



L'enrichissement protéique et les paramètres du procédé influencent-ils la structure chimique, les propriétés d'usage, la digestibilité et l'efficacité métabolique des fractions protéiques de spécialités pastières à base de légumineuses ?

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***ECOLE DOCTORALE SCIENCES DE LA VIE,
SANTE, AGRONOMIE, ENVIRONNEMENT***

Thèse

Présentée à l'Université Blaise Pascal
pour l'obtention du grade de

DOCTEUR D'UNIVERSITÉ

SPECIALITE : Science des Aliments et Nutrition Humaine

Soutenue le 22-09-2016 par

KARIMA LALEG

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la structure chimique, les propriétés d'usage, la digestibilité et l'efficacité métabolique
des fractions protéiques de spécialités pastières à base de légumineuses ?**

Président	M. Jean-Michel Chardigny , INRA, AlimH, Theix.
Examinateur	Mme Ambrogina Pagani , université de milan, DeFENS, Milan.
Rapporteur	M. Didier Dupont , INRA, UMR STLO-1253, Rennes.
Rapporteur	M. Jean François Huneau , INRA, UMR PNCA-914, Jouy-en-Josas.
Directeur de thèse	Mme Valérie Micard , Montpellier-SupAgro, UMR IATE-1208, Montpellier.
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INRA UMR 1208 IATE Ingénierie des Agropolymères et Technologies Emergentes

INRA UMR 1019 Unité de Nutrition Humaine

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Liste des abréviations et symboles

- °H: degree of protein hydrolysis.
- AA: amino acid
- ACC: acetyl-CoA carboxylase
- AD: apparent digestibility
- ANOVA : analysis of variance
- APT: peri-renal tissue
- AST: sub-cutaneous tissue.
- ATGL: adipose triglyceride lipase
- BG: black-gram
- BV: biological value
- CD: control diet
- Cs: chemical score
- CVA: Canonical Variate Analysis
- CypA: cyclophiline
- DAA: dispensable amino acid.
- db: dry basis
- DIAAS: digestible indispensable amino acid score
- DSC: differential scanning calorimetry
- DTE: dithioerythritol
- E: egg white
- EAA: essential amino acid
- EAAS: essential amino acid score
- EFN: endogenous fecal nitrogen
- EUN: endogenous urinary nitrogen
- F: faba bean
- FAS: fatty acid synthase
- FATcd36: fatty acid translocase cluster of differentiation 36
- FM: fat mass
- FN: fecal nitrogen
- FTIR: fourier transformed infrared spectroscopy
- G: gluten
- HOMA-IR: homeostasis model assessment of insulin resistance
- HPRT: hypoxanthine-guanine phosphoribosyltransférase
- L: lentil
- LM: lean mass
- LPL: lipoprotein lipase
- LT: low temperature
- MCP-1: monocyte chemoattractant protein-1;
- NEFA: non esterified fatty acids
- NI: nitrogen intake
- NPU: net protein utilization
- NSP: non starch poly saccharides
- OCT: optimal cooking time
- PCA: principal component analysis
- PDCAAS: protein digestibility-corrected amino acid score
- PFD: protein free diet
- PPAR γ : peroxisome proliferator-activated receptor gamma;
- RAG: rapidly available glucose
- RS: resistant starch
- SAG : slowly available glucose

- SDS: sodium dodecyl sulfate
- SE-HPLC: size-exclusion high performance liquid chromatography
- SERBP1c: sterol regulatory element-binding protein 1
- S-GF: spaghetti gluten-free
- S-WW: spaghetti from whole-wheat
- TD: true digestibility
- TDS: sensory analysis by Temporal Dominance of Sensations
- TIA: trypsin inhibitor activity
- TPA: texture profile analysis
- UN: urinary nitrogen
- UN: urinary nitrogen.
- VHT: very high temperature
- W: wheat
- wb : wet basis

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INTRODUCTION GENERALE

INTRODUCTION GENERALE

Contexte de l'étude

Ces travaux de thèse ont été réalisés dans le cadre du projet VEGAGE intitulé « Vitesse de digestion et intérêt nutritionnel des sources protéiques végétales sur la rétention azotée et le métabolisme protéique au cours du vieillissement : étude de deux nouveaux aliments », avec l'appui financier de l'institut Carnot Qualiment et de la région Auvergne. Les travaux de cette thèse se sont déroulés au sein de l'UMR IATE 1208 (Montpellier SupAgro) et de l'UMR 1019 (INRA/Université d'Auvergne)

Selon certaines études nutritionnelles menées au cours de ces dernières décennies (Expertise scientifique collective INRA, 2010), une des transitions caractérisant les pratiques alimentaires depuis le XX^{ème} siècle est l'évolution vers une alimentation riche en produits animaux. D'après la FAO, la consommation mondiale de viande a atteint 286,2 millions de tonnes en 2010 et devrait progresser de 200 millions de tonnes entre 2010 et 2050, soit pratiquement un doublement des volumes actuellement produits. La place de la viande dans notre assiette est toutefois remise en question, du fait des effets de sa surconsommation sur la santé et de sa surproduction sur l'environnement. La consommation excessive de viande pourrait augmenter la prévalence de certaines maladies chroniques. Il est cependant nécessaire de maintenir un apport en protéines adéquat, c'est-à-dire répondant aux recommandations nutritionnelles. Le développement de nouveaux aliments à base d'ingrédients végétaux, riches en protéines de bonne qualité, pouvant se substituer à la viande, à la fois faciles à préparer, de prix abordable, et appréciés par toutes les catégories de consommateurs semble de ce fait d'un grand intérêt.

Certaines études épidémiologiques ont mis en évidence l'intérêt du régime alimentaire méditerranéen composé d'un mélange de céréales et de légumineuses sur la prévention des maladies cardiovasculaires. Plusieurs aliments céréaliers peuvent servir de base à un enrichissement en légumineuses comme le pain, les biscuits et les pâtes alimentaires et ainsi permettre la production d'aliments nouveaux riches en protéines et à forte densité nutritionnelle. Les pâtes alimentaires, produit de consommation courante apprécié de tous, constituent une base intéressante. D'un point de vue nutritionnel cet aliment possède en effet

un indice glycémique relativement faible. Cette propriété est liée à sa structure particulière, formée au cours du procédé de fabrication, compacte et faite d'un réseau protéique protégeant les grains d'amidon de l'hydrolyse.

Présentation et objectifs du projet VEGAGE

La valeur nutritionnelle des protéines est définie par leur capacité à fournir des acides aminés pour les besoins de l'organisme pour la croissance et le renouvellement des protéines corporelles. La capacité des protéines alimentaires à assurer les besoins en protéines de l'organisme dépend de la biodisponibilité de leurs acides aminés et de leur capacité à être utilisés pour répondre aux besoins spécifiques des tissus de l'organisme. Dans ces conditions, il semble important d'étudier la cinétique de digestion, la biodisponibilité et les qualités nutritionnelles des protéines végétales, en particulier dans les situations où l'utilisation de cette source est d'intérêt, par exemple chez les sujets âgés. Le vieillissement est en effet lié à une perte significative à la fois de la masse et de la fonction musculaires, autrement appelée sarcopénie. Ce syndrome est associé à une augmentation des besoins en protéines. En outre, une meilleure assimilation et une plus grande efficacité métabolique des protéines à digestion rapide, comme certaines protéines laitières (lactosérum) a été observée chez les sujets âgés. L'avancée en âge est également caractérisée par la nécessité de contrôler la consommation de graisses saturées et de cholestérol et d'optimiser l'apport en micronutriments. Les sources alimentaires végétales à la fois pauvres en lipides et riches en micro-constituants présentent donc un intérêt certain pour l'alimentation du sujet âgé. Néanmoins, les protéines végétales doivent être de bonne qualité nutritionnelle (profil en acides aminés correspondant aux besoins) et être facilement et rapidement digestibles. L'objectif général du programme VEGAGE est de concevoir et d'étudier de nouvelles matrices alimentaires enrichies en protéines végétales de légumineuses, de bonne composition en acides aminés rapidement digestibles, afin de les adapter à la nutrition des populations âgées.

Présentation des travaux de thèse

L'objectif principal de cette thèse est de produire des spaghetti caractérisés par des propriétés nutritionnelles améliorées du fait de leur enrichissement en farine de légumineuse. Il s'agit également d'évaluer la relation entre l'état des structures protéiques aux différentes échelles et les propriétés culinaires, texturales, sensorielles et nutritionnelles de ces pâtes. Les travaux de cette thèse s'intéressent en particulier à l'effet de l'augmentation de la teneur en

protéines dans la pâte de blé dur par incorporation de quantités croissantes (de 0 à 100%) de protéines de différentes sources notamment des protéines de légumineuses (fèverole, lentille ou black gram) (figure i), dont la composition en acides aminés indispensables est complémentaire de celle des protéines de blé. L'introduction de ces protéines de légumineuses dans une pâte a fait objet de plusieurs travaux mais sans jamais permettre d'atteindre un score en lysine au-delà de 60% de la quantité conseillée au regard des recommandations pour cet acide aminés indispensable (WHO/FAO/UNU, 2007). A noter que cette teneur en lysine correspond à un taux de substitution de la semoule de blé dur par la farine de légumineuse de 35% (Gimenez et al., 2012). L'incorporation de quantités importantes de farine de légumineuses dans une pâte initialement constituée de blé dur pose plusieurs difficultés technologiques au cours du procédé de production des pâtes, notamment pendant l'étape d'hydratation-malaxage. Aucun travail ne rapporte l'utilisation de presse industrielle pilote pour la production de ces spécialités pastières particulières avec des taux de substitution de semoule de blé par la farine de légumineuse très élevées (au-delà de 35%) pouvant aller jusqu'à 100%. A noter que la production de pâtes 100% légumineuses présenterait un autre intérêt nutritionnel que celui des pâtes mixtes céréale-légumineuse. Elles ne contiennent pas de gluten et pourraient donc être consommées par des sujets souffrant d'intolérance au gluten ou de maladie cœliaque. Dans le cadre de cette thèse, des travaux ont donc été entrepris afin de déterminer les conditions de faisabilité de ces spécialités pastières très riches en légumineuses (taux de substitution du blé dur par la farine de légumineuse de 35 à 100% correspondant à un ratio protéines de légumineuses sur protéines totales de 50 à 100%).

Les protéines globulaires de légumineuses présentent des propriétés différentes de celles du gluten. Leur incorporation dans une pâte de blé dur entraîne une dilution du réseau de gluten qui pourrait se répercuter sur les propriétés culinaires, texturales et nutritionnelles des pâtes. Peu de travaux ont été conduits à ce jour afin de déterminer sur quelles bases et dans quelles mesures cette dilution du réseau de gluten pourrait influencer ces propriétés de la pâte. Afin d'approfondir les connaissances sur cette question, nous avons étudié aux différentes échelles le degré de modification de la structure du réseau protéique engendré par l'incorporation des protéines de légumineuses notamment la fèverole, la lentille ou le black gram, sur le maintien de l'intégrité et de la texture de la pâte, ainsi que sur la digestibilité *in-vitro* de ses constituants majeurs (protéines et amidon).

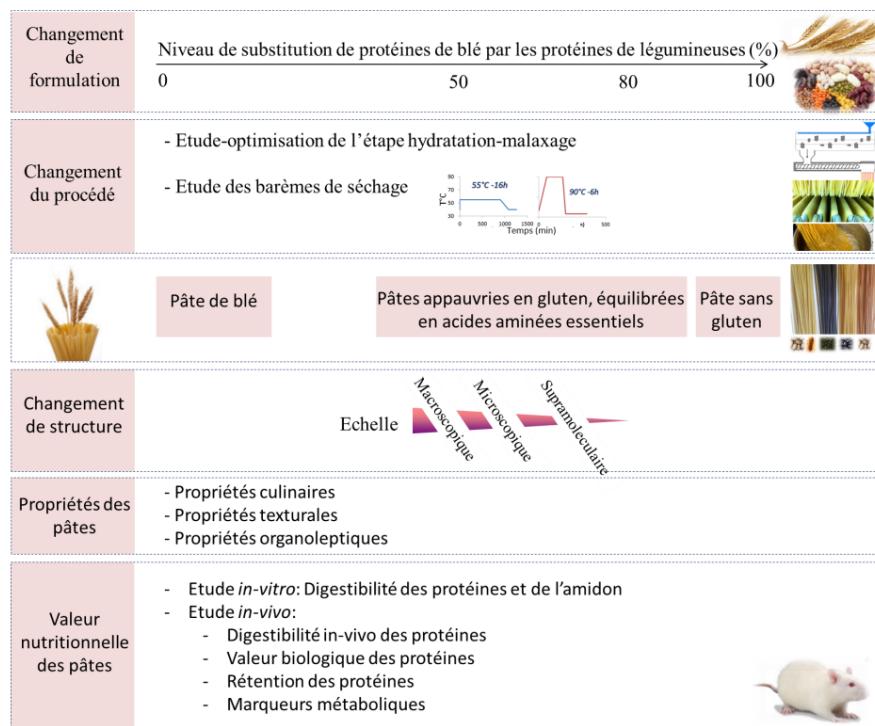


Figure i Schéma général de la thèse.

L’incorporation de farine de légumineuse dans les pâtes peut affecter leurs propriétés culinaires, texturales et sensorielles par modification de leur structure. La modification de certains paramètres du procédé, notamment les températures de séchage engendre des variations structurales qui pourraient améliorer considérablement les propriétés générales des pâtes, mais pourraient également affecter leurs propriétés nutritionnelles, notamment la digestibilité de ses constituants. Si cet effet des températures de séchage est avéré pour les pâtes de blé et pour les pâtes enrichies jusqu’à 35% en farine de légumineuses, il reste à déterminer si l’application des hautes températures de séchage affecte la structure et les propriétés culinaires, texturales, sensorielles et nutritionnelles dans des spaghetti contenant des quantités plus importantes de farine de légumineuses (jusqu’à 100%).

Enfin, le bénéfice nutritionnel engendré par la consommation de ces spécialité pastières riches en légumineuses a été évalué *in-vivo* chez des rats en croissance afin de déterminer les répercussions de l’introduction de légumineuses et/ou de la variation des températures de séchage sur la structure des spaghetti et ses répercussions sur la digestibilité protéique et la rétention azotée. Les effets de la consommation de ces pâtes enrichies en légumineuses sur les marqueurs métaboliques et l’homéostasie glucidique de ces animaux ont également été étudiés.

Ces travaux de thèse participeront à enrichir nos connaissances sur la relation structure du réseau protéique des pâtes en lien avec leurs propriétés culinaires, texturales, sensorielles et nutritionnelles en répondant aux questions suivantes :

Dans quelle mesure et jusqu'à quel point la dilution du réseau de gluten par des protéines de légumineuses permettrait-t-elle de modifier la structure des pâtes ? La nature de la légumineuse importe-t-elle ?

L'utilisation de températures de séchage contrastées affecte-t-elle la structure de pâtes et, dans la positive, dépendamment ou indépendamment du niveau de dilution du réseau de gluten ?

Dans quelle mesure les changements de structure des pâtes et notamment de leur réseau protéique obtenus par changement de leur formulation et/ou de leur température de séchage modifient-ils leurs propriétés culinaires et rhéologiques?

Et enfin, une des principales questions abordées, ces changements de composition et de structure de la pâte ont-ils un effet sur la digestibilité *in-vitro* et/ou *in-vivo* de leurs constituants (protéines et amidon) ?

Ce manuscrit de thèse comporte une synthèse des principales connaissances bibliographiques en la matière, une présentation des principaux résultats, une discussion générale, les conclusions tirées des différents travaux ainsi que les perspectives. Ces parties sont organisées en 5 chapitres. La synthèse bibliographique et les principaux résultats sont présentés sous la forme d'une revue ou d'articles scientifiques acceptés, soumis ou en préparation dans des revues internationales à comité de lecture. Une présentation plus approfondie de l'organisation générale du manuscrit est présentée ci-après.

Chapitre 1. Synthèse bibliographique

Partie 1. Publication 1. *Fortification of pasta with legume flour: processing ease, cooking quality, structure and nutritional properties. Extrait d'une revue intitulée « Wheat-legume mixed food: structure, organoleptic and nutritional properties» en cours de préparation dont les auteurs sont : Laleg K., Monnet A.-F., Petitot M., Walrand S., Michon C. and Micard V.*

Partie 2. *Quality and utilization of protein in human diet*

L'objectif de cette synthèse bibliographique est d'expliquer comment l'incorporation des différentes quantités de farines de légumineuses, aux propriétés distinctes de celles du blé, dans une pâte pourrait entraîner des modifications de sa structure et de ses propriétés culinaires et nutritionnelles. La seconde partie est consacrée à la définition des principaux mécanismes impliqués dans le métabolisme des protéines ainsi que des concepts d'évaluation des propriétés nutritionnelles des protéines, notamment en termes de dégradation, d'absorption et d'utilisation.

Chapitre 2. Pâtes alimentaires enrichies en protéines : influence de la nature des protéines et des températures de séchage sur la structure des protéines et leur digestibilité *in-vitro*

Publication 2. Laleg K., Barron C., Santé-Lhoutellier, V., Walrand S., Micard V. *Protein enriched pasta: structure and digestibility of its protein network.* Food & Function. 2016, 7, 1196-1207.

Ce chapitre est consacré à l'étude de l'effet de l'enrichissement des pâtes par des protéines de natures différentes ainsi que l'utilisation de plusieurs températures de séchage sur la structure du réseau protéique aux différentes échelles et sur sa digestibilité *in-vitro*.

Chapitre 3. Production et caractérisation de la structure, des propriétés culinaires, nutritionnelles et organoleptiques des spaghetti contenant de 0 à 100% de légumineuse

Partie 1. Dépôt de brevet. Laleg K., Cassan D., Abecassis J. et Micard V. *Procédé de fabrication de pâtes destinées à l'alimentation humaine et/ou animale comprenant au moins 35 % de légumineuses.* Institut national de la recherche agronomique (inra)-Centre international d'études supérieures en sciences agronomiques (SupAgro). Brevet N° FR1462811, Publié le 24-06-2016.

Partie 2. Publication 2. Laleg K., Barron C., Cordelle S., Schlich P., Walrand S. and Micard V. *How structure, nutritional and sensory attributes of pasta made from legume can be impacted by the legume protein inclusion level?* Soumise à LWT-Food Science and Technology.

Partie 3. Publication 3. *Laleg K., Cassan D., Barron C., Prabhasankar P. and Micard V. Structural, culinary, nutritional and anti-nutritional properties of gluten-free pasta exclusively made of legume. PlosOne, 2016, 11.*

Ce chapitre est consacré à la description de la production des pâtes contenant de 0 à 100% de farines de légumineuses. Les modifications structurales provoquées par la dilution du réseau du gluten ou par l'utilisation de différentes températures de séchage sont étudiées. Les répercussions de tels changements de structure et de composition de la pâte sur ses qualités culinaires, texturales, organoleptiques et nutritionnelles (digestibilité *in-vitro* des protéines et de l'amidon et facteurs bioactifs) sont étudiées. Une partie de ces travaux portant sur la présentation des conditions du procédé permettant la mise en forme de pâtes aux légumineuses et ce quel que soit le taux d'incorporation de la légumineuse dans la pâte ne pourra être incluse dans ce manuscrit car elle fait l'objet du dépôt de brevet (FR1462811).

Chapitre 4. Etude de l'effet de la consommation de pâtes enrichies en légumineuses sur le métabolisme la digestion et la rétention protéique.

Publication 4. *Laleg K., Micard V., Salles J., Berry A., Giraudeau C., Patrac P., Guillet C., Denis P., Boirie Y. and Walrand S. Biological evaluation of faba bean-wheat mixed pasta in growing rats: Impact of protein source and drying temperature on protein digestibility and retention. À soumettre à J. Agric. Food Chem.*

Ce chapitre est consacré à l'évaluation *in-vivo* de l'impact de la modification de la composition ou de la structure des pâtes par leur enrichissement avec de la farine de fèverole ou par la modification des températures de séchages, sur leur valeur nutritionnelle, avec un focus sur la digestibilité et la rétention de la fraction protéique ainsi que sur l'efficacité alimentaire.

Chapitre 5. Discussion générale

Une discussion de la synthèse des résultats acquis au cours de ces travaux de thèse est présentée dans ce chapitre, afin d'approfondir les connaissances sur les différentes relations formulation/température de séchage et structure des pâtes ainsi que sur les relations entre structure et qualités culinaires, texturales et nutritionnelles.

CHAPITRE 1

Synthèse bibliographique

CHAPITRE 1. SYNTHESE BIBLIOGRAPHIQUE

Partie 1. Publication 1. Fortification of pasta with legume flour: processing ease, cooking quality, structure and nutritional properties.

Extrait d'une revue intitulée « Wheat-legume mixed food: structure, organoleptic and nutritional properties» en cours de préparation dont les auteurs sont :

Laleg K., Monnet A.-F., Petitot M., Walrand S., Michon C. and Micard V.

Abstract

Pasta is a staple food known to have a low glycemic index, but is deficient in some essential amino acids. Incorporation of nontraditional ingredients, such as legume flours is a way to enhance pasta nutritional quality notably in terms of protein, amino acid composition and fiber content, without increasing its glycemic index. However, the production of legume enriched pasta present technological issues encountered during the pasta processing. In addition, legume flour incorporation into pasta can affect its cooking and sensory attributes and bring some anti-nutritional factors. This review focuses on limiting factors (processing ease, consumer acceptability) that must be considered to develop such pasta products. It presents also the nutritional interest of pasta enrichment with legume flours.

Key words: Durum wheat, legumes, pasta processing, nutritional and cooking properties.

1. Introduction

One of the great challenges today is the development of nutritionally enhanced foods which are inexpensive and at the same time acceptable to the intended consumers. Pasta partly meets these specifications. It contains ~13% of proteins and ~70% carbohydrates which are slowly digested, hence promoting a low plasma glucose response named Glycemic Index (Bjorck et al., 2000; Jenkins et al., 1983). Despite this interesting nutritional property, as most cereal products, pasta is deficient in some essential amino acids such as lysine and threonine (Abdel-Aal & Hucl, 2002; Kies & Fox, 1970). The incorporation of nontraditional ingredients into wheat pasta can improve its nutritional properties, notably its protein content and quality (Laleg et al., 2016a; Manthey & Hall, 2007; Nielsen et al., 1980; RayasDuarte et al., 1996) or its fiber content (Ingelbrecht et al., 2001; Manthey & Schorno, 2002; Tudorica et al., 2002). Among these nontraditional ingredients, legumes are a good source of both proteins and fibers but also of minerals and vitamins required for human nutrition (Costa et al., 2006; Tharanathan & Mahadevamma, 2003). Grain legumes would play a key role in the prevention of cardiovascular disease (due to the high fiber content, the low glycemic index and the presence of oligosaccharides), diabetes (due to the low glycemic index and the high content in fibers), digestive tract disease (due to the high content in insoluble fibers which accelerate the intestinal transit), overweight and obesity (due to the satiety effect and low glycemic index) (Duranti, 2006). However, the introduction of high level of legume flour interesting from a nutritional point of view into pasta may cause technological issues and change the culinary properties of pasta. In addition, legume grains contain also a number of anti-nutritional compounds such as α -galactosides, phytic acid and protease inhibitors (Duranti, 2006), susceptible to decrease the bioavailability of nutrients or to cause digestive discomforts. Nevertheless, most of these anti-nutritional factors could be inhibited by a pre-processing (germination, fermentation and cooking) of legume grains or flours. The objective of this review is therefore to determine the impact of wheat and legume co-processing into pasta first on processing itself but also on the properties of mixed pasta obtained, notably on their culinary, rheological, and sensorial properties. We also intend to focus on the nutritional interest of such wheat and legume association in a single product.

2. Composition of durum wheat and some grain legumes

2.1. Global composition

Both wheat and legume grains are composed of protein, starch, fibers and lipids but, in different proportions. Durum wheat semolina is composed of 64-68% starch, 11-18% proteins, ~8% dietary fibers, and 2% lipids (Table 1.1). In comparison, legume grains contain generally lower amounts of starch (30-67%) but higher amounts of proteins (18-41%) and dietary fibers (8-39%) especially insoluble ones. Lupin is different from other legumes as it contains very low amounts of starch (3%) and very high amounts of lipids.

Table 1.1 Global composition of durum wheat and some legume grains (% db)

	Starch	Proteins	Dietary fibers			Lipids
			Total	Insoluble	Soluble	
Durum wheat ^{4, 5, 11}	64-68	11-18	8.1	6.7	1.4	2
Faba bean ^{1, 2}	30-44	28-33	15-24	nd	nd	1-2
Pea ^{1, 2, 3, 11}	32-48	22-26	13-22	20.3	1.7	1-3
Chickpea ^{3, 7, 9, 10, 11}	43-58	18-27	10-18	6-12	3-5	4 -7
Lentil ^{3, 7, 9, 10}	43-65	23-27	8-20	5-17	2-3	2-3
Lupin ^{1, 6, 8, 12}	0-3	30-41	34-39	30-34	4-5	7-15
Black bean ¹³	62-67	22-24	15	nd	nd	1

¹ Colonna and Mercier (1979); ² Cuq and Leynaud-Rouaud (1992); ³ de Almeida Costa et al. (2006); ⁴ Degidio et al. (1990); ⁵ El-Khayat et al. (2003); ⁶ Guillou and Champ (2002); ⁷ Jood et al. (1998); ⁸ Mohamed and Rayasduarte (1995); ⁹ Perez-Hidalgo et al. (1997); ¹⁰ Rehman and Shah (2005); ¹¹ Souci et al. (1989). ¹² Martinez-Villaluenga et al. (2006). ¹³ (Brijesh & Narinder). nd : not determined.

2.2. Proteins

Wheat storage proteins are composed of 80% gliadin (alcohol-soluble) and glutenins (acid/alkali soluble) and 20% albumins (water-soluble) and globulins (salt-soluble) (Osborne, 1907) (Table 1.2). Polymeric glutenins are composed of high-molecular-weight glutenin and of low-molecular-weight glutenin subunits, linked by disulfide bonds into polymers of high molecular weight (>100 kDa) (Schofield, 1986). These large polymers are believed to contribute to the strength and elasticity of wheat dough. Monomeric gliadin (25-75 kDa) can only form intra-chain disulfide bonds whereas glutenins can form both intra and inter-molecular bonds (Kokini et al., 1994; Singh & MacRitchie, 2004). Gliadins are believed to be responsible for dough viscosity and extensibility.

Legume proteins are mainly composed of globulins and albumins (Table 1.2). In pea and faba bean flours, globulins represent 55-80% and 55-75% of proteins, respectively, and albumins represent 21-28% and 2-33% of proteins respectively. Globulins and albumins exhibit good emulsifying and foaming properties (Boye et al., 2010).

Table 1.2 Solubility of wheat and some legume proteins

Proteins	Percent of total protein	Solvent	Molecular weight (kDa)
Wheat ^{2,7}			
Glutenin (insoluble)	30	none	> 1 000
Glutenin	16-40	Acidic/ alkali solution	> 100
Gliadin (prolamin)	35-69	Ethanol 70%	25-75
Globulin	5-10	NaCl 0.5N	20-90
Albumin	3-15	Water	5-30
Pea ^{3, 4, 5, 8}			
Globulin	55-80	NaCl 0.5N	20-70
Albumin	21-28	Water	23-47
Prolamin	nd	Ethanol 70%	nd
Glutelin	nd	Acidic/ alkali solution	nd
Insoluble	14-22	none	
Faba bean ^{1, 6, 8}			
Globulin	55-75	NaCl 0.5N	19-66
Albumin	2-33	Water	17 - 91
Prolamin	3	Ethanol 70%	2.5-21.8
Glutelin	18	Acidic/ alkali solution	nd
Insoluble	2-20	none	nd

¹ El-Fiel et al. (2002), ² Feillet (2000), ³ Gueguen and Barbot (1988), ⁴ Koyoro and Powers (1987), ⁵ Leterme et al. (1990), ⁶ Pasqualini et al. (1991), ⁷ (Lookhart & Bean, 2000), ⁸ (Petitot, 2009) nd: not determined.

2.3. Starch

Starch is a polymeric carbohydrate composed of amylose and amylopectin. Glucose is a basic building unit of these two polyosides. Amylose is a linear polymer mainly linked in the form $\alpha(1-4)$ D-glycopyranosyl whereas the branched amylopectin contains additional $\alpha(1-6)$ linkages. Starch is a semi-crystalline material, i.e., the granules contain alternating crystalline and amorphous regions (Buleon et al., 1998).

Wheat starch presents a bimodal distribution with large (25-40 μ m) lenticular starch granules (A-type) and small (5-10 μ m) spherical starch granules (B-type) (Figure 1.1) and are characterized by a amylose to amylopectin ratio of 25/75 (Buleon et al., 1998). In comparison, legume starches are generally composed of oval or irregularly shaped starch (figure 1.1) granules with average diameter of 17.5 μ m for lentil and 14.6 μ m for black-gram starches (Sandhu & Lim, 2008). Amylose / amylopectin ratio could be higher in legume than in cereal (30-37% amylose) (Hoover & Sosulski, 1985).

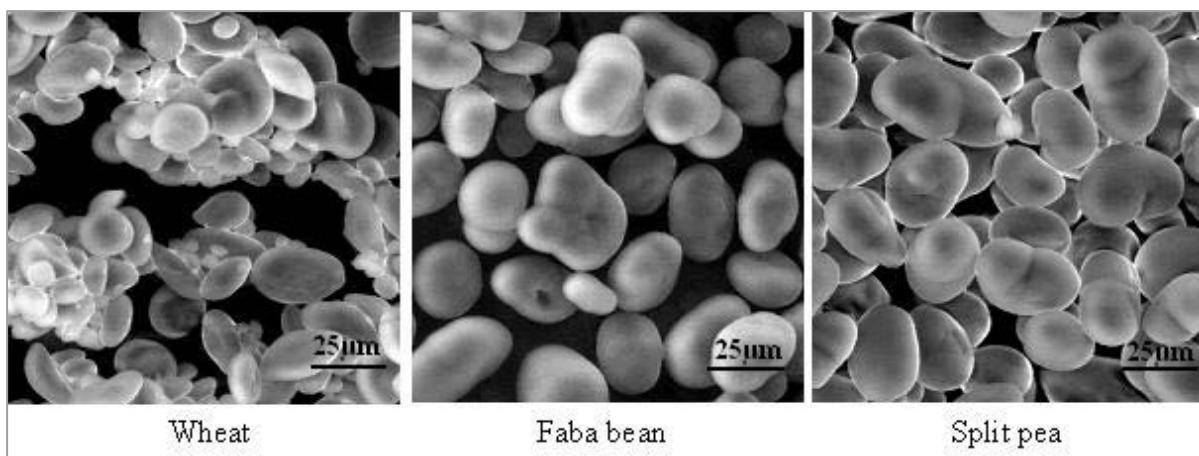


Figure 1.1 Wheat and legume starch granules observed by scanning electron microscopy (Petitot, 2009).

When heated in excess water, starch granules absorb water and swell, this phenomenon, named gelatinization is caused by the disruption of the native crystalline structure of starch granules. As granules continue to expand, amylose is released from the intergranular phase to the aqueous phase. Cooling the gelatinized starch/water mixture to room temperature induces the re-crystallization of starch molecules (mainly amylose) : this process is known as retrogradation (Parker & Ring, 2001). Differential scanning calorimetry (DSC) is used to study different aspects of the gelatinisation process. Based on this technic, durum wheat starch presents generally lower onset, peak and conclusion temperatures compared to legume starches (Table 1.3).

Table 1.3 Thermal characteristic (DSC) of durum wheat and some legume starches: onset (To), peak (Tp) and conclusion (Tc) temperatures

	To	Tp	Tc
Durum Wheat ^{1, 2, 3}	50.9-54.4	56.3-58.7	63.1-63.6
Chickpea ^{4, 5}	57.9-59.3	63.5-65.3	69.8-70.4
Pea ^{4, 5, 6}	55.9-61	61.4-68	66.5-76
Lentil ⁵	57.8	66.8	71.0
Black-gram ⁵	70.5	74.5	78.7

¹ Jacobs et al. (1998), ² Vansteelandt and Delcour (1999), ³ Yue et al. (1999), ⁴ Huang et al. (2007), ⁵ Sandhu and Lim (2008), ⁶ Ratnayake et al. (2001).

3. Pasta formulation and composition

Regular wheat pasta is one of the simplest wheat products in terms of ingredients (Marti & Pagani, 2013). Durum wheat (*Triticum durum*) semolina is recognized as the most suitable ingredient for pasta production due to its unique color, flavor and cooking quality (Feillet & Dexter, 1996). From a statutory point of view, dry pasta intended for the national French market can only be made from durum wheat semolina (by the law n° 99-574, 9 July

1999). Only minimum water required to form crumby dough (47%, in dry basis; $\leq 30\%$ in wet basis) is added to semolina. The dried pasta is mainly composed of carbohydrates and proteins, whereas lipids, fibers and ash remain minor fractions (table 1.4). Each of pasta components plays an important role during the process and on the final pasta quality. A high protein quantity and a visco-elastic gluten network are two main factors required to process semolina into a suitable final pasta product with an optimal cooking quality (Degidio et al., 1990).

Table 1.4 Composition of wheat and legume enriched pasta

Formulation		Dried pasta composition (% db)				
Pasta products	Legume enrichment level (%)	Protein	Fiber	Lipid	Ash	Carbohydrates
Regular-wheat ^{1, 2, 3}	0	10-12	0.9-2.5	0.1-1.7	0.58-0.84	87.1-87.6
Legume-enriched						
Chickpea ^{4, 5}	5-30	14.3-18.1				
Lentil ^{2, 5}	5-40	12.2-18.5	1.51-3.41	0.08-0.18	1.12-2.02	86.3-81.4
White bean ²	10-40	12.2-15.7	1.42-2.13	0.13-0.23	1.22-2.41	86.5-81.7
Yellow pea ²	10-40	12.1-16.0	1.17-1.61	0.09-0.27	1.16-2.21	86.6-81.6
Lupin ³	10-50	16.0-27.0	6.5-17.5			
Black-gram ^{6, 7}	10-50	12.9-16.1	8.41	1.90	1.23-3.24	70.4
Green gram ⁸	60	17.6	6.59	1.68	2.17	78.6
Faba bean ^{1, 9}	10-100	12.4-35.4	2.5-8.7	1.6-2.0	1.0-3.8	84.9-59.3

¹ Gimenez et al. (2012), ² Wojtowicz et al. (2014), ³ Jayasena et al (2012), ⁴ Wood et al. (2009), ⁵ Zhao et al. (2005), ⁶ Madhumitha and Prabhasankar (2011), ⁷ Jyotsna et al. (2015), ⁸ Rajiv et al. (2014), ⁹ Rosa-Sibakov et al. (2015).

Several researches have been undertaken incorporating various natural additives in wheat pasta and noodle products (Li et al., 2014) such as plant and animal proteins (Laleg et al., 2016a; Petitot et al., 2010b), fibers (Tudorica et al., 2002), hydrocolloids and antioxidants (Li et al., 2014). The selection of ingredients used for formulation of pasta depends on the targeted consumers. For people wishing to increase the protein quality and quantity in their diet, legume flours could be a more useful source than other plant or animal proteins. Indeed, at identical protein content, legume enriched pasta have a better amino acid profile than gluten or egg enriched pasta (Laleg et al., 2016a). Legume flours are added in different amounts to wheat semolina, depending on the legume type. The most common legume flours used to fortify pasta are chickpea (Fares & Menga, 2012; Goni & Valentin-Gamazo, 2003; Sabanis et al., 2006; Wood, 2009), lupin (Jayasena & Nasar-Abbas, 2012; RayasDuarte et al., 1996; Torres et al., 2007a), pea (Frijs et al., 1997; Torres et al., 2006; Zhao et al., 2005), faba

(Gimenez et al., 2012; Greffeuil et al., 2015; Laleg et al., 2016a; Petitot et al., 2010a; Petitot et al., 2010b; Petitot & Micard, 2010) and lentil (Bahnassey & Khan, 1986; Wojtowicz & Moscicki, 2014; Zhao et al., 2005). Most of these studies mainly focused on the impact of legume flour addition on the culinary properties of pasta. Recently, more attention has been devoted to the study of structural and nutritional properties of legume fortified pasta (Laleg et al., 2016a; Petitot et al., 2010a; Petitot & Micard, 2010; Rajiv et al., 2014). Legume flour can be incorporated to wheat pasta in different amounts depending on the legume type (table 1.4). Very recently, the production of pasta was also rendered possible from 100% legume flour benefiting therefore for people suffering from celiac diseases (Rosa-Sibakov et al., 2016).

Legume pasta enrichment results in the modification of pasta composition (table 1.4). The major changes occur on protein content and fibers which can be increased by 3 and 10 times (for example for 100% faba bean pasta), respectively depending on the legume type and on the level of substitution; whereas carbohydrates are generally reduced in comparison to regular wheat pasta. The modification of pasta formulation by changing the nature and the proportions of its components can modify the behavior of the dough during the processing steps, and pasta cooking quality. These aspects are discussed in the following sections

4. Pasta processing

Beside the composition, the manufacturing steps play an important role in the determination of the final structure of pasta (Marti & Pagani, 2013). The classical process of pasta production consists of 3 main steps: the hydration and mixing, the extrusion and finally the drying step. First, the water is added to semolina and mixed under low energy input to obtain a crumby dough which is then formed at 40 °C by extrusion or by sheeting (Petitot et al., 2009a). The degree of pasta compactness is higher in extruded than in sheeted pasta (Fardet et al., 1998b) because of the pressure applied during extrusion of 110 bars (Petitot et al., 2010b). Pasta is then dried to reach 12% moisture. Different hydrothermal treatments programs can be applied at the industrial scale to dry pasta, ranging from 40 °C/70% relative humidity (RH) (during 28h) to >84 °C/90% RH (during 2h) (Petitot et al., 2009a). High temperature drying are used to save time, to overcome the strength of gluten network (Degidio et al., 1990) and to improve the textural properties of pasta (Petitot et al., 2009b; Zweifel et al., 2003).

According to most of the authors, the addition of 30% legume flour to wheat semolina does not require essential changes in the pasta production process (RayasDuarte et al., 1996;

Torres et al., 2007a; Zhao et al., 2005). The incorporation of 35% legume flour induces a minor change on the pasta processing, mainly consisting in a decrease in the required amount of water to hydration and in the increase of the mixing speed from 60 to 120 rpm (Petitot et al., 2010b). However, when pasta is produced exclusively from faba bean flour, in addition to the reduction in the water used to hydration and the increase in the mixing speed, the mixing time is also reduced from 20 min for wheat to 10 min for faba bean pasta (Rosa-Sibakov et al., 2016). These changes in the process conditions are necessary to overcome the increased size of dough particles and the decreased the extrusion pressure (Petitot et al., 2010b). When high level of substitution (>30-35%) are used a sticky and heterogeneous large dough lumps are formed and render the extrusion step difficult (Petitot et al., 2010b; Wood, 2009). This increase in dough stickiness is related to the absence of gluten and to the high level of soluble globulins and albumins in legume proteins which weakens the gluten matrix (Wood, 2009). In addition, the fine particles of legume flours in comparison with wheat semolina, and the different composition on protein and fiber which when mixed with water develop a sticky and gummy mass (Jayasena & Nasar-Abbas, 2012), induces a variability in water absorption between dough particles (Petitot et al., 2010b). A reduction of 2-3% of the total water added, and an increase in the mixing speed from 60 to 120 rpm limits the formation of aggregates and dough lumps (Petitot et al., 2010b).

In the case of total substitution of wheat by legume flours (100% legume pasta), authors often change the traditional process steps to prevent the alteration in the rheological behavior of the dough and/or cooked pasta, in order to obtain good quality legume pasta. Particularly, the mixing step is performed in hot suspension of water and emulsifiers to form an elastic dough which is then rolled (Frias et al., 1997). The temperature of extrusion is also increased to 70-135 °C to ensure the integrity of pasta (Wang et al., 1999). Some studies, reported the use of traditional process in pasta with 100% legumes. However, in these studies the dough is processed into spaghetti using a small scale laboratory press (Fares & Menga, 2012; Rosa-Sibakov et al., 2016).

5. Impact of pasta processing and cooking steps on the state diagram and the structure of wheat pasta macro-components

Several studies demonstrated that polymer science principles can be applied to food systems (Brent et al., 1997; Cuq et al., 2003a; Cuq et al., 2003b; Huang et al., 1996; LeMeste et al., 1996; Roudaut & Wallecan, 2015). The development of cellular structure in food products is influenced by the changes in the molecular organization of polymers throughout the processing steps (Cuq et al., 2003a; Thanatuksorn et al., 2007). State diagrams of main polymers in pasta, ie. starch and proteins were established by several authors (Bengoechea et al., 2007; Cuq & Icard-Verniere, 2001; Fujio & Lim, 1989; Hoseney et al., 1986; Huang et al., 1996; Kalichevsky et al., 1992; Micard et al., 2001; Zeleznak & Hoseney, 1987). A plot of the evolution of the physical state of starch and protein during pasta process is presented in figure 1.2 and discussed for each process step i.e. hydration-mixing, extrusion, drying and cooking.

5.1.Hydration-Mixing step

In native wheat semolina, starch polymers (semi-crystalline, with some amorphous zones) and proteins (amorphous) are found in a glassy and stable state. At moisture levels greater than 15% for protein and 22% for starch, glass transition occurs below the room temperature (figure 1.2). During hydration (symbolized by arrow 1 on figure 1.2) the water content is increased from 13 to 47% (db). This increase in semolina hydration leads to an increase in molecular mobility and glass transition of proteins and amorphous regions of starch granules, resulting on a rubbery, soft and reactive material (Landillon et al., 2008; Saad et al., 2011). The increase in wheat components reactivity by hydration is reported to induce various biochemical mechanisms. Some disulfide bounds present in semolina proteins are broken resulting in a slight depolymerization of larger and smaller wheat glutenin polymers ($\simeq 9\%$) (Petitot et al., 2010a). The secondary structure of protein in pasta dough is also affected increasing β -sheet structures at the expanse of α -helix in comparison to raw material (Bock et al., 2015). These modifications of the molecular structure of gluten proteins does not permit the formation of gluten network (Matsuo et al., 1978), due to the limited hydration (13-47%, db) level and low energy input (Icard-Verniere & Feillet, 1999).

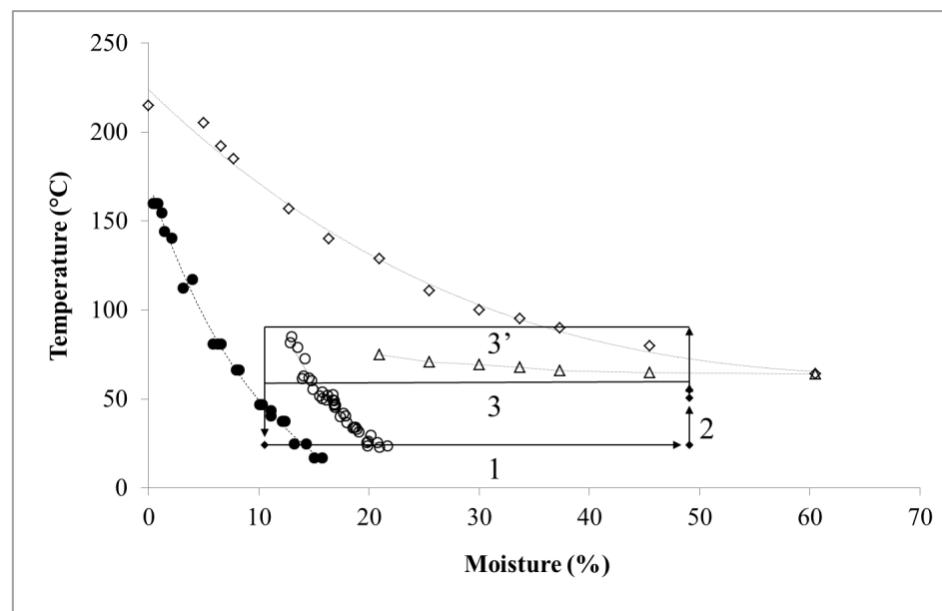


Figure 1.2 State diagram of wheat starch and protein as a function of the temperature and the moisture.

Arrows with numbers indicate various steps of pasta processing. 1, mixing; 2, extrusion (at 40 °C); 3, low temperature drying (55 °C); 3', very high temperature drying (90 °C). o glass transition of native starch (Zeleznak & Hoseney, 1987); • glass transition of gluten proteins (Hoseney et al., 1986); Δ starch gelatinization and ◊ starch melting (Kaletunc & Breslauer, 1996).

5.2.Pasta forming step by extrusion

During pasta forming (symbolized by arrow 2 on figure 1.2), there is no change in the dough water content and the temperature remains lower than 50 °C. Only mechanical energy (66 kJ/kg in the case of extrusion) is transferred to the dough (Abecassis et al., 1994). The fresh pasta obtained after forming step behaves as a visco-plastic and soft product (Cuq et al., 2003b). At the molecular scale, extrusion promotes the formation of larger protein polymers by glutenin aggregation, as result of mechanical and local thermal forces involved (Petitot et al., 2009a). These forces also affects starch fraction reducing its gelatinization enthalpy meaning that some starch damage and/or gelatinization can occur during pasta extrusion (Petitot et al., 2009a). At microscopic scale, the formation of discontinuous proteinaceous particles in the form of platelets and jagged edges is visible; but the entire development of protein network (under sheet and fibril forms) remains unachieved.

5.3.Drying step

During drying (symbolized by arrows 3 and 3' on figure 1.2), around 65% of the total water is removed from fresh pasta. Cuq et al. (2003b) reported that mechanical properties of

pasta transits from a rubbery to a glassy elastic and rigid state for dried pasta. According to the figure 1.2, the decrease in moisture content of starch and gluten induces an increase in their glass transition temperature. Thus, at low temperature drying (40-60 °C) (arrow 3, figure 1.2), the glass transition occurs at 15 to 18% moisture for starch and at 9 to 12% moisture for proteins. At high temperature (90 °C) (arrow 3', figure 1.2) less than 15% and 9% moisture are required for starch and protein (respectively) to reach the glassy state. Whatever the drying temperature (55 or 90 °C), starch passes the glass transition during the drying cycle, whereas for proteins, the glass transition occurs during the end of drying cycle when the temperature decreases from 55 °C to room temperature.

The hydrothermal conditions used to move pasta constituents through glass transition affect the molecular conformation of proteins and starch. Figure 1.3 shows the amount of covalently linked protein (disulfide and others) in semolina (temperature = 25 °C) and in pasta dried using different hydrothermal diagrams. Only a little increase in the degree of protein polymerization in comparison to semolina occurs when pasta is dried at 55-60 °C. Around 30% of total proteins are linked through covalent bounds (counter 15-20% in semolina). The remaining protein fraction is mainly linked by weak bridges (Laleg et al., 2016a; Petitot et al., 2009b). Most changes on protein aggregation occur above 70 °C (figure 1.3). This temperature at which the denaturation of wheat gluten begins to occur is reported to be accompanied by the change of its mechanical properties called “thermosetting” (Kokini et al., 1994). When the drying temperature of pasta is higher than 80 °C, a dramatic increase in protein aggregation occurs (figure 1.3A). At 90 °C more than 70% of total proteins polymerize through covalent bounds, which could be constituted of disulfide and other crosslinks such as isopeptide or Maillard-type-reaction (Petitot et al., 2010a; Petitot et al., 2010b). In addition, secondary structure of dried pasta is reversed to structural distributions similar to those of starting material, by decreasing β -sheet and increasing α -helix structures (Bock et al., 2015).

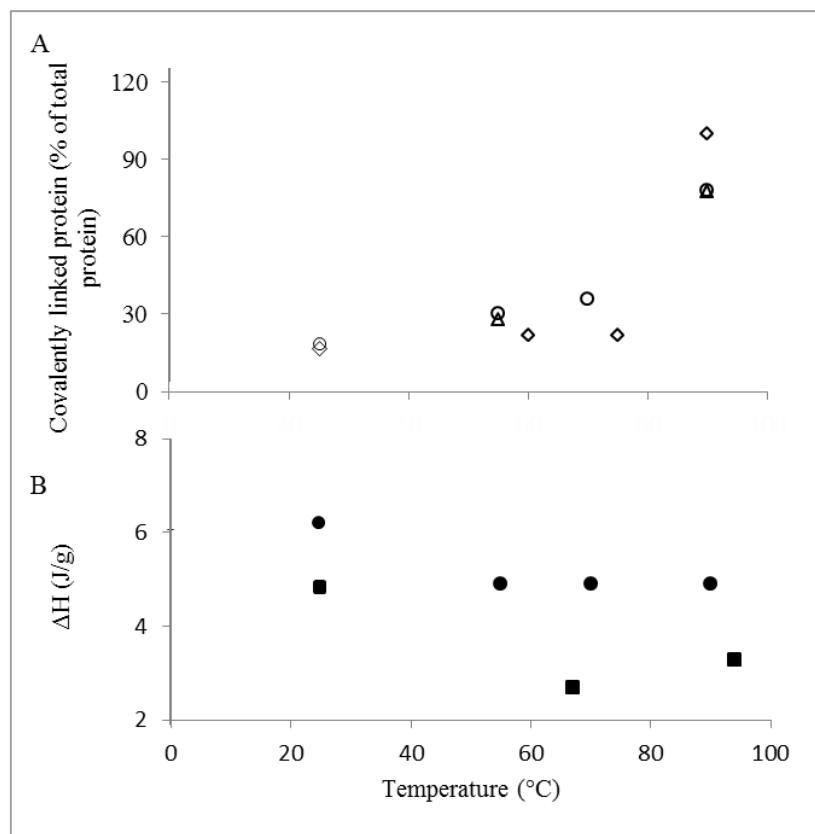


Figure 1.3 Plots of the extent of covalently linked protein non-extractable in sodium dodecyl sulfate (using SE-HPLC, A), and the enthalpy (ΔH) of starch gelatinization (using differential scanning calorimetry, B) in semolina (temperature = 25 °C) and in pasta under different drying temperatures.
A: protein aggregation: ○ adapted from Petitot et al. (2010a), ◇ from Lamacchia et al. (2007) and △ from Stuknyte et al. (2014). B: Starch gelatinization enthalpy ● from Petitot et al. (2009b) and ■ from Guler et al. (2002).

