glucose and maximum ethanol production from wheat grain: application to a complete genetic set of near-isogenic waxy lines. Gadonna-Widehem P., Debiton C., Marier D., **Rhazi L.**, Branlard G. J Agric Food Chem. Feb 1; 60(4), 985-90, (2012).
- A22.** Xylo-oligosaccharide (XOS) in combination with inulin modulates both the intestinal environment and immune status in healthy subjects, while XOS alone only shows prebiotic properties. Lecerf J-M., Dépeint F., Clerc E., Dugenet Y., Niamba C. N., **Rhazi L.**, Cayzeel A., Abdelnour G., Jaruga A., Younes H., Jacobs H., Lambrey G., Abdelnour A. M., Pouillart P.R. British Journal of Nutrition, 1-12, (2012).
- A23.** Amylose and amylopectin in starch by asymmetric flow field-flow fractionation with multi-angle light scattering and refractive index detection (AF4-MALS-RI). Chiamonte E., **Rhazi L.**, Aussenac T., Richard White Jr D. Journal of Cereal Science 56, 457-463, (2012).
- A24.** New insight into puroindoline function inferred from their subcellular localization in developing hard and soft near-isogenic endosperm and their relationship with polymer size of storage proteins. Lesage V. S., Bouchet B., **Rhazi L.**, Elmorjani K., Branlard G., Marion D. Journal of Cereal Science 53, 231-238, (2011).

- A25.** *N*-Carboxymethyl lysine: its origin in selected foods and its urinary and faecal excretions in healthy humans. Tessier F.J., Niquet C., **Rhazi L.**, Hedhili K., Navarro P., Seiquer I. and Delgado-Andrade C. In *The Maillard Reaction: Interface between Aging, Nutrition and Metabolism*. Merlin Christopher Thomas and Josephine Forbes (Eds.): Cambridge: RSC Publishing, Special publication No.332, 144-150, (2010).
- A26.** Effect of the three waxy null alleles on enzymes associated to wheat starch granules using proteomic approach. Debiton C., Bancel E., Chambon C., **Rhazi L.**, Branlard G. *Journal of Cereal Science* 52, 466-474, (2010).
- A27.** Identification of a potential bottleneck in branched chain fatty acid incorporation into triacylglycerol for lipid biosynthesis in agronomic plants. Nlandu Mputu M., **Rhazi L.**, Vasseur G., Vu T.-D., Gontier E., Thomasset B. *Biochimie* 91, 703–710, (2009).
- A28.** High throughput microchip-based separation and quantitation of high-molecular-weight glutenin subunits. **Rhazi L.**, Bodard A.-L., Fathollahi B., Aussenac T. *Journal of Cereal Science* 49, 272–277, (2009).
- A29.** Sulfhydryl-disulfide changes in storage proteins of developing wheat grain: influence on the SDS-unextractable glutenin polymer formation. **Rhazi L.**, Cazalis R., Aussenac T. *Journal of Cereal Science* 38, 3–13, (2003).
- A30.** Changes in the glutathione thiol–disulfide status during wheat grain development. **Rhazi L.**, Cazalis R., Lemelin E., Aussenac T. *Plant Physiology and Biochemistry* 41, 895–902, (2003).
- A31.** Homology modeling and molecular dynamics simulations of the N-terminal domain of wheat high molecular weight glutenin subunit 10. Cazalis R., Aussenac T., **Rhazi L.**, Marin A., Gibrat J-F. *Protein Science* 12, 34–43, (2003).

CONFERENCE PRESENTATIONS

- O1. 2018:** Storage Proteins Accumulation and Aggregation in Developing Wheat Grains. **Rhazi L.** and Aussenac T. ICC International Conference "Grains for Wellbeing. 5-8 November, Taipei, Taiwan.
- O2. 2018:** An integrated approach to investigate the functioning of wheat quality components and their interaction with environment. Daaloul Bouacha O., **Rhazi L.**, Aussenac T. 4th ICC Latin American cereals conference, 11-14 March, Mexico City, Mexico.
- O3. 2017:** Technological characterization of Lebanese wheat bread new cultivars under significant water stress. Sakr N., **Rhazi L.**, Kabalan R., Aussenac T. AACC 2017, San Diego, California, USA.
- O4. 2017:** Bioinspired gluten-chitosan supramolecular assemblies: effect on the functionality, digestibility and release of celiac disease active peptides. Ribeiro, M., Nunes, F.,

Guerreiro, A.C.L.; Domingues, P., Carrillo, J.M., Rodriguez-Quijano, M., Rhazi, L., Branlard, G., Igrejas, G. T. In Livro de Resumos do GLUPOR. 12.^a Reunião do Grupo de Glúcidos. Aveiro, 11-13 September 2017, pp. 35.

- O5. 2017:** Effet des conditions pédoclimatiques et variétales sur la composition des graines de lin de printemps. (Effect of pedoclimatic and varietal conditions on the composition of spring flax seeds.). Lazouk M.-A., Roulard R., Savoie R., **Rhazi L.**, Mesnard F., Petit E., Guillot X., Tavernier R., Thomasset B. FFR-Net, Amiens, France
- O6. 2015:** Metabolic profiling of flaxseed (*Linum usitatissimum*) depending on climatic conditions. Roulard R., Wattier C., Molinié R., Fontaine X., Petit E., **Rhazi L.**, Mesnard F. 11th Annual International Conference of the Metabolomics Society, San Francisco, California, USA.
- O7. 2015:** Impact of environmental conditions on flax seed composition: oil and omega-3. 11th Lipidomics Meeting on Lipids in agronomy, Health and Disease. Thomasset B., Lazouk M.A., Roulard R., **Rhazi L.**, Mesnard F., Guillot X., Tavernier R., Savoie R. GERLI, 2015, Strasbourg-Le Bischenberg, FRANCE.
- O8. 2015:** Impact of environmental conditions on the flaxseed (*Linum usitatissimum*) metabolic profile. Roulard R., Wattier C., Molinié R., Fontaine X., Jamali A., Petit E., **Rhazi L.**, et Mesnard F. 9 JS RFMF 2015, Lille, France.
- O9. 2015:** Modification of the molecular properties of wheat grain storage proteins by ozonation. Gozé P., **Rhazi L.**, Lakahal L., Paus A., and Aussenac T. Ozone 2015, Congress AIO3, Barcelona, Spain.
- O10. 2015:** Combination of Two in vitro Models to Investigate the Impact of a Pesticide (Chlorpyrifos) and a Prebiotic (Inulin) on Intestinal Microbiota and Permeability of the Intestinal Mucosa. Depeint F, Gonzalez-Alvarez DO, Aveline C, Royez-Citerne C, Reygnier J, Joly C, **Rhazi L.**, Delanaud S, Mayeur C, Bach V, Abdennebi-Najar L, Khorsi-Cauet H. International Scientific Conference on Probiotics and Prebiotics 2015, Budapest, Hungary.
- O11. 2014:** Profilage métabolique des graines de lin (*Linum Usitatissimum*) en fonction des conditions pédoclimatiques. (Metabolic profiling of flackseed in pedoclimatic conditions.) Roulard R., Wattier C., Molinie R., Fontaine J.-X., Petit E., **Rhazi L.** & Mesnard F. 8^{ème} Journées scientifiques du réseau français de fluxomique et de métabolomique 2014, Lyon, France. (8th Conference of the French Fluxomics and Metabolomics Network 2014, Lyon, France)
- O12. 2015:** Impact of culture conditions and variety on flaxseed composition and oil extraction processes. Lazouk M.A., Savoie R., Imatoukene N., Mesnard F., Petit E., Roulard R., **Rhazi L.**, Thomasset B. 12th Euro Fed Lipid Congress for Oils, Fats and Lipids: From Lipidomics to Industrial Innovation 2014, Montpellier, France.

- O13. 2013:** Predicting rheological behavior of wheat dough based on machine learning and front-face fluorescence spectroscopy on wheat flour. Lakahal L., **Rhazi L.**, Bonhoure J.-P., Aussenac T. AACC 2013 - Albuquerque, New Mexico, USA.
- O14. 2013:** Coping with wheat quality in a changing environment:—Proteomics evidence of stress caused by the changing environment, Branlard B., Lesage, V.S., Martre P., Méleard B. and **Rhazi L.** In “Advances in Wheat Genetics: From Genome to Field. Proceedings of the 12th International Wheat Genetics Symposium” (Y. Ogihara, S. Takumi, H. Handa Eds), Yokohama, Japan ISBN: 978-4-431-55674-9, 255-264.
- O15. 2013:** Compréhension du rapport Ténacité/ Extensibilité et du volume du pain. Branlard G, Méléard B, Oury FX, **Rhazi L**, Boinot N, (2013) In : « Synthèse du programme de recherche FSOV , actes de la rencontre scientifique 15 mars 2013, Paris, 18-26.
- O16. 2012:** Effects of HMW and LMW-Glutenins and grain hardness on size of wheat storage proteins polymer. Lesage, V.S. **Rhazi, L.**, Aussenac, T., Meleard, B., Branlard, G. In: He Z and Wang D (eds) Wheat Gluten 2012, Proc 11th Int Wheat Gluten Workshop, Beijing, 200-205.
- O17. 2009:** Accumulation of polymeric proteins in developing grains of hexaploid wheats in relation with changes in the glutathione thiol-disulfide status. Aussenac T. and **Rhazi L.** The 10th International Gluten Workshop, 2009, Clermont-Ferrand, France.

POSTERS

- P1. 2018:** Encapsulation of bioactive phenolic extract from brewers’ spent grain for use in functional foods; investigating bioequivalence in a cellular setup. Dépeint F., Socaci SA., Fărcaș AC., **Rhazi L.**, Vodnar DC. 10-13 septembre 2018 Bioavailability conference, Norwich, UK.
- P2. 2018:** Extraction and characterization of starch from old corrugated containers: a new raw material for biorefinery. Walger E., Chirat C., Marlin N., Aussenac T., **Rhazi L.**, Bloch JF. And Peyroux R. Conference: 15th European Workshop on Lignocellulosics and Pulp.
- P3. 2018:** Phytosterols and tocopherols variability in Linsed (*Linum usitatissimum*). **Rhazi L.**, Ayerdi A., Thomasset B., Tavernier R., Guillot X., Petit E. and Mesnard F. The 23rd International Symposium on Plant Lipids, Yokohama, Japan.
- P4. 2018:** New insights into the metabolic pathways of oil synthesis in three oilseed crops using carbon-14 radiolabeling. Belkouch M., Merlier F., Gontier E., **Rhazi L.**, Thomasset B. and Perrin Y. The 23rd International Symposium on Plant Lipids, Yokohama, Japan.
- P5. 2018:** Relationship between physico-chemical and molecular grain quality parameters for Tunisian durum wheat varieties (*Triticum durum l.*) in different environments. Daaloul Bouacha O., **Rhazi L.**, Nouaigui S., Aussenac T. and Rezgui S. 13th International Gluten Workshop. 14-17 March. Mexico, Mexico.

- P6. 2014:** Profilage métabolique des graines de lin (*Linum usitatissimum*) en fonction des conditions pédoclimatiques. Roulard R., Wattier C., Molinié R., Fontaine X., Petit E., **Rhazi L.**, Mesnard F. 8ème JS RFMF 2014, Lyon, France.
- P7. 2014:** Effects of ozone pretreatment on the molecular properties of wheat grain proteins. Gozé P., **Rhazi L.**, Pauss A. and Aussenac T. AACC 2014, Providence, Rhode Island, USA.
- P8. 2014:** Molecular properties of wheat lines containing combinations of three HMW-GS transgenes. **Rhazi L.**, Barro F. and Aussenac T. AACC 2014, Providence, Rhode Island, USA.
- P9. 2012:** Glutenin molecular characterization of three transgenic high molecular weight glutenin subunit events in winter wheat. **Rhazi L.**, Aussenac T. and Graybosch R. AACC 2012, Hollywood, Florida, USA.
- P10. 2011:** Characterization of waxy isogenic lines starch using asymmetric Flow Field-Flow Fractionation. **Rhazi L.**, Branlard G. and Aussenac T. AACC 2011, Palm Springs, California, USA.
- P11. 2012:** Relationship between waxy alleles and the amylose content in wheat isogenic lines. **Rhazi L.**, Aussenac T., and Branlard G. AACC 2012, Savannah, Georgia, USA.
- P12. 2007:** Relationship between the amount of protein-glutathione mixed disulphides in polymeric protein and their molecular weight distribution. Zarai T., **Rhazi L.**, Mejri S., and Aussenac T. AACC 2007, San Antonio, Texas, USA.
- P13. 2007:** High throughput separation and quantification of high-molecular-weight glutenin subunits. Benachour O., **Rhazi L.**, Mejri S., and Aussenac T. AACC 2007, San Antonio, Texas, USA.
- P14. 2002:** Analysis of HMW mRNA from ripening wheat grains. **Rhazi L.**, Cazalis R. and Aussenac T. AACC 2002, Montréal, Québec, Canada.
- P15. 2002:** Redox status changes in proteins of developing wheat grain: influence on the SDS-unextractable glutenin polymer formation. **Rhazi L.**, Cazalis R. and Aussenac T. AACC 2002, Montreal, Québec, Canada.

LANGUAGES

Arabic (Mother Tongue).

English (C1 level, European Framework).

Spanish, beginner.

Summary of research activities

Summary of research activities:

PhD Abstract:

Thesis work was carried out at the laboratory of Agro-Physiology in the Higher National Engineering School of Agriculture, Purpan (ESAP). The thesis was supervised by Thierry AUSSENAC.

PhD title: Formation of the glutenin polymers in common wheat grain (*Triticum aestivum* L.)

Several questions on the formation of glutenin polymers in common wheat and their final structure remain unanswered. Thus, the topic was undertaken to understand the formation and structure of glutenin aggregates.

Therefore, every effort was made to precisely define the formation of glutenin polymers during grain development insofar as protein associations govern the end-use quality of flour. By studying the redox status changes of storage proteins in the development of wheat grain, we demonstrated that the formation of the high molecular polymers is due to the oxidation of free glutenin subunit sulfhydryl groups. This phenomenon, assisted by the closeness of polymer structures during desiccation, leads to the formation of additional intermolecular disulfide bridges and induces an increase in the molecular weight and compactness of the glutenin aggregates.

We also demonstrated that glutathione can be bound to the glutenin fraction which limits its association capacities. This limitation of protein interactions causes a significant decrease in the molecular distribution of glutenin polymers. Therefore, variations in the glutenin polymers bound to glutathione could explain the variation in the technological quality.

Finally, a molecular modelling study was undertaken to identify a methodological process that would lead to understand the interaction capacities of the high molecular weight glutenin subunits.

During my PhD studies, I had the opportunity to showcase the most interesting findings at AACC meetings (P14 and P15). In order to reach a large audience, these posters that had positive impacts, were useful to make scientific publications (A29, A30 and A31).

Post-doctoral research:

This research was conducted in the enzymatic engineering laboratory at Compiègne University of Technology (UTC). It was part of the BIOLUBE research program funded by TOTAL and focused on the functionalization study of genes coding for the synthesis of unusual fatty acids.

Oilseed plants produce a huge amount of fatty acids stored as triacylglycerol (TAG) in seeds. Plants produce a wide variety of fatty acid structures. About 300 kinds of fatty acids are detected many of which exhibit interesting functional groups. Because of their stability, biodegradability and mainly physical-chemical features, these fatty acids are very useful in a variety of industrial applications such as lubricants, plastics, paints, dyes, and coatings. The plant species that accumulate these unusual fatty acids are habitually not appropriate for large scale commercial production. Using metabolic engineering as an approach to create genetically modified oil crops offers the potential to produce specific high yield, low cost fatty acids. Although Genetic modifications of model plant seeds (*Tobacco* and *Arabidopsis thaliana*) and agronomic plants (*Brassica napus*) under control of strong seed specific promoters, the expression of the recombinant enzymes potentially coding for the biosynthesis of unusual fatty acids typically lead to relatively low accumulation of the desired product.

Two genes potentially responsible for the synthesis of a special fatty acid which are important feedstocks for industrial products such as lubricants and functional fluids, were identified and characterized. Thus, genes were isolated and successfully cloned into different biological systems such bacteria, tobacco cells and oilseed plants. However, transgenic systems carrying gene encoding enzymes catalyzing the synthesis of that fatty acid have produced poor and different levels of targeted fatty acid. In order to determine the potential bottleneck, all steps of gene expression were studied. We found that In vivo activities of expressed enzymes were not optimal. These results could be due to specificity and selectivity of acyltransferases which are required for the transfer of our unusual fatty acids into storage triacylglycerols. Therefore, due to a limited knowledge of the lipid metabolism and fatty acid transfer in plants, my work focused on understanding the interactions between the expression of transgene and the endogenous pathways to further enhance the yields of unusual fatty acids in transgenic organisms. Understanding the lipid biosynthesis pathways helped resolve the bottlenecks encountered during plant transformation.

Results are protected by patent number WO2006087364. Unfortunately, I was not a co-author because the program was already in place when I arrived.

Research at UniLaSalle:

In the last 12 years, my research activities have focused on three very specific species: bread wheat (*Triticum aestivum* L.), hard wheat (*Durum aestivum* L.) and flaxseed (*Linum usitatissimum* L.). I will summarize two major topics of my research:

Unusual fatty acids: bottleneck incorporation into plant lipid metabolism

I have been interested in lipid metabolism in plants to understand the difficulties of gene expression in host cells, encountered during my post-doctoral research at UTC. Thus, we have developed several collaborative projects with Dr Brigitte HOMASSET, Dr Yolande PERRIN (UTC) and colleagues at UPJV (Amiens University).

Unusual fatty acids are produced by a limited number of non-agronomic plant species. The industrial applications of these unusual lipids have led several units to focus their research on the production of these structures in transgenic plants. Their research results and ours have led to very low accumulation of these unusual lipids in seeds which was largely due to a limited knowledge of the lipid metabolism and fatty acid transfer in plants. It is largely known that the biosynthesis of triglycerides (TAG) in the endoplasmic reticulum of plants follows by at least two distinguish pathways. The Kennedy pathway known as the acyl-CoA dependent pathway is the classical way for de novo biosynthesis of TAG from glycerol-3-phosphate and acyl-CoAs by a combination of acylation and dephosphorylation involving the action of three acyltransferases (Glycerol-3-Phosphate Acyltransferase G3PAT, Lysophosphatidyl Acid Acyltransferase LPAAT and Diacylglycerol Acyltransferase DAGAT). The second pathway is called an alternative pathway or acyl-CoA independent pathway. In the last, the biosynthesis is catalyzed by phospholipid:diacylglycerol acyltransferase (PDAT) which uses phosphatidylcholine (PC) as an acyl donor and therefore transfers the sn-2 acyl chain to the sn-3 position of diacylglycerol (DAG), resulting in the formation of sn-1 lyso-PC and TAG.

In order to understand the plant lipid metabolism and therefore to find a potential bottleneck in unusual fatty acid incorporation into triacylglycerol in agronomic plants, we developed a series of analytical methods to analyze and follow quantitatively and qualitatively different family of lipids. Enzymatic and molecular biology tools were of great help in understanding some parts of lipid metabolism. More specifically, in order to better understand the substrate specificity and selectivity of acyltransferase enzymes which catalyzes the transfer of these unusual fatty acids into storage TAG. In my first collaborative studies we have investigated the incorporation of C-14 radiolabeled Oleoyl-CoA and Branched Chain Acyls-CoA into [3H] LPA-C18:1 by the Lysophosphatidic acid Acyltransferase (LPAAT) extracted from developing seeds of agronomic plants (flax (*Linum usitatissimum*) and rape (*Brassica napus*)) and from a plant producing a high level of unusual structure such as hydroxy fatty acids from castor bean (*Ricinus communis*).

Our enzymatic assays demonstrated that LPAATs from the three chosen species:

- had a high specificity to incorporate oleyl-CoA,
- could incorporate cyclopropane acyl-CoA when added alone as a substrate,
- had a very weak incorporate cyclopropane acyl-CoA in cases of rapeseed and castor bean seeds,
- presented a low efficiency to incorporate methyl branched acyl-CoA when added alone as a substrate,
- exhibited a very weak incorporation of cyclopropane acyl-CoA and was unable to use methyl branched acyl-CoA when presented with an equimolar mix of oleyl-CoA and branched chain acyl-CoA.

Our results showed that in all cases, the LPAAT enzymes presented a very low affinity for branched chain acyl-CoAs. We concluded that LPAAT activity from agronomic plants is a bottleneck for the incorporation of branched Chain acyl-CoA.

In order to share and make our results accessible to other researchers and have a potential impact on the greater scientific community, a scientific paper was written and published (A27).

More recently, we presented at The 23rd International Symposium on Plant Lipids (Poster P4), the results of the incorporation study of C-14 radiolabeled oleate (C18:1 ω -9), linolenate (C18:3 ω -3) and ricinoleate (C18:1-OH) into the lipid species of homogenates from rape, flax and castor bean embryos, harvested during the highest rate of the TAG accumulation phase. We demonstrated that:

- All tested FA were incorporated into TAG by diverse enzymatic processes involving either the PDAT pathway or the DGAT pathway, or both pathways,
- The selected oilseed plants shared the same enzymatic pathway to incorporate a particular FA into TAG,
- The enzymatic reaction did not depend on the structure of AF (usual or unusual) for the plant species, but only of the type of FA.

Our results came as a complement to other investigations on lipid enzymatic processes by *in vitro*, *in vivo* and *in planta* approaches.

Until now, the understanding of the enzymatic processes of FA incorporation into TAG in oilseed and the bottleneck for the incorporation of unusual FA using genetic modifications is still incomplete.

Summaries and Perspectives of my Research on wheat:

My areas of research have always remained focused on bread wheat. Technological properties, but also the phenotypic evaluation, the agronomic performances as well as the physiological and genetic associated factors are involved in the main fields of my research on wheat. We collaborate and develop scientific projects with all French wheat breeders and technical agronomic institutes (INRA, ARVALIS).

I will provide a general summary of my research activity on wheat followed by summaries of some relevant topics developed in last two decades.

General summary (A3 and O1):

The aggregative properties of wheat grain prolamins are largely responsible for the technological functionalities of flours and doughs. The ability of wheat prolamins to form a plastic three-dimensional network during the mixing depends to a large extent on their ability to interact. These aggregative properties, which can be evaluated by measuring their molecular weight distribution, is dependent on the polymorphism of the protein subunits present but also, on the environmental conditions that are applied during grain development. Much progress has been made in the last 30 years at a genetic level to better understand and/or to favour the interaction properties of the storage proteins. However, these improvements can be severely limited by environmental conditions. Any modification of the redox status of the grain cells in response to an oxidative stress can lead to a decrease in the degree of association of the prolamins by limiting the protein-protein interactions during the grain desiccation. Considering the current and projected environmental impacts (i.e. climate change with increasing heat stress), it is essential to better understand these phenomena to implement new breeding strategies for a sustainable quality.

Effect of HMW-GS and LMW-GS on size of glutenin polymers (A12, A16, A19, O14, O15, O16, P8 and P9):

To better understand relationships between these traits, a statistical study was performed on a set de French varieties cultivated in 3 locations during 2 years providing a set of 240 bread wheat samples. Molecular properties of glutenin polymers, as evaluated by A4F technique, were significantly affected by some glutenin alleles encoding the HMW-GS and LMW-GS. PLS analysis showed that glutenin alleles had different effects on polymer mass, some of them were positive while others were negative. *Glu-B1-7*, had a much more positive effect on the MWD than other alleles. The *Glu-A1* and *Glu-D1* loci influenced also the establishment of glutenin polymers. The two *Glu-D3* alleles exhibited opposite effects on polymer mass, *Glu-D3-b* being positive and *Glu-D3-c* negative. *Glu-B3-b* also showed a negative impact on glutenin polymer mass.

From HMW-GS to technological features (A3, A6, A19, O1, O2, O3, O9, O15, O17, P7 and P9):

It has been well established that baking quality of wheat flour is tightly related to the presence or absence of particular HMW-GS subunits or alleles encoding these proteins. Thus, specific subunits including 1Ax1, 1Ax2*, 1Bx17 + 1By18 and 1Dx5 + 1Dy10 are associated with stronger dough and thus improve breadmaking performance. In addition, HMW-GS play considerably greater roles in determining dough properties of bread wheat flours than LMW-

GS. In order to evaluate the effect of quantitative variation of some specific HMW-GS, we studied three transgenic HMW-GS events in advanced breeding lines of hard winter wheat (collaboration with Dr Graybosch, USDA). We found that the over-expression of both HMW-GS 1Dx5 and or 1Dy10 increased both the amount of HMW-GS and the average ratio of HMW-GS to LMW-GS. As consequence, the over-expression of such HMW-GS increases the glutenin polymer molecular weight distribution. Furthermore, we demonstrated that the HMW-GS to LMW-GS ratio affects the molecular weight distribution (MWD) of the polymeric glutenin proteins. Increasing the HMW-GS to LMW-GS ratio increases the average molecular mass of the polymers. Finally, we proved that HMW-GS led to stronger polymers and large MWD affecting technological properties including mixograph time to peak, mixograph tolerance, bake mix time and loaf volume.