Starch also undergoes some conformational modifications which appear to occur mainly when pasta is dried at temperature higher than 70 °C according to the figure 1.2. Figure 1.3 presents the variation of gelatinization enthalpy of starch obtained from semolina and pasta dried at different temperatures. The gelatinization enthalpy of starch is lower in dried pasta than in semolina. Drying can lead to partial melting of starch (Altan & Maskan, 2005; Guler et al., 2002; Yue et al., 1999) and to the formation of amylose-lipid complexes (Yue et al., 1999). However, the impact of the drying cycle on gelatinization enthalpy is not clear yet (Petitot et al., 2009a). Indeed, according to the figure 1.3, drying pasta at temperature higher than 70° can increase the gelatinization enthalpy (Guler et al., 2002) or not (Petitot et al., 2009b).

5.4.Cooking step

The state diagram of pasta constituents during cooking is presented in figure 1.4. The cooking step is performed in excess and boiling water. Water diffuses concentrically to the pasta core, and increases polymer mobility because of its plasticizing effect. Starch granules swell, become partly soluble, and reach a state of gel (Kaletunc & Breslauer, 1996). Whereas gluten proteins (gliadins and glutenins) acquire a form of an entangled polymer (Kokini et al., 1994) with enough mobility to enter a reaction zone and to aggregate through crosslinks (Kokini et al., 1994), leading to an insoluble matrix (Resmini & Pagani, 1983). However, gluten does not achieve a softening state because the temperature of pasta cooking remained lower than 130 °C (figure 1.4).

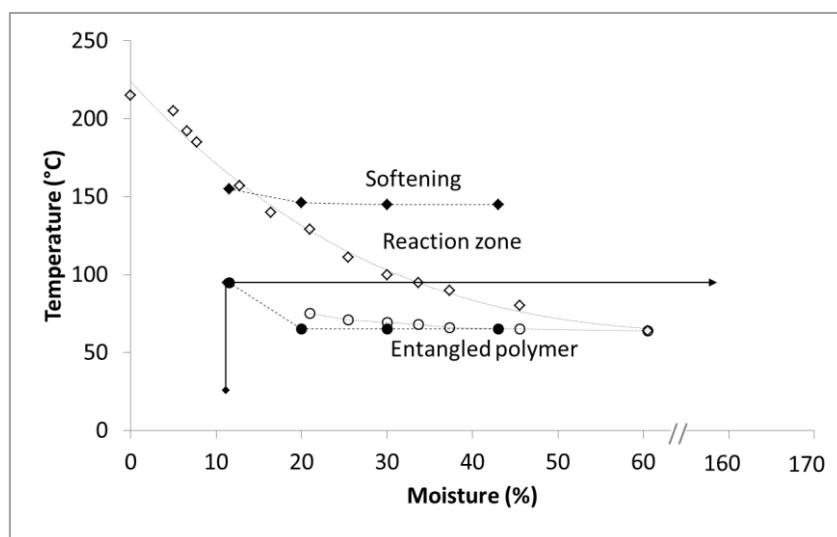


Figure 1.4 State diagram of wheat starch and protein as a function of cooking water temperature and the moisture during pasta cooking step represented by Arrow.

o starch gelatinization and ◊ starch melting (Kaletunc & Breslauer, 1996); ● glutenin thermosetting and ◆ glutenin softening (Kokini et al., 1994).

The change in the physical state of starch and proteins are related to a conformational reorganization at the molecular scale, where an extensive polymerization of protein occurs. As a result, around 85% of pasta proteins are linked through disulfide and other covalent crosslinks after cooking step (Petitot et al., 2010a). Secondary and tertiary conformations of proteins are also affected by the cooking of pasta. Cooked pasta loss all the α -helical structures and some of the β -turns and random structures, whereas increasing the β -sheet related to a gluten protein aggregation (Bock et al., 2015). Regarding starch fraction, the absence of gelatinization endotherm is observed by DSC as pasta is cooked to its optimal cooking time ie. to the entire gelatinization of its starch content (Petitot et al., 2009b).

Starch gelatinization and protein denaturation occur in the same range of temperature (Cuq et al., 2003a; De Noni & Pagani, 2010). These two competing phenomena built the ultimate structure of pasta where starch granules are held into a strong protein network (Cunin et al., 1995). The rate of starch gelatinization and protein aggregation govern the textural and cooking quality of pasta (Resmini & Pagani, 1983).

Each of the structural transformation occurring during the final cooking step is determined by those occurring during previous pasta processing steps and by the pasta composition. The modification of one or both of these parameters, as the variation of drying temperature or the introduction of legume flours in the formulation of pasta, can affect the pasta specific structure and its related nutritional properties.

6. Impact of legume flour substitution on the state diagram and the structure of pasta macro-components

Only few studies describe the glass transition of starch and proteins in legume (pea and soy) (Mizuno et al., 2000; Pelgrom et al., 2013). As for wheat polymers, a reversible glass transition phenomenon occurs near of the ambient temperature in legume starch and proteins depending on the water content and mobility. The two state diagrams of legume and wheat polymers are presented in figure 1.5. As for wheat, the DSC analysis of legume shows that an increase in water content or of the temperature induced the glass transition of protein first followed by the glass transition of starch (Pelgrom et al., 2013). Starch and proteins in legumes undergo glass transition approximately in the same range of water and temperature than starch and protein in wheat. Thus when legume flours are added to pasta, no disruption in the glass transition occurrence is expected to affect the pasta during the mixing, forming and drying steps.

The enrichment of pasta with 35% legume (faba bean or split pea) increases protein solubility by gluten dilution, during mixing and forming steps (Petitot et al., 2010a). Figure 1.6A shows a higher solubility of proteins in mixed wheat-legume than in wheat dried pasta (represented by shaded area in figure 1.6A) whatever the drying temperature. The use of temperatures higher than 80 °C to dry pasta (figure 1.6A) help to recover higher level of covalently linked protein in legume enriched pasta, but it still lower than those observed in wheat pasta (~90% and ~60% of covalently linked protein under drying at 90 °C in wheat and legume enriched pasta, respectively).

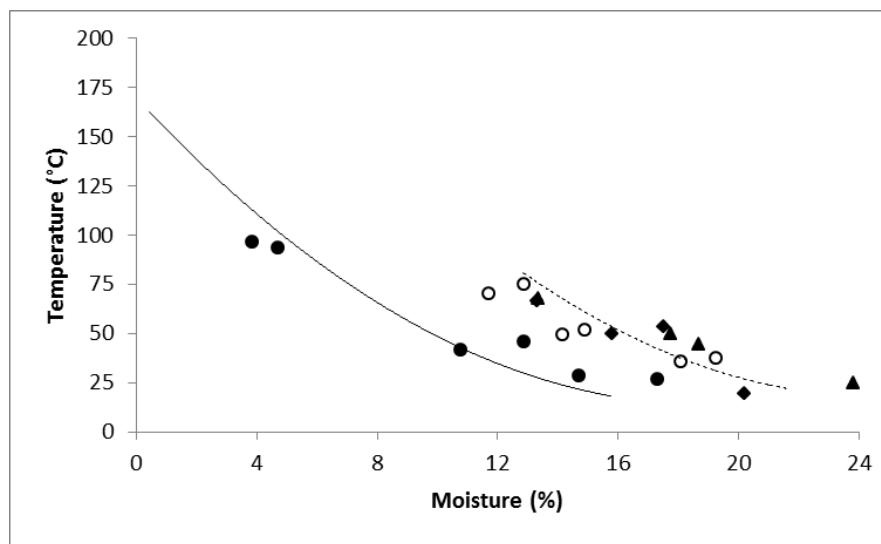


Figure 1.5 Plots of the glass transition of legume starch and proteins and schematic representation of the glass transition of wheat starch and proteins.
○ Pea starch, ● Pea protein (Pelgrom et al., 2013), ◆ Soy globulin 11S and ▲ Soy globulin 7S (Mizuno et al., 2000). The glass transition of wheat protein (—) and starch (....) are drawn according to the figure 1.4.

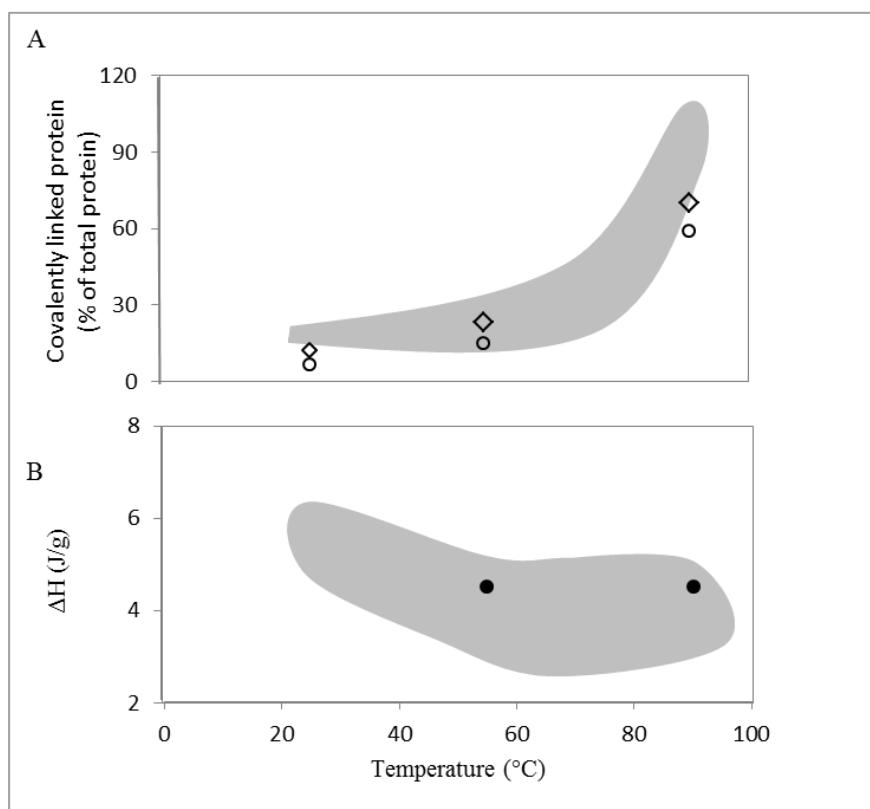


Figure 1.6 Effect of legume flour incorporation (35%) on the extent of protein aggregation (A) and on the enthalpy (ΔH) of starch gelatinization of legume-wheat enriched pasta under different drying conditions, and of raw material used to produce pasta (temperature=25°).

Values are compared to a wheat pasta (shaded area, drawn according to the figure 1.3). ○ Pasta enriched with 35% split pea, ◊ pasta enriched with 35% faba bean, ● pasta enriched with 35% faba bean or split pea. (Petitot et al., 2010a; Petitot & Micard, 2010)

Concerning starch fraction (figure 1.6B), starch gelatinization enthalpy is not affected by the enrichment of pasta with 35% legume flours in comparison to wheat pasta. In addition, no effect of drying temperature is reported on the gelatinization enthalpy of 35% legume enriched pasta (Petitot et al., 2009b; Petitot & Micard, 2010).

7. Structural typology of pasta as affected by its composition and its process conditions

Starch and gluten are the main structuring components in wheat pasta. The microscopic structure of wheat pasta cooked to its optimal cooking time is described as a compact structure where starch granules are confined in a porous (1-40 µm) strong viscoelastic protein network (Cunin et al., 1995). These two immiscible fractions will give a homogenous structure at the macroscopic scale. At the microscopic scale, three main regions are distinguished in cooked wheat pasta depending on the extent of water diffusion (figure 1.7A). An external region, where a thin protein films surround extensively swollen and deformed starch granules, an intermediate region including partly swollen starch surrounded by a dense protein network, and a central region where starch granules are less swollen and compacted in a well discernible protein network (Cunin et al., 1995; Heneen & Brismar, 2003). According to Petitot et al. (2010a), no specific changes in protein and starch distributions are observed by bright field light microscopy when 35% of legume flour replaced wheat in pasta (figure 1.7B). However, pasta made exclusively from legume flour (figures 1.7C and 1.7D) with higher protein content (35%), shows starch embedded in a denser protein matrix compared to that of 100% wheat pasta and 35% enriched pasta with lower protein content (13-17%). In addition, in the pasta core of 100% legume pasta, starch granules are less uniformly surrounded by the protein network and tends to aggregate (figure 1.7C) whereas, the structure of pasta in its outer layer (figure 1.7D) is disturbed leading to solid leaching in the cooking water (Rosa-Sibakov et al., 2016).

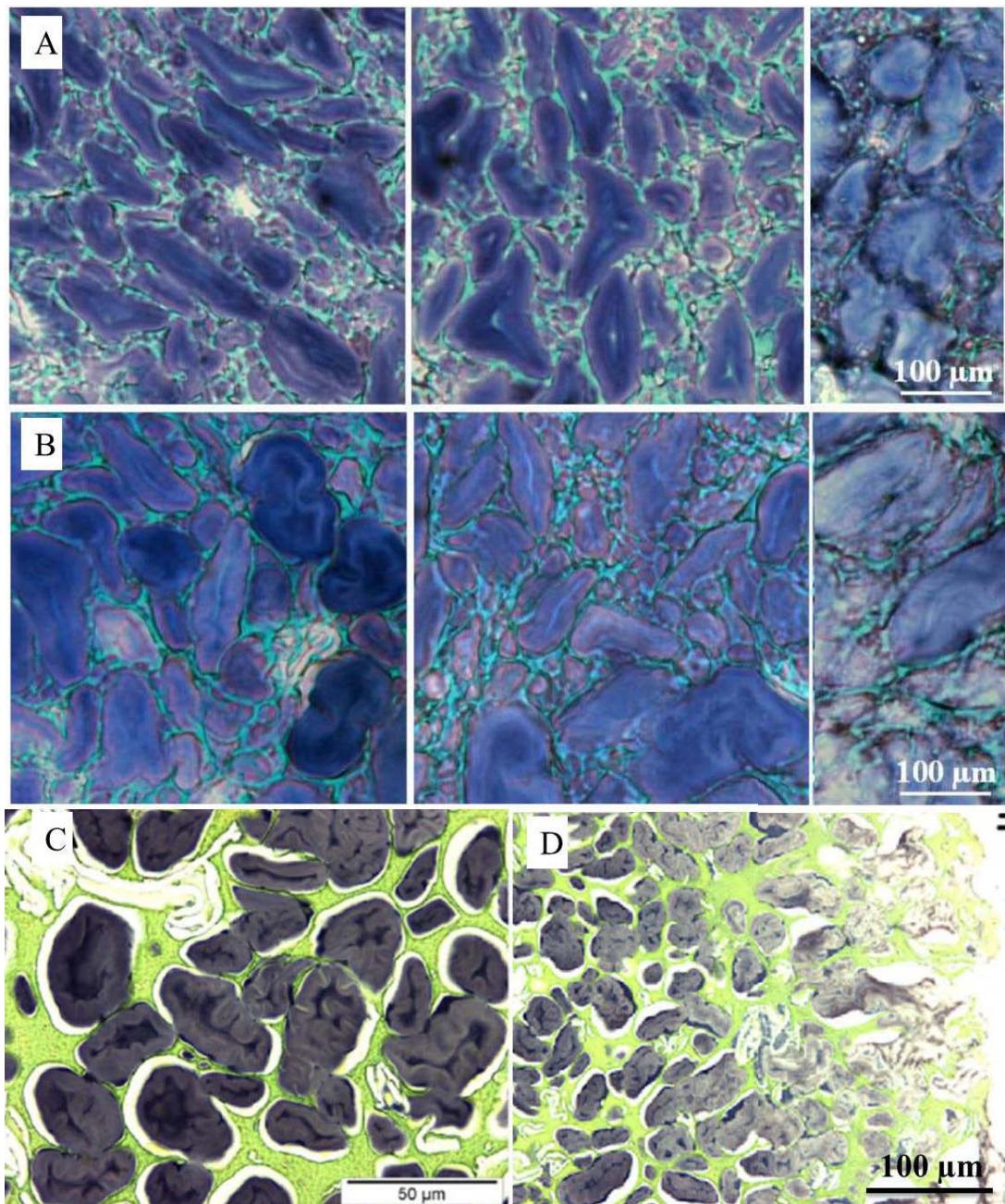


Figure 1.7 Bright field light microscopy images of the internal structure of cooked (optimal cooking time + 1 min) pasta from the central (left) to the external region (right) of (A) durum wheat pasta, (B) 35% faba bean enriched pasta (Petitot et al., 2010a) and (C) 100% faba bean pasta (cooked to its optimal cooking time) (Rosa-Sibakov et al., 2016). Proteins are stained in green while starch granules stained blue.

8. Cooking, textural and sensory properties of legume based pasta

The amount of gluten protein in pasta is believed to be the main factor governing its overall integrity, its cooking and textural properties and its sensory qualities. For this reason, the impact of legume pasta enrichment on their cooking, textural and organoleptic properties is discussed here considering the ratio of protein from legume (used to substitute gluten) to the total protein content of pasta (table 1.5). The limit of protein from legume which could be incorporated to pasta is defined by authors considering the pasta processing abilities, its final properties and consumer acceptability. The incorporation in pasta of legume to reach a concentration of protein coming from legumes higher than 45% began in 2010 (Petitot et al., 2010b). Previously, the level of legume enrichment did not exceed 30% (on protein base). Indeed, technological issues appear when the concentrations of protein from legume are over the limit suggested in table 1.5. These technological issues concern, beside the different steps of pasta processing (section above), the alteration in cooked pasta properties, and the decrease of its general acceptability. High cooking losses are major parameter related to the depreciation of pasta quality and concern most legume species, however lupin at a lesser extent (Jayasena & Nasar-Abbas, 2012; RayasDuarte et al., 1996). The addition of soluble non-gluten protein dilutes the gluten network. This dilution depreciates the strength of protein network and weakens the overall structure of the pasta resulting in leaching of more particles into the cooking water (RayasDuarte et al., 1996). Lost material can be constituted of amylose, salt-soluble proteins or pasta fragments (Petitot et al., 2010b; Rosa-Sibakov et al., 2016). Pasta enrichment is also associated with a decrease cooking time, which is explained by a physical disruption of the gluten matrix by the high quantity of fibers coming from legume flours which have facilitated the diffusion of water to the core of pasta (Petitot et al., 2010b).

Table 1.5 Impact of pasta substitution with different legumes on its cooking, textural, color and sensory properties

Legume type	Substitution level ¹	Impact on pasta properties				Reference
		Cooking	Textural	Color	Sensory	
Lupin ²	18	Increase in cooking loss			Decrease general pasta acceptability	(Torres et al., 2007a)
	28	Slight increase in cooking loss	No change in firmness	Increase in color score	Increased adhesiveness and grittiness without effect on flavor	(Rayas-Duarte et al., 1996)
	43	No effect on cooking loss, Decrease in cooking time	No effect on firmness, Stickiness decreases	Increase in redness and yellowness and a decrease in brightness	Decrease in general pasta appearance taste and overall acceptability	(Jayasena & Nasar-Abbas, 2012)
Lentil ²	23	Increase in cooking loss without effect on cooking time or cooking weight	Increase in firmness	Decrease in brightness and yellowness and increase redness		(Zhao et al., 2005)
	48	Increase in cooking loss	Decrease in hardness	Decrease in brightness increase in green color and yellowness	Poor taste and beany flavor Low score of consistency and high stickiness	(Wojtowicz & Moscicki, 2014)
Chickpea ²	15-29	increase in cooking loss, decrease in water uptake			Decrease in color, flavor and overall acceptability scores	(Sabanis et al., 2006)
	30	Increase in cooking loss without effect on cooking time or cooking weight	Depending on the variety, firmness increases or not	Decrease brightness and yellowness and increase redness		(Zhao et al., 2005)
	29	No effect on cooking loss	Decrease in firmness, resilience and stickiness	Decrease in brightness Increase in redness		(Wood, 2009)

¹ g of legume protein per 100g total proteins, ² the impact given occurs when higher than the suggested limit of legume protein is added to pasta, ³ the impact given concerns the presented pasta.

Legume type	Substitution level ¹	Impact on pasta properties				Reference
		Cooking	Textural	Color	Sensory	
Pea ²	19			Decrease in color score	Unpleasant color and higher firmness, decrease in elasticity, in bulkiness, taste and overall pasta quality	(Padalino et al., 2014)
	29	Increase in cooking loss without effect on cooking time or cooking weight	Depending on the variety, firmness increases or not	Decrease brightness and yellowness without effect on redness		(Zhao et al., 2005)
	45	Increase in cooking loss	Decrease in hardness	Increase in green color and yellowness	Poor taste and beany flavor low score of consistency and high stickiness	(Wojtowicz & Moscicki, 2014)
	47 ³	Increase in cooking loss, decrease in cooking time and water uptake	Increase in hardness, decrease in breaking energy	Decrease in brightness	Increase in fracturability, increase in hardness and elasticity and decrease in surface smoothness	(Petitot et al., 2010b)
Black-gram ²	66	Increase in cooking loss	Decrease in firmness, increase in stickiness	Decrease in redness and yellowness and increase in brightness	Decrease in general appearance and overall quality score and off flavor	(Jyotsna et al., 2015)
white bean ²	46	Increase in cooking loss	Decrease in hardness	Increase in green color	Poor taste and beany flavor Low score of consistency and high stickiness	(Wojtowicz & Moscicki, 2014)
Faba ³	57	Increase in cooking loss, decrease in water uptake			Positive effect on the firmness No changes in the stickiness	(Gimenez et al., 2012)
	54	Increase in cooking loss, decrease in cooking time and water uptake,	Increase in hardness, Decrease in resilience	Decrease in brightness	Increase in fracturability, increase in hardness and elasticity	(Petitot et al., 2010b)
	100	Increase in cooking loss, decrease in cooking time and water uptake	Increase in hardness		decrease in elasticity, increase in hardness and Shewyness and intense flavor	(Rosa-Sibakov et al., 2016)

¹ g of legume protein per 100g total proteins, ² the impact given occurs when higher legume protein than the suggested limit is added to pasta, ³ the impact given concerns the presented pasta.

A decrease in legume pasta resilience and breaking energy, and an increase in stickiness are also generally reported when 29% or greater protein from legume are added to pasta. A less clear relation can be found between the pasta firmness or hardness and the concentration of legume proteins. These parameters increase when incorporating legume from *Vicia* genera (faba bean) in pasta, decrease with *Phaseolus* (white bean and black-gram) genera, whereas remain unchanged with *Lupinus* incorporation into pasta, even when the concentration of lupin protein increases from 28 to 43%. Pasta hardness and firmness can also be related to the amount of water uptake during pasta cooking, to the total pasta protein (Petitot et al., 2010b), to the amylose content (Gianibelli et al., 2005), to the non-starch polysaccharide content or to the gluten concentration (Wood, 2009). In addition pasta firmness may behave differently with respect to the method used to access firmness parameters. Pasta color also changes by increasing protein from legume: brightness generally decreases independently from the legume type (except for black-gram) and the level of enrichment, whereas yellowness and green colors vary according to the legume type and the color of raw material (Zhao et al., 2005).

Sensory properties of legume pasta are one of the major parameters used by the authors to define the critical limit of legume enrichment. The decrease in overall pasta acceptability is related to a poor taste, beany flavor, and to a decrease in the scores of major textural parameters, notably firmness and elasticity. The decrease in sensory acceptability related to taste or flavor concerns all the enrichment levels and legume types except for lupin, where the off taste appears only when the enrichment exceed 43% (on protein basis). In conclusion, the highest gluten substitution levels with legume proteins leading to an acceptable pasta quality would depend on the legume type.

Some technological treatments applied to pasta help for recovering the loss in some pasta properties due to the legume enrichment. The extrusion-cooking (75-98 °C) (Wojtowicz & Moscicki, 2014) and the high temperature drying (70-90 °C) (Petitot & Micard, 2010) techniques are successfully applied to process legume pasta. They help to hinder the cooking loss and to increase the resilience and the breaking energy of pasta made from legume. However, pasta subjected to high thermal treatment pasta have a low color score mainly with higher redness and lower yellowness and brightness, indicating the occurrence of Maillard reactions between sugar and free amino acids which can reduce the nutritional value of proteins (Petitot et al., 2010b).

In addition, some additives such as gluten or hydrocolloids can be added to legume pasta in order to strengthen their structural integrity. Gluten functionality can indeed be mimicked by the formation of a network structure encapsulating starch granules (Sozer, 2009). Two percent of guar gum added to pea enriched pasta enhances the overall sensory quality of pasta and its instrumental adhesiveness, whereas no effect is observed on its cooking loss and hardness (Padalino et al., 2014). The use of 2% gluten and 0.5% glycerol mono stearate or sodium stearoyl lactylate improves the quality of legume enriched pasta with 46% protein from green pea flour reducing its cooking loss, increasing its firmness and improving its sensory texture and taste scores (Sudha & Leelavathi, 2012). However, the utilization of these additives remains limited because consumers often associate their presence in pasta to an “artificial” food (Marti & Pagani, 2013).

9. Impact of legume-wheat substitution on the nutritional value of pasta

9.1. Starch

Dietary carbohydrates are characterized considering their chemical composition and the rate and the extent of their digestion. The glycemic index is used to rank foods based on their postprandial blood glucose response (Jenkins et al., 1981). It is based on the measurement of the postprandial glycemic area of a test meal, expressed as the percentage of the corresponding area of a reference food (glucose or white bread) (Figure 1.8). Increasing the consumption of low glycemic index carbohydrates is associated with the protection against some diseases such as diabetes, coronary heart disease, obesity and even colon cancer (Jenkins et al., 2002; Opperman et al., 2004). Among cereal products, pasta is considered as a source of slow-release carbohydrates, therefore possessing a low glycemic index (Table 1.6) (Fosterpowell & Miller, 1995; Granfeldt & Bjorck, 1991; Jenkins et al., 1983). The slow rate of starch hydrolysis in pasta is related to various parameters such as its compact structure (Fardet et al., 1998a; Granfeldt & Bjorck, 1991), the encapsulation of starch granules by fibers (Tudorica et al., 2002) or by proteins (Colonna et al., 1990; Fardet et al., 1998b), the degree of gelatinization and retrogradation of starch granule and the amylose-to-amylopectin ratio (Akerberg et al., 1998; Holm & Bjorck, 1988).

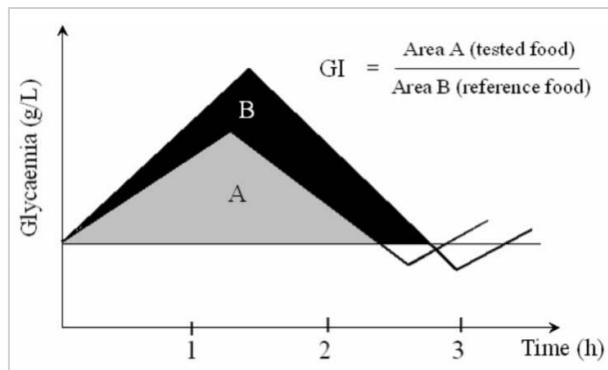


Figure 1.8 Schematic representation of the glycemic index calculation

The substitution of semolina with legume flours dilutes gluten protein and induces structural changes in the pasta (Petitot & Micard, 2010). Despite the weaker protein network in comparison to classical pasta, the *in-vitro* starch digestibility of 35-100% faba bean or split pea flours enriched pasta (Greffeuille et al., 2015; Petitot et al., 2010a; Rosa-Sibakov et al., 2016) is not higher in comparison to wheat pasta. In addition, the *in-vitro* starch digestibility of spaghetti fortified with 25% of chickpea flour is even lower than in wheat spaghetti (Goni & Valentin-Gamazo, 2003; Padalino et al., 2014). *In-vitro* starch digestion is reduced from 84 to 62% in pasta enriched with 40% chickpea flour (Osorio-Diaz et al., 2008) and from 69 to 64% in pasta enriched with 19% protein from pea flour (Padalino et al., 2014). These results are confirmed *in-vivo*: the glycemic index of chickpea fortified pasta (58 ± 6) is significantly lower than the glycemic index of wheat spaghetti (73 ± 5). Recent *in-vivo* studies reported an identical glycemic index of wheat and legume enriched pasta (Greffeuille et al., 2015). The slow rate of legume starch digestion can be explained by several factors. The crystallinity of starch would be important: C-type starch found in legume is generally more resistant to digestion. The starch granule diameter, the molecular weight of amylopectin and amylose, the amylose content (Sandhu & Lim, 2008) and the inhibition of amylase by the presence of some anti-nutritional factors (Deshpande & Damodaran, 1990) are all parameters interfering with the rate of starch digestibility.

Table 1.6 Glycemic index of cereal and legume products, with glucose considered as the standard food

Food product	Glycemic index
French bread ¹	95±15
Couscous ¹	65±4
White rice boiled ¹	64±7
Rice and maiz pasta (gluten free) ¹	76±6
Macaroni ¹	47±2
Barilla durum wheat pasta boiled 10 min ¹	58
Black bean soaked and cooked ¹	20
Kidney bean dried boiled ¹	23±1
Chickpeas, dried, boiled ¹	31
35% faba bean enriched pasta ²	65 ± 3
100% faba bean pasta ³	36±3

¹ adapted from Foster-Powell et al. (2002), ² Greffeuil et al. (2015), ³ Rosa-Sibakov et al. (2016).

9.2. Proteins

Wheat proteins are known to be deficient in some essential amino acids such as lysine, the first-limiting amino acid and threonine, the second-limiting one (Abdel-Aal & Hucl, 2002; Arrage et al., 1992). Pasta processing, drying at low temperature (40 °C) and cooking reduce lysine, methionine, tryptophan, and histidine by 8, 16, 13 and 11%, respectively, but do not affect the *in-vitro* protein digestibility (Abdel-Aal & Hucl, 2002). However, increasing drying temperatures of pasta may lead to a reduced *in-vitro* protein digestibility probably due to the formation during drying of large protein aggregates stabilized by strong irreversible interactions (De Zorzi et al., 2007). High temperature drying also increases the loss of total and available lysine in cooked pasta (Acquistucci, 2000; Anese et al., 1999; Dexter et al., 1984; Resmini & Pellegrino, 1994). This decrease in lysine bioavailability could be due to the protein-carbohydrate Maillard reactions which involve reducing sugars and the terminal amino group of a free amino acid (Acquistucci, 2000; Anese et al., 1999). Moreover, wheat protein can act as food allergen in sensitized individuals (Rasanen et al., 1994; Simonato et al., 2004; Varjonen et al., 2000; Varjonen et al., 1997). All the potential allergens (> 1 kDa) are usually degraded after peptic/pancreatic digestion in both semolina and cooked pasta, although the presence of potentially allergenic protein fragments with very low Mr (< 1 kDa) in digested pasta cannot be excluded (Simonato et al., 2004).

The fortification of wheat pasta with legume flours increases the protein content of pasta up to 19% (Jyotsna et al., 2015; Padalino et al., 2014; Rajiv et al., 2014; RayasDuarte et al., 1996; Zhao et al., 2005). The chemical score of pasta represents the amount of the limiting

amino acid in food to the same amino acid in food reference and is used to evaluate protein quality (Gimenez et al., 2012). It is reported to be enhanced in faba bean enriched pasta (Laleg et al., 2016a). Legumes are an important source of food proteins and are relatively low in sulfur-containing amino acids (methionine, cysteine) and tryptophan but the amounts of lysine are much greater than in cereals grains (Alonso et al., 2000; Carbonaro et al., 1997). Wheat and legume present therefore a complementary amino acid profile.

The fortification of wheat pasta with 30-35% of lupin or faba bean flours increases significantly the *in-vitro* protein digestibility of cooked pasta (Laleg et al., 2016a; RayasDuarte et al., 1996), whereas no effect of cowpea flour (20%) supplementation on *in-vitro* protein digestibility is reported (Herken et al., 2006). The supplementation of pasta with 10% α -galactoside free lupin flour does not change the *in-vivo* protein digestibility in rats (Torres et al., 2007a). However, the protein efficiency ratio (the amount of weight gain per unit of protein consumed) which is used to predict the protein quality of foods, increases by two times (Torres et al., 2007a).

9.3.Others nutritional properties of legume fortified pasta

Fortification of pasta with lupin or pigeon pea flour increases the amount of dietary fiber, mineral, B1, B2 and E vitamin contents and antioxidant capacity in comparison to wheat pasta (Torres et al., 2007a; Torres et al., 2006). Fortification of pasta with legume (navy bean, pinto bean or lentil flour) is also a way to increase the protein, ash and fiber content of pasta (Bahnassey et al., 1986).

10. Impact of legume-wheat substitution on the anti-nutritional factors of pasta

Despite their potential interesting nutritional composition, legume fortified pasta can contain some bioactive compounds generally considered as “anti-nutritional factors” coming from legume flours. These compounds can alter the absorption of nutrients or induce intestinal discomforts. Food processing of legume prior to pasta production as soaking, germination and fermentation, is known to reduce them. In addition pasta processing and cooking can have an impact on these anti-nutritional factors.

The major anti-nutritional factors investigated in legume enriched pasta are protease inhibitor, alpha-galactoside and phytic acid. Phytic acid is a hexaphosphoric acid ester of the hexahydric cyclic alcohol meso-inositol, located in the protein bodies of the endosperm (Campos-Vega et al., 2010). Phytic acid chelates protein, starch and notably minerals (Ca, Fe

and Zn), lowering the bioavailability of those nutrients (Multari et al., 2015). Fermentation pre-process causes a reduction of about 48% of the total phytic acid in the pigeon pea seed by an enzymatic hydrolysis (phytase). Cooked pasta made from flour issued from this fermented legume flour still contains higher phytic acid than wheat pasta (1.8 counter 1 mg/g). 10% Lupin flour enriched pasta have 3 mg/g of phytic acid (Torres et al., 2007a).

Protease inhibitors inhibit the action of various enzymes such as trypsin, chymotrypsin and amylase, reducing therefore the digestibility of starch and protein. Trypsin inhibitory activity is reported to be highly sensitive to the processing and cooking steps of pasta. No residual activity is found in 10-20% pea, lentil and fermented pigeon pea flours enriched pasta (Torres et al., 2006; Zhao et al., 2005), while pasta fortified with 20% chickpea, and macaroni produced exclusively from pea, retains approximately 20-50% of the original trypsin inhibitory activity (Frias et al., 1997; Zhao et al., 2005).

α -galactosides are low molecular weight non-reducing sugars. The best-known α -galactosides are raffinose, stachyose, verbascose, and ajugose. These compounds are $\alpha(1 \rightarrow 6)$ galactosides linked to the C-6 of the glucose moiety of sucrose (Martinez-Villaluenga et al., 2008). Excessive α -galactosides in the diet is believed to induce flatulence or digestive discomfort, as they are not digested in the upper part of the gastrointestinal tract but fermented in the colon and induce the production of gas (CO₂, H₂ and CH₄) and short-chain fatty acids (Guillon & Champ, 2002). α -galactosides are not detected in cooked pasta supplemented with 10% pigeon pea (Torres et al., 2006), because of their enzymatic (α -galactosidase and invertase) hydrolysis during the fermentation preprocess performed prior to pasta production.

Anti-nutritional factors are now recognized to have also beneficial effects on human health. Indeed, phytic acid has a preventive action against cancer by chelating metals involved in DNA damage (Midorikawa et al., 2001), and reduces bioavailability of toxic heavymetals present in the diet such as cadmium (Campos-Vega et al., 2010), whereas α -galactosides stimulate bifidobacteria and present some prebiotic activity (Guillon & Champ, 2002; Martinez-Villaluenga et al., 2008).

11. Conclusion

Wheat and legumes are nutritionally complementary. Their combination in a single food product such as pasta is a way to benefit from the nutritional value of both raw materials notably, high protein quantity and quality, fibers vitamins and minerals. It also permits to decrease its starch digestibility. However, legume flour addition above 10-15% can affect the quality (texture and flavor) of pasta. Moreover, high level of legume flour addition may induce the formation of sticky dough during mixing, and prevent its extrusion. The development of legume fortified pasta requires therefore a compromise between processing ease, consumer acceptability and nutritional gain.

Partie 2. Quality and utilization of protein in human diet

1. Statement on protein consumption and requirements

Proteins are considered to be one of the most important macronutrients of the human diet. The average dietary protein requirement established for healthy adult is 0.66 g/kg of body weight/day, whereas the recommended dietary allowance is 0.83 g/kg of body weight/day (ANSES, 2007b). According to the FAO (Food and Agriculture Organization), the average world consumption of protein was estimated in 2009-2011 to be closed to 79 g/person/day. This consumption is imbalanced in the parts of the word. A consumption of only 74 g/person/day is observed in developing countries, whereas an amount of 103 g/person/day is consumed in economically developed countries. In addition, age and sex can modulate protein intake. In France, averages amounts of 68.5 and 78.9 g/day of dietary protein intake are reported to be consumed by older women and men of 57-80 years-old, versus 85.8 and 107.8 g/day for younger women and men, respectively (ANSES, 2007a). In elderly, the average protein requirement is established to be greater, i.e. 1g/kg/day instead of 0.8 g/kg/day in younger people. Protein requirements for children and infant are also higher in comparison to adults, because of the physiological protein requirements for maintenance and for growth. Protein requirements also increase according to the physiological conditions, such as pregnancy and lactation during which requirements are based on the quantities of nutrients deposited in the foetus, the placenta and the mother's body.

Proteins are composed of amino acids which are involved in the turnover of body proteins. Some of them could not be produced by the body, and have to be inevitably included in the diet. The French Agency for Food, Environmental and Occupational Health and Safety (ANSES, « Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail »), established the “ideal” profile of essential amino acids required in humans (table 1.7). As for proteins, these amino acid requirements are modulated by several metabolic parameters such those related to age. For instance, children require a higher amount of essential amino acids than younger adults. In addition, elderly evidenced a higher need of some essential amino acids such as branched chain amino acids, lysine and methionine, than younger adults (Tuttle et al., 1965). A lack of one or more of these amino acids can affect the utilization of other amino acids by the body, especially for body protein turnover (Rémond, 2015).

Table 1.7 Essential amino acid profile proposed by ANSES (2007a) as a reference profile

Essential amino acid	Infant (mg/g of protein)	Adult (mg/g of protein)
Histidine	28	17
Isoleucine	62	27
Leucine	113	59
Valine	64	25
Lysine	78	45
Methionine + cysteine	40	23
Phenyl alanine + tyrosine	92	41
Threonine	52	25
Tryptophan	24	6

2. Protein and amino acid content of dietary sources

Dietary proteins could be provided by animal and plant foods. In general, the content of proteins in animal products such as meat, egg and fish, is higher in comparison to vegetable proteins (table 1.8). Only 152g of meat could satisfy body requirements for proteins in a 70kg-adult versus 1 kg of wheat pasta or 2.4 kg of cooked potatoes. Some vegetable products such as legumes also provide a protein quantity closed to those observed in animal products.

Table 1.8 Protein content of some vegetable and animal products

Food	Moisture (g/100g)	Protein content (g/100g)
Meat (Beef, lean, fried) ¹	58	30.4
Poultry (Chicken) ²	66	20.0
Fish (fresh) ²	74	18.8
Egg (boiled) ¹	75	12.1
Milk (pasteurized) ¹	88	3.2
Potatoes (cooked) ¹	78	1.9
Faba bean flour ³	10	28.8
Lentil flour ³	11	24.0
Mung bean flour ³	9	23.7
Wheat semolina ⁴	0	13.3
Wheat pasta (cooked) ⁵	67	4.7
35% faba bean enriched pasta (cooked) ⁵	67	6.3

¹ (FAO, 2007), ² FAO (1970), ³ Naivikul and Dappolonia (1978), ⁴ Petitot et al. (2010b), ⁵ Greffeuil et al. (2015).

Both animal and plant proteins contain the 20 amino acids needed for body protein turnover, but their respective proportions differ according to the food product. Essential amino acid composition of some animal and plant products expressed as percent of the ANSES recommendations for adult people are given in table 1.9. Contrary to animal proteins, most plant proteins are poor in some amino acids such as lysine for cereal and cereal products,

and sulfur amino acids for legumes, and are not able to satisfy body requirement for essential amino acids.

Table 1.9 Amino acid content of some plant and animal foods expressed as percent of amino acid requirement recommended by ANSES (presented in table 1.7) for adult people.

	Casein ¹	Egg ¹	Beef ¹	Chicken ¹	Fish ¹	Semolina ²	Pasta ²	Faba bean ³	Lentil ³
Histidine	107	154	237	171	202	124	112	158	151
Isoleucine	208	260	220	223	191	115	111	164	166
Leucine	166	145	148	135	149	122	119	140	132
Valine	282	269	227	227	279	160	152	205	212
Lysine	188	197	216	179	205	49	47	151	152
Methionine + cysteine	137	252	181	169	167	170	161	81	81
Phenyl alanine + tyrosine	278	238	209	210	225	193	195	195	183
Threonine	191	192	189	197	199	124	116	160	161
Tryptophan	385	396	227	203	255	183	167	nd	nd
Total (mg/g protein)	690	658	656	617	663	464	451	510	497

¹Sosulski and Imafidon (1990), ²Abdel-Aal and Hucl (2002), ³Carbonaro et al. (1997). nd, not determined.

3. Protein utilization by the body

3.1.The concept of protein turnover

Body protein metabolism is a dynamic process in which proteins and amino acids are being continuously synthetized (anabolism) and degraded (catabolism) (Committee on Dietary Allowances, 1974). Nitrogen and amino acid homoeostasis is traditionally described in terms of maintenance and growth. Maintenance implies a neutral nitrogen balance between nitrogen input and output, whereas growth involves the deposition of proteins in tissues, i.e. a positive nitrogen balance (Millward & Pacy, 1995). The prevalence of protein synthesis indicates an anabolic state with an increase in lean mass, whereas higher protein breakdown in regard to protein synthesis results in a catabolic state accompanied with a net decrease in body protein content (Mauro, 2008). Amino acids used for protein synthesis are provided both by food and the reutilized body proteins from proteolysis (figure 1.9). In healthy conditions, the body fails to catch all the free amino acids and some of them are irreversibly lost by oxidation, forming metabolic products such as urea. These end-products are mainly excreted in urine. The body losses another fraction of protein in the *feaces*. Considering the total metabolic losses, around 70-90 g of proteins has to be brought by diet to ensure body protein turnover (ANSES, 2007a).

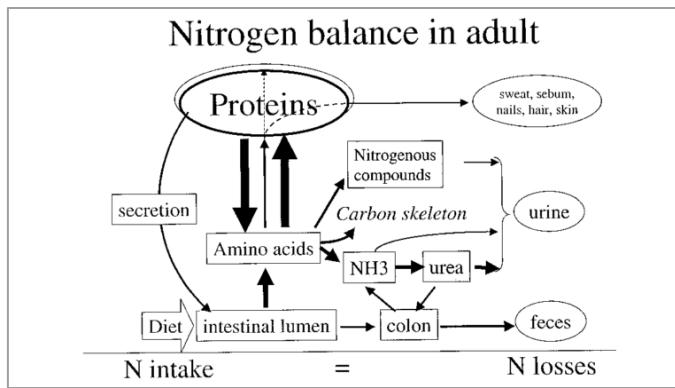


Figure 1.9 schematic representation of amino acid homeostasis (Tome & Bos, 2000)

Most of body proteins are located in skeletal muscles. The rate of muscle turnover depends on various parameters. Dietary amino acids are the most important dietary stimulus of the anabolic drive implying protein deposition and growth (Millward, 1990). However, the fact that the rate of protein turnover is affected by physiological conditions that are characterized by hormonal changes (such as fasting, disease or aging) (Cheng et al., 1987; Rennie, 1985; Welle et al., 1993), suggests that hormones are key regulator of protein metabolism. Some hormones such as insulin, growth hormone, insulin-like growth factor-1, and testosterone play an anabolic role, whereas others such as glucagon, glucocorticoids, and catecholamines are implicated in catabolic mechanisms. Among the anabolic hormone, insulin is able to respond acutely and sensitively to amino acid intake (Millward, 1990). The marked increase in insulin level exert a stimulatory effect on protein synthesis rates in growing rats, but this effect is reported to be attenuated by age (Baillie & Garlick, 1992). Rooyackers and Nair (1997) reviewed the effect of insulin on protein metabolism regulation in adult Humans and concluded that insulin acts mainly by inhibiting muscle protein breakdown without any effect on protein synthesis.

3.2. Protein digestion

Dietary proteins are degraded in the stomach (pepsin) and in the small intestine (pancreatin). The physiological aim of protein digestion and absorption is to provide amino acids to restore the post-absorptive losses of proteins and to ensure body protein maintenance and growth (Mariotti et al., 2000). The digestibility and absorption rate of dietary protein are different according to the protein sources, and they are governed by different parameters. Among these factors, the chemical structure of proteins plays an important role. The *in-vivo* digestibility of the soluble protein fraction of cooked faba and common beans, determined on growing rats, is higher than insoluble fraction (Carbonaro et al., 2000). Legume native protein structure and decreased protein solubility related to protein aggregation through

intramolecular disulfide bonds, electrostatic or hydrophobic forces are reported to be major parameters decreasing *in-vivo* protein digestibility of young rats (Porres et al., 2002). In addition, the steric hindrance of aggregated proteins restricts the access of proteolytic enzymes to peptide bonds and decreases the rate of protein hydrolysis and digestibility in rats (Kim & Barbeau, 1991). Genovese and Lajolo (1996) reported that only 32% of native albumine (*Phaseolus vulgaris*) are hydrolysed during 3 hours pepsin and 4 hours pancreatin, because of the compact structure of legume albumins involving disulfide bonds and the steric hindrance due to glycosylation. Heating (99 °C for 30 min), reduces this protein hydrolysis to 20% because of the creation of new disulfid bounds. The chemical deglycosylation increases proteolysis of both native (to 44.4%) and heated (to 30.7%) albumins. Carbonaro et al. (2012) reported that the β -sheet structures of raw legume proteins and the intermolecular β -sheet aggregates, arising upon heating, are primary factors adversely affecting the *in-vitro* protein hydrolysis.

Beside its effect on protein hydrolysis, the chemical structure of legume proteins can affect the absorption of nutrients. Pea globuline are reported to increase gut permeability toward dietary protein inducing a local exacerbated immune response leading to a digestif disorder (Crevieu-Gabriel, 1999).

Digestive losses can also be caused by various anti-nutritional factors present in foods (Millward et al., 2008; Sarwar Gilani et al., 2012). The presence of high levels of trypsin inhibitors, tannins, phytic acid, or lysoalanine (formed by heat treatment) in lactalbumin, casein, soya or wheat proteins can reduce protein digestibility in rats and pigs (Sarwar Gilani et al., 2012). The increase in endogenous protein excretion with cooked legumes is related to the residual anti-nutritional factors such as protease inhibitors, haemagglutinins (lectins), dietary fiber and tannins (Sarwar et al., 1989). The presence of trypsin inhibitors increases the synthesis and secretion of proteases in humans (trypsin, chymotrypsin and elastase) (Liener et al., 1988). In addition, age-related differences in protein digestibility are reported to be increased in the presence of anti-nutritional factors (Gilani & Sepehr, 2003). In the presence of anti-nutritional factor (trypsin inhibitors, glucosinolates, lysinoalanine, Maillard compounds), protein digestibility is dramatically decreased in 20 months-old rats (up to 17%) in comparison to 5 week-old rats. Of interest, in this later study, an appropriate treatment of these products, such as autoclaving reducing their anti-nutritional factors reduces the age related difference in protein digestibility to 3% (Gilani & Sepehr, 2003). *In-vivo* studies in rats showed that the digestive utilization of lupin protein by growing rats is lower than that of

the casein control group. In addition, a α -galactosides-free lupin diet and the supplementation of the diet with phytase lead to a similar protein utilization than that of raw lupin attributed to the absence or low levels of certain non-nutritional components such as trypsin inhibitors, lectins or tannins in all (treated and raw) lupin diets (Porres et al., 2006).

Lectin anti-nutritional factors can interfere with the absorption of nutrients by interacting with a specific receptor located on the surface of the intestinal epithelial cells (Rubio et al., 1991). Other parameters such as the physical nature of the food matrix (solid or liquide), the non-protein energy intake or the statut of the digestive tract (affected by age, genetic or specific diseases) can also have a significant impact on the bioavailability of food amino acid (Mauro, 2008).

3.3.Protein synthesis

After being absorbed, amino acids are released by the intestinal mucosa in the portal vein to reach the liver (Mariotti et al., 2000). A significant part of these amino acids is retained by the gut and the liver for their own metabolism and protein turnover. Another fraction of amino acids not catabolized in the liver, is transported in the plasma to peripheral organs and tissues. Skeletal muscle is one of the most important tissues implicated in the oxidation of these types of amino acids, mainly composed of branched amino acids (leucine, isoleucine and valine) (Brosnan & Brosnan, 2006). Over 40% of body proteins are found in the muscle. This tissue is a heterogeneous tissue constituted of contractile proteins. Muscle protein synthesis is stimulated in particular by a hyper-aminoacidemia induced by dietary protein intake. A lack of one or more essential amino acids inhibits this step (Kimball, 2002). Branched chain amino acids notably leucine are particularly important because they activate the initiation phase of mRNA translation (Anthony et al., 2001; Crozier et al., 2005). In addition, insulin and high dietary amino acid levels are both demonstrated to be the main inhibitors of protein catabolism (Boirie et al., 1997; Castellino et al., 1987; Tessari et al., 1987). Beside the amino acid content and nature, the muscle protein synthesis may be affected by the rate of protein digestion, as it will be discussed in the following section.