Relationship between the amount of protein-glutathione mixed disulfides in polymeric protein and their molecular weight distribution (A3, A30, O1, O17, P12 and P15):

To understand more precisely the function of GSH in developing wheat kernel for the formation of unextractable polymeric proteins (UPP), we investigated the natural level of polymeric protein linked sulphides (PPSSG) in flours of a large set of French wheat cultivars (130 cultivars, provided by 5 breeding companies, grown at three locations two consecutive years in France). The MWD of polymeric proteins were also measured using A4F measurements to determine relationship between the MWD and the levels of PPSSG in the polymeric protein fractions.

The PPSSG values ranged from 400 to 2400 nmol/g PP which represent approximately 80% total protein-bound glutathione (PSSG). The variance analysis showed significant differences among cultivars. However, the environmental factors presented the most influence on the PPSSG content. Components associated with environmental factors largely exceeded genetic factors. Furthermore, there was no significant relationship between PPSSG and PP content. The polymeric protein content was not a limiting element for the PPSSG accumulation.

Our studies showed that PPSSG content in flour which can be largely modified by environmental factors, induced modification of the MWD of the polymeric proteins. The change in the polymeric protein content characterized by molecular weight above 2×10^6 g/mol was highly correlated with the change in the PPSSG level in the polymeric fraction. Results suggested that PPSSG might be involved in controlling the glutenin polymerization through preventing intermolecular SS bonds. In other words, PPSSG might be acting as a “chain blocker”

In conclusion for these studies, the amounts of PPSSG in flour were negatively correlated with glutenin M_w distribution indicating that glutathione may play a crucial role in controlling the degree of polymerisation of the PP which has been shown to be important in determining baking performance. Thus, the level of PPSSG in flour may be a very important indication in assessing quality attributes in wheat breeding.

MWD of polymeric proteins and critical molecular weights for W index (O1, O13, O15, O17 and P12):

As mentioned above, the polymeric fraction has often been correlated with bread making properties such as mixing time, extensibility, and loaf volume. To elucidate the relationship between glutenin mass distribution and rheological properties, the samples issued from the 130 French common wheat cultivars, grown under conventional conditions in 2004 and 2005 at 3 different locations in France, have been studied.

The results indicated the A4F/MALLS method used in this study offers the means of a rapid procedure for the fractionation and size characterisation of all the wheat storage proteins. Furthermore, the dough strength W which is one of the most used characteristics provided by the Alveograph test to evaluate in many countries the bread-making quality, was related to the proportion of polymeric protein of molecular weight above a critical value (i.e. $M_w > 2 \times 10^6$ g/mol). These results demonstrated that not all polymeric proteins contributed to the W value; rather, a fraction above a certain molecular weight was most important. The proportion of polymeric protein having a molecular weight $> 2 \times 10^6$ g/mol corresponded to approximately 25-35% of total polymeric protein.

We can conclude that the MWD of glutenin plays an important role in the establishment of wheat technological properties. In addition, depending on the MWD, glutenin mass can increase or decrease the breadmaking quality of wheat flours.

***PUBLIC & PRIVATE
FUNDING/PROJECTS***

Projects:

In this part, I would like to present all relevant research projects where I was officially involved as an academic partner and collaborator. In the framework of these projects, I managed and coordinated all of scientific and administrative tasks in UniLaSalle. I will showcase the summary and objective of each project followed by a description of my tasks.

-1- GLUTNSAFE : Vers la sélection de variétés de blés au gluten plus digestible ?

Fonds de Soutien à l'obtention Végétale, AAP 2016.

Plant Protection Support Fund, AAP 2016

(2016-2019)

Coordination: Dr Catherine RAVEL, UMR GDEC, INRA Clermont-Ferrand.

Summary:

The project presented is in response to growing societal concerns about the consumption of products with gluten, a network formed by hydration of grain wheat storage proteins. Several pathologies are associated with its consumption: well-recognised allergies and celiac disease. One hypothesis put forward to explain this sensitivity concerns the molecular weight distribution (MWD) of glutenin polymers which may strongly impact gluten digestibility and induce symptoms. The development of varieties that can be better supported is therefore a priority for breeders. Thus, the project aims to analyze the genetic variability and the effect of its interaction with the agro-climatic environment on the MWD of glutenin polymers in bread and durum wheat.

Objective:

- (1) To evaluate the technological quality, the gluten characteristics, and the polymer size of wheat storage proteins. MWD will be determined for wheat flour and gluten proteins. Fifty lines will be used for this experiment composed of scientific materials (containing genes impacting reserve protein accumulation) and conventional lines (land races and agronomic lines). Experiments will be established on two locations with different climatic conditions.
- (2) To evaluate these results, contrasting material to measure the size of the polymers and the characteristics of the gluten will be used, followed by technological processing, to study the degradation using a tool to simulate human gastrointestinal digestion in vitro (digester);
- (3) Characterize gluten by imaging before digestion and establish the characteristics of polymers in processed products, before studying the size and composition of their degradation products in two phases of the digestion process.

This innovative project will promote the selection of better tolerated varieties through the description of gluten proteolysis in processed products. It will provide methods for characterization of this proteolysis and show the impact of the genetic variability of wheat. The

first elements of understanding the relationship between polymer size and gluten digestibility will then be used in studies relating to human health.

Tasks:

The genesis of this project comes from a long collaboration with Clermont-Ferrand INRA. During our research on glutenins, we found that they could form very large polymers. A hypothesis has emerged: too large sizes could prevent complete degradation of polymers by digestive juices and avoid exposure of epitopes.

Therefore, as an academic partner, my mission in this project was:

Characterization of glutenin polymers in flours and glutens before and after processing using A4F.

- Analysis of 400 wheat samples (100 lines * 2 places * 2 repetitions): determination of the molecular features of the glutenin polymers in flour and gluten.

- Analysis of the size and mass of polymers at the processed products before and after digestion

-Analysis and interpretation of data.

Then, we will be able to suggest phenotyping methodologies based on molecular features of glutenin polymers

However, the molecular weight distribution of glutenin polymers which is changing during 1st and 2nd transformation appears to be the most important think that also we need to take into account.

-2- REGULIPIDE : Régulation métabolique et identification de gènes clés de la synthèse d'huiles inhabituelles dans les plantes oléagineuses, pour une adaptation de systèmes végétaux ou de systèmes alternatifs à la bio raffinerie lipidique.

GENESYS, AAP 2014

(2015-2018)

Coordination: Dr Yolande PERRIN, UMR 6022 - UTC

Summary:

Some non-agronomic oilseeds accumulate unusual fatty acids (AGI), giving oils physicochemical properties suitable for non-food industrial applications. The production of industrial vegetable oils requires the integration, in field crops, of biosynthesis and the transfer of AGI in their triglycerides (TAG).

The repeated failure to modify oils in agronomic plants underline the need to determine the key points involved in the processes of discrimination of the usual and unusual fatty acid (AGU, AGI) in the accumulation of TAG.

The purpose of this study was to measure the incorporation into TAGs and lipid intermediates of AGI and AGU carried by lipid precursors / substrates supplied to embryonic extracts of three oleaginous plants. Cross-experiments between rapeseed, flaxseed and castor oil were conducted to compare the effectiveness of the different pathways of TAG synthesis. Then, the management of AGI and AGU according to plants were established. Moreover, the regulation

of these pathways during embryogenesis were studied in flaxseed and castor oil and identification of key enzymes was done by bioinformatic, molecular and enzymological approaches. Finally, the implication of storage processes, intracellular trafficking and degradation that may lead to the exclusion of AGI from TAG were studied by biochemical and enzymological approaches.

Objective:

The ambition of the project was to advance and improve the knowledge on the biosynthesis of vegetable oils and to identify key genes that could be used in assisted plant breeding for industrial use. Results could be used for the transformation of microorganisms that may constitute an alternative source of oil production for various industrial applications.

Tasks:

As an academic partner (UniLaSalle), I took charge of task 3 entitled: Isolation of Key Genes / Enzymes in Flaxseed and Regulation of TAG Synthetic Pathways during Linseed and Castor Embryogenesis.

My role was the identification in flaxseed of key enzyme genes by comparing the genome of flax (<http://www.linum.ca>) with those of reference plants. The aim was to study the function of these microsomal enzymes by heterologous expression in the yeast *Saccharomyces cerevisiae* based on studies of PDAT, DGAT, PDCT and LPCAT.

This task included 3 sub-tasks on:

- Identification of flax candidate genes (<http://www.linum.ca>) by homology search (BLASTp, BLASTn ...) with known animal and plant sequences.
- The cDNA synthesis of the candidate genes from RNA extracts of developing flax embryos and their cloning in the yeast *S. cerevisiae* and
- The in vitro measurement of the functionality of enzymes on yeast microsomal fractions by providing appropriate labeled and cold substrates and TLC analyzes. This sub-task was done at UTC because UniLaSalle is not allowed to work with labeled products. For information, I participated in the development of the method when I did my postdoc at the UTC.

My role was also to reflect on the strategies adopted to meet the objectives and to discuss the results obtained by the different partners involved in the project. . I was associated at the presentation delivered recently to the 23rd International Symposium on Plant Lipids, Yokohama, Japan in 2018 (P4).

-3- IGE: Compréhension de l'effet des interactions génotype-milieu sur la valeur boulangère.

Fonds de Soutien à l'obtention Végétale, AAP 2014.

Coordination: Benoit MELEARD, Arvalis

Summary:

Despite the progress in the field of genetics over the past 15 years, interannual and inter-location variations in the breadmaking value of French wheats are viewed as problematic for end-users,

who are looking for quality, stability and variety diversification. We aimed (1) to better understand the value of a given location (2) to be able to identify varieties which could exhibit variability and (3) predict the behavior of varieties in a given pedoclimatic situation.

The project was based on a chain of hypotheses, attributing the causal relationships as follows: temperature and water supply regime during grain filling → molecular weight distribution of protein polymers, starch and soluble sugar content → bread volume and “blade shots” → breadmaking value. We assessed 30 winter wheat genotypes in multi-environment trials at 10 locations in France and Europe. In order to study the relationships at each stage of the hypothesis schema, Pedoclimatic parameters (water reserve, precipitation, temperature, radiation), phenological (flowering, physiological maturity), physicochemical (protein and starch polymers, hardness, protein content, etc.) and bread baking value (bread test) were measured. An advanced statistical treatment of the generated data and the historical data of the partners was done using multivariate methods.

Objective:

The aim of the study was to understand the contribution of genotype, environment and genotype-by-environment interaction to wheat grain bread making quality.

Tasks:

Current knowledge suggests that the use value (measured by the breadmaking mark) is dependent on two major parameters measured in the breadmaking test, the bread volume and the development of blade strokes. It is postulated that these two parameters are under the influence of protein aggregates (concentration, size distribution), the size distribution of starch granules, the amylopectin molar mass distribution, and the concentration of simple sugars. Therefore, as an academic partner (UniLaSalle), we focus our analysis on:

- Analysis of the concentration, molecular weight, size and conformation (radius of gyration) of glutenin polymers (A4-MALLS) of mature grains produced in the European test network.
- Analysis of the concentration, molecular weight, size, conformation (radius of gyration) of starch polymers and amylose / amylopectin ratio (A4-MALLS) of mature grains produced in the European test network.

We took advantage of a set of samples to carry out additional analyzes. Thus, simple carbohydrates, thiols, metabolites, proteins, soluble/insoluble proteins have been assayed. Due to the quantity of generated data, all statistical analysis are taking a lot of time.

Scientific publications will be done as soon as possible. At least three articles are envisaged.

-4- GRANOLIN: Bioraffinerie de la graine de lin pour une dynamisation de la filière lin oléagineuse française.

**Fonds Unique interministériel, AAP 2011.
(Single Interministerial Fund)**

Coordination: Sylvie BRETON, Lesieur

Summary:

The methodology implemented in the GRANOLIN project framework GRANOLIN, was based on a preliminary fractionation of flaxseed (almonds, teguments). The work focused on the development of suitable processes to facilitate the extraction of target compounds in the different fractions of this seed. Alternative or innovative technologies (dehulling, microwaves, supercritical fluids...) coupled with conventional technologies (pressing, refining...) were evaluated to improve extraction yields to obtain better selectivity for the separation of molecules while limiting the use of solvents. These processes were then scaled up and their economic viability validated. Work was developed upstream on the impact of pedoclimatic conditions and genotype factor on the synthesis and accumulation of target compounds in flaxseed. To optimize grain filling, the aim of the final part was to determine the key factors regulating these syntheses and determining yields.

Objective:

The objective of the GRANOLIN project was to develop economically viable pre-industrial processes that make it possible to maximize the use of ingredients and molecules derived from the fractionation of flaxseed grains. This was part of a biorefinery concept for the entire plant and aimed to boost the French linseed industry. This project was supported by 3 competitiveness clusters: IAR, Cosmetic Valley and NSL.

Tasks:

As a representative of the UniLaSalle partner in this project, our participation was important and diverse. I was involved in the following tasks:

- Task 1: Choice of molecules and variety choice
 - Under Task 1.1: Evaluation of flax genotypes meeting the needs
 - Under Task 1.2: Impact of pedo-climatic conditions on the production of targeted molecules in selected flax genotypes
- Task 3: Extraction, purification and refining
 - Under Task 3.3: Extraction and purification of mucilage
- Task 4: Quantitative and qualitative analyzes of the targeted compounds of flaxseed and monitoring during the process and / or their culture conditions
 - Under Task 4.1: Determination of the composition of flaxseed in targeted compounds of interest in the different varieties selected
 - Under Task 4.2: Optimization of the analysis protocols of the different fractions of the linseed
 - Under Task 4.3: Impact of processes on the quality and quantity of extracted compounds
- Task 6: Process implementation
 - Under Task 6.1: Production a pilot of dehulling unit

I have developed and optimized the flaxseed Abrasive dehulling system. Then, we studied effects of dehulling on chemical composition of flaxseed.

In this project, I evaluate the impact of environmental conditions on the chemical composition (mainly: minor compounds such as tocopherols and phytosterols; and mucilage) of seeds of different genotypes of flax.

My project participation was valued by the publication of a PhD dissertation, several articles, oral communications and posters (A5, A14, A15, A17, O5, O6, O7, O8, O11, O12, P3 and P6).

-5- METALIPPRO-PL1 : Métabolisme lipidique chez les plantes et les levures.

**Programme de recherche GENESYS, AAP 2011
GENESYS Research Program, AAP 2011
(2012-2015)**

-6- METALIPPRO-PL2 : Métabolisme lipidique chez les plantes et les levures.

**Programme de recherche GENESYS, AAP 2012
(2013-2016)**

Coordination: Dr Jean-Marc NICAUD, INRA-AgroParisTech, UMR1319, Jouy-en-Josas.

Coordination: Dr Brigitte THOMASSET, UMR 6022 - UTC.

Summary:

The MetaLipPro-PL1 and MetaLipPro-PL2 projects were the initial phase of the study in order to further our knowledge of lipid metabolism in both plants and yeasts. Studies were proposed to establish the groundwork for developing a pilot platform for lipid production and extraction.

Objective:

The scientific and technical objectives of both projects were:

- (1) To develop a better understanding of the molecular, cellular and metabolic factors governing the synthesis and accumulation of common and unusual oils in oleaginous plant embryos and yeasts. A study of the metabolic pathways and / or the genes conferring the oleaginous character of the cells and the demonstration of the possible bottlenecks in these mechanisms. A study of the import and export mechanisms of these fatty acids in the yeast and the plant. The production of comparative databases and metabolic models to provide new insights into the functioning of the two systems studied (plant, yeast). The creation of an ORFeome library for suspected genes which could play an active role in lipid production and / or regulation
- (2) The identification and isolation of genes involved in the biosynthesis of unusual fatty acids and lipids in plants. The introduction of these candidate genes into oleaginous yeast to induce new fatty acids synthesis. The exploitation of the varietal biodiversity belonging to the *Yarrowia lipolytica* clade for the evaluation of the accumulation capacities and the profiles of the substrates used for their growth.
- (3) The creation of genetically modified yeast producing high value added fatty acids and structured bio-lipids that could be exploited as valuable chemical compounds in the fields of lubrication, cosmetics, etc., but also as basic molecules for the creation of nano-objects.

Tasks:

In these two big projects, we worked on the characterization of lipid species produced in recombinant yeast. I was involved in the analysis of the localization of the lipid compounds in the different cell fractions. Finally, I participated in the design of conjugated genes and their expression in yeast.

I had the opportunity to supervise two postdoc fellows. We did not publish any articles because we are still waiting for the response of industrial partners to establish a patent. However, we will be able to publish some fundamental results related to these projects.

-7- P/L: Compréhension du rapport “Ténacité/ Extensibilité” et du volume du pain.

Fonds de Soutien à l’obtention Végétale, AAP 2009.

Coordination: Dr Gérard BRANLARD, UMR GDEC, INRA Clermont-Ferrand.

Summary:

The characteristics of wheat frequently selected in Europe and especially in France often show an insufficiency in the extensibility of the dough, as measured with an alveograph. These wheats generally produce dough with very high tenacity, which results in an imbalance of the P / L ratio which is often detrimental to a satisfactory processing in bread making. It is known that the diversity of the storage proteins added to the protein content and the hardness of the grain can explain nearly 70% of the phenotypic variability of the baking strength but is insufficient to explain the bread volume. Few studies have been conducted in the search for molecular markers (other than those for the coding of storage proteins) associated with bread volume. The volume of the bread is a result of many factors thus it was necessary to specify by looking at the effects of the alleles of the storage reserve proteins and their polymerization. The insufficiencies of extensibility and the small volume of the bread are two defects which, if present in the same cultivar, are reasons for exclusion from the Official Catalog. Research has been undertaken to better understand the constituents of grain and the properties of flour, the variables that should be considered to make a selection.

Objective:

- (1) “Increasing” or “Reinforcing” the biochemical and molecular characteristics of wheats with extensible gluten,
- (2) (Deepening of) As above. the biochemical and technological characteristics of wheats with extensible dough,
- (3) Identification of the QTL involved in the size distribution of starch granules,
- (4) Prediction of bread volume.

Tasks:

In order to achieve our objectives, our participation in this projects was divided into three major part:

- A4F-MALLS analysis of the polymer molecular size distribution of the set samples.
- Identification of variables characterizing the state of gluten polymerization.
- Participation in the analysis and determination of the relevant variables defining P / L ratio and bread volume.

I had the opportunity to supervise several training students of Master II. I was associated to the publication presented at the scientific synthesis of the FSOV Program in Paris and at the presentation delivered to the 11th International Wheat Genetic Symposium, Yokohama in 2013 (O14 and O15). I was also associated to other scientific productions (A28 and O13).

-8- METALIPIDE: Régulation de la synthèse d'acides gras inhabituels dans les triglycérides d'espèces oléagineuses, pour une adaptation industrielle des huiles végétales.

**AAP Région Picardie, 2007.
(2008-2011)**

Coordination: Dr Brigitte THOMASSET, UMR 6022 - UTC.

Summary:

The question posed in the METALIPIDE project concerned the proven limitations on the production of modified oils (triglycerides, TAG) by oleaginous crop species in an industrial context where the demand for these optimized oils was more and more marked.

Indeed, certain oleaginous plant species naturally produce oils with physicochemical properties suitable for non-food industrial uses. They may be of interest to sectors currently using petrochemicals such as energy, lubricants / surfactants, materials etc. However, the particular properties of these oils are related to their richness in atypical / unusual fatty acids (AG) which are differentiated from the usual AG by the length of their carbon chain (e.g. short chain lauric acid C12 in coconut oil), their richness in conjugated double bonds (e.g. linolenic acid in linseed oil) or by the presence of functional groups on the hydrocarbon chain (e.g. the hydroxy group of ricin oleic acid in Castor oil). Nevertheless, most of these unusual AGs are produced by plant species not suitable for large-scale agricultural production, thus requiring the transfer of the biosynthetic capacities of these species to agronomic species. This requires knowledge of the key points involved in the metabolic pathway of incorporation of unusual AG in oils in order to better target the adaptation strategies of oleaginous species to produce oils for industrial use. This is important because attempts to produce modified oils in oleaginous species have resulted in an absence of accumulation of unusual AGs in TAGs.

Objective:

The METALIPIDE project aimed to identify the nature and regulation of the mechanisms that cause discrimination between usual GAs and unusual GAs during modified oil synthesis processes in seeds of oleaginous species of agronomic interest.

Tasks:

As an academic representative of UniLaSalle in this program, I was particularly involved in the characterization of the products and enzymes involved in the accumulation of TAG in agronomic plants (flaxseed and rapeseed, task 3.1.2).

I characterized and analyzed the key enzymatic systems responsible of the production of the ricinolenic acid (tasks 3.2.1 and 3.2.2).

The main tasks were as follow:

- Task 3.1.2. Study of mechanisms used by the rapeseed and flax agronomic plants for the management of branched fatty acids and their accumulation.
- Task 3.2.1. Characterization of the transport mechanisms of hydroxy fatty acids put in place by castor bean.
- Task 3.2.2. Study of the management of other types of unusual fatty acids by the enzymatic machinery of castor bean.

To achieve our objectives, several chemical, biochemical and enzymatic techniques were developed.

We had the opportunity to publish our relevant results in a peer-reviewed journal (A27).

-9- Ozonation of bread wheat grains: oxidation effects on the main biochemical components

ANRT 2012

(2013-2016)

Coordination: Dr Thierry AUSSENAC, UP Transformations & Agro-ressources

Summary:

The project focused on the effects of treatment of bread wheat grains with ozone gas on their major biochemical constituents. Two wheat cultivars differing in their HMW-GS composition and technological properties were treated in a semi-industrial ozone reactor. A Box-Behnken experimental design was employed to investigate the effects of (i) humidification rate (during grain preparation), (ii) the ozone concentration in the ozone inlet and (iii) the reaction time. The molecular weight distribution of glutenin polymers, starch and polysaccharides such as β -glucan were studied. Techno-functional properties of flours and dough were analyzed.

Objective:

Investigation of the effects of treatment of bread wheat grains with ozone gas on their major biochemical constituents.

Tasks:

My tasks were:

- The co-supervision of the PhD student.
- Time and analysis management.
- Methodologies development.
- Chemical and biochemical characterization of wheat flour constituents (proteins, starch, polysaccharides, ...)

I was involved in all scientific productions (A6, A13 and P7). In addition, other relevant results will be published soon.

-10- BLE BIO-ETHANOL: Analyse et identification des caractéristiques génétiques influençant la composition du grain de blés destinés à la production de bio-éthanol.

Fonds de Soutien à l'obtention Végétale, AAP 2006.

(2007-2010)

Coordination: Dr Gérard BRANLARD, UMR GDEC, INRA Clermont-Ferrand

Summary:

Varieties created so far for bread-making purposes are not specifically suitable for the objectives of bioethanol production. Several factors such as grain composition can influence the efficiency and speed of starch transformation for bio ethanol production. The program consisted of several phases (1) The establishment of multi-local trials of several tens of cultivars implanted over a period of three years in France (2) Determination of the ethanol processability of wheat cultivars grown each year. A pilot ethanol plant was used to test each of the varieties

that were selected based on several genetic factors of grain composition. (3) The study of genetic characteristics of bread wheat associated with bioethanol production. For this purpose, several genetic parameters of the grain and the size and the composition of the starch granules were analyzed.

Objective:

This research program was conducted to define the genetic characteristics of common wheat required to obtain a high yield of ethanol production.

Tasks:

We were several Unilasalle researchers involved in this project. The first major task was the establishment of a laboratory protocol to assess glucose and ethanol yields from wheat. The impact of the analyzed wholemeal flour quantity and the saccharification on the amount of released glucose was determined. We were also involved in the study of the effect of genotype on bioethanol production.

The second major task was the determination of amylose/ amylopectin ratio of wheat samples and to study the relationship between this ratio and bioethanol production.

We had also the opportunity to develop in our laboratory the analysis of the molecular features of starch using A4F-MALLS.

Thanks to this multidisciplinary collaboration, several scientific publications were achieved (A21, A23, A24, A26, P10 and P11).

-11- INDICE DE QUALITE : Etude et prédiction des fractions protéiques de réserve et indices de sélection pour la qualité.

**Fonds de Soutien à l'obtention Végétale, AAP 2003
(2004-2007)**

Coordination: Dr Gérard BRANLARD, UMR GDEC, INRA Clermont-Ferrand

Summary:

In the context of segmentation of the wheat market end-uses, this program has helped breeders to create varieties adapted to the requirements of industrial processors and to produce good quality flour. We assessed 130 winter wheat cultivars representing a wide range of genetic variations in multi-environment trials at 3 locations over 2 years in France. The work focused on (1) the establishment of breeding indices based on HMW-GS and LMW-GS allelic diversity (2) Determination of protein fractions (gliadins and glutenins) and the measurement of NIRS absorbance parameters (3) Analysis of technological characteristics and (4) Protein Polymer Analysis and Redox Status.

Objective:

The objective was to establish selection indices for the quality of common wheat based on reserve protein analysis (glutenins and gliadins).

Tasks:

I was mainly involved in the analysis of wheat protein distribution. The molecular weight distribution of glutenin polymers was determined by A4F technique.

The second main task was the study of the Redox Status by following the quantitative variations of glutathione (Free reduced and oxidized glutathione and glutathione bound to glutenin polymers).

At the academic level, I had the opportunity to supervise students of Master II.

Most results of this project are presented in the last part of this manuscript (A3, A28, O1, O17, P12 and P13).

-12- BIOLUB : clonage et séquençage de gènes végétaux pour la production de biolubrifiants à partir de plantes oléagineuses transgéniques. (1999-2006)

Coordination: Dr Brigitte THOMASSET, UMR 6022 - UTC.

Summary:

The project consisted of demonstrating the feasibility of a process to produce unusual fatty acids from transgenic flax and rapeseed. These "high-end" oils will be used as a substitution for synthetic oils and will be used as biodegradable industrial lubricants.

This project was based on the significant involvement of both public research actors via the University Organizations (UTC and associated teams), upstream industrial partners (LIMAGRAIN / BIOGEMMA) and downstream (TOTAL for the use of the product) and Societies allowing the transfer of this technology to agriculture for the production of the raw material (LIMAGRAIN for rapeseed).

Objective:

- (1) To isolate the genes from appropriate organisms/plants coding for unusual fatty acids, adapted to our problematic, using all the accumulated knowledge in plant genomics and biochemistry in recent years. The functionality of these genes was then tested in industrial oleaginous plants cultivated in France or in Europe.
- (2) To study the feasibility of production of such unusual fatty acid esters by these genetically modified oilseed plants.
- (3) To demonstrate that these esters offer high-performance biodegradable and renewable industrial products, such as high-end lubricants, cosmetics, detergents, etc.

Tasks:

I was responsible for molecule construction from gene isolation to its insertion into host organisms (prokaryotic and eukaryotic cells).

I made all biochemical, molecular and enzymatic analysis from gene insertion to the target molecule.

I supervised and managed several technical staffs. This project which needed strong organizational, multi-tasking, and problem solving skills, allowed me to improve my oral and written communication skills, including giving presentations to industrial clients.

Important participation:

Since joining UniLaSalle, I have participated in a significant way in several projects. This was made possible thanks to my knowledge acquired during my university, doctoral and postdoctoral studies. My technical and scientific skills such as multi-tasking, and problem solving abilities, allowed me to provide technical and especially scientific assistance to my colleagues. I was also involved in the supervision of training students and management of some tasks. My other role in these projects was to participate in all kinds of scientific communications. The most important of research programs are:

- **PRONAP:** Programme de recherche GENESYS, AAP 2014, (2015-2018).
- **PREBIODIG:** AAP Région Picardie 2011, (2012-2015).
- **APHAR:** Fonds Unique interministériel, AAP 2010, (2011-2015).
- **MUCINOL:** AAP Région Picardie 2005, (2006-2009).
- **QUALITNBLE:** Fonds Unique interministériel, AAP 2007, (2008-2011).
- **PIB:** (2008-2009).
- **PREB:** (2009-2010).
- **PREBIOSE:** (2010-2011).

Thanks to these collaborations, we were able to spread the most important findings through publications in peer-reviewed journals (A4, A8, A9, A10, A11, A22 and A25), oral communication (O10) and poster (P1).

COLLABORATIONS

Internal collaborations

Within UniLaSalle, I had the opportunity to collaborate with many colleagues in different fields. These collaborations were part of either research projects and / or Private contracts. I have worked with many researchers of whom I will mention only a few:

Dr. Thierry Aussenac: Director of Institutional Research, Head of Transformations & Agro-Resources research unit (EA 7519).

Dr. Philippe Pouillard: Associate Professor of Culinary Practice and Health.

Dr. Flore Depeint: Associate Professor of Applied Nutrition.

Dr. Alicia Ayerdi Gotor: Associate Professor of Ecophysiology.

Dr. Celine Leridon: Researcher in Analytical Chemistry.

Dr. Delphine Mareck: Associate Professor of Organic Chemistry.

Dr. Jean-Paul Bonhoure: Associate Professor of Applied Chemistry.

Dr. Robert Ralainirina: Associate Professor of Process Engineering.

National collaborations

Academic collaborations

All academic collaborations were carried out as part of research projects. Most of them were conducted with the French National Institute for Agricultural Research (INRA):

ARVALIS-Institut du Végétal, 91720 Boigneville, France.

CENTRE HOSPITALIER DE BEAUVAIS, 40 Avenue Léon Blum, 60000 Beauvais, France.

CIDAM, Université d'Auvergne Clermont 1, 28 Place Henri Dunant - 63001 Clermont-Ferrand, France.

CNRS-FRE 3580, GEC, Université de Technologie de Compiègne, CS 60319, 60203 Compiègne Cedex, France.

CRRBM (UPJV-CRRBM) UFR des Sciences, UPJV, 33 rue Saint Leu, 80039 Amiens cedex, France.

EA 2222, LMAC (UTC-LMAC) UTC, Centre de Recherche, BP 20529, 60205 Compiègne cedex, France.

EA 4297, TIMR, UTC/ESCOM, CS60319, 60203 Compiègne cedex, France.

EA3900 BIOPI Biologie des Plantes et Innovation UFR de Pharmacie - 1 rue des Louvels
80037 Amiens cedex, France.

LASBA, IUT d'Angers, 4 boulevard Lavoisier, 49016 Angers cedex, France.

INSTITUT PASTEUR DE LILLE, Département de Nutrition, 1 rue du Professeur Calmette,
BP 245, 59019 Lille cedex, France.

INRA, Centre de Recherche de Versailles, Mathématique, Informatique et Génome, 78026
Versailles cedex, France.

INRA, UMR 1095 INRA/UBP, Génétique, Diversité et Ecophysiologie des Céréales
(GDEC), Domaine de Crouël, 234 avenue du Brézet, 63100 Clermont-Ferrand, France.

INRA, Unité de Recherche Biopolymères, Interactions, Assemblages, 44316, Nantes cedex
3, France.

INRA UR 370, PFEM-Plateau Protéomique, 63122 Saint-Genès-Champanelle, France

INRA UMR 1319 (INRA-BIMLip) Micalis, INRA, Domaine de Vilvert, 78352 Jouy-en-
Josas, France

INRA Plateforme d'exploration du métabolisme Theix, Centre de recherche Auvergne
Rhône-Alpes, R454 Microbiologie, 63122 Saint-Genès-Champanelle, France.

INRA Plateforme de Résonance Magnétique des Systèmes Biologiques (RMSB) Theix,
Centre de recherche Auvergne Rhône-Alpes, R454 Microbiologie, 63122 Saint-Genès-
Champanelle, France

INRA, Unité expérimentale DIASCOPE Mauguio, Domaine de Melgueil, 34130 Mauguio,
France.

INRA Unité IATE Montpellier, INRA 2 place Viala 34060 Montpellier cedex, France.

INRA Unité BIA Nantes, INRA Nantes Rue de la Géraudière - BP71627, 44316 Nantes
cedex, France.

ROBERVAL (UTC-Roberval) UTC, Centre de Recherche, BP 20529, 60205 Compiègne
cedex, France.

UMR1095 Génétique, Diversité and Ecophysiologie des Céréales, Université Blaise Pascal,
63173 Aubière, France.

Industrial collaborations

Industrial collaborations were carried out either as part of research projects or as part of service delivery. Some of my research activities allowed me to work with industrialists on applied projects and sometimes, I have worked with industrialists for more targeted requests. The two areas most concerned are lipid and protein sciences.

ANMF (Association Nationale de la Meunerie Française), 66 rue la Boétie 75008 Paris,
France.

AEGILOPS, Rle des saules, 27100 Val de Reuil, France.

ALBAN-MULLER, 8 Rue Charles Pathé, 94300 Vincennes, France.

BANETTE, Le Moulin à Vent, 45250 Briare, France.

CAUSSADE Semences, Zone industrielle de Meaux, 82303 Caussade, France.

CETAC (Centre d'Etudes Techniques pour l'Amélioration des Céréales), 7 rue Coq Héron
75030 Paris cedex, France.

CEO, MDN Group, 26, rue d'Artois 75008 Paris, France.

CERELAB, 4 boulevard de Beauregard - BP 4075 21604 Longvic cedex, France.

FLORIMOND DESPREZ, Lieu dit Aux Wattines 59242 Cappelle en Pevele, France.

GIE CLUB 5, 83 avenue de la Grande Armée, 75782 Paris cedex 16, France.

KWS MOMONT SAS, 7 Rue de Martinval, 59246 Mons-en-Pévèle, France.

LABOULET Semences, 80270 Airaines, France.

LEMAIRE DEFFONTAINES, 180 Rue du Rossignol, 59310 Auchy lez Orchies, France.

LESIEUR, 29 quai Aulagnier 92665 Asnières-sur-Seine, France.

LIMAGRAIN, 5 rue de l'Egalité, 28130 Chatainvilliers, France.

LINEA, 20 avenue Saget, 60210 Grandvilliers, France.

LTR Industries, SWM INTL Usine Le Mans Le grand Plessis 72700 Allonnes cedex France.

NEXIRA, 129 Chemin de Croisset, 76723 Rouen, France.

NUTRISET, Route du Bois Ricard, 76770 Malaunay, France.

RAGT, Rue Emile Singla BP 3331 12033 Rodez cedex 9, France.

ROQUETTE Lestrem, Department of Biology and Nutrition, Bat. L3 F-62 080 Lestrem,
France.

SAATEN UNION, 163 Ter avenue de Flandre BP 6 60190 Estrées-Saint-Denis, France.

SECOBRA, Le Bois Henry, 78580 Maule, France.

SOFIPROTEOL, 11, rue de Monceau, CS 60003, 75378 Paris cedex 08, France.

SOUFFLET, Quai du Général Sarrail 10400 Nogent Sur Seine, France.

TERRES INOVIA, 11 rue Monge, 33600 Pessac, France.

TOTAL S.A., Tour Coupole - 2 place Jean Millier 92078 Paris La Défense cedex, France.

ULICE, Rue Georges Gershwin ZAC les Portes de Riom, 63 200 Riom, France.

UNISIGMA, 2 Rue du Petit Sorri, 60480 Froissy, France.

International collaborations

Working on wheat has allowed me to collaborate with several international researchers and institutions. My publications on proteins and bread wheat starch have given me certain

notoriety and visibility in the field of cereal chemistry with many requests for collaboration from abroad. I have supervised some PhD and Master 2 students and most collaborations were recognized in publications in peer-reviewed journals (A1, A2, A7, A12, A18, A19, A20, A23, A25 and A28). In addition, oral and poster communications were realized (O2, O4, P1, P8, P9, P10, P12 and P13).

CQ-VR, Chemistry Research Centre, Chemistry Department, University of Trás-os-Montes and Alto Douro, 5000-801 Vila Real, Portugal.

Caliper Life Sciences, 605 Fairchild Drive, Mountain View, 94043-2234 CA, U.S.A.

Chemistry Department, University of Aveiro, 3810-193 Aveiro, Portugal.

Cosucra – Groupe Warcoing, 1 rue de la Sucrierie, 7740 Warcoing, Belgium.

Department of Plant Breeding, The Swedish University of Agricultural Sciences, Box 101, SE-23053 Alnarp, Sweden.

Department of Food Science, The Swedish University of Agricultural Sciences, Box 7051, SE-750 07 Uppsala, Sweden.

Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, 5000-801 Vila Real, Portugal.

Functional Genomics and Proteomics Unit, University of Trás-os-Montes and Alto Douro, 5000-801 Vila Real, Portugal.

INAT, 43, Avenue Charles Nicolle 1082, Tunis Mahrajène, Tunisia.

Innventia AB, Box 5604, SE-11486 Stockholm, Sweden.

Instituto de Agricultura Sostenible, CSIC, E-14080-Córdoba, Spain.

Instituto Tecnológico Agrario de Castilla y Leon, Carretera de Burgos Km 119 47071 Valladolid, Spain.

Institut Malgache de Recherches appliquées, Fondation Suzanne et Albert Rakoto-Ratsimamanga, Avarabohitra, Itaosy, Antananarivo, Madagascar.

KTH Royal Institute of Technology, School of Chemical Science and Engineering, Fiber and Polymer Technology, SE-10044 Stockholm, Sweden.

Laboratoire de Chimie et de Valorisation des Produits Naturels (LCVPN), Faculté des Sciences BP 906, Université d'Antananarivo, 101 Antananarivo, Madagascar.

Laboratoire Plante-Santé/Laboratoire de Chimie et de Valorisation des Produits Naturels (LPS/LCVPN) Fanambinantsoa BP: 1264, Faculté des Sciences, Université Fianarantsoa, 301 Fianarantsoa, Madagascar.

Lot IIP109 Tambohobe BP 1264, Faculté des Sciences, Université Fianarantsoa, 301 Fianarantsoa, Madagascar.

MAX-IV Laboratory, Lund University, Box 118, SE-221 00 Lund, Sweden.

The Procter & Gamble Company, 8700 Mason-Montgomery Rd., Mason, OH, U.S.A.

USDA-ARS, 137 Keim Hall, East Campus, University of Nebraska, Lincoln, NE 68583,
U.S.A.

Unidad de Genética y Mejora de plantas Departamento de Biotecnología, E.T.S. Ingenieros
Agrónomos Universidad Politécnica de Madrid, 28040 Madrid, Spain;

UCIBIO-REQUIMTE, Faculty of Science and Technology, University Nova of Lisbon,
Caparica, Portugal.

Witaxos DF3 SAS/BioActor b.v. BioPartner Center, Oxfordlaan 70, 6229 EV Maastricht,
The Netherlands.

MANAGERIAL TASKS

At the infrastructure level:

My first assignment at UniLaSalle in 2006 was the management of the analytical chemistry and molecular biology platform. Two years later due to an increased workload, it was decided to split the platform into an analytical chemistry platform and molecular biology platform. Following a period of transition I was able to devote myself to the management of the increasing number of research projects.

- **2006-2008:** Manager of the analytical chemistry and molecular biology platform.
- **2008-2010:** Manager of the analytical chemistry platform.
- **2006-2010:** Management and maintenance of analytical and molecular devices.

At the institute level:

My project was to train technical staff on the use of different analytical machines and to develop methods for extracting and analyzing simple and complex molecules from agro-resource matrices.

Reporting directly to the Scientific Director Thierry AUSSENAC, I have had various and varied projects over the years:

- Design and implementation of research, studies, consulting and training programs.
- Scientific project management.
- Management of study projects and private contracts.
- Writing Reports and Scientific Articles.
- Member of the Transformations & Agro-resources research unit.
- Member of the scientific council of T&A research unit.
- Member of CHCT taskforce (health and safety at work).
- Supervision and co-supervision of PhD students.
- Supervision and co-supervision of Post-Doctoral fellows.
- Supervision and co-supervision of students within research platforms.

TEACHING ACTIVITIES

Teaching responsibilities:

Teaching activities during thesis:

In parallel with my thesis work, I was asked to provide lectures and lead practical work. My teaching activities were done at the School of Agriculture of Purpan (Toulouse).

- **1999-2003:** Teaching assistant in Biochemistry and Agri-food lab work (64 h per year).
- **1999-2003:** Teaching assistant in Applied Statistics in Agri-food quality (8 h).
- **2001-2003:** Teaching assistant in plant physiology lab work (12 h per year).

Teaching activities at UniLaSalle:

My annual average teaching time is 174 hours face to face. All my teaching interventions are closely connected with my paths of study and research. They are focused on the plant kingdom.

- **2006-2007:** Coordinator of Plant Biology module (1st year Agriculture undergraduate students), Lecture (20 h) and Plant Biology Lab work (48 h).
- **2007-2008:** Coordinator of Plant Biology module (1st year Agriculture undergraduate students), Lecture (20 h) and Plant Biology Lab work (48 h).
- **2008-2009:** Coordinator of Plant Biology (1st year Agriculture undergraduate students), Biotechnology and Plant Improvement (4th year Agriculture graduate students) modules. Lecture (60 h), Plant Biology Lab work (48 h).
- **2009-2010:** Coordinator of Plant Biology (1st year Agriculture undergraduate students), Biotechnology and Plant Improvement (4th year Agriculture graduate students) modules. Lecture (60 h), Plant Biology Lab work (48 h).
- **2010-2011:** Coordinator of Biotechnology and Plant Improvement module (4th year Agriculture graduate students). Lecture (36 h), Plant Biology Lab work for 1st year Agriculture undergraduate students (32 h).
- **2011-2012:** Coordinator of Biotechnology and Plant Improvement module (4th year Agriculture graduate students). Lecture (36 h), lecture in non-food valuations (4 h) (5th year Agriculture graduate students). Plant Biology Lab work for 1st year Agriculture undergraduate students (32 h).
- **2012-2013:** Coordinator of Molecular Biology (3rd year Agriculture undergraduate students) and Biotechnology and Plant Improvement modules (4th year Agriculture graduate students). Lectures (56 h), lectures in non-food valuations (4 h) (5th year Agriculture). Plant Biology Lab work for 1st year Agriculture students (32 h), Molecular Biology Lab work for 3rd year Agriculture undergraduate students (48 h).
- **2013-2014:** Coordinator of Molecular Biology (3rd year Agriculture undergraduate students) and Biotechnology and Plant Improvement modules (4th year Agriculture graduate students). Lectures (56 h), lectures in non-food valuations (4 h) (5th year agriculture). Plant Biology Lab work for 1st year Agriculture (32 h), Molecular Biology Lab work for 3rd year Agriculture undergraduate student (64 h).

- **2014-2015:** Coordinator of Plant Histology (1st year Agriculture undergraduate students), Molecular Biology (3rd year agriculture undergraduate students) and Biotechnology and Plant Improvement modules (4th year Agriculture graduate student). Lectures (62 h), lectures in non-food valuations (4 h) (5th year agriculture). Plant Biology Lab work for 1st year Agriculture (32 h), Molecular Biology Lab work for 3rd year Agriculture undergraduate students (64 h). Plant Histology lab works for 1st year Health & Nutrition undergraduates (8 h).
- **2015-2016:** Coordinator of Plant Histology (1st year Agriculture undergraduate students), Molecular Biology (3rd year Agriculture undergraduate students) and Biotechnology and Plant Improvement modules (4th year Agriculture graduate students). Lectures (62 h), lectures in non-food valuations (4 h) (5th year agriculture). Molecular Biology Lab work for 3rd year Agriculture undergraduate students (64 h). Plant Histology lab work for 1st year Health & Nutrition undergraduates (8 h).
- **2016-2017:** Coordinator of Plant Histology (1st year Agriculture undergraduate students), Molecular Biology (3rd year agriculture undergraduate students) and Biotechnology and Plant Improvement modules (4th year Agriculture graduate students). Lectures (62 h), lectures in non-food valuations (4 h) (5th year Agriculture). Molecular Biology Lab work for 3rd year Agriculture undergraduate students (64 h). Plant Histology lab work for 1st year Health & Nutrition undergraduates (8 h).

Student supervision (Educational activities):

In addition of my teaching activities at UniLaSalle, I have always been involved in the training of our students through coaching, proposals of research topics, tutoring etc.

Thus, every year I am tutor to at least 2 UniLaSalle students during end-of-studies internships. The current academic year (2017-2018) is marked by the follow-up of 2 students in the Agriculture and Territory engineering cycle, 2 students in the Master 2 Plant Breeding cycle and an apprentice student who is working for one of our partners specialized in wheat breeding.

Every year, I participate at 3, 4 or 5 examination committees for end-of-studies reports (MFE) (Engineering and Master 2 Breeding cycles). Last year, in addition to my participation in MFE defenses, I participated in three dissertations of Master 2 Breeding, one as president.

I propose and supervise research work conducted by our 4th year students in Agriculture and Territory within the framework of projects for professional situation scenarios. In the past, I have led topics for 4th year students in connection with their activities "Center of interest (CI)".

***SUPERVISION OF
INTERNSHIPS IN RESEARCH
(YOUNG SCIENTISTS)***

Supervision of PhD students and Post-Doctoral fellows:

PhD students:

Year	PhD student Name	PhD Title
2016-2019	Nada SAKR: Co-supervision 50%. Supervisor: Thierry AUSSENAC (UNILASALLE)	Caractérisation des variétés de blés tendres libanais : approches agronomique, biochimique et technologique <i>“Characterization of Lebanese bread wheat varieties: agronomic, biochemical and technological approaches”</i>
2014-2018	Hiba TORKHANI: Co-supervisor INAT Tunisia, 50% Supervisor: Sleh REZGUI (INAT, TUNISIA)	Caractérisation technologique et moléculaire (QTL et sous unités gluténiques) de quelques cultivars de blé tendre <i>“Technological and molecular (QTL and glutenin subunits) characterization of some wheat cultivars”</i>
2013-2017	Romain ROULARD: Co-supervision 50%. Supervisor Emmanuel PETIT (UPJV)	Impact des conditions climatiques sur le contenu à valorisation non alimentaire de la graine de lin (<i>Linum usitatissimum</i>). <i>“Impact of environment factors on the non-food valuation of flaxseed (Linum usitatissimum)”</i> .
2013-2016	Perrine GOZE: Co-supervision 50%. Supervisor: Thierry AUSSENAC (UNILASALLE) Co-supervisor: André PAUSS (UTC)	Ozonation des grains de blé tendre : étude de l’oxydation des constituants biochimiques majeurs. <i>“Ozonation of bread wheat grains: study of the oxidation of major biochemical constituents.”</i>

2009-2014	Olfa BOUACHA DAALOU :	Contribution à la caractérisation biochimique, physico-chimique et technologique de quelques variétés tunisiennes de blé dur (<i>Triticum durum</i> L.) cultivées dans différents environnements.
	Co-supervision INAT Tunisia, 50%	
	Supervisor: Sleh REZGUI (INAT, TUNISIA)	<i>“Contribution to the biochemical, physicochemical and technological characterization of some Tunisian varieties of durum wheat (Triticum durum L.) grown in different environments”</i>
2006-2009	Madeleine NIANDU MPUTU:	Identification des enzymes intervenant dans le transfert des acides gras dans les végétaux.
	Co-supervision 15%	
	Supervisor: Brigitte THOMASSET (UTC)	Spécificité de ces enzymes vis-à-vis de la prise en charge d'acides gras inhabituels
	Co-supervisor: Eric GONTIER (UPJV)	<i>“Identification of enzymes involved in the transfer of fatty acids in plants. Specificity of these enzymes regarding the management of unusual fatty acids”</i>

Supervision of Post-doctoral fellows:

I had the opportunity in Unilasalle to supervise three postdoctoral fellows in two different areas. Also, I participated in technical supervision of several postdoctoral fellows working on nutritional and health field.

Unfortunately, we did not publish any results because Dr DONOT and Dr ALI-GHARS worked on applied projects. We are still waiting for the response of industrial partners to establish a patent. Dr DONOT had submitted an article on lipid fractions analysis. However, the article was accepted with revision. I am waiting for availabilities to resubmit it. Whereas, Dr ALI-GHARS did not finish the writing of his article on linseed and phytosterols.

However, I co-signed an article with Dr Mimoun-Benarroch postdoctoral fellow supervised by my colleague Dr Depeint.