3.4.Impact of the rate of food digestion and absorption on muscle protein synthesis, the concept of “fast and slow protein”

The concept of dietary fast and slow proteins was developed in milk proteins, i.e. whey and casein fractions. The kinetic and the extent of amino acid delivery in healthy adult subjects is different depending on the ingested protein fraction: whey protein leads to a higher but a shorter in time aminoacidemia, whereas casein induces a prolonged plateau of a

moderate plasma aminoacidemia (Boirie et al., 1997). These differences are related to the lower rate of digestion of casein which clots in the stomach slowing-down the amino acid release in the proximal intestine, contrary to whey proteins which are soluble in acidic gastric conditions and rapidly emptied from the stomach (Mahe et al., 1996). As whey proteins, soy proteins are composed of mainly soluble proteins, even though, no difference in the gastric emptying of soy and milk proteins are reported (Hara et al., 1992). However, soy proteins exhibit higher intestinal transit rate than milk proteins (Hara et al., 1992), resulting in a more rapid digestion by human (Bos et al., 2003). These results showed that soy proteins acts as a “fast” protein and milk protein as a “slow” protein (Bos et al., 2003). Boirie et al. (1997) reported that protein synthesis, breakdown and deposition may be affected by the different rate of protein digestion and absorption. According to these authors, if the stimulation of postprandial protein synthesis is twice greater after the intake of a whey than of a casein meal, whole body protein breakdown is only inhibited after casein intake. Based on these considerations, this concept of slow and fast proteins can be used in the context of functional foods as a mean to improve the physiological conditions of specific populations, such as older people (Boland et al., 2014). Hence, a significant age-related effect is observed on body protein gain induced by slow and fast proteins. In elderly men, a greater utilization of whey proteins than casein is reported compared with younger counterparts. These results suggest that fast proteins can be more adapted to limit body protein losses during aging (Dangin et al., 2003).

4. Evaluation of protein quality

The assessment of protein quality aims to determine whether the protein provided by the diet meets amino acids and nitrogen body requirements and metabolic demands (FAO Expert consultation, 2011). The ability of dietary proteins to ensure body protein requirement depends on their digestibility, on the amount and proportion of essential and non-essential amino acids and on the bioavailability of the constitutive amino acids. These characteristics affect body protein turnover. As the quality of proteins vary considerably according to its dietary source, it is necessary to evaluate it in order to ensure that dietary protein intake corresponds to body requirements. For example, both a rapidly digested protein (Dangin et al., 2003; Gryson et al., 2014) and a high essential amino acid score (Dardevet et al., 2002; Volpi et al., 2003) were demonstrated to be highly relevant for muscle protein synthesis, in particular in older people. Therefore, some characteristics such as amino acid score, protein efficiency ratio, protein digestibility, biological value and protein digestibility-corrected

amino acid score were proposed and validated by the FAO/WHO Expert Consultation (1989) as criteria to evaluate dietary protein quality. Some of them are described below.

4.1.Chemical score

The determination of the chemical score (Cs) requires to quantitatively know the essential amino acid profile of the dietary protein. The content of essential amino acid is then compared with that of a reference protein such as egg protein or the ideal amino acid pattern defined by ANSES or FAO/WHO according to the equation:

$$Cs (\%) = \frac{\text{Essential amino acid in food protein (mg/g)}}{\text{content of same amino acid in reference protein (mg/g)}} \times 100$$

The amino acid characterized by the lowest score is considered to be the first limiting amino acid.

4.2.Protein efficiency

The protein efficiency ratio (PER) is a simple parameter allowing to evaluate protein efficiency considering the body weight gain of growing animals during a specific period of time. Protein efficiency ratio is then calculated according to the following equation:

$$PER = \frac{\text{Gain in body weight (g)}}{\text{protein consumed (g)}}$$

This method is widely criticized because it does not allow to distinguish between the growing rate of fat and lean tissues. In addition to the chemical score and to the protein efficiency ratio, other parameters were proposed and widely used to evaluate dietary protein quality, based on the bioavailability of amino acids for digestion and for the metabolic demands.

4.3.Evaluation of protein bioavailability for the digestive process

As a high chemical score is not predictive of a high digestion and absorption rate, protein digestibility and amino acid availability have to be assessed. For example, the presence in the food matrix of a high content of dietary fibers, phytic acid or protein bonded to carbohydrates can affect dietary protein digestibility. Hence, the first objective of these methods is to evaluate the ability of proteins to be degraded to its constitutive amino acid in the intestine. Different equation with different significance could be used. The apparent (AD) and true digestibility (TD) are calculated according to the following equations:

$$AD (\%) = \frac{NI - FN}{NI} \times 100$$

$$TD (\%) = \frac{NI - (FN - EFN)}{NI} \times 100$$

Where, AD: apparent digestibility, EFN: endogenous fecal nitrogen (determined using the fecal nitrogen of animals fed a protein free diet), FN: fecal nitrogen, NI: nitrogen intake, TD: true digestibility.

The apparent digestibility considers that all the fecal nitrogen originates from the ingested proteins, while the true digestibility takes into account fecal nitrogen of non-dietary origin coming from endogenous secretion, such as proteases. This later parameter is calculated based on the fecal nitrogen released in animals fed with a protein free diet. Therefore, the true digestibility is always greater than the apparent digestibility (Greaves, 1963).

4.4.Evaluation of protein bioavailability for metabolic processes

After the proteins are digested, the released amino acids are absorbed in the intestine and subjected to different metabolic fates, e.g. oxidation, anabolism... The ability of dietary amino acids to be retained in the body for maintenance and growth, i.e. nitrogen retention, could be measured using the biological value (BV) (Gropper et al., 2009). This parameter is calculated according to the following equation:

$$BV (\%) = \frac{NI - (FN - EFN) - (UN - EUN)}{NI - (FN - EFN)} \times 100$$

Where, BV: biological value, EFN: endogenous fecal nitrogen (determined using the fecal nitrogen of animals fed with a protein free diet), EUN: endogenous urinary nitrogen determined using the urinary nitrogen of animals fed with a protein free diet), FN: fecal nitrogen, NI: nitrogen intake, UN: urinary nitrogen.

Net protein utilization is a simpler method comparing to biological value, and consist to determine the amount of nitrogen retained by the body to food nitrogen intake according to the equation:

$$NPU (\%) = \frac{NI - (FN + UN) + EFN + EUN}{NI} \times 100$$

Where, EFN: endogenous fecal nitrogen (determined using the fecal nitrogen of animals fed with a protein free diet), EUN: endogenous urinary nitrogen determined using the urinary nitrogen of animals fed with a protein free diet), FN: fecal nitrogen, NI: nitrogen intake, NPU: net protein utilization, UN: urinary nitrogen.

Another index for the evaluation of protein quality, called protein digestibility-corrected amino acid score (PDCAAS) is used. It take into account the composition of essential amino acids and the digestibility of dietary proteins. This parameter is calculated based on the equation:

$$PDCAAS = \frac{Cs \times TD}{100}$$

Where, Cs: chemical score, TD: true digestibility.

A PDCAAS lower than 100% means that the protein source cannot fully satisfy body amino acids requirement, and have to be completed with another protein source. The use of PDCAAS to evaluate the quality of protein was recommended by ANSES (2007a). In addition to the PDCAAS, the FAO expert consultation on protein quality evaluation in human nutrition concluded that proteins should first be described on the basis of their digestible amino acid contents (Tome, 2013), with each amino acid being treated as an individual nutrient, and proposed another score: “the digestible indispensable amino acid score” (DIAAS), calculated as:

$$DIAAS = \frac{\text{digestible essential amino acid}}{\text{same essential amino acid in reference protein}}$$

The protein quality of some food products were evaluated in the literature using the method described above. Some of the reported results are summarized in table 1.10. Animal proteins notably egg and whey proteins lead to higher protein efficiency ratio, digestibility and biological value in comparison to plant products. Among plant proteins, wheat gluten is

the less efficient for growth and induced the lowest nitrogen retention in the body. Of interest, protein efficiency and digestibility are increased in pasta enriched with legumes (pigeon pea).

Table 1.10 Protein quality evaluation in some food products

Food protein	PER	TD	BV	NPU	PDCAAS
Beef ¹	2.9		80	73	0.92
Black beans ^{1,2}	-	70	-	-	0.53-0.75
Casein ^{1,3}	2.5-2.7	93	77	76	1.00
Egg ¹	3.9	-	100	94	1.00
Soy ¹	2.2	-	74	61	1.00
Wheat gluten ¹	0.8	-	64	67	0.25
Whey protein ¹	3.2	-	104	92	1.00
<hr/>					
Pasta products					
100% corn ⁴	-	91	38	35	0.38
70% corn+30 broad bean ⁴	-	81	69	56	0.50
80% corn+20 quinoa ⁴	-	78	75	59	0.51
100% semolina ³	1.1	85	-	-	-
20% semolina+80% pigeon pea ³	1.9	90	-	-	-

¹ Hoffman and Falvo (2004), ² Pulse Canada (2011), ³Torres et al. (2006) , ⁴Gimenez et al. (2016)

PER: protein efficiency ratio, TD: true digestibility, BV: biological value, NPU: net protein utilization, PDCAAS: protein digestibility-corrected amino acid scores.

5. Future trends for protein quality improvement in food

Because the production of animal proteins impacts negatively the environment and because its durability is questionable, it is of interest to develop nutritionally improved basic plant foods, e.g. pasta products. Previous studies showed that the addition of legume flour (30 - 35%) to pasta increases its protein content from 12 to 19% (Petitot et al., 2010b; Wood, 2009; Zhao et al., 2005) and improves its amino acid chemical score from 40 to 60% due to the complementary amino acid pattern of wheat and legume proteins (Gimenez et al., 2012). However, in these studies, the optimal amino acid score has never been achieved for lysine. In addition, the incorporation of a high amount of legume flours into pasta brings some bioactive compounds such as phytic acid and protease inhibitors (Torres et al., 2007a; Torres et al., 2007b; Zhao et al., 2005). Taken as a whole, these compounds can depreciate the digestibility of proteins. Nowadays, researches focus on the reduction of these anti-nutritional factors by different processing such as germination and precooking treatments of legume prior to pasta production (Boye et al., 2010; Torres et al., 2007b). In addition, the structural transformations induced by the different processing steps, e.g. pasta drying temperatures (Laleg et al., 2016a; Petitot & Micard, 2010) can affect protein network and, consequently protein digestibility.

The relationship between these structural modifications and the rate of digestibility of dietary proteins, remains unclear and requires further investigations, notably *in-vivo* studies.

CHAPITRE 2

Pâtes alimentaires enrichies en protéines : influence de la nature des protéines et des températures de séchage sur la structure des protéines et leur digestibilité in-vitro.

CHAPITRE 2. PATES ALIMENTAIRES ENRICHIES EN PROTEINES : INFLUENCE DE LA NATURE DES PROTEINES ET DES TEMPERATURES DE SECHAGE SUR LA STRUCTURE DES PROTEINES ET LEUR DIGESTIBILITE *IN-VITRO*

La pâte alimentaire de blé est un aliment reconnu pour avoir des propriétés nutritionnelles intéressantes notamment grâce à un faible indice glycémique. Ces propriétés sont principalement attribuées à la structure spécifique de la pâte caractérisée par des grains d'amidon enchaînés dans un réseau protéique dense de gluten limitant l'activité amylolytique. Plusieurs travaux ont démontré l'impact d'une modification de la structure de la pâte, par incorporation d'ingrédients non conventionnels comme les légumineuses ou par modification de leurs températures de séchage, sur la variation de la digestibilité de l'amidon. Cependant peu de travaux ont rapporté l'effet d'une telles modifications de structure sur la digestibilité des protéines. Afin d'approfondir les connaissances sur le lien structure-digestibilité des protéines, nous avons mené une étude sur les pâtes alimentaires enrichies avec des protéines de différentes sources (animale : blanc d'œuf ; végétales : gluten ou fèverole). Ce chapitre est consacré à l'étude de l'impact de la variation de la formulation et de la modification des températures de séchage 90 vs. 55 °C sur la structure multi-échelle des pâtes en utilisant des méthodes de caractérisation structurales peu utilisées sur des matrices pâtes. Les répercussions sur les propriétés culinaires et nutritionnelles des pâtes notamment sur la digestibilité des fractions protéiques *in-vitro* ont été déterminées.

Publication 2. Protein enriched pasta: structure and digestibility of its protein network.

Laleg K., Barron C., Santé-Lhoutellier, V., Walrand S., Micard V.

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Abstract

Wheat (W) pasta was enriched in 6% gluten (G), 35% faba (F) or 5% egg (E) to increase its protein content (13% to 17%). The impact of the enrichment on the multiscale structure of the pasta and on *in-vitro* protein digestibility was studied. Increasing the protein content (W- vs. G-pasta) strengthened pasta structure at molecular and macroscopic scales but reduced its protein digestibility by 3%. Greater changes in the macroscopic and molecular structure of the pasta were obtained by varying the nature of protein used for enrichment. Proteins in G- and E-pasta were highly covalently linked (28-32%) resulting in a strong pasta structure. Conversely, F-protein (98% SDS-soluble) altered the pasta structure by diluting gluten and formed a weak protein network (18% covalent link). As a result, protein digestibility in F-pasta was significantly higher (46%) than in E- (44%) and G-pasta (39%). The effect of low (55 °C, LT) vs. very high temperature (90 °C, VHT) drying on the protein network structure and digestibility was shown to cause greater molecular changes than pasta formulation. Whatever the pasta, a general strengthening of its structure, a 33% to 47% increase in covalently linked proteins and a higher β -sheet structure were observed. However, these structural differences were evened out after the pasta was cooked, resulting in identical protein digestibility in LT and VHT pasta. Even after VHT drying, F-pasta had the best amino acid profile with the highest protein digestibility, proof of its nutritional interest.

Key words: Durum wheat, faba bean, pasta, microscopy, SE-HPLC, FTIR, front face fluorescence, rheology and *in-vitro* protein digestibility.

1. Introduction

Wheat pasta is a widely consumed staple food worldwide. As well as being a source of carbohydrates (74-77%, db) and proteins (11-15%, db), it has other interesting nutritional properties, notably its low glycemic index (Petitot et al., 2009a). The structure of cooked pasta is usually described as a protein network with entrapped swollen starch granules. This specific structure, which is the result of successive changes that occur at different scales throughout pasta processing, has been shown to affect the digestibility of its nutrients, and their metabolic and health effects (Petitot et al., 2009a; Zou et al., 2015).

Plants with high protein content such as legumes, or gluten and animal-based ingredients, such as egg white, are incorporated into pasta to enhance its nutritional or textural and cooking properties (Bustos et al., 2015; Krishnan & Prabhakar, 2012; Li et al., 2014). Enrichment with 10-20% wheat gluten has been shown to reduce cooking loss, increase the firmness of pasta, and to improve the encapsulation of starch, thereby reducing its accessibility to α -amylase (Fardet et al., 1999). Enrichment with 3-18% egg white has been shown to increase the firmness and elasticity (Hager et al., 2012a; Schoenlechner et al., 2010) and to reduce the compressibility of cooked pasta (Matsuo et al., 1972). Split pea (Petitot et al., 2010a), faba bean (Gimenez et al., 2012; Petitot et al., 2010a), fermented pigeon pea (Torres et al., 2006), lupin (Jayasena & Nasar-Abbas, 2012; Rayas-Duarte et al., 1996), black-gram (Madhumitha & Prabhakar, 2011), lentil, pea and chickpea (Zhao et al., 2005) have all been used in different amounts (up to 50%) to enrich pasta to improve its protein content, its essential amino acid (EAA) score (Gimenez et al., 2012), especially lysine and to a lesser extent threonine, its protein digestibility (Torres et al., 2006), and to reduce glycaemia (Capraro et al., 2014). Legumes are also rich in dietary fibers, vitamins, minerals and carbohydrates (Krishnan & Prabhakar, 2012; Petitot et al., 2010b). However, high (>10-30%) legume substitution in pasta may weaken its protein network by decreasing covalent linkages, thereby impairing the overall quality of pasta (Petitot et al., 2010b; Rayas-Duarte et al., 1996). The use of high temperature drying (>70 °C) has been shown to improve pasta properties through the formation of a strong covalently linked protein network in wheat (Zweifel et al., 2003) and legume fortified pasta (Petitot & Micard, 2010). Severe thermal treatment has been shown to reduce the *in-vitro* digestion of protein and starch in wheat pasta (De Zorzi et al., 2007; Petitot et al., 2009b). In pasta fortified with 35% legume, the slowdown of *in-vitro* starch digestion due to high temperature drying was even more pronounced (Petitot & Micard, 2010), and was demonstrated *in-vivo* to improved digestive

comfort and satiety after eating (Greffeuille et al., 2015). However, the digestibility of the pasta protein network according to its protein composition or processing conditions has been less widely studied than starch digestibility. Few data (De Zorzi et al., 2007; Petitot et al., 2009b; Simonato et al., 2015) are available on the relationship between the structure and digestibility of the protein, especially in a multi-protein system pasta. No data are available on the simultaneous effect of protein content and composition and of the drying temperature on the digestibility of the protein in pasta. The aim of the present study on plant and animal protein enriched-pasta was thus to obtain further insight into the multiscale structural modifications in the protein network according to the level and nature of proteins and/or the drying diagrams used for pasta production. The effects of such structural changes on the *in-vitro* protein digestibility of pasta are discussed.

2. Material and methods

2.1. Material

Wheat (*Triticum durum*) (W) semolina, Faba bean (*Vicia faba*) (F) flour, wheat (*Triticum aestivum*) gluten (G) powder and egg white (E) powder were provided by Panzani (Marseille, France), GEMEF industries (Aix-En-Provence, France), Syral, (Aalst, Belgium) and IGRECA (Seiche sur le Loir, France), respectively. The protein contents of all raw materials were analyzed using Kjeldahl procedure (NF V 03-050, 1970) with a conversion factor of 5.7 for W-semolina and G-powder and 6.25 for F-flour and E-powder. Their total starch content was determined using an enzymatic assay kit (Megazyme, Co. Wicklow, Ireland; AACC method 76-13.01). All analyses were conducted in duplicate.

2.2. Pasta manufacturing

All pasta was processed to produce spaghetti using a continuous pilot-scale pasta extruder (Bassano, Lyon, France). W-semolina was enriched with 6% G-powder, 35% F-flour or 5% E-powder to obtain 17% protein content in the pasta. This level is the highest that can be reached in F-pasta without introducing major difficulties in the pilot scale manufacturing process (Petitot et al., 2010b).

W- and enriched-pasta were hydrated to 47% and 45%, respectively, and processed to pasta (Petitot et al., 2010b; Petitot et al., 2009b) then dried at low temperature (LT-55 °C for 15 h) or at very high temperature (VHT-90 °C for 3 h) in a pilot-scale drier (AFREM, Lyon, France) to reach 12% moisture content. The diameter of the dry pasta was 1.56±0.03 mm. Two batches of each kind of pasta were produced and mixed into a single batch prior to pasta

analysis. Pasta protein content of the final batch was measured using the Kjeldahl procedure with a conversion factor of 5.7 for W-semolina and G-powder and 6.25 for F-flour and E-powder according to their respective proportions in pasta.

2.3. Cooking quality of pasta

The optimum cooking time (OCT) of each kind of pasta was determined according to the approved AACC method 66-50.(AACC, 1989). All analyses of cooked pasta were made on pasta cooked at OCT+1 min (Petitot et al., 2009b). Cooking loss and water uptake of cooked (OCT+1 min) pasta were determined in triplicate, as previously described in Petitot et al. (2010b)

2.4. Rheological properties of cooked pasta

The textural properties of cooked (OCT+1 min) pasta were determined using a TA-XTplus (Stable Micro Systems, Scarsdale, USA) texture profile analyzer equipped with a Windows version of Texture Expert software package (Stable Micro Systems, Scarsdale, USA). Prior to measurement, the cooked pasta was equilibrated at ambient temperature for 10 min in a saturated vapor atmosphere container. Nine replicates of three different types of cooking were performed for each kind of pasta.

Pasta elongation. The TA-XTplus analyzer was equipped with tensile grips (ref. A/SPR, Stable Micro Systems). The initial distance between the two tensile grips was 15 mm. The test was performed at a constant rate of deformation at 3 mm/s. Elongation (the ability of pasta to be elongated) was determined as the increase in pasta length (cm) until breakage, and was calculated according to the following equation:

$$\text{Elongation (\%)} = \frac{\text{Final length} - \text{Original length}}{\text{Original length}} \times 100$$

Pasta firmness. was determined based on the AACC approved Method 66-50(AACC, 1989), expressed as the force (g) required to cut five strands of spaghetti positioned adjacent to another at a constant rate of deformation (0.17 mm/sec).

2.5. Pasta structure

Light microscopy of cooked pasta. Cooked (OCT+1 min) pasta sections (8 µm) were cut using a cryoprotector (Cellpath, Newtown, UK), and a microtome (Microm HM 520, Walldorf, Germany). The sections were stained with fast green and lugol (Petitot et al., 2010a). Bright field images were acquired using the multizoom AZ100M microscope (Nikon, Japan) equipped with a Nikon DSRiI (Nikon, Japan) color digital camera. Observations were

made with a plan fluor 5x objective and a fixed optical zoom of 8 leading to a global magnification of 40x.

Protein size distribution of dry and cooked pasta. Sodium dodecyl sulfate (SDS) soluble proteins and dithioerythritol (DTE) soluble proteins (subjected to sonication) were extracted in triplicate from raw blends (used for pasta production) and from dried and freeze-dried cooked (OCT+1 min) pasta. All protein extracts were analyzed by size-exclusion high performance liquid chromatography (SE-HPLC) according to the modified method of Morel et al. (2000) described in Petitot et al. (2009b). The protein fraction that was not extracted in either SDS or in DTE constituted the non-extractable fraction. Once corrected for the different solid-to-solvent ratios used during extraction, areas (in arbitrary units) of SDS-soluble and DTE-soluble proteins were summed and total extractable proteins are expressed as the percentage of the corresponding total area calculated for semolina (for W-pasta) or blends of semolina and high protein powders (35% F, 6% G or 5% E) for protein enriched-pasta.

Protein secondary structure of dry and cooked pasta. Infrared and fluorescence spectroscopies were performed on samples in the same physical state as those used for the study of *in-vitro* protein digestibility. FTIR (Fourier transformed infrared) spectra were recorded on an FTIR Nicolet 6700 spectrometer, equipped with an attenuated total reflectance (ATR) Smart DuraSample IR accessory (ThermoScientific, U.K.) and a Mercury Cadmium-Telluride-High D detector. Interferograms (128) were collected at 2 cm⁻¹ resolution and co-added before Fourier transformation. Spectra were recorded between 800 and 4000 cm⁻¹. In order to standardize their water content, all samples of both dry and cooked pasta were freeze-dried and pressed down against the diamond ATR surface. Nine spectra were recorded for each sample. Spectra were analyzed in the region of the amide I band (1,600-1,700 cm⁻¹) after baseline correction and normalization. Principal component analysis (PCA) was performed on both dry and cooked pasta spectra using the PLS-Toolbox v7.9 (Eigenvector Research, Inc) for Matlab (v10.0, Mathworks).

Protein tertiary structure of both dry and cooked pasta. Fluorescence spectroscopy was performed using a JASCO Spectrofluorometer FP-8300, equipped with spectra manager 2 (version 1.0). All dried and cooked freeze-dried pasta were excited at 290 nm and emission spectra were then recorded at room temperature (25±2 °C) from 300 to 390 nm with the scan rate of 10,000 nm/min at a constant 2.5 nm bandwidth for both excitation and emission, and a data interval of 0.5 nm. Maximum intensity spectra and their corresponding wavelengths (λ_{max}) were collected. Six replicates were performed for each sample.

2.6. Amino acid profile of raw material and dried pasta

The amino acid (AA) profiles of LT and VHT dry pasta and of the raw material used for their production were determined in duplicate at CIRAD (Montpellier, France) according to Moore et al. (1958) using an AA analyzer (Biochrom 30+, Biochrom, France). The AA profile determined separately for each raw material (W-semolina, G- and E-powders and F-flour) was used to calculate the AA composition of blends used for pasta production. Essential AA scores (EAAS) correspond to the ratio of the amount of each AA in each sample to the amount of the same AA in an ideal protein recommended for human adults by ANSES (2007a). EAAS have to reach 100% of the ANSES recommendation for each AA to ensure optimal use of the protein.

2.7. *In-vitro* protein hydrolysis of cooked pasta

In-vitro protein digestion of pasta was performed in triplicate according to Pasini et al. (2001) using pepsin (700 U/mg protein, P7125, Sigma) and Pancreatin (Sigma catalog n° P7545). Both reactions were stopped after 30 min of pepsin hydrolysis or after 30 min pepsin+180 min of pancreatic digestion by adding one volume of 20% (w/v) trichloroacetic acid (TCA). The amount of free amino group was determined on digestion extracts (supernatant) based on the ninhydrin method (Prochazkova et al., 1999). The degree of hydrolysis (°H) was calculated as previously described by Petitot et al. (2009b).

2.8. Statistical analysis

All data (except for FTIR spectroscopy described in the spectroscopy method section) were subjected to analysis of variance (two-way ANOVA) using “formulation” and “drying” as factors. ANOVA was followed by the Fisher’s least significant difference (LSD) test to compare means at the 5% significance level, using Statistica 8.0 software (Tulsa OK, USA).

3. Results

3.1. Composition of raw material and pasta

The composition of the raw material and their blends used for pasta production is provided in table 2.1. Starch was the main constituent of W-semolina and to a lesser extent of F-flour, while proteins were the main constituents of G- and E-powders. The enrichment of W-semolina with 6% G-, 5% E-powders or 35% F-flour increased the protein content of the blend by 30%. G-, F- and E-proteins contributed 28%, 50% and 27%, respectively, of the total protein content of the respective blends (table 2.1). Protein enrichment of W-semolina reduced the proportion of starch by 5% in the G- and E-blends and by 9% in the Fblend. The

protein content of dry pasta determined by Kjeldahl's method was in agreement with the calculated contents, i.e. $13.15 \pm 0.03\%$ (db) for wheat pasta and $16.96 \pm 0.15\%$ (db) for all enriched-pasta whatever the drying profile (LT or VHT) used. Considering the contribution of each added protein to the total protein content in the blend, and the sulfur AA content of each raw material, the E- and to a lesser extent G-blend, were richer in sulfur AA and the F-blend poorer in comparison to W-semolina (table 2.1).

Table 2.1 Composition of wheat- (W) semolina, gluten- (G) powder, faba bean- (F) flour and egg- (E) powder, and the blends of 6%G+94%W (G-blend), 35%F+65%W (F-blend), and 5%E+95%W (E-blend) used for pasta production.

Raw material	Composition				
	Pure	W-semolina	G-powder	F-flour	E-powder
Starch (%, db)	77.8 ± 0.6	10.6 ± 0.1	57.6 ± 0.3		Na ¹
Protein (%, db)	13.1 ± 0.1	79.8 ± 0.1	24.0 ± 0.1	90.9 ± 0.4	
Sulfur AA (mg/g protein)	30.2	34.9	17.4	58.7	
In blend²		G-blend	F-blend	E-powder	
	Starch (%, db)	73.8	70.7	73.9	
Protein (%, db)		17.1	16.9	17.0	
Supplemented protein (% of total protein) ³		28.0	49.7	26.8	
Sulphur AA (mg/g protein)		31.5	23.8	37.8	

¹Not analyzed.

² Result obtained by calculation; G-blend: 6%G+94%W, F-blend: 35%F+65%W and E-powder: 5%E+95%W.

³ Contribution (%) of supplemented protein (G, F or E) as a proportion of the total protein content in each blend.

Results are means of 2 replicates.

3.2. Effect of formulation and drying temperature on the cooking properties of pasta

The main effects of pasta formulation, drying and their interactions on pasta cooking properties are listed in table 2.2. Cooking loss and water uptake were significantly affected by pasta formulation. All protein-enriched pasta had lower water uptake as already reported in 15-35% legume (split pea, faba bean and pea) (Gimenez et al., 2012; Padalino et al., 2014; Petitot et al., 2010b) and 9-20% gluten pasta (Sissons et al., 2005). This could be linked to the lower starch content in all enriched-pasta, and to the introduction of legume starch in F-pasta. In legume enriched-pasta (20 to 60% green gram), starch was previously shown to acquire a different amylograph profile from wheat pasta starch (Rajiv et al., 2014), which could play a role in reducing water uptake in F-pasta.

Table 2.2 Results of two way analysis of variance and of an LSD test of the cooking and rheological properties of wheat- (W) pasta and gluten (G), faba bean (F) and egg (E) enriched-pasta dried at low (LT) and very high temperature (VHT) and cooked to optimal cooking time +1 min.

Pasta properties		Mean effects ¹								F-value ²				
		Pasta formulation		W		G		F		E		Formulation	Drying	Interaction ³
		Drying temperature	LT	VHT	LT	VHT	LT	VHT	LT	VHT	LT			
Cooking	Cooking loss (%), db)	7.5 ^b	6.9 ^{ab}	6.7 ^a	6.7 ^{ab}	8.5 ^d	6.8 ^{ab}	7.2 ^{ab}	5.1 ^c	11	30	7		
	Water uptake (%), dry pasta)	186 ^c	181 ^c	174 ^b	173 ^b	165 ^a	165 ^a	163 ^a	164 ^a	48				
Rheological	Firmness (g)	493 ^a	558 ^b	680 ^c	852 ^d	499 ^a	552 ^b	857 ^d	992 ^e	594	180			
	Elongation (%)	339 ^a	353 ^{ab}	368 ^{ab}	452 ^c	230 ^d	324 ^a	394 ^b	446 ^c	26	23			

¹ Means in the same row with the same letter are not significantly different (p>0.05).² F-value is given only when the effect was statistically significant.³ Interaction between drying and formulation.

Results are means of 3 replicates for cooking properties and of 9 replicates for rheological properties.

Conversely, no decrease in water uptake was reported in rice pasta enriched with 15% liquid egg albumen (Martí et al., 2014). The enrichment of pasta with 6% gluten decreased its cooking loss by 11%. This is consistent with the results of Fardet et al. (1999) (22% decrease after the addition of 10% gluten to wheat-pasta). No significant modification of this parameter was observed in LT E-pasta. Particle losses in cooking water only increased (by 13%) in F-pasta. Most authors reported 7-10% cooking losses in legume (faba bean, lupin, pea, lentil and chickpea) enriched-pasta (Petitot et al., 2010b; RayasDuarte et al., 1996; Zhao et al., 2005) when the substitution level was $\geq 30\%$. Cooking losses were more affected by pasta drying temperature than by formulation (F-values in table 2.2). A slight but significant impact of interaction (formulation \times drying) was also observed. Cooking loss decreased by 20% and 29% in VHT F- and VHT E-pasta, respectively, compared to LT dried pasta, without affecting W- and G-pasta. No modification in water uptake was observed with an increase in the drying temperature.

3.3. Effect of formulation and drying temperature on the rheological properties of pasta

Pasta firmness and elongation, which are good indicators of pasta structure at the macromolecular scale, are presented in table 2.2. The majority of textural properties of cooked pasta were significantly affected by pasta formulation ($p<0.05$). A marked increase in pasta firmness (by 38% and 74%, respectively) was observed in LT cooked G- and E-pasta in comparison to W-pasta. This was accompanied by a 16% increase in elongation in E-pasta. Similar improvements in pasta texture (firmness/compressibility and elasticity) have been reported in 3-18% egg albumen enriched wheat (Hager et al., 2012a; Matsuo et al., 1972) and oat pasta (Hager et al., 2012a), and in 10-20% gluten enriched-pasta (Fardet et al., 1999). Enrichment of pasta with F drastically altered (by 32%) its elongation without affecting its firmness, when dried at LT. Zhao et al. (2005) and RayasDuarte et al. (1996) also reported no change in the firmness of 5-30% lupin, yellow pea and chickpea enriched-pasta. Reduced elasticity in 35% faba and 30% pea enriched-spaghetti was recorded by Petitot et al. (2010b) and Padalino et al. (2014). Drying temperature had a significant effect on the rheological properties of the pasta, even if the effect was lower than that of the formulation (F-value in table 2.2). VHT generally increased pasta firmness and elongation with no interaction with formulation.

3.4. Effect of formulation and drying temperature on the structure of dry and cooked pasta

Microstructural scale, light microscopy of cooked (OCT+1 min) pasta. The microstructure of all LT cooked pasta is presented in figure 2.1 as parts of a diagonal cross section observed by light microscopy. Starch granules colored bluish-purple are surrounded by the protein network stained green. At this scale, the increase in protein content in W- vs. enriched- (G, F and E) pasta was not clearly visible. In all pasta, a gradual increase in starch swelling from the core to the external region resulted in three main regions, as already reported by several authors (Cunin et al., 1995; Petitot et al., 2010a). The protein network appeared to be tighter in the core of G- and E- than in F-pasta. It was possible to differentiate faba bean starch (oval) from wheat starch (elongated) by their shape in the pasta core. In the intermediate region, the starch granules seemed larger and well swollen in all pasta except for E-pasta. In the external region of all pasta, as a result of the high exposure to water, the starch granules were highly swollen or had even disintegrated, creating many empty protein cavities. However, no match could be established between the cooking losses measured on pasta (table 2.2) and their external state. Whatever the pasta formulation, no effect of VHT drying was observed on pasta microstructure (data not shown). This could be related to the same level of water uptake in LT vs. VHT pasta, whatever the formulation (table 2.2).

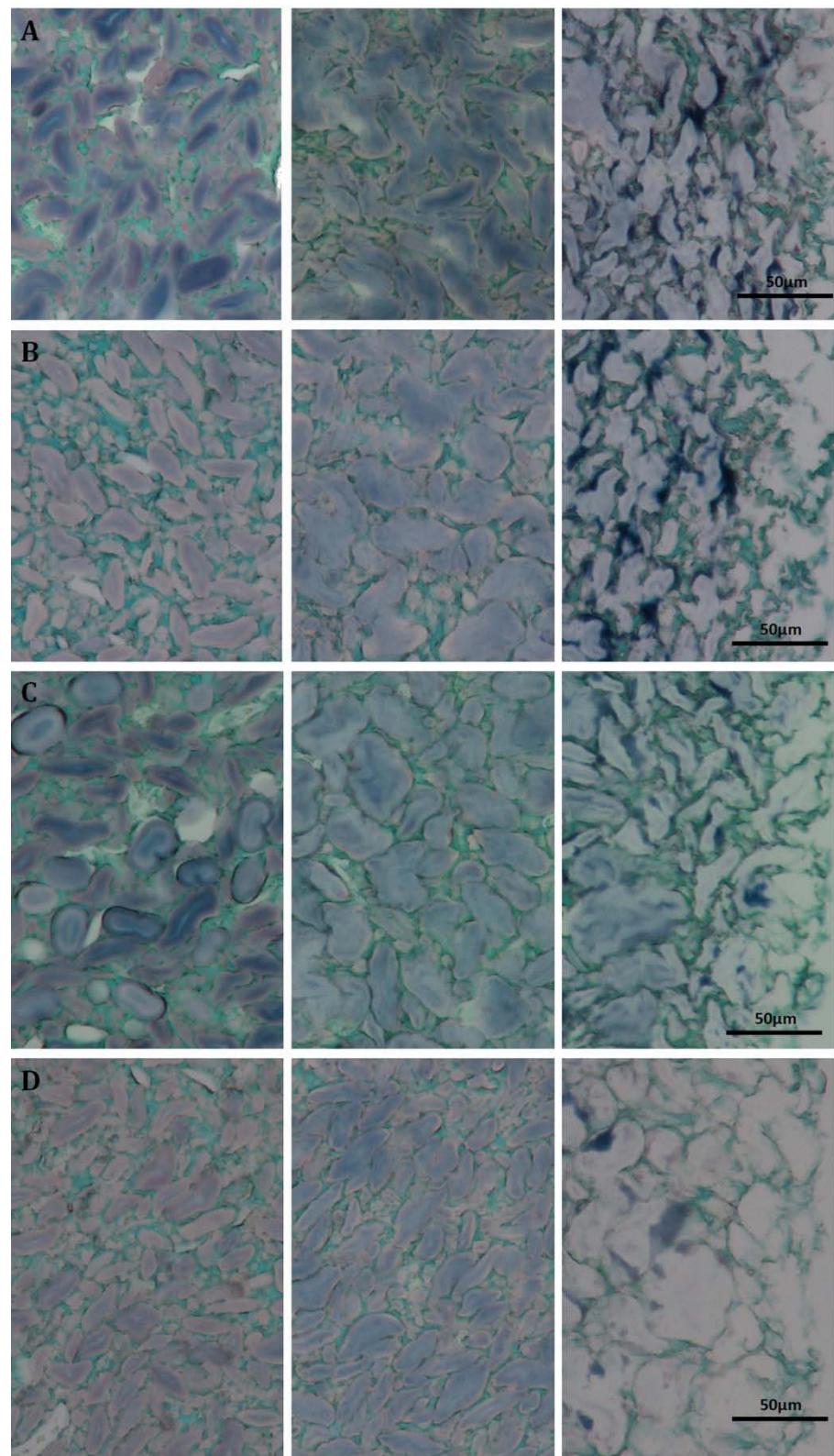


Figure 2.1 Light microscopy image of low temperature wheat- and protein enriched-pasta cooked to their optimal cooking time +1 min, from the central (on the left) to the external region (on the right). A: Wheat-pasta; B: Gluten-pasta; C: Faba bean-pasta and D: Egg-pasta.

Molecular scale, Protein aggregation by SE-HPLC. Results of protein solubility in SDS and DTE (after sonication) in both dry and cooked pasta are presented in figure 2.2. The SDS-soluble fraction presented weakly linked (electrostatic, hydrophobic and hydrophilic) proteins; the DTE-soluble fraction contained disulfide bonded proteins. Proteins that were linked by other covalent interactions than disulfide bridges (i.e. isopeptide bonds) were considered to be the non-extractable fraction. In all LT dry pasta (figure 2.2A), more than 65% of proteins were soluble in SDS. The remaining proteins were linked by disulfide bonds (29, 32, 18 and 28% in W-, G-, F- and E-pasta, respectively). Enrichment of pasta with F-flour whose proteins were 98% SDS-soluble (results not shown) resulted in a noticeable (11%) increase in the weakly linked proteins counterbalanced by a decrease in the DTE-soluble proteins as previously reported by Petitot et al. (2010a). Conversely, the enrichment of pasta with G-powder, which contained 32% of large protein aggregates in SDS-soluble protein fraction vs. only 28% in semolina (result not shown), led to a slight increase in covalently linked proteins in G-pasta. The addition of E-proteins (98% SDS-soluble as F-proteins, result not shown) did not increase SDS-soluble proteins in E-pasta. This could be explained by the greater ability of E-protein (in comparison to F-proteins) to form DTE-soluble protein, which could counterbalance the SDS-solubility of E-pasta proteins. This could be due to the high sulfur AA content in E- vs. F-blends used for pasta production (table 2.1). The temperature (LT vs. VHT) used to dry pasta had a greater impact on protein linkage in pasta than formulation (F -value=17,959 and 71 for drying and formulation effects, respectively; data not shown). VHT drying (figure 2.2B) drastically decreased the SDS and increased DTE protein solubility in all pasta. The formation of covalently aggregated proteins was already demonstrated in high temperature (>70 °C) dried wheat (De Zorzi et al., 2007; Petitot et al., 2009b; Zweifel et al., 2003) and legume pasta (Petitot & Micard, 2010) and in pasteurized (95 °C) fresh egg pasta (Alamprese et al., 2005). The effect of drying temperature we observed in our results varied with the protein used for pasta enrichment (F -value of interaction between drying and formulation=39; data not shown): The evolution of protein behavior with an increase in drying temperature was more intense in E- than in the other pasta reducing by 5.6 fold the SDS-soluble protein in favor of covalently linked proteins due to its higher sulfur AA content (table 2.1). SDS-soluble proteins were reduced by 3.3, 3.1 and 2 fold in W-, G- and F-pasta, respectively. Even dried at VHT, the protein network of F-pasta remained the weakest, with the highest proportion of SDS-soluble proteins, the lowest proportion of DTE-soluble proteins and non-extractable proteins, as already reported in the literature (Petitot et al., 2010a). Results of protein aggregation in cooked pasta are presented

in figures 2.2C and 2.2D. Cooking drastically reduced the difference in protein aggregation between LT and VHT dried pasta (F -value of drying=17,959 and 94 before and after cooking, respectively; data not shown), while those related to pasta formulation remained the same or even increased after cooking (F -value=71 and 100 before and after cooking, respectively; data not shown). Cooking led to a 3 fold increase in the percentage of disulfide bonds in all LT pasta and to a 1.2 fold increase in all VHT pasta. Non-extractable proteins were slightly more numerous in G- and E-cooked pasta. Cooking did not create additional non-extractable proteins when pasta was previously dried at VHT. Even if cooking increased soluble-DTE in F-pasta, the weakly linked proteins were always twice as numerous in this cooked pasta in comparison to W-, G- and E-pasta whatever the temperature used for drying.

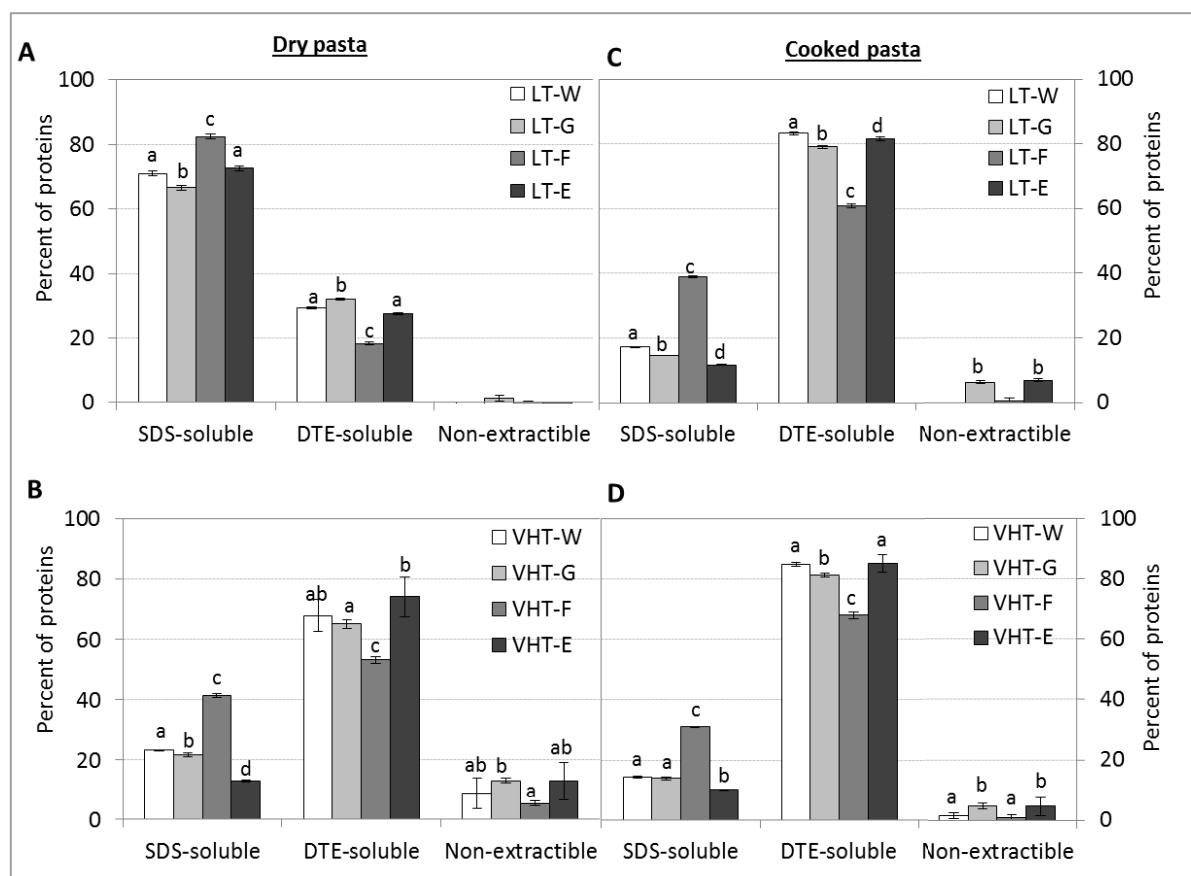


Figure 2.2 SE-HPLC analyses of soluble proteins in sodium dodecyl sulfate (SDS) and dithioerythritol (DTE) and non-extractable proteins in wheat- (W) pasta and gluten (G), faba bean (F) and egg (E) enriched-pasta, dried at low temperature (LT) (A and C) and very high temperature (VHT) (B and D), in the dry state or after cooking to optimal cooking time +1 min. Bars bearing different letters differ significantly from each other ($p<0.05$). Results are means of 3 replicates.

Protein secondary structure by FTIR spectroscopy. Mid-infrared spectroscopy was used to evaluate the protein secondary structure of the pasta in the amid I spectral region (Barth & Zscherp, 2002), even if some contribution of the AA side chain has already been

observed in this spectral region in wheat protein (Wellner et al., 1996). Considering the minor change in spectral intensities, a PCA was performed (Benhdech et al., 1993) using the spectra from both dry and cooked pasta. Figure 2.3A shows the projections of different dry pasta spectra on the first two axes (PC1 and PC2). Scores on PC1 (75.7% of the total variation) separated VHT pasta with positive values from LT pasta with negative values, whatever the formulation of the pasta. PC1 loading showed a positive peak at 1,626 cm⁻¹ and a main negative peak at 1,656 cm⁻¹ (figure 2.3B). VHT pasta spectra thus differ from LT spectra in the higher intensity of the absorbed band at 1,626 cm⁻¹ in comparison to 1,656 cm⁻¹. These two peaks were linked to β -sheet and α -helix (with contribution of random structures), respectively (Georget & Belton, 2006; Wellner et al., 1996). Increasing the pasta drying temperature, thus, increased β -sheet at the expense of α -helix and random coil structures of proteins. Our results are in agreement with those observed in heat treated (25-100 °C) ovalbumine (Ngarize et al., 2004), legume (*Phaseolus vulgaris* globulins and isolate) (Meng & Ma, 2001; Rui et al., 2011) and 47% hydrated gluten (Georget & Belton, 2006). The PC2 (16% of the total variation) axis separated the pasta spectra into two groups based on pasta formulation (figure 2.3A). The first group comprised W- and G-pasta (with negative values) and the second one F- and E-pasta (with positive values). PC2 loading (Figure 2.3B) showed a positive peak at 1,637 cm⁻¹ attributed to β -sheet structures, and a negative peak at 1,608 cm⁻¹ attributed to glutamine side chain vibrations (Wellner et al., 1996). Enrichment of pasta with E-powder or F-flour led to an increase in β -sheet structures in comparison to W- or G-pasta, in which the contribution of glutamine side chain vibrations was greater. Higher glutamine side chain vibration in W- and G-pasta was in accordance with the higher amount of this residue analyzed in the corresponding raw materials: 303 and 229 mg/g of W- and G-protein, respectively vs. 79 and 71 mg/g of F- and E-proteins, respectively (result not shown). Regarding the respective variance of PC1 (75.7%) and PC2 (16.0%), the effect of the drying temperature on the secondary structure of dry pasta observed by FTIR was in fact greater than the nature of the protein used to formulate the pasta, as already observed by SE-HPLC analysis. The spectra of the cooked pasta were analyzed in the same way as those of the dry pasta (Figures 2.3C and 2.3D). Two groups of spectra can easily be identified according to PC1 (73.7%) and PC2 (20.6%): the W- and G-pasta spectra had lower PC1 and higher PC2 scores than F- and E-pasta spectra. Looking at PC1 and PC2 loadings (Figure 2.3D), spectral regions involved in the distinction between (W/G) and (F/E) pasta are observed at 1,610 and 1,628-1,636 cm⁻¹ (related to glutamine side-chain and β -sheet vibration, respectively), as observed for dry pasta. The effect of the formulation is thus mainly related to the nature of

protein used for enrichment and the relative amount of glutamine AA it contains. No clear difference between VHT and LT spectra was observed within each group (figure 2.3C), in contrast to Bock et al. (2015), who distinguished pasta dried at a low temperature ($60\text{ }^{\circ}\text{C}$) from pasta dried at a high temperature ($85\text{ }^{\circ}\text{C}$) by a higher β -sheet and lower β -turn structures in cooked pasta.

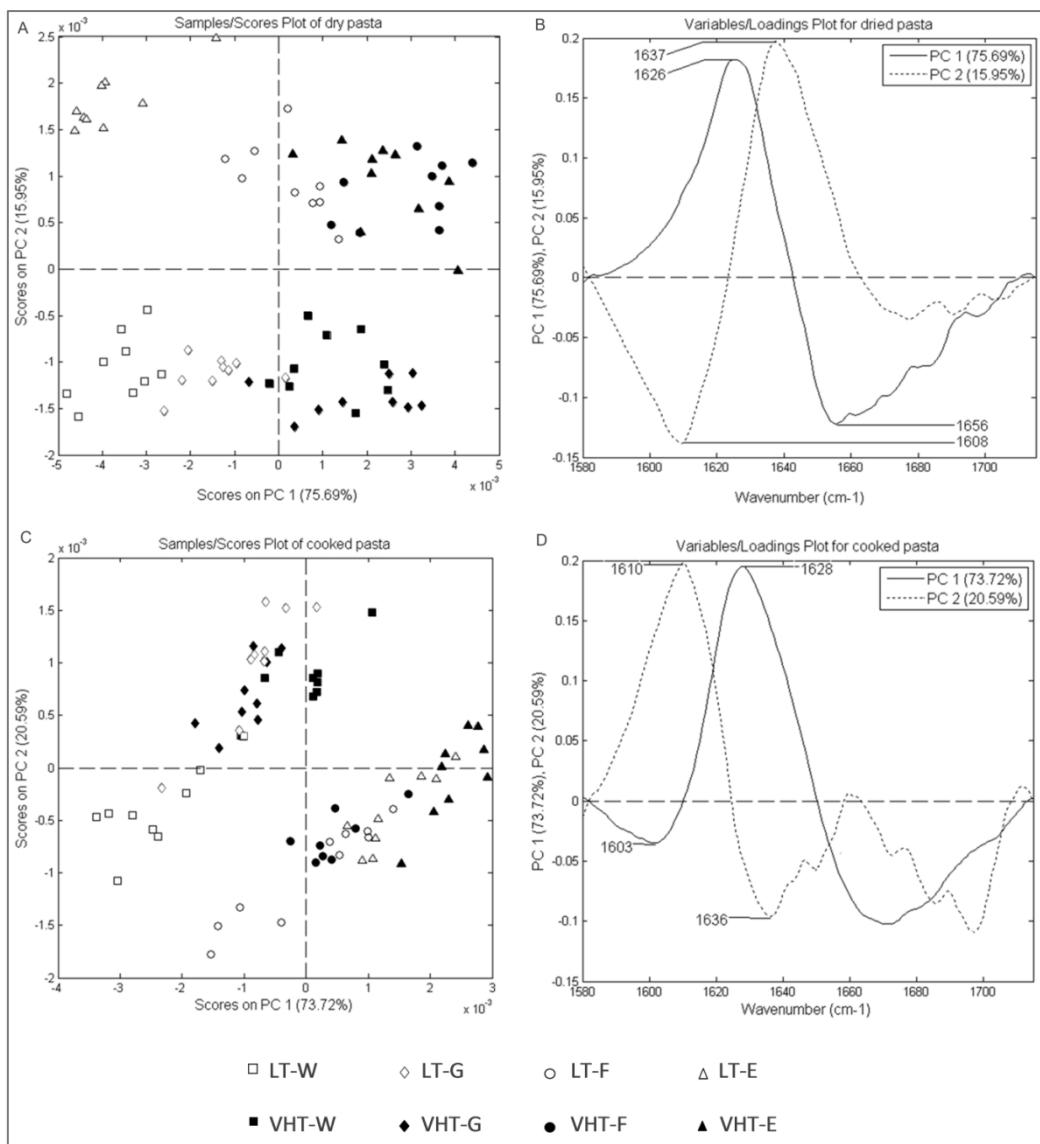


Figure 2.3 PCA analyses (on the left) and the loadings (on the right) corresponding to spectra of dry (A and B) and cooked at optimal cooking time +1 min (C and D) wheat- (W), gluten- (G), faba bean- (F) and egg- (E) pasta.