Year	Post-doc Name	Subject
2012	Juan Rodriguez Gamir	Suivi de l'accumulation des protéines de blé.
	Supervisor 100%	<i>"Follow-up of accumulation of wheat proteins"</i>
2014-2015	Florentin DONOT	Fonctionnalité de voies métaboliques créées génétiquement dans la levure -
	Supervisor 100%	Identification des étapes limitantes. <i>"Functionality of metabolic pathways created genetically in yeast -</i> <i>Identification of limiting steps"</i>
2015-2016	Mohammed ALI-GHARS	Analyse des différentes espèces lipidiques dans les souches de
	Supervisor 100%	<i>Yarrowia lipolytica</i> modifiées par la CFA. <i>"Analysis of the different lipid species in the strains of Yarrowia lipolytica modified by the CFA."</i>

Supervision of Master 2 students:

Within the framework of the collaboration established since 2006 between UniLaSalle and INAT (Tunisia), I have supervised about fifteen internships from which the most recent are quoted below.

I also supervised about ten trainees within the framework of my various collaborative projects. By participating in research projects led by my colleagues, I co-supervised about twenty trainees with levels of study ranging from undergraduate to graduate.

Finally, I introduced several High school students to the practice of research through observation internships.

Year	Student Name	Subject
2018	Md Anisur RAHAMAN M.Sc. Pflanzenwissenschaft (Pflanzenzucht), Universität Hohenheim, Stuttgart, Germany	Heat stress tolerance: Molecular weight distribution of glutenin is genotype and environment dependent
2017	Nesrine BECHRAOUI Master 2 INAT Tunisia	Distribution moléculaire des polymères gluténiques du grain de blé : étude de stabilité. <i>“Molecular weight distribution of glutenin polymers in wheat grain: stability study”</i>
2017	Zeineb KADRI Master 2 INAT Tunisia	Effet des interactions génotypes × milieu sur la distribution granulaire et moléculaire de l’amidon du blé tendre. <i>“Effect of genotype × environment interactions on the granular and molecular distribution of bread wheat starch”</i>
2017	Chaima NEJI Master 2 INAT Tunisia	Indice de solubilité et distribution des fractions protéiques du grain de blé : Effet sur la note finale de panification. <i>“Solubility index and distribution of wheat grain protein fractions: Effect on final breadmaking quality”</i>

2017	Rabeb AOUMI	Etude spectral et métabolomique : interactions génotypes × milieu.
	Master 2	<i>“Spectral and metabolomic study: genotype × location interactions”</i>
	INAT Tunisia	
2016	Serigne Magueye KANE	Régulation métabolique et identification de gènes clés de la synthèse d’huiles inhabituelles dans les plantes oléagineuses pour une adaptation de systèmes végétaux ou de systèmes alternatifs à la bio raffinerie lipidique.
	Master 2	<i>“Metabolic regulation and identification of key genes for the synthesis of unusual oils in oleaginous plants for adaptation of plant systems or alternative systems to lipid bio-refinery”</i>
	Université de Picardie Jules Verne	
2016	Asma MAAROUFI	Etude de la variation des protéines de réserve de blé tendre en fonction de l’interaction génotype-environnement et leurs effet sur la qualité du blé.
	Master 2	<i>“Study of the variation of bread wheat reserve proteins according to the genotype-environment interaction and their effect on wheat quality”</i>
	INAT Tunisia	
2016	Marwa BENSALAH	Effet des interactions génotypes × milieu sur les propriétés techno-fonctionnelles de l’amidon du blé tendre et sa contribution dans la qualité.
	Master 2	<i>“Effect of genotype × location interactions on the technological properties of bread wheat starch and its contribution to quality”</i>
	INAT Tunisia	

RESEARCH PROJECTS

In this part entitled "Research Projects" I made the deliberate choice to present:

(A) As a first step, the international scientific context by including my own contributions (articles published and / or in the course of publication) over the past 15 years,

(B) In a second step, the scientific project of the research unit that houses me since 2016 (EA 7519) and which was recently evaluated by the HCERES during wave C,

(C) In a third and last time, the declination of my scientific implication within my new research unit.

(C) Perspectives

(A) International scientific context of my researches on glutenin polymer aggregates

A large part of this first part has been included as a chapter of a book entitled "Global Wheat Production" (ISBN 978-953-51-6255-1). This recently published chapter makes it possible to resituate my research on understanding the accumulation of glutenin polymer aggregates. All my work has been done in close collaboration with Dr Thierry AUSSENAC. Some essential results presented in this documents are not yet published and noted (Aussenac et al. unpublished data).

1. Introduction

During the last 60 years, in the field of cereal chemistry, the scientific community has been working to determine in an ever more precise way, the nature of the constituents responsible for the acquisition of technological properties (i.e. bread-making properties for common wheat doughs and/or pasta properties in the case of durum wheat). Emphasis has been placed on those whose (quantitative and/or qualitative) variations account for observed and measured changes in processing ability.

As early as the 1950s, thanks to very good recombination experiments with flour constituents, Finney [1] confirms that the baking capacity is essentially conferred by gluten. Gluten, which can be defined as a viscoelastic protein complex formed after hydration and mixing of a flour, consists of a heterogeneous mixture of prolamins (i.e. gliadins and glutenins) associated by covalent (S-S) and non-covalent bonds (hydrogen, hydrophobic, ionic). The specific role of certain protein fractions (monomer to polymeric proteins ratio) in the different properties of wheat doughs was also highlighted.

During the period 1970 - 1990, it became clear that the variation of the baking capacity of a flour is based on the ability of its reserve proteins or storage proteins (i.e. prolamins) to form, during mixing, a three-dimensional plastic structure (Figures 1A and 1B). This remarkable structure creates a cohesive and visco-elastic network, insoluble in water, ensuring the retention of carbon dioxide, ethanol and aromas, during the fermentation of the dough and unlike other cereals for which these properties are non-existent (Figure 2). Consequently, the reserve proteins found in common wheat flour have a strong tendency to aggregate into a three-dimensional viscoelastic network during mixing. Thus, gluten is considered as a transient

network whose mechanical properties depend on the density of the junction zones between the elements that compose it [2].

Since the 1990s, thanks to the integration of many complementary scientific approaches (i.e. molecular biology, biochemistry, analytical chemistry, rheology ...), a clear vision of the transformation processes and the role of the main protein constituents within them has begun to take shape [3]. Thus, attention has been focused on the (polymeric) glutenin fraction because a strong relationship has been established between bread making properties, such as mixing time, extensibility and loaf volume and the molecular weight distribution (MWD) of the polymeric protein components [4].

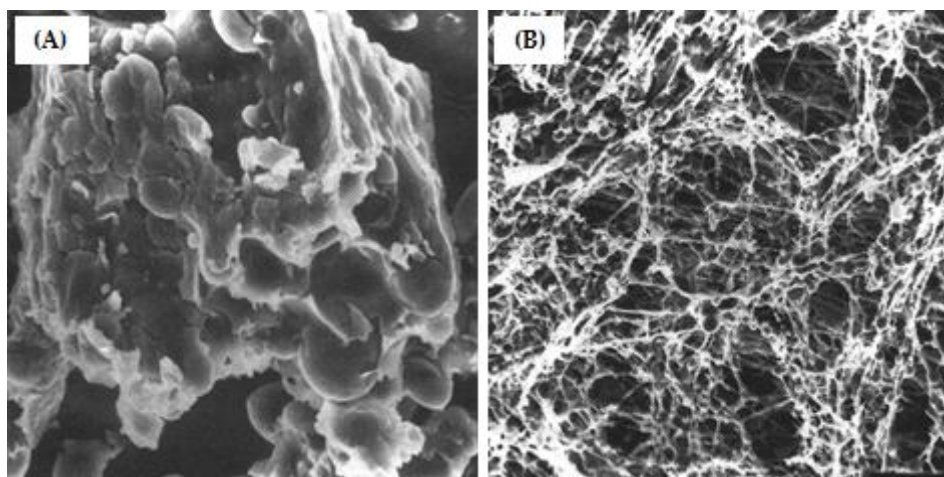


Figure 1. Scanning electron photomicrographs of (A) hard wheat flour particle and (B) a mixed dough that was lyophilized and then rehydrated. (From Hoseny and Rogers [3]).

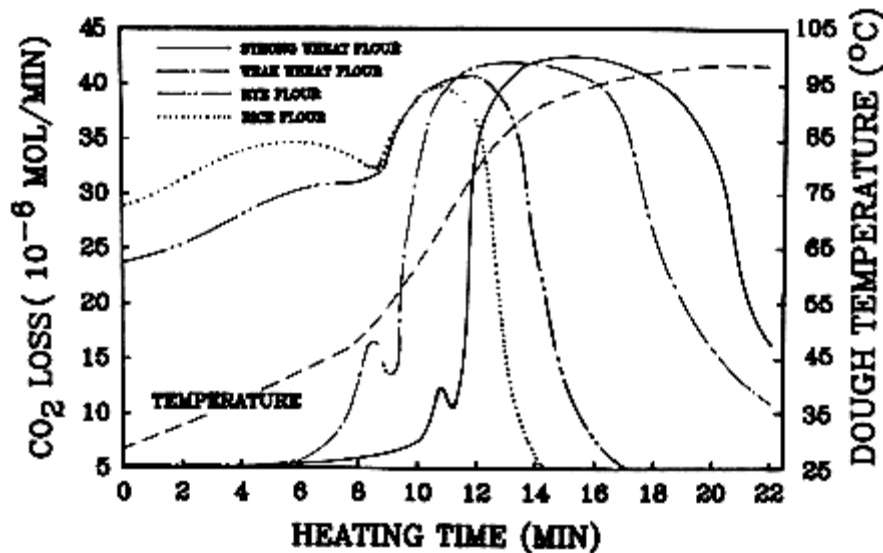


Figure 2. Carbon dioxide loss and temperature increase of dough made from different flours from several grains. (From Hosenev and Rogers [3]).

To the extent that any changes (genetically and/or environmentally controlled) in the molecular size and/or aggregation status of these polymeric proteins can potentially result in very significant changes in the technological properties of the products concerned, it is important to understand how they are synthesized and accumulated in grains of wheat during their development. This knowledge is essential if we are to manipulate wheat quality in the future for traditional or new end-uses.

This chapter reviews the definition of the molecular weight distribution of wheat storage proteins, their changes during grain development and the impacts of environmental factors.

2. Molecular Weight Distribution (MWD) of Wheat Storage Proteins

2.1 Classification and Polymorphism of Wheat Grain Proteins

Like all grain seeds, wheat grain contains a large number of proteins classified as structural proteins, functional proteins and reserve proteins. They are unequally distributed within the different tissues of the grain. A natural gradient of distribution can be highlighted. As a result, the starch to protein ratio significantly increases from the regions to the central regions of the grain. Given the relative weight of these different tissues, 70-80% (w/w) of the proteins are in the albumen.

The classification system for cereal proteins is mainly based on Osborne's historical work, in 1907 [5], based on their differences in solubility later used in sequential extractions

(Table 1). Thus, four major protein fractions have been defined: albumins (soluble in water), globulins (soluble in dilute salt solutions), gliadins (soluble in diluted alcohols, 70% ethanol) and finally, glutenins (residual proteins, partially soluble in diluted acids and bases). Other authors have enriched these classifications based on structural and/or functional properties [6, 7].

Table 1. Classification of Wheat Grain Proteins.

Osborne [5] classification			Shewry et al. [6] classification		Singh and Shepherd [7] classification	
Protein fraction	Solubility	MW (kDa)	Composition	Structure	Gene localization (*)	Function
Albumins and Globulins	Water Neutral salts	5 to 90				Structural and functional proteins
Gliadins				Monomers		
- ω			Poor in S		<i>Gli-1</i> (1A,1B,1D) S	
- α	Diluted alcohols	25 to 75			<i>Gli-2</i> (6A,6B,6D) S	
- β			Reach in S		<i>Gli-2</i> (6A,6B,6D) S	Storage proteins (prolamins)
- γ					<i>Gli-1</i> (1A,1B,1D) S	
Glutenins				Polymers		
- LMW	Acids, Bases, Reductants, Detergents	100 to several millions	Reach in S		<i>Glu-3</i> (1A,1B,1D) S	
- HMW					<i>Glu-1</i> (1A,1B,1D) L	

(*) *Allelic blocks*, Wheat homologous chromosomes (noted 1 to 6), Wheat genomes (A, B and D) and Chromosome position: (S) short arm; (L) long arm.

Within the large family of the storage proteins (prolamins), two main classes can be differentiated due to their degree of aggregation/polymerization. Thus, on the one hand, gliadins (soluble monomeric proteins in aqueous alcohols) which represent approximately 30 to 40% (w/w) of flour proteins and, on the other hand, glutenins representing 40 to 45% (w/w) of the total flour proteins. The latter are polymeric and aggregated proteins, forming a much more complex material than gliadins.

Gliadins correspond to a mixture of monomeric proteins of molecular weight between 25 and 75 kDa and are characterized by their richness in glutamine and proline. They represent 45% (w/w) of the total prolamins. There are four classes based on their electrophoretic behavior (i.e. increasing mobility in acid medium): α/β , γ and ω -gliadins (which respectively represent 44-60%, 30-46% and 6-20% of total gliadins) [8].

Glutenins, for their part, represent 40 to 50% (w/w) of total proteins; they are rich in proline and glutamic acid and their content in basic amino acids is higher than that of gliadins. They constitute a much more complex material formed of an assembly of polypeptide chains, commonly called subunits, linked together mainly by intermolecular disulfide bridges. These subunits have been grouped into two different subgroups: low molecular weight subunits (LMW-GS) and high molecular weight subunits (HMW-GS).

LMW-GS account for an average of two-thirds of total glutenins. They are very polymorphic and have molar masses of between 30 and 50 kDa. Given their similarity to some gliadins, these have sometimes been difficult to quantify. HMW-GS, as their name indicates, have higher molecular weights ranging from 70 to 130 kDa. According to their SDS-PAGE migration, they fall into two groups: HMW-GSy (67 to 74 kDa) and HMW-GSx (83 to 88 kDa).

Gliadins have a large genetic polymorphism, it has been possible to detect between 20 and 40 different constituents for a wheat variety [9]. Within a class of gliadin, it is possible to find several sub-families depending on the composition and richness of certain amino acids (the ω 1 and ω 5 gliadins differ in basic amino acids, glutamine and proline, than that γ 1, γ 2 and γ 3 differ in their richness in tyrosine, lysine and methionine...). Thus, the polymorphism of gliadins is so important that it serves as a basis for the varietal identification of wheat [10].

The polymorphism of low molecular weight glutenin subunits (LMW-GS) is less important than that of gliadins. For a given variety, there are 7 to 16 LMW-GS. But 40 different LMW-GS were found in 222 varieties of bread-wheat [11]. Finally, high molecular weight glutenin subunits (HMW-GS) are the prolamins that have the lowest polymorphism. The association of two genes at each Glu-A1, Glu-B1 and Glu-D1 locus was noted. The x-type genes express subunits of masses greater than those encoded by y-type genes in SDS-PAGE [12]. However, some y-type HMW-GS (notably subunit 12) have been shown to have important immunochemical similarities with α/β and γ -gliadins [13]. In all cases, recombinations between these genes are very rare. The different wheat varieties contain between 3 and 5 HMW-GS. Indeed, when they were listed by Payne et al., 1983 [14] 1Ay gene was not found today this gene is very rarely expressed in wheat cultivars, and 1By and 1Ax genes are only expressed in some varieties [14].

The primary structure of the storage proteins is well understood. They comprise three distinct domains (Figure 3): a central domain made up repeated sequences and two domains

formed of non-repeated sequences at the ends (i.e. C- and N-terminal). The understanding of these sequences has made it possible to locate particularly important cysteine residues because of their ability to form disulfide bonds (intra and/or intermolecular). α -, β - et γ -gliadins are provided with cysteines at their C-terminal domains; these all being involved in the formation of intramolecular disulfide bridges. HMW-GS have unpaired cysteine in their C-terminal domain and several others in their N-terminal domain; LMW-GS carrying 7 C-terminal cysteines and one N-terminal cysteine. Thanks to these unpaired cysteines, unlike gliadins, HMW-GS and LMW-GS can form intermolecular disulfide bridges. Some authors report a globular type structure for the N- and C-terminal and a spiral structure for the repetitive domain (Figure 3).

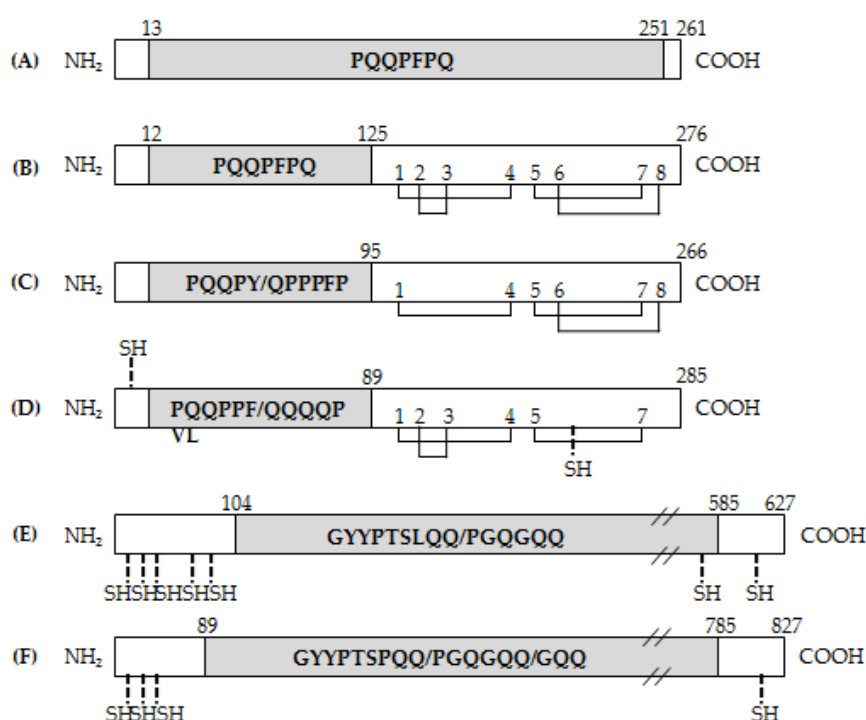


Figure 3. Schematic structures of typical primary structures of (A) ω -gliadin, (B) α -gliadin, (C) γ -gliadin, (D) LMW-GS, (E) HMW-GS γ and, (F) HMW-GS x [15, 16]. Repetitive sequences are shaded and disulfide bonds between conserved cysteine residues (1–8) in the γ -gliadin are shown as lines. SH denotes the positions of cysteine residues in the HMW prolamins. Single letter abbreviations for amino acids: F = phenylalanine; G = glycine; L = leucine; P = proline; Q = glutamine; S = serine; V = valine; Y = tyrosine.

2.2 Gliadin to Glutenin Ratio

Generally, it is accepted that the functional properties of gluten proteins are related to their ability to form a network during technological processes [17, 18]. However, gliadins and glutenins do have not the same effect on the rheological properties of doughs or glutes. Consequently, gliadins explain the viscous nature, while glutenins determine elasticity. In fact,

small quantity of cysteine residues in these storage proteins make it possible to establish an important structural and functional distribution between gliadins and glutenins (Figure 4).

For the former, all cysteine residues are involved in the establishment of intramolecular disulfide bridges while for both high and low molecular weight glutenins, a number of cysteines not involved in intramolecular bonds are therefore available to establish intermolecular links with other subunits. Glutenins are therefore likely to constitute polymers with a real consistency, thanks to the formation of intermolecular disulfide bridges, while gliadins remain in the monomeric state. The latter may, however, be aggregated by weak bonds (hydrogen, hydrophobic). The viscoelasticity of gluten depends on its state of polymerization and the interactions between polymers [2].

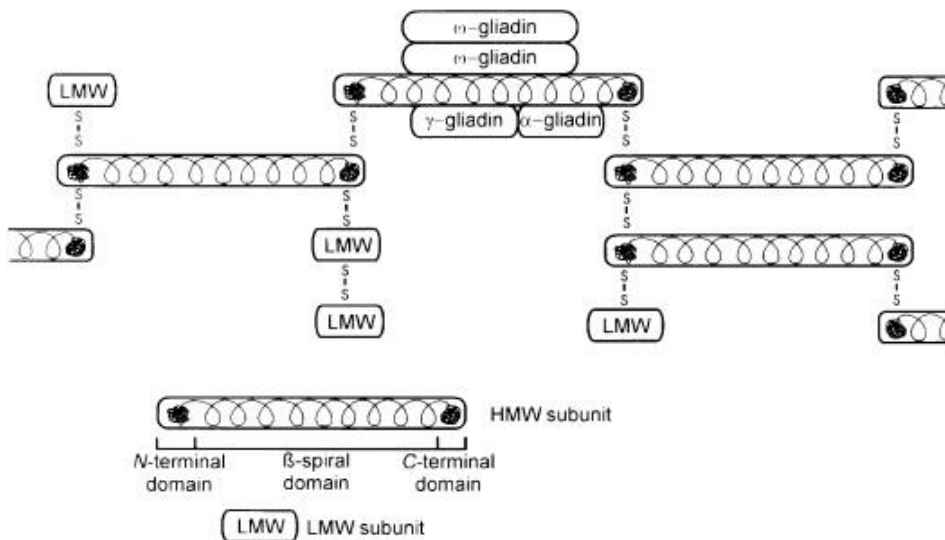


Figure 4. A structural model for wheat gluten in which the HMW subunits provide a disulphide-bonded backbone which interacts with other gluten proteins through disulfide bonds (LMW subunits) and non-covalent interactions (gliadins). (From Shewry et al. [19]).

A large number of conventional fractionation and reconstitution tests have been conducted based on the differential physical properties observed in purified gliadins and glutenins. The aim of these studies was to link variations in molecular weight distribution (i.e. monomer to polymer ratio) with the rheological characteristics of the glutes obtained. In the majority of cases, the results obtained during these different reconstitution studies have demonstrated that the rheological properties of the restructured flours generated are strongly influenced by the ratio of these two protein fractions [20, 21]. With a constant amount of prolamins, the strength of the reconstituted flour, measured by the time of development of the

dough with a mixograph [i.e. Peak Time Value Mix (MPT)], is related to the proportion of polymeric proteins.

The development of original analytical approaches [High Performance Liquid Chromatography of Size Exclusion (SEC-HPLC) for example] during the 1980s confirmed the vast majority of these hypothesis, which were essentially based on results obtained from differential solubility protocols (i.e. gliadins vs. glutenins). Thus, many authors [22-29] have confirmed the existence of a significant relationship between the relative amount of glutenin aggregates and the baking quality of many common wheat genotypes.

The molecular weight distribution (MWD) of prolamins is becoming recognized as the main determinant of physical dough properties [30, 31]. However, in theory, the MWD can be altered from one sample of wheat (or one cultivar) to another by changes in the relative proportions of monomeric proteins and polymeric proteins (gliadins to glutenins ratio) but also by changes in the size distribution of polymeric proteins [32].

2.3 Size Distribution of Polymeric Proteins

Chen and Bushuk [33] revealed that part of the glutenin is soluble in acetic acid, thus making the distinction between an insoluble and a soluble fraction. The importance of this distinction became clear when Orth and Bushuk [34] demonstrated a positive correlation between the amount of acetic acid insoluble glutenin and bread loaf volume. From then on insoluble glutenin became widely recognized as the key protein fraction that can explain differences in dough strength and bread-making quality [35]. The use of detergents (SDS) and organic solvents (propanol) [36] allowed an even better separation and led to the conclusion that insolubility was due to size and a very high degree of polymerization. Other groups developed methodology with propanol to further separate soluble proteins parts from the insoluble glutenin. Currently, two main methods are in use to quantify and characterize this fraction. The first correspond to the so-called UPP method using propanol and during which Unextractable Polymeric Protein (UPP) fraction is obtained. Upon sonication, this fraction becomes soluble in SDS [28, 29] and can be analyzed using Size Exclusion Chromatography [27, 37]. The other method is the SDS method as advanced by Graveland et al. [38] resulting in the SDS insoluble gel protein fraction. This fraction was renamed Glutenin Macro Polymer (GMP) to reflect its highly aggregated nature [39, 40]. Moonen et al. [41] found that the SDS insoluble glutenin-gel protein fraction highly correlated with SDS sedimentation values and

loaf volume. Weegels et al. [40, 42] studied this fraction in great detail and presented firm evidence that GMP quantity correlates to bread loaf volume.

Most authors seem to think that wheat cultivars may be characterized by a variable amount of unextractable polymers which control the rheological properties of dough. However, when we used different extracting systems containing anionic surfactants to dissolve all storage proteins without disrupting any covalent bonds, we showed that all flour protein (> 97%) were solubilized when a sufficient extraction time was used (**unpublished data**). Therefore, the difference in protein solubility between flours could be considered as difference in the rate of glutenin solubilization due to hydrophobic interaction disruption. Thus, this polymer disaggregation could be related to bread-making performances of the wheat cultivar. Our observation were consistent with the hypothesis that dough strength of wheat cultivars depends significantly on the non-covalent aggregative forces of wheat protein polymers.

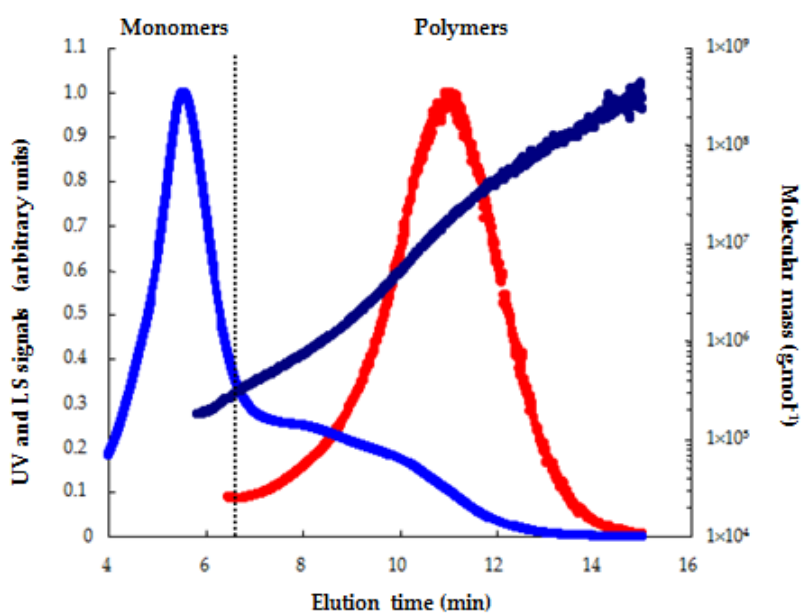


Figure 5. Asymmetrical flow field-flow fractionation (A4F) profiles of total solubilized storage proteins of a common French wheat cultivar (Soissons). UV (blue line), light scattering at 90° (red line), and molecular weight as a function of elution time (dark line). (**O17, P12, Aussenac et al. unpublished data**).

In addition to these classical approaches (UPP and/or GMP), new analytical protocols have been developed since the early 2000s to separate and more accurately characterize the molecular size distribution of the polymeric proteins. Field flow fractionation (FFFF) [43-46], which is a new separation technique without any stationary phase and which is therefore not

hampered by a steric exclusion limit [47-49] has been used successfully to separate a number of HMW fractions [A23, A30, P10, P12, 50-52]. Furthermore, the MALLS technique which is one of the most effective means of determining molecular weight, size, and conformation of glutenin polymers without reference to standards [A29, A30, 48, 53-56] has been applied in combination with the A-FFFF method to accurately measure size and conformation of wheat glutenins [57] (Figure 5).

	Dx5 >	Dx2 >	Dy10 =	Dy12 >	Bx7 >	SG-FPM
Number of intramolecular bonds	5	4	3	3	2	2
Amino acids in the repetitive domain	690	680	481	492	647	200-150
Type of polymer	Branched	Branched	Branched	Branched	Linear	Linear




Figure 6. Hierarchical arrangement of SG-HPM and SG-FPM in relation to their intrinsic contribution to polymer formation (From Kasarda [65]).

The glutenin association level (i.e. the size distribution) is strongly correlated with the HMW-GS / LMW-GS [58-60] ratio and the nature of the HMW-GS present (especially HMW-GS pair 5+10 vs. HMW-GS pair 2+12 coded by Glu-D1). As demonstrated by the different experimental approaches carried out in recent years [61-64], the different glutenin subunits (i.e. HMW-GS, LMW-GS, and HMW-GS x and y type) are unequally distributed within polymers. These results demonstrate the existence of a highly ordered structures in which some subunits play a predominant role, notably because of their difference in functionality (i.e. number and especially position of cysteine residues capable of forming intermolecular bonds) [65] (Figure 6). Using fold-recognition techniques (A31), we identified a fold compatible with the N-terminal domain of HMW-GS Dy10. Then, three models for the N-terminal domain of HMW-GS Dy10 were built and analyzed. We were able to discuss two possible ways of interaction between the N-terminal domains of the y-type HMW glutenin subunits. The first way consists in the creation of interchain disulfide bridges. According to our models, we propose two plausible scenarios:

- (1) The first scenario suggests that only Cys 2 and Cys 3 can create an intrachain disulfide bridge, leaving three cysteines free from making interchain bridges. This scenario is not compatible with data obtained by Köhler et al. (1991), because the intrachain bond involves

two cysteines that are found by them to be engaged in interchain disulfide bridges. Let us note that the involvement of Cys 2 in an interchain bond with Cys 5 of an x-type HMW-GS is only one of three, equally likely, possibilities. the existence of an intrachain disulfide bridge between cysteines 22 and 44, leaving the three other cysteines free of engaging in intermolecular bonds,

- (2) The second scenario we proposed above, namely, that Cys 1–Cys 5 and Cys 2–Cys 3 form intrachain disulfide bonds, leaving Cys 4 free to create an interchain disulfide bridge, is fully compatible with the proposal of Kasarda. However, this scenario is built on the assumption that Cys1 and Cys 5 are able to create an intrachain disulfide bond. ***From the data obtained during the simulations this is possible but not yet proven.***

The second way, although less likely, is nevertheless worth considering. There might exist a possibility for the N-terminal domain of Dy10, Nt-Dy10, to create oligomers, because homologous cereal inhibitor proteins are known to exist as monomers, homodimers, and heterooligomers. As a result of this work, we cloned the truncated gene of the subunit 10 into a bacterial system, the SDS-PAGE data demonstrated that the N-terminal part is able to form a homodimer, homotrimer and homotetramer (unpublished data).

3. Accumulation of Prolamins in Developing Wheat Grains

3.1 Endosperm development

The development of the wheat endosperm which has been well described at the microscopic level, as reviewed by Bechtel et al. [66], can be quite easily characterized by the study of the temporal variations of several quantitative components of it such as the accumulation of the total dry matter, the water content of the grain and the accumulation of total protein and starch [67] (Figure 7).

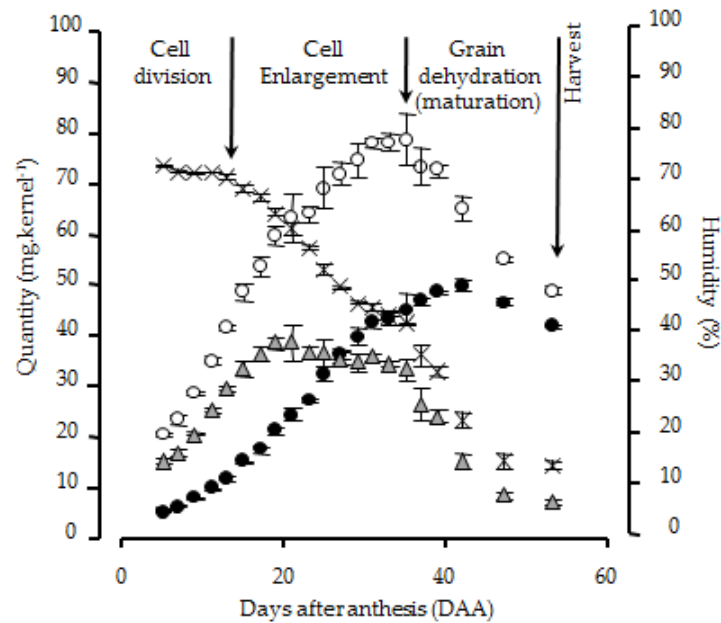


Figure 7. Grain filling period for a common wheat cultivar (*Soissons*). Evolution of (●) dry matter per kernel, (○) fresh matter per kernel, (▲) water quantity per kernel and (X) grain humidity. The vertical bars represent the standard deviation ($n=3$). (From Carceller and Aussenac [67]).

The accumulation of total dry matter in the grain provides a good insight into the functioning of different accumulation metabolisms (i.e. nitrogen translocation and post-flowering photosynthesis) [68]. Thus, after an initial lag phase (up to 10-15 DAA), it is easy to observe a phase of linear accumulation of this dry matter; wheat grains reaching a maximum dry weight from 40 DAA.

During this linear phase, the observed phenomena depend on two main variables: the duration (D) and the speed or flux of assimilates towards the grain (V), so that the weight of a grain (P) is given by the relation $P = V \times D$ [69]. D can be expressed in days or in the sum of average daily temperatures [i.e. degree-days (DD)]. The filling speed is the limiting factor in the development of the weight of a grain. This speed is mainly by the number of grains per m^2 . Finally, under natural conditions, the duration D cannot compensate for the weight loss produced by any reduction in the rate of accumulation. The amount of water per grain that gradually increases to about 20 DAA remains relatively constant up to ≈ 35 DAA (i.e. “water plateau” phase) before decreasing at harvest time.

The higher the rate of water accumulation in the grain, the greater the height of the “water plateau”, and the higher the weight of the grain at maturity [70]. Based on changes in the amount of water and total dry matter per grain after anthesis, three particular phases of grain development can be estimated: the cell division phase, the cell enlargement phase (i.e. grain

filling phase) and, the grain desiccation phase (the beginning of this phase corresponding to the acquisition of physiological maturity) [71] during which protein bodies disappear to form the protein matrix [72, 73].

Since the 1970s, a great deal of work has been done to evaluate the effects of the environment on grain development. Thus, the effects of several environmental variables (i.e. light, temperature, water availability, nutrient availability), taken individually or in combination, have been studied [74-85]. In general, temperature and water availability strongly affect the filling rate (V) and the duration of grain filling (D), although some differences in behavior may exist between wheat genotypes. Consequently, differences in thermal regimes and/or water regimes cause profound changes in the accumulation of the total dry matter (P) by affecting indifferently and without compensation the speed and duration of filling [86].

3.2 Accumulation of Storage Proteins

The accumulation of different protein fractions (albumins-globulins, gliadins, glutenins) is progressive from flowering until the acquisition of the physiological maturity of the grains (\approx 35-40 DAA). However, even if the time of initiation of the biosynthesis of the different proteins of the grain is not significantly different (5-7 DAA) [87], their rate of accumulation varies considerably, suggesting a phenomenon of differential regulation of this biosynthesis (Figures 8A and 8B).

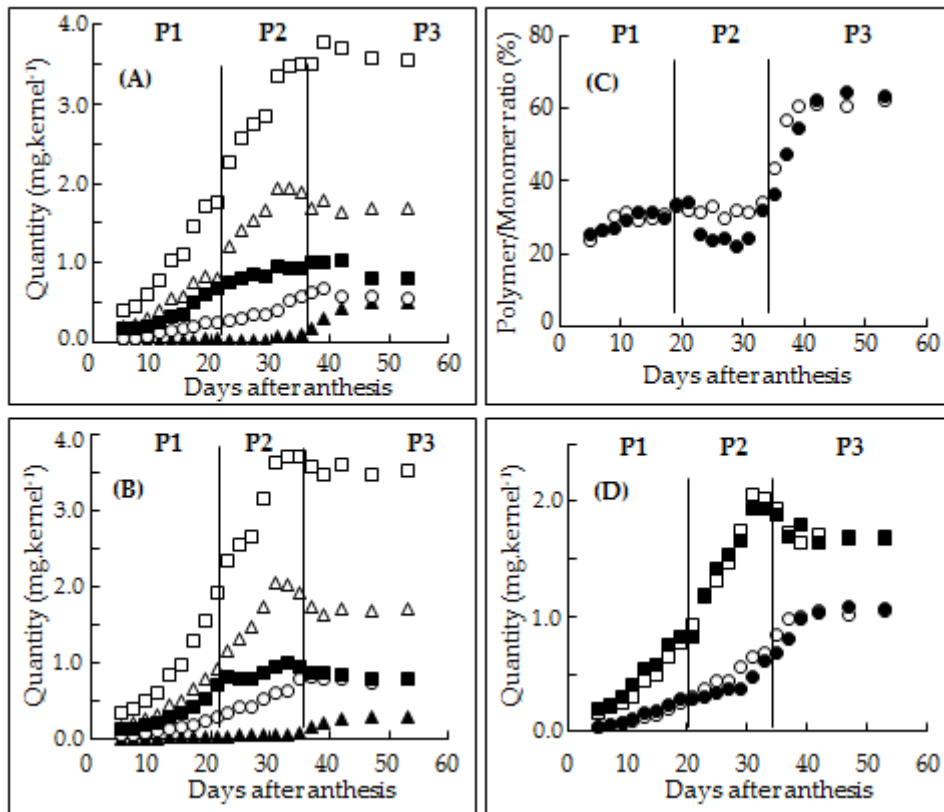


Figure 8. Evolution of the quantity of the different protein fractions (mg.kernel^{-1}) for two common French wheat cultivars (A) Soissons and (B) Thésée, as a function of the days after anthesis. (\blacktriangle) SDS-insoluble polymers; (\circ) SDS-soluble polymers; (\blacksquare) albumins and globulins; (\triangle) monomers and; (\square) total proteins. Evolution of (C) the polymer/monomer ratio (%) and (D) the quantity of monomers and total polymers (mg.kernel^{-1}) as a function of days after anthesis. (\circ, \bullet) total polymers of Thésée and Soissons respectively; (\square, \blacksquare) Monomers of Thésée and Soissons respectively. Stages: (P1) cell division; (P2) cell enlargement and; (P3) grain dessication and maturation. (From Carceller and Aussenac, Rhazi et al. [67, 103]).

Thus, a certain accumulation asynchrony in the protein fractions of the grain can be highlighted. The albumins-globulins accumulate most rapidly in the grain, followed by the monomeric prolamins and finally the polymeric prolamins. As many researchers have shown [88-93], the accumulation of albumins-globulins is maintained only during the cell division phase, contrary to that of prolamins. This confirms the functional and/or structural role of these specific proteins.

While the ratio between polymeric proteins and monomeric proteins is stable during the first stages of grain development (i.e. cell division and cell enlargement), this ratio increases significantly during the grain dessication phase (i.e. after 35 DAA) (Figure 8C). A number of results in the literature are quite contradictory [90, 94, 95]. *In our opinion, and in accordance with the remarks of Stone and Nicolas [92], most of these differences can be explained by the*

fact that the methods of extraction and analysis of the polymeric proteins retained are extremely varied from one research group to another; it is therefore certain that all the researchers did not take into account the same protein entities in the calculation of the polymers/monomers ratio.

The accumulation of SDS-soluble polymers that starts very early in the grain (from 7 DAA), is very slow and continues up to the beginning of the drying phase of the grain. The accumulation of SDS-insoluble polymers (i.e. UPP) is, in turn, really visible only when the grain begins to lose its water balance (i.e. end of the "water plateau") [67, 92, 96] (Figure 9B).

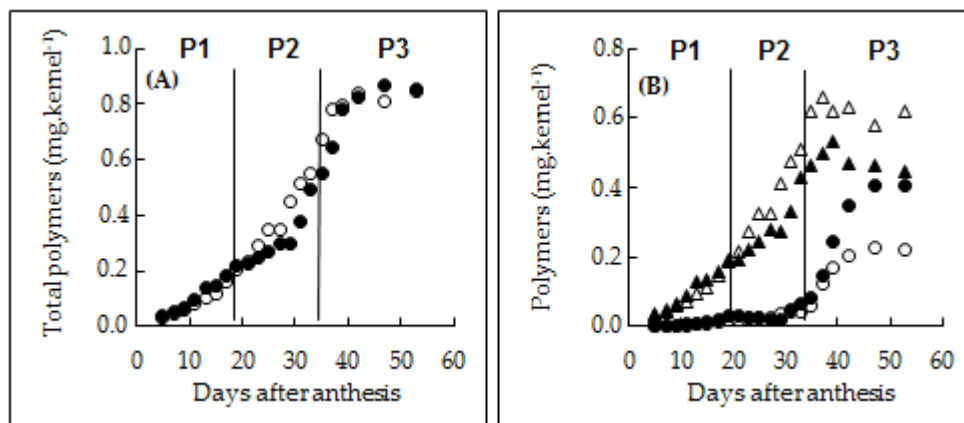


Figure 9. Accumulation of total polymers, SDS-soluble and SDS-insoluble polymers as a function of the days after anthesis. (A) Total polymers (mg.kernel^{-1}) for (●) Soissons and, (○) Thésée. (B) Open symbols are for Thésée and closed symbols are for Soissons. (Δ) SDS-soluble polymers and, (○) SDS-insoluble polymers. Stages: (P1) cell division; (P2) cell enlargement and; (P3) grain desiccation and maturation (From Carceller and Aussenac; Rhazi et al. [67, 103]).

These elements must be compared with the observations of researchers such as Woodman and Engledow who, as early as the 1920s, noted the increase in the ability of proteins to form a coherent mass, gluten, in relation to the beginning of the grain desiccation [97]. The accumulation of the protein polymers in the broad sense coincides perfectly with the accumulation of the different glutenin subunits (LMW-GS and HMW-GS) in the grain [91, 98]; the HMW-GS/LMW-GS ratio being an important parameter for differentiating wheat genotypes from each other. For example, in the framework of our own research [67, 99, 103], we have been able to demonstrate that at harvest time, the association state of polymeric proteins (i.e. SDS-insoluble polymers/total polymers ratio) is strongly correlated with the HMW-GS/LMW-GS ratio. Thus, at maturity, with the same total polymer amount (Figure 9A), the wheat genotype Soissons, which is characterized by a HMW-GS/LMW-GS ratio twice that

of the wheat genotype Thésée, has a SDS-insoluble polymers/total polymers ratio twice as large that of Thésée (Figure 9B).

3.3 Unextractable Polymeric Protein (UPP) accumulation

The formation and accumulation of polymeric protein fractions characterized by high levels of aggregation [indifferently qualified in the literature of SDS-insoluble polymeric proteins, Unextractable Polymeric Proteins (UPP) or Glutenin Macro Polymers (GMP)], have been the focus of attention during the last 15 years because these fractions became widely recognized as the key protein fraction that can explain differences in dough strength and breadmaking quality.

According to the various physiological observations carried out since the early 2000s [67, 93, 100-102], it appears that the UPP accumulation phase coincides very strongly with the grain desiccation phase (Figure 9B), whatever the culture conditions applied (i.e. light, temperature, water availability, nutrient availability). Thus, 95-100% (w/w) of the UPPs present in the grain at harvesting accumulate during the grain desiccation phase. Finally, several experiments of artificial dehydration of wheat grains have confirmed the strong relationship between grain water loss and UPP accumulation [93, 102].

Although today the mechanisms responsible for the formation of UPPs are still the subject of discussions and/or hypotheses, many observations seem to confirm that the strengthening of the aggregation character in these polymeric proteins during grain desiccation results from the reinforcement of intermolecular interactions (mainly covalent interactions) between the different glutenin subunits (HMW-GS and LMW-GS) [103, 104]. This phenomenon has led to a very significant increase in the different molecular dimensions (Mw and Rg) of the glutenin polymers [103].

Studying the function of free glutenin sulfhydryl (SH) and disulfide (SS) groups in glutenins of developing wheat for UPP formation, we showed that the major wheat glutenin subunits residing in the protein bodies undergo redox change during the development and the maturation of the grain [103] (Figure 10). Indeed, during the cell division and grain filling, glutenin subunits and particularly LMW-GS have a large amount of free SH groups and become oxidized during grain desiccation which coincided with the accumulation of UPP. Moreover, monobromobimane (mBBr) derivatization of free glutenin SH groups before the artificial grain desiccation totally inhibits the UPP deposition [104].

In our hypothesis which is very close to the model proposed by Hamer and van Vliet [105] for the gluten structure termed “hyper aggregation” model, the grain desiccation promotes the aggregation of polymers already present (i.e. SDS-soluble polymers or level I aggregates in the “hyper aggregation” model) by facilitating specifically the formation of interchain hydrogen bonding between the repeat regions of glutenin subunits [106-108] which can bring glutenin free accessible SH groups into close proximity to form additional intermolecular disulfide de bridges.

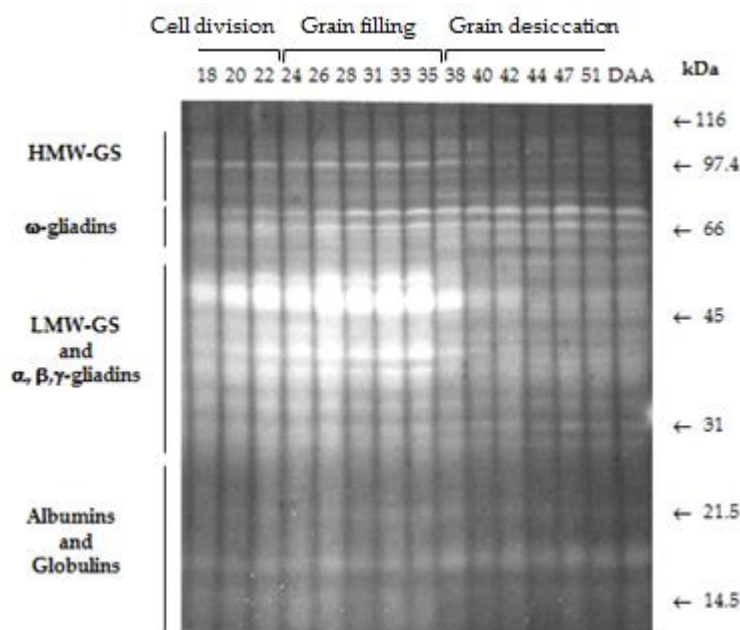


Figure 10. Change in sulfhydryl status of wheat proteins during grain development and maturation. M-BBr-derivatized (fluorescence photography) storage proteins of a common French wheat cultivar (Soissons). Days after anthesis (DAA). (From Rhazi et al. [103]).

Changes in sulfhydryl status of wheat glutenins is a result of changes in protein structure allowing non-covalent interactions which accentuates protein structure modifications (A6). Also, the formation of disulfide bonds in turn causes structural modifications of the glutenins. These all changes lead to the formation of an aggregative structure.

The importance of each kind of interaction have to be investigated more precisely. A physiological and quantitative follow-up of these interactions would make it possible to determine the sequence of these different events. Others covalent bonds could be important such as dityrosine bonds studied in our laboratory. The molecular, nano, micro and macrostructures will be undertaken to get a consensus on the structure of glutenin ‘polymers and aggregates’.

Since, the primary sequence of glutenin subunits are rich in tyrosine residues, and these amino acids are thought to be involved in the formation of covalent bonds, putatively involving tyrosyl residues in unions catalyzed by peroxidases, it would be interesting to study the variation of dityrosine content in the wheat grain during the formation of glutenin polymers. We will be able to determine precisely the role of tyrosine residue in the establishment of the structure of wheat protein network.

To understand the glutenin network conformation and the role of intra-molecular electrostatic repulsion and the molecular aggregation force, such as noncovalent (hydrophobic interactions) in the native aggregation state of glutenin, changes in protein conformation in vivo and vitro were investigated. Variation will be studied using several techniques such as physicochemical properties, SDS-PAGE, LabOnChip, A4F, molecular force change, intrinsic fluorescence emission spectroscopy (IFES) and FTIR. In order to carry out these studies, we intend to establish collaboration with teams that are specialized in physical studies. In addition, we plan to improve some of our techniques that we have used in the earlier past such as FTIR.

4. Impacts of Environmental Factors on MWD of Prolamins

The multiple agronomic studies which were done during the last 25 years, indicate that environmental conditions affect the amount, composition and polymerization of the gluten proteins [109-119]. Furthermore, we found that the impact of environmental components on the molecular weight distribution of the prolamins is significantly greater than that of genetic components (i.e. $\sigma_{2E}/\sigma_{2R} > \sigma_{2G}/\sigma_{2R}$) (Table 2) (**O17, P12, Aussenac et al. unpublished data**). This is complementary to literature results [57, 120, 121]. This is why, in a context of profound environmental changes [122], *it is very important to better understand the mechanisms responsible for these effects in order to better anticipate them.*

The availability of nutrients (nitrogen and/or sulfur availability) and the temperature (thermal regime) are the two main environmental factors responsible for these protein changes.

High nitrogen availability translates into high protein contents in the grain and flour but also by changes in protein composition. With increasing protein content, gliadins tend to increase at a greater rate than other proteins. This can lead to MWD alterations which results from decreases in the polymeric-to-monomeric protein ratio and/or increases in the HMW-GS to LMW-GS ratio [113, 123, 124].