Spectra were means of 9 replicates of each pasta sample. Analysis were performed in the amid I region ($1,580\text{--}1,720\text{ cm}^{-1}$). The two principal components (PC1 and PC2) explained more than 91% of the total variance in both dry and cooked pasta samples.

Protein tertiary structure by front face fluorescence spectroscopy. Intrinsic protein fluorescence is related to the presence of aromatic AA, notably tryptophan, whose emission is highly sensitive to its local environment. Tryptophan fluorescence has been used to monitor the change in protein tertiary structure in complex food system (Bonomi et al., 2012; Karoui et al., 2006). The maximum intensities and their corresponding wavelengths (λ_{max}) of both dry and cooked pasta are presented in table 2.3. The λ_{max} of all the pasta was around 330 nm, indicating that the tertiary structure of the proteins in the pasta created a more hydrophobic environment around tryptophan (Heitz & Van Mau, 2002), probably related to the hydrophobicity of tryptophan microenvironment in gluten protein (Genot et al., 1992). Similar λ_{max} values were observed by Karoui et al. (2006) in wheat pasta. To our knowledge no study has reported the fluorescence properties of tryptophan in legume or egg enriched-pasta. However, a λ_{max} values around 330 nm would be expected for F-pasta, as the λ_{max} of legumin (11S) and vicilin (7S), the main storage proteins in faba bean seed and wheat proteins, were 320-329 and 330 nm, respectively (Genot et al., 1992; Kosinska et al., 2011). Neither VHT drying nor cooking changed the hydrophobicity of tryptophan microenvironment whatever the pasta considered. Tryptophan emission intensities in both dry and cooked pasta were also analyzed (table 2.3). In dry pasta, a significant effect ($p<0.05$) of the drying temperature was observed. VHT drying led to a drastic decrease in emission intensity whatever the pasta considered. Fluorescence quenching by disulfide bonds could be involved, as already reported in gluten after heating (at 70 °C) and cooling steps (Genot et al., 1992) and in commercial pasta dried at high vs. low temperatures (Bonomi et al., 2012). The degree of decrease in emission intensity caused by drying temperature also differed according to the formulation of the pasta (significant effect of interaction). Emission intensity decreased 2.2 times in F- and E-pasta vs. only 1.6 times in W- and G-pasta. Even less pronounced than the effect of drying, pasta formulation had a significant impact on emission intensities (see F-values in table 2.3). The enrichment of LT dried pasta with F-flour resulted in a drastic (50%) decrease in emission intensity, whereas enrichment with E-powders led to a slight increase (7%) in fluorescence intensity. No significant change in this parameter was observed in G-pasta. These differences in emission intensities due to pasta formulation could be related to the difference in protein structure (notably the quantity of disulfide bridge which acts as a quencher of tryptophan residues), without neglecting the possible effect of the AA composition (notably the amount of fluorescent residues: 9, 12 and 16 mg/g for F-, W- and E-proteins, respectively) (FAO, 1970). After cooking, the effect of drying temperature, formulation and their interaction on emission intensity was still observed ($p<0.05$). As

observed in dry pasta, the spectra of cooked VHT had a lower emission intensity than the spectra of LT ones.

Table 2.3 Tryptophan maximum emission wavelengths (λ_{max}) and corresponding fluorescence intensity of wheat- (W) pasta and gluten (G), faba bean (F) and egg (E) enriched-pasta dried at low temperature (LT) and very high temperature (VHT), in the dry state or after cooking to optimal cooking time +1 min.

Pasta formulation and drying		$\lambda_{\text{max}} (\text{nm})$ ¹		Max. intensity (a.u.) ²	
		dry	cooked	dry	cooked
W	LT	332	331	645 ^b	732 ^c
	VHT	334	331	411 ^a	566 ^b
G	LT	332	331	661 ^b	776 ^e
	VHT	334	330	414 ^a	571 ^b
F	LT	331	329	326 ^e	446 ^a
	VHT	331	328	146 ^c	261 ^d
E	LT	332	331	687 ^f	717 ^c
	VHT	335	330	302 ^d	429 ^a
Formulation		-	-	1088	1030
F-value	Drying	-	-	3501	2172
Interaction ³		-	-	97	35

¹Relative standard deviation<0.2%.

² Means in the same column with the same letter are not significantly different ($p>0.05$). a.u.: arbitrary units.

³ Interaction between drying and formulation.

Results are means of 6 replicates.

3.5. Impact of formulation and thermal treatment on the nutritional quality of pasta

AA profile. Total EAA and their scores (EAAS), based on ANSES (2007a) recommendations, in blends of raw materials used for pasta production are listed in table 2.4. Incorporation of G- or E-powder or F-flour in W-semolina resulted in an increase in total EAA of 3, 14 and 21% respectively, counterbalanced by a decrease in DAA compared to W-semolina. Low AA scores for lysine and to a lesser extent for threonine were observed in W-semolina (respectively 56 and 90%) and G-blend (respectively 51 and 95%), both these AA being deficient in wheat protein (Abdel-Aal & Hucl, 2002). W-semolina enrichment with 5% of E-powder may make it possible to recover the required amount of threonine and to increase the lysine score to 81%. The adequate lysine AA score was only achieved in the F-blend (lysine score: 107%).

Table 2.4 Essential amino acid scores (EAAS), total essential amino acid (EAA) and dispensable amino acid (DAA) contents in raw material and in low (LT) and very high temperature (VHT) dried pasta. W, wheat; G, Gluten; F, Faba bean; E, Egg

AA	ANSES Recommendation (mg/g protein)	EAAS (% ANSES recommendation) ¹											
		Blends of raw material				LT dried pasta				VHT dried pasta			
		W	G	F	E	W	G	F	E	W	G	F	E
Histidine	17	159	155	182	169	141	135	158	159	147	133	172	151
Isoleucine	27	111	112	118	121	109	96	111	103	110	98	94	107
Leucine	59	104	107	112	112	108	102	114	107	105	104	106	109
Lysine	45	56	51	107	81	46	41	86	64	45	39	74	64
Sulfur AA	23	131	137	104	165	123	147	115	159	149	132	124	140
Aromatic AA	41	161	176	171	208	172	150	147	177	149	159	159	184
Threonine	25	90	95	116	116	94	97	120	116	88	95	112	115
Valine	27	116	117	127	135	120	119	131	130	115	116	111	137
		AA (mg/g protein)											
EAA		294	303	334	355								
DAA		706	698	666	645								

AA: amino acid, EAA: essential amino acid, DAA: dispensable amino acid, EAAS: essential amino acid score.

¹ Triptophan amino acid was not analyzed.

Pasta processing and drying at LT reduced lysine scores in all the pasta to a similar extent (around 20% loss) compared to blends of raw materials but without decreasing their threonine scores. EAAS still conformed with ANSES recommendations when the pasta was dried at VHT, except for lysine in all pasta, threonine in W- and G-pasta and isoleucine in F-pasta. Concerning lysine content, increasing the pasta drying temperature from 55 to 90 °C, only affected F-pasta (14% decrease in lysine in VHT vs. LT drying).

Protein digestibility of cooked (OCT+1 min) LT and VHT pasta. The amount of hydrolyzed protein after 30 min action of pepsin on LT and VHT cooked pasta is listed in table 2.5. The pasta proteins remained slightly digested (mean °H 4-6%) by pepsin, with no significant effect of the drying profile ($p\text{-value}>0.05$) as already reported by (Petitot et al., 2009b) in wheat pasta. ANOVA revealed a significant effect of pasta formulation ($p\text{-value}<0.05$) with no significant effect of interaction with drying temperature. Pasta protein enrichment with E-powder and especially with F-flour led to a significant increase in the degree of hydrolysis by pepsin in comparison with that in W-pasta (mean °H value of 4.97, 6.40 and 3.82%, respectively) whatever the drying temperature used. No statistical differences in °H were recorded when the pasta was enriched with G-powder. After 180 min of additional pancreatic hydrolysis, pasta proteins were noticeably digested (mean °H of 39-46%), and digestion appeared to be significantly affected by the formulation, with an interaction between formulation and drying. G-pasta was less digested than W-pasta (mean °H of 39.24 and 42.36%, respectively). Protein hydrolysis was still higher in F- and E-pasta compared to W-pasta (mean °H of 46.22, 44.28% vs. 42.36%, respectively).

Table 2.5 Results of two-way analysis of variance and an LSD test of the degree of hydrolysis of proteins (°H) by pepsin for 30 min, and 30 min pepsin +180 min pancreatin of cooked to optimal cooking time + 1min wheat (W) pasta, and gluten (G), faba bean (F) and egg (E) enriched-pasta

Protein hydrolysis	Comparison of means (LSD) test ¹								
	Analysis of variance			Effect of formulation			Effect of drying		
	Effects	F-value	p-value	W	G	F	E	LT	VHT
by pepsin	Formulation	14.3	0.0001	3.82 ^a	4.14 ^{ab}	6.40 ^c	4.97 ^b		
	Drying	0.08	0.7862					4.88 ^a	4.79 ^a
	Interaction	2.4	0.1031						
by pepsin and pancreatin	Formulation	49.7	0.0000	42.36 ^a	39.24 ^b	46.22 ^c	44.28 ^d		
	Drying	1.5	0.2432					43.28 ^a	42.77 ^a
	Interaction ²	8.6	0.0013						

¹ Means in the same row with the same letter are not significantly different ($p>0.05$). ² Interaction between formulation and drying. For each analyzed effect, the mean value for all conditions tested for the other effect is given. Results are means of three replicates.

Our results are consistent with *in-vivo* (Torres et al., 2006) and *in-vitro* (RayasDuarte et al., 1996) studies on 10% legume (fermented pigeon pea and lupin) enriched-pasta. To the best of our knowledge, there is no data in the literature on the impact of structural variation of E-pasta on its protein digestibility.

Even after the pancreatic phase, we reported no impact of drying temperature on protein digestibility. In agreement with our results, no influence of the drying profile of pasta on its digestibility was reported in W-pasta dried at or below 90 °C (De Zorzi et al., 2007; Petitot et al., 2009b). Only drying at VHT (90 °C) applied as a post treatment (after low temperature drying) (Petitot et al., 2009b) or drying above 110-180 °C (De Zorzi et al., 2007) reduced the digestibility of wheat pasta by 14% and 37% respectively.

4. Discussion

The primary objective of this study was to assess the impact of pasta protein enrichment on its structure and nutritional properties. Increasing the protein content of pasta from 13% (W-pasta) to 17% (G-pasta) reduced its water uptake and increased its firmness. This is in agreement with the results of Sissons et al. (2005) who reported that a coupled decrease in gluten and increase in starch content increased pasta water absorption, making it softer. G-pasta underwent less cooking loss. The particular structure of its protein network, notably its higher covalently linked protein network, could partly explain this decrease in cooking loss, and may have reduced the degree of protein hydrolysis in G-pasta. The change in pasta structure and protein digestibility obtained by increasing protein content from 13% (W-pasta) to 17% (G-pasta) were less pronounced than changes occurred in pasta when gluten was replaced by egg or faba proteins. F-pasta enrichment gave the highest lysine score (86% vs. 41% and 64% for G- and E-pasta, respectively), even with high temperature drying. Pasta texture has been shown to be highly dependent on the nature of the protein used for enrichment. E- and G-pasta were characterized by a higher firmness score with a better resistance to elongation (for E-pasta) and less cooking loss (for G-pasta) in agreement with previous studies on 10-20% gluten (Fardet et al., 1999) and 3-15% egg (Marti et al., 2014; Matsuo et al., 1972) enriched-pasta. Conversely, F-pasta had a weakened texture and greater cooking loss, as previously demonstrated by Petitot et al. (2010b) G- and E-pasta presented a more compact microstructure in the center, and, in E-pasta, even in intermediate regions compared to the open microstructure of the core of F-pasta. All these differences in cooking loss and in the textural and microscopic properties of G- and E- vs. F-pasta could be linked

both to the molecular properties of each protein used for the enrichment and to the contribution of each added protein to the total protein content in pasta. Indeed, proteins of F-flour composed of albumins and globulins (Kosinska et al., 2011), are 98% SDS-soluble with a low sulfur AA content (17 mg/g protein) and represented 50% of the total proteins in F-pasta (table 2.1). F-proteins diluted gluten network and reduced the opportunity for disulfide crosslinks to be formed, thereby weakening the protein structure in both dry and cooked F-pasta. Conversely, G- and E-proteins represented 28 and 27% (respectively) of the total protein content of enriched-pasta and both (but especially E-proteins) possessed a higher sulfur AA content (35 and 59 mg/g protein for G- and E-proteins, respectively) able to form disulfide bonds during drying and cooking, thereby strengthening the protein network. In addition to the composition of the proteins, which considerably affected pasta protein digestibility, and among the protein structure parameters we explored, the degree of protein hydrolysis in cooked protein enriched G-, E- or F-pasta appeared to be more related to the percentage of protein covalently linked than to its secondary and tertiary organization. Indeed, all pasta displayed an identical tryptophan environment hydrophobicity (λ_{max} at 330-335 nm). In addition, F-pasta proteins, which contained the highest β -sheets but the lowest covalently linked proteins, resulted in the highest degree of protein hydrolysis. Conversely, G- and E-pasta proteins were more covalently aggregated but G-pasta contained less β -sheet structure, while E-pasta contained the same amount as F-pasta proteins, and both were less hydrolyzed than F-pasta.

In the second step of this study, the effect of drying temperature on pasta structure was investigated on dry and cooked pasta as a function of the protein (G, E or F) used for their enrichment. Considering dry pasta, ANOVA analysis of the results of SE-HPLC, FTIR and fluorescence spectroscopy revealed that more molecular rearrangements of proteins were caused by the increase in drying temperature (55 vs. 90 °C) than by the change in pasta formulation. The denaturation of protein by VHT drying led to extensive β -sheet formation probably at the expense of α -helix unfolding. The resulting structure was stabilized by the formation covalent bridges leading to high covalent protein aggregation in all VHT dry pasta, as already reported in gluten proteins (Bruun et al., 2007; Georget & Belton, 2006) and in wheat pasta subjected to a severe hydrothermal treatment (60-100 °C) (Petitot et al., 2009b). The extensive formation of disulfide bonds was responsible for higher tryptophan emission quenching in VHT pasta in comparison to pasta dried at LT, in agreement with the results of Bonomi et al. (2012). When G-, E- and F-pasta were cooked, the differences between LT and

VHT pasta concerning their protein secondary structure and the density of the covalent protein linkages presented above were drastically reduced. Only a difference in the protein tertiary structure between VHT and LT cooked pasta remained (higher fluorescence quenching in all VHT vs. LT cooked pasta). As this fluorescence quenching was not associated with an increase in covalent bonds between proteins, it may be associated with a different molecular position of disulfide cross-links depending on the temperature used for drying the pasta before the cooking step. Disulfide bridges were probably closer to tryptophan residues in pasta dried at VHT, reflecting a more compact local tryptophan environment than in pasta dried at LT. These conformational changes in the protein network in pasta dried at VHT vs. pasta dried at LT were accompanied by an improvement in their firmness and elongation when cooked. Unlike the effect of formulation, these changes in the protein network structure observed at supramolecular scale were not related to a difference in the microstructure of VHT vs. LT pasta, and did not lead to any difference in protein digestibility. However, VHT drying, although beneficial for the rheological and cooking properties of pasta, decreased the lysine content of dry F-pasta by 14% compared to the same pasta dried at LT, which could negatively affect lysine release and bioavailability (Stuknyte et al., 2014). Interestingly, despite this loss, VHT F-pasta kept a higher lysine score than G- and E-pasta. In addition, no alteration in the *in-vitro* protein hydrolyses was caused by VHT drying making F-pasta interesting from a nutritional point of view. It is now necessary to confirm whether this behavior is maintained *in-vivo*.

5. Conclusion

The present investigation highlighted the impact of plant (i.e. gluten and faba bean) or animal (egg) protein enrichment on pasta structure, with particular emphasis on its protein network, and on the nutritional quality of the pasta. The protein network formed by the addition of egg was tight, β -sheet structured and stabilized through covalent bounds leading to improve textural and cooking properties of the pasta. Gluten or faba bean enrichments resulted in two distinct pasta structures. Like egg proteins, gluten enrichment improved the textural and cooking properties of the pasta by increasing protein covalent links without favoring β -sheet formation. Conversely, even if, like egg, faba bean enrichment of the pasta promoted β -sheet structure, it decreased covalent stabilizing bonds thereby altering pasta textural properties and cooking loss. High drying temperature of faba bean pasta could help recover textural properties, decrease cooking loss and bring them close to those of wheat pasta. In comparison to egg pasta, faba bean pasta presented a better amino acid profile, with

a high lysine content even when dried at very high temperature, and higher protein digestibility proof of its nutritional interest.

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Conclusions

La modification de la formulation des pâtes par augmentation de la quantité totale des protéines engendre des variations plus ou moins importantes de la structure des pâtes en fonction de la nature des protéines incorporées. Parmi les pâtes enrichies étudiées, la pâte contenant 35% de farine de fèverole a conduit à une meilleure composition en acides aminés que les pâtes enrichies avec 5% de blanc d'œuf ou 6% de gluten. Elle présentait également des propriétés culinaires et texturales inférieures à celles des pâtes enrichies au gluten et à l'œuf et une structure microscopique moins compacte. Ces différences aux échelles macroscopique et microscopique étaient liées à la structure moléculaire spécifique des protéines dans les pâtes enrichies avec de la fèverole, caractérisée notamment par un niveau plus élevé de protéines solubles au détriment de celles liées par des interactions covalentes. Cette structure protéique affaiblie de la pâte enrichie en légumineuse a conduit à une meilleure digestibilité protéique que celle enregistrée pour les pâtes enrichies en œuf et notamment en gluten.

L'utilisation des très hautes températures de séchage ont conduit à une amélioration considérable des propriétés culinaire et texturale dans toutes les pâtes, sans effet observé à l'échelle microscopique. A l'échelle supramoléculaire, l'augmentation des températures de séchage engendrait une expansion des structures en feuillet- β au dépend des hélices- α témoignant d'une dénaturation importante des protéines, accompagnée d'une agrégation par des interactions essentiellement de types disulfures. La cuisson des pâtes réduisait ces différences, conduisant à un degré identique de digestibilité *in-vitro* des pâtes séchées à basse et haute température.

Cette étude a démontré l'intérêt nutritionnel, notamment la composition en acides aminés et la digestibilité *in-vitro* des protéines, de l'utilisation de protéines de légumineuse pour l'enrichissement de pâtes par rapport à d'autres protéines de source végétale ou animale.

CHAPITRE 3

Production et caractérisation de la structure, des propriétés culinaires, nutritionnelles et organoleptiques des spaghetti contenant de 0 à 100% de légumineuse

CHAPITRE 3. PRODUCTION ET CARACTERISATION DE LA STRUCTURE, DES PROPRIETES CULINAIRES, NUTRITIONNELLES ET ORGANOLEPTIQUES DES SPAGHETTIS CONTENANT DE 0 A 100% DE LEGUMINEUSE

Le chapitre 2 a pu mettre en évidence l'intérêt nutritionnel de l'enrichissement de la pâte en fèverole en termes de digestibilité des protéines et de composition en acides aminés. Cependant, il a également démontré la nécessité d'augmenter le taux d'incorporation de la farine de légumineuse afin d'atteindre un apport maximal en lysine. De précédentes études ont démontré que l'incorporation de quantités importantes de farines de légumineuses (au delà de 35%) dans une pâte fabriquée à l'échelle pilote posait des problèmes techniques au cours du malaxage et de l'extrusion des spaghetti. De plus elle peut modifier la structure des pâtes à plusieurs niveaux d'échelles se répercutant ainsi sur leurs propriétés culinaires et texturales. Ce chapitre est consacré à l'étude de la processabilité à l'échelle pilote de pâtes enrichies avec une quantité élevée (35 à 100%) de farine de fèverole, de lentille ou de haricot urd (black gram), avec un éclairage particulier mis sur la relation entre la concentration de protéines de légumineuse (exprimée par rapport au protéines totales dans la pâtes, soit de 0 à 100%),et sur l'intensité du séchage utilisé, sur la structure du réseau protéique de la pâte. Les répercussions d'un changement de structure de la pâte sur leurs propriétés culinaires, texturales, sensorielles et sur la digestibilité *in-vitro* des fractions protéines et amidon ont également été étudiées.

Partie 1. Procédé de fabrication de pâtes destinées à l'alimentation humaine et/ou animale comprenant au moins 35 % de légumineuses.

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Le modèle alimentaire des pays méditerranéens, composé d'un mélange de céréales et de légumineuses est reconnu pour ses vertus bénéfiques pour la santé, notamment en termes de prévention des maladies cardiovasculaires, du diabète de type 2, du cancer et de l'obésité. Les aliments mixant les céréales et les légumineuses comme l'Idlis, existent depuis longtemps dans plusieurs pays en développement (Koh & Singh, 2009; Nagaraju & Manohar, 2000; Thakur et al., 1995). Ces deux denrées alimentaires sont complémentaires au niveau nutritionnel, et leur réunion au sein d'un seul et même produit alimentaire permet de bénéficier de la valeur nutritionnelle de chacun des deux composés (richesse en protéines, fibres, composition en acides aminés essentiels améliorée).

Parmi les aliments, les pâtes alimentaires constituent une base extrêmement intéressante pour cette association. C'est en effet un aliment courant, bon marché, facile à préparer et à accommoder, apprécié de toutes les catégories de la population et se conservant très longtemps (pâtes sèches), dont les avantages nutritionnels pourraient ainsi bénéficier à un grand nombre de consommateurs. A ce jour, si l'enrichissement des pâtes en légumineuses a déjà été pratiqué, notamment lors des travaux de recherche antérieurs (Petitot et al., 2010a; Petitot et al., 2010b; Petitot & Micard, 2010), les niveaux de substitution de la céréale par la légumineuse demeurent généralement inférieurs à 50%. Même si ces taux de substitution de la céréale par la légumineuse permettent d'améliorer légèrement sa teneur en protéines, ils ne permettent pas d'atteindre une couverture en acides aminés indispensables dans l'aliment mixte fabriqué. Des taux de substitution en légumineuse supérieurs seraient nécessaires. Cependant, un taux de substitution élevé ($> 30\text{-}35\%$) du blé par la légumineuse dans la pâte peut être à l'origine de la formation de pâtes collantes et hétérogènes, laissant apparaître de grosses particules pendant le malaxage, rendant difficiles voire impossible les premières étapes du processus de fabrication des pâtes, le malaxage et l'extrusion (Petitot et al., 2010b; Wood, 2009). Ce qui explique, que jusqu'à présent ces taux de substitution élevés des céréales par les légumineuses ne soient pas utilisés.

L'intérêt de maîtriser le taux de substitution des céréales par les légumineuses au sein d'une pâte alimentaire peut également concerner, poussé à l'extrême (100% substitution), l'alimentation des personnes souffrant de maladie cœliaque, qui ne peuvent ingérer de gluten (protéines présente dans le blé notamment) et pour lesquelles l'évitement totale à vie du gluten de leur régime alimentaire reste la seule solution. Le développement de ces produits se fait grâce à la modification de certains ingrédients intervenants dans la formulation de ces aliments. Les pâtes sans gluten présentes actuellement dans le commerce sont fabriquées à partir de farine de maïs ou de riz seule ou en mélange avec du millet, du lupin, des protéines de pois, du quinoa (fabriquées par Schar, Gerblé, Ma vie sans gluten...). Ces pâtes sont souvent moins structurées et présentent des propriétés texturales et culinaires différentes des pâtes ordinaires 100% blé dur (Hager et al., 2012b; Lucisano et al., 2012; Marti & Pagani, 2013). La farine de riz donne un résultat très blanc et un peu collant, peu ferme, la farine de maïs donne des pâtes très jaunes, cassantes. Le mélange riz et maïs permet de se rapprocher de la pâte au blé mais les pâtes fabriquées avec ce mélange demeurent toujours plus cassantes et plus « farineuses ». De plus ces pâtes sans gluten sont plus pauvres en minéraux (Marti & Pagani, 2013) et en protéines (3-10% de protéines) (Hager et al., 2012b). Introduits dans le marché depuis plus de 50 ans, les aliments sans gluten, ont connu une expansion en raison des enjeux socioéconomiques importants liés à la maladie cœliaque. Une étude menée par (Lee et al., 2007) a démontré que la disponibilité de ces produits est toujours faible, et leur coût reste supérieur à leur homologue contenant du gluten. Les pâtes 100% légumineuses permettraient de diversifier l'offre actuellement existante du marché en terme de pâtes sans gluten.

Nos travaux ont été conduits dans l'objectif de lever le verrou technologique lié à l'incorporation de plus de 35% de farines de légumineuses quel que soit sa nature, comme ingrédient dans la production des pâtes alimentaires, en utilisant, notamment un procédé industriel classique de pastification, et ceci dans un contexte de durabilité (transition alimentaire animale/végétale) et de nutrition (maladie cœliaque).

Partie 2. Publication 2. How structure, nutritional and sensory attributes of pasta made from legume can be impacted by the legume protein inclusion level?

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Soumise à LWT-Food Science and Technology

Abstract

In the last decades, scientists researched an alternative to meat and wheat protein for both environmental and intolerance concerns. In this study, Faba bean flour, as a rich source in protein with a low glycemic index, was chosen to replace partly or totally wheat in pasta in order to enhance its protein quantity and quality. When increasing the ratio of faba bean/wheat protein from 0 to 100% in pasta, its protein network became more discernable at the microscopic scale. The gluten network of a classical dried wheat pasta, characterized by a high proportion of covalent interactions, was linearly diluted by weakly linked proteins coming from faba bean. Concomitant linear increase of the cooking loss (up to 2.6 fold), decrease of the resilience (up to 1.4 fold) and increase of the *in-vitro* protein digestion (up to 25%) were observed in pasta. The use of VHT (90 °C), instead of LT (55 °C), to dry pasta increased the proportion of protein network covalently aggregated in all dried pasta. However, the impact of the strengthening of protein matrix by VHT drying was more efficient to enhance resilience and to reduce cooking loss in faba bean enriched pasta than in wheat pasta. The degree of protein hydrolysis in legume pasta was not decreased by the use of VHT vs LT drying, despite its stronger protein network. VHT drying slightly decreased the TIA in all pasta legume pasta. Sensory attributes of faba bean enriched pasta were compared to both a commercial cereal gluten-free and a whole-wheat pasta. Liking scores of LT pasta containing 80% faba bean protein were interestingly closed to scores obtained for the whole-wheat pasta. LT or VHT pasta made exclusively from faba bean tended to be more appreciated than their commercial gluten-free counterparts.

Key words: Protein network, SE-HPLC, microscopy, *in-vitro* protein digestion, TIA and TDS.

1. Introduction

Wheat and meat have constituted for a while the main protein source in human diet. Their consumption are planned to reach in 2016, 66.9 and 43.4 kg/hab/year, respectively, according to the FAO report (FAO, 2015). However, WHO (World Health Organisation, 2015) recommended to decrease the consumption of meat because of its ecological and health disadvantages of their excessive consumption. Beside its environmental impact (European Commission, 2006), an excessive meat consumption have been demonstrated to increase the risk of heart diseases (Bernstein et al., 2010). In the last decades, the request for an alternative to meat proteins in the diet is increasing. Various studies demonstrated the beneficial effect of the Mediterranean diet, notably rich in legumes, on the reduction of cardiovascular diseases (Cam & de Mejia, 2012; Turati et al., 2015). The potential of legume such as faba bean to partly replace meat intake in the human diet was also reviewed by Multari et al. (2015). In addition, the association of wheat and legumes in the same food helps to benefit from the nutritional composition of the two crops notably their complementary profile in essential amino acids (Duranti, 2006). Among the several traditional wheat products, pasta constitutes an interesting base for this association because of its palatability, low cost and wide consumption. Legume-wheat mixed pasta with 50% of its protein issued from faba bean was demonstrated to have, at identical protein content, a better essential amino acid profile than classical wheat-gluten or wheat-egg enriched pasta (Laleg et al., 2016a). Such faba bean enriched pasta also got higher fiber (Greffeuille et al., 2015), vitamin and mineral (Petitot et al., 2010b) contents than a classical wheat pasta with a conserved low glycemic index (Greffeuille et al., 2015). However, the total body requirement in essential amino acids (ANSES, 2007a) has not been achieved in pasta because technological issues were encountered when introducing in pasta more than ~50% proteins issued from legumes (Petitot et al., 2010b; Wood, 2009). Two recent studies demonstrated that it is now possible to overcome this previous threshold in legume protein incorporation into pasta and render even possible to produce pasta with legume as a unique source of proteins (Laleg et al., 2016b; Rosa-Sibakov et al., 2016). These totally gluten-free legume pasta could be of interest for celiac patients, or people who want to reduce or to remove gluten from their diet.

However, legume protein incorporation into pasta can also have unexpected nutritional and sensory effects. Legumes contain some protease inhibitors such as trypsin inhibitors, which can alter the digestibility of proteins (Duranti, 2006) but could hopefully be partially or totally inactivated by cooking legume pasta (Zhao et al., 2005). In addition, it has been

reported that beyond 50% of gluten protein substitution with legume protein, culinary properties and sensory acceptance of pasta are depreciated (RayasDuarte et al., 1996). It was attributed to the dilution or the absence of the gluten network responsible for the pleasant organoleptic and cooking quality of pasta. The use of high temperature to dry pasta was demonstrated to prevent the alteration of culinary, textural and organoleptic properties of classical and low legume protein (<50% of total protein) enriched pasta by promoting covalent links between protein (Petitot & Micard, 2010). Whereas drying temperature effect on pasta with higher legume protein substitution degree remains unstudied.

This work aimed to study the impact of the level (0 to 100%) of protein enrichment issued from legume (faba bean) in pasta and the impact of drying temperature (LT vs VHT) on pasta structure and its resulting textural and culinary properties. The effect of change in pasta formulation and/or process on trypsin inhibitory activity and on its protein network structure and the resulting *in-vitro* digestibility was discussed. Pasta with the better textural, cooking and/or nutritional qualities was subjected to consumer acceptance analyses and compared to a commercial gluten-free and a whole wheat counterparts, using for the first time Temporal Dominance of Sensations test.

2. Material and methods

Wheat (W; *Triticum durum*) semolina (Panzani, Aix-en-Provence, France) and Faba bean (F; *Vicia faba*) flour (GEMEF industries, Aix-en-Provence, France) contained 13.1 and 24.0% proteins, 77.8 and 57.6% starch, and 2.4 and 11.7% fibers, respectively. Two commercial pasta were purchased from the local French market and used to evaluate the sensory attributes of our pasta. Celnat (Saint-Germain-Laprade, France) spaghetti from whole-wheat (S-WW), was composed of 70% carbohydrate, 11.5% proteins, 2.4% fat and 0.8% fibers; and Schär (Burgstall, Italy) spaghetti (S-GF) from maize, rice and millet, was composed of 78% carbohydrates, 8.3% protein, 1.5% fat and 2% fibers.

2.1. Pasta production

Pasta containing 0 (F0), 50 (F50), 80 (F80) and 100g (F100) of protein issued from faba bean per 100g of total protein was produced using a mixture of W-semolina and F-flour in the ratio W:F of 100:0, 65:35, 30:70 and 0:100, respectively. Pasta formulation is detailed in table 3.1.

Table 3.1 Formulation of pasta and protein composition of F0, F50, F80 and F100 pasta; and the amino acid content of low (LT) and very high temperature (VHT) dried pasta.

Amounts of Raw materials (g per 100g, db)	F0	F50	F80	F100
W-semolina	100	65	30	0
F-flour	0	35	70	100
Composition in protein (g per 100g of pasta)				
Total protein content	13.1	16.9	20.7	24.0
W-protein ¹	13.1	8.5	3.9	0.0
F-protein ¹	0	8.4	16.8	24
W/F-protein ratio ¹	100/0	50/50	20/80	0/100
Lysine amino acid (mg/g protein)				
LT dried pasta	20.5	38.6	55.0	65.1
VHT dried pasta	20.3	33.4	52.4	60.3
Cysteine amino acid (mg/g protein)				
LT dried pasta	17.2	14.8	10.4	9.6
VHT dried pasta	17.4	15.4	10.4	8.8

¹ Results obtained by calculation according to the raw material composition

F0 and F50 pasta were processed to spaghetti form as described by (Petitot et al., 2010b). F80 and F100 pasta were produced according to the process described by Laleg et al. (2016b) in the FR 14 62811 patent. All pasta were produced in triplicates using a continuous pilot scale pasta extruder (Bassano, Lyon, France), then dried at low temperature 55 °C (LT) or at VHT (90 °C) in a pilot-scale drier (AFREM, Lyon, France) to reach 12% moisture. The diameter of dried pasta was 1.56 mm ± 0.02 for F0 and F50, and 1.47 ± 0.03 mm for F80 and F100. The pasta composition is given in table 3.1.

2.2. Molecular structure of the protein network of dried pasta

Raw materials (W-semolina and F-flour) and dried (LT and VHT) pasta (F0, F50, F80 and F100) were subjected to two sequential protein extractions performed in sodium dodecyl sulfate (SDS, to disrupt the weak interactions) and then in SDS + dithioerythritol (DTE) subjected to sonication to disrupt the disulfides bonds. Protein were extracted in triplicates and then analyzed by size-exclusion high performance liquid chromatography (SE-HPLC) according to the modified method of Morel et al. (2000) as described by Petitot et al. (2009b). Remaining proteins that were not extracted in neither SDS nor in DTE constituted the non-extractable fraction. SDS-soluble and DTE-soluble proteins were expressed as the percentage of the total extractable proteins from W-semolina (for F0), from blends of semolina and F-

flour with 50% and 80% protein from F-flour (for F50 and F80, respectively), or from F-flour (for F100).

2.3. Cooking losses of pasta

Each pasta was cooked to its own optimal cooking time (OCT) according to the AACC approved method (66-50). Cooking losses (% db) was calculated as the difference between the dry matter of each dried and cooked pasta, reported to the dry matter of dried pasta (Petitot et al., 2010b). Experiment was performed in triplicates.

2.4. Resilience of cooked pasta

Texture profile analysis (TPA) was performed, as described by Petitot et al. (2010b) on pasta cooked to OCT using a TA-XTplus (Stable Micro Systems, Scarsdale, USA) texture profile analyzer equipped with Texture Expert software package (Stable Micro Systems, Scarsdale, USA). Pasta resilience was then obtained (Petitot et al., 2010b). Three replicates from 2 different cooking ($n = 6$) were performed.

2.5. Microscopic structure of cooked pasta

F0 (LT) and F100 (LT and VHT) cooked (to OCT) pasta sections (8 μm) were cut at -20 °C using microtome (Microm HM 520, Walldorf, Germany). The sections were stained with 1g/L fast green (Sigma Aldrich Co., USA) and 1:8 (v/v) diluted lugol (Fluka, Buchs, Switzerland) (Petitot et al., 2010a). Bright field images were acquired using the multizoom AZ100M microscope (Nikon, Japan) equipped with a Nikon DSRII (Nikon, Japan) color digital camera. Observations were made with a plan fluor 5x objective and a fixed optical zoom of 8 leading to a global magnification of 40x.

2.6. Trypsin inhibitory activity and *in-vitro* protein digestion of cooked pasta

Trypsin inhibitory activity (TIA) was determined according to the standardized method ISO 14902 (2009) on raw material and OCT cooked pasta. *In-vitro* protein digestion of pasta was performed in triplicate on freeze-dried OCT cooked pasta after 30 min of pepsin and 180 min of pancreatic attack (Pasini et al., 2001). The degree of hydrolysis (°H) of proteins was determined as described by Laleg et al. (2016).

2.7. Statistical analysis

ANOVA two ways was performed using “formulation” and “drying” as factors followed by the Fisher’s least significant difference (LSD) test to compare means at the 5% significance level, using Statistica 8.0 software (Tulsa OK, USA).

2.8. Sensory analysis of cooked pasta by Temporal Dominance of Sensations (TDS)

Six variant of pasta were tested: F0-LT, F80-LT, F100-LT and F100-VHT (cooked to OCT) and two commercial pasta, S-GF and S-WW (cooking time indicated by the manufacturer). Forty-three naïve subjects, aged from 19 to 69 years old were approximately balanced for gender and age. Twenty-one of them were regular consumers of whole-wheat pasta, whereas the twenty-two others were regular consumers of classical pasta. A pre-test with an internal panel permitted a selection of ten attributes (Table 3.2), which were presented and explained to the subjects prior to sensory session. The subjects had to smell food references to familiarize themselves with the unusual flavor attributes. The concept of dominance was explained to the subjects by a simulation of the data acquisition. A dominant sensation was defined as a sensation that triggers the most attention at a point of time (Pineau et al., 2009).

Table 3.2 Temporal dominance of sensation (TDS) list of attributes, with their definitions and/or references

Attributes	Type	Definition	References
Firm	Texture	Firmness, chewing resistance	/
Floury	Texture	Like texture of flour in mouth	/
Sticky, pasty	Texture	Sticks to teeth and palate	/
Elastic	Texture	Deforms without breaking	/
Rough	Texture	Irregularities perceived at the surface of the pasta (not smooth)	/
Salty	Taste	Salty taste	/
Legume	Flavor	Flavor of lentils, beans, chickpeas...	Cooked lentils, cooked beans...
White cereal	Flavor	Flavor of white flour, refined wheat	White flour
Whole cereal	Flavor	Flavor of whole-wheat flour, buckwheat	Whole-wheat flour, cooked buckwheat
Grilled	Flavor	Grilled and toasty flavor	/

For the data acquisition, the attributes were presented simultaneously on the computer screen with corresponding buttons to click on.

Sensory sessions were organized in a sensory room equipped with separate booths, under red lights at 20 °C (ISO 8589, 2007). A computerized system was used to capture the data (TimeSens®, www.timesens.com). The subjects received portions of about 40-50g of each of the six variants of cooked pasta, monadically presented.

For each variant, the consumer had to take one bite, to put it in mouth and to click on a “In mouth” button. Then, he had to focus on which sensation was perceived as dominant and to click on the corresponding button. When the dominant perception changed, the subject had

to score the new dominant sensation. He was free to choose several times the same attribute or conversely to never select an attribute as dominant. He had to carry on scoring dominant sensations after swallowing, until the perception ends. Then, he had to click on a “I no longer perceive anything” button to stop the process. Afterwards, the computer screen displayed a linear scale labeled at the left anchor ‘I do not like it at all’ and at the right “I like it very much” (Meilgaard et al., 2006) and the subject had to score his current liking. He had to do the same for a second, then for a third bite of the variant.

The confidence level was set to 5% for the analysis of variance tests and the comparison of means. For the liking scores, statistical calculations were performed using SAS system (SAS Institute Inc., Cary, NC, USA). The analyses of the TDS measurements were performed using TimeSens® software. The analysis of the liking scores was performed thanks to the MIXED SAS® procedure, with product, bite, type of consumers and their 3 interactions of order 2 as fixed effects. Consumer and its interaction with bite and with product were the three random effects in this model in which bite was a repeated factor within product by consumer with a heterogeneous auto-regressive covariance structure. Afterwards, least square means were calculated for the significant effects in order to analyze the highlighted differences.

For each variant of pasta and each bite, TDS curves were produced (Pineau et al., 2009). Times were standardized between 0 (first score) and 1 (“I no longer perceive anything”) (Lenfant et al., 2009).

3. Results and discussion

3.1. Structure of pasta protein network

Figure 3.1 shows the protein soluble in SDS (weakly linked), in DTE (disulfide linked) and non-extractable (covalently, other than disulfide, linked) protein in dry (LT and VHT) pasta as a function of F-protein concentration.

In LT pasta, increasing F-protein from 0 to 100% linearly increased the weakly linked protein (SDS-soluble) in the rate of 0.24 (figure 3.1A) and linearly decreased disulfide linked (DTE-soluble) proteins in the same rate (-0.25) (Figure 3.1B), with no creation of others covalently linked (non-extractable) proteins (Figure 3.1C). Laleg et al. (2016a) and Petitot et al. (2010b) substituting 50% of wheat protein by two legume flours also detected a decrease in DTE soluble proteins concomitant to a SDS soluble increase. DTE soluble proteins reached also in their study 15% and 20% of total proteins for faba bean and split pea, respectively.

However, we demonstrated here for the first time the linear character of the weakening of protein network when faba bean protein increased in pasta. Only 4.5% proteins were proteins disulfide bounded in a F100-LT, against 30% in F0-LT pasta. In the corresponding raw materials these percentages were 3 and 15%, in F-flour and W-semolina respectively. Only wheat proteins seemed therefore to have reacted under LT drying conditions through disulfide bonds. Even if the F-protein contained non negligible cysteine (9.6 mg/g protein counter 17.2 mg/g wheat protein), they created only minor disulfide linked proteins under LT drying. This fact and the linear character of the decrease of DTE-soluble protein could indicate that F-proteins acted as a diluting agent of gluten in mixed W-F pasta dried in LT conditions. The low water content required to pasta production, the low energy input (during mixing) and the LT drying program used to dry pasta, were probably not enough to force W-F and F-F protein to interact covalently by disulfide bridges.

A VHT drying of pasta, whatever its F-protein content, promoted in comparison to LT drying a higher formation of disulfide and an additional creation of others covalent linkages (as isopeptide or Maillard products) at the expanse of weakly linked proteins (Figure 3.1). The creation of additional disulfide bound under VHT in comparison to LT drying at the expanse of weakly linked proteins was already reported on wheat and protein (legume or egg) enriched pasta (Laleg et al., 2016a; Petitot et al., 2009b; Petitot & Micard, 2010). The formation of covalent bonds others than disulfide under VHT drying was also already reported for wheat pasta (De Zorzi et al., 2007; Stuknyte et al., 2014), and in 35% legume flour enriched pasta (Petitot & Micard, 2010). In our study, covalently linked protein are even detected in a large proportion in F100-VHT in comparison to F100-LT (32 % vs 4%, respectively), showing that high drying temperature allows an additional reactivity of F-proteins. Under VHT drying, disulfide bounded proteins were therefore probably formed through the interaction of free sulfhydryl groups between W-W proteins as in LT pasta but also from F-F protein or even W-F proteins. In VHT pasta, as in LT pasta, the variations of all kinds of protein linkages (weak, disulfide and other covalent) as a function of F-protein concentration were linear. However, the rate of weakening of protein network (increase of SDS-soluble proteins) when increasing F-protein concentration was two time higher in VHT pasta than in LT pasta.

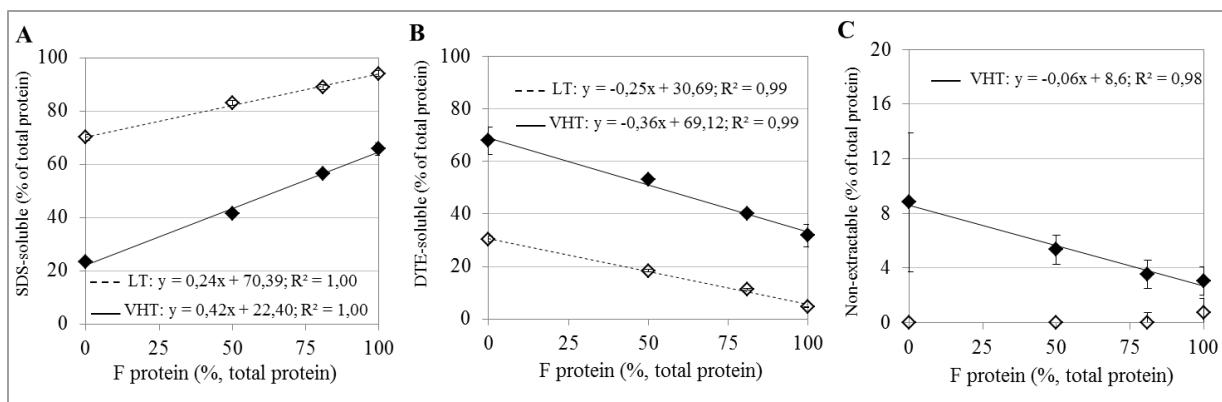


Figure 3.1 Evolution of sodium dodecyl sulphate (SDS), dithioerythritol (DTE) soluble proteins and insoluble protein (non-extractable) from pasta dried at low (LT) or very high temperature (VHT), as a function of the percentage of faba bean (F) protein in pasta.
Experimental data and best fit. \diamond LT ; \blacklozenge VHT.

3.2. Microscopic structure

Figure 3.2 shows the microscopic structure of F0-LT, F100-LT and F100-VHT cooked pasta. The central region of F0-LT presented a compact starch granules (blue) with almost no clearly visible protein network (green). Whatever the drying temperature, F100 presented a more opened structure, with a highly swollen starch granules and visibly thicker protein network. Starch granules were elongated in F0 and round/oval in F100, related to the respective shape of starch in wheat and faba bean raw materials (Petitot et al., 2010a). Internal region of F100-LT and F100-VHT differed slightly on the color of starch granules which were slightly darker in F100-VHT, probably related to a limited gelatinization (Cunin et al., 1995). The external region of F0-LT presented highly swollen and disintegrated starch granules in comparison to the pasta core as already reported in the literature (Petitot et al., 2010a). In F100-LT, the starch granules were in the external region, as swollen as in the pasta core, and coalesce to form a continuous phase in the surface of pasta excluding protein mass. In F100-VHT, starch was more held into a protein network, which was more uniformly dispersed around starch granules than in F100-LT.

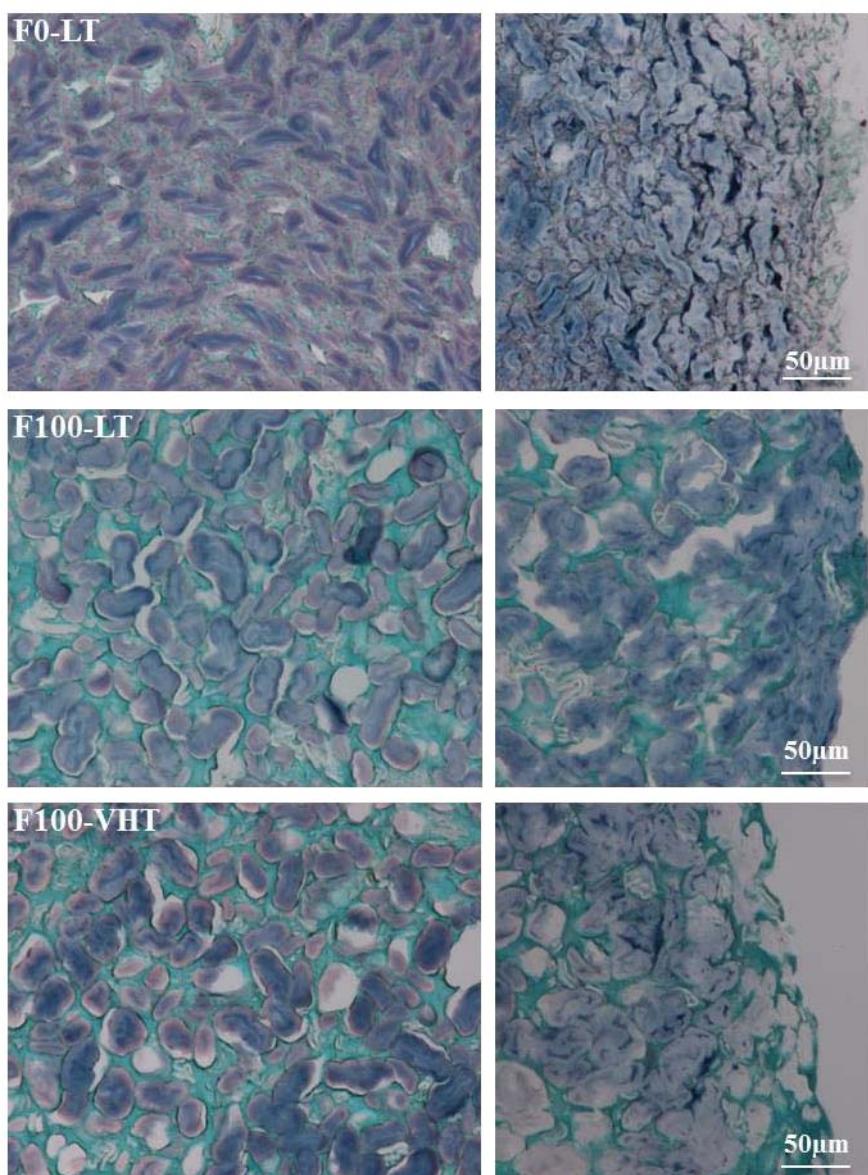


Figure 3.2 Light microscopy of cooked pasta with 100% protein from wheat and dried at low temperature (F0-LT); and pasta with 100% protein from faba bean and dried at low (F100-LT) and very high temperature (F100-VHT).