Table 2. Genetic (G) and Environmental (E) Influence on Molecular Weight Distribution of Storage Proteins Determined by Analysis of Variance (*F*-test) for 130 French common wheat genotypes cultivated in three different locations for 2005 and 2006. (O17, P12, Aussenac et al. unpublished data).

Parameters	Maximum value	Minimum value	Mean value	σ^2_G/σ^2_R	σ^2_E/σ^2_R
Total proteins ⁽¹⁾	8.700	15.100	11.187	11.411***	271.577***
Total polymers ⁽¹⁾	2.785	5.755	4.016	11.104***	187.462***
Polymer/monomer	0.321	0.700	0.561	14.845***	72.611***
Polymer M_n ⁽²⁾	0.730 x10 ⁶	9.609 x10 ⁶	0.972 x10 ⁶	1.068 ^{NS}	4.383***
Polymer M_w ⁽²⁾	1.142 x10 ⁶	22.970 x10 ⁶	7.640 x10 ⁶	3.370***	38.974***

⁽¹⁾Quantity in mg/100g DM. ⁽²⁾ M_n = Molecular weight number-average and, M_w = Molecular weight-average (g.mol⁻¹). NS: Not significant; (***) indicates *F*-test significance at 0.1% level of probability; σ^2_G/σ^2_R = Genetic variance/Residual variance ratio and, σ^2_E/σ^2_R = Environmental variance/Residual variance ratio.

When sulfur fertilization is limited, the molecular distribution of glutenins is strongly affected insofar as this limitation results in a significant modification of the HMW-GS/LMW-GS ratio [109, 125]. The increase in the HMW-GS/LMW-GS ratio which is linked to the fact that the high molecular weight glutenin subunits are much less affected by a sulfur limitation because they are poorer in corresponding amino acids, therefore results in an increase in the average molecular weight of the polymers. Finally, sulfur deficiency is accentuated by higher nitrogen levels [126]. ***Thus, using such experiences we can study and determine more accurately the effect of the HMW-GS/LMW-GS ratio on the molecular weight distribution of glutenin aggregate.***

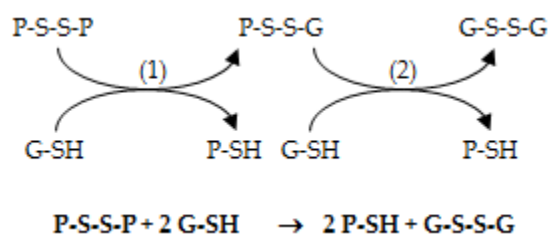
Temperature (i.e. daily mean temperature, temperature regime, temperature application stage) can induce very large changes in the association state of polymers during grain filling [110, 127-129]. Thus, in the great majority of the work carried out in recent years, various researchers have shown that the increase in temperature and/or the sudden change in the thermal regime during grain filling could lead to a significant decrease in the association status of prolamins resulting in a decrease of MWD (or solubility) of glutenins [79, 110, 130].

In the majority of the work to which we have just referred to above, the effects observed are most often attributed to modifications in the synthesis activities of the different reserve proteins (i.e. gliadins vs. glutenins and/or HMW-GS vs. LMW-GS) resulting from modulation of the expression of storage protein genes [85]. ***Today, it seems that other phenomena could also be reasonably involved.*** These phenomena could be based in particular on important

variations in the cellular redox status in response to environmental stimuli (i.e. environmental stress).

It has long been established that desiccation of plant tissues causes the appearance of free radicals. Although this phenomenon is a very general mechanism, a large number of observations have been made from seeds of various species [131-134]. In the majority of these studies the presence of free radicals has been correlated with viability losses [135]. Among these implemented detoxification mechanisms, the ascorbate/glutathione cycle (i.e. trapping of H₂O₂ generated) is one of the most efficient. This essential cycle in chlorophyll tissues [136, 137] has also been studied in seeds [138, 139].

At a cellular level, thiols are the first compounds affected by oxidative stress in general because of the high sensitivity to the oxidation of sulfhydryl (SH) groups. The predominant non-protein thiol in most plant species is glutathione (GSH). This tripeptide ensures the maintenance of the redox status at a cellular level but also the storage and transport of the reduced sulfur necessary for the synthesis of proteins [140-142]. The first compound resulting from the oxidation of glutathione is its dimer (GSSG) which is produced *in vivo* largely thanks to SH/SS exchanges with proteins (noted P) [143]. The reactions below illustrate these exchanges.



The GSSG dimer is normally reduced in GSH by glutathione reductase (GR) activity. Thus, under normal conditions, glutathione is very much present at a cellular level in its reduced form (i.e. high GSH/GSSH ratio) which has the effect both of maintaining the SH status of proteins (to maintain enzymatic activities [144]) and continue to trap H₂O₂.

Under the influence of oxidative stress, the redox status of glutathione will be modified; GSSG dimer will accumulate due to either an increase in GSH oxidation and/or a decrease in GSSG reduction activity (i.e. decrease of GSH/GSSH ratio). Such changes in the SH/SS status have already been widely observed in response to oxidative stress, especially during seed desiccation [145, 146]. Glutathione which is able to bind to protein thiols is considered as a "protective" element of these protein compounds since it prevents the formation of

intramolecular disulfide (S-S) bridges during the desiccation phenomena [147]. In this way, GSH contributes both to limit the protein denaturation phenomena and to modulate enzymatic activity [148]. In contrast to desiccation, the imbibition phenomenon preceding germination causes the reduction of the disulfide bonds (SS) of a large number of compounds such as GSSG [149, 150], protein-SSG conjugates [151], α -amylases [152], or the reserve proteins [153, 154]. A synthesis of the presumed role of glutathione can be postulated, referring in particular to the hypothesis formulated by Kranner and Grill [148] (Figure 11).

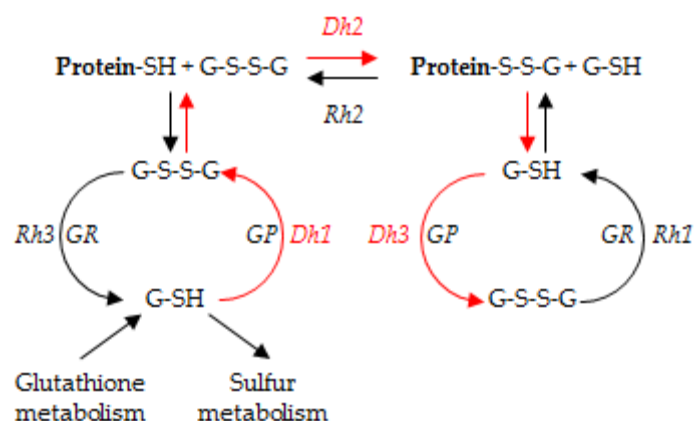


Figure 11. SH/SS interchange during dehydration/rehydration phenomenon. (Dh) Dehydration step, (Rh) Rehydration step, (GP) Glutathione Peroxidase, (GR) Glutathione Reductase. (From Kranner and Grill [148]).

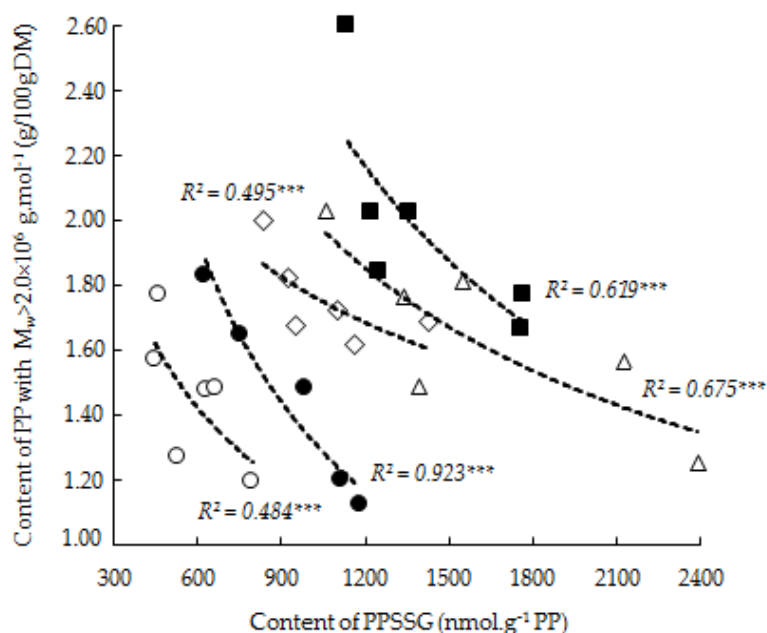


Figure 12. Relationship between the content of high aggregated polymeric proteins (PP with $M_w > 2.0 \times 10^6$ g.mol⁻¹) and the content of polymeric proteins conjugated to glutathione (PPSSG) for five different common French wheat cultivars (harvest 2005 and 2006 in three locations). (O17, P12, Aussenac et al. unpublished data).

Glutathione may occur endogenously in wheat flour in the free reduced glutathione (GSH) and free oxidized glutathione disulphide (GSSG) forms as well as in the form of protein-glutathione mixed disulphides (PSSG) [100, 108, 143-157]. Moreover, approximately 85% of PSSG in mature wheat grains are represented by polymeric proteins (PP) conjugated to glutathione (PPSSG) [156]. Even if glutathione is able to bind to the storage proteins during grain filling, the formation of PSSG is not however correlated with the accumulation of the storage proteins in the grain but coincide rather with the grain desiccation during which the major wheat storage proteins residing in protein bodies undergo redox change (i.e., become oxidized) and UPP are accumulated [100, 103, 156].

In the framework of FSOV projects, we demonstrated that a low-molecular-weight endogenous thiols such as glutathione, which mainly act as "protein protectors" [147] through the formation of PSSG during tissue desiccation, are responsible in wheat grains during its desiccation to a significant reduction of the MWD of the polymeric proteins by the formation of PPSSG (Figure 12). This action is all the more important because it is very targeted because GSH was bound almost exclusively to those cysteine residues that have been proposed to form intermolecular disulfide bonds (in particular, cysteines Cb* and Cx, which are responsible for the aggregative nature of LMW-GS) as Köhler et al. [157] has been able to demonstrate it by using ³⁵Slabelled GSH.

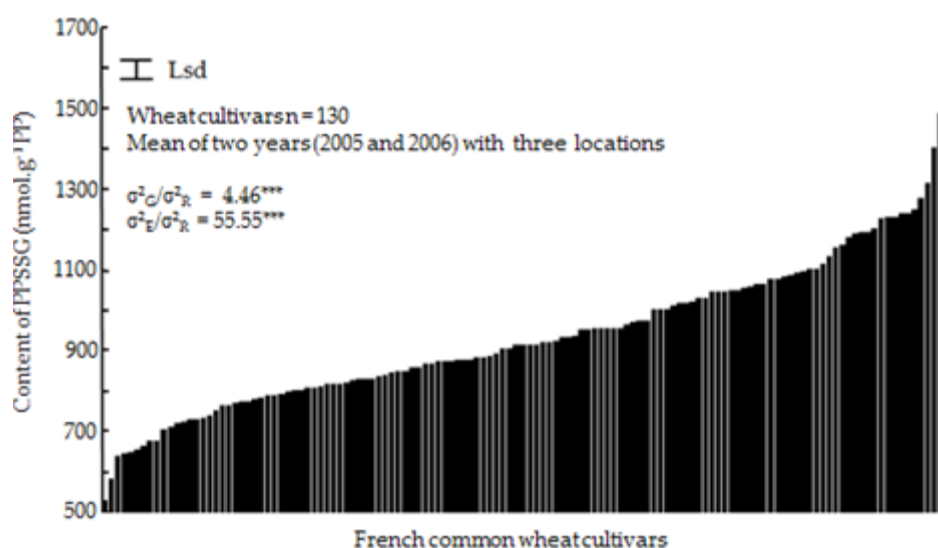


Figure 13. Variation of the content of polymeric proteins conjugated to glutathione (PPSSG) for a significant set of French common wheat cultivars (harvest 2005 and 2006 in three locations). σ^2_G/σ^2_R = Genetic variance/Residual variance ratio and, σ^2_E/σ^2_R = Environmental variance/Residual variance ratio. (O17, P12, Aussenac et al. unpublished data).

Consequently, it is now clear that glutathione conjugation with polymeric proteins during the grain development resulting in drastic changes of the cellular redox status (largely due to environmental factors - Figure 13), play a crucial role in controlling the MWD of the polymeric proteins which has been shown to be important in determining baking performance.

Does GSH be the alone thiol affecting the MWD of glutenins? Certainly no. In our previous studies (A30, O17 and P12), we found some unidentified peaks. One of them is likely to be cysteine. It is important to quantify these molecules and determine their relationship with the glutenin MWD. Since these conjugations coincide with changes in the cellular redox status, the impact of reactive oxygen species (ROS) variation during grain development on the wheat protein accumulation could be interesting to undertake. The role of wheat grain redox systems, which employ a wide spectrum of enzymatic and non-enzymatic antioxidants, could be a crucial in the formation of glutenin polymer aggregates. Therefore, using contrasting environments and genotypes, we will be able to analyze the environment impact on ROS metabolism and study the mechanisms by which ROS molecules and enzymes affect the protein accumulation during wheat grain development. Using proteomic approach it has been shown that grain hardness influence the polymer size (A24), and can be associated to the endosperm stress response. The grain hardness and the field temperature during protein accumulation were seen to significantly influence the polymer mass (A16).

Also, foliar diseases could be considered as environmental effect. Up to now, no or few works were focused on the effect of wheat diseases such as powdery mildew and septoria tritici blotch that have a global distribution in particular Europe, on wheat protein distribution and glutenin aggregates formation that are very important in the breadmaking quality of wheat flours. It will be interesting to collaborate with phytopathologists and propose a common scientific project in the future. Does wheat will use the same mechanism employed in abiotic stress to escape this biotic pressure?

5. Transcriptional regulation of prolamin gene expression

Cereal prolamin gene expression is specifically confined to the endosperm tissue and restricted to specific seed maturation stages which means it is under temporal control [158]. Several molecular genetic mechanisms regulating the expression of grain storage protein genes during grain development have been studied [159-164]. Most of them concerned barley (*Hordeum vulgare*), maize (*Zea mays*), and rice (*Oryza sativa*). Control and coordinate

transcription of the wheat prolamin gene families occurs early in grain development [165-169]. Transcription of storage protein genes is initiated by transcription factors that bind to specific sequences located in promoter regions of prolamin genes [170]. The promoter regions of grain storage protein genes are rich in conserved DNA sequences of 5 to 15 nucleotides called cis-regulatory elements. These are made up of the endosperm box (EB; [171]) and the AACA motif [172]. The endosperm box is composed of two adjacent motifs GCN4-like motif (G-motif, GLM, or N-motif) and the prolamin box (PB, E-motif). The PB and the AACA are found to be essential for endosperm-specific expression of prolamin genes in several cereal seeds [173, 174]. The GCN4 motif in prolamin genes is thought to be involved in the nutritional regulation and in non-cereal organisms and could be involved in the response to nutrients and the environment [175, 176].

These motifs, 5 in number in The 5' proximal flanking regions of prolamin gene promoters are recognized by several transcription factors belonging to four families (the Opaque-2 family, the B3, DOF, and MYB proteins) [177-179]. The GCN4 motif (5'-ATGAG/CTCAT-3') and the G-box motif (5'-TTACGTGG-3') are recognized by the protein family of basic Leu zipper (bZIP) transcription factors termed SPA in wheat [180, 181]. It belongs to the Opaque2 subfamily. Transcription factors interacting with PB (5'-TGTAAG-3'), are composed of proteins in the DNA binding with one finger (DOF) family [182, 183] and called WPBF in wheat and BPBF in barley [184]. GAMYB, a gibberellic acid-dependent MYB TF, interacts with the AACA motif (5'-AACAAC-3') [184]. Other motifs, VP1 recognition sites and basal promoter elements are common with the promoters of other seed storage protein genes [185]. Additional regulatory proteins involved in the transcriptional regulation of grain storage protein genes were reported [186-188]. The cis-element, 5'-TATC/GATA-3' motif, can bind R1MYB (MCB1 and MYBS3) TFs [163,186, 187].

The bZIP transcriptional factor SPA is known to activate and regulate expression of different types of prolamin genes [189]. BPBF was reported to be an activator of endosperm-specific expression of hordein genes during barley grain development and to be a regulator of aleurone-specific hydrolase gene expression during grain germination [184]. Recent reports showed that wheat TaGAMyb activates the HMW-GS genes (TaGLU) through recruiting the histone acetyltransferase GCN5 [190]. Full activation of GSP genes is thought to be achieved by a complex regulatory network where synergetic interactions of different TF play a determinant role.

The available studies on elements regulating the transcription of seed storage protein genes, both of cis-elements and trans-factors involved in regulating tissue-specific expression of wheat storage genes are still not elucidated. *However, the functions of many of these sequences must be experimentally proved, and many of the transcription factors are not yet functionally characterized. This could be achieved in collaboration with our academic partners working on these topics.*

In conclusion, identifying and understanding the factors regulating expression of the wheat storage protein genes is crucial to unraveling the complex effects of the environment on grain development and mainly on the formation of glutenin polymer aggregates.

6. Environmental effect on expression of storage protein genes

Variations in the amount of prolamin transcripts or in the temporal expression of storage protein genes in the endosperm tissue are closely related to environmental conditions during wheat grain development. These changes could explain alterations in the breadmaking quality of flours. Wheat protein distribution could be affected and thus modify the formation of glutenin polymers that determine end use quality, as they are closely related to gluten and dough strength [28, 91, 191]. The transcription of the major gluten storage protein genes is mainly influenced by temperature during grain development [166]. Whatever the environmental conditions, accumulation patterns of transcripts within the α -, γ - and ω -gliadin, LMW-GS, and HMW-GS gene families are similar during grain fill. However, differences between the relative quantities of transcripts within each gene family are observed. Under controlled and moderate conditions mRNAs for the various gene families, are present from 11 days after anthesis [166]. The accumulation of transcripts increases to reach maximum levels of around 34 DAA, then decreases severely to between 36 DAA and 38 DAA. Shift of temporal expression could be observed between studies [192]. This type of difference could be due to genotype and culture conditions. Under heat stress conditions, transcripts within all gene groups are detected earlier, between 5 and 7 DAA and the time frame of their accumulation is shorter compared to optimal conditions. Genes within the α - and γ -gliadin families using hybridization analysis of RNA realized from seven stages of developing kernels between 3 and 31 DPA were highly co-regulated [168]. The accumulation of transcripts encoding HMW subunits occurs later when compared to the expression of the LMW subunit and gliadin genes [93]. The accumulation lag is about 2 days. Differences of temporal regulation could be explained by the fact that the

promoters of the LMW subunit and gliadin genes are tightly related in the endosperm box structure whereas they are different from those found in the sequences of the HMW subunit gene promoters [193, 194]. Moreover, Plessis et al. 2013 [195] found less co-regulation of LMW-GS and HMW-GS than of the gliadin classes. They also observed that the trans-regulation of gliadin classes and glutenin subunits appears to be controlled by different loci and suggested that the mechanisms regulating HMW-GS and LMW-GS are more independent of each other than those regulating the different gliadin classes. Finally, their results strongly suggest that the genes regulating glutenin and gliadin compositions are mostly distinct and operate differently.

It is also well-known that accumulation of storage grain proteins in wheat is affected mainly by nitrogen and sulfur fertilizer [19]. Post-anthesis N deficiency implies a low abundance of ω -gliadin gene transcript while transcript abundance for other classes of gliadin or glutenin genes were unchanged [196]. Post-anthesis nitrogen supply did not affect temporal expression of any of the prolamin genes. Recently, Vincent et al. 2015 [197] studying the influence of nitrogen and sulfur supply on the regulatory network in wheat, showed that absence of N fertilization leads to a low level of the four classes of gliadin and HMW-GS while it shows a high level of LMW-GS. The report showed that a S deficiency implies a low abundance of the S-rich α -gliadins. However, abundance of transcripts was not investigated. Nine TFs were found to be associated with both N and S deficiency and may orchestrate the response to the N-to-S balance. Some of them are involved in the response to hormones, abiotic stress and nitrate transport [197].

Storage grain proteins are synthesized with a signal peptide that is deleted when protein is translocated into the endoplasmic reticulum. The processing and post-translational modification of storage proteins to achieve their 3-dimensional structure are assumed with the help of endosperm genes encoding several proteins. These molecular chaperones (HSP70, binding protein (BiP) and protein disulphide isomerase (PDI), a foldase) are required for synthesis, polymerization, and accumulation of storage proteins in many tissues [198, 199]. However, their true role in glutenin polymerization and aggregation is not yet elucidated.

In conclusion, investigating the regulation network and factors that control expression of the wheat prolamin genes is fundamental to understanding the molecular effects of the environment on the developing kernel. However, the accumulation and conformational modifications of gluten proteins are a very small part of a complex molecular processes

influenced by condition variations taking place during grain development. ***High-throughput qualitative and quantitative analysis of transcripts associated with phenotyping and metabolomic techniques would provide a comprehensive molecular picture of storage product synthesis that are affected by environmental conditions. Finally, the regulation network, taking into account wheat breeding, requires further in-depth study.***

7. Conclusions

Since the 1990s, there has been a broad consensus within the scientific community that the value of using a wheat flour depends mainly on the quality of the assembly of its prolamins (glutenins in particular) which are themselves largely under the control of protein polymorphism (nature and relative abundance of LMW-GS and HMW-GS) and the conditions of development and maturation of the grains from which it is made. Although much progress has been made in the field of characterization of polymeric structures, in particular through the implementation of new analytical approaches (A-4F / MALLS), the fact remains that significant work needs to be done to better understand the structure of its protein assemblies of technological interest (UPP or GMP).

This part of my manuscript demonstrates that to achieve these objectives, ***it is essential to better understand the mechanisms that govern the formation of these polymers and/or protein aggregates in wheat grains during the final stages of their development which are subject to changing environmental conditions (i.e. increasing heat stress due to climate changes)***. In this context, the important role of cellular redox status is addressed by highlighting the significant effects of specific free thiols such as glutathione on the state of association of glutenins. These compounds, of which one of the main functions is to limit the deleterious effects of oxidative stress on protein structures by combining with them, will at the same time reduce the inter-prolamin interactions in the grain thus limiting their technological functionalities. ***The current improved understanding of these cellular mechanisms will undoubtedly open up new avenues for exploring redox strategies for wheat improvement necessary for a sustainable quality.***

(B) Scientific Program of my research unit

My research project is part of the new research unit “Transformations and Agro-Resources” that has just been officially accredited by the ministry of higher education and research as a host laboratory under the number EA7519.

The research unit and my integration follow the new strategy of the direction of our UniLaSalle, an agricultural engineering school founded in January 2016. This last was born following the merger between the Institut Polytechnique LaSalle Beauvais and ESITPA Rouen.

My request to take part in this research unit was a logical continuum in adequacy with my scientific background in the field of agro-resources. The scientific director and myself saw it as an opportunity to progress in this field by integrating an accredited research unit as well as contributing to the overall scientific project.

The scientific program focuses on the processing of whole crops with a view to sustainable development. The program can target different mechanisms impacting molecular composition of the crop (from growth to post-harvest transformation) in relation to specific food or non-food applications (structure function relationships). The unit comprises two teams:

- The VAM²IN team: Processing for non-food uses.
- The PETALES team: Processing for food uses and effects on health, which is further detailed below as I am member of this team.

Industrial process leads to modifications of the intrinsic molecular assembly potentially generating new (functional) structures and/or new compounds. Upon consumption these molecules go through the digestive tract, with or without changes, inducing biological effects.

In this purpose the scientific program of the PETALES team develops two complementary objectives:

1. - Characterization of agro-resources during processing

Quality of the initial raw materials is paramount to the later stage outcomes. In addition we evaluate the effects of process on the food matrix by identification and quantification of the newly-formed compounds. We thus aim at:

1.1 - Understanding and characterizing factors inducing variability in the composition of unprocessed raw materials;

1.2 - Characterizing the potential impact of heat-treatment and other types of processing on the food matrix;

1.3 - Implementing new dimensions of characterization to identify changes in the structure/conformation of the biopolymers present.

2. - Analyze how the processing of agro-resources affects intestinal homeostasis following early exposure

The development and maintenance of intestinal homeostasis have a major impact on health. As such processed or unprocessed foods can have a direct positive or negative impact on health. We aim to shed light on biological effects and molecular and cellular mechanisms and to measure their potential impact through:

2.1 - Evaluating technological, organoleptic, health and nutritional functions following processing techniques,

2.2 - Determining the origins of variability in raw materials and the role of this variability in the changes observed during processing;

2.3 - Identifying the conditions needed for these molecules to affect the behavior of the various cells in the gut region (microbiota, mucosal and immune cells);

2.4 - Estimating the impact of these molecules on intestinal homeostasis.