3.3. Cooking and textural properties

Pasta cooking losses and resilience are presented in figure 3.3A and 3.3B, respectively. In LT pasta increasing F-protein from 0 to 100% linearly increased the cooking loss, and linearly decreased the pasta resilience. As in our results, Petitot et al. (2010b) reported 7% cooking loss and 0.53-0.54 resilience in pasta containing 50% of its total protein from faba bean. Rosa-Sibakov et al. (2016) found 11% cooking loss in pasta made exclusively from faba bean, slightly lower than in our study (14%), whereas an identical pasta resilience of 0.41. The unique ability of gluten to form a protein network is the primary factor

responsible for the lower cooking loss and the good textural properties of wheat pasta (Matsuo et al., 1972; Matsuo & Irvine, 1970). The incorporation of non-gluten material, as faba bean proteins, in pasta dilutes the gluten network and weakens its overall structure as seen in SE-HPLC section explaining at least partially the decrease in pasta resilience and cooking loss. (RayasDuarte et al., 1996); Torres et al. (2007a) also attributed the higher release of solids into the cooking water to the dilution of gluten network in a 5-30% lupin enriched pasta. In addition, fibers (higher in faba bean flours than in wheat semolina 11.7%, db against 2.4%) can also have contributed to the weakening of pasta structure when introducing increasing amount of faba bean in pasta. According to Padalino et al. (2014) and Petitot et al. (2010b), the inclusion of pea fibers promoted indeed the formation of discontinuities or cracks inside the pasta strand weakening its structure, resulting therefore in a low sensory elastic recovery.

In comparison to LT drying, VHT drying allowed to decrease the cooking loss and to increase the pasta resilience in all faba bean enriched pasta (F50, F80 and F100). These variations are linear when F-protein increased in pasta. The strengthening of pasta protein network demonstrated in the SE HPLC section could contribute to this overall improvement of pasta quality. Petitot et al. (2010b) also attributed the changes in quality of wheat/legume mixed pasta equivalent to our F50 pasta to the formation of a strong protein network entrapping starch granules through protein aggregation. We demonstrated that the reduction of cooking loss and improvement of pasta resilience under VHT vs LT drying were even more pronounced as the concentration of F-protein increased in pasta. VHT drying in comparison to LT drying led to an additional reactivity of faba bean proteins notably through disulfide linked protein creation (SE-HPLC section). At the opposite, VHT did not efficiently contribute to enhance pasta cooking loss and resilience when pasta is made exclusively from wheat protein (F0); even if pasta protein network has a higher proportion of covalently (disulfide and others) linked proteins. Petitot (2009) also observed this higher VHT effect on 35% faba bean and split pea enriched pasta in comparison to 100% wheat pasta. Even if VHT allowed faba bean enriched pasta to recover a higher resilience, this resilience reached a threshold corresponding to the value reached for a 100% wheat pasta whatever its drying temperature.

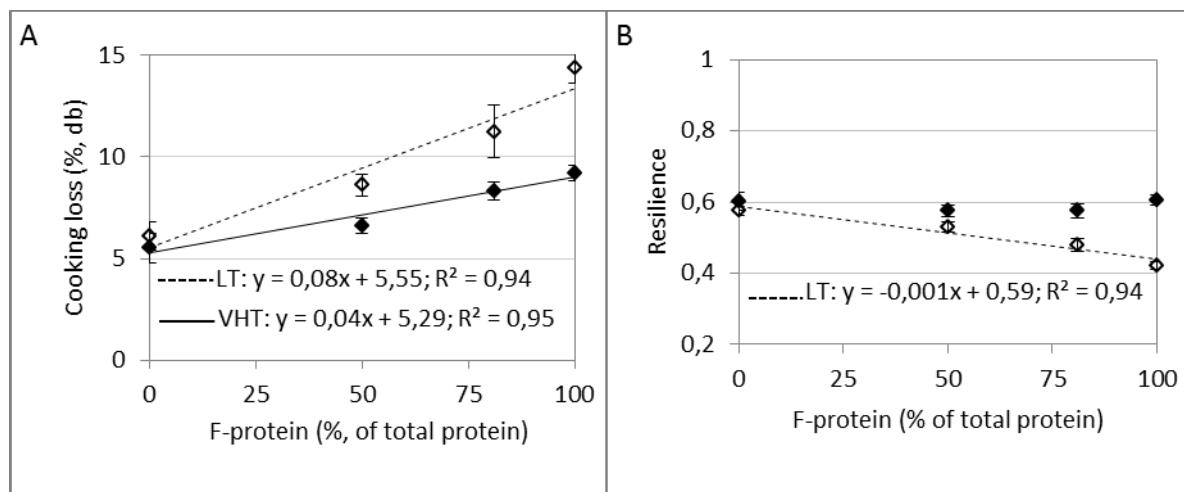


Figure 3.3 Evolution of cooking loss (A) and resilience (B) of cooked pasta dried at low (LT) or very high temperature (VHT) as function of the percentage of faba bean (F) protein in pasta. \diamond LT ; \blacklozenge VHT.

3.4. Trypsin inhibitory activity and *in-vitro* protein digestibility

The initial TIA measured in raw blends used for pasta production was 0, 3.92, 6.35 and 7.84 mg/g (db) respectively for F0, F50, F80 and F100 (result not shown). In cooked pasta (table 3.3), TIA increased significantly ($p<0.05$) following the order of F-protein incorporation. However, the amount of TIA reached a maximal value of 2.28 mg/g of cooked pasta for F100. Therefore, pasta processing and cooking steps decreased dramatically TIA (3.4 to 6.4 fold depending on the pasta considered), as already reported in the literature (Zhao et al., 2005). The decrease in TIA was more pronounced in F50 and F80 (both 84%) than in F100 (71%). Trypsin inhibitors are proteins with a compact structure stabilized through disulfide bounds (Mueller & Weder, 1989). The lower free sulphydryl in gluten-free F100 pasta than in F0 pasta, probably led to a lower rearrangement and unfolding of trypsin inhibitor during the pasta processing and cooking (Friedman et al., 1982).

Due to the multiplicity of methods used to assess TIA reported in the literature, it remains difficult to make a comparison with our own values. Nevertheless, TIA reported in the literature ranged from 2 to 18 mg/g for lentil (Ma et al., 2011; Vasagam & Rajkumar, 2011; Wang et al., 2009), 15 mg/g for black-gram (Vasagam & Rajkumar, 2011) and 3 mg/g for faba bean (Hove & King, 1979), and were all higher than those obtained for our cooked pasta.

The use of VHT drying had a lower ($F\text{-value} = 12$) but a significant effect on TIA than F-protein concentration ($F\text{-value} = 168$). VHT drying decreased slightly but significantly ($p=0.005$) TIA in pasta. Sulfhydryl-disulfide interchanges under VHT drying could probably

modify the structure of the trypsin inhibitor. No effect of the interaction between drying temperature and the pasta formulation was observed on TIA.

Table 3.3 Two-way analysis of variance of TIA (mg/g, db) and the degree of *in-vitro* protein hydrolysis ($^{\circ}\text{H}$; % of total protein) in cooked pasta containing different faba bean protein concentration. F0, F50, F80 and F100 present pasta in which 0, 50, 80 and 100% (respectively) of total proteins were issued from faba bean.

Analysis of variance ¹			Comparison of means-LSD test ²						
			Effect of F-protein concentration			Effect of drying			
	Effects	p-value	F-value	F0	F50	F80	P100	LT	VHT
TIA (n=3)	A : F-protein concentration	0.000	168	ND ³	0.61 ^a	1.03 ^b	2.28 ^c		
	B : Drying	0.005	12					1.44 ^a	1.17 ^b
	Interaction : A×B	0.480	0.8						
$^{\circ}\text{H}$ (n=3)	A : F-protein concentration	0.000	75	41.87 ^a	45.47 ^b	47.48 ^c	52.24 ^d		
	B : Drying	0.054	4					46.26 ^a	47.27 ^a
	Interaction : A×B	0.416	1						

¹ ANOVA two ways was performed using F-protein concentration and pasta drying temperature as factors

² Within the same row, the values with the same lower case letters are not significantly different (P>0.05). For each analyzed effect, the mean value for all conditions tested for the other effect is given.

³ Not detected by the method used.

Results of *in-vitro* protein digestibility of cooked pasta are presented in table 3.3. Increasing F-proteins in pasta increased significantly ($p<0.05$) the $^{\circ}\text{H}$ of proteins in pasta from 42 to 52%, in the order F0<F50<F80<F100. This could be related to the linear weakening of F-protein network as the F-protein content increased in pasta observed by SE-HPLC analyses. VHT tended to increase the $^{\circ}\text{H}$ in all F-protein containing pasta ($p=0.054$) which was unexpected as it increased the percentage of covalently linked proteins in all pasta. This could however be explained by the lower TIA registered in the VHT pasta than in LT pasta.

3.5. Sensory evaluation

Liking scores. The analysis of variance showed that the variants of pasta, F0-LT, F80-LT, F100-LT and F100-VHT and two commercial pasta, S-GF (gluten-free) and S-WW (whole wheat) were perceived significantly different in terms of liking ($F=3.56$, $p=0.04$). The average liking scores for each variant are displayed in figure 3.4. Overall, the hedonic scores were quite low, which can be explained by the unusual presentation of the products (no butter, no sauce). F0-LT was the preferred one, whereas S-GF pasta was the less appreciated one. The other pasta were close to each other. F80-LT and S-WW pasta tended to be more liked than F100-LT and F100-VHT. F80-LT seems to be the best compromise in terms of preferences.

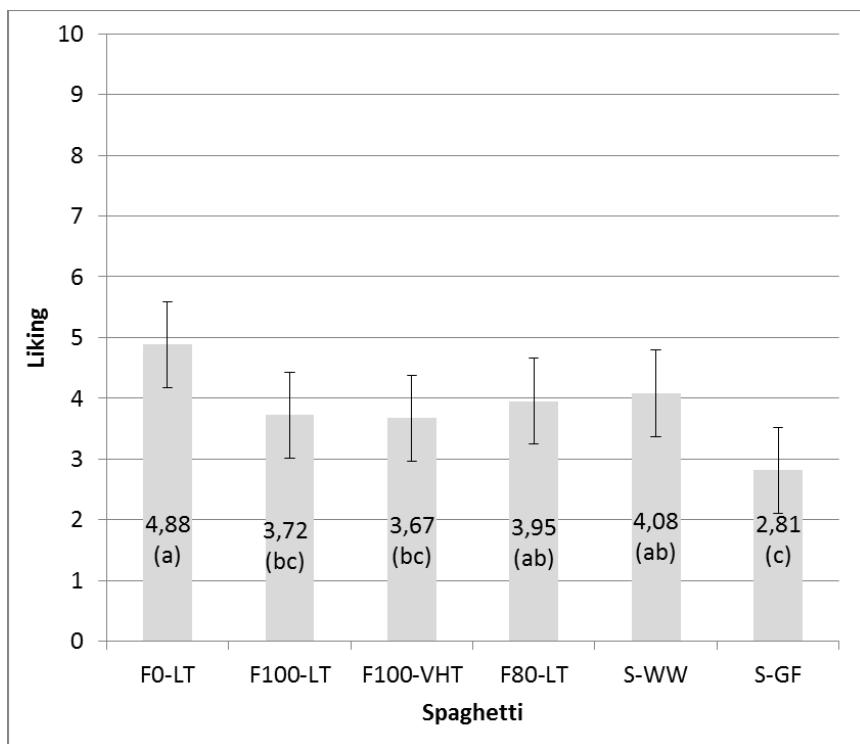


Figure 3.4 Average liking scores and their 95% confidence intervals of the different variants of pasta (LSMeans). Variants with a same letter are not significantly different

The analysis also underlined a significant Bite effect ($F=8.99$, $p=0.0005$). For all pasta the liking scores decreased over the bites (the LSMeans of liking for the first, the second and the third bites are respectively equal to 3.98, 3.85 and 3.73). However, this evolution was less obvious for F0-LT.

There was also a significant effect of the type of consumers on the liking scores ($F=7.33$, $p=0.0073$): in average, regular consumers of whole-wheat pasta gave higher liking scores (LSMeans of liking = 4.25) than regular consumers of classical pasta (LSMean of liking = 3.46). The product by type of consumer interaction was not significant, showing that the overall ranking of the products based on the liking scores was consistent between the two types of consumers. However, S-WW pasta was more appreciated by the regular consumers of whole-wheat pasta (LSMean of liking = 5.06) than by the regular consumers of classical pasta (LSMean of liking = 3.10).

TDS measurements. The TDS curves (“Annexe 1”) show very different temporal profiles. The most appreciated pasta (F0-LT) and the least appreciated one (S-GF) differed mainly in texture: the S-GF pasta had a firmer and more elastic attack and no stickiness, contrarily to the F0-LT pasta. The most appreciated F-pasta (F80-LT) seems to be more complex than F100-LT pasta and was not dominated by firmness, contrarily to F100-VHT

pasta. In addition, the legume flavor was less dominant in F80-LT pasta. F100-LT and F80-LT pasta differed in texture from S-GF. They had a less firm and a less elastic texture. This could be explained by the lack in resilience measured by the instrumental method in F100-LT and F80-LT (textural property section). F100-VHT recovered in elasticity in comparison to F100-LT but remained less elastic than S-GF pasta. The F100-VHT had a firmer attack and dominance of legume and slight grilled flavor than F100-LT.

Figure 3.5 shows the Canonical Variate Analysis. The projections of the three bites of each variant are close to each other, showing very few differences of perception of each product along bites. S-GF pasta is characterized by longer dominance duration of white cereal flavor and of elastic and firm texture. F0-LT pasta is essentially characterized by longer dominance duration of white cereal flavor and of stickiness. F100-VHT pasta is longer dominated by the legume flavor and by a firm texture. F80-LT pasta, F100-LT and S-WW whole pasta are close to each other. They are longer dominated by whole-wheat and legume flavors, but the variant F80-LT is longer perceived stickier than the two other variants.

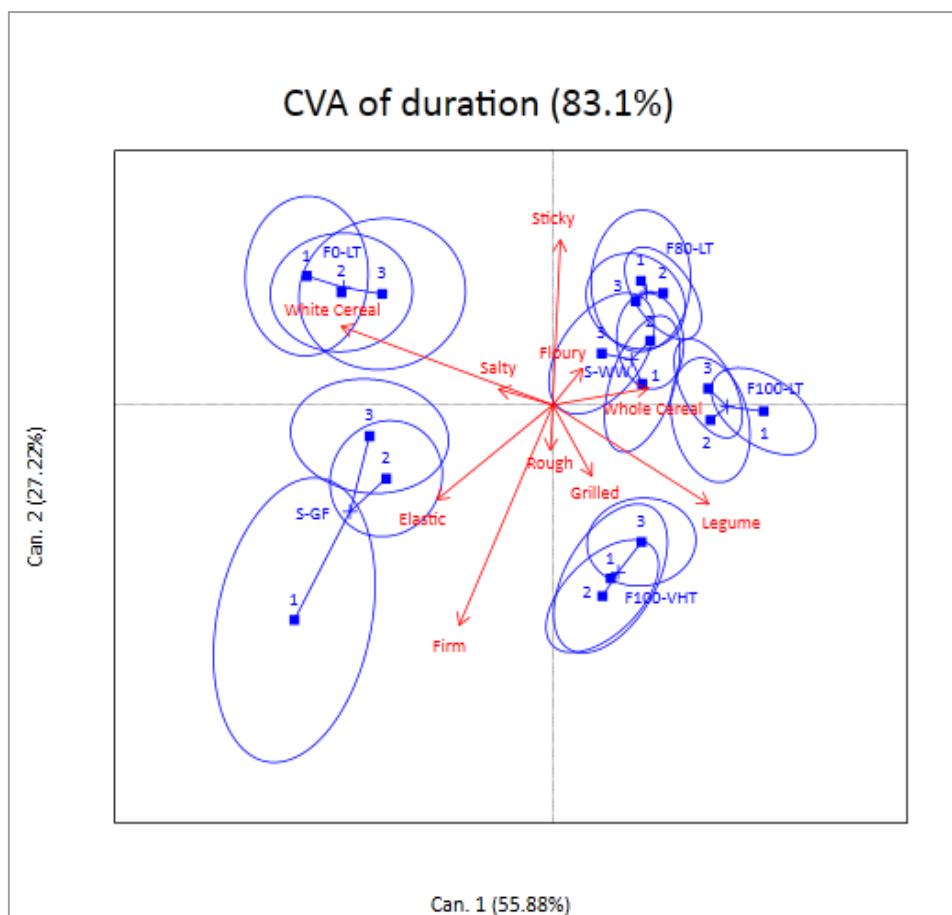


Figure 3.5 Canonical Variate Analysis (CVA) based on the dominance durations of the TDS attributes.

4. Conclusion

In this study, the impact of F-protein concentration and drying temperature on the structure of pasta was investigated and put in relation with the modification of its culinary and rheological properties and the *in-vitro* digestion of its protein network. F-protein incorporation linearly weakened the structure of protein network of LT pasta, by diluting gluten network without to create any additional covalent interactions between gluten and F-protein. The increase in F-protein concentration linearly increased cooking loss and decreased pasta resilience. VHT drying strengthened the protein structure of pasta aggregating protein through covalent bounds, and resulted in a better resilience and lower cooking loss in all enriched pasta. From a nutritional point of view, most TIA coming from faba bean were inhibited in cooked pasta. The presence of gluten and the application of VHT drying usefully intensified the reduction of TIA, by some sulphydryl-disulfide interchanges leading to a modification of the structure of the trypsin inhibitor. A noticeable enhancement (by 9 to 25%) of the *in-vitro* protein digestion as the concentration of legume protein increased was observed and related to the weakening in pasta protein network structure. The use of VHT drying did not alter the protein digestibility of pasta, and could be therefore used to recover the pasta quality (cooking loss and resilience) altered by legume protein enrichment.

If faba bean appeared therefore promising for the formulation of a high protein mixed or even gluten-free pasta processing, their sensory acceptation have to be taken into consideration. The liking score of mixed pasta made from 80% protein from faba bean was interestingly similar to that of whole-wheat pasta, whereas, gluten-free pasta made from 100% faba bean tended to be even more appreciated than a cereal gluten-free commercial pasta. In conclusion, the promising nutritional and sensory qualities of legume pasta could be used to develop mixed pasta with high protein quality and gluten-free pasta suitable for gluten intolerant people.

Acknowledgments

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Partie 3. Publication 3. Structural, culinary, nutritional and anti-nutritional properties of gluten-free pasta exclusively made of legume.

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Abstract

Wheat pasta has a compact structure built by a gluten network entrapping starch granules resulting in a low glycemic index, but is nevertheless unsuitable for gluten-intolerant people. High protein gluten-free legume flours, rich in fibers, resistant starch and minerals are thus a good alternative for gluten-free pasta production. In this study, gluten-free pasta was produced exclusively from faba bean, lentil or black-gram flours. The relationship between their structure, their cooking and rheological properties and their *in-vitro* starch digestion was analyzed and compared to cereal gluten-free commercial pasta. Trypsin inhibitory activity, phytic acid and α -galactoside were determined in flours and in cooked pasta. All legume pasta were rich in protein, resistant starch and fibers. They had a thick but weak protein network, which is built during the pasta cooking step. This particular structure altered pasta springiness and increased cooking losses. Black-gram pasta, which is especially rich in soluble fibers, differed from faba bean and lentil pasta, with high springiness (0.85 vs. 0.75) and less loss during cooking. In comparison to a commercial cereal gluten-free pasta, all the legume pasta lost less material during cooking but was less cohesive and springy. Interestingly, due to their particular composition and structure, lentil and faba bean pasta released their starch more slowly than the commercial gluten-free pasta during the *in-vitro* digestion process. Anti-nutritional factors in legumes, such as trypsin inhibitory activity and α -galactosides were reduced by up to 82% and 73%, respectively, by pasta processing and cooking. However, these processing steps had a minor effect on phytic acid. This study demonstrates the advantages of using legumes for the production of gluten-free pasta with a low glycemic index and high nutritional quality.

Key words: faba bean, lentil, black-gram, gluten-free pasta, multi-scale structure, *in-vitro* starch digestion and bioactive factors.

1. Introduction

Wheat semolina has long been considered a central component of food, notably in pasta products. It contains gluten proteins, mainly composed of gliadins and glutenins (80%) that coagulate and form a strong viscoelastic protein network while the pasta is cooking, thereby trapping starch material inside. This unique feature of gluten proteins is responsible for the firm consistency and overall structure of pasta (Marti & Pagani, 2013). The particularly compact structure of wheat pasta is responsible for the slow digestibility of its starch content, hence promoting a low plasma glucose response resulting in a low glycemic index (Bjorck et al., 2000; Jenkins et al., 1983). However in some individuals, gluten ingestion causes a range of clinical disorders named “gluten-related-disorders” with a world prevalence of 5% (Elli et al., 2015). These disorders including celiac disease, wheat allergy and non-celiac gluten sensitivity, manifest closed clinical symptoms. A restriction or total omission of gluten from the diet is the sole alternative (Ludvigsson et al., 2014; Mansueto et al., 2014). Thus, the demand and consumption of gluten-free food has increased (Lee et al., 2007). However, in 2015, a study of 303 pasta products available in the Australian market revealed that commercial gluten-free pasta presented low nutritional properties, as they are generally poorer in protein (mean 6.1 g per 100 g) than classical no gluten-free pasta (mean of 12.6 g per 100 g) (Wu et al., 2015). In addition, the removal of gluten from wheat flour was shown to increase the rate of amylolytic digestion *in-vitro*, and to enhance the glycemic response in bread *in-vivo* (Jenkins et al., 1987).

Legumes are gluten-free ingredients that could be used to produce gluten-free pasta of high nutritional quality. Legumes are rich in proteins (20-37%, w/w) (FAO, 1970) easily available (Asif et al., 2013), rich in dietary fibers (3-31% w/w) (Sanchez-Chino et al., 2015) and in resistant starch (RS, 11-20% w/w) (Piecyk et al., 2012). The *in-vitro* and the *in-vivo* glycemic index can be reduced or maintained at its low value in legume enriched pasta (Giuberti et al., 2015; Goni & Valentin-Gamazo, 2003; Greffeuille et al., 2015; Petitot et al., 2010a) in comparison to their no-legume counterparts. In addition, legumes are also known to lower blood cholesterol and triglycerides (Asif et al., 2013). Despite these interesting nutritional properties, raw legumes contain certain bioactive compounds considered as “anti-nutritional factors”. These compounds include protease inhibitors, phytic acid and α -galactosides, able to reduce protein digestibility, nutrient absorption and may be responsible for intestinal discomfort, respectively (Gupta et al., 2015; Martinez-Villaluenga et al., 2008; Multari et al., 2015). Despite these disadvantages, some of these anti-nutritional factors are

now recognized to have beneficial effects on human health. Indeed, phytic acid has a preventive action against cancer by chelating metals involved in DNA damage (Midorikawa et al., 2001); whereas α -galactosides present some prebiotic activity (Martinez-Villaluenga et al., 2008). The amount of bioactive compounds in legumes can be significantly reduced by food processing (soaking, fermentation and germination) and cooking (Boye et al., 2010; Brijesh & Narinder; Martinez-Villaluenga et al., 2008; Multari et al., 2015; Torres et al., 2006). However, some studies reported residual activity of trypsin inhibitors after cooking 20% chickpea enriched pasta (Zhao et al., 2005). In addition, even if richer in protein than cereals, legumes are mainly composed of soluble proteins (albumins and globulins). The incorporation of such soluble proteins in pasta weakens the protein network of legume enriched pasta, increases cooking loss and reduces resistance to breaking (Petitot et al., 2010b). To the best of our knowledge, only one study has reported the use of legumes as the sole ingredient in gluten-free pasta (Rosa-Sibakov et al., 2016), focused on the use of faba bean and on the impact of bioprocessing and fractionation of its grain on the textural and sensory properties of pasta and its *in-vitro* starch hydrolyses.

The objective of the present investigation was to explore and compare pasta made from three different types of legume, and particularly the link between their multiscale structure and their rheological, cooking, nutritional and anti-nutritional properties, notably the digestibility of their starch content and their anti-trypsic factors, phytic acid and α -galactoside contents. We also compared all three legume pasta with commercial gluten-free pasta made from cereals.

2. Materials and methods

Faba bean (F, *Vicia faba*), lentil (L, *Urvum lens L.*) and black-gram (BG, *Vigna mungo*) flours were supplied by GEMEF industries (Aix-en-Provence, France), Celnat (Saint-Germain-Laprade, France) and Pondicherry University (Pondicherry, India), respectively.

2.1. Chemical and nutritional composition

Total starch content was determined with an enzymatic assay kit (Megazyme, Co. Wicklow, Ireland; AACC method 76-13.01). Individual neutral sugar composition was determined at INRA “BIA” (Nantes, France), after acid hydrolysis (Saulnier et al., 1995) of the samples, by gas chromatography of alditol acetates (Englyst & Cummings, 1988). Uronic acid content was determined at INRA “BIA” (Nantes, France) in acid hydrolysis supernatant using an automated m-phenylphenol method according to Thibault (1979) and galacturonic

acid as standard. Total protein content was determined using the Kjeldahl procedure (NF V 03-050, 1970) with a conversion factor of 6.25. Amino acid profiles were determined by Agrobio (Rennes, France) according to the CEE method 152/2009 (2009). Lipid content and fatty acid profiles were determined according to the French NF ISO 6492 and NF-EN-ISO 12966-2 norms (respectively) by Inovalys (Nantes, France). Total, soluble and insoluble fibers were determined by ISHA (Lonjumeau, France) according to the JORF (1986) method. All analyses were performed in duplicate on raw materials.

2.2. Pasta production

One hundred percent legume flours (F, L or BG) were processed into spaghetti with a lab-scale pasta extruder (Sercom, Montpellier, France) as described in the FR 14 62811 patent (Laleg et al., 2016b), and dried (AFREM, Lyon, France) at 55 °C for 12 hours to reach 11% moisture. All pasta was produced in triplicate, and the three batches of each kind of pasta were pooled into a single batch for further analysis. Pasta made from legume flours was compared to Schär (Burgstall, Italy) commercial gluten-free (S-GF) spaghetti (8% protein, 78% starch and 2% fiber). The S-GF was composed of a mixture of maize, millet, rice flours and cane sugar syrup. The diameter of the dried S-GF was 1.85 ± 0.03 mm and that of the legume pasta was 1.47 ± 0.03 mm. Cooked pasta was subjected to starch and protein analyses as described for raw materials.

2.3. General appearance and color of the pasta

A picture of dried pasta was taken using a Samsung camera with a resolution of 16 megapixels. The color of dry spaghetti was determined with a Minolta Chroma Meter (Model CR-400, Minolta Co., Osaka, Japan) using the Hunter L*, a*, b*. L* values measure black to white (0–100); a* values measure redness when positive, and greenness when negative; b* values measure yellowness when positive, and blueness when negative. Four measurements were made of each kind of pasta (n=4).

2.4. Pasta cooking properties

The optimum cooking time (OCT) of each legume pasta was determined according to the AACC approved method (66-50). S-GF was cooked for 11 min, the OCT suggested by its manufacturer. Water uptake (% dry pasta) of each pasta was calculated as the difference in weight between cooked and dried pasta relative to the weight of dried pasta. Cooking losses (%, db) were calculated as the difference between the dry matter of each dried and cooked pasta, relative to the dry matter of dried pasta (Petitot et al., 2010b). All the experiments were performed in triplicate.

2.5. Changes in protein structure during pasta processing, including the cooking step

Two sequential protein extractions were performed in triplicate on raw material (F, L and BG flours), dry and freeze-dried cooked legume pasta. Proteins were first extracted in sodium dodecyl sulfate (SDS) to disrupt the electrostatic, hydrophobic and hydrophilic interactions between proteins. The second extraction was conducted in SDS/dithioerythritol (DTE) and then subjected to sonication to disrupt disulfide bonds. All protein extracts were analyzed by size-exclusion high performance liquid chromatography (SE-HPLC) according to the modified method of Morel et al. (2000). In our experiment, total protein extractability after the two extraction steps was found to be equal to the total protein content obtained with the Kjeldhal procedure in each of the analyzed fractions (flour, dried or cooked then freeze-dried pasta), which means that no remaining non-extractable proteins were found in all samples after the two extractions. Once corrected for the different solid-to-solvent ratios used during extraction, areas (in arbitrary units) of SDS-soluble and DTE-soluble proteins were expressed as the percentage of the corresponding total area calculated for F, L or BG flour proteins. SE-HPLC analyses of the commercial S-GF were performed on dried and cooked then freeze-dried pasta, and the results are expressed as a percentage of total extractable proteins (in SDS/DTE) of dried pasta.

2.6. Light microscopy of cooked pasta

Cooked (OCT) pasta sections (8 µm) were cut at -20 °C using a microtome (Microm HM 520, Walldorf, Germany) with a cryoprotector (Cellpath, Newtown, UK). The sections were stained with 1 g/L fast green (Sigma Aldrich Co., USA) and 1:8 (v/v) diluted lugol (Fluka, Buchs, Switzerland) (Petitot et al., 2010a). Bright field images were acquired using the multi-zoom AZ100M microscope (Nikon, Japan) equipped with a Nikon DSRi1 (Nikon, Japan) color digital camera. Observations were made with a plan fluor 5x objective and a fixed optical zoom of 8, resulting in a magnification of 40x.

2.7. Rheological properties of cooked pasta

Texture profile analysis (TPA) was performed, as described in Petitot et al. (2010b) on pasta cooked to OCT using a TA-XTplus (Stable Micro Systems, Scarsdale, USA) texture profile analyzer equipped with Texture Expert software (Stable Micro Systems, Scarsdale, USA). Pasta cohesiveness and springiness were then obtained (Petitot et al., 2010b). The firmness of pasta was determined using the same texturometer, according to the AACC approved method (66-50), and is expressed as the maximum force (N) required to cut five strands of spaghetti positioned adjacent to another at a constant rate of deformation (0.17

mm/sec). Three replicates of two different cooking steps ($n = 6$) were performed for each experiment.

2.8. Anti-nutritional factors

Trypsin inhibitor activity (TIA) was determined according to the standardized ISO 14902 method. Phytic acid was extracted and determined colorimetrically (Latta & Eskin, 1980). α -galactosides (raffinose, verbascose and stachyose) were extracted according to Hou et al. (2009) and determined by Agrobio (Rennes, France) based on the AOAC 980.13 and AOAC 982.14 methods. All analyses were performed in duplicate on raw legume flours and on freeze-dried cooked pasta, except for TIA, which was performed in triplicate.

2.9. *In-vitro* starch digestion of cooked pasta

In-vitro starch digestion was performed on pasta cooked to OCT by Englyst Carbohydrates LTD (Englyst et al., 1999). The glucose released from starch was measured by HPLC during timed incubation with digestive enzymes under standard conditions. The glucose released up to 20 min incubation corresponds to rapidly available glucose (RAG), and is expressed in g per 100 g available carbohydrates (total carbohydrates minus resistant starch). This fraction was found to be positively correlated with the postprandial glycemic index (Englyst et al., 1999). The glucose released from starch between 20 and 120 min incubation corresponds to slowly available glucose (SAG). The starch not digested after 120 min corresponds to resistant starch (RS). Non-starch polysaccharide (NSP) content was also determined on cooked pasta according to Englyst et al. (1994).

2.10. Statistical analysis

All data were subjected to analysis of variance followed by Fisher's least significant difference (LSD) test to compare means at the 5% significance level using Statistica 8.0 software (Tulsa OK, USA). Correlation coefficients (r) are given.

3. Results and discussion

3.1. Composition of legume flours used for pasta production

Table 3.4 shows the composition of legume flours (faba bean, lentil and black-gram), used to produce our pasta. Starch (46 - 58%, db), proteins (24 - 28%, db) and fibers (11 - 31%, db) were the main components of all legume flours. This composition differs from that of the wheat semolina usually used to produce pasta, which contains 13% protein, 78% starch and 2% fibers (Petitot et al., 2010b). Among the legume flours, F contained the least protein (24%, db) but the most starch (58%, db). BG had the highest fiber content, the fibers being

90% insoluble the same as in F and L flours, and contained 2 - 3 times more soluble fibers than F and L flours. Considering the high galactose and arabinose measured, BG was probably made of mucilaginous polymers such as arabinogalactans, in accordance with reports in the literature (Susheelamma & Rao, 1974). In BG, like in F flour, the measured glucose mainly originated from starch. In lentil flour, 16% of the total glucose did not originate from starch but could originate from cellulosic material or hemicellulose such as xyloglucans, as higher xylose content was found in L than in F and BG flours. Uronic acid, composed of 75% galacturonic acid (result not shown) in L, was also higher than in F and BG flours and could originate from pectic substances (MacGregor & Greenwood, 1980). The smaller size of lentil grains and hence its higher surface to volume ratio could increase the proportion of the seed coat to the cotyledon and the cell wall materials in the flour. Regarding amino acid composition (table 3.4), all the legume flours had higher essential amino acid contents than those recommended by the French governmental agency ANSES for human adults (ANSES, 2007a), except for sulfur amino acids in F proteins which were lower than those recommended by ANSES. Sulfur amino acid is known to be a limiting amino acid in legume proteins (*Lathyrus*, *Lens*, *Pisum* and *Faba* genera) (Pastor-Cavada et al., 2014). However, in our study, proteins in L and notably in BG flours presented a complete essential amino acid profiles compared to ANSES recommendations. Amino acid composition of legumes can differ depending on the legume species, genotype, environmental conditions and biotechnological treatments. Lipids in legume flour were low, ranging from 1.91 to 2.25% (db) mostly composed of unsaturated fatty acids that are susceptible to lipid peroxidation thus rendering several changes in lipids and other constituents (proteins, carbohydrates minerals and vitamins) possible during storage and processing (Brijesh & Narpinder). The composition of unsaturated fatty acids differed depending on the legume flour considered. F flour and to a lesser extent L flour, were particularly rich in omega-6 fatty acids. Conversely, BG flour was rich in omega-3 fatty acids. A diet with a high ratio of omega-3 to omega-6 (higher than $\frac{1}{4}$) is associated with a reduced risk of many chronic diseases of high prevalence (Simopoulos & Cleland, 2003).

Table 3.4 Composition of legume flours

Composition	Legume flour		
	F	L	BG
Starch (%), db)	57.6	48.6	45.7
Total fibers (%), db)	11.7	16.5	31.3
Insoluble fibers (%), db)	10.5	14.6	27.4
Soluble fibers (%), db)	1.2	1.9	3.9
Monosaccharides (%), db)			
Rhamnose	0.16	0.17	0.30
Fucose	0.00	0.06	0.05
Arabinose	2.36	2.38	5.06
Xylose	0.74	1.07	0.37
Mannose	0.38	0.29	0.54
Galactose	2.63	3.93	5.46
Glucose	57.54	57.69	47.49
Uronic acid	0.61	2.08	0.55
Proteins (%), db)	24.0	28.1	28.1
Essential amino acids (mg/g protein)	ANSES recommendation (mg/g protein)		
Histidine	17	34.6	26.4
Isoleucine	27	33.7	46.1
Leucine	59	71.1	78.1
Valine	27	37.1	52.6
Lysine	45	71.1	72.2
Cysteine + methionine	23	17.4	23.3
Tyrosine + Phenylalanine	41	74.0	79.0
Threonine	25	35.9	37.9
Tryptophan	6	10.8	8.9
Total lipids (%), db)		1.91	2.25
Fatty acids (% total lipid)			2.22
Saturated fatty acids	15.0	13.4	20.1
Unsaturated fatty acids	85.1	86.7	79.9
Omega 3 fatty acids	3.0	10.9	50.4
Omega 6 fatty acids	51.9	46.9	11.4

All analyses were performed in duplicate.

F: faba bean, L: lentil and BG: black-gram.

Concerning the composition of cooked pasta (results not shown), no change in the protein and starch contents was observed in comparison to legume flours except for a 3 g per 100 g (db) increase in the starch content of BG pasta (48.7% in BG cooked pasta vs. 45.7% in BG flour) probably due to a higher loss of some soluble components of the pasta, particularly fibers, during cooking.

3.2. Appearance and color of the dried pasta

Figure 3.6 shows strands of dry pasta. All the legume pasta had a homogeneous appearance with a smooth surface. Their diameter was identical (1.47 ± 0.03 mm). Legume pasta differed in color. BG pasta had the highest brightness (L^*) and yellowness (b^*) followed by F pasta. L was the darkest pasta with the lowest yellowness and redness scores resulting in a green pasta. Redness (a^*) was higher in F pasta followed by BG pasta. The color of pasta partially depends on the color of raw material used to produce the pasta (Zhao et al., 2005). Pasta color may also be related to enzymatic oxidation during mixing and the first steps of pasta drying (Troccoli et al., 2000) and to the vacuolar pigments (anthocyanin) present in legume flours (Takeoka et al., 1997). S-GF had a rough surface and a larger diameter than legume pasta. Its color was characterized by higher L^* and b^* scores than all legume pasta with a lower a^* score than BG and F pasta, resulting in a bright yellow pasta (figure 3.6). Color scores of wheat pasta (results not shown) differed from all the gluten-free pasta with higher brightness ($L^* = 67.9$) and lower redness ($a = -0.3$). The yellowness ($b^* = 25.1$) was higher than F and L pasta, but lower than BG and notably S-GF.

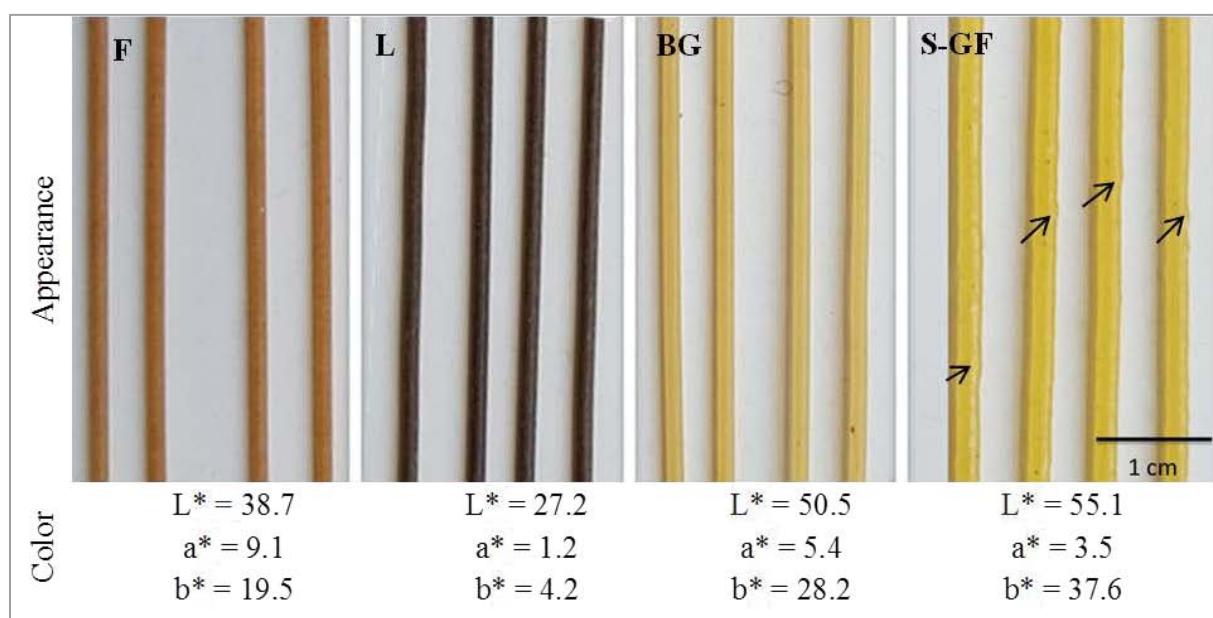


Figure 3.6 General appearance and color scores of dried pasta. F: faba bean, L: lentil, BG: black-gram and S-GF: commercial gluten free spaghetti. Arrows denote bumps on the surface of S-GF.

3.3. Protein aggregation throughout the pasta process

In order to determine the nature (strong or weak) of the protein network and the step at which it was built during the processing of gluten-free pasta, the degree of protein aggregation was investigated in all three legume (F, L and BG) pasta. It was determined on flours before processing and after drying and cooking of pasta, two steps known to determine the protein structure in wheat and wheat/legume mixed pasta (Petitot et al., 2009b; Petitot & Micard, 2010). The gluten-free F, L and BG pasta were compared to gluten-free S-GF (dry and cooked) pasta. The results obtained by SE-HPLC are presented in figure 3.7. In all legume flours and pasta, the whole proteins were extracted in SDS and DTE with no non-extractable proteins recorded, meaning that the proteins were linked by electrostatic, hydrogen or disulfide bonds. Before processing (figure 3.7A), all legume flours (F, L and BG) showed a similar protein solubility profile; more than 97% being soluble in SDS with only 2-3% DTE-soluble proteins. Storage proteins in legumes are mainly composed of soluble globulins and albumin with minor glutelin and protamine fractions (Petitot et al., 2010a), which could explain their high SDS solubility. After pasta processing and drying (figure 3.7B), only minor additional DTE-soluble proteins (+2.1, +4.5 and +1.1 additional percentage in F, L and BG, respectively) were formed at the expense of SDS-soluble proteins. Cooking the pasta (figure 3.7C) resulted in higher molecular changes in the protein solubility profiles of legume pasta than those observed during drying, especially in F and L pasta, where +21.2 and +23.4 % (respectively) additional DTE-soluble proteins were formed at the expense of SDS-soluble protein. The same effect was observed but to a lesser extent in BG cooked pasta (+9 % DTE-soluble proteins only). The low reactivity of BG pasta to binding through disulfide bonds was probably related to the smaller quantity of cysteine residue able to bind and to form cystine through the disulfide bond in BG protein than in F and L proteins (8.7, 9.6 and 11.2 mg/g; respectively; result not shown). In addition, the higher fiber content of BG (table 3.4) could probably disrupt the protein network (Petitot et al., 2010a). Whatever the legume pasta considered, the protein solubility profile differed significantly from that of regular pasta made from wheat semolina, where disulfide linked proteins represent 20% in raw semolina, 30% in dried pasta and 72 to 82% in cooked pasta (Laleg et al., 2016a; Petitot et al., 2010a).

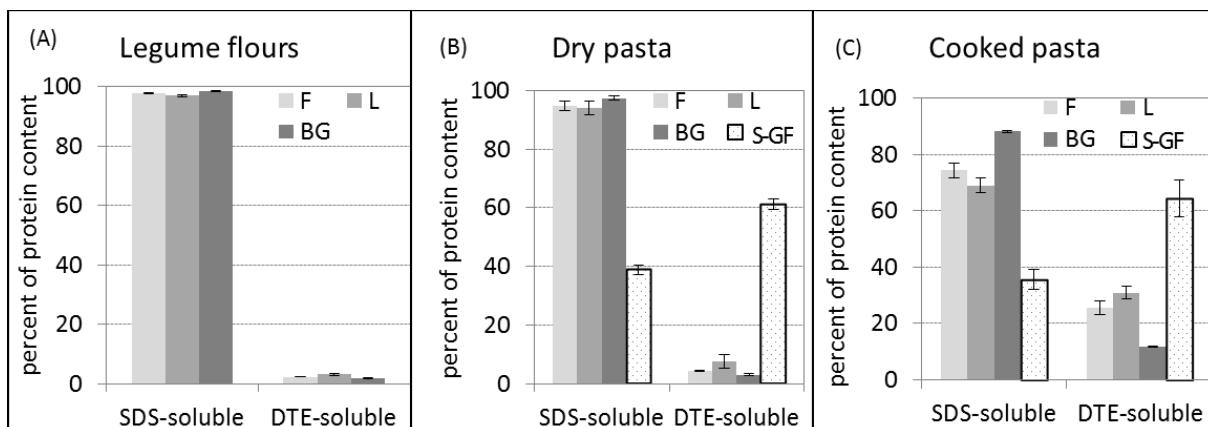


Figure 3.7 Changes in protein solubility in sodium dodecyl sulfate (SDS) and dithioerythritol (DTE). (A) Legume flours, (B) dry and (C) cooked pasta. F: faba bean, L: lentil and BG: black-gram flours. S-GF: gluten-free commercial spaghetti. Results are means of three replicates, standard errors shown as vertical bars.

The results of protein aggregation in all gluten-free dried legume pasta demonstrated that cooking was the crucial step for the strengthening of the protein network. This result was different for dried S-GF based on cereals. The disulfide bonded protein (61%) pre-existed in dried S-GF (figure 3.7B), with no additional change observed during cooking (figure 3.7C). A stronger disulfide linked protein network governed the protein linkage in cooked S-GF and was 2.5, 2.1 and 5.5 fold higher than in F, L and BG pasta, respectively. This could be partly explained by the different nature of the proteins in legume (albumins and globulins) than in cereal (mainly glutelins and/or prolamins). Proteins in S-GF could have a higher cysteine content (15-25 mg/g protein (Bailey et al., 1979; Houston et al., 1969; Keeney, 1970)) than in legume pasta (8.7-11.2 mg/g, result not shown). A difference in drying temperature used for S-GF (not known) could also partly explain these results. Over a 90 °C drying temperature, Petitot et al. (2009b) indeed reported similar 64% disulfide bonded protein in wheat pasta.

3.4. Microscopic structure of cooked pasta

The microscopic structure of faba bean, lentil and black-gram legume pasta is depicted in figure 3.8. In all legume pasta, swollen starches (blue-violet) were included in a protein network (green), as reported by Rosa-Sibakov et al. (2016) in 100% faba bean pasta. The protein (P) networks in all the legume pasta were more apparent and thicker than those reported in the literature for wheat pasta (Petitot et al., 2010a), in accordance with their higher protein content (24% for F and 28% for L and BG pasta versus 13% for wheat pasta). No distinction between F, L or BG pasta was found in the perceptible thicknesses of their protein network (P). The protein network of BG pasta appeared as small round structures as previously described in the literature (Brijesh & Narpinder).

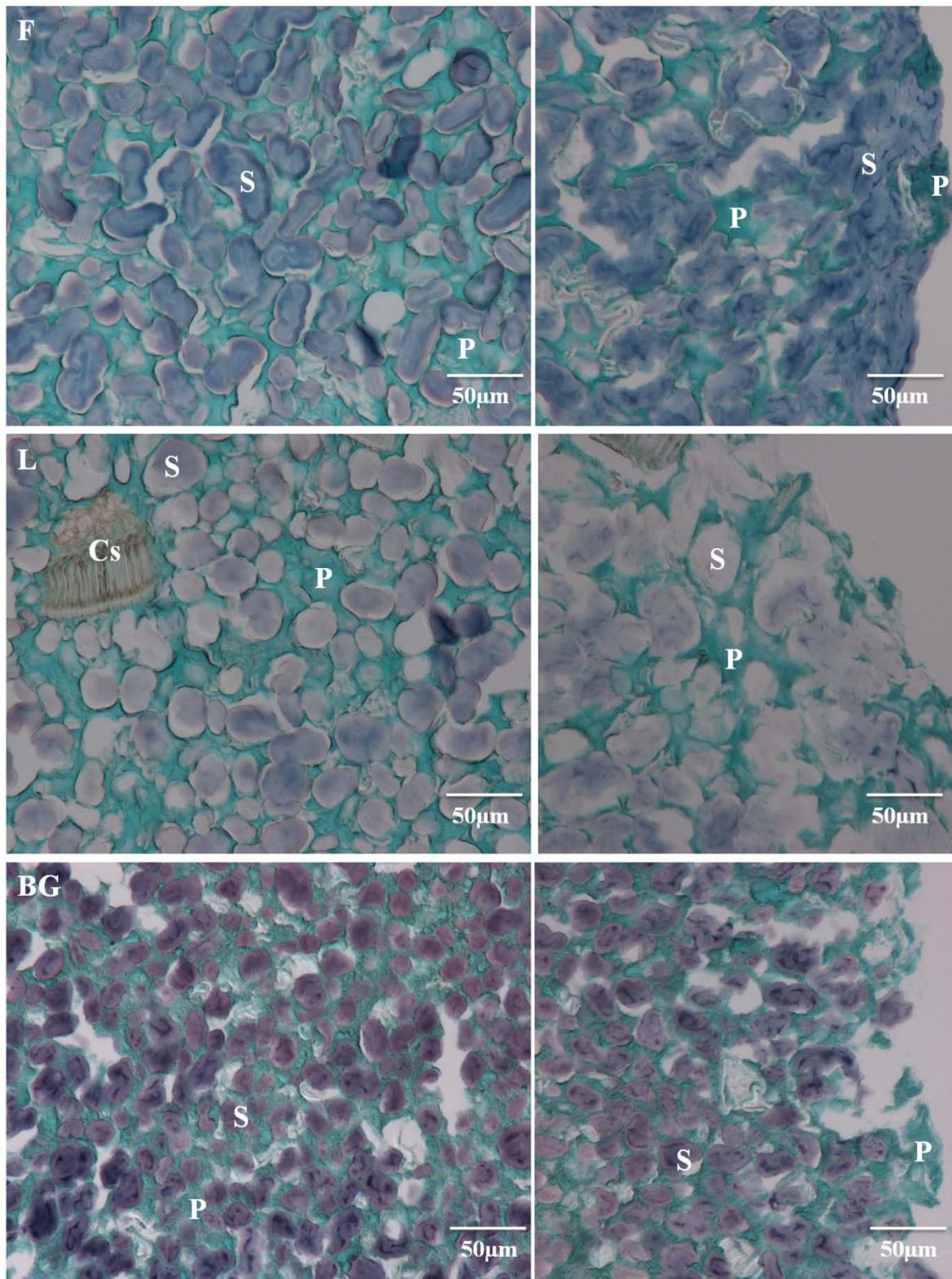


Figure 3.8 Light microscopy image of 100% legume cooked pasta. F: faba bean, L: lentil and BG: black-gram. The internal regions of the pasta are in the left panels and external regions in the right, panels. S, starch; P, protein and Cs, cell structure.