(C) Declination of my scientific implication within my new research unit.

Afin de respecter le cahier des charges de l'école doctorale concernant la rédaction du manuscrit HDR en anglais, la dernière partie de ce manuscrit sera rédigé en français (les deux dernières propositions de projets). Puisque un certain nombre de réponses aux appels à projets sont exigés en anglais, j'ai gardé mes deux premières propositions en Anglais.

Avant d'intégrer la nouvelle unité de recherche EA7519 Transformations & Agro-ressources, j'ai collaboré avec un certain nombre de mes collègues dans différents programmes de recherche (**A4, A8, A9, A10, A11, A21, A22, O10 and P1**). Ainsi, mon intégration est facilitée par ces collaborations et je compte m'impliquer davantage dans les programmes à venir.

Cependant, je souhaiterai focaliser mes travaux de recherche sur la compréhension et la caractérisation des facteurs induisant la variabilité de la matière première. Ceci correspond à l'objectif 1.1 de l'équipe PETALES.

Cereals are a vital part of human daily diet, on average about 53% of our daily caloric intake comes from cereal-based products. Wheat is one of the cereals that contribute the most to the world's food supply chain. The role of wheat is the most prominent, 31% of all cereals produced is wheat. *Therefore, a major part of my research project will focus on wheat such as described previously and will try to answer the various questions raised previously.*

C-1 Celiac disease and gluten proteins

Recently, we were several Unilasalle researchers involved in scientific projects dealing with the topic of gluten celiac-immunotoxicity (FSOV GlutNSafe, ProtalSafe). Therefore, we would like to maintain these kind of research themes using two different technologies as pretreatment to prevent immunogenic properties of wheat proteins.

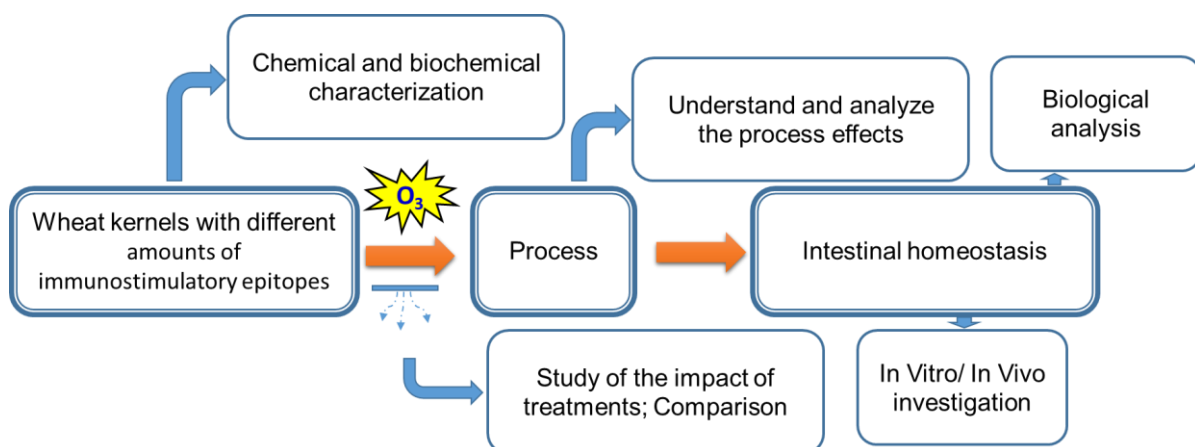
Celiac disease is a complex disorder characterized by an immune-mediated reaction to gluten that occurs in genetically predisposed individuals. Celiac patients react to dietary proteins, named prolamins, which are rich in proline and glutamine residues, including wheat gliadins, rye secalins and barley hordeins [200]. Statistics indicate that approximately 1% of the global population suffers from celiac disease. In some countries like Algeria, the prevalence of CD is even higher reaching approximately 5.6% [201]. Its prevalence continues to be on the rise and patients suffering from celiac disease face with the daunting task of completely eliminating gluten from their diets in order to ameliorate or normalize symptomatology, as this is the only treatment available. This task is made even more burdensome by the high cost of gluten-free foods as well as their technological performances that are barely satisfactory for this category of consumers [202, 203]. Moreover, the gluten free diet can cause nutrient deficiencies because gluten-free products often contain less fiber, magnesium and folic acid than gluten products [204] and higher amounts of saturated fat, total fat, sugar and complex carbohydrates [205].

In this context, some research studies are being conducted aiming the improvement of technological, nutritional and organoleptic properties of gluten-free foods [206-208], whereas other studies point to the modification of the immunological and toxic properties of wheat gluten by chemical and enzymatic means, in particular the transamidation of glutamine residues [209, 210], or the proteolysis of immunodominant epitopes [211]. Physical and chemical treatments like microwave treatment and zone.

Microwave treatment of wheat kernels were proposed to abolish and or reduce immunogenic properties of gluten proteins for celiac patients [212], nevertheless this subject remains controversial as there are some scientific evidences showing inefficiency of microwave treatment to reduce the gluten celiac-toxicity [213, 214]. The aim would be to gain a deeper understanding about the effect of microwave treatment of gluten on its functionality, structure, conformation and more importantly on gluten celiac-related toxicity.

At the opposite, the effect of ozone treatment of wheat kernels on immunogenic properties of wheat proteins are not studied.

Both technologies used as pretreatment are well mastered in our research unit. The effect of ozone on the major components of wheat kernels have been investigated (A6, A13). Studies showed a strong impact of ozone treatment on wheat protein molecular features which modify protein behavior during wheat process and consequently could change positively their effect on intestinal homeostasis and immunogenic properties. The expected and targeted changes will be oxidation of prolamins, especially the amino acids of the epitopes responsible for the immune response against gluten. Change in structure of glutenin polymers may also play a role in reducing immunogenic properties by preventing digestion of protein into peptides with immunostimulatory activity in the intestinal lumen. The deal would be to prevent the occurrence of adverse reactions during ingestion while maintaining good technological and organoleptic qualities.



The scheme above summarize our approach where several colleague would be implied. Our collaborators from University of Trás-os-Montes and Alto Douro will be invited to participate.

C-2 Sourdough and health benefits

As part of a GoLasalle thesis "call for projects" of the Golasalle international network, our project on fermentation was accepted. Several colleagues from our research team will be involved at different levels.

Nowadays, the consumer's demands for a healthier diet have become more evident and most consumers are searching for functional food with for example fewer calories, more dietary fibers, plant proteins, less salt and fewer additives... Baked goods being an essential constituent in the diet of most populations, their improvement and fortification could be considered as an effective approach for developing high quality and healthy products.

Owing to the specific food needs of the consumers even for products as simple as bread, there is a necessity for careful optimization of the food processing methods. The intensity of food transformation could result in different properties of the food itself, its molecular composition and also different « health » properties for the consumer. As an example, in traditional French baguette, we have already shown the impact of baking time on the production of Maillard reaction products (MRP) [215], revealing different organoleptic properties and modulating sub-species of the intestinal microbiota leading to potential health impact.

Interestingly, microorganisms, such as yeast and bacteria present in sourdough are widely used in food formulation due to their implication in transformation processes (fermentation) as well as their potential health benefits (probiotics) [215-220]. Sourdough fermentation is an important traditional process in wheat baking because it improves bread quality by prolonging shelf life, increasing loaf volume, delaying staling, as well as by improving bread flavour and nutritional properties [221]. For instance, it is known that microorganisms used in the fermentation process of bread-making can affect the structure of both the proteic and glucidic fractions of the dough. During further process, like kneading and baking, those macromolecular networks will be further affected, creating notably MRP. Similarly, bacterial proteolytic or saccharolytic metabolism can influence the production of by-products with organoleptic (different aroma profile) and nutritional consequences (decrease in antinutritional components) [222-224]. Finally, those bacterial modulations throughout process can affect the technological properties of the bread and its overall quality and health outcomes [225].

Fermentation of wheat flour with selected sourdough bacteria and other microorganisms following an optimized protocol may decrease and or abolish the gluten toxicity [226]. It could also optimizing the bread-making quality to get superior nutritional, sensory and rheological characteristics.

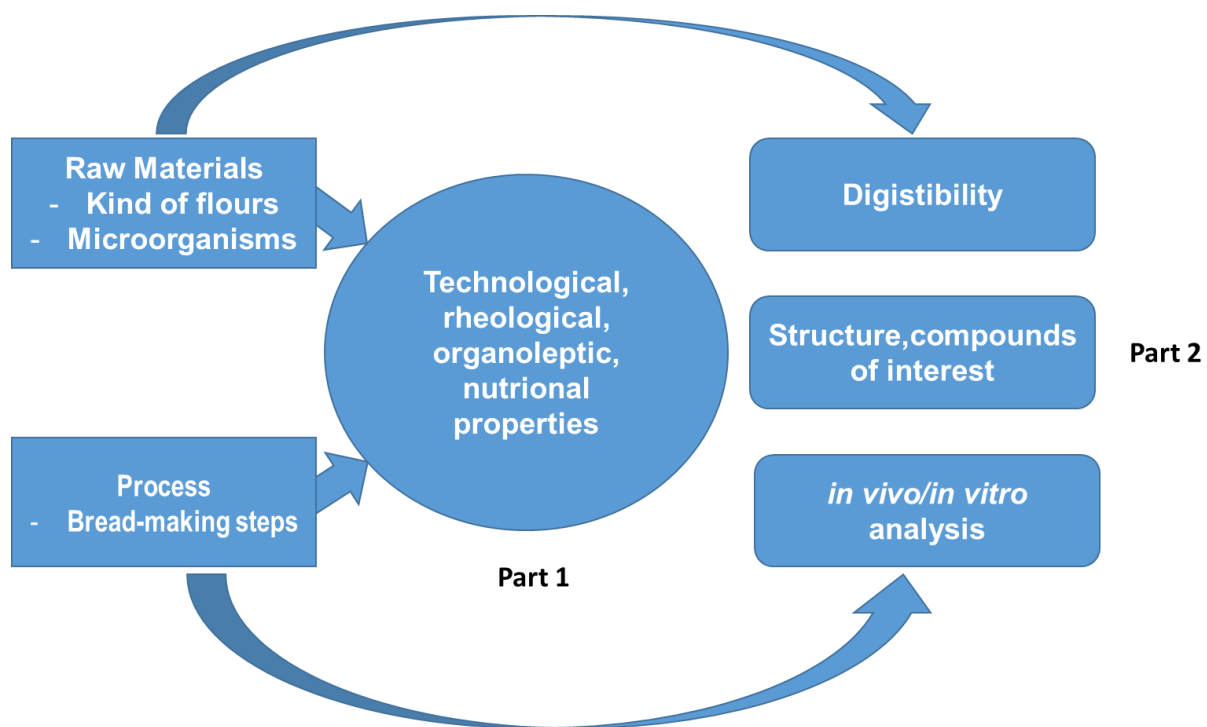
The objective of this project is thus to investigate, through modulation of different parameters in bread making (e.g. choice of starting material such as microbial populations, microbial enzymatic activity, flour source), the impact of processing (kneading, baking: conventional, combined microwave/hot air,..) on the technological, organoleptic, nutritional properties of the bread as well as the impact on digestibility by intestinal microbiota and the modulation of this microbiota and metabolic luminal environment as early markers of health outcomes. The objectives can be divided in 2 axes (Scheme below).

C-2-1- Evaluate the properties of a “model Bread”

Different parameters of the dough’s composition (e.g. sourdough vs baking yeasts, choice of enzymes supplemented) will be standardized and crossed with parameters of processing (e.g. fermentation, kneading, baking conditions, temperature and time) so as to evaluate the impact of these modifications to the properties of the final product, the reference bread being the French baguette. Output parameters include technological, organoleptic, nutritional or protein molecular structure properties as well as in vitro digestibility in the intestinal environment. Changes in the structure of wheat protein network during all steps will be investigated.

C2-2- Health impact

During digestibility studies, modulation of the intestinal microbiota (composition and metabolism of known bacterial species) will be investigated. Those output parameters will be compared to the literature for health benefits and correlated with In vitro and/or in vivo studies. In this part, we will investigate a limited number of downstream health outcomes (e.g. impact on gut mucosal or immune response), depending on the hypotheses arising from the first set of experiments.



C-3 Stress abiotique pendant le développement du grain de blé:

Le stress thermique est souvent défini comme une élévation significative de la température au-delà d'un certain seuil pendant une certaine période de temps suffisante pour induire une altération irréversible de la fonction et du développement de la plante [227-229]. Une augmentation transitoire et ponctuelle de quelques degrés (10 à 15) au-dessus de la température ambiante est considérée comme un stress thermique [227-229].

En tant que stress abiotique, le stress thermique survenant pendant les phases de remplissage du grain est une contrainte majeure pour la production de blé (*Triticum aestivum* L.). Ce type de stress pourrait déstabiliser la balance économique de la filière en réduisant la rentabilité de nombreuses régions productrices de blé. Par conséquent, des réductions supplémentaires de rendement résultant de l'augmentation prévue des températures annuelles mondiales seront critiques et impacteront la sécurité alimentaire, la viabilité économique de l'agriculture et les activités agricoles annexes. Ainsi, la tolérance à la chaleur est et restera considérée comme l'une des priorités les plus importantes.

Chez les génotypes de blé sensibles à la chaleur, le stress thermique pendant la période de remplissage du grain régule directement ou indirectement un certain nombre de voies métaboliques différentes. Les processus les plus affectés sont étroitement liés à la photosynthèse. Suite à l'évanescence de cette dernière, le stress thermique pendant le développement du grain provoque l'accélération de la sénescence. Quand la période du stress thermique est centrée sur l'anthèse (la phase la plus critique), la stérilité du pollen et l'avortement des embryons augmentent, entraînant une réduction du rendement, du nombre et de la qualité des grains [230-234]. Par la suite, un choc thermique réduit les paramètres agronomiques tels que le nombre d'épillets, le nombre de grain par épillet, le poids de mille grain, le poids spécifique [235], le rendement en farine....Les qualité technologiques et rhéologiques de la pâte sont également affectées. Les génotypes de blé sensibles aux températures élevées connaissent une baisse de 3% de rendement en grain pour chaque degré enregistré au-delà de 15°C [236].

La diversité génétique du blé avec une certaine tolérance à la chaleur a été identifiée. Ces génotypes de blé sont capables de maintenir une certaine stabilité de leurs processus de photosynthèse, leur teneur en chlorophylle, leur taux d'accumulation d'amidon et de protéines, poids de mille grain et d'autres caractéristiques agronomiques. Ils se caractérisent également

par une durée de remplissage du grain prolongée, même à des températures élevées [237]. Bien que les recherches sur ces caractères phénotypiques chez le blé aient récemment attiré une attention croissante, la compréhension fondamentale de certaines bases moléculaires et physiologiques de la réponse au stress thermique a encore besoin d'être déterminée.

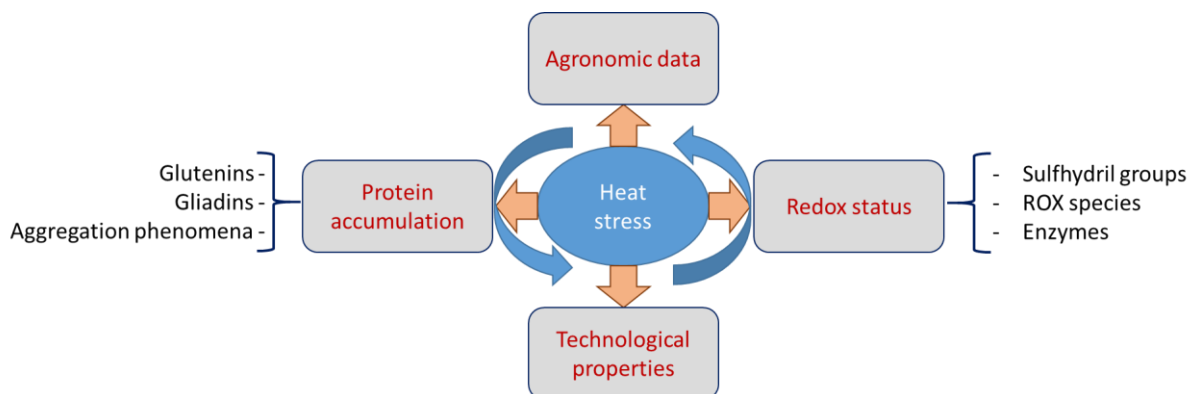
Les températures élevées sont connues pour causer une multitude de dommages aux structures et fonctions de la plante. Le stress thermique affecte la structure cellulaire et diverses voies métaboliques, principalement celles liées à la fluidité et à la thermostabilité des membranes, à la photosynthèse et à la synthèse de l'amidon [238-241]. Une température élevée conduit à une dénaturation des protéines et, par conséquent, altère la fonction enzymatique dans de nombreux cas. Cette dernière associée à un niveau élevé d'accumulation d'acides gras insaturés affecte la perméabilité de la membrane cellulaire et par le fait même les fonctions cellulaires [238-241]. On pense que le stress thermique est responsable de l'augmentation des espèces d'oxygène réactives qui induisent par conséquent un stress oxydatif [242-244]. Il peut également induire un certain nombre de dommages tels que la mort cellulaire programmée, une translocation d'assimilation réduite, une croissance et une reproduction altérées et une sénescence prématurée [245-247]. Le stress thermique accélère le processus de développement du grain. Ce phénomène est associé à une maturation plus précoce des grains et à une phase de reproduction plus courte. Par conséquent, une perte de rendement en grain importante est souvent observée.

Les plantes réagissent et peuvent s'adapter aux conditions de stress abiotiques par le biais de divers mécanismes physiologiques et biochimiques. Ainsi, Les phytohormones jouent un rôle essentiel dans la régulation de ces réponses au stress. Ils jouent un rôle central dans la capacité d'adaptation des plantes aux changements environnementaux en assurant la médiation de la croissance, du développement, de la répartition des éléments nutritifs et des transitions source / puits. En particulier, l'acide abscissique (ABA) et l'éthylène ont été considérés comme les principaux signaux endogènes permettant de déclencher diverses acclimations. Une augmentation accrue de la production d'éthylène endogène se traduit par une croissance réduite, une sénescence accélérée et des réactions des plantes à différents stress environnementaux. Chez le blé, il a été démontré que la synthèse accrue d'éthylène réduit les phases de développement du grain, le poids de 1 000 grains, accélère la maturité du grain et déclenche une sénescence précoce [248]. Une surproduction d'éthylène a également été observée pendant ou après un stress hydrique [249-251]. De même, la production d'éthylène induit également la formation d'espèces réactives de l'oxygène, la peroxydation des lipides et une réduction de la

fluidité membranaire [252]. L'augmentation de la production d'éthylène dans les tiges et les racines a également été constatée dans le cas d'une diminution de la tolérance au sel chez une variété de blé sensible à ce type de stress [253]. Des travaux de recherche sur des variétés sensibles et résistantes au stress thermique ont montré que l'apparition des symptômes liés au stress chez les variétés de blé sensibles étaient associés à une production accrue d'éthylène [254]. Ils ont également montré que la température élevée n'affectait pas la production d'éthylène chez un cultivar tolérant à la chaleur. Ainsi, tous les symptômes liés au stress thermique ont disparu.

En collaboration avec le Dr. Dirk B. Hays, j'ai reçu 800 lignées issues d'un croisement de deux plantes parentales particulières, «Halberd» (blé de force blanc résistant à la chaleur) et «Karl 92» (blé dur rouge) sensible à la chaleur.

Les premières épurations variétales sont en cours et nous pourrons tester un certain nombre de lignées pour leurs caractéristiques physiologiques. L'impact de la contrainte thermique sur la régulation de la période de remplissage du grain et les mécanismes de réponse seront étudiés comme décrit dans le schéma ci-dessous



Pour répondre aux objectifs de cette collaboration, les travaux seront structurés en plusieurs tâches principales et seront réalisés sur plusieurs lignées différentes (sensibles et résistantes):

- Screening variétal :

Dans le cadre des travaux de 4^{ème} année cycle ingénieur « Agronomie et Territoire », les étudiants ont débuté des travaux de sélection. A la suite de ces travaux, un certain nombre de lignées sera sélectionné.

- Caractérisation agronomique:

Les lignées seront jugées sur leurs données agronomiques en conditions d'absence et présence de stress thermique. Les essais seront réalisés en condition contrôlée sous serre. Les différents stades de développement des plantes seront notés. Les paramètres suivants seront pris en

compte : nombre de talles, nombre d'épis, nombre de grain par épis, nombre d'épillets fécondés par épis, poids spécifiques,...

- Caractérisation génétique et biochimique:

Dans un premier temps, l'ensemble des phytohormones sera suivi avant et après l'application du stress thermique. La quantité et la qualité des protéines seront déterminées au cours du développement du grain dans les deux conditions. La distribution moléculaire des agrégats de polymères gluténiques sera déterminée. Le statut d'oxydoréduction permettra d'évaluer l'état de stress de la plante. Aussi, les molécules cibles d'oxydoréduction seront suivies par voie chimique et moléculaire.

- Caractérisation technologique:

Les lignées élites après multiplication seront analysées par l'alvéographe, texturomètre et autres types de méthodologies de caractérisation indirecte de la qualité pour évaluer l'impact du stress thermique sur le potentiel de qualité des lignées sensibles et tolérantes.

- Analyse des données:

Les analyses de données seront ensuite réalisées par le logiciel SPSS afin d'étudier et comprendre les mécanismes de réponse du blé face au stress thermique.

C-4 Etude des mécanismes d'adaptation du blé tendre à des contraintes abiotiques de nature hydrique et thermique :

Le projet suivant pourrait s'intégrer dans le cadre d'un appel à projet régional structurant faisant appel à des partenaires de la région Haut de France. Il sera structuré selon les exigences des appels à projets régionaux.

Il nécessitera des compétences diversifiées allant de l'agronomie à la génétique en passant par la biochimie, la physiologie, l'histologie...les objectifs seront également multiples et pluridisciplinaires.

C-4-1. Quelle est la question traitée dans ce projet ?

Les contraintes climatiques auxquelles les plantes cultivées sont et seront confrontées dans les années à venir peuvent influencer leur rendement. Chez le blé un stress hydrique influence la phénologie (feuillaison, floraison,...), la croissance et le remplissage du grain. Il en est de même pour les variations thermiques qui peuvent altérées la phénologie (durée du cycle...) mais aussi les rendements potentiels (nombre de fleurs ou d'épis fertiles, taille des fruits ou des grains, qualité... ; [255]). De ce fait il devient primordial d'identifier et de mieux comprendre les mécanismes d'adaptabilité et de tolérance aux changements climatiques chez les plantes cultivées en prenant l'exemple du Blé, quatrième culture mondiale. Nous nous intéresserons plus particulièrement à la phase de post-floraison correspondant au remplissage de la graine.

C-4-2. Pourquoi cette question est-elle importante ?

C-4-2.1. Enjeux scientifiques

L'augmentation de la productivité chez le blé est une priorité. En effet, les prévisions démographiques pour 2050 montrent une augmentation importante de la population mondiale. Cette augmentation se fera dans les pays en voie de développement. Cependant, ces pays ne pourront pas produire suffisamment de céréales, donc les pays exportateurs devront augmenter leur production. La France, grâce à ses conditions pédoclimatiques favorables, est le 5ème exportateur de blé au niveau mondial. Elle est donc concernée par cette nécessité de produire plus. Cependant, le rendement en France stagne et parfois connaît des chutes considérables de l'ordre de 50%. Ceci est dû au fait que le climat de notre pays s'est caractérisé dernièrement par des épisodes de déficit hydrique et des élévations de température, d'une façon progressive

ou brutale pendant les saisons de culture. En Picardie, Beauvais a connu en 2012 un déficit hydrique qui s'est étalé sur plusieurs mois (Avril, Mai, Juin). Ces mois correspondent aux phases critiques d'épiaison et de floraison.