The size and the shape of starch (S) differed depending on the raw material used to produce the pasta. BG presented smaller starch granules than F and L pasta. All the legume pasta had a high starch swelling rate even in the center of the pasta, with no major change in starch swelling from the center to the external region of the pasta strand. This result differs from results reported in the literature on wheat or wheat/legume mixed pasta, in which three main regions were distinguished from the outer to the central pasta strand according to the gradual starch swelling state (Laleg et al., 2016a; Petitot et al., 2010a). The lack of a strong covalently linked protein network in F, L and BG pasta, as revealed by SE-HPLC analysis, could have facilitated penetration of water to the core of the pasta and hence starch swelling. In the external region of F and L pasta, swollen starch granules met to form a continuous phase. Large cellular structures (Cs) were also observed in L pasta resulting from the cell wall, which is in accordance with the larger quantities of cellulosic and hemicellulosic materials in L in comparison to F and BG flours (Table 3.4).

3.5. Cooking properties

Cooking properties (OCT, water uptake and cooking loss) of gluten-free legume pasta are listed in table 3.5. OCT differed significantly according to the legume pasta considered. Two main groups were distinguished: the first one is formed by BG, which cooked in 3 min less than the second group formed by F and L pasta. The diameter of the pasta, which was identical in F, L and BG pasta, could not explain this huge difference. The more soluble and weakly linked protein network (Figure 3.7C) and the higher fiber content, especially the water-soluble arabinogalactans (table 3.4), of BG pasta in comparison to F and L pasta, could have facilitated penetration of the water to the core of the pasta. Rosa-Sibakov et al. (2016) reported 9 min cooking time for 100% faba bean pasta with similar starch and protein composition (23.3 and 58.7%, respectively) to that of our F pasta. Around 10 min cooking time was reported for wheat pasta (Rosa-Sibakov et al., 2016). Pasta produced from 35% or 100% legumes (faba bean or split pea) reduced the pasta cooking time by 1 min in comparison to wheat pasta (Petitot et al., 2010b; Rosa-Sibakov et al., 2016). The OCT of commercial S-GF (11 min) was higher than the OCT of all legume pasta. This could be explained by two parameters: the bigger diameter and the higher starch content of S-GF (78%).

Table 3.5 Cooking and rheological properties of gluten-free legume and commercial pasta.

		F	L	BG	S-GF
Cooking properties	OCT (min)	9.5 ^a	9.8 ^b	6.6 ^c	11*
	Water uptake (% dry pasta)	165 ^a	154 ^b	124 ^c	103 ^d
	Cooking loss (%, db)	14.35 ^a	12.96 ^a	10.35 ^b	18.34 ^c
Rheological properties	Firmness (N)	4.37 ^a	5.92 ^b	6.86 ^c	10.13 ^d
	Cohesiveness	0.70 ^b	0.64 ^a	0.64 ^a	0.86 ^c
	Springiness	0.75 ^a	0.75 ^a	0.85 ^b	0.97 ^c

Means with the different superscripts within a row are significantly different. F: faba bean, L: lentil, BG: black-gram and S-GF: commercial gluten-free pasta cooked to their optimal cooking time (OCT).

*OCT of S-GF was not subjected to variance analysis, as it was provided by the manufacturer.

Water uptake also differed significantly between F, L and BG pasta in the following order F > L > BG pasta (165, 154 and 124%, respectively). Water uptake of pasta is governed by different parameters including starch and fiber contents, the strength of protein network, OCT and the swelling power of its constituents. BG pasta had higher fiber, but lower starch content (table 3.4) and a lower OCT, resulting in lower water absorption. L pasta had higher fiber but lower starch content than F pasta (table 3.4), which could explain its lower water uptake than F pasta. S-GF absorbed less water (103%) than all the legume pasta during cooking. Its bigger diameter (1.85 mm) could delay the swelling of all its higher (78%) starch content. Indeed Laleg et al. (2016a) reported 186% water absorption for wheat pasta with the same (78%) starch content and a diameter of 1.56 mm when the pasta was cooked to its optimal cooking time + 1 min.

F and L pasta presented a statistically similar loss during cooking (table 3.5). These values are higher than those reported in the literature for wheat pasta (5.6%) and for 35% or 100% legume (faba bean and split pea) pasta (7 to 12%) (Petitot et al., 2010b; Rosa-Sibakov et al., 2016), but lower than losses recorded for 15 to 30% common bean enriched wheat pasta (15-21% cooking loss) (Gallegos-Infante et al., 2010). The lack of strong covalently linked protein network in our legume pasta, as demonstrated by SE-HPLC analysis, resulted in a weak overall pasta structure resulting in significant leaching out of particles during cooking. However, BG pasta, which had the most weakly linked protein network, also had the lowest cooking loss. This could be due to the lower OCT of BG pasta leading to less exposure of the surface of the pasta to the cooking water. In addition, this legume could contain a larger amount of arabinogalactan polymers (table 3.4), which could play the role of stabilizer in pasta. The addition of 2% of hydrocolloids (pectin, guar or agar) to 15% chickpea pasta

indeed reduced its cooking loss by slowing down the diffusion of the amylose molecules from the core to the surface of spaghetti (Padalino et al., 2015).

All the legume flours presented a significantly lower cooking loss than S-GF. This could be due to the higher OCT of S-GF and to its lower protein content (8%) compared to legume pasta (24-28%). In addition, the tightly associated protein network pre-formed during the drying step (figure 3.7B) in S-GF could be less elastic and hence less deformable and breakdowns during starch swelling resulting in high cooking loss, as already reported in the literature (Bock et al., 2015). Gluten-free pasta made from rice, from a mixture of rice and bean flour or from a mixture of chickpea unripe plantain and maize, lost 11-15% material (Flores-Silva et al., 2014; Giuberti et al., 2015; Marengo et al., 2015).

3.6. Rheological properties

Firmness (cutting force), cohesiveness (ability of the pasta to stick to itself) and springiness (ability of pasta to regain its original shape after compression) of legume (F, L and BG) and S-GF pasta are listed in table 3.5. F, L and BG pasta presented a significantly different firmness with the highest firmness score for BG followed by L and F pasta. Pasta firmness was significantly ($p<0.05$) negatively correlated ($r = 0.91$) with water uptake. Pasta firmness is determined by the water taken up by the pasta and by the presence or the absence of a strong protein network. BG and L presented a similar cohesiveness, which was slightly lower than in F pasta. This lower cohesiveness could be mainly due to the presence of the cell wall structures observed by light microscopy in L pasta, and to the more weakly linked protein network in BG pasta (in comparison to L and F pasta). Like our results, Rosa-Sibakov et al. (2016) reported a score of 0.74 cohesiveness for 100% F pasta, whereas higher cohesiveness (0.8) was reported for wheat pasta (Petitot et al., 2009b). Springiness was higher in BG than in F and L pasta even though BG cooked pasta presented less disulfide linked proteins (figure 3.7C). This means that another type of interaction may be involved between the components of BG pasta. Indeed, arabinogalactan-rich carbohydrates, which were higher in BG than in F and L flours (table 3.4), could form a network that improves the structure of the pasta, notably its elasticity, as reported in the literature on the use of hydrocolloids (Padalino et al., 2015; Sozer, 2009).

All texture scores were significantly higher in S-GF than in all the legume pasta we tested. The lack of disulfide bonds (figure 3.7C) in F, L and BG pasta in comparison to S-GF explained the reduced springiness of the legume pasta. The lack of pasta elasticity is a major textural disadvantage associated with the depreciation of organoleptic properties in 30-35%

legume (faba bean, pea, chickpea and lentil) enriched wheat pasta (Petitot et al., 2010b; Zhao et al., 2005). The springiness of S-GF is in agreement with that of wheat pasta containing the same amount of disulfide bounds (Petitot et al., 2009b).

3.7. Anti-nutritional factors

Trypsin inhibitory activity (TIA), phytic acid and α -galactosides were determined in raw legume flours and in cooked pasta. The results are presented in table 3.6. TIA ranged from 7.8 to 11.3 mg/g (db). BG flour contained 1.4 fold more TIA than F and L flours. TIA reported in the literature ranges from 2 to 18 mg/g for lentil (Ma et al., 2011; Vasagam & Rajkumar, 2011; Wang et al., 2009), 15 mg/g for BG (Vasagam & Rajkumar, 2011) and 3 mg/g for faba (Hove & King, 1979), depending on the type and variety of legume. Cooking pasta reduced TIA 3 fold in F and 5 fold in L and BG cooked pasta. The effectiveness of thermal treatment on TIA reduction is widely reported in the literature. Heat treatment (roasting, boiling or cooking) of legume seeds or flours reduced TIA by 38-96% (Ma et al., 2011; Vasagam & Rajkumar, 2011; Wang et al., 2009). Contrary to our results, Zhao et al. (2005) reported no residual TIA in 15-20% spaghetti fortified with pea and lentil, because of the lower legume content and the higher drying temperature used, 70 °C, versus 55 °C in our study. The same author reported that pasta fortified with 20% chickpea retained 30% of its original TIA, in agreement with our results (18-32% residual TIA). The residual TIA in our pasta is in accordance with that reported for 100% pea macaroni which retained 21 to 51% of the initial TIA activity after 30 min cooking (Friis et al., 1997). Phytic acid contents (table 3.6) of legume flours ranged from 14.5 to 18 mg/g in the order L > F > BG. Ranges of 8-12, 6-9 and 2-9 mg/g of phytic acid are reported in the literature (Honke et al., 1998; Vidal-Valverde et al., 1998; Wang et al., 2009; Yadhu et al., 2011) for faba bean, lentil and black-gram seeds, respectively. The difference in legume cultivars, the climatic conditions and the application of fertilizers can affect the amount of phytic acid (Brijesh & Narpinder; Honke et al., 1998). Dried wheat pasta and S-GF contained 6.5 and 7.25 mg/g (db) phytic acid, respectively (results not shown). Only a slight reduction in phytic acid content was observed in all cooked pasta in comparison to the corresponding flours. The pasta processing steps were less efficient in reducing phytic acid than fermentation and germination pre-processing (Torres et al., 2006; Torres et al., 2007b).

Table 3.6 Trypsin inhibitory activity (TIA), phytic acid and α -galactosides content of legume flours and cooked pasta.

	Anti-nutritional factor (mg/g, db)	Legume flours	Cooked pasta
TIA ¹ (n=3)			
F		7.84 ^a	2.48 ^a
L		8.24 ^a	1.52 ^b
BG		11.26 ^b	2.13 ^a
Phytic acid (n=2)			
F		15.31	14.91
L		17.97	14.76
BG		14.49	12.79
α -galactosides (n=2)			
F	Raffinose	3.34	1.75
	Stachyose	8.75	4.86
	Verbascose	32.14	5.52
	Total	44.23	12.13
L	Raffinose	3.03	2.49
	Stachyose	37.86	10.88
	Verbascose	8.25	3.27
	Total	49.14	16.64
BG	Raffinose	1.25	1.10
	Stachyose	7.90	6.03
	Verbascose	47.50	26.50
	Total	56.66	33.63

¹ Means with the different superscripts within a column are significantly different. F: faba bean, L: lentil and BG: black-gram (BG)

Total α -galactoside ranged from 44 to 57 mg/g (db) in the order BG>L>F. Raffinose was a minor oligosaccharide in all legume flours. The concentration of stachyose and verbascose varied with the type of legume. Verbascose accounted for 73% and 84% of the total α -galactosides in F and BG flours, respectively, whereas the main oligosaccharide in L was stachyose with 77% of total α -galactoside. Our results concerning total α -galactoside contents are in agreement with those reported in the literature for faba bean (10 to 45 mg/g), lentil (18 to 75 mg/g) and for black-gram (33 to 81 mg/g) seeds (Girigowda et al., 2005; Guillou & Champ, 2002; Martinez-Villaluenga et al., 2008; Vidal-Valverde et al., 1998). The percentage of each oligosaccharide is also reported to vary depending on the type of legume and agricultural factors such as irrigation conditions (Guillon & Champ, 2002).

Pasta processing and cooking dramatically reduced the total amount of α -galactoside in F, L and BG by 73, 66 and 41%, respectively, which could be the result of heat hydrolysis or leaching in cooking water. This reduction was more efficient in L and F than in BG pasta

probably because BG pasta was cooked for a shorter time (OCT of 6 vs. 9 min for the two others). The effect of cooking on the concentration of α -galactoside in legume seeds is well documented in the literature, and is reported to decrease α -galactosides considerably (from 25 to 100%), depending on the type of legume (faba bean, black-gram) and on the cooking conditions (20 to 60 min) (Girigowda et al., 2005; Vidal-Valverde et al., 1998). α -galactoside were not detected in cooked pasta supplemented with pigeon pea, because of the very low level of substitution (10%) and the fermentation preprocess applied to the legume seed prior to pasta manufacturing (Torres et al., 2006).

The effectiveness of pasta processing and cooking in our results also varied depending on the oligosaccharide considered, and was stronger in reducing verbascose in F (by 83%) and stachyose in L pasta (71%). This resulted in different oligosaccharide profiles in the cooked pasta than in the legume flours, notably in cooked F pasta, where the contribution of stachyose (40%, vs. 20% in legume flour) and Verbascose (46% vs. 73% in legume flour) to total α -galactoside was similar. Like in our study, verbascose oligosaccharide was reported to be subject of the largest reduction (36%) in soaked and cooked faba bean seed (Vidal-Valverde et al., 1998).

3.8. *In-vitro* starch digestibility of cooked pasta

The results of non-starch polysaccharides (NSP) of legume and commercial gluten-free pasta cooked to OCT are presented in table 3.7. BG pasta presented the highest soluble NSP followed by lentil and faba bean, in accordance with the fiber content of the raw flours (table 3.4). Total NSPs of 3.8% and 4.6% are reported in the literature for wheat and of 35% for faba bean enriched pasta, respectively (Greffeuille et al., 2015); 27.3, 18.2 and 59.1% of the total NSP were soluble ones in F, L and BG pasta, respectively. The percentage of soluble NSP was higher than the percentage of soluble fibers in raw legume flours (around 10%). This could be related to the solubilization of some insoluble fibers during cooking of the pasta, or to the different method used to assay the fibers in the two cases. S-GF cooked pasta presented 3 to 6 fold less total NSP than legume pasta, in agreement with the low fiber content of dried S-GF (2% fibers). Soluble NSP accounted for 50% of the total NSP of cooked S-GF pasta. A slight but significant difference was found in the amount of resistant starch (RS) between F, L and BG pasta (F>BG>L). RS was twice higher in all legume pasta than in S-GF. S-GF had a similar RS to RS values reported in the literature for wheat pasta (Greffeuille et al., 2015). A range of 0.88 to 1.69 g/100g (wb) is reported in 35% faba bean and split pea enriched pasta (Greffeuille et al., 2015; Petitot et al., 2010a). The amount of

available carbohydrates was slightly but significantly higher in F (20.5%) than in L and BG pasta (19.1 and 18.9%, respectively). All legume pasta presented lower available carbohydrates than S-GF (34%), in accordance with their lower starch content (48-58% in legume pasta versus 78% in S-GF), and higher RS. F and L presented the lowest RAG (61.8 and 62.5%, respectively) and the highest SAG (36.4 and 36.0%, respectively). RAG values were lower than those reported in the literature for wheat pasta ($67 \pm 0.7\%$ of available carbohydrates) (Greffeuille et al., 2015), but close to those reported for 35% faba bean enriched pasta ($64.9 \pm 3.2\%$ of available carbohydrates). BG pasta presented a significantly higher RAG and lower SAG than F and L pasta. This could be related to its weaker protein network (SE-HPLC results) and smaller starch granules (microscope observation) than in F and L pasta, as reported in the literature (Greffeuille et al., 2015; Sandhu & Lim, 2008). Starch digestibility is reported to decrease when starch granules are large (Sandhu & Lim, 2008) and in the presence of a highly aggregated protein network (Greffeuille et al., 2015). F and L pasta had lower RAG and higher SAG than S-GF. SAG was lower in BG pasta than in S-GF. S-GF presented similar *in-vitro* starch digestion parameters to those reported in the literature for wheat pasta (Greffeuille et al., 2015). The absence of gluten network did not increase the rate of hydrolysis in S-GF. In addition, even if legume pasta presented a very weakly linked protein network, the RAG was either reduced (for F and L pasta) or closer to that of S-GF (for BG pasta). The presence of a higher amylose:amylopectin ratio and anti-nutritional factors (such as phytic acid, which could inhibit α -amylase activity) (Deshpande & Cheryan, 1984; Sandhu & Lim, 2008; Thompson & Yoon, 1984), could affect the rate of starch digestion. The lower RAG values obtained *in-vitro* for L and F pasta could be a predictor of a lower glycemic index.

Table 3.7 Non-starch polysaccharides (NSP), resistant starch (RS), available carbohydrates, rapidly available glucose (RAG), and slowly available glucose (SAG) in cooked pasta

Pasta	Dry matter (%)	NSP (% wb)		RS (% wb)	Available carbohydrates (% wb)	RAG (% available carbohydrates)	SAG (% available carbohydrates)
		Total	Soluble				
F	31.93	2.2	0.6	1.16 ± 0.01^b	20.50 ± 0.09^b	61.83 ± 0.95^b	36.43 ± 0.95^b
L	36.15	3.3	0.6	0.99 ± 0.02^c	19.14 ± 0.14^c	62.53 ± 1.61^b	35.99 ± 1.61^b
BG	36.39	4.4	2.6	1.05 ± 0.03^d	18.90 ± 0.15^c	68.64 ± 2.26^a	29.12 ± 2.26^c
S-GF	38.07	0.8	0.4	0.58 ± 0.02^a	33.88 ± 0.66^a	66.64 ± 1.34^a	32.81 ± 1.34^a

*Means with the different superscripts within a column are significantly different.

Analyses of starch digestibility were performed in triplicate and for NSP in duplicate.

F: faba bean, L: lentil, BG: black-gram and S-GF: commercial gluten-free cooked pasta

4. Conclusion

In this study, the structure and properties of gluten-free pasta made using 100% faba bean, lentil and black-gram flours were investigated. Whatever the legume used, the cooking and Rheological properties of pasta, its microscopic structure and the strength of its protein network clearly differed from those of gluten-free cereal pasta. Legume pasta is notably richer in fiber and produced a weaker protein network, which was mainly formed during the cooking step in comparison to the cereal gluten-free pasta in which drying is the step that leads to the creation of the protein network. The texture of the legume pasta was therefore weaker with notably low springiness scores. Interestingly, all legume pasta, which is three times richer in protein than gluten-free cereal pasta, lost less material during cooking. If most legume pasta structure and properties were similar, some differences were noticeable depending on the legume used for pasta production. Black-gram pasta had better Rheological and cooking properties related to its particular richness in arabinogalactan fibers.

From a nutritional point of view, in addition to their high protein content, the legume pasta also contained higher resistant starch than gluten-free cereal pasta. Furthermore, the remaining available starch in faba bean and lentil pasta was more slowly digested than the starch of gluten-free cereal pasta. This interesting *in-vitro* starch digestion profile could predict a lower glycemic index. However, even if legumes appear to be promising for the production of gluten-free pasta, their anti-nutritional factors need to be known. Trypsin inhibitory activity and the α -galactoside content can be drastically reduced during legume processing into pasta that includes a cooking step. Phytic acid was less affected by pasta processing, making germination, fermentation of legume grains or flours prior to pasta processing a promising option (Torres et al., 2006; Torres et al., 2007b).

In conclusion, the promising nutritional quality of legume pasta, notably their richness in protein and fibers and their potential low glycemic index could be of interest to expand the range of gluten-free cereal pasta available on the market today.

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Conclusions

Ce chapitre a permis de mettre au point un protocole de production de spécialités pastières contenant des concentrations de légumineuses allant de 0 à 100% pour au moins trois légumineuses différentes, riches en protéines, en acides aminés essentiels, notamment en lysine, et en fibres. L'enrichissement des spaghetti par une quantité croissante de protéines de fèverole induisait des changements de structure de la pâte aux trois niveaux d'échelle, macro, microscopique, supramoléculaire. Un affaiblissement de la structure du réseau protéique de la pâte par effet de dilution du gluten proportionnel à la quantité de protéines de légumineuses incorporées a notamment été observé. Ce changement de structure se répercutait sur la texture et les propriétés culinaires des pâtes. La substitution de 80% des protéines de blé par des protéines de légumineuses a conduit à l'obtention d'une pâte enrichie en protéines (20%), plus équilibrées en acides aminés essentiels. D'un point de vue sensorielle, cette pâte a été certes globalement moins appréciée qu'une pâte classique au blé mais autant appréciée que des spaghetti de blé complet du marché. Des spaghetti produits exclusivement de fèverole ont également été obtenus, qui peuvent convenir aux personnes intolérantes au gluten. Ces spaghetti ont été mieux appréciés que leurs homologues sans gluten à base de céréales du commerce. La nature de la légumineuse utilisée pour la production de ces spécialités pastières sans gluten permettait de diversifier leur couleur, leurs propriétés de texture et de résistance à la cuisson. La texture et la résistance à la cuisson des spécialités pastières à base de légumineuse ont pu être améliorées par l'utilisation de très hautes températures de séchage grâce à un renforcement de la structure de leur réseau protéique. Cette amélioration était notamment plus efficace dans les spaghetti enrichis en fèverole que dans une pâte de blé classique. Du point de vu nutritionnel l'affaiblissement de la structure du réseau protéique, observé lors de l'enrichissement des pâtes aux légumineuses, a induit une augmentation du degré d'hydrolyse *in-vitro* des protéines, proportionnelle à la concentration en protéine de fèverole incorporée, non modifiée par l'application de hautes températures de séchage. La structure affaiblie du réseau protéique dans les spaghetti 100% légumineuses n'a cependant pas conduit à une augmentation de la digestibilité de l'amidon. Certaines pâtes comme celles faites exclusivement de lentille ou de fèverole présentaient même un indice glycémique *in-vitro* plus faible que celui des spaghetti de céréales. Concernant les facteurs antinutritionnels, si une réduction appréciable de la quantité d'inhibiteurs de trypsines et des α -galactosides a été obtenue lors des différentes étapes de production et de cuisson des spaghettis, l'acide phytique quant à lui reste peu affecté par le procédé.

CHAPITRE 4

*Etude de l'effet de la consommation de pâtes enrichies en
légumineuses sur le métabolisme, la digestion et la rétention
protéique*

CHAPITRE 4. ETUDE DE L'EFFET DE LA CONSOMMATION DE PATES ENRICHIES EN LEGUMINEUSES SUR LE METABOLISME, LA DIGESTION ET LA RETENTION PROTEIQUE

Le chapitre précédent a montré que l'enrichissement des pâtes avec 35% de farine de fèverole augmente la teneur en protéines, améliore l'équilibre en acides aminés essentiels, ainsi que la digestibilité des protéines *in-vitro*. L'augmentation de la digestibilité de la fraction protéique semble être expliquée en partie par un affaiblissement de la structure chimique du réseau protéique. De plus, la variation des températures de séchage des pâtes n'avait engendré aucun effet sur la digestibilité protéique mesurée *in-vitro*.

L'objectif du travail décrit dans le chapitre 4 était d'étudier *in-vivo* l'intérêt de la consommation des pâtes enrichies avec 35% de farine de fèverole et séchées à température basse ou élevée en comparaison à d'autres régimes composés de pâtes traditionnelles (protéine de blé) ou de protéines de caséine. L'influence de la consommation de tels régimes sur la digestibilité et la rétention des protéines ainsi que sur la croissance et certains marqueurs nutritionnels ont été étudiés chez des rats jeunes.

**Publication 4. Biological evaluation of faba bean-wheat mixed pasta in growing rats:
Impact of protein source and drying temperature on protein digestibility and retention**

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En préparation, à soumettre à J. Agric. Food Chem.

Abstract

This study was undertaken to evaluate the nutritional value of diets containing faba bean-wheat mixed pasta and wheat pasta dried either at low (LT-55 °C) or very high (VHT-90 °C) temperature in comparison to a control diet composed of casein with the same protein content. One month-old rats consumed these experimental diets during 21 days. Body composition, food efficiency, protein digestibility and utilization, protein digestibility-corrected amino acid scores (PDCAAS) and biological value were determined. Metabolic and inflammatory markers and the expression level of adipose tissue genes were analyzed. Pasta enriched with faba bean had 97% higher chemical score and 10% higher branched amino acid content than gluten pasta. The true digestibility was similar in faba bean and gluten pasta but net protein utilization, PDCAAS and food efficiency were twice higher in rats fed faba bean pasta. The use of a VHT to dry pasta reduced the chemical score up to 6% and the true digestibility by 3% notably in gluten pasta but did not affect net protein utilization. Faba bean pasta led to a 3.5% decrease in protein digestibility and a 8.8% decrease in net protein utilization in comparison to control diet, but did not provoke a significant decrease in the rate of growth or lean body mass. Blood albumin and proteins, triglycerides, non-esterified fatty acids and free glycerol were also identical in faba bean pasta compared to control diet. However, faba bean pasta increased body fat mass by 45%, blood leptin by 42%, blood adiponectin by 31% and blood orosomucoids by 76% in comparison to control diet. Accordingly, mRNA expression levels of some markers associated to lipid metabolism, such as lipoprotein lipase and peroxisome proliferator-activated receptor gamma were also higher in rats fed with faba bean pasta in comparison to control diet. Of note, despite significant changes in adiposity, the HOMA-IR index was identical in all rats. This study clearly evidenced the efficiency of feeding legume enriched pasta regardless of its drying temperature on protein digestibility and utilization, growth and body composition.

Key words: Pasta, faba bean, protein digestibility, protein retention, growing rats, metabolic and inflammatory markers.

1. Introduction

Several investigations conducted over the past decades (WHO/FAO, 2003), reported that trends in food supply are transiting from a traditional diet, largely made of vegetable proteins towards a diet rich in animal products. However, WHO (World Health Organisation, 2015) recommended to decrease the consumption of meat because of its adverse ecological and health effects, e.g. increased risk of cardiovascular diseases (Bernstein et al., 2010). Proteins are one of the most important macronutrient of the human diet. Their nutritional value is defined by their ability to meet the specific needs for the growth and maintenance of body tissues (FAO, 2013). Dietary proteins of a good quality have to be provided in adequate amounts to meet body amino acid requirements. It is believed that the ability of dietary proteins to ensure the body protein requirement depends on their digestibility, on the amount and proportion of essential and non-essential amino acids and on the bioavailability of the constitutive amino acids. These parameters affect tissue protein turnover, i.e. protein synthesis and degradation rates, and the ability of body proteins to ensure their biological functions. Both a rapidly digested protein (Dangin et al., 2003; Gryson et al., 2014) and a high essential amino acid score (Dardevet et al., 2002; Volpi et al., 2003) were demonstrated to be highly relevant for muscle protein synthesis, underlying the importance of the quality of dietary proteins for their metabolic function.

It is of interest to develop a vegetable based food, partially replacing meat that is easy to prepare, rich in proteins of a good quality and appreciated by consumers. Pasta, a highly popular wheat product, partly meets these specifications. It is worldwide consumed and appreciated because of its low cost, ease preparation and long shelf-life. From a nutritional point of view, dry pasta is a source of protein (12-13%) and carbohydrates (78%) which are slowly digested resulting in a low glycemic index (Bjorck et al., 2000; Jenkins et al., 1983). Despite this interesting nutritional quality, as most cereal products, pasta is deficient in lysine and threonine (Abdel-Aal & Hucl, 2002) and generally has a lower protein content than animal products. Several studies have been carried out to improve the nutritional quality of pasta by the incorporation of non-traditional ingredients such as legume flours (Gimenez et al., 2012; Jayasena & Nasar-Abbas, 2012; Laleg et al., 2016a; Padalino et al., 2014; Petitot & Micard, 2010; Wood, 2009). Legumes are a valuable source of dietary proteins (20-37%) (FAO, 1970) and fibers (3-31% w/w) (Sanchez-Chino et al., 2015). They are rich in resistant starch (11-20% w/w) (Piecyk et al., 2012), and in slowly digested carbohydrates and are known to lower blood cholesterol and triglycerides (Asif et al., 2013). In addition, legumes

are rich in lysine and as a result, are characterized by a complementary amino acid profile to wheat proteins (Duranti, 2006). The incorporation of 35% faba bean flour increased the pasta protein content and enhanced the lysine score by 52% in comparison to the classical wheat pasta (Laleg et al., 2016a). The *in-vitro* digestibility of the protein fraction was also reported to be increased by legume (faba bean and lupin) pasta enrichment (Laleg et al., 2016a; RayasDuarte et al., 1996), related to a specific weaker structure of pasta (Laleg et al., 2016a).

Beside the pasta composition, the variation in the process parameters, notably temperature of drying, was also reported to affect pasta protein digestibility due to a modification of its protein network structure (De Zorzi et al., 2007; Petitot et al., 2009b). In the industry, pasta is generally dried at high temperature ($> 65 - 94^{\circ}\text{C}$) (Guler et al., 2002) in order to save time and energy. Moreover, it has been demonstrated to improve the quality of pasta enriched with legumes, as firmness, and overall integrity (Petitot et al., 2010b; Petitot et al., 2009b). However, the application of temperatures higher than 85°C , strongly aggregates protein inducing therefore a lower *in-vitro* protein digestibility. Of note, this phenomena is not observed when using a low temperature to dry pasta (De Zorzi et al., 2007; Petitot et al., 2009b). Few data are available on the impact of the enrichment of pasta with legume on the *in-vivo* protein digestibility (Torres et al., 2007a; Torres et al., 2006; Torres et al., 2007b). In addition, no data is available on the effect of both improving protein composition and using different drying temperature on the *in-vivo* digestibility and nutritional value of pasta proteins.

This investigation aimed to compare in growing rats the nutritional value, the degree of protein digestibility and retention of wheat-legume (faba bean) mixed pasta with those of wheat pasta and casein diet characterized by the same protein content. The effect of two (low and very high) drying temperatures was also studied for each pasta. The consequences of such protein composition and drying temperatures on body composition, metabolic and inflammatory markers and the expression level of key genes were analyzed and discussed.

2. Material and methods

2.1. Raw material

Wheat (*Triticum durum*) semolina, faba bean (*Vicia faba*) flour (F) and wheat (*Triticum aestivum*) gluten powder (G) were provided by Panzani (Marseille, France), GEMEF industries (Aix-En-Provence, France) and Syral, (Belgium), respectively. The composition of raw materials was previously published (Laleg et al., 2016a).

2.2. Pasta manufacturing

Pasta manufacturing and cooking was performed as recently described by Laleg et al. (2016a). Briefly, wheat semolina was enriched with 6% gluten powder or 35% faba bean flour to obtain the same protein content in the blend, and then processed into spaghetti form using a continuous pilot-scale pasta extruder (Bassano, Lyon, France). All spaghetti were dried at low temperature (LT-55 °C for 15h) or at very high temperature (VHT-90 °C for 3h) in a pilot-scale drier (AFREM, Lyon, France). Four types of pasta were then obtained:

- 35% faba bean: 65% semolina dried at low temperature (FLT)
- 35% faba bean: 65% semolina dried at very high temperature (FVHT)
- 6% gluten: 94% semolina dried at low temperature (GVHT)
- 6% gluten: 94% semolina dried at very high temperature (GVHT)

Each pasta was cooked to its own optimal time + 1 min, and then dried at 40 °C during 24h to reach 12% moisture. Cooked and dried pasta was grounded into a fine powder, and served as base to animal diet.

2.3. Amino acid composition of pasta

Amino acid profile of the different dry pasta was determined by Agrobio (Nantes, France) in duplicates. Chemical scores (Cs) were calculated as done by Gimenez et al. (2012), using the equation:

$$Cs = \frac{\text{limiting amino acid in pasta}}{\text{limiting amino acid in reference protein}} \times 100$$

The ANSES (2007a) recommendations on amino acids for adult people were taken as reference.

2.4. Biological essays

2.4.1. Ethical considerations

All procedures with animal were approved by the institution's animal welfare committee (Comité d'Ethique en Matière d'Expérimentation Animale Auvergne: CEMEAA; Permit number: C2EA-02) and were conducted in accordance with the European's guidelines for the care and use of laboratory animals (2010-63UE). Animals were housed in the animal facility of the research unit (Agreement N°: C6334514). At the end of the experiment, rats were sacrificed after isoflurane anesthesia. All efforts were made to minimize animal suffering.

2.4.2. Experimental Design and diet

One-month-old male Wistar rats were purchased from JANVIER (Le Genest St Isle, France), and housed in individual cages in an environmentally controlled (temperature of 22.0 ± 0.8 °C, humidity of $50 \pm 5\%$ and 12/24h light cycle) room, and given *ad libitum* access to standard chow and water. During the acclimatization period, the animals were fed a control diet (AIN-93M, Safe diets) until the study began. Rats were then randomly separated into six groups of ten animals for the pasta and casein diets (CD) and 14 animals for the protein free diet (PFD). Each group received one experimental diet composed of pasta (GLT, GVHT, FLT or FVHT), of CD, or of PFD (table 4.1). All diets were supplemented with 4.5% vitamin mix, and with the appropriated amount of cellulose and soy fat, to provide 14% protein in the diet, except for the protein free diet (table 4.1). The study lasted 21 days during which the food intake of rats was recorded every week. During the three last days of the study, rats were transferred into metabolic cages where food intake was weighed every day. *Faeces* and urines were also collected, and weighed, then stored at 4 °C for a further nitrogen determination according to Dumas' method. Twelve hours prior to sacrifice, the animals were fasted. Blood samples were collected into EDTA tubes (Venosafe®, Terumo, France) at the end of the experiment and centrifuged at 1300 g for 10 min at 4 °C to separate the plasma which was then rapidly frozen in liquid nitrogen and stored at -80 °C until analysis. Blood was also collected into dry tubes (Venosafe®, Terumo, France), and after incubation for 20 min at room temperature they were centrifuged at 1300 g for 10 min at 4 °C to separate the serum which was then rapidly frozen in liquid nitrogen and stored at -80 °C until analysis.

Table 4.1 Composition of experimental and control diets

Ingredient (%)	Experimental diet				Protein-free diet (PFD)	Casein diet (CD)
	GLT	GVHT	FLT	FVHT		
Proteins	15.7	15.7	15.8	15.9	0.0	15.3
Amidon	65.2	65.8	63.2	62.9	83.5	68.1
Cellulose	0.9	0.9	1.0	1.0	6.1	6.1
Lipid (soy)	4.5	4.5	4.6	4.6	6.0	6.0
Minerals and vitamins*	4.5	4.5	4.5	4.5	4.5	4.5
Energy (kcal/100g)	349.8	349.8	358.4	358.4	363.0	365.2

Muscles *plantaris*, *soleus* and *tibialis*, were immediately removed from each side. Liver and perirenal (APT) and subcutaneous (AST) adipose tissues were also collected. All tissues were weighed and frozen in liquid nitrogen and stored at -80 °C until analyses.

2.4.3. Body weight and composition

Body weight was recorded in the beginning and on the end of the experiment. In addition, all rats were subjected to magnetic resonance imaging (MRI) using Echo MRI (Echo Medical Systems, Houston, TX) to determine body composition at the beginning and the end of the experiment. Lean mass (LM) and fat mass (FM) were determined.

2.4.4. Nutritional value of the diet

The food intake (g/j) and the rate of growth (g/j) were determined during the study. The food efficiency (g of weight gain/g food intake) was calculated according to Proll et al. (1998) by the equation:

$$- \text{ Food efficiency} = \frac{\text{Rate of growth}}{\text{Food intake}}$$

Protein quality of each diet was evaluated. The apparent and true digestibility, the net protein utilization and the biological value were calculated according to Proll et al. (1998). The protein digestibility corrected-amino acid score were calculated as described by Gimenez et al. (2016). The following equations were used:

$$\begin{aligned} - \text{AD} &= \frac{\text{NI}-\text{FN}}{\text{NI}} \times 100 \\ - \text{TD} &= \frac{\text{NI}-(\text{FN}-\text{EFN})}{\text{NI}} \times 100 \\ - \text{NPU} &= \frac{\text{NI}-(\text{FN}+\text{UN})+\text{EFN}+\text{EUN}}{\text{NI}} \times 100 \\ - \text{BV} &= \frac{\text{NPU}}{\text{TD}} \times 100 \\ - \text{PDCAAS} &= \frac{\text{Cs} \times \text{TD}}{100} \end{aligned}$$

AD: apparent digestibility, BV: biological value, Cs: chemical score, EFN: endogenous fecal nitrogen (determined using the fecal nitrogen of GFP group rat), EUN: endogenous urinary nitrogen (determined using the urinary nitrogen of GFP group rat), FN: fecal nitrogen, NI: nitrogen intake, NPU: net protein utilization, PDCAAS: protein digestibility-corrected amino acid score, TD: true digestibility, UN: urinary nitrogen.

2.4.5. Biochemical analyses

Tissue protein content was measured using a PCA kit. Blood glucose, non-esterified fatty acids (NEFA), total cholesterol, triglycerides, albumins and total proteins were measured using an automat Konelab 20 (Thermo Scientific, MA, United States). Plasma inflammation markers, i.e. monocyte chemoattractant protein-1 (MCP-1), fibrinogen and orosomucoid and plasma metabolic markers, i.e. insulin, adiponectin and leptin, were measured using an ELISA

kit (PromoKine, France) according to the manufacturer's instructions. HOMA-IR (Homeostasis model assessment of insulin resistance) was calculated using glucose and insulin fasting values (Matthews et al., 1985) by using the equation:

$$\text{HOMA - IR} = \frac{\text{glucose (nmol/L)} \times \text{insulin (mU/mL)}}{22.5}$$

2.4.6. Tissue transcript levels

Total RNA was extracted using Tri-Reagent according to the manufacturer's instructions (Euromedex, Mundolsheim, France) from 50 mg of frozen tissue. RNA was quantified by measuring optical density at 260 nm. The concentrations of mRNAs corresponding to genes of interest were measured by reverse transcription followed by PCR using a Rotor-Gene Q system (Qiagen, Courtaboeuf, France). Five µg of total RNA was reverse-transcribed using SuperScript III reverse transcriptase and a combination of random hexamer and oligo dT primers (Invitrogen, Life Technologies, Saint-Aubin, France). cDNAs were diluted 1:60 before PCR analysis. PCR amplification was performed in a 10 µL total reaction volume. The PCR mixture contained 2.5 µL diluted cDNA template, 5 µL 2×Rotor-Gene SYBR Green PCR master mix (Qiagen, Courtaboeuf, France), and 0.5 µM forward and reverse primers. The amplification profile was initiated by a 5-min incubation at 95°C to activate HotStarTaq Plus DNA Polymerase, followed by 40 cycles of two steps: 95°C for 5 s (denaturation step) and 60°C for 10 s (annealing/extension step). Relative mRNA concentrations were analyzed using Rotor-gene software. Seven-fold serial dilutions from a mix of all undiluted cDNA were used for each target gene to construct linear standard curves from which the concentrations of the test sample were calculated. Table 4.2 lists the primers used for quantitative real-time PCR amplification. mRNA levels were normalized to RPLP0 mRNA expression values.

Table 4.2 Primer sequences for the quantitative analysis of gene expression

Gene name	Forward and reverse primers
FAS (Fatty Acid Synthase)	<i>For</i> 5'-GGCCACCTCAGTCCTGTTAT-3' <i>Rev</i> 5'-AGGGTCCAGCTAGAGGGTACA-3'
SREBP1c (Muscle RING Finger-1)	<i>For</i> 5'-ACAAGATTGTGGAGCTCAAGG-3' <i>Rev</i> 5'-TGCAGAAGACAGCAGATTAA-3'
Leptin	<i>For</i> 5'-TTGTCACCAGGATCAATGACATT-3' <i>Rev</i> 5'-GACAAACTCAGAATGGGGTGAAG-3'
PPAR γ (Peroxisome proliferator-activated receptor gamma)	<i>For</i> 5'-GATCCTCTGTTGACCCAGA-3' <i>Rev</i> 5'-AGCTGATTCCGAAGTTGGTG-3'
Adiponectin	<i>For</i> 5'-AATCCTGCCAGTCATGAAG-3' <i>Rev</i> 5'-CATCTCCTGGGTACCCCTTA-3'
ACC1 (Acetyl-CoA Carboxylase 1)	<i>For</i> 5'-GACGAAGGCGAGTCAGGTAT-3' <i>Rev</i> 5'-GAAGCCTCTCCTGCAATCAT-3'
MCP1 (Monocyte Chemoattractant Protein 1)	<i>For</i> 5'-GCTGCTACTCATTCACTGGCAA-3' <i>Rev</i> 5'-TGCTGCTGGTATTGTCTTGTA-3'
LPL (LipoProtein Lipase)	<i>For</i> 5'-CAGCAAAACCTTGTGGTAT-3' <i>Rev</i> 5'-CATTGGAGTCAGGTTCTTTGG-3'
ATGL (Adipose TriGlyceride Lipase)	<i>For</i> 5'-CAACGCCACTCACATCTACG-3' <i>Rev</i> 5'-GAGCCTCCTGGACACCTC-3'
FAT/CD36 (Fatty Acid Translocase)	<i>For</i> 5'-TTACTGGAGCCGTTATTGGTG-3' <i>Rev</i> 5'-TCCTTCTTCAAGGACAACCTCC-3'
RPLP0 (Ribosomal Protein, Large, P0)	<i>For</i> 5'-GTGTTGACAATGGCAGCAT-3' <i>Rev</i> 5'-ACAGACGCTGGCCACATT-3'

2.4.7. Statistical analysis

All data were subjected to analysis of variance followed by Fisher's least significant difference (LSD) test to compare means at the 5% significance level using Statistica 8.0 software (Tulsa OK, USA). Correlation coefficients (*r*) are given.

3. Results

3.1. Amino acid composition of pasta

The amino acid composition of pasta is presented in table 4.3. The essential amino acid profile differed considerably according to the protein source. GLT presented a conform amino acid profile to those generally recommended for human adult people (ANSES, 2007a) except for lysine, which was 2.4 fold lower than the recommendations. The enrichment of pasta with faba bean (FLT) enhanced the lysine content of pasta by 97%. It also increased histidine by 18%, threonine by 23% and branched amino acids by 10% (isoleucine, leucine and valine); whereas decreased sulfur amino acid by 27% as expected. Regarding the non-essential amino acids, FLT is composed with a higher arginine, alanine, glycine, serine and

aspartic acid content in comparison to GLT, and a lower proline and glutamic acid contents. The same results of pasta enrichment with faba bean on amino acid composition were observed in VHT dried pasta. In addition, the use of VHT drying decreased the amount of lysine by 6% and 4% and the amount of tryptophan by 17% and 7% in GVHT and FVHT pasta in comparison to GLT and FLT pasta, respectively. The highest chemical score was obtained with FLT followed by FVHT. The chemical scores of both GLT and GVHT was two fold lower than FLT and FVHT.

Table 4.3 Amino acid composition of pasta (mg/g of protein)

	FLT	FVHT	GLT	GVHT	Recommendation (mg/g of protein) ¹
Histidine	25.8	25.4	21.9	21.8	17.0
Isoleucine	39.2	39.9	36.5	37.3	27.0
Leucine	78.4	80.0	73.6	72.6	59.0
Valine	46.9	48.0	39.9	40.7	27.0
Lysine	37.4	35.9	19.0	17.9	45.0
Sulfure-AA	32.1	31.7	43.8	44.0	23.0
Aromatic-AA	77.4	77.2	75.4	76.2	41.0
Threonine	33.1	33.7	26.9	26.4	25.0
Tryptophan	11.4	10.6	10.9	9.1	6.0
Aspartic acid	75.3	76.6	38.2	38.6	
Serine	49.5	49.1	47.0	46.5	
Glutamic acid	273.5	269.0	352.7	351.5	
Proline	80.1	83.4	117.9	116.5	
Glycine	38.8	39.2	31.9	33.1	
Alanine	37.6	37.8	28.4	29.9	
Arginine	63.4	62.5	36.1	37.9	
Branched AA	164.5	167.9	150.0	150.6	
Chemical score	83.1	79.8	42.2	39.8	

All analysis were performed in duplicates; ¹ proposed as a reference profile for adult people (ANSES, 2007a).
FLT and FVHT: wheat-mixed faba bean pasta dried at low temperature and very high temperature, respectively;
GLT and GVHT: gluten wheat pasta dried at low temperature and very high temperature, respectively.

3.2. Impact of the type of the diet on growth parameters and body composition

Throughout the study, rats ate an equivalent amount of diet (table 4.4). The food efficiency, the rate of growth and the final body weight of rats are given in table 4.4. Regardless of the drying temperature (LT or VHT), rats receiving a diet based on faba bean pasta were characterized by a 2 fold higher food efficiency, rate of growth and by a 1.3 fold higher body weight in comparison to a diet composed of gluten pasta. Faba bean pasta was as efficient as a CD and resulted in a similar rate of growth and body weight than CD. No effect of the drying temperature was observed between rats nourished LT and VHT pasta regardless of the protein source (faba bean/gluten or gluten).

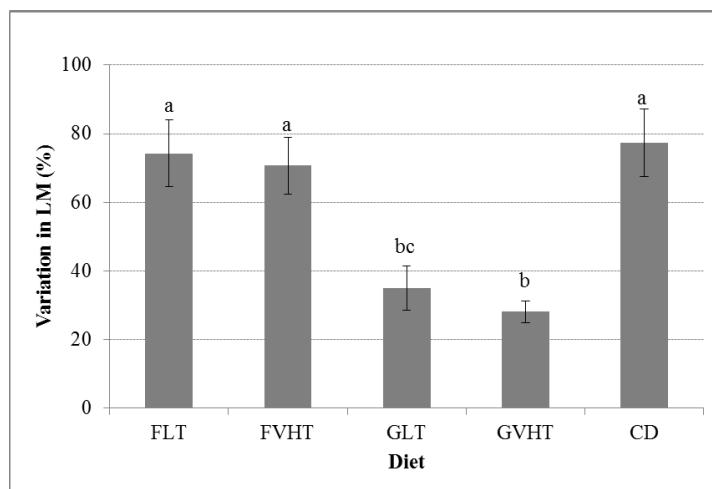
Table 4.4 Food intake, rate of growth, body weight and body composition

Diet	Food intake (g/j)	Food efficiency (g gain/g feed intake)	Rate of growth (g/j)	Body weight (g)	FM (% of body weight)	LM (% of body weight)
FLT	28.68 ^a	0.33 ^a	9.30 ^a	326.9 ^b	14.14 ^a	78.74 ^a
FTHT	29.09 ^a	0.32 ^a	9.30 ^a	328.2 ^b	15.76 ^a	77.21 ^a
GLT	31.24 ^a	0.18 ^b	5.42 ^b	257.0 ^a	14.24 ^a	78.42 ^a
GVHT	28.44 ^a	0.17 ^b	4.64 ^b	236.9 ^a	11.21 ^b	81.25 ^b
CD	27.34 ^a	0.33 ^a	8.86 ^a	319.6 ^b	9.83 ^b	82.90 ^b
p-value	0.4	<0.01	<0.01	<0.01	<0.01	<0.01

Means with the different superscripts within a column are significantly different.

The rats were fed with wheat-mixed faba bean pasta dried at low temperature (FLT) or very high temperature (FVHT), with gluten wheat pasta dried at low temperature (GLT) or very high temperature (GVHT), or casein diet (CD). LM: lean and FM: fat mass.

FLT and GLT rat groups presented similar amounts of lean (LM) and fat mass (FM, table 4.4), whereas GVHT rats had a significantly lower fat mass and higher lean mass in comparison to the FVHT counterparts. Body composition of all rats fed with pasta, except for GVHT, was characterized by a higher fat mass and a lower lean mass than in rats fed the CD. A significant drying effect was only observed in gluten pasta group with a lower fat mass and an increased lean mass in GVHT in comparison to GLT. The Figure 4.1 shows the variation of lean mass between the beginning and the end of the study. The lean mass increased similarly in FLT and FVHT rats and in CD rats. In contrast, the increase in lean mass was drastically lower in GLT and GVHT rats.

**Figure 4.1** Variation in lean mass (LM) of rats between the beginning and the end of the study.

Bars wearing different letters are significantly different ($p<0.05$). Diets were composed of wheat-mixed faba bean pasta dried at low temperature (FLT) or very high temperature (FVHT), gluten wheat pasta dried at low temperature (GLT) or very high temperature (GVHT) or casein diet (CD).

The weight of muscles of each group is presented in table 4.5. Regardless of the pasta drying temperature, the weight of *plantaris*, *tibialis anterior* and *soleus* was higher in rats fed with faba bean pasta in comparison to the rats fed with gluten pasta. The weights of the three muscles were generally similar in rats fed with faba bean pasta or with CD. On the contrary, rats fed with gluten pasta were characterized by a significantly lower muscle weight. No clear difference in the muscle weight to body weight ratio was found between the different groups (table 4.5). FLT and FVHT rats were characterized by a significantly enhanced liver weight to body weight ratio in comparison to GLT and GVHT. CD led to a 7% and 17% higher liver weight to body weight ratio than in faba bean and control pasta groups, respectively. No effect of the drying temperature was recorded regardless of the parameters presented above.

Table 4.5 Muscle, adipose tissues peri-renal, and sub-cutaneous and liver weights

Diet	Plantaris ¹	Soleus ¹	Tibialis ¹	Total muscle ²	APT ²	AST ²	Liver ²
FLT	0.59 ^a	0.29 ^{ab}	1.05 ^a	5.92 ^{ab}	11.21 ^a	13.84 ^a	29.98 ^b
FVHT	0.56 ^a	0.27 ^a	1.06 ^a	5.77 ^a	11.82 ^a	15.91 ^a	29.89 ^b
GLT	0.44 ^b	0.24 ^c	0.86 ^b	6.00 ^{abc}	10.68 ^a	14.89 ^a	26.70 ^a
GVHT	0.44 ^b	0.23 ^c	0.81 ^b	6.26 ^c	8.49 ^b	13.65 ^a	26.75 ^a
CD	0.58 ^a	0.31 ^b	1.07 ^a	6.15 ^{bc}	7.44 ^b	9.79 ^b	32.10 ^c
p-value	<0.01	<0.01	<0.01	0.04	<0.01	<0.01	<0.01

¹ Results are expressed in g; ² results are expressed as g per g of body weight $\times 10^3$

Means with the different superscripts within a column are significantly different.

APT, peri-renal tissue; AST, sub-cutaneous tissue.