Les températures optimales de culture de blé permettant un rendement maximum sont comprises entre 15 et 20°C [256, 257]. De ce fait des changements climatiques pendant la période du remplissage du grain impactent le rendement. Des températures comprises entre 25 °C et 35 °C ou de courtes périodes de fortes températures (> 35°C) lors du remplissage du grain ont souvent été associée à une augmentation de la concentration de protéines dans le grain et à une diminution de la masse de grain sec finale et donc une perte de rendement en grains [258-260]. Ceci est principalement dû à l'action de la température sur la vitesse et la durée de remplissage du grain et par l'abaissement de la synthèse d'amidon [261]. A ce jour, cette diminution de la durée de remplissage du grain contrôlée par la température est généralement compensée par un taux plus élevé d'accumulation de l'azote dans le grain. Ainsi, la quantité d'azote par grain est généralement peu affectée et la concentration en protéines du grain augmente avec la température [262-264]. Par contre, la composition des protéines de stockage et leurs fonctionnalités peuvent être sensiblement modifiées par la température [265-267]. De plus, une culture en condition de température élevée favorise la synthèse des gliadines par rapport à celle des gluténines [268] ou encore une réduction significative des polymères insolubles [266, 269-272]. Il en est de même pour un stress hydrique qui va également influencer le rendement [273]. Bien que ces études aient permis d'obtenir des informations sur l'influence des contraintes climatiques sur le remplissage du grain, les mécanismes moléculaires restent encore peu connus.

Chez des plantes tel que le blé, un stress abiotique pendant la phase de post-floraison écourte la période du remplissage du grain et réduit la vitesse d'accumulation des réserves provoquant une baisse importante du rendement [274-276]. Ce processus pourrait être initié et contrôlé par l'intermédiaire des hormones et leurs interactions chez les plantes [277]. L'éthylène et l'acide abscissique (ABA) sont les deux majeures hormones les plus induites dans le cas d'une réponse aux stress [277, 278]. Une surproduction de l'éthylène induite par la sécheresse chez le blé a été reliée à une diminution du poids de mille grains, une accélération de la maturité et un déclenchement de la sénescence prématurée [279, 280]. Chez d'autres espèces, la surproduction a eu comme effet l'avortement des embryons [280]. Il est important de signaler que la surexpression de cette hormone est également induite par un déficit hydrique, une augmentation thermique et une forte concentration en sel [254, 281]. Sa production a été

reliée à la formation des espèces réactives d'oxygène (ROS), la peroxydation lipidique et à la réduction de la fluidité membranaire [282]. Certaines espèces ROS peuvent directement inactiver les enzymes métaboliques et endommager fortement les acides nucléiques ce qui pourrait conduire à la mort cellulaire [283].

L'acide abscissique produit suite à des stress hydriques, ralentit la croissance des grains et la division cellulaire dans l'endosperme des grains en développement [284-286]. L'augmentation du taux de l'ABA endogène a été également associée à la sénescence des feuilles [287]. Malgré que certains aspects de la régulation hormonale ont été étudiés, les effets physiologiques, enzymatiques et moléculaires de ces hormones ainsi que leurs voies d'actions restent à explorer.

Le projet étudiera à la fois les mécanismes physiologiques, biochimiques, moléculaires et enzymatiques de plusieurs variétés ayant des tolérances différentes aux changements des conditions environnementales. Ceci permettra d'alimenter le modèle général des réponses aux stress abiotiques chez les plantes et en particulier chez le blé (figure ci-dessous). L'étude permettra, après compréhension et établissement des voies de réponse aux stress abiotiques, la mise en place des programmes de sélection pour améliorer nos variétés françaises qui sont connues pour leurs qualités technologiques. La sélection pour améliorer simultanément le rendement en grains et l'adaptation à des contraintes abiotiques de nature hydrique et calorique devrait donc être assistée par des approches physiologiques, biochimiques et enzymatiques.

C-4-2.2. Développement durable : progrès en terme

Sur le plan scientifique, ce projet apportera des éléments primordiaux pour la compréhension des mécanismes d'adaptabilité et de tolérance aux changements climatiques chez le blé en particulier et chez les plantes cultivées en général. Il permettra également l'identification des étapes clés intervenant dans l'augmentation du rendement.

Sur le plan technique, le projet permettra d'identifier des marqueurs physiologiques, biochimiques, enzymatiques et moléculaires pour une sélection assistée de plantes adaptées aux variations climatiques Ceci pourrait participer à la compréhension vis-à-vis de la réduction des intrants de type azote et contribuer donc à l'application du plan Ecophyto Europe.

Augmenter le rendement équivaut à stabiliser et assurer aux agriculteurs un revenu correct.

Le programme prévoit de recruter un doctorant et des Masters participant ainsi à leur formation dans le domaine des agro-ressources.

C-4-3. Comment la question sera-t-elle traitée ?

Pour répondre aux objectifs affichés de notre projet, ce dernier sera structuré en plusieurs tâches principales et sera réalisé sur plusieurs génotypes différents:

I. Caractérisation de différences génotypiques pour la tolérance à un déficit hydrique et/ou à une augmentation de la température.

Sur un large panel de variétés (~20), différents paramètres seront analysés afin de caractériser et sélectionner des génotypes tolérants et sensibles aux changements climatiques

i) Evaluation de la densité stomatale et de l'index stomatal

L'ouverture et le nombre de stomate sont des critères primordiaux dans la réponse au stress hydrique chez le blé [288, 289]. De ce fait, l'évaluation des différents critères tels que la densité stomatale et l'index stomatal seront évalués en condition normale, de forte température et en déficit hydrique chez les différents génotypes de blé.

ii) Mesure des teneurs endogènes en phytohormones liées aux réponses induites par les changements climatiques

Les différences entre la tolérance et la sensibilité à un déficit de stress ont mis en évidence des changements dans l'expression des gènes liés aux phytohormones (éthylène, cytokinines, acide salicylique, gibbérellines, acide abscissique et auxine), incluant les gènes liés à la biosynthèse, la signalisation et la réponse [290]. Ainsi une analyse par LC-MS-MS sera entreprise afin d'évaluer le contenu endogène et induit en hormone chez les différents variétés de blé.

iii) Profil racinaire des différents génotypes choisis (~6),

Le système racinaire permettant à la plante d'absorber les éléments nutritifs ainsi que l'eau représente un organe essentiel dans la compréhension des différentes réponses des génotypes de blé aux changements climatiques [291]. Pour limiter les effets d'un déficit hydrique, la plante peut développer son système racinaire afin d'avoir une meilleure capacité hydrique malgré une réduction des disponibilités en eau [292]. De ce fait une évaluation du

système racinaire (profondeur, masse et volume, ramification) permettrait de mettre en relation l'adaptabilité aux changements climatiques et l'architecture racinaire.

La plante peut également moduler son potentiel hydrique en ajustant sa capacité d'accumuler, au niveau symplasmique et de manière active certains solutés [293-295]. La recherche de ce type de composé pourra être évaluée.

En utilisant plusieurs variétés ayant des tolérances différentes aux changements des conditions environnementales il sera possible de réaliser des corrélations entre le rendement observé, la résistance aux changements climatiques et les caractéristiques stomacales, hormonales et ou racinaires.

II. Mise en évidence des mécanismes d'adaptation aux changements climatiques

i) Etude transcriptomique intervenant lors de la réponse au stress

- Mise en évidence des gènes ou voies de signalisation clés intervenant dans la plasticité de la tolérance au stress observé dans plusieurs variétés de blé sur plante entière et au cours du développement du grain

- Etude par microarray de la variabilité génotypique de la tolérance au stress hydrique (comparaison variété sensible/tolérant dans deux conditions normal/stress à la fois au niveau de la plante entière et sur la graine uniquement)

- En rapport avec les teneurs endogènes et induites en phytohormones par les changements climatiques, une étude de l'expression des gènes liés à ces phytohormones pourra être réalisée afin de mieux comprendre comment la tolérance s'établit. Ces données permettront également de comprendre comment les facteurs de l'environnement peuvent affecter le rendement du blé en modulant l'expression des gènes relatifs aux phytohormones.

- Les petits ARN non codant et particulièrement les microARN ont été récemment impliqués comme régulateurs post-transcriptionnels du développement et de la réponse au stress chez les plantes [296, 297]. A partir des données du transcriptome provenant de plante tolérante et de plante sensible, il serait possible d'identifier des voies de régulation impliquant des microRNA spécifiques des changements climatiques. Ces microRNA pourraient également être impliqués dans le développement de la graine. De ce fait, il serait intéressant d'évaluer

comment les contraintes de l'environnement affectent leur expression et donc pourraient par cette voie affecter le rendement du blé.

- L'analyse des promoteurs des gènes affectés par le stress hydrique ou l'élévation de la température pourrait permettre la réalisation de promoteurs inductibles aux changements climatiques chez le blé. De même, l'identification d'éléments cis spécifiques de la réponse aux changements climatiques permettra par des expériences d'interaction promoteur-protéine dans des protoplastes d'identifier les facteurs de transcription intervenant dans la réponse à ce type de stress. Ces gènes pourraient ensuite être évalués dans le cadre du développement de la graine et aider à mieux comprendre comment les facteurs de l'environnement peuvent affecter les différentes phases de développement de la graine.

- Mise en relation des données transcriptomiques-protéomiques afin d'identifier des processus de régulation post transcriptionnelle associées à la tolérance au stress hydrique.

- ii) Etude protéomique ciblée des grains de blé en cours du développement et suivi de l'accumulation des réserves :

- L'objectif est de déceler les enzymes clefs qui interviennent dans la protection de plante et l'accumulation des réserves sous un stress abiotique.

- Changements des profils protéiques qui en résultent et les modifications post-traductionnelles (ubiquitination, sumoylation, phosphorylation) de ces protéines.

- Identifier les enzymes responsables de ces modifications post-transcriptionnelles et leur mode de régulation associé aux stress abiotiques.

- Réalisation de complexes protéiques intervenant lors du développement des grains de blé et lié à la tolérance au stress hydrique. Evaluation des variations entre les génotypes.

- Mise en évidence de marqueur moléculaire permettant la sélection

- Associer l'accumulation des réserves à une étude histologique et idéalement immunohistophysiological en fonction des résultats du protéome.

- iii) Suivi du statut d'oxydo-réduction dans les grains de blé en cours du développement :

- L'objectif est de suivre les radicaux libres et les différentes espèces d'oxydo-réduction formés dans les grains en voie de développement pendant un stress abiotique.

- Etant donné que le ROS est lié à la dormance des grains, il serait bon d'évaluer ce caractère et l'associer à la perte de rendement.

iv) Identification des facteurs physiologiques, histologiques et biochimiques de la plante intervenant dans la réponse de la plante soumise à des stress abiotiques.

v) Étude des différentes sources de variation de l'accumulation des réserves protéiques et amylacées :

- Nous allons réaliser un approfondissement sur la compréhension du remplissage de la graine en composés pour découvrir les phases clés de l'accumulation; enfin la dernière approche portera sur une étude comparative entre conditions stressantes et non stressantes.

- Le stress hydrique va perturber les métabolismes carboné (C) et azoté (N) (e.g. activités photosynthétiques, absorption) et donc les sources de C et N disponibles pour le grain ainsi que leur capacité de transfert (remobilisation). Une étude physiologique (plante entière) au cours de la phase de remplissage du grain peut être menée chez deux Cvs (sensible Vs. Résistant) sous deux conditions (métabolites C et N / sucres, AA, hormones, aquaporines, osmoprotecteur (proline, glycine betaine, manitol), ions ; activités enzymatiques / NR, GS ; activités photosynthétique, transpiration) associées à des études histologiques (tissus, stomates..).

- On peut envisager une étude cycle J/N, l'ouverture des stomates et donc la photosynthèse peut être plus ou moins longue (i.e. quelques heures) entre Cv. Tolérant et les autres.

vi) Étude de l'impact des différentes molécules intrinsèques identifiées sur les performances et la stabilité de productivité de la graine.

L'originalité du programme réside dans l'utilisation de plusieurs variétés connues pour leurs tolérances au stress abiotique et leurs stratégies d'adaptation. De plus, nous réaliserons ces stress abiotiques en champs.

C-5 Perspectives

En parallèle et en collaboration avec mes partenaires académiques voire industriels, je souhaiterai développer une recherche fondamentale sur le blé pour améliorer notre connaissance du domaine de la science des céréales en apportant des réponses à de nombreuses questions précédemment mentionnées dans le présent document.

Les travaux de recherche effectués par des généticiens et chimistes des céréales ont abouti à la publication de milliers d'articles scientifiques basés sur un paradigme selon lequel les protéines de réserve du blé tendre (gluténines et gliadines) possèdent des caractéristiques primordiales dans l'établissement des propriétés technologiques et rhéologiques des pâtes, grâce au réseau protéique tridimensionnel qu'elle forment au cours de leur hydratation et des étapes de pétrissage dans le process de panification. Aussi, ces propriétés de la pâte sont étroitement liées à la composition protéique des sous-unités gluténiques de haut et faible poids moléculaire.

Les scientifiques du domaine se mettent tous d'accord sur le fait que ces relations mises en évidence sont extrêmement complexes et que la structure du réseau protéique formé par les gluténines est le plus important. En dépit des efforts considérables déployés par les chercheurs pour mieux comprendre la structure des gluténines, la base structurale des propriétés du réseau gluténique reste encore une question ouverte. Ces connaissances ne peuvent pas être atteintes que par l'étude accrue des différents mécanismes qui contrôlent la formation et l'accumulation des protéines de réserve dans le grain. Ainsi, nous nous attacherons au cours de nos futurs programmes de recherche à :

- “Quelles sont les bases structurales et fonctionnelles qui contrôlent les assemblages du réseau de protéines de blé et comment ces assemblages sont-ils liés à la qualité technologique et de santé?”

- “Quels sont les facteurs qui impactent la composition chimique et comment les molécules cibles sont impactées?”

- “Quels sont les mécanismes physiologiques, moléculaires et biochimiques adoptés durant le développement du grain?”

Nous nous baserons sur les modèles d'agrégation existants comme support pour aborder ces objectifs de recherche. Cela nécessitera l'exploration approfondie des propriétés d'agrégation de polymères à différents niveaux. Par conséquent, nous mettrons des efforts pour tenter de :

- identifier les caractéristiques du réseau protéique formé par les polymères agrégés.
- comprendre dans quelle mesure les changements de structure du réseau protéique peuvent être utilisés pour expliquer les différences dans les exigences rhéologiques de la pâte entre des variétés de différentes qualités technologiques.
- comprendre les mécanismes présidant l'accumulation des réserves dans le grain de blé.
- Comprendre la relation entre la distribution moléculaire de masse et de taille des agrégats et les propriétés rhéologiques.
- clarifier davantage les modifications physiques et chimiques des agrégats de gluténines au cours du processus de fabrication de panification;
- intégrer les résultats de l'ensemble de ces études dans un modèle reliant la composition et la structure à la fonctionnalité.

Acknowledgments

The authors would like to thank the FSOV (French Support Fund for Plant Breeding) and the Conseil Régional HdF (Regional Council, Hauts de France) for supporting part of our research.

Conflict of Interest

The authors report no conflict of interest.

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RESUME:

Afin de respecter le cahier des charges de l'école doctorale, le résumé en français reprend d'une façon succincte certaines parties de mon mémoire.

Ainsi, mon document est organisé en plusieurs parties dont les plus importantes sont résumées comme suit:

❖ *Curriculum vitae* :

Cette partie détaille mon parcours universitaire et professionnel. Elle fait état de mes connaissances et compétences acquises dans les domaines de la biochimie, physiologie, biologie et génétique moléculaire. Une seconde partie de mon CV est consacrée à ma production scientifique au nombre d'une soixantaine d'actes montrant mon implication dans différents domaines de recherche. Une trentaine d'articles dans des revues scientifiques à comité de lecture a été publiée.

❖ **Activités de recherche :**

Cette partie regroupe l'ensemble de mes activités de recherche allant de ma thèse jusqu'à mon dernier projet en cours. Je résumerais cette partie en fonction des espèces moléculaires les plus étudiées à savoir les protéines de blé et les lipides.

- Accumulation des protéines de blé :

De nombreuses questions sur la formation des polymères gluténiques dans le grain de blé tendre et leur structure finale demeurent sans réponse. Ainsi, l'objectif de mes nombreuses études en particulier mes études doctorales était de mieux comprendre la formation et la structure des agrégats gluténiques. Nous nous sommes attachés à préciser les mécanismes de formation des polymères gluténiques durant le développement du

grain dans la mesure où ces associations protéiques conditionnent en grande partie la qualité d'utilisation de la farine de blé tendre. En étudiant l'évolution du statut rédox des protéines de réserve au cours du développement du grain, il nous a été notamment possible de démontrer que la formation des très hauts polymères est liée à un phénomène d'oxydation des groupements sulfhydryls libres portés par les sous-unités gluténiques. Ce phénomène, favorisé par le rapprochement des structures polymériques au cours de la déshydratation, conduit à l'apparition de nouvelles liaisons disulfures intermoléculaires et se traduit à la fois par une augmentation de la masse moléculaire et une augmentation du degré de compacité des agrégats gluténiques. Nous avons également pu démontrer que le glutathion était capable de se lier de manière prépondérante à la fraction gluténique limitant ainsi ses capacités d'association lors de la dessiccation. Enfin, une étude exploratoire de modélisation moléculaire a été entreprise afin de proposer une démarche méthodologique permettant d'appréhender les capacités d'interaction des sous-unités gluténiques de haut poids moléculaire.

- Accumulation et synthèse des acides gras usuels et inhabituels

En collaboration avec mes partenaires académiques (UTC, UPJV, INRA...) et industriels (Lesieur, Linéa, Alban Muller..., nous avons mis en place cinq projets de recherche dans le cadre des appels à projets régionaux, FUI et PIVERT avec des objectifs divers et variés. Grace à ces collaborations, nous avons pu établir une meilleure compréhension des facteurs génotypiques, environnementaux, moléculaires, cellulaires et métaboliques gouvernant la synthèse et l'accumulation des acides gras conventionnels et non usuels dans les embryons de plantes oléagineuses (en particulier le lin) et chez les levures oléagineuses. Nous avons étudié les voies métaboliques et/ou les gènes conférant le caractère oléagineux aux cellules et la mise en évidence des

goulots d'étranglement possibles dans ces mécanismes. Une étude des mécanismes d'importation et d'exportation de ces acides gras chez les organismes oléagineux a été réalisée. Ces études nous ont permis de mettre en place une base de données comparative et de modèles métaboliques permettant d'apporter de nouveaux éclairages sur le fonctionnement des systèmes oléagineux (dans notre cas plante et levure).

Nous nous sommes également intéressés à l'identification et l'isolement de gènes impliqués dans la biosynthèse d'acides gras inhabituels et de lipides dans les plantes. L'étude de fonctionnalisation de ces gènes dans différents organismes (allant de procaryotes aux eucaryotes) a été réalisée avec de succès pour plusieurs de ces gènes.

L'objectif de ces études est de créer des organismes génétiquement modifiés producteurs d'acides gras à haute valeur ajoutée et des bio-lipides structurés pouvant être exploités comme composés chimiques valorisables dans les domaines de la lubrification, cosmétique, ...

❖ **Financements & Projets :**

Ce chapitre de mon HDR reprend l'ensemble des projets de recherche développés avec mes différents partenaires. Le résumé, les objectifs ainsi que mon rôle dans ces projets ont été abordés. Plus d'une dizaine de projets ont été mentionnés. UniLasalle était partenaire sur l'ensemble de ces projets. Le financement a été accordé dans le cadre des appels à projets régionaux (1 projet), FUI (1projet), ANRT (1 projet), PIVERT (3 projets) et FSOV (5 projets). Les budgets globaux varie entre 200 K€ et 3500 K€. Les projets de recherche peuvent être répartis en deux thématiques (métabolisme lipidiques et protéiques) et concernent deux espèces végétales le lin (*Linum usitatissimum*) et le blé tendre (*Triticum eastivum* L.).

❖ Encadrements :

J'ai eu l'occasion d'encadrer plusieurs étudiants de différents niveaux d'études. J'ai encadré deux post-doctorants dans le cadre des appels à projets PIVERT et quatre thèses avec différents niveaux d'implication allant de 25% à 50%. Je présenterais le résumé de la thèse dont l'étude est en étroite relation avec le sujet de mon mémoire d'HDR:

Dans cette étude, nous rapportons les effets du traitement des grains de blé tendre par l'ozone gazeux sur leurs constituants biochimiques majeurs. Les grains de deux variétés de blé tendre, choisies pour leur composition en sous-unités gluténiques de haut poids moléculaire (SG-HPM) différente, ont été traités grâce à un dispositif pilote semi-industriel. Un plan d'expérience de type de Box-Benhken a ainsi permis d'étudier les effets de la concentration d'ozone utilisée, du temps de traitement et du niveau de pré-humidification des grains avant traitement. L'ozonation des grains en amont de leur mouture entraîne des modifications significatives des propriétés techno-fonctionnelles des farines et/ou pâtes fabriquées (baisse de la force et la consistance des pâtes, augmentation importante de rapport P/L). Ces modifications résultent majoritairement d'un renforcement de l'état associatif des prolamines en raison de : la formation de nouvelles liaisons disulfures intermoléculaires au niveau des sous-unités gluténiques, la formation de ponts dityrosine au niveau des domaines répétables de ces mêmes sous-unités et, des changements significatifs de leur structure secondaire (baisse du ratio hélices α /feuillet β). Dans un même temps, l'ozone gazeux ne provoque pas de modifications moléculaires significatives au niveau de la population des granules d'amidon présents. Enfin, il semble que l'ozone soit susceptible de modifier quelque peu la signature nutritionnelle des produits fabriqués en entraînant, notamment, une augmentation de la solubilité et disponibilité des fibres totales et une dépolymérisation des β -glucanes totaux.

❖ **Projet de recherche :**

Dans cette partie, je présente l'ensemble de mes travaux de recherche sur l'accumulation des protéines de blé tendre dans un contexte international valorisés sous forme d'un chapitre. Elle a été complétée par des perspectives de recherche et quatre propositions de sujets de recherche. Le résumé de cette partie est le suivant :

Les propriétés agrégatives des prolamines de blé sont largement responsables de l'établissement des propriétés rhéologiques de la pâte. La capacité des prolamines de blé à former un réseau tridimensionnel visco-élastique au cours de l'étape de pétrissage dépend dans une large mesure de leur capacité à interagir. Ces propriétés agrégatives, qui peuvent être évaluées en mesurant leur distribution de masse moléculaire, dépendent du polymorphisme des sous-unités protéiques présentes mais également des conditions environnementales qui surviennent lors du développement de grains de blé. Beaucoup de progrès ont été réalisés au cours des 30 dernières années au niveau génétique pour mieux comprendre et / ou favoriser les propriétés d'interaction des protéines de réserve. Cependant, ces améliorations peuvent être sévèrement limitées par les conditions environnementales. Toute modification de l'état redox des cellules céréalières en réponse à un stress oxydatif peut conduire à une diminution du degré d'association des prolamines en limitant les interactions protéine-protéine lors de la phase de dessiccation des grains. Compte tenu des impacts environnementaux actuels et prévus (c'est-à-dire du changement climatique avec un stress thermique croissant), il est essentiel de mieux comprendre ces phénomènes pour mettre en œuvre de nouvelles stratégies de sélection pour une qualité durable.

Abstract:

The aggregative properties of wheat grain prolamins are largely responsible for the technological functionalities of flours and doughs. The ability of wheat prolamins to form a plastic three-dimensional network during the mixing depends to a large extent on their ability to interact. These aggregative properties, which can be evaluated by measuring their molecular weight distribution, is dependent on the polymorphism of the protein subunits present but also, on the environmental conditions that are applied during grain development. Much progress has been made in the last 30 years at a genetic level to better understand and/or to favour the interaction properties of the storage proteins. However, these improvements can be severely limited by environmental conditions. Any modification of the redox status of the grain cells in response to an oxidative stress can lead to a decrease in the degree of association of the prolamins by limiting the protein-protein interactions during the grain desiccation. Considering the current and projected environmental impacts (i.e. climate change with increasing heat stress), it is essential to better understand these phenomena to implement new breeding strategies for a sustainable quality.

ACKNOWLEDGEMENT

I wish to express my sincere gratitude to Dr. Thierry AUSSENAC, head director of the Transformations and Agro-Resources Research Unit for providing me the opportunity to accomplish this manuscript and for his advices throughout this work. The collaboration with him is still a great experience in many aspects and allows me to meet, learn and to develop my skills for a research career.

I sincerely thank Marie-Odile BANCAL, Jean-Michel CHARDIGNY, Gilles CHARMET, Michèle BOITEL and Emmanuel PETIT who generously gave their time to review and evaluate my HDR manuscript.

I would like to convey my gratefulness to Alicia AYERDI GOTOR for her reactivity and support when needed.

I take this advantage to express my sincere thanks to Gérard BRANLARD and Brigitte THOMASSET for their support and guidance all along the collaboration.

I won't forget all my colleagues for the pleasant and rich moments we passed together, Camille BOROCH, Flore DEPEINT, Laure ANCELET, Perrine GOZE, Lyes LAKHAL and Céline LERIDON...