Diets were composed of wheat-mixed faba bean pasta dried at low temperature (FLT) or very high temperature (FVHT), gluten wheat pasta dried at low temperature (GLT) or very high temperature (GVHT) or casein diet (CD).

3.3. Protein quality evaluation of pasta based diet

The fecal (FN) and urinary nitrogen (UN) to nitrogen intakes ratio are presented in table 4.6. Regardless of the drying temperature, rats fed with faba bean pasta lost higher fecal nitrogen than rats fed with gluten pasta. CD rats were characterized by the lowest fecal nitrogen, which was similar to that of rats fed with GLT. The effect of drying temperatures was only observed in gluten pasta increasing the fecal nitrogen losses in the *feaces* in rats fed with GVHT pasta. Regarding urinary nitrogen, FLT and FVHT rats were characterized by a 32% and 22% lower urinary nitrogen than in GLT and GVHT, respectively. Rats fed with CD maintained the lowest urinary nitrogen which was 2 fold lower than in the rats fed with gluten pasta and 1.3 fold lower than faba bean pasta rats. No effect of drying temperature was observed on urinary nitrogen whatever the pasta considered.

Table 4.6 Evaluation of protein quality

Diet	FN /NI	UN /NI	AD	TD	NPU	BV	PDCAAS
FLT	0.15a	0.51a	85.3 a	98.3 a	76.4 b	77.9 ab	81.71 d
FTHT	0.15a	0.53a	85.1 a	97.2 ab	72.3 b	74.3 b	77.6 c
GLT	0.09b	0.75b	91.1 b	98.5 a	43.7 a	44.3 a	41.6 b
GVHT	0.11c	0.68b	88.5 c	95.6 b	46.6 a	48.7 a	38.1 a
CD	0.08b	0.39c	92 b	101.9 c	83.8 c	82.2 b	101.9 e
p-value	<0.01	<0.05	<0.01	<0.01	<0.01	<0.01	<0.01

Means with the different superscripts within a column are significantly different.

NI: Nitrogen intake, FN: fecal nitrogen, UN urinary nitrogen, AD: apparent digestibility, TD: true digestibility, NPU: net protein utilization and PDCAAS: protein digestibility-corrected amino acid score.

The diets contained wheat-mixed faba bean pasta dried at low temperature (FLT) or very high temperature (FVHT), gluten wheat pasta dried at low temperature (GLT) or very high temperature (GVHT), or casein diet (CD).

The higher fecal nitrogen of rats fed with faba bean pasta led to a lower apparent digestibility than in rats fed gluten pasta (table 4.6). All pasta, except for GLT, resulted in a lower apparent digestibility in comparison to CD. Drying pasta at a VHT led to a slight but significant decrease in the apparent digestibility in gluten pasta in comparison to LT drying, without impact on faba bean pasta. The true digestibility (table 4.6) did not vary according to the protein composition of pasta, meaning that the higher fecal nitrogen in rats fed faba bean pasta was induced by a greater endogenous fecal nitrogen loss compared to gluten pasta. This result suggested that faba bean pasta, likely containing protease inhibitors could involve a higher excretion of digestive enzymes leading to an increase in endogenous fecal nitrogen. Regardless of the pasta considered, the true digestibility was lowered by 3 to 6% in rats fed with pasta than those fed with CD. The use of VHT drying decreased the true digestibility in pasta, but the effect was only significant in the case of gluten pasta.

The net protein utilization and biological value (table 4.6) were considerably increased (1.6 folds) in FLT and FVHT compared to GLT and GVHT diets, without any effect of the drying temperature. This was related to the lower urinary nitrogen displayed by the rats fed with faba bean pasta compared with gluten pasta. CD conserved the highest net protein utilization and the better biological value. PDCAAS was greatly enhanced (two times) in faba bean pasta related to its relatively higher chemical score in comparison to gluten pasta. LT dried pasta were characterized by a higher PDCAAS compared to VHT dried pasta with the respect to the chemical scores assessed for each pasta (table 4.3). As expected, the highest PDCAAS was recorded for CD proteins.

3.4. Metabolic and inflammatory parameters

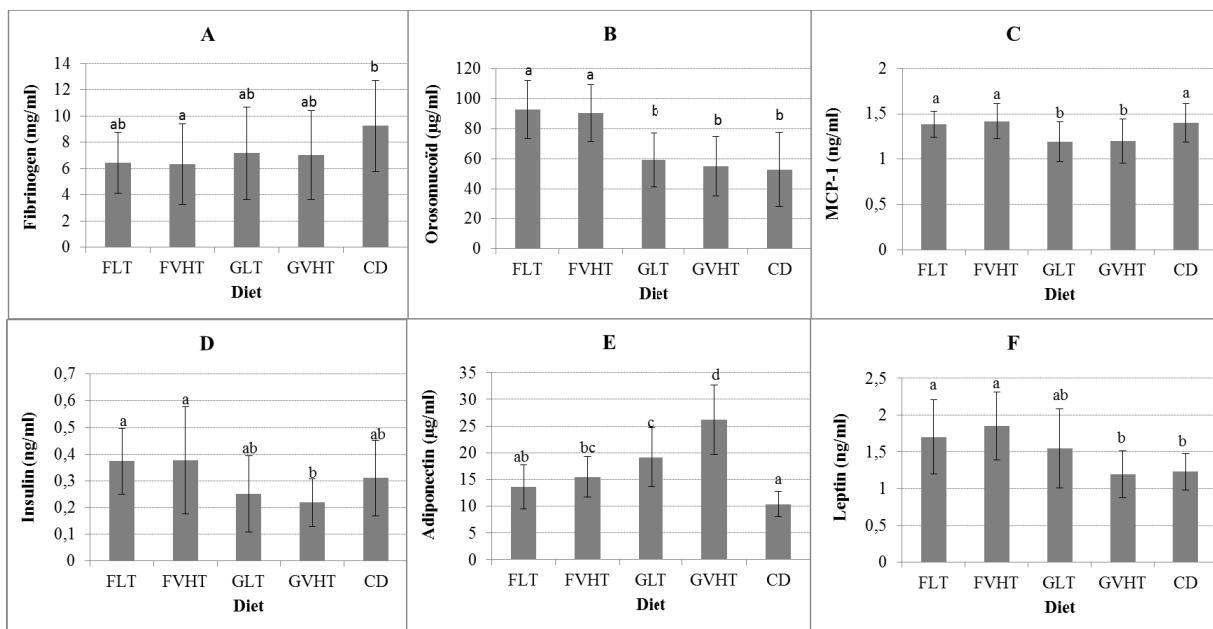
Blood parameters are presented in table 4.7. Higher blood albumin and total proteins were observed in rats fed FLT and FVHT than in rats fed GLT or GVHT. FLT and FVHT rats had similar blood albumin and total protein concentration than CD rats, contrary to GLT and GVHT groups. Glucose was generally higher in rats fed with pasta, notably in FLT and FVHT. Blood total cholesterol (table 4.7) was lowered by the enrichment of pasta with faba bean in comparison to gluten pasta, notably in VHT pasta (a 22% decrease), although blood cholesterol of rats receiving faba bean pasta remained significantly higher than in those receiving the CD. Feeding rats with FLT and FVHT led to two fold higher blood triglyceride content than GLT and GVHT rats, but was still conformed to the triglyceride level observed in CD rat. No significant effect ($p>0.05$) of the type of diet was observed on blood NEFA and free glycerol. Concerning the pasta drying temperatures, VHT drying tended to increase the NEFA content, but the effect was not statistically significant ($p=0.1$), whereas no drying effect was recorded on the other parameters.

Table 4.7 Serum composition

Diet	Albumin (g/l)	Total protein (g/l)	Glucose (g/l)	Cholesterol (g/l)	Triglyceride (g/l)	NEFA (mg/l)	Free glycerol (mg/l)
FLT	33.42 ^b	56.42 ^a	1.06 ^b	0.74 ^a	0.81 ^a	276 ^a	243 ^a
FTHT	33.44 ^b	56.12 ^a	1.04 ^b	0.74 ^a	0.85 ^a	299 ^{ab}	255 ^a
GLT	31.41 ^a	50.45 ^b	0.99 ^{ab}	0.86 ^{ab}	0.43 ^b	281 ^a	243 ^a
GVHT	31.27 ^a	51.23 ^b	0.92 ^a	0.95 ^b	0.47 ^{bc}	331 ^b	252 ^a
CD	33.71 ^b	58.31 ^a	0.89 ^a	0.54 ^c	0.70 ^{ac}	314 ^{ab}	236 ^a
p-value	<0.01	<0.01	0.01	<0.01	<0.01	0.1	0.8

Means with the different superscripts within a column are significantly different. NEFA: non esterified fatty acids. The diets were based of wheat-mixed faba bean pasta dried at low temperature (FLT) or very high temperature (FVHT), wheat pasta dried at low temperature (GLT) or very high temperature (GVHT), or of casein diet (CD).

Blood fibrinogen (figure 4.2A) tended to be lower in all rats fed with pasta than control diet, without significant statistical difference ($p=0.3$). Blood orosomucoid (figure 4.2B) was higher in rats fed with faba bean pasta than gluten pasta and CD. No difference in blood orosomucoid content was found in rats fed CD, GLT or VHT diets. Orosomucoid content was significantly positively correlated with the total body weight ($r=0.55$) and with the FM ($r=0.59$), and negatively correlated with the LM ($r=-0.59$).

**Figure 4.2** Plasma markers.

A: fibrinogen, B: orosomucoid, C: insulin, D: Adiponectin, E: leptin and F: MCP-1 (monocyte chemoattractant protein 1). Bars wearing different letters are significantly different ($p<0.05$). The diet were composed of wheat-mixed faba bean pasta dried at low temperature (FLT) or very high temperature (FVHT), wheat pasta dried at low temperature (GLT) or very high temperature (GVHT), or of casein diet (CD).

MCP-1 (figure 4.2C) was decreased in rats fed with gluten pasta in comparison to faba bean pasta which were characterized by a similar MCP-1 values than the CD rats. A significant correlation was found between MCP-1 and body weight ($r= 0.58$), and between MCP-1 and FM ($r=0.30$).

Blood insulin (figure 4.2D) measured in rats fed with FLT tended to be higher than in the GLT group. This effect was significant in particular when pasta were dried at VHT. Overall, pasta led to closed blood insulin to that of CD rats. Eating gluten pasta provoked an increase in blood adiponectin content (figure 4.2E) notably in GVHT in comparison to faba bean pasta (an increase by 160%). Overall, rats fed with CD had a lower adiponectin concentration than other groups except for FLT. Blood leptin concentration (figure 4.2F) was equivalent in FLT and GLT, whereas FVHT presented a significantly higher (58%) leptin concentration than GVHT. Leptin was higher in faba bean pasta in comparison to CD (42 and 58% for FLT and FVHT, respectively), but it was similar in gluten pasta and CD. Blood leptin was significantly and positively correlated with FM ($r=0.81$), with adipose tissue weights (0.63 for adipose peri-renal tissue and 0.62 for adipose sub-cutaneous tissue), with blood insulin (0.63), and with blood triglycerides (0.67) and negatively correlated with LM ($r=-$

0.80). No effect of the pasta drying temperature was observed except for adiponectin level, 36% higher in GVHT rats than in GLT rats.

HOMA-IR index is presented in figure 4.3. FLT tended to increase HOMA-IR in comparison to GLT, with a significant effect only in the case of VHT pasta. Nevertheless, no statistical difference was noticed between the HOMA-IR in rats fed with pasta compared to rats fed with CD. The change in drying temperatures did not bring any statistical differences on this index.

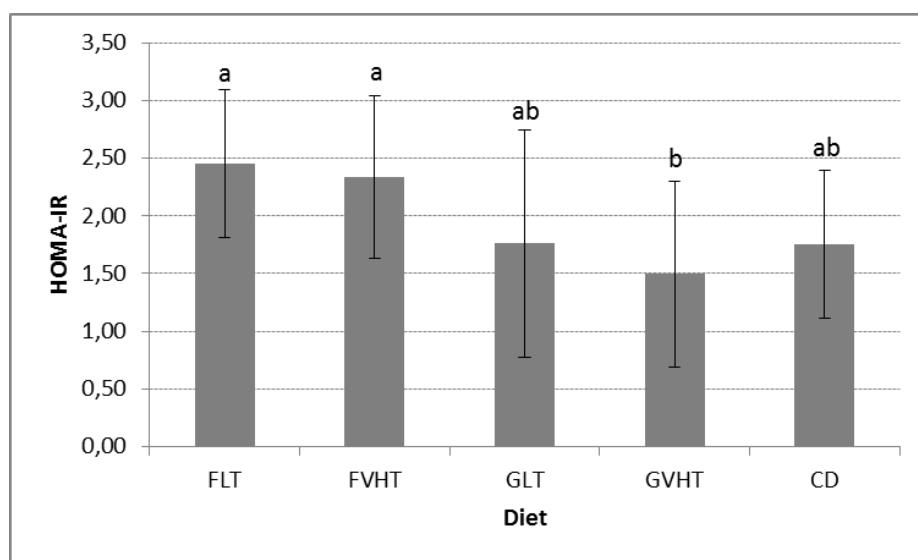


Figure 4.3 HOMA-IR index.

Homeostasis model assessment of insulin resistance. Bars wearing different letters are significantly different ($p<0.05$). The diets were based of wheat-mixed faba bean pasta dried at low temperature (FLT) or very high temperature (FVHT), wheat pasta dried at low temperature (GLT) or very high temperature (GVHT), or of casein diet (CD).

3.5. Tissue protein content

The protein content of muscles and liver is presented in table 4.8. No significant differences were observed in the protein content of *soleus*, *tibialis* muscles and liver regardless of the group ($p=0.2$). However, significantly higher protein content was observed in *plantaris* muscle in FLT and FVHT rats (16 and 11%, respectively) in comparison to GLT and GVHT animals. Faba beans also led to higher protein content in *plantaris* (10 and 6% for FLT and FVHT, respectively) in comparison to CD. The drying temperature of pasta did not change any of the muscle or liver protein contents.

Table 4.8 Protein composition of muscles (soleus, tibialis, plantaris) and liver

Diet	Soleus (%ms)	Tibialis (%ms)	Plantaris (%ms)	Liver (%ms)
FLT	14.84 ^{ab}	17.97 ^b	17.67 ^c	18.78 ^{ab}
FTHT	15.67 ^b	17.06 ^{ab}	16.99 ^{bc}	19.64 ^b
GLT	15.33 ^{ab}	17.87 ^{ab}	15.28 ^a	19.24 ^{ab}
GVHT	15.06 ^{ab}	17.17 ^{ab}	15.26 ^a	18.90 ^{ab}
CD	15.36 ^{ab}	17.29 ^{ab}	16.09 ^{ab}	19.00 ^{ab}
p-value	0.2	0.2	<0.05	0.2

Means with the different superscripts within a column are significantly different.

The diets were based of wheat-mixed faba bean pasta dried at low temperature (FLT) or very high temperature (FVHT), wheat pasta dried at low temperature (GLT) or very high temperature (GVHT), or of casein diet (CD).

3.6. mRNA expression

Among mRNA expression of the genes related to lipid metabolism (table 4.9), only the expression of peroxisome proliferator-activated receptor-gamma (PPAR γ), Monocyte chemoattractant protein-1 (MCP-1) and lipoprotein lipase (LPL) differed significantly ($p < 0.05$) between groups. The expression of PPAR γ was similar in all rats fed pasta, and was generally higher in these animals (1.3 to 1.8 fold) than in CD rats. The transcript level of MCP-1 was twice lower in FLT than in GLT, but no difference was observed between FVHT and GVHT. In addition, VHT drying of pasta resulted in a 38% decreased MCP-1 transcript level only in the case of gluten pasta without effect on faba bean pasta. Regardless of the drying temperature, faba bean pasta had similar MCP-1 than CD rats. FLT and GLT had a similar LPL gene levels. LPL expression was 78% higher in FVHT than in GVHT. No effect of drying temperature was observed on this parameter. In addition faba bean pasta led to higher LPL than CD. Finally, the expression of leptin tended to be increased in all the groups receiving pasta, notably in GLT in comparison to control diet but without significant difference ($p = 0.06$).

Table 4.9 mRNA expression in adipose tissue

Diet	FAS/ RPLP0	SERBP1c/ RPLP0	leptin/ RPLP0	PPAR γ / RPLP0	Adiponectine /RPLP0	ACC/ RPLP0	MCP-1/ RPLP0	LPL/ RPLP0	ATGL/ RPLP0	FATcd36/ RPLP0
FBT	2.60 ^a	1.60 ^a	1.49 ^{ab}	1.33 ^a	2.03 ^a	1.78 ^a	0.87 ^a	1.45 ^a	1.53 ^a	0.93 ^a
FTHT	2.55 ^a	1.25 ^a	1.58 ^{ab}	1.36 ^a	1.62 ^a	2.01 ^a	1.03 ^a	1.48 ^a	1.44 ^a	0.95 ^a
GBT	1.64 ^a	1.14 ^a	1.91 ^b	1.34 ^a	2.28 ^a	1.46 ^a	2.06 ^b	1.24 ^{ab}	1.68 ^a	1.15 ^a
GTHT	1.01 ^a	0.86 ^a	0.91 ^a	0.93 ^{ab}	1.51 ^a	0.98 ^a	1.27 ^a	0.83 ^b	1.07 ^a	0.94 ^a
CS	0.64 ^a	0.58 ^a	0.71 ^a	0.74 ^b	1.46 ^a	0.35 ^a	1.02 ^a	0.89 ^b	0.90 ^a	0.78 ^a
p-Value	0.35	0.50	0.06	0.01	0.32	0.57	0.02	0.02	0.39	0.49

Means with the different superscripts within a column are significantly different.

The diets were based of wheat-mixed faba bean pasta dried at low temperature (FLT) or very high temperature (FVHT), wheat pasta dried at low temperature (GLT) or very high temperature (GVHT), or of casein diet (CD)

ACC, Acetyl-CoA carboxylase; ATGL, Adipose triglyceride lipase; CypA, Cyclophiline; FAS, fatty acid synthase; FATcd36, fatty acid translocase cluster of differentiation 36; HPRT, hypoxanthine-guanine phosphoribosyltransferase; LPL, lipoprotein lipase; MCP-1, Monocyte chemoattractant protein-1; PPAR γ , peroxisome proliferator-activated receptor *gamma*; SERBP1c, Sterol regulatory element-binding protein 1.

4. Discussion

The primary objective of this study was to assess the impact of a diet based on wheat-faba bean mixed pasta on key indicators of dietary protein quality, body composition, blood metabolic and inflammatory markers on tissue protein content in growing rats. Diets composed of either plant proteins, i.e. wheat gluten pasta, or animal proteins, i.e. casein, with the same protein contents, were considered as controls.

Despite an equivalent dietary intakes, diets based on faba bean pasta (FLT and FVHT) were two fold more efficient and resulted in two times higher rate of growth than gluten pasta, meaning that the protein efficiency was greater when feeding rats with pasta containing faba bean flour. Our results confirm those of Torres et al. (2006) and Torres et al. (2007a) performed on pasta supplemented with 10% of lupin or pigeon pea flours. These authors showed that the enrichment of pasta with legume flours doubled the protein efficiency ratio in comparison to control pasta. The effect of legume flour incorporation into pasta seems to increase the efficiency of protein regardless of the legume type considered or the enrichment level.

Rats fed with faba bean pasta displayed a similar true protein digestibility than those fed with gluten pasta. However, a lower urinary nitrogen excretion and higher net protein utilization, biological value and PDCAAS indexes were seen in these animals compared to rats fed with gluten pasta. This noticeable improvement of the efficiency and of the retention and biological quality of pasta proteins enriched with faba bean could be explained by a better nitrogen balance related to the higher amino acid score, in particular the significant increase in lysine content. The improved nitrogen balance in rats fed with faba bean pasta was accompanied by higher weight of muscles and a greater increase in lean mass between the beginning and the end of the study. However, the protein content of muscles did not differ between pasta except for *plantaris* muscles, which were characterized by a significantly higher protein content in the rats fed with in faba bean pasta. This could be related to the more balanced essential amino acid profile in faba bean pasta. In addition, these pasta contained 9-10% higher branched chain amino acids than gluten pasta. These amino acids were reported to be able to dose-dependently stimulate muscle protein synthesis in growing rats (Garlick & Grant, 1988; Li & Jefferson, 1978). Blood parameters such as total proteins and albumin concentrations were also higher in rats fed faba bean pasta than in rats receiving gluten pasta. Overall, these observations could imply that dietary proteins are more available when rats

were fed with faba bean pasta, leading to increased efficiency of protein metabolism. The bioavailability of dietary proteins is one of the main factors that regulate body protein synthesis and oxidation (Volpi et al., 1998). Moreover, postprandial protein gain was already reported to vary according to the nitrogen source (Boirie et al., 1997; Dangin et al., 2001). The different chemical scores of the two proteins in gluten and faba bean pasta likely led to different amino acid bioavailability, and therefore to a different protein retention rate. In addition, the strength of protein linkages between wheat and wheat/legume pasta proteins was reported to affect the *in-vitro* protein digestion of pasta (De Zorzi et al., 2007; Laleg et al., 2016a; Petitot et al., 2009b). We recently showed that pasta enriched with 35% legume flours (used in this study) has a specific structure mainly composed of weakly bonded proteins in comparison to gluten pasta structured through covalently disulfide linked proteins (Laleg et al., 2016a). The weakened structure in faba bean pasta resulted in a 18% higher *in-vitro* protein digestibility in comparison to gluten pasta, and could probably increase the availability of amino acids in rats fed with faba bean pasta, as shown in the present study.

Remarkably, feeding rats with faba bean pasta resulted in the same growing efficiency than in rats fed with casein (CD). However, faba bean pasta increased fecal nitrogen and urinary nitrogen compared to CD, and lowered protein digestibility and retention parameters (true digestibility, apparent digestibility, net protein utilization) and the PDCAAS, which resulted in a lower nitrogen balance compared to CD. This lower nitrogen balance in faba bean pasta could be related to its lower chemical score (80-83%) in comparison to casein composed of a suitable amino acid profile. Nevertheless, FLT rats were characterized by the same muscle weight and even higher protein content in *plantaris* muscle than CD rats. Taken as a whole these data could indicate that dietary proteins were more efficient in the FLT group and the constitutive amino acids were probably more available for muscle protein synthesis. This could be explained by the higher solubility of faba bean pasta proteins, and their weakly chemical structure mainly linked through hydrogen, hydrophobic, and ionic bounds with a very low fraction of covalently linked protein (Laleg et al., 2016a). These intrinsic chemical characteristics could increase the rate of protein digestion. It was previously reported that the rate of protein digestion affected the post prandial protein gain (Dangin et al., 2003). A rapid aminoacidemia period was reported to enhance the muscle protein synthesis and the anabolic signaling to a greater extent than an identical amount of slowly digested protein (Macotela et al., 2011). In addition, soy proteins, considered as “fast proteins” induced a greater

stimulation of muscle protein synthesis in young men (64%) than casein (slowly digested protein) (Tang et al., 2009).

The second objective of our study was to evaluate whether the use of VHT drying during pasta processing affected the protein digestibility and retention and the subsequent metabolic parameters. The use of a VHT drying process did not affect the food efficiency and the growth parameters of the animals, whereas it was responsible for increased urinary nitrogen and a decreased protein digestibility notably in rats fed gluten pasta. PDCAAS was also decreased in both faba bean and gluten pasta after VHT drying. The decrease in protein quality parameter by VHT drying was likely related to the alteration of some essential amino acid during the drying process through Maillard reactions. Maillard reactions are able to decrease the *in-vitro* release of amino acids (Stuknyte et al., 2014), and to negatively affect the *in-vivo* protein digestibility and utilization (Seiquer et al., 2006).

As the adipose tissue masses increased in rats fed pasta, we have measured the mRNA and the adipocytes implicated in the principle regulating pathways of lipolysis and lipogenesis in order to clarify the phenomenon involved. In the present study, eating pasta, except for GVHT, increased the expression of PPAR γ (peroxisome proliferator-activated receptor gamma) and LPL (lipoprotein lipase). PPAR γ regulates adipocyte differentiation in response to endogenous lipid activators (Tontonoz et al., 1994), whereas, LPL regulates the triglyceride concentration of the plasma by monitoring the penetration of fatty acids into adipose tissues and muscles (Kageyama et al., 2003). Eating pasta also increased the leptin content, and tended to increase the expression of its gene in adipose tissue in comparison to CD. In addition, leptin was positively correlated with body fat mass, adipose tissue weight, and blood triglyceride content. Leptin contributes to the regulation of body weight by modulating appetite and energy expenditure (Yadav et al., 2013). It stimulates fatty acid oxidation and prevent the accumulation of lipid in non-adipose tissues (Shimabukuro et al., 1997). High adipose level was demonstrated to raise blood leptin in mice (Frederich et al., 1995). In addition, obese human presented higher leptin level suggesting a resistance to its action (Considine et al., 1996). As obesity progression is associated with an increased risk for insulin resistance, leptin is thought to contribute to the mechanism linking obesity and insulin resistance. Results found in our study could indicate that eating pasta increased the resistance to insulin in comparison to conventional diet (CD). This phenomenon could be related to the higher rate of digestibility of their gelatinized starch in comparison to the non-gelatinized

starch in CD. However, the HOMA-IR index of all rats eating pasta remained conform to that of CD. In addition, gene encoding for adiponectin which is another adipokine implicated in insulin resistance, was expressed similarly in rats eating pasta and CD, although blood adiponectin was higher in rats eating pasta. Adiponectin stimulates the free fatty acids oxidation in muscles, thereby reducing muscle triglyceride content. Increased tissue triglyceride content interferes with insulin-signaling pathway leading to insulin resistance. On the contrary, a decreased triglyceride content in muscle contributed to improve insulin signal transduction, and enhanced insulin sensitivity (Yamauchi et al., 2001). Concerning inflammatory markers, orosomucoide was higher in rats eating FLT and FVHT than control diet. Orosomucoides, fibrinogen and MCP-1 are acute-phase plasma proteins exclusively synthesized in the liver. The concentration of these molecules can be increased or decreased by at least 50% in inflammatory disorders, they restore homeostasis after the inflammation (Liaskou et al., 2012). The increase of some of these inflammatory markers in rats eating faba bean pasta in our study could be related to the increase in fatty mass in this group of rats. Indeed, MCP-1 and orosomucoides were found to be positively correlated to body weight ($r=0.58$ and $r=0.55$, respectively), and to fatty mass ($r=0.30$, and $r=0.59$, respectively).

5. Conclusion

The present investigation evaluated the nutritional effect of mixing legume and wheat proteins in the same food. We clearly demonstrated that it is possible to improve protein digestibility and nitrogen retention, body muscle weight and composition, and blood nutritional markers, by enriching wheat pasta with an adequate amount of faba bean flour with a complementary amino acid profile, and highly soluble protein. We also showed that some process parameters such as pasta drying temperature can slightly monitor the protein digestibility but does not affect protein retention. Pasta drying at low temperature can be preferentially used to promote protein digestibility. Wheat/legume mixed pasta can be nutritionally adapted to people who need to eat high protein diet with a balanced amino acid profile notably the elder people.

Conclusions

Ce travail a clairement démontré l'intérêt de l'enrichissement des pâtes avec 35% de farine de fèveroles, quelles que soient les températures utilisées pour le séchage, sur la croissance, la composition corporelle et la digestibilité, ainsi que sur l'efficacité et la rétention corporelle des protéines. Un accroissement d'un facteur 2 de l'utilisation nette des protéines, de la valeur biologique et du score PDCAAS a été observé chez les rats ayant consommé la pâte contenant 35% de farine de fèveroles en comparaison avec les rats nourris avec la pâte 100% blé. L'amélioration de ces paramètres est associée à la composition plus équilibrée en acides aminés essentiels, ainsi qu'à la structuration par des interactions majoritairement faibles du réseau protéique des pâtes mixtes blé-fèverole. Les modifications de la composition ainsi que l'affaiblissement de la structure du réseau protéique de la pâte ont également permis d'atteindre des taux de croissance et de masse maigre équivalents à ceux observés chez les rats consommant un régime standard contenant une protéine de source animale (caséine).

CHAPITRE 5

Discussion générale

CHAPITRE 5. DISCUSSION GENERALE

Contexte, objectifs et démarche expérimentale de l'étude

La pâte alimentaire est l'un des aliments céréaliers les plus simples présentant des propriétés nutritionnelles d'intérêt. Son indice glycémique est faible (autour de 32-65) (Foster-Powell et al., 2002) du fait de sa structure compacte, faite d'un réseau dense de protéines encapsulant les granules d'amidon (Cunin et al., 1995; Pagani et al., 1986) limitant ainsi leur susceptibilité à l'hydrolyse enzymatique (Colonna et al., 1990; Granfeldt & Bjorck, 1991). Cette structure spécifique est le résultat de transformations structurales aux différentes échelles de l'amidon et des protéines au cours des étapes successives (hydratation-malaxage, extrusion, séchage, cuisson) du procédé de pastification. La pâte alimentaire est également une source non négligeable de protéines (13%), mais ces dernières sont pauvres en certains acides aminés essentiels, comme la lysine et la thréonine (Abdel-Aal & Hucl, 2002). De plus, comme tout aliment contenant du gluten, la pâte peut provoquer des réactions allergiques chez les individus intolérants au gluten. Pour cette population, d'autres ingrédients pouvant se substituer partiellement ou intégralement aux protéines de gluten dans les pâtes doivent être recherchés. Les légumineuses, riches en protéines (entre 18 et 30%) (Brijesh & Narpinder) et en acides aminés essentiels (lysine et thréonine), constituent une source d'intérêt. Utilisées en mélange avec le blé, elles peuvent permettre la production de spécialités pastières enrichies en protéines et équilibrées en acides aminés essentiels (Gimenez et al., 2012; Petitot et al., 2010b). Cependant, l'introduction au-delà d'un certain pourcentage (35%) de farines de légumineuses dans la pâte peut poser des problèmes techniques au moment de la pastification (Petitot et al., 2010b; Wood, 2009) et changer la structure finale des pâtes altérant de fait leurs propriétés texturales et culinaires. La modification de certains paramètres du procédé de fabrication des pâtes telles que les températures de séchage peut prévenir une telle altération (Petitot et al., 2010b) mais pourrait, au même titre que le changement de formulation consistant à incorporer des légumineuses, modifier la structure de la pâte et affecter ainsi la digestibilité de ses principaux nutriments, l'amidon et les protéines (De Zorzi et al., 2007; Greffeuil et al., 2015; Petitot et al., 2009b).

L'objectif principal de cette thèse était d'étudier l'effet d'un enrichissement en protéines, notamment par apport de farines de légumineuses, sur la structure aux différentes

échelles de la pâte et ses répercussions sur leurs propriétés culinaires, texturales et nutritionnelles. Une attention particulière a été portée sur l'évolution de la nature et du degré d'interactions entre les protéines suite : i) à la dilution du réseau de gluten par l'ajout de protéines globulaires de légumineuses, ii) à la modification des températures de séchage de la pâte. La relation entre la structure du réseau protéique de la pâte et le maintien de son intégrité lors de sa cuisson ou sa texture (fermeté, élasticité) a été établie. L'effet de la nature et du degré d'interactions entre protéines sur la digestibilité *in-vitro* des protéines et de l'amidon de la pâte a également été étudié. La qualité nutritionnelle de spécialités pastières mixtes céréale-légumineuse notamment la digestibilité *in-vivo* des protéines a ensuite été évaluée sur des rats en cours de croissance. Les répercussions de la composition et de la structure des pâtes sur la rétention protéique, les paramètres métaboliques et l'homéostasie glucidique de ces animaux ont été étudiées. Afin de mieux répondre aux objectifs de la thèse, les travaux ont été divisés en quatre tâches principales (figure ii).

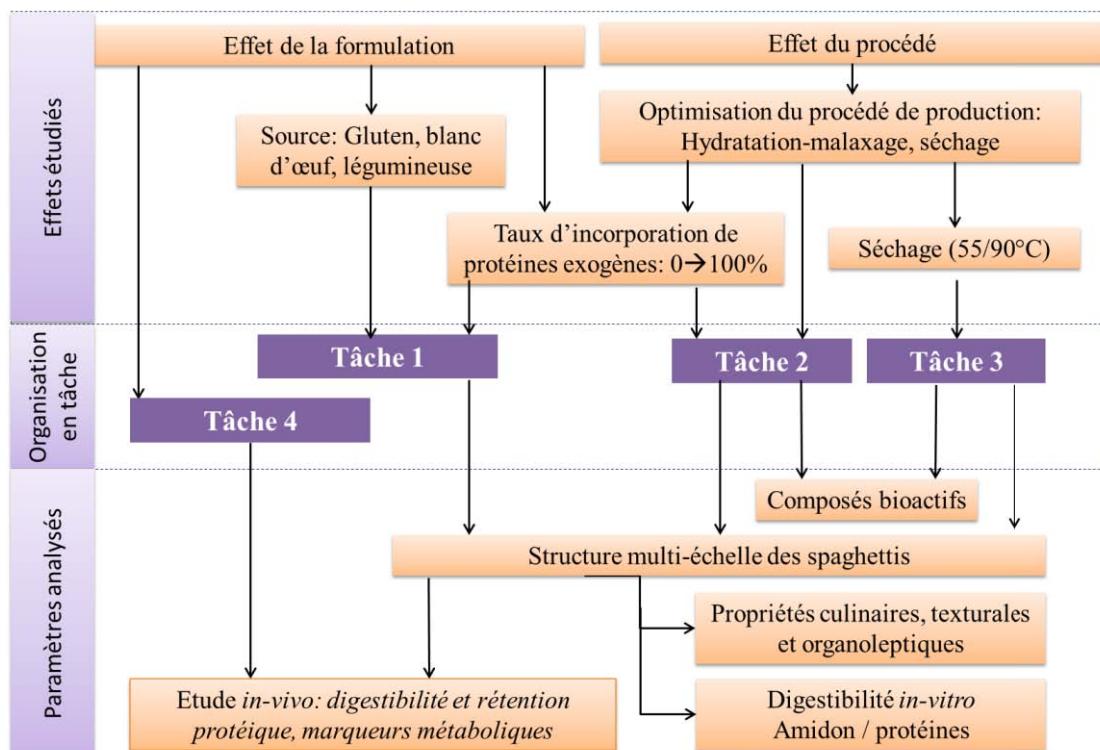


Figure ii Organisation générale du travail de thèse

Dans la première tâche, les effets de la nature et de la teneur en protéines de la pâte sur sa structure et ses répercussions sur ses propriétés culinaires et texturales ont été étudiés. Des farines de légumineuses (fèverole, 35%), du blanc d'œuf en poudre (5%) ou du gluten de blé (6%) ont substitué une partie de la semoule de blé, afin de produire une pâte enrichie en

protéines (17% vs 13% pour une pâte classique 100% blé dur). Le lien entre la structure des différents réseaux protéiques et leur digestibilité *in-vitro* a été établi. Cette tâche visait à évaluer le bénéfice potentiel, en terme de digestibilité *in-vitro* des protéines, à enrichir une pâte en protéines provenant notamment de légumineuse, par rapport à d'autres sources protéiques possibles, animale (blanc d'œuf) ou végétale (gluten).

Pour bénéficier d'une complémentarité céréale-légumineuse optimale au niveau nutritionnel, notamment en termes de composition en acides aminés indispensables (lysine), la farine de légumineuse doit représenter plus de 35 % dans la formule de la pâte mixte blé dur-légumineuse. Une pâte contenant 35% de farine de fèverole et 65% de semoule de blé dur ne couvre en effet que 86% des besoins en lysine (Laleg et al., 2016a) vs 36-46% pour la pâte au blé dur (Abdel-Aal & Hucl, 2002; Laleg et al., 2016a). Par ailleurs, une substitution complète de la semoule de blé dur par une légumineuse permettrait d'obtenir des pâtes à la fois très enrichies en protéines et dépourvues de gluten à destination des personnes intolérantes au gluten ou souffrant de maladie cœliaque. Une incorporation de légumineuse au-delà de 35% engendre cependant des problèmes d'agglomération au cours de la première étape d'hydratation-malaxage du procédé de pastification (Petitot et al., 2010b; Wood, 2009). La tâche 2 a donc eu pour objectif de lever le verrou technologique rencontré lors de la production de spécialités pastières aux légumineuses et rendre ainsi possible l'incorporation de farine de légumineuse au taux souhaité (de 0 à 100%) en fonction des propriétés nutritionnelles recherchées et ce quel que soit le type de légumineuse¹. L'effet du taux d'enrichissement de la pâte et de la nature de la légumineuse (fèverole, lentille ou haricot urd) sur la teneur en composé bioactifs dans la pâte, sur sa structure et ses répercussions sur ses propriétés culinaires et organoleptiques a été étudié. Les liens entre la structure de ces spécialités pastières et la digestibilité *in-vitro* de leurs fractions protéique et amylacée ont été également été déterminés.

L'incorporation de quantités importantes de légumineuses dans les pâtes, en diminuant ou même en éliminant le gluten, engendre également une baisse de ses attributs culinaires et texturaux (Padalino et al., 2014; RayasDuarte et al., 1996; Torres et al., 2007a; Zhao et al., 2005). L'utilisation de très hautes températures ($> 70^{\circ}\text{C}$) pour sécher les pâtes peut être une

¹ Cette partie des travaux ayant fait l'objet d'un dépôt de brevet (n° FR 14 62811) ne pourra faire l'objet d'une discussion dans ce manuscrit.

adaptation intéressante du procédé pour structurer le réseau protéique comme précédemment démontré sur une pâte 100% blé dur (Guler et al., 2002; Petitot et al., 2009b; Zweifel et al., 2003). Cependant, de tels barèmes de séchage peuvent, en augmentant le taux d'agrégations covalentes des protéines, diminuer leur digestibilité (De Zorzi et al., 2007; Petitot et al., 2009b) et leur teneur en acides aminés indispensables, notamment en lysine biodisponible (Greffeuille et al., 2015). L'utilisation d'un séchage à basse température à l'inverse peut être recommandé lorsque la digestibilité des protéines doit être maximale et la composition en acides aminés indispensables conservée. Peu de travaux ont été réalisés pour évaluer l'effet des températures de séchage sur la structure de spaghetti enrichis avec des légumineuses. L'effet des températures de séchage est plus important sur la structure des pâtes cuites mixtes (35% farine de fève+65% semoule de blé) que sur une pâte de blé (Petitot et al., 2009b; Petitot & Micard, 2010). Aucune étude ne rapporte l'effet des températures de séchage sur la structure de ces spécialités pastières et sur la digestibilité de leurs protéines lorsqu'elles contiennent plus de 35% de légumineuse. La troisième tâche visait donc à comprendre l'impact des modifications de structure de la pâte engendrées par de hautes températures de séchage (90 °C) vs. basses températures (55 °C) sur les propriétés culinaires, organoleptiques et nutritionnelles des spaghetti et notamment sur la digestibilité *in-vitro* des protéines.

La tâche 4 visait à étudier *in-vivo* l'effet sur la digestibilité et sur certains marqueurs nutritionnels de l'enrichissement protéique des pâtes avec de la farine de légumineuse (17% de protéines) couplé à un séchage soit à haute température, soit à basse température. La digestibilité et la rétention des protéines ainsi que leur efficacité sur la croissance ont été étudiées sur des rats jeunes nourris exclusivement avec ces pâtes pendant 21 jours. Les résultats ont été comparés à ceux obtenus chez des rats consommant des pâtes 100% blé contenant la même teneur en protéines (grâce à un enrichissement en gluten) ou un régime standard dont la composante protéique était représentée par de la caséine.

Synthèse et discussion des principaux résultats

Tâche 1 : effet de la nature et de la teneur en protéines d'une pâte sur sa structure aux différentes échelles ; Conséquences sur ses propriétés texturales, culinaires et la digestibilité *in-vitro* de son réseau protéique.

L'enrichissement de 13 à 17% en protéines d'une pâte (séchée à basse température), obtenu par substitution de 6% de semoule de blé dur par une poudre de gluten, a provoqué une réduction de la prise en eau pendant la cuisson et une augmentation de la fermeté de la pâte, liée à une réduction de la quantité d'amidon susceptible d'absorber de l'eau, comme démontré par Sissons et al. (2005). La pâte au gluten contenant 17% de protéines perdait également moins de matières à la cuisson grâce à sa structure spécifique et notamment à la nature des interactions entre ses protéines caractérisées par un taux élevé d'agrégation covalentes. Cette structure du réseau protéique qualifiée de « forte » était également à l'origine de la réduction de sa digestibilité *in-vitro* (7.4%).

Contrairement à l'utilisation du gluten, l'enrichissement en protéines de la pâte par le blanc d'œuf et notamment la fèverole améliorait la balance des acides aminés par rapport à celle d'un pâte de blé, avec notamment un doublement du score chimique en lysine (respectivement 64 et 86 pour la pâte enrichie en blanc d'œuf et fèverole contre 41 pour celle enrichie en gluten). De plus, la source de protéines utilisée pour l'enrichissement affectait la texture des pâtes. Comme les pâtes enrichies en gluten, les pâtes enrichies avec du blanc d'œuf étaient caractérisées par une meilleure fermeté et une meilleure résistance à l'élongation, et par une perte moindre à la cuisson comme démontré dans la littérature (Fardet et al., 1999; Marti et al., 2014; Matsuo et al., 1972). A l'inverse, la pâte enrichie en fèverole présentait des pertes à la cuisson plus importantes en accord avec les résultats de la littérature (Gimenez et al., 2012; Petitot et al., 2010b). Les spaghetti enrichis en gluten et en œuf présentaient une structure microscopique plus compacte, notamment au centre de la pâte, par comparaison avec la structure plus ouverte des spaghetti enrichis avec 35% de fèverole. Ces différences entre spaghetti pourraient être liées à la structure moléculaire des protéines utilisées pour l'enrichissement, ainsi qu'au pourcentage de protéines exogènes introduites par rapport aux protéines totales de la pâte. Dans une pâte contenant 35% de farine de féverole, les protéines de féverole représentaient 50% des protéines totales. Ces protéines composées d'albumines et de globulines (Kosinska et al., 2011), très solubles dans l'eau et le SDS (Petitot et al., 2010a), pauvres en acides aminés soufrés (17mg/g de protéines) diluaient le

réseau de gluten et diminuaient la quantité de protéines susceptibles de se lier par des ponts disulfures dans la pâte. A l'inverse, les protéines de gluten et de blanc d'œuf constituaient, dans les pâtes enrichies en ces deux matières premières, respectivement 28 et 27% des protéines totales de la pâte. Ces deux types de protéines étant de plus toutes deux riches en acides aminés soufrés (respectivement 35 et 59 mg/g de protéines pour les protéines du gluten et de l'œuf) capables de former des ponts disulfures pendant le séchage et la cuisson des pâtes, elles ont ainsi induit un renforcement de la structure du réseau protéique de la pâte. Parmi les paramètres de structure des pâtes que nous avons étudiés, le degré d'hydrolyse des protéines dans les pâtes cuites s'est révélé plus lié à la nature des interactions entre les protéines et notamment à la teneur en ponts covalents qu'à un changement de structure secondaire ou tertiaire des protéines. En effet, toutes les pâtes enrichies en protéines présentaient une organisation tertiaire similaire. Au niveau de la structure secondaire des protéines, celles de la pâte enrichie avec 35% de farine de fèverole, plus riches en feuillets- β mais moins riches en ponts covalents, étaient les plus digérées. A l'inverse, les protéines des spaghetti enrichis en gluten et en blanc d'œuf riches en ponts covalents, mais avec des niveaux différents de feuillets- β , étaient moins hydrolysées que les pâtes de fèverole (notamment pour la pâte enrichie en gluten).

Peu d'études reportent l'effet de l'enrichissement de pâtes en protéines de légumineuses sur la digestibilité protéique. RayasDuarte et al. (1996) ont montré, comme dans notre étude, une augmentation de leur digestibilité avec un taux d'enrichissement en lupin de 5 à 30%, mais aucune explication à ce phénomène n'a été apportée, notamment en lien avec la structure des protéines dans les pâtes mixtes produites.

En conclusion l'enrichissement de pâtes de blé de 13 à 17% de protéines, par incorporation de gluten, s'il engendrait une amélioration des propriétés texturales et culinaires des pâtes, n'augmentait pas leur score chimique en lysine, et diminuait de plus le degré d'hydrolyse des protéines dans la pâte. Les spaghetti enrichis avec de la fèverole afin d'atteindre 17% de protéines, contenaient plus de 50% de protéines de fèverole et présentaient de fait un profil plus équilibré en acides aminés essentiel que les pâtes à 17% de protéines enrichies avec du gluten ou du blanc d'œuf. Le réseau protéique plus faiblement structuré des pâtes à la fèverole altérait partiellement leurs propriétés culinaires et texturales, mais favorisait la digestibilité de leur réseau protéique (augmentation de 18% du taux d'hydrolyse par rapport à une pâte enrichie en gluten).

La tâche 1 de ce travail de thèse a clairement montré l'intérêt nutritionnel de l'utilisation de farine de légumineuse, à raison de 35%, comme source d'enrichissement protéique des pâtes. Cependant, cette pâte mixte (35 : 65 légumineuse : blé dur) étant toujours déficitaire en lysine (score chimique de 86%), il était nécessaire de calculer le pourcentage en fèverole à incorporer dans une pâte pour atteindre 100% des besoins en lysine, à savoir 60%. Un tel taux d'enrichissement en légumineuse d'une pâte fabriqué sur une presse à pâte pilote n'avait jamais été décrit jusqu'à présent dans la littérature.

Tâche 2 : faisabilité technologique des spaghetti contenant plus de 50% de protéines de légumineuses. Etude du lien entre la structure, les propriétés texturales, culinaires, organoleptiques et nutritionnelles des pâtes en fonction du taux d'enrichissement en légumineuse.

Faisabilité technologique

De nombreux travaux visant à développer des spécialités pastières mixtes blé-légumineuses ont été menés ces dernières années. Jusqu'au démarrage de ce travail de thèse, les niveaux d'incorporation des protéines de légumineuses par rapport aux protéines totales dans la pâte variaient généralement entre 18 et 54% (Padalino et al., 2014; Petitot et al., 2010b; Wojtowicz & Moscicki, 2014; Zhao et al., 2005). Ces taux d'enrichissement en protéines de légumineuses inférieurs à 50% ne permettaient pas d'atteindre un score chimique optimal en lysine (Laleg et al., 2016a). L'incorporation dans une pâte de quantités plus importantes de légumineuse, si elle est intéressante d'un point de vu nutritionnel, s'est révélée problématique d'un point de vue technologique au cours notamment de l'étape d'hydratation-malaxage du procédé de fabrication (Petitot et al., 2010b; Wood, 2009). En effet, lors de cette étape, des phénomènes d'agglomérations accrus des particules de farine et semoule se produisent, rendant difficile voire impossible l'étape d'extrusion ultérieure. Deux études ont été conduites sur des pâtes hautement enrichies en légumineuses (dans lesquelles 66 à 100% des protéines totales provenaient de légumineuses), notamment celle de Jyotsna et al. (2015) et de (Rosa-Sibakov et al., 2016). La fabrication de ces pâtes n'a été possible que parce qu'elle était menée sur une mini-presse de laboratoire et non sans rencontrer cependant de difficultés au cours des étapes de malaxage et d'extrusion (Rosa-Sibakov et al., 2016). Un travail a donc été entrepris au cours de cette thèse afin de lever ce verrou technologique et de permettre la production à l'échelle pilote de ces spécialités pastières hautement enrichies en légumineuses (taux de protéines de légumineuses supérieures à 50% des protéines totales).

Ces travaux ont fait l'objet d'un dépôt de brevet en décembre 2014 (FR 14 62811). En utilisant le protocole expérimental décrit dans ce brevet, il est aujourd'hui possible de produire sur une presse pilote ou industrielle, sans rencontrer de problème d'agglomération, des pâtes contenant jusqu'à 100% de protéines de légumineuses.

Effet de la concentration en protéines de légumineuses dans une spécialité pastière sur sa structure et ses propriétés culinaires, organoleptiques et nutritionnelles

Des spaghetti enrichis avec de la farine de féverole ont été produits, dans lesquelles les protéines de féverole représentaient 0, 50, 80 et 100% (respectivement, F0, F50, F80 et F100) des protéines totales, puis séchés à basse température. L'augmentation de la concentration de farine de féverole dans une spécialité pastière avait pour premier objectif d'améliorer le score chimique en lysine, acide aminé indispensable déficitaire chez le blé. Un score de 122% de lysine a pu être atteint dans les spaghetti F80 contre seulement 86% pour les spaghetti F50 (pâtes faites respectivement par une substitution de 70% et 35% de la semoule de blé par de la farine de féverole dans la pâte).

La substitution des protéines de blé par des protéines de féverole a modifié la structure moléculaire du réseau protéique des pâtes. Une diminution des protéines liées par des liaisons covalentes, notamment disulfures, et une augmentation des protéines liées par interactions faibles ont été observées ; toutes deux linéaires et proportionnelles à la proportion de protéines de féverole incorporée dans la pâte par rapport aux protéines totales de la pâte. Le caractère linéaire de l'affaiblissement du réseau protéique en fonction du taux d'enrichissement en protéines de légumineuses a été démontré ici pour la première fois. De plus, seules les protéines de blé semblaient réagir pour former des ponts disulfures. Nous avons démontré que cette fragilisation linéaire de la structure du réseau protéique, notamment la diminution de la quantité de ponts disulfures, conduisait à une altération linéaire des propriétés culinaires (augmentation des pertes à la cuisson) et texturales (altération de la résilience) des spécialités pastières aux légumineuses selon le taux d'enrichissement en protéines de féverole utilisé.

La réduction marquée de la résilience des spaghetti F80 et F100 s'est traduite lors d'un test de préférence sensorielle de ces spaghetti par les consommateurs, par une appréciation diminuée de ces spaghetti par rapport aux pâtes de blé classiques. Leur texture, qualifiée de

moins élastique (en accord avec l'analyse instrumentale) et leur arôme de légumineuse ont été mis en cause. Néanmoins, les spaghetti F80 ont été autant appréciés qu'une pâte du commerce au blé complet de marque Celnat.

Nous avons également démontré que l'enrichissement en protéines de féverole des pâtes, engendrait une augmentation respective de 8.6, de 13.4 et de 24.8% de leur digestibilité *in-vitro* dans les spaghetti F50, F80 et F100 par rapport à une pâte F0. Et ce alors même qu'une élévation de l'activité anti-trypsique de la pâte cuite a été enregistrée lorsque la teneur en féverole augmentait, jusqu'à atteindre 2.3 mg/g de pâtes cuites F100. Ce niveau d'activité anti-trypsique restait cependant 4 fois inférieur à celui mesuré initialement sur la farine de féverole. L'augmentation du taux de protéines hydrolysées en réponse à la fragilisation de la structure du réseau protéique des pâtes était donc probablement plus important comme phénomène que le taux d'inhibition de la digestion protéique par la quantité croissante de facteurs anti-trypsiques.

Si l'utilisation de pâtes contenant des quantités importantes de farine de légumineuses, notamment les spaghetti F80 ou F100, semble intéressante d'un point de vue de la nutrition protéique, il était cependant nécessaire de vérifier que cette propriété ne s'exerçait pas au détriment d'une augmentation de l'indice glycémique de ces pâtes. En effet, le faible indice glycémique des pâtes alimentaires de blé est relié à la fois à sa structure compacte (Granfeldt & Bjorck, 1991) et à l'emprisonnement de son amidon au cœur d'un réseau protéique le protégeant de l'action des enzymes amylolytiques (Colonna et al., 1990; Fardet et al., 1998b). Il a été démontré que la vitesse de digestion *in-vitro* de l'amidon et la réponse glycémique *in-vivo* d'un pain sans gluten était augmentée (Jenkins et al., 1987). A l'inverse une réduction de la quantité de gluten, ou son élimination dans la pâte par introduction de 35% ou 100% de farine de féverole n'a pas engendré de modification de la digestibilité *in-vitro* de l'amidon. (Greffeuille et al., 2015; Petitot et al., 2010a; Rosa-Sibakov et al., 2016).

Dans le cas des pâtes 100% féverole, le réseau de gluten était totalement absent et remplacé par un réseau de protéines globulaires peu liées (25%) par des interactions covalentes, formé principalement au cours de l'étape de cuisson des pâtes. Ce changement drastique de la structure du réseau protéique n'a pourtant pas non plus engendré d'augmentation de la vitesse de digestion *in-vitro* de l'amidon dans notre étude. Au contraire, une diminution du glucose disponible total, une augmentation de l'amidon résistant et une diminution du glucose rapidement disponible (communément nommé « RAG » pour Rapidly

Available Glucose) ont même été rapportées allant pour le seul RAG de 67% dans une pâte 100% blé (Greffeuille et al. (2015) à 62% dans un spaghetti 100% fèverole. La quantité importante de protéines (24-28%) dans les spaghetti faits exclusivement de fèverole constituait une matrice plus épaisse autour des grains d'amidon pouvant effectivement ralentir leur hydrolyse (Rosa-Sibakov et al. (2016). Dans notre étude, le réseau protéique de pâtes 100% fèverole apparaissait effectivement plus épais que dans une pâte de blé lors des observations microscopiques. La composition particulière (notamment le ratio amylose / amylopectine) et la taille plus importante des grains d'amidon de légumineuses pourraient également être à l'origine de leur plus grande résistance à l'hydrolyse (Sandhu & Lim, 2008). La teneur des légumineuses en certains facteurs bioactifs, comme l'acide phytique, qui complexe l'amylase affectant ainsi son activité (Deshpande & Cheryan, 1984; Thompson & Yoon, 1984), pourrait également favoriser le ralentissement de la digestion de l'amidon observé dans les spaghetti 100% fèverole. Si les propriétés nutritionnelles de la pâte 100% fèverole sont intéressantes du point de vue des protéines (meilleure digestibilité) et de l'amidon (indice glycémique plus faible), l'absence de réseau de gluten a conduit à diviser par 1,5 l'élasticité de la pâte et a augmenté par deux les pertes à la cuisson par rapport à celles obtenues avec une pâte classique au blé. Néanmoins, les pertes à la cuisson enregistrées sur ces spaghetti sans gluten (13%) demeuraient largement inférieures à celle mesurées pour des spaghetti de céréales sans gluten du commerce (marque Schär) et leur appréciation par un test de préférence sensorielle (par les consommateurs de pâtes classiques et complètes) mieux notée. Selon ce test, les spaghetti 100% fèverole différaient des spaghetti Schär par une texture moins élastique, moins ferme et un arôme de légumineuse.

Si certaines propriétés organoleptiques et nutritionnelles de la pâte 100% fèverole s'avèrent intéressantes, il nous est apparu nécessaire de vérifier si elles pouvaient être conservées avec d'autres types de légumineuses. A ce jour, aucune étude de ce type n'avait été entreprise. Pour ce faire, des spaghetti faits exclusivement de farine de lentille ou d'haricot urd (black gram) ont été produits. Leurs structures, leurs propriétés culinaires et nutritionnelles ont été comparées à celles d'une pâte 100% fèverole. A l'échelle microscopique, la quantité importante de protéines dans les spaghetti de lentille et d'haricot urd (28% contre 24% pour la fèverole) n'a pas engendré de différence perceptible d'épaisseur du réseau protéique par rapport à un spaghetti 100% fèverole. La structure moléculaire, en termes de protéines liées par des ponts covalents ou par des interactions faibles, des spaghetti faits de lentille était similaire à celle des spaghetti de fèverole. En conséquence, ces deux

types de spaghetti présentaient des propriétés culinaires (pertes à la cuisson) et texturales (élasticité) similaires. Ce résultat confirmait bien le lien, précédemment mis en évidence sur des spaghetti enrichis avec des concentrations croissantes de protéines de fèverole, entre la diminution des protéines liées par des ponts disulfure et l'altération de l'élasticité et l'augmentation des pertes à la cuisson de la pâte. Cependant, ce lien n'existe pas pour toutes les légumineuses. Les spaghetti faits d'haricot urd, présentaient ainsi une meilleure élasticité (0.85 pour le haricot urd contre 0.75 pour la lentille et la fèverole) et moins de pertes à la cuisson (10% contre 13-14% pour la lentille et fèverole). Ceci n'a pu être expliqué par la nature des interactions entre les protéines, puisque les protéines des pâtes 100% haricot urd étaient principalement liées par des liaisons faibles (90%, contre 75% pour la fèverole et la lentille). Ceci suggère que d'autres types d'interactions sont impliquées dans le maintien de la structure et de l'intégrité des spaghetti 100% urd. Leur teneur plus importante en fibres solubles, en particulier de type arabinogalactane, pourrait être impliquée. En effet, ces polysaccharides sont connus pour leur capacité à stabiliser les émulsions de protéines formées dans les aliments traditionnels à base de haricot urd comme l'idlis (Nisha et al., 2005; Sathe & Salunkhe, 1981; Susheelamma & Rao, 1979).

La structure proche des pâtes 100% fèverole et 100% lentille, quelle que soit l'échelle d'observation, s'est traduite d'un point de vue nutritionnel par des vitesses similaires de digestion de l'amidon. A l'inverse, l'amidon des spaghetti 100% haricot urd entouré d'un réseau protéique moins structuré par des interactions covalentes, était digéré plus rapidement (RAG : 68.6%) que celui des spaghetti 100% fèverole (RAG : 61.8%). Un lien entre le caractère plus ou moins covalent du réseau de protéine des pâtes et la digestibilité de leur amidon semble donc exister. Un réseau protéique structuré par un niveau important d'interactions covalentes, engendrées par de hautes températures de séchage ou par ajout de transglutaminase, a été effectivement impliqué dans la réduction de la vitesse d'hydrolyse de l'amidon de spaghetti enrichis avec 35% de farine de fèverole (Greffeuille et al., 2015) ou 100% fève (Rosa-Sibakov et al., 2016). Néanmoins, l'amidon rapidement digestible de pâtes 100% haricot urd reste similaire à celle d'une pâte de blé (RAG : 67%), montrant que la structure seule du réseau protéique d'une pâte ne peut expliquer totalement les différences de RAG.

Les légumineuses, outre leurs avantages nutritionnels (richesse en protéines, composition en acides aminés indispensables, amidon lentement digestible), contiennent des

composés bioactifs qui peuvent exercer une action anti-nutritionnelle. Certains de ces facteurs, comme l'acide phytique, les facteurs anti-trypsiques et les α -galactosides, réduisent la digestibilité ou la biodisponibilité des nutriments notamment celle des protéines et des minéraux, ou sont sources d'inconfort digestif. Au cours de ces travaux de thèse, nous avons démontré pour la première fois que les spaghetti cuits faits exclusivement de légumineuses (fèverole, lentille ou haricot urd) contiennent jusqu'à 81% moins de facteurs anti-trypsiques, et jusqu'à 73% moins d' α -galactosides que les farines de légumineuse utilisées pour la production de ces spécialités pastières. Quant à l'acide phytique, il n'a été par contre que très peu touché par le procédé de pastification.

Par ailleurs, la modification de la structure de la pâte 100% légumineuse, si elle n'induit pas d'augmentation potentielle de leur indice glycémique, quel que soit le type de légumineuse étudiée, peut par absence du réseau de gluten, engendrer une baisse des attributs culinaires et texturaux des pâtes. Une solution potentielle consiste à utiliser de hautes températures de séchage des pâtes qui améliorent la structuration du réseau protéique de pâte 100% blé dur (Petitot et al., 2009b) ou 35% légumineuses (Petitot & Micard, 2010). En outre, si ce type de traitement peut se révéler intéressant d'un point de vue culinaire et sensoriel, il convenait de vérifier dans quelle mesure il modifiait la teneur de la pâte en composés d'intérêt nutritionnel (acides aminés essentiels) ou au contraire en composés bioactifs antinutritionnels (facteurs anti-trypsiques).

Tâche 3 : effet des hautes températures de séchage (*vs* basse température) sur la relation structure-propriétés culinaires, -texturales ou -nutritionnelles de spécialités pastières de légumineuses

Des spaghetti enrichis avec de la farine de fèverole, dans lesquelles les protéines de fèverole représentaient 50 (F50), 80 (F80) et 100% (F100) des protéines totales, ont été produits puis séchés à basse température (LT ; 55 °C) ou à très haute température (VHT ; 90 °C). A l'échelle microscopique aucune différence visuelle n'a été observée entre les deux modes de séchage LT et VHT concernant les pâtes 100% blé (F0) ou mixtes blé-fèverole (F50). Sur le spaghetti 100% fèverole séché à 90 °C, nous avons toutefois constaté une amélioration de l'intégrité du réseau protéique à la surface de la pâte. A l'échelle supramoléculaire, le séchage à 90 °C (*vs* 55 °C) des spécialités pastières aux légumineuses conduisait à un renforcement de la structure de leur réseau protéique et ce quelles que soient leurs teneurs en protéines de légumineuses (0 à 100%). Ce renforcement se caractérisait par

l'augmentation du pourcentage de protéines liées par des ponts disulfures entre un séchage LT et VHT (de respectivement 30 à 68%, de 18 à 53%, de 11 à 40 et de 4 à 31% dans les pâtes F0, F50, F80 et F100). Parallèlement à l'augmentation des disulfures, le séchage VHT provoquait également une apparition de protéines liées par d'autres types de liaisons covalentes (ex : les ponts isopeptidiques) atteignant respectivement 8%, 5%, 4 et 3% pour les pâtes F0 et F50, F80 et F100). Que ce soit avec un séchage LT ou un séchage VHT, la proportion de liaisons covalentes (disulfures et autres) diminuait linéairement avec l'augmentation de la concentration de protéines de fèverole dans la pâte. L'agrégation des protéines par des ponts covalents (disulfures et autres) semble donc diminuer en intensité au fur et à mesure que la concentration de fèverole s'élève dans la pâte. Selon Zheng et al. (1998), la dénaturation thermique des protéines de légumineuses se fait par agrégation hydrophobe des chaînes polypeptidiques. Dans une pâte aux légumineuses ce type d'agrégation participerait donc plus activement au maintien du réseau protéique au côté des interactions covalentes que dans les pâtes aux céréales où les ponts disulfures prédominent.

Selon de précédentes études, des températures de séchage de 90-100 °C vs 55 °C engendraient effectivement une agrégation covalente massive des protéines dans les pâtes de blé (Lamacchia et al., 2007; Zweifel et al., 2003) ou enrichies en légumineuses (35% farine de fève-65% blé soit l'équivalent de notre F50) (Petitot & Micard, 2010), comme nous avons pu l'observer dans cette thèse. Toutefois ces différences massives de structure du réseau protéique de la pâte en sortie de séchage à très haute température vs basse température se réduisaient considérablement après leur étape de cuisson (Petitot et al., 2009b). Dans notre étude, la cuisson des pâtes F0, F50 engendrait effectivement une réduction des différences de structure du réseau protéique initialement observées entre un séchage LT et VHT, notamment pour la pâte de blé F0. Des différences plus marquées dans la structure du réseau protéique subsistaient dans la pâte mixte cuite F50. En effet, dans les spaghetti F50 séchés à 55 °C et cuits, 60% des protéines étaient liées par des ponts disulfures contre 68% pour un spaghetti séché à 90 °C puis cuit. Cette différence de structure du réseau protéique des pâtes F50 après cuisson, liée à l'étape antérieure de séchage, pourraient expliquer en partie l'amélioration plus conséquente de leurs propriétés culinaires (pertes à la cuisson diminuée de 23%) et texturales (résilience et résistance à l'elongation augmentée respectivement de 9% et 41%) par rapport aux modifications mineures de ces propriétés pour une pâte 100% blé. Une amélioration considérable de ces propriétés a également été obtenue sur le spaghetti F80 et F100 cuits quand les températures de séchage étaient augmentées de 55 à 90 °C (pertes à la

cuisson diminuée de 26 et 36% et résilience augmentée de 21 et 45% pour respectivement F80 et F100). Petitot et al. (2010b) et Petitot et al. (2009b) ont également observé une amélioration plus importante des paramètres de texture (comme la résilience) de pâtes enrichies avec 35% de fève par rapport à celle de blé par effet des hautes température de séchage (90 °C vs. 55 °C).

Afin de savoir si l'amélioration des propriétés texturales des pâtes à la fèverole séchées VHT, mesurées par la méthode instrumentale, se traduisait par une amélioration de leur appréciation par le consommateur, nous avons conduit une étude sensorielle en comparant les spaghetti 100% fèverole séchées LT et VHT. Même si l'analyse du test de préférence n'a révélé aucune différence statistique d'appréciation globale entre les deux , les spaghetti VHT étaient déclarés plus fermes et élastiques que les spaghetti LT. Comme dans notre étude, Petitot et al (2010b) avaient montré par une évaluation sensorielle l'amélioration des critères d'élasticité et de fermeté des spaghetti de blé dur enrichis avec 35% de farine de fève après un séchage VHT Petitot et al. (2010b). Le séchage à très haute température donnait par contre, aux spaghetti séchés VHT de notre étude, une note aromatique de type « grillé » peu appréciée des consommateurs.

D'un point de vue nutritionnel, la température de séchage peut par contre diminuer la teneur en inhibiteurs trypsiques des pâtes à la fève. Ces facteurs antinutritionnels sont des protéines caractérisées par une structure compacte stabilisée par un pont disulfure (Mueller & Weder, 1989). Un séchage des pâtes à haute température pourrait favoriser un réarrangement sulfhydryl-disulfide modifiant ainsi la structure de l'inhibiteur et perturbant son activité. L'activité anti-trypsique de la pâte à la fèverole F100 cuite était ainsi respectivement réduite de 69% et 74% après un séchage LT ou VHT par rapport à l'activité anti-trypsique de la farine de fèverole. Le séchage VHT des spaghetti induisait également une perte respective de 13, 5 et 7% de lysine dans les spaghetti secs (avant cuisson) F50, F80 et F100, probablement par implications dans les réactions de Maillard ou la formation de ponts isopeptidiques. Malgré cette perte, les pâtes enrichies en fèverole étaient 1.6 à 3 fois plus riches en lysine en fonction des teneurs en légumineuse incorporées, qu'une pâte de blé classique séchées à basse température.

Le séchage VHT, par rapport à un séchage LT, augmentait la part de protéines liées par interactions covalentes dans les pâtes sèches, cette différence étant toutefois considérablement diminuée après cuisson des pâtes (notamment pour F0 et F50). C'est sans

doute pour cette raison qu'aucune modification du degré d'hydrolyse des protéines des spécialités pastières cuites n'a été observée dans notre étude entre un séchage LT et VHT et ce quelle que soit la concentration de fèverole utilisée pour l'enrichissement. Petitot et al. (2009b) n'obtenaient pas non plus de différence du degré d'hydrolyse de pâtes cuites 100% blé préalablement séchées à 55 °C, 70 °C ou 90 °C. Un effet de la modification de structure du réseau protéique sur la digestion des pâtes cuites n'a pu être observé par ces auteurs que lors de l'application d'un traitement de séchage sévère. Ce séchage, de type post-traitement (séchage à 55 °C pendant 15 heures suivi de 3 heures à 90 °C), engendrait alors une baisse du degré d'hydrolyse des protéines, passant de 40% à 34%. Cette diminution du taux d'hydrolyse des protéines était liée à l'apparition d'agrégats plus large lors du séchage VHT de type post-traitement qu'en séchages LT ou VHT classiques (cycle simple de 90 °C pendant 3 heures comme le nôtre). De Zorzi et al. (2007) obtenaient également une baisse de la digestibilité des protéines dans les spaghetti de blé lorsque ceux-ci étaient séchés à des températures plus élevées (180 °C) que celles utilisées dans notre étude.

Tâche 4 : Effet de l'incorporation de farine de fèverole et de la modification des températures de séchage des pâtes sur la digestibilité, la rétention et l'efficacité protéiques : étude chez le rat

L'enrichissement des spaghetti avec 35% de farines de légumineuses permettait d'améliorer les propriétés nutritionnelles de l'aliment. La quantité de protéines dans la pâte était augmentée passant ainsi de 13 à 17%. De plus, la teneur en lysine s'élèvait de 21 à 39 mg/g de protéines. En outre, la digestibilité *in-vitro* des protéines était améliorée grâce à leurs protéines plus solubles et leur structure spécifique où les interactions faibles sont plus importantes que dans la pâte 100% blé. En effet, dans une pâte au blé, les protéines de gluten constitutives, de type fibrillaires peu solubles, sont essentiellement structurées par des interactions covalentes de type disulfures. Si les propriétés culinaires et structurales des spaghetti mixtes légumineuses-blé ont fait l'objet de recherche ces dernières années, la digestibilité de leur fraction protéique reste peu étudiée. Ainsi la digestibilité *in-vitro* des protéines d'une pâte enrichie avec 20% de dolique asperge (*Vigna unguiculata*, natif, germé ou fermenté) était identique à celle d'une pâte de blé (Herken et al., 2006). D'autres travaux notamment ceux de RayasDuarte et al. (1996) rapportaient une augmentation de 2% de leur digestibilité suite à l'enrichissement de la pâte par 5 à 30% de farine de lupin. Les travaux réalisés dans la présente thèse confirment ces résultats (9% d'augmentation de la digestibilité

in-vitro par rapport à une pâte traditionnelle) qui seraient probablement liés à la nature plus soluble du réseau protéique et à sa structure plus faible (interactions covalentes réduites).

L'effet de la consommation des pâtes mixtes blé/légumineuses *in-vivo* sur des marqueurs nutritionnels, notamment la digestibilité et la rétention protéique corporelle, reste à ce jour très peu étudié. Certains travaux (Torres et al., 2007a; Torres et al., 2006; Torres et al., 2007b) ont évalué la digestibilité et l'efficacité protéique *in-vivo* de spaghetti enrichis avec des farines de lupin et de pois d'Angole sur des rats, mais les taux d'incorporation de ces farines ne dépassaient pas 10% (soit 17 à 22% des protéines correspondant à des protéines de légumineuses, contre au moins 50% dans nos travaux). Ce taux d'incorporation est insuffisant pour garantir un équilibre en acides aminés essentiels par rapport aux besoins tels que décrits dans la protéine dite idéale (ANSES, 2007a). De plus, aucune information n'a été fournie concernant l'effet de la composition ou de la structure de ces aliments mixtes ainsi que l'effet de la variation de leur procédé de fabrication, notamment les températures de séchage, sur la composition corporelle ou sur la rétention azoté des animaux.

Pour répondre à ces questions, nous avons réalisé une étude chez des rats en croissance ayant consommé pendant 21 jours des pâtes contenant 17% de protéines. Ce taux protéique a été obtenu en substituant une partie de la semoule de blé dans la pâte par 35% de farine de fèveroles, ou par 6% de poudre de gluten (groupe contrôle isoprotéique). Ces pâtes ont été séchées soit à basse (LT ; 55 °C) soit à très haute température (VHT ; 90 °C).

Le taux de croissance des rats nourris avec le régime composé des pâtes contenant des protéines mixtes blé/fèverole a été accéléré (multiplication par un facteur 2) par rapport à un régime de pâtes contenant seulement des protéines de gluten. Ces résultats confirment ceux de la littérature chez des rats consommant des régimes isoprotéiques (10%) de pâtes enrichies avec 10% de farine de pois d'Angole germés ou fermentés (Torres et al., 2006; Torres et al., 2007b), ou avec 8 à 10% de farine de lupin (Torres et al., 2007a). Cependant, les pertes azotées fécales dans notre étude étaient supérieures chez les rats recevant les pâtes de fèverole comparativement à ceux consommant les pâtes de gluten. La présence de facteurs bioactifs dans les spaghetti contenant de la farine de fèverole (acide phytique ou inhibiteurs trypsiques) et d'une quantité importantes fibres réduisant l'accessibilité des protéines aux enzymes digestives, a probablement été à l'origine de l'augmentation de l'excrétion d'azote endogène, telle qu'observée dans les *fécès* des rats consommant des pâtes contenant de la farine de fèverole. En effet, les enzymes digestives (dans la salive, les sucs gastriques, pancréatiques et

biliaires) constituent une source importante d'azote endogène, qui peut être augmentée par la présence de facteurs antinutritionnels dans les aliments (Nyachoti et al., 1997). De plus, Shah et al. (1982) rapportaient que l'apport de fibres (comme la cellulose, la pectine, la lignine et la gomme de guar) dans un régime composé de 10% de caséine réduisait la digestibilité des protéines par augmentation de l'excrétion d'azote endogène chez des rats en croissance. La digestion des protéines dans la pâte de fèverole a donc nécessité un plus fort taux de production de protéases endogènes du fait de l'effet inhibiteur des facteurs bioactifs et des fibres ingérées. L'azote endogène sécrété est soumis au processus de digestion, une partie (70-80%) étant réabsorbée au niveau intestinal et une partie étant excrétée dans les fèces (Nyachoti et al., 1997) expliquant les plus forts taux d'azote dans ce compartiment après ingestion d'une pâte mixte blé-fèverole.

Dans notre étude, la quantité totale d'azote hydrolysée et absorbée (digestibilité vraie) était néanmoins la même quel que soit le type de pâte consommée (pâtes au gluten ou mixte blé-légumineuse). Ce résultat est différent de celui obtenu par Torres et al. (2007b) et Torres et al. (2006) qui rapportaient une augmentation de 6 à 13% de la digestibilité en substituant 10% de la semoule de blé par de la farine de pois d'Angole. Cette différence pourrait être liée à l'effet des prétraitements (germination ou fermentation) du pois qui pourraient avoir favorisé la digestibilité des protéines dans les légumineuses dans ces deux études. En effet, les mêmes auteurs rapportaient que la digestibilité d'une pâte de blé et d'une pâte enrichie avec 8 à 10% de lupin (sans prétraitement de germination ou de fermentation) demeurait par contre identique.

Par ailleurs, les vitesses de dégradation et d'absorption, ainsi que l'utilisation des protéines obtenues dans les deux régimes (pâtes au gluten ou mixtes blé-légumineuses) pourraient différer en fonction de la structure respective de ces pâtes. En effet, une rétention protéique plus importante était observée chez les rats ayant consommé des spaghetti à la fèverole. A apports protéiques équivalents, le taux de rétention des protéines alimentaires peut être affecté par plusieurs paramètres comme la composition en acides aminés essentiels, mieux équilibrée dans les spaghetti de fèverole, et la vitesse de digestion des protéines (Bos et al., 2003; Dangin et al., 2003). Dans le cas de notre étude, les protéines de type globulaire des spaghetti à la fèverole étaient plus solubles (dans le SDS et dans l'eau) car principalement liées par des interactions faibles. De ce fait, elles ont probablement pu être digérées plus rapidement que les protéines fibrillaires du gluten peu solubles (dans le SDS et dans l'eau) et

liées par plus d’interactions covalentes engendrant une moindre rétention protéique. En effet, le degré d’hydrolyse des protéines *in-vitro* était plus important pour les spaghetti de fèverole que pour les spaghetti de gluten. La rétention protéique plus élevée obtenue avec les spaghetti contenant de la farine de fèverole se traduisait par une augmentation plus importante de la masse maigre chez les rats en croissance. Ce phénomène pourrait être dû à une augmentation des taux de synthèse protéique tissulaire en particulier au niveau des muscles squelettiques. En effet, une stimulation plus importante de la synthèse protéique musculaire chez des sujets adultes était obtenue après la consommation d’une boisson contenant 4% d’isolat protéique de soja, plus rapidement digérée par rapport à une boisson contenant 4% de caséine micellaire (Tang et al., 2009).

L’utilisation de très hautes températures, plutôt que des basses températures, pour sécher les pâtes réduisait la quantité totale de protéine hydrolysée et absorbée par l’organisme (digestibilité vraie) notamment pour les spaghetti enrichis en gluten. A noter que la réduction de la digestibilité protéique des spaghetti séchés à très haute température, n’était pas observée *in-vitro*. La dégradation de certains acides aminés comme la lysine et la présence de sous-produits des réactions de Maillard (Seiquer et al., 2006) pourraient avoir contribué à une réduction de la digestibilité totale des protéines telles que mesurée *in-vivo*. Si la digestibilité protéique était réduite dans notre étude entre un séchage LT et un séchage VHT, le taux de rétention protéique par l’organisme n’était néanmoins pas modifié. L’efficacité alimentaire, le taux de croissance et l’évolution de la quantité de masse maigre entre le début et la fin de l’étude étaient également identiques entre une pâte séchée à 55 °C ou à 90 °C, que celle-ci soit enrichie en gluten ou en fèverole. Cette dernière observation signifie que l’assimilation des protéines issues des spaghetti enrichis en fèverole était plus efficace que celle du spaghetti enrichi en gluten et ce quelle que soit leur température de séchage. Le meilleur équilibre en acides aminé indispensables (notamment la lysine, la thréonine et les acides aminés soufrés) et le réseau protéique moins structuré des pâtes aux légumineuses y compris séchées à très haute température était sans doute à l’origine de la meilleure efficacité protéique de la pâte à la fève.

Ces premiers résultats ont permis de mettre en évidence le bénéfice de l’enrichissement des pâtes en farine de fèverole sur la digestibilité protéique, les paramètres de croissance et la rétention protéique chez le rat. Il nous semblait intéressant de comparer également l’intérêt de ces pâtes mixtes enrichies en protéines de légumineuses à celui d’une

protéine animale. Pour cela nous avons comparé les résultats obtenus à ceux issus de la consommation d'un régime équivalent mais dont la composante protéique était constituée de caséine.

Les indices de digestibilité protéique obtenus avec les pâtes enrichies en fèverole étaient légèrement inférieurs (3,5 et 4,6% pour les pâtes séchées en conditions respectivement de basse ou de haute température) à ceux obtenus pour le groupe de rats consommant la caséine. En revanche, l'efficacité alimentaire et le taux de croissance étaient similaires pour le régime contenant la caséine et les pâtes enrichies en fèverole. Des travaux antérieurs rapportaient que le taux de croissance des rats pouvait être réduit suite à la consommation d'un régime composé de 20% de protéines de soja par rapport à un régime standard composé de 20% de protéines de caséine. Ce phénomène était expliqué par l'apport de lectines et d'inhibiteurs trypsiques par le soja (Gu et al., 2010). Dans notre étude, les quantités d'inhibiteurs trypsiques ingérées par les rats (1,44 mg/g de pâte cuite en base sèche) étaient beaucoup trop faibles pour engendrer un ralentissement de la croissance des animaux. En effet, selon des études antérieures, l'inhibition de la croissance chez le rat s'observerait au-delà d'un apport de 2 mg d'inhibiteurs trypsiques par gramme de régime (Li, 2003). La présence de facteurs bioactifs dans les spaghetti de fèverole a néanmoins engendré dans notre étude des pertes azotées endogènes plus importantes. De plus, le score de rétention protéique était plus faible de 9% suite à l'ingestion de pâtes contenant de la farine de fèverole par rapport au régime contenant la caséine. Ce résultat pourrait être expliqué par la composition en acides aminés essentiels, du spaghetti de fèverole qui reste déficitaire en lysine (score de 86%), contrairement à la caséine qui présente un profil équilibré en acides aminés essentiels. A noter cependant, que la plus faible rétention protéique n'affectait pas l'augmentation de la masse maigre au cours de la croissance. Cette observation pourrait être associée à la solubilité plus élevée dans l'eau des protéines de fèverole comparativement à celle des caséines, facilitant leur hydrolyse. Cette propriété pourrait augmenter la vitesse de digestion et d'absorption des protéines et induire, au final, une libération plus rapide des acides aminés dans le sang. Bos et al. (2003) ont effectivement démontré chez des sujets consommant des régimes composés de 0,41g/kg (de poids corporel) de protéines de lait (totales) ou de soja, que la vitesse de libération des acides aminés dans le sang était plus importante pour les protéines de soja. Ces auteurs ont d'ailleurs associé ce phénomène à la forte solubilité des protéines de soja par comparaison avec les protéines laitières. Une libération plus rapide des acides aminés dans le sang est à l'origine d'une stimulation plus importante de la synthèse protéique au

niveau tissulaire, en particulier dans le muscle squelettique (Macotela et al., 2011; Tang et al., 2009). Ce phénomène pourrait avoir contribué à maintenir au même niveau, le taux de production de masse maigre chez les rats nourris de fèverole, avec les rats ayant consommés le régime de caséine dans notre étude.

Si la consommation de spaghetti contenant de la fèverole a été bénéfique sur l'absorption et la rétention protéiques chez les rats jeunes quelle que soit leur température de séchage, une augmentation de la masse grasse et du taux de leptine sanguin a été notée dans ce groupe. Ce phénomène pourrait être associé à une structure différente de l'amidon dans les deux régimes (gélatinisé dans la pâte et natif dans le régime de caséine). En outre, le profil des acides aminés constitutifs, différents entre les protéines de blé et de fèverole et la caséine, pourrait également expliquer cette observation. Par exemple, la caséine est plus riche en leucine (~89 mg/g caséine contre 78 mg/g dans la pâte contenant de la farine de fèverole). Or cet acide aminé possède des actions biologiques multiples dont certaines sont associées à l'augmentation de l'adiposité et à ses effets métaboliques (Macotela et al., 2011).

Nos résultats démontrent que l'enrichissement des spaghetti en protéines de fèverole, à un taux correspondant à 50% des protéines totales d'une pâte, améliore considérablement la digestibilité et rétention protéique chez le rat en croissance, quelle que soit sa température de séchage. Un tel régime constitué d'un mélange de protéines végétales complémentaires en acides aminés, faiblement structurées au sein de la matrice alimentaire, permet d'améliorer la qualité de l'apport protéique.

CONCLUSIONS ET PERSPECTIVES

CONCLUSION ET PERSPECTIVES

Ces travaux de thèse avaient pour objectif de produire des pâtes à haute valeur nutritionnelle, riches en protéines rapidement digestibles, avec un apport équilibré en acides aminés essentiels. Ces pâtes sont produites par substitution de la semoule de blé par des quantités importantes de farine de légumineuses. Au démarrage de ce projet, d'après des travaux antérieurs (Petitot et al., 2010b), il n'était pas possible d'incorporer plus de 35% de farine de légumineuses dans une pâte produite à l'aide d'équipements pilotes sans provoquer de problèmes technologiques au moment de l'étape d'extrusion. Ce seuil de 35% de farine de légumineuses correspondait à une pâte au sein de laquelle les protéines de légumineuse représentaient 50% des protéines totales. L'objectif de cette thèse était donc de permettre la production de spaghetti contenant plus de 35% de légumineuses, quelle que soit leur nature, voire même une substitution totale de la semoule de blé par la légumineuse permettant de ce fait l'obtention de spaghetti sans gluten. Trois types de légumineuses ont été incorporés dans la pâte lors de notre étude : la fèverole, la lentille et le haricot urd. Les pâtes ont été séchées à basse ou très haute température, ce dernier paramètre du procédé étant utilisé dans l'industrie pour palier à des altérations des propriétés culinaires et de texture des pâtes. A noter que dans notre cas ces altérations peuvent être associées à la modification de la formulation des pâtes par l'introduction de farine de légumineuses. L'effet de l'incorporation de légumineuses de natures différentes et en quantités croissantes dans la pâte, ainsi que celui des changements des températures de séchage sur la structure de la pâte ont été étudiés à différentes échelles. Les répercussions du changement de la structure des pâtes sur leurs propriétés culinaires et sensorielles, leur teneur en facteurs bioactifs ainsi que sur leur valeur nutritionnelle, notamment la digestibilité *in-vitro* et *in-vivo* (rats en croissance) des fractions protéines et amidon ont été déterminées.

L'incorporation de 35 à 100% de farines de fèverole (représentant plus de 50% des protéines de la pâte) engendre des changements de structure du réseau protéique à différentes échelles. A l'échelle macroscopique, les propriétés texturales et les pertes à la cuisson témoignent d'un affaiblissement linéaire de la structure générale de la pâte en fonction du taux d'incorporation des protéines de fèverole. À l'échelle microscopique le film protéique entourant les granules d'amidon devient visiblement plus épais dans un spaghetti 100%

légumineuses (fèverole, lentille ou haricor urd) contenant deux à trois fois plus de protéines qu'une pâte de blé. A l'échelle supramoléculaire, l'enrichissement des pâtes avec des protéines globulaires de fèverole, très solubles dans l'eau et dans le SDS, s'est traduit par une diminution linéaire des interactions covalentes de type disulfure (stabilisant les protéines de gluten dans une pâte classique) concomitante à une augmentation des interactions faibles entre protéines. Les protéines de fèverole ont donc agit comme un agent diluant du gluten. Cette modification structurale du réseau protéique dans les pâtes s'est traduite par une augmentation de la digestibilité *in-vitro* de la fraction protéique proportionnelle à l'enrichissement en protéines de fèverole (50, 80 et 100%). Cette augmentation de la digestibilité des protéines semblait moins liée aux variations des structures tertiaires et secondaires des protéines des pâtes qui demeuraient tout à fait mineures même si une augmentation des feuillets- β dans la pâte enrichie avec de la farine de fèverole par rapport à une pâte de blé a pu être observée. Les inhibiteurs trypsiques présents en quantité plus élevée dans les pâtes à mesure que l'enrichissement en farine de fèverole est élevé n'ont semble-t-il pas déprécié la digestibilité *in-vitro* des protéines. La quantité restante de ces inhibiteurs dans une pâte après séchage et cuisson demeurait certes faible (29% de la valeur initiale dans la farine de fèverole). La dilution du réseau de gluten par les protéines de légumineuses, s'il a modifié la structure du réseau protéique des pâtes et leur digestibilité *in-vitro*, n'a pas engendré d'augmentation de la digestibilité *in-vitro* de l'amidon. La cinétique de libération du glucose issu des pâtes sans gluten constituées de farine de lentille ou de fèverole était même inférieure à celle des pâtes de blé ou de céréales.

In-vivo, l'intérêt de la consommation de spaghetti mixtes constitués de semoule de blé et de farine de fèverole (65/35) par rapport à celle d'une pâte composée uniquement de blé (enrichie en gluten), contenant toutes deux 17% de protéines, a clairement été démontré sur le taux de croissance, sur la digestibilité, l'efficacité et la rétention des protéines, ainsi que sur l'augmentation de la masse maigre chez des rats jeunes. L'amélioration de ces paramètres physiologiques observée avec le régime contenant les spaghetti mixtes blé-légumineuse peut être en partie expliquée par leur composition plus équilibrée en acides aminés, ainsi que par la structuration du réseau protéique par des interactions majoritairement faibles. A noter que l'amélioration de la composition en acides aminés de la pâte aux légumineuses ainsi que l'affaiblissement de la structure du réseau protéique ont permis d'atteindre des niveaux de croissance et de masse maigre équivalents à un régime contenant des protéines animales (caséines).

Le second objectif de cette thèse était d'éclaircir l'effet de la variation des températures de séchage sur la structure des spaghetti enrichis avec des taux croissants de farine de légumineuses. L'augmentation des températures de séchage est utilisée dans l'industrie pour améliorer les propriétés culinaires et texturales des pâtes. L'utilisation de températures de séchage élevées a participé à la structuration du réseau protéique en augmentant notamment la part des feuillets- β au dépend des hélices- α , et en créant des ponts covalents de type disulfures entre les protéines. Les changements de structure observés au sein des pâtes en sortie de séchage en fonction de la température utilisée ont été toutefois réduits après cuisson des pâtes. Néanmoins, les différences de structure demeuraient plus élevées au sein des pâtes mixtes séchées à basse ou haute température comparativement aux pâtes au blé, pour lesquelles ces différences structurales restaient négligeables. Ce phénomène s'est traduit par une moindre amélioration des propriétés texturales et culinaires pour la pâte au blé par comparaison aux pâtes enrichies en fèverole. D'un point de vue nutritionnel, l'augmentation des températures de séchage dans les pâtes enrichies en fèverole permettait de réduire l'activité anti-trypsique induite par l'addition de la farine de légumineuses dans la pâte. Les fortes températures de séchage induisaient par ailleurs une légère réduction de la teneur en lysine, probablement impliquée dans les réactions de Maillard. L'augmentation du taux d'interactions covalentes dans une pâte du fait de l'augmentation de la température de séchage a conduit à une légère réduction de la digestibilité des protéines *in-vivo* chez les rats jeunes, effet qui a été moins perceptible *in-vitro*. La diminution de la digestibilité n'a pas modifié le taux de croissance ou l'évolution de la masse maigre des rats au cours de la croissance.

D'un point de vue sensoriel, les spaghetti contenant 80% de protéines de fèverole, enrichis en protéines et plus équilibrés en acides aminés indispensables, ont été autant appréciés qu'une pâte de blé complet et ceci indépendamment de leur température de séchage. Les spaghetti sans gluten faits exclusivement de farine de fèverole tendaient eux à être mieux appréciés que leurs homologues sans gluten mais produites à base de céréales et disponibles sur le marché. L'utilisation des températures élevées pour sécher les spaghetti 100% fèverole n'a pas amélioré l'appréciation globale du consommateur mais a permis d'améliorer leurs scores de fermeté et d'élasticité. Les propriétés sensorielles prometteuses des spaghettis riches en légumineuses, appauvris en gluten voire sans gluten vont de pair avec des propriétés nutritionnelles intéressantes notamment un apport considérable en lysine, de fortes teneurs en protéines rapidement digestibles, jusqu'à trois fois plus élevées que dans une pâte classique.

Ces pâtes pourraient être utilisées pour diversifier l'offre de spécialités pastières à haute valeur nutritionnelle sans gluten ou appauvries en gluten, pouvant convenir à tous les consommateurs.

Perspectives

Afin de mieux comprendre comment les protéines de blé et de légumineuses se structurent conjointement, et quelle serait leur contribution dans la détermination de la texture de la pâte par rapport à d'autres composants comme l'amidon, il serait intéressant d'étudier la structure et la texture de matrices modèles avec des teneurs réduites en amidon, faites d'isolats de protéines de légumineuses et de gluten de blé. Les propriétés de texture de ce type de matrices seraient ainsi plus directement reliées à la structure des protéines.

L'introduction de quantités importantes de légumineuses allant jusqu'à 100% dans les pâtes s'est avérée possible. Si la perception sensorielle de ces spécialités pastières est relativement bonne, elle peut sans doute encore être améliorée. Ceci pourrait être réalisé par un choix approprié des températures de séchage et par ajouts de texturants ou de protéines d'œuf qui sont connues pour améliorer la texture et les propriétés culinaires des pâtes.

De plus, les étapes de production et de cuisson des pâtes ne permettant pas d'éliminer tous les facteurs antinutritionnels issus des légumineuses, notamment les phytates, l'utilisation de farines prétraitées pour la production de pâtes pourraient être intéressante à condition toutefois de vérifier que les prétraitements utilisés ne modifient pas la capacité des farines à être pastifiées. La germination des graines de légumineuses, leur fermentation, voire le traitement des farines par des enzymes (phytases) pourraient constituer des prétraitements d'intérêt pour réduire les taux de phytates au sein des pâtes et optimiser la digestion protéique.

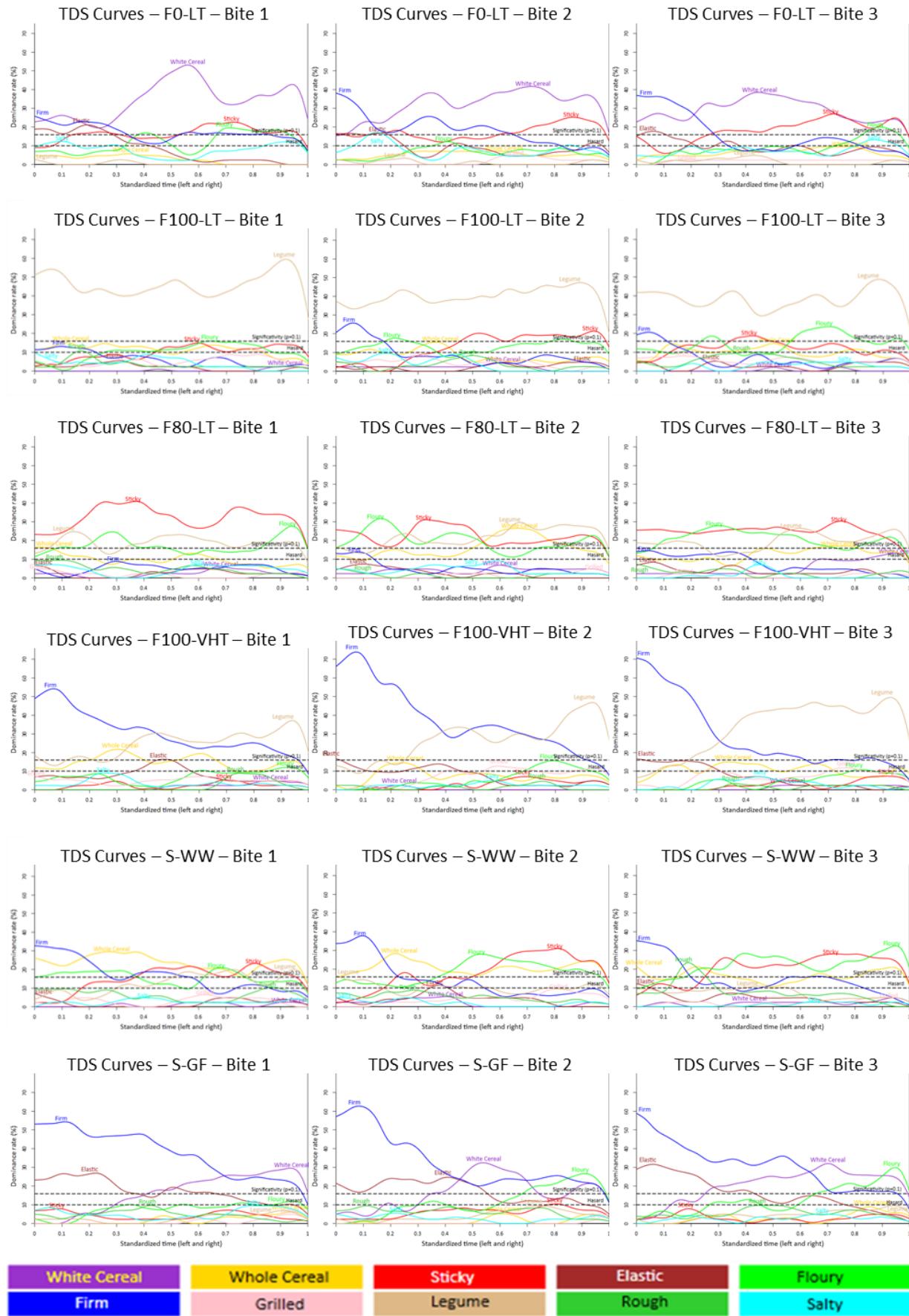
Enfin les pâtes aux légumineuses, intéressantes d'un point de vue nutritionnel grâce à leur teneur élevée en protéines (pouvant aller jusqu'à trois fois celle du blé), leur bonne digestibilité et leur composition équilibrée en acides aminés, devraient faire objet d'étude *in-vivo* à l'aide de différents modèles animaux (rats âgés), mais également d'études cliniques. Les séniors, dont les besoins à la fois quantitatifs et qualitatifs en protéines sont plus élevés que la population générale, pourraient constituer une population cible. Dans cette optique, une étude chez des rats âgés fait actuellement l'objet d'une seconde thèse et permettra de déterminer l'impact de l'enrichissement de pâtes alimentaires en légumineuses sur la

digestibilité et la rétention protéique ainsi que sur la synthèse protéique musculaire au cours du vieillissement.

ANNEXES

ANNEXES

Annexe 1: TDS curves of the six variants of pasta, for each bite



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TRAVAUX RELATIFS A CETTE ETUDE

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Laleg K., Barron C., Sante-Lhoutellier V., Walrand S., Micard V. Protein enriched pasta: structure and digestibility of its protein network. *Food & Function.* 2016, 7, 1196-1207.

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Laleg K., Cassan D., Barron C., Cordelle S., Schlich P., Walrand S. and Micard V. Qualités culinaires, sensorielles et nutritionnelles des pâtes alimentaires sans gluten à base de légumineuses, 31 mai et 1^{er} juin, Dijon, France.

Abstract

In order to reinforce our knowledge about the links between structural and nutritional properties of pasta, pasta with contrasting structures were produced by modifying the formulation and/or the manufacturing process. Pasta structure was then characterized using a multiscale approach and linked to the digestibility of protein and starch fractions. The modification of pasta formulation by partial or total substitution of durum wheat semolina by legume flours changed pasta structure at various scales. A marked weakening in the structure of pasta protein network proportional to the level of legume protein incorporated was observed and related to an improvement of the *in-vitro* digestibility of pasta proteins. Interestingly the *in-vitro* starch digestibility was maintained at its low level or even reduced in mixed pasta. Changing pasta drying temperature amplified changes in the pasta structure. These differences, however, were reduced after cooking pasta resulting in unchanged *in-vitro* protein digestibility between cooked pasta dried at low or very high temperature. Anti-nutritional factors were also reduced by the manufacturing and cooking steps. *In-vivo* study revealed a better growth and nitrogen efficiency in rats consuming the same quantity of proteins from wheat/faba (65/35) mixed pasta in comparison with animals fed with 100% wheat pasta. This was probably related to the better amino acid balance, and to the weakened protein network in mixed pasta. The change in drying temperatures did not allow modifying protein retention.

Pasta developed in this thesis are up to three fold richer in proteins than in traditional wheat pasta, and more balanced in essential amino acids. Produced pasta are appreciated by the consumer as well as their counterparts found on the market. Mixed wheat/legume may be adapted to the elderly people, whereas 100% legume pasta may be consumed by gluten intolerant subjects. Produced pasta may therefore find wide application at the industrial scale.

Keywords: Pasta, legumes, structure, protein network, *in-vitro* and *in-vivo* digestibility.

Résumé

Afin d'approfondir les connaissances sur les liens entre la structure et les propriétés nutritionnelles de pâtes alimentaires, des pâtes de structures contrastées ont été produites en modifiant la formulation et/ou le procédé de fabrication. Leur structure a été caractérisée aux différentes échelles, puis les caractéristiques définies ont été reliées à la digestibilité des protéines et de l'amidon. La substitution partielle ou totale de la semoule de blé dur par des farines de légumineuses lors de la formulation des pâtes a engendré des changements structuraux à plusieurs niveaux d'échelles. Un affaiblissement marqué de la structure du réseau protéique proportionnel à la quantité de légumineuses incorporées a notamment été observé conduisant à une amélioration de la digestibilité *in-vitro* des protéines. La digestibilité *in-vitro* de l'amidon était par contre maintenue à son faible niveau voir même réduite. L'application de différentes températures de séchage a amplifié les modifications de la structure des pâtes enrichies en légumineuses. Ces différences s'atténuait toutefois après cuisson n'engendrant de fait aucune modification marquée de la digestibilité *in-vitro* des protéines entre une pâte cuite préalablement séchée à basse ou à très haute température. Les teneurs des facteurs antinutritionnels étaient réduites par le procédé de fabrication et de cuisson des pâtes. A quantité égale de protéines consommées, l'étude *in-vivo* a révélé une augmentation de la croissance et de l'efficacité azotée chez les rats nourris avec les pâtes mixtes blé/fèverole (65/35) par comparaison aux rats nourris aux pâtes 100% blé. L'assemblage blé/fèverole constituait un mélange protéique efficace pour la croissance des rats jeunes, probablement du fait de son profil équilibré en acides aminés, et de la structure affaiblie du réseau protéique. Par ailleurs, les différentes températures de séchage appliquées aux pâtes n'ont pas engendré de différence au niveau de la rétention azotée *in-vivo*.

Les spécialités pastières développées au cours de ce travail de thèse sont jusqu'à trois fois plus riches en protéines et mieux équilibrées en acides aminés comparativement à une pâte alimentaire classique. Ces spécialités pastières sont aussi bien appréciées que leurs homologues qui se vendent sur le marché. En outre, la recette mixte blé/légumineuses peut être adaptée à tout consommateur souhaitant diversifier ses apports en protéines, mais également à certaines populations spécifiques, notamment aux personnes âgées. La recette 100% légumineuses peut concerner les personnes intolérantes au gluten. Ces spécialités pastières pourraient de ce fait, trouver une large application à l'échelle industrielle.

Mots clés : Pâtes, légumineuses, structure, réseau protéique, digestibilité *in-vitro* et *in-vivo*.